THE CHEMICAL CONSTITUENTS OF

RAYLESS GOLDENROD

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By

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HISTORICAL INTRODUCTION

The poisonous nature of rayless goldenrod (Aplopappus heterophyllus Blake), which was established by the experiments of Marsh, Roe and Clawson (11), confers upon the plant considerable economic importance. The disease resulting from ingestion of the plant is known as trembles, or alkali disease, or to the medical profession as milksickness. Couch (4, 5) isolated from rayless goldenrod a substance that he proved to produce trembles in sheep. He considered this substance a pure compound, named it tremetol, and assigned it a formula. Lathrop (10), however, succeeded in further fractionating both a sample of tremetol which he isolated from rayless goldenrod by the general method of Couch and a sample which Couch himself supplied for comparison. It is thus from a toxicologic point of view that the chemistry of this plant has been studied: we have found no report of further chemical investigation.

Buehrer, Mason and Crowder (3), however, studied a related organism, Aplopappus hartwegi Blake. They reported the presence of unidentified but typical plant alkaloids, the presence of pyridine to the extent of 2% of the fresh plant, and the properties of an essential oil obtained from the fresh plant. This oil was found to consist entirely of one or more hydrocarbons of formula $C_{10}H_{18}$; two fractions were found to have the following properties: boiling-point (26 mm.) 72°C., 85°C.; density (25°C.) 0.7791, 0.8056; index of refraction 1.670, 1.663; specific rotation (30°C.) -0.40°, -0.55° .

In order to obtain sufficient tremetol for chemical study, Lathrop exhaustively extracted with 95% alcohol 225 pounds of dried, ground plant bodies of A. heterophyllus. The plant was collected while in bloom in August, 1938, near Hagerman, New Mexico. He removed the waxy materials from the alcohol extract by chilling and filtering through glass wool. He

reduced the filtrate to a small volume by distillation of the alcohol and subjected the residue to steam distillation to remove as much as possible of the essential oil and all traces of alcohol. He extracted the condensate with benzene, and upon evaporation of the benzene obtained an impure essential oil. This oil he subjected to distillation in the Hickman vacuum still. The temperature was maintained at 100°C. by means of a steam bath, and the pressure at approximately one mm. of mercury by means of a Hyvac oil pump. He so obtained no more than 10 ml. of a clear, light yellow oil of strong aromatic odor.

Since no chemical investigation of either the oil or the wax from this plant had been made, the gummy wax-impregnated glass wool and the essential oil were retained for study.

By the general procedure of Couch, which consisted in selective extraction with aqueous alcohol, saponification, and extraction with ether, Lathrop then isolated about 40 ml. of tremetol from the black gummy residue. A part of this tremetol he distilled in the Hickman vacuum still on the steam bath at 1 mm. pressure. He thus obtained four liquid fractions of unequal volume, collected over equal time periods, and a fifth dark brown resinous fraction as a residue. After about a week, fraction 2 deposited a few white crystals. All other fractions were then seeded, whereupon fractions 1 and 3 also yielded the same crystalline material. About 50 mgm. was collected and purified from petroleum ether.

A second portion of the tremetol he placed in the Hickman still. At 80°C. and 1 mm. pressure there distilled about 6 ml. of a light yellow oil of spicy odor; at 100°C. about 5 ml. of a somewhat more viscous liquid distilled; and at 170°C. there distilled a resinous orange solid without the spicy odor. A red tarry residue was removed from the still with ether and preserved for study.

The objectives of this work on some of the alcohol-soluble constituents of A. heterophyllus are threefold: (a) to continue the study of chemical and toxic properties of tremetol and fractions thereof; (b) to study the properties of the essential oil; and (c) to study the properties of the plant wax.

All of the source materials were supplied to the author by Lathrop (10).

EXPERIMENTAL

Part I. Tremetol and Fractions Thereof.

A. Animal Inoculation Experiments.

Healthy, approximately year-old guinea pigs were used as test animals in the hope of establishing the toxicity of the individual fractions of tremetol. Guinea pigs were chosen because they were on hand, they had been previously used, and the quantity of any fraction of tremetol was so small that even had sheep been available no reliable results could have been obtained. Each animal was weighed frequently, although almost daily fluctuations in weight are observed in healthy animals.

