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The NMR Assignments of Anthraquinones from *Cassia tora*

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The ^{1}H- and ^{13}C-NMR spectra of alaternin, aurantio-obtusin, chryso-obtusin, obtusin and 2-glucosyl obtusifolin isolated from the seeds of *Cassia tora* have been assigned based on HMBC, long-range HETCOR, fully ^{1}H-coupled ^{13}C-NMR, deuterium isotope experiment, and by comparison with the model compounds.

Key word : *Cassia tora*, Leguminosae, anthraquinones, HMBC, long-range HETCOR, Deuterium isotope effect

INTRODUCTION

In the course of investigating biologically active natural compounds from the Korean medicinal plants, we reported that the methanolic extract of the seeds of *Cassia tora* (Leguminosae) exerts a radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (Choi *et al.*, 1993, 1994) and antimutagenic activity in *Salmonella* assay system (Choi *et al.*, 1996). From this methanolic extract, 2-hydroxyemodin (alaternin) and methoxylated anthraquinones such as chryso-obtusin and aurantio-obtusin were isolated as the active principles (Choi *et al.*, 1994, 1996).

Though the structure of these anthraquinones have already been elucidated (Nikaido, *et al.*, 1984, Kitanaka and Takido, 1984, Danielsen *et al.*, 1992), the assiagnments of the ^{13}C resonances to their respective carbon atoms has not been established yet. The ^{13}C-NMR assignment of anthraquinones from *C. tora* was obtained by utilizing DEPT, HMBC, long-range HETCOR, fully ^{1}H-coupled ^{13}C-NMR, and deuterium isotope experiment, and by comparisons with the model compounds (Stothers, 1972). The spectra of chrysophanol (**1**), physcion (**2**), and emodin (**3**) have been examined previously (Danielsen *et al.*, 1992).

MATERIALS AND METHOD

The ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75.5 MHz, respectively on a Bruker AM 300 spectrometer with tetramethylsilane as the internal standard. In the case of compound **5**, the chemical shifts were referenced to residual solvent peak {(2.49 ppm in ^{1}H-NMR (500 MHz) and 39.5 ppm in ^{13}C-NMR (125 MHz) for DMSO-d_6}. Multiplicities of ^{1}H- and ^{13}C-NMR signals are indicated as s (singlet), d (doublet) and t (triplet). The samples were examined in DMSO-d_6, except for physcion (**2**), which was examined in $CDCl_3$.

Plant material

The seeds of *C. tora* were purchased from a commercial supplier in 1993, and authenticated by Prof. H. J. Chi of Natural Products Research Institute, Seoul National University. A voucher specimen has been deposited in the herbarium of the Natural Products Research Institute, Seoul National University.

Isolation of compounds

The powdered seeds (3.0 kg) of *C. tora* were extracted with MeOH and concentrated to give a dark residue, which was successively extracted with dichloromethane, ethyl acetate, *n*-butanol, and water. The anthraquinones were thereafter isolated from the dichloromethane and ethyl acetate fractions according to the procedure reported in the previous paper (Choi *et al.*, 1994).

RESULTS AND DISCUSSION

The ^{1}H and ^{13}C chemical shifts of the anthraquinones chrysophanol (**1**), physcion (**2**), emodin (**3**), alaternin (**4**), aurantio-obtusin (**5**), chryso-obtusin (**6**), obtusin (**7**), and obtusifolin-2-glucoside (**8**) are presented in Tables 1 and 2, respectively. The ^{1}H sig-

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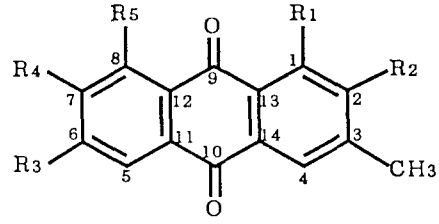
nals were assigned by comparison of spectral data with those reported in literature (Nikaido, *et al.*, 1984, Kitanaka and Takido, 1984, Danielsen *et al.*, 1992). The numbering adopted in this paper is the usual numbering of anthraquinone derivatives (Fig. 1).

