STRUCTURE OF MORACENIN D, A HYPOTENSIVE PRINCIPLE OF *MORUS* ROOT BARKS[1]

Yoshiteru Oshima, Chohachi Konno and Hiroshi Hikino*

Pharmaceutical Institute, Tohoku University, Aoba-yama, Sendai, Japan

Abstract — A new isoprenoid flavone derivative, moracenin D, showing hypotensive activity has been isolated from the crude drug "sōhakuhi", the root barks of *Morus* sp. Chemical and physico-chemical studies have established the structure of moracenin D as shown in formula I.

We have previously reported the isolation and structural study of three new flavone derivatives, moracenin A, B and C, as the hypotensive principles of the oriental medicine "sōhakuhi", the root barks of certain species of *Morus* plants (Moraceae).[2-4] In our further survey on the constituents of the material, another novel isoprenoid flavone showing hypotensive activity has been isolated from the fraction more polar than moracenin B and is now referred to as moracenin D.

Moracenin D, a yellow amorphous powder, $[\alpha]_D$ -388° (*c* 0.25, MeOH), has the molecular formula of $C_{40}H_{38}O_{12}$ (FD-MS spectrum: *m/e* 710 (M^+)). The ^{13}C NMR spectrum indicated the presence of fourteen aliphatic carbons (CH_3- × 3, $-CH_2$- × 3, >CH- × 3, >C-O- × 1, >C=CH- × 1, >C=C-O- × 1), twenty-four aromatic carbons (CH × 10, C × 5, C-O × 9) and two carbonyl carbons. The fact that moracenin D was positive to ferric chloride test and magnesium-hydrochloric acid reaction, showed moracenin D to be a flavonoid. The UV spectrum of moracenin D (λ_{max}^{MeOH} 209, 230 (sh), 264.5, 280 (sh) and 320 nm (log ε 4.75, 4.50, 4.45, 4.27 and 4.14)) matched the data for moracenin B (λ_{max}^{MeOH} 209, 264, 280 (sh) and 320 nm (log ε 4.80, 4.49, 4.31 and 4.18)).[3] Furthermore, the IR, ^1H and ^{13}C NMR spectra (Table I) of moracenin D resembled those of moracenin B. These findings suggested that moracenin D is an analog of moracenin B with an additional H_2O moiety.

The IR spectrum (KBr) revealed the presence of hydroxyls (3350 cm^{-1}) and a carbonyl at C-4 which is conjugated and hydrogen-bonded (1650 cm^{-1}). On addition of aluminum chloride, there was a bathochromic shift (7.5 nm) of the absorption at 264.5 nm due to the A-ring in the flavone skeleton, indicating the presence of a C-5 hydroxyl group. The ^1H and ^{13}C NMR spectra of moracenin D exhibited signals at δ 6.00[5] and 97.5, respectively (hydrogen and carbon at the 6 position in a 5,7-dihydroxyflavone).[3] ^1H NMR signals due to hydrogens of the B-ring in the flavone skeleton appeared at δ 6.64 (1H, doublet, *J* 2 Hz), 6.56 (1H, doublet of doublets, *J* 2 and 8 Hz) and 7.30 (1H, doublet, *J* 8 Hz), whose parameters showed that hydroxyls exist at the 2' and 4' positions. On the other hand, the ^1H NMR spectrum lacked signals originating from the hydrogens at the 3 and 8 positions. These data, as well as the concurrence of the ^{13}C NMR resonances of the flavone skeleton of moracenin D and of moracenin B (Table I), suggested that moracenin D is a 5,7,2',4'-tetrahydroxy-3,8-disubstituted-flavone like moracenin B.

