

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

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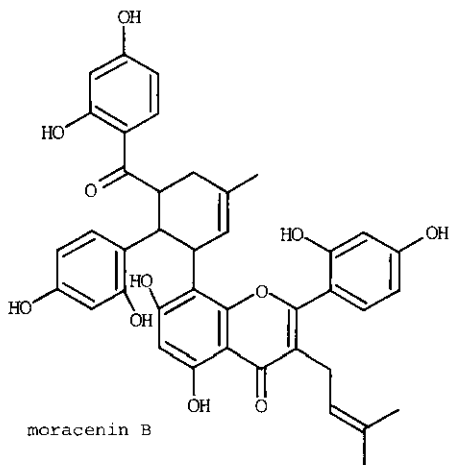
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Abstract — From the crude drug "sōhakuhi", the root barks of *Morus* plants, a novel isoprenoid flavone derivative, moracenin A, showing hypotensive activity has been isolated. The structure has been determined, as indicated by formula I, on the basis of chemical and physical evidence.

From the oriental medicine "sōhakuhi", the root barks of *Morus* plants (Moraceae), we have recently isolated the hypotensive principle moracenin B and deduced its structure.² During the course of the isolation of moracenin B, the acidic fraction from the extract was subjected to chromatography and the hypotensive activity of the resultant fractions monitored. From the fraction less polar than that containing moracenin B, another amorphous isoprenoid flavone was obtained and named moracenin A. Dosing of moracenin A to rats (*i. v.*) induced a significant hypotension. The present paper describes the structural elucidation of the active principle, moracenin A.



Moracenin A, $[\alpha]_D^{25} -427^\circ$ (*c* 0.15, MeOH), was shown to have molecular weight 760 (FD-MS) which, along with the results of ¹³C NMR spectroscopy, indicated its composition to be C₄₅H₄₄O₁₁. Namely, the ¹³C NMR spectrum demonstrated the presence of nineteen aliphatic carbons (CH₃- × 5, -CH₂- × 3, >CH- × 3, >C=CH- × 3, >C=C-O × 1), twenty-four aromatic carbons (CH × 9, C × 6, C-O × 9) and two carbonyl carbons. The ¹H NMR spectrum of moracenin A was very similar to that of moracenin B, as will be discussed in detail below. These data, together with the fact that moracenin A coexists with

moracenin B, led to the supposition that moracenin A is a homolog of moracenin B, with the extra C₅H₈ moiety constituting an isopentenyl group.

As for the case of moracenin B, the structure determination of moracenin A was made solely from analysis of the spectral data.

Its polyphenolic nature, which was suggested by the fact that moracenin A has a number of aromatic carbons of the C-O type (¹³C NMR), was substantiated by the occurrence of an intense IR band at 3360 cm⁻¹ (KBr) and a positive ferric chloride test.

That moracenin A is a flavonoid was deduced from a positive reaction with magnesium and hydro-

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

	moracenin A	moracenin B
C-2	156.7 s	157.3 s
C-3	121.7 s	121.6 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	160.9 s	160.9 s
C-8	108.0 s	108.0 s
C-9	160.8 s	160.9 s
C-10	105.7 s	105.7 s
C-11	24.5 t	24.5 t
C-12	122.4 d	122.4 d
C-13	132.9 s	132.9 s
C-14	25.8 q	25.8 q
C-15	17.8 q	17.7 q
C-1'	113.4 s	113.4 s
C-2'	161.1 s	161.3 s
C-3'	103.7 d	103.7 d
C-4'	162.1 s	162.2 s
C-5'	108.3 d	108.3 d
C-6'	132.3 d	132.3 d
C-1"	115.4 s	115.5 s
C-2"	162.1 s	165.1 s
C-3"	115.1 s	103.7 d
C-4"	163.4 s	165.7 s
C-5"	108.0 d	108.3 d
C-6"	130.8 d	133.8 d
C-7"	210.0 s	209.8 s
C-8"	38.6 d	38.5 d
C-9"	38.0 t	38.0 t
C-10"	134.1 s	134.1 s
C-11"	23.0 q	23.0 q
C-12"	123.9 d	124.0 d
C-13"	38.6 d	38.5 d
C-14"	47.8 d	47.9 d
C-15"	122.4 s	122.4 s
C-16"	156.7 s	156.7 s
C-17"	103.7 d	103.0 d
C-18"	156.6 s	156.6 s
C-19"	107.5 d	108.3 d
C-20"	129.6 d	130.3 d
C-21"	22.0 t	
C-22"	122.7 d	
C-23"	132.5 s	
C-24"	25.8 q	
C-25"	17.9 q	

