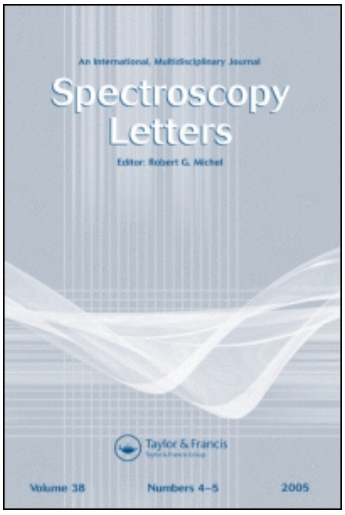

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CARATUBERSIDE A2: A NEW PREGNANE FROM *CARALLUMA TUBERCULATA*

KEY WORDS: Caratuberside A2, *Caralluma tuberculata*, Asclepiadaceae, Pregnane.

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ABSTRACT:

A medicinal herb, *Caralluma tuberculata* (Asclepiadaceae), furnished a pregnane type compound, caratuberside A2. The sugar linkage was at C-14 which is a rare site of substitution. The structure was elucidated by spectroscopic means, including COSY-45, J-resolved, HMBC, HMQC, C-H correlation experiments and derivatization.

INTRODUCTION:

Our further studies on *Caralluma tuberculata*, family Asclepiadaceae, resulted in the isolation of a new pregnane, caratuberside A2. Previously reported compounds, caratuberside A and B[1], and this compound showed similarities in structure, but different behaviour on HPTLC and HPLC. This communication deals with the structural relationship among caratubergenin A1, caratuberside A & B and caratuberside A2.

RESULTS AND DISCUSSION:

Caralluma tuberculata is a medicinal herb of folkloric medicine[2]. From the chemistry of natural products point of view no work has been done on its

chemical constituents except that described in our previous report[1]. In continuation of our research work on *C. tuberculata* we are now reporting another pregnane glycoside which was not isolated previously from this plant. This compound had two basic differences in configuration when compared with caratuberside A[1], that is i) a double bond at C-5 and ii) attachment of sugars at C-14. The structure was elucidated with the help of spectroscopic techniques including DEPT, COSY-45, J-resolved, HMBC and HMQC.

This compound was isolated from the butanol fraction as a white amorphous powder through HPLC. Electron ionization (EI) mass spectrum showed the molecular ion peak at m/z 654, corresponding to the molecular formula C_{34} H_{54} O_{12}, and the FAB mass spectrum confirmed the molecular weight i.e 654 with signals at m/z 653 $[M-H]^-$ and 745 $[M+glycerol]^-$. The IR spectrum exhibited the diagnostic peaks of hydroxyl, carbonyl groups and double bond at 3430, 1700 and 1615 cm^{-1},respectively. In comparison to our previously isolated compound, caratuberside A, there was no double bond peak in the IR spectrum. Further information for double bond was obtained from ^1H- and ^{13}C-NMR spectra. The ^1H-NMR spectrum showed a signal at 5.40 ppm (dd, J= 4.9, 1.2 Hz) which was assigned to H-6 on the basis of H-C correlation and HMBC spectra assignments (123.07 ppm). Another signal in ^{13}C-NMR spectrum at 140.76 ppm was assigned to C-5 due to the fact that no signal for proton of the C-5 was observed in the ^1H-NMR spectrum. In DEPT and HMQC experiments its signal appeared as a quaternary carbon. The effects of a double bond were observed on the neighbouring carbons, C-4 and C-8. The chemical shift for C-4 was found at 39.61 ppm (4.89 ppm downfield) due to a β-effect. Both β- and γ-effects were noted on C-8 (2.82 ppm upfield) due to the substitution at C-14 and a double between C-5 and C-6. The other signals of aglycone in ^1H- and ^{13}C-NMR were found to be identical with caratubergenin A1[1].

