Pepper Paper: Pepper-Black, approximately 1954-1956

PEPPER PAPER

PEPPER - BLACK

the substrate and the reaction mixture chromatographed (collidine–NH_3–water), a metabolite (R_f 0.15) migrating slower than thyroxine (R_f 0.49) was detected by radioautography. Thyroxine was liberated from this compound after treatment with β-glucuronidase. In its chromatographic behavior and the results of enzymatic hydrolysis, this metabolite resembles the thyroxine conjugate found in rat bile by Taurog, *et al.*[2b] (Cpd. U), and Roche, *et al.*[2c] (Cpd. A), and considered by them to be thyroxine glucuronide.

The glucuronide conjugating reaction involving UDPGA and the microsomal system appears to be a general mechanism for the formation of phenolic and alcoholic glucuronides. However, demonstration of the specificity of the enzyme system involved must await purification of the microsomal preparation.

NATIONAL INSTITUTE OF ARTHRITIS AND
METABOLIC DISEASES, AND KURT J. ISSELBACHER
NATIONAL INSTITUTE OF MENTAL HEALTH
NATIONAL INSTITUTES OF HEALTH JULIUS AXELROD
BETHESDA, MARYLAND

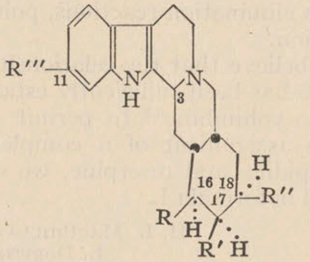
RECEIVED DECEMBER 16, 1954

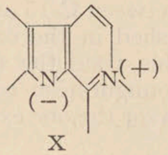
RAUWOLFIA ALKALOIDS. XVIII.[1] ON THE CONSTITUTION OF DESERPIDINE AND RESERPINE

Sir:

In a recent publication[2] we have proposed a structure for deserpidine, a minor alkaloid of many Rauwolfia species, and have pointed out its close chemical and biological similarly to reserpine.[3] We have now been able to convert this alkaloid to α-yohimbine (rauwolscine)[4] by the following series of reactions. Methyl deserpidate tosylate (II) on treatment with sodium iodide or lithium bromide yielded the corresponding 18-halogen compounds III (bromide, m.p. 179–182°, calcd. for $C_{22}H_{27}BrN_2O_3$: C, 59.06; H, 6.08; N, 6.26. Found: C, 58.98; H, 6.31; N, 6.43). These we dehalogenated with zinc in acetic acid to the 18-desoxy compound IV, m.p. 272–275°, (calcd. for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.94; H, 7.49; N, 7.67). Cleavage of the 17-methoxy group with hydrobromic acid and re-esterification of the resulting hydroxyamino acid V with diazomethane afforded α-yohimbine (VI), m.p. 240–242° $[\alpha]^{23}D\ -22 \pm 2$ (ethanol) (calcd. for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.08; H, 6.96; N, 7.77). The infrared spectrum was identical with that of α-yohimbine isolated from *Rauwolfia canescens* leaves.[5]

A further linking of the two alkaloids was accomplished by treatment of the tosylate II with lithium aluminum hydride to form deserpidinol VII, m.p. 232–236° (calcd. for $C_{21}H_{28}N_2O_2$: C, 74.08; H, 8.29; N, 8.23. Found: C, 73.78; H, 8.38; N, 8.29). This compound when subjected to ether cleavage gave rauwolscinyl alcohol (VIII),[6] m.p. 229–231° (calcd. for $C_{20}H_{26}N_2O_2$: C, 73.59; H, 8.03. Found: C, 73.55; H, 7.96); infrared spectrum identical with that of a sample of rauwolscinyl alcohol prepared by the reduction of α-yohimbine (rauwolscine).





X

	R	R′	R″	R‴	C-3
I	$COOCH_3$	OCH_3	3,4,5-Trimethoxybenzoyloxy	H	α
II	$COOCH_3$	OCH_3	Tosyloxy	H	α
III	$COOCH_3$	OCH_3	Br or I	H	α
IV	$COOCH_3$	OCH_3	H	H	α
V	COOH	OH	H	H	β
VI	$COOCH_3$	OH	H	H	β
VII	CH_2OH	OCH_3	H	H	α
VIII	CH_2OH	OH	H	H	β
IX	CH_2OH	OCH_3	OH	OCH_3	α
XI	CH_2OH	OCH_3	OH	OCH_3	β

Since α-yohimbine has been shown to have the alloyohimbane structure[7] it would seem logical on the basis of the above interconversion to assign the same stereochemical configuration to the basic ring system of deserpidine. However we have accumulated considerable evidence which shows that resperine and its derivatives, (methyl reserpate, reserpinediol, reserpinol) and with less ease also deserpidine and its derivatives undergo an acid or base catalyzed epimerization at the C_3 center. That this and only this center is involved is shown by the successive lead tetraacetate oxidation of (for example) reserpinediol (IX) to tetradehydroreserpinediol (X) [isolated as the hydrochloride: m.p. 280–282°; calcd. for $C_{22}H_{27}N_2O_4Cl\cdot{}^1/_2H_2O$: C, 61.75; H, 6.34; N, 6.55. Found: C, 62.07; H, 6.63; N, 6.59; ultraviolet maxima at 251–2 mμ (log ε 4.52) and 325 mμ (log ε 4.33)] and reduction with sodium borohydride to 3-iso-reserpinediol (XI), m.p. 220–222° (calcd. for $C_{22}H_{30}N_2O_4\cdot{}^1/_2H_2O$: C, 66.83; H, 7.59. Found: C, 66.35; H, 8.06. Diacetate: m.p. 210–212°, calcd. for $C_{26}H_{34}N_2O_6$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.40; H, 7.41; N, 5.91), which was identical with a sample prepared by the acid or base catalyzed epimerization of reserpinediol. Therefore, it seemed probable that in the conversion of deserpidine to α-yohimbine inversion has taken place. Evidence to show that this was actually so was obtained by treating 3-epi-α-yohimbine[1] under the demethylation condition described above. After reesterification we obtained α-yohimbine, a transformation which has also been accomplished by oxidation and subsequent reduction of ring C[1].

---

[1] Paper XVII, F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, THIS JOURNAL, in press.

[2] E. Schlittler, P. R. Ulsafer, M. L. Pandow, R. M. Hunt and L. Dorfman, *Experientia*, **11**, in press (1955).

[3] L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Müller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

[4] A. Chatterjee, A. K. Bose and S. Pakrashi, *Chemistry and Industry*, 491 (1954).

[5] A. Mookerjee, *J. Indian Chem. Soc.*, **18**, 33 (1941).

[6] A. Chatterjee and S. Pakrashi, *Science and Culture (India)*, **19**, 109 (1953).

[7] A. Le Hir, *Compt. rend.*, **234**, 2613 (1952); A. Le Hir, M. M. Janot and R. Goutarel, *Bull. soc. chim., France*, **20**, 1027 (1953).

We therefore propose that deserpidine and reserpine are derivatives of 3-epi-α-yohimbine.

Regarding the stereochemistry of the substituents in ring E of these alkaloids, we feel that the formation of the γ-lactone of reserpic acid[3] and of deserpidic acid together with other evidence which we have obtained from elimination reactions, point to an all *cis* configuration.

Although we do not believe that the relationship between C-15 and C-16 has been sufficiently established in the case of α-yohimbine[4,7] to permit at this time the definite assignment of a complete configuration to deserpidine and reserpine, we do favor the one expressed in formula I.

RESEARCH DEPARTMENT
CIBA PHARMACEUTICAL PRODUCTS, INC.
SUMMIT, NEW JERSEY

H. B. MACPHILLAMY
L. DORFMAN
C. F. HUEBNER
E. SCHLITTLER
A. F. ST. ANDRÉ

RECEIVED JANUARY 21, 1955

RESOLUTION AND SYNTHESIS OF AN OPTICALLY ACTIVE FLUORO COMPLEX

Sir:

The role of fluoride ion in complex formation has been of especial interest in studies on bond type in coördination compounds.[1,2] Magnetic evidence has indicated that fluoride is capable of forming bonds with tervalent cobalt of the extreme ionic type, *viz.*, in $[CoF_6]^{\equiv}$, but is also able to enter into covalent bond formation when present in partially substituted cobalt ammines.[3] Except for the information inferred from the fact that the latter type of complexes are diamagnetic, little is known about the nature of the Co–F bond in these complexes.

We have recently succeeded in resolving the complex, *cis*-$[Co\ en_2F_2]^+$ (I) by use of *l*-dibenzoyltartaric acid (II) and have prepared (I), as well as *cis*-$[Co\ en_2NH_3F]^{+2}$ (III), in active form through reactions of the analogous chloro complexes. This is believed to be the first instance in which a complex containing coördinated fluoride has been resolved.

The resolution consisted in bringing together stoichiometric quantities of *cis*-$[Co\ en_2F_2]I$, Ag_2CO_3 and (II), removing AgI and precipitating the *d*-$[Co\ en_2F_2]^+$ salt of (II) with acetone. Purification of the latter was effected by dissolving it in a small quantity of water and chilling sharply, whereby a crystalline product was obtained having $[\alpha]^{28}D$ +120°. The resolving agent was removed by triturating the diastereomer with acetone containing a little concd. HNO_3, yielding thereby *d*-$[Co\ en_2F_2]NO_3$ with $[\alpha]^{28}D$ +220.

Active (I) was also prepared by the reaction of *l*-$[Co\ en_2Cl_2]Cl$ ($[\alpha]_D$ +610) in 1:1 ethanol–HF, in which an excess of Ag_2CO_3 had been dissolved. For the purified substance, isolated as the nitrate, $[\alpha]^{28}D$ +220. The *dextro* isomer of (III) was prepared in like manner by starting with *d*-$[Co\ en_2NH_3Cl]Cl_2$ ($[\alpha]_D$ +140°). For the bromide of (III), $[\alpha]^{28}D$ +170. The salts of (I) and (III) were isolated in microcrystalline form, that of (I) being red, and that of (III) being salmon in color.

Kinetic studies now in progress on a number of cobalt fluoro complexes indicate that the rates of racemization and aquation are slower than those of the analogous chloro complex. At 35° in 0.1 *N* HNO_3 (I) mutarotates to about one half the original rotation at a moderate rate (half-life, 1 hr.) and then loses its remaining activity over a period of several days. A study of the reactions undergone by the active fluoro complexes with a number of reagents to determine the nature of active products is also being undertaken.

A more detailed account of this work as well as other results will be communicated in the near future.

The authors gratefully acknowledge the support of grants from the National Science Foundation and the Atomic Energy Commission. They wish also to express thanks to Dr. F. P. Dwyer for his helpful suggestions and interest in this work.

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

WILLIAM R. MATOUSH
FRED BASOLO

RECEIVED JANUARY 10, 1955

---

(1) W. C. Fernelius, *Record Chem. Progress* (*Kresge-Hooker Sci. Lib.*), **2**, 17 (1950).

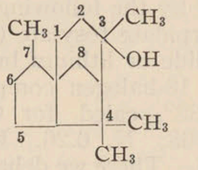
(2) H. Taube, *Chem. Rev.*, **50**, 69 (1952).

(3) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1945, pp. 116–117.

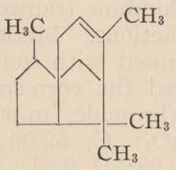
THE TOTAL SYNTHESIS OF CEDROL AND CEDRENE

Sir:

We have recently outlined the considerations which led us to propose structure I for the tricyclic sesquiterpene cedrol[1] and II for the related cedrene.

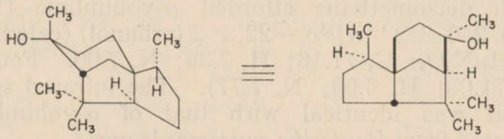


I



II

Our communications on the subject did not deal with the stereochemistry of the five asymmetric centers present in I, but various considerations have led us to consider III the most likely representation of the stereochemistry of cedrol.



III

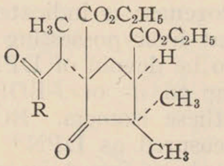
We have now completed an unambiguous, stereospecific total synthesis of cedrol which confirms the stereochemistry illustrated by formula III and incidentally provides unambiguous proof that no rearrangement is involved in the dehydration of cedrol to cedrene:

Diethyl 4,4-dimethyl-5-keto-1,3-cyclopentane dicarboxylate[2] was alkylated with benzyl α-bromopropionate, and the resulting triester was hydrogenolyzed over palladium charcoal to the acid IV, m.p. 113–115°. (Found: C, 58.47; H, 7.43).

---

(1) G. Stork and R. Breslow, THIS JOURNAL, **75**, 3291 (1953).

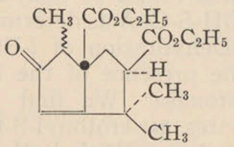
(2) *Cf.* C. S. Gibson, K. V. Hariharan and J. L. Simonsen, *J. Chem. Soc.*, **3009** (1927).



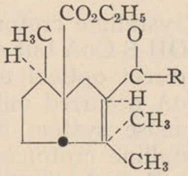
IV, R = OH
V, R = CH_3

The dry sodium salt of IV was transformed by oxalyl chloride into the corresponding acid chloride which reacted readily with diazomethane to give a crystalline diazoketone. The diazoketone was converted into the chloromethyl ketone, m.p. 72–73° (Found: C, 56.41; H, 7.08), which was readily reduced to the crystalline methyl ketone (V) (Found: C, 62.34; H, 7.92. Semicarbazone, m.p. 191–193°; Found: C, 56.41; H, 7.91).

Short treatment with potassium *t*-butoxide changed the diketone (V) into the bicyclic aldol which was readily dehydrated by heating with *p*-toluene sulfonic acid in benzene. The cyclopentenone so obtained (VI) is a mixture of C_7 epimers, one of which had m.p. 65–67°. (Found: C, 66.21; H, 7.87). Epimerism at C_7 at this stage of the synthesis is irrelevant, and the 65° isomer was reduced with lithium and liquid ammonia, or with palladium on charcoal, to the *cis* bicyclic ketone (VII) m.p. 33.5–35.0°; dinitrophenylhydrazone, m.p. 160–161° (Found: C, 56.26; H, 6.04). The stereochemistry at C_7 in this ketone corresponds to that of the more stable epimer since base hydrolysis, followed by reesterification gave back unchanged VII. Transformation of VII into the thioketal, m.p. 75–76° (Found: C, 59.23; H, 7.77) and Raney nickel desulfurization of the latter gave *dl*-diethyl norcedrenedicarboxylate, hydrolyzed to *dl*-norcedrenedicarboxylic acid VIII, m.p. 221–223°. (Found: C, 65.02; H, 8.36). The infrared spectrum of the anhydride of VIII was indistinguishable from that of *l*-norcedrenedicarboxylic anhydride obtained by the degradation of natural cedrene.[3]



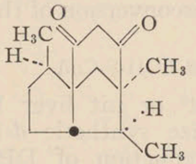
VI



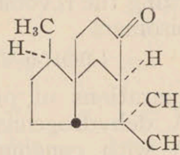
VIII, R = OH
IX, R = CH_3

Resolution of the *dl*-acid was readily effected by means of the quinine salt, m.p. 209–210°; $(\alpha)^{27}\text{D}$ $-122°$ (*c* 1.00 in chloroform). (Found C: 70.13; H, 8.18), which was decomposed to produce *l*-norcedrenedicarboxylic acid, m.p. 212–213°, undepressed on mixture with the natural isomer; the rotation, $(\alpha)^{27}\text{D}$ $-38.9°$ ± (*c* 1.08 in acetone), was identical with that of the acid from natural sources.

Conversion of the *l*-acid by established paths[4] into the methyl ketone (VIII), followed by treatment of the ester with potassium *t*-butoxide led to the cyclohexane dione (X), m.p. 202–204°. (Found: C, 76.66; H, 9.07). Reduction of X with lithium aluminum hydride gave a mixture of saturated and unsaturated alcohols which, on oxidation with chromic acid, followed by catalytic hydrogenation, gave rise to the saturated ketone (XI); 2,4-dinitrophenylhydrazone, m.p. 146–147°. Reaction of the ketone (XI) with methyl lithium gave *l*-cedrol (III), identical in all respects with the natural product (m.p. and mixed m.p. 86.5–87.5°, identical infrared absorption curves).



X



XI

The stereochemically simpler cedrene is obtainable by dehydration of cedrol with formic acid, and the synthesis of cedrol is also a synthesis of cedrene.

CHANDLER LABORATORY
COLUMBIA UNIVERSITY
NEW YORK 27, NEW YORK
GILBERT STORK
FRANK H. CLARKE, JR.

RECEIVED JANUARY 28, 1955

[3] We thank Dr. T. F. Gallagher for carrying out this comparison.

[4] G. Stork and R. Breslow, THIS JOURNAL, **75**, 3292 (1953).

ENZYMATIC RACEMIZATION OF β-HYDROXYBUTYRYL-S-CoA AND THE STEREOSPECIFICITY OF ENZYMES OF THE FATTY ACID CYCLE[1]

Sir:

Some evidence has been presented that the enzymes crotonase[2,3] and β-hydroxybutyryl-S-CoA dehydrogenase (β-keto reductase)[4,5,6] which catalyze reactions (1) and (2) are specific for *d*-BOH-S-CoA.[5,6]

$$\text{Crotonyl-S-CoA} + H_2O \rightleftarrows d\text{-BOH-S-CoA} \quad (1)$$

$$d\text{-BOH-S-CoA} + \text{DPN}^+ \rightleftarrows \text{acetoacetyl-S-CoA} + \text{DPNH} + H^+ \quad (2)$$

However, an "activating" enzyme of liver forms both the *d*- and *l*-isomers of BOH-S-CoA from the corresponding free acids,[5] probably according to reaction (3).

$$\text{BOH} + \text{CoA-SH} + \text{ATP} \rightleftarrows \text{BOH-S-CoA} + \text{AMP} + \text{PP} \quad (3)$$

By use of synthetically prepared *l*-BOH-S-CoA and *d*-BOH-S-CoA[7] we have been able to establish directly the stereospecificity of enzymes catalyzing reactions (1) and (2), and have found that liver and other organs contain an enzyme or enzyme system

---

[1] Supported by grants from the U. S. Public Health Service, the American Cancer Society (recommended by the Committee on Growth National Research Council), the Nutrition Foundation, Inc., and by a contract (N6onr279, T.O. 6) between the Office of Naval Research and New York University, College of Medicine. Abbreviations: Coenzyme A (reduced), CoA-SH; acylcoenzyme A derivatives, acyl-S-CoA; β-hydroxybutyric acid, BOH; β-hydroxybutyryl-S-CoA, BOH-S-CoA; *d* and *l* refer to direction of rotation; DPN^+ and DPNH, oxidized and reduced diphosphopyridine nucleotide; 2-amino-2-methyl-1,3-propanediol, Diol.

[2] J. R. Stern and A. del Campillo, THIS JOURNAL, **75**, 2277 (1953).

[3] S. J. Wakil and H. R. Mahler, *J. Biol. Chem.*, **207**, 125 (1954).

[4] F. Lynen, L. Wessely, O. Wieland and L. Rueff, *Angew. Chem.*, **64**, 687 (1952).

[5] A. L. Lehninger and G. D. Greville, *Biochim. Biophys. Acta*, **12**, 188 (1953).

[6] S. J. Wakil, D. E. Green, S. Mii and H. R. Mahler, *J. Biol. Chem.*, **207**, 631 (1954).

[7] Prepared from *l*-BOH and *d*-BOH (donated by Dr. G. D. Greville) by the general method of T. Wieland and L. Rueff, *Angew. Chem.*, **65**, 186 (1952).

catalyzing the reversible interconversion of the two stereoisomers

$$l\text{-BOH-S-CoA} \rightleftarrows d\text{-BOH-S-CoA} \qquad (4)$$

Preparations of pig heart[8] or rat liver BOH-S-CoA dehydrogenase oxidize synthetic *d*-BOH-S-CoA with concomitant reduction of DPN as determined spectrophotometrically. *l*-BOH-S-CoA is not oxidized by the dehydrogenase, nor does it inhibit the oxidation of *d*-BOH-S-CoA (Fig. 1).

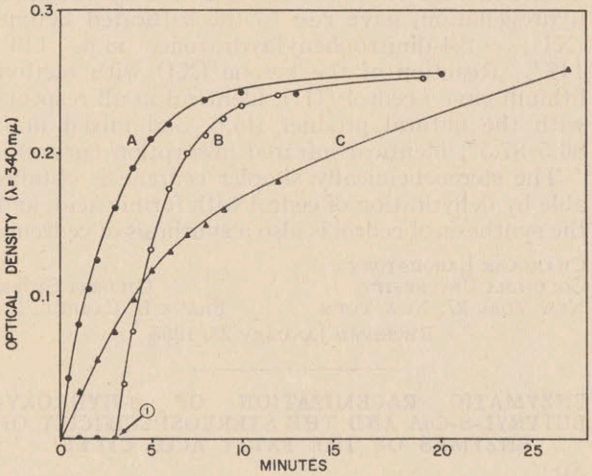


Fig. 1.—The experimental cell (d = 0.5 cm.) contained initially 100 μM. Diol buffer *p*H 9.5, 0.27 μM. DPN^+ and 0.14 μM. *d*-BOH-S-CoA (A), or 0.13 μM. *l*-BOH-S-CoA (B), or 0.21 μM. *l*-BOH-S-CoA (C). The reaction was started at zero time by addition of 20 γ of pig heart fraction (A and B) or 600 γ of ox liver fraction (C). At Arrow 1, 0.14 μM. *d*-BOH-S-CoA was added. A concomitant increase in O.D. at λ310 mμ (not shown) due to acetoacetyl-S-CoA formation was recorded in Experiments A and B, but was partly obscured in Experiment C by contamination of liver fraction with thiolase; volume, 1.5 ml.; temp., 25°.

Hence the dehydrogenase is stereospecific for the *d*-isomer. However, certain enzyme fractions from ox and rat liver which oxidize *d*-BOH-S-CoA were also found to oxidize *l*-BOH-S-CoA (*cf.* Figure 1), but at much slower rates. This suggested the presence either of a racemase or of a DPN^+-linked dehydrogenase specific for *l*-BOH-S-CoA which in concert with the *d*-BOH-S-CoA dehydrogenase could effect racemization. As shown in Table I, the dialyzed ox liver fraction catalyzes the conversion of *l*-BOH-S-CoA to *d*-BOH-S-CoA, measured with *d*-BOH-S-CoA dehydrogenase, and conversely the conversion of the *d*-isomer to a form which no longer reacts directly with DPN^+ in the presence of *d*-BOH-S-CoA dehydrogenase but which does react on addition of the ox liver fraction. Since (a) the liver fraction contains only 0.5–1 γ of DPN^+ per mg. protein[9] (final DPN^+ concentration $\leq 1.3 \times 10^{-6}$ *M*), and (b) the rate of interconversion of the isomers is not affected by addition of excess DPN^+ although the K_M for DPN^+ of *d*-BOH-S-CoA dehydrogenase is 4.3×10^{-5} *M*,[6] the presence of a racemase rather than a specific *l*-BOH-S-CoA dehydrogenase is indicated. Preparations of ox and rat liver possessing racemase activity were found to be devoid of DPN^+-linked dehydrogenases acting on *d*- or *l*-BOH and of enzymes racemizing these isomers. BOH-S-CoA racemase activity, measured as DPN^+ reduction by *l*-BOH-S-CoA, has been found in extracts of mitochrondria from rat liver, kidney, heart and brain and in extracts of *R. rubum* and *Cl. acetobutylicum*, all of which contain the *d*-BOH-S-CoA dehydrogenase.

TABLE I

100 μM. diol buffer, *p*H 9.5, BOH-S-CoA (as indicated) and 1 mg. of ox liver fraction (final volume, 1.0 ml.) were incubated 30 minutes at 25°. Assays performed after acid heat deproteinization.

	μM. recovered	
Addition	*d*-BOH-S-CoA[b]	*l*-BOH-S-CoA[c]
l-BOH-S-CoA[a] (0.21 μM.)	0.06	0.13
d-BOH-S-CoA[a] (0.20 μM.)	0.10	0.12

[a] Assayed by conversion to citrate in a multi-enzyme system.[2] [b] Assayed with heart *d*-BOH-S-CoA dehydrogenase fraction. [c] Measured by reduction of DPN on addition of ox liver fraction after oxidation of *d*-BOH-S-CoA by heart dehydrogenase fraction.

