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

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The crude drug "maō", the aerial part of *Ephedra* plants (Ephedraceae), is famous for containing the alkaloids of the ephedrine series[2] which are responsible for the perspiratory, antitussive and anti-allergic effects of the crude drug. While the crude drug "maō-kon", the underground part of the *Ephedra* plants, is said to have the therapeutic effects opposite to those of "maō" and has been used as an antiperspirant in Oriental Medicine. However, the chemical and pharmacological study of *Ephedra* roots has been reported only by Fujii[3] who examined some pharmacological actions of a preparation and demonstrated the presence of a hypotensive principle which was not characterized. We have thus performed elucidation of the active principle.

The methanol extract of a preparation of "maō-kon" was dosed to rats (2 g (crude drug)/kg, *i.v.*) to cause a marked hypotension. The extract was then partitioned with dil. hydrochloric acid and *n*-butanol, and the acidic solution made alkaline with ammonia and extracted with *n*-butanol to yield the alkaloid fraction which was pharmacologically active. The fraction was repeatedly chromatographed (alumina/AcOEt-MeOH-H_2O), by monitoring the hypotensive activity, furnishing, together with the new betaine, maokonine, with a weak hypertensive activity,[4] the crystalline alkaloid, now designated as ephedradine A, m.p. 166° (dec.).

Ephedradine A was characterized as the dihydrochloride, m.p. 222-225°, $C_{28}H_{36}N_4O_4 \cdot 2HCl \cdot H_2O$ (elemental analysis), and as the dihydrobromide, m.p. 233-235°, $C_{28}H_{36}N_4O_4 \cdot 2HBr \cdot H_2O$ (FD-MS (*m/e* 493, M^++1 as free base) and elemental analysis). Administration of these salts to rats (1.5-1.(mg/kg, *i.v.*) elicited a significant blood pressure fall.

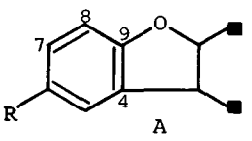
The ^{13}C NMR spectrum showed the presence of fourteen aliphatic carbons ($CH_2 \times 11$, $CH \times 2$, $CH\text{-}O \times 1$), twelve aromatic carbons ($CH \times 7$, $C \times 3$, $C\text{-}O \times 2$) and two carbonyl carbons.

Two ^{13}C NMR signals occurred at δ 171.1 and 175.5 and the IR spectrum disclosed a carbonyl band only at 1631 cm^{-1}, demonstrating that the two carbonyls constituted two amide groups.

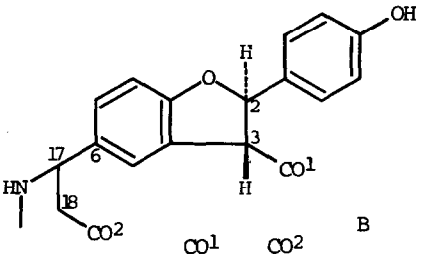
The presence of a *p*-substituted phenol moiety was indicated by a positive $FeCl_3$ test, UV maxima at 229 and 283 nm (the former showed a red shift by 14 nm on addition of alkali), ^1H NMR signals at δ 6.77 and 7.26 (2H each) in an A_2B_2 type, a ^{13}C NMR signal at δ 156.8 and addition o bromine to yield the dibromide, m.p. 288° (dec.) (^1H NMR signal at δ 7.42 (2H singlet)).

Acetylation of ephedradine A with acetic anhydride in methanol gave the N,N-diacetate (II) (an IR band at 1617 cm^{-1} (N-acyl), $FeCl_3$ test: violet), whereas acetylation with acetic anhydrid in pyridine afforded the N,N,O-triacetate (III), m.p. 167-175° (IR bands at 1745 (O-acetyl) and 1612 cm^{-1} (N-acyl), $FeCl_3$ test: negative), showing that ephedradine A has, besides the phenolic hydroxyl, two acetylatable amino groups (primary and/or secondary). This coincided with the above findings that ephedradine A formed a dihydrochloride and a dihydrobromide.

