The Constituents of the Volatile Oil of Catnip. II. The Neutral Components. Nepetalic Anhydride

By S. M. McElvain, Philip M. Walters1 and Robert D. Bright2

In the first paper3 of this series the structures of the compounds, nepetalic acid (III) and nepetalactone (VI), that are extractable from the volatile oil of catnip by dilute alkali were discussed. These alkali-soluble components comprise 85–90% of the oil, depending upon the method of extraction; when the oil is shaken vigorously with a 10% solution of sodium hydroxide at 60° for fifteen minutes only about 10% of the oil remains undissolved. It is with this alkali-insoluble portion that the present paper deals.

Hixon4 has reported an investigation of this alkali-insoluble portion of the oil. He subjected it to steam distillation and found that only about half of it was readily volatile and that a considerable portion of it did not distill with the steam. Fractionation of the steam-distilled material yielded a main fraction that boiled at 245–255° (11 mm.) and which from its odor and physical properties was thought to be a dicyclic sesquiterpene.

In the present work careful fractioning of this neutral portion of the oil divided it into a number of fractions that appeared to have constant boiling points and that attained maxima or minima in their refractive indices. The lowest boiling of these fractions was found to be a colorless azeotropic mixture that was composed of about 70% of a C15H24 hydrocarbon and 30% of nepetalactone (VI). The lactone was readily removed from the hydrocarbon when the mixture was refluxed with 10% aqueous sodium hydroxide. The properties of this hydrocarbon and its dihydrochloride correspond to those of the sesquiterpene, p-caryophyllene (I), that has been isolated from oil of cloves by Ruzicka.

The next fraction that distilled from the neutral portions of the oil was nepetalactone (VI) which was readily identified as the semicarbazone of nepetalic acid. After the nepetalactone two very small fractions (see Table I) were obtained. The first of these was colorless and from analyses and physical properties appeared to have the molecular formula, C14H26O. The oxygen of this compound seemed to be in the form of an ether since it shows no hydroxyl or carbonyl function. The other of these fractions is deep yellow in color and has a tea-like odor. Analyses show it to have an empirical formula of C9H14O2; saponification in standard alkali indicates that it is an ester with a saponification equivalent of 176. On account of the scarcity of both of these materials, no further work was done on them.

The last fraction was relatively large and was obtained as a yellow, viscous oil that boiled at 200–210° (0.1 mm.). On standing this oil slowly crystallized. The crystalline material, after recrystallization from petroleum ether, melted at 139–140° and was optically active. [α]D 136° in chloroform. Analyses and molecular weight determinations showed it to have the molecular formula, C20H30O5. It contained no readily oxidizable or titratable groups, but hydrolysis with dilute hydrochloric acid converted it into nepetalic acid. These facts lead to the conclusion that the compound is the anhydride (V) of nepetalic acid formed by the loss of a molecule of water between two molecules of the hydroxylactone form (I) of the acid. It is obvious that an anhydride formed from either of the other two tautomeric forms of nepetalic acid (III and IV) would contain highly reactive functional groups.

Nepetalic anhydride (V) is formed in considerable amounts together with the acetate of II when nepetalic acid is treated with acetyl chloride. In this connection it is interesting to note that acetic anhydride converts nepetalic acid quantitatively into the acetate. The anhydride, V, slowly forms at ordinary temperatures in non-crystalline samples of nepetalic acid on standing; such samples, which originally were completely alkali soluble, after a year or more of standing may con-
tain as much as 4% of this anhydride. Nepetalic anhydride may also be prepared by heating nepetalic acid under 0.2 mm pressure; at this pressure as high as 30% intermolecular dehydration occurs with the formation of V. At atmospheric pressure the dehydration of nepetalic acid is intramolecular with the formation of nepetalactone as pure crystalline material or as the viscous oil, this neutral oil comprises 10% of the original oil of catnip. Since this compound appears to be an ester it is likely that its molecular formula is double this empirical formula to account for the relatively high boiling point of the compound.

