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A new geniculatoside from aerial parts of *Euphorbia geniculata* Linn.

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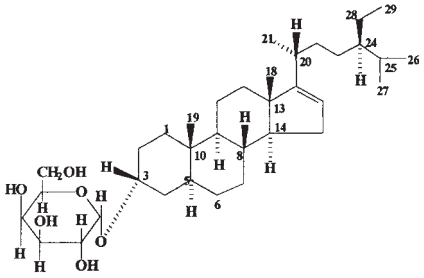
The phytochemical investigation of the aerial parts of *Euphorbia geniculata* Linn. has resulted in the isolation of a new steroidal galactoside, stigma 16-en-3α-O-(β-D-galactopyranoside) designated as geniculatoside F. The structure was elucidated by spectroscopic and chemical methods.

1. Introduction

Euphorbia geniculata, commonly known as Dudhi, belongs to the family Euphorbiacea. It is native to tropical America and is naturalised as a weed in many parts of India up to an altitude of 800 m [1]. It is also found in Dehradun (Uttranchal) and in Gujarat [2]. The extract of *E. geniculata* shows antifungal activity against *Aspergillus flavus* [3]. Some compounds such as kaempferol and its rutinoside, quercetin and its 3-rhamnoside, quercitrin, (-amyrin acetate, (-sitosterol, campesterol, stigmasterol, cholesterol [1], geniculatin, 10,10-dimethyl-hexacosan-7-one, (-amyrin, euphyl acetate and mortenone [4] have been previously reported. The present paper deals with the isolation and characterization of a new galactoside stigma 16-en-3α-O-(β-D-galactopyranoside) from the aerial parts of this plant.

2. Investigations, results and discussion

The compound named as geniculatoside-F was obtained as colourless crystals and had the molecular composition of $C_{35}H_{60}O_6$ on the basis of high resolution MS (M^+ 576) and ^{13}C NMR spectral data. It gave a positive Liebermann Burchard test [5] for steroid and Molisch's test [6] for glycoside indicating it to be a steroidal glycoside. Its IR spectrum exhibited a band at 3550–3450 cm^{-1} (OH), 1550 (C=C), 1080–1040 (C−O, alcoholic) which indicated the presence of hydroxyl group and a double bond in the compound. The presence of the double bond was further substantiated by its ^1H NMR spectrum which showed a doublet at δ 5.333 (J = 6.0 Hz). The acetate of the compound also confirmed the presence of hydroxyl groups which indicated a sharp peak at (ν_{max} 1750, due to C=O group and 1240 due to C−O, ester linkage in the IR spectrum. The ^1H NMR spectrum of the acetate exhibited four singlets at (2.055 (3 H, s), 2.045 (3 H, s), 2.027 (3 H, s), 2.011 (3 H, s) due to four acetoxyl groups of the sugar moiety which indicated the presence of only one sugar unit in the molecule. The ^1H NMR spectrum also showed the presence of six methyl fractionalities all attached to saturated carbons which showed the six signals at δ 0.642 (3 H, s, Me-18), 0.986 (3 H, s, Me-19), 0.952 (3 H, d, J = 7.8 Hz, Me-21), 0.797 (3 H, d, J = 6.6 Hz, Me-26), 0.841 (3 H, d, J = 6.9 Hz, Me-27), 0.819 (3 H, d, J = 6.6 Hz, Me-29). The ^1H NMR spectrum showed a broad multiplet at δ 3.591 due to a carbinolic proton at position-3. The coupling constant (dddd, J = 4.2, 7.2, 5.7, 5.4 Hz) or 1/2 width was found to be 11.7 Hz which indicated the ß-orientation of the carbinolic proton [7, 8]. Biogenetically and on the basis of The MS the hydroxyl group was assigned to position-3 which was found to be linked with the sugar moiety. The presence of a double bond was found at $\Delta^{16(17)}$ on the basis of the MS fragmentation pattern of the aglycone which exhibited a sharp peak at m/z 260. Hydrolysis of the compound with 10% HCl afforded an aglycone. The ratio of the aglycone obtained to the glycoside was found to be about 60% indicating only one sugar unit per molecule [9]. The sugar was identified as galactose by the use of co-paper chromatography. The sugar was found to be linked with the aglycone moiety through a β-linkage as evidenced by a doublet at δ 4.793 and the coupling constant 8.1 Hz of the anomeric proton of the sugar moiety in the ^1H NMR spectrum of the compound. The other signals of the sugar were found to be consistent with the galactose protons in the ^1H NMR spectrum (Table 1). The characteristic ions of the MS suggested a stigmastane skeleton [10, 11], being found at 396 $(M\text{-}H_2O)^+$, 329 (M-side chain, C_6H_{13}, 85)$^+$, 301 (M-side chain, C_8H_{17}, 113)$^+$, 273 (M-side chain, $C_{10}H_{21}$, 141)$^+$. The MS clearly proved the absence of a double bond in the side chain and ring A, B, C. The ions at m/z 85 and 71 indicated the presence of a hydroxyl group in ring A which was assigned to position-3. The aglycone obtained on hydrolysis was acetylated with acetic anhydride and pyridine. This afforded a mono acetate as confirmed by its ^1H NMR spectral data which exhibited a singlet at (2.022 (3 H, s) due to one acetoxyl groups, indicating only one hydroxyl group in the aglycone. The carbinolic proton at C-3 was shifted down field at δ 4.499 in the ^1H NMR spectrum of the acetate of the aglycone. The ^{13}C NMR spectrum displayed signals for olefinic carbons at δ 141.26 (C-17) and 122.13 (C-16), anomeric carbon at δ 106.8 (C-1^1), C-3 carbinol carbon at δ 79.51 and sugar carbons at δ 75.81 (C-2), 78.7 (C-3), 71.80 (C-4^1), 71.27 (C-5^1) and 63.02 (C-6^1). Thus on the basis of the above spectral and chemical studies the structure of the aglycone compound was established as stigma 16-en-3α-ol and its glycoside was found to be stigma 16-en-3α-O-(β-D-galactopyranoside).



