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SULPHATED STEROIDAL DERIVATIVES FROM *RUSCUS ACULEATUS*

ALI OULAD-ALI, DOMINIQUE GUILLAUME,* RENE BELLE,† BRUNO DAVID‡ and ROBERT ANTON‡

Laboratoire de Pharmacognosie, Faculté de Pharmacie de Strasbourg, B.P. 24, 67401 Illkirch Cédex, France; *Laboratoire de Chimie Thérapeutique, URA 1310 du CNRS, Faculté des Sciences Pharmaceutiques et Biologiques, 4, Av. de l'Observatoire, 75270 Paris Cédex 06, France; †Centre de Recherche des Substances Naturelles Institut de Recherche Pierre Fabre, B.P. 31319 Labège Cédex, France

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Key Word Index—*Ruscus aculeatus*; Liliaceae; therapeutic drug; cardiovascular disease; veinous ailments; sulphated spirostanol saponins; sulphated furostanol saponins; 1β-hydroxyruscogenin 1-sulphate; 26-*O*-β-D-glucopyranosyl furost-5-en-1β,3β,22ζ-triol 1-sulphate.

Abstract—Two novel steroidal derivatives, (1β-hydroxyruscogenin 1-sulphate and 26-*O*-β-D-glucopyranosyl furost-5-en-1β,22ζ-triol 1-sulphate) were isolated from the rhizome of *Ruscus aculeatus*. The structures were established by spectroscopic methods.

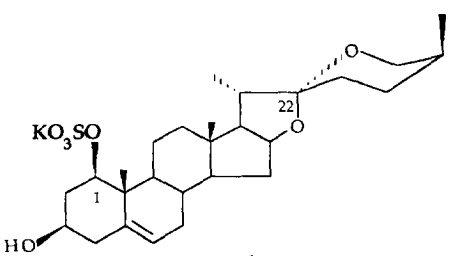
INTRODUCTION

Preparations elaborated from an alcoholic extract of the rhizome of *Ruscus aculeatus* L. have been prescribed for veinous ailments (haemorrhoids, capillary fragility) for decades. Previous studies of this alcoholic extract led to the isolation of steroidal saponins [1–4] and flavonoids [5] as major constituents. The recent discovery of the hypoglycaemic [6] and antitumor [7, 8] activity of steroidal saponins encouraged us to reinvestigate the composition of this extract and, in particular, to study the minor steroidal saponins. In this paper, we describe the isolation and structural determination of two new sulphated compounds: spirost-5-en-1β,3β-diol 1-sulphate (**1**) and 26-*O*-β-D-glucopyranosyl furost-5-en-1β,3β,22ζ-triol 1-sulphate (**2**).

RESULTS AND DISCUSSION

The dried and finely powdered rhizome was treated with methanol. The extract was partitioned between *n*-butanol and water. The butanol layer was evaporated *in vacuo* to dryness. The residue, dissolved in a minimum volume of methanol, was poured into diethyl ether, yielding the saponin mixture as a whitish precipitate. Final purification was achieved using a combination of column chromatography on silica gel and preparative HPLC to provide **1** and **2**.

The positive FAB mass spectrum of **1** displayed two molecular peaks at *m/z* 587 $[M + K]^+$ $[(C_{27}H_{41}O_7SK + K)]^+$ and 571 $[M + Na]^+$ $[(C_{27}H_{41}O_7SK + Na)]^+$ which indicated the presence of a sulphate group. The ^{13}C NMR spectrum displayed signals for 27 carbons identified as four CH_3, nine CH_2, 10 CH and four C quaternary resonances from the DEPT spectrum. From the latter resonances, carbons at δ 139.0 and 109.2 were assigned directly to an olefinic and spirostanic function, respectively. The methyl groups at δ 14.8 and δ 16.2 were shown to be at angular position (^1H–^{13}C spectrum) while the two others (δ 15.0 and 17.3) correlated with the methyl doublet at δ 0.85 ($J = 5.4$ Hz) and 1.03 ($J = 6.9$ Hz),



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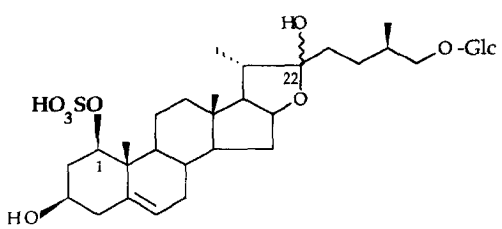

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‡Author to whom correspondence should be addressed.

respectively. Overall, the chemical shifts of the carbons of **1** were very similar to those of 3-*O*-α-L-rhamnopyranosyl ruscogenin 1β-sulphate [9] except for C-3 which did not experience the glycosylation shift. Thus, **1** is 1β-hydroxyruscogenine 1-sulphate.

