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ON THE STRUCTURE OF STEPHANTHRANILINE C

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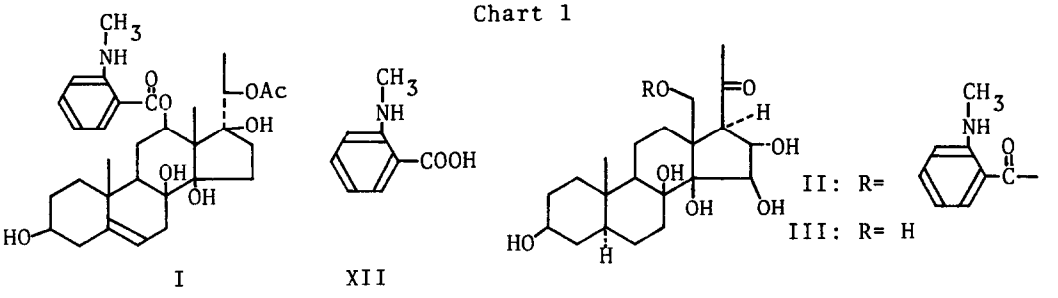
Previously, we reported the isolation of stephanthraniline A (I) from the stem of *Stephanotis japonica* Makino (Asclepiadaceae).[1] Stephanthraniline A is the first example of the presence of N-methylanthranilic acid (XII) as an acid moiety in C/D-*cis*-polyoxypregnane esters. It appeared of interest to investigate the further components of the same source for the chemotaxonomy of Asclepiadaceae.

Chart 1



By a combination of column chromatography and repetitive TLC, a new polyoxypregnane ester derivative, named stephanthraniline C (II), amorphous $[\alpha]_D$ +4.2 (c = 0.58 in $CHCl_3$), $C_{29}H_{41}O_8N$, mass spectrum; m/e 531 (M^+), was isolated from the fraction eluted by 30% acetone in benzene. UV spectrum of II showed absorption maxima at 222 nm (log ε 3.53) and 253 (3.04), which indicates the presence of N-methylanthraniloyl group (Chart 1).

^{13}C-nuclear magnetic resonance (CMR) have proved to be a useful tool for the structure analysis of C/D-*cis* polyoxypregnane derivatives by our laboratory.[2,3,4] CMR spectrum of II (Table 1) revealed that carbinyl carbon of C-12, which is normally observed at 71-75 ppm, and C-18 methyl carbon are absent, and that six carbinyl carbons are present at 63.06, 70.92, 76.09, 83.13, 83.37, and 84.13. Further, the three signals at 70.92, 83.13, and 83.37 were assignable to tertiary carbons, the resonance at 63.06 was due to a secondary carbon, and the rest were quaternary carbons by off-resonance decoupling technique. Comparison of CMR spectra of benzoyl-isoramanon (IV)

purpnigenin (VIII), and 5α,14β-androstan-14-ol[5] shows the very little dependence of a hydroxy group bearing on C-12 or C-15 to chemical shifts of C-14 and C-20. According to Yamagishi *et al*'s reports,[2,3,4] the chemical shifts of C-14 and C-20 are hardly affected by the presence of 8β-OH and acylation of 12β-OH. These evidences suggest that the shft values of C-14 and C-20 are nearly independent of neighboring environment. Thus, on the basis of above data,[2,3,4] the configuration of C-14 and C-17 were determined as both β. The signal at 69.81 was assigned to C-17 which is shifted downfield by the hydroxyl substituent effect of C-16 on β-carbon resonance. This assignment was established by comparison of CMR spectra of benzoylisoramanon (IV), pregnenolone (V), 16α-methoxy-pregnenolone (VI), and benzoyldihydrosoladulcidine (VII) (Chart 2, Table 1). From these results, one of two signals at 83.13 and 83.37 might be assignable to C-16 carbinyl-carbon. These shift values, 83.13 and 83.37, are larger than those of normal carbinyl carbons of hydroxy-steroids. Therefore, it seems that II has a 1,2 glycol system in ring D. In order to confirm this speculation, CMR spectra of purpnigenin[6] (VIII) and 3β,15ξ,16β-trihydroxy-iminocholestane (IX) were measured. Shift values of C-14 and C-15 of VIII were found to be 85.18 and 80.65, respectively. In the case of IX, carbon signals of C-15 and C-16 were observed at 84.84 and 81.62. These findings suggest that the carbinyl carbon resonances of glycol system in ring D of steroids are shifted downfield from those of glycol system in a six-membered ring. In fact, CMR spectrum of cholestane-3β,5α,6β-triol (X) shows signals of C-5 and C-6 at 76.08 and 67.38, respectively. These results indicate that stephanthraniline C is a monoester of 3β,8β,14,15ξ,16ξ,18-hexahydroxy-5α,14β,17α-pregnan-20-one with N-methylanthranilic acid.

In order to investigate the configuration at C-15 and C-16, proton nuclear magnetic resonance (PMR) spectrum of II was determined. PMR spectrum of II shows the following signals, δ ($CDCl_3$): 1.08 (3H, s, 19-CH_3), 2.30 (3H, s, 21-CH_3), 2.81 (1H, d, j = 2.9 Hz, 17α-H), 2.90 (3H, d, j = 6 Hz, NH-CH_3), 3.66 (1H, m, 3α-H), 3.97 (1H, t, j = 2.9, 3.6 Hz, 16β-H), 4.18 (1H, d, j = 3.6 Hz, 15α-H), 4.45 and 5.27 (2H, AB quartet, j = 11 Hz, 18-CH_2), 6.52, 6.63, 6.71, 7.29, and 7.78 (4H, m, aromatic protons).

