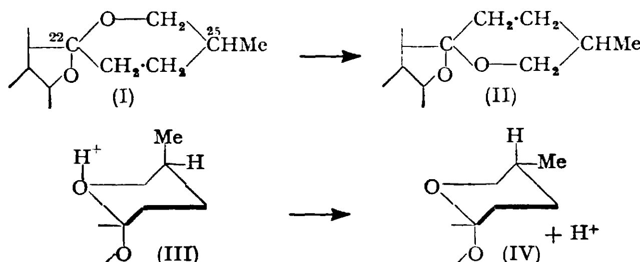
Epimerisation at $C_{(25)}$ of Steroid Sapogenins: Sarsasapogenin, neo*Tigogenin, and Sisalagenin.*

By R. K. Callow and V. H. T. James.

[Reprint Order No. 6052.]

The transformation of three " normal " sapogenins, sarsasapogenin, *neo*tigogenin, and sisalagenin, into the " *iso*- "isomerides, smilagenin, tigogenin, and hecogenin, respectively, has been examined, and the inversion of configuration at $C_{(25)}$ confirmed. Sisalagenin, the $C_{(25)}$-epimer of hecogenin, has been isolated from the sapogenins of *Agave sisalana.*

The work of Scheer, Kostic, and Mosettig (*J. Amer. Chem. Soc.*, 1953, **75**, 4871) appears to establish that sarsasapogenin and smilagenin differ in configuration at $C_{(25)}$ and hence to disprove Marker's hypothesis that " normal " and "*iso*-"sapogenins differ only in their steric arrangement at $C_{(22)}$ (Fieser and Fieser, " Natural Products related to Phenanthrene," Reinhold, New York, 1949, 3rd edn.). This revision naturally provoked a re-examination of the reported conversion of sarsasapogenin into smilagenin (Marker and Rohrmann, *J. Amer. Chem. Soc.*, 1939, **61**, 846), of yamogenin into diosgenin, and of *neo*tigogenin into tigogenin (Marker, Wagner, Ulshafer, Wittbecker, Goldsmith, and Ruof, *ibid.*, 1947, **69**, 2167). This reaction was previously explained by the opening and closing of an oxide ring at $C_{(22)}$ [(I) ⟶ (II)] but now requires, to explain it, a mechanism of inversion of configuration at $C_{(25)}$ such as that suggested by Cornforth [(III) ⟶ (IV)] (*Ann. Reports*, 1953, **50**, 219).



We were able to confirm the occurrence of this isomerisation, brought about by boiling a solution of the steroid in ethanol and concentrated hydrochloric acid for 120 hr., with sarsaspogenin and *neo*tigogenin and extended the investigation to a new " normal " sapogenin, sisalagenin. *neo*Tigogenin and sisalagenin have been obtained from the crystallisation residues of the acetate of hecogenin extracted from sisal, *Agave sisalana* Perrine (Callow, Cornforth, and Spensley, *Chem. and Ind.*, 1951, 699; Spensley, *ibid.*, 1952, **426**), made available to us by the kindness of Messrs. T. and H. Smith of Edinburgh with the permission of the National Research Development Corporation.

Sisalagenin, which has not been described before, is a ketonic sapogenin, isomeric with hecogenin, which we find to be the " normal " analogue of the latter, epimeric with it at $C_{(25)}$. Sisalagenin acetate was separated * in small amount from the more soluble fractions of the ketonic part of the crystallisation residues, obtained by the use of Girard's reagent T. Sisalagenin,† obtained by hydrolysis, had an optical rotation slightly more negative than that of hecogenin $\Delta([M_D]_{S-H} = -48°)$: this is comparable with sarsasapogenin–smilagenin, $\Delta[M_D] = -42°$, and *neo*tigogenin–tigogenin, $\Delta[M_D] = -33°$. Its infrared absorption spectrum showed the bands typical of " normal " sapogenins, in particular one

* In the course of this investigation Professor F. S. Spring kindly informed us that he had isolated the " normal " isomer of hecogenin, but, in view of our work, he most courteously refrained from continuing his own investigation.