The ^{13}C-NMR assignments of the 2-hydroxyemodin (alaternin, **4**) shown in Table 2, was obtained by comparisons of those of emodin (**3**) and by assuming that the 2-hydroxy substituent effects on the anthraquinone nucleus are additive (Berger and Castonguay, 1978). In comparison between the spectra of **3** and **4**, considerably large differences in the chemical shifts were discernible for C-1 (+12.14), C-2 (-26.2), C-3 (+12.5) and C-14 (+9.74). Such differences clearly show the presence of the hydroxyl group at C-2.

Substituent chemical shift from emodin (**3**) and alat-



Compound	R_1	R_2	R_3	R_4	R_5
1 (chrysophanol)	OH	H	H	H	OH
2 (physcion)	OH	H	OMe	H	OH
3 (emodin)	OH	H	OH	H	OH
4 (alaternin)	OH	OH	OH	H	OH
5 (aurantio-obtusin)	OMe	OH	OH	OMe	OH
6 (chryso-obtusin)	OMe	OH	OMe	OMe	OMe
7 (obtusin)	OMe	OH	OMe	OMe	OH
8 (obtusifolin-2-glucoside)	OMe	O-Glucose	H	H	OH

Fig. 1. Structures of *Cassia* anthraquinones.

Table I. ^{1}H-NMR chemical shifts of *Cassia* anthraquinones

Compound Proton	**1**[1]	**2**[1]	**3**	**4**	**5**	**6**	**7**	**8**
H-2	7.22	7.07	7.11					
4	7.55	7.62	7.43	7.47	7.72	7.68	7.81	7.86
5	7.71	7.36	7.07	7.08	7.13	7.46	7.31	7.64
6	7.80	-						7.74
7	7.38	6.68	6.56	6.52				7.33
CH_3	2.44	2.44	2.39	2.34	2.26	2.26	2.29	2.44
1-OCH_3					3.78	3.81	3.81	3.90
6-OCH_3		3.93				3.97	3.98	
7-OCH_3					3.82	3.86	3.83	
8-OCH_3						3.87		

[1]Data taken from (Danielsen *et al*, 1992)

Table II. ^{13}C-NMR chemical shifts of *Cassia* anthraquinones

Compound Carbon	**1**[1]	**2**[1]	**3**	**4**	**5**	**6**	**7**	**8**
C- 1	161.67	162.51	161.33	149.19	147.16	147.38	146.98	153.19
2	124.16	124.51	123.94	150.14	155.44	155.10	155.28	154.65
3	149.26	148.44	148.08	135.58	131.96	130.52	131.93	132.46
4	120.64	121.29	120.35	122.86*	125.84	125.89	125.76	125.13
5	119.41	108.22	108.74	108.51	107.62	105.26	102.50	118.28
6	137.41	166.56	165.51	165.60	156.59	156.51	155.86	136.34
7	124.49	106.78	107.79	107.15	139.36	146.16	140.45	141.39
8	161.41	165.20	164.37	164.35	156.94	153.34	157.32	161.34
9	191.72	190.82	189.54	190.09	187.13	180.94	187.39	187.87
10	181.57	182.05	181.13	179.89	180.33	180.69	179.96	181.28
11	133.40	135.27	134.93	131.28	128.47	129.60	128.30	129.59
12	115.94	110.27	108.74	108.98	111.07	122.43	112.31	116.80
13	113.85	113.69	113.19	113.92	123.68	124.10	123.24	124.01
14	133.10	133.23	132.66	122.92*	124.84	124.71	124.62	124.48
3-CH_3	21.71	22.15	21.42	16.20	16.48	16.20	16.24	17.54
1-OCH_3					59.99	60.71	59.93	60.86
6-OCH_3		56.07				56.11	55.90	
7-OCH_3					61.19	61.23*	60.90	
8-OCH_3						61.38*		
G-1								103.74
2								74.01
3								77.34
4								69.82
5								76.40
6								61.40

[1]Data taken from (Danielsen *et al*, 1992)
*Assignment may be reversed in each column

