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

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

Abstract—Aerial parts of *Bauhinia candicans* afforded a novel steroidal glycoside identified as sitosterol 3-*O*-α-D-xyluronofuranoside. This is the first report on a naturally occurring glycoside with a xyluronofuranose as the sugar moiety.

INTRODUCTION

In continuation of our work on medicinal plants we now report the isolation and identification of a new glycoside, sitosterol 3-*O*-α-D-xyluronofuranoside (**1**), from *Bauhinia candicans*, an Argentinian species with hypoglycaemic and hypocholesterolaemic properties. We have previously identified other steroidal glycosides isolated from this plant, all of them with the widespread sitosterol as aglycone but with the following sugars: glucopyranose [1], xylopyranose [2] and riburonofuranose [3].

This paper and our previous results show the unusual accumulation of sitosterol glycosides in this species as well as the occurrence of uronic acid moieties (riburonofuranose and now xyluronofuranose), which are reported for the first time as naturally occurring sugars.

RESULTS AND DISCUSSION

Upon column chromatography of the chloroform percolate of the methanolic extract of *B. candicans* a fraction rich in steroidal glycosides was obtained. This fraction was methylated and acetylated to isolate its components by chromatographic methods and yield **1a**. A survey of its ^{1}H NMR spectrum indicated the expected signals for a penturonoglycoside of a Δ^5-sterol. In fact, it was similar to that of sitosterol 3-*O*-α-D-riburonofuranoside [3]. Since direct hydrolysis of these uronic acid derivatives led to degradation of the glycoside as we had earlier observed, **1a** was first reduced with lithium aluminium hydride and tetrahydrofuran and subsequently hydrolysed. Mild conditions were required for both procedures to prevent thermal decomposition. The low activation energy of hydrolysis shown by furanosides [4] prompted us to use more dilute acid and a short time for hydrolysis. Otherwise, the autoxidation of sitosterol [5] is favoured and only stigmasta-3,5-dien-7-one is obtained. Under the appropriate conditions, sitosterol and xylose were detected by chromatographic methods and GC/MS of the hydrolysis products.

The ^{1}H NMR spectrum of **1a** confirmed these results. The upper-field signals ($\delta < 1$) were assigned to Me-18, Me-19, Me-21, Me-26, Me-27, and Me-29, [6] of sitosterol and the signal at δ5.32 to the olefinic H-6.

The presence of only two acetate (singlets at δ1.96 and 2.02) and one carboxymethyl signal (δ3.48) confirmed the pentofuranose uronic acid structure. Evidence about the glycosidic linkage and the identity of the sugar were obtained from its four methinic proton signals: H-1' appeared as a doublet at δ4.44; the coupling constant $J_{1',2'} = 8$ Hz indicated an α-linkage of the sugar to the aglycone (a β-configuration would have given a $J_{1',2'}$ value < 0.5 Hz [7, 8]); H-2' and H-3' gave double doublets at δ5.04 ($J_{1',2'} = 8$, $J_{2',3'} = 7.5$ Hz) and 4.88 ($J_{2',3'} = 7.5$, $J_{3',4'} = 5$ Hz), respectively; whilst H-4' was observed as a doublet at δ4.15 ($J_{3',4'} = 5$ Hz).

The conformational study of **1a** using the experimental coupling constants suggested the preferred conformation ^{4}E. Moreover, a study of the internal angles and the amount of buckle of the furanose ring was also carried out [9].

The ^{13}C NMR spectral data were decisive in the identification of the aglycone since neither chromatographic methods nor other spectroscopic techniques (MS, ^{1}H NMR at 100 MHz) distinguish between epimers at C-24 of ethylcholesterols (sitosterol and clionasterol). Therefore, the signals of C-20, C-22, C-23, C-24 and C-25 confirmed the presence of sitosterol [10]. The lack of ^{13}C NMR literature data of pentaglycofuranose uronic acids and their derivatives led us to calculate the δ_C values of different methylated and peracetylated sitosterol 3-*O*-penturonoglycosides following the method we have previously reported [3]. Analysis of these results allowed us to assign the signals at δ99.5, 78.6, 72.1 and 75.1 to C-1', C-2', C-3' and C-4' (C-5' appeared in the carbonyl region), respectively. Calculated values: α-xyluronofuranoside: δ97.3, 77.1, 71.9 and 75.2, respectively; β-xyluronofuranoside: δ105.6, 80.8, 73.0 and 81.3, respectively.

