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SPIROSTANIC DIOSGENIN PRECURSORS FROM *DIOSCOREA COMPOSITA* TUBERS

OFELIA ESPEJO, JESÚS CAMPOS LLAVOT,* HELGI JUNG and FRANCISCO GIRAL†

Departamento de Química Farmacéutica y Productos Naturales, División de Estudios de Posgrado, Facultad de Química, Universidad Nacional Autónoma de México, México 20, D.F., México

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Key Word Index—*Dioscorea composita*; Dioscoreaceae; ^{13}C NMR spectra of diosgenin glycosides; diosgenin glycosides; saponins.

Abstract—The main saponin from the fresh tuber of *Dioscorea composita* was dioscin and from the fermented material 3-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl]diosgenin. The ^{13}C NMR chemical shifts of saponins were used in the determination of their structure. No free sapogenin was isolated from the fresh tuber.

INTRODUCTION

For more than 35 years, Mexican barbasco, *Dioscorea composita* Hemsl, has been used as a source of diosgenin for the preparation of steroid hormones. For this purpose the rhizomes are fragmented, fermented, and directly hydrolysed to give diosgenin. Its precursors, the corresponding saponins, have merely been assumed to be the same and present in the same proportion as in other Dioscoreaceae. This assumption is mainly based on the work of Tsukamoto *et al.* [1] with *D. tokoro*. In the present study, we describe the isolation and structural elucidation of some diosgenin precursors of *D. composita*, as well as the composition and proportion of saponins present in our material.

RESULTS AND DISCUSSION

Different types of material were used: (a) 'fermented' material collected and fermented in Chiapas, Mexico, in the central part of the Tehuantepec isthmus; (b) fresh material collected in Los Tuxtlas, Veracruz, near the region where the former material was gathered, which was analysed immediately; and (c) fresh material of a special clone of *D. composita* with a high content of pennogenin, collected in the Chiapas region.

Sapogenins

MeOH extraction of each material (a, b, c) followed by further purification yielded a crude mixture of glycosides. Fermented material (a) contained a mixture of free diosgenin and pennogenin (1.35%), and no yamogenin was detected. No free sapogenins were found in the other samples. Total hydrolysis of each of these mixtures of glycosides with ethanolic 2N HCl yielded diosgenin, yamogenin and pennogenin, initially identified by TLC and comparison with authentic samples, and further characterized by IR and ^{13}C NMR spectroscopy. The ^{13}C NMR spectral values were obtained by comparison with values for sapogenins reported by Eggert and Djerassi [2] and are essentially in accord with those published by Marquardt [3]. Even though diosgenin was always accompanied by yamogenin when the crude saponins were hydrolysed with ethanolic acid, the ^{13}C NMR chemical shifts of the isolated purified saponins showed no evidence of yamogenin (see values for C-23, C-25, C-27; Table 1). The amount of pennogenin was greater in the special clone of *Dioscorea* (c).

Saponins

As expected, the furostanols which were detected with Ehrlich reagent [4] were present in greater proportion and variety in the fresh (b, c) than in the fermented (a) material. We observed that mild hydrolysis carried out on a two-dimensional chromatogram using 1N H_2SO_4, converted some of the furostanols into spirostanols that no longer gave a coloured compound with Ehrlich reagent. Total hydrolysis of the purified glucosides yielded only diosgenin.

From the fermented material were isolated 3 - *O* - [β - D - glucopyranosyl] - diosgenin (trillin) (**1**) [5], 3 - *O* - [α - L - rhamnopyranosyl - (1→4) - β - D - glucopyranosyl] - diosgenin (**2**) (*ca* 50% of saponin mixture), and 3 - *O* - {[α - L - rhamnopyranosyl - (1→4)] - [α - L - rhamnopyranosyl - (1→2) - β - D - glucopyranosyl]} - diosgenin (dioscin) (**4**) [1, 6].

