

STRUCTURE OF MORACENIN C, A HYPOTENSIVE PRINCIPLE OF *MORUS* ROOT BARKS¹

Yoshiteru Oshima, Chohachi Konno and Hiroshi Hikino*

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

and

Kazuhiro Matsushita

JEOL Ltd., Nakagami, Akishima, Tokyo, Japan

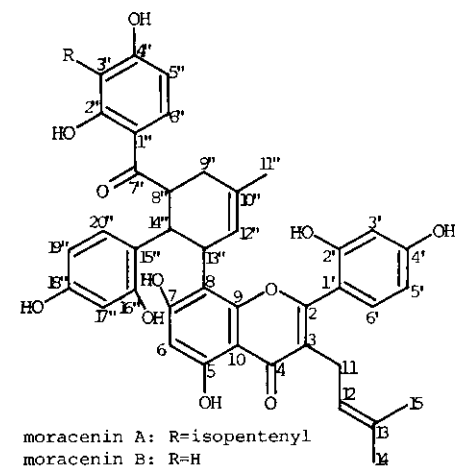
Abstract — From the crude drug "sōhakuhi", the root barks of *Morus* plants, a novel isoprenoid flavone, moracenin C, having hypotensive activity has been isolated and the gross structure has been determined as represented by formula I (exclusive of stereochemistry). The absolute stereostructures of moracenin A, B and C have been deduced on the basis of physico-chemical evidence.

During our investigations on the hypotensive principles of the oriental medicine "sōhakuhi", the root barks of certain species of *Morus* plants (Moraceae), we have recently isolated two isoprenoid flavones, moracenin A and B, as the hypotensive principles, and determined their structures.^{2,3} Further survey for active principles has

led to the isolation of another novel isoprenoid flavone having hypotensive activity named moracenin C. This paper deals with the elucidation of the gross structure of moracenin C and the deduction of the absolute stereostructures of moracenin A, B and C.

Moracenin C, $[\alpha]_D^{25} -445^\circ$ (*c* 0.16, MeOH), gave the FD-MS spectrum which showed the molecular ion peak at *m/e* 760 and the ¹³C NMR spectrum which indicated the presence of forty-five carbons (nineteen aliphatic carbons (CH₃- × 5, -CH₂- × 4, >CH- × 3, >C × 1, >C=CH- × 2, >C=C-O- × 1), twenty-four aromatic carbons (CH × 9, C × 6, C-O × 9) and two carbonyl carbons), so that the composition of moracenin C was determined to be C₄₅H₄₄O₁₁.

Moracenin C was considered to be a flavonoid from



a positive magnesium-hydrochloric acid reaction. The fact that the UV, IR, ¹H and ¹³C NMR spectra of moracenin C, as described in detail below, were very similar to those of moracenin A, and that moracenin C coexists with moracenin A and B in the same plant, suggested that moracenin C was a congener of moracenin A and B. The IR spectrum (KBr and THF) disclosed a characteristic band at 1650 cm⁻¹ (hydrogen-bonded C-4 carbonyl in the flavone skeleton) as well as a strong band at 3350 cm⁻¹ (hydroxyls), the UV spectrum of moracenin C ($\lambda_{\text{max}}^{\text{MeOH}}$ 208, 264, 280(sh) and 320(sh) nm (log ϵ 4.79, 4.50, 4.34 and 4.20)) closely resembled that of moracenin A ($\lambda_{\text{max}}^{\text{MeOH}}$ 206, 264, 286 and 330 nm (log ϵ 4.86, 4.49, 4.32 and 4.15)), and the UV maximum at 264 nm (A-ring benzoyl system) was bathochromically shifted by 8 nm on addition of aluminum chloride, indicating that moracenin C has a

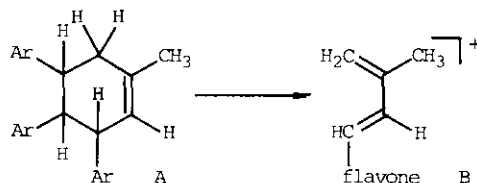
Table I. Carbon-13 shieldings in moracenin C and moracenin A (δ in CD_3CN)

