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STRUCTURES OF MAHUANNIN A AND B, HYPOTENSIVE PRINCIPLES OF EPHEDRA ROOTS[1]

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Abstract —— Two novel bisflavanols, mahuannin A and B, exhibiting the hypotensive activity have been isolated from the crude drug "maō-kon", the roots of Ephedra plants. The stereostructures of mahuannin A and B, advanced on the basis of chemical and physical evidence, have been elucidated as those represented by formulas I and II.

A search for hypotensive principles of the crude drug "maō-kon", the underground part of Ephedra plants (Ephedraceae), earlier resulted in the isolation of the macrocyclic spermine alkaloids, ephedradine A, B, C and D,[2-5] and the flavano-flavonol, ephedrannin A.[6]

From the same sourse, we have further isolated two new bisflavanols possessing the hypotensive activity designated as mahuannin A and B.

Mahuannin A, a colorless amorphous powder, $C_{30}H_{24}O_{10}$ (FD-MS: m/e 544 (M^+)), ν_{max} 3280 cm^{-1}, was revealed to be phenolic in nature from its positive ferric chloride test. Methylation with dimethyl sulfate gave the non-phenolic pentamethyl ether (III) (HRMS: m/e 614.2141 (M^+)), ν_{max} 3480 cm^{-1}. The ether (III) was acetylated to yield the pentamethyl ether diacetate (HRMS: m/e 698.2364 (M^+)) which disclosed no hydroxyl band in the IR spectrum.[7] Of the ten oxygen atoms present in the molecule, five were thus proved to be phenolic hydroxyls, two as alcoholic hydroxyls and the remaining three were considered to be present as ether functions since mahuannin A possesses no carbonyl as revealed by the spectral data.

The ^{13}C NMR spectrum of mahuannin A disclosed the presence of six aliphatic (CH_2 x 1, CH x 4, C x 1) and twenty-four aromatic carbons (CH x 11, C x 5, C-O x 8) (Table I).

The ^1H NMR spectrum of mahuannin A displayed 1H doublets (J 2 Hz) at δ 5.88 and 6.07, and a 1H singlet at δ 6.08. These chemical shifts of the three hydrogens matched with that of the aromatic hydrogens of phloroglucinol (δ 5.94[8]). It was thus logically inferred that mahuannin A beared in its molecule a tetrasubstituted and a pentasubstituted benzene having three oxygen functions at the 1,3,5-positions.

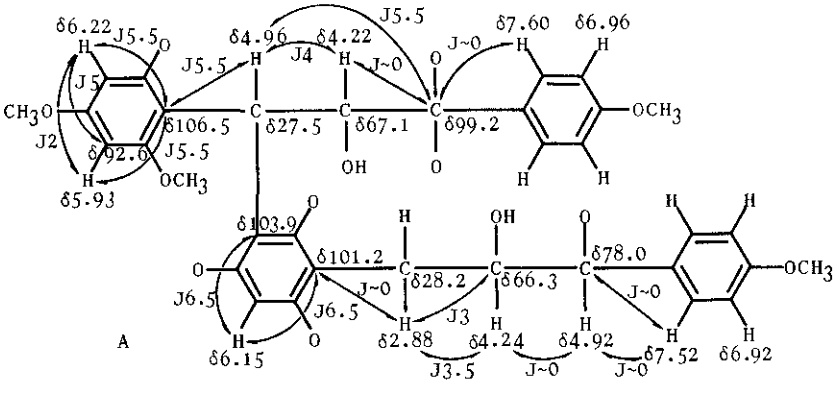
The remaining eight aromatic hydrogen signals which appeared as a pair of A_2B_2 type (δ 6.82 and 7.45, and 6.84 and 7.50, 2H d each, J 8 Hz) in the ^1H NMR spectrum of mahuannin A demonstrated the presence of two p-substituted phenyl nuclei. These were further clarified to be p-hydroxyphenyl systems from the chemical shifts of the carbon signals at δ 115.5, 116.1, 129.1 and 129.4 (2C each) which fairly corresponded with those of hydrogen-carrying aromatic carbons of p-cresol (δ 115.3, 130.2[9]).

In the aliphatic region of the ^1H NMR spectrum of mahuannin A, there were signals at δ 4.40 (1H) and 4.24 (1H) in an AB type (J 4 Hz) and those at δ 2.90 (2H) and 4.26 (1H) in an A_2X type, the latter (δ 4.26) being further coupled to a signal at δ 5.10 (1H) (J ca. 0 Hz).