Crystalline crotonase[10] was found to dehydrate *d*-BOH-S-CoA but not *l*-BOH-S-CoA as determined by direct optical test.[11] Dehydration of *l*-BOH-S-CoA occurred only in the presence of the liver racemase system and crotonase. We find that crystalline crotonase hydrates *cis*-crotonyl-S-CoA as well as *trans*-crotonyl-S-CoA. With both isomers the product of hydration has been identified as *d*-BOH-S-CoA by enzymatic assay.

The enzymatic racemization of BOH-S-CoA may play a special role in linking ketone body metabolism to the fatty acid cycle. The properties of the racemase system and its significance are currently being investigated.

DEPARTMENT OF PHARMACOLOGY
NEW YORK UNIVERSITY
COLLEGE OF MEDICINE
NEW YORK 16, N. Y.
JOSEPH R. STERN[12]
ALICE DEL CAMPILLO[13]
DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
THE JOHNS HOPKINS SCHOOL OF MEDICINE
BALTIMORE 5, MD.
ALBERT L. LEHNINGER

RECEIVED NOVEMBER 1, 1954

---

[8] J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).

[9] Assayed catalytically according to an unpublished procedure of Dr. S. Korkes.

[10] J. R. Stern, I. Raw and A. del Campillo, *Fed. Proc.*, **13**, 304 (1954).

[11] Compare F. Lynen and S. Ochoa, *Biochim. Biophys. Acta*, **12**, 299 (1953).

[12] Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland 6, Ohio.

[13] Department of Biochemistry, University of Puerto Rico, San Juan, Puerto Rico.

A MECHANISM FOR THE PHOTO-OXIDATION OF WATER BY CERIC ION

Sir:

Chemical evidence has been obtained for the intermediate formation of OH radical in the photoreduction of ceric ion as proposed by Weiss and Porret.[1] The evidence is the effect of added solutes which have been demonstrated to react with OH radical in aqueous solution. Photoreduction of ceric ions by ultraviolet radiation, previously re-

---

[1] J. Weiss and D. Porret, *Nature*, **139**, 1019 (1937).

significance of a mean and of a correlation coefficient. *J. Roy. Stat. Soc.,* Vol. C, 69 (1937).

3. Hanson, H. L., Davis, J. G., Campbell, A. A., Anderson, J. H., and Lineweaver, Hans. Sensory test methods. II. Effects of previous tests on consumer response to foods. *Food Technol.,* **9**, 56 (1955).
4. Ishler, Norman H., Laue, Elsie A., and Janisch, Anna J. Reliability of taste testing and consumer testing methods. II. Code bias in consumer testing. *Food Technol.,* **8**, 399 (1954).
5. Langwill, Katheryn. Taste perceptions and taste preferences of the consumer. *Food Technol.,* **3**, 136 (1949).
6. United States Department of Agriculture, Agricultural Marketing Service. "U. S. Standards for Grades of Canned Green Beans and Canned Wax Beans." March 2 (1953).

A Chemical and Flavor Study of Off-Flavor Due to Artificial Pepper[a]

Emily L. Wick and Stanley E. Cairncross
Arthur D. Little, Inc., Cambridge, Mass.

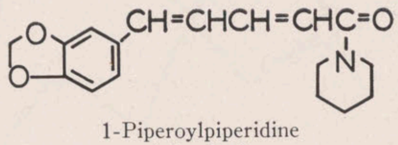
(Manuscript received June 14, 1956)

A SINGLE OFF-FACTOR in a highly blended food product can upset the entire flavor, changing the character completely.

The work carried out in our laboratories on black pepper resulted from the use of an abnormally compounded pepper in the preparation of a high quality commercial product. This paper has two main objectives: (a) to describe the method we used in detecting, isolating, and characterizing the pepper compound responsible for the flavor upset in a high-quality food product—thereby showing the effectiveness of integrating flavor and chemical analyses in solving such a problem; and (b) to outline an expedient method of determining the suitability of any black pepper oleoresin for use in a good product.

Composition of black pepper oleoresin. The principal components of pepper flavor are its aromatic or odor-bearing constituents and its mouth-feeling factors, referred to as "bite". The aromatic factors are contained in the volatile oils which can be obtained from pepper oleoresin by distillation. The "bite" principle is found in its non-volatile components.

Recently most of the chemical components of pepper oil have been identified by Hasselstrom *et al. (7, 8)*. Some of these compounds are α-pinene, β-pinene, 1-α-phellandrene, DL-limonene, piperonal, dihydrocarveol, β-caryophyllene, various oxygenated compounds and a piperidine complex. The chemical investigation of pepper bite has been the subject of a major body of research since the first isolation of crystalline piperine in 1820 *(13)*. It is generally believed that the active principle of black pepper consists of a mixture of piperine and a very pungent resin—chavicine, a sterioisomer of piperine.



1-Piperoylpiperidine
(piperine)

An excellent review of the elucidation of the structure of these compounds has been made by Newman *(11)*. Investigations of the other components of the non-volatile pungent fraction of black pepper and of the relative contribution of piperine and chavicine to pepper "bite" are currently in progress *(16)*. A comprehensive review of synthetic bite compounds with melting or boiling points, degree of pungency and taste, and literature references to their preparation has been published by Newman *(12)*.

Since piperine is universally accepted as the predominant pungent principle in black pepper, piperine content is generally used as a basis for judgment of pepper quality. Almost identical spectrophotometric methods of piperine assay have recently been published by the research laboratories of the William J. Stange Co. *(16)* and of the Kraft Foods Co. *(5)*. These methods are based upon the ultraviolet absorption spectrum of piperine and are therefore superior to the Kjeldahl *(14)* determination of total nitrogen in which piperine could not be distinguished from other nitrogenous materials present either as natural components or as intentional adulterants.

EXPERIMENTAL

The present investigation was initiated when a normally high-quality food product was found to have developed unusual and somewhat disturbing flavor characteristics. This change was defined by an experienced flavor panel using the Flavor Profile method *(1, 3)*. The intentionally high blends of aroma and flavor in the normal product were upset, and a foreign character note described as "woody-raisin" dominated the profiles of both the aroma and the flavor.

Although it was possible that the off-flavor was the result of some reaction occurring during the preparation of the product, careful flavor analysis of each ingredient that had gone into the product pointed to the black pepper oleoresin. Subsequently, the product was prepared in the laboratory with various known quantities of the suspect oleoresin and with duplicate quantities of an authentic oleoresin. Flavor panel findings showed that the black pepper oleoresin was undoubtedly the sole source of the foreign note in the final product.

The next step in the investigation was to find out the nature of the abnormality of the offensive black pepper oleoresin. Three major physicochemical approaches were employed, each involving parallel treatment of the abnormal sample and an authentic sample of black pepper oleoresin: (a) spectrographic determination of piperine content; (b) chromatographic separation; (c) fractional distillation of volatile oils and purification of nonvolatile residue.

Throughout the physicochemical investigation, the flavor panel served to evaluate the isolates. For aroma, the panel analyzed the isolates suspended in odorless mineral oil. For flavor, the panel tasted specially prepared samples of the final product from which pepper had been omitted and in which were

---

[a] Presented at Sixteenth Annual Meeting of the Institute of Food Technologists, St. Louis, Missouri, June 13, 1956.

incorporated known amounts of the isolates to be evaluated. In this manner the direction of the chemical investigation was guided by the findings of the panel, and unnecessary chemical work was avoided.

Methods. *Spectrophotometric Determination of Piperine.* The method of Fagan, Kolen, and Hussong *(5)* was used to determine piperine in the oleoresins of black pepper. Piperine was isolated from oleoresin of black pepper and purified to constant melting point (m.p. 132-133°) by recrystallization from absolute ethanol.

All spectrophotometric measurements were made on a Beckman Model DU Spectrophotometer with ultraviolet attachment. One-centimeter matched silica cells were used.

Chromatographic Separation of Authentic and Abnormal Oleoresins. Each oleoresin (0.250 g.) was added to a column (1 x 30 cm.) prepared from a slurry of 8 g. of untreated Merck "Aluminum Oxide Suitable for Chromatographic Use" in Skellysolve B. The alumina was Brockmann grade V activity *(2)* and had pH 9-10. The columns were run concurrently and were eluted with two 5 ml.-portions of each of the following solvents: Skellysolve B, skellysolve-benzene (4:1), skellysolve-benzene (3:2), skellysolve-benzene (2:3), skellysolve-benzene (1:4), benzene, benzene-ethyl ether (4:1), benzene-ethyl ether (3:2), benzene-ethyl ether (2:3), benzene-ethyl ether (1:4), ethyl ether, ethyl ether-ethanol (4:1), ethyl ether-ethanol (3:2), ethyl ether-ethanol (2:3), ethyl ether-ethanol (1:4), ethanol, 80% aqueous ethanol, 60% ethanol, 40% ethanol, 20% ethanol and water. General examination of the physical properties and odor of each of the resulting 42 fractions was carried out after removal of solvent under reduced pressure. Fractions 12-20 from the authentic oleoresin were combined and recrystallized from hot absolute ethanol. Piperine (m.p. 132-133°) was obtained as pale yellow crystals; λmax 344 mμ. Fractions 12-17 from the abnormal oleoresin were combined. Purification by sublimation under reduced pressure yielded a white crystalline compound, m.p. 122-123°; λmax 275 mμ.

Separation of Volatile Oils. A. Abnormal Oleoresin. A suspension of 11.4 g. of abnormal oleoresin in 300 ml. of water was distilled in the normal manner in a Clevenger still *(10)*. The distillate thus obtained was extracted with ether and the ether extract containing the volatile oils was dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure yielded a clear liquid, 1.4729 g. (12.8%), $n_D^{25^\circ}$ 1.4948. This liquid exhibited the sought for off-flavor. Vapor chromatograms were obtained by means of a Perkin-Elmer Vapor Fractometer, Model 154. The column temperature used was 150° C.

Distillation of the volatile oils in a semimicro column *(6)* yielded the following fractions: 1) b.p. 75-79° (1 mm.); 0.3334 g.; $n_D^{24^\circ}$ 1.4934. 2) b.p. 79-83° (1 mm.); 0.5912 g.; $n_D^{24^\circ}$ 1.4962. Redistillation of the second fraction yielded an analytically pure sample $n_D^{24^\circ}$ 1.4964; $[\alpha]_D^{25^\circ}$ — 12.5°; sp. gr. 25°/4° 0.8810. This liquid when incorporated in the original product at a concentration of 2 p.p.m. (0.0002%) exhibited the typical unpleasant flavor and aroma first noted.

Analysis. Calculated for $C_{15}H_{24}$: C, 88.16; H, 11.84; mol. wt. 204. Found: C, 88.19; H, 12.01; mol. wt. 217 (Rast method in camphor). Sodium fusion disclosed no other elements.

The infrared spectrum of the off-flavor liquid was typical of that of terpenes in that the presence of a terminal isopropyl group and an exocyclic methylene group was indicated.

B. Authentic Oleoresin. The volatile oils were removed in the manner described above from 30 g. of authentic oleoresin. A colorless liquid which exhibited no off-flavor or aroma was obtained in 2.7% yield: 0.8291 g.; n_D^{24} 1.4890. Vapor chromatograms were obtained at a column temperature of 150° C. Distillation in a semimicro column *(6)* yielded the following fractions: 1) b.p. 62-75° (1 mm.); 0.0637 g.; $n_D^{24^\circ}$ 1.4938. 2) b.p. 75-76° (1 mm.); 0.2030 g.; $n_D^{24^\circ}$ 1.4942; $[\alpha]_D^{25^\circ}$ — 12.6°; sp. gr. 25°/4° 0.8930. Neither fraction exhibited off-flavor.

Investigation of Nonvolatile Portions. A. Abnormal Oleoresin. The aqueous suspension remaining after distillation of the volatile oils contained an insoluble resinous material and a white crystalline solid. The suspension was extracted with ether, the yellow ether extract dried, concentrated under reduced pressure and the residue was sublimed at 60-70° (1-2 mm.). A white crystalline sublimate was obtained in 25% yield. Resublimation yielded an analytically pure sample, m.p. 122-123°, which had no odor but provided a sharp pepper-bite.

Analysis. Calculated for $C_{14}H_{17}NO$: C, 78.10; H, 7.96; N, 6.51; mol. wt. 215. Found: C, 78.18; H, 8.19; N, 6.56; mol. wt. 242 (Rast method in camphor). Sodium fusion disclosed no other elements. λmax 275 mμ. (See Figure 2).

This compound was proved to be 1-cinnamoyl piperidine by comparison with an authentic sample[b] of this substance. The

B. Authentic Oleoresin. The aqueous suspension remaining after removal of the volatile oils contained an insoluble resinous layer. There was no evidence of any compound similar to 1-cinnamoyl piperidine described above. The dark resinous layer was separate and treated with boiling ethanol. Crystalline piperine (m.p. 132-133°) was obtained. No attempt to further investigate this fraction was made.

RESULTS

As depicted in Table 1 spectrographic determinations showed a significant lack of piperine in the abnormal oleoresin. It contained less than half the amount of piperine found in the authentic oleoresin. Evidence that the off-flavor oleoresin contained an abnormal constituent or adulterant was provided by its ultraviolet spectrum. It showed, in addition to the usual piperine maximum at 344 mμ, an additional strong peak at 275 mμ. (See Figure 1.) This finding suggested that

TABLE 1

Comparison of general characteristics of authentic and abnormal pepper oleoresins

	Authentic Oleoresin	Abnormal Oleoresin
Aroma	Typical pepper	Woody in addition to pepper
Flavor	Typical pepper	Woody-raisin
Color	Yellow	Tan to pale yellow
pH (of aqueous suspension of pepper dispersion)	7.9	7.3
Piperine content (%) (5)	46.4	19.0
Ultraviolet spectrum (λmax in chloroform)	344 mμ	344 mμ 275 mμ

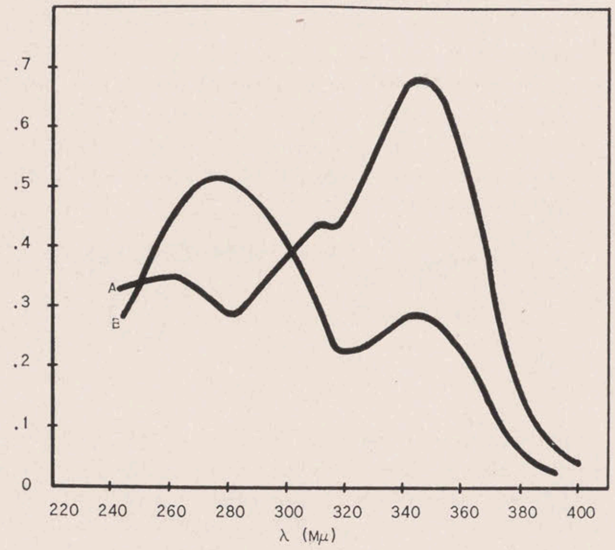


Figure 1. Ultraviolet spectrum of authentic oleoresin (A) and abnormal oleoresin (B) in chloroform.

---

[b] Kindly provided by Dr. L. C. King, Northwestern University.

melting point, mixed melting point and infrared spectrum of both were identical.

the compound responsible for the abnormal spectrum was probably a nitrogeneous substance added to mask the lack of a normal piperine content. Since the Kjeldahl total nitrogen determination *(14)* of piperine could not have provided this information the value of the spectrophotometric piperine determinations is well illustrated.

Initial chemical evidence concerning the apparent abnormal composition of the adulterated oleoresin was provided by chromatographic separation of the oleoresins on alumina. The use of column chromatography in problems involving the separation of complex flavorful materials is often very helpful since in favorable cases it provides, in addition to chemical separation, a general odor and flavor spectrum of the material. Chromatographic separation of the authentic and abnormal pepper oleoresins was followed by odor and flavor evaluation and qualitative chemical examination of the resulting fractions. The results summarized in Table 2 showed that the oleoresins were apparently similar with the exception that the abnormal oleoresin lacked piperine but contained a white crystalline solid which was not present in the authentic oleoresin. This compound when evaluated for odor and flavor provided pepper-bite but had none of the aromatic odor common to pepper. As shown in Figure 2, its ultraviolet spectrum showed a strong peak at 275 mμ. This compound was undoubtedly the cause of the abnormal spectrum noted in the spectrophotometric piperine determination of abnormal oleoresin. Examination of all the chromatographic fractions failed to turn up any which exhibited the characteristic unpleasant "woody-raisin" off-flavor, the isolation of which was the goal of the investigation.

TABLE 2

Chromatographic separation of oleoresins

Fraction No.	Eluant	Authentic Oleoresin	Abnormal Oleoresin
1	Skellysolve B	......	......
2	Skellysolve B	Colorless liquid	Colorless liquid
3	Skellysolve-benzene (4:1)	Colorless liquid	......
4	Skellysolve-benzene (4:1)	Colorless liquid	......
5	Skellysolve-benzene (3:2)	Colorless liquid	......
6	Skellysolve-benzene (3:2)	Yellow liquid	......
7	Skellysolve-benzene (2:3)	Yellow liquid	Colorless liquid
8	Skellysolve-benzene (2:3)	Yellow liquid	Colorless liquid
9	Skellysolve-benzene (1:4)	Yellow liquid	Colorless liquid
10	Skellysolve-benzene (1:4)	Yellow liquid	Colorless liquid
11	Benzene	Yellow liquid	Colorless liquid
12	Benzene	Yellow solid	White crystals
13	Benzene-ethyl ether (4:1)	Yellow solid	White crystals
14	Benzene-ethyl ether (4:1)	Yellow solid	White crystals
15	Benzene-ethyl ether (3:2)	Yellow solid	White crystals
16	Benzene-ethyl ether (3:2)	Yellow solid	White crystals
17	Benzene-ethyl ether (2:3)	Yellow solid	White crystals
18	Benzene-ethyl ether (2:3)	Yellow solid	Yellow liquid
19	Benzene-ethyl ether (1:4)	Yellow solid	Yellow liquid
20	Benzene-ethyl ether (1:4)	Yellow solid	Yellow liquid
21	Ethyl ether	......	......
22	Ethyl ether	......	......
23	Ethyl ether-ethanol (4:1)	......	......
24	Ethyl ether-ethanol (4:1)	Yellow liquid	Yellow liquid
26	Ethyl ether-ethanol (3:2)	Yellow liquid	Yellow liquid
25	Ethyl ether-ethanol (3:2)	......	......
27	Ethyl ether-ethanol (2:3)	......	......
28	Ethyl ether-ethanol (2:3)	......	......
29	Ethyl ether-ethanol (1:4)	......	......
30	Ethyl ether-ethanol (1:4)	......	......
31	Absolute ethanol	......	......
32	Absolute ethanol	......	......
33	80% ethanol	......	......
34	80% ethanol	Yellow liquid	Yellow liquid
35	60% ethanol	Yellow solid	Yellow solid
36	60% ethanol	Yellow solid	Yellow solid
37	40% ethanol	Yellow solid	Yellow solid
38	40% ethanol	Yellow solid	Yellow solid
39	20% ethanol	White solid	White solid
40	20% ethanol	White solid	White solid
41	Water	White solid	White solid
42	Water	White solid	White solid

Fractions 12-20 from authentic oleoresin contained piperine.

Fractions 12-17 from abnormal oleoresin contained 1-cinnamoyl piperidine.

Fractions 18-20 are believed to have contained crude piperine.

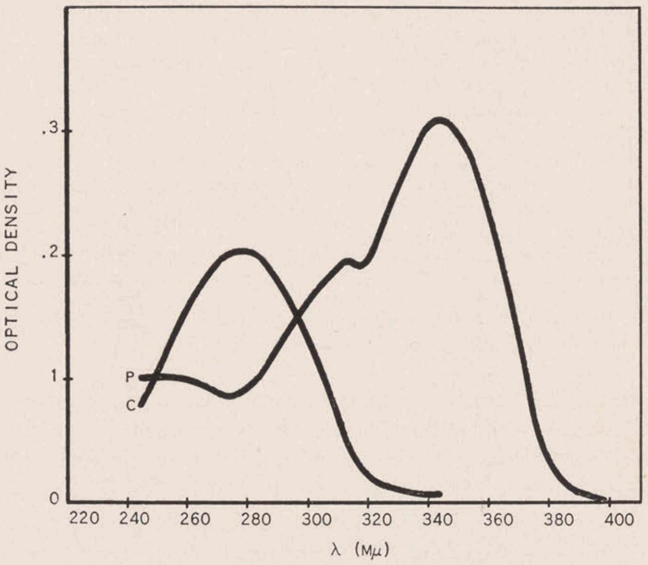


Figure 2. Ultraviolet spectrum of piperine (P) and abnormal pepper-bite compound (1-cinnamoyl piperidine [C]).

TABLE 3

Physical properties of isolates from authentic and abnormal pepper oleoresins

	Authentic Oleoresin	Abnormal Oleoresin
Volatile Oils		
Yield (%)	2.3	12
Refractive index ($n_D^{24°}$)	1.4890	1.4948
Flavor	Good	Woody-raisin
Distillation of Volatile Oils		
Fraction 1		
Refractive index ($n_D^{24°}$)	1.4938	1.4934
Fraction 2		
Refractive index ($n_D^{24°}$)	1.4942	1.4964
Specific rotation $^{25°}_{D}$	−12.6°	−12.5°
Specific gravity (25°/4°)	.8930	.8810
Flavor	Good	Woody-raisin
Nonvolatile Fraction		
Piperine content (%)	46.4	19.0
1-Cinnamoyl piperidine (%)	......	25

Real progress toward isolation of the substance responsible for off-flavor was accomplished by routine removal of the volatile oils by distillation of the oleoresins *(10)*. The physical properties of the oils thus obtained (See Table 3) varied slightly. The yield of oil from the abnormal oleoresin was much larger than from the standard. Flavor evaluation of these oils, however, immediately revealed the presence of the off-flavor in the sample derived from abnormal oleoresin. Physical evidence that the oils differed fundamentally was provided by vapor phase chromatography. In Figure 3 the chromatogram of the volatile oils from the abnormal oleoresin is completely blank while the corresponding chromatogram of oils from the authentic oleoresin

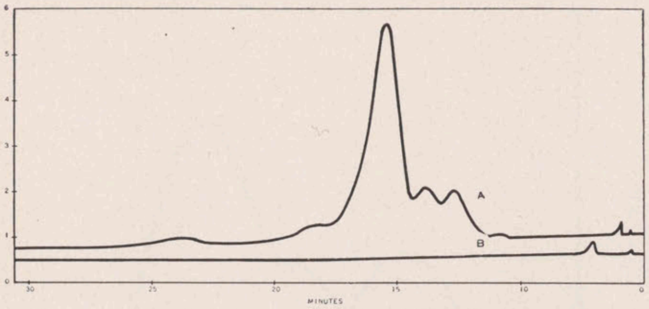


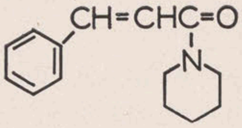
Figure 3. Vapor chromatogram of volatile oils from authentic oleoresin (A) and abnormal oleoresin (B).

shows the presence of at least three volatile components. Although this indicated that the component producing off-flavor was too low in volatility to be detected by vapor phase chromatography at 150° C. it illustrated the absence of several apparently normal volatile components in the abnormal oleoresin.

Isolation of the component of the abnormal pepper oil which was responsible for its unpleasant flavor was accomplished by vacuum fractional distillation of the oil. A colorless liquid fraction was obtained (b.p. 79-80 (1 mm.); $n_D^{24°}$ 1.4964; $[\alpha]_D^{25°}$ — 12.5°) which when incorporated at a concentration of 2 p.p.m. (0.0002%) in the original product exhibited the typical unpleasant flavor and aroma first noted. Elemental analysis showed it to be a hydrocarbon of the composition $C_{15}H_{24}$. Its infrared spectrum indicated a probable mixture and the presence of such typical terpene groups as an isopropyl and an exocyclic methylene group. It was thus concluded that the liquid which had been the cause of unpleasant flavor was a mixture of isomeric sesquiterpene hydrocarbons. Further identification of the liquid was not attempted.

Fractional distillation of the volatile oils of the authentic pepper oleoresin yielded a liquid quite similar in physical properties (b.p. 75 (1 mm.); $n_D^{24°}$ 1.4942; $[\alpha]_D^{25°}$ — 12.6) but which did not exhibit the unpleasant off-flavor. No attempt was made to identify this material. It was undoubtedly also a terpene hydrocarbon mixture.

During the removal of the volatile oils from the abnormal oleoresin, quantities of the unknown white crystalline pepper-bite compound were obtained. Purification of this substance by sublimation resulted in the isolation of a pure compound (m.p. 122-123°) having the composition $C_{14}H_{17}NO$. This compound was identified as 1-cinnamoyl piperidine by comparison with an authentic sample (see footnote [b]) of this substance.