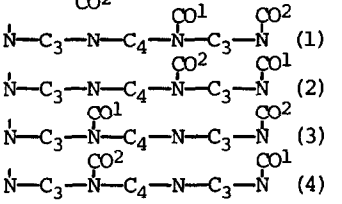
A second aromatic ring in ephedradine A indicated by the ^{13}C NMR spectrum was deduced to be a 1,2,4-trisubstituted benzene by three ^{1}H NMR signals at δ 6.75, 7.12 and 7.18 in an ABX type. Since the triacetate (III) had no more phenolic hydroxyls ($FeCl_3$ test) and a ^{13}C NMR signal appeared at δ 160.2 in ephedradine A, an ethereal oxygen must be attached to the benzene ring. The ^{1}H NMR signal for $H_{(8)}$ resonated higher by 0.37 ppm than that for $H_{(7)}$ in ephedradine A (both constituted the AB part of an ABX type and the former was sharper than the latter). Therefore, the ethereal oxygen should be attached to $C_{(9)}$ in part structure A. In the ^{1}H NMR spectrum of ephedradine A, two signals at δ 4.60 and 6.02 in an AB type were also observed. The lower field position of the former (δ 6.02) along with a ^{13}C NMR signal at δ 88.7 indicated that a methine is attached to the ether oxygen and to the phenyl or a carbonyl. Since no more quaternary carbons are present in the molecule (^{13}C NMR spectrum), the splitting pattern of the signal at δ 4.60 showed that a methine is α to $C_{(4)}$ and to the remaining carbonyl or phenyl, thereby indicating the presence of a dihydrobenzofuran moiety (A) being deduced. Hydrogenation of ephedradine A over palladium in acetic acid gave the hydrogenolysis product (no ^{1}H NMR signals in an AB type), demonstrating that $C_{(2)}$ is located at a benzylic position and consequently that $C_{(3)}$ carries a carbonyl. The large coupling constant (*J* 11 Hz) between $H_{(2)}$ and $H_{(3)}$ indicated that the *p*-hydroxyphenyl group and the amide group (CO^1) are situated in the *trans* configuration on the dihydrobenzofuran ring (formula B).



A



B



In order to cleave the amide linkages, ephedradine A was hydrolyzed with hydrochloric acid to give a tetramine, $C_{10}H_{26}N_4$ (the tetrahydrochloride, m.p. 312-314°). From the composition (no double bond equivalent) and the spectral properties (no ^{1}H NMR signals for a C-terminal or for $N-CH_2-N$ or $N-CH_2-CH_2-N$ moieties were visible and the ^{13}C NMR spectrum showed the presence of five kinds of methylenes (a symmetrical molecule) in which there were three C-C-N and two C-C-C carbons), the tetramine was deduced to be spermine. Confirmation was obtained by direct comparison.

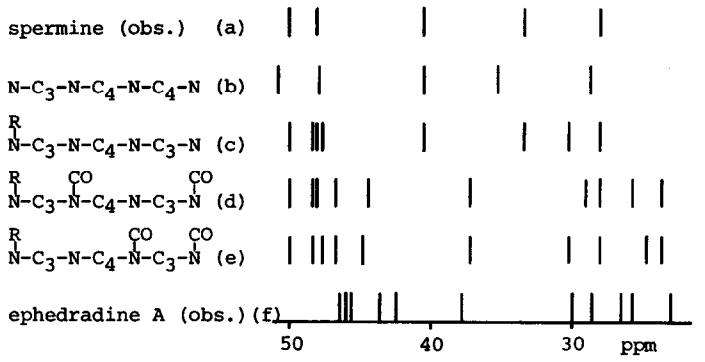
A methine and a methylene remained to be assigned. The ^{1}H NMR signals for the methine hydrogens in the diacetate (II) and the triacetate (III) appeared at a lower field region (δ 5.48 and 5.52, respectively) as a doublet of doublets (*J* 11 and 6 Hz), showing that the methine bears the remaining methylene, a nitrogen atom and $C_{(6)}$ as in formula B. The relatively facile cleavage of the $N-C_{(17)}$ bond in the acid treatment of ephedradine A to spermine is well rationalized by this location of the nitrogen atom (a benzylic position and β to the carbonyl). One of the two carbonyls (CO^2) was therefore concluded to be next to the methylene ($C_{(18)}$) (formula B).

Among the four nitrogen atoms in the spermine part, two constituted the amide groups in ephedradine A. Since ephedradine A forms the N,N-diacetate (II) and since no primary amine group is present in ephedradine A (it gave a weak purple coloration with ninhydrin only under forcing conditions), an ω-amino group must be attached to $C_{(17)}$ and another ω-amino group must constitute an amide group. The two carbonyls (CO^1 and CO^2) are not equivalent, so that potentially four combinations 1-4 (formula B) could be considered in which the combination 4 was

thought to be unlikely due to the severe compression of the molecule.

The remaining problem to be settled was therefore the combination of the diacid and the spermine units. For this purpose, the observed chemical shifts of the ^{13}C NMR signals of the methylene carbons of ephedradine A were to be compared with the calculated ones of those of appropriate model compounds. Thus, addition of the substituent parameters of the NH_2- and RNH-groups to the chemical shifts of the carbons of *n*-propane and *n*-butane led to the calculated values for those of spermine which were compatible with the observed values for spermine (Fig. 1, a and b). The calculated values for those of ω-N-alkyl spermine were deduced in a similar way (Fig. 1, c). Further addition of the substitution parameters of the N-acylation, estimated from those of *n*-propylamine and acetyl-*n*-propylamine, gave calculated values for those of the two model compounds, N,N-diacyl-ω-N-alkyl spermines (Fig. 1, d and e). Neither set of the calculated values, however, was consistent with the set of the observed values for those of ephedradine A (the methylene part, Fig. 1, f). The failure of a combination mode is probably due to the difference of the stereochemical environments of ephedradine A and the model compounds.