Table I. Carbon-13 shieldings in mahuannin A and B, and related substances (δ)

	mahuannin A (methanol-$\underline{d}_4$)	mahuannin B (methanol-$\underline{d}_4$)	ephedrannin A (acetone-$\underline{d}_6$)	epiafzelechin (acetone-$\underline{d}_6$)
C-2	100.5 s	100.2 s	100.6 s	
C-3	66.9 d	66.9 d	67.2 d	
C-4	29.2 d	29.2 d	29.1 d	
C-5	156.4 s*	156.9 s*	157.4 s*	
C-6	98.0 d	98.3 d	97.2 d	
C-7	156.4 s*	156.4 s*	157.4 s*	
C-8	96.6 d	96.5 d	95.9 d	
C-9	158.0 s*	158.1 s*	158.0 s*	
C-10	104.0 s	104.2 s	105.1 s	
C-11	131.5 s	131.7 s	130.7 s	
C-12	129.4 d	129.3 d	129.2 d	
C-13	116.1 d	115.9 d	116.1 d	
C-14	151.9 s*	152.1 s*	152.6 s*	
C-15	116.1 d	115.9 d	116.1 d	
C-16	129.4 d	129.3 d	129.2 d	
C-2'	80.8 d	81.6 d	147.5 s	79.4 d
C-3'	67.5 d	67.9 d	136.7 s	66.3 d
C-4'	29.5 t	29.9 t	176.5 s	29.0 t
C-5'	154.0 s*	154.1 s*	160.1 s*	157.0 s
C-6'	96.6 d	96.5 d	98.9 d	96.4 d
C-7'	158.6 s*	158.7 s*	158.6 s*	157.0 s
C-8'	101.9 s	102.3 s	102.6 s	95.8 d
C-9'	151.2 s*	152.1 s*	154.0 s*	157.0 s
C-10'	106.8 s	107.1 s	107.4 s	99.7 s
C-11'	130.6 s	130.5 s	123.4 s	131.3 s
C-12'	129.1 d	129.9 d	131.1 d	128.9 d
C-13'	115.5 d	115.5 d	115.4 d	115.5 d
C-14'	157.9 s*	157.9 s*	159.3 s*	157.0 s
C-15'	115.5 d	115.5 d	115.4 d	115.5 d
C-16'	129.1 d	129.9 d	131.1 d	128.9 d

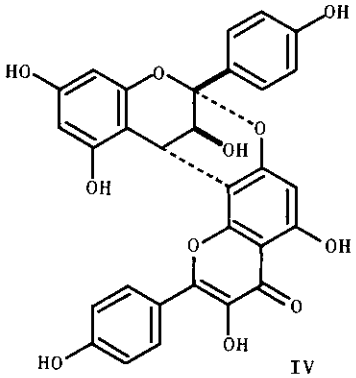
Abbreviations: s=singlet, d=doublet, t=triplet
*The assignments of the asterisked signals are ambiguous and might have to be reversed.



A

These data taken in conjunction with the findings made by double resonance experiments with the ether (III) permitted settling the mode of linkages between the aromatic and aliphatic parts of the molecule to build up the part structure A for the ether (III).

This part structure A together with the existence of three ether linkages in the molecule showed that mahuannin A was similar in structure to ephedrannin A (IV).[6] Comparison of the ^{13}C NMR spectrum of mahuannin A with that of ephedrannin A (IV) revealed that the parameters of the signals due to $C_{(2)}$–$C_{(16)}$ were in good agreement in both the substances (Table I), showing that the $C_{(2)}$–$C_{(16)}$ portion of mahuannin A constituted the same flavanol structure as in ephedrannin A (IV). As for $C_{(2')}$–$C_{(16')}$, although the chemical shifts of the signals attributed to $C_{(5')}$–$C_{(16')}$ were in good accord in both the substances, the signals associated with $C_{(2')}$–$C_{(4')}$ in ephedrannin A (IV)



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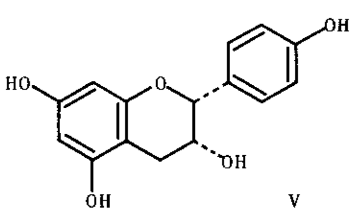


V

were not observed and instead those at δ 80.8 (d), 67.5 (d) and 29.5 (t) were found in mahuannin A (Table I). The chemical shifts of these three signals coincided with those of the ^{13}C NMR signals of $C_{(2)}$–$C_{(4)}$ in (–)-epiafzelechin (V)[10] (Table I). Combined evidence showed that mahuannin A is a flavanol dimer.