3 - *O* - [α - L - Rhamnopyranosyl - (1→2) - β - D - glucopyranosyl] - diosgenin (**3**) was not obtained in pure form and was identified only by partial hydrolysis and comparison with the literature [2]. Of these saponins, only dioscin (**4**) could be isolated from fresh material (b, c) in which it constituted the major glycoside [*ca* 70% in (b)].

*Present address: Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana Xochimilco, Calz. del Hueso 1100, México 22, D.F.

†Present address: Facultad de Farmacia, Universidad de Salamanca, Spain.

Table 1. ^{13}C NMR chemical shifts (ppm) of sapogenins, aglycones of saponins and aglycones of saponin acetates

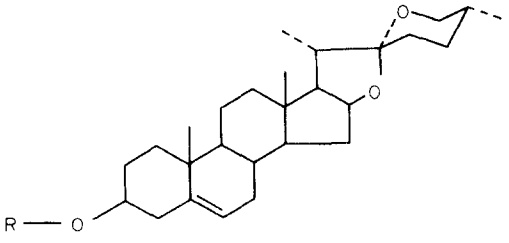
Carbon	Diosgenin	Yamogenin	Pennogenin	1	2	4	Ac(1)	Ac(4)
1	37.8	37.8	37.9	37.5	37.4	37.5	37.4	37.4
2	32.5	32.6	32.4	32.2	32.2	32.3	31.7	32.2
3	71.2	71.2	71.2	78.5	78.4	78.7	79.7	78.4
4	43.4	43.4	43.4	39.3	39.2	39.0	39.3	38.5
5	142.0	142.0	142.0	141.0	140.9	140.9	140.6	140.4
6	121.0	121.0	121.0	121.7	121.7	121.8	122.1	122.5
7	32.3	32.4	32.4	32.2	32.2	32.2	32.3	32.2
8	31.8	31.8	31.8	31.7	31.6	31.7	31.8	31.7
9	50.5	50.5	50.4	50.3	50.2	50.4	50.3	50.3
10	37.0	37.0	37.0	37.1	37.0	37.1	37.0	37.1
11	21.2	21.2	21.0	21.1	21.1	21.1	21.1	21.5
12	40.0	40.0	32.4	39.9	39.9	39.9	39.9	39.9
13	40.5	40.5	44.8	40.5	40.4	40.4	40.5	40.5
14	56.8	56.8	53.2	56.7	56.6	56.7	56.7	56.6
15	32.2	32.2	32.1	31.8	32.2	31.8	30.0	31.7
16	81.1	81.1	90.0	81.1	81.1	81.1	81.1	81.1
17	62.9	62.8	90.0	62.9	62.8	62.9	63.0	62.9
18	16.4	16.4	17.2	16.4	16.3	16.3	16.4	16.4
19	19.6	19.6	19.6	19.4	19.4	19.4	19.4	19.4
20	42.0	42.5	45.2	42.0	41.9	42.0	42.0	42.0
21	15.0	15.0	9.7	15.0	15.0	15.0	15.0	15.0
22	109.2	109.7	109.9	109.3	109.4	109.3	109.3	109.3
23	31.8	26.2	31.8	30.2	30.0	30.2	32.3	29.9
24	29.3	27.6	28.8	29.3	29.2	29.3	29.3	29.3
25	30.6	26.4	30.4	30.6	30.5	30.6	30.6	30.6
26	66.9	65.1	66.7	66.9	66.9	66.9	66.9	66.9
27	17.3	14.9	17.3	17.3	17.3	17.3	17.3	17.3

1: 3 - *O* - [β - D - glucopyranosyl] - diosgenin; **2**: 3 - *O* - [α - L - rhamnopyranosyl - (1→4) - β - D - glucopyranosyl] - diosgenin; **4**: 3 - *O* - {[α - L - rhamnopyranosyl - (1→4)] - [α - L - rhamnopyranosyl - (1→2) - β - D - glucopyranosyl]} - diosgenin; Ac**1**, Ac**4**: Acetates of **1** and **4**, respectively.