Luer syringes of 10 ml. capacity and 18 or 20 gauge hypodermic needles were used in injecting the olive oil solutions of the two resinous fractions: (a) the glassy orange solid that distilled at 100°-170°C. at 1 mm. pressure, and (b) the reddish tarry residue. Olive oil was chosen as a menstruum for these resinous fractions because it is non-toxic and dissolves the fractions satisfactorily. The pure virgin olive oil was sterilized in pressure bottles in the autoclave at 115°C. for 20 minutes. A control animal received 4.0 ml. of sterile olive oil.

A standard "tuberculin" syringe of 1 ml. capacity was used to inject the liquid fractions: (a) undistilled tremetol isolated by Lathrop, and (b) the mother liquid of the fraction from which crystals first deposited.

All syringes and needles used were chemically cleaned, wrapped separately in heavy brown paper and baked in an oven at 150°C. for 2 1/2 hours. All injections were made intraperitoneally after thorough swabbing of the area with alcohol. Injections were continued until the supply of tremetol was exhausted. Table I shows dates of injection, amounts of inocula, and clinical findings.

Table I.* Cumulative Record of Animal Inoculation Experiments.

Number of animal	Identity of Inoculum	Clinical Findings* Amounts of inocula, in g. or ml. Dates of injections 1939									
		3-18	4-1	4-9	4-14	4-27	5-2	5-3	5-10	5-20	7-12**
1.	A	0.1	n	n	n	n	n	n	n	n	
2.	A	0.2	n 0.3	s	n 0.2	n 0.1	n 0.1	n 0.1	n 0.1	n	
3.	B	0.1	n	n	n	n 0.2	s 0.2	s 0.3	s 0.2	n	n
4.	B	0.3	n 0.3	d***							
5.	C	4.0	n								
5.	D			n 0.3	s	n	n	n	n	n	
6.	E			0.2	n 0.3	s	s 0.1	s 0.1	s 0.2	s 0.2	n
7.	F					0.2	n 0.1	n 0.1	n 0.2	n 0.1	n

* Explanation of abbreviations in the table:
Inocula: A--distillate at 100°C. (1 mm.) after crystals had been removed.
B--orange resinous solid distilling from 100°-170°C. (1 mm.).
C--sterile olive oil.
D--essential oil.
E--tarry residue from vacuum fractionations of tremetol.
F--whole tremetol isolated by Lathrop.
Clinical findings: n--normal irritability.
s--recognizably subnormal irritability.
d--death

** Clinical findings the day the animals were destroyed.

*** Saprophytic decomposition had advanced too far for necropsy.

On July 12, animals 3, 6 and 7 were destroyed for necropsy, because animals 3 and 6 had shown clinical symptoms of poisoning, and animal 7 was expected to show organologic symptoms.

Animal 3. Weight at beginning of test period, 670 g., at end of test period, 610 g. Viscera and peritoneal cavity normal. No evidence of cirrhosis or fatty degeneration found in liver.

Animal 6. Weight at beginning, 650 g., at end of test period, 580 g. Peritoneal cavity normal. Liver considerably enlarged, but no evidence of generalized cirrhosis or fatty degeneration found. Several localized abscesses present. Other viscera normal.

Animal 7. Weight at beginning of test, 700 g., at end of period, 690 g. No pathological findings.[1]

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1. The author is indebted to Dr. Lewis H. Moe, of the Department of Bacteriology, Physiology and Veterinary Science, for his assistance in interpreting the conditions found at necropsy.

B. Chemical Investigation of Tremetol and its Fractions.

The white crystals.

The purified crystals obtained by Lathrop, although preserved in a tightly stoppered flask, underwent decomposition in 2 months, as evidenced by their acquiring a yellow color and a sticky consistency and the presence of peroxides.

Detection of peroxides. A few of the crystals were dissolved in carbon tetrachloride. A few ml. of 15% potassium iodine solution was added and the mixture warmed slightly. The carbon tetrachloride layer took on a purple tinge. A control tube containing no crystals, but otherwise identical to the test tube showed no tinting of the carbon tetrachloride layer.

