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STEROIDAL SAPONINS FROM *DIOSCOREA FLORIBUNDA*: STRUCTURES OF FLORIBUNDASAPONINS A AND B

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Key Word Index—*Dioscorea floribunda*; Dioscoreaceae; steroidal saponins; floribundasaponin A; floribundasaponin B.

Abstract—Two steroidal saponins, floribundasaponins A and B isolated from the yams of *Dioscorea floribunda*, have been characterized as pennogenin-3-*O*-β-D-glucopyranoside and pennogenin-3-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranoside.

INTRODUCTION

Dioscorea floribunda is a newly introduced species in India. Its high diosgenin content and particularly its adaptability to the agro-climatic conditions of the vast Indian plains attracted attention for its use in production of diosgenin in India [1]. A strain of this species with high diosgenin content is being maintained at the Institute of Horticultural Research, Bangalore. Hoyer *et al.* [2] reported the isolation of a number of saponins from the yams of *D. floribunda*. In a recent communication we reported the isolation of six steroidal saponins from the yams, collected from Bangalore [3]. Four of these saponins, floribundasaponins C, D, E and F were completely characterized. The present study is concerned with the isolation and structure elucidation of the two uncharacterized saponins, floribundasaponins A and B.

RESULTS AND DISCUSSION

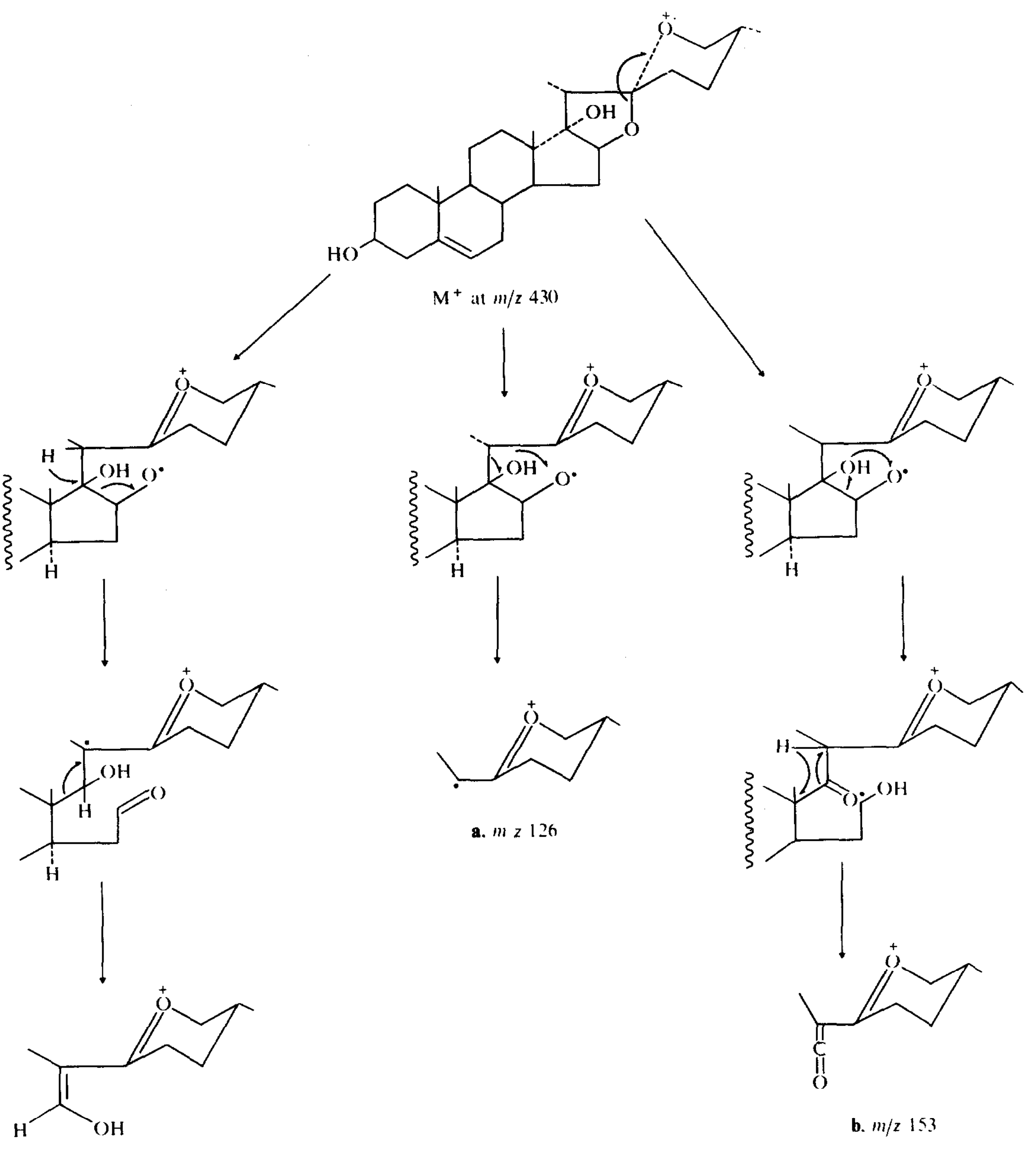
Repeated column chromatography of the methanol extract of the yams led to the isolation of six saponins including floribundasaponins A (**1**) and B (**2**). Compound **2** crystallized from MeOH and was negative to the Ehrlich test [4]. It showed spiroketal absorptions in its IR spectrum. On acid hydrolysis **2** yielded a sapogenin (**3**) and sugar constituents identified as D-glucose and L-rhamnose. Moreover, GLC analysis revealed that D-glucose and L-rhamnose were present in the ratio 1:1. The sapogenin **3** showed IR absorptions characteristic of the (25*R*)-configuration [5]. The ^{1}H NMR spectrum of the sapogenin acetate (**4**) displayed signals which revealed it to be identical to pennogenin acetate. The identity of **3** with pennogenin was finally confirmed by direct comparison with an authentic sample [6]. The mass spectrum of pennogenin shows some characteristic peaks whose geneses have not yet been explained. For example, it does not show the base peak at *m/z* 139 which is characteristic of the spirostane sapogenins [7, 8] having no substitution in rings E and F. Instead, the base peak is exhibited at *m/z* 126. The other significant peaks appear at *m/z* 153 and 155. Although the genesis of the peak at *m/z* 126 resulting from (25*R*)-5α-spirostane has been rationalized, it seems reasonable that the appearance of this ion peak as the base peak in the mass spectrum of pennogenin may best be rationalized as shown in Scheme 1. It is noteworthy that the existence of the peak at *m/z* 153 ($C_9H_{13}O_2$) has been mentioned by Howe *et al.* [9] while reporting the mass spectrum of a metabolite (**5**) with a 17α-hydroxyl group formed by microbial modification of diosgenin. However, the structure of the fragment ion has not been deduced. The geneses of the peaks at *m/z* 126, 153 and 155 are rationalized in Scheme 1.

