matographic determination of hippuric acid, which emerged at about 245 ml. from the Dowex 2 column. Normal adult males may excrete 1.0 to 2.5 g. of hippuric acid per day, a value which is higher than others previously reported and accounts for 65 to 75% of the observed conjugated glycine in 24 hour urines.[2] Hippuric acid and PAG together account for about half of the 2 g. of bound amino acids excreted daily.

The quantities of PAG and hippuric acid observed in the urines from fasting individuals, were similar to those reported above, indicating that both compounds probably are normal metabolic products, and do not arise only as a result of the "detoxication" of dietary precursors. In phenylpyruvic oligophrenia, PAG excretion was 2.4 g. per day (*cf.* 7) whereas the quantity of hippuric acid was diminished to about 0.3 g. In Wilson's disease, urinary hippuric acid was similarly reduced in amount but PAG excretion was normal.

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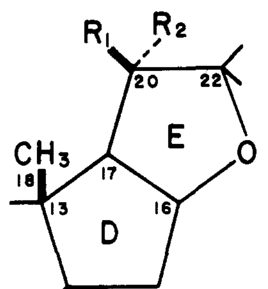
(7) L. I. Woolf, *Biochem. J.*, **49**, ix (1951).

(8) Rockefeller Foundation Fellow, 1951–1953.

STEROIDAL SAPOGENINS. XIX. STEREOCHEMISTRY OF SAPOGENINS AND CHOLESTEROL AT CARBON 20[1]

Sir:

The configuration of the methyl and hydrogen groups attached to the asymmetric C_{20} of steroid sapogenins has never been determined. We have established that naturally occurring sapogenins of both the 22b- and 22a-spirostane series have structure I at C_{20}. We have also prepared for the first time a new series of 20-isosapogenins with structure II. The evidence for these formulations follows. PSa[2,3] (m.p. 169–170°, $[\alpha]^{25}D$ +12°. Found: C, 77.79; H, 10.63) and PSm (m.p. 161°, $[\alpha]^{25}D$ +20°) on brief treatment at room temperature with alcoholic hydrochloric acid or 24 hours in ethanol–acetic acid form two new compounds which we have designated as 20-iSa and 20-iSm, respectively. The new compounds are isomeric with Sa and Sm; 20-iSa (m.p. 176–177°, $[\alpha]^{25}D$ +31.9°; Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.70; H, 10.62); 20-iSm (m.p. 185°, $[\alpha]^{25}D$ −60°; Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.92; H, 10.74). Refluxing 20-iSa and 20-iSm in alcoholic hydrochloric acid gave, respectively, Sa and Sm. Thus as we previously indicated[4] and later was shown by another group,[5] PSa and PSm are not identical as claimed by Marker and co-workers.[6] On acetylation at room temperature in pyridine–acetic anhydride, 20-iSa and 20-iSm both form monoacetates; 20-iSa acetate (m.p. 167°, $[\alpha]^{25}D$ +30°; Calcd. for $C_{29}H_{46}O_4$: C, 75.95; H, 10.11. Found: C, 75.94; H, 9.94); 20-iSm acetate (m.p. 160°, $[\alpha[^{25}D$ −49°; Calcd. for $C_{29}H_{46}O_4$: C, 75.95; H, 10.11. Found: C, 75.77; H, 10.05). Infrared spectra of both compounds showed a peak at 1732–1735 kr.[7] of strength corresponding to a monoacetate. Treatment of 20-iSa and 20-iSm with acetic anhydride at *reflux* or at 200° in a sealed tube resulted in smooth formation of PSa and PSm (after hydrolysis of the acetates).



I = Natural Sapogenins
R_1 = H, R_2 = CH_3

II = 20-Isosapogenins
R_1 = CH_3, R_2 = H

Fig. 1.—Configuration of natural and 20-isosapogenins at C_{20}.