1-cinnamoyl piperidine

The melting points, mixed melting point and infrared spectrum of each were identical. 1-Cinnamoyl piperidine is known *(9, 15)* to be a pepper-bite compound and can be easily prepared synthetically *(4)*.

SUMMARY AND CONCLUSIONS

The suitability of a black pepper oleoresin for use in a food product can only be determined by flavor analysis. If the pepper is found unsuitable the source of its abnormal flavor can be traced by the coordinated use of flavor evaluation and chemical investigation. The spectrophotometric determination *(5, 16)* of piperine is an excellent method for evaluating the composition of the oleoresin. This method in conjunction with flavor evaluation of the oleoresin should reveal the presence of a potential off-flavor producing pepper "bite" substance.

Evaluation of the suitability of the aromatic volatile oils of pepper is more complicated since it requires their separation from the oleoresin before their real contribution to flavor can be judged. Judgment on the volatile oil fractions must be based on flavor evaluation and on the general physical properties and yield of the oils. Due to the general over-all similarity of the terpenes in spite of possessing many detailed differences, a general quick method of evaluating their chemical identity is not possible. Flavor evaluation of the oils must therefore be depended upon more fully than in the case of the nonvolatile pungent principle.

On the basis of this investigation it was concluded that the abnormal black pepper oleoresin contained chemical constituents normal to black pepper plus abnormal constituents. Abnormal chemical compounds were found both in the aroma-bearing volatile oil and the nonvolatile pungent principle.

A sesquiterpene hydrocarbon fraction isolated from the volatile portion of the pepper oleoresin was the source of off-flavor detected in a commercial product processed with the pepper oleoresin. This off-flavor could be reproduced by adding 0.0002% of the fraction to the commercial product specially prepared to contain all ingredients except pepper.

The nonvolatile portion of the oleoresin contained considerably less than the normal quantity of piperine, which is a pungency principle. In addition, the nonvolatile portion contained 1-cinnamoyl piperidine, a synthetic pepper bite compound not known to be a constituent of natural black pepper oleoresin. It is, therefore, concluded that the black pepper oleoresin was adulterated.

LITERATURE CITED

1. Anonymous. Flavor profile describes food flavors in easily understandable terms. *Food Processing*, **11** (5) 30 (1950).
2. Brockmann, H., and Schodder, H. *Ber.*, **74**, 73 (1941).
3. Cairncross, S. E., and Sjöström, L. B. Flavor profiles—a new approach to flavor problems. *Food Technol.*, **4**, 308 (1950).
4. Cromwell, N. H., and Coughlan, J. A. *J. Am. Chem. Soc.*, **67**, 903 (1945).
5. Fagan, H. J., Kolen, E. P., and Hussong, R. V. Spectrophotometric methods for determining piperine in oleoresins of black pepper. *J. Agri. Food Chem.*, **3**, 860 (1955).
6. Gould, C. W., Jr., Holzman, G., and Niemann, C. *Anal. Chem.*, **20**, 361 (1948).
7. Hasselstrom, T. *Activities Report*, The Research and Development Associates, Food and Container Institute, Inc., **5** (2) July, 1953.

8. Hasselstrom, T., Ritter, J. J., Königsbacher, K. S., and Hewitt, E. J. Papers presented at XIIth International Congress for Pure and Applied Chemistry, New York, September, 1951 and before the Division of Agricultural and Food Chemistry, American Chemical Society, New York, September 1954.
9. Herstein, B. *Ber.*, **22**, 2265 (1899).
10. Jacobs, M. B. *The Chemical Analysis of Food and Food Products.* 1951, Van Nostrand Co., Inc., New York.
11. Newman, A. A. Natural and synthetic pepper-flavored substances (2). *Chem. Prod.*, Lond., **27**, 379 (1953).
12. Newman, A. A. Natural and synthetic pepper-flavored substances (5). *Chem. Prod.*, Lond., **28**, 14 (1954).
13. Oerstedt. "Uber das Piperin, ein neues Pflanzenalkaloid." *Schweiger's J. Chem. Phys.*, **29**, 80 (1820).
14. *Official Methods of Analysis.* 1950, 7th Ed. Association of Official Agricultural Chemists, Washington, D. C.
15. Staudinger, H., and Müller, F. *Ber.*, **56**, 712 (1923).
16. Tausig, F., Suziki, J. F., and Morse, R. E. Observations on black pepper. I. Analysis of bite principles. *Food Technol.*, **10**, 151 (1956).

The Breakdown of Strawberry Anthocyanin Pigment

(Manuscript received April 9, 1956)

A. Lukton, C. O. Chichester, and G. Mackinney
Food Technology Department,
Berkeley and Davis

The breakdown of anthocyanins in natural and model systems has been studied previously by several investigators. These studies were generally conducted in the presence of hydrogen peroxide or ascorbic acid. Sondheimer and Kertesz *(16)* presented a hypothesis for the breakdown of anthocyanins by the addition of hydrogen peroxide to pelargonidin-3-monoglucoside as occurring through the carbonium ion. This is in accord with the conclusions of Dilthey *et al.* *(2, 3)*, who studied the oxidation of pyrenium salts by hydrogen peroxide.

Karrer and De Meuron *(5)* demonstrated the formation (65% yield) of malvone from malvidin chloride in the presence of hydrogen peroxide. On the basis of the proposed structure of malvone, he assumed position 2 of the flavylium salt to be the site of hydrogen peroxide addition. Leffler *(6)* noted that hydroperoxides of anthocyanins gave rearranged products due to the cleavage of a neighboring carbon to carbon bond.

The importance of peroxides in the degradation of anthocyanin in natural products, as distinct from model systems, has not yet been established, although it is known that hydrogen peroxide is produced by the reaction of ascorbic acid and oxygen in the presence of cupric ion *(12)*. Beattie *et al.* *(1)* suggested that ascorbic acid might play a role in the deterioration of pigments in fruit juices. Pederson *et al.* *(9)* observed parallel losses of anthocyanin and ascorbic acid in juices, and suggested a possible interaction between these compounds. Pratt *et al.* *(10)* and Meschter *(8)* found that ascorbic acid and anthocyanin pigments interact, with the subsequent destruction of the color. Livingston *et al.* *(7)* studied several factors including ascorbic acid which affect the discoloration of pectin gels.

Sondheimer and Kertesz *(17)* demonstrated that ascorbic acid induces the destruction of anthocyanin pigments in strawberry juice, both aerobically and anaerobically. They noted an increase in the rate of destruction in the presence of ascorbic acid if cupric ion were present. Meschter *(8)* observed a marked difference in the effects of ascorbic acid and dehydro-ascorbic acids on pigment loss in buffered solutions.

Huang *(4)* determined the mechanism of decoloration of anthocyanin by fungal enzymes. He regarded it as a two-step process, involving enzymatic hydrolysis of the anthocyanin and a decoloration of the liberated aglucone by transformation into one of several possible pseudobase modifications *(11)*. There was an increase of decolorizing activity with increase in pH in the range 3.0 to 4.5. Few data have been accumulated on the breakdown of anthocyanins in the absence of oxidizing agents. The nature of the breakdown products and the reactions causing the breakdown are not known.

The present study is concerned with the effect of pH on the rate of pelargonidin-3-glucoside destruction in pure pigment solutions and strawberry juice. Spectral characteristics of the pigment breakdown products have also been studied.

EXPERIMENTAL

I. RATE STUDIES

(a) *Pure pigment solutions:* Pure pigment solutions of pelargonidin-3-glucoside were obtained from strawberries (Shasta variety) by extraction with n-butanol. Crude pigment was precipitated by addition of diethyl ether. The precipitate was then dissolved in water, isolated as the crystalline picrate, and then converted to the chloride. Before use, the chloride was re-crystallized from a solution of ethyl alcohol-5% hydrochloric acid *(14)*.

A series of 0.10 molar citrate buffer solutions with pH values of 1.25, 2.0, 2.7, 3.25, 3.40, 3.85, and 4.50 was prepared. To 50 ml. of each, 2 ml. of an aqueous solution of pigment containing 52 mg. per 100 ml. were added to give a final pigment concentration of 4.27×10^{-5} moles per liter. The buffered solutions were introduced into 100 ml. tubes through which oxygen or nitrogen (after passing through pyrogallol) could be bubbled via a side arm. To prevent loss of water, the tubes were equipped with reflux condensers. The pigment solutions were maintained at 45° C. in a constant temperature water bath over a period of 10 days in the oxygen samples and 32 days in the nitrogen samples.

(b) *Strawberry juice:* A water extract of Shasta strawberries was prepared by macerating frozen berries in a Waring blender. A clear solution was obtained by filtration through a layer of Hyflo Super Cel. The extract was divided into 3 portions and each adjusted to a different pH value with concentrated HCl or concentrated NaOH. The juice was introduced into tubes as in (a). Oxygen was bubbled through one set of tubes and nitrogen through another. The samples were heated for 5 days at 45° C. ± 0.5 in a constant temperature water bath.

(c) *Pigment determination:* The pure pigment solution and the strawberry juice were periodically sampled. Pigment concentration was determined in both cases by the method of Sondheimer and Kertesz *(15)*. A simpler method applicable only to the pure pigment solution gave identical results. It is discussed later.

II. STUDIES OF MECHANISM

Pure pigment was dissolved in 0.02 M citrate buffer at pH 3.4 and heated at 55° C. under oxygen for 10 days in the apparatus described above. Samples were removed every 2 days after correction of minor volume losses due to evaporation. During the heating, a red-brown precipitate formed. To insure homogeneity, before a sample was removed, the solution was stirred vigorously and aliquots taken rapidly. Samples thus obtained were cooled and filtered on a sintered glass filter. The absorption spectrum of the filtrate was measured in 0.1 *M* citrate buffer at pH 3.4. The material remaining on the sinter after filtering was washed 3 times with water, then with ether, and dissolved in absolute ethanol-0.001 *M* HCl. The absorption spectrum was then measured.

(a) *Chromatography:* Samples were chromatographed on paper using n-butanol-2 *N* HCl[a] as solvent.

To obtain well defined spots on chromatograms, it was necessary to remove the salt. This was accomplished by extracting the solution with n-butanol. The n-butanol solution was evaporated to a small volume and streaked across Whatman No. 1 filter papers, 18 x 22 in. After removal of the solvent, strips containing the isolated materials were cut out and eluted with a mixture of water, acetic acid and hydrochloric acid (30:10:3). The solvent was removed in vacuum and the residues dissolved in ethyl alcohol (0.001 *M* HCl). The spectrum of the red-brown material was determined after corrections had been made for absorption of contaminants eluted from the filter paper.

(b) *Degradation of the aglucone:* The aglucone was prepared by boiling the glucoside in 5 *N* HCl for 3 minutes and collecting the resulting crystalline precipitate which formed on cooling. The aglucone was washed with cold 1% hydrochloric acid, then with ether and air-dried. It was recrystallized from hot dilute hydrochloric acid. The absorption spectrum in ethanol containing 0.001 *M* HCl was identical with that given by Sondheimer and Kertesz *(14)*.

The aglucone was dissolved in 2 ml. 0.1 *M* HCl to give a saturated solution, 50 ml. of 0.1 *M* citrate buffer at pH 3.4 were added and the solution held at room temperature until completely decolorized.

(c) *Insoluble pigment breakdown product in strawberry juice:* Free-run strawberry juice was prepared from frozen Shasta strawberries by macerating in a blender, squeezing through 4 layers of cheesecloth and filtering, using Hyflo Super Cel as filter aid. The juice was heated under reflux in air at 55° C. Samples were removed periodically, filtered on a sintered glass filter, and the precipitate washed thoroughly with cold 0.1 *M* HCl, water, then ether. It was then dried.

RESULTS

Rate studies. On heating, the buffered pigment solutions became brown as the pigment disappeared. Concurrently a red-brown precipitate formed in oxygen. Aliquots of each solution were removed periodically and the optical density at 440, 500, and 700 $m\mu$ was determined. The value at 700 $m\mu$ (a measure of the turbidity of the solution) was subtracted from that at 440 and 500 $m\mu$. The optical density of the pigment at 500 $m\mu$ was calculated from the following equations, which take into account the absorbance of pigment breakdown products:

$$D^{500} = D_B{}^{500} + D_P{}^{500}$$
$$D^{440} = 0.48\, D_P{}^{500} + 2.3\, D_B{}^{500}$$

where D_P is the optical density of the pigment and D_B is that of the brown breakdown products. The value 0.48 is the ratio of absorption of the pigment at 500 $m\mu$ to that at 440 $m\mu$ at pH 1.25 and 2.70. However, at pH 3.25, 3.85, and 4.50 the ratios are 0.49, 0.57, and 0.69, respectively. The ratio of absorption at 440 to that at 500 $m\mu$ of the breakdown products was determined from the absorption spectrum of the brown soluble material which had been separated and characterized by paper chromatography (Section II). The formation of soluble breakdown products (which is expressed as the non-anthocyanin pigment absorption at 440 $m\mu$) was also determined (Table 1).

TABLE 1

Change in optical density 440 $m\mu$. Corrected for pigment absorption

Time in hours	Optical density pH				
	1.25	2.70	3.25	3.85	4.50
0	0	0	0	0	0
96	0	.005	.012	.030	.026
124	0	.018	.030	.030	.035
172	0	.028	.041	.036	.036
220	0	.034	.035	.039	.034

The change in pigment concentration was calculated by solving the simultaneous equations for $D_P{}^{500}$. The specific rate constants, k, at various pH values for the first order reaction by which the pigment is destroyed were derived from plots of the values thus determined (Tables 2, 2a).

Sondheimer *(13)* determined the relation between spectral changes and pH of pelargonidin-3-monoglucoside. Evidence was presented for the hypothesis that there is an equilibrium between hydronium ions and the anthocyanin in the forms of a red cation, R^+, and a

TABLE 2

Changes in optical density at 500 $m\mu$ of buffered pigment solutions as a function of pH, corrected for non-anthocyanin absorption

Atmosphere	pH	Optical density 500 $m\mu$							
		Time in hours							
		0	96	124	172	220			
Oxygen	1.25	1.025	1.00	0.990	0.970	0.960			
	2.70	0.600	0.496	0.470	0.431	0.407			
	3.25	0.309	0.247	0.219	0.205	0.167			
	3.85	0.130	0.094	0.0822	0.0695	0.067			
	4.50	0.080	0.0537	0.0499	0.0403	0.0361			
		Time in hours							
Atmosphere	pH	0	24	48	96	168	312	480	768
Nitrogen	2.10	2.10	2.09	2.04	2.01	1.81	1.77	1.70	1.53
	3.40	0.630	0.593	0.594	0.577	0.537	0.489	0.443	0.319
	4.50	0.176	0.165	0.165	0.160	0.140	0.133	0.116	0.091

---

[a] This solvent was made by equilibrating equal volumes of n-butanol and 2 *N* hydrochloric acid. The upper layer was used as the solvent.

PD

MEMORANDUM

To: S. E. Cairncross
L. B. Sjöström

Case: C-59074 Date: July 28, 1954 Page: 1

Subject: Flavor Evaluation of Production Samples of Miracle Whip Containing the Isolated $C_{15}H_{24}$ Liquid

Samples of Miracle Whip prepared by the Kraft Foods Company--which contained no pepper but in which had been incorporated the $C_{15}H_{24}$ liquid believed to be the cause of off-flavor--have been tested by our flavor panel. This liquid was isolated from a sample of Long's pepper dispersion (416-3) and a purified sample was submitted to Dr. Yanick. Dr. Yanick prepared special batches of Miracle Whip by incorporation of the $C_{15}H_{24}$ liquid in the oils prior to emulsification.

The samples tested (628-2 and 628-3) contained the liquid at a concentration of 0.0002% (calculated normal level) and at 0.007% (threshold for recognition of off-flavor in hand-mixed samples). When tested immediately after their arrival, the samples did not in general exhibit the characteristic "woody-raisin" off-note. A second panel study of the same materials was carried out twelve days later. Unopened jars of the salad dressing which had been stored in a refrigerator were used. In both samples the "woody-raisin" flavor was clearly and immediately recognized by all the panel members. There was no evidence of rancidity or that any major change other than the production of the woody flavor had occurred.

To summarize, these panel findings show that the hydrocarbon, $C_{15}H_{24}$, isolated from suspected samples of Long's black pepper dispersion (416-3, 203-19) can produce the type of "woody-raisin" off-note in Miracle Whip which was found in the complaint samples submitted to us on February 12, 1954. There was no indication that this hydrocarbon was present in any pepper dispersion examined other than the suspected samples of Long's pepper dispersion. Assuming that all samples examined are authentic and connected, it must be concluded that this $C_{15}H_{24}$ hydrocarbon was the sole cause of the off-flavor note in Miracle Whip and that it came from the pepper dispersion in use at the time.

Emily L. Wick
Emily L. Wick

From ELW:efd:4

FORM NO. 101

Arthur D. Little, Inc.

PD

MEMORANDUM

To:
S. E. Cairncross
CC: L. B. Sjöström
P. T. Durand

Case: C-59074
Date: July 16, 1954
Page: 1

Subject: Flavor Evaluation of Production Samples of Miracle Whip Containing the Isolated $C_{15}H_{24}$ Liquid

Samples of Miracle Whip prepared by the Kraft Foods Company which contained no pepper but in which had been incorporated the $C_{15}H_{24}$ liquid believed to be the cause of off-flavor have been tested by the panel. This liquid was mixed with the oils before preparation of the salad dressing emulsion was accomplished.

The samples tasted contained the liquid at a concentration of 0.0002% (calculated normal level) and at 0.007% (threshold for recognition of off-flavor in hand mixed samples). When tested immediately after their arrival the samples did not, in general, exhibit the characteristic "woody-raisin" off-note although one panel member indicated it was present by flavor in the 0.007% sample but not by aroma.

A second panel study of the same materials was carried out twelve days later. Fresh jars of the salad dressing which had been stored in a refrigerator were used. In both samples the characteristic "woody-raisin" flavor was clearly and immediately recognized by all the panel members (RLS, PTD, and ELW). Only one person detected the offness by aroma. There was no evidence of rancidity or that any change other than the production of the woody flavor had occurred.

It can be concluded that the $C_{15}H_{24}$ liquid isolated from Long's pepper dispersion was indeed the cause of off-flavor in complaint samples of Miracle Whip.

In a letter to Dr. Mottern on July 6th which acknowledged receipt of the samples, it was indicated that final results of the above flavor evaluation would be sent to him during the week of July 19th. It was agreed that the attached spectra would be sent to Kraft at that time.

Emily L. Wick
E. L. Wick

From
E. L. Wick/hmn:4

FORM NO. 101

Arthur D. Little, Inc.

Figure 1

<u>Curve A</u> Infrared spectrum of $C_{14}H_{17}NO$ compound isolated from Long's pepper dispersion (416-3) by ADL and identified as cinnamoyl piperidide. Sample run in a mineral oil mull.

<u>Curve B</u> Infrared spectrum of cinnamoyl piperidide synthesized by Kraft Foods Company. Sample run in a mineral oil mull.

<u>Curve C</u> Infrared spectrum of the $C_{15}H_{24}$ liquid isolated from Long's pepper dispersion (416-3).

These spectra were determined using a modified Perkin-Elmer Infrared Spectrometer fitted with a calcium fluoride prism for the range of 3600-1300 cm^{-1} and a sodium chloride prism for the range of 1500-700 cm^{-1}.

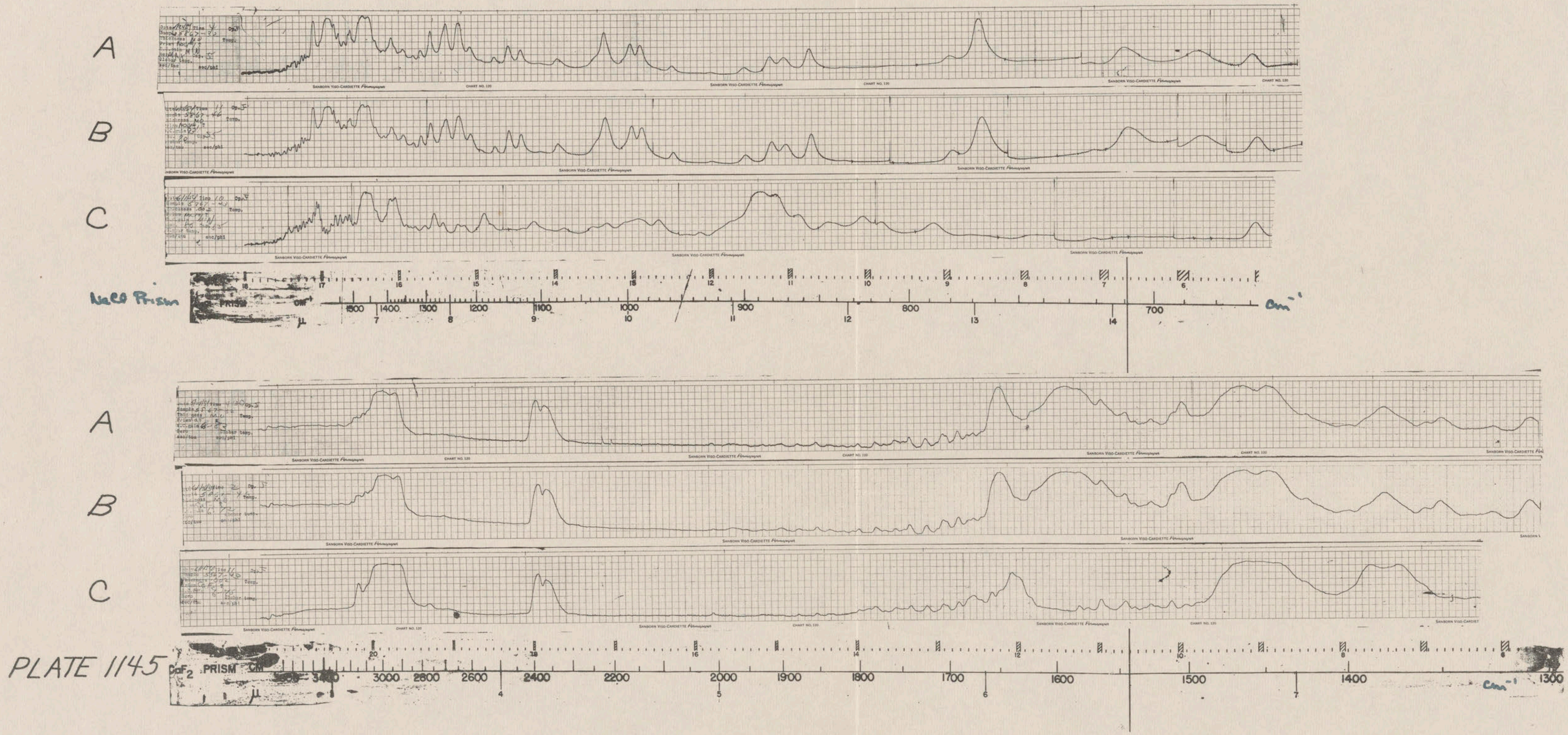

A
B
C
NaCl Prism
PRISM
cm⁻¹
µ
1500
1400
1300
1200
1100
1000
900
800
700
7
8
9
10
11
12
13
14
A
B
C
PLATE 1145
CaF2 PRISM
3400
3000
2800
2600
2400
2200
2000
1900
1800
1700
1600
1500
1400
1300
4
5
6
7
SANBORN VISO-CARDIETTE Permapaper
CHART NO. 120

Figure 1

Figure 2

Ultraviolet spectrum of the $C_{14}H_{17}NO$ compound isolated from Long's pepper dispersion (416-3) by ADL and identified as cinnamoyl piperidide. Sample run in ethanol, 10^{-5}M.

Figure 3

Ultraviolet spectrum of the $C_{15}H_{24}$ liquid isolated from Long's pepper dispersion (416-3). Sample run in ethanol, 0.0024M.