The methyl hydrogen signal at δ 1.14 (6H, singlet) and the methylene hydrogen signals at δ 1.72 (2H, triplet, *J* 6 Hz) and 2.54 (2H, triplet, *J* 6 Hz) were due to a 3-hydroxy-3-methylbutyl group. In the ^{13}C NMR spectrum, signals at δ 20.0 (t), 41.9 (t), 70.2 (s), 28.2 (q) and 28.1 (q) attributed to a 3-hydroxy-3-methylbutyl group agreed well with those of morusinol (δ 21.3 (t), 43.0 (t), 69.9 (s), 29.4 (q) and 29.4 (q) (C_5D_5N)).[6] In order to determine the position of the 3-hy-

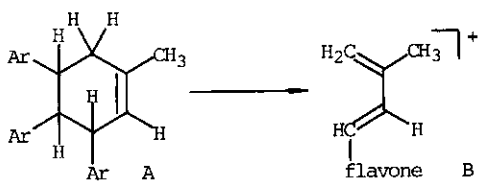
Table I. Carbon-13 shieldings in moracenin D and moracenin B (δ in CD_3CN)

	moracenin D	moracenin B
C-2	156.3 s	157.3 s
C-3	121.5 s	121.6 s
C-4	182.5 s	183.3 s
C-5	154.9 s	155.8 s
C-6	97.5 d	98.5 d
C-7	160.0 s	160.9 s
C-8	107.0 s	108.0 s
C-9	160.0 s	160.9 s
C-10	104.7 s	105.7 s
C-11	20.0 t	24.5 t
C-12	41.9 t	122.4 d
C-13	70.2 s	132.9 s
C-14	28.2 q	25.8 q
C-15	28.1 q	17.7 q
C-1'	112.4 s	113.4 s
C-2'	160.2 s	161.3 s
C-3'	102.6 d	103.7 d
C-4'	161.0 s	162.2 s
C-5'	107.1 d	108.3 d
C-6'	131.2 d	132.3 d
C-1"	114.5 s	115.5 s
C-2"	164.0 s	165.1 s
C-3"	102.8 d	103.7 d
C-4"	164.7 s	165.7 s
C-5"	107.3 d	108.3 d
C-6"	132.7 d	133.8 d
C-7"	208.8 s	209.8 s
C-8"	47.0 d	47.9 d
C-9"	37.6 d	38.5 d
C-10"	122.9 d	124.0 d
C-11"	133.2 s	134.1 s
C-12"	22.0 q	23.0 q
C-13"	37.1 t	38.0 t
C-14"	37.6 d	38.5 d
C-15"	121.5 s	122.4 s
C-16"	155.8 s	156.7 s
C-17"	102.0 d	103.0 d
C-18"	155.8 s	156.6 s
C-19"	107.0 d	108.3 d
C-20"	128.8 d	130.3 d

droxy-3-methylbutyl group, long range selective proton decoupling technique was used. As a result it was found that the methylene hydrogen signal at δ 2.54 and the carbonyl carbon signal at δ 182.5 were spin coupled (J 4.8 Hz), leading to the conclusion that the 3-hydroxy-3-methylbutyl group was attached to the 3 position.

In the aromatic region of the ^{1}H NMR spectrum, a set of signals of an ABC pattern were seen at δ 6.20 (1H, doublet, J 2 Hz), 6.06 (1H, doublet of doublets, J 2 and 8 Hz) and 6.78 (1H, doublet, J 8 Hz), the parameters being similar to those of a 2,4-dihydroxyphenyl group in moracenin B (δ 6.14 (1H, doublet, J 2 Hz), 6.02 (1H, doublet of doublets, J 2 and 8 Hz) and 6.71 (1H, doublet, J 8 Hz)).[3] These findings, along with the fact that the ^{13}C NMR spectrum of moracenin D showed signals corresponding to those of a 2,4-dihydroxyphenyl group in moracenin B (Table I), indicated the presence of a 2,4-dihydroxyphenyl group. Furthermore, there were another set of ^{1}H NMR signals at δ 5.98 (1H, doublet, J 2 Hz), 5.94 (1H, doublet of doublets, J 2 and 8 Hz) and 7.40 (1H, doublet, J 8 Hz), whose parameters were in good accord with those of the 2,4-dihydroxybenzoyl group in moracenin B (δ 5.92 (1H, doublet, J 2 Hz), 5.90 (1H, doublet of doublets, J 2 and 8 Hz) and 7.32 (1H, doublet, J 8 Hz)).[3] The presence of a 2,4-dihydroxybenzoyl group was also supported by the following evidence: 1) the UV maximum at *ca.* 280 nm was attributed to a 2,4-dihydroxybenzoyl group, 2) the mass fragment peak of $C_7H_5O_3^+$ was observed at *m/e* 137.0246 (calcd. *m/e* 137.0237), 3) there were ^{13}C NMR signals corresponding to those of the 2,4-dihydroxybenzoyl group in moracenin B (Table I) and 4) in addition to the ^{1}H NMR signal attributed to the C-5 hydroxyl hydrogen, another ^{1}H NMR signal due to a hydrogen-bonded hydroxyl hydrogen was visible.