The linkage between the aglycone and sugar was also found unusual in that no evidence was detected for linkage between C-3 and sugar. In the ^1H-NMR spectrum the signal for the proton of C-3 appeared at 3.41 ppm as a multiplet. In our pregnane compounds there were only two positions of hydroxyl groups, i.e. C-3 and C-14. Whereas the signals for C-3 (71.47 ppm) were clear in ^{13}C-, C-H correlation, HMBC and HMQC there was now only the C-14 position left for the linkage. It was found there because C-14 showed its signal at 86.89 ppm in the ^{13}C-NMR spectrum, which was a clear indication of substitution at this position. The β-effects of this substitution were found on C-8, 13, 15 & 16. In case of C-15 and 16 signals were observed downfield (3.07 and 0.42 ppm) and upfield in C-8 and 13 (2.82 and 2.05 ppm).

The sugars were identified as 3-O-methyl fucose and glucose on the basis of acid hydrolysis and comparison on HPLC, TLC and HPTLC plates with authentic samples. The linkage between sugars were detected as 1→4 by the hydrolysis of the compound and spectroscopic data[1]. The sugar, glucose, was found as the terminal sugar and 3-O-methyl fucose as the bridge between glucose and aglycone. The evidences of C-H correlation, HMBC, HMQC and J-resolved spectra were in favour of our proposed structure. This linkage is also identical to caratuberside A[1]. Therefore, this compound was identified as 14-*O*-[β-d-glucopyranosyl(1–4)-(3-*O*-methylfucose)]-3β-hydroxyl-14β-pregn-20-one. The acetylation product and its spectroscopic data also confirmed this structure.

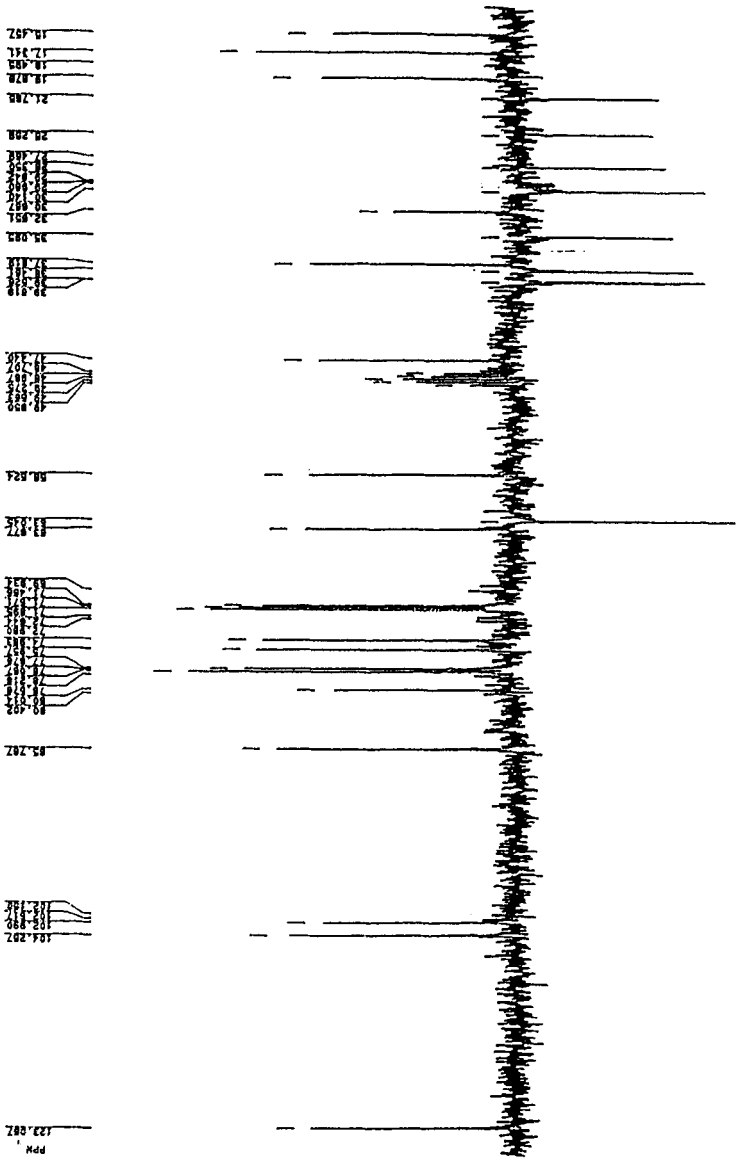


Fig. 1 A ^{13}C-NMR DEPT spectrum of caratuberside A2, measured in CD_3OD (75.42 MHz), shows CH_3 & *CH* signals up and CH_2 down.