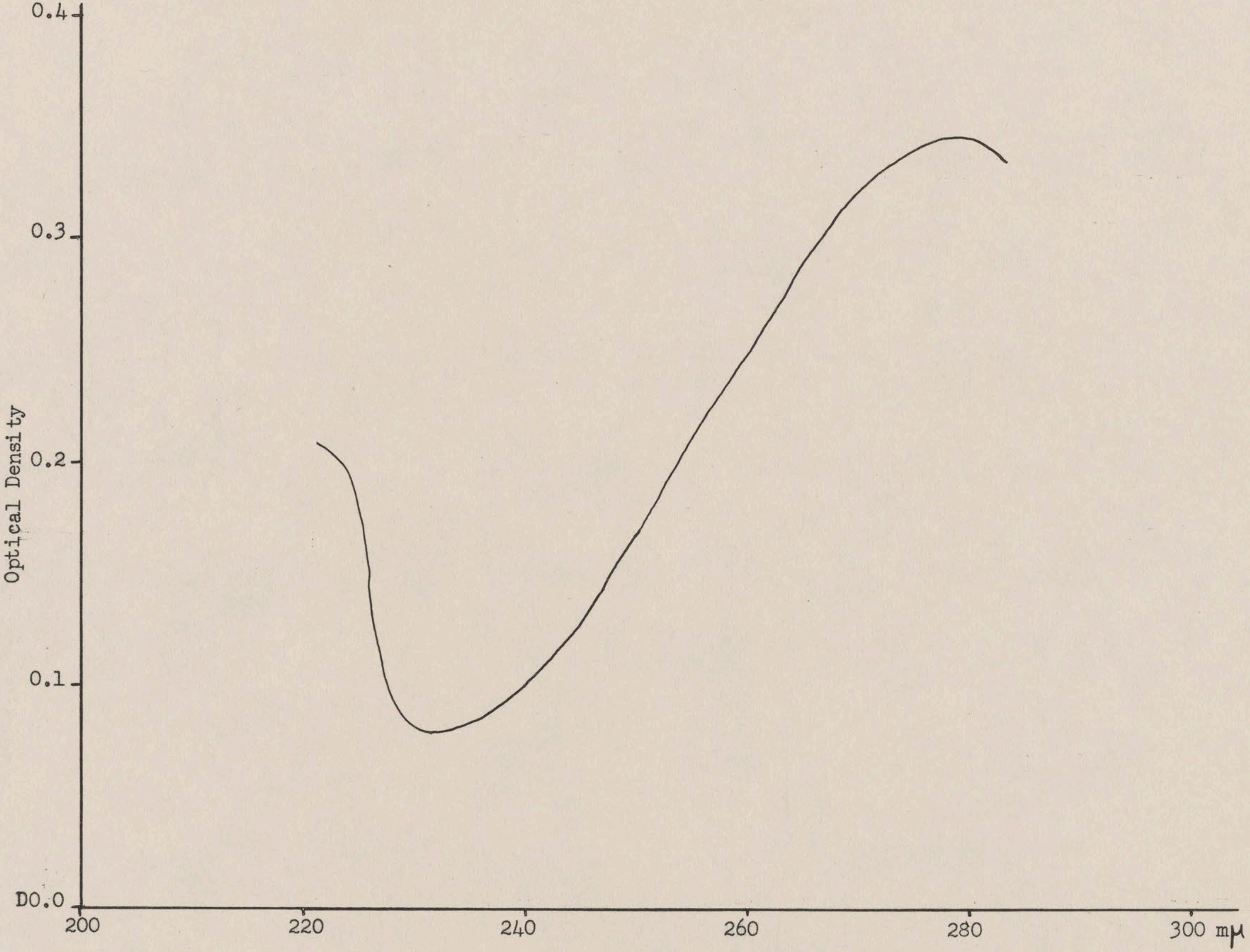


Figure 2

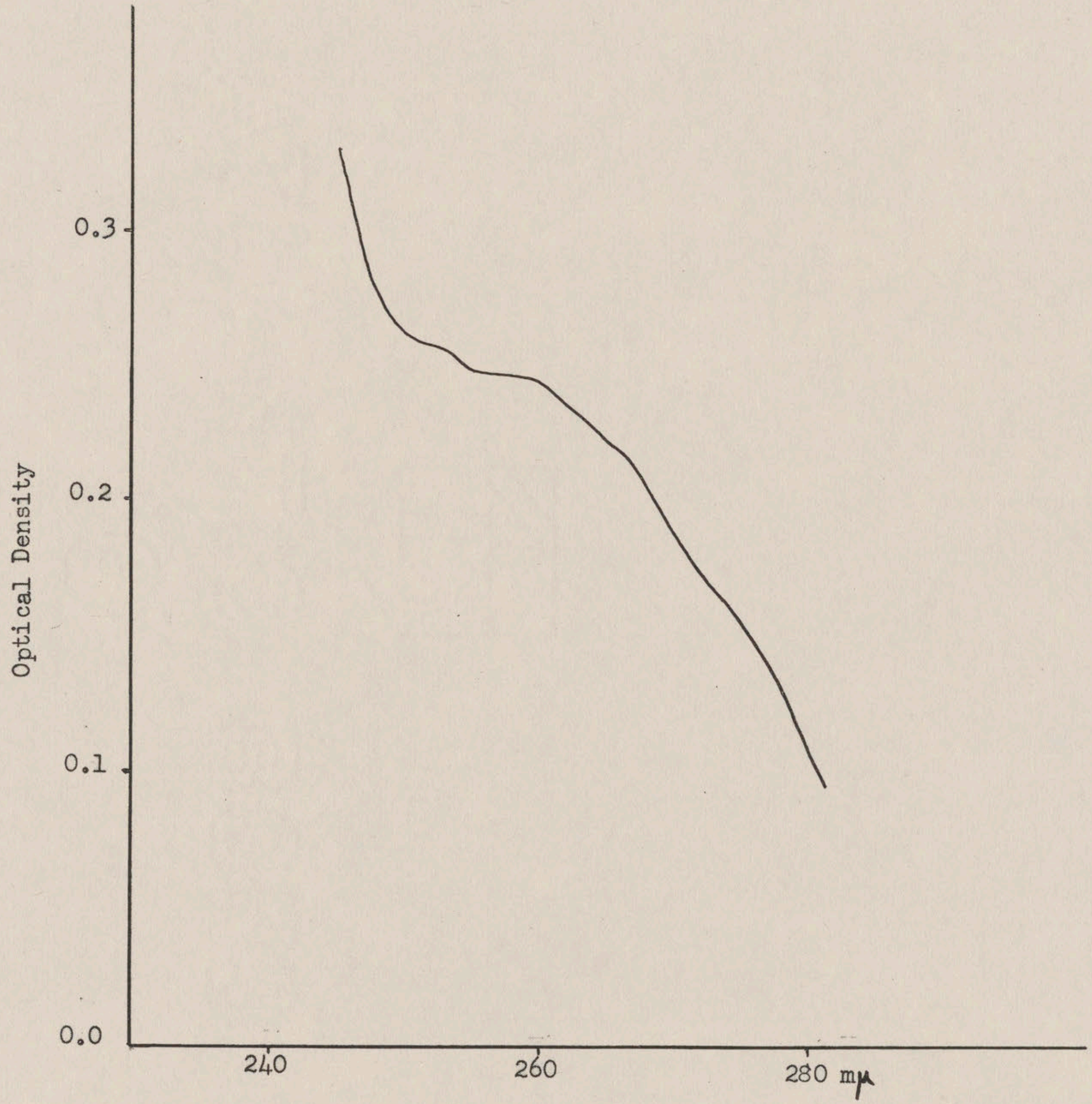


Figure 3

PTD

ELW:hmn:4 June 22, 1954

Dr. H. H. Mottern
Research Laboratories
Kraft Foods Company
923 Waukegan Road
Glenview, Illinois

Dear Dr. Mottern: <u>C-59074</u>

We are sending you today, under separate cover, approximately 0.6g of a pure fraction of the $C_{15}H_{24}$ liquid isolated from Long's pepper dispersion (416-3), which we believe provided the off-flavor to Miracle Whip. This sample is for your use in the preparation under normal conditions of reconstituted "complaint" samples of Miracle Whip which may be tested by our taste panel for the presence of the "woody-raisin" off-flavor.

Our panel has studied the effect of the $C_{15}H_{24}$ "off"-liquid in the test Miracle Whip which contained no pepper (610-3). We find that the "woody-raisin" note is readily recognized when the "off"-liquid is present in a .007% concentration. This is thirty-five times the calculated normal level of .0002%. This figure is based on the fact that Miracle Whip contains .057% of #222 spice mix which is made up of 87% pepper dispersion. The dispersion contains 5.25% of oleoresin from which we isolated 7.2% of the $C_{15}H_{24}$ "off"-liquid. The panel could not detect the characteristic off-flavor when the liquid was added at the normal level. We believe this to be due to the difficulty in accomplishing complete dispersion of the "off"-liquid in a large quantity of salad dressing. We feel that evaluation by our panel of samples of Miracle Whip prepared by Kraft in the usual manner but containing the "off"-liquid at the normal level of .0002% and at our experimental "threshold" level of .007% should definitely determine the effect of the $C_{15}H_{24}$ isolate from Long's dispersion.

The sample of the "off"-liquid which we have sent you has been divided into three weighed vials so that one may be used in the .0002% preparation, another in the .007% preparation, and the third will serve as a reference sample for the "woody-raisin" note.

The immediate preparation and shipment of these samples would enable the panel to complete their evaluation by July 2nd. After that time, vacation schedules interfere so that completion of this work would be postponed several weeks. In this case, it would be better if the samples were not prepared until July 19th thus insuring the availability of fresh material for panel evaluation.

Sincerely yours,

ARTHUR D. LITTLE, INC.

By: ELW

Emily L. Wick

MEMORANDUM

PTD

To: L. B. Sjöström
CC: S. E. Cairncross
P. T. Durand

Case: 59074

Date: June 16, 1954

Page: 1

Subject: Report of Meeting with Kraft Foods Company on June 9, 1954 in Glenview, Illinois

Personnel Present: Dr. H. H. Mottern, Dr. N. S. Yanick, Dr. L. C. King of the Chemistry Department of Northwestern University, Dr. R. V. Hussong, Chief Bacteriologist at Kraft, Mr. Partyka and Mr. Fagan also of Kraft. Dr. S. E. Cairncross and E. L. Wick of Arthur D. Little, Inc.

The work carried out at ADL and reported in the memorandum of June 9, 1954, entitled "The Isolation of Off-Flavor in Miracle Whip Salad Dressing" was described.

Dr. Mottern reported the work which had been carried out concurrently at Kraft. They are using ultraviolet absorption to detect adulterated peppers and have found that Long's pepper dispersion and pepper samples from Norda showed similar spectra which were completely different from the spectra of any other pepper samples. Kraft has surveyed most of the commercially available pepper oleoresins in this manner. The spectrum found for the Long and Norda samples is characteristic of the white crystalline $C_{14}H_{17}NO$ compound isolated by the ADL group and believed by them to be cinnamoyl piperidide. Positive identification of this compound was prevented by anomalous melting point (113-114°C) and infrared data.

Dr. King reported that this same crystalline material (m.p. 122°) had been isolated by the Kraft group and definitely identified as cinnamoyl piperidide by comparison of its melting point, infrared and ultraviolet spectra with those of an authentic sample of synthetic cinnamoyl piperidide prepared in his laboratory. The infrared spectra of the synthetic and isolated compounds were identical. The spectrum of piperine was also found to be practically identical to that of cinnamoyl piperidide. The apparent lack of a carbonyl band in the infrared curve determined by the ADL group was clarified by the Kraft curves which showed that this band had been shifted in position due to conjugation in the molecule. In all respects the ADL infrared specturm was similar to those of Kraft after allowance was made for the fact that the ADL sample was done in a Nujol mill while the Kraft samples were done in a solvent.

The discrepancy in melting points between the Kraft and ADL samples of white solid has been resolved by a comparison of their melting points as determined in the ADL apparatus. Both melting points were found to be 114-115° indicating a serious inaccuracy in the ADL equipment. There is no doubt that the ADL and Kraft samples of cinnamoyl piperidide are identical.

From ....
E. L. Wick/hmn:4

FORM NO. 101

Arthur D. Little, Inc.

The Kraft group gave no details of their investigation of Long's pepper dispersion. They gave no indication that they had isolated the $C_{15}H_{24}$ terpene which produces the "off" flavor in Miracle Whip. Dr. Cairncross pointed out that the ultraviolet method developed by Kraft for determining an adulterated pepper did not give any protection against the presence of the compound which was actually the cause of all their trouble with Miracle Whip. The fundamental importance of this fact did not appear to be recognized by the Kraft group.

A discussion of the possibility of future work by ADL on the identification of the terpene responsible for off-flavor in Miracle Whip resulted in the following decisions:

1. Kraft would identify the $C_{15}H_{24}$ terpene.
2. ADL should determine whether the terpene produced off-flavored Miracle Whip when used at normal levels.
3. ADL should obtain more pure $C_{15}H_{24}$ terpene and send it to Kraft for use in the preparation of test samples of off-flavor Miracle Whip.
4. Copies of the infrared and ultraviolet spectra of the two compounds isolated at ADL should be sent to Kraft.

All of the above projects have been initiated and should be completed within a week.

ELW
E. L. Wick

PD

MEMORANDUM

From: E. L. Wick C-59074 June 9, 1954

To: Kraft Foods Company Subject: The Isolation of Off-Flavor in Miracle Whip Salad Dressing

The chief purpose of the work undertaken since the last meeting with Kraft Foods Company on April 14, 1954, has been the isolation and identification of the off-flavor found in complaint samples of Miracle Whip. This off-note has been described as "woody-raisin" and is readily detectable in salad dressing in aroma as well as flavor. Isolation of the off-flavor has been successfully accomplished from Long's pepper dispersion (416-3) and has been identified as a sesquiterpene, $C_{15}H_{24}$, $n_D^{24°}$ 1.4964, b.p. 79-80° C (1 mm). In addition to the "off"-flavor liquid there was isolated a white crystalline solid having a peppery bite, $C_{14}H_{17}NO$, m.p. 113-114°. Piperine was also found. The details of this work will be described under the following headings.

I. Chemical Separation of Long's Pepper Dispersion:--Isolation of the "Woody-raisin" Off-flavor, and a Peppery-bite Characteristic.

II. Possible Identity of the Off-liquid and the Peppery-bite Compound.

I. Chemical Separation of Long's Pepper Dispersion (416-3):--Isolation of the "Woody-raisin" Off-flavor and a Peppery-bite Compound.

Long's pepper dispersion was chosen as a source material for the isolation of off-flavor since it provided fewer variables than #222 spice mix and since after being heated at 55°C in the presence of moisture it produced the "woody-raisin" note in mineral oil and to a slight degree in salad dressing which contained no spices. Several separations were carried out during the investigation but the most satisfactory method is outlined in Figure 1, page 3.

Isolation of the "Woody-raisin" Off-flavor.

Long's dispersion (416-3) was treated with petroleum ether (b.p. 30-60°C) at room temperature for 18 hours. The resulting mixture was separated by filtration. The yellow filtrate (A) was concentrated under reduced pressure and the residue distilled as a water suspension in order to remove volatile oils (C). The yellow solid (B) was treated with ethyl ether at room temperature for 18 hours to remove organic material from the salt and phosphate dispersion base (F).

The volatils oils (C) were obtained in 12% yield and had a peppery and aromatic odor. Fractional distillation of the volatile oils at 1 mm yielded an aromatic fraction, $n_D^{24°}$ 1.4953, and two "woody-raisin" fractions, $n_D^{24°}$ 1.4962; 1.4963. Redistillation of the woody fractions at 79-80°C (1 mm) yielded an analytically pure sample, $n_D^{24°}$ 1.4964, in 7.2% yield. This liquid when tested in mineral oil and in salad dressing which contained no spices disclosed the "woody-raisin" odor and flavor typical of that found in the complaint samples of Miracle Whip. Samples of the pure fraction were submitted for the following analyses.

Elemental analysis: Calculated for $C_{15}H_{24}$: C, 88.16; H, 11.84; mol. wt. 204. Found: C, 88.19; H, 12.01; mol. wt. 217 (Rast method in camphor). Sodium fusion disclosed no other elements.

Infrared spectrum: The spectrum indicates the presence of a terminal isopropyl group $[(CH_3)_2CH-]$, and an exocyclic methylene group ($CH_2=$). Absorption typical of terpenes is exhibited.

On the basis of the above results, it is concluded that the material which contributed the off-flavor to Miracle Whip is a sesquiterpene found in

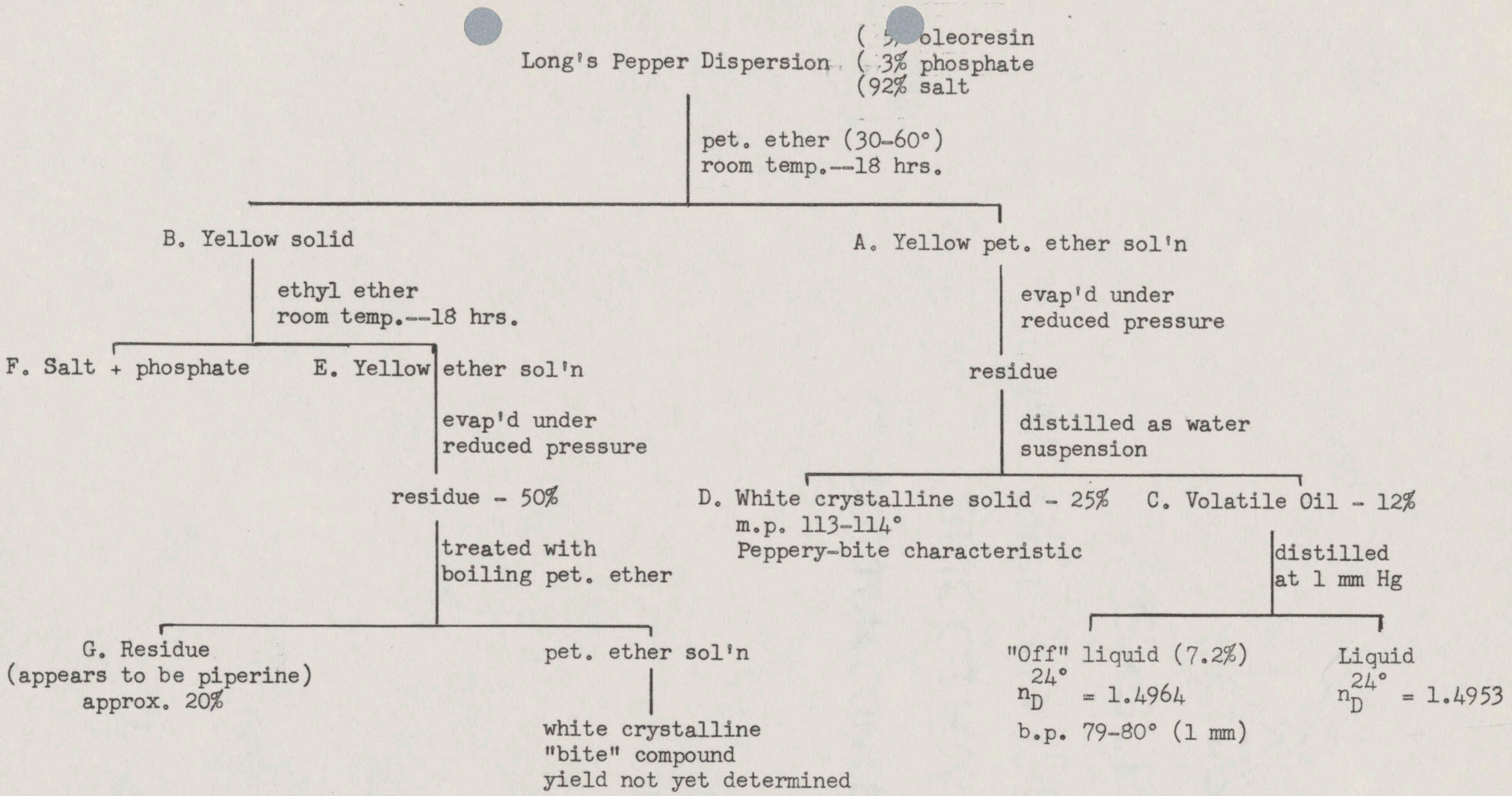


FIGURE 1. CHEMICAL SEPARATION OF LONG'S PEPPER DISPERSION (416-3)
Yields are based on oleoresin present in dispersion

the volatile oils present in Long's pepper dispersion (416-3). The fact that the off-flavor is exhibited only in heated moist dispersion and in #222 spice mix prepared last summer under unusual conditions of heat and moisture is consistent with this finding. It is reasonable to believe that sufficient heat and moisture were present to cause distillation of the volatile oils in the degree necessary to accentuate the off-flavor.

Isolation of a Peppery-bite Compound

The aqueous suspension remaining after distillation of the volatile oils (C) was extracted with ether. The yellow ether solution was dried, concentrated under reduced pressure and the residue was sublimed at 60-70°C (1-2 mm). A white crystalline sublimate (D) was obtained in 25% yield. Resublimation yielded an analytically pure sample, m.p. 113-114°, which when tested in mineral oil and in salad dressing containing no spices gave no odor but provided a pepper-like flavor. When tasted *per se* a very sharp biting sensation is produced. Slight woodiness is noted if an impure sample is tasted *per se*. Samples of the pure sublimate were submitted for the following analyses.

Elemental analysis: Calculated for $C_{14}H_{17}NO$: C, 78.10; H, 7.96; N, 6.51; mol. wt. 215. Found: C, 78.18; H, 8.19; N, 6.56; mol. wt. 242 (Rast method in camphor). Sodium fusion disclosed no other elements.

Infrared spectrum: The spectrum indicates the presence of an aromatic ring and a *cis*-double bond of the $R_1CH=CHR_2$ type. There is no evidence for the presence of the -NH-, -OH, or -C=O groups.

Ultraviolet spectrum: The presence of a conjugated system containing an aromatic ring is indicated by the spectrum which contains three maxima. λ 200 mμ, ϵ 20500; 282, 33800; 378, 34500.

Qualitative color tests recommended by the U. S. Dispensatory, 22nd Edition, for the identification of piperine were found to give positive results when applied to the isolated $C_{14}H_{17}NO$ "bite" compound. This would indicate similarities in the structure of these two compounds.

Further separation of the pepper dispersion (416-3) was accomplished by brief investigation of the ether solution (E). (See Figure 1, page 3). This yellow solution was concentrated under reduced pressure. The residue (50% yield based on oleoresin) was treated with boiling petroleum ether to remove all of the already partially isolated $C_{14}H_{17}NO$ compound. Time did not permit determination of the total yield of this "bite" characteristic. The petroleum ether insoluble residue G is a yellow solid which appears to be impure piperine. This material is present in approximately 20% yield. This figure corresponds to the results of The Emulsol Corporation which reported a 20% yield of piperine in the oleoresin from suspected spice mixture using a modified AOAC method of determination.

II. Possible Identity of the Off-liquid and the Peppery-bite Compound

Since any ingredient of a commercially available abnormally compounded pepper would undoubtedly be easily obtainable and its properties well known, it was felt that a brief search of the literature might result in identification of the isolated compounds described above.

Hasselstrom[1] and Greunther[2] have described various imitation black pappers and report that common volatile oils used as adulterants are caryophyllene, phellandrene, and dipentene (limonene). The physical properties and molecular weight of the isolated $C_{15}H_{24}$ "woody-raisin" off-liquid rule out its identification as phellandrene or dipentene. That it is γ-caryophyllene is a possibility though the odors of the isomeric caryophyllenes are not known to be woody. Its identification as α-cedrene is a possibility since this compound has a woody odor and physical properties quite similar to those of the isolated off liquid. Table I contains the properties, molecular weights, and other pertinent data characteristic of these compounds.

It is believed that the identification of the $C_{15}H_{24}$ off-liquid may be quite possible if time were made available for: (1) the determination of its density and optical rotation to complete the knowledge of its physical properties; (2) accomplishment of a thorough literature search of known hydrocarbon sesquiterpenes; (3) the procurement of pure samples of compounds which might be identical to the off-liquid; and (4) the comparison of physical properties and infrared spectra of these compounds.

Various "peppery-bite" chemicals have been described by Staudinger[3] and Hasselstrom[1,4]. Several of the most common of these and those most similar to the "bite" compound isolated from Long's dispersion are shown in Table II. Compounds II and IV were used in Germany during World War I in imitation black pepper[1] while in World War II only compound II was used. This latter compound II, β-cinnamenyl acryloyl piperidide was selected[1] as the synthetic peppery-bite material to be used in the black pepper substitute developed by the Quartermaster Research and Development Laboratories.

It is reported by Hasselstrom[1] that most domestic imitation peppers contain the oleoresin of capsicum as the biting ingredient. Compound V in Table II is capsaicine the pungent principle of red pepper. Its physical properties and molecular weight are not at all similar to those of the "bite" compound from Long's dispersion.