Microanalysis and molecular weight. Dr. Ogden Baine (1) of Southwestern University, who made the analyses for carbon and hydrogen and the determinations of molecular weight (Rast), also noted evidence of decomposition. Analysis: Calculated for $C_{14}H_{14}O_4$: molecular weight 246.1, carbon 68.22%, hydrogen, 5.69%; calculated for $C_{15}H_{15}O_4$: molecular weight 259.1, carbon 69.08%, hydrogen 5.87%; calculated for $C_{16}H_{16}O_3$: molecular weight 256.1, carbon 75.34%, hydrogen, 6.25%. Found: molecular weight 259, 262; carbon 66.46%, 67.95%, 68.54%; hydrogen 5.89%, 5.57%, 5.83%.

Examination with the polarizing microscope.[2] Because of the state of disrepair of the available polarizing microscope, most of the usual determinations could not be made. The crystals were found to be acicular, anisotropic, and pleochroic. The index of refraction was found to be 1.59 through the short axis. An average of 12 readings gave 55.6° as the angle of extinction parallel to the long axis.

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2. The author is indebted to Professor Ray L. Six, of the Department of Geology for determining the physical constants of the crystals.

Permanganate oxidation. Since Couch had obtained a crystalline acid by permanganate oxidation, it was wondered if the crystalline material was not the compound from which the acid was derived. Accordingly, a few crystals were heated under reflux for half an hour with alkaline potassium permanganate. The mixture was then cooled, acidified with sulfuric acid, and again refluxed for half an hour. The manganese dioxide was destroyed by adding sodium bisulfite to the cooled mixture. This was then repeatedly extracted with ether. Evaporation of the ether yielded no product.

Hydrogenation with phosphorus and hydrogen iodide (9, 13). A small amount of the crystalline material, about 0.1 g. red phosphorus, and about 0.7 ml. hydriodic acid (specific gravity 1.7) were sealed in a heavy-walled test tube. Two such tubes were prepared and placed in the bomb furnace that was heated slowly to 250°C. and held at that temperature for 13 hours. One tube was shattered by the internal pressure. The other tube was cooled, opened with caution and the contents washed out with water and ether. The water was extracted repeatedly with ether. The combined ether layers were shaken with dilute aqueous alkali. The water was removed, acidulated and again extracted with successive portions of ether. Evaporation of the combined ether layers yielded only a small amount of a yellow oily material of a phosphorous odor.

Dehydrogenation with selenium (13). A few crystals and about 1.0 g. grey selenium were sealed in a thick-walled test tube which was then placed in the bomb furnace. The temperature was slowly raised and maintained at 260°C. for 18 hours. The tube was then removed, cooled and opened with caution. The glass was broken into small pieces, and the grey-black contents of the tube pulverized. This mass of glass fragments and blackish powder was heated under reflux with successive 25 ml.

portions of ether. Evaporation of the combined ether solutions yielded only a few yellowish crystals which in the desiccator melted to an oil of disagreeable odor.

The liquid fraction, distilling at 100°C. (1 mm.) freed of crystals.

The yellow oily mother liquid from which the crystals previously described were removed yielded no derivatives in the following reactions: oxidation by alkaline permanganate, hydrogenation with phosphorus and hydriodic acid, and dehydrogenation with selenium.

The fraction of Couch's tremetol distilling at 100°C. (1 mm.).

This yellow oily liquid formed no derivative when subjected to hydrogenation with red phosphorus and hydriodic acid, and only a small amount of a yellow oil when dehydrogenated with selenium. Oxidation with alkaline permanganate yielded no acid.

Lathrop's undistilled tremetol.

Molecular weight determination (Rast) for tremetol isolated by Lathrop gave the value 260. Attempts to derive the acid of Couch by alkaline permanganate oxidation, and to dehydrogenate with selenium and to hydrogenate with phosphorus and hydriodic acid were unsuccessful.

Part II. The Essential Oil.

The essential oil which was obtained by Lathrop in the manner already described was used in this study. Determinations of some physical and chemical properties were made. The values obtained are shown in Table II.

Table II. Properties of the Essential Oil.

