STRUCTURE OF SANGGENON D, A NATURAL HYPOTENSIVE DIELS-ALDER ADDUCT FROM CHINESE CRUDE DRUG "SĀNG-BÁI-PÍ" (MORUS ROOT BARKS)[+]

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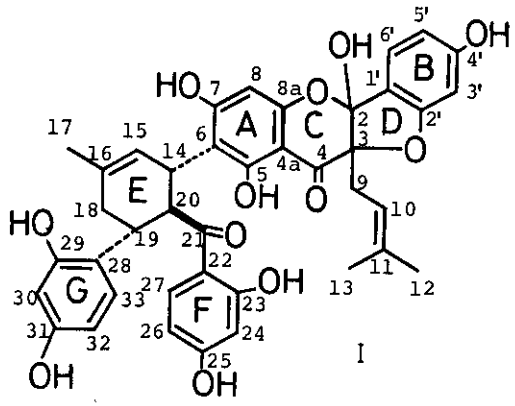
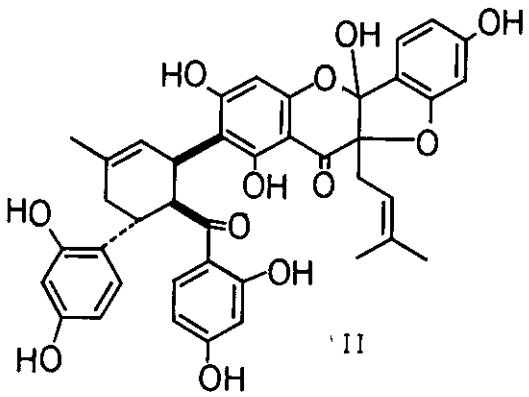
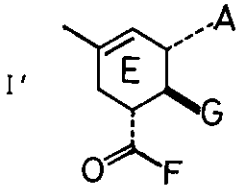
Abstract ——— From the ethyl acetate extract of the Chinese crude drug "Sāng-Bái-Pí" (Japanese name Sōhakuhi), the root barks of Morus sp. (Moraceae), a new flavanone derivative with a fused dihydrochalcone partial moiety was isolated and named sanggenon D. The structure was shown to be I on the basis of spectral data. Sanggenon D (I) is a stereoisomer at the C-14 position on the cyclohexene ring of sanggenon C (II) obtained from the same source, and is regarded biogenetically as a Diels-Alder adduct of chalcone derivative and a dehydroprenylflavanone derivative. The nmr variable temperature studies on I and II are depicted in Figures 2 and 3.

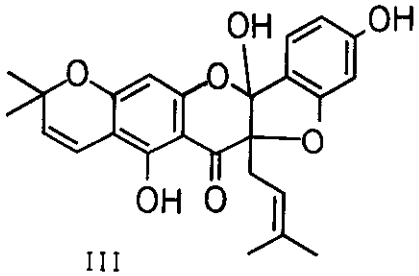
In the previous communications,[1,2] we reported that a natural hypotensive Diels-Alder adduct, *sanggenon C (II), as well as an isoprene-substituted flavanone* derivative, sanggenon A (III), were isolated from the Chinese crude drug "Sāng-Bái-Pí" (Japanese name Sōhakuhi"), the root barks of Morus sp. (Moraceae), and the structures were shown to be II and III, respectively. In this paper, we report the isolation and structure determination of a new flavanone derivative, sanggenon D (I), isolated from the methanol extract of the same drug.

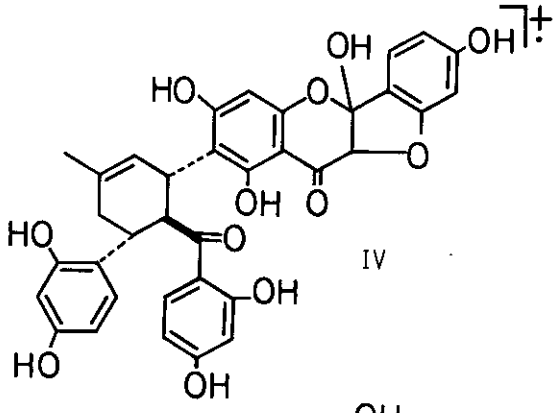
The crude drug "Sāng-Bái-Pí" (8 Kg) imported from the People's Republic of China was extracted successively with n-hexane, benzene, and methanol. The methanol extract was dissolved in ethyl acetate. The ethyl acetate extract was fractionated sequentially by the column and the preparative thin-layer chromatography over silica gel to give sanggenon D (I) in 1.3×10^{-2}% yield from the crude