On the basis of the facts known about the "bite" characteristic isolated from Long's dispersion, its structure is most probably of the type represented by compound I, cinnamoyl piperidide. This material is known[5,6], is easily prepared, and has a strong pepper "bite"[4b]. Facts which support identification of the isolate as I are the agreement of the analytical data, ultraviolet data and most of the infrared data. The fact that the infrared spectrum lacks evidence for a carbonyl group and that the melting point of I is 122° while that of the isolate is 113-114°, does not support structure I. This discrepancy in melting points may be explained by the possibility of *cis*-*trans* isomers of I. *Trans*-isomers are known to have higher melting points than do *cis* isomers and the infrared data indicates that the isolate from Long's pepper contains a *cis*-double bond.

Whether or not the isolated "bite" compound has structure I should be rather easily proved since this compound would be expected to be hydrolyzed to the well known and easily identified compounds of piperidine and cinnamic acid.

Emily L. Wick

E. L. Wick

TABLE I

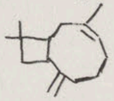
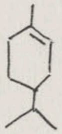
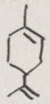
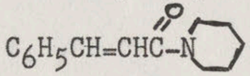
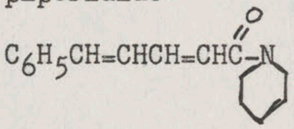
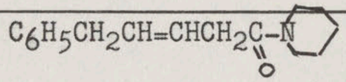
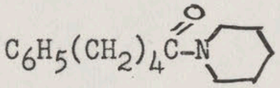
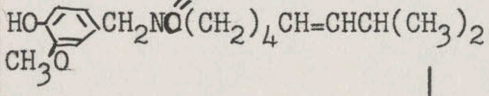
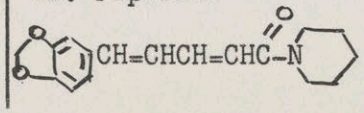
	Empirical Formula	Analytical Data Calculated	 Found	Physical Properties	Infrared Data
"Woody-raisin" off-liquid	$C_{15}H_{24}$	C, 88.16 H, 11.84 Mol. Wt. 204 Sodium fusion disclosed no other elements.	C, 88.19 H, 12.01 Mol. Wt. 217	$n_D^{24^\circ}$ 1.4964 b.p. 79-80°(1 mm)	Terminal isopropyl group Exocyclic methylene group General terpene absorption
α- Caryophyllene 	$C_{15}H_{24}$	C, 88.16 H, 11.84 Mol. Wt. 204		$n_D^{19^\circ}$ 1.4966; b.p. 125-125.5° (14.5 mm); d^{15° 0.8995; $[\alpha]_D$ -26.17° from oil of cloves	
α- Cedrene (Structure not proved)	$C_{15}H_{24}$	C, 88.16 H, 11.84 Mol. Wt. 204		$n_D^{20^\circ}$ 1.4982; b.p. 100° (3.5 mm); $d_{4^\circ}^{20^\circ}$ 0.9342; $[\alpha]_D$ -91.3° from oil of cedar wood	
α- Phellandrene 	$C_{10}H_{16}$	C, 88.16 H, 11.84 Mol. Wt. 136		$n_D^{20^\circ}$ 1.4777; b.p. 66-68° (16 mm) $d_{4^\circ}^{25^\circ}$ 0.8463; $[\alpha]_D$ +45° from oil of bitter fennel, ginger grass oil, aniseed oil	
Dipentene (limonene) 	$C_{10}H_{16}$	C, 88.16 H, 11.84 Mol. Wt. 136		$n_D^{20^\circ}$ 1.4730; b.p. 176-178° $d_{4^\circ}^{20^\circ}$ 0.840	

TABLE II

	Empirical Formula	Analytical Data		Physical Properties	Infrared Data	Ultraviolet Data	
		Calculated	Found			λmμ	ε
"Bite" compound from Long's dispersion	$C_{14}H_{17}NO$	C, 78.10 H, 7.96 N, 6.51 Mol. Wt. 215 Sodium fusion disclosed no other elements.	C, 78.18 H, 8.19 N, 6.56 Mol. Wt. 242	m.p. 113-114°C	Aromatic ring cis $R_1CH=CHR_2$ no - NH no - OH no - C=O	220 282 378	20500 33800 34500
I. Cinnamoyl piperidide 	$C_{14}H_{17}NO$	C, 78.10 H, 7.96 N, 6.51 Mol. Wt. 215		m.p. 122°			
II. β-Cinnamenyl acryloyl piperidide 	$C_{16}H_{19}NO$	C, 79.63 H, 7.94 N, 5.80 Mol. Wt. 241		m.p. 92°			
III. 	$C_{16}H_{21}NO$	C, 78.96 H, 8.70 N, 5.76 Mol. Wt. 243		are 3 isomers - all are liquids			
IV. n-Valeroyl piperidide 	$C_{16}H_{23}NO$	C, 78.32 H, 9.45 N, 5.71 Mol. Wt. 245		liquid			
V. Capsaicine 	$C_{18}H_{27}NO_3$	C, 70.78 H, 8.91 N, 4.59 Mol. Wt. 305		m.p. 65°			
VI. Piperine 	$C_{17}H_{19}NO_3$	C, 71.55 H, 6.11 N, 4.91 Mol. Wt. 285		m.p. 130°			

BIBLIOGRAPHY

1. T. Hasselstrom, Activities Report, The Research and Development Associates, Food and Container Institute, Inc., Vol. 5, No. 2, July 1953.

2. E. Greunther, Am. Perfumer, 44, No. 8, 39-40, 43 (1942).

3.a. H. Staudinger and H. Schneider, Ber., 56, 705 (1923); b. H. Staudinger and F. Müller, Ber., 56, 712 (1923).

4.a. T. Hasselstrom, Science 112, 626 (1950); b. Am. Perfumer Essent. Oil Rev., 60, 430 (1952); c. with H. W. Coles and N. E. Kennedy, Science, 116, 204 (1953).

5. B. Herstein, Ber., 22, 2265 (1899).

6. N. H. Cromwell, J. A. Coughlan, J. Am. Chem. Soc., 67, 903 (1945).

COPY

PD

KRAFT FOOD COMPANY

Research Laboratories - 923 Waukegan Road

Glenview, Illinois

April 19, 1954

Dr. S.E. Cairncross
Arthur D. Little, Inc.
Cambridge 42, Massachusetts

Dear Dr. Cairncross:

In accordance with your request, we are shipping to your attention the following materials referred to during our meeting here last week:

✓ 415-4 11 pts. Miracle Whip, regular formula
✓ 415-5 11 pts. Miracle Whip, without spices
✓ 416-2 ½ gal. COSP, Lot 1258, Drum 552 currently used Stange's black pepper dispersion.
✓ 416-3 ½ gal. Long's "suspected" dispersion, Drum (1) same as the sample previously submitted.
✓ 416-4 ½ gal. Pepperoyal P-114--1 drum Black Pepper seasoning from another source, intended for use by Kraft
✓ 416-5 1 qt. Long's soluble black pepper, sample received 4-16-54
✓ 416-6 8 oz. Long's oleoresin black pepper, sample received 4-16-54. This is said to be the oleoresin used in the preparation of 416-5.
✓ 416-7 8 oz. Stange's oleoresin black pepper, lot No. 78441 30# received 4-16-54
419-6 1 qt. Stange's COSP, lot 1270 said to be made out of oleoresin of type 416-7
✓ 416-8 1 pt. "bad" #222 spice mix, drum #D2, Hillside, N.J. 11-27-53. This is the same as sample previously submitted.
✓ 824-1 1 pt. COSP(Stange's) used by Kraft on or about 8-24-53
✓ 925-10 1 pt. COSP (Stange's) used by Kraft on or about 9-25-53.

When you review the list you will note that you have:

Stange's recent oleoresin in 416-7
Stange's recent dispersions in 416-2 and 419-6
Stange's old dispersions in 824-1 and 925-10
Long's suspected dispersion of 1953 in 416-3
Long's recent oleoresin in 416-6
Long's recent dispersion in 416-5.

Pepperoyal, a dispersion from third source
in 416-4
"Bad" #222 spice mix in 416-8

We regret that we are unable to send you more of the "bad" #222 spice mix, as we have only two pints left. The Stange's old dispersions 824-1 and 925-10 may be of interest to you to compare the aroma with the "bad" #222 spice mix and for microscopic observation against the #222 spice mix and against the "suspected" Long's dispersion.

Very truly yours

KRAFT FOODS COMPANY
Research Laboratories

N.S. Yanick

NSY:bw

P.D.

MEMORANDUM

To: S. E. Cairncross
L. B. Sjöström

Case: 59074 Date: April 16, 1954 Page: 1

Subject: Report of Meeting with Kraft Foods Company on April 14, 1954 in Glenview, Illinois

Personnel present: Dr. H. H. Mottern, Dr. N. Yannick, Mr. T. Partyka of Kraft Foods Company
Dr. S. E. Cairncross and E. L. Wick of Arthur D. Little, Inc.

The major purpose of this meeting was to acquaint the ADL group with the results of the investigations carried out by The Emulsol Corp. on the good and bad #222 spice mixtures, pepper dispersions and oleoresins. This work consisted of chemical analysis of the #222 spice mixtures, chromatographic separation of the #222 spice mixtures and good and bad pepper dispersions, and piperine determinations on the good and bad pepper oleoresins. Copies of the report of this work submitted to Kraft by Emulsol Corp. are attached to this memorandum. The results may be summarized as follows:

Good pepper oleoresin and good #222 spice mixture contain more than twice as much piperine as do the corresponding suspected materials. The essential oil isolated from bad #222 spice mixture is composed of at least 50% of some component other than pepper or mustard oil. The obvious conclusion to be reached is that the bad pepper has been adulterated with a nitrogen containing organic compound in an amount required to provide a total nitrogen content comparable to that of piperine.

The chromatographic work of Emulsol was brief and not particularly definitive since individual fractions were not isolated and only the number of colored bands was observed. Results were compatible with the chemical analysis carried out.

The work of The Emulsol Corporation does not in any way contradict the work of the ADL group and does, in fact, supplement it very well. The chemical analyses and piperine determinations are of particular interest.

Dr. Cairncross summarized the recent work of the ADL group which indicated that the "woody-raisin" off-note characteristic of the bad #222 spice mix, may be produced from Long's pepper dispersion by heating it at 55°C in the presence of moisture. It was emphasized that this finding must be considered tentative until the treated dispersion can be tested in the salad dressing taste medium.

The results of the examination of the Kraft paper spice drum liner were reported. It was felt that the liners did not contribute to the production of the "woody-raisin" note although wet liner in the presence of the #222 spice mix did produce a typical "Kraft paper" aroma.

From E. L. Wick/clf:4

Mr. Partyka reported that it had not been possible to obtain any of Long's pure pepper oleoresin from which the bad dispersion and bad #222 spice mix had been prepared. It was decided that samples of Long's current production of pepper oleoresin and pepper dispersion would be obtained and sent to ADL. Additional samples which were to be sent immediately to ADL are Long's bad pepper dispersion (203-19), Stange's pure pepper oleoresin, Stange's pepper dispersion, bad #222 spice mix, good #222 spice mix and 12 jars of Miracle Whip salad dressing containing no spices.

There was a general discussion of methods used in processing pepper. Chlorophyll is removed by a bleaching process in which is included an alkaline treatment. It was suggested that the green color of bad pepper dispersion in mineral oil might be due to chlorophyll or to the bleaching process. Dr. Yannick said that Stange and Long use different bleaching methods. A peroxide determination on the peppers might be of value in our investigations.

Mr. Steve Harrison who has directed the organization of a taste panel for the evaluation of pepper at Kraft Foods Company, described their methods and the progress made. On the basis of "pepper strength" their panel selects from four samples of varying concentrations a "match" for a control sample. Differences in flavor are noted. If a panel member indicates "offness" in a given sample, this sample is used in a triangle test. If it is not picked for "offness" this second time, it is considered to be acceptable. The Kraft panel has existed for less than a month and it was felt that with experience it can do a completely adequate job. Dr. Cairncross felt that a conference with Mr. Sjöström about flavor and its evaluation would be of interest and value to Mr. Harrison.

It was agreed that as soon as the material responsible for the "woody-raisin" off-note is isolated, a sample would be sent to Kraft to be used as a reference standard for "offness" by the panel. The general impression gained from the Kraft group was that they were not sure of the identity of the "woody-raisin" off-note and were not confident that they could recognize it in a given sample.

Plans for future work were discussed in relation to the program outlined in Dr. Cairncross' letter of March 25, 1954. It was decided that major emphasis would be placed on the isolation and identification of the material causing the off-flavor, although it was understood that attempts to produce the off-note in the various spices were necessary to a limited degree. The matter of quality control of the spices used was to be left mainly to Kraft.

Suggestions from ADL as to methods particularly helpful to control would be welcomed and incorporated into their work.

From ELN/clf:4

MEMORANDUM

To: Case: Date: Page: 3

Subject:

Dr. Mottern requested that the procedure used at ADL in chromatographing the pepper dispersions be sent to him.

The meeting concluded at approximately 2:30 P.M.

ELW

E. L. Wick

From

C O P Y

The Emulsol Corporation
59 East Madison Street
Chicago 3, Illinois

January 5, 1954

Mr. C. E. Nelson
Kraft Foods Company
500 Peshtigo Court
Chicago 90, Illinois

Dear Mr. Nelson:

The two samples of spice mix submitted to us November 30, 1953, during our visit to your office, were subjected to various tests with the following results:

	Old 222	New 222
Salt	88.0%	83.5%
Dextrose	0.0%	5.5%
Fixed oleoresin	5.90%	6.30%
Essential oil (volatile)	0.90%	0.75%
Piperine	1.1%	2.35%
Allyl isothyocyanate content in the distilled essential oil	6.0%	10.5%
Refractive index of essential oil N^{20}_{C}	1.4949	1.4864
Analysis of essential oil	At least 50% of the essential oil is composed of some component other than pepper oil or mustard oil.	Typical pepper oil with small percent of mustard oil.
Taste evaluation	Flavor harsh. Something foreign to pepper or mustard.	Typical pepper-mustard combination.

The allyl isothyocyanate values reflect the presence of mustard in both mixes. The amounts indicate concentrations of approximately 10%, although, because of natural variations ordinarily found in such values, the concentration may very well be in the range of 5% to 15%.

C O P Y

Mr. C. E. Nelson
Kraft Foods Company
Chicago 90, Illinois

January 5, 1954

In the Old 222 we find 1.1% piperine and 0.90% essential oil, a ratio of 1.2 parts of piperine to 1.0 part essential oil. In black pepper the piperine content ranges from 2 to 3 times the essential oil content, that is, the piperine value runs between 5 to 9% and the essential oil ranges from 1 to 4%. These results would seem to indicate that some other essential oil or volatile substance is present in the old 222 which is not present in the new. You will note that in the latter, the ratio of the piperine content (2.35%) to the essential oil content (0.75%) is 3.1 to 1.0 which is in the normal range.

Taste evaluation showed that the Old 222 had a harsh, strong flavor which is not pepper-like, whereas the New 222 has a typical pepper flavor.

It is our opinion that the essential oil from the Old 222 contains some constituents other than black pepper or mustard oil. The sample we had was too small to determine the character of the adulterant. With a large enough sample it should be possible to identify this adulterant.

Even without doing this additional work we now have enough information to recommend a change in your specifications for the pepper spice mix. You must realize by now that setting a minimum oleoresin content is not enough. We suggest that you establish the ratio of the piperine content to the content of essential oil, along with flavor and taste characteristics.

We will be glad to cooperate with you in every way possible in any additional studies you care to conduct.

Very truly yours,

THE EMULSOL CORPORATION

MHJ:jc

M. H. Joffe

COPY 12/9/53

Differences Observed in Spice and Pepper Mixes

Submitted by Kraft

Chromatographic separation of "Old" and "New" mixes (222) received 11/30/53 revealed the following differences:

1. Two pink bands appeared in the "New" sample during the first stage of development. Only one appeared in the "Old" sample, and only in trace quantities.

2. A wide bright yellow band was observed in the "New" sample during the second stage of development, which was absent in the "old".

3. The "Old" sample seemed to contain more green material than the "New" as observed in the fourth stage of development.

A qualitative examination of the residue remaining after extraction of the sample with organic solvents showed the presence of reducing sugar in the "New" but none in the "Old". The original samples contained approx. 85% salt.

A similar chromatographic separation of the two pepper mixes rec'd 12/4/53 revealed nearly the same band differences as was observed in the "Old" and "New" spice mixes.

1. Pink bands present in Spice No. 1 do not appear to be present in Spice No. 2.

2. The wide bright yellow band observed during the second stage of development in Spice No. 1 did not appear in Spice No. 2.

A similar examination of the residue after extraction of the sample with organic solvents showed the presence of reducing sugar in Spice No. 1, but only a trace in Spice No. 2. The original sample contained approx. 88% salt.

Summary:

The extractable material from the pepper mixes gave nearly the same sequence and type of bands when treated and developed in the same manner chromatographically as did the "Old" and "New" spice mixes. The additional bands found in pepper No. 1 seem to be the same as those found in the "New" mix 222. These bands were found absent in pepper No. 2 and "Old" spice mix 222.

Reducing sugar was present in both "New" - 222 and No. 1 pepper. It was found absent in "Old" - 222 and present in only a trace in pepper No. 2.

The differences noted in the "Old" and "New" spice mixes appear to be related to the pepper used.

D. Elkind

C O P Y

Mr. Joffe of Emulsol Company phoned to state that their chemists had found 65% piperine in the oleoresin from the suspected spice mixture by the AOAC method and only 20% by a modification of the AOAC method which is not inferferred with by the presence of organic nitrogen compounds other than piperine.

New oleoresin showed 46% by both methods.

From this it is concluded that the old oleoresin has been adulterated by adding an organic nitrogen compound in an amount to yield approximately the same nitrogen as piperine.

H. H. MOTTERN/pmf

P D

E. L. Wick 59074 March 24, 1954

S. E. Cairncross
L. B. Sjöström

Report of Meeting at Kraft Foods Company

Personnel present: Dr. H. H. Mottern, Mr. T. Partyka, and Dr. N. Yannick of Kraft Foods Company; Dr. S. E. Cairncross, Mr. L. B. Sjöström, and E. L. Wick of Arthur D. Little, Inc.

On Thursday, March 18, the above group met at the Research Department of the Kraft Foods Company in Glenview, Illinois, to discuss results of the preliminary work carried out at Arthur D. Little, Inc., to "determine the source or sources of off-flavor in Miracle Whip salad dressing by means of screening raw materials, examination of control samples, and checking of current theories regarding possible contaminants."

The meeting opened with Dr. Cairncross' summary of the general problem and of our results. These results generally confirmed the conclusions of Kraft that the trouble was due to the spice mixture used and that major responsibility rested on the type of pepper dispersion used. Other ingredients used in the production of Miracle Whip were found to be satisfactory although it was pointed out that all must be of high quality in order to produce an acceptable product. Mr. Partyka asked in particular whether the corn sugar vinegars were found to be acceptable since these samples had at one time been suspected. Samples of Miracle Whip made with and without corn sugar vinegar were brought in and tasted by Mr. Sjöström and Dr. Cairncross. Both were found to be acceptable.

General agreement existed that the "woody-raisin" off-note was produced in salad dressing by only the Kraft prepared #222 bad spice mixture. Neither the Kraft group nor the ADL group were able to prepare in the laboratory a similarly bad spice mixture.

The possible effect of salt on the acceptability of the spice mixture was discussed. It was pointed out that salt could not be disregarded as a source of the off-flavor since no free pepper oleoresin had been available for study. A discussion of the preparation of the spice mixture revealed that Kraft buys the pepper oleoresin already on a salt base and uses Diamond Crystal or Morton's salt in the mixture. Dr. Yannick described the difficulties encountered last summer in preparation of the spice mix. A Day mixer was used and the mixture attained a temperature of approximately 110° F before it was completely emptied into the paper-lined storage drums. The mixture was not free flowing and was "gooey" and much wetter than under normal conditions. These difficulties were experienced when either Stange (good) or Long (suspected) pepper dispersions were used. Portions of both types were returned to the suppliers because of unusual wetness and stickiness. The possibility that the warm spice mixture may have become "bad" due to contact with "bad" salt or with the paper liners in the drums was considered. "Used" and "unused" drum liners were provided for investigation by the ADL group.

Dr. Mottern felt that the possibility that the paper liners had contributed the off-character to the spice mixture was remote and that the investigation of the spice mix, the pepper oleoresin, and the effect of salt on these spices was of immediate interest. It appeared to be possible that, as indicated by chromatographic work, the oleoresin had been accidentally or purposefully adulterated or contaminated. The development of a quality control method adaptable to any pepper oleoresin would be of great value.

The methods currently in use for the determination of piperine were discussed. Dr. Yannick felt that none were adequate and that there was a great need for a really good quantitative method. He pointed out the possibility that the use of a determination of piperine as a control for the quality of pepper to be used in salad dressing might not be sufficient for the prevention of the recurrence of off-flavor since many adulterants might be used in the oleoresin which did not cause off-flavor. He felt that the most important aim was to determine the source of the off-flavor.

General discussion was held of methods applicable to the most efficient and rapid determination of the reason for the "badness" of the bad spice mix. It was felt that the possibility that the paper liners were the cause of "off-ness" could be investigated and their contribution to the problem determined rather quickly. If the liners were found to have no effect, investigation of the spices themselves should be undertaken. Distillation and chromatographic techniques should be applied to the "good" and "bad" spice mixtures, pepper dispersions, and pepper oleoresins. Of great importance to the solution of the problem would be the production of a "bad" spice mix from good ingredients and from the "bad" oleoresin since, if this were accomplished, definite knowledge as to the source of the "off-ness" would be attained.

The results of the screening of the mayonnaise and sandwich spread samples submitted to ADL by Kraft were reported by Mr. Sjöström. Dr. Yannick indicated that three additional samples of mayonnaise may be sent to ADL for a complete profile determination.

The meeting was concluded at 3:00 P.M. Copies of the memorandums of Dr. Cairncross, Mr. Sjöström, and E. L. Wick were left with Dr. Mottern, Mr. Partyka, and Dr. Yannick.

ELW

Emily L. Wick

/dkh

MEMORANDUM

From: Emily L. Wick　　Case No.: C-59074　　March 16, 1954

To:　　Subject: The Determination of the Source of Off-Flavor in Miracle Whip Salad Dressing

The determination of the source of the off-flavor which has appeared in Miracle Whip salad dressing has been carried out by the examination of raw materials from which the product is made, the study of especially prepared samples of salad dressing, and by a brief chromatographic investigation of "good" and "suspected" #222 spice mixtures and of "good" and "suspected" oleoresins of black pepper. The work carried out is described in this report under the following headings:

I. Screening of Raw Materials of Miracle Whip Salad Dressing and Examination of Control Samples.

II. Chromatographic Investigation of "Good" and "Suspected" Oleoresins of Black Pepper and #222 Spice Mixtures.

I. Screening of Raw Materials of Miracle Whip Salad Dressing and Examination of Control Samples.

Initial work consisted of flavor panel evaluation of samples of regular Miracle Whip prepared from "good" ingredients and of samples prepared from "suspected" ingredients. It was found that the offending characteristic "woody-fruity-dried raisins" off-note was easily recognized. This note will be referred to as the "woody-raisin" note. Further orientation in the flavor of Miracle Whip was obtained by the study of samples which contained in the one case no pepper (202-7), and in the other case no mustard flour (126-3). In each instance, the clean blended flavor of Miracle Whip salad dressing was destroyed. The resulting sour unblended flavor was different and easily distinguishable from the off-note observed in samples prepared from suspected ingredients.

Having become familiar with the "woody-raisin" off-flavor found in samples prepared from suspected ingredients, and having learned to differentiate it from the off-flavor resulting from the absence of certain spices in salad dressing, the raw materials used in Miracle Whip were screened systematically. With the exception of the spices, all ingredients were tasted *per se*. Questionable ingredients were then tested in salad dressing containing no spices. None of the materials thus screened were found to be unacceptable. Examination of the starches (203-5, SH; 203-6, AO; 203-7, K-35) indicated that the jars in which they were shipped may at one time have contained "suspected" pepper seasoning since their aroma was very like that of this spice.

Screening of the spices was carried out by tasting them at four times the level used in regular Miracle Whip in salad dressing which contained no spices (202-6). All the spices with the exception of Long's straight peppercream "suspected" (203-19) and the "bad" #222 spice mix (203-21) prepared from this pepper were found to be completely acceptable. The addition of "suspected" pepper (203-19) to the taste medium caused a sharp sour flavor which was not outstanding and was definitely not similar to the "woody-raisin" off-note under consideration. The addition of mustard to this mixture similarly did not produce the off-flavor. An ADL #222 spice mix prepared from "suspected" pepper (203-19) and mustard flour (203-8) was tested in the medium. In this case, the normal flavor and aroma of Miracle Whip was lacking, but the "woody-raisin" note did not develop. However, only the Kraft sample (now aged) of "bad" #222 spice mix prepared from "suspected" pepper, was capable of producing the full "woody-raisin" off-flavor which is the subject of this investigation.

