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STRUCTURE OF EPHEDRANNIN A, A HYPOTENSIVE PRINCIPLE OF EPHEDRA ROOTS[1]

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Abstract —— A novel flavano-flavonol ephedrannin A showing the hypotensive activity has been isolated from the crude drug "maō-kon", the roots of Ephedra plants. The stereostructure of ephedrannin A has been elucidated as that represented by formula I on the basis of chemical and physical evidence.

The crude drug "maō-kon", the underground part of Ephedra plants (Ephedraceae), has been employed as an antiperspirant in Oriental medicine. The chemical and pharmacological investigation of the crude drug, Ephedra roots, was previously carried out by Fujii who revealed that it possessed the hypotensive activity.[2] Recently we have reconfirmed the hypotensive effect of the crude drug and fractionated the extract by monitoring this pharmacological activity to obtain from the basic fraction the novel macrocyclic spermine alkaloids, ephedradine A, B, C and D, as the active principles.[3-6]

During the course of the above fractionation, we have found that the acidic fraction of the extract also exhibited the hypotensive activity. Therefore, the acidic fraction was fractionated by monitoring the hypotensive activity to give a polyphenol which was termed as ephedrannin A. The i. v. dosing induced a significant hypotension similar to that by the ephedradines in rats.

Ephedrannin A, m.p. >360°, $[\alpha]_D$ -357° (MeOH), analyzed for $C_{30}H_{20}O_{11}\cdot 2H_2O$ which was substantiated by the presence of molecular ion peak at m/e 556 in the FD-mass spectrum.

The ^{13}C NMR spectrum exhibited the presence of three aliphatic carbons (CH x 1, CH-O x 1, O-C-O x 1), twenty-six olefinic and aromatic carbons (CH x 11, C x 5, C-O x 10) and one carbonyl carbon (Table I).

Ephedrannin A showed in the IR spectrum[7] a band at 1655 cm^{-1} assigned to a carbonyl group conjugated and chelated which should be ascribed to the one deduced from the above ^{13}C NMR evidence. The IR spectrum also displayed a strong band at 3380 cm^{-1}, indicating it to have hydroxyl groups. Because ephedrannin A gave a positive ferric chloride test, it was concluded that some of the hydroxyls were phenolic. Methylation of ephedrannin A with dimethyl sulfate and potassium carbonate in acetone afforded the pentamethyl ether (II) and the hexamethyl ether (III), a fact which demonstrated that ephedrannin A possessed six phenolic (enolic) hydroxyls. When the ether (III) was acetylated with acetic anhydride in pyridine, the hexamethyl ether monoacetate (IV) was obtained, showing that ephedrannin A had one alcoholic hydroxyl. Since the IR spectrum of the ether acetate (IV) disclosed no band associated with hydroxyl, the remaining three oxygen atoms should constitute oxide linkages.

In agreement with the above ^{13}C NMR data, the ^{1}H NMR spectrum of ephedrannin A indicated the presence of two methine hydrogens (δ 4.19 and 4.96 in an AB type, J 4.0 Hz, >C-CH-CH-C<) and eleven aromatic (olefinic) hydrogens (δ 5.94-8.48). When the seven hydroxyl hydrogens are

Table I. Carbon-13 shieldings in ephedrannin A and related substances (δ)