Saponin **2** showed a quasi-molecular ion peak at *m/z* 713 $[M+Na]^+$ $[(C_{33}H_{54}O_{13}S+Na)^+]$ in the positive FAB mass spectrum, indicating, as in the case of **1**, the presence of a sulphate function. The ^1H NMR spectrum of **2** showed signals for four methyl groups [δ 0.89 (*s*), 1.00 (*d*, *J* = 6.6 Hz), 1.05 (*d*, *J* = 6.9 Hz), 1.15 (*s*)], one olefinic proton (δ 5.65) and one anomeric proton (δ 4.29, *d*, *J* = 7.7 Hz). Its ^{13}C NMR spectrum exhibited 33 separated signals; three sp^3 quaternary carbons (δ 43.0, 43.7, 111.0), a couple of olefinic carbons (δ 126.7, 138.8), seven hydroxyl-bearing methines (δ 68.8, 71.6, 75.1, 77.8, 78.1, 82.3 and 85.7), two hydroxyl-bearing methylenes (δ 62.7 and δ 75.9), four methyls (δ 14.8, 15.9, 17.0 and 17.4) and one anomeric carbon (δ 104.6). Comparison of the NMR data of **2** with the literature [1, 9–12] showed that **2** was substituted at its C-1 position by a sulphate group and at C-26 by a β-D-glucopyranosyl moiety. Thus **2** is 26-*O*-β-D-glucopyranosyl furost-5-en-1β,3β,22ζ-triol 1-sulphate.

Saponins containing sulphated sugar residues have frequently been identified [13, 14], but direct esterification of the aglycone by a sulphate function is much less common.

Table 1. ^{13}C NMR data for compounds **1** (pyridine-d_5) and **2** (CD_3OD)

C	1	2
1	84.1	85.7
2	39.8	36.9
3	68.0	68.8
4	40.1	41.5
5	139.0	138.8
6	125.2	126.7
7	33.0	34.0
8	31.9	32.7
9	49.8	50.6
10	43.7	43.7
11	23.6	24.3
12	41.9	39.0
13	40.6	43.0
14	56.7	57.5
15	32.8	33.1
16	81.1	82.3
17	63.1	63.9
18	16.6	15.9
19	14.8	17.4
20	43.1	41.3
21	15.0	14.8
22	109.2	111.0
23	31.9	30.7
24	29.2	28.5
25	30.7	34.9
26	66.8	75.9
27	17.3	17.0
26-*O*-Glucose		
C-1		104.6
C-2		75.1
C-3		78.1
C-4		71.6
C-5		77.8
C-6		62.7

EXPERIMENTAL

General. FAB mass spectra were obtained on a Kratos MS 80 spectrometer. ^1H (200.13 or 300.13 MHz) and ^{13}C (50.2 or 75.4 MHz) NMR spectra were performed using Bruker library of microprograms. Chemical shifts (δ) are reported in ppm by setting the ref. on CD_3OD residual solvent peak at δ 3.4 and 49.0 for ^1H and ^{13}C, respectively. Coupling constants (*J*) are given in Hz.

Extraction and isolation. Rhizome material of *Ruscus aculeatus* was collected in the south-western France. Ground rhizome (5 kg) was defatted by CH_2Cl_2 and treated with MeOH. The extract was partitioned between *n*-BuOH and water. The BuOH layer was evapd *in vacuo* to dryness. The residue, dissolved in a minimum volume of MeOH was poured into Et_2O to provide the saponin mixture as a white ppt. (334 g). A portion of the ppt. (20 g) was fractionated on a silica gel column eluated with a discontinuous gradient of $CHCl_3$–MeOH–H_2O. The saponin frs were rechromatographed and purified by prep. HPLC equipped with a variable wavelength UV detector operating at 203 nm and a Microsorb column (50 × 21.4 mm i.d.; C18, 3 μm). Elution with a MeCN–H_2O gradient yielded compounds **1** (8 mg) and **2** (10 mg).

Compound **1**. Amorphous powder from MeOH, $[\alpha]_D^{24}$ −45.7° (MeOH; *c* 0.03). FAB-MS (positive) *m/z*: 587 $[M+K]^+$ and 571 $[M+Na]^+$; ^1H NMR: δ 0.85 (3H, *d*, *J* = 5.4 Hz, Me-27), 0.88 (3H, *s*, Me-18), 1.03 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.17 (3H, *s*, Me-19), 5.69 (1H, *d*, *J* = 5.1, H-6); ^{13}C (Table 1).

Compound **2**. Amorphous powder from MeOH, $[\alpha]_D^{24}$ −86° (MeOH; *c* 0.01). FAB MS (positive) *m/z*: 713 $[M+Na]^+$; ^1H NMR: δ 0.89 (3H, *s*, Me-18), 1.00 (3H, *d*, *J* = 6.6 Hz, Me-27), 1.05 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.15 (3H, *s*, Me-19), 4.29 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.65 (1H, *d*, *J* = 5.1, H-6); ^{13}C (Table 1).

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