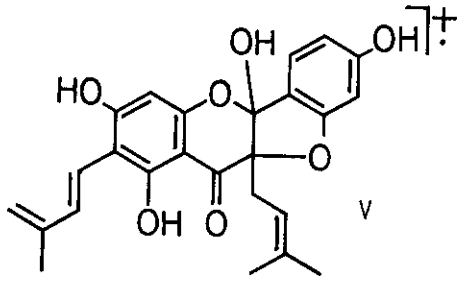


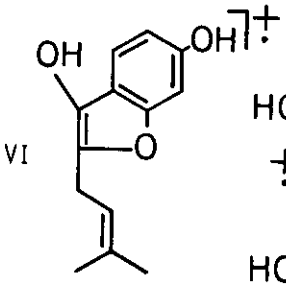


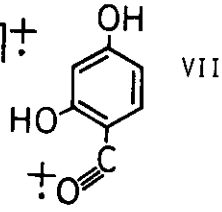


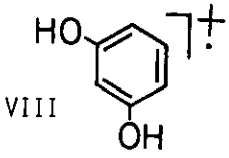












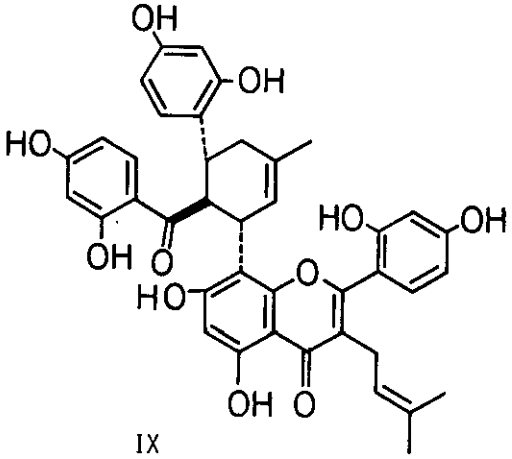


Fig. 1

drug. The compound (I) showed a marked hypotensive effect (0.5-2.0 mg/Kg, i.v.) in rat.

Sanggenon D (I), amorphous powder,[3] $[\alpha]_D^{26}$ - 145° (c=0.17 in methanol), gave the FD-MS spectrum which showed the molecular ion peak at m/e 708, and the ^{13}C nmr spectrum[4] (100° C, ^{12}C-DMSO-d_6) indicated the presence of forty carbons [fourteen aliphatic carbons (3xCH_3, 2x-CH_2-, 3x>CH-, 1x>C-O-, 1x>C<O, 2x>C=CH-), twenty four aromatic carbons (10xCH, 5xC, 9xC-O) and two carbonyl carbons]. The elemental analysis gave the following result: Anal. Calcd. for $C_{40}H_{36}O_{12}\cdot 2H_2O$: C, 64.51; H, 5.41. Found: C, 64.75; H, 5.20. These results suggest the composition of sanggenon D (I) to be $C_{40}H_{36}O_{12}$. The compound (I) showed the following color reactions: Mg-HCl test (orange), $NaBH_4$ test (violet),[5] $FeCl_3$ test (reddish violet), and showed the following spectra: ir ν_{max}^{Nujol} cm^{-1}: 3300, 1660 (sh), 1655 (sh), 1640 (sh), 1625, 1580; uv λ_{max}^{MeOH} nm (log ε): 220 (sh 4.65), 226 (sh 4.57), 283 (4.33), 288 (sh 4.32), 309 (4.30); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 225 (4.62), 292 (sh 4.28), 309 (4.39), 352 (sh 3.94), 400 (sh 3.38). These finding indicate that I is a flavanone derivative. The uv spectra were similar to those of sanggenon C (II).[2]

The mass spectrum of sanggenon D (I) was also similar to that of sanggenon C (II),[2] and showed the following characteristic fragments[6]: m/e 708 (M^+), 640 (IV), 598 ($M^+-C_6H_6O_2$), 436 (V),[2] 421 (436-CH_3),[2] 353 (421-C_5H_8),[2] 218 (VI),[2] 137 (VII),[2] 110 (VIII).[2] This result suggests that sanggenon D (I) may be a Diels-Alder adduct such as kuwanon G[7] (IX) (= albanin F[8] = moracenin B[9]) regarded as a cyclo-addition product with the chalcone and the dehydroprenylflavanone derivative.

