STRUCTURE OF EPHEDRADINE C, A HYPOTENSIVE PRINCIPLE OF *EPHEDRA* ROOTS[1]

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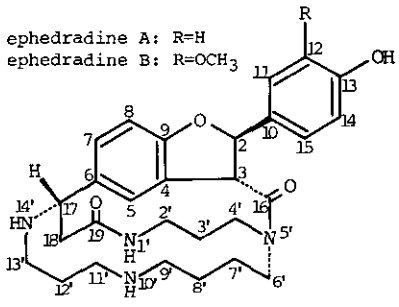
Abstract — From the crude drug "mao-kon", the roots of *Ephedra* plants, a novel macrocyclic spermine alkaloid ephedradine C exhibiting the hypotensive activity has been isolated whose stereostructure has been established as represented by formula I on the basis of chemical and physical evidence. Flexibility of the molecules of the ephedradines has been noticed by means of NMR and CD spectroscopy.

The crude drug "mao-kon", the underground part of *Ephedra* plants (Ephedraceae), has been used as an antiperspirant in Oriental medicine. From the crude drug, we have recently isolated two hypotensive principles ephedradine A and B, their stereostructures being clarified.[2,3] During the course of the isolation of ephedradine A and B, the alkaloid fraction from the extract was submitted to repeated chromatography (alumina/AcOEt-MeOH-H_2O) by monitoring the hypotensive activity. From the fraction eluted before ephedradine A, another alkaloid was obtained and designated as ephedradine C.

ephedradine A: R=H
ephedradine B: R=OCH_3



Although ephedradine C has not yet been crystallized, *it was characterized as the dihydrobromide, m.p. 224-225°,* $[\alpha]_D$ -100.7° (H_2O), $C_{30}H_{40}N_4O_5 \cdot 2HBr \cdot H_2O$ (FD-MS (*m/e* 536, M^++1 as free base) and elemental analysis). Administration of the salt to rats (2.0 mg/kg, *i.v.*) produced a significant hypotension.

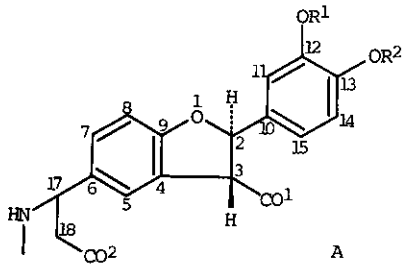
The ^1H NMR spectrum of ephedradine C was quite similar to the spectra of ephedradine A and B with the exception in the methoxyl region and the aromatic region as *will be discussed in detail* below. The ^{13}C NMR spectrum exhibited the presence of sixteen aliphatic carbons (CH_3-O × 2, CH_2 × 11, CH × 2, CH-O × 1), twelve aromatic carbons (CH × 6, C × 3, C-O × 3) and two carbonyl carbons. Further, the chemical shifts and the splitting patterns of most of the ^{13}C NMR signals of ephedradine C also corresponded to those of ephedradine B (Table I). These data, together with the fact that ephedradine C coexists with ephedradine A and B, led to the supposition that ephedradine C is an analog of ephedradine B and has an extra CH_2 moiety as a methoxyl group.

The presence of a dihydrobenzofuran moiety ($C_{(2)}$-$C_{(9)}$ in formula A) as in ephedradine B was indicated by the UV spectrum of ephedradine C dihydrobromide (maxima at 231 (log ε 4.16) and 280 nm (log ε 3.62) in MeOH) which closely resembled that of ephedradine B dihydrobromide (maxima at 232 (log ε 4.32) and 281 nm (log ε 3.92) in MeOH), by three 1H signals in the ^1H NMR spectrum of ephedradine C at δ 7.05 , 7.13 and 6.75 in an ABC type which were consistent with the corresponding signals in that of ephedradine B (δ 7.02, 7.10 and 6.75 for $H_{(5)}$, $H_{(7)}$ and $H_{(8)}$), by two 1H signals in ephedradine C at δ 6.02 and 4.59 in an AB type which were in agreement with the corresponding

