diluted, extracted with ether and the camphononic acid was crystallized by dissolving in benzene, evaporating in a test tube on the water bath till crystallization began and adding an equal volume of petroleum ether. The following comparisons with the camphononic acid from camphanamide (see above) establish its identity:

CAMPHONONIC ACID.

	From camphanamide by Lapworth's method.	Mixture.	From our hydroxy acid by oxidation.
Melting point	229°–230°	227°–228°	227–228°
$[\alpha]_D$ in benzene	+17.8°		+17.0°
(0.024 g. in 1 cc.)	at 27.5°		at 27.5°
$[\alpha]_D$ in alcohol	—3.9°		—3.9°
(0.02 g. in 1 cc.)	at 28°		at 29°

Conclusions.

1. The lactone obtained by Taveau by the decomposition of the nitroso derivative of the anhydride of aminolauronic acid with sodium hydroxide is identical with cis-camphononololactone obtained by Bredt by the reduction of camphononic acid and the corresponding hydroxy acids, camphonolic acid, are also identical.

2. Camphonolic acid has been oxidized to camphononic acid, establishing more completely that this acid contains the hydroxyl group in the same position as the secondary carboxyl of camphoric acid.

3. The amyl ether of camphonolic acid, $C_8H_{14}(OC_5H_{11})CO_2H$, and its copper and silver salts have been prepared. The free acid is a viscous liquid.

4. Cis-camphononololactone melts at 165–167°; $[\alpha]_D$ in alcohol (0.05 g. in 1 cc.) at 28° is —20.2° or at 26° (0.1 g. in 1 cc.) it is —22.3°.

Cis-camphonolic acid melts at 202–203° when rapidly heated; $[\alpha]_D$ in alcohol (0.1 g. in 1 cc.) at 28° is +29.2°.

Camphononic acid melts at 229–230°; $[\alpha]_D$ in benzene (0.024 g. in 1 cc.) at 27.5° is +17.8°; $[\alpha]$ in alcohol (0.02 g. in 1 cc.) at 28° is —3.9°.

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THE ROLE OF OXIDASES IN THE FORMATION OF CERTAIN CONSTITUENTS OF ESSENTIAL OILS.

[PART I.]

BY BENJAMIN T. BROOKS.

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The work described in the present paper was undertaken with the object of throwing some light on the manner in which ketones and aldehydes are formed in certain essential oils.

In the study of the essential oil of *Michelia champaca* L.[1] it was noted that an energetic oxidase was present in the flowers, and on investigating

[1] Brooks, THIS JOURNAL, 33, 1763 (1911).

the oil it was found that a considerable quantity of a crystallin ketone, $C_{18}H_{20}O_5$, separated from the oil and that, among other constituents, benzyl alcohol, benzaldehyde and benzoic acid were present. It is believed that these oxidation products owe their existence to the activity of the oxidizing enzyme present in the flowers. Thus, Batteli and Stern[1] and Jaquet[2] have shown that benzyl alcohol is readily oxidized by an oxidase prepared from certain animal tissues.

According to Lecomte[3] the vanilla plant contains an oxidase and the pods richest in vanilla showed the strongest oxidase reactions. He considers that the aldehyde is formed by the oxidation of coniferyl alcohol. Anisic alcohol and anisic aldehyde are present in very small proportions.

The work of the chemists of Roure-Bertrand Fils on oil of peppermint and that of Schimmel and Company on oil of caraway is particularly suggestive. The former chemists have shown[4] that menthone is formed in *Mentha piperita* chiefly when the plant blossoms and that it is apparently formed at the expense of menthol. Plants systematically deprived of their inflorescences, or affected by parasites so that the blossoms do not develop normally, contain only a very low per cent. of menthone, approximately 3 per cent. instead of 10 to 15 per cent., which is the proportion of the ketone contained in the oil distilled from normal healthy plants. Similar relations were found to hold in the case of absinth,[5] *Artemisia absynthum* L., and verbena, *Verbena triphylla* L.[6]

It was shown by the chemists of Schimmel and Company[7] that oil of caraway consists principally of limonene and carvone. Plants deprived of their inflorescences gave neither of these substances. Plants in flower yielded an oil containing limonene and a little carvone while in the oil distilled from mature plants carvone predominated.