Structure determination was made by total and partial hydrolysis, quantitative sugar determination in the case of **1** and **2**, and ^{13}C NMR spectra. ^{13}C NMR spectral values were assigned by comparison with the ^{13}C data of the corresponding sapogenins (Table 1) and sugars [7] (Table 2). In the case of dioscin (**4**), chemical shifts were assigned taking into account those of **2** as well as the data obtained by partial hydrolysis. However, the value of 78.8 ppm given to the C-2 of glucose was considerably lower than the value expected for a carbon in a glycosidic bond (displacement of 7 ppm from the corresponding value for C-2 in **2** would give *ca* 82 ppm). The ^{13}C NMR spectral values for acetates (Table 2) confirmed those of the free glycosides. For anomeric carbons in the case of **1** and **2**, further confirmation was obtained from the calculated molecular rotation differences [8].

EXPERIMENTAL

General. IR spectra were recorded using KBr discs and ^1H NMR spectra in $CDCl_3$ with TMS as the int. standard. Mps were uncorr.; samples were dried at 40°, −0.3 torr, and kept



1 R = β - D - Glucopyranosyl

2 R = α - L - Rhamnopyranosyl - (1→4) - β - D - glucopyranosyl

3 R = α - L - Rhamnopyranosyl - (1→2) - β - D - glucopyranosyl

4 R = [α - L - Rhamnopyranosyl - (1→4)] - [α - L - rhamnopyranosyl - (1→2)] - β - D - glucopyranosyl

Table 2. ^{13}C NMR chemical shifts of the saccharide portion of saponins of *D. composita*

	Saponin									
	1	2		4			Ac(1)	Ac(4)		
					Rhamnose				Rhamnose	
Carbon	Glucose	Glucose	Rhamnose	Glucose	(1→4)	(1→2)	Glucose	Glucose	(1→4)	(1→2)
1	102.6(104.2)*	102.5	102.3(94.9)	102.9	102.0	100.3	99.8	99.9	99.2	98.0
2	75.3(74.1)	75.2	72.5(71.9)	78.8	74.1	72.5	72.2	78.0	72.7	70.5
3	78.4(76.9)	76.5	72.3(70.8)	76.9	72.8	72.7	72.3	75.9	70.9	69.4
4	71.7(70.7)	78.4	73.7(73.2)	78.1	73.7	73.9	69.3	78.0	71.6	71.6
5	78.1(76.8)	76.8	70.2(69.1)	77.9	70.4	69.6	73.6	75.9	68.4	67.1
6	62.9(61.9)	61.4	18.3(17.8)	61.3	18.6	18.5	62.5	62.9	17.7	17.5

1: 3 - *O* - [*β* - D - glucopyranosyl] - diosgenin; **2**: 3 - *O* - [*α* - L - rhamnopyranosyl - (1→4) - *β* - D - glucopyranosyl] - diosgenin; **4**: 3 - *O* - {[*α* - L - rhamnopyranosyl - (1→4)] - [*α* - L - rhamnopyranosyl - (1→2) - *β* - D - glucopyranosyl]} - diosgenin; Ac(**1**), Ac(**4**): Acetates of **1** and **4**, respectively.

*Data in parenthesis are those reported for glucose and rhamnose [7].

in a desiccator over P_2O_5. Literature methods were followed to prepare peracetates [9] and to perform quantitative sugar determinations [10, 11]. ^{13}C NMR spectra were recorded in a XL-100 with FT mode in C_5D_5N with TMS as the int. standard.

Isolation. Fermented material (a). Fermentation was carried out according to published methods [12]. Dried material was defatted with hexane and extracted ×3 with hot MeOH for 8 hr. The extracts were combined, concentrated, and filtered. The solid was purified by successive precipitation from MeOH (yield: 4.6% consisting exclusively of saponins).

Fresh material (b). Fresh rhizomes (H_2O content *ca* 80%) were peeled, chopped, and extracted with MeOH in a Soxhlet for 14 hr. The extract was concentrated, and the resulting solid was filtered and washed with hexane (yield: 9.87% consisting of saponins).

