

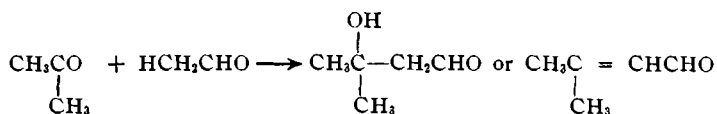
STUDIES IN THE GENUS MENTHA.*

12. THE SIGNIFICANCE OF THE PRESENCE OF METHYL-2-BUTANOL-4 IN MENTHA PIPERITA L. AND ITS IDENTIFICATION.**†

BY SAMUEL M. GORDON.^{1,2}

The peculiar structure of certain plant constituents with five carbon atoms, also the more striking configuration of numerous phytochemical compounds, both chain and cyclic, with ten carbon atoms, have years ago suggested an hypothesis for their biogenesis.

Given acetaldehyde and acetone, both of which have been found widely distributed in plants, their condensation to iso-compounds with five carbon atoms readily suggests itself.

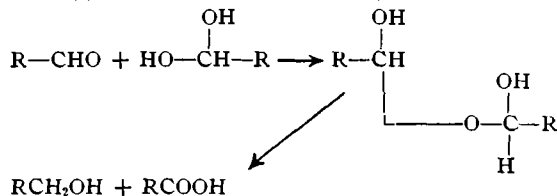


Reactions of this type are assumed in the explanation of numerous biochemical processes. Either of these condensation products, whether resulting from aldol or true condensation, may be expected to yield an isovaleric aldehyde upon reduction. This, in turn, upon auto-oxidation and auto-reduction will yield the corresponding primary isoamyl alcohol on the one hand and the corresponding isovaleric acid on the other.³

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† Since this report was completed, Neuberg and Windisch (*Biochem. Z.*, 166, 478 (1926)), have shown that isovaleric aldehyde undergoes auto-oxidation and reduction by means of *B. ascendens* in the presence of CaCO_3 and salt solution. It is interesting to note that the authors have found auto-oxidation and reduction of acetaldehyde to ethyl alcohol and acetic acid to take place both in the absence and presence of atmospheric oxygen. Thus KCN, when added to the reaction mixtures did not hinder the course of the reaction. This behavior is in agreement with the newer views on the Cannizzaro reaction recently advanced by Meerwin and Schmidt (*Ann.*, 444, 229 (1925)). It is supposed that the intermediate product is of a half-acetal nature, thus

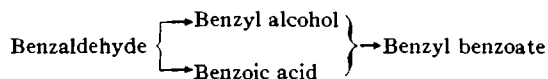


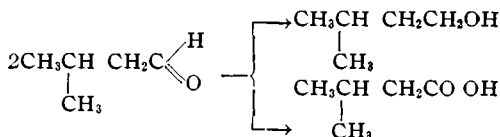
The further bearing of this reaction upon the formation of alcohols and acids in connection with the biochemistry of the Mints is now being studied in this laboratory.

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² Wrigley Fellow, 1924-1926.

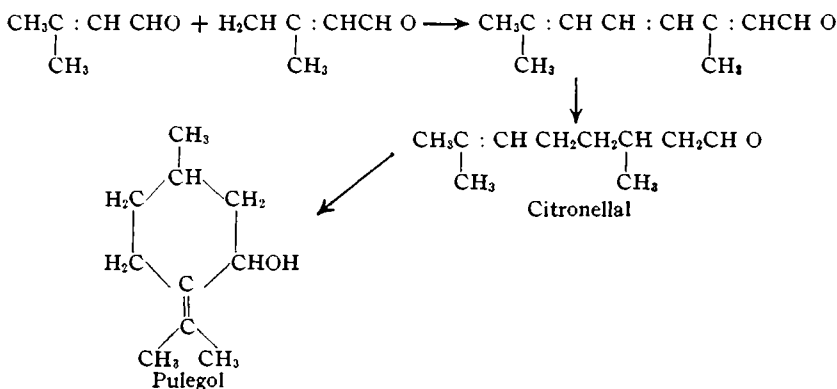
³ Other illustrations observed in plant biochemistry involving the simultaneous oxidation and reduction of an aldehyde are:





These supplementary reactions are assumed in not a few instances as the most rational explanation of the formation of certain substances, existing side by side, or in the form of esters resulting upon condensation of alcohol and acid.