† In view of the confusion of prefixes in this group, the impossibility of legitimately maintaining the name " *neo*hecogenin " used for an imperfectly characterised compound by Marker and Lopez (*J. Amer. Chem. Soc.*, 1947, **69**, 2373), and the uncertainty attaching to certain details of configuration, it has been thought preferable to invent a new trivial name for this compound.

at 921 cm.$^{-1}$ some 4 times the height of one at 897 cm.$^{-1}$, and a band at 851 cm.$^{-1}$, but only a weak one at 863 cm.$^{-1}$. Converted into a *pseudo*sapogenin in the usual way it yielded, by opening of ring F, *pseudo*sisalagenin which, in a potassium bromide disc, has an infrared absorption practically indistinguishable from that of *pseudo*hecogenin. Oxidation of this with chromic acid yielded (+)-α-methylglutaric acid and *allo*pregn-16-ene-3 : 12 : 20-trione, proving that $C_{(25)}$ has the configuration opposite to that of $C_{(25)}$ of hecogenin which yields the same pregnene derivative and (—)-α-methylglutaric acid (cf. James, *J.*, 1955, 637).

Treatment of sisalagenin with boiling ethanolic hydrochloric acid yielded hecogenin, characterised by m. p., optical rotation, infrared absorption, and conversion into the acetate.

*neo*Tigogenin, first reported by Goodson and Noller (*J. Amer. Chem. Soc.*, 1939, **61**, 2420) as accompanying tigogenin from *Chlorogalum pomeridianum* Kunth, is also a companion of tigogenin in the non-ketonic fraction of sisal sapogenins and is separable by a rather tedious fractional crystallisation of the acetates. *pseudoneo*Tigogenin, prepared by the usual method, had an infrared absorption in potassium bromide practically indistinguishable from that of *pseudo*tigogenin. Oxidation with chromic acid yielded (+)-α-methylglutaric acid. Epimerisation of *neo*tigogenin by boiling ethanolic hydrochloric acid yielded tigogenin, characterised by its physical properties, and, to remove all possible doubt that inversion at $C_{(25)}$ actually occurs, the tigogenin obtained in this way was degraded to give the α-methylglutaric acid: this was lævorotatory. In this instance alternative conditions of epimerisation were investigated. Transformation of *neo*tigogenin into tigogenin took place in hot dioxan containing concentrated hydrochloric acid. Under similar conditions epimerisation occurred in the presence of acetic anhydride, a circumstance which seems definitely to preclude any mechanism in which an oxide ring is opened giving a hydroxy-compound as an intermediate.

EXPERIMENTAL

M. p.s were determined in a Kofler apparatus, with polarised light, and are corrected. Optical rotations are, unless otherwise specified, in chloroform and measured in a 4-dm. tube. Infrared absorption spectra were measured in potassium bromide by the technique of Schiedt and Reinwein (*Z. Naturforsch.*, 1952, **7b**, 270; cf. Schiedt, *ibid.*, 1953, **8b**, 66; Franck, *ibid.*, 1954, **9b**, 276) with a Perkin–Elmer double-beam instrument with rock-salt prism.

Isomerisation of Sarsasapogenin.—Sarsasapogenin [1·5 g. of a commercial product, m. p. 191—198°, $[\alpha]_D^{25}$ −77°, $[\alpha]_{5461}^{25}$ −90° (*c*, 1·2) (infrared absorption as reported by Jones, Katzenellenbogen, and Dobriner, *J. Amer. Chem. Soc.*, 1953, **75**, 158)], was dissolved in 95% ethanol (150 ml.), and a mixture of 95% ethanol (100 ml.) and concentrated hydrochloric acid (45 ml.) was added. Boiling was continued for 96 hr., then a further 40 ml. of hydrochloric acid were added and the mixture was boiled for 24 hr. longer. The product was isolated by addition of water and extraction of the mixture with ether. Evaporation of the washed extract yielded a solid which, recrystallised from acetone, gave 0·42 g. of crystals, m. p. 157—160°, $[\alpha]_D$ − 64° (*c*, 0·48), with the infrared absorption spectrum of smilagenin (C.S. No. 189).* After trials, satisfactory purification was accomplished by conversion into the acetate by acetic anhydride in pyridine and passing a solution of this material (0·3 g.) in benzene through a column (15 × 2 cm.) of neutral alumina. After a trace of oil, a fraction came through which crystallised, on evaporation and rubbing with methanol, in long, silky needles, m. p. 150—153°, not depressing the m. p. of authentic material (m. p. 146—151°), $[\alpha]_D^{19}$ −62°, $[\alpha]_{5461}^{19}$ −71° (*c*, 0·881) (Found: C, 75·9; H, 10·1. Calc. for $C_{29}H_{46}O_4$: C, 75·9; H, 10·1%); the infrared absorption spectrum (C.S. No. 190) was identical with that of authentic material (C.S. No. 191). Authentic smilagenin (8 g.) was prepared from grey Jamaica sarsaparilla (23 kg.) essentially as described by Askew, Farmer, and Kon (*J.*, 1936, 1399). The physical constants of the alcohol and of the acetate agreed with those recorded (*loc. cit.*) and the infrared absorption of the acetate was in agreement with the curves published by Eddy, Wall, and Scott (*Analyt. Chem.*, 1953, **25**, 266) and by Dobriner, Katzenellenbogen, and Jones ("Infrared Absorption Spectra of Steroids. An Atlas," Interscience, New York, 1953).

