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TRITERPENOIDS IN THE BARK OF ELDER (*SAMBUCUS NIGRA*)

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Abstract—The non-saponifiable fractions from petroleum and ether extracts of the bark of *Sambucus nigra* contain α-amyrenone, α-amyrin, betulin, oleanolic acid and β-sitosterol. This is the first recorded instance of α-amyrenone occurring in Nature.

THE only comprehensive examination of elder bark (*Sambucus nigra*) reported in the literature is that of Zellner,[1] who obtained ceryl alcohol and three other components which were unidentified. We have repeated Zellner's work and from the non-saponifiable portion of the light petroleum extract have isolated α-amyrenone, α-amyrin, betulin and β-sitosterol. α-Amyrenone has not previously been found in nature. The bark which had been extracted with petroleum, was subsequently extracted with ether and furnished a brown tar which on alkaline hydrolysis gave acidic and neutral fractions. The acidic fraction contained oleanolic acid while the neutral fraction yielded further quantities of α-amyrin, betulin and β-sitosterol. All the compounds isolated were identified by comparison with the relevant authentic specimens.

EXPERIMENTAL

Specific rotations were measured in chloroform solution, ultraviolet spectra in ethanol, and i.r. spectra in chloroform. Petrol refers to light petroleum (b.p. 60–80°).

Examination of Petrol Extract

Dry crushed elder bark (1·7 kg) was extracted (17 hr) with hot petrol in a Soxhlet apparatus. Removal of the solvent gave a greenish-brown resin (44·5 g) which was refluxed (5 hr) in benzene (500 ml) and methanol (1750 ml) with potassium hydroxide (75 g) in water (250 ml). Working up through ether gave the non-saponifiable material (19·0 g) which was dissolved in petrol–benzene (4:1, 1500 ml) and chromatographed on alumina (600 g). Successive elution with petrol–benzene mixtures, benzene and benzene–ether (4:1) yielded the following fractions:

(*a*) Colourless gum (2·8 g), (*b*) crystalline solid (1·1 g), m.p. 81-122°, (*c*) orange gum (1·3 g), (*d*) orange gum (3·6 g), (*e*) low melting solid (2·5 g), (*f*) crystalline solid (1·7 g), m.p. 225-227° and (*g*) crystalline solid (1·9 g), m.p. 120-135°.

Fractions (*a*) and (*c*) did not yield crystalline material and showed no triterpenoid characteristics (i.r. and Liebermann–Burchardt test).

α-Amyrenone. Fraction (*b*), which showed strong carbonyl absorption in the infrared, was recrystallized from chloroform–methanol and then from aqueous acetone to give colourless blades (302 mg), m.p. 124-126°, $[\alpha]_D$ +106·5° (*c*, 1·6), λ_{max} 2040 Å (ε, 5600), ν_{max} 1701 cm^{-1}, identical (mixed m.p. and infrared spectrum) with authentic α-amyrenone.

α-Amyrin. Fraction (d) after standing (6 weeks) in methanol slowly deposited an orange gum and clusters of needles, m.p. 161-175°. The crystals were acetylated with acetic anhydride–pyridine (1:1) at 100° for 1 hr, and a solution of the acetylated product (1·3 g) in petrol (50 ml) was chromatographed on alumina (30 g). Elution with the same solvent (1400 ml)

[1] J. ZELLNER, *Monatsh.* 47, 151 (1926).

afforded fractions which crystallized from chloroform–methanol as plates (645 mg), m.p. 224-225°, $[\alpha]_D$ +80° (*c*, 1·6), λ_{max} 2050 Å (ϵ, 3800), identical with an authentic sample of α-amyrin acetate. Hydrogenolysis of the acetate (192 mg) in ether (125 ml) with lithium aluminium hydride (200 mg) and crystallization of the product from aqueous acetone gave the alcohol as needles, m.p. 188-190°, $[\alpha]_D$ +90° (*c*, 1·2), identical with authentic α-amyrin. Oxidation of the alcohol (50 mg) in dry pyridine (1·2 ml) with the chromium trioxide–pyridine complex (100 mg in 1 ml) at 20° for 17 hr gave the corresponding ketone as plates (from aqueous acetone), m.p. 125-127°, $[\alpha]_D$ +115° (*c*, 1·0), identical with α-amyrenone.

