Studies in the Synthesis of Cortisone. Part IX. Infra-red Absorption of Polymorphic Steroids and Steroidal Sapogenins.*

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[Reprint Order No. 5662.]

Six steroids and steroidal sapogenin derivatives have been isolated in more than one crystalline form. The infra-red absorption spectra of Nujol mulls and potassium bromide discs of a steroid's polymorphic forms differ considerably, but the spectra of dilute solutions in either carbon disulphide or bromoform are identical. The differences in crystal form have been confirmed by *X*-ray powder photography. It is desirable, whenever possible, to record for interpretation purposes the spectrum of the solution rather than, or in addition to, that of either the Nujol mull or the potassium bromide disc of an unknown steroid.

Infra-red examination of several hundred steroids and steroid sapogenins encountered during synthetic work revealed that certain steroid samples, though known to be identical chemically, gave infra-red absorption spectra that were different when examined in the solid state as either Nujol mulls or potassium bromide discs, but identical when examined as dilute solutions in either carbon disulphide or bromoform. The spectral differences were attributed to polymorphism; this was confirmed by *X*-ray diffraction.

Polymorphic forms of steroids have been recorded, *viz.*, progesterone (Slotta, Ruschig, and Blanke, *Ber.*, 1934, **67**, 1947; Butenandt and Schmidt, *Ber.*, 1934, **67**, 2088), cholest-4-en-3-one (Barton and Jones, *J.*, 1943, 602), 3β-acetoxypregn-5-en-20-one (Gould and Tarpley, *Science*, 1951, **113**, 417), 5-hydroxycholestan-3-one (Fudge, Shoppee, and Summers, *J.*, 1954, 958), 3β-hydroxy*ætio*cholan-17-one (Marker and Rohrmann, *J. Amer. Chem. Soc.*, 1940, **62**, 900), 3β-acetoxy*allo*pregnane-11 : 20-dione (Chamberlin, Ruyle, Erickson, Chemerda, Aliminosa, Erickson, Sita, and Tishler, *ibid.*, 1953, **75**, 3477), cortisone acetate (Merck & Co., Inc., B.P. 694,280), and smilagenin and sarsasapogenin acetates (Marker, Wagner, Ulshafer, Wittbecker, Goldsmith, and Ruof, *J. Amer. Chem. Soc.*, 1943, **65**, 1199; 1947, **69**, 2167), but Merck & Co., Inc., alone presented *X*-ray diffraction evidence for polymorphism (cortisone acetate); no-one has reported infra-red measurements on these compounds.

We believe that infra-red spectroscopy provides a valuable method for identifying polymorphic steroids and is more rapid and more informative than *X*-ray powder photography. Its value for crystalline polymorphs of organic compounds has been demonstrated by Ebert and Gottlieb (*ibid.*, 1952, **74**, 2806), Kendall (*Analyt. Chem.*, 1953, **25**, 382), Ross (*ibid.*, 1953, **25**, 1288), and Plattner, Keller, and Boller (*Helv. Chim. Acta*, 1954,

* Part VIII, *J.*, 1954, 1739.

37, 1379) and of inorganic compounds by Hunt, Wisherd, and Bonham (*Analyt. Chem.*, 1950, **22**, 1478), Keller and Halford (*J. Chem. Phys.*, 1949, **17**, 26), and Wagner and Hornig (*ibid.*, 1950, **18**, 296); Mochel and Hall (*J. Amer. Chem. Soc.*, 1949, **71**, 4082) have observed absorption bands in the spectrum of crystallised neoprene, which are absent from that of the amorphous polymer.

However, care must be exercised in interpreting Nujol mull spectra. Solvent of crystallisation and crystal orientation effects (sometimes complicated by the small inherent polarization of radiation in a prism spectrometer) can produce appreciable changes. Solvent of crystallisation can be detected by analysis, optical rotations, and solution spectra. Crystal orientation effects may be eliminated by fine grinding.

The infra-red measurements discussed below indicate that the two forms of each compound differ in their crystal form. This is confirmed by the *X*-ray data summarised in the Table. *pseudo*Tigogenin illustrates the case of solvent of crystallisation: the other five examples are of true polymorphism.