Fractionation of Sapogenin Acetates from Sisal.—Residues (200 g.), consisting of acetates of

* Infrared spectra thus marked have been deposited with the Society. Photocopies, price 3*s.* 0*d.* per copy per spectrum, may be obtained from the General Secretary, the Chemical Society, Burlington House, Piccadilly, London, W.1, on application stating the C.S. No.

mixed sapogenins after collection of most of the hecogenin, derived from Kenya sisal, were boiled under reflux with Girard's reagent T (100 g.) and acetic acid (100 g.) in ethanol (2 l.) for 2 hr. The solution was filtered hot. Material which separated in the filter (fraction A) (10 g.), m. p. 176—190°, consisted of tigogenin acetate, recognisable by the infrared absorption. Fraction B (56 g.), m. p. 168—184°, which separated on cooling, was, judged from the relative height of the absorption bands at 920 and 900 cm.$^{-1}$, a mixture of tigogenin and *neo*tigogenin acetates in about equal amounts; and fraction C (45 g.; m. p. 152—164°), which separated after addition of 2N-sodium carbonate solution (750 ml.) and water (2 l.), was, by the same criterion, mostly *neo*tigogenin acetate. A ketonic fraction (88 g.), m. p. 217—230° (mostly hecogenin acetate), was obtained by acidification of the filtrate from fraction C. The first *neo*tigogenin acetate obtained actually separated from mother-liquors of a non-ketonic acetate fraction in the compact octahedra first described by Goodson and Noller (*loc. cit.*), having m. p. 174—180°, $[\alpha]_D$ $-79°$, $[\alpha]_{5461}$ $-91°$ (*c*, 0·312). Systematic separation was, however, achieved by fractionation from *iso*propanol. Fraction B was added to the mother-liquors of fraction A and, after collection of the crystals, the mother-liquors were used to recrystallise fraction C. The solid which separated (37·5 g.; m. p. 168—175°) was largely *neo*tigogenin acetate and the m. p. rose to 175—181° on repeated recrystallisation. Hydrolysis of the acetate by sodium hydroxide in aqueous ethanol (15% of H_2O) yielded *neo*tigogenin in thin hexagonal plates, m. p. 197—203°, $[\alpha]_D$ $-75°$, $[\alpha]_{5461}$ $-84°$ (*c*, 0·659), the infrared absorption spectrum closely matching that given in the literature (Jones *et al.*, and Dobriner *et al.*, *locc. cit.*).

A ketonic fraction obtained as described above from a cruder, yellow, crystallisation residue derived from Jamaica sisal was the source of sisalagenin. This fraction (16·8 g.), recrystallised from *iso*propanol, yielded three successive crops on cooling and evaporation: (*a*) (6 g.) hecogenin acetate, (*b*) (6·3 g.) m. p. 196—200°, with infrared absorption at 918 cm.$^{-1}$ higher than at 897 cm.$^{-1}$ (ratio of heights above intermediate minimum 22/9), and (*c*) (3·2 g.) pasty. Fractional crystallisation of (*b*) with the addition of a fraction from (*c*) yielded, with some careful manipulation in the way of seeding and separating rapidly the first crops of crystals, 1·15 g. of *sisalagenin acetate*, m. p. 226—229°, $[\alpha]_D$ $-12°$, $[\alpha]_{5461}$ $-13°$ (*c*, 1·05). Recrystallised to constant m. p. from ethanol–light petroleum (b. p. 100—120°) and ethyl acetate, the substance formed prisms, m. p. 228—232°, $[\alpha]_D^{22}$ $-12°$, $[\alpha]_{5461}^{22}$ $-12°$ (*c*, 1·006) (Found: C, 74·0; H, 9·5. $C_{29}H_{44}O_5$ requires C, 73·7; H, 9·5%). The infrared absorption (C.S. No. 192) generally resembles that of hecogenin acetate, but there are differences between 1100 and 950 cm.$^{-1}$, where the strong bands are at 1068, 1038, and 986 cm.$^{-1}$: the "normal" sapogenin characteristics are shown in the much greater height of the band at 918 relative to that at 898 and in the presence of a band at 850 and only a weak maximum at 865 cm.$^{-1}$. Hydrolysis by aqueous-ethanolic sodium hydroxide yielded *sisalagenin*, which formed prisms (from ethyl acetate), m. p. 244—246°, $[\alpha]_D^{22}$ $-4·5°$, $[\alpha]_{5461}^{22}$ $-2°$ (*c*, 1·408) (Found: C, 75·2; H, 10·2. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%). The infrared absorption spectrum (C.S. No. 193) was concordant with that of the acetate, allowing for removal of the acetoxyl group.

