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

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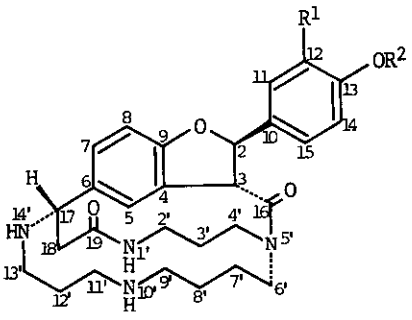
Abstract — From the crude drug "maō-kon", the roots of Ephedra plants, a new macrocyclic spermine alkaloid ephedradine D exhibiting the hypotensive activity has been obtained whose stereostructure has been elucidated as shown in formula I based on chemical and physical evidence.

The crude drug "maō-kon", the underground part of Ephedra plants (Ephedraceae), has been utilized for antiperspiratory purpose in Oriental medicine. We have recently isolated from the crude drug three hypotensive principles ephedradine A, B and C whose stereostructures have been established.[2-4] Further survey of the alkaloid fraction from the extract of the crude drug resulted in the isolation of another alkaloid which was termed as ephedradine D.



ephedradine A: $R^1=R^2=H$
ephedradine B: $R^1=OCH_3$, $R^2=H$
ephedradine C: $R^1=OCH_3$, $R^2=CH_3$

Ephedradine D was characterized as the dihydrobromide, m.p. 219—221°, $[\alpha]_D$ -85.3° (H_2O), $C_{29}H_{38}N_4O_5 \cdot 2HBr$ (FD-MS: m/e 522, M^+ as free base). When ephedradine D dihydrobromide was administered to rats (1.5 mg/kg, i.v.), a significant hypotension was observed.

The IR spectrum of ephedradine D displayed bands at 3370 cm^{-1} for hydroxyl and/or amino groups and at 1620 cm^{-1} for amide groups, these bands being compatible with those of ephedradine A (3380 and 1631 cm^{-1}). In accord with this observation, ephedradine D on treatment with acetic anhydride in pyridine afforded the N,N,O-triacetate (II) (m.p. 167-173°, IR bands at 1751 (O-acetyl), 1640 cm^{-1} (N-acetyl)).

The ^1H NMR spectrum of ephedradine D disclosed signals at δ 3.86 (3H singlet), 4.62, 6.01 (1H doublet each in an AB type (J 11 Hz)), 6.77, 7.30 (2H multiplet each in an A_2B_2 type), 6.79 and 6.89 (1H singlet each).

The ^{13}C NMR spectrum of ephedradine D showed the presence of fifteen aliphatic carbons (CH_3-O x 1, CH_2 x 11, CH x 3), twelve aromatic carbons (CH x 6, C x 3, C-O x 3) and two carbonyl carbons (Table I).

The UV spectra of ephedradine D in methanol and in methanol in the presence of alkali (λ_{max}^{MeOH} 228, 283 nm, $\lambda_{max}^{MeOH-NaOH}$ 248, 291 nm) were similar to those of ephedradine A (λ_{max}^{MeOH} 229, 283 nm, $\lambda_{max}^{MeOH-NaOH}$ 243, 286 nm)[2]. The ^1H NMR parameters of all the signals in ephedradine D and its triacetate (II) also resembled those of ephedradine A and its triacetate except for those of the methoxyl and aromatic regions as will be discussed in detail later. Furthermore, the ^{13}C NMR parameters of the aliphatic and carbonyl carbon signals in ephedradine D and its triacetate (II) were in good agreement with those in ephedradine A and its triacetate (Table I[5]). These data,

