STRUCTURE OF MAHUANNIN C, A HYPOTENSIVE PRINCIPLE OF EPHEDRA ROOTS[1]

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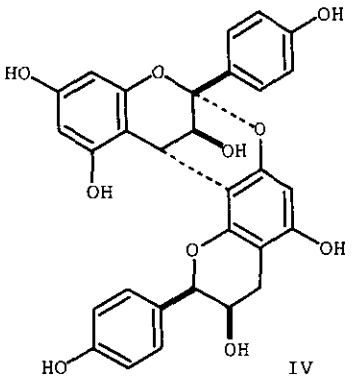
Abstract —— A new bisflavanol, mahuannin C, showing the hypotensive activity has been isolated from the crude drug "maō-kon", the roots of Ephedra plants. The stereostructure of mahuannin C has been deduced as shown in formula I on the basis of chemical and physical evidence.

From the crude drug "maō-kon", the underground part of Ephedra plants (Ephedraceae), we have previously isolated the macrocyclic spermine alkaloids, ephedradine A, B, C and D,[2-5] a flavano-flavonol, ephedrannin A,[6] and the bisflavanols, mahuannin A (IV) and B (V),[7] as the hypotensive principles.

Further survey of the phenolic fraction from the extract of the crude drug resulted in the isolation of a novel bisflavanol, mahuannin C, also possessing hypotensive activity.



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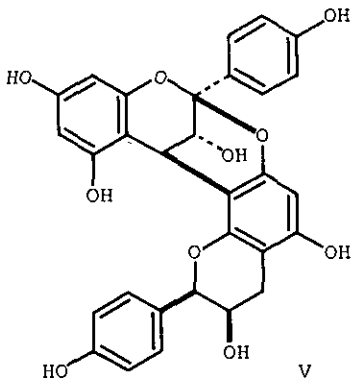

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Mahuannin C, a colorless amorphous powder, $C_{30}H_{24}O_{10}$ (FD-MS: m/e 567 ($M + Na^+$), 583 ($M + K^+$), ν_{max} 3380 cm^{-1}), was revealed to be phenolic in nature from its positive ferric chloride test.

Methylation of mahuannin C with dimethyl sulfate and potassium carbonate in acetone afforded the pentamethyl ether (II) (MS: m/e 614 (M^+), ν_{max} 3450 cm^{-1}), and acetylation of mahuannin C with acetic anhydride in pyridine gave the heptaacetate (III) (FD-MS: m/e 838 (M^+)) which showed no hydroxyl band in the IR spectrum,[8] demonstrating that mahuannin C possessed five phenolic and two alcoholic hydroxyls. Since ten oxygen atoms exist in the molecule, the remaining three were considered to be present as ether functions.

The ^{13}C NMR spectrum of mahuannin C showed the presence of six aliphatic carbons (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). The chemical shifts and splitting patterns of these ^{13}C NMR signals were closely related to those of mahuannin A (IV) and B (V) (Table I).

The ^1H NMR spectrum of mahuannin C exhibited a singlet (δ 6.08, 1H) and two doublets (δ 6.04, 6.07, each 1H, J 2 Hz) which corresponded to those of the aromatic hydrogens of phloroglucinol (δ 5.94[9]). These data suggested that mahuannin C possessed a pentasubstituted and a tetrasubstituted benzene having oxygen functions at the 1, 3 and 5-positions. Two pairs of

Table I. Carbon-13 shieldings in mahuannin C and related substances (δ)