	moracenin C	moracenin A
C-2	156.7 s	156.7 s
C-3	121.5 s	121.7 s
C-4	183.3 s	183.3 s
C-5	155.8 s	155.8 s
C-6	98.5 d	98.5 d
C-7	160.8 s	160.9 s
C-8	107.9 s	108.0 s
C-9	160.8 s	160.8 s
C-10	105.6 s	105.7 s
C-11	24.5 t	24.5 t
C-12	122.4 d	122.4 d
C-13	132.8 s	132.9 s
C-14	25.7 q	25.8 q
C-15	17.7 q	17.8 q
C-1'	113.3 s	113.4 s
C-2'	161.2 s	161.1 s
C-3'	103.6 d	103.7 d
C-4'	162.0 s	162.1 s
C-5'	108.2 d	108.3 d
C-6'	132.2 d	132.3 d
C-1''	114.5 s	115.4 s
C-2''	161.1 s	162.1 s
C-3''	109.0 s	115.1 s
C-4''	163.1 s	163.4 s
C-5''	109.2 d	108.0 d
C-6''	130.3 d	130.8 d
C-7''	209.9 s	210.0 s
C-8''	38.6 d	38.6 d
C-9''	37.9 t	38.0 t
C-10''	134.1 s	134.1 s
C-11''	23.0 q	23.0 q
C-12''	124.0 d	123.9 d
C-13''	38.6 d	38.6 d
C-14''	47.7 d	47.8 d
C-15''	122.4 s	122.4 s
C-16''	156.7 s	156.7 s
C-17''	103.6 d	103.7 d
C-18''	156.6 s	156.6 s
C-19''	107.9 d	107.5 d
C-20''	129.2 d	129.6 d
C-21''	16.7 t	22.0 t
C-22''	32.1 t	122.7 d
C-23''	76.5 s	132.5 s
C-24''	26.8 q	25.8 q
C-25''	26.6 q	17.9 q

There are seven signals in the ^{13}C NMR spectrum of moracenin C whose parameters coincided with those of the C-8''-C-14'' signals in moracenin A (Table I), suggesting that moracenin C has part structure A. This suggestion was confirmed by 1H NMR double resonance experiments. Furthermore, in the mass spectrum, the appearance of a fragment peak at m/e 420.1598 (calc. m/e 420.1571 for $C_{25}H_{24}O_6^+$ (B)), formed by retro Diels-Alder cleavage, demonstrated the flavone skeleton to be situated at C-13'' in the cyclohexene ring. The 1H NMR spectrum of moracenin C showed signals at δ 6.19 (1H, doublet, J 2 Hz), 6.04 (1H, doublet of doublets, J 2 and 8 Hz) and 6.73 (1H, doublet, J 8 Hz) due to H-3, H-5 and H-6 in a 2,4-dihydroxyphenyl group, which corresponded to those for H-17'', H-19'' and H-20'' in moracenin A. This observation, along

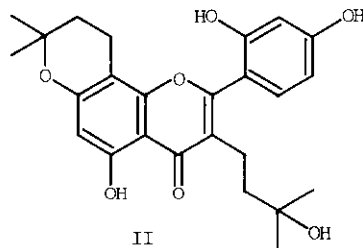
carbonyl at C-4 and a hydroxyl at C-5. The 1H and ^{13}C NMR spectra of moracenin C showed signals at δ 5.99⁴ and 98.5 respectively (hydrogen and carbon at the 6 position in a 5,7-dihydroxyflavone²). Further, the absence of 1H NMR signals for H-3 and H-8 indicated that the 3 and 8 positions were substituted in moracenin C. Three 1H NMR signals at δ 6.65 (1H, doublet, J 2 Hz), 6.54 (1H, doublet of doublets, J 2 and 8 Hz) and 7.27 (1H, doublet, J 8 Hz) in an ABC pattern were observed which, together with the coincidence of the chemical shifts of the ^{13}C NMR signals for C-1'-C-6' in moracenin C and moracenin A, demonstrated that the B-ring of moracenin C has a 2',4'-dihydroxy arrangement as in moracenin A. Thus, these accumulated data indicated moracenin C to be a 5,7,2',4'-tetrahydroxy-3,8-disubstituted-flavone.

1H NMR signals at δ 1.48 (3H, singlet), 1.60 (3H, singlet), 3.17 (2H, doublet, J 6 Hz) and 5.18 (1H, triplet, J 6 Hz) and ^{13}C NMR signals at δ 17.7 (q), 25.7 (q), 24.5 (t), 122.4 (d) and 132.8 (s) suggested the presence of an isopentenyl moiety. The chemical shifts (δ 3.17 and 24.5) of the methylene signals indicated the isopentenyl to be situated at the 3 position,² and this was verified by the observation of ^{13}C - 1H spin coupling (J 3.8 Hz) between the C-4 carbonyl signal at δ 183.3 and the methylene hydrogen signal at δ 3.17.



with the fact that the parameters of the ^{13}C NMR signals for six aromatic carbons in moracenin C were in accord with those for C-15"-C-20" in moracenin A (Table I), suggested that a 2,4-dihydroxyphenyl group is attached to the cyclohexene ring in moracenin C.

