842. *Non-saponifiable Constituents of Spanish Broom.*

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The non-saponifiable material from the concrète (extract) of Spanish broom has been investigated and the following constituents identified : a mixture of higher paraffins, a mixture of higher aliphatic alcohols, α-, β-, and δ-amyrin, lupeol, β-sitosterol, *n*-octadecane-1 : 18-diol, and *n*-hexacosane-1 : 26-diol. In addition, two unidentified alcohols have been isolated.

EXTRACTS of Spanish broom (*Spartium junceum* L.) are widely used in the French perfume industry. Little is known of their constituents (Naves and Mazuyer, " Les Parfums Naturels," Paris, 1939), the only recorded investigation being that of Sabetay and Igolen (*Ann. Chim. anal.*, 1946, **27**, 224), which was mainly concerned with the alkali-soluble fractions.

The extract available to us was obtained by digestion of the flowers with cold light petroleum followed by low-temperature removal of the solvent. The total extract (concrète) was an oily wax (0·09—0·18% of the dried flowers). Treatment of it with cold ethanol gives the alcohol-soluble " absolue " (approximately 40% of the concrète) and an insoluble wax.

The non-saponifiable matter from the broom wax is only partly soluble in cold ether. The ether-insoluble fraction was separated by chromatography into a saturated paraffin and a saturated alcohol. Chibnall, Piper, Pollard, Williams, and Sahai (*Biochem. J.*, 1934, **28**, 2189) have shown that the higher paraffins present in plant waxes are usually inseparable mixtures of straight-chain hydrocarbons which contain an odd number of carbon atoms and that naturally occurring higher alcohols are usually inseparable mixtures of homologues each containing an even number of carbon atoms. Consideration of the physical and analytical data suggests that the broom-wax paraffin is a mixture containing approximately 60% of *n*-nonacosane and 40% of *n*-hentriacontane. The broom-wax alcoholic fraction is probably a mixture of alcohols with a mean chain-length of C_{24}.

The ether-soluble fraction from broom wax was separated by chromatography into a mixture of paraffins, a mixture of alcohols similar to that obtained from the ether-insoluble fraction, a mixture of triterpenoid alcohols, from which a small amount of β-amyrin was isolated, and β-sitosterol.

The broom absolue contains a high proportion (46%) of free fatty acids. After removal of these the neutral fraction was hydrolysed, and the non-saponifiable material, which constituted approximately 20% of the absolue, was separated by chromatography on alumina. Elution of the chromatogram with benzene gave an alcohol, $C_{22}H_{46}O$ or $C_{22}H_{44}O$, m. p. 65—66°, $\alpha = 0°$, which is saturated to tetranitromethane and has no selective absorption of high intensity above 2200 Å. Since it contains one active hydrogen atom yet fails to afford acyl derivatives, and was recovered unchanged after attempted oxidation, it is probably a tertiary alcohol.

Elution of the chromatogram with benzene–ethanol gave a mixture of alcohols, chromatography of the benzoates from which gave a mixture of α- and β-amyrin benzoates, separated by crystallisation; a second benzoate fraction, which was not readily purified, was converted into the corresponding acetate which after chromatography was identified as δ-amyrin acetate [olean-13(18)-en-2"β"-yl acetate]. δ-Amyrin has not previously been isolated directly from natural sources so far as we are aware; it has been prepared from β-amyrin (Ruzicka and Jeger, *Helv. Chim. Acta*, 1941, **24**, 1236; Ruzicka, Jeger, and Norymberski, *ibid.*, 1942, **25**, 457), from lupenyl acetate by acid isomerisation, and also by a similar isomerisation of β-amyrenone followed by reduction of the carbonyl group (Ames, Halsall, and Jones, *J.*, 1951, 450). Since the last two methods require strongly acidic conditions, it is unlikely that the δ-amyrin isolated from broom has been produced from β-amyrin or from lupeol during the isolation procedure. A third benzoate fraction from the chromatogram was identified as lupenyl benzoate.

