0.96 (6H, *d*, $J = 6$ Hz, H-4', H-5'), 2.17 (2H, *m*, H-2'). The ^{1}H NMR signals arising from the triterpene moiety of this ester and **4c** were assigned with the aid of the lanthanide-induced-shift techniques [unpublished results]. *Taraxasteryl 3'-methylbutanoate* (**4c**). Mp 200–204°; $RR_t = 4.73$; MS *m/z* (rel. int.): 510.4459 ($C_{35}H_{58}O_2$) $[M]^+$ (19), 409 (43), 407 (20), 393 (10), 291 (8), 257 (6), 229 (7), 218 (7), 205 (19), 204 (17), 203 (20), 191 (33), 189 (100), 175 (17), 151 (14); ^{1}H NMR: δ0.85 (9H, *s*, H-23, H-24, H-28), 0.87 (3H, *s*, H-25), 0.93 (3H, *s*, H-27), 1.02 (3H, *s*, H-26), 1.02 (3H, *d*, $J = 6$ Hz, H-29), 4.5 (1H, *m*, H-3α), 4.61 (2H, *m*, H-30), 0.96 (6H, *d*, $J = 6$ Hz, H-4', H-5'), 2.17 (2H, *m*, H-2'). *Lupeyl 3'-methylbutanoate* (**5c**). $RR_t = 3.64$; MS *m/z* (rel. int.): 510 $[M]^+$ (21), 495 (14), 409 (10), 408 (10), 393 (10), 365 (6), 217 (44), 203 (41), 189 (100), 175 (27), 161 (30)

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SITOSTEROL 3-*O*-β-D-XYLOPYRANOSIDE FROM *BAUHINIA CANDICANS*

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Key Word Index—*Bauhinia candicans*; Leguminosae; structure elucidation; steroidal glycoside; sitosterol 3-*O*-β-D-xylopyranoside.

Abstract—A novel steroidal glycoside was isolated from aerial parts of *Bauhinia candicans*. Its structure was determined as sitosterol 3-*O*-β-D-xylopyranoside by chemical and spectral methods.

INTRODUCTION

In continuation of our work on *Bauhinia candicans* Benth. (Leguminosae; subfamily Caesalpinioideae), a medicinal plant from Argentina we now report the isolation and identification of a novel steroidal glycoside, sitosterol 3-*O*-β-D-xylopyranoside (**1**). We have previously described [1] the isolation and identification of Δ^5-sterols, flavonoid glycosides, sitosterol 3-*O*-β-D-glucopyranoside, D-pinitol and trigonelline from this plant.

RESULTS AND DISCUSSION

Upon chloroform percolation of the methanolic extract of *Bauhinia candicans* and further chromatographic purification of this material a fraction was obtained rich in steroidal glycosides. Since the separation of its components was not possible, the fraction was methylated to yield a mixture of compounds which exhibited considerable R_f differences. The main component (**1**) was not methylated while the other two minor glycosides gave methylated derivatives. Further purification of **1** was achieved by acetylation and column chromatography of the acetylated products.

Upon acid hydrolysis of the acetyl derivative **1a** the chloroform phase provided an aglycone that was identified as sitosterol by mass spectrometry and by capillary column GLC. The aqueous phase gave a sugar that was characterized as xylose by preparing its alditol acetate.

The ^{1}H NMR spectrum of **1a** showed the following signals: an olefinic proton (H-6) at δ 5.35 (*m*), Me-18 at 0.68 (*s*), Me-19 at 0.99 (*s*), Me-26 and Me-27 at 0.83 (*d*, $J = 7$ Hz), Me-29 at 0.84 (*t*, $J = 6.5$ Hz) and Me-21 at 0.92 (*d*, $J = 3$ Hz). These are the typical signals for sitosterol. The anomeric proton of the xylose (H-1') was present as a doublet at δ4.56 with a $J_{1',2'} = 8$ Hz due to axial–axial coupling thus showing that the xylose was β-linked to the aglycone. Protons 2' and 3' were superimposed triplets ($J_{aa} = J_{1',2'} = J_{2',3'} = J_{3',4'} = 8$ Hz) at 4.94 and 5.12, respectively. The H-4' appeared as a superimposed multi-