The above considerations suggested a search for an oxidizing enzyme in peppermint, caraway and certain other plants which yield essential oils containing ketones or aldehydes as important constituents.

The method of procedure was practically the same for all the plants examined. The fresh plants were ground with clean quartz sand and a little water to a paste, the juice squeezed out through a cloth and then filtered through paper. The reagents used in testing for the oxidase were tincture of guaiac resin, the indophenol reagent of Röhmann and

[1] *Biochem. Z.*, 28, 145.

[2] *Arch. exper. Path. Pharm.*, 29, 386 (1892).

[3] *Compt. rend.*, 133, 745.

[4] *Bull. Roure-Bertrand Fils.*, [1] 1, 16 (1900); [1] 5, 22 (1902); [1] 8, 31 (1903). Also Charabot, *Compt. rend.*, 130, 518; *Bull. soc. chim.*, [3] 23.

[5] *Bull. Roure-Bertrand Fils*, [2] 3, 5 (1906).

[6] *Ibid.*, [2] 4, 9 (1906).

[7] Schimmel and Co., *Semi-annual Rep.*, 2, 24 (1896).

Spitzer, phenolphthalin and pyrogallol. Phenolphthalin often gave uncertain results, owing to the dark brown color of the extracts, which color was intensified by the addition of alkali, thus obscuring the pink or red color developed by the alkali and the phenolphthalein formed.

The Oxidase in Carum carvi L.—In the examination of caraway, the green seeds were used. The extract, made as described above, gave positive tests for an oxidase although the reactions were not as pronounced as in the case of the mints, probably owing to the comparatively dry, ripe condition of the seeds employed.

The Oxidase of Peppermint.—The oxidizing enzyme in *Mentha piperita* is localized chiefly in the inflorescence. This is strictly parallel with the results of Charabot on the distribution of menthone in the peppermint plant. The plants were examined for oxidases before and during the flowering period and in the latter case the leaves and inflorescences were tested separately. Before flowering, feeble but positive reactions were obtained. The flower stalks, on the other hand, gave beautiful positive results, the extracts being much more active than extracts of the leaves alone or of the whole plants before the flowering period. Colorimetric comparison of equivalent quantities of extract with equal quantities of reagent was the nearest approach to a quantitative method of several methods that were tried. Pyrogallol was not oxidized by peppermint extract so the quantitative method proposed by Chodat and Bach, depending upon weighing the purpurogallin formed, was not applicable.

In every case in which active solutions were obtained, the activity was lost after standing ten or twelve hours. This behavior is similar to that of the zymase of pressed yeast juice in that the loss in activity of the enzyme is accompanied by a proteolytic digestion of the soluble proteins in the extract. Thus a freshly prepared extract of green peppermint gave a heavy precipitate on heating to 100°, whereas a portion of the same extract, after standing eight hours with a little chloroform as an antiseptic, yielded only a slight turbidity on heating to the same temperature. No evidence was obtained of the presence of a cyano-genetic glucoside. The extract from 400 grams of peppermint flower stalks was allowed to stand 24 hours and then distilled with steam.. No trace of hydrocyanic acid was detected in the concentrated distillate by the ferric thiocyanate test.

The instability of the enzyme, in the form of its aqueous extract, rendered uncertain an attempt to oxidize menthol. Fifty cubic centimeters of extract were prepared from 100 grams of peppermint blossoms and to one-half of the extract 2 grams of menthol crystals were added. Both portions of the solution were then shaken in a shaking machine, the air in the apparatus being displaced by oxygen. The absorption of the oxygen was measured by means of gas burettes connected with the ap-

paratus. Both solutions absorbed practically the same amount of oxygen. During the first ten minutes, 8.3 cc. of oxygen were absorbed by each solution, 5.2 cc. during the second ten minutes and thereafter the rate of absorption diminished steadily until after three hours all absorption of gas ceased and the solutions were found to be inactive to the oxidase reagents. The added menthol was recovered by steam distillation and the presence of menthone could not be proven. The action of other oxidases on terpene alcohols will be tested in the continuation of the investigation.

Heating to 100° for a few moments completely destroyed the activity of all the extracts which showed positive reactions for an oxidase.

