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

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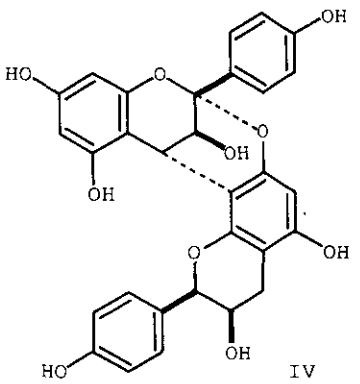
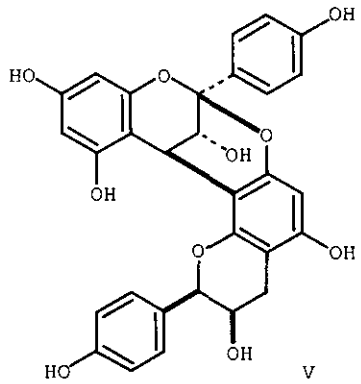
Abstract —— From the crude drug "maō-kon", the roots of Ephedra plants, a new flavano-flavanol, mahuannin D, exhibiting the hypotensive activity has been isolated. The stereostructure of mahuannin D has been determined as represented by formula I based on chemical and physical evidence.

We have hitherto isolated the macrocyclic spermine alkaloids, ephedradine A, B, C and D,[2-5] an imidazole alkaloid, feruloylhistamine,[6] a flavano-flavonol, ephedrannin A,[7] and the bisflavanols, mahuannin A (IV), B (V) and C (VI),[8-9] as the hypotensive principles from the crude drug "maō-kon", the underground part of Ephedra plants (Ephedraceae).

In a continuation of our survey of the phenolic fraction from the extract of the crude drug, a new flavano-flavanol, mahuannin D, also having hypotensive activity was further isolated.



IV



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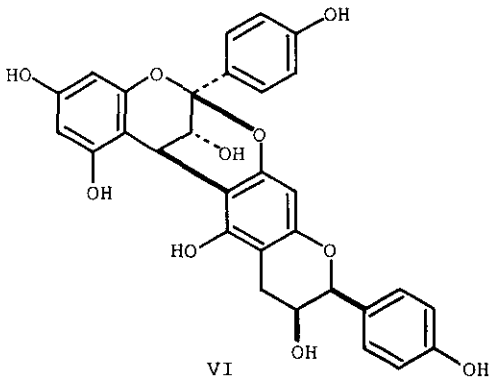


VI

Mahuannin D, a colorless amorphous powder, $C_{30}H_{24}O_9$ (FD-MS: m/e 528 (M^+), $[\alpha]_D$ -104° (methanol), ν_{max} 3370 cm^{-1}), was indicated to be phenolic in nature from its positive ferric chloride test.

On methylation with dimethyl sulfate and potassium carbonate in acetone, mahuannin D gave the pentamethyl ether (II) (MS: m/e 598 (M^+), ν_{max} 3470 cm^{-1}), which was acetylated with acetic anhydride in pyridine to furnish the pentamethyl ether monoacetate (III) (MS: m/e 640 (M^+)) disclosing no hydroxyl band in the IR spectrum.[10] These observations showed mahuannin D to have five phenolic and one alcoholic hydroxyls. Because nine oxygen atoms are present in the molecule, the remaining three oxygen atoms were thought to exist as involved in ether functions.

Table I. Carbon-13 shieldings in mahuannin D and A (δ)