The ^1H and the ^{13}C nmr spectra of sanggenon D (I) observed at room temperature showed the complex patterns, and the signals appeared broad. These phenomena suggest that sanggenon D (I) exists as an equilibrium mixture of conformational isomers in the solution. The ^1H and the ^{13}C nmr variable temperature studies were then carried out on I, and the former study on sanggenon C (II) was also performed. At higher temperature, the ^1H and the ^{13}C nmr spectra of I showed the simple patterns, and the signals appeared more sharply owing to the rapid conversion of the isomers (Fig. 2 and Table 1). At lower temperature, the ^1H nmr spectrum of I showed a more complex pattern than the spectrum at room temperature (Fig. 2). On the other hand, the ^1H nmr spectrum of II did not show such a remarkable variation as of I (Fig. 3). In the ^{13}C nmr spectrum of I (100° C, ^{12}C-DMSO-d_6), the chemical shift values of the carbon atoms of the flavanone skeleton of I were similar to those of sanggenon A (III)[1] except the signals of carbon atoms (C-6 and

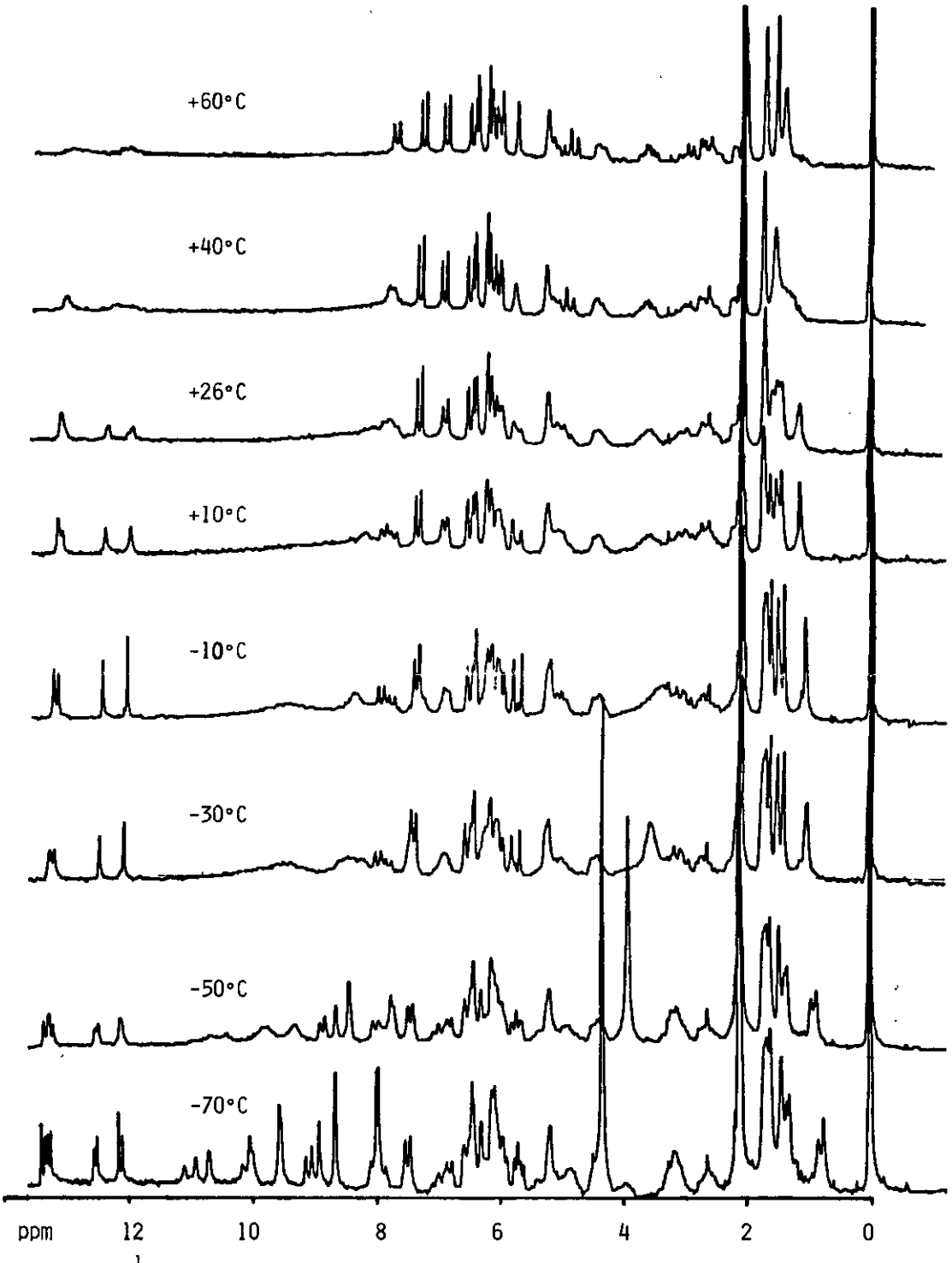


Fig. 2 ^{1}H nmr spectra of I in acetone-d_6

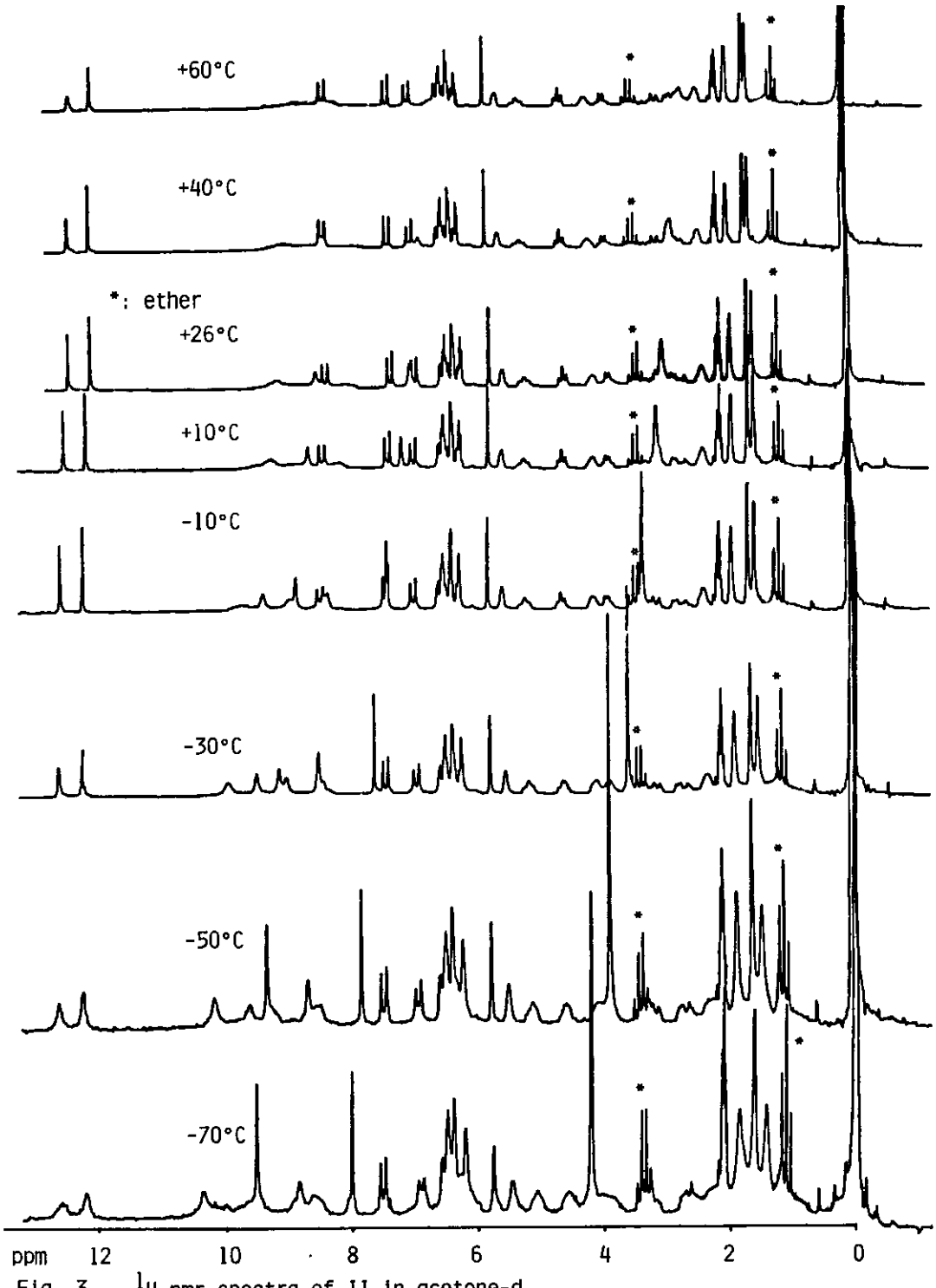


Fig. 3 ^{1}H nmr spectra of II in acetone-d_6

Table 1 ^{13}C nmr chemical shifts

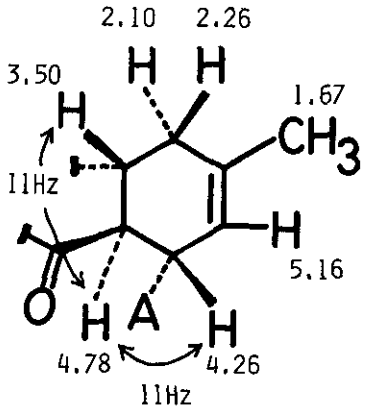
compound	I	I	III	II		I	I	II	IX
C-2	90.6	90.8	92.6	90.4	C-14	38.5 br	39.7	33.1	[38.5]
C-3	101.7	102.2	102.5	101.7	C-15	124.1 br	124.4	121.4	123.2
C-4	187.1	187.1	188.6	187.2	C-16	131.7	132.0	132.6	132.8
C-4a	98.7	99.6	100.5	98.8	C-17	22.8	22.9	23.3	22.5
C-5	162.8, 160.7	162.1 br	163.3	163.3	C-18	36.2 br	37.4	32.8	[37.9]
C-6	108.6	109.5	103.3	107.5	C-19	36.2 br	36.8	33.1	[38.5]
C-7	167.3, 165.6	166.9	164.4	167.0	C-20	44.3	45.7	47.2	45.8
C-8	94.4 br	95.1	96.5	94.1	C-21	208.3	208.6	206.2	208.1