Continued elution of the original chromatogram with benzene–ethanol gave a fraction separated by crystallisation into a C_{24}-alcohol mixture and an alcohol, $C_{16}H_{32}O$. The latter contains one active hydrogen (Zerewitinoff) and its infra-red absorption spectrum shows bands at 3214 and 1064 cm.$^{-1}$. The acetate of the alcohol could not be obtained crystalline. The presence of one double bond was established by microhydrogenation.

The last fraction to be eluted with benzene–ethanol readily afforded β-sitosterol. The remaining material was held tenaciously by the alumina; partial separation was effected by mechanical division of the column. From the upper part of the chromatogram, *n*-octadecane-1 : 18-diol was isolated. Identity was established by comparison of the natural diol with a specimen prepared by lithium aluminium hydride reduction of ethyl *n*-octadecane-1 : 18-dioate and by oxidation of the natural diol to *n*-octadecane-1 : 18-dioic acid (cf. *Nature*, 1951, **168**, 298). From the lower part of the column, a second diol, $C_{26}H_{54}O_2$, was isolated. It was identified as *n*-hexacosane-1 : 26-diol by synthesis. Electrolysis of a mixture of sebacic acid and two molecular proportions of ethyl hydrogen sebacate gave a readily separable mixture of ethyl *n*-octadecane-1 : 18-dioate and ethyl *n*-hexacosane-1 : 26-dioate. Reduction of the last ester with lithium aluminium hydride gave *n*-hexacosane-1 : 26-diol, identical with the diol from broom.

Experimental

The alumina used for chromatography was Grade II (Brockmann). The light petroleum employed was a fraction of b. p. 60—80°, unless otherwise stated. Specific rotations were measured in chloroform (unless otherwise specified) in a 1-dm. tube at room temperature (18—20°).

Broom wax.

Paraffin and C_{24}-Alcohol Mixtures.—A solution of the dark brown wax (97 g.) in ethanolic potassium hydroxide (7·5%; 1150 ml.) was heated under reflux for 7 hours, then diluted with water (4 l.) and extracted with ether. On concentration and cooling, the extract deposited a waxy solid (fraction A; 53·9 g.; m. p. 58—64°). Evaporation of the ethereal mother-liquors gave a brown waxy solid (fraction B; 12·6 g.). A solution of fraction A (2·5 g.) in benzene (200 ml.) and light petroleum (100 ml.) was passed through alumina (20 × 3 cm.), and the chromatogram was developed with the same solvent mixture (1000 ml.) to give fraction (i) (2·1 g.) as a waxy solid, m. p. 62°. Continued washing with the same solvent (1800 ml.) gave fraction (ii) (0·32 g.) as a waxy solid, m. p. 75°. Crystallisation of fraction (i) from ethyl acetate gave a higher paraffin mixture as plates, m. p. 64—65° (1·6 g.) (Found: C, 85·4; H, 14·6. Calc. for $C_{29}H_{60}$: C, 85·2; H, 14·8. Calc. for $C_{31}H_{64}$: C, 85·2; H, 14·8%). Piper, Chibnall, Hopkins, Pollard, Smith, and Williams (*Biochem. J.*, 1931, **25**, 2072) record m. p. 64·8—65° for

a mixture of $C_{29}H_{60}$ (60%) and $C_{31}H_{64}$ (40%). Crystallisation of fraction (ii) from ethyl acetate gave the C_{24}-alcohol mixture (0·22 g.) as brittle rods, m. p. 75·5° (Found: C, 81·3; H, 14·0. $C_{24}H_{50}O$ requires C, 81·3; H, 14·2%). The mixture of acetates obtained by heating the alcohol under reflux with acetic anhydride separated from light petroleum (b. p. 40—60°) as plates, m. p. 59—60° (Found: C, 79·0; H, 13·2. Calc. for $C_{26}H_{52}O_2$: C, 78·7; H, 13·2%).