Boiling-point (Siwoloboff)	238°C.
Density (22°C.)	0.9942
Index of refraction (20°C.)	1.4940
Molecular weight (Rast)	264, 292 (278)
Specific rotation (20°C.) (0.50 g. oil in 10 ml. alcohol observed in a 10 cm. tube.)	$[\alpha]_D^{22°}$ + 9.54°
Saponification value (8)	0.12
Iodine value (8)	103.5, 107.5

From the average iodine number, and the average molecular weight, it was calculated that each molecule of oil added 2.31 atoms of iodine. From the average iodine value, and the lower molecular weight found, it was calculated that each molecule of oil absorbed 2.02 atoms of iodine. This indicates one active double bond in each molecule.

The very low saponification value precludes the presence of appreciable free acid.

Analysis was made for free alcoholic hydroxyl by acetylation in the presence of pyridine (12). Based on the assumptions that the molecular weight was 278 (as determined above), and that each alcohol molecule contained but one hydroxyl group, calculations indicated the presence of about 3% such alcohol molecules.

Analysis was made for aldehydic or ketonic carbonyl by a modified hydroxylamine method (6). Based on assumptions analogous to those for alcohol analysis, calculations indicated the presence of about 2.2% carbonyl compound.

Fractionation. In order to obtain the individual components relatively uncontaminated, a portion of the oil was subjected to qualitative vacuum fractionation. For this purpose a pyrex fractionating tube was constructed similar to the one described by Benedetti-Pichler and Schneider (2). The bulb of the tube was immersed in a bath of glycerol. At a pressure of about 1 mm., about 3 grams of the oil was divided into four liquid fractions of approximately equal volume, and an amber resinous fraction that was not volatile at the boiling point of glycerol. After removal of each fraction with a micropipet from the L-shaped tip of the tube, the area was cleansed with pipe-cleaners. This fraction was removed from the bulb with ether. The physical constants which were determined on these fractions are shown in Table III.

Table III. Physical Constants of Essential Oil Fractions.

Fraction	Boiling-point °C. (Siwoloboff)	Refractive index, 22°C. (Abbe)	Molecular Weight (Rast)
1.	237	1.4888	218, 225
2.	248	1.4955	270, 244
3.	228?	1.5040	238, 241
4.	275	1.5161	322, 335
5.	--	--	473

Chemical investigations of these fractions yielded the following results: All fractions turned a carbon tetrachloride solution of iodine brown, indicating the presence of an oxygen-containing compound. Fractions 1 and 2 absorbed bromine readily from carbon tetrachloride, but no solid bromides were isolable. Fractions 3 and 4 did not absorb bromine from carbon tetrachloride. Fractions 1 and 2 reacted violently, with darkening and thickening, even in the cold, when dry HCl gas was introduced. No solid hydrochloride was isolated. Fractions 1 and 2 reacted vigorously with sodium.

Saponification:

The mixture obtained from the determination of the saponification value was made alkaline and placed on a water bath to evaporate the alcohol. The water insoluble products rose to the top and were pipetted off. This oil, of brown color and of an odor reminiscent of the original oil, was subjected to qualitative fractional distillation at 1 mm. in the same manner as before. There were so isolated four liquid fractions of approximately equal volume and a small amount of resinous material which was removed from the still with difficulty. Some physical constants were determined for the liquid fractions. They are shown in Table IV.

Table IV. Physical Constants of Fractions of Water-insoluble Saponification Products of Essential Oil.

Fraction	Boiling-point °C.* (Siwoloboff)	Refractive index 25°C. (Abbe)
1.	238	1.4954
2.	235?	1.4985
3.	230?	1.500
4	268	1.5058

*During the determinations of the boiling-points, the contents of the capillary tubes underwent darkening and the volumes diminished.

Attempts to obtain phenylhydrazones and solid hydrochlorides of fractions 1, 2 and 3 were fruitless.

The water layer from which the water-insoluble saponification products were removed was acidified and extracted repeatedly with isopropyl ether. Upon evaporation of the ether, there remained a small amount of an amber oil possessing an odor strongly suggestive of spoiled citrus fruits. In a semi-micro Siwoloboff determination, this oil boiled at 119°C. with much decomposition and with escape of volatile material.

Part III. The Wax.

