^{13}C-NMR Data of Tetraoxygenated Xanthones from *Centaurium pulchellum* Druce.

[1]H.BIBI, [1]I. ALI*, [2]M. I. CHOUDHARY AND [1]G. A. MIANA
[1]*Department of Chemistry*
Gomal University, Dera Ismail Khan, Pakistan
[2] *H.E.J.Research Institute of Chemistry,*
University of Karachi, Karachi-75270,Pakistan

(Received 24th March, 2004, revised 22nd May, 2004)

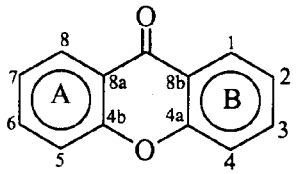
Summary:Three tetraoxygenated xanthones have been isolated from Centaurium pulchellum namely 1, 8-dihydroxy 3, 5-dimethoxy xanthone **1**, 1, 8-dihydroxy 3, 7-dimethoxy xanthone **2** and 1-hydroxy 3, 7, 8-trimethoxy xanthone **3**, respectively. As the ^{13}C-NMR data of these xanthones have not been reported earlier, we measured and multiplicity assignments made using polarization transfer pulse sequences (DEPT) and HMQC .

Introduction

C. pulchellum Druce. belongs to the family of Gentianaceae. Members of this family have been used in the indigenous systems of medicine [1-3]. Various xanthones [4,5], alkaloids [6,7], and glucosides [8,9] have been isolated from the plants of this family. Xanthones are perhaps one of the most abundant group of secondary metabolites in nature and have been isolated from various species of 30 genera. The majority of natural xanthones have been found in the members of Gentianaceae and Guttifiereae [10]. Simple oxygenated xanthones are found in both the families whereas more highly oxygenated xanthones are only found in Gentianaceae. Xanthones have been reported as mutagenetic [11,12], as well as producing varying responses to the central nervous and cardio vasculaer systems [13-15].

Results and Discussion

Three tetraoxygenated xanthones have been isolated from the $CHCl_3$ fraction. A comparison of spectral data (mass, UV, ^{1}H-NMR) and m.p.of these three tetraoxygenated xanthones (1, 8-dihydroxy-3, 5-dimethoxy xanthone, 1, 8-dihydroxy-3-7-dimethoxy xanthone and 1-hydroxy-3, 7, 8-trimethoxy xanthone) were consistent with those reported in literature [16]. As the ^{13}C-NMR of these xanthones have not so far been described, therefore it was decided to report this data. The ^{13}C resonance of xanthone **1** afforded 15 signals. The carbonyl carbon (C-9) which resonated at δ 184.6, was characteristic of a double chelated carbonyl carbon (1, 8-dihydroxyl) [14]. The two-methoxy carbons were resonated at δ 55.9 (C-3-OMe) and δ 57.4 (C-5 -OMe). The four methine aromatic carbons were resonated at δ 93.1 (C-4), δ 97.9 (C-2), δ 109.3 (C-7) and δ 120.4 (C-6). The long range coupling of methine carbon (C-6) was characteristic of the 1, 3 dioxy polyketide ring B with a hydroxyl group at C-1 [14-17]. The xanthone **2** also showed 15 signals in ^{13}C-NMR spectrum. The signal at δ 185.1 was character-istic of a double chelated carbonyl carbon (1, 8-dihydroxyl) [14]. The four methine aromatic carbons were resonated at δ 92.9 (C-4), δ 97.2 (C-2), δ 105.6 (C-5) and δ 120.5 (C-6). Two methoxy carbons resonated at δ 55.9 (3-OMe) and δ 57.1 (7-OMe). The xanthone **3** afforded 16 signals in ^{13}C-NMR spectrum. Three methoxy carbons occured at δ 55.8 (3-OMe), δ 57.2 (7-OMe) and δ 61.8 (8-OMe). Four aromatic methine carbons resonated at δ 92.1 (C-4), δ 96.9 (C-2), δ 112.8 (C-5), and δ 120.6 (C-6). The assignments of the carbons and ^{1}H-^{13}C connecti-vities were confirmed by HMQC and are presented in Tables 1-3.