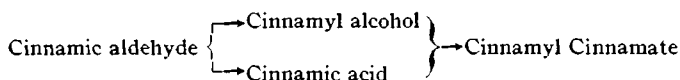
The further condensation of two molecules of the pentenoic aldehyde in question, of the partial reduction of the aldehyde with ten carbon atoms resulting, also the cyclization of the reduced aldehyde to a compound of the striking configuration of many volatile constituents of plants, may receive mention here merely as further indication of the biochemical importance of the reactions indicated above.



Without pointing out how the reactions, merely indicated here, can be utilized in the rational explanation of numerous biochemical phenomena, the few data given may suffice to suggest the importance of any and all experimental facts contributory to a verification of the biochemical hypothesis involved.

Peppermint oil has been known to contain isovaleric aldehyde, isovaleric acid and presumably amylvalerate (1). The presence of an amyl alcohol in the plant, therefore, seemed well nigh a foregone conclusion. However, definite proof of this assumption has hitherto been wanting. This proof has been made possible by the cooperation of A. M. Todd and Co., who in 1922 contributed about 150 pounds of a "Vorlauf" from not less than 12,000 pounds of peppermint oil to the study of the "Biogenesis of the Mints" by the Wisconsin Pharmaceutical Experiment Station. But even with this large amount of material, the amyl alcohol fraction amounted to not many cubic centimeters.

The real significance of this bit of chemical research into the life processes of the peppermint plant, consisted, not in proving the presence of any one of the



The reaction appears to be of widespread importance in the plant kingdom.

eight isomeric amyl alcohols, but in demonstrating the identity of the alcohol in question with the one having the peculiar structure used in the above formulas expressive of the biochemical synthesis hypothetically assumed. Inasmuch as the several isomeric amyl alcohols have all been known for some time, and since the presence of the alcohol has repeatedly been demonstrated in plants, the task would seem very easy provided a reasonable amount of material were available. Yet a merely casual perusal of the literature, both structural and phytochemical, will reveal that the task of definite identification is by no means as easy as might be assumed. Indeed, the satisfactory accomplishment of this apparently simple task seemed to call for a reëxamination of the entire group of amyl alcohols and their derivatives. Having indicated the fundamental biochemical significance of the several related groups, the statement that our knowledge of these carbon compounds with five carbon atoms is in need of revision may not seem exaggerated. Such work as has been done in this direction will be reported in another place.

In 1893 Halsey (2) suspected the presence of an amyl alcohol in peppermint oil, suggested by the peculiar odor of the fraction boiling below 140°. In the following year, Power and Kleber (3) likewise observed the odor of "amyl alcohol" in fraction 100° to 155°, also that it reacted with metallic sodium. In 1919, E. R. Miller (4) suspected the presence of an amyl alcohol in the oil cohobated from the aqueous distillate. All of these observations were made in connection with American oil.

So far as French oil is concerned, Roure-Bertrand Fils (5) in 1909 supply somewhat better evidence of the presence of a primary amyl alcohol in fraction 131-132°, since upon oxidation with chromic acid mixture it gave the characteristic odor of valeric aldehyde. The boiling point of the alcohol favors the assumption that the alcohol in question was $\text{CH}_3\text{CHCH}_2\text{CH}_2\text{OH}$ without, however, being positive proof.



The 100 pounds or more of "Vorlauf" from A. M. Todd and Company having been deprived of their aldehydes (6), the aldehyde-free portion was redistilled with steam into eleven large fractions. The first of these, in quantities of about 500 cc., was subjected to ordinary fractionation rather rapidly in order to prevent resinification as much as possible. All fractions collected below 140° and those collected between 140° to 150° were bulked and subjected to a more careful fractionation with the following results:

TABLE I.

Fraction.	Boiling point.	Volume.
1	88-90°	71.0 cc.
2	90-93	154.0
3	93-95	104.0
4	95-96	7.0
5	96-100	0.0
6	100-110	0.0
7	110-120	11.0
8	120-130	83.5
9	130-140	74.5
10	140-150	107.5

Since the amyl alcohol sought for boils at 131.0°, the ester values of Fractions 8, 9 and 10 were determined before and after acetylation.

