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SAPOGENINS FROM *SOLANUM MERIDENSE*

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Key Word Index—*Solanum meridense*; Solanaceae; steroidal sapogenins; (25*R*)-3β-acetoxy-5α-spirostan-6-one; chlorogenone; chlorogenin; diosgenin; sitosterol.

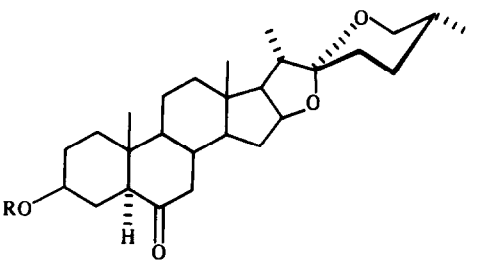
Abstract—In addition to chlorogenin, chlorogenone, diosgenin, and sitosterol a new sapogenin has been isolated from the green berries of *Solanum meridense*. Its structure was established by spectral data and chemical degradation as (25*R*)-3β-acetoxy-5α-spirostan-6-one.

INTRODUCTION

There are no previous reports on the chemical constituents of *Solanum meridense* Bitter et Pittier. This small tree is widely distributed around Mérida and its fruits are known for their foam forming properties, indicating the presence of sapogenins.

RESULTS AND DISCUSSION

Dried and powdered berries were treated as described in the Experimental to yield a chloroform extract. Repeated preparative TLC over silica gel plates yielded



1a R = Ac
1b R = H

(25*R*)-3β-acetoxy-5α-spirostan-6-one (**1a**), mp 159–162°. The mass spectrum of **1a** gave a $[M]^+$ peak at *m/z* 472 ($C_{29}H_{44}O_5$), prominent peaks at *m/z* at 139 and 115 were indicative of a spiroketal moiety [1], and a fragment representing $[M-59]^+$ revealed the existence of an acetoxy group.

The ^1H NMR spectrum of **1a** showed two singlets at δ 0.75 and 1.20 indicating the presence of two angular methyl groups, two doublets ($J=7$ Hz) at 0.73 and 0.95 which were assigned to secondary methyl groups, one singlet at 1.98 attributed to an acetate, a multiplet centred at 3.35 which was assigned to the hydrogen at C-16, and another multiplet centred at 5.10 which was assigned to the hydrogen geminal to the acetoxy group.

The (25*R*)-configuration was established because the IR spectrum showed bands at 980, 960, 920, and 900 cm^{-1} (the band at 900 cm^{-1} was more intense than the one at 920 cm^{-1}) [2, 3]. This was confirmed by the ^{13}C NMR spectrum of **1a** (Table 1) because the signals corresponding to the carbons of the side chain were in complete aggreement with the values reported by Tori *et al.* [4] for (25*R*)-spirostanes.

The ^{13}C NMR signals of **1a** were almost identical with those of chlorogenone [5] with the exception of carbons belonging to ring A of the steroid nucleus. Based on this comparison it was assumed that the acetoxy group was located at C-3.

To prove this assumption, compound **1a** was subjected to hydrolysis to yield the deacetylated derivative **1b**. The ^{13}C NMR spectrum of this compound (Table 1) is almost identical to the ^{13}C NMR spectrum of **1a** with the exception of the signals for C-2, C-3 and C-4. The signal for C-3 appears at δ 70.6, shifted upfield from the value observed in the acetylated compound. As expected C-2 and C-4 appear downfield from the corresponding values in **1a**.

The deacetylated product **1b** was dissolved in acetone and treated with Kiliani's reagent leading to a diketone, mp 233–236°. This compound was found to be identical to chlorogenone (TLC, mp, mmp, IR, ^1H NMR, and ^{13}C NMR). Based on biogenetic considerations the acetoxy group at C-3 should be equatorial and β-oriented. In accord with this a sample of chlorogenone was partially reduced with PtO_2 in MeOH yielding **1b**. Based on the above evidence, the structure of **1a** was established as (25*R*)-3β-acetoxy-5α-spirostan-6-one. The following substances were also isolated as described in the Experimental and identified by comparison with authentic samples: chlorogenone, chlorogenin, diosgenin and sitosterol.