The structural feature of the carbohydrate moiety of **2** was demonstrated as follows. When **2** was subjected to acid hydrolysis under controlled conditions pennogenin and a prosaponin (**1**) were isolated. The prosaponin on hydrolysis yielded D-glucose as the only monosaccharide constituent. Moreover, **1** on permethylation by the Hakomori method [10] followed by hydrolysis liberated 2,3,4,6-tetra-*O*-methyl-D-glucose. The methylated derivative (**6**) showed signals in its ^{1}H NMR spectrum at δ 4.35 (1 H, *d*, *J* = 7 Hz) attributable to H-1 of glucose and indicating a β-linkage ($^4C_1$ conformation). Moreover, the prosaponin was identical to a synthetic sample of pennogenin-3-*O*-β-D-glucopyranoside (**1**). Consequently, it is demonstrated that in **1** and hence in **2**, the carbohydrate moiety is attached at the C-3 hydroxyl. Methylation of **2** gave **7** which on hydrolysis resulted in the formation of 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose. Thus, it was evident that in **2** rhamnose is linked to glucose through a (1 → 4)-linkage.

The configurations at the anomeric centres of glucose and rhamnose were revealed as β and α respectively by application of Klyne's rule [11] of molecular rotation. The calculated and observed values for $[M]_D$ of **2** were of the same order of magnitude. The ^{1}H NMR spectrum of **6** displayed signals at δ 4.36 (1 H, *d*, *J* = 7 Hz) and 5.21 (1 H, *br s*) ascribable to H-1 of glucose (β-linkage) and H-1 of rhamnose (α-linkage) respectively. Consequently, floribundasaponin B is pennogenin-3-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranoside (**2**).

Floribundasaponin A (**1**) on hydrolysis liberated **3** as the sapogenin and D-glucose as the only sugar ingredient. Its mp and $[\alpha]_D$ value indicated its identity with pennogenin-3-*O*-β-D-glucopyranoside (**1**), the prosapogenin obtained as mentioned earlier.

Although pennogenin-3-*O*-β-D-glucopyranoside has already been reported [6] to be obtained by partial hydrolysis of another saponin, this is the first report of its isolation from a natural source.

