STUDIES ON THE CHEMICAL CONSTITUENTS OF RAYLESS GOLDENROD (APLOPAPPUS HETEROPHYLLUS)

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The poisonous nature of rayless goldenrod, *Aplopappus heterophyllus* Blake, which was established by the experiments of Marsh, Roe and Clawson (1926), 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 (1929, 1930) 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 even assigned it a formula. Lathrop (1939), however, succeeded in fractionating both a sample of tremetol that he isolated from rayless goldenrod by the general method of Couch and also 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 (1939) studied a related species, *Aplopappus hartwegi* (Gray) Blake. They reported the composition of the plant in detail; it contains much rubbery resin, some unidentified but typical plant alkaloids, pyridine (the most unusual component), and an essential oil. This oil, obtained from the fresh plant by steam distillation, had the formula $C_{10}H_{12}$, and two fractions had the following constants: boiling point (26 mm) 72°, 85°; density (25°) 0.7791, 0.8056; index of refraction 1.670, 1.663; specific rotation (30°) —0.40°, —0.55°.

Since the material for the present study was supplied by Lathrop, his procedure will be outlined. *A. heterophyllus* was collected by mowing it while in bloom in August, 1938, near Hagerman, New Mexico; after sun-drying, it was baled and shipped to Stillwater. Here 225 pounds of it were ground and exhaustively extracted with 95% alcohol; the chilled alcohol extract deposited a wax which was filtered out. Most of the alcohol was removed by distillation, and the rest, along with the essential oil, by steam distillation. This essential oil was extracted with benzene and distilled at about 1 mm pressure in a Hickman vacuum still; about 10 ml of a light yellow aromatic oil was so obtained.

By the general procedure of Couch, which consisted in selective extraction of the tarry residue with aqueous alcohol, saponification, and extraction with ether, Lathrop then isolated about 40 ml of tremetol. Part of this he distilled in the Hickman still on the steam bath at 1 mm pressure, and thereby obtained several liquid fractions over successive time intervals. After standing about a week several fractions deposited a white highly crystalline material, which was isolated and recrystallized from petroleum ether; about 100 mg were secured. Lathrop found this substance to melt sharply at 84.0-84.5°, to be unsaturated toward bromine, and to yield a phenylhydrazone, m.p. 164°, and a semicarbazone, m.p. 203-204°; no solid oxime could be obtained.

When the present study was begun, some two months later, these crystals had decomposed perceptibly even in a stoppered flask; they had become yellow and sticky, and peroxides were proved present by the iodide test. A microanalysis was nevertheless arranged.[1]

Found: C 66.5, 67.9, 68.5; H 5.9, 5.6, 5.8; mol. wt. (Rast) 259, 262. Calcd. for $C_{14}H_{14}O_4$: C 68.2, H 5.7, mol wt. 246; for $C_{15}H_{18}O_4$, C 69.1, H. 5.9, mol. wt. 259.

Although it is not possible to decide between these formulas, it is interesting to compare them to the formula found by Couch for tremetol: $C_{16}H_{22}O_5$. There was too little material to permit biological assay of toxicity, but it is not believed that these crystals are toxic or represent anything but a terpenoid ketone that was not removed with the essential oil.

Examination of the crystals with a polarizing microscope gave the following information:[2] they were acicular, anistropic, and pleochroic; index of refraction through the short axis 1.59; angle of extinction parallel to the long axis 55.6°,

The following standard methods in structural proofs failed to yield positive results with either the crystals or the distillate whence they separated: oxidation with alkaline permanganate solution, hydrogenation with red phosphorus and hydriodic acid in a sealed tube, and dehydrogenation with selenium in a sealed tube.

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[1] Microanalyses and molecular weight determinations by Dr. Ogden Baine, Southwestern University, Memphis, Tenn., arranged by Dr. Paul Arthur of this department. Our thanks are due these men for their assistance.

[2] We are indebted to Prof. Ray L. Six, Department of Geology, for these determinations.