There were three possible modes in which the two flavanol moieties may be linked up to build the structure: 1) $C_{(2)}$-O-$C_{(5')}$ and $C_{(4)}$-$C_{(6')}$, 2) $C_{(2)}$-O-$C_{(7')}$ and $C_{(4)}$-$C_{(8')}$, and 3) $C_{(2)}$-O-$C_{(7')}$ and $C_{(4)}$-$C_{(6')}$. A choice in these three possibilities was made from a study of the ^{1}H NMR spectrum of the ether (III) in which NOE's were observed between the methoxyl hydrogens (δ 3.69) and the insulated aromatic hydrogen (δ 6.15), and between the methoxyl hydrogens (δ 3.69) and the methylene hydrogens (δ 2.88). It was thus established that the structure of mahuannin A was represented by formula I (stereochemistry not implied).

Mahuannin B, a colorless amorphous powder, had the same composition, $C_{30}H_{24}O_{10}$ (FD-MS: m/e 544 (M^+)) as mahuannin A. In the IR spectrum, a hydroxyl band at 3410 cm^{-1} was found. The ^{1}H NMR spectrum exhibited 1H doublets (J 2 Hz) at δ 5.99 and 6.08, 1H singlet at δ 6.09 and 2H doublets (J 8 Hz) at δ 6.80, 6.81, 7.44 and 7.50 (a tetrasubstituted and a pentasubstituted benzene bearing three oxygen functions at the 1,3,5-positions, and two p-hydroxyphenyls). Further, there were signals at δ 4.41 (1H) and 4.10 (1H) in an AB type (J 4 Hz) and those at δ 2.88 (2H), 4.27 (1H) and 4.97 (1H) in an A_2XY type, these signals being very much like those of mahuannin A. Comaprison of the ^{13}C NMR spectra of mahuannin B and A revealed a near coincidence of the chemical shifts of the signals for all the carbons of the two substances excepting those of $C_{(2')}$ and $C_{(9')}$ which showed displacements of about 1 ppm. In the ^{1}H NMR spectrum of the pentamethyl ether diacetate prepared from mahuannin B, NOE's were observed between the methoxy hydrogens (δ 3.67) and the isolated aromatic hydrogen (δ 6.13), and between the methoxy hydrogens (δ 3.67) and the methylene hydrogens (δ 2.81), indicating identical mode of attachment of two flavanol moieties in mahuannin B and A. These data suggested that both the substances are diastereoisomers.

The close parameters of the ^{1}H NMR signals for $H_{(3)}$ and $H_{(4)}$ in mahuannin A (δ 4.24 (d, J 4 Hz), 4.40 (d, J 4 Hz)), mahuannin B (δ 4.10 (d, J 4 Hz), 4.41 (d, J 4 Hz)) and ephedrannin A (δ 4.19 (d, J 4 Hz), 4.96 (d, J 4 Hz)), and the close chemical shifts of the ^{13}C NMR signals for $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$ in the above three substances (Table I) settled that three of them had the same relative configurations at $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$; the phenyl at $C_{(2)}$ and the hydrogen at $C_{(3)}$, and also the hydrogens at $C_{(3)}$ and $C_{(4)}$ being in the trans-arrangement.

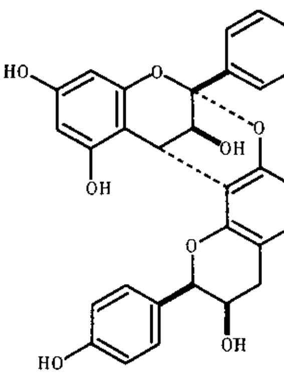
The absolute configuration at $C_{(4)}$ in the mahuannins was elucidated from the sign of the Cotton effect discernible at ca. 200-220 nm on the basis of the report[11] that such type of compounds having 4R-configrations exhibit a positive couplet and 4S-configurations a negative couplet in this wavelength region. Mahuannin A exhibited a negative couplet ($[\theta]_{228}$ -111200, $[\theta]_{202}$ +87100) while mahuannin B showed a positive couplet ($[\theta]_{220}$ +62800, $[\theta]_{204}$ -102000), indicating that the substances have 4S- and 4R-configuration, respectively. Mahuannin A and B thus proved to have 2S,3S,4S- and 2R,3R,4R-configuration, respectively. This deduction was further substantiated by application of Horeau method. Thus, esterification of mahuannin A pentamethyl ether 3'-monobenzoate with (±)-2-phenylbutanoic acid permitted recovery of levorotatory 2-phenylbutanoic acid.[12]

The hydrogens at $C_{(2')}$ and $C_{(3')}$ in mahuannin A and B were concluded to be cis-oriented in view of the small coupling constants between the $H_{(2')}$ and $H_{(3')}$ signals in the ^1H NMR spectra of both the substances.[13] Based on the above data, the absolute configurations at $C_{(2')}$ and $C_{(3')}$ of mahuannin A and B were concluded to be the same.

