Summary

1. The substances designated by Butenandt and Hilgetag as toxicarol hydrate and dihydrotoxicarol hydrate have been shown, as a result of their formation in various ways, their reactions and their analyses, to be respectively 7-hydroxytoxicarol and 7-hydroxydihydrotoxicarol.

2. A summary of the reactions employed to demonstrate this statement is presented diagrammatically in the text.

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[CONTRIBUTION FROM THE MACKAY CHEMICAL LABORATORY OF THE UNIVERSITY OF NEVADA]

Essential Oil in Desert Plants. IV. Examination of the Oil of Tetrademia Glabrata*

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The occurrence and physical properties of the essential oil of *Tetrademia Glabrata* has been given in a previous article[1] and the poisonous properties have been studied by Fleming, Miller and Vawter[2] in connection with the livestock industry of the Lahontin Basin. Tetrademia is known locally as "Yellow Star Sage" and as "Spring Rabbit Brush." There are six varieties identified in the intermountain region of the United States, two of which, Glabrata and Spinosa (only oil from Glabrata being used in this study), are found growing in the vicinity of Reno, Nevada. During the flowering period several hundred pounds of the small branches, leaves and flowers were collected near the Black Panther Mine north of Reno, Nevada, and steam distilled according to the method described for the preparation of the oil of *Chrysothamnus Nauseosus*.[3] One charge of twenty kilograms yielded 61 g. of oil, or about 0.03%; one small selected sample of leaves yielded more than 0.1%. The colorless oil turns yellowish-brown on standing, and if exposed to the air becomes viscous. The oil has the pungent and somewhat characteristic odor of the plant. The plant contains a poisonous principle, the exact nature of which is undetermined, but range sheep die after feeding upon it in early spring. A petroleum ether extract of the leaves when fed to rabbits produced death but the volatile oil showed no toxic effect.

Two hundred grams of the redistilled volatile oil was extracted with dilute sodium carbonate according to the usual method and the extract acidified with dilute sulfuric acid, made basic with ammonium hydroxide, and silver nitrate added. Considerable silver oxide was formed, and from the filtrate a small yield of a salt which proved to be silver acetate, crystallized out.

The oil from which the fatty acids had been removed with sodium carbonate was now extracted with a 1% solution of sodium hydroxide. The extract thus obtained was washed with ether to remove adhering oil, made acid with sulfuric acid, and the resulting solution extracted with ether. When the ether evaporated there remained a very small yield of a thick, viscous, ill-smelling oil, which was extracted from the salt crystals with alcohol, the alcohol removed by evaporation, the residual oil treated with dilute ammonium hydroxide, and converted into the silver salt by the addition of silver nitrate. An organic silver compound was formed, which, after repeated crystallizations from alcohol, gave colorless crystals which darkened in the light, showed an indistinct melting point at 143°, and decomposed at 162°. The crystals, upon analysis, contained 14.77% silver, which indicated a compound with a molecular weight of about 616, if it is a pure substance and monovalent to silver. The sample was too small for a more detailed examination. After the removal of the acid and phenolic bodies the oil was fractionated three times at a pressure of 18 mm. using a Glinsky ball and bulb fractionating column. After the final fractionation the optical rotation was determined for each fraction, as had previously been done for the original oil.

Fraction	Dist. temp., °C.	Percentage of total	Color	Opt. rotation
Original oil			Yellowish	+17.60
No. 1	50 to 60	55	Colorless	+ 7.98
No. 2	60 to 70	20	Tinge of green	− 0.40
No. 3	70 to 80	10	Pale yellow	− .52
No. 4	80 to 105	5	Yellow	+ 2.10
No. 5	105 to 120	2	Brown	No test
Residue		8	Black	

It was found that fractions one and two could be distilled at ordinary pressure without apparent decomposition and they were further fractionated by this method. A sample which boiled at 149 to 156° at atmospheric pressure (649 mm.) was distilled over sodium and gave a small yield of pinene hydrochloride melting at 125°, and pinene nitrosochloride melting at 103°, indicating the presence of α-pinene. The metallic sodium combined with much of this fraction suggesting that there had been present in the original oil a considerable proportion of terpene derivatives,

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(1) Adams, THIS JOURNAL, **49**, 2895 (1927).

(2) Bulletin 104, University of Nevada Agricultural Experiment Station.

(3) Adams, THIS JOURNAL, **54**, 2448 (1932).

or that the pinene had been oxidized by repeated fractionations.

From the original fractions one and two there was obtained a part that distilled between 167 and 171° at atmospheric pressure. After this fraction had been digested over metallic sodium for an hour and then distilled, it was dissolved in an equal volume of dry ether and to this solution was added drop by drop a solution of bromine in dry ether as long as the bromine was completely absorbed. By completing the procedure for the preparation of limonene tetrabromide according to the method described by one of us,[3] an almost quantitative yield of striated fragile crystals of dipentene tetrabromide was obtained which, when recrystallized several times from ethyl acetate, melted sharply at 125°.