Values obtained for some physical and chemical properties of the vacuum-distilled essential oil are: boiling point (Siwoloboff) 238°; density (22°) 0.9942; index of refraction (Abbe, 20°) 1.4940; molecular weight (Rast method) 264, 292; specific rotation (20°, D line; 0.50 g oil in 10 ml ethanol in a 10 cm tube) + 9.54°; saponification value (Jamieson 1932) 0.12: iodine value (Jamieson 1932) 103.5, 107.5. From the iodine value and the molecular weight, it was calculated that each molecule of oil contained one double bond, on the average. By quantitative acetylation in the presence of pyridine (Smith and Bryant 1935) and use of 278 as average molecular weight, it was similarly possible to deduce that only about 3 per cent of the molecules could be even monohydroxy alcohols or phenols. A similar procedure for carbonyl compounds by the hydroxylamine method of Dermer, Wilson, Johnson, and Dermer (1941) indicated the presence of about 2 per cent monoaldehydes or —ketones.

To gain further information, a vacuum fractionation of about 3 g of the oil was performed in a fractionating tube like that of Benedetti-Pichler and Schneider (1931). Succeeding fractions showed increased boiling points, refractive indices, and molecular weights, but none gave any evidence of being a pure compound. All fractions contained oxygenated compounds (iodine solution test), and the earlier ones reacted readily with sodium, bromine, or dry hydrogen chloride, though not so as to give crystalline derivatives.

Intraperitoneal injection of the whole essential oil into guinea pigs produced no symptoms of poisoning; this is in agreement with Couch's statement that the essential oil is non-toxic.

All these data indicate the essential oil to be mainly a mixture of sesquiterpenes or polyterpenes. The discrepancy between our findings and those of Buehrer, Mason, and Crowder (1939) is due to the fact that they isolated the oil from fresh plants, whereas we used air-dried material that had lost its more volatile components.

The crude green-black wax was freed from mechanical impurities by dissolving it in hot benzene, filtering, and evaporating the benzene. The removal of chlorophyll proved far more difficult; indeed, it appears that in most studies of plant waxes the wax has simply been extracted with an organic solvent and the chlorophyll content disregarded. Recrystallization from a variety of solvents failed to accomplish decolorization, even when charcoal was used. High-vacuum distillation of the wax in the Hickman still was prevented by uncontrollable foaming, which was probably due to decomposition of impurities.

Sucrose has been reported as a satisfactory adsorbent for chlorophyll in chromatographic analysis (Morton 1938: 186-193), but a petroleum ether solution of the crude wax remained colored when passed through a tube packed with commercial powdered sugar.

Chlorophyll is easily saponified at room temperature with alcoholic alkali, whereas waxes require 3 hours' boiling with this reagent for the same reaction.[3]

In a fractional saponification procedure, the wax was dissolved in isopropyl ether, shaken for 20 minutes with a little concentrated alcoholic alkali, and afterward isolated from the ether layer. This produced a relatively hard greenish-yellow or brown wax melting at about 80°, of average molecular weight (Rast) 470. About 25 g of wax were so purified.

[3] The possible utility of this difference in rate of reaction was suggested by Professors J. E. Webster and Orville C. Schultz.

As further purification, vacuum distillation was again attempted, and again abandoned because of frothing. Three recrystallizations of the partly purified wax from ethanol gave a brittle tan product melting at about 60°, molecular weight 404. This decrease in molecular weight might be due to alcoholysis but is probably better attributed to concentration of the simpler molecules by the alcohol. The alcohol-insoluble residue, recrystallized from acetone, showed a melting point of 90° and a molecular weight of 485.

All wax fractions were soluble or at least dispersed in warm concentrated sulfuric acid, for filtration through sintered glass plates yielded no residual alkanes. The Hanus iodine number (Jamieson 1932) of the wax from the partial saponification procedure was 5.4, and two saponification values found were 6.1 and 8.1. These low saponification values indicate a relatively high concentration of nonsaponifiable substances, but no sterols could be detected by the Liebermann-Burchard test. It may be concluded that the wax consists largely of hydrocarbons and alcohols of high molecular weight, although it must be remembered that even the mild differential saponification used in purifying the wax may have altered its ester content, and certainly must have introduced phytyl alcohol from the chlorophyll.

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