Table 1. ^{13}C NMR chemical shifts of (25*R*)-3β-acetoxy-5α-spirostan-6-one (**1a**) and its deacetylated derivative, **1b**

C	**1a**	**1b**
1	38.4	36.7
2	29.6	30.2
3	72.7	70.6
4	30.2	30.8
5	56.5	56.7
6	209.1	209.2
7	46.7	46.9
8	37.4	37.4
9	54.0	54.2
10	36.5	36.5
11	21.3	21.4
12	39.5	39.6
13	40.8	40.8
14	56.6	56.9
15	31.4	31.6
16	80.4	80.4
17	62.4	62.4
18	16.6	16.3
19	13.0	13.1
20	41.7	41.7
21	14.3	14.3
22	109.2	109.1
23	31.6	31.8
24	28.8	28.8
25	30.2	30.3
26	66.9	66.9
27	16.9	16.9
3(O-Ac)	170.1	—
	21.0	—

EXPERIMENTAL

Green berries of *Solanum meridense* (600 g) were dried at 45° and ground to a powder. The powdered material was first treated with H_2O at room temp. for 24 hr and then hydrolysed with 2 M HCl under reflux for 2 hr. The suspended solid was filtered off, washed with H_2O and NH_4OH soln, and dried at 80°. This produced 265 g of solid which was extracted with $CHCl_3$ in a Soxhlet. The solvent was evapd under vacuum to yield 210 g of extract which was applied to 500 silica gel plates (20 × 20 × 0.5 mm) and separated into 4 bands using Cl_2CH_2–MeOH–formamide (93:6:1) [6]. The upper band (R_f, *ca* 1.0–0.8) was scraped off and rechromatographed on silica gel plates (20 × 20 × 0.25) using C_6H_6–EtOAc (5:1) as a solvent. After three developments the upper band (R_f, *ca* 0.65) was scraped off and extracted to render 110 mg of residue. Crystallization from $CHCl_3$–MeOH yielded 93 mg of **1a**, mp 159–162° (uncorr.). IR ν_{max}^{KBr} cm^{-1}: 1740, 1250 (OAc), 1700 (C=O), 980, 960, 920 and 900 (band at 900 more intense than 920) EIMS (70 eV) *m/z*: 472 $[M]^+$ ($C_{29}H_{44}O_5$), 413 $[M-59]^+$, 139 (base peak, $C_9H_{15}O$), 115 (43%). ^1H NMR ($CDCl_3$): δ 0.73 (3H, *d*, $J=7$ Hz, H-27), 0.75 (3H, *s*, H-18), 0.95 (3H, *d*, $J=7$ Hz, H-21), 1.20 (3H, *s*, H-19), 1.98 (3H, *s*, OAc), 3.35 (1H, *m*, 16-H), 5.10 (1H, *m*, 3-H). ^{13}C NMR (see Table 1).

The lower band (R_f, 0.47 of plates developed with C_6H_6–EtOAc) was scraped off and extracted. The residue crystallized as white needles (20 mg) from $CHCl_3$–MeOH, mp 233–236°. This substance was identified as chlorogenone by comparison with an authentic sample (TLC, mmp, IR and ^{13}C NMR).

The second band (R_f, *ca* 0.8–0.6 of first prep. TLC) was extracted and rechromatographed over silica gel plates using the same solvent (Cl_2CH_2–MeOH–formamide). The second band was extracted with $CHCl_3$, and the residue showed on TLC to be a mixture of two substances which were separated by prep TLC on 0.25 mm thick silica gel plates using $C_6H_6 \times 3$ as a solvent. The upper band yielded 62 mg of a substance which crystallized from $CHCl_3$–MeOH as needles, mp 133–136°, and was identified as sitosterol. The lower band rendered diosgenin (45 mg) mp 200–203°, identified by comparison with an authentic sample (TLC, mmp, IR, ^{13}C NMR).

