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THE STRUCTURE OF MONNIERIN

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In our previous communication (1), the isolation of monnierin and an approach for the determination of its molecular formula, $C_{51}H_{82}O_{21}$, were reported. The present communication contains data which can serve a basis for suggesting structure I for monnierin.

It has already been shown (1), that monnierin (I) is a triterpene tetroside, containing residues of one glucose and three arabinoses per molecule of the triterpene. The molecular formula and the physical constants of the acid hydrolysed sapogenin, $C_{30}H_{48}O_4$, corroborated fairly well with the same of bacogenin A, isolated from the same plant by Chatterjee et al (2).

On keeping monnierin suspended in 0.01(N) aqueous hydrochloric acid at room temperature for 21 days arabinose was eliminated from the molecule as was noted from paper chromatograms. A further treatment of the isolated product under similar conditions did not indicate the presence of any sugar in the hydrolysate. This easy elimination of arabinose indicates that either a part or whole of the arabinose molecule is present in furanose form. The prosapogenin, m.p., 303-5°, $[\alpha]_D$, +10.7° (C, 2.23 in 70% aq.methanol) on hydrolysis with 5% methanolic hydrochloric acid for 6 hrs. gave bacogenin A and the hydrolysate indicated and identified glucose only on paper chromatograms. The above observation clearly led to the conclusion that whole of the arabinose residue present in monnierin was linked through the furanose form. Furthermore identification of glucose only in the hydrolysate of the prosapogenin conclusively indicated that glucose was linked directly to the sapogenin.

Monnierin was found to consume 5 moles of periodate and liberated one mole of formic acid. This is quite consistent with the contention that arabinose is present in the furanose form and glucose in the pyranose form.

The periodate oxidation product was degraded according to the methods of Barry (3). The crude product after removal of the sapogenin part, was chromatographed on alumina and two fractions were isolated in almost equal proportions. The first one eluting with benzene was identified as glyoxal-bis-phenyl hydrazone, m.p., 166-8° (Found, C, 70.71; H, 5.53; N, 22.8%; Calc. for $C_{14}H_{14}N_4$, C, 70.59; H, 5.88; N, 23.53) and the second one eluting out with benzene: ether (1:1), as glycerosazone, m.p., 130-2° (Found, C, 67.6; H, 5.63; N, 19.8; Calc. for $C_{15}H_{16}ON_4$, C, 67.17; H, 5.97; N, 20.89). Isolation of glycerosazone and glyoxal-bis-phenyl-hydrazone supported the previous observation of the furanosidic and pyranosidic nature of arabinose and glucose respectively.

I was methylated with CH_3I in presence of Ag_2O in dimethyl formamide (4) and the resulting product was chromatographed over alumina. The benzene: Chloroform (3:7) eluate afforded crystalline permethyl-I, m.p.,144-6°, $[\alpha]_D$, -32.31° (C, 1.47 in $CHCl_3$), ν_{max} 1096 cm^{-1} (ether) and absence of -OH bands; (Found, C, 62.40; H, 8.81; $-OCH_3$, 28.11; Calc. for $C_{62}H_{104}O_{21}$, C, 62.84; H, 8.78; $-OCH_3$, 28.80). The acid hydrolysis of permethyl derivative (5% methanolic hydrochloric acid for 12 hrs. at 100°) furnished a mono-methyl sapogenin, m.p., 208-10°; $[\alpha]_D$, +26.63° (C, 1.35 in $CHCl_3$); (Found, C, 76.78; H, 9.89; $-OCH_3$, 5.98; Calc. for $C_{31}H_{50}O_4$, C, 76.54: H, 10.29; $-OCH_3$, 6.38) as well as a water soluble part containing a mixture of the methylated sugars. The paper chromatography of the partially methylated sugars following the method of Hirst et al (5) showed 3 spots, two of which comparing favourably well with authentic specimens of 2,3,5-tri-O-methyl-arabinose (R_G,0.97) and 2,3,4-tri-O-methyl-glucose (R_G, 0.80). The partially methylated sugars were inert to periodate and it, therefore, followed that the methylated sugar arising from the acid hydrolysis of the permethyl derivative definitely possessed a $-OCH_3$ group in C-2. This result excluded the possibility of all the

glycoside linkages implicating the C-2 of the tetrasaccharide.