The original oil and each of the above four lower boiling fractions gave an aldehyde test with Schiff's reagent but all efforts to separate the aldehyde by fractional distillation either at ordinary or diminished pressure, failed. The concentration of the aldehyde in any sample obtained was so low that all efforts to separate the oxime or the semicarbazone failed.

Fraction four, which at 18 mm. distilled over at 80 to 105° and boiled under ordinary pressure at 260°, was tested for sesquiterpenes and found by the hydrochloride and iodine tests to contain a considerable amount of caryophyllene.

The residue from the original oil proved to be one of the most interesting fractions because when treated with absolute ethyl alcohol there was a considerable portion which failed to dissolve but which dissolved readily in petroleum ether, from which it may be again precipitated by absolute alcohol. When this compound was further purified by crystallization from carbon tetrachloride, there separated out pearly-white, scale-like crystals, which had a melting point of 64°. The substance was readily soluble in benzene, carbon disulfide and chloroform, and very slightly soluble in glacial acetic acid, methyl and ethyl alcohols. It was soluble in neither dilute hydrochloric acid, potassium hydroxide, concentrated sulfuric acid nor dimethyl sulfate, which showed it to be a member of the saturated paraffin series of hydrocarbons. The molecular weight, determined by the freezing point method of a solution in benzene, was 404.6. The melting point, molecular weights and general properties agree with those thus far recorded for nonacosane, $C_{29}H_{60}$. This substance was first obtained from beeswax by Gascard and Damoy[4] and later by Anderson[5] in the pollen of white flint corn. The sample obtained from beeswax had a melting point of 63.5°. Levene, West and Van der Scheer[6] prepared the even-numbered hydrocarbons from 22 to 34 carbon atoms and determined their melting points. By interpolation, they concluded that the melting point of nonacosane should be 67 to 68°. Hildebrand and Wachter[7] report that theoretically the melting point of nonacosane should be 64°. This prediction agrees completely with our results. Neither Levene nor Hildebrand had a sample of the compound for experimental verification. In the study of the roots of *Oenanthe Crocota*, Tutin[8] found in the water extract of the insoluble residue, the hydrocarbons tricontane and hentriacontane, which are the nearest members of the series to nonacosane. These were found during the study of the poisonous principle of the plant known as Poehl's enantho toxin, and were believed to be the result of the decomposition of the toxin by heat. This fact may indicate that the toxic principle of *Tetrademia Glabrata* furnishes the nonacosane through the decomposition of the toxic principle by the heat of distillation. This would agree with the conclusions arrived at by Miller[2] in his study of the toxic properties of this plant, *viz.*, that the poison of *Tetrademia* may be extracted by petroleum ether in the cold, but is destroyed by heat.

In addition to the nonacosane the residue from fractional distillation contained a small amount of a compound which was soluble in alcohol; a very small amount of the same substance separated from a sample of the original oil upon standing for two years in the laboratory. When dissolved in alcohol and allowed to stand, tetrahedral crystals separated which melted sharply at 155°. When finely pulverized they give the characteristic odor of the original plant. This compound was readily soluble in ethyl alcohol, ethyl acetate and acetic acid, slightly soluble in chloroform, carbon disulfide and ether; not soluble in ligroin, dilute potassium hydroxide nor dilute hydrochloric acid; readily soluble in concentrated sulfuric acid. These tests indicate an oxygen-containing compound. It gave no aldehyde test with Schiff's reagent but the formation of a hydrazone with phenylhydrazine was indicated, and with 2,4-dinitrophenylhydrazine a derivative was formed which melted at 132–133°, indicating a ketone, but none of the dinitrophenylhydrazones listed by Allen[9] correspond closely in melting points to this substance, indicating that it is a hitherto unobserved ketone. A complete study of the properties of this compound will not be possible until a larger sample is available.

Summary

This investigation has shown that the oil of *Tetrademia Glabrata* extracted by steam distillation contains nonacosane, a high melting hydrocarbon of the paraffin series; α-pinene, dipentene and caryophyllene, hydrocarbons of the terpene series; a small amount of free fatty acids; a phenol or combined acid of a high molecular weight; small amounts of an unidentified aldehyde and an unidentified ketone. The approximate percentage composition of the oil based upon the amount of the separate fractions is as follows: α-pinene 15%, dipentene 25%, aldehyde fractions 10%, caryophyllene 8%, nonacosane 7%, ketone 1%, colophonium and unidentified terpenes 34%.

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(4) Gascard and Damoy, *Compt. rend.*, **177**, 1442 (1923).

(5) Anderson, *J. Biol. Chem.*, **55**, 611 (1923).

(6) Levene, West and Van der Scheer, *ibid.*, **20**, 521 (1915).

(7) Hildebrand and Wachter, THIS JOURNAL, **51**, 2487 (1929).

(8) Tutin, *J. Pharm.*, **87**, 296 (1911).

(9) Allen, THIS JOURNAL, **52**, 2955 (1930).