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URBANA, ILLINOIS,
AUGUST, 1924.

(Part II will appear in a succeeding issue.)

PHYTOCHEMICAL NOTES.*

No. 94. Oil of *Echinacea angustifolia*.¹

BY FRITZ BISCHOFF.

Two samples of a volatile oil, recently discovered by Professor Lloyd in the root of *Echinacea angustifolia*, were submitted by him to this laboratory. He had observed that the yield from the fresh roots was somewhat greater, *viz.*, 1.5 p. c., than that from the dry roots, *viz.*, 1.25 p. c. However, the physical constants of both oils were found to be very nearly identical, the most characteristic being the remarkably low density of 0.80. The results of this preliminary study indicate that the oils consist for the most part of a single constituent, which is very probably a tetrahydrosesquiterpene with two double bonds. Over 80% of the oil, either from the fresh or dried roots, boils within a range of 8°. By fractionation it was possible to obtain 40% of the total oil boiling within 2°. The analysis for carbon and hydrogen, the boiling point range (at 260° C.) and the fact that the substance adds on an amount of bromine equivalent to two double bonds pointed to the empirical formula

* From the laboratory of Edward Kremers.

¹ History of the Discovery of the Volatile Oil of *Echinacea* Root.

In the working of *Echinacea* on a large scale by means of the extracting apparatus employed in my laboratory, the content of the concentrator gradually changes from a highly alcoholic menstruum to one which, if the drug be fresh, and not fully dried, becomes practically an aqueous liquid.

It was noticed in the manipulation that when this liquid was withdrawn from the concentrator and allowed to stand, an oily substance separated and floated on the surface, this being in considerable amount when large batches (1000 lbs. each) were manipulated.

In the finishing of the preparation for use of physicians, alcohol was employed as a diluent of the mixture as a whole, the amount being established by another constituent of *Echinacea* quite different from this oily separate, which, but without any recognition whatever of its presence, became a part of the final product.

On further examination and separation of the oil, it was found that on standing it separated an extraneous coloring material, the greater part of it becoming an overlying, practically transparent oil. This, decanted and filtered, but without further manipulation, constitutes the oil you have received, and which so excited my interest as to lead me to feel assured it was worthy of scientific examination.

Very truly yours,

JOHN URI LLOYD, May 17, 1924.

$C_{15}H_{28}$. The density (0.797 at 15°) indicates that there are no cycles in the compound. The relationship existing between density and number of cycles is shown in Table I. It will be noticed that the difference in density between the compound in question and that for aliphatic sesquiterpenes ($0.84 - 0.80 = 0.04$) is about the same as the difference between the densities of cadinene and tetrahydrocadinene ($0.921 - 0.888 = 0.033$).

TABLE I.

Formulas.	No. of cycles.	Density.	Index of refraction.
$C_{15}H_{24}$	3	0.935-0.910	1.50-1.49
$C_{15}H_{24}$	2	0.92-0.90	1.5-1.50
$C_{15}H_{28}$	2	0.888	..
$C_{15}H_{24}$	1	0.873	1.49
$C_{15}H_{24}$	0	0.84	1.53
$C_{15}H_{32}$	0	0.77	..

Dehydrogenation of the hydrocarbon $C_{15}H_{28}$ with sulphur did not yield either the cadalene or eudalene of Rusicka (*Helv. Chim. Acta*, 5, 344, 1922), and while this is not conclusive proof in itself that the compound contains no cycles it is additional evidence for the chain structure and also shows that the compound does not belong to the eudalene or cadalene groups of sesquiterpene derivatives.

