The Configuration at $C_{(25)}$ in the Natural Spirostan Sapogenins.

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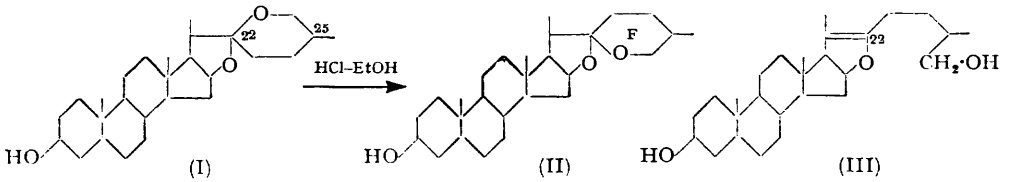
*pseudo*Hecogenin and *pseudo*diosgenin have been degraded to (−)-α-methylglutaric acid, which contains the original asymmetric carbon atom $C_{(25)}$. (−)-α-Methylglutaric acid has been configuratively related to (+)-methylsuccinic acid and so to glyceraldehyde.

The difference between the isomeric sapogenins sarsasapogenin and smilagenin was originally shown to be confined to the side chain by Farmer and Kon (*J.*, 1937, 414), and these authors considered it to be one of configuration of the asymmetric carbon atom in the terminal ring. Marker and Rohrmann (*J. Amer. Chem. Soc.*, 1939, **61**, 846) proposed the spiroketal formulation (I) for sarsasapogenin, and (II) for an isomeric transformation product, later accepted as being identical with smilagenin.



(I) (II) (III)

The instability of sarsasapogenin to ethanolic hydrochloric acid was interpreted as an opening of the terminal oxide ring, with subsequent reclosure in the opposite direction to give smilagenin. Marker and Rohrman (*ibid.*, 1940, **62**, 518) also found that treatment of a sapogenin with acid anhydrides converted it into an isomer, designated the *pseudo*-sapogenin and formulated as (III). The reported observation that *pseudo*sarsasapogenin and *pseudo*smilagenin were identical further supported the spiroketal formulation, since the asymmetry at $C_{(22)}$ would be lost when ring F opens.

On the basis of experiments inter-relating various sapogenins, and their stability towards ethanolic hydrochloric acid, Marker and his co-workers established the existence of two series of compounds: (*a*) the " normal " sapogenins, substituted at various carbon atoms in the steroid nucleus, but having the same side chain as sarsasapogenin, and (*b*) their corresponding isomers, differing only at $C_{(22)}$, the " *iso* "-sapogenins.

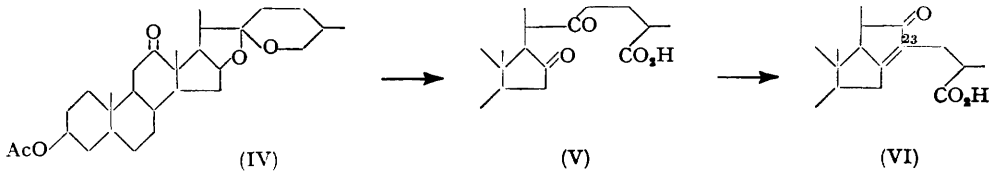
The other asymmetric centre in ring F, $C_{(25)}$, was neglected, and the present work was undertaken in order to ascertain the configuration of this carbon atom. However whilst this was in progress, Scheer, Kostic, and Mosettig (*ibid.*, 1953, **75**, 4871) reported that, by preparing derivatives of *pseudo*sarsasapogenin and *pseudo*smilagenin, they had established a difference between these compounds. They also found that on oxidation these *pseudo*-compounds gave respectively (+)- and (−)-α-methylglutaric acid.

Experimental work in this laboratory involved the degradation of the sapogenins diosgenin and hecogenin, both members of the " *iso* "-series. By isomerisation to the corresponding *pseudo*-compound, followed by oxidation, hecogenin yielded a neutral fraction, *allo*pregn-16-ene-3 : 12 : 20-trione, and an acidic fraction containing carbon atoms $C_{(22)}$ to $C_{(27)}$ inclusive as (−)-α-methylglutaric acid. Diosgenin similarly treated also gave the lævorotatory α-methylglutaric acid. Hence with respect to configuration at $C_{(25)}$, the three " *iso* "-sapogenins, smilagenin, diosgenin, and hecogenin, all belong to one series, whilst sarsasapogenin and *neo*tigogenin (Callow and James, *Chem. and Ind.*, 1954, 691) belong to the other.