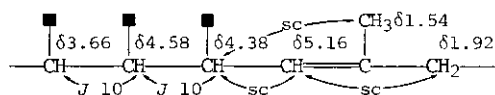
chloric acid. The location of the C-4 carbonyl and the C-5 hydroxyl was shown by the presence of an IR band at 1650 cm^{-1} (KBr and THF), associated with a carbonyl conjugated and hydrogen-bonded, and by the occurrence of a red shift of the UV maximum at 264 nm, by 7 nm, on addition of aluminum chloride. An 1H NMR singlet (1H) at δ 5.98³ and a ^{13}C NMR doublet at δ 98.5 were attributed to the hydrogen and the carbon, respectively, at the 6 position in a 5,7-dihydroxyflavone.² In the 1H NMR spectrum, 1H signals at δ 6.62 (doublet, J 2 Hz), 6.53 (doublet of doublets, J 2 and 8 Hz) and 7.24 (doublet, J 8 Hz) in an ABC pattern whose parameters are in agreement with those for the C-3', C-5' and C-6' hydrogens of the B-ring of moracenin B, indicated that moracenin A also possesses a 2,4-dihydroxyphenyl moiety. These findings, together with the lack of 1H NMR signals for hydrogens at C-3 and C-8, showed moracenin A to be a 5,7,2',4'-tetrahydroxy-3,8-disubstituted flavone. Consistent with this, the UV spectrum (λ_{max}^{MeOH} 206, 264, 286 and 330 nm ($\log \epsilon$ 4.86, 4.49, 4.32 and 4.15)) was almost superimposable on that of moracenin B (λ_{max}^{MeOH} 209, 264, 280 (sh) and 320 nm ($\log \epsilon$ 4.80, 4.49, 4.31 and 4.18)). Furthermore, the parameters of the ^{13}C NMR signals for the fifteen carbons constituting the flavone skeleton of moracenin A (C-2-C-10 and C-1'-C-6') coincided with those for the corresponding carbons of moracenin B (Table I).

In the 1H NMR spectrum, two vinyl methyl signals at δ 1.48 and 1.59, and a vinyl hydrogen signal at δ 5.12 which was further spin coupled (J 6 Hz) to a methylene hydrogen signal at δ 3.10, demonstrated the presence of an isopentenyl group. The ^{13}C NMR spectrum exhibited a set of signals (δ 17.8 and 25.8 for $CH_3 \times 2$, δ 132.9 and 123.9 for $>C=CH-$ and δ 24.5 for $-CH_2-$) ascribed to an isopentenyl group. Because the chemical shifts of the above NMR signals agree well with those originating from the C-3 isopentenyl group in moracenin B, the location of this isopentenyl at C-3 was suggested. This was confirmed by the observation of $^{13}C-^1H$ spin coupling (J 4.2 Hz) between the C-4 carbonyl carbon signal at δ 183.3 and the methylene hydrogen signal at δ 3.10.

The problem remaining to be solved was the constitution of the C-8 side chain with the composition $C_{25}H_{27}O_5$. The 1H NMR spectrum showed a set of 1H signals for three aromatic hydrogens (doublet at δ 6.18 (J 2 Hz), doublet of doublets at δ 6.05 (J 2 and 8 Hz) and doublet at δ 6.76 (J

8 Hz) and another set of 1H signals for two aromatic hydrogens (doublet at δ 5.96 (J 8 Hz) and doublet at δ 7.24 (J 8 Hz)). The parameters of the former set of signals were in accord with those of the 2,4-dihydroxyphenyl system (C-15"-C-20") in moracenin B (doublet at δ 6.14 (J 2 Hz), doublet of doublets at δ 6.02 (J 2 and 8 Hz) and doublet at δ 6.71 (J 8 Hz)), indicating the presence of a 2,4-dihydroxyphenyl moiety in this side chain, as in moracenin B. The chemical shifts of the ^{13}C NMR signals originating from this 2,4-dihydroxyphenyl group also coincided with those of the corresponding system in moracenin B (Table I). The splitting pattern, an AB type (J 8 Hz), of the latter set of ^1H NMR signals demonstrated that the hydrogens in question were vicinal. The chemical shifts of these two signals were consistent with those of the C-5" and C-6" hydrogens of the 2,4-dihydroxybenzoyl system in moracenin B (δ 5.90 and 7.32), suggesting that moracenin A also has a 2,4-dihydroxybenzoyl moiety. The presence of a benzoyl grouping was confirmed by the following observations: 1) the UV spectrum agreed well with that of moracenin B and disclosed an extra absorption around 280 nm in comparison with that of kuwanon C, 5,7,2',4'-tetrahydroxy-3,8-diisopentenyl-flavone,^{2,4} and 2) no IR band attributable to carbonyl was visible other than that at 1650 cm^{-1} , indicating the second carbonyl to be also conjugated. As there were no other signals ascribable to an aromatic hydrogen, substitution of the benzoyl system by an isopentenyl group would make up the required atom count, and this extra isopentenyl unit would constitute the difference between moracenin A and moracenin B. The presence of this isopentenyl group was shown by the ^1H NMR spectrum (singlets at δ 1.54 and 1.64 for $\text{CH}_3 \times 2$, triplet at δ 5.02 (J 6 Hz) for $>\text{C}=\text{CH}-$ and doublet at δ 3.10 (J 6 Hz) for $-\text{CH}_2-$) and the ^{13}C NMR spectrum (Table I). A dihydroxy-isopentenyl-benzoyl moiety was confirmed by the mass spectrum which exhibited a peak at m/e 205.0802 associated with the ion $\text{C}_{12}\text{H}_{13}\text{O}_3^+$ (calc. m/e 205.0863).