With the exception of the hydrogenation product no solid derivatives have thus far been obtained from the hydrocarbon $C_{15}H_{28}$ with bromine, hydrobromic acid, acetic acid, nitrosyl chloride, sulphuric acid, etc. Oxidation with dilute permanganate yielded carbon dioxide and hexoic acid (b. p. $180^\circ C.$, liquid at $-17^\circ C.$). Four out of the seven possible hexoic acids are eliminated by these constants. The acids which remain as possibilities are diethyl acetic acid, methyl *n* propyl acetic acid and methyl isopropyl acetic acid. The isolation of the hexoic acid, as one of the abba products, proves conclusively that the hydrocarbon $C_{15}H_{28}$ is not a straight chain compound, for normal hexoic acid boils at $205^\circ C.$ and has a melting point of $8.0^\circ C.$

On hydrogenating the $C_{15}H_{28}$ hydrocarbon catalytically in the presence of Pahl's palladium, a saturated hydrocarbon was obtained, the carbon and hydrogen analysis checking for $C_{15}H_{32}$. The compound is insoluble in concentrated sulphuric acid and melts sharply at $8.2^\circ C.$ Normal pentadecane melts at $10.0^\circ C.$ The densities (0.7727 and 0.7724 at $\frac{15^\circ}{4}$) for the hydrocarbon $C_{15}H_{32}$ under observation and for normal pentadecane are the same out to the third decimal places. The difference in melting points is additional proof that the hydrocarbon $C_{15}H_{32}$ and its parent $C_{15}H_{28}$ are not straight chain compounds.

As to the reason why the fresh roots yield more oil than the dry, it has been observed that on exposure to the air the oil from the fresh (or dry root) becomes viscous and finally hard and it is therefore very probable that the tetrahydro-sesquiterpene is slowly oxidized by the air when the roots are dried, thus cutting down the yield.

EXPERIMENTAL PART.

Constants of Echinacea Oil.—The indices of refraction, densities and boiling point ranges for the oils from the fresh and dried roots are very nearly the same. $n_{25^\circ} = 1.4490$ for both oils. $d_{15^\circ} = 0.802$ for the oil from the fresh roots and

0.792 for the oil from the dry roots. Both are optically inactive. B. p. (740 mm.) fractions for 100 grams of oil from fresh roots: 9.7 grams below 252° C. (uncor.), 79.5 grams between 252–257° C., and 6.4 grams between 257–260° C. $n_{25}^{\circ} \text{C.} = 1.4492$, $d_{15}^{15} = 0.797$ for portion distilling between 252–257° C. By repeated fractionation it was found possible to obtain 40 grams boiling at 258.5–260° C. and 4 grams boiling at 256–258.5° C., 60 per cent of the oil from the dried roots boiled over 257° C. on fractionation. The oils contain no free acids. The neutrality equivalent of the fresh oil was found to be over 3000, so that the amount of esters present is negligible. A combustion for carbon and hydrogen was made on the fraction distilling 252–257° C. Found 85.7% C, 14.4 per cent H₂. Theory for C₁₅H₂₈, 86.5% C, 13.6% H. Theory for C₁₅H₃₀, 85.7% C, 14.3% H.

Bromination.—Samples of the higher boiling fractions were suspended in glacial acetic acid and cooled in an ice-bath. Bromine was slowly added from a burette until a positive test for bromine was obtained with starch iodide paper. In order to prevent loss of bromine vapors, a layer of conc. H₂SO₄ was used over the bromine in the burette.

18.44 grams oil absorbed 28.0 grams Br.

Theory for C₁₅H₂₈ is 28.3 grams Br.

The brominated oil was separated by pouring the suspension in water, and was dried over CaCl₂. It was light yellow in color. At about –80° C. (solid CO₂) it turns to a glass which fractures conchoidally, but does not crystallize. At about –20° C. the glossy mass turns viscous. Preliminary experiments indicate the dehydrogenation of this tetrabromide may be brought about by heating with aniline at 100° C., although the reaction is accompanied by decomposition. Aniline hydrobromide crystals (soluble in water) were obtained.