β-Amyrin and β-Sitosterol.—A solution of fraction B (12·1 g.) in benzene (250 ml.) and light petroleum (b. p. 40—60°; 250 ml.) was filtered through alumina (20 × 3 cm.). The column was washed with the same solvent mixture (1300, 2200, and 400 ml.) to give fractions, (i) a waxy solid (5·7 g.) m. p. 63°, (ii) a solid (3·4 g.), m. p. approx. 140°, and (iii) a sticky solid (0·15 g.). Extraction of the lower portion of the chromatogram with hot benzene–ethanol (9 : 1) yielded fraction (iv), a sticky solid (0·8 g.), m. p. approx. 130°. Crystallisation of fraction (ii) from ethyl acetate gave the C_{24}-alcohol mixture (1·2 g.) as needles, m. p. 75·5° alone or mixed with the specimen described above. Concentration of the mother-liquors from this crystallisation yielded a solid (1·65 g.), m. p. 147—169°, which gave a magenta Liebermann–Burchard colour. Part of this material (0·14 g.) was acetylated under reflux with acetic anhydride (3 ml.) for 3 hours. The product, crystallised successively from acetic acid and ethanol, yielded β-amyrin acetate (0·02 g.) as long needles, m. p. 235°, $[\alpha]_D$ +78° (*c*, 1·6 in benzene) (Found: C, 81·9; H, 11·4. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%); it did not depress the m. p. of an authentic specimen of β-amyrin acetate, m. p. 236°, $[\alpha]_D$ +79° (*c*, 1·0 in benzene). Crystallisation of fraction (iv) from methanol gave β-sitosterol as plates, m. p. 136·5°, $[\alpha]_D$ −34·4° (*c*, 0·6).

Broom absolue.

Alcohol $C_{22}H_{46}O$ *or* $C_{22}H_{44}O$.—A solution of the dark brown absolue (194 g.) in ether (1200 ml.) was extracted with potassium hydroxide solution (4%; 5 × 200 ml.), washed with water, and dried (Na_2SO_4). Removal of the solvent gave a dark brown oil (105 g.) with a raisin-like odour which was heated under reflux with ethanolic potassium hydroxide (5%; 1 l.) for 2 hours on the water-bath. The bulk of the ethanol was removed under reduced pressure, and replaced simultaneously by water (2 l.). The mixture was extracted with ether, and the solvent evaporated, to give the non-saponifiable fraction (40 g.) as a pleasant-smelling, clear red, viscous oil. A solution of this fraction (40 g.) in hot benzene (1 l.) was cooled and the solid separating (0·38 g.; later identified as *n*-octadecane-1 : 18-diol) collected. The benzene solution was filtered through alumina (35 × 6 cm.). Benzene (3·8 l.) eluted fraction 1 (yellow oil; 4·6 g.). Continued washing with benzene (6·8 l.) gave fraction 2 (brown wax; 2·6 g.). Benzene–ethanol (9·8 l.; 200 : 1) eluted fraction 3 (pale yellow solid, m. p. 140°; 10·4 g.). Washing with benzene–ethanol (8 l.; 200 : 1) yielded fraction 4 (waxy solid, m. p. 50°; 8·1 g.), and continued washing with the same solvent mixture (4 l.) gave fraction 5 (m. p. 120°; 4·1 g.). Fractions 6 (brown oil; 3·0 g.), 7 (partly crystalline; 1·1 g.), 8 (partly crystalline; 1·6 g.), and 9 (brown oil; 0·5 g.) were obtained from zones occupying 4, 10, 8, and 13 cm. respectively of the column, by extraction with boiling benzene–ethanol (3 : 1).