C-8a	160.0	160.4	161.4	160.1	C-22	114.0	114.8	113.8	114.0
C-1'	119.5	120.8	121.2	122.4	C-23	164.1	164.4	164.0*1	164.2
C-2'	159.4	159.9	161.4	159.5	C-24	102.4	103.5	102.6	102.6
C-3'	98.2	98.9	99.6	98.3	C-25	164.1	164.4	164.3*1	164.2
C-4'	159.9	160.2	159.5	159.8	C-26	107.0	107.4	105.9*3	107.2
C-5'	108.6	109.0	109.9	108.7	C-27	130.1, 129.3, 128.2	129.2	128.2	130.8
C-6'	124.7	125.0	125.9	124.9	C-28	119.4	119.9	119.5	120.7
C-9	30.5	31.6	32.1	30.9	C-29	155.5	155.8	155.5*2	155.8
C-10	117.5, 117.1	117.9	118.7	117.5	C-30	101.7	102.2	102.2	102.0
C-11	135.2	135.2	136.9	135.3	C-31	155.9	156.4	155.8*2	155.8
C-12	25.3, 24.8	25.3	25.9	25.5	C-32	105.7	106.5	107.5*3	106.8
C-13	17.4	17.8	18.1	17.7	C-33	132.4	132.7	132.6	132.4
solvent	a	b	c	d		a	b	d	d

a: ^{12}C-DMSO-d_6 at 26°C, b: ^{12}C-DMSO-d_6 at 100°C, C: acetone-d_6 at 25°C, d: DMSO-d_6 at 35°C,

[ ]: CD_3CN, *: Assignments may be reversed.