Similar screening of the pepper oleoresins and of the #222 spice mixes was carried out in mineral oil. The aroma of good #222 spice mix in this medium was that of black pepper. The bad #222 spice mix produced the characteristic "woody-raisin" note. Suspected pepper (203-19) alone or with the addition of fresh mustard did not produce the off-aroma. Good pepper (203-18) alone or with the addition of fresh mustard produced the black pepper aroma. Only the aged bad #222 spice mix produced the off-note under consideration.

On the basis of these observations, it must be concluded that the off-flavor noted in Miracle Whip is caused by compounding and mixing the suspected pepper (203-19) with mustard and salt and not from pepper alone, and that a time factor appears to be important in its production since only the Kraft prepared #222 spice mix causes its appearance in oil or in salad dressing.

A more detailed description of the screening of the raw materials and of the control samples is given in outline form in the supplement to this report.

II. Chromatographic Investigation of "Good" and "Suspected" Oleoresins of Black Pepper and "Good" and "Suspected" #222 Spice Mixtures.

In an attempt to determine and define the differences between the "good" and "suspected" #222 spice mixes and thus clarify the cause of off-flavor in Miracle Whip salad dressing, a preliminary chromatographic study was undertaken. The "good" (203-20) and "bad" (203-21) spice mixes were adsorbed on columns of alumina (pH 9-10) and eluted in forty-two 5 ml fractions with skellysolve 'B', skellysolve-benzene (4:1, 3:2, 2:3, 1:4), benzene, benzene-ether (4:1, 3:2, 2:3, 1:4), ether, ether-ethanol (4:1, 3:2, 2:3, 1:4), ethanol, aqueous ethanol (80%, 60%, 40%, 20%) and water. As a control a comparable quantity of mustard flour (203-8) was chromatographed in similar fashion. General examination of the physical properties and odor of the fractions obtained after removal of solvents under reduced pressure showed that the two spice mixes were similar to each other and only slightly different from mustard flour. On the basis of physical properties and odor the spice mixes yielded only two fractions which differed noticeably from the mustard. These fractions were 19 and 20 and contained yellow solids which had pepper-like aromas and which, when tasted, had the strong pepper "bite" characteristic.

In a further effort to determine the difference between "good" and "bad" spices, chromatographic analysis of Long's "suspected" peppercream (203-19) and Stange's COSP "good" pepper seasoning (203-18) was carried out. The two oleoresins were removed from their salt bases by ether extraction and chromatographed according to the method described above. Separation by the columns appeared to be efficient and the two peppers yielded generally similar fractions with the following outstanding exceptions: The "good" pepper yielded in fraction 2 a yellow oily solid having the good aromatic black pepper character, while none of this substance was obtained from the "suspected" sample. The "suspected" pepper yielded in fractions 9 through 13, a white crystalline solid, m.p. 114-115°, which, when tasted in mineral oil, provided the pepper "bite" but had none of the aromatic character. This substance was not obtained from the "good" pepper.

On the basis of these preliminary examinations, it appears that chromatographic technique offers an efficient method for separating and demonstrating differences in the "good" and "suspected" oleoresins of black pepper. It is entirely possible that further examination of the "good" and "suspected" #222 spice mixtures by modified procedures will illustrate differences between them also. Results obtained indicate the possibility that the "suspect" pepper may be "bad" due to the absence of a requisite component rather than to the presence of a contaminant.

Whether the white crystalline material found in the "suspect" pepper may result from a chemical reaction of the yellow aromatic substance derived from "good" pepper, or whether the two oleoresins differ even more fundamentally might be answered by the chemical identification of the two unknown compounds. Since both compounds are obtainable in good yield it seems reasonable to believe that their identification can be accomplished and that tests for their quick identification may be developed.

The results of this introductory examination of the pepper oleoresins illustrate definite differences between them and indicate that investigation of these differences may be expected to be of value in the determination of the cause of the off-flavor found in Miracle Whip salad dressing.

Supplement: Screening of Raw Materials and Control Samples

The screening of the raw materials of Miracle Whip and the examination of control samples is summarized in the following outline. In each case, the code number, a brief description of the sample and its treatment, and the resulting comments on its flavor, are given.

I Comparison of various samples of regular Miracle Whips made at various times from good ingredients

1 Received December, 1953 - is excellent sample
126-1 Prepared January 26, 1954 - almost as good as sample 1
202-4 Prepared February 2, 1954 - is slightly sour
929-7 Prepared September 29, 1953 - has "Waldorf salad" note

Conclusion: Each sample differed though made from good ingredients. Samples 1 and 126-1 were selected as examples of standard Miracle Whip.

II Effect of using cottonseed oil versus soya oil in Miracle Whip

126-1 Contains soya oil
126-2 Contains cottonseed oil - is sweeter and more oily than 126-1. Cottonseed oil does not improve the flavor.
The oils (203-10, 203-22, 203-23) were tasted per se and no off-notes were evident.

Conclusion: Oils are not suspected as the source of the "woody-raisin" off-flavor.

III Effect of the absence of a single spice on the flavor of Miracle Whip

Samples are made from good ingredients
202-6 Contains no spices - is bland, sweet
202-7 Contains no pepper - blend broken, strong vinegar
126-3 Contains no mustard flour - blend broken, strong vinegar

Conclusion: All spices must be present for a successful blended flavor.

IV Effect of the addition of spices (at four times the level used in regular Miracle Whip) to 202-6 (contains no spices)

(1) 202-6 + mustard flour - has clean strong flavor, little aroma
(2) 202-6 + paprika - clean, waxiness is cut
(3) 202-6 + wet PL (203-9) - fresh, clean, sweet spices
(4) 202-6 + good pepper (203-18) - fresh, clean
(5) 202-6 + suspected pepper (203-19) - sour, "woody-raisin" note is not present
(6) 202-6 + good spice mix (203-20) - clean, good

(7) 202-6 + suspected spice mix (203-21) - "woody-raisin" note is very evident
(8) 202-6 + good pepper + mustard - clean, good
(9) 202-6 + suspected pepper + mustard - "woody-raisin" note is not present though flavor is not that of Miracle Whip
(10) 202-6 + ADL prepared good spice mix - clean, good
(11) 202-6 + ADL prepared bad spice mix - "woody-raisin" note absent though flavor is not that of Miracle Whip

V Effect of vinegar versus spices on the flavor of Miracle Whip

929-6 Bad vinegar + good spices
929-8 Bad vinegar + bad spices
929-9 Good vinegar + bad spices

Conclusion: Sample 929-8 is worse than 929-9 and both 929-8 and 929-9 are much worse than 929-6. Thus bad spices have a more devastating effect on the flavor than bad vinegar alone. The combination is very bad.

VI Screening of vinegars

All the vinegar samples were screened by tasting per se and appeared to be acceptable. Sample 201-34 Cider 425-7, which was "originally suspected to be bad", was tasted in salad dressing containing no spices. No evidence of the "woody-raisin" off-flavor was noted.

Emily L. Wick

PD

E. L. Wick 59074 March 8, 1954

S. E. Cairncross
L. B. Sjöström
R. L. Swaine

Progress Report on Exploratory Work for Kraft Foods Company

Progress toward the "determination of the source or sources of off-flavor in Miracle Whip salad dressing by means of screening of raw material, examination of control samples, and checking of current theories regarding possible contaminants" is summarized in the following outline. This preliminary work is scheduled to be completed March 12, one month after the receipt of the samples.

I. Comparison of various samples of regular Miracle Whips made at various times from good ingredients.

a.
#1 Received December, 1953 - is "super" sample
126-1 Prepared January 26, 1954 - almost as good as #1
202-4 Prepared February 2, 1954 - sour
929-7 Prepared September 29, 1953 - "Waldorf salad" note

Conclusion: Each batch differs though made from good ingredients.

b. Color has no effect since all four samples above differed.

II. Effect of using soya oil versus cottonseed oil in Miracle Whip.

a.
126-1 Soya oil
126-2 Cottonseed oil: did no harm but did not help, higher sweet, more oily

b. The oils were tasted per se and no off-notes were evident.

Conclusion: Oils are not suspected as source of off-flavor.

III. Effect of spices

a. Samples made from good ingredients

202-6 No spices: bland, sweet, not much body
202-7 No pepper: strong vinegar, blend broken
126-3 No mustard flour: strong vinegar, blend broken

Conclusion: All spices must be present for successful blended flavor.

b. Effect of addition of spices (at four times the level used in regular Miracle Whip) to 202-6 (no spices).

1. 202-6 + mustard flour: clean, strong, little aroma
2. 202-6 + paprika: clean, cuts waxiness
3. 202-6 + wet PL (203-9): fresh, clean, sweet spices
4. 202-6 + good pepper (203-18):) little difference in aroma,
) bad spice
5. 202-6 + suspected pepper (203-19)} causes sharper sour, effect
) not outstanding
6. 202-6 + good spice mix (203-20):)Difference is marked!
7. 202-6 + suspected spice mix (203-21):)Off-note very evident
8. 202-6 + good pepper + mustard:)Off-flavor less noticeable
9. 202-6 + suspected pepper + mustard:)than with prepared spice mixes
10. 202-6 + lab prepared good spice mix:)Off-note definitely
11. 202-6 + lab prepared suspected spice mix:)present though not as
)bad as in Kraft prepared
)samples

Conclusion: Bad pepper appears to be the fundamental source of the off-flavor even though it must be combined with mustard and salt in a spice mix in order to exhibit the total off effect in salad dressing. The fact that a less bad effect was observed with laboratory prepared spice mix than with Kraft prepared spice mix, may be due to the use of different mustards in the Kraft samples or due to incomplete mixing of the spices and dressing in the laboratory prepared samples. The laboratory prepared samples will be tasted again in several days in order to clarify this point.

c. Chromatography of 203-18 Stange COSP "good" pepper versus 203-19 Long's straight peppercream (suspected).

Ether extraction of 5 grams of the "good" and "suspect" peppers in order to remove the oleoresins from their salt base was carried out and the residues (approximately 0.25 grams) were chromatographed on columns containing 8 grams of untreated (pH 9-10) Merck alumina. Alkaline alumina was chosen as the adsorbent in order to minimize losses on the columns since the active principles of black pepper are known to be slightly basic. Elution solvents used were 100% skellysolve 'B', skellysolve-benzene (4:1, 3:2, 2:3, and 1:4 proportions), 100% benzene, benzene-ether (4:1, 3:2, 2:3 and 1:4), ether, ether-ethanol (4:1, 3:2, 2:3 and 1:4), ethanol, aqueous ethanol (80%, 60%, 40%, 20%) and water.

Separation by the columns appeared to be quite efficient and the peppers yielded similar fractions with the following outstanding exceptions:

1. "Good" pepper gave in fraction 2 a yellow solid having the good aromatic black pepper character, while none of this substance was obtained from the "suspect" sample.

2. "Suspect" pepper gave a white crystalline solid in fractions 9-13 which, when tasted in mineral oil, provided the pepper "bite" but had none of the aromatic characters. This substance was not obtained from the "good" pepper.

Conclusions: On the basis of this first preliminary examination it appears that chromatographic technique offers a simple and efficient method for separating and illustrating differences in the pepper samples. Results obtained indicate the possibility that the "suspect" pepper may be "bad" due to the absence of a requisite component rather than to the presence of a contaminant. It would seem of value to determine the previous history and processing of the suspect pepper sample since it seems unlikely that two natural and similar oleoresins would differ in this manner.

IV. Effect of vinegar versus spices

a. 929-6 Bad vinegar - good spices) 929-8 is worse than 929-9
929-8 Bad vinegar - bad spices) Both 929-8 and 929-9 are much
929-9 Good vinegar - bad spices) worse than 929-6

Conclusions: Bad spices have more devastating effect than just bad vinegar. The combination is very bad.

V. Screening of vinegars (tasted per se)

a. Bad vinegar (201-34) is easily distinguished from the good sample (201-35)

b. The vinegars can be catagorized by groups (corn sugar, apple, white, etc.) easily. Twenty-five vinegars have been screened to date and all appear to be "good".

VI. Plans for completion by March 12 of the exploratory work for Kraft Foods Company.

a. Fifteen vinegars remain to be screened.

b. Representative "good" and "bad" vinegars will be tasted in salad dressing to determine their effect.

c. It would be desirable to chromatograph "good" Kraft prepared spice mix (203-20) and "bad" spice mix (203-21) in order to compare them. Mustard should be chromatographed at the same time so that its contribution to the components found in the spice mixes may be recognized and evaluated. This work will be carried out -- money and time permitting.

VII. Evaluation of mayonnaise samples

A general examination of these samples was made. A more careful study by the senior staff members would probably be advisable.

204-1	blended, bland
204-2	off-character, unblended
204-3	bland, nothing bad
204-4	slight sour
204-5	toasted chicken salad sandwich note

ELW

Emily L. Wick

/dkh

PTD

C
O
P
Y

C
O
P
Y

KRAFT FOODS COMPANY

Research Laboratories - 923 Waukegan Road
Glenview, Illinois

February 26, 1954

Dr. Robert L. Swaine
ARTHUR D. LITTLE, INC.
Memorial Drive
Cambridge 42, Massachusetts

Dear Dr. Swaine: C-59074

The five samples of mayonnaise of the #204 series--1, 2, 3, 4, and 5 represent a competitor's, two Production formulas for Kraft, and two Experimental formulas.

We would like to have you evaluate them on the basis of the code numbers supplied.

Very sincerely yours,

s/H. H. Mottern

H. H. Mottern
Manager

HHM/pmf

PTD

SEC:mc

February 17, 1954

Dr. H. H. Mottern, Manager
Research Laboratories
Kraft Foods Company
P. O. Box 187
Glenview, Illinois

Dear Dr. Mottern:

C-59074

We wish to acknowledge receipt of the very comprehensive set of samples of materials used in preparing Miracle Whip. All of the samples mentioned in Mr. Stevens' letter of February 8 arrived in good condition. In addition to those listed, we received five samples of two pints each of mayonnaise, which are numbered 204-1, 204-2, 204-3, 204-4 and 204-5.

The work of screening the raw materials, examination of control samples and evaluation of current theories regarding possible contaminants in Miracle Whip is underway, and we look forward to discussing our findings with you.

As we indicated in our first conversation and also in the proposal, we are going to attempt to carry out this preliminary study in one month. It is obvious, however, that the effective working period must begin with receipt of samples and not with the date of authorization. The project was authorized on January 18; the samples were received on February 12 and work was begun on that day.

Very sincerely,

ARTHUR D. LITTLE, INC.

By:

Stanley E. Cairncross

PD

C
O
P
Y

KRAFT FOODS COMPANY

Research Laboratories - 923 Waukegan Road
Glenview, Illinois

C
O
P
Y

February 8, 1954

Dr. Stanley E. Cairncross
Arthur D. Little, Inc.
Chemists-Engineers
Cambridge 42, Massachusetts

Dear Dr. Cairncross:

In reply to your telephone message of January 25, to Mr. Partyka and your letter of the same date addressed to Dr. Mottern, we are submitting to your attention numerous samples of Miracle Whips and raw materials that go to make up this product.

We would like to submit the samples identical with your requests, however, this being impossible, we are sending you those which comply as closely as possible.

For example, with regard to pepper, we do not have what you term one of "doubtful" quality, but are sending you what we believe is "good" and a suspected one which we call "bad".

The oldest pepper sample we have available is that which dates to March 3, 1953.

Similarly, with respect to the vinegars, we are sending 4 ounce samples of vinegars used from September, 1953 to the present. July and August samples were not available. We are sending you larger quantities of vinegars but the quantities you request are not available. The oldest oil we have is that used in November, 1953.

We have coded all the individual samples which are listed below, together with whatever description we believe you may need in aiding you in the solution of our problem.

The samples are as follows:

<u>Vinegars</u>:

The codes 201-1 to 201-40 are the vinegars that were used in Production during 1953 (except 201-35). The codes 203-13 and 203-14 are the vinegars used currently in production.

C
O
P
Y

C
O
P
Y

Vinegar Samples

✓201-1	SBIX-1609 Apple Ind. Cider Vin.	August 18-53 Chi-TKE
✓201-2	SBIX-1645 " " "	August 21-53 " "
✓201-3	SBIX-1644 " " "	August 26-53 " "
✓201-4	SBIX-1609 " " "	Sept. 1-53 " "
✓201-5	Steffen's " " "	Sept. 3-53 East TK
✓201-6	" Cider 506 Sept. 4-53 (49.8-49.8) TKD	
✓201-7	SBIX-1645 Apple Cider	Sept. 8-53 Chi-TKE
✓201-8	SBIX-1644 Apple Ind. Cider	Sept. 18-53 Chi-TKE
✓201-9	SBIX-1609 " " "	Sept. 25-53 58.0 Chi-TKE
✓201-10	SBIX-1645 " " "	Oct. 1-53 Chi-TKE
✓201-11	SBIX-1644 Cider	Oct. 8-53 Chi-TKE
✓201-12	SBIX-1609 Apple Cider	Oct. 21-53 Chi-TKE
✓201-13	Car SBIX-1644	Nov. 1-53 645
✓201-14	SBIX-1644 Cider	Nov. 5-53 Chi
✓201-15	Corn Sugar 671	Nov. 17-53 Chi-West TK
✓201-16	SBIX-1609 Apple Cider	Nov. 18-53 Chi-TKE
✓201-17	SBIX-1576 Cider	Dec. 1-53 Chi-Must-TK
✓201-18	SBIX-1609 Apple Cider Apple Ind.	Dec. 15-53 Chi-TKE
✓201-19	SBIX-1576 Apple Cider	Dec. 13-53 Chi-TKE
✓201-20	Cons White 622:	Nov. 12-53 Chi-TKE
✓201-21	Stan Brands White	Nov. 13-53 Chi-TKE
✓201-22	Composite White 676 89.4	Nov. 18-53 5660 gal. EK-2
✓201-23	Composite White 680 (90.0) 90.0	Nov. 19-53 8633 gal. TK-1
✓201-24	" " 90	Nov. 23-53; 8573 gal TK-2
✓201-25	White (15.2-91.2) 113 H_2O -8460 gal-TK-2	
✓201-26	Steffen Corn Sugar 668	Nov. 16-53 Chi-East
✓201-27	Corn Sugar 689	Nov. 24-53 Chi-ETK
✓201-28	Comp. Corn Apple (45.1) Nov. 1-53 Nov. 19-53 4514 gal TK-East	
✓201-29	Comp. Corn Apple (45.0) Nov. 24-53 4625 gal. TK East	
✓201-30	Corn Sugar 8-25-53 M. Steffen & Co. - 58.8	
201-31	Consolidated White Aug 26-53 Chi-Tank 1	
201-32	SBIK-1644 Apple Ind.	Aug. 26-53 Chi Tank E

✓201-33	45 grain brought from Peshtigo 9-25-53.
✓201-34	Cider 925-7 (43.6) stored in Glenview originally marked 45 grain presumably used during summer of 1953.
✓201-35	Heinz Cider Vinegar 925-8 diluted to 45-grain used in experimental lots of Miracle Whip at Glenview 925 and 929 series
✓201-36	Vinegar from Glenview - marked "84" presumably used during summer of 1953.
✓201-37	90-grain white vinegar - first suspected as "bad" used at Glenview in 910 series of Miracle Whips
✓201-38	45-grain used in 910 & 922 series in Glenview originally suspected as "bad"
✓201-39	Vinegar stored in Glenview marked 50-grain presumably used in production in summer of 1953.
✓201-40	Vinegar stored in Glenview marked 59-grain presumably used in production in summer 1953.

203-13	Apple Cider Vinegar 45 grain as was used in Production on 2-1-54.
203-14	90-grain distilled vinegar as used in Production on 2-1-54.

COPY

The following are the single raw materials used in the manufacture of Miracle Whip:

✓ 203-2 sugar ok
✓ 203-3 salt ok
203-4 peppercream as currently used ok
✓ 203-5 starch (SH)
203-6 " (AO)
203-7 " (K-35)
✓ 203-8 mustard flour (SDM)
✓ 203-9 spice mixture (wet PL)
✓ 203-10 vegetable oil
✓ 203-11 liquid sugar (invert) ok
✓ 203-12 water
203-13 cider vinegar listed above
203-14 distilled vinegar listed above
✓ 203-15 paprika

The egg yolk that is used is not included in this shipment, because it has to be kept in the frozen state prior to use. If this material is desired we will send you a sample packed in dry ice.

✓ 203-22 K.C. oil (soya oil) as was used in production on 11-23-53
✓ 203-23 K.C. oil (soya oil) as was used in production on 2-2-54
✓ 203-24 corn oil, which is not used in Miracle Whip, but is included in this shipment for your possible interest (fresh).

Of the peppers and pepper-spice mixtures we are submitted sic the following:
203-16 COSP (3-5-53) The oldest black pepper seasoning we have available this was used in Production in March, 1953.
203-17 Hillside, N.J. 11-27-53 #222 Spice Drum D2 (bad) This #222 spice mixture is composed of: black pepper seasoning 87.5%
mustard flour 6.25%
salt 6.25%
203-18 Stange COSP "good." This is black pepper seasoning on a salt base which we consider as of <u>good</u> quality.
203-19 Long's straight peppercream (suspected) This is black pepper seasoning which is suspected as contributing the "off flavor" to Miracle Whip. It was used in Production sometime during July and August, 1953.
203-20 #222 spice mixture made of "good" COSP (ie 203-18)
203-21 #222 Spice mixture made of "suspected peppercream (ie 203-19)

When you compare the above list of ingredients with that which you have requested in your letter, you will note that:

1) Pepper:
We can submit only what we term "good" and what we suspect as "bad" but there is no doubtful quality pepper.
2) Control samples:
There are none available which date back to May, 1952.
3) All Single Raw Materials:
We submit all the single raw materials as are used currently in

C
O
P
Y

the manufacture of Miracle Whip.

4) Vinegars:
We are submitting numerous samples, however, we do not include any comments as to their "extreme" or "middle range" qualities as you requested. We are shipping you one-half of our supply of these vinegars and retaining the other half.

5) Spices and other Ingredients:
Samples submitted are those representing the current usage, unless otherwise indicated.

With regard to your request for the rough formulation of the Miracle Whip product, we can only give you a general method of its compounding. This method is as follows:

A. Preparation of "Cooked Starch"
The three starches, water, some 90-grain vinegar, salt, paprika, wet PL, and liquid sugar are mixed into a starch slurry. The slurry is cooked to properly gelatinize the starch which is subsequently cooled in a vacuum pan and is ready for use.

B. Preparation of the Mayonnaise emulsion:
The egg yolks, some water, pepper, some sugar, some salt, K.C. oil, SOE, are pre-mixed. The mixture is then emulsified by a continuous process where the vinegar and water mixture is proportioned.

C. Blending of A and B:
The Mayonnaise emulsion (B) is then added to the "cooked starch" in suitable proportions and a "mustard mix" is added to the mixture. Mustard mix is composed of mustard flour, sugar, salt and water. The ingredients A, B and the mustard mix are blended for a definite length of time and the product "miracle Whip" is packed in a customary manner.

We regret we are not prepared to disclose to you the exact formulation, or the details of the manufacturing procedure. We feel that should you desire to formulate samples of Miracle Whip from any combinations of the individual ingredients listed above, we shall be happy to prepare these for you in the exact manner as per your directions and forward them to you for your examination.

You have requested for thefollowing samples of Miracle Whip:
1) Miracle Whip made with cottonseed oil.
2) Miracle Whip made without mustard.

These two were requested during your visit in December. In your recent letter you request:
3) Miracle Whip without spice mixture
4) Miracle Whip without pepper oleoresin.
5) Samples of off-flavored product.

Accordingly, we are submitting the following;

C
O
P
Y

126-1: six pints of laboratory prepared Miracle Whip using the regular formula.
126-2: five pints of Miracle Whip prepared using cottonseed oil, instead of soya oil.
126-3: four pints of Miracle Whip containing no mustard flour.

These were prepared on January 26, 1954.

202-4: eight pints of laboratory prepared Miracle Whip using the regular formula, similar to samples coded 126-1.
202-5: eight pints Miracle Whip made with "suspected" black pepper seasoning (coded 203-19 above)
202-6: four pints Miracle Whip containing no spices.
202-7: four pints Miracle Whip containing no pepper. These were prepared on Feburary 2nd.