The CD spectra of mahuannin A, B and their monomeric unit, (-)-epiafzelechin (V) showed Cotton effect at ca. 270 nm (mahuannin A: $[\theta]_{270}$ +9270; mahuannin B: $[\theta]_{269}$ -14900; (-)-epiafzelechin: $[\theta]_{270}$ -3100[14]). As the asymmetric field due to the $C_{(2)}$–$C_{(4)}$ part is spatially located apart from that due to the $C_{(2')}$–$C_{(3')}$ part, it was assumed that they do not interfere with each other and the ellipticities manifested by the CD spectrum are the summation effect of the two asymmetric moieties. Hence, the sum of the molecular ellipticities at ca. 270 nm of mahuannin A and B (-5630) was thought to be due to the asymmetric moieties at $C_{(2')}$–$C_{(3')}$ of the two molecules. In fact, this value was found to be approximately double the value of the molecualr ellipticity of (-)-epiafzelechin (V) (-3100). Consequently, the absolute configurations at $C_{(2')}$ and $C_{(3')}$ of mahuannin A and B were assumed to be both R. In support of this supposition, the CD spectrum of mahuannin B was found to resemble that of procyanidin A-2 ($[\theta]_{287}$ -2800, $[\theta]_{271}$ -16700, $[\theta]_{237}$ +45500(sh), $[\theta]_{221}$ +73300, $[\theta]_{205}$ -125400[11]). The absolute configuration at $C_{(3')}$ of mahuannin A was finally confirmed by esterification of its pentamethyl ether 3-benzoate according to Horeau (the recovered 2-phenylbutanoic acid was dextrorotatory).[12]

The absolute stereostructures of mahuannin A and B were thus established to be those represented by formulas I and II, respectively.

It is interesting to note that the diastereoisomeric bisflavanols, mahuannin A and B, occur in the same plant.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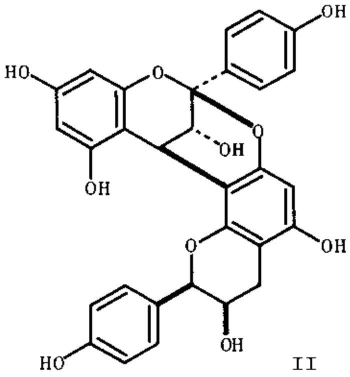
II

NOTES AND REFERENCES

1. Studies on the constituents of Ephedra. 11. This paper also constitutes Part 35 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. H. Hikino, M. Ogata and C. Konno, Heterocycles, 1982, **17**, 155.
6. H. Hikino, M. Takahashi and C. Konno, Tetrahedron Letters, 1982, **23**, 673.
7. CD, UV and IR spectra were determined in methanol, methanol and KBr disks, respectively. ^1H NMR were measured in methanol-d_4 (mahuannins) and chloroform-d (derivatives).
8. P. G. Waterman and D. F. Faulkner, Planta medica, 1979, **37**, 178.
9. C. J. Pouchert and J. R. Campbell, 'The Aldrich Library of NMR Spectra', Vol. IV, Aldrich Chemical Co., Milwaukee, 1974, p. 144.
10. E. Breitmaier, G. Haas and W. Voelter, 'Atlas of Carbon-13 NMR Data', Heyden & Son, London, 1975, Compd. 630.
11. M. W. Barrett, W. Klyne, P. M. Scopes, A. C. Fletcher, L. J. Porter and E. Haslam, J. Chem. Soc. Perkin I, 1979, 2375.
12. Horeau, 'Stereochemistry Fundamentals and Methods', Vol. 3, ed. by H. B. Kagan, Georg Thieme Publishers, Stuttgart, 1977, p. 52.
13. R. S. Thompson, D. Jacques, E. Haslam and D. Tanner, J. Chem. Soc. Perkin I, 1972, 1387.
14. O. Korver and C. K. Wilkins, Tetrahedron, 1971, **27**, 5465.

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