Table III. ^{13}C 2-hydroxy substituent chemical shifts from **3** and **4**

Substituent	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14
Calculated*	-14.37	+28.76	-12.84	+3.18	-0.07	+0.02	-0.49	-0.07	0	-1.50	+0.02	+0.16	+2.17	-7.84
Observed	-12.14	+26.20	-12.50	+2.51	-0.23	+0.09	-0.64	-0.02	+0.05	-1.24	-3.65	+0.24	+0.73	-9.74
Difference	+2.23	+2.56	+0.34	+0.67	+0.16	-0.07	+0.15	-0.05	-0.05	-0.26	+3.67	-0.08	+1.43	-1.90

Plus sign denotes a downfield shift (△ δ in ppm)
*Berger and Castonguay, 1978

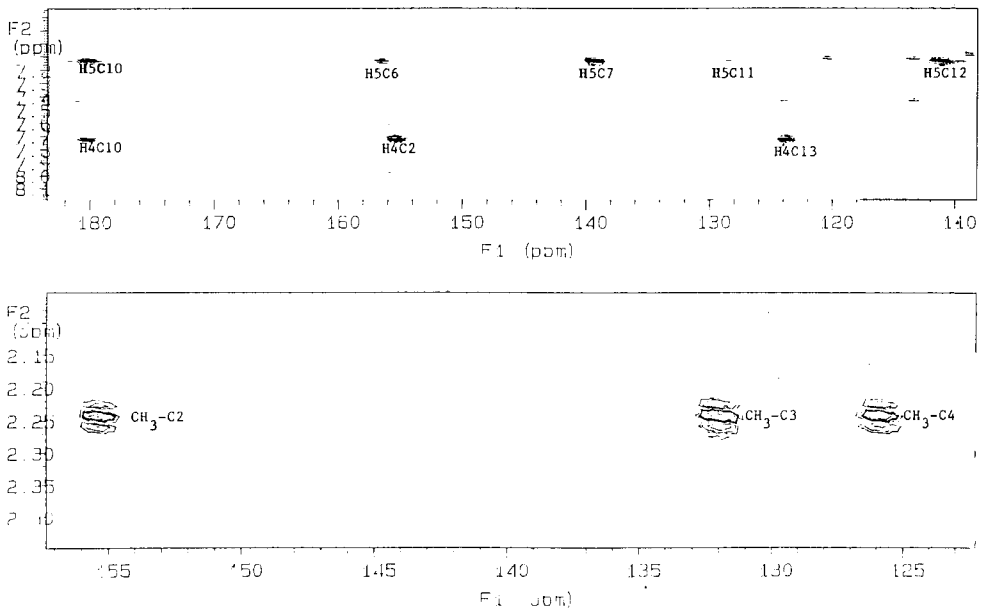


Fig. 2. HMBC spectrum of aurantio-obtusin (**5**)

ernin (**4**) have been established, and compared with the calculated shifts (Table 3). As shown in Table 3, the deviation between the calculated and observed shifts on C-1, C-2, C-4, C-11, C-13, and C-14 are more than ± 0.5 ppm. This may be explained that when a second hydroxy substituent is introduced on to the same moiety of the anthraquinone nucleus an additional perturbation must be considered.

The ^{13}C signal assignment of aurantio-obtusin (**5**) was not all straightforward since most of them were those of quaternary carbons. For the aid of the signal assignment, HMBC, long-range HETCOR, fully ^{1}H-coupled ^{13}C-NMR, and deuterium isotope experiment were performed. The signal assignment of C4 (δ 125. 84), C5(δ 107.62), and CH_3 (δ 16.48) were straightforward by analysis of DEPT spectrum. C4 showed coupling with CH_3 protons in HMBC (Fig. 2). Two carbonyl carbon signals (C9, C10) were assigned based on the long range ^{1}H-^{13}C couplings. (Fig. 2). The signal of C10 (δ 180.33) showed coupling with H5 and H4 in HMBC. In the ^{1}H-coupled ^{13}C-NMR spectrum, the signal of C10 appeared as a triplet ($^{3}J_{C,H}$=4.4 Hz) while that of C9 (δ 187.13) appeared as a broad singlet. The assignment of C9 and C10 signals was further confirmed by deuterium isotope (Jung and Mclaughlin, 1990). When a drop of 1 : 1 (v/v) D_2O/H_2O was added to the sample in DMSO-d_6, aurantio-obtusin (**5**) existed as a mixture of (A) and (B) (Fig. 3). The ex-