It is seen from the above table that \( \beta \)-caryophyllene, nepetalactone and nepetalic anhydride comprise over 90% of the alkali-insoluble portion of the oil of catnip. Since this portion represents only about 10% of the original oil it follows that over 99% of the volatile oil of catnip is accounted for as nepetalic acid (I) and its derivatives, nepetalactone (VI) and nepetalic anhydride (V), which comprise about 98% of the oil, and \( \beta \)-caryophyllene (I) which is present to the extent of about 1.4%. The ether, \( \text{C}_{14} \text{H}_{24} \text{O} \), and ester, \( \text{C}_{9} \text{H}_{14} \text{O}_2 \), together represent only about 0.5% of the oil.

Since both nepetalic acid and its anhydride are odorless, it seemed probable that nepetalactone and possibly the caryophyllene are the constituents of the oil that are responsible for the odor of the catnip plant that is so attractive to many species of the cat family. Through the courtesy and cooperation of Mr. Fred Winkelmann, superintendent of the Vilas Zoo at Madison, it was possible to test this supposition. Small pledges of cotton soaked in a dilute alcoholic solution of each of these constituents were placed, after the evaporation of the alcohol, in the different cages of the African lions at the Zoo. The nepetalactone that was used was prepared from the odorless nepetalic acid. There were ten of these animals available for the test. They represented both sexes and varied in age from cubs a few months old to a lioness so old (25-30 years) that she was partially blind. With the exception of the three cubs that were present, all of the

<table>
<thead>
<tr>
<th>Component</th>
<th>% of neutral oil</th>
<th>C. p.</th>
<th>n</th>
<th>d144</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )-Caryophyllene</td>
<td>14</td>
<td>59-60</td>
<td>0.03</td>
<td>1.4902</td>
</tr>
<tr>
<td>Nepetalactone</td>
<td>42</td>
<td>70-71</td>
<td>0.03</td>
<td>1.4870</td>
</tr>
<tr>
<td>CnHnO</td>
<td>3</td>
<td>85-87</td>
<td>0.03</td>
<td>1.4942</td>
</tr>
<tr>
<td>CnHnO</td>
<td>2</td>
<td>115-117</td>
<td>1</td>
<td>1.4790</td>
</tr>
<tr>
<td>Nepetalic anhydride</td>
<td>30</td>
<td>200-210</td>
<td>.1</td>
<td></td>
</tr>
</tbody>
</table>

* This neutral oil comprises 10% of the original oil of catnip. ° [\( \alpha \)] indentation of this hydrocarbon melts at 68-69°. Since this compound appears to be an ester it is likely that its molecular formula is double this empirical formula to account for the relatively high boiling point of the compound. ° The melting point of the crystalline material from this fraction is 139-140°.

In Table I are listed the various components that have been isolated from the portion of the volatile oil of catnip that is insoluble in aqueous alkali at 60°, the percentage of this neutral portion which each component comprises and certain of the physical properties of the pure compound. The percentage values in the second column of this table include both the pure material, having the properties shown, and that estimated to be present in the intermediate fractions from the chemical and physical properties of these fractions.

(6) A compound with a structure analogous to V has been reported by Carrier (Ann. chim., 17, 84 (1922)). It was formed to the extent of about 25% when the trimer of the half-aldehyde of succinic acid was subjected to distillation and depolymerized into the monomer.
Lions responded immediately to nepetalactone in the same manner that they do to the oil of catnip and to the fresh plant, the latter of which is given to them regularly during the summer. They showed no interest in the caryophyllene. The reaction of the lions to either nepetalactone or the catnip plant is quite similar to that of an ordinary house cat. They can be aroused immediately from a state of lethargy to one of intense excitement by the odor of the lactone, and will follow the odor to its source. When they acquire the material with the odor they become ludicrously playful and their main interest seems to be to get the odoriferous material transferred to their fur. They show no desire to eat the material, nor is there any evidence of sexual stimulation.

