Asian Journal of Chemistry; Vol. 28, No. 3 (2016), 517-519

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19380

Oblongiside: A New Steroidal Glycoside from Aerial Parts of *Croton oblongifolius* Roxb.

BAHAR AHMED[1], TANVEER ALAM[1], LUBNA NAJAM[2] and SHAH ALAM KHAN[3,*]

[1]Antihepatotoxic Research Lab, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110 062, India
[2]Department of Chemistry, D.A.V. College, Muzaffar Nagar, India
[3]Department of Pharmacy, Oman Medical College, Muscat, Sultanate of Oman

*Corresponding author: E-mail: shahalamkhan@yahoo.com

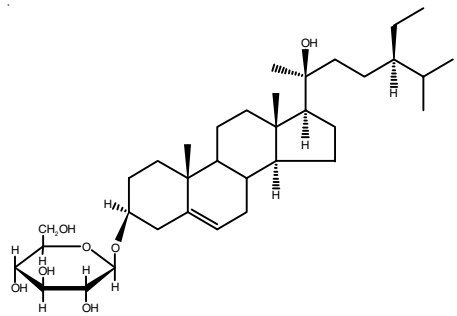
Received: 28 May 2015; *Accepted*: 25 July 2015; *Published online*: 5 December 2015; AJC-17642

A new steroidal glycoside, stigma 5(6)-ene-3-β-O-(β-D-glucopyranoside)-20-β-ol (**1**) along with two known steroids namely β-sitosterol and β-sitosterol-3-O-β-D-glucopyranoside were isolated from the aerial parts of *Croton oblongifolius* Roxb. The chemical structure of new steroidal glycoside was elucidated by chemical and spectroscopic evidences.

Keywords: *Croton oblongifolius* Roxb, Steroidal glycoside, Stigmastane, Euphorbiaceae.

INTRODUCTION

The plant of *Croton oblongifolius* Roxb belongs to the family Euphorbiaceae and is commonly known as 'Chucka' in Hindi. The bark of this plant is used in reducing chronic enlargement of the liver and in remittent fever. It is applied externally to the hepatic region in chronic hepatitis, sprains, bruises and in rheumatic swellings [1,2]. We have earlier reported that various extracts of aerial parts of *Croton oblongifolius* (COB) possess varied degree of antihepatotoxic activity which could be due to the presence of different biologically active phytoconstituents in them [3]. We therefore, investigated the methanol extract which was observed to be the most active extract in bringing down the elevated liver biomarker enzymes to the normal level, to isolate the chemical compounds responsible for exhibiting antihepatotoxic activity. We report herein, the isolation and characterization of a new steroidal glycoside namely Stigma 5(6)-ene-3-β-O-(β-D-glucopyranoside)-20-β-ol (**1**) from the aerial parts of *Croton oblongifolius* Roxb.



Chemical structure of isolated compound, oblongiside (**1**)

EXPERIMENTAL

Melting points were determined by open capillary method and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on 300 MHz (Bruker model DRX-300) NMR spectrometer in $CDCl_3$ using TMS as an internal reference. Chemical shifts are expressed in δ (ppm) and coupling constants are in Hz. The mass spectra were recorded on Jeol-JMS DX-303 spectrometer and IR spectra on Hitachi IR 270-30 spectrometer in KBr pellets. Column chromatography was carried out using silica gel (60-120 mesh) and TLC was performed on silica gel G. The aerial part of *Croton oblongifolius* were procured from Herba Indica, Chandigarh and identified by the taxonomist Dr. H.S. Puri. A voucher specimen was deposited in the herbarium of Department of Pharmaceutical Chemistry, Jamia Hamdard for future reference.

The air dried plant material (8 kg) was crushed to a coarse powder and was exhaustively extracted with ethanol by cold percolation method. The crude alcoholic extract was concentrated to small volume under reduced pressure to obtain a viscous mass (1 kg). It was then fractionated into petroleum ether (60-80 °C), acetone and methanol. The concentrated methanol extract (400 g) was chromatographed on silica gel column prepared in petroleum ether (60-80 °C). The column was eluted successively with petroleum ether and mixture of petroleum ether, benzene and methanol in increasing order of their polarity. The elutent benzene:methanol (9:1) afforded colorless solid powder of steroidal glycoside **1** (100 mg). R_f; 0.783 (benzene:petroleum ether, 1:1), m.p. 135 °C, IR (KBr, ν_{max}, cm^{-1}): 3500-3440 (OH), 2950, 2850 (CH_3,CH_2), 1670