Theoretically, there are six possibilities for the arrangement of the three substituents, two hydroxyls and an isopentenyl, in the benzoyl system. The four possibilities where the two hydroxyls are located in ortho and para relations were eliminated because the ^{13}C NMR signals due to the carbons bearing the hydroxyls are expected in these cases to appear at δ 140-150, whereas in fact they occurred at δ 162.1 and 163.4. For choosing between the remaining two possibilities, the ^{13}C NMR spectrum was again examined. As a result it was found that the chemical shifts of the signals for the six aromatic carbons were in agreement with the calculated values for a 2,4-dihydroxy-3-isopentenyl-benzoyl grouping (δ 115.1, 166.0, 113.1, 167.0, 109.0 and 131.7 for C-1-C-6) but not with those for a 2,6-dihydroxy-3-isopentenyl-benzoyl grouping (δ 111.6, 163.2, 117.6, 138.5, 108.6 and 159.6 for C-1-C-6).^{2,5,6}

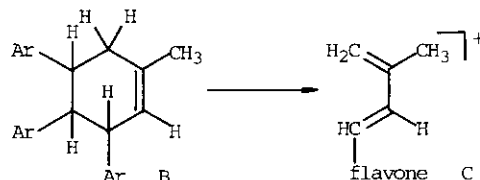


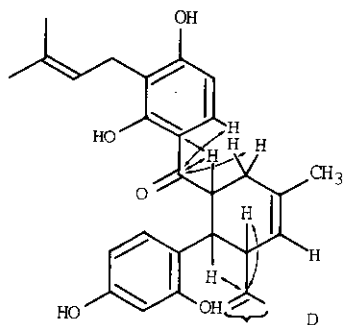
A ■=quaternary carbon, sc=small coupling

with the aid of double resonance experiments which revealed the presence of part structure A. Since the methine hydrogen signal at δ 3.66 and the methylene hydrogen signal at δ 1.92 were spin coupled to each other, part structure A may be extended to part structure B.

The location of the flavone skeleton was deduced by the observation of the mass fragment at m/e 420.1567 originating from the ion $\text{C}_{25}\text{H}_{24}\text{O}_6^+$ (C) (calc. m/e 420.1571).

The remaining part of the C-8 side chain consisted of C_7H_9 which, based on the NMR evidence, was allotted to three methines, one methylene, one methyl and one ethylene bond. In order to completely clarify the nature of the C-8 side chain, the ^1H NMR spectrum of moracenin A was analyzed





The allocation of the 2,4-dihydroxy-3-isopentenyl-benzoyl group, the 2,4-dihydroxyphenyl group and the flavone skeleton to the three substitution positions in part structure B was accomplished from the ^{13}C - ^1H spin couplings illustrated in formula D.

The ^{13}C NMR spectrum of moracenin A could be completely rationalized on the conclusion that moracenin A is the 3''-isopentenyl derivative of moracenin B (Table I).

The structure of moracenin A has thus been deduced to be that represented by formula I.

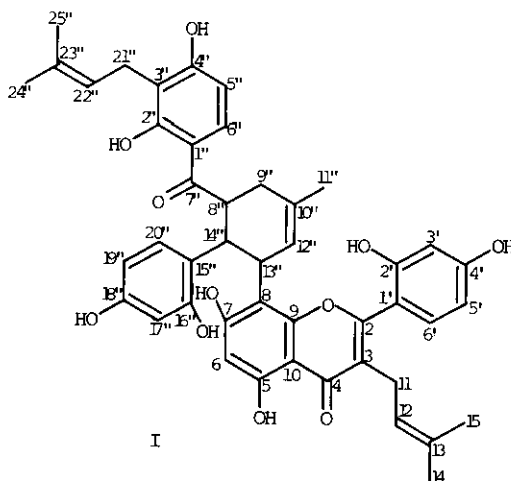
The coupling constants between the C-8'' and C-14'' hydrogens and between the C-14'' and C-13'' hydrogens in the ^1H NMR spectrum

were both 10 Hz, a fact which indicated that the C-8'' hydrogen, C-14'' hydrogen and C-13'' hydrogen were quasi-axially oriented in the cyclohexene ring, thereby allowing assignment of the *trans*-orientation of the C-8'' and C-14'' hydrogens and of the C-14'' and C-13'' hydrogens.

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NOTES AND REFERENCES

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