

819. *The Chemistry of Gum Labdanum. Part I. Some Acidic Constituents.*

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The acid fraction from gum labdanum has been examined. *p*-Anisic acid, β -phenylpropionic acid, and two new diterpene acids have been isolated, in addition to straight-chain fatty acids and long-chain hydroxy-acids. One of the diterpenes, labdanolic acid, is a bicyclic hydroxy-acid, $C_{20}H_{36}O_3$. The other is a bicyclic compound, $C_{20}H_{32}O_3$, which contains an $\alpha\beta$ -unsaturated ketone grouping.

SPANISH gum labdanum, which is obtained from *Cistus ladaniferus* ("rock-rose")¹ by boiling the wood of the bramble in water and collecting the resin from the surface,^{2,3,4,5} has been little investigated in the past. It is dark brown to black and sold as pitch-like blocks. Considerable quantities are produced in Spain (50 tons a year⁶) and it is used as a fixative in perfumery. Hence the use also of names "black amber" and "black balsam." The gum consists mainly of water, resin, and impurities such as earth⁶ and contains only little volatile oil (1—2%). The few past investigations have been concerned with the characteristics of this oil^{7,8} and even here very little work of a truly chemical nature has apparently been carried out. Probably the most important is that of Masson⁸ who isolated acetophenone and 2:2:6-trimethylcyclohexanone. We have commenced an investigation of a commercial sample of the gum and in this paper describe the isolation of some of its acidic constituents.

The gum, which contained 1.6% of steam-volatile material, was only partially soluble in light petroleum, giving an almost colourless extract from which a crystalline saturated paraffin, m. p. 63—64°, was obtained by chromatography. The melting point and analytical data suggest that this is very similar to that of the broom-wax paraffin, m. p. 64—65°, isolated by Musgrave, Stark, and Spring,⁹ who suggest that their material is a mixture of approximately 60% of *n*-nonacosane and 40% of *n*-hentriacontane.

Most of the gum was soluble in ether, and the ether-soluble material was separated into acidic, phenolic (and acidic), and neutral fractions by extraction with potassium hydrogen carbonate solution and sodium carbonate solution. The examination of the phenolic and the neutral fractions is not yet complete.

The acidic portion was esterified with ethereal diazomethane and the resulting methyl esters were taken up in light petroleum and separated by chromatography into five broad fractions (*A*, 10%; *B*, 25%; *C*, 25%; *D*, 13%; and *E*, 20%) of increasing polarity.

The infrared spectrum of fraction *A* in "Nujol" was typical of that of the methyl esters of straight-chain saturated fatty acids, e.g., it showed the typical band progression¹⁰ between 1180 and 1295 cm^{-1} . By repeated crystallisation crystals, m. p. 43—44°, were obtained with analytical figures best in agreement with the formula $C_{22}H_{44}O_2$ or $C_{23}H_{46}O_2$. The latter corresponds to methyl docosanoate. The melting point, however, is best in agreement with that (m. p. 46—47°)¹¹ of the methyl ester of the lower homologue, methyl eicosanoate. It is probable that the substance obtained from gum labdanum is a mixture of the methyl esters of these two acids.

Fraction *B* was separated by chromatography into a number of fractions, which formed three groups. Hydrolysing each group with aqueous methanolic potassium hydroxide gave

¹ Montero, *Ion*, 1951, **12**, 565 (*Chem. Abs.*, 1952, **46**, 6331).

² Howes, "Vegetable Gums and Resins," Chronica Botanica Co., Waltham, Mass., 1949, p. 158.

³ Howes, *Econ. Bot.*, 1950, **4**, 307.

⁴ Geradin, *Bull. Sci. pharmacol.*, 1919, **26**, 289 (*Chem. Abs.*, 1919, **13**, 2104).

⁵ Igolen, *Parfums France*, 1937, **15**, 164 (*Chem. Abs.*, 1937, **31**, 7602).

⁶ Paltre, *Ion*, 1952, **12**, 132 (*Chem. Abs.*, 1952, **46**, 10546); see also Emmanuel, *Arch. Pharm.*, 1912, **250**, 111.

