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# DIOSGENIN SAPONINS FROM *DIOSCOREA FLORIBUNDA*

GEORG-ALEXANDER HOYER

Schering AG, Berlin

and

WOLFGANG SUCROW and DIETER WINKLER

Institut für Organische Chemie, Technische Universität Berlin D-1000 Berlin 12 / Germany

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**Key Word Index**—*Dioscorea floribunda*; Dioscoreaceae; saponins; diosgenin glycosides; spirostanols; furostanols; NMR spectra of saponins.

**Abstract**—Five spirostanol glycosides and two furostanol glycosides were isolated from *Dioscorea floribunda*. In addition to the IR spectra of the free glycosides and the MS of the peracetates and permethyl ethers, the most effective method for structural determination proved to be the NMR spectra of the free saponins in pyridine-$d_5$.

## INTRODUCTION

In recent papers, Kawasaki [1] and Tschesche [2] have independently described the isolation and structural elucidation of a diosgenin tetraglycoside from Liliaceae species. This prompts us to report our own examinations of the saponins obtained from *Dioscorea floribunda* Mart. et Gal.

Previously, dioscin (**4**) [3], its prosapogenin B (**2**) [4] and diosgenin have been isolated from *Dioscorea* species. The partial hydrolysis [3, 4] of dioscin (**4**) gave trillin (**1**), prosapogenin A (**3**) and smaller amounts of prosapogenin B (**2**). All these compounds belong to the spirostanol series with an intact ring *F*. Furthermore, compounds giving a red colour with Ehrlich reagent have been isolated which possess a furostanol structure, whereas spirostanol glycosides do not show an Ehrlich reaction [5].

Marker and Lopez [6] have pointed out that saponins of the spirostanol type may be artefacts formed from furostanol glycosides by hydrolysis during isolation. In the natural state, the sugar linked to the C-26 hydroxyl group protects the furostanol saponins from ring closure, but rupture of the C-26 glycosidic bond then causes the formation of spirostanol glycosides. It is equally possible, however, that furostanol glycosides represent the "transport form" and spirostanol glycosides the "depot form" in the root.

## RESULTS AND DISCUSSION

We examined roots about 4 weeks after their harvest in Central America. They were mostly intact, but parts were rotten and these were separated. After extraction of the intact parts with EtOH and repeated chromatography with $CHCl_3$–MeOH–$H_2O$ over silica gel the following saponins were isolated:

Spirostanol saponins: 3-*O*-[β-D-Glucopyranosyl]-diosgenin (trillin) (**1**) [3]; 3-*O*-[α-L-Rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl]-diosgenin ('prosapogenin B') (**2**) [4]; 3-*O*-[α-L-Rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-diosgenin ('prosapogenin A') (**3**) [3, 4]; 3-*O*-{[α-L-Rhamnopyranosyl-(1 → 4)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl}-diosgenin (dioscin) (**4**) [3, 7]; 3-*O*-{[α-L-Rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl}-diosgenin (**5**) [1, 2].

Furostanol saponins (isolated as the 22-methyl ethers): 26-[β-D-Glucopyranosyloxy]-(25*R*)-furost-5-en-3β,22α-diol (**6**); 3β-{[α-L-Rhamnopyranosyl-1 → 4)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyloxy} - 26 - [β - D - glucopyranosyloxy] - (25*R*)-furost-5-en-22α-ol (**7**) [5, 8].

As expected, the amount of the more polar furostanol glycosides was rather high in well-preserved roots, but low in less-well-preserved samples, where they were replaced by the spirostanol