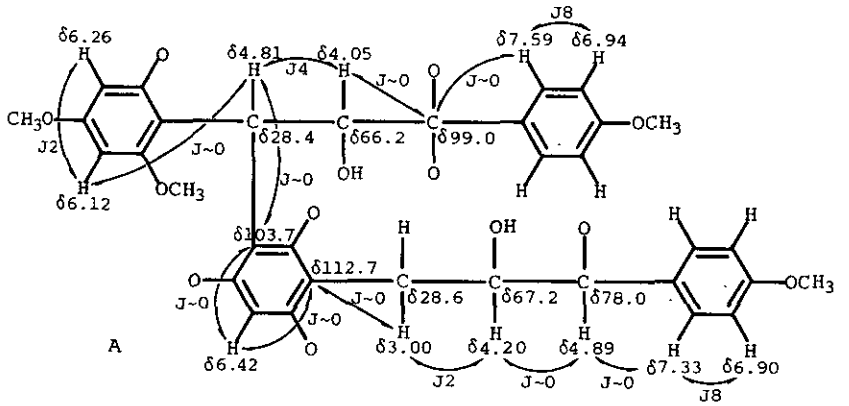
	mahuannin C (methanol-d_4)	mahuannin A (methanol-d_4)	mahuannin B (methanol-d_4)
C-2	100.6 s	100.5 s	100.2 s
C-3	67.2 d	66.9 d	66.9 d
C-4	29.6 d	29.2 d	29.2 d
C-5	155.2 s*	156.4 s*	156.9 s*
C-6	96.9 d	98.0 d	98.3 d
C-7	155.2 s*	156.4 s*	156.4 s*
C-8	96.6 d	96.6 d	96.5 d
C-9	157.9 s*	158.0 s*	158.1 s*
C-10	104.2 s	104.0 s	104.2 s
C-11	131.2 s	131.5 s	131.7 s
C-12	129.5 d	129.4 d	129.3 d
C-13	115.7 d	116.1 d	115.9 d
C-14	151.5 s*	151.9 s*	152.1 s*
C-15	115.7 d	116.1 d	115.9 d
C-16	129.5 d	129.4 d	129.3 d
C-2'	79.7 d	80.8 d	81.6 d
C-3'	67.4 d	67.5 d	67.9 d
C-4'	29.6 t	29.5 t	29.9 t
C-5'	154.2 s*	154.0 s*	154.1 s*
C-6'	102.9 s	96.6 d	96.5 d
C-7'	158.7 s*	158.6 s*	158.7 s*
C-8'	96.9 d	101.9 s	102.3 s
C-9'	152.6 s*	151.2 s*	152.1 s*
C-10'	108.8 s	106.8 s	107.1 s
C-11'	131.5 s	130.6 s	130.5 s
C-12'	129.0 d	129.1 d	129.9 d
C-13'	115.5 d	115.5 d	115.5 d
C-14'	157.6 s*	157.9 s*	157.9 s*
C-15'	115.5 d	115.5 d	115.5 d
C-16'	129.0 d	129.1 d	129.9 d

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

signals of the A_2B_2 type (δ 6.75 and 7.26, and 6.83 and 7.50, each 2H, J 8 Hz) in the ^1H NMR spectrum of mahuannin C could be attributed to two p-substituted benzene systems. The existence of these systems as p-hydroxyphenyl systems was established by the two pairs of signals for two carbons (δ 115.5 and 129.0, and 115.7 and 129.5, each 2C) which consisted with those of hydrogen-bearing aromatic carbons of p-cresol (δ 115.3, 130.2[10]). These data therefore rationalized the presence of twenty-four aromatic carbons associated with four benzene rings in mahuannin C.

In the aliphatic region of the ^1H NMR spectrum of the ether (II), signals at δ 4.05 (1H) and δ 4.81 (1H) in an AB type (J 4 Hz) and those at δ 3.00 (2H) and δ 4.20 (1H) in an A_2X type were present, and the signal at δ 4.20 (1H) was further coupled to that at δ 4.89 (1H) (J ca. 0 Hz).

Further examination of the NMR spectra of the ether (II) by means of double resonance experiments revealed the presence of ^1H-^1H and ^{13}C-^1H spin couplings as shown in partial structure A. These data suggested that mahuannin C, similar to mahuannin A and B, was composed of two flavanols. There were three possible linkages considered in which the two flavanol moieties could be joined to construct the molecule: 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 $C_{(4)}$-$C_{(6')}$. In order to establish the linkage, acetylation shifts of the ^1H and ^{13}C NMR signals for the isolated aromatic hydrogen and the carbon attached to this hydrogen in A' ring were calculated in mahuannin C and its heptaacetate (III) ($\Delta\delta_{H(8')}$ +0.50, $\Delta\delta_{C(8')}$ +6.0), and in mahuannin A and its heptaacetate ($\Delta\delta_{H(6')}$ +0.35, $\Delta\delta_{C(6')}$ +7.0),