Fresh material (c). Rhizomes of a high pennogenin-containing clone (H_2O content *ca* 80%) were peeled, chopped, and macerated with MeOH at room temp. for 2 weeks to avoid possible decomposition of the pennogenin glycosides; the extract was concentrated and the resulting solid filtered (yield: 2.07%).

Solids from (a) were chromatographed on a Si gel column and eluted with CH_2Cl_2–MeOH–H_2O (80:20:0.1), yielding a mixture of free sapogenins (diosgenin and pennogenin, 1.35%) and various mixtures of saponins. Pure free glycosides were obtained from (a), (b) and (c) after repeated CC using this solvent system. The purity of the sapogenins and free glycosides was confirmed by TLC using (A) C_6H_6–EtOAc (80:20), (B) toluene–EtOAc (80:20), (C) CH_2Cl_2–MeOH–H_2O (80:20:0.1) and (D) $CHCl_3$–MeOH–H_2O (75:15:15) respectively; C_6H_6–EtOAc (80:20) was used for acetates. Spirostanol glycosides were identified by TLC with 5N H_2SO_4, followed by heating and UV examination; furostanol glycosides were detected with Ehrlich reagent.

Hydrolysis. Refluxing the saponins with 2N HCl–95% EtOH yielded diosgenin, yamogenin, pennogenin, small amounts of spirosta-3,5-diene, glucose, and rhamnose; these were compared with authentic samples on TLC plates.

Hydrolysis on two-dimensional TLC plates. The purified saponin mixture was applied in duplicate on a 20×20 cm plate and the chromatogram was developed in one dimension in the usual manner. One of the runs was used as a reference while each of the 4–5 spots of the other was treated with one drop of 1N H_2SO_4. The chromatogram was run at 90° to the first dimension. Colour was developed first with Ehrlich reagent and then with 5 N H_2SO_4 and viewed with UV light.

3 - O - [*β* - D - *Glucopyranosyl*] - *diosgenin* (**1**) (*trillin*). Compound **1** was obtained free from fermented material (a) and from partial hydrolysis of the total glucoside mixture with 1 N H_2SO_4–95% EtOH, purified by Si gel CC using CH_2Cl_2–MeOH–H_2O (80:20:0.1), and further crystallized from MeOH–H_2O. Mp 263–264° ([3] 250–255° dec.); R_f 0.79 and 0.83 in solvent systems A–B and C–D, respectively, $[\alpha]_D^{23} = -94.3°$ ($CHCl_3$; *c* 1.5); IR ν_{max}^{KBr} cm^{-1}: 3400 (*br*), 1650, 900, 920; (Found: C, 67.19; H, 9.17; O, 23.48. Calc. for $C_{33}H_{52}O_8$: C, 68.77; H, 9.08; O, 22.19%.) Quantitative sugar determination: 29.0%. (Calc. for diosgenin–glucose: 31.2%.)

Tetraacetate of (**1**) (*trillin acetate*). Mp 208–210° ([1] 203–205°); $[\alpha]_D^{24} = -89.8°$ ($CHCl_3$; *c* 10) [[1] $[\alpha]_D^{30} = -77.0°$ ($CHCl_3$; *c* 0.61)]; IR ν_{max}^{KBr} cm^{-1}: 2980, 1750, 1690, 900, 920; (Found: C, 66.64; H, 8.27; O, 25.24. Calc. for $C_{41}H_{60}O_{12}$: C, 66.10; H, 8.11; O, 25.77%.) ^1H NMR: *δ*0.7 (*s br*, Me-27), 0.88 (*s*, Me-18), 0.90 (*s*, Me-19), 1.01 (*d*, Me-21), 1.9–2.1 (12 H, 4 signals, –OAc), 3.4 (*m*, $-CH_2-O-$), 4.1–5.1 (*m*, –CH–O–, sugar moiety), 5.4 (*m br*, –CH=CH–). ^{13}C NMR, see Tables 1 and 2.

