23. *Melanthigenin and its Identity with Hederagenin.*

By (Miss) Zahira Mustafa and Gabra Soliman.

Melanthigenin, the acidic sapogenin obtainable by hydrolysis of an alcoholic extract of the defatted seeds of *Nigella sativa* L. (Ranunculaceæ), is shown to have the formula $C_{30}H_{48}O_4$ and to be identical with hederagenin.

The seeds of *Nigella sativa* L. were first examined by Greenish (*Pharm. J.*, 1880, **10**, 909, 1013), who found that the alcoholic extract contained, apart from a small quantity of resin, a saponin, which was precipitated on dilution with water and after purification melted at 205°; it was named melanthic acid, $C_{20}H_{33}O_7$. Greenish hydrolysed melanthic acid with hydrochloric acid to melanthigenin, to which he assigned the formula $C_{14}H_{23}O_2$ without giving a description of its chemical properties. Kobert and Kruskal (*Arb. Pharmacol. Inst. Dorpat*, 1891, **6**, 29) and Kobert and Schulz (*ibid.*, 1896, **14**, **37**, 112; see Abderhalden, "Biochemisches Handlexicon," 1912, VII, 205) gave the formulæ $C_{30}H_{52}O_{10}$ and $C_{29}H_{50}O_{10}$, respectively, to melanthic acid.

Greenish found that the saponin content of the seeds was variable, amounting to 1% and 0·1% in seeds of Russian and German origin, respectively. The seeds of Egyptian origin examined in the present work contained no water-insoluble saponin. The alcoholic extract gave no precipitate on dilution with water. When the alcohol was completely removed, a brownish water-soluble syrup was obtained, which on hydrolysis with hydrochloric acid in presence of alcohol gave a dark brownish, crystalline precipitate of melanthigenin. After purification through its sodium salt melanthigenin crystallised from dilute pyridine in white prisms, m. p. above 325°, which gave the general colour reactions of sapogenin acids.

Analysis indicated that melanthigenin has the formula $C_{30}H_{48}O_4$ and contains a free carboxyl group as indicated by titration with standard alkali. The presence of two hydroxyl groups was indicated by the formation of a diacetate, m. p. 170°, and a dibenzoate. Further, the acid was converted into its methyl ester by the action of methyl sulphate, and the ester converted into its diacetate and dibenzoate. The formulæ, melting points, and properties of these derivatives are in agreement with those of hederagenin (Jacobs, *J. Biol. Chem.*, 1925, **63**, 621; Winterstein and Meyer, *Z. physiol. Chem.*, 1931, **199**, 37; Kitasato and Sone, *Acta Phytochim.*, 1932, **6**, 179). Only the methyl ester was not obtained directly pure, but on treatment with hydrochloric acid in presence of dry acetone, it was converted into the acetonyl derivative, which was hydrolysed to the pure ester, m. p. 237—238° (Jacobs, *loc. cit.*, p. 631). These derivatives were shown to be identical with authentic specimens prepared from hederagenin isolated from the leaves of *Hedera helix* (Van der Haar, *Biochem. Z.*, 1916, **76**, 342). The sapogenin was shown to contain one double bond by titration with standard perbenzoic acid, and its position with respect to the carboxyl group was indicated by the formation of a diformyl-lactone. This was deformylated, and the free lactone converted into its diacetate (cf. Winterstein and Meyer, *loc. cit.*; Winterstein and Wiegand, *Z. physiol. Chem.*, 1931, **199**, 46).

Experimental.

Isolation of the Sapogenin.—The crushed seeds of *Nigella sativa* L. were extracted three times with light petroleum (b. p. 60—80°), dried, finely ground, and extracted with hot light petroleum, which removed the last trace of fatty matter (loss in weight, about 30%). The defatted meal (1 kg.) was refluxed with alcohol for 6 hours, and the liquid filtered while luke warm; this process was repeated twice and the combined filtrates were evaporated under diminished pressure on the water-bath. The brownish syrup obtained was dissolved in water, a trace of fatty matter removed by extraction with light petroleum, and the dark aqueous solution triturated with freshly precipitated lead hydroxide (100 g.), kept overnight, and filtered. Lead was removed from the limpid yellow filtrate by hydrogen sulphide, and the excess of the gas expelled under reduced pressure on the water-bath. The solution containing the saponin was mixed with alcohol and hydrochloric acid so that it contained about 40% of alcohol and 10% of hydrogen chloride; when it was heated on the water-bath for 2 hours, a whitish gelatinous precipitate separated, which gradually changed into a dark brownish, crystalline mass.