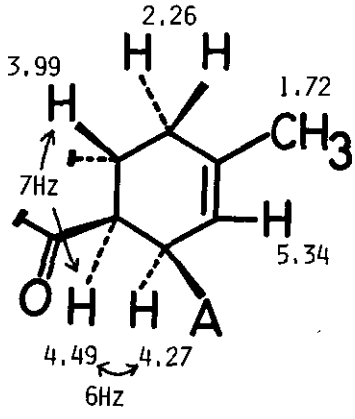
C-7) which were affected by the additional substituent effect (Table 1). The chemical shift values of the carbon atoms of the 2,4-dihydroxybenzoyl moiety and of the 2,4-dihydroxyphenyl moiety were similar to those of the relevant carbon atoms of IX (Table 1).[7a] The carbon atoms of I, except those of the cyclohexene ring and the C-21 carbonyl carbon, showed the chemical shift values similar to those of the relevant carbon atoms of II (Table 1).[2] All these results indicate that the structure of sanggenon D is possibly represented by I or I' (except stereochemistry). The location of the 2,4-dihydroxyphenyl and the 2,4-dihydroxybenzoyl moiety on the cyclohexene ring of sanggenon D (I) was supported by comparison of the ^{13}C nmr spectrum of I with that of sanggenon C (II)[2] and kuwanon G (IX).[7a] In respect to the chemical shifts of the carbon atoms of the relevant cyclohexene ring, I was more similar to IX than to II (Table 1). These results suggest that sanggenon D (I) and kuwanon G (IX) have the same disposition concerning the location of the 2,4-dihydroxyphenyl and the 2,4-dihydroxybenzoyl moiety on the cyclohexene ring, and also have the same relative configuration, so that the structure of sanggenon D is possibly represented by the formula (I).

The formula (I) for sanggenon D was substantiated by detailed analysis of the ^1H nmr spectrum (120° C, DMSO-d_6) using sequential decoupling and by comparison of



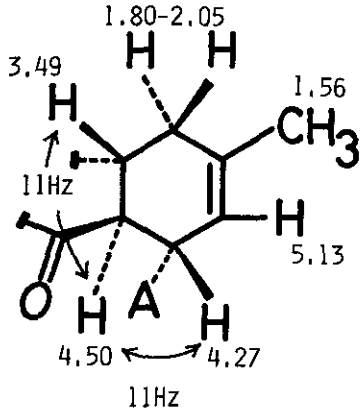
I (DMSO-d_6 at 120°C)

Fig. 4



II (DMSO-d_6 at 120°C)

Fig. 5



IX (DMSO-d_6 at 100°C)