Both 20-iSa and 20-iSm have complex infrared spectra in the region 650-1400 kr. associated with the spiroketal linkage at C_{22}.[8,9] The spectra of these two steroids are completely different from each other and also from Sa and Sm; among others 20-iSa has strong bands at 985, 965, 951, 917 and 905 kr.; 20-iSm at 974, 964, 920 and 897 kr.

Catalytic hydrogenation[10] of 20-iSa and 20-iSm resulted in formation of D20-iSa (m.p. 167°, $[\alpha]^{25}D$ −8°; Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.57; H, 10.98; diacetate, m.p. 96°, $[\alpha]^{25}D$ −3°; Calcd. for $C_{29}H_{50}O_5$: C, 74.06 H, 10.025. Found: C, 74.08; H, 10.17) and D20-iSm (m.p. 161°, $[\alpha]^{25}D$ +3°; Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.73; H, 11.02; diacetate, m.p. 96°, $[\alpha]^{25}D$ −4°; Calcd. for $C_{29}H_{50}O_5$: C, 74.06; H, 10.025. Found: C, 74.36; H, 10.09). As with DSa and DSm,[8,9] D20-iSa and D20-iSm do not have complex infrared spectra in the region 650–1400 kr. and have essentially identical spectra, which differ from that of DSa.[11] However, their respective X-ray diffraction powder patterns are completely different.

(1) Paper XVIII. M. M. Krider and M. E. Wall, THIS JOURNAL, **76**, in press (1954).

(2) Abbreviations used in this paper: Sa = sarsapogenin; Sm = smilagenin; P = pseudo; D = dihydro; 20-i = 20-iso. Tbus PDSa = pseudodihydrosarsasapogenin.

(3) All melting points obtained with Kofler micro hot stage. Rotations in chloroform with exception of PSa and PSm which were in dioxane. Infrared spectra were obtained with CS_2 solvent.

(4) M. E. Wall, C. R. Eddy, S. Serota and R. F. Mininger, THIS JOURNAL, **75**, 4437 (1953).

(5) I. Scheer, R. B. Kostic and E. Mosettig, *ibid.*, **75**, 4871 (1953).

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(8) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).

(9) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953).

(10) R. E. Marker and E. Rohrmann, *ibid.*, **61**, 846 (1939).

(11) We have found that compounds isomeric at carbon 25 cannot be distinguished by infrared spectra which are essentially identical. However, their X-ray diffraction patterns are markedly different. Compounds differing both at C_{22} and C_{25} can be distinguished by infrared spectra. Thus in the case of the infrared spectra of PSa, PSm; DSa, DSm; D20-iSa, D20-iSm; each pair has essentially identical spectra characteristically different from every other pair. Each individual compound has a characteristically different X-ray diffraction pattern. Full details of these findings will be presented in a detailed paper which will be submitted to THIS JOURNAL.

Catalytic hydrogenation of PSa and PSm diacetates followed by alkaline hydrolysis yielded the known DPSa and DPSm[12] which were found to be *identical* to D20-iSa and D20-iSm, respectively.

Mild oxidation of 20-iSa and 20-iSm with CrO_3-pyridine[13] gave the respective 3 keto derivatives; 3 keto-20-iSa (m.p. 151°, $[\alpha]^{25}D$ +20°, strong ketonic band at 1714 kr.; Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.24; H, 10.04); 3 keto-20-iSm (m.p. 162°, $[\alpha]^{25}D$ −55°; ketonic band at 1714 kr.; Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.14; H, 10.18). Reflux with alcoholic HCl resulted in formation of the known 3 keto-Sa (sarsasapogenone), m.p. 223° and 3 keto-Sm (smilagenone), m.p. 188° identical with the products of CrO_3–pyridine oxidation of Sa and Sm.