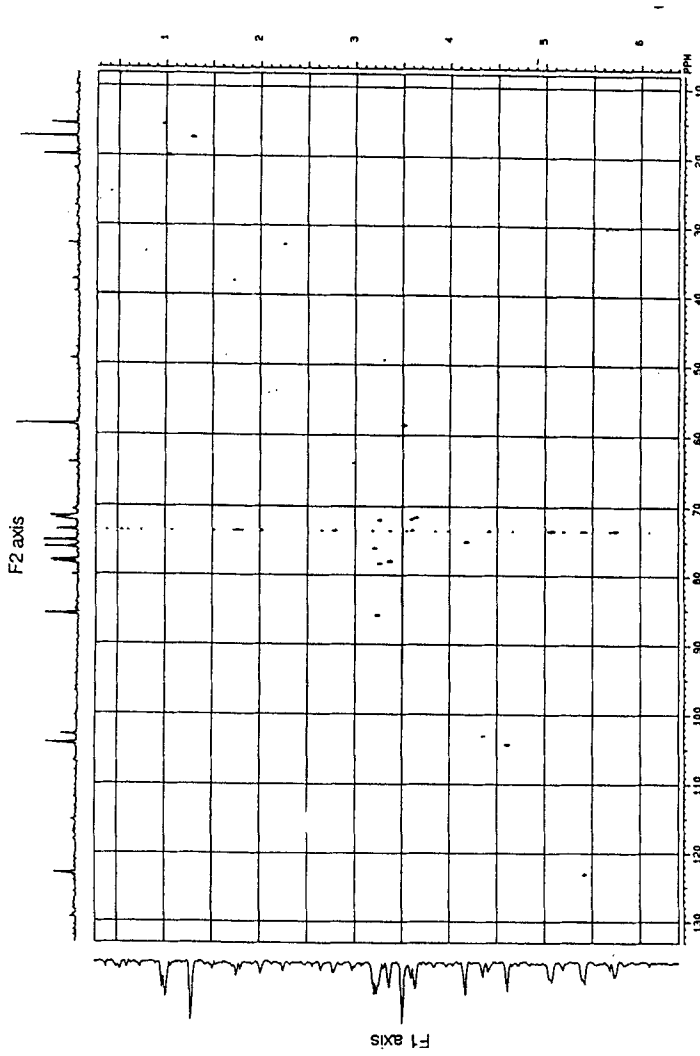


Fig. 2 Two-dimensional ^{1}H-^{13}C chemical shift correlation ship spectrum of caratuberside A2 in CD_3OD at 300/75.42 MHz presented a normal high resolution proton spectrum at F1 axis while 90°C projection to recover the protonated carbon spectrum along F2 axis.