The crude sapogenin (13·5 g.) was dissolved in a mixture of alcohol (200 c.c.) and 10% sodium hydroxide solution (200 c.c.), refluxed (animal charcoal) for 2 hours, and filtered while hot. The filtrate was gradually diluted with sodium hydroxide solution until a permanent white precipitate began to separate; it was then cooled, and the white plates of the sodium salt collected. A solution of the salt in alcohol was heated with animal charcoal, and the hot filtrate acidified with acetic acid. Instantly the solution was transformed into a gelatinous mass, which gradually changed into a microcrystalline precipitate (10 g.). This crystallised from dilute pyridine in white prisms, m. p. above 325°, not depressed by hederagenin [Found: C, 76·3, 76·2; H, 10·2, 10·1; CO_2H (by titration), 9·6, 9·6; *M*, 467·2, 463·1. Calc. for $C_{29}H_{47}O_2(CO_2H)$: C, 76·2; H, 10·2; CO_2H, 9·5%; *M*, 472·4].

O-Acyl Derivatives.—A solution of the sapogenin (1 g.) in pyridine (10 c.c.) and acetic anhydride (10 c.c.) was heated for an hour. The product, isolated in the usual manner, crystallised from dilute methanol in needles, m. p. 170°, identical with hederagenin diacetate [Found: C, 73·3; H, 9·3. Calc. for $C_{29}H_{45}O_2(CH_3{\cdot}CO)_2(CO_2H)$: C, 73·3; H, 9·4%].

A solution of the sapogenin (1 g.) in pyridine (20 c.c.) was heated with benzoyl chloride (2 c.c.) for an hour at 90° and poured into cold water. The resinous product, after being washed with water acidified with sulphuric acid, was heated with a small volume of methanol; the insoluble portion crystallised from alcohol in platelets, m. p. 292°, identical with hederagenin dibenzoate (Found: C, 77·4; H, 8·0. Calc. for $C_{44}H_{56}O_6$: C, 77·6; H, 8·3%).

The Methyl Ester.—Methyl sulphate (8 c.c.) was gradually added to a solution of the sapogenin (3 g.) in alcohol (30 c.c.) and 10% sodium hydroxide solution (30 c.c.); after addition of more alkali and methyl sulphate, the ester was collected. It crystallised from methanol in needles, m. p. 228°, identical with an authentic specimen. The m. p. was gradually raised by successive crystallisations, yet the pure ester was directly obtained by hydrolysis of its acetonyl derivative prepared by the action of a few drops of hydrochloric acid on a solution of the impure ester in dry acetone. The acetonyl derivative crystallised from absolute alcohol in platelets, m. p. 250° (cf. Jacobs, *loc. cit.*) (Found: C, 77·3; H, 10·3. Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·3%). An analytical specimen of the ester was prepared when the acetonyl derivative was hydrolysed by heating with hydrochloric acid in alcohol for 20 minutes. It crystallised from methanol in needles, m. p. 237—238° (Found: C, 76·6; H, 10·2. Calc. for $C_{31}H_{50}O_4$: C, 76·5; H, 10·4%).

O-Acyl Derivatives of the Ester.—The methyl ester reacted with acetic anhydride in pyridine to give the diacetyl derivative, which crystallised from methanol in needles, m. p. 192°, identical with an authentic specimen (Found: C, 73·7; H, 9·5. Calc. for $C_{35}H_{54}O_6$: C, 73·6; H, 9·5%).

The dibenzoate was prepared by heating a solution of the ester (1 g.) in pyridine with benzoyl chloride (2 c.c.) for 20 hours at 40°. The mixture was poured into water, and the resinous product washed and crystallised from methanol and alcohol; it formed plates, m. p. 195—197° (Found: C, 77·9; H, 8·2. Calc. for $C_{45}H_{58}O_6$: C, 77·8; H, 8·4%).

The Unsaturated Centre.—The diacetate of the methyl ester was titrated with a standard solution of perbenzoic acid (Levy and Lagrave, *Bull. Soc. chim.*, 1925, **37**, 1598) and the values found were 1·01 and 1·0 atom of oxygen, indicating the presence of one double bond.

The position of the double bond with respect to the carboxyl group became obvious from the formation of a diformyllactone when the sapogenin (1 g.) was refluxed with anhydrous formic acid (30 c.c.) for 7 hours. After dilution with water, the product was isolated; it crystallised from methyl alcohol–ethyl alcohol in needles, m. p. 265° (Found: C, 72·6; H, 9·0. Calc. for $C_{32}H_{48}O_6$: C, 72·7; H, 9·2%). It was deformylated by heating with 5% alcoholic potassium hydroxide for 45 minutes; the alcohol was then removed by distillation, and the residue extracted with chloroform after dilution with water. The neutral chloroform solution left on evaporation a white residue, which crystallised from chloroform–alcohol in plates, m. p. above 350° (Found: C, 76·5; H, 10·4. Calc. for $C_{30}H_{48}O_4$: C, 76·2; H, 10·2%). Acetylation of the free lactone with acetic anhydride in presence of pyridine gave a diacetyl-lactone, which crystallised from methanol in plates, m. p. 251° (Found: C, 73·5; H, 9·4. Calc. for $C_{34}H_{52}O_6$: C, 73·3; H, 9·4%). None of these lactones was hydrolysed by treatment with alakli.

Melting points are not corrected, analyses are by Dr. Weiler, and the specimens were dehydrated in a high vacuum before analysis.

Fouad I University, Abbassia, Cairo.

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