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

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Abstract — From the crude drug "sōhakuhi", the root barks of *Morus* plants, a new derivative of isoprenoid flavones, moracenin B, showing hypotensive activity has been isolated and the structure has been determined as I on the basis of chemical and physical evidence.

The crude drug "sōhakuhi" is prepared from the root barks of certain species of *Morus* plants (Moraceae) and has been employed as a diuretic, an antitussive, an expectorant and a tonic in Oriental medicine. A number of pharmacological investigations have shown that it has a hypotensive effect. Although many substances, including isoprenoid flavones,[2] have been isolated from the crude drug, no hypotensive principle has been reported, except for Tanemura's pharmacological examination of a hypotensive principle which, however, was not chemically characterized.[3] Since we have found that a marked hypotension was induced when the methanol extract of a preparation was dosed to rats, we have carried out the elucidation of an active principle.

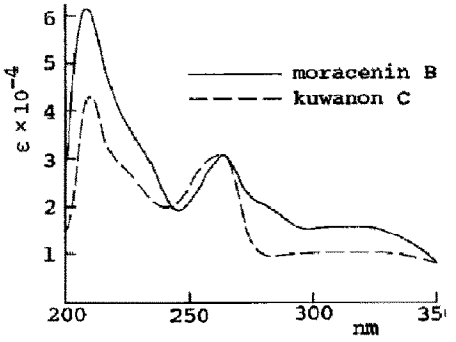
The extract was separated into acidic, neutral and basic portions. As the acidic portion was found to be active, it was repeatedly chromatographed, while monitoring hypotensive activity of the fractions, to give the amorphous flavonoid, now designated moracenin B, the administration of which to rats (*i.v.*) produced a significant hypotension. The present report deals with the structure determination of this active principle, moracenin B.

Moracenin B, $[\alpha]_D$ -466° (*c* 0.13, MeOH), was found to have molecular weight 692 (FD-MS) which, together with the results of ^{13}C NMR spectroscopy, showed its composition to be $C_{40}H_{36}O_{11}$. Thus, the ^{13}C NMR spectrum indicated the presence of fourteen aliphatic carbons (CH_3- × 3, $-CH_2-$ × 2, >CH- × 3, >C=CH- × 2, >C=C-O × 1), twenty-four aromatic carbons (CH × 10, C × 5, C-O × 9) and two carbonyl carbons. Further, it exhibited an intense band at 3350 cm^{-1} in the IR spectrum (KBr) and gave a positive ferric chloride test, indicating that it is a polyphenol.

Moracenin B gave a positive reaction with magnesium and hydrochloric acid, indicating it to be a flavonoid. In support of this, an IR band at 1650 cm^{-1} (KBr and THF), for a carbonyl conjugated and hydrogen-bonded, determined the location of the C-4 carbonyl and the C-5 hydroxyl, which was further confirmed by a red shift of the UV maximum at 264 nm by 9 nm on addition of aluminum chloride. Although an ^1H NMR singlet (1H) appeared at δ 5.96,[4] its line position provided no decisive evidence for the location of this hydrogen at C-6 or C-8 in a 5,7-dihydroxyflavone. However, the ^{13}C NMR signal at δ 98.5 for the aromatic carbon carrying the hydrogen in question showed that this carbon is situated at the 6 position (C-6 carbons occur at δ 97-100 while C-8 carbons appear at δ 94-96[2,5]). ^1H NMR signals (1H each) occurred at δ 6.58 (doublet,

J 2 Hz), 6.50 (doublet of doublets, J 2 and 8 Hz) and 7.20 (doublet, J 8 Hz) in an ABC pattern whose chemical shifts and splitting patterns were in accord with those for the C-3', C-5' and C-6' hydrogens of kuwanon C, 5,7,2',4'-tetrahydroxy-3,8-diisopentenyl-flavone,[6] pointing to the presence of a 2,4-dihydroxyphenyl as the B-ring of moracenin B. These data showed that moracenin B is a 5,7,2',4'-tetrahydroxy-3,8-disubstituted-flavone. In agreement with this conclusion, the UV spectrum (λ_{max}^{MeOH} 209, 264, 280 (sh) and 320 nm (log ε 4.80, 4.49, 4.31 and 4.18, respectively) resembled that (λ_{max}^{EtOH} 210, 264.5 and 315 nm (log ε 4.63, 4.49 and 4.06, respectively[6]) of kuwanon C. Further, the parameters of the ^{13}C NMR signals for the fifteen carbons of the flavone skeleton of moracenin B (C-2—C-10 and C-1'—C-6') fit well with those of kuwanon C (Table I).