Crystallisation of fraction 2 from ethyl acetate and then from methanol gave the *alcohol* as clusters of needles (0·6 g.), m. p. 65—66° [Found: C, 81·4; H, 13·8; active H, 0·36%; *M* (Rast), 318. $C_{22}H_{46}O$ requires C, 80·9; H, 14·2; active H, 0·31%; *M*, 327. $C_{22}H_{44}O$ requires C, 81·4; H, 13·7%; *M*, 325].

β- and α-Amyrin Benzoates.—Fraction 3 was treated with pyridine (15 ml.) and benzoyl chloride (3·5 ml.) at 100° for 4 hours. A solution of the mixed benzoates (12 g.) (isolated in the usual manner) in light petroleum (b. p. 40—60°; 300 ml.) was filtered through alumina (5 × 30 cm.). Fraction (i) (3·2 g.; m. p. 190°) was obtained by washing the chromatogram with light petroleum–benzene (19 : 1; 2·2 l.). Continued washing with the same solvent mixture (1·3 l.) gave fraction (ii) (1·3 g.; m. p. 150°). Fraction (iii) (0·8 g.; m. p. 190°) was obtained by washing the chromatogram with a mixture of the same solvents (9 : 1; 800 ml.), and final washing with light petroleum–benzene (6 : 1; 1·2 l.) gave fraction (iv) (2·3 g.; m. p. 220°). Fractional crystallisation of fraction (i) from benzene–acetone gave β-amyrin benzoate (690 mg.) as rectangular plates, m. p. 235°, $[\alpha]_D$ +97° (*c*, 1·2), which did not depress the m. p. of an authentic specimen (Found: C, 83·8; H, 10·4. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·25%), and α-amyrin benzoate (120 mg.) as large prisms, m. p. 198°, $[\alpha]_D$ +92° (*c*, 1·1), undepressed in m. p. when mixed with an authentic specimen (Found: C, 83·3; H, 10·0%).

δ-Amyrin Acetate.—Fraction (ii) was crystallised from benzene–acetone, and the crude benzoate (1·25 g.; m. p. 184°) hydrolysed with ethanolic potassium hydroxide (4%; 50 ml.) under reflux for 10 hours. The product, isolated in the usual manner, was heated with acetic anhydride (10 ml.) and pyridine (5 ml.) for 6 hours at 100°. Crystallisation of the acetate from

chloroform–methanol gave β-amyrin acetate {382 mg.; m. p. 235°; $[\alpha]_D$ +79° (*c*, 1·3)}. The mother-liquor was evaporated, the residue dissolved in light petroleum, and the solution filtered through alumina (2 × 20 cm.). Washing with the same solvent (300 ml.) eluted a fraction (112 mg.; m. p. 194—205°). Continued washing with light petroleum (800 ml.) gave a fraction which on crystallisation from chloroform–methanol gave δ-amyrin acetate (265 mg.) as hexagonal plates, m. p. 206·5—207·5°, $[\alpha]_D$ −30° (*c*, 1·3) (Found: C, 81·9; H, 10·9. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). A specimen prepared according to Ruzicka and Jeger's method (*loc. cit.*) had $[\alpha]_D$ −35°, m. p. 208·5—209·5° alone or mixed with the specimen described above. Light absorption (in ethanol): ε_{2050} 4200, ε_{2100} 6600, ε_{2150} 5000, ε_{2200} 3500, ε_{2250} 1750. These extinction coefficients are in good agreement with those for authentic δ-amyrin acetate.

Lupenyl Benzoate.—Crystallisation of fraction (iv) of the benzoate chromatogram from benzene–acetone gave lupenyl benzoate (0·7 g.) as plates, m. p. 265°, $[\alpha]_D$ +60·4° (*c*, 2·0), which did not depress the m. p. of an authentic specimen (Found: C, 83·6; H, 10·3. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·25%).