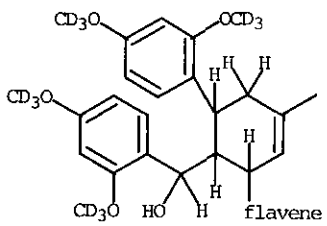
Part structure A was derived from the decoupling experiments in the aliphatic region of the ^{1}H NMR spectrum. In the mass spectrum, the fragment peak at *m/e* 438.1705 (calcd. *m/e* 438.1678) is characteristic of an ion (B) formed by a retro Diels-Alder type fragmentation at a cyclohexene ring, showing that a flavone moiety is located at the 9" position.



Recently, Nomura and Fukai,[7] and Masamune *et*

al.[8] isolated from similar sources the comparable substances, kuwanon G and albanin F, respectively, and proposed formula III for their structures.[9]

From the fact that the parameters of ^{1}H and ^{13}C NMR signals attributed to the cyclohexene ring of moracenin D were essentially identical with those of moracenin B, it is evident that the locations of the two substituents, the 2,4-dihydroxyphenyl group and the 2,4-dihydroxybenzoyl group, in moracenin D are the same as those of moracenin B. In order to establish the locations of the two substituents, the subsequent experiments were carried out on moracenin B instead of moracenin D. Thus, methylation of moracenin B with dimethylsulfate-d_6 and potassium carbonate in acetone afforded the deuterated hexamethyl ether (*m/e* 794 (M^+), $FeCl_3$ test: positive), the deuterated heptamethyl ether (*m/e* 811 (M^+), $FeCl_3$ test: positive) and the deuterated octamethyl ether (*m/e* 828 (M^+), $FeCl_3$ test: negative). Reduction of the deuterated octamethyl ether with lithium aluminum hydride in ether gave two epimeric alcohols. One of the epimers (II) was concluded to be a reduced product not only at the 7" position but also at the 4 position by the following data: 1) in the mass spectrum, there were the molecular ion peak ($C_{48}H_{32}D_{24}O_{10}^{+}$) at *m/e* 816 and the fragment ion peak ($C_{29}H_{22}D_{12}O_5^{+}$) at *m/e* 474 arising from the retro Diels-Alder cleavage in the cyclohexene ring, 2) ^{1}H NMR signal (benzene-d_6) due to the newly-formed methylene hydrogens at C-4 was seen at δ 3.54, 3) the IR spectrum ($CHCl_3$) showed the absence of carbonyl groups.



II

In the ^{1}H NMR spectrum (benzene-d_6) of the alcohol (II), irradiation of the hydroxyl hydrogen signal at δ 3.36 (disappeared with D_2O) changed the signal at δ 4.98 which was assigned to a carbinyl hydrogen, and alternative irradiation of the latter altered the former. The carbinyl hydrogen signal was found to couple to the methine hydrogen signal at δ 3.84 which shows vicinal coupling to the signal at δ 4.70 for the methine hydrogen adjacent to the flavene nucleus but not to the signal at δ 3.88 for the methine hydrogen next to the methylene in the cyclohexene ring. From the results of these decoupling experiments, the locations of the 2,4-dihydroxyphenyl group and the 2,4-dihydroxybenzoyl group were concluded to be as shown in formula II.

Erroneous conclusions drawn from NMR evidence on the locations of these groups in the previous papers[2-4] appear to be due to inappropriate irradiation powers in the ^{13}C-^{1}H decoupling experiments.

On the basis of the above evidence, the structure of moracenin D is represented by formula I.

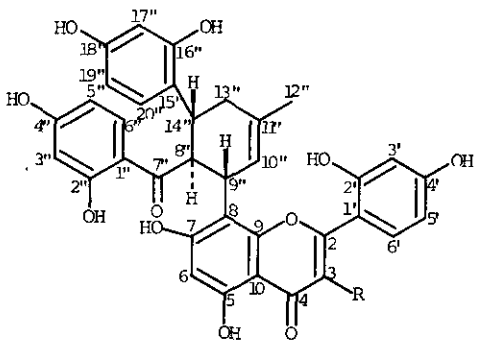
As a consequence, the locations of the phenyl group and the benzoyl group in the cyclohexene ring in moracenin A-C[2-4] have to be revised.

