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A New Cardenolide from the Seeds of *Pennisetum spicatum* Roem

RAJ NATH YADAVA* and KAVITA RATHORE
Natural Products Laboratory, Department of Chemistry
Dr. H.S. Gour University, Sagar 470 003, India

Pennisetum spicatum belongs to family Gramineae which is commonly known as 'Bajara' in Hindi. It is useful in diseases of heart and also used as an appetizer. Earlier workers have reported various constitutents from this plant. In the present paper we report the isolation and structural elucidation of a new cardenolide, m.f. $C_{34}H_{48}O_{14}$, m.pt. 162–163°C, which was identified as 6,7-dehydro-strophanthidin-3-O-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranoside from the seeds of this plant by various chemical degradations and spectral analysis.

INTRODUCTION

Pennisetum spicatum[1-3] belongs to family Gramineae which is commonly known as 'Bajara' in Hindi. It is cultivated in numerous forms in India. It is used as tonic. It is also useful in diseases of heart and also used as an appetizer. In the present paper we report the isolation and structural elucidation of a new cardenolide 6,7-dehydro-strophanthidin-3-O-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranoside from the seeds of this plant by various chemical degradations and spectral analysis.

RESULTS AND DISCUSSION

The acetone soluble fraction of the seeds of *Pennisetum spicatum* afforded a new compound 1, molecular formula, $C_{34}H_{48}O_{14}$, m.p. 162–163°C, $[M]^+$ 680. Compound 1 gave a positive response for Kedde test[4] confirming it to be a cardenolide. Its IR spectrum showed absorption peaks at 3445 ν(—OH), 1735 ν(>C=O), 1720 (butanolide), 1640, 1445, 1050, 785 cm^{-1}. Its UV spectrum showed a band at 216 nm with MeOH, which is a characteristic of a carbonyl gorup conjugated with a double bond.

^{1}H-NMR spectrum of compound 1 showed two doublets at δ 4.96 and δ 4.82 assigned for H-21α and H-21β and one proton singlet at δ 5.86 assigned for H-22. Two proton doublets appeared at δ 6.18 (J = 3.5 Hz) and δ 6.36 (J = 5.1 Hz) attributed for H-6 and H-7. A broad singlet appeared at δ 4.41 assigned for carbinylic proton at H-3. A singlet at δ 1.02 was assigned for H-18. In ^{1}H-NMR spectrum of 1, two anomeric proton signals at δ 5.32 and δ 4.31 (d, J = 7.5 Hz) were assigned for H-1′, H-1″ of rhamnose and xylose respectively and a complex signal at δ 1.02 was due to rhamnosyl methyl group.

In the EIMS of **1**, characteristic ions appearing at m/z 547 and 401 were generated by subsequent losses from the molecular ions of one rhamnose and one xylose units, suggesting that the xylose is the terminal sugar.

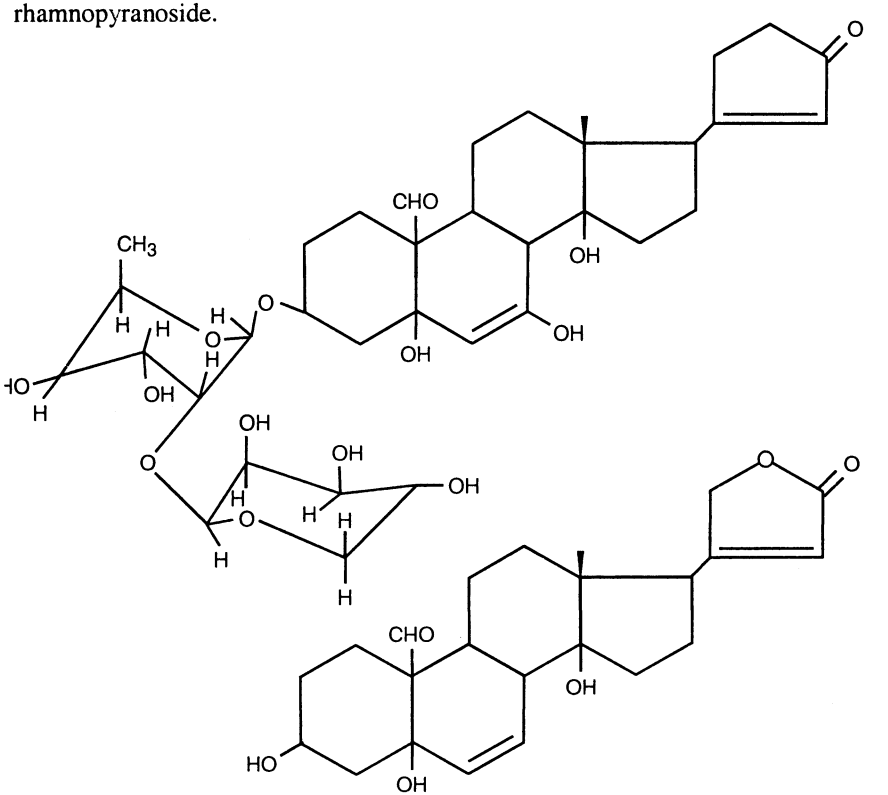
Acid hydrolysis of **1** with 10% H_2SO_4 gave a glycone (**2**), m.f. $C_{23}H_{29}O_6$, m.p. 154–156°C, $[M]^+$ 401. It responded to the characteristic reactions of cardenolide and identified as 6,7-dehydro-strophanthidin by its spectral analysis (see Experimental).