Fig. 1 ^{13}C NMR of spermine analogs and ephedradine A



In order to establish the structure of ephedradine A, a single crystal X-ray analysis was performed. The crystals of the dihydrobromide were found to belong to a rather unusual tetragonal form, and the systematic extinctions (001. absent if 4n+1, 4n+2 and 4n+3) indicated the space group of $P4_1$ or $P4_3$. Precise measurement of 2θ for 15 high angle reflections gave a=10.450 (2) and c=28.130 (4) Å. A total of 3129 independent reflections (within 2θ=140°) were measured on an automatically controlled four cycle diffractometer using Cu-K_α radiation. The structure was solved by the heavy atom methods. Positions for the two bromine atoms were deduced from a sharpened Patterson map and eventually determined by means of structure factor calculations. A series of successive Fourier maps located other non-hydrogen atoms and finally one water molecule. All atoms were initially identified as carbons, and careful inspection of the isotropically refined temperature factors and molecular geometry allowed unambiguous assignment of oxygen and nitrogen atoms except for one nitrogen atom which could be at either 7' or 14', and the choice was made by the previous chemical information that spermine was obtained as a degradation product. All hydrogen atoms except for the amide group at 1' were readily located from a difference map, although some of them were not well refined. The block-diagonal least-squares refinement of non-hydrogen atomic parameters with fixed contribution from the hydrogen atoms resulted in the successful convergence of the crystal structure of ephedradine A as in the ORTEP drawing (Fig. 2).

At this stage, identification of the space group was tested by two independent refinements with the anomalous dispersion correction for the bromine atoms.[5] Refinement of the structural parameters for the space group of $P4_1$ converged the R-factor to 0.0724, while the refinement with the inverted coordinate for the enantiomorphic space group of $P4_3$ converged the value of 0.0741.

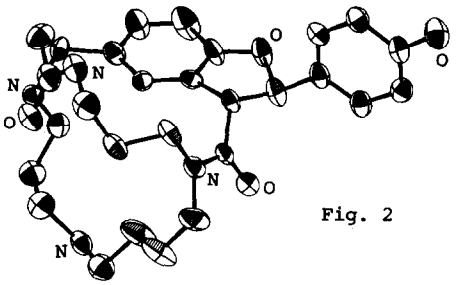
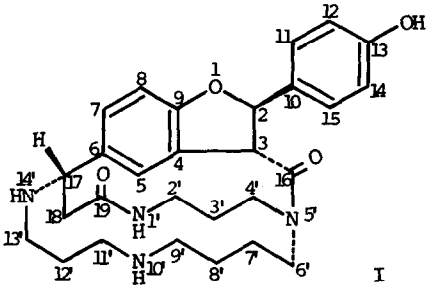


Fig. 2



I

According to the Hamilton's R-factor significance test,[6] the ratio of the agreement factor ($R(P4_3)/R(P4_1)=1.023$) favored the space group of $P4_1$, which corresponded to the absolute stereochemistry of the 2*R*,3*R*,17*S*-configuration. This was substantiated by the observation that the dihydrobromide exhibited the Cotton effects at 281 ([θ] 7.5×10^3) and 233 nm ([θ] 4.9×10^4) in the CD spectrum (MeOH). These chiroptical properties are in good accord with those of the model compound, (-)-homopterocarpin, having the 2*R*,3*R*-configuration.[7] The absolute stereostructure of ephedradine A was thus established as in formula I.

One of the interesting features of this structure is the existence of strong intramolecular hydrogen bonding between the amide carbonyl group at 19 and the amino group at 14'. The distance of 2.26 Å between these atoms is one of the shortest values found in similar situations.[8] A relatively large contribution from the resultant resonance form O-C=NH- is evidenced by the significant elongation of the C=O bond length (1.247 Å) and the shortening of the C=N bond length (1.292 Å). The crystal structure of the dihydrobromide is constructed by the network of hydrogen bondings. The amino group at 14' is associated through a hydrogen bond with a bromine atom at a distance of 3.23 Å. A remarkable feature is that the amino group at 10' is associated with two atoms of bromine which are shared by the same nitrogen atom of another molecule situated in a symmetrical position. The interatomic distances of 3.16 and 3.29 Å between the nitrogen and the bromines indicate the presence of effective hydrogen bondings, since these values are in good agreement with those found in other structures containing bromine which vary from 3.12 to 3.48 Å.[9] A water molecule also serves as hydrogen bonding bridge between the two bromine atoms, thereby increasing the stability of the crystalline state of ephedradine A. There were no other abnormally short intermolecular distances found in the crystal structure.

The biosynthesis of ephedradine A may be envisaged as the union of the base spermine with a phenolic diacid which appears to be biosynthesized from two molecules of *p*-coumaric acid.

NOTE AND REFERENCES

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