The next problem was to determine the nature of the remaining substituent on the cyclohexene ring. The ^1H NMR spectrum showed a pair of doublets in an AB pattern at δ 5.81 and 7.27 (1H each, J 8 Hz), indicating that two *ortho* aromatic hydrogens were present. Also in the ^1H NMR spectrum, no signals for an isopentenyl group located at C-3", as in moracenin A, were found. Instead, signals at δ 1.20 (6H, singlet), 1.65 (2H, triplet, J 6 Hz) and 2.38 (2H, triplet, J 6 Hz) were observed, suggesting the presence of a 2,2-dimethyldihydropyran ring. This suggestion was verified by the observation that ^{13}C NMR signals at δ 16.7 (t), 32.1 (t), 76.5 (s), 26.8 (q) and 26.6 (q) were consistent with signals at δ 16.2 (t), 31.7 (t), 75.9 (s), 26.5 (q) and 26.5 (q) ($\text{C}_5\text{D}_5\text{N}$) due to the aliphatic carbons in the dimethylchroman ring of dihydromorusinol (II). Furthermore, 1) the UV spectrum was in agreement with that of moracenin A while exhibiting an extra absorption at ca. 280 nm as compared with that of kuwanon C, 5,7,2',4'-tetrahydroxy-3,8-diisopentenylflavone,⁵ 2) in the ^{13}C NMR spectrum, a carbonyl carbon signal appeared at δ 209.9, the chemical shift of which was similar to that for the C-7" carbonyl carbon in moracenin A, and 3) the carbonyl carbon signal was spin coupled to the methylene hydrogen signal



at δ 1.96 in the cyclohexene ring. These observations showed that the remaining substituent on the cyclohexene ring was a 2,2-dimethyldihydropyrano-benzoyl system. The composition ($\text{C}_{12}\text{H}_{13}\text{O}_3$) of this substituent and the existence of only two aromatic hydrogens in it indicated that one hydroxyl was attached to the 2,2-dimethyldihydropyrano-benzoyl system. This was confirmed by the observation of the mass fragment peak at m/e 205.0870 (calc. m/e 205.0863 for $\text{C}_{12}\text{H}_{13}\text{O}_3^+$).

Six possibilities exist for the substitution pattern of the hydroxy-2,2-dimethyldihydropyrano-benzoyl moiety. However, the cases where the hydroxyl and the oxygen of the pyran ring were *ortho* and *para* were eliminated, because the ^{13}C NMR signals of the C-O type aromatic carbons should appear at δ 140-150 in these cases, whereas in moracenin C they occurred at δ 161.1 and 163.1. From the remaining three possibilities, the two where the hydroxyl and the carbonyl are situated in an *ortho* relationship were deduced to be correct from the fact that the IR spectrum disclosed a carbonyl band at 1650 cm^{-1} and that another 1H hydrogen-bonded hydroxyl signal was seen in the ^1H NMR spectrum of moracenin C besides the 1H signal due to the C-5 hydroxyl. Since an ^1H NMR aromatic hydrogen signal appeared in the lower field region (δ 7.27) and was spin coupled to the benzoyl carbonyl carbon signal, this hydrogen was concluded to be present in a position *ortho* to the benzoyl carbonyl group. These data established the substitution pattern of the hydroxy-2,2-dimethyldihydropyrano-benzoyl ring to be as shown in formula I.

Allocation of the 2,4-dihydroxyphenyl group and the 2,2-dimethyldihydropyrano-benzoyl group to C-14" and C-8" respectively, was made on the observation of ^{13}C - ^1H spin coupling between the C-7" carbonyl signal and the H-9" methylene signal, as mentioned above.

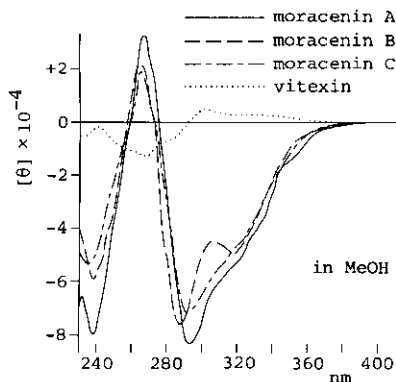
The structure of moracenin C has thus been established to be I (without stereochemistry).