Table I. Carbon-13 shieldings in ephedradine C and related substances (δ)

	ephedradine C dihydrobromide (D_2O)	ephedradine B dihydrobromide (D_2O)	ephedradine C diacetate ($CDCl_3$)	ephedradine B triacetate ($CDCl_3$)
C-2	88.0 d	88.7 d	87.6 d	86.5 d
C-3	52.0 d	52.5 d	53.8 d	54.1 d
C-4	125.8 s	125.3 s	125.4 s	125.0 s
C-5	133.4 d	134.3 d	132.7 d	132.3 d
C-6	126.1 s	126.9 s	130.6 s	131.0 s
C-7	121.0 d	121.5 d	124.2 d	124.4 d
C-8	111.3 d	111.1 d	111.3 d	110.2 d
C-9	159.1 s	159.9 s	159.3 s	159.0 s
C-10	130.7 s	130.8 s	132.4 s	139.2 s
C-11	110.4 d	111.1 d	110.3 d	110.2 d
C-12	148.4 s*	147.9 s	149.2 s*	151.0 s
C-13	148.0 s*	145.9 s	149.1 s*	139.8 s
C-14	109.8 d	115.7 d	109.7 d	122.9 d
C-15	119.7 d	120.5 d	118.8 d	117.6 d
C-16	170.7 s*	171.1 s*	171.5 s*	171.3 s*
C-17	58.7 d	59.2 d	57.5 d	57.0 d
C-19	174.5 s*	175.2 s*	172.0 s*	171.9 s*
C-18 &	21.4 t	21.8 t	26.2 t	26.0 t
C-2'-	22.8 t	23.1 t	26.2 t	26.3 t
C-13'	25.2 t	25.7 t	27.9 t	28.1 t
	25.4 t	25.7 t	29.4 t	29.6 t
	37.7 t	38.0 t	37.4 t	37.0 t
	37.9 t	38.0 t	39.4 t	39.0 t
	41.9 t	42.3 t	44.1 t	44.2 t
	42.6 t	42.7 t	44.7 t	44.6 t
	44.2 t	44.8 t	45.3 t	45.3 t
	46.0 t	46.5 t	46.5 t	46.6 t
	46.0 t	46.5 t	51.0 t	51.0 t
$\underline{C}H_3O$	55.5 q	56.4 q	56.0 q	56.0 q
	55.5 q		56.1 q	
$\underline{C}H_3CO$			21.8 q	20.5 q
			22.6 q	21.7 q
				22.6 q
$CH_3\underline{C}O$			169.6 s*	169.4 s*
			170.6 s*	169.7 s*
				170.4 s*

Abbreviations: s=singlet, d=doublet, t=triplet, q=quadruplet
The assignments of the asterisked signals are ambiguous and might have to be reversed



A

signals in ephedradine B (δ 6.02 and 4.61 for $H_{(2)}$ and $H_{(3)}$), and by the occurrence of the ^{13}C NMR signals for $C_{(2)}$-$C_{(9)}$ in ephedradine C which were coincident with those attributed to the same moiety in ephedradine B (Table I). The above coincidence of the ^1H NMR signals for $H_{(2)}$ and $H_{(3)}$, along with the splitting patterns of these signals, showed that $C_{(2)}$ carries a phenyl and $C_{(3)}$ bears a carbonyl (CO^1) in ephedradine C. The large coupling constant (J 11 Hz) between $H_{(2)}$ and $H_{(3)}$ indicated that the phenyl group and the carbonyl group are located in the *trans* configuration on the dihydrofuran ring (formula A).