(-C=O ester), 1610 (C=C), 1450, 1390, 1260, 1030 (C-O alcoholic), 820 cm^{-1}. IR (KBr, acetate, ν_{max}, cm^{-1}): 2950, 2850 (CH_3, CH_2), 1720 (-C=O ester), 1610 (C=C), 1450, 1390, 1260, 1220 (C-O ester), 1030 (C-O alcoholic), 820 cm^{-1}. EIMS (relative intensity) *m/z*: 430 [M^+; $C_{29}H_{50}O_2$ (16.1)], 396 [M^+-2xH_2O (29.3)], 381 [396-CH_3 (9.2)], 367 [381-CH_3 (7.5)], 345 [M^+- side chain, fission *via* 23(24) (8)], 331 [M^+- side chain, fission *via*, 22(23), (3)], 317 [M^+- side chain, fission *via*, 20(22), (3)], 301 [M^+- H_2O- side chain, fission *via*, 20(22) (5)], 288 [301-CH_3 (8.5), (4.5)], 273 [M^+- side chain, fission *via*, 19(20) (5)], 255(7.4), 228 (5), 231 [$C_{16}H_{23}O$, (5)], 213 [231-H_2O, (6.6)], 177 [$C_{12}H_{17}$, (6.6)], 163 [$C_{11}H_{15}$, (6.1)], 159 [177- H_2O, (6.6)], 145 [163- H_2O, (6.0)], 129 (18), 111 (24), 95 (40), 83 (43), 69 (65), 57(100). ^{1}H NMR and ^{13}C NMR ($CDCl_3$, δ ppm) (Table-1).

RESULTS AND DISCUSSION

Compound **1** was obtained as colorless, amorphous powder which gave a positive Liebermann-Burchard test for steroid and Molisch's test for glycosides [4] indicating it to be a steroidal glycoside. The IR spectrum showed a band at 3450- 3500 cm^{-1} (OH), 1620 cm^{-1} (C=C) and 1020 (C-O, alcoholic), which indicated the presence of hydroxyl group and a double bond in the compound. The presence of double bond was further

TABLE-1
^{1}H NMR AND ^{13}C NMR SPECTRAL DATA OF COMPOUND **1** AND ITS ACETATE **1a**

Position	Compound **1**		Acetate **1a**	
	^{1}H NMR*	^{13}C NMR	^{1}H NMR*	^{13}C NMR
1	**	37.22	**	36.97
2	**	31.89	**	31.79
3	4.107 *dddd* (6.9,7.2, 7.2, 9.6) 1/2w = 16.05, α-H	73.6	3.489 *dddd* (6.9,7.2, 7.2, 9.6) 1/2w = 16.05, α-H	73.97
4	**	42.2	**	42.29
5	-	138.5	-	138.84
6	5.356 *d* (5.7)	129.5	5.337 *d* (5.7)	129.6
7	**	23.0	**	23.04
8	**	50.0	**	51.22
9	**	40.5	**	40.5
10	-	36.6	-	36.57
11	**	30.1	**	30.1
12	**	33.9	**	33.9
13	-	39.8	-	40.0
14	**	38.1	**	38.5
15	**	25.5	**	25.5
16	**	28.2	**	28.2
17	**	56.0	**	56.01
18	0.679 *s* (Me)	11.8	0.675 *s* (Me)	11.97
19	1.019 *s* (Me)	19.3	1.000 *s* (Me)	19.29
20	-	71.5	-	71.6
21	0.934 *s* (Me)	18.7	0.945 *s* (Me)	18.76
22	**	39.7	**	39.69
23	**	26.0	**	26.03
24	**	45.8	**	45.80
25	**	29.1	**	29.11
26	0.804 *d* (6.0) (Me)	19.7	0.803 *d* (6.0) (Me)	19.81
27	0.855 *d* (6.0) (Me)	19.0	0.802 *d* (6.0) (Me)	19.09
28	**	31.9	-	31.87
29	0.877 *d* (6.0) (Me)	11.8	0.855 *d* (6.0) (Me)	11.84
			2.01 OCO***C***H_3	28.90
			O***C***OCH_3	170.54
	**2.436-2.373 *brm* (1H)		**2.225-2.175 *brm* (1H)	
	**2.300-2.272 *brm* (6H)		**1.876-1.831 *brm* (6H)	
	**2.025-1.692 *brm* (8H)		**1.567-1.478 *brm* (8H)	
	**1.303-1.101 *brm* (13H)		**1.285-1.085 *brm* (13H)	
	Sugar protons			
1'	4.519 *d* (7.5), β-linkage	93.4	4.632 *d* (7.5), β-linkage	104.2
2'	3.862 *d* (6.0)	73.98	4.935 *d* (6.0)	70.4
3'	4.425 *d* (6.0)	76.10	5.215 *d* (6.0)	74.7
4'	4.401 *d* (6.0)	69.84	5.041 *d* (6.0)	64.5
5'	3.758 *m* (1/2w = 14.25)	77.10	3.748 *m* (1/2w = 14.25)	77.5
6'a	3.482 *dd* (5.1, 4.8)	56.0	4.251 *dd* (5.1, 4.8)	56.8
6'b	3.302 *dd* (2.1, 2.4)	-	4.104 *dd* (2.1, 2.4)	-
			Sugar acetoxyls	
			2.055 *s* (3H, O*COCH*$_3$)	28.6, 170.4
			2.046 *s* (3H, O*COCH*$_3$)	28.5, 171.1
			2.027 *s* (3H, O*COCH*$_3$)	27.9, 171.3
			2.011 *s* (3H, O*COCH*$_3$)	27.6, 171.5

