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SITOSTEROL 3-*O*-α-D-RIBURONOFURANOSIDE FROM *BAUHINIA CANDICANS*

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Key Word Index—*Bauhinia candicans*; Caesalpinioideae; Leguminosae; structure elucidation; steroidal glycoside; sitosterol 3-*O*-α-D-riburonofuranoside.

Abstract—From the aerial parts of *Bauhinia candicans* a novel steroidal glycoside was isolated and identified as sitosterol 3-*O*-α-D-riburonofuranoside.

INTRODUCTION

We have previously reported [1, 2] on compounds isolated from *Bauhinia candicans* Benth., an anti-diabetic plant native to Argentina. This paper deals with the isolation and identification of sitosterol 3-*O*-α-D-riburonofuranoside (**1**) from the aerial parts of this plant by chemical and spectral data. Although sitosterol is widespread in nature as the free sterol, its naturally occurring 3-*O*-glycosides are limited to the glucoside, galactoside, mannoside [3], xyloside [2] and di- and triglucosides [4, 5]. As far as we know, this is the first report on the isolation and identification of sitosterol 3-*O*-α-D-riburonofuranoside.

RESULTS AND DISCUSSION

The chloroform percolate of the methanolic extract of *Bauhinia candicans* provided by chromatographic methods a fraction containing Liebermann–Burchardt positive components. To enable their separation, methylation with diazomethane and acetylation were performed. Upon column chromatography of the methylated and peracetylated products, compound **1a** was obtained. Acid hydrolysis of **1a** provided a non-resolvable mixture. Therefore, **1a** was reduced with $LiAlH_4$–THF and subsequently hydrolysed to give sitosterol and ribose. The latter was identified as its acetate alditol (ribitol).

The ^{1}H NMR spectrum of **1a** showed the typical signal for sitosterol [6]: δ0.68 (*s*), 0.82 (*d*), 0.84 (*t*), 0.92 (*d*) and 1.00 (*s*) assigned to Me-18, Me-26 and Me-27, Me-29, Me-21 and Me-19, respectively. The olefinic proton (H-6) appeared at δ5.36 and H-3 at 3.50. The presence in **1** of two acetylable hydroxyls and of a carboxyl group was demonstrated by the singlets at δ2.09, 2.10 (Ac) and 3.39 (COOMe). Apparently the sugar attached to the aglycone was a pentafuranoside uronic acid. This assumption was supported by four signals in the region δ4.00–5.00, and the hydrolysis products. The doublet at δ4.50 was assigned to H-1′ ($J_{1',2'}$ = 7.5 Hz). This coupling constant value is in agreement with those of α-glycosides. If the configuration were β the coupling constant would have been less than 0.5 Hz [7–10]. The double-doublet at δ5.05 was ascribed to H-2′ ($J_{1',2'}$ = 7.5 and $J_{2',3'}$ = 10 Hz), while that at δ4.97 to H-3′ ($J_{2',3'}$ = 10 and $J_{3',4'}$ = 5 Hz). Finally, the doublet at δ4.16 was attributed to H-4′ ($J_{3',4'}$ = 5 Hz). These experimental coupling constants and the conformational analysis [11] using the Karplus equation led to the preferred conformation (°*E*) of the riburonofuranose moiety.

The ^{13}C NMR spectrum of **1a** showed the 29 aglycone signals in accordance with those of sitosterol, including the C-24 configuration (24*R*) [12]. Signals at δ99.8, 71.9, 69.0 and 81.1 were assigned to C-1′, C-2′, C-3′ and C-4′, respectively. C-5′ appeared in the carbonyl region. Since no ^{13}C NMR data had previously been reported on pentaglycofuranose uronic acids, their free or acetylated methyl glycofuranosides and free or acetylated methyl pentaglycofuranosides, the assignments had to be performed on the basis of calculated δ_C values. This was achieved taking into account the Δδ values obtained by the peracetylated ribofuranose → peracetylated ribopyranose variation [7, 13] and addition of these Δδ values to the δ_C of the acetylated methyl ribopyranoside [13]. Thus, the δ_C values of the acetylated methyl ribofuranoside were obtained.

