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

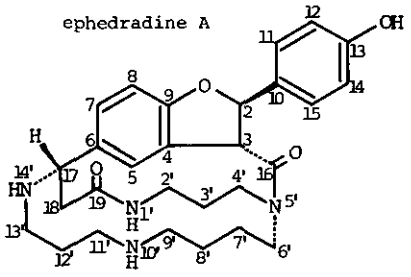
Mitsuru Tamada, Katsuya Endo and Hiroshi Hikino*

Pharmaceutical Institute, Tohoku University, Aoba-yama, Sendai, Japan

Abstract — From the crude drug "maŏ-kon", the roots of *Ephedra* plants, a novel macrocyclic spermine alkaloid ephedradine B showing the hypotensive activity has been isolated whose stereostructure has been elucidated as represented by formula I on the basis of chemical and physical evidence.

The crude drug "maŏ-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 a hypotensive principle ephedradine A and established its stereostructure.[2] During the course of the isolation of ephedradine A, the alkaloid fraction from the extract was subjected to repeated chromatography (alumina/AcOEt-MeOH-H_2O) by monitoring the hypotensive activity. From the fraction eluted after ephedradine A, another alkaloid was obtained and termed as ephedradine B.

ephedradine A



Although ephedradine B has not yet been crystallized, it was characterized as the dihydrobromide, m.p. 219-221°, $[\alpha]_D$ -101.5° (H_2O), $C_{29}H_{38}N_4O_5 \cdot 2HBr \cdot H_2O$ (FD-MS (*m/e* 523, M^++1 as free base) and elemental analysis). Administration of the salt to rats (2.0 mg/kg, *i.v.*) induced a significant hypotension.

The ^1H NMR spectrum of ephedradine B was quite similar to that of ephedradine A 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 fifteen aliphatic carbons (CH_3-O × 1, 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 B also corresponded to those of ephedradine A (Table I). These data, together with the fact that ephedradine B coexists with ephedradine A, led to the supposition that ephedradine B is an analog of ephedradine A and has an extra CH_2O moiety as a methoxyl group.

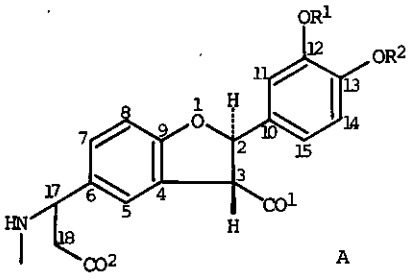
In support of this supposition, acetylation of ephedradine B with acetic anhydride in methanol afforded the N,N-diacetate (II) (an IR band at 1629 cm^{-1} (N-acyl), $FeCl_3$ test: violet), while its acetylation with acetic anhydride in pyridine yielded the N,N,O-triacetate (III), m.p. 170-183° (a MS peak at *m/e* 648.3206 (M^+), IR bands at 1758 (O-acetyl) and 1613 cm^{-1} (N-acyl), $FeCl_3$ test: negative), indicating that ephedradine B possesses, besides a phenolic hydroxyl, two acetylatable amino groups (primary and/or secondary) as in ephedradine A.

The presence of a 1-oxygenated-2,4-dialkylbenzene moiety ($C_{(4)}$-$C_{(9)}$ in formula A) was shown by three 1H signals in the ^1H NMR spectrum of ephedradine B dihydrobromide at δ 6.96, 7.40 and 7.16 in an ABC type which were consistent with the corresponding signals in that of ephedradine A dihydrobromide (δ 7.01, 7.35 and 7.16) and by the occurrence of the ^{13}C NMR signals for $C_{(4)}$-$C_{(9)}$ in ephe-

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

	ephedradine B dihydrobromide (D_2O)	ephedradine A dihydrochloride (D_2O)	ephedradine B triacetate ($CDCl_3$)	ephedradine A triacetate ($CDCl_3$)
C-2	88.7 d	88.7 d	86.5 d	86.7 d
C-3	52.5 d	52.6 d	54.1 d	54.2 d
C-4	125.3 s	125.2 s	125.0 s	125.1 s
C-5	134.3 d	134.8 d	132.3 d	132.8 d
C-6	126.9 s	127.0 s	131.0 s	130.9 s
C-7	121.5 d	121.6 d	124.4 d	124.3 d
C-8	111.1 d	111.3 d	110.2 d	110.4 d
C-9	159.9 s	160.2 s	159.0 s	159.3 s
C-10	130.8 s	130.3 s	139.2 s	138.2 s
C-11	111.1 d	129.2 d	110.2 d	127.2 d
C-12	147.9 s	111.6 d	151.0 s	121.9 d
C-13	145.9 s	156.8 s	139.8 s	150.5 s
C-14	115.7 d	111.6 d	122.9 d	121.9 d
C-15	120.5 d	129.2 d	117.6 d	127.2 d
C-16	171.1 s*	171.1 s*	171.3 s*	170.5 s*
C-17	59.2 d	59.3 d	57.0 d	57.2 d
C-19	175.2 s*	175.5 s*	171.9 s*	172.1 s*
C-18 &	21.8 t	22.0 t	26.0 t	26.2 t
C-2'-	23.1 t	23.2 t	26.3 t	26.3 t
C-13'	25.7 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	37.0 t	37.5 t
	38.0 t	38.6 t	39.0 t	39.4 t
	42.3 t	42.1 t	44.2 t	44.3 t
	42.7 t	42.7 t	44.6 t	44.8 t
	44.8 t	45.0 t	45.3 t	45.3 t
	46.5 t	46.5 t	46.6 t	46.6 t
	46.5 t	46.7 t	51.0 t	51.1 t
$\underline{C}H_3O$	56.4 q		56.0 q	
$\underline{C}H_3CO$			20.5 q	21.1 q
			21.7 q	21.8 q
			22.6 q	22.6 q
$CH_3\underline{C}O$			169.4 s*	169.6 s*
			169.7 s*	170.5 s*
			170.4 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.