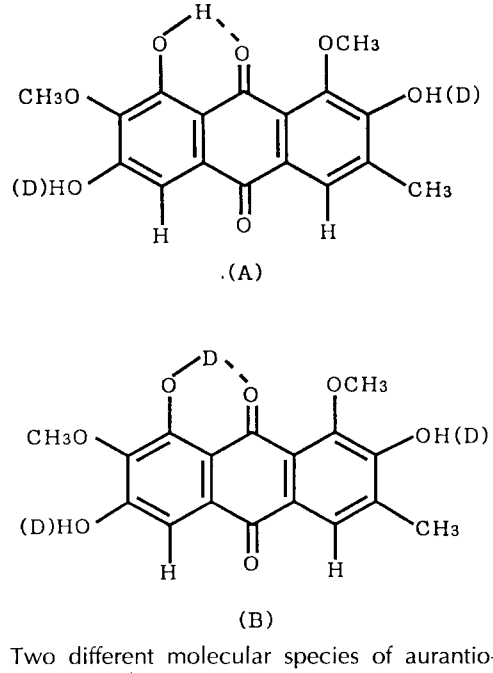


Fig. 3. Two different molecular species of aurantio-obtusin in DMSO-d_6+D_2O/H_2O (1 : 1).

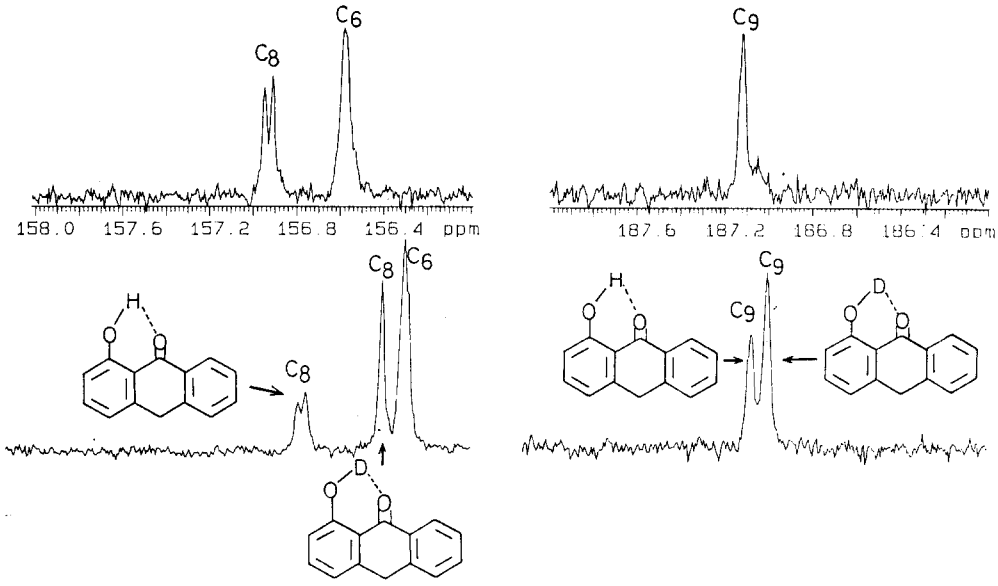


Fig. 4. The ^{1}H-coupled ^{13}C-NMR spectra of the D_2O/H_2O (1 : 1) mixture of aurantio-obtusin and deuteriated aurantio-obtusin.