According to the above data, the natural glycoside **1** is

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sitosterol 3-*O*-α-D-xyluronofuranoside. As far as we know, this is the first report on this glycoside and on this sugar moiety from a natural source.

EXPERIMENTAL

Aerial parts of *Bauhinia candicans* Benth. were collected in Buenos Aires, Argentina. A voucher specimen (No. SI 27581) has been deposited at the Instituto de Botánica Darwinion, Buenos Aires, Argentina.

General experimental details have been reported previously [2]. Percolation of the methanol extract and CC purification of the fraction containing the steroidal glycosides were performed as detailed previously [3].

The methylation (CH_2N_2, Et_2O) of this fraction provided a mixture which could be separated. TLC (silica gel G) using Cl_2CH_2–EtOAc (4:1) as solvent showed three spots (R_f 0.50, 0.32 and 0.07). Two of them had been previously identified as sitosterol 3-*O*-β-D-xylopyranoside (R_f 0.07) [2] and methyl (sitosterol 3-*O*-α-D-riburonofuranoside) uronate (R_f 0.50) [3]. In order to identify the third minor compound, the whole fraction was chromatographed on a silica gel H column ($CHCl_3$–EtOAc, 4:1, and EtOAc). The subfraction containing the compound with R_f 0.32 was further purified by acetylation (Ac_2O–pyridine) to give the peracetylated methyl ester of sitosterol 3-*O*-α-D-xyluronofuranoside (**1a**).

Methyl (sitosterol 3-O-α-di-O-acetyl-D-xyluronofuranoside) uronate (**1a**). ^{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), 0.93 (3H, *d*, J = 3 Hz, Me-21), 1.00 (3H, *s*, Me-19), 1.96 (3H, *s*, MeCO), 2.02 (3H, *s*, MeCO), 3.48 (3H, *s*, CO_2Me) 4.15 (1H, *d*, $J_{3',4'}$ = 5 Hz, H-4'), 4.44 (1H, *d*, $J_{1',2'}$ = 8 Hz, H-1'), 4.88 (1H, *dd*, $J_{2',3'}$ = 7.5, $J_{3',4'}$ = 5 Hz, H-3'), 5.04 (1H, *dd*, $J_{1',2'}$ = 8, $J_{2',3'}$ = 7.5 Hz, H-2'), 5.32 (1H, *m*, H-6). ^{13}C NMR (80 MHz, $CDCl_3$): δ174.1, 169.4 and 169.2 (CO_2R), 140.5 (C-5), 122.0 (C-6), 99.5 (C-1'), 80.0 (C-3), 78.0 (C-2'), 75.1 (C-4'), 72.1 (C-3'), 56.8 (C-14), 56.1 (C-17), 50.2 (C-9), 45.9 (C-24), 42.3 (C-13), 39.9 (C-12), 38.9 (C-4), 37.3 (C-1), 36.8 (C-10), 36.2 (C-20), 34.3 (C-22), 31.9 (C-7 and C-8), 29.5 (C-2), 29.3 (C-25), 28.1 (C-16), 26.2 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 20.9 ($\underline{C}H_3CO$); 20.7 ($\underline{C}H_3CO$), 19.3 (C-26), 19.1 (C-19), 18.8 (C-21 and C-27), 11.8 (C-18 and C-29).

Reduction and acid hydrolysis of **1a**. Compound **1a** was reduced with $LiAlH_4$ in dry THF for 3 hr at 60° under reflux. The product was worked up in the usual manner, followed by acid hydrolysis with 0.2 M HCl in MeOH and drops of H_2O under reflux at 60° for 90 min. After neutralization and evaporation 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 GC/MS (OV-17 3‰ length: 1.8 m, 200 → 290°, 6°/min). The aq. layer was desalted using successively a weak basic resin, Dowex-3 (HO-form), and a cationic resin, AG-50 W X8 (H-form). After evaporation of the H_2O the sugar was characterized by TLC (cellulose), BuOH–pyridine–H_2O (6:4:3).

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