Ceryl alcohol. Several recrystallizations of fraction (*e*) from aqueous acetone yielded ceryl alcohol, plates, m.p. 67-71° $[\alpha]_D \pm 0°$, which gave no colour in the Liebermann–Burchardt test. The corresponding acetate, m.p. 59-60°, $[\alpha]_D \pm 0°$ was obtained by acetylation.

Betulin. Fraction (*f*) yielded betulin (needles from chloroform–methanol), m.p. 256-259°, $[\alpha]_D$ +16° (*c*, 1·2), which showed i.r. and mixed m.p. identity with authentic betulin. Acetylation of the material and filtration of the acetylated product in petrol through alumina gave betulin diacetate as needles (from chloroform–methanol), m.p. and mixed m.p. 223-224°, $[\alpha]_D$ +22° (*c*, 1·7), λ_{max} 2070 Å (ϵ, 2150).

β-Sitosterol. Fraction (*g*) on recrystallization from chloroform–methanol gave β-sitosterol as plates, m.p. and mixed m.p. 138-140°, $[\alpha]_D$ −31° (*c*, 1·4), λ_{max} 2040 Å (ϵ, 2300). Acetylation of the alcohol furnished β-sitosteryl acetate, m.p. and mixed m.p. 128-130°, $[\alpha]_D$ −38° (*c*, 1·2).

Examination of the Ether Extract

Oleanolic acid. The defatted bark was extracted (83 hr) with ether. Evaporation of the solvent left a brown tar (18 g) which was refluxed (5 hr) in benzene (200 ml) and methanol (1150 ml) with potassium hydroxide (75 g) in water (150 ml). The solution was diluted with water (1·5 l.) before being concentrated (to about 2 l.) and extracted with ether. The interfacial solid (770 mg), which separated was collected, washed, dried, and dissolved in the minimum of hot methanol. The solution was acidified with concentrated hydrochloric acid and after 3 days the crystalline precipitate was collected and recrystallized from chloroform–methanol to give oleanolic acid (needles), m.p. 310-312°, $[\alpha]_D$ +73° (*c*, 0·9), λ_{max} 2060 Å (ϵ, 3600), identical in all respects with an authentic specimen. Treatment of the acid with an excess of ethereal diazomethane afforded methyl oleanolate as needles (from methanol), m.p. 198-200°, $[\alpha]_D$ +72·5° (*c*, 1·6) which showed mixed m.p. and i.r. identity with authentic ester. Acetylation of the methyl ester gave methyl oleanolate acetate as plates (from chloroform–methanol), m.p. and mixed m.p. 221-223°, $[\alpha]_D$ +71° (*c*, 0·5).

The ether solution containing the non-saponifiable fraction was concentrated and yielded a further quantity (85 mg) of oleanolic acid.

α-Amyrin, Betulin and β-Sitosterol

The above ether solution on evaporation to dryness gave the non-saponifiable fraction (3·2 g) which was chromatographed on alumina (120 g) from petrol–benzene (4:1, 500 ml). Development of the column with petrol–benzene, benzene, and benzene–ether mixtures gave fractions which were individually acetylated and chromatographed on alumina to give α-amyrin acetate (841 mg), m.p. and mixed m.p. 222-224°, $[\alpha]_D$ +77° (*c*, 1·6), ceryl acetate, m.p. 59-60°, $[\alpha]_D \pm 0°$, β-sitosteryl acetate (43 mg), m.p. and mixed m.p. 130-132°, $[\alpha]_D$ −36° (*c*, 0·9), and betulin diacetate, m.p. and mixed m.p. 223-224°, $[\alpha]_D$ +31° (*c*, 1·4).

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