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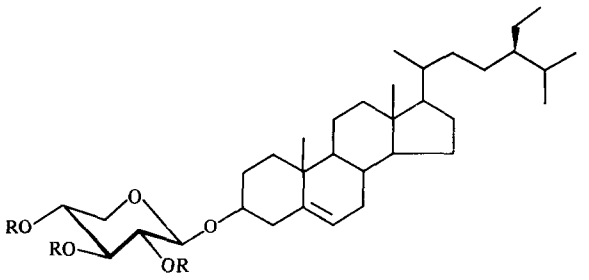

R
1 H
1a Ac

plet in the same region (4.85–5.23) as shown by double resonance experiments. H-5′$_{eq}$ and H-5′$_{ax}$ appeared as multiplets at δ4.19 and 3.71, respectively.

The noise-decoupled ^{13}C NMR spectrum showed a signal at δ99.5 assigned to C-1′ indicating a β-configuration for the xylose. If the configuration were α the signal would have appeared at 94.3. This value was calculated taking into account that the anomeric carbon of the methyl tri-*O*-acetylxylopyranoside appears at 96.4 and the shift due to the substitution of the methyl groups by the aglycone is $\Delta\delta \simeq -2$ ppm [2].

The expected δ_C values for C-3′, C-4′ and C-5′ for a xylopyranose [3] and xylofuranose [4] are 71.0, 68.3 and 61.3; 74.3, 79.9 and 62.3, respectively. The experimental values (71.4, 68.6 and 61.9) are in agreement with those of the pyranose form.

The ^{13}C NMR signals of the aglycone were also appropriate for the determination of the configuration at C-24 of the sterol moiety. The δ_C values of the diastereoisomer 24β (*S*) are quite different from those of its epimer [5]. The experimental δ_C values of **1a** confirmed that the side chain of the aglycone was that of sitosterol, that is 24α (*R*).

Finally, the Klyne rule was used to determine the glycosidic linkage (calculated value for acetyl derivative of sitosterol 3-*O*-α-D-xylopyranoside: +346.55°; calculated value for acetyl derivative of sitosterol 3-*O*-β-D-xylopyranoside: −176.32°. Observed value for **1a**: −108.04°). According to these results sitosterol is linked to a β-D-xylopyranose in **1**.

As far as we know this is the first report of the isolation and structure elucidation of sitosterol 3-*O*-β-D-xylopyranoside. However, there has been a report [6] of the isolation of a sitosterol glycoside from *Maytenus senegalensis* (Celastraceae) which was only identified by acid hydrolysis (xylose and sitosterol), mp and fragment at *m/z* 414 in the mass spectrum of the glycoside.

EXPERIMENTAL

Aerial parts of *Bauhinia candicans* Benth. were collected in Buenos Aires (Argentina). A voucher specimen was deposited at the Darwinian Institute, Buenos Aires, under Nr: SI 27581.

Dried ground aerial parts were successively extracted in a Soxhlet with petrol (60–70°) and MeOH. The methanolic extract was percolated on polyamide with $CHCl_3$, H_2O and finally MeOH.

The $CHCl_3$ fraction was chromatographed on a silica gel H column under pressure using gradients of $CHCl_3$–MeOH as eluents. Three main fractions were obtained. Only fractions 1 and 2 gave a positive Liebermann–Burchard test.