**Experimental**

**Fractionation of the Neutral Portion of the Oil of Catnip.**
—A 100-g. sample of the neutral fraction of oil of catnip (which represented the undissolved material from 1 kg. of the oil of catnip that had been shaken with 10% aqueous sodium hydroxide at 60° for fifteen minutes) was distilled through an 8-cm. still-head at 0.1 mm. This crude fractionation separated the lower boiling fractions, 63 g., from the higher boiling material, principally nepetalic anhydride, 36 g., which remained as the residue. Careful fractionation of the distillate from the above distillation gave four fractions, (a) 23 g., b. p. 55-60° (0.03 mm.); $n_{D}^{20} = 1.4900-1.4921$; (b) 31 g., b. p. 61-71° (0.03 mm.); $n_{D}^{20} = 1.4870-1.4900$; (c) 5 g., b. p. 72-110° (0.03 mm.); $n_{D}^{20} = 1.4900-1.4920$; (d) 3 g., b. p. 110-120° (0.03 mm.); $n_{D}^{20} = 1.4883-1.4880$. Refractionation of each of the above fractions gave the quantities of material with a constant refractive index that are shown in Table II.

**Table II**

**Fractions of the Lower Boiling Neutral Components of Oil of Catnip**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight, g</th>
<th>$n_{D}^{20}$</th>
<th>B. p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>20</td>
<td>0.03</td>
<td>1.4931</td>
</tr>
<tr>
<td>b</td>
<td>29</td>
<td>0.03</td>
<td>1.4878</td>
</tr>
<tr>
<td>c</td>
<td>8-95</td>
<td>0.03</td>
<td>1.4830</td>
</tr>
<tr>
<td>d</td>
<td>2</td>
<td>1.11-117</td>
<td>1.4790</td>
</tr>
</tbody>
</table>

**Isolation and Identification of β-Caryophyllene (Fraction a).**—Carbon-hydrogen analyses and molecular weight determinations indicated fraction a to be a constant boiling mixture of about 70% of a C₂₀ hydrocarbon and 30% of an oxygen-containing component. On the assumption that the oxygen compound was nepetalactone which had not been completely removed by the alkaline extraction of the oil, fraction a (20 g.) was refluxed with 10% sodium hydroxide for one hour, and then extracted thoroughly with ether. The ether extracts were combined and distilled. There was obtained 12 g. of a hydrocarbon which proved to be β-caryophyllene, $\beta$-C₁₅H₂₄, b. p. 112-113° (10 mm.); $n_{D}^{20} = 1.4902$; $d_{25}^{20} = 0.9074$; $[\alpha]_{D}^{20} = -11.42$ (in chloroform); mol. wt. (Beckmann), 195 (calcd. 204); $M_{D}$, 66.13 (calcd. 66.16).

**Anal.** Calcd. for C₁₅H₂₄: C, 82.0; H, 11.8. Found: C, 88.0; H, 11.8.

β-Caryophyllene was further identified by conversion to its dihydrochloride; m. p. 68-69°. This was done by saturating a solution of 1 g. of the hydrocarbon in 10 ml. of anhydrous ether with dry hydrogen chloride. This solution was allowed to stand in the refrigerator for four days, after which time the ether was removed by evaporation under diminished pressure. The residual oil was dissolved in 2 ml. of absolute alcohol and crystallization induced by the introduction of a crystal of dry-ice into the alcoholic solution. The white crystalline dihydrochloride was filtered off and recrystallized from absolute alcohol. The yield of β-caryophyllene dihydrochloride so obtained amounted to 0.2 g. It melted at 68-69° and contained 25.0% chlorine (calcd. 25.0%).

Ruzicka has reported the following properties for β-caryophyllene that was isolated from the oil of cloves, b. p. 110-121 (12 mm.): $d_{10}^{20} = 0.9074$; $n_{D}^{10} = 1.5000$; $[\alpha]_{D}^{10} = 1.4733$; $[\alpha]_{D}^{20} = -8.9°$; $M_{D}$, 66.14; m. p. of dihydrochloride, 69°.