3. Experimental

3.1. General procedure

The melting point of the compound was determined by the open capillary method and is uncorrected. The IR spectra were recorded on a Perkin

Table 1: ^{1}H NMR data of glycoside and its acetate

Position	Glycoside	Acetate
3	3.591 (1 H, m, 1/2w = 11.7 Hz, β-H) (or dddd, J = 4.2, 7.2, 5.7, 5.4 Hz)	3.510 (1 H, m, 1/2w = 11.6 Hz, β-H)
16	5.333 (1 H, d, J = 6.0 Hz)	5.353 (1 H, d, J = 6.0 Hz)
18	0.642 (3 H, s, Me)	0.675 (3 H, s, Me)
19	0.986 (3 H, s, Me)	0.987 (3 H, s, Me)
21	0.952 (3 H, d, J = 7.8 Hz, Me)	0.920 (3 H, d, J = 7.8 Hz, Me)
26	0.797 (3 H, d, J = 6.6 Hz, Me)	0.800 (3 H, d, J = 6.6 Hz, Me)
27	0.841 (3 H, d, J = 6.9 Hz, Me)	0.869 (3 H, d, J = 6.9 Hz, Me)
29	0.819 (3 H, d, J = 6.6 Hz, Me)	0.845 (3 H, d, J = 6.6 Hz, Me)
	* 2.579–2.573 (1 H, m)	* 2.226 (1 H, m)
	* 2.377–2.366 (1 H, m)	* 2.084 (1 H, m)
	* 2.212–1.69 (6 H, bm)	* 1.875–1.473 (6 H, bm)
	* 1.668–1.417 (8 H, bm)	* 1.320–1.001 (21 H, bm)
	* 1.366–1.040 (13 H, bm)	
Sugar Proton		
1^1	4.793 (1 H, d, J = 8.1 Hz, β-linkage)	4.606 (1 H, d, J = 8.1 Hz, β-linkage)
2^1	4.164 (1 H, d, J = 5.7 Hz)	4.965 (1 H, dd, J = 7.8, 9.9 Hz)
3^1	4.677 (1 H, d, J = 3.9 Hz)	5.208 (1 H, dd, J = 9.6, 9.3 Hz)
4^1	4.331 (1 H, d, J = 7.8 Hz)	5.080 (1 H, dd, J = 9.9, 9.3 Hz)
5^1	3.747 (1 H, dd, J = 5.2, 5.1 Hz)	** 3.665 (1 H, m, 1/2w = 9.6 Hz)
6^1a	3.227 (1 H, dd, J = 5.4, 4.8 Hz)	4.250 (1 H, dd, J = 4.5, 4.8 Hz)
6^1b	3.112 (1 H, dd, J = 2.1, 2.4 Hz)	4.125 (1 H, dd, J = 2.4, 2.1 Hz)
		# 2.055 (3 H, s, $OCOCH^3$)
		2.045 (3 H, s $OCOCH^3$)
		2.027 (3 H, s $OCOCH^3$)
		2.011 (3 H, s $OCOCH^3$)

* Assignable to methylene and methine protons; ** m low value of coupling constant; # Assignments of acetoxy groups are interchangeable

Elmer 1600 IR spectrometer in KBr pellets, ^{1}H NMR and ^{13}C NMR spectra on a Brucker 300 MHz and 75 MHz, respectively, in $CDCl_3$. Coupling constants are in Hz. The high resolution MS were obtained on an HMGM mass spectrometer. CC was carried out using silica gel (60–120 mesh). TLC was performed on silica gel G.