Substantiation of the presence of a spermine moiety as in ephedradine B was performed by the following facts. 1) Ephedradine C possesses two amide carbonyl groups (an IR band at 1630 cm^{-1} and ^{13}C NMR signals at δ 170.7 and 174.5). 2) Although ephedradine C has no hydroxyl group, acetylation of ephedradine C with acetic anhydride in pyridine gave the N,N-diacetate (II) (a MS peak

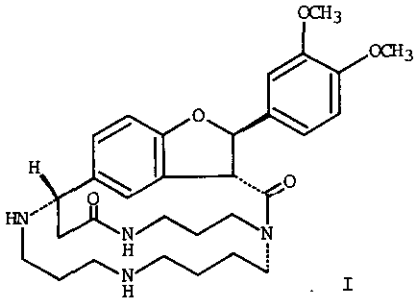
at *m/e* 620.3188 (M^+), an IR band at 1625 cm^{-1} (N-acetyl)), demonstrating that ephedradine C has two acetylatable amino groups as in ephedradine B. 3) The chemical shifts and splitting patterns of the ^{13}C NMR signals for $C_{(2')}$-$C_{(13')}$ in ephedradine C were identical with those associated with the same moiety in ephedradine B (Table I).

In the ^1H NMR spectrum of ephedradine C diacetate (II), a signal for the methine hydrogen appeared at a lower field region (δ 5.45) as a doublet of doublets (*J* 5 and 3 Hz) which was in accord with that (a doublet of doublets at δ 5.47 (*J* 5 and 3 Hz)) of ephedradine B triacetate, a fact which showed that the methine bears the methylene ($C_{(18)}$), a nitrogen atom and $C_{(6)}$ as in formula A. One of the two carbonyls (CO^2), the last carbon to be allocated, was therefore concluded to be next to the methylene ($C_{(18)}$) (formula A). The coincidence of the chemical shifts and splitting patterns of the ^1H NMR signals for the methine hydrogens ($H_{(17)}$) in the acetates of ephedradine C and ephedradine B, as mentioned above, and that of the chemical shifts of the ^{13}C NMR signals for the methylene carbons of the spermine parts ($C_{(2')}$-$C_{(13')}$) and for the methine ($C_{(17)}$) and methylene ($C_{(18)}$) carbons in ephedradine C and ephedradine B (Table I), when the substitution effects and environmental effects were taken into account, indicated that the mode of the linkages of the diacid parts and the spermine parts is identical in both the substances.

Ephedradine C and ephedradine B were therefore concluded to be different in their phenyl side chain ($C_{(10)}$-$C_{(15)}$). In fact, the UV maxima in methanol of ephedradine C dihydrobromide did not exhibit on addition of alkali bathochromic shifts as observed in the case of ephedradine B dihydrobromide. Six ^{13}C NMR signals ascribed to this phenyl group ($C_{(10)}$-$C_{(15)}$) in ephedradine C revealed the presence of three CH's, one C and two C-O's. Further, three 1H signals at δ 6.89, 6.95 and 7.13 in an ABC type together with a 6H singlet at δ 3.80 in the ^1H NMR spectrum of ephedradine C demonstrated the existence of another 1,2,4-trisubstituted benzene moiety for which three substitution patterns were possible, *i. e.* a 2,4-dimethoxy-1-alkylbenzene, a 2,5-dimethoxy-1-alkylbenzene and a 3,4-dimethoxy-1-alkylbenzene. More precise inspection of the chemical shifts of the above three ^1H NMR signals suggested that it was a 3,4-dioxygenated-1-alkylbenzene. In confirmation of this assumption, the observed chemical shifts of the six ^{13}C NMR signals for $C_{(10)}$-$C_{(15)}$ (Table I) were consistent with the calculated shifts of $C_{(1)}$-$C_{(6)}$ in a 3,4-dimethoxy-1-alkylbenzene (Table II, 3) but not with those of a 2,4-dimethoxy-1-alkylbenzene and a 2,5-dimethoxy-1-alkylbenzene (Table II, 1 and 2).[4]

Table II. Carbon-13 shieldings in the dimethoxy-alkylbenzenes (δ)

	C-1	C-2	C-3	C-4	C-5	C-6
2,4-dimethoxy-1-methylbenzene (1)	115.3	161.6	99.6	158.0	106.3	131.2
2,5-dimethoxy-1-methylbenzene (2)	124.0	152.9	115.0	112.2	152.1	115.8
3,4-dimethoxy-1-methylbenzene (3)	130.7	115.8	145.4	142.6	115.0	122.5



