

STRUCTURE OF MORACENIN D, A HYPOTENSIVE PRINCIPLE OF MORUS ROOT BARKS<sup>1</sup>

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**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).<sup>2-4</sup> 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,  $[\alpha]_D^{25} -388^\circ$  (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 ( $CH_3 \times 3$ ,  $-CH_2- \times 3$ ,  $>CH- \times 3$ ,  $>C-O- \times 1$ ,  $>C=CH- \times 1$ ,  $>C=C-O- \times 1$ ), twenty-four aromatic carbons ( $CH \times 10$ ,  $C \times 5$ ,  $C-O \times 9$ ) 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 ( $\lambda_{max}^{MeOH}$  209, 230 (sh), 264.5, 280 (sh) and 320 nm ( $\log \epsilon$  4.75, 4.50, 4.45, 4.27 and 4.14)) matched the data for moracenin B ( $\lambda_{max}^{MeOH}$  209, 264, 280 (sh) and 320 nm ( $\log \epsilon$  4.80, 4.49, 4.31 and 4.18)).<sup>3</sup> 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 ( $3350\text{ cm}^{-1}$ ) and a carbonyl at C-4 which is conjugated and hydrogen-bonded ( $1650\text{ cm}^{-1}$ ). 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  $\delta$  6.00<sup>5</sup> and 97.5, respectively (hydrogen and carbon at the 6 position in a 5,7-dihydroxyflavone).<sup>3</sup>  $^1H$  NMR signals due to hydrogens of the B-ring in the flavone skeleton appeared at  $\delta$  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  $\delta$  1.14 (6H, singlet) and the methylene hydrogen signals at  $\delta$  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  $\delta$  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 ( $\delta$  21.3 (t), 43.0 (t), 69.9 (s), 29.4 (q) and 29.4 (q) ( $C_5D_5N$ )).<sup>6</sup> In order to determine the position of the 3-hy-

Table I. Carbon-13 shieldings in moracenin D and moracenin B ( $\delta$  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

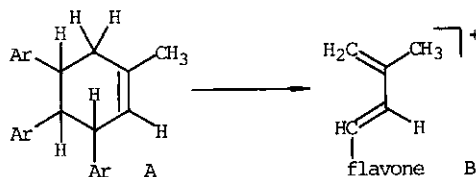
droxy-3-methylbutyl group, long range selective proton decoupling technique was used. As a result it was found that the methylene hydrogen signal at  $\delta$  2.54 and the carbonyl carbon signal at  $\delta$  182.5 were spin coupled ( $J$  4.8 Hz), leading to the conclusion that the 3-hydroxy-3-methylbutyl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

In the aromatic region of the  $^1H$  NMR spectrum, a set of signals of an ABC pattern were seen at  $\delta$  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 ( $\delta$  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)).<sup>3</sup> 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  $^1H$  NMR signals at  $\delta$  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 ( $\delta$  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)).<sup>3</sup> 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  $^1H$  NMR signal attributed to the C-5 hydroxyl hydrogen, another  $^1H$  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  $^1H$  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,<sup>7</sup> and Masamune et



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

From the fact that the parameters of <sup>1</sup>H and <sup>13</sup>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<sub>6</sub> and potassium carbonate in acetone afforded the deuterated hexamethyl ether (*m/e* 794 (M<sup>+</sup>), FeCl<sub>3</sub> test: positive), the deuterated heptamethyl ether (*m/e* 811 (M<sup>+</sup>), FeCl<sub>3</sub> test: positive) and the deuterated octamethyl ether (*m/e* 828 (M<sup>+</sup>), FeCl<sub>3</sub> 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<sub>48</sub>H<sub>32</sub>D<sub>24</sub>O<sub>10</sub><sup>+</sup>) at *m/e* 816 and the fragment ion peak (C<sub>29</sub>H<sub>22</sub>D<sub>12</sub>O<sub>5</sub><sup>+</sup>) at *m/e* 474 arising from the retro Diels-Alder cleavage in the cyclohexene ring, 2) <sup>1</sup>H NMR signal (benzene-d<sub>6</sub>) due to the newly-formed methylene hydrogens at C-4 was seen at δ 3.54, 3) the IR spectrum (CHCl<sub>3</sub>) showed the absence of carbonyl groups.