3 - O - [*α* - L - *Rhamnopyranosyl* - (1→4) - *β* - D - *glucopyranosyl*] - *diosgenin* (**2**) (*prosapogenin B*) [6]. Isolated free by CC from fermented material (a) with *ca* 50% yield, purified by Si gel CC using CH_2Cl_2–MeOH–H_2O (80:20:0.1), and crystallized with MeOH–H_2O. Mp 230–231° ([9] 215–220°); R_f 0.69 and 0.68 in solvent systems A–B and C–D, respectively; $[\alpha]_D^{28} = -89°$ (pyridine; *c* 0.93), identical with the corresponding spot of the partial hydrolysis of **4**. Partial hydrolysis of **2** gave **1** and diosgenin. IR ν_{max}^{KBr} cm^{-1}: 2400 (*br*), 1635, 895, 920; (Found: C, 62.06; H, 8.68; O, 29.17. Calc. for $C_{39}H_{62}O_{13}$. CH_3OH: C, 62.31; H, 8.62; O, 29.05%.) Quantitative sugar determination: 41.8%. (Calc. for diosgenin–glucose rhamnose: 42.6%.) ^{13}C NMR, see Tables 1 and 2.

Hexaacetate of (**2**). Purified by crystallization from MeOH–H_2O. Mp 110° ([9] 115–120°); IR ν_{max}^{KBr} cm^{-1}: 1750, 1650, 895, 920; (Found: C, 62.85; H, 7.62. Calc. for $C_{51}H_{74}O_{18}$: C, 62.83; H, 7.59%.) ^1H NMR: *δ*0.81 (*s br*, Me-27), 1.03 (*s*, Me-18), 1.1 (*s*, Me-19), 1.27 *d*, Me-21), 1.9–2.1 (18 H, 5 signals, –OAc), 3.4 (*m*, $-CH_2-O-$), 4.2–5.2 (*m*,

-CH-O-, sugar moiety), 5.4 (*m br*, -CH=CH-). ^{13}C NMR, see Tables 1 and 2.

3 - O - {[α - L - *Rhamnopyranosyl* - (1→4)] - [α - L - *rhamnopyranosyl* - (1→2) - β - D - *glucopyranosyl*]} - *diosgenin* (**4**) (*dioscin*) [1]. Obtained from fresh material (*ca* 70%) by Si gel CC using CH_2Cl_2–MeOH–H_2O (80:20:0.1), and crystallized with aq. MeOH. Mp 274–275° ([1] 275–277°); R_f 0.36 and 0.28 in solvent systems A–B and C–D, respectively; $[\alpha]_D^{23}$ −107° (pyridine; *c* 0.85) ([1] $[\alpha]_D^{13}$ −115°, EtOH; *c* 2.72). Identical to an authentic sample by TLC and mmp. Partial hydrolysis of **4** with 1 N H_2SO_4–95% EtOH gave diglycosides **2** and **3**, monoglucoside **1** diosgenin, glucose, and rhamnose. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 892, 920; (Found: C, 59.84; H, 8.05; O, 32.10. Calc. for $C_{45}H_{72}O_{16}$. $2H_2O$: C, 59.71; H, 8.46; O, 31.82%.)

Triglycoside (**4**) *octaacetate*. Mp 133–134°; IR ν_{max}^{KBr} cm^{-1}: 1750, 1650, 895, 920; (Found: C, 60.54; H, 7.34; O, 32.12, Calc. for $C_{61}H_{88}O_{24}$: C, 60.78; H, 7.36; O, 32.86%.) ^1H NMR: δ0.75 (*s br*, Me-27), 1.03 (*s*, Me-28), 1.1 (*s*, Me-19), 1.25 (*s br*, Me-21), 1.9–2.2 (24 H, 5 signals, -OAc), 3.4 (*m*, $-CH_2-O-$), 4.2–5.5 (*m*, -CH-O-, sugar moiety and -CH=CH-). ^{13}C NMR, see Tables 1 and 2.

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