⁷ Anon, *Roure-Bertrand Fils, Sci. Ind. Bull.*, [4], 1920, No. 2, p. 25 (*Chem. Abs.*, 1921, **15**, 2149).

⁸ Masson, *Compt. rend.*, 1912, **154**, 517.

⁹ Musgrave, Stark, and Spring, *J.*, 1952, 4393.

¹⁰ Jones, McKay, and Sinclair, *J. Amer. Chem. Soc.*, 1952, **74**, 2575.

¹¹ Adams and Dyer, *J.*, 1925, **127**, 70.

dihydrocinnamic acid, *p*-anisic acid, and a new acid, $C_{20}H_{32}O_3$. The infrared spectrum of the last acid had a band at 1668 cm.^{-1} indicative of an $\alpha\beta$ -unsaturated ketone grouping. This was confirmed by its ultraviolet absorption spectrum with a maximum at 2385 \AA (ϵ 11,470). After allowance for one double bond and two carbonyl groups the formula of this new unsaturated keto-acid indicates that it is bicyclic. In view of the isolation also of a bicyclic diterpene hydroxy-acid (see below) it is probable that the acid is a diterpene. If so, it is the first diterpene containing an $\alpha\beta$ -unsaturated ketone grouping to be isolated. From the ultraviolet data the double bond of the $\alpha\beta$ -unsaturated system must have two alkyl substituents.

Fraction *C* was mainly composed of the methyl ester of a new hydroxy-acid, $C_{20}H_{36}O_3$, named labdanolic acid. The infrared spectrum showed the presence of methoxycarbonyl and hydroxyl groups and gave no indication of the presence of a double bond. Hydrolysis gave the free acid which was characterised as its *cyclohexylamine* salt. The equivalent weight confirmed the formula $C_{20}H_{36}O_3$ which, after allowance for the carbonyl group, indicated that labdanolic acid was bicyclic. The presence of twenty carbon atoms suggested that it was a diterpene. This is shown to be correct in the following paper.

The infrared spectra of fractions *D* and *E* suggest that they are composed of long-chain hydroxy-acids. They have not been examined in detail.

So far no evidence of the presence of triterpenes in gum labdanum has been obtained. In Nature triterpenes and diterpenes do not appear to be found together and the results with gum labdanum are in line with this. Triterpenes and steroids have oxygen at $C_{(3)}$ and are formed by the cyclisation of squalene initiated by an oxidative electrophilic reagent.¹² Diterpenes, on the other hand, have no oxygen at $C_{(3)}$ * and their formation appears to be initiated in quite a different manner. It may well be that the enzyme systems leading to steroids and triterpenes on the one hand and to diterpenes on the other are incompatible.

EXPERIMENTAL

Rotations were determined for $CHCl_3$ solutions at room temperature. *M. p.*s were determined on a Kofler block and are corrected. The alumina used for chromatography had an activity I—II unless otherwise stated. Light petroleum refers to the fraction with *b. p.* 60—80°. Unless otherwise stated infrared data refer to carbon disulphide solution.

Steam-distillation of Gum Labdanum.—Gum labdanum (135 g.) was steam-distilled until 4 l. of distillate had been collected. Ether-extraction of the distillate yielded a pleasant-smelling oil (2.0 g.), distillation of which gave fractions (each 300 mg.), *b. p.* 125—130° (bath-temp.)/14 mm., n_D^{20} 1.4838, and *b. p.* 135—150° (bath-temp.)/14 mm., n_D^{18} 1.4933. The residue was non-volatile.

Extraction of Gum Labdanum with Light Petroleum.—The gum (60 g.), a plastic material at room temperature, became brittle at 0°. It was powdered and treated with light petroleum (*b. p.* 40—60°; 400 c.c.) for 2 days with occasional shaking. After filtration, evaporation afforded a pale yellow gum (6.8 g.) which was absorbed from light petroleum (40 c.c.) on alumina (200 g.). Elution with light petroleum yielded a crystalline fraction (0.75 g.), *m. p.* 59—63°. Crystallisation of this material from ethyl acetate afforded a saturated paraffin as plates, *m. p.* 63—64° (Found: C, 85.4, 85.2; H, 14.8, 14.9. Calc. for $C_{29}H_{60}$: C, 85.2; H, 14.8. Calc. for $C_{31}H_{64}$: C, 85.25; H, 14.75%). Continued elution of the column with solvents of increasing polarity yielded only intractable gums.

