Int. J. Chem. Sci.: 8(2), 2010, 1231-1239

DAMMARANE AND CEANOTHANE TRITERPENES FROM *ZIZYPHUS XYLOPYRA*

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ABSTRACT

From the roots of *Zizyphus xylopyra*, a new dammarane-type triterpene, pseudojujubogenin-3-O-β-D-glucopyranoside, along with the known ceanothane triterpenes, ceanothic acid and daucosterol were isolated. The structures of the compounds were fully characterized by detailed NMR investigations including ^{1}H and ^{13}C NMR, HSQC, COSY, HMBC and NOESY experiments. In addition, the dammarane glycoside was tested for its potential in inhibiting various bacteria and was found to possess significant bactericidal activity. This is the first report on the chemical constituents of the roots of *Zizyphus xylopyra*.

Key words: *Zizyphus xylopyra*, Rhamnaceae, Dammarane, Ceanothane triterpenes, Antimicrobial activity.

INTRODUCTION

Zizyphus xylopyra willd (Family Rhamnaceae) is a large shrub or a small tree armed with spines up to 4 mm height. Leaves broadly elliptic, obovate (or) orbicular, serrulate, glabrous. Flowers in compact cymes. Fruits grobose, 2 (or) 4 celled with usually a seed in each cell, very heart and woody. The plant is found in North – Western India, Uttar Pradesh, Bihar and Central and South India[1]. The tree is one of the chief hosts for the propagation of lac.[2] A number of species belonging to genus *Zizyphus* are used in the Indian system of medicine for treatment of bilious affections, diarrhoea, delirium, pectoral complains, boils, abscesses, carbuncles and ulcers.[3] A survey of available literature indicated that pharmacological studies were not reported on *Zizyphus xylopyra* and hence, the present study was conducted on the roots of the above plant.

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EXPERIMENTAL

General experimental procedures

Melting points were measured on a Cipla I-28 digital melting point apparatus and are reported uncorrected. The IR spectra were recorded on a Buck Scientific 500 infrared spectrophotometer. Silica gel (Acme, 60-120 mesh) for column chromatography and silica gel (Acme) was used for preparative thin layer chromatography. Spots on chromatogram were detected under UV light and by spraying with 5% H_2SO_4 in methanol. The NMR experiments were performed on a Bruker AVANCE DRX-500 spectometer operating at 500.13 MHz and 125.77 MHz, respectively. Mass spectra were obtained using an Agilent 1100 series LC/MSD in APCI or API-ES mode.

Plant material

The roots of *Zizyphus xylopyra* (1.5 kg) were collected at the Khailasa Hills, India, in April 2009. The sample was authenticated by Dr. M. Venkaiah, Taxonomist, Botany Department, Andhra University, Visakhapatnam. A voucher specimen (SG/ZGL/03/345) has been deposited at the Herbarium, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam India (Herbarium Code = SKU)

Extraction and isolation

Powered plant material (900 g) was extracted in a Soxhlet apparatus, successively with hexane, $CHCl_3$ and MeOH and the extracts were concentrated using a rotary evaporator at a maximum temperature of 45^0C. The dark viscous green residue (11 g) from the methanol extract was separated over silica gel eluting with different mixtures of petroleum ether-chloroform and chlororom-methanol to give 25 fractions. Fraction 12-18 were combined, purified by repeated preparative TLC and then recrystallized from methanol to give 1(38.0 mg). Fraction 23 from the original column was crystallized using methanol to give **(2)** (14.0 mg).

Pseudojujubogenin-3-O-β-D-glucopyranoside **(1)** : Pale green amorphous powder, m.p. 241-243°C. – IR (KBr): v = 3200, 3640 (OH), 1465, 1285, 1078, 1012, cm^{-1}, - ^{1}H NMR (500.13 MHz, d_5-pyridine, ^{13}C NMR (128.77 MHz, d_5 – pyridine) COSY, HMBC and NOESY (Tables 1 and 2 and structure 1) HR-EIMS : m/z (%) = 650 (18) (M^+). – $C_{36}H_{58}O_{10}$ (650.85) : Calcd.C 66.43, H 8.98, O 24.58; Found C 66.32, H 8.94, O 24.54.