The latter data were corrected ($\Delta\delta_C$: C-1′, −0.5; C-2′, 0.6; C-3′, −1.8; and C-4′, 0.8) to give the δ_C of the methyl (methyl ribofuranoside) uronate. The effect on $\delta_{C\text{-}1'}$ due to the attachment of the aglycone was also estimated [15]. Calculated δ_C values for peracetylated methyl (sitosterol 3-*O*-α-D-ribofuranoside) uronate: C-1′, 99.9; C-2′, 71.0; C-3′, 69.8; C-4′, 82.0; for the β-anomer: C-1′; 104.4; C-2′, 74.8; C-3′, 68.9; C-4′, 81.3. Calculated $\Delta\delta_C$ for α-glycoside → **1a**: C-1′, 0.1; C-2′, −0.9; C-3′, 0.8; C-4′, 0.9. Consequently, α-D-riburonofuranose is attached to sitosterol. The same procedure was used satisfactorily to calculate the δ_C values of the methyl β-D-ribofuranoside, which is the only methyl pentaglycofuranoside with reported ^{13}C NMR data [14]. Therefore, **1** was identified as the novel 3-*O*-α-D-riburonofuranoside.

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EXPERIMENTAL

General experimental details have previously been reported [2].

The defatted methanolic extract of aerial parts of *Bauhinia candicans* Benth. (voucher specimen No. SI 27581) was successively percolated on polyamide with $CHCl_3$, H_2O and MeOH. The $CHCl_3$ percolate was chromatographed as previously described [2] to give three main fractions. The separation of the components of fraction 1 was only possible by methylation (CH_2N_2–Et_2O) and further silica gel H CC ($CHCl_3$–EtOAc, 3:2, and EtOAc). Thus, the main component remained unmethylated and was identified as sitosterol 3-*O*-*β*-D-xylopyranoside [2] while the minor glycoside **1** was isolated as the methylated derivative. Further acetylation of the latter provided the peracetylated methyl ester of sitosterol 3-*O*-*α*-D-riburonofuranoside (**1a**). Methyl (sitosterol 3-*O*-*α*-D-di-*O*-acetylribofuranoside)uronate (**1a**), $[\alpha]_D^{25}$ −18.26° (*c* 0.23; $CHCl_3$).^{1}H NMR (100 MHz, $CDCl_3$): δ0.68 (3H, *s*, Me-18), 0.82 (6H, *d*, J = 7 Hz, Me-26 and Me-27), 0.84 (3H, *t*, J = 6.5 Hz, Me-29), 0.92 (3H, *d*, J = 3 Hz, Me-21), 1.00 (3H, *s*, Me-19), 2.09 (3H, *s*, MeCO), 2.10 (3H, *s*, MeCO), 3.39 (3H, *s*, CO_2Me), 3.50 (1H, *m*, H-3), 4.16 (1H, *d*, $J_{3',4'}$ = 5 Hz, H-4'), 4.50 (1H, *d*, $J_{1',2'}$ = 7.5 Hz, H-1'), 4.97 (1H, *dd*, $J_{2',3'}$ = 10, $J_{3',4'}$ = 5 Hz, H-3'), 5.05 (1H, *dd*, $J_{1',2'}$ = 7.5, $J_{2',3'}$ = 10 Hz, H-2'), 5.36 (1H, *m*, H-6). ^{13}C NMR (20.15 MHz, $CDCl_3$): δ173.4, 169.2 and 169.1 (CO_2R), 140.4 (C-5), 121.8 (C-6), 99.8 (C-1'), 81.1 (C-4'), 79.8 (C-3), 71.9 (C-2'), 69.0 (C-3'), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.7 (C-24), 42.2 (C-13), 39.7 (C-12 and C-4), 37.1 (C-1), 36.6 (C-10), 36.0 (C-20), 33.8 (C-22), 31.8 (C-7 and C-8), 29.1 (C-25), 28.2 (C-16 and C-2), 26.0 (C-23), 24.1 (C-15), 23.0 (C-28), 21.0 (C-11), 20.8 and 20.7 (MeCO), 19.7 (C-26), 19.2 (C-19), 18.9 (C-27), 18.7 (C-21), 11.9 (C-29), 11.7 (C-18).

Reduction and acid hydrolysis of **1a**. Compound **1a** was reduced with $LiAlH_4$ in dry THF overnight under reflux. The product was worked up in the usual manner, followed by acid hydrolysis with 6% HCl in MeOH and drops of H_2O in a sealed tube at 75° for 2 hr. After neutralization and evapn of the solvent, the hydrolysate was extracted with $CHCl_3$–H_2O (1:1). The aglycone was obtained from the organic layer and identified as sitosterol by capillary GC (SP-2100, 12 m length, 200 → 280°, 10°/min, R_t 8.1 min). The sugar isolated from the aq. layer was reduced and acetylated to give acetate ribitol (GC: 3% ECNSS, 3 m length, 180° isothermal, R_t 6.89 min).

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