3.2. Plant material

The plant was collected from Dehradun (Uttranchal) and indentified by Dr. M. P. Saharma, Department of Botany, Hamdard University, New Delhi, 110062, India, where a voucher specimen has been kept for reference.

3.3. Extraction and isolation

The plant material (3.5 kg) was dried in the shade, crushed to a coarse powder and exhaustively extracted with ethanol by cold percolation. The crude alcoholic extract was concentrated under reduced pressure to get a viscous mass (150 g). It was then fractionated into ethyl acetate and methanol soluble portions. The ethyl acetate fraction was dissolved in hot methanol and allowed to cool, after which the fatty material was filtered off. The filtrate was concentrated to dryness to get a viscous mass (60 g). The dried extract was dissolved in the minimum amount of methanol and adsorbed on silica gel to form a slurry. The slurry was dried and chromatographed over a silica gel column prepared in petroleum ether. The column was eluted with petroleum ether-chloroform and chloroform-methanol in increasing order of polarity. Only one pure and single compound was obtained.

3.4. Acetylation

The compound (50 mg) was acetylated with acetic anhydride-pyridine (1 : 1) which on the usual work-up afforded the mono acetate (45 mg); m.p. 175 °C.

3.5. Characterization of compound

Elution of the ethyl acetate fraction with chloroform-methanol (93 : 7) furnished colourless crystals, yield 80 mg, soluble in chloroform, R_f value 0.29 (chloroform-methanol, 95 : 5), m.p. 200 °C, IR ν_{max} (KBr): 3550–3450 (OH), 2950, 2850 (CH_3, CH_2), 1550 (C=C), 1480, 1300, 1080–1040 (C–O, alcoholic), 800 cm^{-1}. IR ν_{max} (KBr) (Acetate): 2950, 2850 (CH_3, CH_2), 1750 (C=O), 1550 (C=C), 1460, 1370, 1240 (C–O, ester) 1060–1040 (C–O, alcoholic), 800 cm^{-1}. ^{1}H NMR (Table 1 & 2), EIMS m/z: 414 [M^+ $C_{29}H_{50}O$, (30)], 396 [M^+-H_2O, (9)], 329 [M^+-side chain, fission

Table 2: ^{1}H NMR data of aglycone and its acetate

Position	Aglycone	Acetate
3	3.586 (1 H, m, 1/2w = 11.7 Hz, β-H)	4.499 (1 H, m, 1/2w = 11.6Hz, β-H)
16	5.336 (1 H, d, J = 6.0 Hz)	5.352 (1 H, d, J = 6.0 Hz)
18	0.641 (3 H, s, Me)	0.674 (3 H, s, Me)
19	0.988 (3 H, s, Me)	0.986 (3 H, s, Me)
21	0.950 (3 H, d, J = 7.8 Hz, Me)	0.919 (3 H, d, J = 7.8 Hz, Me)
26	0.796 (3 H, d, J = 6.6 Hz, Me)	0.801 (3 H, d, J = 6.6 Hz, Me)
27	0.840 (3 H, d, J = 6.9 Hz, Me)	0.868 (3 H, d, J = 6.9 Hz, Me)
29	0.818 (3 H, d, J = 6.6 Hz, Me)	0.846 (3 H, d, J = 6.6 Hz, Me)
	* 2.578–2.574 (1 H, m)	* 2.227 (1 H, m)
	* 2.376–2.367 (1 H, m)	* 2.085 (1 H, m)
	* 2.212–1.60 (6 H, brm)	* 1.876–1.474 (6 H, brm)
	* 1.667–1.418 (8 H, brm)	* 1.320–1.002 (21 H, brm)
	* 1.366–1.040 (13 H, brm)	
	1.615 (1 H, brs, OH) exchangable with D_2O	2.022 (3 H, s, OCOCH3)

* Assignable to methylene and methine protons

via 23 (24), (15)], 315 [M^+-side chain, fission via 22 (23), (5)], 301 [M^+-side chain, fission via 20 (22), (15)], 273 [M^+-side chain, fission via 19 (20), (15)], 260 [$C_{18}H_{28}O$, (15)], 233 [$C_{17}H_{28}$, (15)], 247 [$C_{17}H_{27}O$, (20)], 218 [$C_{16}H_{26}$, (25)], 203 [$C_{15}H_{23}$, (25)], 167 [$C_{11}H_{19}O$, fission of ring C, via (11)–8 (14), (50)], 149 [$C_{11}H_{17}$, (100)], 139 (5), 99 (10), 85 (15), 71 (15), 43 (10).

Acknowledgement: The authors are thankful to the Hamdard National Foundation for financial support in this work and to RSIC, CDRI, Lucknow for recording spectral data.

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Received February 14, 2002
Accepted March 30, 2002

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