ASPERAGENIN, A RARE TYPE OF STEROIDAL SAPOGENIN WITH 25-HYDROXYL GROUP

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SMILAX ASPERA LINN. a drug used in Indian Medicine as a substitute for sarsaparilla is rich in saponins. The crude saponin obtained by the butanol method was hydrolysed with 2 N alcoholic HCl. Column chromatography of the crude genin mixture on neutral alumina yielded three crystalline compounds referred to as A, B and C (yields 0·01%, 0·0001% and 0·0007–0·002% respectively).

Compound A, m.p. 200–3°, $[\alpha]_D$ — 68·°*, $C_{27}H_{44}O_3$† was identified as sarsasapogenin (I) by its acetate $C_{29}H_{46}O_4$, m.p. 141–3°, $[\alpha]_D$ — 60·5°, and its pseudogenin $C_{27}H_{44}O_3$, m.p. 166–8°, $[\alpha]_D$ + 4·2° and the I.R. spectra of all the three compounds.

Compound B, m.p. 110–2°, $[\alpha]_D$ — 68·0° analysed for $C_{27}H_{42}O_3$. With Ac_2O/Py (40°) it gave an acetate m.p. 74–5°. The acetate gave a pale yellow colour with tetranitromethane. The IR spectrum of B and its acetate showed absorptions at 852, 898, 921 and 989 cm.$^{-1}$, the intensity of the 921 cm.$^{-1}$ band being *ca.* four times that of the 898 cm.$^{-1}$ band. Hence it is a steroid sapogenin of the *neo* series. It could not be studied closely because of the extremely poor yields.

Compound C, m.p. 264–8, $[\alpha]_D$ — 135·9° analysed for $C_{27}H_{44}O_5$. It was not identical with any of the known sapogenins and has been named asperagenin. It gave a diacetate with Ac_2O/Py (40°), m.p. 185–6°, $[\alpha]_D$ — 89·0° analysing for $C_{31}H_{48}O_7$. Asperagenin and its diacetate show IR absorptions at *ca.* 850, 900, 920 and 987 cm.$^{-1}$. The band at 920 cm.$^{-1}$ region is *ca.* 3–4 times more intense than that at 900 cm.$^{-1}$ Thus asperagenin seems to belong to the *neo*(25 L) series.[1,2] The nature of the fifth oxygen atom as a tertiary hydroxyl was indicated by the observation that the TNM-negative diacetate gave a TNM-positive reaction product on heating with $POCl_3$-Py at 100°.

Asperagenin gave a digitonin precipitate 3–4 times more copious than sarsasapogenin under identical conditions. This is taken as evidence for a 3β-hydroxyl. A complex peak at *ca.* 1250 cm.$^{-1}$ in the IR spectrum of asperagenin diacetate indicated the presence of an axially oriented acetoxyl group at C_3.[3] Hence 5-H should be β-oriented.

Further information about the hydroxyl groups was obtained by a study of the NMR spectrum of asperagenin diacetate (taken in $CDCl_3$ on a Varian A-60 instrument with TMS as internal standard, *see* Fig. 1 and Table I) and comparing it with the data for sarsasapogenin acetate[4,5] (ref. 4 for the C_3-H and ref. 5 for the other protons).

TABLE I

NMR spectral data for sarsasapogenin acetate and asperagenin diacetate

(Values in ppm.)

	C–18	C–19	C–21	C–27	C-3H	C–3 OAc	C–26	C–16	C–6H	C–6 OAc
Sarsasapogenin (I) acetate	0·77 (s)*	1·00 (s)	1·00 (d)*	1·08 (d)	5·1	2·06	3·3 – 3·95	4·4	..	..
Asperagenin (II) diacetate	0·77 (s)	1·04 (s)	0·95 (d)	1·14 (s)	5·33	2·06	3·28 - 3·96	4·4	4·84	1·96

* s = singlet; d = doublet.

The assignments of signals of asperagenin diacetate appearing at 0·77, 2·06, 4·4 and 3·28–3·96 ppm were made in analogy with the signals of protons in sarsasapogenin acetate. Of the remaining signals to be accounted for in asperagenin diacetate the singlet at 1·04 ppm should be due to C_{19} methyl protons; the explanation for down-field shift by 0·04 ppm is given later. The signal at 1·14 ppm may be ascribed with greater probability to the methyl protons of C_{27} and with lesser probability to those of C_{21}, the downward shift in either case being attributed to the influence of a tertiary hydroxyl group on the adjacent carbon atom, *viz.*, C_{25} or C_{20} respectively, and in either case the signal will be expected to appear as a singlet. However, a choice between the two

* All rotations were taken in chloroform solution.