In the <sup>1</sup>H NMR spectrum (benzene-d<sub>6</sub>) of the alcohol (II), irradiation of the hydroxyl hydrogen signal at δ 3.36 (disappeared with D<sub>2</sub>O) changed the signal at δ 4.98 which was assigned to a carbinylic hydrogen, and alternative irradiation of the latter altered the former. The carbinylic 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<sup>2-4</sup> appear to be due to inappropriate irradiation powers in the <sup>13</sup>C-<sup>1</sup>H decoupling experiments.

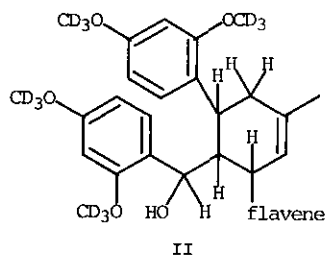
On the basis of the above evidence, the structure of moracenin D is represented by formula I.

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

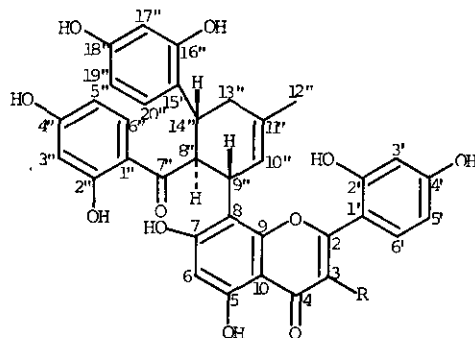
From the fact that the coupling constant between the H-9" and H-8" signals, and that between the H-8" and H-14" signals were both 10 Hz, it is clear that the three hydrogens, H-9", H-8" and H-14", are all quasi-axially situated in the cyclohexene ring, leading to the conclusion that the three substituents, 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 ([θ]<sub>380</sub> 0, [θ]<sub>315</sub> -46600, [θ]<sub>304</sub> -42200, [θ]<sub>288</sub> -71300, [θ]<sub>270</sub> 0, [θ]<sub>264</sub> +30600, [θ]<sub>255</sub> 0, [θ]<sub>238</sub> -59700, [θ]<sub>228</sub> -39300 in MeOH) almost overlapped on the CD curves of moracenin A-C<sup>4</sup>, a fact which demonstrates that the absolute configuration of moracenin D is the same as that of moracenin A-C.

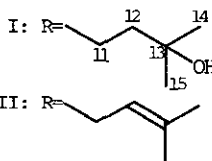
Because of the parallelism of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of moracenin D with those of moracenin A-C with respect to the cyclohexene moiety, it is concluded that the conformation of moracenin D is identical to that of moracenin A-C. The chemical shifts of the H-3', H-5' and H-6'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



moracenin D ( $\delta$  6.64, 6.56 and 7.30, respectively) were compatible with those of kuwanon C ( $\delta$  6.52, 6.43 and 7.20, respectively), whereas the chemical shift of the H-6 signal ( $\delta$  6.00) of moracenin D was shifted relative to that of kuwanon C ( $\delta$  6.31).<sup>10</sup> In addition, the H-3", H-5" and H-6" signals of moracenin D ( $\delta$  5.98, 5.94 and 7.40, respectively) showed upfield shifts in comparison with the corresponding signals of 2',4'-dihydroxyacetophenone ( $\delta$  6.32, 6.44 and 7.76, respectively), and the chemical shifts of the H-17" and H-19" signals of moracenin D ( $\delta$  6.20 and 6.06) were displaced relative to those of 4-ethylresorcinol ( $\delta$  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.



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#### NOTES AND REFERENCES

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