Table 1. NMR spectra of saponins and sapogenins in pyridine-$d_5$

| | Yamogenin | Diosgenin | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|
| Me-18 | 0·85*s* | 0·85*s* | 0·83*s* | 0·83*s* | 0·82*s* | 0·83*s* | 0·83*s* | 0·83*s* | 0·83*s* |
| Me-19 | 1·04*s* | 1·04*s* | 0·92*s* | 0·92*s* | 1·04*s* | 1·04*s* | 1·04*s* | 1·03*s* | 1·04*s* |
| Me-21 | 1·14*d* | 1·13*d* | 1·13*d* | 1·13*d* | 1·12*d* | 1·13*d* | 1·13*d* | 1·17*d* | 1·18*d* |
| | (6) | (6) | (6) | (6) | (6) | (6) | (6) | (7) | (7) |
| Me-27 | 1·08*d* | 0·70*m* | 0·70*m* | 0·70*m* | 0·70*m* | 0·70*m* | 0·70*m* | 1·00*d* | 1·00*d* |
| | (6) | (8) | (8) | (8) | (8) | (8) | (8) | (7) | (7) |
| $CH_2$-26 | 4·04*dd* | | | | | | | | |
| | (11, 3) | 3·52*m* | 3·52*m* | 3·53*m* | 3·53*m* | 3·54*m* | 3·53*m* | 3·61*m* | 3·61*m* |
| | 3·35*d* | (11) | (11) | (11) | (10) | (11) | (11) | (12) | (12) |
| | (11) | | | | | | | | |
| H-6 | 5·36*d* | 5·36*d* | 5·28*m* | 5·29*m* | 5·28*m* | 5·29*m* | 5·29*m* | 5·36*d* | 5·30*m* |
| | (5) | (5) | (9) | (9) | (9) | (9) | (9) | (5) | (9) |
| $CH_2$-4 | 2·58*d* | 2·58*d* | | | 2·75*m* | 2·73*m* | 2·73*m* | 2·58*d* | 2·75*m* |
| | (7) | (7) | | | (12) | (14) | (12) | (7) | (14) |
| H-1′ | | | 4·95*d* | 4·98*d* | 4·95*m* | 4·87*m* | 4·87*m* | | 4·87*m* |
| | | | (7) | (7) | | | | | |
| H-1″ | | | | | 6·28*m* | 6·27*m* | 6·27*m* | | 6·28*m* |
| | | | | | (4) | (4) | (4) | | (4) |
| H-1‴ | | | | 5·78*m* | | 5·74*m* | 5·71*m* | | 5·74*m* |
| | | | | (4) | | (4) | (4) | | (4) |
| H-1⁗ | | | | | | | 6·15*m* | | |
| | | | | | | | (4) | | |
| H-1$^V$ | | | | | | | | 4·77*d* | 4·78*d* |
| | | | | | | | | (7) | (7) |
| Me″ | | | | | 1·73*d* | 1·73*d* | 1·72*d* | | 1·73*d* |
| | | | | | (6) | (6) | (6) | | (6) |
| Me‴ | | | | 1·67*d* | | 1·58*d* | 1·55*m* | | 1·58*d* |
| | | | | (6) | | (6) | | | (6) |
| Me⁗ | | | | | | | 1·55*m* | | |
| OMe | | | | | | | | 3·23*s* | 3·24*s* |

* Chemical shifts in $\delta$ (ppm); *s* = singlet, *d* = doublet, *dd* = double doublet, *m* = multiplet; the figures in parentheses give the coupling constants, *J*, in Hz, after *m* the half-height width is given; ′ means glucose in C-3 position of the sapogenin, $^V$ means glucose in C-26 position of the sapogenin, ″ means rhamnose in C-2 position of the C-3 glucosyl group, ‴ means rhamnose in C-4 position of the C-3 glucosyl group, ⁗ means rhamnose in C-4 position of rhamnose.

glycosides. These compounds, particularly the saponin **2** [4], are formed by enzyme catalysed reactions occurring in the roots.

Acid hydrolysis of all the saponins gave diosgenin as the only sapogenin. The compounds were characterized as far as possible by m.p. and IR spectral comparison with literature data, by TLC comparison with authentic samples (**1** to **5**) and by the MS molecular ions of the permethylated and/or peracetylated (**1** to **5** and **7**) derivatives.

Particularly suited for structural determination were the NMR spectra of the free glycosides in pyridine-$d_5$ (Table 1). The anomeric protons of the carbohydrate residues were clearly separated from the normal sugar protons at lower field, especially those of the rhamnose groups. Saponins of the yamogenin type with their typically split signal for Me-27 (Table 1) can be excluded. The NMR spectra of the permethyl ethers and peracetates were less informative.

In pyridine-$d_5$ compounds **1**–**5** showed the Me-27 multiplet at $\delta$ 0·70 ppm characteristic for diosgenin, a relatively narrow methylene multiplet for $CH_2$-26 and an olefinic proton at $\delta$ 5·29 ppm for H-6 (Table 1). Additionally, trillin (**1**) showed the anomeric proton of the glucoside as a doublet at $\delta$ 4·95 ppm.