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

	ephedradine D dihydrobromide (D_2O)	ephedradine A dihydrochloride (D_2O)	ephedradine D triacetate ($CDCl_3$)	ephedradine A triacetate ($CDCl_3$)
C-2	88.9 d	88.7 d	87.1 d	86.7 d
C-3	53.1 d	52.6 d	54.4 d	54.2 d
C-4	126.2 s	125.2 s	125.8 s	125.1 s
C-5	113.6 d	121.6 d	115.7 d	124.3 d
C-6	127.3 s	127.0 s	131.7 s	130.9 s
C-7	117.3 d	134.8 d	116.6 d	132.8 d
C-8	144.7 s	111.3 d	144.5 s	110.4 d
C-9	148.4 s	160.2 s	147.7 s	159.3 s
C-10	129.9 s	130.3 s	138.1 s	138.2 s
C-11	128.8 d	129.2 d	127.2 d	127.2 d
C-12	115.7 d	116.0 d	121.8 d	121.9 d
C-13	156.5 s	156.8 s	150.4 s	150.5 s
C-14	115.7 d	116.0 d	121.8 d	121.9 d
C-15	128.8 d	129.2 d	127.2 d	127.2 d
C-16	170.7 s*	171.1 s*	171.5 s*	170.5 s*
C-17	59.4 d	59.3 d	57.4 d	57.2 d
C-19	175.2 s*	175.5 s*	172.0 s*	172.1 s*
C-18 &	21.7 t	22.0 t	26.1 t	26.2 t
C-2'–	23.0 t	23.2 t	26.3 t	26.3 t
C-13'	25.3 t	25.9 t	28.1 t	28.0 t
	25.7 t	25.9 t	29.6 t	29.5 t
	38.0 t	38.1 t	37.1 t	37.5 t
	38.3 t	38.6 t	39.1 t	39.4 t
	42.0 t	42.1 t	44.4 t	44.3 t
	42.5 t	42.7 t	44.9 t	44.8 t
	44.7 t	45.0 t	45.3 t	45.3 t
	46.6 t	46.5 t	46.7 t	46.6 t
	46.6 t	46.7 t	51.1 t	51.1 t
$\underline{CH_3}O$	56.4 q		56.4 q	
$\underline{CH_3}CO$			21.1 q	21.1 q
			21.7 q	21.8 q
			22.6 q	22.6 q
$CH_3\underline{CO}$			169.5 s*	169.6 s*
			169.9 s*	170.5 s*
			170.7 s*	172.1 s*

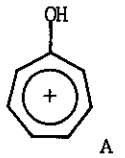
Abbreviations: s=singlet, d=doublet, t=triplet, q=quadruplet
The assignments of the asterisked signals are ambiguous and might have to be reversed

together with the fact that ephedradine D coexists with the other ephedradines in the same Ephedra, indicated that ephedradine D possesses the common structural feature including the spermine moiety as in known ephedradines. The ^1H NMR doublets at δ 4.62 and 6.01 in an AB pattern (J 11 Hz) and the ^{13}C NMR doublets at δ 53.1 and 88.9 were consistent with the signals originating from the hydrogens and carbons at the 2 and 3 positions of ephedradine A (Table I), demonstrating ephedradine D to have the dihydrobenzofuran moiety with the phenyl and carbonyl substituents at $C_{(2)}$ and $C_{(3)}$ in the trans-configuration as in ephedradine A.

Ephedradine D in its ^1H NMR spectrum showed a methoxy methyl signal and six aromatic hydrogen signals while ephedradine A exhibited no methoxy methyl signal and instead seven aromatic hydrogen signals. Ephedradine D gave a positive reaction for $FeCl_3$ test as ephedradine A. These findings along with the molecular formula of ephedradine D which possesses an extra CH_2O moiety as a methoxyl group as compared with ephedradine A indicated that ephedradine D might be a methoxy derivative of ephedradine A.

The location of the free phenolic hydroxyl group and the methoxyl group came into question. In the ^1H NMR spectrum, two signals (2H each) appeared at δ 6.77 and 7.30 in an A_2B_2 type, a fact

which revealed the presence of a p-substituted phenyl grouping. The observed chemical shifts of ^{13}C NMR signals associated with the six aromatic carbons in the p-substituted phenyl group (δ 129.9, 128.8, 115.7, 156.5, 115.7, 128.8 for $C_{(1)}$—$C_{(6)}$) were comparable with the chemical shifts calculated by addition of the substituent parameters of the hydroxyl or methoxyl group and the hydroxymethyl group to the chemical shift of the carbons of benzene (δ 128.7)[6] (δ 133.1, 129.1, 114.7, 154.2, 114.7, 129.1 for $C_{(1)}$—$C_{(6)}$ in p-hydroxybenzyl alcohol or δ 132.1, 127.3, 111.8, 157.5, 111.8, 127.3 for $C_{(1)}$—$C_{(6)}$ in p-methoxybenzyl alcohol), confirming the presence of a p-substituted phenol or p-substituted anisole moiety in ephedradine D. Further, the six ^{13}C NMR resonances in ephedradine D were in accord with those for $C_{(10)}$ $C_{(15)}$ in ephedradine A (Table I). These observations led to the conclusion that the substituent at $C_{(2)}$ in the dihydrobenzofuran system is a p-hydroxyphenyl grouping, which was substantiated by the fact that the mass spectrum of ephedradine D exhibited the base peak at m/e 107 due to the ion A.