† All the compounds whose formulæ are given in this communication analysed correctly for elements and functional groups.

seems to be possible. It may be recalled that treatment of asperagenin diacetate with $POCl_3$-Py yielded a mixture of two dehydration products. The formation of two products can only be explained if the tertiary hydroxyl is at C_{25}, as it can give rise to both Δ 24 : 25 and Δ 25 : 26 compounds.[6] On the other hand a tertiary hydroxyl at C_{20} has been known to undergo dehydration in only one direction yielding a single product with the double bond at 20 : 21,[7] and this is not what has happened with asperagenin diacetate. The singlet at 1·14 ppm may therefore be assigned to C_{27} methyl protons. This conclusion seems to derive support from the fact that in the closely analogous case of reineckiagenin (25L, 5β-H-spirostan-1β, 3β, 25-triol) the C_{27} methyl signal has been assigned a value 1·27 ppm.[6]

asperagenin is inert towards periodate, positions 2 and 4 are ruled out. Position 15 is also ruled out because in the NMR spectrum the signal due to C_{16} α-proton appears at the same position as in sarsasapogenin acetate. The exact location of the hydroxyl was deduced by taking into consideration the difference in the chemical shift of the C_{19} methyl proton signal as between asperagenin diacetate and sarsasapogenin acetate. It is known that in steroids the resonance frequencies of C_{18} and C_{19} methyl protons are dependent on the nature and orientation of the substituents in rings A, B, C and D.[8] An equatorial acetoxyl function if present at position 7 or 11 affects the resonance frequency of both the C_{18} and C_{19} methyls; if it is present at 6 only the C_{19} methyl signal is affected and its presence at C_{12} affects neither