The following samples of Miracle Whip submitted were prepared on September 29, 1953:

929-6 Miracle Whip made with "good" black pepper seasoning and 201-34 cider vinegar which was suspected to be bad.
929-7 Miracle Whip made with "good" black pepper seasoning and 201-35 Heinz cider vinegar which was considered to be good.
929-8 Miracle Whip made with "Garland" suspected #222 Spice mix and 201-34 cider vinegar above.
929-9 Miracle Whip made with Garland "suspected" #222 spice mix and 201-35 Heinz cider vinegar.

These represent the samples which you were tasting here two months ago. They are the laboratory prepared samples made with combinations of good ingredients and suspected ingredients. The "Garland" #222 spice mix was suspected to be the cause of the off-flavor in the Miracle Whip. The 201-35 cider vinegar is considered to be of inferior quality as compared to Heinz's vinegar.

With reference to your request for samples of the off-flavored product, we wish to call your attention to the fact that the so-called "typical complaint" samples received from the consumers are now over six months old. After such a storage period, even the normal product will have deteriorated to a certain extent as it normally does. It is hoped that you will not be led to confuse the normal off-flavor and typical "off-flavor" which was the cause of the complaints.

We are not submitting any of the 6-month old "complaint" samples that were obtained from the consumers, however, if you believe that you will need them we shall be glad to send them to you.

While we cannot give you the exact formula of the product, we submit only its approximate percentage composition:

COPY

Soy oil	40-50%
Egg yolk	4-8%
H_2O	less than 20%
Sugar	1-3%
Salt	1-2%
#222 Spice Mix	less than 0.1%
90-grain dist. vinegar	5-9%
45-grain cider vinegar	4-7%
mustard flour	less than 0.5%
starches	3-6%
Liquid sugar	7-12%
wet PL	less than 0.5%
paprika	less than 0.1%

This will give you an idea of the relative levels of the individual ingredients going to make up the Miracle Whip formula.

We are looking forward to your visit to our laboratories to discuss further details of the problem.

Yours very truly,

KRAFT FOODS COMPANY
Research Laboratories

s/Harold H. Stevens

HHS:bw

Harold H. Stevens

MEMORANDUM

From: E. L. Wick　　C-59074　　June 9, 1954

To: Kraft Foods Company　　Subject: The Isolation of Off-Flavor in Miracle Whip Salad Dressing

The chief purpose of the work undertaken since the last meeting with Kraft Foods Company on April 14, 1954, has been the isolation and identification of the off-flavor found in complaint samples of Miracle Whip. This off-note has been described as "woody-raisin" and is readily detectable in salad dressing in aroma as well as flavor. Isolation of the off-flavor has been successfully accomplished from Long's pepper dispersion (416-3) and has been identified as a sesquiterpene, $C_{15}H_{24}$, $n_D^{24°}$ 1.4964, b.p. 79-80° C (1 mm). In addition to the "off"-flavor liquid there was isolated a white crystalline solid having a peppery bite, $C_{14}H_{17}NO$, m.p. 113-114°. Piperine was also found. The details of this work will be described under the following headings.

I. Chemical Separation of Long's Pepper Dispersion:--Isolation of the "Woody-raisin" Off-flavor, and a Peppery-bite Characteristic.

II. Possible Identity of the Off-liquid and the Peppery-bite Compound.

I. Chemical Separation of Long's Pepper Dispersion (416-3):--Isolation of the "Woody-raisin" Off-flavor and a Peppery-bite Compound.

Long's pepper dispersion was chosen as a source material for the isolation of off-flavor since it provided fewer variables than #222 spice mix and since after being heated at 55°C in the presence of moisture it produced the "woody-raisin" note in mineral oil and to a slight degree in salad dressing which contained no spices. Several separations were carried out during the investigation but the most satisfactory method is outlined in Figure 1, page 3.

Isolation of the "Woody-raisin" Off-flavor.

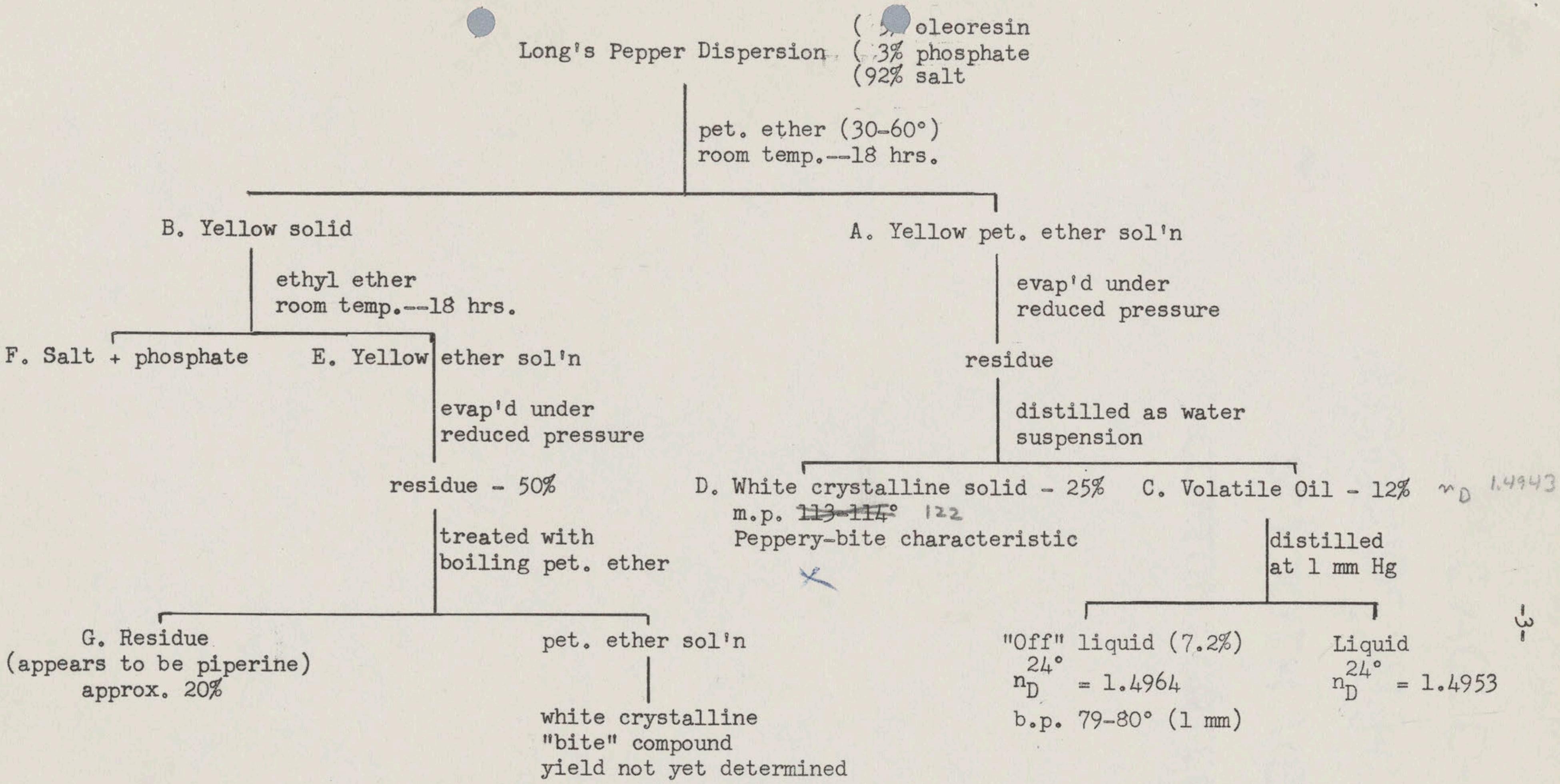
Long's dispersion (416-3) was treated with petroleum ether (b.p. 30-60°C) at room temperature for 18 hours. The resulting mixture was separated by filtration. The yellow filtrate (A) was concentrated under reduced pressure and the residue distilled as a water suspension in order to remove volatile oils (C). The yellow solid (B) was treated with ethyl ether at room temperature for 18 hours to remove organic material from the salt and phosphate dispersion base (F).

The volatil~~s~~e oils (C) were obtained in 12% yield and had a peppery and aromatic odor. Fractional distillation of the volatile oils at 1 mm yielded an aromatic fraction, $n_D^{24°}$ 1.4953, and two "woody-raisin" fractions, $n_D^{24°}$ 1.4962; 1.4963. Redistillation of the woody fractions at 79-80°C (1 mm) yielded an analytically pure sample, $n_D^{24°}$ 1.4964, in 7.2% yield. This liquid when tested in mineral oil and in salad dressing which contained no spices disclosed the "woody-raisin" odor and flavor typical of that found in the complaint samples of Miracle Whip. Samples of the pure fraction were submitted for the following analyses.

Elemental analysis: Calculated for $C_{15}H_{24}$: C, 88.16; H, 11.84; mol. wt. 204. Found: C, 88.19; H, 12.01; mol. wt. 217 (Rast method in camphor). Sodium fusion disclosed no other elements.

Infrared spectrum: The spectrum indicates the presence of a terminal isopropyl group $[(CH_3)_2CH-]$, and an exocyclic methylene group ($CH_2=$). Absorption typical of terpenes is exhibited.

On the basis of the above results, it is concluded that the material which contributed the off-flavor to Miracle Whip is a sesquiterpene found in



Our results fit those of Emulsol almost exactly.

FIGURE 1. CHEMICAL SEPARATION OF LONG'S PEPPER DISPERSION (416-3)
Yields are based on oleoresin present in dispersion

the volatile oils present in Long's pepper dispersion (416-3). The fact that the off-flavor is exhibited only in heated moist dispersion and in #222 spice mix prepared last summer under unusual conditions of heat and moisture is consistent with this finding. It is reasonable to believe that sufficient heat and moisture were present to cause distillation of the volatile oils in the degree necessary to accentuate the off-flavor.

Isolation of a Peppery-bite Compound

The aqueous suspension remaining after distillation of the volatile oils (C) was extracted with ether. The yellow ether solution was dried, concentrated under reduced pressure and the residue was sublimed at 60-70°C (1-2 mm). A white crystalline sublimate (D) was obtained in 25% yield. Resublimation yielded an analytically pure sample, m.p. 113-114°, which when tested in mineral oil and in salad dressing containing no spices gave no odor but provided a pepper-like flavor. When tasted *per se* a very sharp biting sensation is produced. Slight woodiness is noted if an impure sample is tasted *per se*. Samples of the pure sublimate were submitted for the following analyses.

Elemental analysis: Calculated for $C_{14}H_{17}NO$: C, 78.10; H, 7.96; N, 6.51; mol. wt. 215. Found: C, 78.18; H, 8.19; N, 6.56; mol. wt. 242 (Rast method in camphor). Sodium fusion disclosed no other elements.

Infrared spectrum: The spectrum indicates the presence of an aromatic ring and a *cis*-double bond of the $R_1CH=CHR_2$ type. There is no evidence for the presence of the -NH-, -OH, or -C=O groups.

Ultraviolet spectrum: The presence of a conjugated system containing an aromatic ring is indicated by the spectrum which contains ~~three~~ two maxima. λ 200 mμ, ε 20500; 282, 33800; ~~378, 34500~~.

Qualitative color tests recommended by the U. S. Dispensatory, 22nd Edition, for the identification of piperine were found to give positive results when applied to the isolated $C_{14}H_{17}NO$ "bite" compound. This would indicate similarities in the structure of these two compounds.

Further separation of the pepper dispersion (416-3) was accomplished by brief investigation of the ether solution (E). (See Figure 1, page 3). This yellow solution was concentrated under reduced pressure. The residue (50% yield based on oleoresin) was treated with boiling petroleum ether to remove all of the already partially isolated $C_{14}H_{17}NO$ compound. Time did not permit determination of the total yield of this "bite" characteristic. The petroleum ether insoluble residue G is a yellow solid which appears to be impure piperine. This material is present in approximately 20% yield. This figure corresponds to the results of The Emulsol Corporation which reported a 20% yield of piperine in the oleoresin from suspected spice mixture using a modified AOAC method of determination.

II. Possible Identity of the Off-liquid and the Peppery-bite Compound

Since any ingredient of a commercially available abnormally compounded pepper would undoubtedly be easily obtainable and its properties well known, it was felt that a brief search of the literature might result in identification of the isolated compounds described above.

Hasselstrom[1] and Greunther[2] have described various imitation black pappers and report that common volatile oils used as adulterants are caryophyllene, phellandrene, and dipentene (limonene). The physical properties and molecular weight of the isolated $C_{15}H_{24}$ "woody-raisin" off-liquid rule out its identification as phellandrene or dipentene. That it is γ-caryophyllene is a possibility though the odors of the isomeric caryophyllenes are not known to be woody. Its identification as α-cedrene is a possibility since this compound has a woody odor and physical properties quite similar to those of the isolated off liquid. Table I contains the properties, molecular weights, and other pertinent data characteristic of these compounds.

It is believed that the identification of the $C_{15}H_{24}$ off-liquid may be quite possible if time were made available for: (1) the determination of its density and optical rotation to complete the knowledge of its physical properties; (2) accomplishment of a thorough literature search of known hydrocarbon sesquiterpenes; (3) the procurement of pure samples of compounds which might be identical to the off-liquid; and (4) the comparison of physical properties and infrared spectra of these compounds.

Various "peppery-bite" chemicals have been described by Staudinger[3] and Hasselstrom[1,4]. Several of the most common of these and those most similar to the "bite" compound isolated from Long's dispersion are shown in Table II. Compounds II and IV were used in Germany during World War I in imitation black pepper[1] while in World War II only compound II was used. This latter compound II, β-cinnamenyl acryloyl piperidide was selected[1] as the synthetic peppery-bite material to be used in the black pepper substitute developed by the Quartermaster Research and Development Laboratories.

It is reported by Hasselstrom[1] that most domestic imitation peppers contain the oleoresin of capsicum as the biting ingredient. Compound V in Table II is capsaicine the pungent principle of red pepper. Its physical properties and molecular weight are not at all similar to those of the "bite" compound from Long's dispersion.

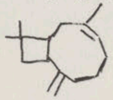
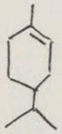
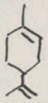
On the basis of the facts known about the "bite" characteristic isolated from Long's dispersion, its structure is most probably of the type represented by compound I, cinnamoyl piperidide. This material is known[5,6], is easily prepared, and has a strong pepper "bite"[4b]. Facts which support identification of the isolate as I are the agreement of the analytical data, ultraviolet data and most of the infrared data. The fact that the infrared spectrum lacks evidence for a carbonyl group and that the melting point of I is 122° while that of the isolate is 113-114°, does not support structure I. This discrepancy in melting points may be explained by the possibility of _cis_-_trans_ isomers of I. _Trans_-isomers are known to have higher melting points than do _cis_ isomers and the infrared data indicates that the isolate from Long's pepper contains a _cis_-double bond.

Whether or not the isolated "bite" compound has structure I should be rather easily proved since this compound would be expected to be hydrolyzed to the well known and easily identified compounds of piperidine and cinnamic acid.

Emily L. Wick

E. L. Wick

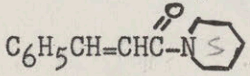
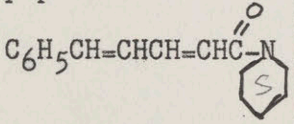
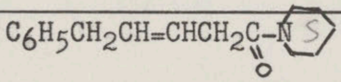
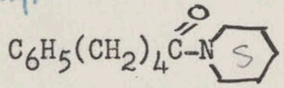
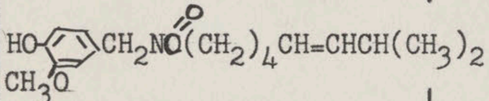
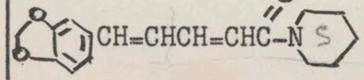
TABLE I

	Empirical Formula	Analytical Data Calculated	Analytical Data Found	Physical Properties	Infrared Data
"Woody-raisin" off-liquid	$C_{15}H_{24}$	C, 88.16 H, 11.84 Mol. Wt. 204 Sodium fusion disclosed no other elements.	C, 88.19 H, 12.01 Mol. Wt. 217	$n_D^{24^\circ}$ 1.4964 b.p. 79-80° (1 mm)	Terminal isopropyl group Exocyclic methylene group General terpene absorption
γ- Caryophyllene 	$C_{15}H_{24}$	C, 88.16 H, 11.84 Mol. Wt. 204		$n_D^{19^\circ}$ 1.4966; b.p. 125-125.5° (14.5 mm); d^{15° 0.8995; $[\alpha]_D$ -26.17° from oil of cloves	
α- Cedrene (Structure not proved)	$C_{15}H_{24}$	C, 88.16 H, 11.84 Mol. Wt. 204		$n_D^{20^\circ}$ 1.4982; b.p. 100° (3.5 mm); $d_{4^\circ}^{20^\circ}$ 0.9342; $[\alpha]_D$ -91.3° from oil of cedar wood	
α- Phellandrene 	$C_{10}H_{16}$	C, 88.16 H, 11.84 Mol. Wt. 136		$n_D^{20^\circ}$ 1.4777; b.p. 66-68° (16 mm) $d_{4^\circ}^{25^\circ}$ 0.8463; $[\alpha]_D$ +45° from oil of bitter fennel, ginger grass oil, aniseed oil	
Dipentene (limonene) 	$C_{10}H_{16}$	C, 88.16 H, 11.84 Mol. Wt. 136		$n_D^{20^\circ}$ 1.4730; b.p. 176-178° $d_{4^\circ}^{20^\circ}$ 0.840	

Long's 20% piperine
Stang's 46% "

Mineral Oil as disclosing medium { Aged mix gave 'off' at room temp
Bad cleansing OK till heated H₂O

TABLE II

	Empirical Formula	Analytical Data Calculated	 Found	Physical Properties	Infrared Data	Ultraviolet Data λmμ	 ε
"Bite" compound from Long's dispersion	$C_{14}H_{17}NO$	C, 78.10 H, 7.96 N, 6.51 Mol. Wt. 215 Sodium fusion disclosed no other elements.	C, 78.18 H, 8.19 N, 6.56 Mol. Wt. 242	m.p. 113-114°C	Aromatic ring cis $R_1CH=CHR_2$ no - NH no - OH no - C=O	220 282 ~~378~~	20500 33800 ~~34500~~
I. Cinnamoyl piperidide 	$C_{14}H_{17}NO$	C, 78.10 H, 7.96 N, 6.51 Mol. Wt. 215		m.p. 122°			
II. β-Cinnamenyl acryloyl piperidide ✓ 	$C_{16}H_{19}NO$	C, 79.63 H, 7.94 N, 5.80 Mol. Wt. 241		m.p. 92°			
III. 	$C_{16}H_{21}NO$	C, 78.96 H, 8.70 N, 5.76 Mol. Wt. 243		are 3 isomers - all are liquids			
IV. n-Valeroyl piperidide 5-Phenyl- 	$C_{16}H_{23}NO$	C, 78.32 H, 9.45 N, 5.71 Mol. Wt. 245		liquid			
V. Capsaicine 	$C_{18}H_{27}NO_3$	C, 70.78 H, 8.91 N, 4.59 Mol. Wt. 305		m.p. 65°			
VI. Piperine 	$C_{17}H_{19}NO_3$	C, 71.55 H, 6.11 N, 4.91 Mol. Wt. 285		m.p. 130°			

Chavicine is stereoisomer of this

BIBLIOGRAPHY

1. T. Hasselstrom, Activities Report, The Research and Development Associates, Food and Container Institute, Inc., Vol. 5, No. 2, July 1953.

2. E. Greunther, Am. Perfumer, 44, No. 8, 39-40, 43 (1942).

3.a. H. Staudinger and H. Schneider, Ber., 56, 705 (1923); b. H. Staudinger and F. Müller, Ber., 56, 712 (1923).

4.a. T. Hasselstrom, Science 112, 626 (1950); b. Am. Perfumer Essent. Oil Rev., 60, 430 (1952); c. with H. W. Coles and N. E. Kennedy, Science, 116, 204 (1953).

5. B. Herstein, Ber., 22, 2265 (1899).

6. N. H. Cromwell, J. A. Coughlan, J. Am. Chem. Soc., 67, 903 (1945).

MEMORANDUM

From: Emily L. Wick   Case No.: C-59074   March 16, 1954

To: Subject: The Determination of the Source of Off-Flavor in Miracle Whip Salad Dressing

The determination of the source of the off-flavor which has appeared in Miracle Whip salad dressing has been carried out by the examination of raw materials from which the product is made, the study of especially prepared samples of salad dressing, and by a brief chromatographic investigation of "good" and "suspected" #222 spice mixtures and of "good" and "suspected" oleoresins of black pepper. The work carried out is described in this report under the following headings:

I. Screening of Raw Materials of Miracle Whip Salad Dressing and Examination of Control Samples.

II. Chromatographic Investigation of "Good" and "Suspected" Oleoresins of Black Pepper and #222 Spice Mixtures.

I. Screening of Raw Materials of Miracle Whip Salad Dressing and Examination of Control Samples.

Initial work consisted of flavor panel evaluation of samples of regular Miracle Whip prepared from "good" ingredients and of samples prepared from "suspected" ingredients. It was found that the offending characteristic "woody-fruity-dried raisins" off-note was easily recognized. This note will be referred to as the "woody-raisin" note. Further orientation in the flavor of Miracle Whip was obtained by the study of samples which contained in the one case no pepper (202-7), and in the other case no mustard flour (126-3). In each instance, the clean blended flavor of Miracle Whip salad dressing was destroyed. The resulting sour unblended flavor was different and easily distinguishable from the off-note observed in samples prepared from suspected ingredients.

Having become familiar with the "woody-raisin" off-flavor found in samples prepared from suspected ingredients, and having learned to differentiate it from the off-flavor resulting from the absence of certain spices in salad dressing, the raw materials used in Miracle Whip were screened systematically. With the exception of the spices, all ingredients were tasted *per se*. Questionable ingredients were then tested in salad dressing containing no spices. None of the materials thus screened were found to be unacceptable. Examination of the starches (203-5, SH; 203-6, AO; 203-7, K-35) indicated that the jars in which they were shipped may at one time have contained "suspected" pepper seasoning since their aroma was very like that of this spice.

Screening of the spices was carried out by tasting them at four times the level used in regular Miracle Whip in salad dressing which contained no spices (202-6). All the spices with the exception of Long's straight peppercream "suspected" (203-19) and the "bad" #222 spice mix (203-21) prepared from this pepper were found to be completely acceptable. The addition of "suspected" pepper (203-19) to the taste medium caused a sharp sour flavor which was not outstanding and was definitely not similar to the "woody-raisin" off-note under consideration. The addition of mustard to this mixture similarly did not produce the off-flavor. An ADL #222 spice mix prepared from "suspected" pepper (203-19) and mustard flour (203-8) was tested in the medium. In this case, the normal flavor and aroma of Miracle Whip was lacking, but the "woody-raisin" note did not develop. However, only the Kraft sample (now aged) of "bad" #222 spice mix prepared from "suspected" pepper, was capable of producing the full "woody-raisin" off-flavor which is the subject of this investigation.

Similar screening of the pepper oleoresins and of the #222 spice mixes was carried out in mineral oil. The aroma of good #222 spice mix in this medium was that of black pepper. The bad #222 spice mix produced the characteristic "woody-raisin" note. Suspected pepper (203-19) alone or with the addition of fresh mustard did not produce the off-aroma. Good pepper (203-18) alone or with the addition of fresh mustard produced the black pepper aroma. Only the aged bad #222 spice mix produced the off-note under consideration.

On the basis of these observations, it must be concluded that the off-flavor noted in Miracle Whip is caused by compounding and mixing the suspected pepper (203-19) with mustard and salt and not from pepper alone, and that a time factor appears to be important in its production since only the Kraft prepared #222 spice mix causes its appearance in oil or in salad dressing.

A more detailed description of the screening of the raw materials and of the control samples is given in outline form in the supplement to this report.

II. Chromatographic Investigation of "Good" and "Suspected" Oleoresins of Black Pepper and "Good" and "Suspected" #222 Spice Mixtures.

In an attempt to determine and define the differences between the "good" and "suspected" #222 spice mixes and thus clarify the cause of off-flavor in Miracle Whip salad dressing, a preliminary chromatographic study was undertaken. The "good" (203-20) and "bad" (203-21) spice mixes were adsorbed on columns of alumina (pH 9-10) and eluted in forty-two 5 ml fractions with skellysolve 'B', skellysolve-benzene (4:1, 3:2, 2:3, 1:4), benzene, benzene-ether (4:1, 3:2, 2:3, 1:4), ether, ether-ethanol (4:1, 3:2, 2:3, 1:4), ethanol, aqueous ethanol (80%, 60%, 40%, 20%) and water. As a control a comparable quantity of mustard flour (203-8) was chromatographed in similar fashion. General examination of the physical properties and odor of the fractions obtained after removal of solvents under reduced pressure showed that the two spice mixes were similar to each other and only slightly different from mustard flour. On the basis of physical properties and odor the spice mixes yielded only two fractions which differed noticeably from the mustard. These fractions were 19 and 20 and contained yellow solids which had pepper-like aromas and which, when tasted, had the strong pepper "bite" characteristic.