By chromatography on cellulose column, following the method of Jones et al (6), of the partially methylated sugars obtained by the hydrolysis of permethyl I it had been possible to separate, isolate and identify, the following:

(a) 2,3,5-tri-O-methyl-L-arabinose - This constituted the first part eluted from the column. It was obtained in the form of a colourless syrup (Found, C, 49.64; H, 8.70; $-OCH_3$, 47.45; Calc. for $C_8H_{16}O_5$, C, 50.00; H, 8.33; $-OCH_3$, 48.44).

(b) 2,3,4-tri-O-methyl-D-glucose - This constituted the second component eluted from the column and was obtained as a colourless syrup; $[\alpha]_D$, $+68^\circ$ (C, 0.83 in water) (Found, C, 48.1; H, 8.44; $-OCH_3$, 40.30; Calc. for $C_9H_{18}O_6$, C, 48.64; H, 8.11; $-OCH_3$, 41.89); anilide, m.p., 145-6° (cf. literature).

(c) 2,3-di-O-methyl-L-arabinose - The third component eluted from the column and was obtained as a colourless syrup, $[\alpha]_D$, $+101^\circ$ (C, 0.92 in water) (Found, C, 46.65: H, 7.22: $-OCH_3$, 33.2: Calc. for $C_7H_{14}O_5$, C, 47.2; H, 7.87; $-OCH_3$, 34.83); anilide, m.p., 137-8° (cf. literature).

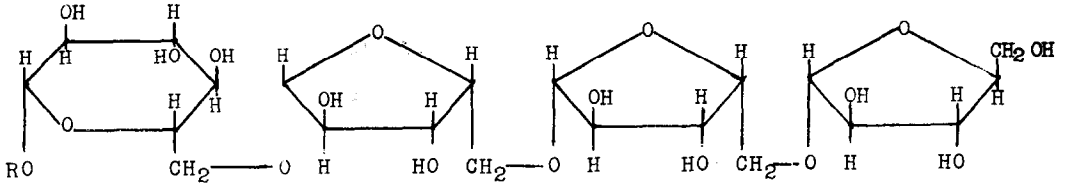
It has been shown by Chatterjee et al (2) that the tertiary hydroxyl group at C-20 of bacogenin A is a hindered hydroxyl group as is also the case with 3:20-dihydroxy-dammar-24-ene (7). This hindered C-20 hydroxyl group thus would not allow any attachment of either a methoxyl radical or a sugar residue. Isolation of a mono-methyl sapogenin from the acid hydrolysis of permethyl monnierin clearly indicates that one of the hydroxyl group other than the hindered one at C-20 is free. The possibility of the attachment of the sugar residue may thus be at C-3 or C-10 - hydroxymethyl. Again the same authors (2) have demonstrated the hindrance of bacogenin A to tritylation which distinctly eliminates the possibility of attachment of the sugar residue to the hydroxymethyl group at C-10. Thus it can be assumed that

the sugar chain is attached to C-3 hydroxyl group. The previous observations corroborate the idea that the tetrasaccharide residue of monnierin is a linear chain and is attached to the C-3 hydroxyl group.

An analysis of the molecular rotations showed that all the arabinofuranoside residues are present in α-configuration. Molecular rotation of the prosapogenin and the sapogenin indicate that in I the glucose residue is attached to the sapogenin by means of β-glycosidic bond.

All the characteristics so far studied indicate to establish that monnierin is best represented as 3-O-[α-L-arabinofuranosyl (1→5)-O- α -L-arabinofuranosyl (1→5)-O- α -L-arabinofuranosyl (1→6)]-O- β -D-glucopyranosyl-20-hydroxy-10-hydroxymethyl-16-keto-dammar-24-ene (I).

An examination of the infra red spectra of monnierin would clearly indicate the absence of any band specific for a carbonyl function. But the acid hydrolysis of the saponin always gave a keto compound. This probably indicates that the keto group is not present in the molecule as such but is present as a ketol function implicating the C-20 hydroxyl and C-16-keto group. Further work on the enzymatic hydrolysis of the saponin as well as chemical evaluation of the actual nature of the sapogenin are in progress.



R = Bacogenin

(I)

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