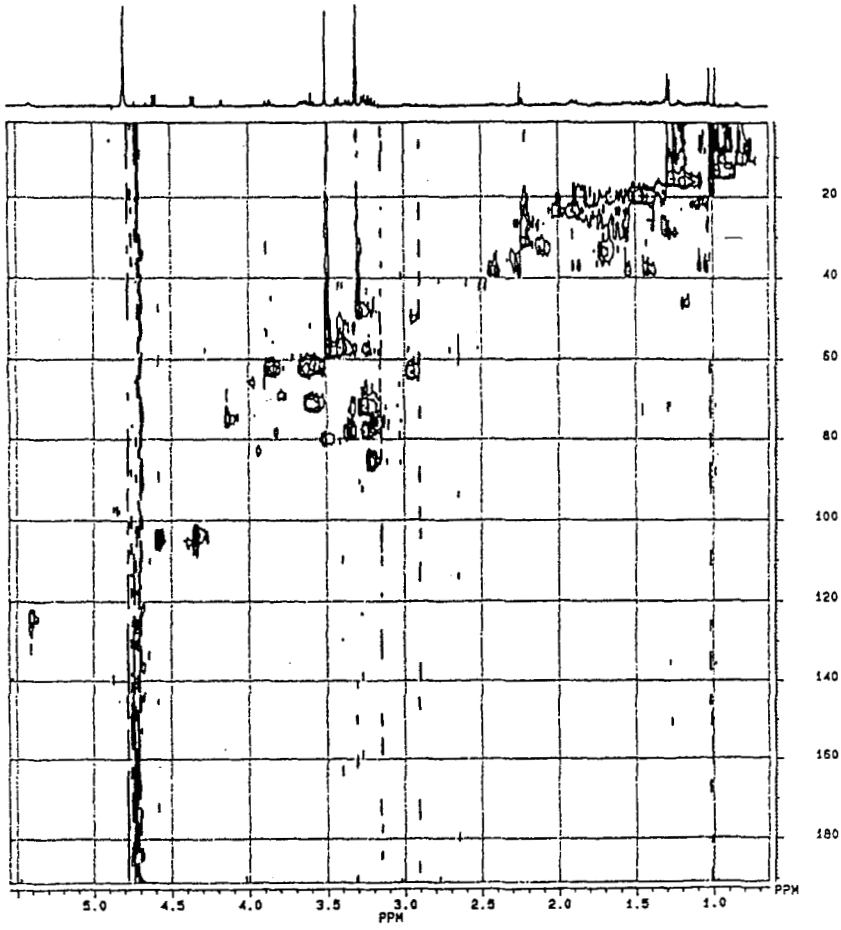
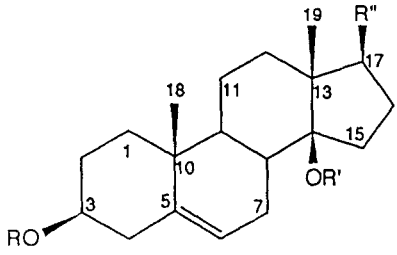


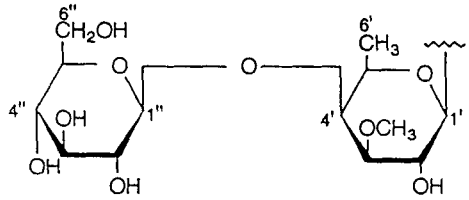
Fig.3 HMQC spectrum of caratuberside A2, measured in CD_3OD, shows a correlation among protons and carbons at high resolution.

EXPERIMENTAL:

The plant material was purchased from local vegetable market in Karachi and identification was kindly carried out by Prof. Dr. S.I. Ali, Department of Botany, University of Karachi. A voucher specimen of plant material was deposited in the Department of Pharmacognosy, University of Karachi. Extraction and fractionation procedure has already been described in our previous publication[1]. The butanol fraction was concentrated under reduced pressure and after the butanol was completely evaporated, it was dissolved in methanol. Later



Caratubergenin A1, R = R '= H, R" = CH_3 -C=O
Caratuberside A, R = Glc.(1 – 4) -3-O-methyl fucose, R' = H, R" = CH_3 -C=O
Caratuberside B, R = Glc.(1 – 4) -3-O-methyl fucose, R' = H, R" = CH_3 -HCOH
Caratuberside A2, R = H, R' = Glc.(1 –4) -3-O-methyl fucose, R" = CH_3 -C=O