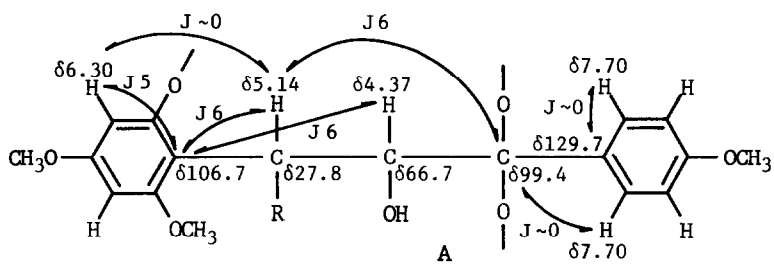
	ephedrannin A (acetone-d_6)	ephedrannin A hexamethyl ether ($CDCl_3$)	ephedrannin A hexamethyl ether monoacetate ($CDCl_3$)	catechin (CD_3OD)	kaempferol (DMSO-d_6)
C-2	100.6 s	99.4 s	98.0 s	82.7 d	
C-3	67.2 d	66.7 d	67.1 d	68.6 d	
C-4	29.1 d	27.8 d	26.0 d	28.4 t	
C-5	157.4 s*	160.2 s*	160.0 s*	157.3 s	
C-6	97.2 d	93.2 d	93.0 d	96.2 d	
C-7	157.4 s*	158.8 s*	158.2 s*	156.7 s	
C-8	95.9 d	93.3 d	93.4 d	95.5 d	
C-9	158.0 s*	160.4 s*	160.3 s*	157.6 s	
C-10	105.1 s	106.7 s	106.1 s	100.7 s	
C-11	130.7 s	129.7 s	129.3 s	131.9 s	
C-12	129.2 d	128.1 d	128.0 d	116.0 d	
C-13	116.1 d	113.9 d	113.9 d	146.0 s	
C-14	152.6 s*	152.5 s*	152.6 s*	146.0 s	
C-15	116.1 d	113.9 d	113.9 d	115.1 d	
C-16	129.2 d	128.1 d	128.0 d	119.9 d	
C-2'	147.5 s	152.5 s	152.6 s		146.8 s
C-3'	136.7 s	140.9 s	141.0 s		135.6 s
C-4'	176.5 s	174.0 s	174.0 s		175.9 s
C-5'	160.1 s*	159.6 s*	159.9 s*		160.7 s
C-6'	98.9 d	95.6 d	95.6 d		98.2 d
C-7'	158.6 s*	156.5 s*	156.3 s*		163.9 s
C-8'	102.6 s	102.7 s	103.0 s		93.5 d
C-9'	154.0 s	153.9 s	153.8 s		156.2 s
C-10'	107.4 s	109.9 s	110.2 s		103.1 s
C-11'	123.4 s	123.4 s	123.4 s		121.7 s
C-12'	131.1 d	130.2 d	130.2 d		129.5 d
C-13'	115.4 d	113.9 d	113.5 d		115.4 d
C-14'	159.3 s*	161.2 s*	161.2 s*		159.2 s
C-15'	115.4 d	113.9 d	113.5 d		115.4 d
C-16'	131.1 d	130.2 d	130.2 d		129.5 d
$\underline{C}H_3O$		54.7 q 55.4 q x 3 56.4 q 59.8 q	54.7 q 55.4 q x 3 56.5 q 59.9 q		
$\underline{C}H_3COO$			20.8 q		
$CH_3\underline{C}OO$			169.4 s		

The assignments of the asterisked signals are ambiguous and might have to be reversed.

taken into account, all the twenty hydrogens in the molecule have been allocated.

In the aromatic hydrogen region, the ^1H NMR spectrum further exhibited a signal (δ 6.28) due to an insulated hydrogen, two signals (δ 5.94 and 6.04) originating from two aromatic hydrogens situated *meta* to each other, and two sets of signals of A_2B_2 type (δ 6.84 and 8.48, and 6.92 and 7.52) attributed to two *p*-substituted benzene systems. The presence of the last systems was also verified by the ^{13}C NMR spectrum of ephedrannin A which showed two sets of signals for two carbons each (δ 115.4 and 129.2, and 116.1 and 131.1). The chemical shifts of these ^1H and ^{13}C NMR signals together with the fragment ion peak at *m/e* 107, 94 and 93 in the mass spectrum of ephedrannin A demonstrated the *p*-substituted benzene systems to be further defined as 4-hydroxyphenyl groupings.

Examination of the NMR spectra of the hexamethyl ether (III) by means of double resonance experiments revealed the presence of ^{13}C-^{1}H spin couplings as shown in formula A where the chemical shift of $C_{(2)}$ (δ 99.4) suggested that two oxygen functions were linked to this carbon. Further, the ketal carbon signal (δ 99.4) was shifted to higher field (δ 98.0) by acetylation of the hexamethyl ether (III) to its monoacetate (IV), demonstrating that the ketal carbon was adjacent to the hydroxyl-carrying carbon. These observations coupled with biogenetic considerations led to the supposition that these carbons constituted a flavane moiety. In support of this supposition that half of the molecule of ephedrannin A is constructed by a flavane, the chemical shifts of the hydrogens (δ 5.94 and 6.04) and carbons (Table I) in the tetrasubstituted benzene system of the A ring were in accord with those of the A ring of catechin (δ 5.85 and 5.93, and Table I).