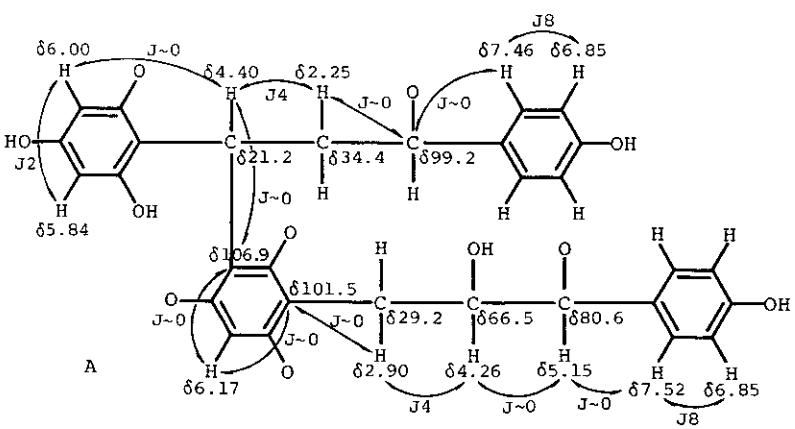
	mahuannin D (acetone-$\underline{d}_6$)	mahuannin A (methanol-$\underline{d}_4$)
C-2	99.2 s	100.5 s
C-3	34.4 t	66.9 d
C-4	21.2 d	29.2 d
C-5	154.2 s*	156.4 s*
C-6	96.3 d	98.0 d
C-7	155.1 s*	156.4 s*
C-8	96.8 d	96.6 d
C-9	158.0 s*	158.0 s*
C-10	106.7 s	104.0 s
C-11	134.0 s	131.5 s
C-12	127.8 d	129.4 d
C-13	115.8 d	116.1 d
C-14	158.4 s*	158.6 s*
C-15	115.8 d	116.1 d
C-16	127.8 d	129.4 d
C-2'	80.6 d	80.8 d
C-3'	66.5 d	67.5 d
C-4'	29.2 t	29.5 t
C-5'	155.8 s*	154.0 s*
C-6'	97.5 d	96.6 d
C-7'	152.1 s*	151.9 s*
C-8'	106.9 s	106.8 s
C-9'	150.8 s*	151.2 s*
C-10'	101.5 s	101.9 s
C-11'	130.3 s	130.6 s
C-12'	129.0 d	129.1 d
C-13'	115.5 d	115.5 d
C-14'	158.0 s*	157.9 s*
C-15'	115.5 d	115.5 d
C-16'	129.1 d	129.1 d

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

The ^{13}C NMR spectrum of mahuannin D demonstrated the presence of six aliphatic carbons (CH_2 x 2, CH x 3, C x 1) and twenty-four aromatic carbons (CH x 11, C x 5, C-O x 8). The parameters of these ^{13}C NMR signals closely resembled those of mahuannin A (IV) (Table I)[11].

The ^1H NMR spectrum of mahuannin D displayed two doublets (δ 5.84, 6.00, each 1H, $\underline{J}$ 2 Hz) and a singlet (δ 6.17, 1H) whose chemical shifts coincided with those of the aromatic hydrogens of phloroglucinol (δ 5.94[12]), showing that mahuannin D had a tetrasubstituted and a pentasubstituted benzene possessing oxygen functions at the 1, 3 and 5-positions. Further two pairs of signals of the A_2B_2 type (δ 6.85 and 7.46, and 6.85 and 7.52, each 2H, $\underline{J}$ 8 Hz) in the ^1H NMR spectrum of mahuannin D revealed the presence of two $\underline{p}$-substituted benzene systems. The existence of these systems was substantiated by the two pairs of signals in the ^{13}C NMR spectrum of the mahuannin D (δ 115.2 and 127.8, and 115.8 and 129.0, each 2C), these chemical shifts being consistent with those of hydrogen-bearing aromatic carbons of $\underline{p}$-cresol (δ 115.3, 130.2[13]). Allocation of twenty-four aromatic carbons involved in four benzene rings in mahuannin D was thus unambiguously settled.

In the aliphatic region of the ^1H NMR spectrum of mahuannin D, there were signals at δ 2.25 (2H) and 4.40 (1H) in an A_2X type ($\underline{J}$ 4 Hz) and those at δ 2.90 (2H) and 4.26 (1H) in an A_2X type in which the latter signal at δ 4.26 (1H) was further coupled to that at δ 5.15 (1H) ($\underline{J}$ ca. 0 Hz).



Further examination of the NMR spectra of mahuannin D by means of double resonance experiments demonstrated the presence of ^1H-^1H and ^{13}C-^1H spin couplings as indicated in partial structure A, suggesting that mahuannin D consisted of a flavane and a flavanol. Therefore, three possible linkages remained where the flavane and flavanol moieties could be linked to form the molecule as follows: 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 $C_{(4)}$-$C_{(6')}$.