Separation of the Acidic Fraction of Gum Labdanum.—Gum labdanum (4 kg.) was taken up in ether (10 l.) and the insoluble material, which causes formation of emulsions during the extractions, was removed by centrifugation. The black ether solution was then washed with potassium hydrogen carbonate solution until the washings were colourless. The total extract was then acidified to pH 5 with 50% aqueous acetic acid, and the liberated acids (X) were taken up in ether. Removal of the ether under reduced pressure gave the crude acids (1 kg.) as a viscous black tar. Further extraction of the ethereal solution of the gum with sodium hydroxide

* The numbering system used is the same as that used for triterpenes and steroids (see following paper).

¹² Tchen and Bloch, *J. Amer. Chem. Soc.*, 1956, **78**, 1516, and the refs. cited therein.

solution gave a phenolic fraction (2.2 kg.). The extracted ether solution was then pale yellow and was evaporated to give the neutral fraction as a pale yellow solid (700 g.).

The acids (X) were dissolved in ether (1 l.) and methanol (1 l.) and treated with an ethereal solution of diazomethane until evolution of nitrogen ceased. The solution of methyl esters was then washed twice with ice-cold *N*-potassium hydroxide (500 c.c.) and once with saturated brine. After removal of the solvents the esters were extracted with boiling light petroleum (1 l.) three times. The combined extracts were made up to a total volume of 8 l. with light petroleum and kept overnight. The solution was then decanted from the precipitated tar and evaporated under reduced pressure to give a clear red viscous syrup (760 g.). This was chromatographed in 150 g. portions, being adsorbed from light petroleum on alumina (3 kg.) which had been deactivated with 3% of 10% acetic acid. The column was eluted as follows: light petroleum (1 l.) gave fraction *A* (15 g.); light petroleum–benzene (3 : 1; 6 l.) gave fraction *B* (ca. 40 g.); benzene (19 l.) gave fraction *C* (ca. 40 g.); ether (5 l.) gave fraction *D* (ca. 20 g.); and methanol (3 l.) gave fraction *E* (ca. 30 g.).

Fraction A. This consisted of the methyl esters of normal long-chain acids. The infrared spectrum was typical of such esters. Infrared absorption (melt): bands at 717 and 728 (split), 1466, 1476, and 1747 cm^{-1} . Repeated crystallisation from chloroform–methanol gave plates, m. p. 43–44° (Found: C, 77.95, 77.85; H, 12.95, 13.05. Calc. for $\text{C}_{23}\text{H}_{46}\text{O}_2$: C, 77.9; H, 13.1. Calc. for $\text{C}_{21}\text{H}_{42}\text{O}_2$: C, 77.25; H, 12.95%).

Fraction B. A portion of fraction *B* (14 g.) was adsorbed from light petroleum on alumina (600 g.) which had been deactivated with 10% aqueous acetic acid (18 c.c.). Elution with the solvent as indicated gave fractions: (a) With 200 c.c. portions of light petroleum: (1) nil, (2) 0.04 g., (3) 0.02 g., (4) 0.07 g., (5) 0.66 g., (6) 0.61 g., (7) 0.41 g., (8) 0.28 g., (9) 0.23 g., (10) 0.27 g., (11) 0.17 g., (12) 0.16 g., (13) 0.16 g., n_D^{25} 1.4932. (b) With 400 c.c. portions of light petroleum: (14) 0.28 g., (15) 0.26 g., n_D^{15} 1.4970, (16) 0.22 g., n_D^{15} 1.4970, (17) 0.17 g., (18) 0.14 g., n_D^{15} 1.4990, (19) 0.13 g., (20) 0.12 g. (c) With 400 c.c. portions of light petroleum–benzene (4 : 1): (21) 0.20 g., (22) 0.14 g., (23) 0.12 g. (d) With 400 c.c. portions of light petroleum–benzene (2 : 1): (25) 0.02 g., (26) 0.50 g., n_D^{15} 1.4942, (27) 0.65 g., (28) 0.62 g., (29) 0.51 g., (30) 0.45 g., (31) 0.36 g. (e) With 400 c.c. portions of light petroleum–benzene (1 : 1): (32) 0.54 g., (33) 0.40 g., (34) 0.55 g. (f) With 400 c.c. portions of benzene: (35) 0.82 g., (36) 0.37 g., (37) 0.23 g., (38) 0.18 g. (g) With 400 c.c. benzene–ether (20 : 1): (39) 0.54 g.