In the ^1H NMR spectrum of moracenin C, the coupling constants between the H-8" and H-14" signals, and between the H-14" and H-13" signals were both 10 Hz, demonstrating that these three hydrogens are quasi-axially situated in the cyclohexene ring. Therefore, the three substituents, the flavone skeleton, the 2,4-dihydroxyphenyl group and the 2,2-dimethyldihydropyrano-benzoyl group are located in *trans* orientation to each other.

The chemical shifts of the H-3', H-5' and H-6' signals in moracenin C (δ 6.65, 6.54 and 7.27

respectively) were similar to those in kuwanon C (δ 6.52, 6.43 and 7.20 respectively), while the chemical shift of the H-6 signal in moracenin C (δ 5.99) differed from that in kuwanon C (δ 6.31).⁵ Furthermore, the chemical shifts of the H-5" and H-6" signals in moracenin C (δ 5.81 and 7.27) were shifted as compared with those of the corresponding signals in 1-acetyl-2,4-dihydroxybenzene (δ

Fig. 1 The CD spectra

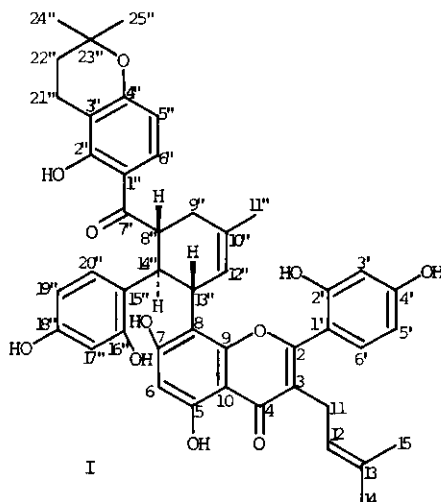


6.44 and 7.76). These upfield shifts of the H-6, H-5" and H-6" signals in moracenin C appear to be caused by the anisotropic shielding effect of the 2,4-dihydroxyphenyl group. Hence, in the preferred conformation of moracenin C, the C-6, C-5" and C-6" hydrogens are situated close to and perpendicular to the plane of the 2,4-dihydroxyphenyl group, where the phenyl moiety of the benzoyl group is nearly eclipsed to the C-8" hydrogen which is the thermodynamically most stable conformation.⁶

The CD curve of moracenin C exhibited a positive Cotton effect at 265 nm and a negative Cotton effect at 292 nm (Fig. 1) which are ascribed to the flavone chromophore and the benzoyl chromophore respectively, since moracenin C showed UV maxima at 264 nm for the flavone chromophore and

at ca. 280 nm for the benzoyl chromophore. The molecular ellipticities of these Cotton effects were much stronger than that of a flavone possessing an asymmetric carbon α to C-8 (e.g., $[\theta]_{269} -12800$ for vitexin⁷ (Fig. 1)) and that of a benzoyl derivative having an asymmetric carbon adjacent to it (e.g., $[\theta]_{280} -317$ for (*S*)-2-methylbutyrophenone⁶), indicating coupling between the dipole moments of the two chromophores. The signs of these Cotton effects demonstrated that the two dipole moments are arranged in an *S*-configuration. Thus, the absolute configurations at C-8", C-13" and C-14" were deduced to be *R*, *S* and *R* respectively. On the basis of the above evidence, the stereostructure of moracenin C is as represented by formula I.

Since the ¹H and ¹³C NMR, and CD spectra of moracenin A and B are essentially identical to those of moracenin C (Fig. 1), the absolute configurations at C-8", C-13" and C-14" in moracenin A and B are concluded to be the same as in moracenin C.



Acknowledgment Thanks are due to Prof. T. Tomimori, Hokuriku University, for a gift of vitexin. 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.

NOTES AND REFERENCES

- 1) Part 26 in the Tohoku University series on the validity of Oriental medicines.
- 2) Y. Oshima, C. Konno, H. Hikino and K. Matsushita, *Tetrahedron Letters*, in press
- 3) Y. Oshima, C. Konno, H. Hikino and K. Matsushita, *Heterocycles*, in press
- 4) ¹H NMR spectra were determined in acetone-*d*₆.
- 5) T. Nomura, T. Fukai and M. Katayanagi, *Chem. Pharm. Bull.*, **26**, 1453 (1978)
- 6) O. Korver, *Tetrahedron*, **27**, 4643 (1971)
- 7) R. M. Horowitz and B. Gentili, *Chem. Ind. (London)*, 498 (1964)

Received, 11th July, 1980