The knowledge of the direction of the optical rotation of the α-methylglutaric acid which can be derived from a sapogenin does not enable an absolute assignment of configuration to be made to ring F, since the configurations of the α-methylglutaric acids are not known with certainty. The configuration of methylsuccinic acid has been determined by Fredga and Leskinen (" The Svedberg, 1884 $\frac{30}{8}$ 1944," Almquist and Wiksells, Uppsala, 1944, p. 261;

Arkiv Kemi, Min., Geol., 1944, **19**, *B*, No. 1) and Freudenberg and Hohmann (*Annalen*, 1953, **584**, 54); further, Fredga (*ibid.*, 1947, **24**, *A*, No. 32) has obtained an indication by the method of quasiracemic compounds that (+)-methylsuccinic acid and (−)-α-methylglutaric acid are configuratively related. Fredga points out, however, that a more direct means of obtaining this correlation would be desirable.

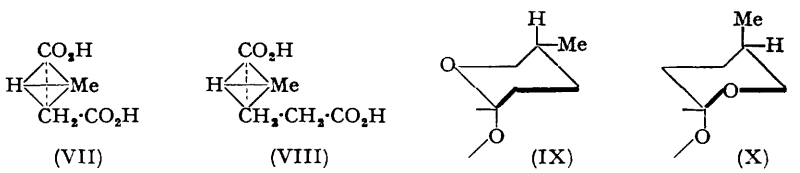
The obvious methods of inter-relating the two homologous acids, *i.e.*, by one-carbon degradation of α-methylglutaric acid, or by synthesis from methylsuccinic acid meet difficulties, and experiments in this direction were not successful. However, oxidation of hecogenin acetate (IV) directly with chromic oxide (cf. Fieser and Jacobsen, *J. Amer.*



(IV) (V) (VI)

Chem. Soc., 1938, **60**, 28) gives hecogenoic acid (V), and this condenses under the influence of alkali to anhydrohecogenoic acid (VI). Ozonolysis of (VI) removes carbon atoms $C_{(23)}$ to $C_{(27)}$ inclusive, which are isolated as (+)-methylsuccinic acid. The acid obtained was compared with authentic (+)-acid kindly provided by Professor Arne Fredga and was identical in melting point, optical rotation, and infra-red spectrum. A mixture of the two substances showed no depression of the melting point. Since it is possible to obtain either (−)-α-methylglutaric acid or (+)-methylsuccinic acid from the same part of the sapogenin, these two acids are obviously related stereochemically, and the conclusion reached by Fredga using physical methods is unambiguously confirmed.

The configuration of (+)-methylsuccinic acid is represented by (VII), so (−)-α-methylglutaric acid is (VIII). If it is assumed that ring F of the sapogenins is in the " chair "



(VII) (VIII) (IX) (X)

conformation in a position which appears to be comparatively unhindered, the terminal rings of hecogenin, diosgenin, and smilagenin will be shown by either (IX) or (X), according to the configuration assigned to $C_{(22)}$.

Experimental

Infra-red absorption spectra were determined on a Perkin–Elmer double-beam instrument by Miss Patricia Dodson.

Oxidation of pseudo*Sapogenins.*—The *pseudo*-compound was prepared by heating the sapogenin at 200° with acetic anhydride for 5 hr. in a sealed tube, followed by removal of excess of anhydride, hydrolysis, and crystallisation from acetone.

*pseudo*Hecogenin (2·0 g.) was dissolved in glacial acetic acid (100 ml.) and to this solution at room temperature was added a solution of chromium trioxide (2·5 g.) in 25 ml. of aqueous acetic acid (80%). The temperature rose to 24° and the solution was set aside for 1 hr. before dilution with water. The product was extracted with ether (5 times), and the ether was washed once with water, dried, and evaporated. The non-crystalline residue was warmed with 20 ml. of N-sodium hydroxide on a steam-bath for 15 min. and then diluted to 300 ml. with water. Extraction (4 times) with ether removed non-acidic material (A), and the aqueous layer was then acidified with dilute hydrochloric acid and ether-extracted (5 times). The ether was dried and evaporated, to yield a brown gum (1 g.). This was chromatographed on a partition column containing " Celite " (100 g.) made up in chloroform equilibrated with 0·5N-sulphuric acid. Some brown material which ran with the solvent front was eluted with equilibrated chloroform, and then elution with chloroform containing 2·5% of *n*-butanol gave the only other acidic

fraction obtained. Extraction of this material with aqueous sodium hydrogen carbonate, followed by acidification and ether-extraction, gave α-methylglutaric acid. Crystallised twice from ether–pentane, the acid (80 mg.) had m. p. 78·5—83°, $[\alpha]_D^{22}$ −17° (in EtOH) (Found: C, 49·8; H, 7·2. Calc. for $C_6H_{10}O_4$: C, 49·3; H, 6·9%). The infra-red spectrum is given in C.S. no. 167.*

Similar treatment of *pseudo*diosgenin gave α-methylglutaric acid, m. p. 82—84°, $[\alpha]_D^{23}$ −21° (in EtOH).