The aqueous hydrolysate obtained after the acid hydrolysis of the glycoside 1 was neutralised with $BaCO_3$ and $BaSO_4$, filtered off and subjected to Co-PC. The presence of sugars was identified as L-rhamnose (R_f 0.36) and D-xylose (R_f 0.26).

Permethylation[5] of **1** followed by acid hydrolysis yielded permethylated aglycone (3) and permethylated sugars, which were identified as, 3,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-xylose according to Petek[6] showing that the C-1″ of xylose was linked with C-2′ of L-rhamnose. The inter linkage (1→2) between both the sugars was further confirmed by its ^{13}C-NMR spectrum (see Experimental). Periodate oxidation[7] of compound **1** confirmed that both the sugars were present in pyranose form.

Enzymatic hydrolysis of compound **1** with equal volume of almond emulsion liberated D-xylose confirming the presence of β-linkage between D-xylose and L-rhamnose. It is also hydrolysed by enzyme Takadiastase liberating L-rhamnose, confirming that L-rhamnose was linked with aglycone through α-linkage[8].

On the basis of above evidences the structure of the compound **1** was established as 6,7-dehydro strophanthidin-3-O-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranoside.



EXPERIMENTAL

General m.p. are uncorrected. IR spectra were measured in KBr disc. ^{1}H-NMR spectra were recorded at 300 MHz and $CDCl_3$ as solvent, ^{13}C-NMR spectra were recorded at 400 MHz and DMSO-d_6 as solvent.

Plant Material

The seeds of *Pennisetum spicatum* were collected locally in Sagar and Taxonomically authenticated by the Department of Botany, Dr. H.S. Gour University, Sagar. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, of this University.