Fraction.	Saponification values. Before acetylation.	After acetylation.
120-130°	46.5	240.5
130-140	45.4	218.4
140-150	15.1	33.8

These figures reveal the presence of considerable free alcohol (an average of 36% computed as $C_5H_{11}OH$) in Fractions 8 and 9, whereas the last fraction contains but little, approximating the terpene fractions as to boiling point.

Inasmuch as Fractions 8 and 9 contained small amounts of ester (10.6% computed as amyl acetate) they were saponified with twice the calculated amount of KOH, without the use of solvent. The oil darkened as the reaction took place, becoming black after two hours. The saponified liquid was subjected to steam distillation. The aqueous distillate¹ was extracted with ether and the ether residue (1.0 cc.) added to the oily distillate. Two fractionations of this oil gave the following results:

Fraction.	Boiling point.	Volume.	Index refraction.	Density.
1	80-128°	13.5 cc.
2	128-129	9.1	1.4230	0.8253
3	129-130	7.0	1.4241	0.8254
4	130-131	3.7	1.4261	0.8258
5	131-132	3.4	1.4280	0.8244
6	132-133	1.9	1.4300	0.8272

Comparison of the constants here recorded with those recorded in the literature for the isomeric amyl alcohols did not afford much satisfaction. Several derivatives from the different fractions were prepared.

AMYL PHENYLURETHANE.

Attempts to react with phenylisocyanate at first gave liquid products only. Finally very cold weather was productive of better results in the crystallization of the products. The 3.7 cc. of fraction 130-131° when treated with 5 Gm. of phenyl isocyanate yielded but 1.4 Gm. of crystals as contrasted with a theoretical yield of 8.7 Gm. These melted at 40°. Crystallization from petroleum ether raised the melting point to 41° and several subsequent crystallizations from dilute alcohol to 43°.

Of the eight isomeric alcohols, the phenol urethanes of five have been reported:

<i>n</i> -butyl carbinol ²	46°
diethyl carbinol ³	48-49
isobutyl carbinol ⁴	55
methyl ethyl carbinol ⁴	30
dimethyl ethyl carbinol ⁵	42

These figures, likewise, do not afford much satisfaction for the melting point agrees best with that of the tertiary dimethyl ethyl carbinol, and is 12 degrees lower than the melting point of the urethane of isobutyl carbinol.

¹ "Isoamyl" alcohol is soluble in water 1:200.

² E. Blaise and L. Picard, *Ann. chim. phys.*, Ser. 8, 25, 261 (1912).

³ C. Mannich, *Arch. für Pharm.*, 246, 182 (1904).

⁴ W. Marckwald, *Ber.*, 37, 1049 (1904).

⁵ E. Lambling, *Bull. soc. chim.*, Ser. 3, 19, 777 (1898).

3,5-DINITRO-BENZOATE.

Three and one-tenth Gm. of fraction 129–130° and 7.8 Gm. of 3,5-dinitro-benzoyl chloride were heated in a tube placed in a boiling water-bath for thirty minutes, and the reaction product poured in water. The crystals obtained upon standing and filtering, when dried, weighed 7.0 Gm. against 9.8 Gm., the theoretical yield. Recrystallized several times from dilute alcohol, the product melted at 55–56°. No change in the melting point was observed after several more crystallizations from ligroin. The ester prepared from a cabinet specimen of "isoamyl alcohol" melted at 61° after several crystallizations from alcohol.

Nitrogen determinations of both esters were made.

a.—0.1500 Gm. of ester from the peppermint oil yielded 12.1 cc. of N₂ at 24° and 741 mm. pressure.

b.—0.4000 Gm. of ester from the cabinet specimen yielded 36.2 cc. of N₂ at 21° and 746 mm. pressure.

Calculated for C₁₂H₁₄O₆N₂: 9.94%. Found: *a.*—9.94%. *b.*—10.14%.

Saponification of the ester from peppermint oil gave a value of 302, whereas the computed number is 282.