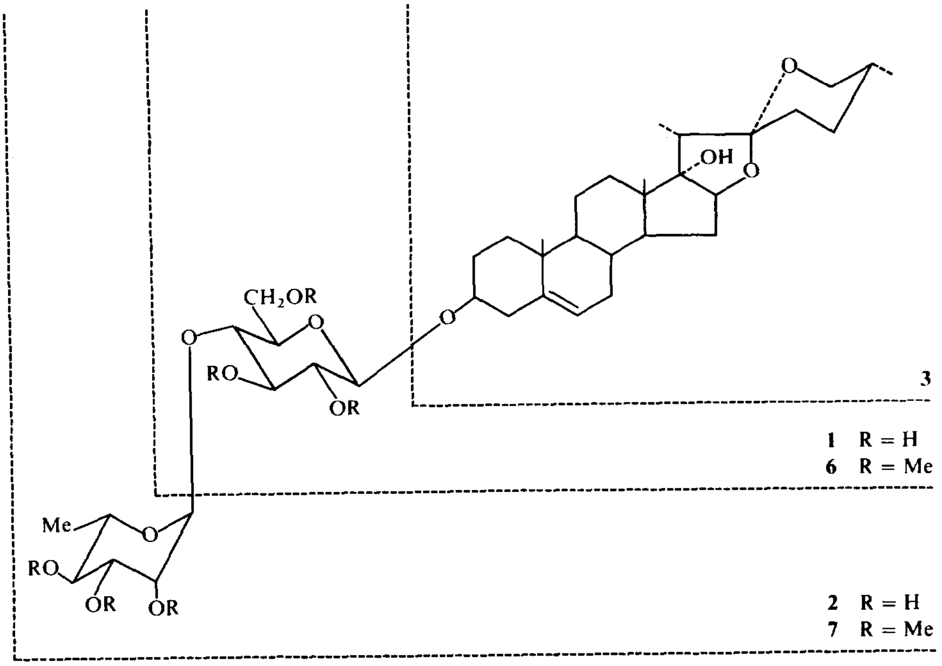


Scheme 1.

EXPERIMENTAL

Mps are uncorr. TLC was performed on Si gel G (BDH) using solvent system (a) C_6H_6–$CHCl_3$–EtOAc (1:1:0.5) and (b) $CHCl_3$–MeOH–H_2O (7:3:0.5). Paper chromatography of sugars was carried out on Whatman paper No. 1 using the solvent systems (c) n-BuOH–C_5H_5N–H_2O (6:4:3) and (d) n-BuOH–EtOH–H_2O (4:1:5, upper layer); a saturated soln of aniline oxalate in water [12] was used for staining. GLC employed the columns (i) 3% ECNSS-M on Gas Chrom Q at 190° for alditol acetates and (ii) 3% OV-225 on Gas Chrom Q at 195° for partially methylated alditol acetates. Optical rotations were taken at 15–25°. ^1H NMR spectra were recorded at 90 MHz in $CDCl_3$ and chemical shifts are given in δ (ppm) with TMS as internal standard. Mass spectra were recorded with an ionizing potential of 70 eV.

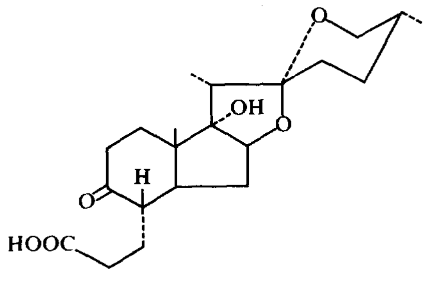
Isolation of saponins. Air-dried, powdered yams of *D. floribunda* Mart. & Gal. (1.5 kg), collected from Bangalore, were successively extracted with petrol (60–80°) and MeOH. The MeOH extract on removal of solvent gave a dark brown, semi-solid mass (200 g). It showed on TLC six distinct spots (floribundasaponins A, B, C, D, E and F). Part (40 g) of the residue was placed on a Si gel (800 g) column. Successive elution with $CHCl_3$, $CHCl_3$–MeOH (90:10), (85:15), (80:20) and (75:25) afforded different saponins and their mixtures in the increasing order of their polarities. Fractions eluted with $CHCl_3$–MeOH (90:10) yielded the mixture of floribunda saponins A and B with some gummy material (300 mg). This was



3

1 R = H
6 R = Me

2 R = H
7 R = Me



Metabolite 5

rechromatographed over Si gel (15 g), eluting the column with $CHCl_3$–MeOH (95:5). The chromatography was monitored by TLC. Earlier fractions yielded floribundasaponin A (35 mg) and the later fractions afforded floribundasaponin B (68 mg).

Floribundasaponin B (**2**). It crystallized from MeOH as needles, mp 251–253°, $[\alpha]_D$ −86.5° (pyridine), negative to Ehrlich reagent, IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (br, OH), 980, 920, 900, 800 (900 > 920). (Found: C, 63.53; H, 8.38. $C_{39}H_{62}O_{13}$ requires: C, 63.39; H, 8.45%).