The gummy wax-impregnated glass wool used by Lathrop to collect the precipitate from the cooled alcohol extract of rayless goldenrod was the source of the wax used in this work. The mass of glass wool was of a green color so intense that it appeared black. Solutions of this material in benzene and other organic solvents were bright green.

Extraction of the crude wax from the glass wool.

To obtain the crude wax, this mass of glass wool was exhaustively extracted with benzene, the solvent in which the material showed greatest solubility. The hot benzene extract was filtered through several folds of gauze to remove plant fibers and fragments of glass wool and the benzene was distilled from the filtrate until the mass was thick and gummy. This residue was poured into shallow dishes and the rest of the benzene evaporated by leaving for several days in an oven at about 70°C. The resulting benzene-free mass was moldable and of a green-black color.

Extraction of the wax from the crude material.

The following methods were applied in attempts to obtain the wax free from pigment.

Elutriation. Since chlorophyl possesses a greater solubility in water than wax, an attempt was made to precipitate the wax, free from pigment, by washing repeatedly a small amount of the crude material in a large volume of boiling water. Although the water was greenish in color, indicating some removal of pigment, no appreciable change in color was observed when the treated material was dried in the oven.

Crystallization. Since waxes and chlorophyl show solubility differences, attempts were made to purify the wax by crystallizing from ethyl alcohol, methyl alcohol, ethyl acetate, butyl acetate, acetone, sulfuric ether,

isopropyl ether, ligroin, and chloroform. In each case the resulting wax was but slightly less green in color. Mixtures of solvents were tried but as little success was attained as with the single solvents.

Ligroin extraction. It is the usual practice to extract wax from plant bodies with common organic solvents and use it without further purification. Accordingly, a small amount of the crude waxy material was placed in the porous cup of a small Soxhlet continuous extractor provided with sufficient petroleum ether. After extraction for 3 hours, the petroleum ether was evaporated. The wax so extracted was no less deeply pigmented than the untreated material.

Charcoal decolorization. Decolorizing charcoal was degassed and somewhat activated by heating strongly under reduced pressure. A relatively large amount of the charcoal was added to a dilute benzene solution of the crude wax and the resulting mixture refluxed for half an hour. Filtration and subsequent evaporation of the filtrate yielded a wax of a green color as intense as that of the crude substance. Similar vain attempts were made to decolorize with charcoal in alcohol and acetone.

Distillation in vacuum. A small amount of crude wax was placed in a large Erlenmeyer flask and heated for 8 hours on a water bath under reduced pressure. Abundant and persistent foaming that completely filled the flask with bubbles discouraged making an attempt to distill the wax in the Hickman still.

Chromatographic adsorption by powdered sugar. Powdered sugar has been reported as a satisfactory adsorbent for chlorophyl. A tube similar to the one employed by Tswett (14) was packed with commercial powdered sugar. A petroleum ether solution of the crude wax was drawn through the tube by slight suction. The results were negative.

Saponification of the chlorophyl. Waxes are saponified by boiling 3 hours with alcoholic alkali; chlorophyl is easily saponified at room temperature with alcoholic alkali. Dr. James E. Webster and Professor Orville C. Schultz suggested that some application of these facts be made. Accordingly, about 400 ml. of isopropyl ether was saturated with a few grams of crude wax. To this solution about 10 ml. of 30% alcoholic potassium hydroxide was added. The resulting mixture was shaken vigorously in a separatory funnel for 20 minutes. Then about 500 ml. of a nearly saturated aqueous solution of sodium nitrate was added (for salting out) and separation of layers allowed to occur. It was often necessary to wait overnight for distinct layers to form, owing to the formation of persistent emulsions. The deep green water layer was repeatedly extracted with isopropyl ether, and the ethereal layers combined. The ether extract was greenish yellow in color. After removal of the ether by distillation, a relatively hard wax of greenish yellow to brownish color remained. This substance melted about 65 or 70°C. This fractional saponification was repeated until about 25 grams of wax was obtained.

Purification of the ether extracted waxy material.

To obtain the wax in a higher state of purity, the following methods were attempted.

