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Argyroside from *Argyreia nervosa* seeds

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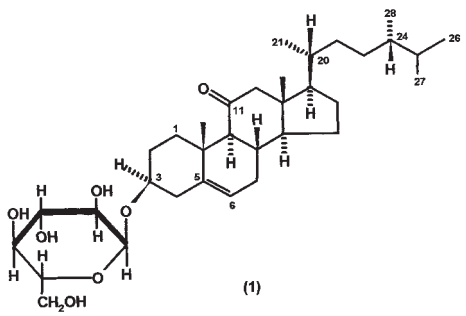
The phytochemical investigation of the seeds of *Argyreia nervosa* has resulted in the isolation of a new steroidal glycoside, (24*R*)-ergost-5-en-11-oxo-3β-ol-α-D-glucopyranoside, designated as argyroside. The structure has been elucidated by spectroscopic and chemical methods.

1. Introduction

Argyreia nervosa, commonly called as Samundar-ka-pat, belongs to the family Convolvulaceae. It is found throughout India up to an altitude of 300 m as a woody climber, stem stout, white tomentose, leaves ovate-cordate, glabrous above, persistently white, tomentose beneath, flowers rose-purple, stout, white tomentose peduncles, fruit globose, apiculate [1]. In the indigenous system of medicine, the plant is prescribed in gleet, gonorhoea, strangury and chronic ulcers. The leaves are used externally in the treatment of ringworm, eczema, itch and other skin diseases. The root is used in gonorhoea, rheumatism, in the diseases of nervous system and as tonic and diuretic [1]. Compounds such as eragine, isoeragine, penniclavine [2], 1-triacontanol, β-sitosterol, epifriedelinol and its acetate, ergometrine, caffeic acid, ethyl caffeate and quercetin [3] have been previously identified in the plant. The present paper deals with the isolation and characterization of a new glycoside, argyroside, from the alcoholic extract of the seeds of *A. nervosa*.

2. Investigations, results and discussion

Compound **1**, named as argyroside, was obtained as colourless crystals and had a molecular composition of $C_{34}H_{56}O_7$ as established on the basis of high resolution mass spectroscopy (M^+576) and ^{13}C NMR spectrum. It gave a positive Liebermann Bruchard test [4] for steroid and Molisch's test [5] for glycoside indicating it to be a steroidal glycoside. Its IR spectrum exhibited absorption bands at 3400–3500 cm^{-1} (OH), 1050 (C–O, alcoholic), 1600 (C=C), 1705 (C=O) which indicated the presence of hydroxyl group, a double bond and a ketonic group in the compound. The presence of the double bond was further substantiated by its ^1H NMR spectrum which showed a doublet at δ 5.343 (J = 4.8 Hz). The IR spectrum of its acetate showed the presence of ester groups as a sharp bands at ν_{max} 1740, due to C=O group and 1210–1260 due to C–O. The ^1H NMR spectrum of the acetate exhibited three-proton each four singlets at δ 2.085, 2.067, 2.028 and 2.011 due to four acetoxyl groups of the sugar moiety which indicated the existence of only one sugar unit in the molecule. The ^1H NMR signals for six methyl fractionalities, all attached to saturated carbons, appeared as three-proton singlets at δ 0.675 (Me-18), 1.169 (Me-19) and as doublets at 0.998 (J = 6.8 Hz, Me-21), 0.766 (J = 6.6 Hz, Me-26), 0.843 (J = 6.8 Hz, Me-27) and 0.930 (J = 6.6 Hz, Me-28). A one-proton broad multiplet at δ 3.513 was due to 3 β-carbinolic proton. The coupling constant (dddd, J = 5.0, 10.9, 11.0, 5.1 Hz) or 1/2 width was found to be 23.0 Hz which indicated the α-orientation of the carbinolic proton. The hydroxyl group was assigned at position-3 biogenetically and on the basis of MS which was found to be linked with the sugar moiety. The presence of the double bond at Δ^5 and an oxo group at position-11 was inferred on the basis of the MS fragmentation pattern of the aglycone which exhibited a sharp peak at m/z 83, 51 and 194. The hydrolysis of the compound with 10% HCl afforded an aglycone. The ratio of the aglycone obtained to glycoside was found to be about 60% indicating only one sugar unit per molecule [6]. The sugar was identified as glucose with the help of co-paper chromatography, linked with the aglycone moiety through an α-linkage as evidenced by a doublet at δ 4.652 and coupling constant 3.0 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 consistent with the glucose protons in the ^1H NMR spectrum (Table 1). The characteristic ions of the MS of **1** were (7,8) at 396 [M-H_2O]$^+$, 344 [M-side chain, C_5H_{11}, 71]$^+$, 315 [M-side chain, C_7H_{15}, 99]$^+$ and 287 [M-side chain, C_9H_{19}, 128]$^+$. The MS also clearly proved the absence of double bond in side chain and ring A, C and D.