A

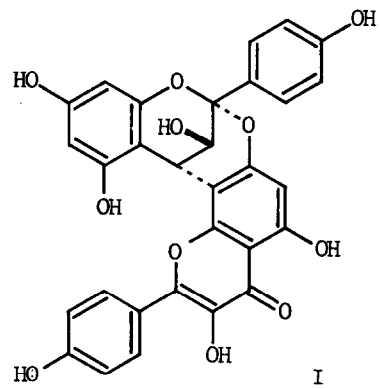
Ephedrannin A afforded a positive magnesium-hydrochloric acid test for flavonoids. Its UV spectrum (λ_{max} 224, 273, 324, 373 nm (ε 54300, 29500, 14400, 18400, respectively)) indicated the presence of a flavonol moiety (e.g., λ_{max} 265, 296(sh), 323(sh), 365 nm (ε 17500, 9900, 11100, 19500, respectively) for kaempferol). The previously deduced carbonyl group should be located at $C_{(4')}$. The UV absorption at 373 nm due to the band I and a bathochromic shift of this UV maximum by 59 nm on addition of aluminum chloride substantiated that a hydroxyl was present at $C_{(3')}$.[8] The UV absorption at 273 nm associated with the band II revealed the situation of a hydroxyl at $C_{(5')}$.[8] The location of a free hydroxyl at $C_{(5')}$ in the pentamethyl ether (II) was confirmed by a bathochromic shift by 8 nm on addition of aluminum chloride of the maximum at 273 nm in the UV spectrum of the pentamethyl ether (II) and by a band at 1647 cm^{-1} attributed to the $C_{(4')}$ conjugated carbonyl still chelated in the IR spectrum. When sodium hydroxide was added, the maximum at 373 nm in the UV spectrum of ephedrannin A displayed a displacement towards longer wave-length (419 nm), a fact which showed that the B-ring of the flavonol moiety beared a free hydroxyl at the p-position. Because 4-hydroxyphenyl group was only possible for the side chain, the feature of the B-ring was thus established. That ephedrannin A possesses a kaempferol moiety as deduced above was substantiated by its ^{13}C NMR spectrum in which the parameters of certain signals coincided with those for the carbons in the B- and C-rings of kaempferol (Table I).

The flavonol moiety should be linked with the above flavane moiety through the C-O-C linkage and the C-C linkage ended at $C_{(2)}$ and $C_{(4)}$ in formula A. As was described above, the ^{13}C NMR resonance for $C_{(2)}$ occurred at δ 99.4, demonstrating that the terminal of the C-O-C linkage must be located at $C_{(2)}$ and, hence, the C-C linkage should be attached to the remaining position $C_{(4)}$ whose ^{13}C NMR resonance appeared at δ 27.8. Now, the other end of the C-O-C linkage should be attached to $C_{(6')}$, $C_{(7')}$ or $C_{(8')}$ in the flavonol moiety. If the oxygen function is situated at $C_{(7')}$, the chemical shifts of the three oxygen-carrying carbons should appear in a lower field region than δ 150, while if it is located at $C_{(6')}$ or $C_{(8')}$, those of the three oxygen-bearing carbons should occur at δ 140—150. In fact, the three carbon

resonances in question were observed at δ 154—160, showing that the oxygen function is present at $C_{(7')}$. Consequently, the other end of the C-C linkage should be terminated at either $C_{(6')}$ or $C_{(8')}$. In accord with this deduction, the chemical shifts of the hydrogen and carbon signals for the hydrogen-carrying carbon in the A-ring in the NMR spectra of ephedrannin A (δ 6.28 and 98.9) were compatible with those at $C_{(6')}$ (δ 6.2—6.4 and 97—100) but not at $C_{(8')}$ (δ 6.5—6.9 and 94—96) in the spectra of the related flavonoids,[8,9] and an intramolecular nuclear Overhauser effect was found between the methoxyl hydrogens at $C_{(5')}$ and the hydrogen in question (30%). That the C-C linkage was placed between $C_{(4)}$ in the flavane moiety and $C_{(8')}$ in the flavonol moiety was further substantiated by the observation of a ^{13}C-^{1}H spin coupling between the $C_{(4)}$ hydrogen signal and the $C_{(8')}$ carbon signal.

The gross structure of ephedrannin A was thus deduced to be that shown by formula I (exclusive of stereochemistry).

The displacement due to the O-benzoyl group at $C_{(3)}$ on passing from the hexamethyl ether (III) to its benzoate was larger in the $H_{(6)}$ signal (Δδ -0.10 ppm) than in the $H_{(6')}$ signal (Δδ -0.01 ppm), revealing that the $C_{(3)}$ hydroxyl is oriented closer to the A-ring of the flavane moiety rather than the A-ring of the flavonol moiety, the relative stereochemistry being thus elucidated.



I

The absolute configuration at $C_{(3)}$ carrying the secondary hydroxyl was deduced to be S by means of esterification with (±)-2-phenylbutanoic acid according to Horeau (the recovered 2-phenylbutanoic acid was levoratatory).[10]

On the basis of the above evidence, the absolute stereostructure of ephedrannin A has been established to be as shown in formula I.

To the best of our knowledge, the known bisflavonoids formed by this type of condensation are constructed from the same monomeric molecules. Ephedrannin A thus constitutes the first example of the bisflavonoids which consist of different monomeric molecules (a flavane and a flavonol).

NOTES AND REFERENCES

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