pseudo*Sisalagenin*.—Sisalagenin acetate was converted by the method of Cameron, Evans, Hamlet, Hunt, Jones, and Long (*J.*, 1955, in the press) into pseudo*sisalagenin* which separated from acetone in platy crystals, m. p. 188—191° after a transition at about 100°, $[\alpha]_D^{21}$ $+92°$, $[\alpha]_{5461}^{21}$ $+112°$ (*c*, 0·830) (Found: C, 75·7; H, 10·0%). The infrared absorption spectrum (C.S. No. 194) in potassium chloride was typical of a *pseudo*sapogenin and practically indistinguishable from that of *pseudo*hecogenin (C.S. No. 195), with a prominent, blunt maximum at 1043 cm.$^{-1}$. The band at 1708 cm.$^{-1}$ (CO) obscured the vinyl ether band only indicated by a bulge on the low-frequency side. Acetylation by boiling with acetic anhydride yielded pseudo*sisalagenin diacetate*, plates (from methanol), m. p. 111—113°, $[\alpha]_D^{20}$ $+70°$, $[\alpha]_{5461}^{20}$ $+87°$ (*c*, 0·453; *l* 2 dm.) (Found: C, 72·4; H, 9·3. $C_{31}H_{46}O_6$ requires C, 72·3; H, 9·0%). The infrared absorption spectrum in potassium chloride (C.S. No. 196) showed the characteristics to be expected from the spectrogram of the alcohol.

Degradation of pseudo*Sisalagenin*.—*pseudo*Sisalagenin (0·43 g.) was dissolved in glacial acetic acid (50 ml.), and to this solution at 22·5° was added a solution of chromic oxide (0·6 g.) in 80% aqueous acetic acid (6 ml.). The temperature rose immediately to 25·5° and some greenish solid appeared. After 1 hr. at room temperature, the solid had dissolved to give a clear solution. Water was then added and the mixture extracted five times with ether. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residual green gum (0·36 g.) was warmed on the steam-bath for 30 min. with N-sodium hydroxide (5 ml.). The alkaline solution was diluted with water and extracted four times with ether. Washed, dried, and evaporated, the ether yielded 0·13 g. of crystals (A). The alkaline liquors from the

extraction were acidified with dilute hydrochloric acid and extracted five times with ether. Drying and evaporation of the ether gave a brown gum (30 mg.) which was sublimed at 100° *in vacuo* to give α-methylglutaric acid (9 mg.), m. p. 70—75° $[\alpha]_D^{22}$ +18° (*c*, 0·9 in EtOH; *l* 1 dm.). The neutral solid (A) was put on an alumina column in benzene. Elution with chloroform–benzene (1 : 4) gave 110 mg. of crystals, which on crystallisation from ether had m. p. 223·5—229° and were identified as *allo*pregn-16-ene-3 : 12 : 20-trione by the infrared absorption spectrum and comparison with an authentic specimen prepared from hecogenin (James, *loc. cit.*). A further few mg. of solid, m. p. 202—222°, was eluted from the column by chloroform–benzene (1 : 1), but was not further investigated.