Fig. 6

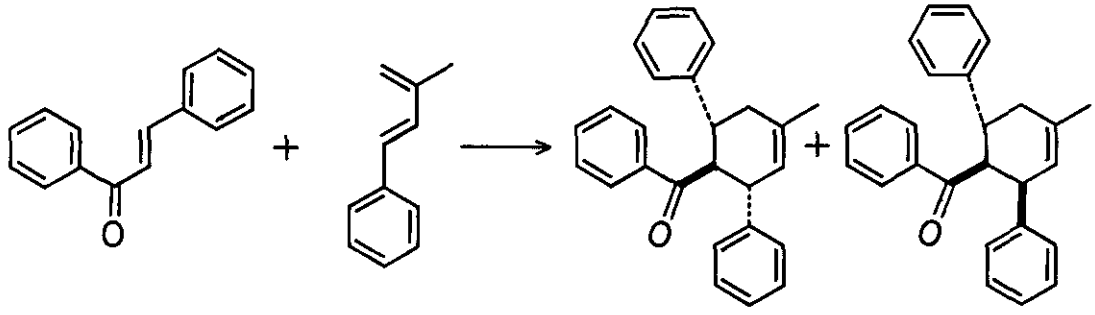


Fig. 7 X XI

the ^{1}H nmr spectra of sanggenon C (II)[2] and other Diels-Alder adducts obtained from Morus species.[7-12] The chemical shifts (δ) and coupling constants (Hz) of protons of the relevant cyclohexene ring are shown in Fig. 4, while the remaining protons are summarized as follows: protons in flavanone moiety, 5.69 (1H, s, C_8-H), 6.41 (1H, dd, J = 2 and 8, $C_{5'}$-H), 6.33 (1H, d, J = 2, $C_{3'}$-H), 7.21 (1H, d, J = 8, $C_{6'}$-H); aromatic protons in a 2,4-dihydroxyphenyl moiety, 5.95 or 6.14 (1H, d, J = 2, C_{30}-H), 5.98 (1H, dd, J = 2 and 8, C_{32}-H), 6.76 (1H, d, J = 8, C_{33}-H); aromatic protons in a 2,4-dihydroxybenzoyl moiety, 5.95 or 6.14 (1H, d, J = 2, C_{24}-H), 6.06 (1H, dd, J = 2 and 8, C_{26}-H), 7.60 (1H, d, J = 8, C_{27}-H); γ,γ-dimethylallyl moiety, 1.46, 1.37 (each 3H, s, C_{11}-CH_3), 2.50-2.70 (1H, m, C_9-H), 2.89 (1H, dd, J = 8 and 17, C_9-H), 5.10 (1H, m, C_{10}-H). As the methylene protons of γ,γ-dimethylallyl group appeared to be nonequivalent, it is suggested that the

γ,γ-dimethylallyl group is located at the asymmetric center.[1] Detailed comparative examination of the ^{1}H nmr spectra of sanggenon D (I), C (II)[2] and kuwanon G (IX)[7a] revealed that the chemical shifts and coupling constants of protons of the cyclohexene ring of I resembled those of IX better than those of II (Figs. 3-5). From these results, we propose the formula (I) for the structure of sanggenon D.

Sanggenon D (I) is optically active, and is considered to be formed by a Diels-Alder type of enzymatic process of a chalcone derivative and a dehydroprenylflavanone derivative. In the previous communication,[7b] we reported that two cycloadducts (X and XI) were prepared by cycloaddition of *trans*-chalcone and 3-methyl-1-phenyl-1,3-butadiene, and that no other cycloadducts were detected in the reaction mixture (Fig. 7). It is interesting that two stereoisomers, sanggenon C (II) and D (I), coexist in the Morus root barks suggesting the biosynthetic process analogous to the cycloaddition reaction.

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REFERENCES AND FOOTNOTES

+ Dedicated to the 75th birthday anniversary of Dr. K. Tsuda, President, Kyoritsu College of Pharmacy.

1 T. Nomura, T. Fukai, Y. Hano, Y. Sugaya, and T. Hosoya, Heterocycles, 1980, 14, 1785.

2 T. Nomura, T. Fukai, Y. Hano, and J. Uzawa, Heterocycles, in press.

3 Although only one spot was detected on tlc, this compound could not be isolated as crystalline form.

4 Assignments of the carbon atoms were performed by comparison of the ^{13}C nmr spectra of model compounds, sanggenon A (III),[1] C (II)[2], and the other Diels-Alder adducts obtained from Morus root barks.

5 R.M. Horowitz, J. Org. Chem., 1957, 22, 1733.[7,10-12]

6 The fragments (m/e 708, 640, 598) could not be detected in EI-MS and only detected in FD-MS.

7a) T. Nomura and T. Fukai, Chem. Pharm. Bull., 1980, 28, 2548; b) T. Nomura, T. Fukai, T. Narita, S. Terada, J. Uzawa, Y. Iitaka, M. Takasugi, S. Ishikawa, S. Nagao, and T. Masamune, Tetrahedron Lett., 1981, 22, 2195.

8 M. Takasugi, S. Ishikawa, S. Nagao, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett., 1980, 1577.

9a) Y. Oshima, C. Konno, H. Hikino, and K. Matsushita, Tetrahedron Lett., 1980, 21, 3381; b) Y. Oshima, C. Konno, and H. Hikino, Heterocycles, 1981, 16, 979.
10a) T. Nomura, T. Fukai, and T. Narita, Heterocycles, 1980, 14, 1943; b) Y. Oshima, C. Konno, H. Hikino, and K. Matsushita, Heterocycles, 1980, 14, 1287.
11a) M. Takasugi, S. Nagao, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett., 1980, 1573; b) T. Nomura, T. Fukai, J. Matsumoto, K. Fukushima, and Y. Momose, Heterocycles, 1981, 16, 759.
12 T. Nomura, T. Fukai, E. Sato, and K. Fukushima, Heterocycles, 1981, 16, 983.

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