A

dradine B which were coincident with those attributed to the same moiety in ephedradine A (Table I). Extension of the 1,2,4-trisubstituted benzene moiety to a dihydrobenzofuran moiety ($O_{(1)}$-$C_{(9)}$ in formula A) was performed by the absence of phenolic hydroxyls ($FeCl_3$ test) in the N,N,O-triacetate (III) which had an acetoxyl group at the other position of the molecule as will be revealed below, by the UV spectrum of ephedradine B dihydrobromide (maxima at 232 (log ε 4.32) and 281 nm (log ε 3.92) in MeOH) which closely resembled that of ephedradine A dihydrobromide (maxima at 229 (log ε 4.40) and 281 nm (log ε 3.76) in MeOH), and by two 1H NMR signals in ephedradine B at δ 6.00 and 4.62 in an AB type which were in accord with the corresponding signals in ephedradine A (δ 6.02 and 4.60 for $H_{(2)}$ and $H_{(3)}$). The above coincidence of the ^1H NMR signals for $H_{(2)}$ and $H_{(3)}$, along with the splitting patterns of these signals, indicating that $C_{(2)}$ carries a phenyl and $C_{(3)}$ bears a carbonyl (CO^1) in ephedradine

B. The large coupling constant (J 11 Hz) between $H_{(2)}$ and $H_{(3)}$ showed that the phenyl group and the carbonyl group are located in the *trans* configuration on the dihydrofuran ring (formula A).

In order to prove the presence of a spermine moiety which might be connected to a diacid moiety in amide linkages as in ephedradine A, ephedradine B was hydrolyzed with potassium hydroxide solution to furnish spermine (the tetrahydrochloride, m.p. 311-312°, a MS peak at *m/e* 203 (M^++1)).

A methine and a methylene remained to be allotted. The ^{1}H NMR signals for the methine hydrogens in the diacetate (II) and the triacetate (III) occurred at a lower field region (δ 5.52 and 5.47, respectively) as a doublet of doublets (J 5 and 3 Hz) which were in agreement with those (a doublet of doublets at δ 5.48 and 5.52 (J 5 and 3 Hz)) of the corresponding acetates of ephedradine A, a fact which indicated that the methine carries 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 hence concluded to be adjacent to the methylene ($C_{(18)}$) (formula A). The relatively facile fission of the N-$C_{(17)}$ bond by the alkali treatment of ephedradine B to spermine is well explained by this situation of the nitrogen atom (a benzylic position and β to the carbonyl).

The coincidence of the chemical shifts and splitting patterns of the ^{1}H NMR signals for the methine hydrogens ($H_{(17)}$) in the acetates of ephedradine B and ephedradine A, as mentioned above, and that of the chemical shifts of the ^{13}C NMR signals originating from the methylene carbons of the spermine parts ($C_{(2')}$-$C_{(13')}$) in ephedradine B and ephedradine A (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 B and ephedradine A were therefore concluded to be different in their phenyl side chain ($C_{(10)}$-$C_{(15)}$). In fact, the UV spectrum in methanol in the presence of alkali of ephedradine B dihydrobromide (maxima at 238 (sh, log ε 4.24), 249 (sh, log ε 4.21) and 288 nm (log ε 3.94) was not compatible with that of ephedradine A dihydrobromide (maxima at 243 (log ε 4.38) and 286 nm (log ε 3.84)) although the UV spectrum in methanol of ephedradine B resembled that of ephedradine A as described above. Six ^{13}C NMR signals ascribed to this phenyl group ($C_{(10)}$-$C_{(15)}$) in ephedradine B revealed the presence of three CH's, one C and two C-O's. Further, three 1H NMR signals at δ 6.94, 6.87 and 7.11 in an ABC type in ephedradine B dihydrobromide indicated the existence of another 1,2,4-trisubstituted benzene moiety for which two substitution patterns were possible, *i.e.* a 3,4-dioxygenated-1-alkylbenzene and a 2,4-dioxygenated-1-alkylbenzene. The chemical shifts of the above three ^{1}H NMR signals showed that it was more precisely defined as a 3,4-dioxygenated-1-alkylbenzene. In accordance with this deduction, the observed chemical shifts of the six ^{13}C NMR signals for $C_{(10)}$-$C_{(15)}$ (Table I) were also consistent with the calculated shifts of $C_{(1)}$-$C_{(6)}$ in a 3,4-dioxygenated-1-alkylbenzene (Table II, 1 or 2) but not with those of a 2,4-dioxygenated-1-alkylbenzene (Table II, 3 or 4).[3] In the ^{1}H NMR spectrum of ephedradine B triacetate (III), an intramolecular nuclear Overhauser effect was found between the methoxyl hydrogen signal at δ 3.81 and the $H_{(11)}$ signal at δ 7.03, an observation which demonstrated that a methoxyl and a hydroxyl are situated at $C_{(12)}$ and $C_{(13)}$, respectively. This substitution pattern was further confirmed by