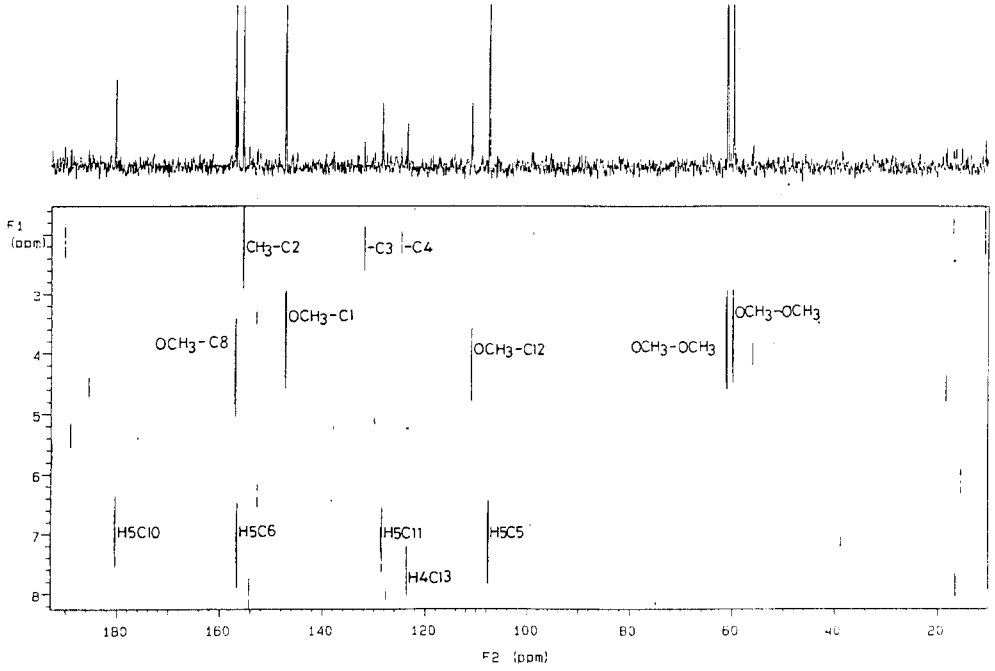


Fig. 5. Long-range HETCOR spectrum of aurantio-obtusin (**5**)

change rates of intramolecular hydrogen-bonded proton or deuterium were relatively slow relative to the magnitude of the isotope effect (=Requisite for detectability of such isotope shift is that the life time of the proton at a particular site is longer than $(\Delta\nu_{HD})^{-1}$, the inverse of the isotope shift in Hz); thus the signal of C9 was resolved into two separate peaks each representing the carbons of C9-OH and C9-OD (Fig. 4).

Of the carbons bearing hydroxyl function (C2, C6, and C8), C6 (δ 156.59) and C2 (δ 155.44) showed couplings with H5 and H4, respectively in HMBC (Fig. 2). Good resolution between the signals of C6 (δ 156.59) and C8 (δ 156.94) was achieved in the long range HETCOR spectrum (Fig. 5). A coupling between C2 and CH_3 protons was also dectected. Accordingly, the remaining carbon signal (δ 156.94) was assigned to C8. In the ^{1}H-coupled ^{13}C-NMR spectrum, the signal of C8 appeared as a doublet ($^{2}J_{C,H}$=5.1 Hz) suggesting a coupling with the intramolecular hydrogen bonded OH proton. In the deuterium isotope experiment, the signal of C8 was resolved into two

separate peaks each representing the carbons of C8-OH (doublet) and C8-OD (singlet). The intrinsic isotope effect for C8 was -0.35 ppm. Between the two methoxylated carbons (C1 and C7), C7 (δ 139.36) showed coupling with H5 in HMBC. Among the four quaternary carbons at the ring junctions (C11, C12, C 13, and C14), C12 (δ 111.07, $^{3}J_{C,H}$=7.3 Hz) and C13 (δ 123.68, $^{3}J_{C,H}$=7.0 Hz) showed strong couplings with H5 and H4, respectively in HMBC. And C11 (δ 128. 47) showed weak coupling with H5. Of the two methoxyl protons (A,B), A (δ 3.82) and B (δ 3.78) showed couplings with C8 and C1, respectively in long-range HETCOR. Thus, the ^{13}C signals of aurantio-obtusin (**5**) were all assigned as shown in Table 2.

The assignments of **7** (obtusin, 6-methyl ether of **5**) and **6** (chryso-obtusin, 6,8-dimethyl ether of **5**) to their carbon atoms, shown in Table 2, was obtained by comparisons with **5** and by assuming that alkylation effects on the anthraquinone nucleus are additive (Berger and Castonguay, 1978). And, the assignments of **8** to their respective carbon atoms, shown in Table 2, was also obtained by comparison of spectral data with those of **1** and by assuming that alkylation effects on the anthraquinone nucleus are additive.

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