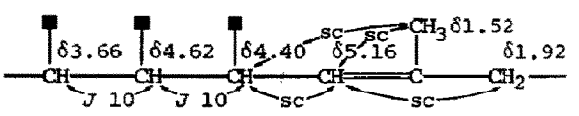
Fig. 1 The UV spectra



In the ^1H NMR spectrum, there was long range coupling between two vinyl methyl signals at δ 1.48 and 1.60 and a vinyl hydrogen signal at δ 5.12, which was further coupled (J 6 Hz) with a methylene hydrogen signal at δ 3.14, indicating the presence of an isopentenyl system. This was substantiated by the occurrence of a set of ^{13}C NMR signals (δ 17.7 and 25.8 for CH_3- ×2, δ 132.9 and 124.0 for >C=CH- and δ 24.5 for $-CH_2-$). Hydrogenation of moracenin B over platinum in methanol afforded a dihydro-derivative whose ^1H NMR spectrum revealed the disappearance of the isopentenyl function with the formation of an isoamyl function (6H doublet at δ 0.84). This isopentenyl group could be attached to either C-3 or C-8. In kuwanon C, a model compound bearing isopentenyls at both C-3 and C-8, the NMR signals for the methylene of the C-3 isopentenyl appear at δ_H 3.12 and δ_C 24.9, while those for the C-8 isopentenyl occur at δ_H 3.35 and δ_C 22.3.[6] In moracenin B, these signals appeared at δ_H 3.14 and δ_C 24.5, showing the isopentenyl group to be at C-3. This was conclusively proved by the fact that a ^{13}C-^1H spin coupling (J 4.2 Hz) was observed between the C-4 carbonyl carbon signal at δ 183.3 and the methylene hydrogen signal at δ 3.14.

The remaining problem was to settle the arrangement of the C-8 side chain of composition $C_{20}H_{19}O_5$. The ^1H NMR spectrum of moracenin B exhibited two sets of signals for the C-3, C-5 and C-6 hydrogens in 2,4-dihydroxyphenyl groupings (doublet at δ 5.92 (J 2 Hz), doublet of doublets at δ 5.90 (J 2 and 8 Hz) and doublet at δ 7.32 (J 8 Hz), and doublet at δ 6.14 (J 2 Hz), double of doublets at δ 6.02 (J 2 and 8 Hz) and doublet at δ 6.71 (J 8 Hz)). The ^{13}C NMR spectrum also revealed the presence of two 2,4-dihydroxyphenyl groups (Table I).

The side chain moiety left to be assigned beared the composition C_8H_9O which, on the basis of the NMR evidence, was allocated to three methines, one methylene, one vinyl methyl, one ethylenic linkage and a carbonyl. Analysis of the ^1H NMR spectrum of moracenin B with the aid of double resonance experiments showed the presence of part structure A. Coupling was observed



A ■=quaternary carbon, sc=small coupling

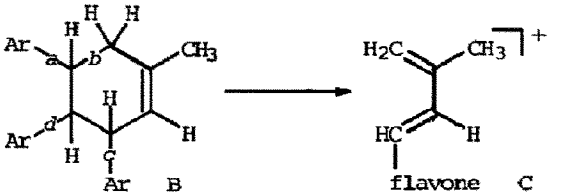


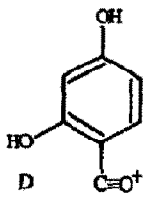
Table I. Carbon-13 shieldings in moracenin B and related substances (δ)

	moracenin B (CD_3CN)	moracenin B (C_5D_5N)	kuwanon C (C_5D_5N)[2]	1-acetyl-2,4-dihydroxybenzene (CD_3CN)	1-isopropyl-2,4-dihydroxybenzene*
C-2	157.3 s	157.9 s	158.5 s		
C-3	121.6 s	120.7 s	120.9 s		
C-4	183.3 s	183.0 s	183.3 s		
C-5	155.8 s	157.2 s	151.2 s		
C-6	98.5 d	98.4 d	98.9 d		
C-7	160.9 s	162.7 s	162.9 s		
C-8	108.0 s	108.3 s	106.9 s		
C-9	160.9 s	162.4 s	162.9 s		
C-10	105.7 s	105.1 s	105.1 s		
C-11	24.5 t	24.8 t	24.9 t		
C-12	124.0 d	——†	123.2 d		
C-13	132.9 s	131.6 s	131.7 s		
C-14	25.8 q	25.8 q	25.8 q		
C-15	17.7 q	17.8 q	17.9 q		
C-1'	113.4 s	113.2 s	113.2 s		
C-2'	161.3 s	160.9 s	161.0 s		
C-3'	103.7 d	103.9 d	104.4 d		
C-4'	162.2 s	162.0 s	162.4 s		
C-5'	108.3 d	108,1 d	108,0 d		
C-6'	132.3 d	132,0 d	132,3 d		
C-1"	115.5 s	115.5 s		115.2 s	
C-2"	165.1 s	165.9 s		165.3 s	
C-3"	103.7 d	103.9 d		104.2 d	
C-4"	165.7 s	165.9 s		166.3 s	
C-5"	108.3 d	107.7 d		109.1 d	
C-6"	133.8 d	133.6 d		134.6 d	
C-7"	209.8 s	209.4 s		204.3 s	
C-8"	38.5 d	38.9 d			
C-9"	38.0 t	38.4 t			
C-10"	134.1 s	133.6 s			
C-11"	23.0 q	23.1 q			
C-12"	122.4 d	122.9 d			
C-13"	38.5 d	38.9 d			
C-14"	47.9 d	47.7 d			
C-15"	122.4 s	122.9 s			127.4 s
C-16"	156.7 s	157,6 s			153.9 s
C-17"	103.0 d	103.1 d			102.7 d
C-18"	156.6 s	157.2 s			153.3 s
C-19"	108.3 d	107.3 d			108.3 d
C-20"	130.3 d	128.8 d			128.0 d