The ether solution of the material (A) was washed with water, dried, and evaporated, to give white crystals (600 mg.). These were chromatographed on alumina (Savory and Moore) and 1 : 4 ether–benzene eluted solid, which crystallised from benzene–light petroleum (b. p. 60—80°) as needles of allo*pregn*-16-*ene*-3 : 12 : 20-*trione*, m. p. 225—230°, $[\alpha]_D^{20}$ +124° (in MeOH), $\lambda_{max.}$ (in MeOH) 227—228 mμ (log ε 3·9) (Found: C, 76·7; H, 8·5. $C_{21}H_{28}O_3$ requires C, 76·8; H, 8·6%). The infra-red spectrum (C.S. no. 168) of this compound in compressed potassium bromide showed bands at 1711 (ketone) and 1675 and 1596 cm.$^{-1}$ (αβ-unsaturated ketone).

Hecogenoic Acid.—To hecogenin acetate (20 g.) in glacial acetic acid (distilled from chromic oxide, 400 ml.), kept at 60—65°, was added during 4 hr., with constant stirring, a solution of chromic oxide (12 g.) in 80% aqueous acetic acid (200 ml.). After a further 1 hr., the solution was set aside overnight. Ethanol (5 ml.) was added and the solution was concentrated at reduced pressure and diluted with water, then extracted with ether. After being washed with water, the ether was extracted with 3% sodium hydroxide solution, and the alkali extract washed with ether; warming the alkali solution on the steam-bath for 15 min., followed by acidification with dilute sulphuric acid, precipitated solid. This was collected (4·17 g.); crystallised from aqueous acetone it gave *hecogenoic acid* as hexagonal plates, m. p. 242·5—244·5°, $[\alpha]_D^{22}$ −73° (in $CHCl_3$) (Found: C, 70·6; H, 8·8. $C_{27}H_{40}O_6$ requires C, 70·4; H, 8·8%). The infra-red absorption spectrum (C.S. no. 169) of the compound in compressed potassium bromide showed strong bands at 3300, 1738, 1713, and 1692 cm.$^{-1}$.

Anhydrohecogenoic Acid.—Hecogenoic acid (5 g.), dissolved in ethanol (50 ml.) and water (20 ml.), was treated with sodium hydroxide (2 g.). After refluxing for 1 hr., the solution was diluted with water (50 ml.) and acidified with dilute sulphuric acid. The precipitated solid was collected and dried (4·4 g.). Trituration with ether and filtration, followed by crystallisation from chloroform–acetone, gave *anhydrohecogenoic acid* as needles, m. p. 278—280°, $[\alpha]_D^{19}$ −30° (in $CHCl_3$), $\lambda_{max.}$ (in MeOH) 242—243 mμ (Found: C, 73·1; H, 8·5. $C_{27}H_{38}O_5$ requires C, 73·3; H, 8·7%). The infra-red absorption spectrum (C.S. no. 170) of the compound as a Nujol mull showed strong bands at 3290, 1724, 1703, 1693, and 1657 cm.$^{-1}$.

Ozonolysis. Anhydrohecogenoic acid (1·0 g.) in chloroform (500 ml.), cooled in alcohol–carbon dioxide, was ozonised for 2 hr. The solution, then blue, was distilled, to give a residual gum. This was heated on the steam-bath with water (10 ml.) and 30% hydrogen peroxide (10 ml.) for 15 min. The material was then extracted with ether, and the ether extracted with 2N-sodium hydroxide. Acidification, ether-extraction, drying, and evaporation yielded 1·0 g. of brown gum. Of this, 800 mg. were chromatographed on " Celite " as for the α-methylglutaric acid. Two fractions were obtained: the more polar, eluted with chloroform containing 10% of *n*-butanol and crystallised from benzene, gave (+)-methylsuccinic acid (41·2 mg.), m. p. 114—115°, $[\alpha]_D^{23}$ +10° (in H_2O) (Found: C, 45·4; H, 6·4. Calc. for $C_5H_8O_4$: C, 45·4; H, 6·1%). The infra-red absorption spectrum (C.S. no. 171) of the acid was identical with that (C.S. no. 172) of an authentic specimen of (+)-methylsuccinic acid, and the two samples showed no depression of m. p. on admixture.

For both methylsuccinic and α-methylglutaric acid, the spectra of the optically active isomers differed from those (C.S. nos. 173 and 174 respectively) of the racemic (±)-acids in minor points.

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* Spectra thus designated have been deposited with the Chemical Society. Copies price 3*s.* 0*d.* per copy per spectrum, may be obtained on application, quoting the C.S. no., from the General Secretary.