The alkaline extract of fraction a was acidified to congo red with 10% sulfuric acid, and thoroughly extracted with ether. The ether extracts were combined and on distillation yielded 5 g. of nepetalic acid, identified by its semicarbazone, m. p. 158-159°, which showed no depression of melting point when mixed with an authentic sample of nepetalic acid semicarbazone.

**Nepetalactone (Fraction b).**—Analysis and physical constants of fraction b indicated it probably was composed of approximately 85% nepetalactone and 15% of β-caryophyllene. Accordingly a 12-g. sample of fraction b was shaken with 25 g. of 10% aqueous sodium hydroxide at 80° for thirty minutes, and then extracted thoroughly with ether. The ether extracts were combined and distilled to yield 1.8 g. of a material boiling from 112 to 135° (12 mm.) and which had the characteristic odor of β-caryophyllene. The alkaline extract was acidified with 10% sulfuric acid to congo red and thoroughly extracted with ether. The ether extracts were combined and on distillation yielded 9.6 g. of nepetalic acid.

**Fractions c and d.**—A 4-g. sample of fraction c on alkaline extraction with hot aqueous alkali solution yielded 2 g. of a neutral oil which showed no reaction with semicarbazone or with acetanilide in refluxing dioxane after two hours. This compound, which appears to be an ether, C₁₅H₂₉O₄, boils at 130-138° (0 mm.). $n_{D}^{20} = 1.4942$; $d_{24}^{20} = 0.9092$.

**Anal.** Calcd. for C₁₅H₂₉O₄: C, 80.7; H, 11.6. Found: C, 80.9; H, 11.2.

The alkaline extract of this fraction yielded 2 g. of nepetalic acid.

Fraction d was rich yellow in color and had a faint tea-like odor. It failed to give an oxime or semicarbazone when treated with hydroxylamine or semicarbazide. Its yellow color suggested a 1,2-diketone structure, but it failed to show any reduction of Fehling solution, a fact which would seem to eliminate that type of structure. Saponification with alcoholic alkali indicated that it is an ester with a saponification equivalent of 176. This compound boiled at 115-117° (0.1 mm.); $n_{D}^{20} = 1.4790$. Analyzes indicated it to have an empirical formula of C₁₅H₂₉O₄.
**Summary**

An investigation of the alkali-insoluble portion (10%) of the volatile oil of catnip shows that it consists of β-caryophyllene (14%), nepetalactone (42%) and nepetalic anhydride (36%). This latter compound is the anhydride of the hydroxylactone form of nepetalic acid.

In addition to these compounds two other substances which comprise not more than 0.5% of the oil have been isolated but not identified.

It is shown that nepetalactone is the component of the oil, the odor of which makes the catnip plant so attractive to certain species of the cat family.

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**α-Alkoxyvinyl- and α-Alkoxyethylbarbituric Acids**

By S. M. McElvain and Howard Burkett

The formation of (α-ethoxyethyldiene)-malonic ester (II, R is ethyl) from the reaction of ketene diethyl acetal and malonic ester suggests a study of this reaction with other ketene dialkylacetals (I) and the conversion of the resulting α-alkoxyethylidenemalonic esters into the corresponding 5-(α-alkoxyvinyl)-5-alkyl-barbituric acids (VI) by the sequence of reactions shown below. It seemed possible also that the vinyl group could be hydrogenated at the malonic ester stage (IV) and a series of 5-substituted (α-alkoxyethyl)-barbituric acids (VII) prepared from the saturated malonic esters (V).

In the original study of the reaction between ketene diethyl acetal and malonic ester the (α-ethoxyethyldiene)-malonic ester (II), m. p. 26–27°, was obtained in 55% yield. It has been found possible in the present work to increase the yield of this product to 66% and to obtain in addition the isomeric (α-ethoxyvinyl)-malonic ester (III, R is ethyl) in 11% yield. The structure of the latter ester, which is a liquid, is shown by its ozonolysis into formaldehyde, and by the fact that the two isomers yield the same malonic ester and barbituric acid in the reactions shown below. By long heating (125° for twenty hours) with a trace of sodium ethoxide the liquid ester (III) may be converted into its solid isomer (II).