Vacuum distillation. It was thought that the waxy substance, now freed of most of the chlorophyl and perhaps other substances which thermally decompose with the evolution of gaseous products, could be distilled under reduced pressure. For this purpose a horizontal still was constructed of large heavy-walled pyrex test tubes. The apparatus was patterned after that of Freudenberg (7). A small amount of the greenish-yellow wax was desiccated for 2 hours in an oven at 110°C. and then placed in the still and the pressure lowered to about 1 mm. Heat was applied by means of a

bunsen burner until the wax was melted. On the internal water-cooled condenser there appeared a distillate of dirty yellow color. After a short period of uneventful distillation, large bubbles began to form and hopelessly to contaminate the condensate. The residue was brown in color and of a disagreeable odor, as was the condensate. Moderate heating for 2 hours in this still did not reduce the amount of bubble formation.

Crystallization. Since vacuum distillation did not prove satisfactory, purification by crystallization in alcohol was undertaken. The wax was found to be partially soluble, yielding a residue which melted to an oil in the hot alcohol. The hot alcohol solution was decanted from the residue and chilled to about -10°C. in an acetone-dry-ice bath. The waxy precipitate was collected on linen filter pads in a Buchner funnel surrounded by acetone chilled to about -10°C. A portion of the alcohol-soluble wax was recrystallized three times from alcohol. The alcohol solution was chilled by means of an ice-salt mixture. Each time the oil appeared as a residue. These residues were collected and found to be recrystallizable from acetone containing a small amount of alcohol. The alcoholic mother liquors yielded a yellow wax upon evaporation.

Determination of Physical Constants of the Wax Fractions.

Molecular weight determinations (Rast) were made of the wax extracted with isopropyl ether from the saponification mixture, of the wax obtained by three recrystallizations from alcohol, and of the acetone-recrystallized residue. Table V shows that recrystallization from alcohol lowered both the melting-point and molecular weight of the wax, and that the molecular weight of the oily residue was greater than that of the alcohol-soluble portion. Table V also records the appearance and the melting point of each fraction after drying in the oven at 110°C. for an hour.

Table V.

Fraction	Origin	Appearance	Melting-point °C.	Molecular Weight (Rast)
1.*	Ether extraction of differential saponification mixture.	Greenish yellow; semi-soft	ca. 80	470
2.	One recrystallization of fraction 1 from alcohol.	Tannish, solid	ca. 75	
3.	Three recrystallizations of fraction 1 from alcohol.	Tan, brittle, glassy	ca. 60	404
4.	Alcohol-insoluble, acetone-soluble residues collected while obtaining fraction 3.	Greenish, hard	ca. 90	485
5.	Evaporation of alcoholic mother liquors.	Soft flaky wax	ca. 65	

* Crystallization from ethyl acetate did not change the melting-point.

Chemical investigation of the wax fractions.

All the materials were soluble or at least dispersed in warm concentrated sulfuric acid, for filtration through sintered glass plates yielded no residual alkanes.

The Hanus iodine value (8) of the wax extracted with isopropyl ether from the fractional saponification mixture was found to be 5.4; saponification values (8) of the same fraction were found to be 6.05 and 8.13.

The low saponification values indicate a relatively high concentration of nonsaponifiable substances. In order to obtain these substances, the saponification mixture was made alkaline and extracted repeatedly with isopropyl ether. Evaporation of the solvent yielded a tannish waxy substance partially soluble in alcohol. After recrystallization from alcohol,

this wax melted at about 80°C. The alcohol-insoluble fraction was crystallized from hexane. It melted at about 70°C. Neither of these substances responded to the Liebermann-Burchard test for sterols.

DISCUSSION OF RESULTS

I.

The results of animal experiments confirm the observation of Couch that guinea pigs show a typical and inconsistent susceptibility to poisoning by tremetol. Our work suggests that the most toxic fraction of tremetol is the residue, buththis cannot be stated positively because guinea pigs were used. Future biological essays should be made of tremetol soon after extraction. Sheep are known to be satisfactory experimental animals, but large amounts of tremetol would be required. Thus, experimentation to determine the susceptibility of rats or mice seems advisable.

