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CARDENAGENIN, A STEROIDAL SAPOGENIN FROM *CALIBANUS HOOKERII**

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Key Word Index—*Calibanus hookeri*, Liliaceae; sacamecate; steroidal sapogenins; cardenagenin.

Abstract—One new steroidal sapogenin with an open side chain was isolated from the rhizome of *Calibanus hookerii*. The structure has been determined by spectroscopic studies as 14α-hydroxy-calibagenin.

In a previous paper [1] we reported the structure of calibagenin as the major sapogenin isolated from the rhizome of *Calibanus hookerii*. Five additional sapogenins have since been isolated from the same material. The present communication reports the characterization of one of them, cardenagenin which has the structure **1** and mp 204–205°.

In the mass spectrum the molecular ion at *m/z* 434 was in accordance with $C_{27}H_{46}O_4$, corresponding to a steroidal sapogenin with an open side chain, four hydroxyl groups and a Δ^5-double bond. The IR spectrum showed a double bond (1630 and 820 cm^{-1}). The ^1H NMR spectrum (60 MHz) showed only a vinylic proton at δ5.1 which corresponds to a Δ^5-double bond. Signals were also observed at δ3.15 (1H) and 3.88 (2H) corresponding to protons bound to the carbon atoms with secondary alcohol groups (C-22, C-16, C-3). The EIMS spectrum showed loss of water from the molecular ion, *m/z* 416 with a metastable transition at 398.7 and loss of the side chain (*m/z* 129). The side chain structure was also revealed by the ion at *m/z* 269 (398 − 129) and it is the same as in calibagenin [1]. The second hydroxyl group was at C-16 (*m/z* 185 and cleavage of C-14/C-15 and C-13/C-17). The peak at *m/z* 243 arises by cleavage of C-8/C-14 and C-12/C-13 showing that one hydroxyl group is at C-14 (434 − 191). The fourth hydroxyl group is at C-3 because of the loss of 72 mu from *m/z* 269 (A ring) and *m/z* 145 (269 − 124). All these fragmentations were demonstrated by the corresponding metastable transitions and are described in the literature [1, 2].

In conclusion, the structure of cardenagenin is cholest-5-en-3β,14α,16ξ,22ξ-tetrol (**1**) and it corresponds to 14α-hydroxy-calibagenin.

EXPERIMENTAL

Plant material. Rhizomes of *Calibanus hookerii* were collected by the authors in San Luis Potosí, Mexico and kindly identified by Prof. Dr. Arturo Gómez Pompa of the Instituto Nacional de Investigaciones Bióticas in Jalapa, Veracruz. A voucher specimen is on deposit at the Jardín Botanico Exterior of the Instituto de Biología (U.N.A.M.), México.

The bark was separated and cardenagenin was obtained, together with the other sapogenins by the method in ref. [3] and it was purified by column chromatography on silica gel G with C_6H_6–EtOAc (6:4) and fractions of 150 ml were collected. The fractions 41–57 were collected and separated by a second column chromatography on silica gel G with *n*-BuOH–$CHCl_3$–hexane (2:8:2). Fractions of 20 ml were collected and the fractions 33 to 77 subjected to a third column chromatography eluted with $CHCl_3$–MeOH–Me_2CO (9:1:1) to yield cardenagenin which was obtained after crystallization in Me_2CO–hexane, mp 204–205°. The purity was confirmed by two-dimensional TLC on silica gel 60 with $CHCl_3$–MeOH–Me_2CO (9:1:1) and BuOH–$CHCl_3$–hexane (2:8:2) and revealed with 5 N H_2SO_4. By GLC only one peak was obtained with a silylated sample on a 1.0% SE-30 column packed with chromosorb WHP 80/100, col. temp 235°, det. temp. 250°, inj. temp. 260°, flow rate 40 ml/min, R_t 15 min IR ν_{max} cm^{-1}: 3400–3260, 2930, 2882, 1630, 1460, 1377, 1055, 1020, 820; ^1H NMR (60 MHz, CD_3OD): δ0.48 (*s*), 0.5 (6H, *d*, *J* = 8 Hz), 0.8 (*m*), 1.57 (*m*), 3.15 (1H, *m*), 3.88 (2H, *m*), 5.1 (1H, *m*). EIMS, *m/z*: 434 $[M]^+$, 416 (base peak), 398, 383, 372, 365, 298, 287, 280, 269, 260, 255, 243, 229, 211, 197, 185, 175, 159, 145, 133, 121, 105, 93, 81, 69, 55, 43. *m**, *m/z*: 398.7, 380.7, 368.5, 234.2, 198 0, 166.7, 154 6, 151.4, 144 2, 136.05, 82.2.