Ceanothic acid **(2)**: Colourless needles from Me_2CO-methanol m.p. 356-357^{0}C Lit.

m.p. 333-335^{0}C dec. –[α]24 = -51.5^0 (c, 1.01 in $CHCl_3$) IR and MS in agreement with the published data[4], ^{1}H NMR (500.13 MHz, d_5-pyridine) δ = 1.09, 1.17, 1.29, 1.41, 1.44, 1.68 (6 x S, 18H, CHMe) 1.71, (m, 1H, 18-H), 3.22 (s, IH, 1-H), 4.84 (s, 1H, 3-H), 2.23 (d, 1H, J = 2.8 Hz, 5/9-H), 1.45, 1.54(m, 2H, 6/7-H), 1.60, (m, 1H, 11-H), 2.11 (d, 1H, J = 11.4 Hz, 11-H), 1.34, (m, 1H, 12-H), 1.98 (d, 1H, J = 10.5 Hz, 12-H), 2.79 (dd, 1H, J = 2.8, 8.5 Hz, 13-H), 1.25, (m, 1H, 15-H), 1.92 (dd, 1H, J = 2.8, 10.5Hz, 15-H), 1.50, 2.61 (d, 2H, J = 11.4 Hz, 16=H), 3.51 (d, 1H, J = 2.3, Hz, 19-H), 1.50, 2.23 (m, 1H, 21-H), 1.50, 2.23 (m, 1H, 22-H), 4.68, (s, 1H, 30-H (CH_2=C), 4.87 (d, 1H, J = 10.0Hz, 30-H (CH_2=C). ^{13}C NMR (d_5-pyridine) δ = 20.7 (24-CH_2), 15.5, 17.4, 19.2, 20.0, 31.9 (all CHMe), 67.4 (C-1), 85.1 (C-3), 44.2 (C-4), 57.4 (C-5), 19.5 (C-6), 35.1 (C7), 42.5 (C-8), 45.5 (C-9), 50.0 (C-10), 24.6 (C-11), 26.6 (C-12), 39.5 (C-13), 43.9 (C-14), 30.9 (C-15), 33.3 (C-16), 57.0 (C-17), 50.1 (C-18), 48.0 (C-19), 31.7 (C-21), 38.0 (C-22), 110.1 (30CH_2) 178.4 (2-COOH), 179.3 (28-COOH), NOESY correlations: H-1 ↔H-3, H-19↔H_2-30, H-5 ↔ H-3, H_3-29↔H_2-30A/B, H_3-23↔H-3, H_3-24↔H-3, H-13↔H_3-26, H-13↔H_2-12 and H_3-29↔H-19.

Daucosterol **(3)** : White powder, m.p. 279-281 ^{0}C. Lit. m.p. 287-289^{0}C. ^{1}H NMR (500.13 MHz, d_5-pyridine), ^{13}C NMR (125.77 MHz, d_5-pyridine) data were in agreement with the literature.[5]

RESULTS AND DISCUSSION

The roots of *Zizyphus xylopyra* were extracted successively with hexane, chloroform and methanol, which on concentration afforded three dark viscous semisolids. The methanolic residue was separated by silica gel column chromatography to furnish new ceanothic acid and daucosterol.

Compound **(1)** was the major isolate obtained in this investigation as pale green amorphous powder, m.p. 241-243^{0}C. It gave a positive for Liebermann-Burchard test for triterpenes and Molisch test for sugars. The IR spectrum indicated the presence of a tertiary hydroxyl at 3460 cm^{-1} and the absence of a conjugated system in the molecule. The high resolution mass spectrum showed a molecular ion peak at m/z 650.85[M]$^+$, supporting the molecular formula of $C_{36}H_{58}O_{10}$ for **(1)**, deduced from the mass spectrum in conjunction with the ^{13}C NMR spectrum. The NMR spectrum (Table 1) exhibited signals for 36 carbons: nine methylene [two of them bearing oxygen atoms (δ = 66.2 and 68.9)], seven methnines [one oxymethine (δ=89.0)], seven methyl carbons, an anomeric carbon δ=107.2 bound to δ= 4.97 (1H, d, 7.7) according to the HSQC spectrum. Comparison of the NMR data for **(1)**

(Table 1) with the COSY 45^0 spectrum, revealed the sugar (pyranose form) to be glucose. The coupling constant of the anomeric proton i.e δ=4.97 (1H, d, 7.7) indicated β-configuration of glucopyranosyl moiety. A 1H double doublet at δ = 3.38 (J = 4.7, 11.5 Hz) characteristic for H-3α having a sugar linked at C-3 was supported by ^{2}J HMBC correlations with the anomeric carbon 107.2 (G-1), and the geminal methyls [28.4 (C-28), 16.6 (C-29)] located at C-4, NOE correlation between H-3 of the genin and G-1 of the glucose confirmed the attachment of the sugar at position C-3 of the aglycone. These signals resembled a dammarane type triterpene having a single sugar unit in the A ring at 3-O-β-position and a free tertiary hydroxyl group[6-9].