In a further effort to determine the difference between "good" and "bad" spices, chromatographic analysis of Long's "suspected" peppercream (203-19) and Stange's COSP "good" pepper seasoning (203-18) was carried out. The two oleoresins were removed from their salt bases by ether extraction and chromatographed according to the method described above. Separation by the columns appeared to be efficient and the two peppers yielded generally similar fractions with the following outstanding exceptions: The "good" pepper yielded in fraction 2 a yellow oily solid having the good aromatic black pepper character, while none of this substance was obtained from the "suspected" sample. The "suspected" pepper yielded in fractions 9 through 13, a white crystalline solid, m.p. 114-115°, which, when tasted in mineral oil, provided the pepper "bite" but had none of the aromatic character. This substance was not obtained from the "good" pepper.

On the basis of these preliminary examinations, it appears that chromatographic technique offers an efficient method for separating and demonstrating differences in the "good" and "suspected" oleoresins of black pepper. It is entirely possible that further examination of the "good" and "suspected" #222 spice mixtures by modified procedures will illustrate differences between them also. Results obtained indicate the possibility that the "suspect" pepper may be "bad" due to the absence of a requisite component rather than to the presence of a contaminant.

Whether the white crystalline material found in the "suspect" pepper may result from a chemical reaction of the yellow aromatic substance derived from "good" pepper, or whether the two oleoresins differ even more fundamentally might be answered by the chemical identification of the two unknown compounds. Since both compounds are obtainable in good yield it seems reasonable to believe that their identification can be accomplished and that tests for their quick identification may be developed.

The results of this introductory examination of the pepper oleoresins illustrate definite differences between them and indicate that investigation of these differences may be expected to be of value in the determination of the cause of the off-flavor found in Miracle Whip salad dressing.

Supplement: Screening of Raw Materials and Control Samples

The screening of the raw materials of Miracle Whip and the examination of control samples is summarized in the following outline. In each case, the code number, a brief description of the sample and its treatment, and the resulting comments on its flavor, are given.

I Comparison of various samples of regular Miracle Whips made at various times from good ingredients

1 Received December, 1953 - is excellent sample
126-1 Prepared January 26, 1954 - almost as good as sample 1
202-4 Prepared February 2, 1954 - is slightly sour
929-7 Prepared September 29, 1953 - has "Waldorf salad" note

Conclusion: Each sample differed though made from good ingredients. Samples 1 and 126-1 were selected as examples of standard Miracle Whip.

II Effect of using cottonseed oil versus soya oil in Miracle Whip

126-1 Contains soya oil
126-2 Contains cottonseed oil - is sweeter and more oily than 126-1. Cottonseed oil does not improve the flavor.
The oils (203-10, 203-22, 203-23) were tasted *per se* and no off-notes were evident.

Conclusion: Oils are not suspected as the source of the "woody-raisin" off-flavor.

III Effect of the absence of a single spice on the flavor of Miracle Whip

Samples are made from good ingredients
202-6 Contains no spices - is bland, sweet
202-7 Contains no pepper - blend broken, strong vinegar
126-3 Contains no mustard flour - blend broken, strong vinegar

Conclusion: All spices must be present for a successful blended flavor.

IV Effect of the addition of spices (at four times the level used in regular Miracle Whip) to 202-6 (contains no spices)

(1) 202-6 + mustard flour - has clean strong flavor, little aroma
(2) 202-6 + paprika - clean, waxiness is cut
(3) 202-6 + wet PL (203-9) - fresh, clean, sweet spices
(4) 202-6 + good pepper (203-18) - fresh, clean
(5) 202-6 + suspected pepper (203-19) - sour, "woody-raisin" note is not present
(6) 202-6 + good spice mix (203-20) - clean, good

(7) 202-6 + suspected spice mix (203-21) - "woody-raisin" note is very evident
(8) 202-6 + good pepper + mustard - clean, good
(9) 202-6 + suspected pepper + mustard - "woody-raisin" note is not present though flavor is not that of Miracle Whip
(10) 202-6 + ADL prepared good spice mix - clean, good
(11) 202-6 + ADL prepared bad spice mix - "woody-raisin" note absent though flavor is not that of Miracle Whip

V Effect of vinegar versus spices on the flavor of Miracle Whip

929-6 Bad vinegar + good spices
929-8 Bad vinegar + bad spices
929-9 Good vinegar + bad spices

Conclusion: Sample 929-8 is worse than 929-9 and both 929-8 and 929-9 are much worse than 929-6. Thus bad spices have a more devastating effect on the flavor than bad vinegar alone. The combination is very bad.

VI Screening of vinegars

All the vinegar samples were screened by tasting *per se* and appeared to be acceptable. Sample 201-34 Cider 425-7, which was "originally suspected to be bad", was tasted in salad dressing containing no spices. No evidence of the "woody-raisin" off-flavor was noted.

Emily L. Wick

Crystalline Solid   m.p. 113-114°

Analysis

Found		Calcd for $C_{14}H_{17}NO$		Calcd for $C_{15}H_{19}NO$	
C =	78.18	C =	78.10	C =	78.56
H =	8.19	H =	7.96	H =	8.35
N =	6.56	N =	6.51	N =	6.11
Mol. wt	242 Rast method in camphor	Mol. wt.	215.3	Mol. wt.	229.3

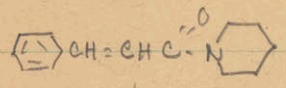
Infrared: Hainer says
no C=O
is aromatic
cis $R_1CH=CHR_2$
no OH
no NH

Ultraviolet

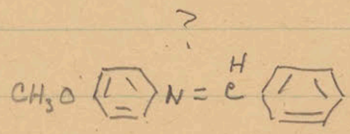
λmax	282	ε	33800
λmax	378	ε	34500
?	(220)	ε	(20500)

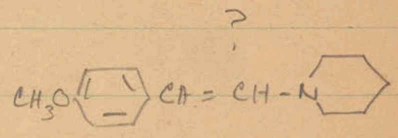
Indicates conjugated system
+ aromatic ring

Possible structures:


I
$C_{14}H_{17}NO$ m.p. 122°
?
Does not fit IR

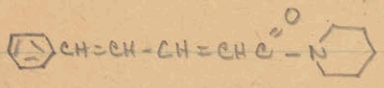
piperine
cis tr
cis vs trans

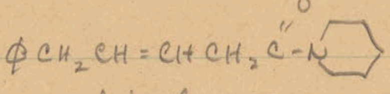

$C_{14}H_{13}NO$

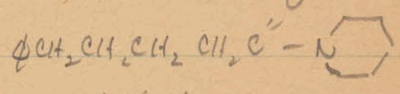

$C_{14}H_{19}NO$

capsicum like cpds.

Known pepper like cpds

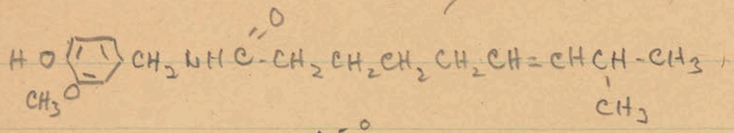

II
Staudinger - Hasselstrom
m.p 92°
$C_{16}H_{19}NO$


III
liquid
$C_{16}H_{21}NO$
Staudinger


IV
liquid
$C_{16}H_{23}NO$
Staudinger

Known pepper like cpds. cont'd

V



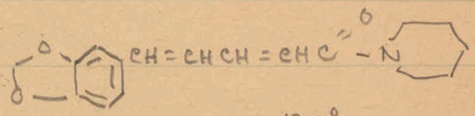
m.p. 65°

$C_{18}H_{27}NO_3$

C, 70.78; H, 8.91; N, 4.59

capsaicine

VI



m.p. 130°

$C_{17}H_{19}NO_3$

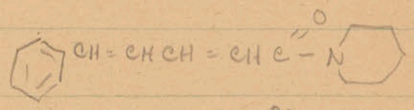
C, 71.55; H, 6.11; N, 4.91

piperine

Various pyrrolidides & pipecolides described by Hasselstrom — none of which fit the facts for the isolated solid.

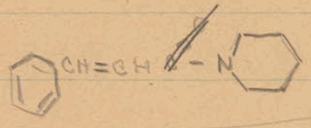
.0580
241.32) 14.01 00
120660
194400
193056
134400

$C_{16}H_{19}NO$		Calc.
C_{16} =	192.16	79.63 % C
H_{19} =	19.15	7.94 % H
N =	14.01	5.80 —
O =	16.	
	241.32	


m.p. 92
Staudinger

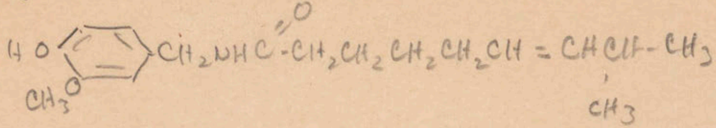
$C_{18}H_{21}NO$		
C_{18} =	216.18	80. — %
H_{21} =	21.17	
N =	14.01	
O =	16.	
	267.36	

$C_{14}H_{17}NO$		Calcd.	Found	IR	U.V.
C_{14} =	168.14	78.10 %	C = 78.18	no C=O	λ_{max} 282
H_{17} =	17.14	7.96 %	H = 8.19	is aromatic	ϵ_{max} 33800
N =	14.01	6.51 %	N = 6.56	cis RCH=CHR₂	log ϵ 4.529
O =	16.00			no OH no NH	
	215.29	215.29	M.Wt 242		λ_{max} 378
					ϵ 34500
					log ϵ 4.538
					λ_{max} (220)
					ϵ 20500
					log ϵ 4.312



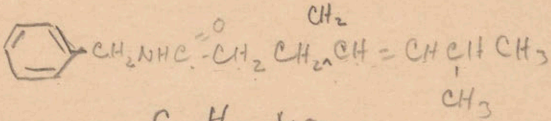
$C_{15}H_{18}NO$		
C_{15} =	180.15	78.9 — %
H_{18} =	18.14	7.0 — %
N =	14.01	
O =	16.00	
	228.30	

capsaicine



$C_{18}H_{27}NO_3$

$\boxed{C_{17}H_{25}NO \quad M.W.\ 257}$



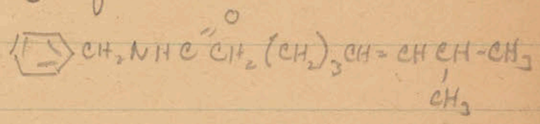
$C_{16}H_{23}NO$

Piperine det'n ?

$C_{17}H_{25}NO$

$C_{17}H_{25}NO$ Calcd.

Analog of capsaicin



C_{17} = 204.17 78.71%

H_{25} = 25.20 9.–

N = 14.01

O = 16.

259.38

$C_{16}H_{23}NO$ Calcd

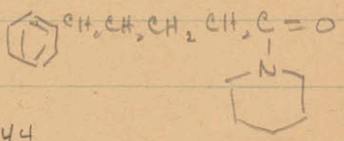
C_{16} = 192.16 78.32%

H_{23} = 23.18 9.45

N = 14.01 5.71

O = 16.

245.35



```
         .0944
245.35 | 23.18,00
         22081 5
           109850
            98140
            117100
             98140
             189600
```

```
          .0571
245.35 | 14.01,00
         12 2675
           174250
           171745
             25050
```

$C_{15}H_{21}NO$ Calcd. Found

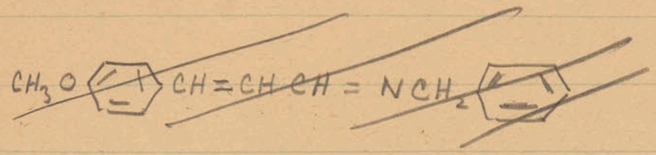
C_{15} = 180.15 77.87 78.18

H_{21} = 21.17 9.–

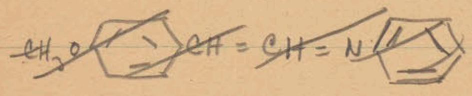
N = 14.01

O = 16.00

231.33



$C_{15}H_{19}NO$		Calcd.	Fd
C_{15}	180.15	78.56	78.18
H_{19}	19.15	8.35	8.19
	14.01	6.11	6.56
	16.00		
	229.31		



$C_{16}H_{21}NO$

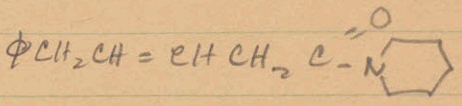
C_{16} 192.16 C - 78.9

H_{21} 21.17

14.01

16.

243.34

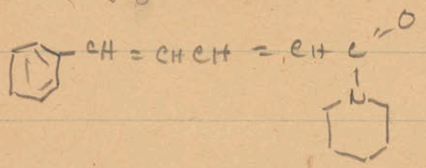


.7896
243.34 | 192.16,0
170338
218220
194672
235480
219006
164740

.0869
243.34 | 21.17,00
194672
170280
146004
242760
219006
237540

.05757
243.34 | 14.01,00
121670
184300
170338
139620
121670
17950

cinnamenyl acrylyl
piperidide



$C_{16}H_{19}NO$

$C_nH_{2n-13}NO$

Beil XXI	×	328
XXI ^{1}	×	
XXI ^{2}	×	

Staudinger - Ber 56B 699 (1923
says m.p. 92°
very sharp.

C.Z: 1922-24 Staudinger ref.
23 I 1089, 1091
23 II 486

1924-29 - 26 II

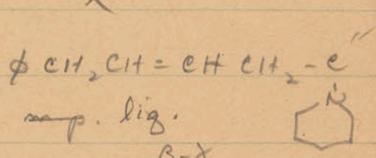
30-34	×
36	×
37	×
38	×
39	×
C.A. 47	×
48	×
49	×
50 -	~~2521b~~
51	×
52	×

Piperidine

$C_{16}H_{21}NO$

$C_nH_{2n-11}NO$

XXI	321	×
		×
^{2}		×

Staudinger: 

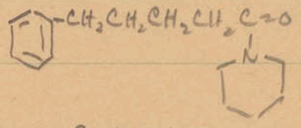
b.p ~~map.~~ liq.
α-β, β-γ + γ-δ - are liquids. β-γ
sharp pepper taste

C.Z. 1922-24 36I 3580
25-29 ↗

30-34	×
36	×
37	×
38	×
39	×

F.I. 17 2582 ^{2}

C.A. 47	×
48	×
49	×
50	×
51	×
52	×



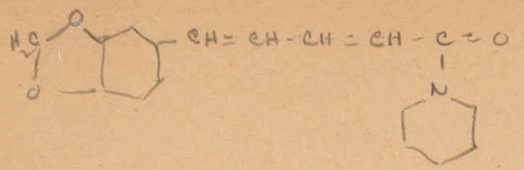
$C_{16}H_{23}NO$

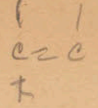
$C_nH_{2n-9}NO$

XXI	×	299
	×	
	×	

Staudinger - liquid
C.Z. Same as above
1922-24

25-29	×
30-34	×
36	II - 628 (b.p 205-8 (10mm)
37	×
38	×
39	×
C.A. 47	×
48	×
49	×
50	~~[illegible]~~
51	×
52	×

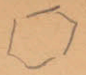




C = O

OH

NH



C = 78.18 / 12 = 6.52

H = 8.19 / 1. = 8.19 ✓

N = 6.56 / 14 = .47 ✓

O = 7.07 / 16 = .44 ✓

12 | 78.18 = 6.51
72
61
60
18
12
60

14 | 6.56 = .47
56
96
84
120

16 | 7.07 = .44
64
67

8.19
78.18
6.56
92.93

$C_{14}H_{18}NO$

$C_{16}H_{20}NO$

.5 | 6.52 = 13.0

.5 | 8.19 = 16.3
~~45~~
69

216

100
92.93
7.07

1.01 | 8.19 = 8.1
808
110
101
9

12
14
48
12
168
18
14
16
216

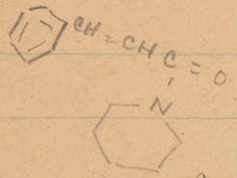
12
13
36
12
156
16
14
16
202

242
216
26

1.008
16
6048
1008
16.128

136
16
152

19

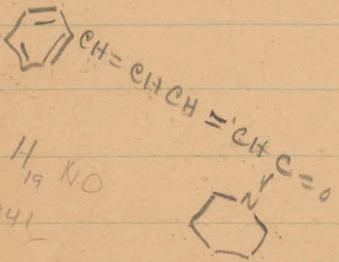


$C_{14}H_{17}NO$

12
16
72
12
192
19
14
16
241

.45 | 8.19 = 18.2
45
369
360
90

.45 | 6.52 = 14.5
45
202
180
220
~~180~~
400



$C_{16}H_{19}NO$

241

192
12
204

$C_nH_{2n-13}NO$

Peppermint

1937-46 ~~adsorption by SiO$_2$ gel~~

~~37 8998^{4}~~

40 4581^{4} ✓

Color reaction c̄ 2 furaldehyde

33 1356^{7}

on pepper. oil 36 6309^{4} ✓

Am Perf. 48 No 7, 60, 62-3 (1946)

rev. on 5879^{6} 40 ✓

prepn of 31 6816^{8}.

pharmacol actn of 32 8063^{9}

Am Perf. 44 No 8 39-40, 43 (1942)

E. Guenther - most dangerous adulterants are phellandrene, dipentene, limonene, caryophyllene most of which occur naturally in the oil.

1953 – 8746a – irritants related to piperine – c̄ Coles H.W & Kennedy N.E.

1951 4249f – pungency taste characteristics of piperazine derivs,

---

1950 – 10685f. – J.C.S. $\frac{1950}{1177\text{-}80}$.

1949 x

1948 – 8564c ✓

✓3504b

✓Subl of. 2729b. – sublimes at 104° (0.02 mm Hg.)

1947. x

Pyperine –

1952 ×

1951 2133i ✓

xxn of. ~~6451d~~

~~9966d, 9967d~~

Plead, – detn & detn of 3994i ✓

J. Pharm. Assoc.

in oils of pepper & cubebs

5881d ✓

spectrum of 9806c

J.A.C.S. 73 305-8 (1951)

L. Marion, N. Jones et al

C.A. 25 4972 - Zach

20 3516 Griebel

Aerstäd method

unangenehm

fest scharf

I - XXVII -

$C_{14}H_{17}NO$

<u>9</u> 640

→ <u>20</u> 48(16)

Beil. N- Cinnamoyl-piperidin

<u>XX</u> 48

✓ Herstein. Ber <u>22</u> 2265 (1899)

✓ Vorlander, Herrmann, A. 3<u>20</u> 91

Needles from alcohol m.p. 122°

insol cold alkali, sol. in HCl

adds Br_2

<u>XX</u>' Pfeiffer. A. <u>383</u>, 149 - 119-122°

✓ <u>XX</u>'' Staudinger, Schneider B <u>56</u> 705 (1923)

m.p. 127° Muller <u>56</u> 712 (1923)

1929

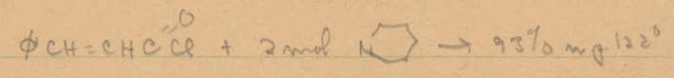
C.A. Form. Index - 1946

~~Acrylophenone, B-1-piperidyl <u>24</u> 4508~~ ¹

Piperidine, 1- cinnamoyl 3<u>9</u> 3272⁶

✓ N.H. Cromwell, J.A. Caughlan,

JACS. 6<u>7</u> 903-5 (1945)



93% m.p. 122°

C.A.	47	x	C.Z. 30-34	x
	48	x	35	x
	49	x	36	x
	50	x	37	x
	51	x	38	x
	52	x	39	x

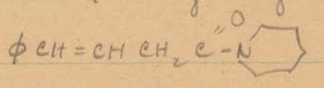
$C_{15}H_{19}NO$

<u>9</u> 640

~~<u>21</u> 321~~ ←

<u>Beil</u>

δ-φ-vinylessigsäure-piperidid



m.p. 64-65

Staudinger, Muller B 64-65

Beil <u>XX</u>'' has good list of all piperidides of acids -

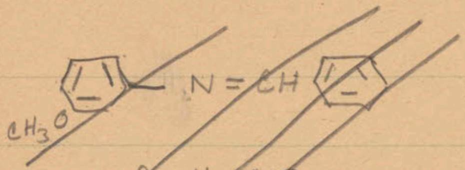
C.A. Form. Ind - 1946

<u>27</u> 2941². Piperidine, ✓

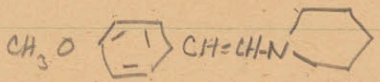
1-[3-(p-CH_3O - φ)-2-propynyl

Piperidine, 1-γ-φ crotonyl <u>17</u> 2582⁵

47	X	C.Z. 30-34 X
48	X	35 X
49	X	36 X
50		37 X
51	X	38 X
52	X	39 X

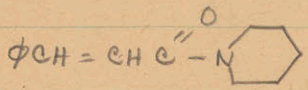


~~$C_{15}H_{15}NO$~~



$C_{14}H_{19}NO$

Search $C_{14}H_{17}NO$

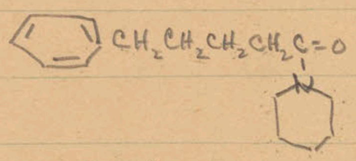


Hexahydrogenated methyl-pyridines are pipecolines (α, β, γ)

Piperic acid α-trans γ trans
iso " " α-cis γ trans
chavicinic " α-cis γ cis
iso " " α-trans γ cis

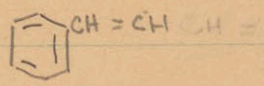
Piperine is a piperidide of piperic acid

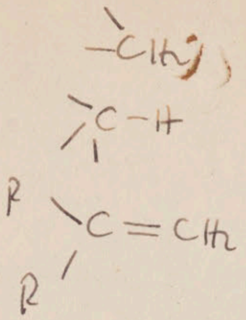
W.W. I & II
5-phenyl-n-valeroyl piperidide

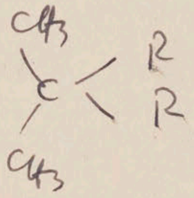


$C_{16}H_{23}NO$

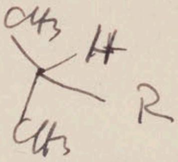
Germans W.W II
β-cinnamenyl acryloyl piperidide



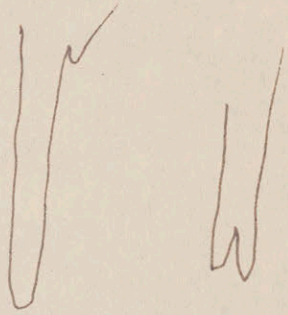


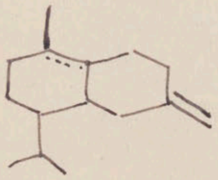


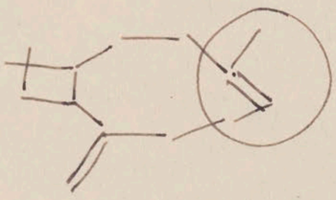
or



gem di Me







840

Optical Rotation - see physical properties -

Clovene

For caryophyllenes - industrial distillations

Physical properties

✓ Ruzicka et al. Helv. Chim. Acta 24 (1941) 1219

Fortsch und Untersuchung

Simonsen has phys properties of isomers.

What about IR ?

Absorption Spect : caryophyllenes -

✓ Naves, Perrottet, Helv. Chim Acta 24, 789 (1941)

✓ Goodway, West, J Chem Soc. 1939 1855 U.V. max 264-265

εmax 142 (alcohol)

Look up cedrene -

✓ JACS 75 3291 (1953) - G. Stork, Breslow - refer to Simonson 45/

Chem Products 17 14-18 ; 103-6 (1954) HRE P 660.52 C51p

16 379-82 ; 413-17 ; 467-71 (1953)

Chemical Products & the Chemical News, Science Services, Ltd,

Temple Chambers, Temple Ave. London EC4, Eng.

U.V. max humulene 215.5 log ε 3.69 (alc.)

J. Chem. Soc. 1954 4345, ✓2669, 2673

good IR → 1952 665

1953 184 ✓ ~~get this~~

General Terpene IR - Barnard, Harding et al JCS 1950 915

Spring & Stork J. Chem Soc 1950 1177-80.