Permanganate Oxidation.—To 39.0 grams of the higher boiling fraction of the Echinacea oil, suspended in 600 cc. of water, 48 grams of KMnO₄ were added in 3.0-gram portions. Mechanical agitation was used. Each portion of KMnO₄ was added only after the preceding portion had disappeared, the presence of permanganate being indicated by a pink ring on spotting out with filter paper. The oxidation was carried out at room temperature and required three days. The final mixture was alkaline to phenolphthalein. One portion was steam-distilled in its original state, the remainder was filtered free of MnO₂, rendered acid to H₂SO₄ and steam-distilled. Steam distillation from alkaline solution gave an oil, free from aldehydes or ketones (negative tests with neutral sulphite solution on shaking over night and with p. nitro phenyl hydrazine), which was probably unchanged Echinacea oil. The acid distillation also yielded unchanged oil, but in addition an oily acid, partly soluble in water, and which had an odor reminiscent of butyric acid but not so strong, was obtained. The silver salt of this acid was made by adding silver nitrate to a solution of the acid which had been neutralized with ammonium hydroxide.

Found, 46.1% Ag. Theory for silver caproate, 45.9% Ag. All of the acid, which was undoubtedly one of the hexoic acids, was separated from the remaining portions of the oxidation mixture as the silver salt. The acid was then reliberated through the boiling with Na₂CO₃, followed by acidification. The acid tends to form an emulsion with water, which was very difficult to separate. One gram of dry acid was finally obtained. It did not solidify at –17° C. The b. p. was 189° C. (cor.), using the equalized vapor-tension method.

Outside of this hexoic acid and a large amount of carbon dioxide, which was evolved when the oxidation mixture was acidulated, no other products of oxidation could be isolated.

Hydrogenation, Using Palladium.—Attempts at hydrogenation, using Adams' method for the preparation of the catalyst, were unsuccessful both at atmospheric and 30 lbs. pressure. By using Pahl's palladium, hydrogen was readily introduced into the compound at room temperature. The mixture for hydrogenation consisted of 20.0 grams of oil, b. p. 257–260° C., 20 cc. alcohol, 1.0+ gram of Pahl's palladium and 50 cc. of water. Four liters of hydrogen (washed with alkaline pyrogallol and conc. H₂SO₄) were absorbed, one liter the first hour, and the remaining three liters in eight hours. The reaction mixture was poured into water, and repeatedly extracted with ether. The ether was evaporated, the residual oil placed in a separatory funnel and washed twice with water, shaken up three times with conc. H₂SO₄, and finally washed successively with water, dilute ammonia and water, and dried over CaCl₂. (The sulphuric acid used in washing the oil becomes dark brown in color.) The oil so obtained is colorless and odorless. It solidifies readily in an ice-bath. M. p. 8.2° C.; $d_{4}^{15^{\circ}} = 0.7727$; $d_{4}^{24^{\circ}} = 0.7681$; $n_{25^{\circ}} = 1.4323$. A combustion for carbon and hydrogen was made.

Found, 84.3% C and 15.5% H.

Theory for C₁₅H₃₂ is 84.8% C and 15.2% H, b. p. 259–262° C. (uncor.). There was a slight decomposition during boiling, the distillate being yellow and having acquired a pungent odor. By washing with conc. H₂SO₄ the distillate lost its yellow color, also its odor. This indicates that distillation should be carried on at reduced pressure and undoubtedly also applies to the original Echinacea oil.

Dehydrogenation with Sulphur.—Forty grams of oil (b. p. 252–257° C.) were heated with 25 grams sulphur, in a round-bottom flask fitted with condenser. The reaction began at about 210° C., with evolution of gas. In two hours the sulphur layer had disappeared. The brown liquid residue was distilled, the following cuts being made: 5 cc. below 292° C., and 20 cc. at 292–305° C. (uncor.). There remained a tar. To remove sulphur compounds the fractions were treated with alcoholic mercuric chloride, white precipitates forming. The latter were filtered off. The oil remaining in the alcohol solution was separated out by the addition of water. It was dried over K₂CO₃ and redistilled. Two fractions, 204–290° C. and 290–300° C., were cut. They still contained some sulphur compounds. Neither fraction yielded a picrate, either immediately from a saturated alcoholic solution or upon standing. On evaporation pure picric acid (m. p. 122° C.) was obtained. The cadalene and eudalene of Rusicka (*Helv. Chim. Acta*, 5, 344, 1922) were therefore absent.