Precipitation of the enzyme with alcohol, or alcohol and ether gave precipitates which, when filtered off and shaken with water, still showed the oxidase reactions but so greatly diminished in intensity that further purification by this means was not attempted.

It is probable that all of the mints contain such an oxidase, since active extracts were prepared from the flower stalks of *Mentha piperita*, *Mentha viridis*, *Mentha crispa*, *Mentha sylvestris* and *Mentha gentilis*. According to Schimmel and Company,[1] the oil of *Mentha sylvestris* contains a relatively large amount of pulegone. The oil of curly mint, or *Mentha crispa*, contains carvone. It should be noted too that, in addition to menthone, American oil of peppermint has been shown to contain acetaldehyde, isovaleraldehyde and the two acids corresponding to these aldehydes.[2]

Peppermint extracts which gave strong oxidase reactions contained no peroxides in amounts sufficient to be detected by the starch iodide reaction.

In addition to an oxidase, peppermint plants contain catalase. An extract amounting to 60 cc. was prepared from 25 grams of peppermint blossoms. Five cc. of this extract liberated 42 cc. of oxygen in six minutes from 10 cc. of a 3 per cent. hydrogen peroxide solution.

The presence of catalase in peppermint and other plants considered in this paper makes it extremely improbable that the ketones and aldehydes under discussion result from ordinary hydrogen peroxide oxidation. The occurrence of traces of hydrogen peroxide in green plants is a point in dispute. At any rate it is a fact that when hydrogen peroxide is decomposed by catalase, molecular or inactive oxygen is liberated.[3] Thus, tincture of guaiac resin is not colored nor can uric acid or xanthine be oxidized by hydrogen peroxide in the presence of catalase. H. D. Dakin[4] has recently prepared ketones by oxidation with hydrogen peroxide of saturated fatty acids. He prepared in this way methylnonyl

[1] Schimmel and Co., *Semi-annual Rep.*, 1, 126 (1910).
[2] Power and Kleber, *Arch. Pharm.*, 232, 639 (1894).
[3] Shaffer, *Am. J. Physiol.*, 14, 299 (1905).
[4] *Am. Chem. J.*, 44, 41 (1910); *J. Biol. Chem.*, 4, 221.

ketone from lauric acid, methylheptyl ketone from caproic acid and methylamyl ketone from caprylic acid. The first two ketones are found as the major constituents in oil of rue and Dakin tentatively suggested that the aldehydes and ketones found in essential oils might be formed in the manner just indicated. The evidence against this hypothesis, for the case under consideration, is as follows: Neither lauric nor caproic acids have been found in oil of rue. Power and Lees[1] found acetic and valeric acids and Houben[2] detected the presence of a small proportion of caprylic acid. Moreover, Power and Lees found the two corresponding alcohols, n. methylheptyl carbinol and n. methylnonyl carbinol, in oil of rue. Finally, I have shown that ordinary rue, *Ruta graveolens*, contains catalase. The aqueous extract from two grams of the leaves, prepared by grinding in a glass mortar, liberated 65 cc. of oxygen from 10 cc. of 3 per cent. hydrogen peroxide in the short interval of 60 seconds. The formation of these ketones in *Ruta graveolens* by hydrogen peroxide oxidation according to Dakin's hypothesis is clearly not in accord with the facts. It should be noted also that the presence of catalase does not diminish the oxidizing power of the oxidizing enzymes.[3]

As in the case of the two ketones in oil of rue, the ketones and aldehydes of essential oils are usually found associated with the corresponding alcohols or acids, as menthone and menthol, thujone and thujyl alcohol, citral and linalool or geraniol, benzaldehyde and benzyl alcohol, isovaleraldehyde and isovaleric acid, valeraldehyde and valeric acid[4] and so on. Also, only open chain ketones or aldehydes may be obtained by Dakin's method, and one carbon atom is split off from the parent substance.

That the oxidation products under consideration may be formed, in certain cases, by autoxidation independent of the action of an oxidizing enzyme, is not entirely excluded, since the formation of aldehydes by autoxidation of the corresponding alcohols, particularly in direct sunlight, has been observed by Neuberg[5] and others. However, the reaction is quite slow unless catalyzed in some way. This is undoubtedly the function of the oxidases. According to Bach[6] and others the substance upon which the oxidase acts must be capable of slow oxidation by the oxygen of the air, and the enzyme is assumed to accelerate this reaction. That sunlight alone is not this accelerating factor is highly probable in

[1] *Proc. Chem. Soc.*, **18**, 192.