The spectrum also revealed an olefinic methane, δ = 5.42 (δ = 127.2) along with signals typical to that of an isobutenyl side chain. The COSY 45^0 spectrum revealed that the methyls δ = 1.64 and 1.72 and resonances at δ = 25.9 and 18.7 ascribed to C-26 and C-27 were coupled to the unsaturated methane at δ = 5.42 and were assignable to H-24. The placement of the side chain at C-22, was accomplished through the HMBC experiment. The olefinic methane resonating at δ = 5.42 (H-24) showed a ^{2}J correlation with the carbon δ = 46.9 (C-22) and 3J long-range couplings with the carbons δ = 68.9 (C-23), 25.9 (C-26), 18.9 (C-27) supporting that the side chain was located at C-22 of the three oxygen functions in **(1)**, one was assigned to a tertiary hydroxyl group δ = 69.1 (C-20), while the two other oxygens were directly involved in ethers of a ketal group δ = 5.03 and 4.28 (d, J = 8.6 Hz, H-30) with resonances δ = 68.9 and 66.2 assignable to sp^3 carbons C-23 and C-30, respectively. The relative stereochemistry at C-3/5/28 and C-18/19 were confirmed by means of the NOESY spectrum. The H - 3α proton showed strong NOE interactions with H_3-28 resonance and H-5 methine suggesting that they were α - oriented and the H-24 olefinic methane showed two interactions with the angular methyls H_3-18 and H_3-19 establishing β-orientation of the methyls.

Some key HMBC correlations (Table 2) observed were between the methyls (δ = 1.34 and 0.74) that exhibited ^{3}J coupling between themselves indicating their geminal nature and ^{2}J coupling with the oxymethine C-3 and the methane C-5, while the methyl at δ = 1.44 showed ^{2}J correlation with the methane at C-22. The angular methyl, H_3-18 (δ = 1.02) showed ^{2}J correlation to the methylene at C-7 and the quaternary carbon at C-14 and ^{3}J couplings with the quaternary carbon C-10. On the basis of the above spectral data, compound **(1)** was identified as psedujujubogenin –3 – O-β-D-glucopyranoside, a new natural product. ^{1}H and ^{13}C NMR resonances were assigned using COSY, HMBC and

NOESY spectra and are presented in Tables 1 and 2 and in Fig. 1.

Table 1: ^{1}H, ^{13}C NMR and COSY spectral data for dammarane triterpene glycoside (1)

Position	δH	δC	COSY	Position	δH	δH	COSY*
1	a) 0.81 (m, 1H)	39.0	H-1b, H-11a	16		110.6	
	b) 1.49 (m, 1H)		H-1a, H-2b				
2	a) 1.77-1.90 (m, 1H)	26.9	H-3α	17α	1.72 (m,1H)	53.9	H-13β
	b) 2.29 (m, 1H)		H-1b				
3α	3.38 (dd, 1H, 4.7, 11.5)	89.0	H-2a, H-2b	18β	1.02 (s, 3H)	19.0	H-7
4		37.6[a]		19β	1.02 (s, 3H)	17.1	H-7
5α	0.70 (m, 1H)	56.3	H-28α	20		69.1	
6	1.37 (m, 2H)	18.5	H-15b	21	1.44 (s, 3H)	29.8	H-13β
7	1.54 (m, 2H)	36.2	H-18, H-19	22	2.16 (m,1H)	46.9	
8		37.4		23	5.03 (m, 2H)	68.9	H-15a, H-24
9	0.81 (m, 1H)	53.2		24	5.42 (d,1H,8.0)	127.2	H-26, H-27
10		37.4[a]		25		135.4	
11	a) 1.37 (m, 1H)	21.8	H-17α	26	1.64 (s, 3H)	25.9	
	b) 1.49 (m, 1H)		H-11a, H-17α				
12	a) 1.77-1.90 (m, 1H)	28.7		27	1.72 (m,3H)	18.7	H-23
	b) 1.97 (m, 1H)		H-11a				
13β	2.72 (m, 1H)	38.6		28α	1.34 (m, 3H)	28.4	H-5α
14		53.5		29β	0.74 (s, 3H)	16.6	H-1b

Cont...