β-D-glucopyranosyl-(1– 4)-3-O-methyl fucose

on ether was added for the formation of precipitate, and this precipitate contained glycosidic constituents, which were separated via Buchner funnel. This fraction was subjected to silica gel column chromatography eluted with ethyl acetate-methanol in increasing order of polarity. A fraction collected with eluent ethyl acetate-methanol (90:10) yielded two major fraction **I & II**. The fraction **II** was subjected to RP-HPLC (semi-preparative) with methanol-water (65:35), flow rate 4 ml/min., RI detector, and a pure compound was collected which yielded a white amorphous powder on lyophilization. It showed the following characteristic features: molecular weight 654; IR (KBr) 3430 (O-H), 2950 (C-H), 1700 (C=O), 1615 (C=C), 1040 (C-O-C) cm^{-1}: ^{1}H-NMR (300 MHz, CD_3OD) ppm; 0.98 (s, H-19), 1.02 (s, H-18), 1.26 (d, J=6.1 Hz, H-6'), 2.24 (s, H-21), 3.41 (m, H-3), 2.96 (dd, J=4.4, 8.8 Hz, H-17), 3.50 (s, OCH3), 3.18-3.70 (m, H-2',3',5',2",3",4",5"), 3.87 (dd, J= 11.6, 2.2 Hz, H-6"), 4.16 (d, J= 2.6 Hz, H-4'), 4.35 (d, J= 7.7 Hz, H-1'), 4.58 (d, J= 7.8 Hz, H-1"), 5.40 (dd, J= 4.9, 1.2 Hz, H-6): FAB-MS (-ve ion mode) at m/z 653 $[M-H]^-$, 745 $[M+glycerol]^-$, 421 [aglycone+glycerol-2H]$^-$,325 [glucose+3-O-methylfucose]$^-$: ^{13}C-NMR; see table 1.

Acid hydrolysis of caratuberside A2: 10 mg compound was used for acid hydrolysis as described in the literature[1,3,4]. Two different sugars were isolated and their type, structures & cofigurations were determined on the basis of comparison with standard sugars on TLC, HPTLC, HPLC and spectroscopic measurements. They were identified as glucose and 3-O-methyl fucose.

Table 1: ^{13}C-NMR chemical shifts of caratubergenin A1 (C_5D_5N), caratuberside A2 (CD_3OD), caratuberside A and caratuberside B (C_5D_5N).

C-atom	Caratubergenin	Caratubersides		
	A1	A2	A	B
1	37.00	38.46, t	37.49	37.63
2	28.91	30.66, t	29.96	30.16
3	72.40	71.47, d	76.53	77.03
4	32.62	39.61, t	34.74	34.88
5	43.40	140.76, s	44.48	44.65
6	28.13	123.07, d	29.21	29.40
7	27.10	28.34, t	28.14	28.18
8	39.25	37.81, d	40.63	40.91
9	47.69	47.74, d	49.65	50.05
10	35.42	38.29, s	36.07	22.86
11	20.00	21.78, t	21.63	22.86
12	38.50	39.52, t	39.44	40.91
13	48.30	47.44, s	49.49	49.09
14	82.52	86.89, s	84.76	83.83
15	33.00	35.09, t	32.02	33.01
16	24.40	25.26, t	24.84	18.85
17	61.48	63.04, d	63.01	57.08
18	12.00	15.45, q	12.22	12.36
19	14.99	17.34, q	15.73	15.48
20	218.39	219.90, s	216.39	65.30, d
21	31.07	32.65, q	32.37	21.53
1'	-	102.99, d	102.41	102.51
2'	-	75.95, d	76.80	76.84
3'	-	85.79, d	85.50	85.58
4'	-	77.87, d	77.30	77.48
5'	-	71.67, d	70.48	70.55
6'	-	19.87, q	17.76	17.82
1"	-	104.26, d	105.59	105.58
2"	-	74.98, d	76.12	76.10
3"	-	80.42, d	78.34	78.41
4"	-	71.89, d	71.94	71.61
5"	-	78.21, d	78.51	78.59
6"	-	63.87, t	63.18	63.25
OCH_3	-	58.52, q	59.50	59.05

Acetylation of caratuberside A2: With the standard method of acetylation the compound caratuberside A2 was acetylated[1, 3,4]. The compound showed acetyl groups, five acetate groups on the sugar portion and one at the C-3 position. ^1H-NMR (300 MHz, $CDCl_3$) ppm: 0.84 (s, H-19), 1.12 (s, H-18), 2.16 (s, H-21), 2.20 (s, OAc), 4.65 (m, H-3), 5.41(dd, J= 5.0, 2.1 Hz, H-6). EI-MS at m/z 906 (M^+, 1.5%), 549 (0.5%, 3-O-methyl fucose+glucose acetate), 533 (0.6%, 3-O-methyl fucose+glucose acetate without oxygen) , 516 (6%, M-glucose acetate), 373 (32%, aglycone), 357 (10%, aglycone without oxygen).

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