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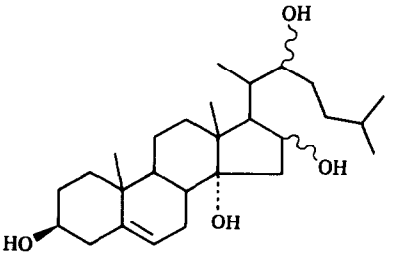

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*Dedicated to the president of Mexico, Lázaro Cárdenas, who invited the Spanish republicans to emigrate to México making possible the integration of this scientific group.

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A PYRONE GLYCOSIDE FROM *ERIGERON KARWINSKYANUS*

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Key Word Index—*Erigeron karwinskyanus*; Compositae; pyrone glucoside; 3-hydroxy-4-pyrone 3-α-D-glucopyranoside.

Abstract—The aerial parts of *Erigeron karwinskyanus* yielded 3-hydroxy-4-pyrone 3-α-D-glucopyranoside, a new glycoside. The structure was elucidated from a combination of spectral data.

INTRODUCTION

Erigeron karwinskyanus DC. (Compositae) is a perennial herb, found in the mountainous region of Nainital. Some *Erigeron* species have been found to show biological activity and the pyrone β-glycosides, erigeroside and 4-pyrone-3-β-D-glucopyranoside have been reported in their extracts [1, 2]. No previous chemical studies have been reported on *E. karwinskyanus*. This communication reports 3-hydroxy-4-pyrone 3-α-D-glucopyranoside (**1**), a new glycoside isolated from an ethanolic extract of *E. karwinskyanus*.

RESULTS AND DISCUSSION

The ethanolic extract of aerial parts of *E. karwinskyanus* was subjected to column chromatography (silica gel). Elution with methanol yielded an amorphous solid which was purified by HPLC and recrystallized from methanol to white needles. This compound ($C_{11}H_{14}O_8$) was found to be a 3-hydroxy-4-pyrone 3-α-D-glycopyranoside ($M^+ + 1$ at *m/z* 275) and its structure was established on the basis of spectral data. Complete structural information was obtained from the IR, ^{1}H NMR and mass spectra, and hydrolysis of the glycoside. IR ν_{max}^{KBr} cm^{-1}: 3550–3320 indicated the presence of a polymeric hydroxyl group. Diagnostic bands of γ-pyrone were at 1662 and 1610 [3]. Absorptions at 1240, 1150–1130 and 780 are due to =C–O– and C–O–C in the pyrone ring. Its UV absorptions at 216 and 262 nm also indicated an α,β-unsaturated keto group in the pyrone ring. Bands at 770 and 840 in the IR spectrum suggested the presence of an α-glucoside. The protons at δ6.8 (*d*, 1H, H-5), 8.32 (*d*, 1H, H-6) and 8.5 (*s*, 1H, H-2) in the 90 MHz ^{1}H NMR spectrum of the glycoside also indicated a pyrone aglycone. MS: *m/z* 275 $[M+1]^+$ and base peak at 112. The fragments at 112, 86, 85, 84 and 55 showed a γ-pyrone, the degradation occurring with the loss of C_2H_2 or CO [4]. The ion peaks at 163, 145, 127, 73 and 57 are from the sugar part of the molecule.

The fragment ion *m/z* 331 in the MS of the acetylated glycoside ($[M]^+$ 442) indicated the formation of a tetra-acetyl glucose oxonium ion after separation of the aglycone. Further fragmentation at C-1 would give fragments at 169 and 109 by the loss of acetic acid or ketene and 43 due to an acylium ion. The fragments at 169 and 109 confirmed the pyranose carbohydrate ring [5, 6] and the high intensity of *m/z* 331 (87%) suggested an α-glucoside. This is also supported by the observation of Biemann *et al.*, who have reported that the intensity of fragment *m/z* 331 is very low in a β-anomer but high in the α-anomer, and they have also used this observation for deducing the stereochemistry at C-1 in anomeric acetates [5].

Failure of **1** to undergo emulsin hydrolysis indicated an α-linkage between sugar and aglycone. Compound **1** $[\alpha]_D^{20}$ +162° (H_2O), showed a 'downward' mutarotation on addition of 6% methanolic hydrochloric acid to an aqueous solution, further confirming that the glycoside is the α-anomer.

EXPERIMENTAL

Mps are uncorr. The UV spectrum was recorded in EtOH and the IR spectrum in KBr. ^{1}H NMR spectra were obtained at 90 MHz in D_2O with TMS as int. standard. MS were recorded at 70 eV.

Plant material. The plants were collected locally from Nainital. A voucher No. H 257/79, has been deposited at the Herbarium of the Royal Botanic Gardens, Kew.

Isolation of 3-hydroxy-4-pyrone 3-α-D-glucopyranoside (**1**). Air dried plants (aerial parts, 3 kg) were ground to a fine powder which was extracted with EtOH in a Soxhlet. The extract was