From their infrared spectra and refractive indices fractions (14)–(21) appeared to be identical and homogeneous. Hydrolysis of them with aqueous-methanolic potassium hydroxide gave β -phenylpropionic acid, m. p. 42–46°, which from light petroleum (b. p. 40–60°) afforded needles, m. p. and mixed m. p. 48–49° (Found: C, 71.65; H, 6.4. Calc. for $\text{C}_9\text{H}_{10}\text{O}_2$: C, 72.0; H, 6.7%). The infrared spectrum was identical with that of an authentic sample.

Fractions (22)–(27) appeared to be identical and homogeneous and were hydrolysed with aqueous-methanolic potassium hydroxide to *p*-anisic acid, m. p. 176–183°, which on repeated crystallisation from aqueous methanol formed needles, m. p. and mixed m. p. 177–184° with a change in crystalline shape at 117° (Found: C, 63.25; H, 5.2. Calc. for $\text{C}_8\text{H}_8\text{O}_3$: C, 63.15; H, 5.3%). The infrared spectrum was identical with that of *p*-anisic acid.

Fractions (28)–(35) appeared to be identical and homogeneous and were hydrolysed with aqueous-methanolic potassium hydroxide to give an *acid*, m. p. 94–110°. Crystallisation from methanol–water (6 : 4) gave prisms (2.0 g.), m. p. 114–115°, $[\alpha]_D +13.5^\circ$ (*c*, 1.7) (Found: C, 74.85, 74.95; H, 10.1, 10.2. $\text{C}_{20}\text{H}_{32}\text{O}_3$ requires C, 74.95; H, 10.05%). Light absorption max. in EtOH at 2385 Å (ϵ 11,470). Infrared absorption bands at 1668 and 1703 cm^{-1} .

Fraction C. Fraction *C* (40 g.) when kept became semisolid and on repeated recrystallisation from light petroleum (b. p. 40–60°) (cooling to –5°) gave *methyl labdanolate* (24 g.), white needles, m. p. 73–74°, $[\alpha]_D -8.0^\circ$ (*c*, 2.0) (Found: C, 74.35, 74.35; H, 11.3, 11.4%; sap. val., 336, 340. $\text{C}_{21}\text{H}_{38}\text{O}_3$ requires C, 74.5; H, 11.3%; sap. val., 338.5). Infrared absorption bands at 1365, 1386, 1738, and 3560 cm^{-1} .

Fractions D and E. A preliminary examination of the infrared spectra of these fractions (bands at 1738 and 3480 cm^{-1}) suggested that they were mainly composed of the methyl esters of long-chain hydroxy-acids.

Labdanolic Acid (8 α -Hydroxylabdan-15-oic Acid).—Methyl labdanolate (630 mg.) was hydrolysed under reflux for 1 hr. with a solution from sodium (410 mg.) in ethanol (12 c.c.) and water (0.2 c.c.). The resulting *acid* was a gum, $[\alpha]_D -7^\circ$ (*c*, 0.98) (Found: C, 73.5; H, 11.15. $\text{C}_{20}\text{H}_{36}\text{O}_3$ requires C, 74.0; H, 11.2%). The acid (580 mg.) was treated in hot ethyl acetate (5 c.c.) with cyclohexylamine (0.3 c.c.). On cooling the cyclohexylamine salt separated. When crystallised from ethyl acetate to constant rotation it formed needles, m. p. 115–135° (dependent upon the

rate of heating), $[\alpha]_D - 12^\circ$ (c , 1.4) (Found : C, 73.5; H, 11.65; N, 3.5. $C_{26}H_{49}O_3N$ requires C, 73.7; H, 11.65; N, 3.3%).

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