(1)

Table 1: NMR data of glycoside 1 and its acetate

Position	^{1}H NMR of **1** (DMSO-d_6)	^{13}C NMR of **1** (DMSO-d_6)	Acetate ($CDcl_3$)
3	3.513 (m, 1/2w = 23.0 Hz, α-H) or (dddd, J = 5.0, 10,9, 11.0, 5.1 Hz)	73.35	3.507 (m, 1/2w = 22.0 Hz, H-α)
5	–	140.31	
6	5.343 (d, J = 4.8 Hz)	121.16	5.355 (d, J = 4.5 Hz)
11	–	202.60	
18	0.675 (3 H, s, Me-18)	11.66	0.674 (3 H, s, Me-18)
19	1.169 (3 H, s, Me-19)	19.61	1.213 (3 H, s, Me-19)
21	0.998 (3 H, d, J = 6.8 Hz, Me-21)	18.32	0.987 (3 H, d, J = 6.8 Hz, Me-21)
26	0.766 (3 H, d, J = 6.6 Hz, Me-26)	19.57	0.823 (3 H, d, J = 6.6 Hz, Me-26)
27	0.843 (3 H, d, J = 6.8 Hz, Me-27)	19.06	0.845 (3 H, d, J = 6.8 Hz, Me-27)
28	0.930 (3 H, d, J = 6.6 Hz, Me-28)	12.61	0.929 (3 H, d, J = 6.6 Hz, Me-28)
	* 1.059–1.305 (10 H, m)	56.14	* 1.042–1.190 (7 H, m)
		55.38	
		49.58	
	* 1.417–1.636 (9 H, m)	45.14	* 1.532–1.420 (5 H, m)
		40.45	
		45.11	
	* 1.829–1.987 (5 H, m)	36.80	* 1.614 (10 H, brs)
		35.45	
		40.32	
	* 2.23–2.45 (2 H, m)	31.37	* 1.781–1.880 (2 H, m)
		20.21	* 2.182–2.246 (2 H, m)
Sugar proton			
1′	4.652 (d, J = 3 Hz, sugar anomeric proton, α-D-linkage)	100.81	4.606 (d, J = 3.1 Hz)
2′	4.422 (d, J = 7.5 Hz)	79.23	4.961 (1 H, dd, J = 8.1, 9.0 Hz)
3′	4.539 (d, J = 2.1 Hz)	78.31	5.210 (1 H, dd, J = 9.3, 8.0 Hz)
4′	4.088 (d, J = 3.0 Hz)	78.76	5.082 (1 H, dd, J = 9.6, 8.5 Hz)
5′	3.799 (m, 1/2w = 7.5 Hz)		3.684 (1 H, m, 1/2w = 8.4 Hz)
6′a(α)	3.587 (dd, J = 5.1, 4.8 Hz)	61.25	4251 (dd, J = 5.1, 4.8 Hz)
6′b(β)	3.281 (dd, J = 2.1, 2.4 Hz)		4.093 (dd, J = 2.1, 2.4 Hz)
			# 2.085 (3 H, s, 2′-$OCOCH_3$)
			2.067 (3 H, s, 3′-$OCOCH_3$)
			2.028 (3 H, s, 4′-$OCOCH_3$)
			2.011 (3 H, s, 6′-$OCOCH_3$)