Experimental

The ^{1}H - NMR spectra were recorded at 300 MHz in $CDCl_3$ on a Bruker AM-300 NMR spectrometer while the ^{13}C-NMR spectra were recorded at 75 MHz and assignments made by using gated spin echo (ATP) and polarization transfer (DEPT) pulse

*To whom all correspondence should be addressed.

Table 1. ^{13}C-NMR Data and ^{1}H/^{13}C Connectivities in **1**

Carbon No.	Chemical Shift (δ)	Multiplicity (DEPT)	Proton Chemical Shift (δ)	Heterocosy (^{13}C /^{1}H Connectivity)
1.	162.9	C	-	-
2.	97.9	CH	6.32 (IH,d,J=2.3Hz,H-2)	97.9 / 6.32
3.	167.5	C	-	-
4.	93.1	CH	6.51 (1H,d,J=2.3Hz,H-4)	93.1 / 6.51
5.	139.9	C	-	-
6.	120.4	CH	7.21 (1H,d,J=8.9Hz,H-6)	120.4 / 7.21
7.	109.3	CH	6.69 (1H,d,J=8.9Hz,H-7)	109.3 / 6.69
8.	154.2	C	-	-
8a.	108.1	C	-	-
8b.	102.8	C	-	-
4a.	157.8	C	-	-
4b.	145.5	C	-	-
9.	184.6	C	-	-
3-OMe	55.9	OCH_3	3.88 (s)	55.9 / 3.88
5-OMe	57.4	OCH_3	3.94 (s)	57.4 / 3.94
1	-	OH	11.94 (s)	-
8	-	OH	11.35 (s)	-

Table-2: ^{13}C-NMR Data and ^{1}H/^{13}C Connectivities in **2**

Carbon No.	Chemical Shift (δ)	Multiplicity (DEPT)	Proton Chemical Shift (δ)	Heterocosy (^{13}C/^{1}H Connectivity)
1.	163.0	C	-	-
2.	97.2	CH	6.28 ((1H,d,J=2.3Hz,H-2)	97.2 / 6.28
3.	167.5	C	-	-
4.	92.9	CH	6.34 ((1H,d,J= 2.4Hz,H-4)	92.9 /6.34
5.	105.6	CH	6.81 ((1H,d,J= 9.6Hz,H-5)	105.6 /6.81
6.	120.5	CH	7.24 ((1H,d,J= 9.1Hz,H-6)	120.5 / 7.24
7.	143.0	C	-	-
8.	150.2	C	-	-
8a.	107.8	C	-	-
8b.	102.3	C	-	-
4a.	158.1	C	-	-
4b.	149.7	C	-	-
9.	185.1	C	-	-
3-OMe	55.9	OCH_3	3.87 (s)	55.9 / 3.87
7-OMe	57.1	OCH_3	3.92 (s)	57.1 / 3.92
1	-	OH	11.48 (s)	-
8	-	OH	11.62 (s)	-

Table-3: ^{13}C-NMR Data and ^{1}H/^{13}C Connectivities in **3**

Carbon No.	Chemical Shift (δ)	Multiplicity (DEPT)	Proton Chemical Shift (δ)	Heterocosy (^{13}C / ^{1}H Connectivity)
1.	163.9	C	-	-
2.	96.9	CH	6.29 (1H,d,J= 2Hz,H-2)	96.9 / 6.29
			-	
3.	166.5	C	6.31 ((1H,d,J= 2Hz,H-4)	-
4.	92.1	CH	7.14 (1H,d,J= 9.1Hz,H-5)	92.1 / 6.31
			7.39 (1H,d,J= 9.1Hz,H-6)	
5.	112.8	CH	-	112.8 / 7.14
			-	
6.	120.6	CH	-	120.6 / 7.39
			-	
7.	149.0	C	-	-
8.	151.1	C	-	-
8a.	115.8	C	-	-
8b.	104.1	C	3.86 (s)	-
4a.	157.2	C	3.91 (s)	-
4b.	149.4	C	3.99 (s)	-
9.	181.3	C	13.22 (s)	-
3-OMe	55.8	OCH_3		55.8 / 3.86
7-OMe	57.2	OCH_3		57.2 / 3.91
8-OMe	61.8	OCH_3		61.8 / 3.99
1	-	OH		-