A

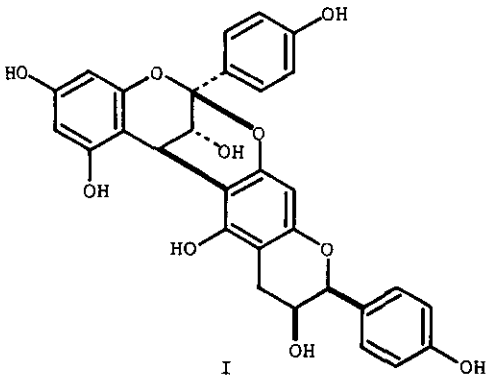
and compared with the known acetylation shifts in phenol (o-position of the phenolic hydroxyl: $\Delta\delta_H$ +0.29[11], $\Delta\delta_C$ +6.4[12], p-position of the phenolic hydroxyl: $\Delta\delta_H$ +0.40[11], $\Delta\delta_C$ +4.8[12]). This comparison pointed to the isolated aromatic hydrogen of the A' ring to be located at the position para to the phenolic hydroxyl.

Further, in the ^{1}H NMR spectrum of the pentamethyl ether (II), an intramolecular NOE was found between the methylene hydrogens (δ 3.00) and the methoxyl hydrogens (δ 3.80), but was not observed between the isolated aromatic hydrogen (δ 6.42) and the methoxyl hydrogens (δ 3.80). Moreover, in the ^{1}H non-decoupling ^{13}C NMR spectrum of mahuannin C, the splitting pattern of the carbon (δ 96.9) attached to the isolated aromatic hydrogen of ring A' was not altered when heavy water was added. From the above evidence, the absence of an isolated aromatic hydrogen at the position ortho to the free phenolic hydroxyl group was confirmed. Therefore, it was concluded that the linkage in mahuannin C is $C_{(2)}$-O-$C_{(7')}$ and $C_{(4)}$-$C_{(6')}$, and its gross structure is represented by formula I (exclusive of stereochemistry).

The chemical shifts and coupling constants of $H_{(3)}$ and $H_{(4)}$ (δ 4.08, d, J 4 Hz, 4.58, broad d, J 4 Hz), and of $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$ (δ 100.6, s, 67.2, d, 29.6, d) in the ^{1}H and ^{13}C NMR spectrum of mahuannin C were compatible with those of mahuannin A ($H_{(3)}$ (δ 4.24, d, J 4 Hz), $H_{(4)}$ (δ 4.40, d, J 4 Hz), $C_{(2)}$ (δ 100.5, s), $C_{(3)}$ (δ 66.9, d), $C_{(4)}$ (δ 29.2, d)), a fact which demonstrated that mahuannin C possesses the same relative configuration as mahuannin A at $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$, the two hydrogens at $C_{(3)}$ and $C_{(4)}$ positions being consequently oriented in the trans-stereochemistry.

The absolute configuration at $C_{(4)}$ in the bisflavanols like mahuannin C was established by the sign of the Cotton effect at ca. 200-220 nm on the basis of the report[13] that such type of compounds having 4R-configurations exhibit a positive couplet and those with 4S-configurations a negative couplet in this wave-length region. The fact that mahuannin C showed a positive couplet ($[\theta]_{216}$ +28000, $[\theta]_{198}$ -44800) indicated the absolute configuration at $C_{(4)}$ to be R, and allowing the 2R,3R-configuration to be deduced. This was further confirmed by esterification of mahuannin C pentamethyl ether 3'-monobenzoate with (±)-2-phenylbutanoic acid by application of Horeau method which led to recovery of dextrorotatory 2-phenylbutanoic acid.[14]

The stereochemistry at $H_{(2')}$ and $H_{(3')}$ was next examined. Since the coupling constant between $H_{(2')}$ and $H_{(3')}$ was ca. 0 Hz in the ^{1}H NMR spectrum, the hydrogens at $C_{(2')}$ and $C_{(3')}$ in the ether (II) were concluded to be cis-oriented. The absolute configuration at $C_{(3')}$ was substantiated to be R by means of esterification of mahuannin C pentamethyl ether 3-monobenzoate with (±)-2-phenylbutanoic acid according to Horeau[14] (the recovered 2-phenylbutanoic acid was dextrorotatory), thus 2'R-configuration was determined.



I

On the basis of the above evidence, the absolute stereostructure of mahuannin C was thus established to be that represented by formula I.

It is biogenetically interesting to note that as well as the two isomeric bisflavanols, mahuannin A and B, the third isomer, mahuannin C, was also obtained from the same plant.

NOTES AND REFERENCES

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