Miscellaneous Tests and Reactions.—The fraction (b. p. 252–257° C.) was used. Addition of chromyl chloride results in an explosion of great violence. Conc. H₂SO₄ dissolves the oil at 0° C., a brown color resulting. The product is a liquid. Nitrosyl chloride failed to give a solid product. Glacial acetic acid containing a little conc. H₂SO₄ also failed to give a solid product. Dry hydrobromic acid in glacial acetic acid reacted with the hydrocarbon to some extent, but it was not found possible to saturate it completely, as some bromine was still absorbed after the hydrobromic acid treatment.

Summary.—This preliminary investigation of the volatile oil of *Echinacea*

angustifolia roots is to be followed by further studies on a larger amount of material kindly supplied by Professor Lloyd.

The chief constituent of the oil appears to be a tetrahydrosesquiterpene with two double bonds, hence a chain hydrocarbon, which, however, is not straight chained.

By catalytic hydrogenation a new pentadecane, which also, of necessity, is not a straight chain hydrocarbon, has been obtained.

A preliminary suggestion as to the possible structure of the tetrahydrosesquiterpene under consideration may be found in the formula:



in which the pentyl radicles are of an iso character. Thus far, however, there is nothing to show that the molecule is as symmetric as the formula would indicate.

PRESERVATIVE EFFECT OF CERTAIN SUBSTANCES UPON CARREL-DAKIN SOLUTION.*

BY JOSEPH W. E. HARRISSON.

Much has been written about the preparation and manufacture of Carrel-Dakin Solution and the number of ways of obtaining a suitable product is legion. A general review in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION (Vol. 13, Nos. 1, 2, 3, 4) is undoubtedly the best available discussion on the history, manufacture and preserving of Dakin's Solution.

It occurred to the writer after reviewing the literature that some substance might be found that would not disturb the reaction of the solution and yet would stabilize the available chlorine. The addition of 0.5 per cent. of borax or 0.5 to 1.0 per cent. of sodium carbonate has been suggested (*Jour. Biol. Chem.*, 1919) and also the addition of sodium chloride (*Bulletin Cincinnati General Hospital*).

As to the stability of a 0.5 per cent. available chlorine solution we find according to Johannesen (*Arch. Pharm. Chem.*, 27) such a solution when kept at 0° C. in amber bottles will deteriorate over a period of 8 weeks at a loss of 0.006 per cent. chlorine per week, and when kept at 18° C. a loss of 0.011 per cent. chlorine per week.

According to an investigator in the department of pathology of the University of Edinburgh a 2.92 per cent. available chlorine solution kept in clear glass bottles assayed 2.62 per cent. available chlorine after a period of two years.

Borax, sodium bicarbonate, sodium chloride, benzosulphinide and acetanilide were selected as substances which might have a preservative effect upon Dakin's Solution. The Dakin Solution was made according to the Daufresne formula and also according to the disodium phosphate formula suggested by E. F. Kelly for inclusion in the Tenth Revision of the United States Pharmacopœia.

<i>Daufresne Formula.</i>		<i>Kelly Formula.</i>	
CaOCl	70.8 Gm.	CaOCl	40.0 Gm.
Na ₂ CO ₃	36.8 Gm.	Na ₂ HPO ₄	40.0 Gm.
NaHCO ₃	30.4 Gm.	Water	
Water		To make	1000 cc.
To make	2000 cc.		

The technic of manufacture being the usual procedure in both.

*Section on Practical Pharmacy and Dispensing, A. Ph. A., Buffalo meeting, 1924.