The extract obtained from the fourth band (lowest band of first prep. TLC) was treated over a neutral alumina column which was eluted with petrol, C_6H_6, $CHCl_3$. Elution with $CHCl_3$–MeOH (1:1) produced a residue which on recrystallization from MeOH yielded 95 mg of chlorogenin, mp 264–268°, identified by comparison with an authentic sample (TLC, mmp, IR, ^{13}C NMR).

Hydrolysis of compound **1a**. A soln of **1a** (50 mg) in MeOH was treated under reflux for 1 hr with NaOH. Upon cooling H_2O was added and the product extracted with $CHCl_3$. Crystallization from $CHCl_3$–MeOH yielded 38 mg of **1b**, mp 202–204°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1710 (CO), 900>920. ^1H NMR ($CDCl_3$): δ0.75 (6H, *s*, H-18 and H-19), 0.78 (3H, *d*, $J=7$ Hz, H-27), 0.94 (3H, *d*, $J=7$ Hz, H-21), 3.35 (1H, *m*, 16-H), 3.40 (1H, *s*, OH), 4.35 (1H, *m*, 3-H). ^{13}C NMR (see Table 1).

(25R)-5α-*Spirostane-3,6-dione (chlorogenone) from compound* **1b**. Kiliani's reagent was added drop by drop to a soln of 30 mg of **1b** in Me_2CO. After 30 min at room temp the reaction was complete and a few drops of MeOH were added to destroy excess reagent. The soln was diluted with H_2O and shaken with $CHCl_3$. The organic layer was washed with H_2O and taken to dryness. The product (28 mg) crystallized from Me_2CO, mp 233–236°. It was found to be identical to an authentic sample of chlorogenone (TLC, mmp, IR ^{13}C NMR).

Catalytic reduction of chlorogenone. Chlorogenone (100 mg) obtained from diosgenin as described by Marker *et al.* [7] dissolved in 50 ml of EtOH was mixed with 50 mg of pre-reduced PtO_2 and shaken for 1 hr in a Parr hydrogenator at 50 PSIG of H_2. The reaction product showed two spots (R_f, 0.40 and 0.15) on TLC (C_6H_6–EtOAc, 1:1). CC over silica gel with C_6H_6–EtOAc (20:1) eluted the less polar substance (R_f, 0.40, 64 mg), mp 202–204°, which was found to be identical to **1b** by TLC, mmp, IR and ^{13}C NMR.

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CLEMONTANOSIDE-A, A BISGLYCOSIDE FROM *CLEMATIS MONTANA*

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Key Word Index—*Clematis montana*; Ranunculaceae; saponins; triterpenic bisglycosides.

Abstract—Clemontanoside-A, a new triterpenic bisglycoside was isolated from the methanolic extract of the leaves of *Clematis montana* and its structure established.

INTRODUCTION

Clematis montana Buch.-Hem. (Ranunculaceae), a woody climber is distributed in temperate Himalaya up to an altitude of 4000 m [1]. The leaves of this plant are used in skin diseases and the seeds have purging properties [1, 2]. A survey of the literature revealed that no phytochemical work has been reported on this plant. This prompted us to investigate this plant for saponins. This paper describes the isolation and characterization of a new oleanolic acid based bisglycoside from the methanolic extract of the leaves of *Clematis montana*.

RESULTS AND DISCUSSION

Column chromatography of the saponin mixture of the leaves of *Clematis montana* gave compound **1** (positive to the characteristic tests for triterpenic saponin). It also gave sugars (glucose and rhamnose (co-PC) on complete hydrolysis with 7% alcoholic sulphuric acid. Thus, the compound was anticipated to be a triterpenic saponin, which was later confirmed by spectral (^1H NMR, ^{13}C NMR, EIMS and FABMS) and hydrolytic studies. The ^1H NMR spectrum of **1** showed the presence of seven tertiary methyls (δ0.88, 0.96, 1.07 and 1.27), one second-