Those fractions of the "Vorlauf" which originally distilled below 140° were bulked and fractionated through a four bulbed column. This distillation caused a sharp break into two main portions, *viz.*, one set of fractions boiling between 88–96° and another boiling between 150–172°, with no intermediate fractions. The higher boiling fractions were reserved for later examination. The lower boiling portions gave positive tests for aldehydes with Schiff's reagent. Accordingly fractions boiling between 88–96° (total volume 227 cc.) were shaken for four days with 375 cc. of a saturated solution of NaHSO₃. No crystals separated. The aqueous layer was separated and treated as follows: extraction with ether and subsequent evaporation of the ether gave no residue. The liquor after the ether extraction was treated with NaHCO₃, steam distilled, and the distillate extracted with ether. No odor or residue of the amyl aldehydes was apparent.

The oily layer was treated with NaHCO₃, steam distilled, separated from water and dried over Na₂SO₄ for 24 hours and distilled with the following results:

TABLE II.

Fraction.	Boiling point.	Volume.
1	83–115°	48.2 cc.
2	115–125	27.0
3	125–135	lost
4	135–145	13.3
5	145–155	19.5
6	155–170	34.3

The odor of Fraction 2 suggested amyl alcohol, and esterification determinations gave the following results:

Saponification no. before acetylation	31.3 = 4.9% C ₆ H ₁₁ OH
Saponification no. after acetylation	226.0 = 51.38% C ₆ H ₁₁ OH. ¹

Meanwhile careful dephlegmation of the fractions boiling at 150–170° was made. This yielded 170 cc. of an oil boiling below 140° in which the odor of amyl alcohol was very apparent.

¹ The acetylated oil had a decided odor of amyl acetate.

A redistillation of this oil through a fifteen-inch Vigreux column yielded:

TABLE III.

Fraction.	Boiling point.	Volume.
1	83-120°	19.4 cc.
2	120-130	26.4
3	130-132	19.8 ¹
4	132-135	14.3
5	135-140	16.1
6	140-150	14.1
7	150-160	28.1
8	160+	12.7

¹ Odor of amyl alcohol very apparent.

Fraction 130-132° was dried over Na₂SO₄ and treated with 5 Gm. of α -naphthyl-isocyanate according to Neuberg and Kansky (7). After standing for twelve hours, 4.0 Gm. of a crystalline product were obtained. Crystallization from petroleum ether yielded a product melting at 61°. Repeated crystallization caused no change in the melting point.

Neuberg and Kansky state that the α -naphthyl-urethane of isobutyl carbinol (isoamyl alcohol) melts at 67-68°. Hence a urethane was prepared from a sample of isoamyl alcohol from Eastman Kodak Company and found to melt at 61°.

A mixture of the two melts at 60-61°, hence it may be concluded that isobutyl carbinol is present in American peppermint oil.

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- (3) F. B. Power and C. Kleber, *loc. cit.*
- (4) E. R. Miller, *Wis. Pharm. Expt. Station, Circular No. 7*, 8 (1919).
- (5) Anon, *Report Roure-Bertrand Fils*, p. 40 (April 1919).
- (6) R. E. Kremers, *Am. J. Pharm.*, 98, 85 (1926).
- (7) C. Neuberg and E. Neumann, *Biochem. Z.*, 20, 445 (1909).

THE BOTANICAL IDENTITY OF MA HUANG.

BY OLIVER ATKINS FARWELL.

In the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION for August 1926, Vol. XV, pages 625 to 639, Chen and Kao present a review of the work that has been done on *ephedrine* and *pseudoephedrine*; the former is obtained from Ma Huang which has been known in Chinese medicine for 5000 years and which is identified as *Ephedra vulgaris* Rich, var. *Helvetica* Hook. f. and Thompson and the latter is obtained from a European plant identified by Arthur Meyer as *Ephedra vulgaris* var. *Helvetica*. Also, they say: "It appears, therefore, probable that the plant *Ephedra vulgaris* var. *Helvetica* yields *ephedrine* when grown in China, but *pseudoephedrine* when grown in Europe. Such analogy can be found in Oil of Turpentine, for the French and Spanish oils contain 1- α -*pinene* whereas the American and Greek oils the dextrogyrate modification."