sequences. The UV spectra were recorded in MeOH on Shimadzu UV 240 instruments. The mass spectra were recorded on a Varian MAT 312 double focusing mass spectrometer connected to DEC–PDP 11/34 computer system. Melting points were determined with a Gallenkamp melting point apparatus. Thin layer chromatrography was carried out on silica gel GF–254 percoated plates from E. Merck, columns were packed by silica gel type-60 (70-230 mesh).

C. pulchellum was collected from Dera Ismail Khan in March-April, 2003, and was identified by Prof. Iftekhar Hussain Shah, Faculty of Pharmacy, Gomal University, where the voucher specimen was deposited.

Method

The air dried plant (6.45 kg) of *Centaurium pulchellum* was powdered and extracted in MeOH (17.5 L) for three days. The extract was filtered and concentrated under vacuum to yield a gummy mass (81 g). It was dissolved in water (750 mL) and extracted with petroleum ether (b.p. 60°-69°) to remove the fatty substances. The aqueous layer was extracted with $CHCl_3$ (3 L). The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and freed of solvent (12.6 g). The material was subjected to flash chromatography over silica gel (130 g). Elution was first carried out with petroleum ether (b.p. 60°-69°) to wash the remaining oily substances. Subsequent elution with $CHCl_3$, EtOAc and EtOH (2 L each) afforded three fractions. The $CHCl_3$ fraction (2.8 g) was loaded a column packed with silica gel (60 g) and elution carried out with gradually increasing the polarity.

Xanthone 1

The eluate obtained with pet ether $CHCl_3$ (6: 4, 300 ml) was subjected to preparative TLC plates (silica gel) in petroleum ether-Me_2CO (3:1) to afford a yellow crystalline, xanthone **1** (14 mg). M. p. 190-191°C. UV (MeOH): λ_{max} 239, 256, 370 and 372 nm. EI MS: *m/z* 288 (M^+, 24%, $C_{15}H_{12}O_6$), 273 (30%), 230 (100%), 211 (15%), 203 (40%), 127 (24%), 111 (22%) and 85(28%). The ^{1}H-NMR ($CDCl_3$,300 MHz) showed four aromatic protons at δ 6.32 (1H,d, *J*= 2.3 Hz, H-2), δ 6.51 (1H,d, *J* = 2.3Hz,H-4), δ 7.21 (1H,d, *J*= 8.9Hz, H-5), and δ 6.69 (1H,d, *J*= 8.9 IIz,H-6). Two methoxyl group at δ 3.88 (s, 3-OMe), δ 3.94 (s, 5-OMe) and two chelated hydroxyl group at δ 11.35 (s) and δ 11.94 (s). ^{13}C- NMR ($CDCl_3$, 75 MHz) δ : see Table-1.