* Assignable to methylene and methine protons
# Assignment of acetoxy groups are interchangeable

^{13}C NMR spectrum of **1** showed the prescence of 34 carbon atoms. The signals at δ 140.31, 121.16, 100.81 and 73.35 were assigned correspondingly to vinylic C-5, C-6, anomeric H-1 and carbinolic C-3. The remaining carbon signals appeared between δ 79.23–61.25. A deshielded signal at δ 202.60 was attributed to oxygenated C-11 carbon. (Table 2). The aglycone obtained on hydrolysis was acetylated with acetic anhydride and pyridine which afforded a monoacetate as confirmed by its ^{1}H NMR spectral data which exhibited a singlet at δ 2.025 (3 H, s) due to one acetoxyl group. The carbinolic proton at C-3 was shifted downfield at δ 4.499 in the ^{1}H NMR spectrum of acetate of aglycone. The methine proton at C-3 was found to be in α-configuration.

On the basis of above spectral and chemical studies the structure of compound **1**, was established as (24*R*)-ergost-5-en-11-oxo-3β-ol-α-D-glucopyranoside.

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

Position	Aglycone ($CDcl_3$)	Acetate ($CDcl_3$)
3	3.515 (m, 1/2w = 22.0 Hz, α-H)	4.499 (m, 1/2w = 21.0 Hz, α-H)
6	5.351 (d, J = 4.8 Hz)	5.354 (d, J = 4.4 Hz)
18	0.674 (3 H, s, Me-18)	0.675 (3 H, s, Me-18)
19	1.168 (3 H, s, Me-19)	1.214 (3 H, s, Me-19)
21	0.998 (3 H, d, J = 6.8 Hz, Me-21)	0.987 (3 H, d, J = 6.7 Hz, Me-21)
26	0.767 (3 H, d, J = 6.6 Hz, Me-26)	0.823 (3 H, d, J = 6.7 Hz, Me-26)
27	0.842 (3 H, d, J = 6.8 Hz, Me-27)	0.846 (3 H, d, J = 6.8 Hz, Me-27)
28	0.931 (3 H, d, J = 6.6 Hz, Me-28)	0.928 (3 H, d, J = 6.5 Hz, Me-28)
OH	2.52 (brs, exchangeable with D_2O)	OAc, 2.025 (3 H, s, at C-3)
	* 1.059–1.306 (10 H, m)	* 1.042–1.191 (7 H, m)
	* 1.416–1.635 (9 H, m)	* 1.532–1.421 (5 H, m)
	* 1.828–1.986 (5 H, m)	* 1.613 (10 H, brs)
	* 2.23–2.45 (2 H, m)	* 1.781–1.879 (2 H, m)
		* 2.182–2.245 (2 H, m)

* Assignable to methylene and methine protons

3. Experimental

3.1. General procedure

Melting points of compounds were determined by the open capillary method and are uncorrected. The IR spectra were recorded on a Perkin Elmer 1600 IR spectrometer in KBr pellets,^{1}H NMR and ^{13}C NMR spectra on a Brucker 300 MHz and 75 MHz, respectively.Coupling constants are in Hz. The high resolution mass spectra was screened on HMGM mass spectrometer. Column chromatography was carried out using silica gel (60–120 mesh). TLC was performed on silica gel G.

3.2 Collection of the seeds

Seeds of *Argyreia nervosa* were procured from Herba Indica, Chandigarh.

3.3 Extraction and isolation

The seeds of *Argyreia nervosa* (5 kg) were dried in the shade and crushed to a coarse powder. The powder was exhaustively extracted with ethanol by cold percolation. The crude alcoholic extract was concentrated under reduced pressure to get a viscous mass (250 g). It was than fractionated into chloroform and methanol soluble fractions. No significant amount of compound was obtained from the chloroform fraction. The methanol fraction was concentrated to dryness to get a viscous mass (50 g) which was dissolved in minimum amount of methanol and adsorbed on a small amount of silica gel (20 g) to get a slurry. It was dried in air and loaded on to the top of the column of silica gel packed with chloroform. The different fractions were obtained during the elution from the column. The polarity was gradually increased. Only one pure compound was obtained from the methanol extract of the seeds.