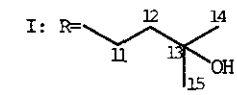
From the fact that the coupling constant between the H-9" and H-8" signals, and that between the H-8" and H-14" signals were both 10 Hz, it is clear that the three hydrogens, H-9", H-8" and H-14", are all quasi-axially situated in the cyclohexene ring, leading to the conclusion that the three substituents, the flavone moiety, the 2,4-dihydroxyphenyl group and the 2,4-dihydroxybenzoyl group, are *trans* to each other.

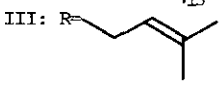
The CD curve of moracenin D ($[\theta]_{380}$ 0, $[\theta]_{315}$ -46600, $[\theta]_{304}$ -42200, $[\theta]_{288}$ -71300, $[\theta]_{270}$ 0, $[\theta]_{264}$ +30600, $[\theta]_{255}$ 0, $[\theta]_{238}$ -59700, $[\theta]_{228}$ -39300 in MeOH) almost overlapped on the CD curves of moracenin A-C[4], a fact which demonstrates that the absolute configuration of moracenin D is the same as that of moracenin A-C.

Because of the parallelism of the ^{1}H and ^{13}C NMR spectra of moracenin D with those of moracenin A-C with respect to the cyclohexene moiety, it is concluded that the conformation of moracenin D is identical to that of moracenin A-C. The chemical shifts of the H-3', H-5' and H-6' signals of

moracenin D (δ 6.64, 6.56 and 7.30, respectively) were compatible with those of kuwanon C (δ 6.52 6.43 and 7.20, respectively), whereas the chemical shift of the H-6 signal (δ 6.00) of moracenin D was shifted relative to that of kuwanon C (δ 6.31).[10] In addition, the H-3", H-5" and H-6" signals of moracenin D (δ 5.98, 5.94 and 7.40, respectively) showed upfield shifts in comparison with the corresponding signals of 2',4'-dihydroxyacetophenone (δ 6.32, 6.44 and 7.76, respectively), and the chemical shifts of the H-17" and H-19" signals of moracenin D (δ 6.20 and 6.06) were displaced relative to those of 4-ethylresorcinol (δ 6.36 and 6.26). These upfield shifts were considered to be originating from the anisotropic shielding effects of the adjacent benzene rings, showing that the planes of the flavone skeleton, the 2,4-dihydroxybenzoyl group and the 2,4-dihydroxyphenyl group are parallel to one another. However, the conformation of moracenin D was not established by the above NMR data. Therefore, no conclusion can be drawn about the absolute configuration of moracenin D which awaits further clarification.







Acknowledgment This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, which is gratefully acknowledged. Thanks are also due to Dr. K. Matsushita, JEOL Ltd., for some NMR data.

NOTES AND REFERENCES

1) Part 28 on the validity of Oriental medicines.
2) Y. Oshima, C. Konno, H. Hikino and K. Matsushita, *Heterocycles*, 14, 1287 (1980)
3) Y. Oshima, C. Konno, H. Hikino and K. Matsushita, *Tetrahedron Letters*, 21, 3381 (1980)
4) Y. Oshima, C. Konno, H. Hikino and K. Matsushita, *Heterocycles*, 14, 1461 (1980)
5) Unless stated otherwise, ^{1}H NMR spectra were taken in acetone-d_6.
6) C. Konno, Y. Oshima and H. Hikino, *Planta Medica*, 32, 118 (1977)
7) T. Nomura and T. Fukai, *Chem. Pharm. Bull.*, 28, 2548 (1980)
8) M. Takasugi, S. Ishikawa, S. Nagao, T. Masamune, A. Shirata and K. Takahashi, *Chem. Letters*, 1577 (1980)
9) These substances were quite recently identified as moracenin B.
10) T. Nomura, T. Fukai and M. Katayanagi, *Chem. Pharm. Bull.*, 26, 1453 (1978)

Received, 14th March, 1981