The possibility of polymorphism provides an additional reason for recording for interpretation purposes, whenever possible, the solution spectrum rather than, or in addition to, the Nujol mull or potassium bromide disc spectrum of a compound. Jones and Dobriner (*Vitamins and Hormones*, 1949, **7**, 293; see also Cole, *Chem. and Ind.*, 1954, 661) stressed the desirability of examining steroids as dilute solutions and, from a study of such spectra, have drawn up frequency correlation tables for the principal oxygen-containing groups, unsaturated linkages, and stereochemical configurations in steroids (see Jones and Herling, *J. Org. Chem.*, 1954, **19**, 1252). Because of intermolecular hydrogen-bonding effects, however, these correlations cannot be trusted if the spectral measurements are conducted on samples in the solid state. Thus the absence of a band at 1746 cm.$^{-1}$ and the presence of one at 1730 cm.$^{-1}$ in the Nujol mull spectrum of the B-form of *allo*-**4** : 5-dihydrocortisone acetate (see Figure) suggests that the compound does not contain a 21-acetate group but possibly contains a 3-acetate group. Again the 1000—800-cm.$^{-1}$ region of the Nujol mull spectrum of the B-form of *pseudo*tigogenin (C.S. no. 151 *) suggests a new type of sapogenin rather than a *pseudo*sapogenin. Solution spectra show that these interpretations are incorrect.

Experimental

The spectroscopic measurements were made with a Perkin–Elmer Corporation model 21, double-beam, infra-red spectrophotometer fitted with a sodium chloride prism and were conducted over the spectral range 4000—650 cm.$^{-1}$. The accuracy of frequency measurements for sharp maxima was about ± 3 at 1700 and $\pm$ 2 cm.$^{-1}$ at 800 cm.$^{-1}$.

The Nujol mulls were prepared by grinding about 5 mg. of the compound to a fine powder and mulling it with one drop of Nujol to give a thick smooth paste, and the potassium bromide discs by grinding about 2 mg. of the compound with 300 mg. of dry powdered "AnalaR" potassium bromide and then pressing the mixture under a vacuum to give a transparent disc (cf. Stimson and O'Donnell, *J. Amer. Chem. Soc.*, 1952, **74**, 1805). Compounds that were sufficiently soluble were examined as 1·0% (w/v) carbon disulphide and carbon tetrachloride solutions in 1·0-mm. cells; the less soluble compounds were examined as 1·0% (w/v) redistilled bromoform solutions in 1·0-mm. cells. Compensation for solvent absorption was achieved by passing the reference beam of the instrument through pure solvent in a matched cell.

The *X*-ray diffraction patterns were photographed with a Unicam 19-cm. powder camera by means of copper $K\alpha$ radiation. The interplanar spacings in Å for the three strongest diffraction lines are listed in the Table in order of decreasing intensity.†

All the compounds examined had been prepared in these laboratories (see previous and subsequent papers in the present series). Optical rotations were measured for chloroform solutions in a 1-dm. tube at room temperature (20—25°) unless otherwise stated.

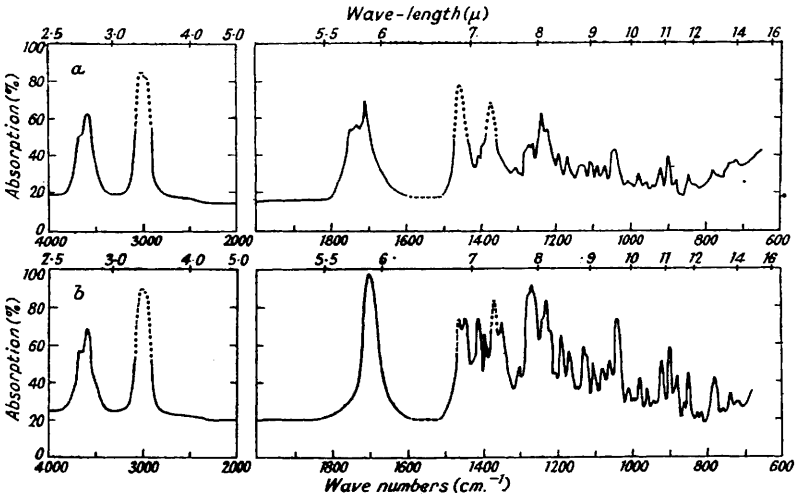
* Spectra thus marked are deposited with the Chemical Society. Photocopies may be obtained, price 3*s.* 0*d.* per copy per spectrum, on application, quoting the C.S. no., to the General Secretary, The Chemical Society, Burlington House, Piccadilly, London, W.1.