Xanthone 2

The eluates obtained from $CHCl_3$ (300 mL) was evaporated, and the crystalline mass thus obtained, filtered and crystallized from Me_2CO to produce yellowish crystals of xanthone **2** (19 mg). M. p. 191-192°C, UV (MeOH): λ_{max} 331, 263, 239 and 203 nm., EI MS: m/z 288 (M^+,18% $C_{15}H_{12}O_6$), 281 (25%), 373 (100%), 259 (14%), 246 (16%), 214 (24%), 201 (20%), 144 (25%), 130 (15%). 97 (22%), 85 (26%), and 71 (15%). The ^{1}H-NMR ($CDCl_3$, 300 MHz) showed four aromatic protons at δ 6.28 (1H,d, J= 2.3 Hz,H-2), δ 6.34 (1H,d, *J*=2.4 Hz,H-4), δ 6.81 (1H,d, *J*= 9.6 Hz,H-5) and δ 7.24 (1H,d, *J* = 9.1Hz,H-6). Two methoxyl groups at δ 3.87 (s, 3-OMe), δ 3.92 (s, 7-OMe) and chelated hydroxyl group at δ 11.48 (s), δ 11.62 (s). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ : see Table-2.

Xanthose 3

The eluates from $CHCl_3$-ether (8:2) and $CHCl_3$-ether (7:3) gave similar spot on TLC plates. The combined semi solid mass afforded yellow crystals, which were washed from ether to obtain xanthone **3** (21 mg).). M. p. 160–161°C, UV (MeOH): λ_{max} 239, 256, 370 and 372 nm. EI MS: *m/z* 302 (M^+, 36%, $C_{16}H_{14}O_6$), 288 (18%), 279 (100%), 259 (45%), 168 (32%), 143 (15%), 113 (20%), 104 (16%), 84 (34%), 69 (25%). ^{1}H-NMR ($CDCl_3$, 300 MHz): showed four aromatic protons at δ 6.29 (1H,d, *J*=2 Hz, H-2), δ 6.31 (1H,d, *J*=2 Hz, H-4), δ 7.14 (1H,d, *J*=9.1Hz, H-5), and δ 7.39 (1H,d, *J*=9.1Hz, H-6), 3-methoxyl groups at δ 83.86 (s, 3-OMe), δ 3.91 (s, 7-OMe), δ 3.99 (s, 8-OMe) and one chelated hyroxyl group at δ 13.22 (s). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ : see Table-3.

References

1. R. Dalal and R.C. Shah, *Chemistry and Industry*, **66**, 664 (1956).
2. F. Hussain, M. I. Khattak and S. A. Warsi, *Pak. J. Secientific and Industrial Research*, **11** (4), 352 (1968).
3. Sumb, Hj. Idris, A. Jafferson and F. Scheinmann, *J. Chem. Soc. Perkin Trans*, 2158 (1977).
4. Gunter and J. Andere, *Helvetica Chimica Acta*, **56**, 284 (1973).

5. Pierra, M. Jaeques, G. Michele, P. Victor, *Phytochemistry*, **8**, 1533 (1969).
6. Brindly, *J. Chem. Soc*, 1067 (1972).
7. Oshkurnina, *J. Gen. Chem. USSR*, **14**, 1148 (1944).
8. Tsuyoshi, and K. Manki, *Yakugaku Zasshi*, **89** (3), 410 (1969).
9. Orge, H. M. Lawrence, *Taxonomy of vascular plants* 673 (1970).
10. M. G. Al-Hazimi and G. A. Miana. *J. Chem. Soc. Pak*, **12** (2), 174 (1990).
11. Morimoto, Watanake, T. Osawa and T.Okatsu, *Org. Mag. Res.* **97**, 81 (1982).
12. Kanamori, I. Sakanoto, M. Mizuta, K. Hashimoto and O. Tanak, *Chem. Pharm. Bull*, **32**, 2290 (1984).
13. S. K. Bhattacharya, A. K. Sanayal and S.Ghosal, Drugs and Central. Synaptic Transmission P.3 33, Macmillan London (1976) .
14. Ghosal, K. Biswas and R.K. Chaudhry, *J. Pharm. Sci*, **17**, 721 (1978).
15. Ghosal and R. K. Chaudhry. *J. Pharm. Sci*, **64**, 888 (1975).
16. A. Miana and H. M. G. Al-Hazimi, *Phytochemistry*, **23** (8), 1637 (1984).
17. U. S. Sultanbawa, *Tetrahedron*, **36**, 1465 (1980).