Position	$^{\delta}$H	$^{\delta}$C	COSY	Position	$^{\delta}$H	$^{\delta}$H	COSY*
15	a) 1.77-1.90 (m, 1H)	39.9	H-13β	30	4.28 (d, 2H, 8.6)	66.2	
	b) 2.20 (d, 1H, 8.3)						
β-D-Glucose							
G-1	4.97 (d, 1H, 7.7)	107.2	G-2	G-4	4.20 (dd,1H,8.6,9.1)	72.2	
G-2	4.07 t, 1H, 8.7)	76.0	G-5, G-6b	G-5	4.25 (t, 1H, 8.6)	79.0	
G-3	4.02 (m, 1H)	78.6	G-4	G-6	a) 4.63 (dd,1H, 2.4,11.7)	63.4	G-6b
					b) 4.42 (dd,1H, 5.5,11.7)		

*Assignments were confirmed by 2D NMR experiments (HSQC, HMBC and 2D-NOESY); [a]Signals are interchangeable, coupling constants 'J" in Hertz.

Table 2: Key HMBC correlations observed for the dammarane triterpene glycoside (1)

Position	^{2}J	^{3}J
H-1	26.9 (C-2)	
H-2	89.0 (C-3)	
H-3	107.2 (G-1), 28.4 (C-28), 16.6 (C-29)	
H-5		89.0 (C-3)
H-12	38.6 (C-13)	53.9
H-13	53.5 (C-14)	(C-17)
H-18	36.2 (C-7), 53.5 (C-14)	69.1 (C-20)

Cont...

Position	**^{2}J**	**^{3}J**
H-19	56.3 (C-5)	37.6 (C-10)
H-21	46.9 (C-22)	38.6 (C-4), 21.8 (C-11)
H-22	53.9 (C-17), 69.1 (C-20)	
H-23	110.6 (C-16)	127.2 (C-24)
H-24	46.9 (C-22)	68.9 (C-23), 25.9 (C-26)
		18.9 (C-27)
H-26/27	127.2 (C-24)	
H-28	89.0 (C-3), 56.3 (C-5)	16.6 (C-29)
H-30	53.5 (C-14)	38.6 (C-13)
G-1	89.0 (C-3)	78.6 (G-3)
G-3	78.6 (G-4)	107.2 (G-1), 79.0 (G-5)
G-4	79.0 (G-5)	63.4 (G-6)

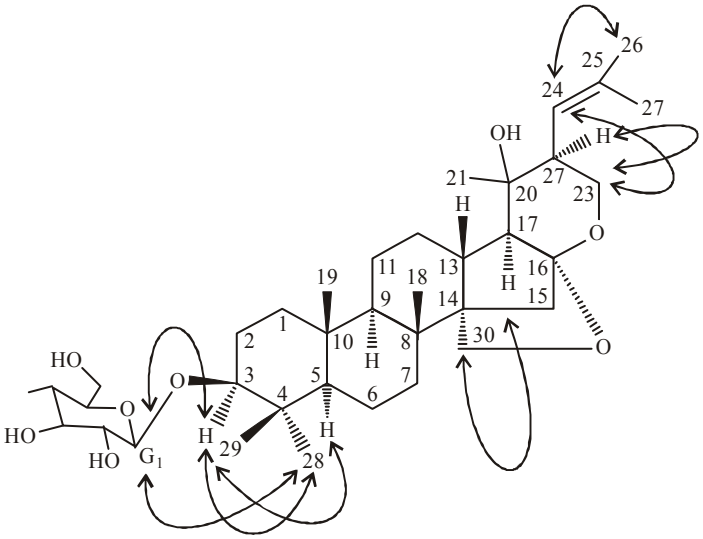

Fig. 1: Important NOESY interactions of (1)

Compound **(2)** : Ceanothic acid and Compound **(3)** daucosterol were characterized by analysis of NMR spectra and comparison with the published data[5,10,11]. The dammarane type triterpene glycoside is the major compound in *Zizyphus xylopyra*. Jujubogenin glycosides, jujuboside A,C and lotoside I, II have been reported from Zizyphus lotus;[12] however, this is the first report of a pseudojujubogenin glycoside isolated from the genus, Zizyphus. The ceanothane triterpene, 3-O-protocatechuoylceanothic acid has been reported from Zizyphus jujuba[4]. The isolation of the dammarane – type glycoside from a plant of the Zizyphus genus is not surprising, but it is remarkable to note that *Zizyphus xylopyra* produces both dammarane and the ceanothane class of terpenoids. Compound **(1)** was tested for its potential to inhibit various bacteria by established methods[13]. It inhibited the growth of *Bacillus pumilus*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* with MICs being 51.2, 102.2, 12.8 and 25.6 μg/mL, respectively. The dammarane and ceanothane terpenoids have been reported to possess potent anti-inflammatory activity[14]. The biological activities of these three compounds are of interest and are presently taken up for investigation.

ACKNOWLEDGEMENTS

Authors are thankful to A. S. N. Pharmacy College, Tenali, Andhra Pradesh for providing necessary support for this research programme.

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Accepted : 06.03.2010