I

The CD curve of ephedradine C dihydrobromide (Cotton effects at 290 ([θ] $+8.1\times10^3$) and 234 nm ([θ] -6.9×10^4) in MeOH) was very similar to that of ephedradine A dihydrobromide (Cotton effects at 281 ([θ] $+7.5\times10^3$) and 233 nm ([θ] -4.9×10^4) in MeOH) and that of ephedradine B dihydrobromide (Cotton effects at 288 ([θ] $+8.1\times10^3$) and 234 nm ([θ] -4.5×10^4) in MeOH), establishing the absolute configurations at $C_{(2)}$ and $C_{(3)}$ of ephedradine C as being both *R*.

Accumulated data point to ephedradine C to be ephedradine B O-methyl ether.

In order to confirm this conclusion, ephedradine B was treated with diazomethane to furnish the methylated derivative whose dihydrobromide was identified as ephedradine C dihydrobromide.

The absolute stereostructure of ephedradine C was thus elucidated as in formula I.

While the structures of the ephedradines were under investigation, it was observed that the resonance peaks in their ^{1}H NMR spectra were rather broad making analysis of the spectral patterns of certain signals difficult. It was also found that the resonance peaks in the ^{13}C NMR spectra of the ephedradine derivatives were broadened. This broadening of the NMR signals was considered to be due to thermal vibration of the molecules, which was also indicated, even in the crystalline state, by the computer-drawn atomic diagram of ephedradine A dihydrobromide.[2]

In order to examine further the flexibility of the molecular conformation of the ephedradines in solution, temperature dependent changes in the ^{1}H NMR spectra, as well as in the CD spectra were determined, although the observations were made only at temperature between room temperature and -68°. Measurements at lower temperatures could not be made because of the low solubility of the substances. As a result, displacements of the chemical shifts as well as line broadening of certain signals in the ^{1}H NMR spectra were found as the temperature was lowered (Table III). In the

Table III. ^{1}H NMR signals in ephedradine A triacetate (δ in acetone-d_6)

	2-H	3-H	5-H	7-H	8-H	11-H	12-H	17-H	N_1-H
+21°	6.31	4.44	7.51	6.97	6.72	7.27	6.97	5.28	7.63
0°	6.29	4.45	7.47	6.99	6.73	7.27	6.98	5.26	7.72
-30°	6.29	4.46	7.42	7.02	6.78	7.29	7.00	5.19	7.88
-60°	6.24	4.52	7.26	7.06	6.81	7.34	7.05	5.08	8.18

Table IV. CD spectra of the ephedradine dihydrobromides

	ephedradine A[a]		ephedradine B[b]		ephedradine C[c]	
	$[\theta]_{235\ nm}$	$[\theta]_{283\ nm}$	$[\theta]_{237\ nm}$	$[\theta]_{289\ nm}$	$[\theta]_{235\ nm}$	$[\theta]_{289\ nm}$
+24°	-33,000	+6,750	-42,100	+7,470	-36,500	+7,610
0°	-31,500	+6,780	-45,000	+7,710	-38,600	+8,100
-30°	-31,100	+7,060	-45,200	+8,490	-39,700	+9,340
-68°	-22,800	+6,790	-36,100	+7,930	-32,500	+8,310

[a] $c=1.185\times10^{-4}$, EtOH [b] $c=1.930\times10^{-4}$, EtOH [c] $c=0.954\times10^{-4}$, EtOH

CD spectra, systematic changes of the molecular ellipticity at about 235 nm were obvious, while less significant deviations of the molecular ellipticity at about 286 nm were also noticed (Table IV). These results demonstrate that the molecular conformation of the ephedradines is temperature dependent.

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NOTE AND REFERENCES

1. Studies on the constituents of *Ephedra*. VII. This paper also constitutes Part 20 in the series on the validity of the Oriental medicines.
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