*Coupling constants in Hertz are given in parentheses.

substantiated by its ^{1}H NMR spectrum which exhibited a doublet at δ 5.356 (J = 5.7 Hz). The acetate of the compound also confirmed the presence of carbonyl group (C=O, 1740 cm^{-1}) and C-O ester linkage (1220 cm^{-1}) in its IR spectrum. The ^{1}H NMR spectrum of the acetate exhibited four singlets at δ 2.011, 2.027, 2.046 and 2.055, which could be assigned to four acetoxyl groups of the sugar moiety and that indicated the presence of only one sugar unit and one hydroxyl group in the molecule. The proton spectrum also showed the presence of six methyl β-functionalities all attached to saturated carbon, obtained as six signals of three protons each at δ 0.679 (Me-18), 1.019 (Me-19), 0.934 (Me-21), 0.804 (*d*, *J* = 6.0, Me-26), 0.855 (*d*, *J* = 6.0, Me-27) and 0.877 (*d*, *J* = 6.0, Me-29). A broad multiplet at δ 4.107 was also obtained due to carbinolic proton at position-3. The half width value (1/2 w = 16.05) indicated the α-orientation of the carbinolic proton [5,6]. The hydroxyl group was assigned at position 3, biogenetically and on the basis of mass spectrum that revealed it to be linked with sugar moiety. The presence of double bond at $\Delta^{5(6)}$ was ascertained by mass spectrum fragmentation of the aglycone, which displayed sharp peaks at *m/z* 69 and 83.

The hydrolysis of the compound with 10 % HCl afforded an aglycone. The ratio of the aglycone obtained with glycoside was found to be about 60 %, indicating only one sugar unit per molecule [7]. The sugar was identified as glucose with the help of co-paper chromatography. The sugar was found to be linked with the aglycone moiety through β-linkage as evidenced by a doublet at δ 4.519 (J = 7.5 Hz) of the anomeric proton of the sugar unit moiety in the proton spectrum [8]. The other signals of the sugar were also found consistent with the glucose protons in the spectrum (Table-1). The aglycone obtained on hydrolysis gave molecular ion peak at *m/z* 414 corresponding to the molecular formula $C_{29}H_{50}O$, having the stimgastane skeleton [9,10], as the mass spectrum exhibited the characteristics peaks at *m/z* 396 (M^+-H_2O), 331 (M-side chain, C_6H_{13}, 85)$^+$, 301(M-side chain, C_8H_{17}, 113)$^+$ and 273(M-side chain, $C_{10}H_{24}$, 141)$^+$ *etc*. The mass spectrum also clearly proved the absence of double bond in side chain and ring A, C and D. The ions at *m/z* 69 and 83 further indicated the presence of double bond at $\Delta^{5(6)}$ position and presence of hydroxyl group in ring A assigned at position 3.

The aglycone obtained by hydrolysis was acetylated with acetic anhydride in pyridine to yield a diacetate as confirmed by its ^{1}H NMR spectral data which exhibited singlets at δ 2.01, 1.99 due to two acetoxyl groups. The carbinolic proton at C-3 was shifted downfield at δ 3.489 of its acetate. On the basis of above chemical and spectral studies, the structure of aglycone was established as stigma 5(6)-ene-3,20-β-diol and its glycoside *i.e.* compound (**1**) as stigma 5(6)-ene-3-β-O-(β-D-glucopyranoside)-20-β-ol. The isolated compound was named as oblongiside and it is the first report of its occurrence in *Croton oblongifolius* Roxb aerial parts.

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Pharmaceutical Chemistry for providing necessary research facilities and to UGC, New Delhi, for awarding JRF scholarship to one of the authors (TA).

REFERENCES

1. K.R. Kirtikar and K.D. Basu, Indian Medicinal Plants, Lalit Mohan Basu and Co., Allahabad, India, edn 2, vol. 2, p. 1125 (2000).
2. K.M. Nadkarni, Indian Materia Medica, Popular Prakashan, Mumbai, India, edn 3, Vol. 1, p. 268 (1979).
3. B. Ahmed, T. Alam, M. Varshney and S.A. Khan, *J. Ethnopharmacol.*, **79**, 313 (2002).
4. H. Molisch, *Monatsh. Chem.*, **7**, 198 (1886).
5. T. Itoh, T. Ishii, T. Tamura and T. Matsumoto, *Phytochemsitry*, **17**, 971 (1978).
6. F. De Simone, A. Dini, L. Minale, C. Pizza and R. Riccio, *Tetrahedron Lett.*, **11**, 959 (1979).
7. W. Rahman and M. Ilyas, *J. Org. Chem.*, **27**, 153 (1962).
8. Vogel's Text Book of Practical Organic Chemistry, Revised by B.S. Furniss, A.J. Hannaford and A.R. Smith Tatchell, Longman (ELBS), UK (1989).
9. J.W. Clark-Lewis and I. Dainis, *Aust. J. Chem.*, **20**, 1961 (1967).
10. P. Reichstein, H. Kaufmann, W. Stoklin and T. Reichstein, *Helv. Chim. Acta*, **50**, 2114 (1967).