Table II. Carbon-13 shieldings in the hydroxy-methoxy-methylbenzenes (δ)

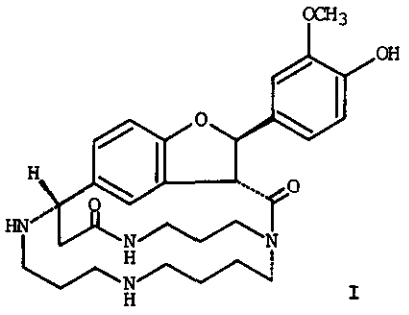
	C-1	C-2	C-3	C-4	C-5	C-6
4-hydroxy-3-methoxy-1-methylbenzene (1)	131.1	116.2	147.1	138.1	116.7	122.9
3-hydroxy-4-methoxy-1-methylbenzene (2)	131.1	117.5	140.9	144.3	115.4	122.9
4-hydroxy-2-methoxy-1-methylbenzene (3)	115.7	162.0	101.3	153.5	108.0	131.6
2-hydroxy-4-methoxy-1-methylbenzene (4)	117.4	157.1	101.3	158.4	106.7	131.6

the following evidence. Thus, the assignments of the $C_{(10)}$-$C_{(15)}$ resonances of ephedradine B which had either the 13-hydroxy-12-methoxy arrangement or the 12-hydroxy-13-methoxy arrangement were performed (Table III) by the chemical shift considerations in comparison with the calculated

Table III. Carbon-13 shieldings of the phenyl side chain in ephedradine B and its acetate

	C-10	C-11	C-12	C-13	C-14	C-15
in the case of the 13-hydroxy-12-methoxy derivative						
δephedradine B (free, obs)	130.8	111.1	147.9	145.9	115.7	120.5
δephedradine B triacetate (calc)	138.7	109.1	158.2	139.6	126.0	118.5
δephedradine B triacetate (obs)	139.2	110.2	151.0	139.8	122.9	117.6
Δδtriacetate-free (obs)	+8.4	-0.9	+3.1	-6.1	+7.2	-2.9
in the case of the 12-hydroxy-13-methoxy derivative						
δephedradine B (free, obs)	130.8	115.7	145.9	147.9	111.1	120.5
δephedradine B triacetate (calc)	128.8	126.0	139.6	158.2	109.1	128.4
δephedradine B triacetate (obs)	139.2	117.6	139.8	151.0	110.2	122.9
Δδtriacetate-free (obs)	+8.4	+1.9	-6.1	+3.1	-0.9	+2.4

resonances for $C_{(1)}$-$C_{(6)}$ in 4-hydroxy-3-methoxy-1-methylbenzene (Table II, 1) and 3-hydroxy-4-methoxy-1-methylbenzene (Table II, 2). The predicted resonances for $C_{(10)}$-$C_{(15)}$ in ephedradine B triacetate (III) were then calculated by adding the acetylation effects on the carbon resonances in ephedradine B, the paramagnetic shifts of the carbon bearing the phenolic hydroxyl, and its *o*-, *m*- and *p*-carbons on going from ephedradine A to its triacetate being -6.3, +10.3, -2.0 and +7.9 ppm, respectively. Comparison of the predicted resonances with the observed ones for $C_{(10)}$-$C_{(15)}$ in ephedradine B triacetate (III) clearly demonstrated the fair identity for the two sets of data if ephedradine B were the 13-hydroxy-12-methoxy derivative but the significant discrepancy for two sets of data if it were the 12-hydroxy-13-methoxy derivative (Table III). Furthermore, the observed acetylation displacements of the $C_{(10)}$-$C_{(15)}$ resonances on passing from ephedradine B to its triacetate (III) distinctly verified the validity of the above conclusion that ephedradine B possessed the 13-hydroxy-12-methoxy arrangement (Table III).



I

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

On the basis of the above evidence, ephedradine B is concluded to have the stereostructure I.

NOTE AND REFERENCES

1. Studies on the constituents of *Ephedra*. V. This paper also forms Part 14 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. G. L. Nelson, G. C. Levy and J. D. Cargioli, *J. Amer. Chem. Soc.*, 1972, 94, 3089.

Received, 20th March, 1979