Isomerisation of Sisalagenin.—Sisalagenin acetate (2·25 g.) was dissolved in boiling ethanol (300 ml.); concentrated hydrochloric acid (50 ml.) was added and boiling continued for 120 hr. Water was added to the boiling solution to precipitate the product, which separated as plates (1·77 g.), m. p. 224—237°, which appeared by the infrared absorption spectrum to consist of hecogenin contaminated with a little sisalagenin. Acetylation (acetic anhydride in pyridine) and purification of the acetate by chromatography on neutral alumina and by crystallisation from *iso*propanol and from petroleum gave a product of m. p. 234—237°, or 237—243° mixed with authentic hecogenin acetate (m. p. 241·5—243°). It had $[\alpha]_D^{22}$ −4°, $[\alpha]_{5461}^{22}$ −3° (*c*, 1·002). The anomalous rotatory dispersion is also found in authentic hecogenin acetate. The free sapogenin obtained by hydrolysis crystallised from light petroleum in elongated plates, m. p. 250—257°, or 257—259° mixed with authentic hecogenin (m. p. 259—260°), $[\alpha]_D^{22}$ +4°, $[\alpha]_{5461}^{22}$+8° (*c*, 0·661). The infrared absorption spectrum (C.S. No. 197) was similar in every detail to one of authentic hecogenin (C.S. No. 198).

pseudoneo*Tigogenin.*—*neo*Tigogenin acetate was converted into *pseudoneo*tigogenin by the method of Cameron *et al.* (*loc. cit.*). The product formed plates (from acetone), m. p. 180—184° $[\alpha]_D^{21}$ +10°, $[\alpha]_{5461}^{21}$ +13° (*c*, 0·93) (Found : C, 77·8; H, 10·8. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·65%). The infrared absorption spectrum (C.S. No. 199) was almost indistinguishable from that of *pseudo*tigogenin : it was of the usual type for a *pseudo*sapogenin with a blunt maximum at 1040 cm.$^{-1}$ and a peak of medium strength at 1693 cm.$^{-1}$ (C=C·O·).

Degradation of pseudoneo*Tigogenin.*—*pseudoneo*Tigogenin (1 g.) was suspended in ethylene dichloride (10 ml.), and a solution of chromic acid in 90% acetic acid added until excess was present (about 1·5 g. in 30 ml.). After 2 hr. methanol was added to destroy the remaining chromic acid, organic solvent was removed by distillation and steam-distillation, and the product worked up as described previously. The (+)-α-methylglutaric acid obtained (17·6 mg.) had m. p. 74—80°, $[\alpha]_D$ +16·5° (*c*, 0·16 in EtOH), after sublimation. Again crystallised from ether and pentane its m. p. rose to 83—85°.

Isomerisation of neo*Tigogenin.*—*neo*Tigogenin acetate (2·26 g.) was treated with hydrochloric acid in ethanol in the same way as in the isomerisation of sisalagenin (above). The crude product (1·75 g.), m. p. 185—194°, had infrared absorption indicating that it was tigogenin slightly contaminated with *neo*tigogenin. Acetylation and recrystallisation of the acetate from ethyl acetate yielded a product, m. p. 195—202°, m. p. 197—207° when mixed with tigogenin acetate (m. p. 206—209°), $[\alpha]_D^{21}$ −72°, $[\alpha]_{5461}^{21}$ −84° (*c*, 1·121). The infrared absorption spectrum (C.S. No. 200) showed complete similarity in all details with one of authentic tigogenin acetate (C.S. No. 201).

Another isomerisation experiment yielded 4·6 g. of crude tigogenin, m. p. 191—199°; this was converted into the *pseudo*sapogenin which was then oxidised. (−)-α-Methylglutaric acid, m. p. 80—85°, $[\alpha]_D^{20}$ −18° (*c*, 1·11 in EtOH), was separated in the usual way. An authentic specimen of natural tigogenin yielded (−)-α-methylglutaric acid, m. p. 74—82°, $[\alpha]_D$ −16° (*c*, 0·5 in EtOH).

Epimerisation under different conditions was carried out by heating *neo*tigogenin acetate (0·5 g.) in dioxan (80 ml.) and concentrated hydrochloric acid (15 ml.). After 48 hr. on the steam-bath the product was examined and the infrared absorption indicated that it was mostly *neo*tigogenin. However, after a further 64 hr. 0·3 g. of crystals, m. p. 195—198·5°, separated when the solution was cooled, and infrared examination showed an absorption spectrum identical with that of tigogenin. Repetition of this experiment with the addition of acetic anhydride (25 ml.) to the other reagents gave a similar result, the tigogenin which separated having m. p. 194—199° after crystallisation from *iso*propanol, and an infrared absorption spectrum indistinguishable from that of authentic tigogenin.

NATIONAL INSTITUTE FOR MEDICAL RESEARCH,
THE RIDGEWAY, MILL HILL, LONDON, N.W.7.
[*Received, January 21st*, 1955.]