Alcohol, $C_{16}H_{32}O$.—Fraction 4 from the broom absolue chromatogram was crystallised from methanol, to yield a solid (3·5 g.; m. p. 50—56°). On slow cooling of a solution of this solid in methanol the C_{24}-alcohol mixture separated as fine needles (0·36 g.), m. p. 71—73°, undepressed when mixed with the specimen described above (Found: C, 81·4; H, 14·2. Calc. for $C_{24}H_{50}O$: C, 81·4; H, 14·1%). The mother-liquor from the C_{24}-alcohol mixture slowly deposited a second crop of fine needles which after recrystallisation from the same solvent gave the *alcohol*, $C_{16}H_{32}O$ (1·45 g.), m. p. 56—57° [Found: C, 79·7; H, 13·7; active H, 0·44%; *M* (Rast), 227. $C_{16}H_{32}O$ requires C, 79·9; H, 13·4; active H, 0·42%; *M*, 240]. The compound gave a pale yellow colour with tetranitromethane in chloroform and on microhydrogenation absorbed 0·7 mole of hydrogen. In the Liebermann–Burchard test, an intense royal-blue colour developed in the chloroform layer if care was taken to avoid mixing with the acid layer. The alcohol is optically inactive, does not absorb bromine in methanolic solution, and does not react with osmium tetroxide in ether.

β-*Sitosterol.*—Fraction 5 separated from methanol, to give β-sitosterol as large hexagonal plates, m. p. 137°, $[\alpha]_D$ −33·4° (*c*, 1·8). The m. p. was undepressed on admixture with an authentic specimen, m. p. 137°, $[\alpha]_D$ −35·6°. Acetylation with acetic anhydride afforded β-sitosteryl acetate as prisms (from ethanol), m. p. 127·5°, $[\alpha]_D$ −37·5° (*c*, 1·0). Benzoyl chloride and pyridine gave the benzoate as leaflets (from benzene–ethanol), m. p. 143—144·5°, $[\alpha]_D$ −14·4° (*c*, 1·1). The 3 : 5-dinitrobenzoate, prepared by using 3 : 5-dinitrobenzoyl chloride and pyridine, separated as pale yellow plates, m. p. 198—200°, $[\alpha]_D$ −10·1° (*c*, 0·5), from ethyl acetate–ethanol.

n-*Octadecane*-1 : 18-*diol.*—(*a*) Crystallisation of fraction 8 from methanol and then from benzene gave *n*-ocatadecane-1 : 18-diol (800 mg.) as soft plates, m. p. 98—99° (Found: C, 75·5; H, 13·2; active H, 0·6. Calc. for $C_{18}H_{38}O_2$: C, 75·5; H, 13·4; active H, 0·7%).

(*b*) A solution of ethyl *n*-octadecane-1 : 18-dioate (10 g.; Greaves, Linstead, Shephard, Thomas, and Weedon, *J.*, 1950, 3326) in dry ether (150 ml.) was added to a solution of lithium aluminium hydride (1·5 g.) in the same solvent (200 ml.). Working up in the usual manner gave *n*-octadecane-1 : 18-diol (sparingly soluble in ether) as plates (6·8 g.; m. p. 98—99°) from benzene. Chuit and Hausser (*Helv. Chim. Acta*, 1929, **12**, 850) record m. p. 98·6—99° for a specimen prepared by Bouveault–Blanc reduction of the same ester. A mixture with the natural diol had m. p. 98—99°; the infra-red spectra of specimens obtained by methods (*a*) and (*b*) were identical.

Heating the natural diol with acetic anhydride at 100° during 2 hours gave n-*octadecane*-1 : 18-*diol diacetate* which separated from methanol as needles, m. p. 59—60° (Found: C, 71·5; H, 11·5. $C_{22}H_{42}O_4$ requires C, 71·3; H, 11·4%); the m. p. was undepressed when mixed with a specimen prepared by acetylation of the synthetic diol.