The diglycosides **2** and **3** possessed an additional anomeric proton and a methyl doublet for the rhamnose moiety. The NMR spectrum of **2** was rather similar to that of **1**, whereas **3** differed markedly from **1**, e.g. in the low field shift of Me-19 to $\delta$ 1·04 ppm. This is caused by the paramagnetic influence of the rhamnose hydroxyls [9] which in **3** are more adjacent to the diosgenin skeleton than in **2**. Thus, the rhamnose group in **3** was linked to

the C-2 position of glucose (prosapogenin A), but in **2** it was attached to the C-4 position (prosapogenin B). This result was consistent with the structures given by Kawasaki and Yamauchi [4], as were the m.ps, the relative polarities of **2** and **3** and the fact that **2** was formed from the crude extract under fermenting conditions. In dioscin (**4**) the anomeric protons of the rhamnose units appeared in the same positions as in the rhamnose groups in **2** and **3** (Table 1).

In the tetraglycoside **5**, three rhamnose and one glucose unit were clearly distinguished by NMR. Two of the rhamnoses have the same linkages as found in dioscin (**4**), this was indicated by the signals of the anomeric protons and by partial hydrolysis to give dioscin (**4**). The position of the third rhamnose could not be located by NMR. However, **5** was identical by m.p., optical rotation, IR and NMR with the tetraglycoside isolated by Kawasaki and co-workers [1] which bears the third rhamnose at position C-4 of the rhamnose moiety more distant from the diosgenin residue. Finally, degradation of the permethyl ether [2] indicated that the saponin described by Tschesche and co-workers [2] is likewise identical with the previously described compounds.

The glycosides **6** and **7** did not contain diosgenin as sapogenin, as the multiplet for Me-27 at $\delta$ 0·7 ppm was missing. Since acidic hydrolysis gave diosgenin and the Ehrlich reaction was positive, the sapogenin must represent a precursor of diosgenin in the furostanol form. The two glycosides exhibited a methoxyl signal at $\delta$ 3·2 ppm since treatment of the naturally occurring furostanols with MeOH will convert the 22$\alpha$-hydroxyl group of the semiketal into a 22$\alpha$-methoxy group [5, 10].

For glycoside **6** the anomeric proton of a glucoside group at $\delta$ 4·77 ppm, the doublet for the Me-27 at $\delta$ 1·0 ppm and the signals for Me-19 and H-4 as found in free diosgenin ($\delta$ 1·03 and 2·58 ppm) (Table 1) established the structure conclusively as 26-[$\beta$-D-glucopyranosyloxy]-(25*R*)-22$\alpha$-methoxyfurost-5-en-3$\beta$-ol. Unfortunately, the small amount of this compound available did not permit a more detailed characterization.

The furostanol **7** had sugar residues at positions C-3 and C-26. The NMR spectrum was quite similar to that of dioscin (**4**) (Table 1). The two rhamnose anomeric protons had the same shifts observed in **4**. Therefore, **7** was a furostanol compound with ring *F* open as in **6** and with the same arrangement of the sugar residues at position C-3 as found in dioscin (**4**). While the spirostanol glycosides **1**–**5** showed the typical IR pattern of the spiroketal side chain in the 840–980 $cm^{-1}$ region [3, 11], this was quite different [12] in **6** and **7**. Compound **7** was thus identical with a saponin previously described by Kiyosawa and Hutoh [5].

The furostanol glycosides corresponding to saponins **2** and **5** were probably also present as evidenced by TLC, but these could not be isolated. Structural determination of these compounds by NMR should be possible since the sugars at positions C-3 and C-26 are sufficiently far apart not to interact; the NMR spectra can then be predicted by simple addition of the values given in Table 1.

## EXPERIMENTAL

*General.* IR were recorded in KBr discs, NMR in pyridine-$d_5$ with TMS as an internal standard, MS were recorded at 70 eV and an ion source temperature of 230–300°. M.ps were uncorrected, samples were dried at 80° −0·1 torr. Literature methods were used to prepare peracetates [13] and permethyl ethers [14].

*Isolation procedure.* The roots were freed from the cork bark and from rotten parts, chopped and disintegrated at 10° under EtOH in a Starmix. The residue was extracted for 60 hr with EtOH in a Soxhlet, the extracts were combined, conc and the residue dried for 5 hr as above. It constituted 2–5% of the root and contained *ca.* 50% saponins. After washing with $CHCl_3$ it was repeatedly chromatographed over silica gel deactivated with 5 to 15% $H_2O$. The eluent was $CHCl_3$–MeOH–$H_2O$ (55:10:1 v/v); the polarity was increased by diminishing the amount of $CHCl_3$. For the isolation of pure saponins up to 12 separations were necessary. The purity of the saponins was examined by TLC with $CHCl_3$–MeOH–$H_2O$ (14:6:1 v/v) for the free glycosides, $C_6H_6$–$Me_2CO$ (21:4 v/v) developed twice, for peracetates and petrol.–acetone (19:6 v/v) for permethyl ethers. Detection of spirostanol glycosides was with $CrO_3$–$H_2SO_4$ at 120°, furostanol glycosides was with Ehrlich reagent [15].