A

The last problem to be solved was the situation of the methoxyl group. 1) The ^{1}H NMR signal attributed to the methoxyl hydrogens occurred at δ 3.86 which was consistent with that for a methoxyl on aromatic carbon but not with that for a methoxyl on aliphatic carbon and 2) the ^{13}C NMR spectrum showed that the parameters for the aliphatic carbons were in good accord with those in ephedradine A and the benzene nucleus in the dihydrobenzofuran moiety had one extra carbon carrying an oxygen function instead of a carbon bearing a hydrogen in ephedradine A. In support of these observations, the ^{1}H NMR spectrum exhibited only two signals at δ 6.79 and 6.89 ascribable to aromatic hydrogens on the dihydrobenzofuran system. These data indicated that the methoxyl must be located at the benzene nucleus in the dihydrobenzofuran. In order to select the three possible substitution patterns, B, C and D, the chemical shifts were calculated on the basis of the carbon resonance for benzene (δ 128.7) by adding the substitution effects for the methyl and the methoxyl[6] (Table II). As a result it was found that the observed resonances which

Table II. Carbon-13 shieldings in dimethyl-dimethoxybenzenes (δ)

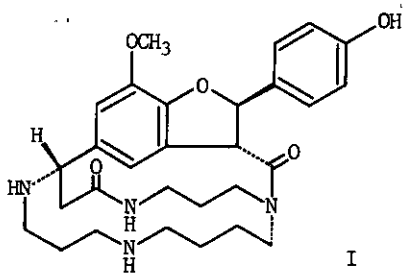
	C-1	C-2	C-3	C-4	C-5	C-6
1,3-dimethyl-5,6-dimethoxybenzene (B)	122.0	121.0	128.6	111.4	143.0	141.6
1,3-dimethyl-4,6-dimethoxybenzene (C)	113.1	129.9	113.1	157.1	97.3	157.1
1,3-dimethyl-2,6-dimethoxybenzene (D)	106.5	160.1	113.1	126.9	103.9	157.1

occurred in a rather higher field region (δ 144.7 and 148.4) for oxygen-bearing carbons could be rationalized only if the two oxygen functions were located at the ortho-positions (Table I, II). On the other hand, if they were situated at the meta-positions, two resonances for the oxygen-carrying carbons should appear at δ 157-160 and one resonance should occur around δ 100 which were not consistent with the observed shifts (Table I, II). Based on the above evidence, it was concluded that the methoxyl group is located at $C_{(8)}$ in the dihydrobenzofuran moiety. This was further verified by the observation that the two aromatic hydrogens at δ 6.79 and 6.89 appeared as singlet-like signals in the ^{1}H NMR spectrum.

Comparison of the CD curve of ephedradine D dihydrobromide (Cotton effects at 292 ([θ] -2.0×10^3), 283 ([θ] $+1.3 \times 10^3$) and 242 nm ([θ] -4.2×10^4) in MeOH) with that of ephedradine A dihydrobromide (Cotton effects at 302 ([θ] -8.5×10^2), 281 ([θ] $+7.5 \times 10^3$) and 233 nm ([θ] -4.9×10^4) in MeOH) revealed that their patterns are identical although their rotatory strengths are different. This discrepancy in the rotatory strengths is considered to be originating from the decrease of the strengths at 281 and 233 nm due to the alteration of the strength of the dipole coupling between the transition moment of the dihydrobenzofuran moiety and that of the phenyl

moiety induced by the introduction of the methoxyl group at $C_{(8)}$. Because the CD curve of ephedradine D is essentially the same as the CD curves of the other ephedradines, it follows that the absolute configuration at $C_{(2)}$ is R and consequently that at $C_{(3)}$ is also R in ephedradine D.

The absolute stereostructure of ephedradine D has thus been elucidated as represented by formula I.



I

NOTES AND REFERENCES

※. Dedicated to Prof. K. Tsuda on his 75th birthday.

1. Studies on the constituents of Ephedra. IX. This paper also forms Part 31 in the series on the validity of the Oriental medicines.
2. M. Tamada, K. Endo, H. Hikino and C. Kabuto, Tetrahedron Letters, 1979, 873.
3. M. Tamada, K. Endo and H. Hikino, Heterocycles, 1979, **12**, 783.
4. C. Konno, M. Tamada, K. Endo and H. Hikino, Heterocycles, 1980, **14**, 295.
5. The previous assignments of certain ^{13}C NMR resonances of ephedradine A and its triacetate[2] have now been revised as shown in Table I, because on determination of the ^{13}C NMR spectra of ephedradine D and its triacetate, we became to know that they are more reasonably rationalized by the present assignments.
6. J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, p. 197; p. 201.

Received, 10th August, 1981