[2] *Ber.*, **35**, 3587.

[3] Abderhalden, *Handbuch der Biochem. Arbeitsmethoden*, Berlin, **3**, 68 (1910).

[4] Henderson and Sutherland, *J. Chem. Soc., London*, **99**, 1541 (1911), found that isocamphenilic acid could not be formed by oxidation of the corresponding aldehyde by hydrogen peroxide.

[5] *Biochem. Z.*, **17**, 270 (1909).

[6] *Ber.*, **43**, 368 (1910).

view of the results of Ciamician and Silber[1] who have shown that cyclic ketones are invariably split by autoxidation in direct sunlight yielding a series of products not found in natural oils. The readiness with which unsaturated organic compounds undergo autoxidation with the intermediate formation of organic peroxides,[2] and the rapidity with which essential oils deteriorate through oxidation when kept in poorly stoppered bottles, is well known. Autoxidation alone, however, cannot explain all of the facts observed.

Charabot considered that the more pronounced oxidation, which took place in the inflorescences of the peppermint, was due to the greater respiratory activity of this part of the plant. However, many biological oxidations are known to take place through the medium of oxygen "carriers" and the reactions under discussion are apparently examples of such a process.

The Enzymes in Valerian Root.—Valerian oil contains isovaleraldehyde and isovaleric acid.[3] The borneol ester of this acid is one of the major constituents of the oil. Carles[4] showed that the root also contains an oxidase. An aqueous extract of the ground root does not develop the valerian odor, on standing, if previously boiled. The addition of a little extract brings out the odor and Carles attributed this behavior to the oxidase. I have investigated the question further and have confirmed the experimental work of Carles but I have found that the development of the rancid valerian odor, in the root extract, is due to the liberation of isovaleric acid by a fat splitting enzyme, or lipase. This is shown by the following experiments. On standing a few hours the uncooked extract develops a rancid odor suggestive of free isovaleric acid. As shown by Carles, the odor is not produced in a cooked extract. Addition of emulsin or of potato oxidase does not develop the odor. Dilute sulfuric acid slowly brings out the odor and finally it was found that a small quantity of fresh extract splits ethyl isovalerate, yielding the free acid. Three grams of fresh roots were crushed in a mortar and shaken with 25 cc. of water and two grams of ethyl isovalerate. Two drops of chloroform were added as an antiseptic. After twenty-four hours at approximately 20° the solution was titrated and 0.141 gram free isovaleric acid was found, 16 cc. of $N/10$ alkali being required for neutralization. A control experiment made with a cooked extract showed no free acid. The liberation of the vile and rancid smelling isovaleric acid is, therefore, due to the action of a lipase on the ester and it is probable that one effect

[1] *Ber.*, 40, 2419 (1907); 41, 1071, 1928 (1908); 42, 945, 1510 (1909).

[2] Engler and Weissberg, *Ber.*, 31, 3046 (1898); 33, 1090 (1900). Dunlop and Schenk, THIS JOURNAL, 25, 826 (1903). Brooks, *Phil. J. Sci., Sec. A.*, 219 (1910); *Chemical News*, Jan. 19, 1910.

[3] Bertram and Gildemeister, *Arch. Pharm.*, 228, 483 (1890).

[4] *J. Pharm.*, [4] 12, 148.

of the oxidase is the formation, in the growing plant, of isovaleric acid from isovaleraldehyde, or yet more complicated substances. The free acid must then combine with borneol, since, as shown by the chemists of Roure-Bertrand Fils,[1] esterification of terpene alcohols takes place with *much* greater rapidity in the growing plant than when a mixture of the pure alcohol and acid is allowed to stand. These authors considered that the rapid esterification in the plant was caused by dehydration conditions in the chlorophyll grains and the presence of an enzyme which enabled a state of equilibrium to be quickly reached. They do not mention having investigated any of the plants studied by them for a lipase. The finding of a lipase in valerian supports their hypothesis, although it is possible that the root is not the seat of formation of the borneol isovalerate.