PMR spectrum, as well as CMR spectrum, showed the absence of 18-CH_3. Remarkably deshielding of 19-CH_3 should be attributed to the 8β-OH, which is in the relation of 1,3-diaxial to 19-CH_3. Further, the appearance of signals assignable to 18-CH_2 at 4.45 and 5.27 indicates that hydroxymethyl group is esterified with N-methylanthranilic acid (XII). In the PMR spectrum of 3β,20-diacetoxy-14β,18-dihydroxy-5α,17α-pregnane[7] (XI), resonances of 18-CH_2 have been observed at 3.46 and 4.16 (AB quartet, j = 12 Hz). Therefore, it is reasonable that 18-hydroxymethyl is shifted downfield by the

Table 1

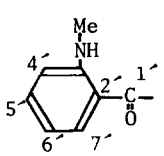
^{13}C Chemical Shifts

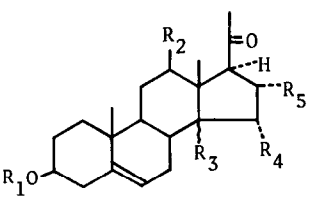
	II		IV	V	VI	VII	VIII	IX
C-1	38.04		37.62	37.32	37.32	36.99	38.94	38.25
C-2	30.94		26.65	31.89	31.74[c]	27.78	32.60	32.06
C-3	70.92		77.82	71.49	71.73	74.56	71.29	71.68
C-4	38.80		43.13	42.22	42.40	34.31	43.47	38.84
C-5	45.21		140.39	140.91	140.91	44.94	141.02	46.05
C-6	32.93		121.53	121.19	121.33[c]	28.81	122.26	28.51
C-7	24.42		27.78	31.83	31.86[c]	32.17	27.39	32.55
C-8	76.09		36.94	31.57	31.66	35.29[d]	38.64	34.60
C-9	50.08		43.56	50.03	50.03	54.68	45.86	55.16
C-10	39.16		37.33	36.56	36.71	35.77[d]	37.67	36.65
C-11	19.32		34.35	21.12	20.94	21.30	21.20	21.98
C-12	34.46		71.05	38.88	38.99	40.65	37.77	41.47
C-13	47.61		54.28	44.04	44.60	43.23	48.39	44.54
C-14	84.13[a]		85.62	56.93	54.61	54.52	85.18	55.60
C-15	83.13[a]		32.36	22.88	32.16	36.16	80.65	84.84
C-16	83.37[a]		24.22	24.52	81.64	71.40	36.89	81.62
C-17	69.81		58.38	63.71	71.73	63.11	62.81	61.60
C-18	63.06		10.82	13.25	14.63	12.53	17.30	12.86
C-19	18.85		19.54	19.44	19.56	13.94	19.15	13.94
C-20	216.35		214.35	209.63	208.22	35.87[d]	214.11	36.40
C-21	33.35		31.92	31.57	31.74	19.69[e]	32.11	18.76
C-1'	168.91[b]	C-22				60.04		60.82
C-2'	114.37	C-23				33.87		33.19
C-3'	152.18	C-24				27.49		29.87
C-4'	110.49	C-25				31.68		29.97
C-5'	134.51	C-26				54.22		52.73
C-6'	110.89	C-27				19.42[e]		14.77
C-7'	131.28							
N-CH_3	29.65							

δ ppm from internal TMS; JEOL JNM-FX 100 FT-NMR spectrometer
a, c, d, e) values may be reversed

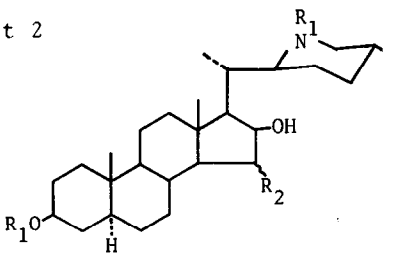
II, V, VII, VI were measured in $CDCl_3$ soln.; IV, VIII in C_5D_5N; IX in CD_3OD.

Chart 2



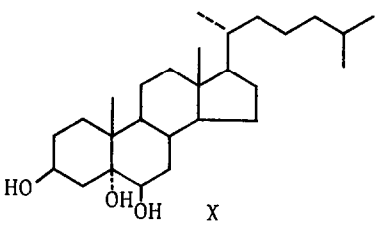


IV: R_1= benzoyl, R_2= OH, R_3= β-OH
R_4=R_5= H
V: R_1=R_2=R_4=R_5= H, R_3= α-H
VI: R_1=R_2=R_4= H, R_3= α-H, R_5= OMe
VIII: R_1=R_2=R_5= H, R_3= β-OH, R_4= α-OH

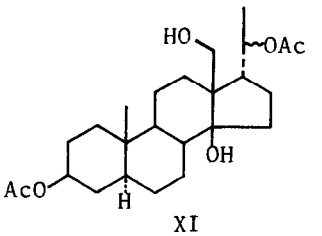


VII: R_1= benzoyl, R_2= H
IX: R_1= H, R_2= OH

presence of an acyl group. From the half-width of 3α-H signal, 22 Hz, the configuration of C-5 was determined as α. Irradiation of absorption at 3.97 collapsed the doublet at 2.81 to a singlet, so that the hydroxymethines at 3.97 and 4.18, in agreement with coupling constants, are assigned to 16β-H (α-OH) and 15α-H (β-OH), respectively. From these evidences, the structure of stephanthraniline C was determined as 18-N-methylanthraniloxy-3β,8β,14,15β,16α-pentahydroxy-5α,14β,17α-pregnan-20-one (II). To the author's knowledge, this is the first example of a novel structure of II, 3β,8β,14,15β,16α,18-hexahydroxy-5α,14β,17α-pregnan-20-one, found in nature. We suggest the name of shitakigenin for this structure (III).



X



XI

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