Components of fraction 1 could not be separated by direct chromatography but after methylation with CH_2N_2–Et_2O, silica gel H column chromatography ($CHCl_3$–EtOAc, 3 : 2, and EtOAc) of the methylated mixture gave three fractions. The first two were in small quantities whilst the third one corresponded to an unmethylated glycoside, the main component of this mixture

The ^{1}H NMR spectrum of this crude compound showed impurities, and the absence of acetyl groups. Upon acetylation of this compound with Ac_2O–pyridine in the usual manner and column chromatography, the acetate (**1a**) and a non-steroidal fraction were obtained.

Sitosterol 3-O-β-D-tri-O-acetylxylopyranoside (**1a**). $[\alpha]_D^{25°} = -15.75°$ (*c* 0.34, $CHCl_3$) ^{1}H NMR (100 MHz, $CDCl_3$): δ0.68 (3H, *s*, Me-18); 0.83 (6H, *d*, *J* = 7 Hz, Me-26 and 27); 0.84 (3H, *t*, *J* = 6.5 Hz, Me-29); 0.92 (3H, *d*, *J* = 3 Hz, Me-21); 0.99 (3H, *s*, Me-19); 1.98 (3H, *s*, MeCO); 2.00 (3H, *s*, MeCO); 2.03 (3H, *s*, MeCO); 3.48 (1H, *m*, H-3); 3.71 (1H, *m*, H-5′$_{ax}$); 4 19 (1H, *m*, H-5′$_{eq}$), 4.56 (1H, *d*, *J* = 8 Hz, H-1′); 4.94 (1H, *t*, *J* = 8 Hz, H-2); 5.12 (1H, *t*, *J* = 8 Hz, H-3′); 5.35 (1H, *m*, H-6). ^{13}C NMR (20.15 MHz, $CDCl_3$): δ 173.2 (MeOCO); 170.1 (MeOCO); 169.1 (MeOCO); 140 2 (C-5); 121.9 (C-6): 99.5 (C-1′); 79.9 (C-3); 71.6[a] (C-2′); 71.4[a] (C-3′); 68.6 (C-4′); 61 9 (C-5′); 56.6 (C-14); 55.9 (C-17); 50.0 (C-9); 45.7 (C-24); 42.2 (C-13); 39 6 (C-12); 38.7 (C-4); 37.0 (C-1); 36.6 (C-10); 36.0 (C-20); 34.0 (C-22); 31.7 (C-7 and 8); 29.5 (C-2); 29.2 (C-25); 28.1 (C-16); 26.0 (C-23); 24 2 (C-15); 23.0 (C-28); 21.0 (C-11); 20.4 (AcO); 19.6 (C-26); 19.2 (C-19); 18.9 (C-27); 18.6 (C-21); 11.8 (C-29); 11.7 (C-18). [a]These assignments may be interchanged. MS 70 eV *m/z* (rel. int): 414 $[M_{agl}]^+$ (3.6); 396 $[M_{agl}-18]^+$ (37); 381 $[M_{agl}-18-15]^+$ (4.9); 275 $[M_{agl}-139]^+$ (2.2); 273 [M_{agl} − side chain] (4.3); 213 $[273-42-18]^+$ (19 6); 199 $[M-M_{agl}+1-60]^+$ (2.2); 187 $[199-43]^+$ (3.8); 145 $[(MeCO)_3O]^+$ (3.8); 139 $[199-60]^+$ (2.7); 128 $[C_8H_{10}O_4]^+$ (4.9); 103 $[(MeCO)_2OH]^+$ (1.1); 43 $[MeCO]^+$ (100).

Acid hydrolysis of **1a** Compound **1a** was hydrolysed in a sealed tube with 6% HCl in MeOH and drops of water, at 75° for 2 hr The hydrolysate was neutralized and the solvent was evaporated. Extraction with $CHCl_3$–H_2O (1 : 1) provided an organic layer that contained the aglycone and an aq. layer with the sugar. The aglycone was identified as sitosterol by GLC (SP-2100 capillary column, 12 m length, 200–280°, 10°/min, R_t 8.06 min).

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