† The *X*-ray powder values for each of these substances are being included in full in a forthcoming supplement to the A.S.T.M. Index. Until this is published the values may be had from the authors upon request.

Compound	Spacings in Å of three strongest *X*-ray diffraction bands in order of decreasing intensity: A-form	B-form
allo-4 : 5-Dihydrocortisone acetate	5·99, 5·02, 5·61	5·92, 5·56, 4·36
11β-Hydroxytigogenin	6·47, 5·73, 5·31	6·29, 5·62, 5·19
3β-Acetoxy-23*a*-bromo-11β-hydroxy-5α : 22*a*-spirostan-12-one	5·38, 5·71, 6·93	6·62, 5·17, 4·84
11-Oxo*pseudo*tigogenin	5·71, 6·34, 4·57	5·23, 5·75, 4·13
*pseudo*Tigogenin	5·10, 6·39, 5·87	6·03, 5·06, 4·76

3β-*Acetoxy*allo*pregnane*-11 : 20-*dione*.—3β-Acetoxy*allo*pregnane-11 : 20-dione obtained by partial synthesis from hecogenin and recrystallised from aqueous methanol originally gave needles (A-form), m. p. 127—128°, which remelted at 143·5°, $[\alpha]_D$ +88·5°. Material obtained by partial synthesis from ergosterol and recrystallised from aqueous methanol gave plates (B-form), m. p. 142—144°, $[\alpha]_D$ +88·5°. Samples recently prepared from hecogenin were of the B-form. The infra-red spectra of Nujol mulls [see C.S. nos. 152(A) and 153(B)] and of potassium bromide discs of the two forms differ significantly. Thus C=O stretching bands for the acetate and ketone groups of the A-form appear at about 1728 and 1700 cm.$^{-1}$ and those for the B-form

allo-4 : 5-*Dihydrocortisone acetate* :
a, *A-form* (C.S. no. 154); *b*, *B-form* (C.S. no. 155).



at about 1718 and 1706 cm.$^{-1}$, respectively; the C–O stretching bands for the acetate group in the two forms appear at about 1254 and 1244 cm.$^{-1}$ respectively. Further absorption changes are observed between 1200 and 700 cm.$^{-1}$. Nevertheless, both forms gave identical spectra when examined as 1·0% solutions in carbon disulphide and carbon tetrachloride, and had the same optical rotation and gave the same microanalysis.

When the A-form was fused at 135° and recrystallised from aqueous methanol, plates, m. p. 142—144°, were isolated : mulled with Nujol they gave an absorption spectrum identical with that of the B-form; the spectra of carbon disulphide solutions were identical.

The polymorphism of 3β-acetoxy*allo*pregnane-11 : 20-dione has been reported independently by Chamberlin *et al.* (*loc. cit.*), who obtained needles, m. p. 124—127°, and plates, m. p. 135—136°, from aqueous methanol. Their samples had the same optical rotation, $[\alpha]_D$ +86°, and in carbon tetrachloride solution gave identical infra-red spectra. However they did not report infra-red measurements on solid samples. Djerassi, Batres, Romo, and Rosenkranz (*J. Amer. Chem. Soc.*, 1952, **74**, 3634) obtained crystals, m. p. 143—145°, $[\alpha]_D^{20}$ +86·5°, from hexane–acetone.