*Hydrolysis.* Refluxing of the saponins in 5N HCl 95% EtOH gave diosgenin, $R_f$: 0·88, rhamnose, $R_f$: 0·22, glucose, $R_f$: 0·09 and small amounts of spirosta-3,5-diene, $R_f$: 0·95.

*Trillin* (**1**). Obtained in small amounts as crystals sensitive to hydrolysis, from MeOH m.p. 196° (dec.) ([3] 250–255°, dec.), $R_f$: 0·63. IR: 3400 (broad), 1240, 1055, 980, 960, 920, 898, 865, 840 $cm^{-1}$. Identical by TLC, IR and NMR with synthetic material [16] prepared from diosgenin and $\alpha$-acetobromoglucose with subsequent saponification (m.p. 275–277°, dec.).

*Prosapogenin B* (**2**). Obtained in traces from the extract of intact roots, but more from the rotten parts or after fermentative degradation of the extract. Crystallized from EtOH m.p. 189–191° ([4] 215–220°), $R_f$: 0·50, identical with the corresponding spot of the partial hydrolyzate of **4**. IR: 3395 (broad), 1635, 1370, 1040, 986, 923, 902, 870 $cm^{-1}$. (Found: C, 62·9; H, 8·5. Calc. for $C_{39}H_{62}O_{12}.H_2O$: C, 63·2; H, 8·7%).

*Hexaacetate of* **2**. Purified by chromatography with $C_6H_6$–acetone over silica gel and crystallized from EtOH, m.p. 104–106° ([4] 115–120°). MS: *m/e* 974 ($M^+$), 685 ($M^+$-triacetylrhamnose +H), 561 ($M^+$-diosgenin +H), 396/397 (spirostadiene),

273 (triacetylrhamnose-HO). (Found: C, 62·4; H, 7·6. Calc. for $C_{51}H_{74}O_{18}$: C, 62·8; H, 7·7%).

*Prosapogenin A* (**3**). This compound represented about 5% of the extract of the rotted roots, crystals from MeOH m.p. 244° (230–245° [3], 225–235° dec. [4]). $R_f$: 0·42, identical with the corresponding spot of the partial hydrolyzate of **4**. IR: as for **2**. (Found: C, 62·6; H, 9·0. Calc. for $C_{39}H_{62}O_{12}.H_2O$: C, 63·2; H, 8·7%).

*Hexaacetate of* **3**. Purified as for the acetate of **2**, crystals from MeOH–$H_2O$ m.p. 207–209° (208–210° [3], 198–200° [4]). MS: *m/e* 974 ($M^+$), 702 ($M^+$-triacetylrhamnose $+H_2O$) and the peaks of **2**-acetate. (Found: C, 62·4; H, 7·6. Calc. for $C_{51}H_{74}O_{18}$: C, 62·8; H, 7·7%).

*Hexamethyl ether of* **3**. After chromatography with petrol.–acetone on silica gel gave crystals from heptane m.p. 164–170° ([4] 154–156°). MS: *m/e* 806 ($M^+$), 397 (spirostadiene +H). (Found: C, 67·0; H, 9·4. Calc. for $C_{45}H_{74}O_{12}$: C, 66·7; H, 9·2%).

*Dioscin* (**4**). Up to 70% in the EtOH extract of rotted roots, crystals from EtOH, m.p. 281° ([3] 275–277°). $R_f$: 0·33. IR: 3400 (broad), 1630, 1365, 1040, 986, 966, 926, 902, 870, 842, 818 $cm^{-1}$. Identical by TLC, IR and NMR with authentic dioscin. (Found: C, 62·0; H, 8·2. Calc. for $C_{45}H_{72}O_{16}$: C, 62·2; H, 8·4%).

*Partial hydrolysis of* **4**. Hydrolysis [4] afforded spots at $R_f$ 0·88 (diosgenin), 0·63 (**1**), 0·42 (**3**) and a faint one at $R_f$ 0·50 (**2**).