Extraction and Isolation

The air-dried and powdered seeds (2.5 kg) of *Pennisetum spicatum* were extracted with 95% EtOH in a Soxhlet extractor. The total ethanolic extract was concentrated under reduced pressure to give a light brown coloured mass and was then successively extracted with petroleum ether, chloroform, benzene, ethyl acetate, acetone and methanol. The acetone solution fraction was concentrated under reduced pressure to viscous mass which was subjected to column chromatography over a Si-gel G column using $CHCl_3$-MeOH (3:2) to gave compound **1** crystallised from MeOH as white solid, m.f. $C_{34}H_{48}O_{14}$, m.p. 162–163°C, $[M]^+$ 680 (Elemental analysis: found (%) C, 59.98, H, 7.03, calcd. (%) C, 60.0, H, 7.05). ν_{max}^{KBr} 3445, 2840, 1735, 1720, 1610, 1445, 1050 and 785 cm^{-1}. UV λ_{max}^{MeOH} 216 nm. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.82 (1H, brt.) H-1α; 2.59 (1H, td.) H-1β; 2.46 (1H, tt.) H-2α; 2.34 (1H, br.d) H-2β; 4.41 (1H, br.s) H-3; 2.01 (1H, dd) H-4α; 2.26 (1H, br.d) H-4β; 6.18 (1H, dd, I = 3.5 Hz) H-6; 6.36 (1H, dd, J = 5.1 Hz) H-7; 2.74 (1H, br.t) H-8; 2.51 (1H, dd) H-9; 4.32 (1H, m) H-11α; 4.52 (1H, m) H-11β; 1.94 (1H, m) H-12α; 1.85 (1H, m) H-12β; 2.06 (1H, m) H-15α; 1.95 (1H, m) H-15β; 2.02 (1H, m) H-16α; 1.91 (1H, m) H-16β; 3.42 (1H, dd) H-17; 1.02 (1H, s) H-18; 10.42 (1H, s) H-19; 4.96 (1H, d) H-21α; 4.82 (1H, s) H-21β; 5.86 (1H, s) H-22; 5.32 (1H, br, s), H-1′; 4.31 (1H, d, 5–7.5 Hz) H-1″, 1.02 (3H, Complex signal) rhamnosyl methyl. ^{13}C-NMR (300 MHz, DMSO-d_6) 24.21 (C-1), 26.09 (C-2), 72.87 (C-3), 36.96 (C-4), 73.18 (C-5), 136.21 (C-6), 126.10 (C-7), 42.15 (C-8), 39.35 (C-9), 54.65 (C-10), 21.80 (C-11), 39.65 (C-12); 48.85 (C-12), 48.70 (C-13), 32.50 (C-15), 26.15 (C-16), 48.85 (C-17), 16.65 (C-18), 208.35 (C-19), 174.55 (C-20), 73.90 (C-21), 117.05 (C-22), 174.05 (C-24), 103.05 (C-1′), 83.10 (C-2′), 72.15 (C-3′), 73.40 (C-4′), 71.80 (C-5′), 17.20 (C-6′), 107.65 (C-1″), 75.05 (C-2″), 76.10 (C-3″), 70.15 (C-4″), 67.20 (C-5″).

Acid Hydrolysis of Compound 1

The compound **1** was dissolved in EtOH and treated with 10% H_2SO_4 and refluxed on water bath for 10 h. The contents were concentrated and allowed to cool and the residue was extracted with Et_2O. The aqueous layer was studied separately for the identification of sugars. The ethereal layer was washed with water and evaporated to dryness and the residue was subjected to column

chromatography over a Si-gel G column using $CHCl_3$:MeOH (4:2) to gave aglycone (**2**) m.f. $C_{23}H_{29}O_6$, m.p. 154–156°C, $[M]^+$ 401 (elemental analysis: found (%) C 68.80, H, 7.21; calcd. (%) C, 68.82, H, 7.23). IR ν_{max}^{KBr} 3442, 2845, 1735, 1715, 1705, 1615, 1445, 1055 and 782 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) 4.42 (1H, br, S) H-3, 6.19 (1H, dd, J = 3.5 Hz) H-6, 6.34 (1H, dd, J = 5.1 Hz) H-7, 2.08 (1H, m) H-15α, 1.94 (1H, m) H-15β, 204 (1H, m) H-16α, 1.92 (1H, m) H-16β, 3.41 (1H, dd) H-17, 1.03 (1H, s) H-18, 10.48 (1H, s) H-19, 4.95 (1H, d) H-21α, 4.84 (1H, s) H-21β, 5.87 (1H, s) H-22.

The aqueous hydrolysate was neutralised with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination (n-BCl : AcOH : H_2O, 4 : 1 : 5) revealed the presence of L-rhamnose (R_f 0.36) and D-xylose (R_f 0.26).

Permethylation followed by Hydrolysis of Compound 1

Compound **1** (40 mg) in MeI (5 mL) and Ag_2O (50 mg) in DMF (5 mL) were refluxed for 24 h at room temperature. The total reaction mixture was diluted with H_2O and extracted with $CHCl_3$, gave permethylated aglyonce (**3**) and methylated sugars which were identified as 3-4-di-O-methyl-1-rhamnose and 2,3,4-tri-O-methyl-D-xylose (by Co-PC and Co-TLC) according to Petek[6].

Periodate Oxidation of Compound **1**: Compound **1** was dissolved in MeOH and treated with sodium metaperiodate for 40 h. The liberation of formic acid and consumed periodate were estimated by Jone's method.

Enzymatic Hydrolysis of Compound **1**: The compound **1** (50 mg) in MeOH (20 mL) was treated with equal volume of almond emulsion, liberating D-xylose and was also hydrolysed by enzyme Takadiastase liberating L-rhamnose and aglycone.

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