allo-4 : 5-*Dihydrocortisone Acetate* (21-*Acetoxy*-17α-*hydroxy*allo*pregnane*-3 : 11 : 20-*trione*).—Crystallisation of *allo*-4 : 5-dihydrocortisone acetate from benzene yields long flat needles, m. p. 228—231°, $[\alpha]_D$ +107° (A-form), and from ethyl acetate elongated plates, m. p. 235—236°, $[\alpha]_D$ +109° (B-form); sublimation of either form yields the A-form. Romo, Rosenkranz, Djerassi, and Sondheimer (*ibid.*, 1953, **75**, 1277) obtained crystals, m. p. 232—235°, $[\alpha]_D^{20}$ +82° (in acetone), from acetone. The Nujol mull (see Figure) and potassium bromide

disc spectra of the two forms differ appreciably in the C=O and C–O stretching regions. The A-form shows the characteristic bands for 21-acetate at about 1746 and 1238, for 20-ketone at 1730, and for 3- and 11-ketones at 1700 cm.$^{-1}$; in the B-form, the 21-acetate bands are displaced to about 1730 and 1272 cm.$^{-1}$ and the 3-, 11-, and 20-ketone bands appear at the same frequency, *i.e.*, about 1710 cm.$^{-1}$. Both forms give identical spectra, when examined as 1·0% solutions in bromoform. The displacement of the 21-acetate bands in the Nujol mull spectrum of the B-form is probably due to intermolecular hydrogen-bonding.

11β-*Hydroxytigogenin* (5α : 22*a*-*Spirostan*-3β : 11β-*diol*).*—The A-form of 11β-hydroxytigogenin, obtained as small needles, m. p. 207—208°, $[\alpha]_D$ −55°, from hexane–ether [cf. Djerassi, Batres, Velasco, and Rosenkranz (*ibid.*, 1952, **74**, 1712), who obtained crystals, m. p. 202—204°, $[\alpha]_D^{20}$ −49°, from hexane–ether], gives a Nujol mull spectrum (C.S. no. 156) that differs in the region between 1300 and 700 cm.$^{-1}$ from the corresponding spectrum of the B-form (C.S. no. 157), which is obtained as needles, m. p. 208—212°, $[\alpha]_D$ −56°, from acetone; dilute carbon disulphide solutions of the two forms yield identical spectra. All the spectra contained bands at about 980, 918, 898, and 865 cm.$^{-1}$ characteristic of an *iso*sapogenin (cf. Wall, Eddy, McClennan, and Klumpp, *Analyt. Chem.*, 1952, **24**, 1337; Jones, Katzenellenbogen, and Dobriner, *J. Amer. Chem. Soc.*, 1953, **75**, 158).

3β-*Acetoxy*-23a-*bromo*-11β-*hydroxy*-5α : 22a-*spirostan*-12-*one*.†—This ketone crystallises as plates, m. p. 209—212°, $[\alpha]_D$ +3° (A-form), from aqueous ethanol and as needles, m. p. 209—212°, $[\alpha]_D$ +3° (B-form), from acetone; the A-form may be converted into the B-form by recrystallisation with rapid cooling from aqueous acetone. In the Nujol mull spectrum of the A-form (C.S. no. 158), the acetate bands appear at the expected frequencies, *i.e.*, 1738 and 1242 cm.$^{-1}$, and in that of the B-form (C.S. no. 159) at 1710 and 1265 cm.$^{-1}$; the displacement is attributed to intermolecular hydrogen-bonding. When examined as 1·0% solutions in bromoform, the two forms yield identical spectra. Both the Nujol mull and solution spectra of the two forms contain the expected absorption peaks at about 1012, 946, 918, 862, and 726 cm.$^{-1}$ for a 23*a*-bromo-22*a*-spirostan side-chain (see Dickson and Page, following paper).