3.4. Acetylation

Compound **1** (50 mg) was acetylated with acetic anhydride-pyridine (1 : 1) which on usual work-up afforded monoacetate (45 mg); m.p. 130 °C.

3.5. Characterization of the compound

Elution of methanol fraction with chloroform-methanol (90 : 10) furnished colourless crystals, yield 85 mg, soluble in methanol, R_f value 0.48 (chloroform and methanol, 90 : 10), m.p. 270 °C, IR ν_{max} (KBr): 3400–3500 (OH), 3100, (=C–H), 2950, 2850 (CH_2, CH_3), 1705 (C=O), 1600 (C=C), 1480, 1380, 1250, 1080, 1050 (C–O, alcoholic), 900, 910, 790 (=C–H), 710, 605, 550 cm^{-1}. IR ν_{max} (KBr) (Acetate): 2850, 2950, 1750 (C=O), 1740 (C=O), 1650, 1520, 1450, 1380, 1210–1260 (C–O, ester), 1160, 1100, 1060, 1040, 980, 905, 825, 700, 600 cm^{-1}. IR ν_{max} (KBr) (Aglycone): 3400–3500 (OH), 2950, 2850 (CH_3, CH_2) 1720 (C=O), 1650 (C=C), 1480, 1380 1240, 1050 (C–O, alcoholic), 920, 790, 610 cm^{-1}. IR ν_{max} (Acetate of aglycone): 2950, 2850 (CH_3, CH_2) 1750 (C=O), 1650 (C=C), 1490, 1375, 1250, 1050 (C–O, alcoholic), 920, 790, 610 cm^{-1}. EIMS (70 ev) (glycoside) m/z: 576 (M^+, $C_{34}H_{56}O_7$, 10.5%), 414 (M^+-$C_{28}H_{46}O_2$, 15), 396 (15), 382 (5), 344, 330 (5), 299 (7), 288, 281, 255, 194, 163, 149, 138, 128, 67, 57, 44 (100). EIMS (70 ev, glycoside (acetate) m/z: 744 (M^+, $C_{42}H_{64}O_{11}$), 572, 508, 416, 396, 382, 344, 299, 288, 255, 194, 163, 149, 138, 128, 67, 44. EIMS (70 ev) (aglycone) m/z: 414 [M^+, $C_{28}H_{46}O_2$, (15%)], 396 [M-H_2O, (43)], 382 [396 Me, (14)], 354 [382-CO, (5)], 344 [M^+-C_5H_{11}, side chain, (15)], 330 [M^+-85, C_6H_{13}; side chain, (13)], 315 [M-99, C_7H_{15}, (11)], 299 [315-Me, (13)], 288 [M-128, C_9H_{19}, (15)], 281 [288-H_2O, (9)], 267 [281-Me, (23)], 255 [288-Me-H_2O, (16)], 194 [$C_{12}H_{18}O_2$, fission via, 11(12)–8(14), (10)], 163 [$C_{11}H_{16}O$, fission, via 9(11)–8(14), (36)], 149 [$C_{11}H_{17}$, (15)], 146 [163-H_2O, (13)], 128 [C_9H_{19}, fission of side chain via 17(20), (37)], 105 [fission of ring B via 6(7)–9(10) eliminating H_2O, (35)], 99 [C_7H_{15}, fission via 20(22), (32)], 71 [C_5H_{11}, (30)], 67 [fission of ring A and B via 2(3)–6(7), (25)], 57, 44 [fission via 24(25), 100%, base peak]. EIMS (70 ev) (Aglycone acetate) m/z: 456 (M^+, $C_{30}H_{48}O_3$), 413, 396, 382, 354, 344, 330, 315, 299, 288, 281, 267, 255, 194, 163, 149, 138, 128, 105, 99, 71, 67, 44.

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