*Octaacetate of* **4**. Crystals from acetone m.p. 142–148° (143–145° [3], 145–147° [13]). MS: *m/e* 1204 ($M^+$), 915 ($M^+$-triacetylrhamnose +H), 791 ($M^+$-diosgenin +H), 396 (spirostadiene). (Found: C, 60·6; H, 7·3. Calc. for $C_{61}H_{88}O_{24}$: C, 60·8; H, 7·4%).

*Octamethyl ether of* **4**. After chromatography with petrol.–acetone over silica gel gave crystals from heptane m.p. 114° ([7] 130–135°). MS: *m/e* 980 ($M^+$), 775 ($M^+$-tri-*O*-methylrhamnose +H), 586 ($M^+$-2 tri-*O*-methylrhamnose $+H_2O$), 567 ($M^+$-diosgenin +H), 449 ($C_{21}H_{37}O_{10}^+$), 397 (spirostadiene +H). (Found: C, 65·0; H, 9·2. Calc. for $C_{53}H_{88}O_{16}$: C, 64·9; H, 9·0%).

*Tetraglycoside* **5**. Contained in small amounts in the EtOH extract, crystals from MeOH m.p. 216° (203–206° dec. [1], 222–231° [2]). $[\alpha]_D$ −103° (*c* 0·56 in MeOH), −112° (*c* 0·87 in pyridine) (−113° (MeOH) [1], −136° (pyridine) [1], −100° (pyridine) [2]). $R_f$ 0·45. TLC and IR identical with that of an authentic sample [1]. NMR: the signals from Table 1 are identical with those of the authentic sample [1]. (Found: C, 59·9; H, 8·1. Calc. for $C_{51}H_{82}O_{20}.1/2\ H_2O$: C, 59·8; H, 8·2%). Partial hydrolysis as described in ref. 4 gave a spot at $R_f$ 0·33 (**4**) and those found from **4**.

*Decaacetate of* **5**. Crystals from hexane–acetone m.p. 162°. MS: *m/e* 1434 ($M^+$), 1145 ($M^+$-triacetylrhamnose +H), 1021 ($M^+$-diosgenin +H), 931 ($M^+$-pentaacetylrhamnosylrhamnose +HO), 915 ($M^+$-pentaacetylrhamnosylrhamnose +H), 503 (pentaacetylrhamnosylrhamnose −OH), 397 (spirostadiene +H), 273 (triacetylrhamnose −OH). (Found: C, 59·4; H, 7·3. $C_{71}H_{102}O_{30}$ requires: C, 59·4; H, 7·2%).

*Decamethyl ether of* **5**. Crystals from heptane m.p. 146–158° (dec.) ([1] 138–140°). MS: *m/e* 1154 ($M^+$), 949 ($M^+$-tri-*O*-methylrhamnose +H, 791/792 ($M^+$-penta-*O*-methylrhamnosylrhamnose $+H_2O$), 775 ($M^+$-penta-*O*-methylrhamnosylrhamnose +H), 397 (spirostadiene +H), 379 (penta-*O*-methylrhamnosylrhamnose −H). (Found: C, 63·6; H, 9·0. Calc. for $C_{61}H_{102}O_{20}$: C, 63·4; H, 8·9%).

*22-Methyl ether of* **6**. Only 26 mg were isolated from 70 g of root extract, crystals from MeOH–isopropanol m.p. 182° (dec.). $R_f$: 0·57. IR: 3460 (broad), 1040 (broad), 918, 814 $cm^{-1}$.

*22-Methyl ether of* **7**. The EtOH extract of an unusually well-preserved sample of roots contained up to 50% of **7**: isolated as the 22-methyl ether, after chromatography gave crystals from MeOH–isopropanol m.p. 181–189° ([5] 200–202°). $R_f$: 0·16. IR: 3400, 1040, 930 (broad), 918 (weak), 840 (weak), 810 $cm^{-1}$. (Found: C, 57·9; H, 7·9. Calc. for $C_{52}H_{86}O_{22}.H_2O$: C, 57·8; H, 8·2%).

*Dodecaacetate of* **7**-*methyl ether*. White decomposible powder m.p. 150° ([5] 125–127°). MS: *m/e* 1566 ($M^+$), 1534 ($M^+$-MeOH), 1204 (M-MeOH-tetraacetylglucose $+H_2O$), 1187 (M-MeOH-tetraacetylglucose +H), 331 (tetraacetylglucose −OH).

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