11-*Oxo*pseudo*tigogenin* (3β : 26-*Dihydroxy*-5α : 25D-*furost*-20(22)-*en*-11-*one*).‡—11-Oxo-*pseudo*tigogenin crystallises as prisms, m. p. 188—191°, $[\alpha]_D$ +73° (A-form), from acetone and as flat needles, m. p. 194—196°, $[\alpha]_D$ +75° (B-form), from methanol. If the A-form is recrystallised from alkaline methanol, the B-form is obtained; the latter, recrystallised from acetone, gives the A-form. The Nujol mull spectra of the two forms [see C.S. nos. 160(A) and 161(B)] differ considerably between 1300 and 700 cm.$^{-1}$, but the spectra of dilute carbon disulphide solutions are identical. Both the Nujol mull and the solution spectra of the two forms exhibit the characteristic C=C and C–O stretching bands for a *pseudo*sapogenin at about 1690 and 1025 cm.$^{-1}$, respectively (cf. Hayden, Smeltzer and Scheer, *Analyt. Chem.*, 1954, **26**, 550).

pseudo*Tigogenin* (5α : 25D-*Furost*-20(22)-*ene*-3β : 26-*diol*).—*pseudo*Tigogenin crystallises from methanol as needles, m. p. 185—187°, $[\alpha]_D$ +24° (A-form), or plates, m. p. 185—187°, $[\alpha]_D$ +21° (B-form). Marker and Rohrmann (*J. Amer. Chem. Soc.*, 1940, **62**, 898) obtained crystals, m. p. 193—196°, from aqueous acetone. The Nujol mull spectra of both forms [see C.S. nos. 150(A) and 151(B)] contain C=C stretching bands at about 1690 cm.$^{-1}$ characteristic of a *pseudo*-sapogenin, but differ considerably between 1050 and 800 cm.$^{-1}$, where absorption bands characteristic of the spirostan side-chain appear. The spectrum of the A-form contains a strong C–O stretching band at about 1025 cm.$^{-1}$ and no prominent bands between 1000 and 800 cm.$^{-1}$, indicating an open sapogenin side-chain; that of the B-form reveals strong peaks at 983 and 938 cm.$^{-1}$ and was at first believed to represent a new type of sapogenin derivative. However, the two forms when examined as 1·0% solutions in either pyridine or chloroform yield spectra that differ in the intensity of the absorption band at about 1010 cm.$^{-1}$. Bromoform solutions of the two forms yield spectra that also differ only in the intensity of the peak at about 1015 cm.$^{-1}$,

* Scheer, Kostic, and Mosettig (*J. Amer. Chem. Soc.*, 1953, **75**, 4871) have shown that sarsasapogenin and smilagenin differ in their configurations at $C_{(25)}$ and possibly not, as earlier thought, at $C_{(22)}$. Although this finding casts some doubt on the nature of the isomerism of other sapogenins the conventional name " 22*a*-spirostan " has been used for the *iso*sapogenins.

† Since there is no convention on the representation of steroisomerism at $C_{(23)}$ in the 23-bromo-22*a*-spirostan, the nomenclature proposed by Mueller and Norton (*J. Amer. Chem. Soc.*, 1954, **76**, 749) has been adopted; thus 23-bromo- and 23-*iso*bromo-*iso*sapogenin are designated 23*a*-bromo- and 23*b*-bromo-22*a*-spirostan, respectively.

‡ In order to represent stereoisomerism at $C_{(25)}$ n the furostendiols, the configuration, 25*D*, determined for hecogenin, diosgenin, and smilagenin (James, *Chem. and Ind.*, 1953, 1388), has been assumed for 22*a*-spirostan derivatives; consequently, 22*b*-spirostan derivatives would be 25*L* (cf. Scheer, Kostic, and Mosettig, *loc. cit.*).

but are characteristic of *ana*tigogenin (cf. Dickson, Elks, Evans, Long, Oughton, and Page, *Chem. and Ind.*, 1954, 692) rather than *pseudo*tigogenin; thus the band at about 1690 cm.$^{-1}$ has been replaced by new bands at about 956, 920, and 895 cm.$^{-1}$, the change being induced by a trace of acid in the bromoform. The intensity of the 1010—1015 cm.$^{-1}$ band is greater in the spectrum of the B-form and is attributed to methanol of crystallisation. This deduction is supported by small differences in optical rotation and microanalysis. The methanol of crystallisation must therefore cause the new crystal structure and, through intermolecular hydrogen-bonding, induce the modified spirostan spectrum.

We are indebted to Miss I. E. Lewis for the *X*-ray measurements.

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CARDIFF. [*Received, August 18th*, 1954.]