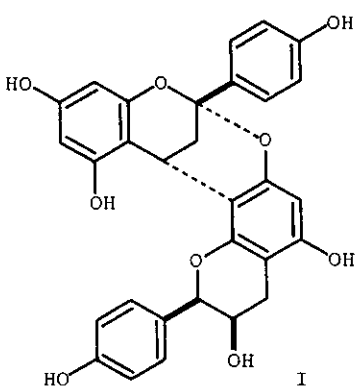
In the ^1H NMR spectrum of the pentamethyl ether (II), intramolecular NOE's were observed

between the methylene hydrogens (δ 2.85) and the methoxyl hydrogens (δ 3.75), and between the isolated aromatic hydrogen (δ 6.15) and the methoxyl hydrogens (δ 3.75). Further, in the ^{1}H non-decoupling ^{13}C NMR spectrum of mahuannin D, the signal of the carbon (δ 97.5) attached to the insulated aromatic hydrogen of ring A' was sharpened when heavy water was added, indicating the presence of a considerably large coupling between the aromatic carbon in question and the phenolic hydroxyl hydrogen. Therefore, an isolated aromatic hydrogen was confirmed to be present at the ortho position to the free phenolic hydroxyl group. Consequently, it was concluded that the flavane and flavanol moieties in mahuannin D are joined in the following linkages: $C_{(2)}$-O-$C_{(7')}$ and $C_{(4)}$-$C_{(8')}$.

Further comparison of the ^{13}C NMR spectrum of mahuannin D with that of mahuannin A (IV) revealed that the chemical shifts and coupling patterns of the signals originating from $C_{(2')}$-$C_{(16')}$ were consistent in both the substances (Table I). Concerning $C_{(2)}$-$C_{(16)}$, although the parameters of the signals associated with $C_{(2)}$ and $C_{(5)}$-$C_{(16)}$ were compatible in both the substances, the signal attributed to $C_{(3)}$ carrying a hydroxyl group in mahuannin A (IV) was not found and instead that at δ 34.4 (t) was visible in mahuannin D. Concurrently, the signal for $C_{(4)}$ adjacent to $C_{(3)}$ was shifted to higher field on passing from mahuannin A to mahuannin D (Table I). The gross structure was thus determined as shown in formula I (exclusive of stereochemistry).

It is known that in the bisflavonoids like mahuannin D, compounds possessing 4R-configurations show a positive couplet and those with 4S-configurations a negative couplet in the wave-length region 200-220 nm in the CD spectra.[14] The absolute configuration at $C_{(4)}$ to be S in mahuannin D was thus determined by the observation that mahuannin D exhibited a negative couplet ($[\theta]_{229}$ -101800, $[\theta]_{202}$ +76900), which in turn established the 2S-configuration upon inspection of the Dreiding model.

The next problem was the stereochemistry at $H_{(2')}$ and $H_{(3')}$. The very small coupling constant between $H_{(2')}$ and $H_{(3')}$ (ca. 0 Hz) in the ^{1}H NMR spectrum of the pentamethyl ether (II) demonstrated the hydrogens at $C_{(2')}$ and $C_{(3')}$ to be situated in the cis-orientation. R-Configuration at $C_{(3')}$ was verified by means of esterification of the ether (II) with (±)-2-phenylbutanoic acid by application of Horeau method[15] (the recovered 2-phenylbutanoic acid was dextrorotatory), and R-configuration at $C_{(2')}$ was consequently deduced.



I

Accumulated data have thus rigorously established the absolute stereostructure of mahuannin D as that represented by formula I.

It is biogenetically of interest to mention that besides this crude drug "maō-kon" contains the three isomeric bisflavanols, mahuannin A, B and C, it has also the fourth analog, mahuannin D, which, to the best of our knowledge, formes the first example of the bisflavonoids consisting of different monomeric molecules, a flavane and a flavanol.

NOTES AND REFERENCES

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10. CD and IR spectra were determined in methanol and KBr disks, respectively. ^{1}H NMR spectra were measured in acetone-d_6 (mahuannin D) and chloroform-d (derivatives).
11. After the present unambiguous assignments of the signals in the ^{13}C NMR spectrum of mahuannin D by means of ^{13}C-^{1}H spin decoupling experiments, the assignments of the $C_{(8')}$ and $C_{(10')}$ signals in that of mahuannin A has been revised.
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