Hydrolysis of **2**. Compound **2** (30 mg) was hydrolysed with 5% MeOH–H_2SO_4 (10 ml) for 5 hr at waterbath temp. and worked up as usual. The residue was purified by chromatography to yield pennogenin (**3**) which crystallized from MeOH as colourless needles (11 mg), mp 234–236°, $[\alpha]_D$ −104° ($CHCl_3$). IR ν_{max}^{KCl} cm^{-1}: 3550, 980, 920, 900, 820; MS *m/z* (rel. int.): 430 $[M]^+$ (9), 415 $[M - Me]^+$ (2), 412 $[M - H_2O]^+$ (1), 359 (2), 277 (5), 259 (4), 257 (5), 239 (4), 232 (7), 214 (12), 199 (10), 155 $[c]^+$ (32), 153 $[b]^+$ (72), 139 (16), 127 (38) and 126 (100). (Found: C, 75.20; H, 9.89. Calc. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83%). Acetate (**4**) was prepared from **3** with Ac_2O and pyridine and crystallized from MeOH to give micro-needles, mp 197–198°, $[\alpha]_D$ −101° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1725, 1240 (acetate carbonyl), 1060, 1040, 980, 920 and 900. MS *m/z* (rel. int.): 472 $[M]^+$ (2), 457 $[M - Me]^+$ (4), 454 $[M - H_2O]^+$ (2), 439 $[M - Me - H_2O]^+$ (2), 412 $[M - HOAc]^+$ (86), 394 $[M - HOAc - H_2O]^+$ (4), 340 (8.6), 299 (21), 298 (77), 281 (5), 280 (15), 269 (5), 259 (23), 258 (14), 257 (14), 251 (5), 239 (1), 219 (1), 215 (15), 214 (52), 203 (30), 202 (48), 155 (35), 153 (84), 139 (22), 127 (51) and 126 (100); ^1H NMR: δ 0.82 (3 H, *s*, H-18), 1.03 (3 H, *s*, H-19), 0.90 (3 H, *d*, $J = 6$ Hz, H-21), 0.80 (3 H, *d*, $J = 6$ Hz, H-27), 1.97 (3 H, *s*, -OCOMe), 3.95 (1 H, *t*, $J = 7$ Hz, H-16), 5.30 (1 H, *m*, H-6). (Found: C, 73.81; H, 9.90. Calc. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.83%).

The filtrate of the hydrolysate was worked up as usual and divided into two parts. The first part was examined for sugars by PC (solvent system c), leading to the identification of D-glucose and L-rhamnose using authentic specimens. The second part was converted to alditol acetates in the usual way and analysed by GLC using column i. The areas of peaks corresponding to the alditol acetates of glucose and rhamnose disclosed that these were present in the ratio 1:1.

Partial hydrolysis of **2** *and formation of* **1**. Compound **2** (100 mg) was hydrolysed with 1% H_2SO_4 in MeOH (15 ml) on a waterbath for 25 min and worked up in the usual manner. The residue was subjected to prep. TLC to yield the sapogenin, pennogenin (**3**) and the prosaponin (**1**). Compound **1** crystallized from MeOH to give colourless needles (35 mg), mp 275–278° (dec.), $[\alpha]_D$ −117.4° (pyridine). (Found: C, 67.71; H, 7.92; Calc. for $C_{33}H_{52}O_9$: C, 67.80; H, 8.04%).

Methylation of **1**. Compound **1** (12 mg) was methylated by the Hakomori method and worked up as usual. The methylate (**6**) was obtained as a brownish amorphous powder; ^1H NMR: δ 4.35 (1 H, *d*, $J = 7$ Hz, H-1 of glucose unit). Hydrolysis of **6** with 1.5 N HCl in MeOH gave pennogenin (**3**) and 2,3,4,6-tetra-*O*-methyl-D-glucose identified by PC (solvent d) and GLC (column ii) by comparison with an authentic sample.

Methylation of **2**. Compound **2** (20 mg) was methylated as in the case of **1** and the methylate **7** was obtained as a colourless powder (16 mg); ^1H NMR: δ 4.36 (1 H, *d*, $J = 7$ Hz, H-1 of glucose unit) and 5.21 (1 H, *br s*, H-1 of rhamnose unit). On hydrolysis as described above, **7** furnished **3**, 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose (identified by PC and GLC with the authentic samples).

Floribundasaponin A (**1**). The fractions eluted before **2** and found homogeneous by TLC were mixed and crystallized from MeOH to yield **1** as colourless needles (16 mg), mp 276–279° (dec.), $[\alpha]_D$ −117° (pyridine). It was found to be identical with the prosaponin **1**.

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