Mild oxidation of 20-iSa and 20-iSm with CrO_3–acetic acid yielded amorphous acids which on treatment with KOH in *t*-butyl alcohol were smoothly *cleaved* to the known 16-pregnen-3,20-dione, (m.p. 200–201°, $[\alpha]^{25}D$ +69.3°, $\lambda_{max.}$ 239 mμ, log ε 3.98). Similar treatment of D20-iSa and D20-iSm also resulted in formation of 16-pregnen-3,20-dione. Under similar oxidative conditions the linkage between C_{20} and C_{22} in Sa, Sm, DSa, DSm is not affected.

The data presented permit a reasonably certain assignment of configuration of steroidal sapogenins at C_{20}. Molecular models constructed for the two possible geometrical isomers show that I is under relatively little strain whereas in II the methyl groups attached to carbons 13 and 20 put a tremendous strain on ring E. The configuration II is assigned to 20-isosapogenins. It is in accord with the *facile oxidative cleavage* of such compounds and their dihydro analogs, and with the formation of pseudosapogenins on *refluxing* with acetic anhydride. Configuration I is assigned to the more stable naturally occurring steroidal sapogenins. Formation of 20-isosapogenins is not confined to sarsasapogenin and smilagenin but has been observed with diosgenin, tigogenin and hecogenin indicating it is a general reaction.

The configuration of cholesterol and related sterols and bile acids at C_{20} is still unsettled. Fieser and Fieser assigned the non-relative designations 20-a or 20-b to differentiate the side chains of such steroids.[14] Based largely on optical rotation differences, they later assigned (in terms of their C_{20} convention) the relative configuration 20-beta to the side chains of cholesterol and bile acids.[15,16] Klyne[17] deduced from the X-ray studies of Carlisle and Crowfoot[18] that the cholesterol side chain has the 20-alpha configuration.

There is now available direct chemical evidence which completely substantiates Klyne's formulation for cholesterol. Marker and Turner[19] converted diosgenin to cholesterol by a route which could not affect the acid stable C_{20} configuration (I) found in all natural steroidal sapogenins. Marker and coworkers[20,21] also showed that diosgenin, tigogenin and smilagenin all have the same side chain, a fact also confirmed by infrared studies.[8,9] Consequently the side chain configurations of cholesterol and smilagenin at C_{20} are identical. We have shown that the C_{20} configuration of smilagenin is 20-alpha. Hence cholesterol and most other natural sterols and bile acids which have been related to it have the 20-alpha configuration with respect to the rest of the molecule.

These findings confirm by an independent route the previous conclusions of Wieland and Miescher.[22] These workers showed that Δ^5-3β-acetoxy-bisnorcholenic acid could be converted to Δ^5-pregnen-3β,-20α-diol as a result of the action of perbenzoic acid. Turner[23] later showed that this type of reaction proceeds with retention of configuration. Hence the bisnor-cholenic acid and the longer chain bile acids from which it can be derived have the 20-alpha configuration.

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STEROIDAL SAPOGENINS. XX. CONFIGURATION OF SPIROKETAL SIDE CHAIN AT CARBON 22[1]

Sir:

In a recent communication Scheer, Kostic and Mosettig[2] state that Sa[3] and Sm are not isomeric at both C_{22} and C_{25} as previously believed[4] but differ only at C_{25}. We feel this view is incorrect. Not only is there excellent evidence available to show that Sa and Sm are isomeric at C_{22}, but in view of the establishment of the configuration of steroidal sapogenins at C_{20}[1] it is now possible for the first time to designate the actual configuration of Sa and Sm at C_{22}.

The evidence that Sa and Sm are isomeric at C_{22} is convincing: (a) Sa and Sm have different infrared spectra in the region 850–1350 K.[5,6] These are believed to be due to the vibrations of the –C–O–C–O–C– spiroketal system constrained by the two E and F rings. When this system is disrupted, as in

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(2) I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **75**, 4871 (1953).
(3) Abbreviations used in this paper: Sa = sarsasapogenin, Sm = similagenin, P = pseudo, D = dihydro, 20-i = 20-iso. Thus D20-iSa = dihydro 20-isosarsasapogenin.
(4) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846 (1939).
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