Treatment of the natural diol with 3 : 5-dinitrobenzoyl chloride and pyridine at 100° during 1 hour gave n-*octadecane*-1 : 18-*diol bis*-3 : 5-*dinitrobenzoate* as yellow plates [from light petroleum (b. p. 100—120°)], m. p. 95° (Found: N, 7·8. $C_{32}H_{42}O_{12}N_4$ requires N, 8·3%); it was undepressed in m. p. on admixture with a specimen, m. p. 95°, prepared from the synthetic diol.

The natural diol (75 mg.) with chromium trioxide (200 mg.) in glacial acetic acid (5·5 ml.) gave *n*-octadecane-1 : 18-dioic acid which separated from chloroform as small prisms (30 mg.), m. p. 124—125° alone or mixed with a specimen of m. p. 125°, prepared by the hydrolysis of ethyl *n*-octadecane-1 : 18-dioate.

n-*Hexacosane*-1 : 26-*diol.*—(*a*) A solution of fraction 7 in hot methanol deposited a colourless solid (0·13 g.) on cooling. Repeated crystallisation from benzene afforded n-*hexacosane*-1 : 26-

diol as small prisms (40 mg.), m. p. 102—105° (Found : C, 78·2; H, 13·7. $C_{26}H_{54}O_2$ requires C, 78·3; H, 13·65%).

(*b*) (With H. GIBSON.) A solution of sebacic acid (13·2 g.) and ethyl hydrogen sebacate (30 g.) in anhydrous methanol (120 ml.) in which sodium (0·1 g.) had been dissolved was electrolysed by the procedure described by Greaves, Linstead, Shephard, Thomas, and Weedon (*loc. cit.*). The neutral fraction (39·0 g.), obtained by extraction with chloroform, was distilled under reduced pressure. After the removal of an unsaturated fraction (b. p. up to 120°/3 × 10^{-3} mm.; 5·0 g.), ethyl *n*-octadecane-1 : 18-dioate (b. p. 124—126°/3 × 10^{-3} mm.) was collected; it separated from methanol as a microcrystalline powder (9·0 g.), m. p. 44—46°. Bowman and Mason (*J.*, 1951, 2748) record m. p. 47°. Hydrolysis of the ester with methanolic sodium hydroxide gave *n*-octadecane-1 : 18-dioic acid, m. p. 124—125° alone or mixed with an authentic specimen. The less volatile residue in the still crystallised from methanol, giving ethyl *n*-hexacosane-1 : 26-dioate (6·0 g.), m. p. 64—66° (Found : C, 74·9; H, 12·15. Calc. for $C_{30}H_{58}O_4$: C, 74·6; H, 12·1%). Fairweather (*Proc. Roy. Soc., Edinburgh*, 1926, **46**, 71) records m. p. 66° for a specimen of the ester prepared by the electrolysis of ethyl sodium *n*-tetradecane-1 : 14-dioate. Ethyl *n*-hexacosane-1 : 26-dioate (2·0 g.) was added to a solution of lithium aluminium hydride (1 g.) in dry ether (60 ml.). The mixture was treated with water and with dilute mineral acid, and the solid collected. Crystallisation from benzene gave *n*-hexacosane-1 : 26-diol (0·85 g.) as plates, m. p. 102—105°, undepressed when mixed with the diol obtained as described under (*a*) (Found : C, 78·15; H, 13·5%).

The diol with benzoyl chloride and pyridine at 100° during 1 hour gave n-*hexacosane*-1 : 26-*diol dibenzoate* as small needles, m. p. 67—70°, from methanol (Found : C, 78·9; H, 10·3. $C_{40}H_{62}O_4$ requires C, 79·15; H, 10·3%). With 3 : 5-dinitrobenzoyl chloride and pyridine at 100° during 1 hour it gave the *bis*-3 : 5-*dinitrobenzoate* as pale yellow platelets, m. p. 91—93·5°, from ethanol (Found : N, 7·6. $C_{40}H_{58}O_{12}N_4$ requires N, 7·1%).

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