It should be noted also that when old valerian roots are distilled with steam the aqueous distillate contains a considerable quantity of free isovaleric acid, and indeed the acid owes its discovery to this fact.

Oils Containing Thujone.—Oil of tansy, distilled from *Tanacetum vulgare* L., contains a high per cent. of thujone and probably also thujyl alcohol.[2] An oxidase was prepared from the leaves of this plant by grinding with sand as described above. The reactions with guaiac and the indophenol reagent were particularly sharp.

The essential oil of *Thuja occidentalis* contains thujone and fenchone.[3] The leaves also contain an oxidase, as was shown by employing the same methods and reagents noted above. The aqueous extracts contained a large quantity of mucilaginous material and consequently the method of partial precipitation with magnesium sulfate and alcohol, recently recommended by Bach,[4] was employed to advantage.

Oils Containing Pulegone.—In addition to *Mentha sylvestris*, mentioned above, an oxidase was found in *Satureja montana* L., and *Calamintha officinalis* Muh. The compositions of the essential oils of the two latter species are not accurately known, but as pennyroyal was not available the closely related species *Satureja montana*, having an odor similar to pennyroyal, was tested for oxidases and with positive results. The inflorescences gave the strongest reactions.

Calamintha nepeta yields an essential oil containing a large proportion of pulegone and menthone, but as it was not available the closely related species *Calamintha officinalis* Muh. was examined. The latter species smelled strongly of pulegone and gave positive results when examined for an oxidase. The strongest reactions were obtained with extracts of the inflorescences.

[1] *Bull. Roure-Bertrand Fils*, [1] 4, 18 (1901).

[2] Bruylants, *Ber.*, 11, 449 (1878).

[3] Wallach, *Nachr. K. Ges. Wiss. Göttingen*, 1, 11 (1901).

[4] *Ber.*, 43, 362 (1910).

Two distinctly negative results were obtained, which are interesting in that they are in harmony with the theory proposed. No reaction for an oxidase was obtained with aqueous extracts from two varieties of cultivated roses. German otto of rose contains a very small proportion of citral and a trace of nonyl aldehyde. The extracted oil may contain as much as 75 per cent. phenylethyl alcohol[1] but it is not known to contain phenylacetaldehyde or phenylacetic acid.

Negative results were also obtained with *Andropogon schoenanthus*, which yields palma rosa oil. This oil may contain as high as 93 per cent. of geraniol in addition to citronellol and dipentene. This is in marked contrast with the very closely related *Andropogon* grasses which yield lemon grass and citronella oils, both of which contain citral and citronellal as their major constituents. These species will be examined when the material is available.

The presence of an oxidase probably accounts for the fact that the perfumes of certain flowers deteriorate rapidly after picking, particularly if bruised, as is the case with champaca and gardenia flowers. The author ventures the opinion that flowers showing marked reactions for oxidases are not treated to the best advantage by the enfleurage method.

The action of oxidases from various sources on terpene alcohols will be taken up in the second paper on this subject.

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ILLINOIS.]

CORRELATION OF IONIZATION AND STRUCTURE. II. NEGATIVELY SUBSTITUTED BENZOIC ACIDS.

BY C. G. DERICK.

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TABLE OF CONTENTS.—1. Introduction. 2. Place Factors for Negative Radicals in Aromatic Acids. 3. Calculation of Ionization Constants of Substituted Benzoic Acids. 4. Determination of Structure of Substituted Benzoic Acids from their Ionization Constants by Means of Place Factors. 5. Limitations in the Use of Place Factors for the Determination of Structure in the Aromatic Series. 6. A New Proof of the Equivalence of the Positions 3 and 5 with Respect to Position 1 in the Benzene Ring. 7. Summary.

1. Introduction.

In a previous article,[2] the author has shown that the free energy of ionization for negatively substituted monobasic paraffin acids in water solution at 25° is made up additively of the separate effects of each atom within the molecule. Because of this fact, it was shown that the position of a negative substituent in a paraffin acid could be determined with certainty if its α place factor and the ionization constant of this nega-

[1] v. Soden, *J. prakt. Chem.*, [2] 69, 265 (1904).

[2] THIS JOURNAL, 33, 1181 (1911).