The crystalline material isolated from tremetol fractions is evidently labile; in a 2 months the crystals became yellow and gummy, undoubtedly because of peroxidation. Molecular weight and carbon and hydrogen analyses of the yellow crystals indicates a compound of formula $C_{14}H_{14}O_4$ or $C_{15}H_{15}O_4$ but oxidation probably increased the oxygen content. It is interesting to note that Couch's analyses of the mixture he called tremetol indicated a formula of $C_{16}H_{22}O_3$. At least one atom of oxygen in the molecule of the crystalline substance is ketonic, for Lathrop prepared a semicarbezone and a phenylhydrezone. Analyses of the crystals, both for composition and structure, should be made as soon as possible after isolation in order to obtain valid information.

It was thought that the crystals might represent the substance from which Couch prepared an acid by oxidation with alkaline permanganate. Failure to obtain this acid, as well as failures to obtain parent hydrocarbon nuclei by dehydrogenation with selenium and hydrogenation with phosphorus and hydriodic acid, may be attributed to the use of two small amounts of substance or failure otherwise to obtain optimum reaction conditions. Couch did not reveal the details of his permanganate oxidation

and it is possible that the technic used in our work was unsuitable. Hydrogenation with phosphorus and hydriodic acid is best carried out in acid of specific gravity 2.; that available for our work was of specific gravity 1.7.

II.

The essential oil is believed to consist mainly of high molecular weight hydrocarbons. The similarities of boiling-point, refractive index and molecular weight of the essential oil to those of its saponification products support this belief. The hydrocarbons are thought to be sesquiterpenes or polyterpenes since the refractive indices, boiling points and molecular weights are too high for simpler ones. Analyses indicated the presence of small amounts of alcohols and carbonyl compounds. Ethers may be present. From the iodine number and molecular weight, it was calculated that each molecule of oil absorbed one mole of iodine, indicating the presence of one active double bond. The discrepancy between our findings and those of Buehrer, Mason and Crowder is attributed to the fact that they isolated the essential oil from fresh plants, whereas we used air-dried material which had lost its more volatile components.

III.

The common practice of studying plant waxes after merely extracting with common organic solvents is considered unsatisfactory since the saponifiability and lability of chlorophyl would markedly alter chemical determinations. Even though solubility differences were greater, simple extraction would not serve, probably because surface forces operate to hold the pigment tenaciously to the wax. However, chlorophyl is about as soluble in most organic solvents as are waxes.

Charcoal adsorption of the chlorophyl is not successful, probably because the charcoal is as efficient an adsorbent for the wax as for the chlorophyl, or because the wax adsorbs chlorophyl very readily.

Vacuum distillation proved unsatisfactory probably because the plant pigments thermally decomposed, even at reduced pressures, to evolve gaseous materials. Only a molecular still could be expected to serve for distillation of heavy wax molecules. In such stills the distance from evaporating surface to condenser is too small to prevent gross contamination of distillate by foaming of the liquid.

The method of differential saponification used here to obtain the wax free from chlorophyl is not thought to be new, although no report of its use has been found.

The wax is shown to consist largely of unsaponifiable compounds, probably alcohols and hydrocarbons of relatively high molecular weights. Sterols are absent. Appreciable changes in composition, however, may have taken place during even the mild saponification. This would be especially true if free acids were present.

SUMMARY

1. Guinea pigs were used as test animals to establish the toxicity of various tremetol fractions. It is believed that the fractions of higher molecular weight are more toxic, but this cannot be stated with certainty, owing to the refractory nature of the test animals.

2. Microanalysis yielded the formula $C_{14}H_{14}O_4$ for the crystals isolated from tremetol by Lathrop. The oxygen content, however, is unreliable, because of peroxidation which had taken place.

3. Some standard methods of investigation of structure (hydrogenation, dehydrogenation and oxidation) were applied to the crystals and liquid fractions of tremetol with consistently negative results.

4. Most of the usual constants were determined for an essential oil of Aplopappus heterophyllus. These investigations show the presence of small amounts of alcohols, carbonyl compounds and esters. It is believed that the oil consists mainly of sesquiterpenes or polyterpenes and that ethers may be present.

5. The method of differential saponification used here for extracting the wax is only partially satisfactory. The wax so extracted was found to consist largely of unsaponifiable material (alcohols and hydrocarbons), accompanied by a small amount of esters.

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