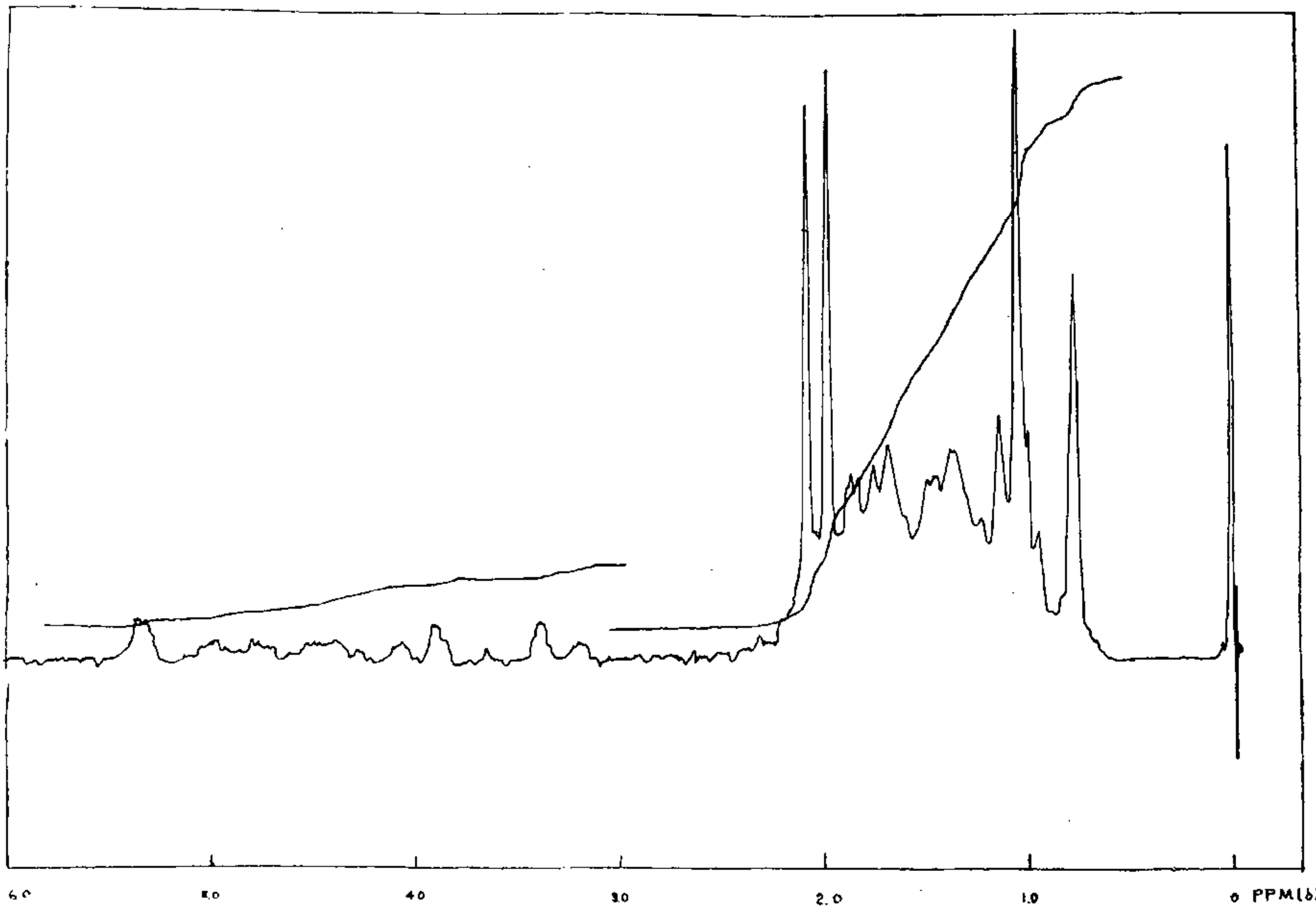


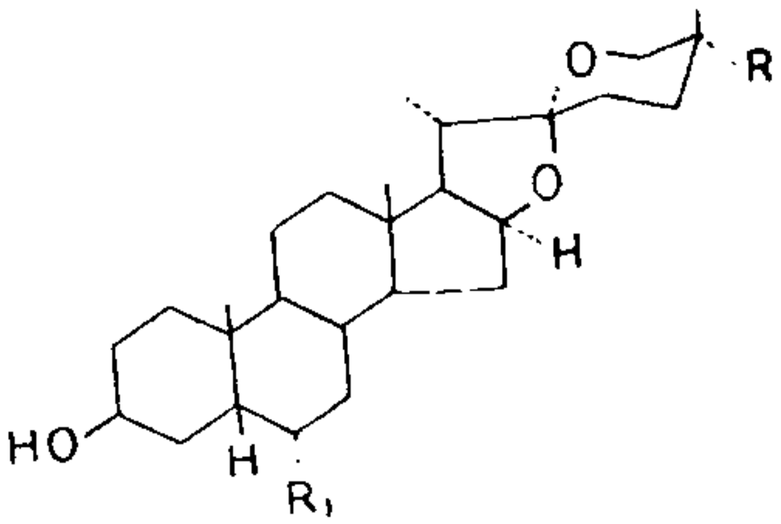
FIG. 1. NMR spectrum of asperagenin diacetate.

Regarding the remaining hydroxyl group the appearance of four C-methyl peaks in the NMR rules out the presence of any primary hydroxyl. Its ease of acetylation suggests that it is most probably an equatorially oriented secondary hydroxyl. Of the various positions which could be considered for it position 1 is eliminated since it would make asperagenin identical with reineckiagenin, which is not the case. As C_{18} nor C_{19} methyl signal. In asperagenin diacetate the signal due to proton at C_{18} appears in the same position as in sarsasapogenin acetate but the signal due to C_{19} methyl which is at 60 cps in sarsasapogenin acetate has been shifted by 2·4 cps in the case of asperagenin diacetate. Hence the acetoxyl group does not seem to be at 7, 11 or 12 but at C_6. This would also explain the value 5·33 ppm for the proton

at C_3 of asperagenin diacetate as compared to 5·1 ppm for sarsasapogenin acetate. The signal at 1·96 ppm is due to the acetoxyl protons at C_6 and the signal at 4·84 ppm to the proton α to this acetoxyl. Thus asperagenin may be assigned the structure of 25L, 5β-H-spirostan-3β, 6α, 25-triol (II). The only point for which it has not been possible to find an explanation is the upfield shift of the C_{21} protons signal by 0·05 ppm.

The molecular rotation of asperagenin diacetate seems to support the above structural assignment.

Calculated :		M_D
Sarsasapogenin acetate[9]		— 323°
Contribution of 6α-OAc[10]		— 87°
Contribution of equatorial	25-OH	
Reineckiagenin diacetate[6]	— 436°	
Rhodeasapogenin diacetate[11]	— 366°	
	— 70°	— 70°
		— 480°
Observed :		— 473°



(I) $R = R_1 = H$, Sarsasapogenin
(II) $R = R_1 = OH$, Asperagenin

Asperagenin thus belongs to the rare type of 25-hydroxy steroidal sapogenin of which the only representative so far known is reineckiagenin.[6]

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ULTRASONIC VELOCITY AND ABSORPTION MEASUREMENTS IN BINARY LIQUID MIXTURES

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RAO AND RAO[1] reported the variation of ultrasonic velocity with molar concentration in binary liquid mixtures of pyridine with some carboxylic acids. Tunin *et al.*[2] studied the ultrasonic velocity and absorption in pyridine-benzene liquid mixture and showed a linear variation of these values with concentration. As pyridine is a polar liquid with a large dipole moment, a study of binary liquid mixtures with pyridine as a common component, is likely to throw light on the nature of molecular association in these liquids. The authors have therefore taken up the study of the binary liquid mixtures of pyridine with benzene, carbon tetrachloride, carbon disulphide and chloroform.

Ultrasonic pulse technique as developed by Pinkerton,[3] was used to obtain the absorption