*calculated from the shieldings of 1-isopropyl-2-hydroxybenzene[7] and the additive substituent parameters for the additional hydroxyl[8] †undeterminable due to overlapping

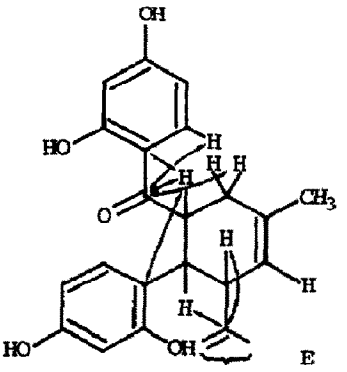
between the methine hydrogen signal at δ 3.66 and the methylene hydrogen signal at δ 1.92, although the coupling constants could not be estimated. Part structure A may thus be expanded to part structure B where the carbonyl is located at the *a*, *b*, *c* or *d* position.

The *c* position for the carbonyl was excluded by the occurrence in the mass spectrum of moracenin B of a peak at *m/e* 420.1558 due to the ion $C_{25}H_{24}O_6^+$ (C) (calc. *m/e* 420.1571).



The location of the carbonyl at the *b* position was eliminated by the following facts: 1) the mass spectrum of moracenin B showed a peak at *m/e* 137.0222 for the ion $C_7H_5O_3^+$ (D) (calc. *m/e* 137.0237), 2) comparison of the UV spectrum of moracenin

B with that of kuwanon C (Fig. 1) disclosed an extra absorption in the former at ~280 nm which must be ascribed to a conjugated carbonyl, 3) no carbonyl band is seen other than the band at 1650 cm^{-1} in the IR spectrum of moracenin B, indicating the second carbonyl to be also conjugated, 4) in the ^{1}H NMR spectrum of moracenin B, one signal for the C-6 hydrogen in a 2,4-dihydroxyphenyl occurred at δ 6.71 which is consistent with that of a 1-alkyl-2,4-dihydroxybenzene (*e.g.*, δ 6.85 (DMSO-d_6+$CDCl_3$)[9] for 1-ethyl-2,4-dihydroxybenzene), while the other signal attributed to another hydrogen of the same situation appeared at δ 7.32 which is consistent with that of a 1-(1-oxo-alkyl)-2,4-dihydroxybenzene (*e.g.*, δ 7.52 (DMSO-d_6+$CDCl_3$)[10] and 7.76 for 1-acetyl-2,4-dihydroxybenzene), 5) the ^{13}C NMR spectrum of moracenin B showed two sets of signals for two 2,4-dihydroxyphenyls which are in accord with the calculated shifts of 1-isopropyl-2,4-dihydroxybenzene and the observed shifts of 1-acetyl-2,4-dihydroxybenzene, respectively (Table I) and 6) a ^{13}C-^{1}H spin coupling was found between the carbonyl carbon signal at δ 209.8 and the C-6 hydrogen signal at δ 7.32 in a 2,4-dihydroxyphenyl.

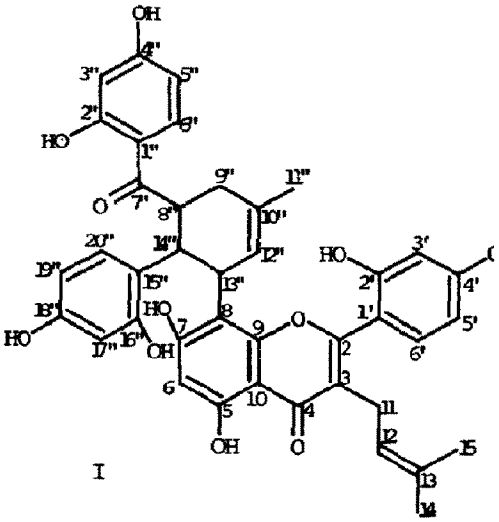


E

Further examination revealed the presence of ^{13}C-^{1}H spin couplings as in formula E. Among these couplings, those between the signal for the carbonyl carbon at δ 209.8 and that for the methylene hydrogens at δ 1.92, and between that for the aromatic carbon α to the carbonyl at δ 115.5 and that for the methine hydrogen at δ 3.66, eliminated the *d* position for the carbonyl. The observed ^{13}C-^{1}H spin couplings are rationalized by the *a* position for the carbonyl.

The structure of moracenin B was thus established to be that represented by formula I.

Moracenin B, having a unique carbon skeleton, appears to be biosynthesized from two units of chalcone and two isoprene units.



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NOTES AND REFERENCES

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