

STRUCTURE OF SANGGENON D, A NATURAL HYPOTENSIVE DIELS-ALDER  
ADDUCT FROM CHINESE CRUDE DRUG "SĀNG-BĀI-PÍ" (MORUS ROOT BARKS)<sup>†</sup>

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**Abstract** — From the ethyl acetate extract of the Chinese crude drug "Sāng-Bāi-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,<sup>1,2</sup> 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-Bāi-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-Bāi-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  $1.3 \times 10^{-2}\%$  yield from the crude

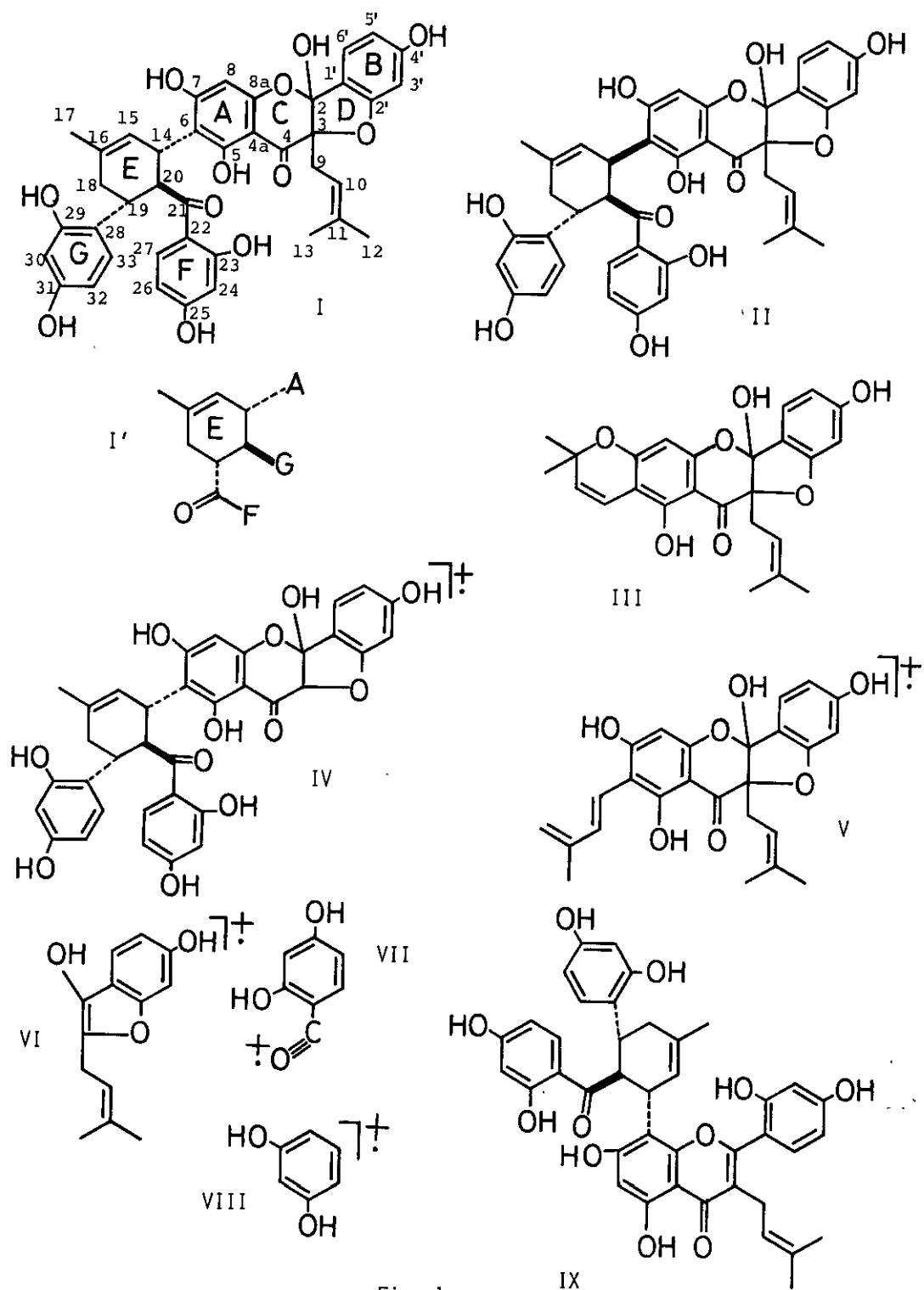


Fig. 1

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

Sanggenon D (I), amorphous powder,<sup>3</sup>  $[\alpha]_D^{26} - 145^\circ$  ( $c=0.17$  in methanol), gave the FD-MS spectrum which showed the molecular ion peak at  $m/e$  708, and the  $^{13}\text{C}$  nmr spectrum<sup>4</sup> (100° C,  $^{12}\text{C}$ -DMSO- $d_6$ ) indicated the presence of forty carbons [fourteen aliphatic carbons ( $3\times\text{CH}_3$ ,  $2\times\text{-CH}_2\text{-}$ ,  $3\times\text{CH-}$ ,  $1\times\text{C-O-}$ ,  $1\times\text{C=O}$ ,  $2\times\text{C=CH-}$ ), twenty four aromatic carbons ( $10\times\text{CH}$ ,  $5\times\text{C}$ ,  $9\times\text{C-O}$ ) and two carbonyl carbons]. The elemental analysis gave the following result: Anal. Calcd. for  $\text{C}_{40}\text{H}_{36}\text{O}_{12}\cdot 2\text{H}_2\text{O}$ : C, 64.51; H, 5.41. Found: C, 64.75; H, 5.20. These results suggest the composition of sanggenon D (I) to be  $\text{C}_{40}\text{H}_{36}\text{O}_{12}$ . The compound (I) showed the following color reactions: Mg-HCl test (orange),  $\text{NaBH}_4$  test (violet),<sup>5</sup>  $\text{FeCl}_3$  test (reddish violet), and showed the following spectra: ir  $\nu_{\text{max}}^{\text{Nujol}}$   $\text{cm}^{-1}$ : 3300, 1660 (sh), 1655 (sh), 1640 (sh), 1625, 1580; uv  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 220 (sh 4.65), 226 (sh 4.57), 283 (4.33), 288 (sh 4.32), 309 (4.30);  $\lambda_{\text{max}}^{\text{MeOH+AlCl}_3}$  nm (log  $\epsilon$ ): 225 (4.62), 292 (sh 4.28), 309 (4.39), 352 (sh 3.94), 400 (sh 3.38). These findings indicate that I is a flavanone derivative. The uv spectra were similar to those of sanggenon C (II).<sup>2</sup>

The mass spectrum of sanggenon D (I) was also similar to that of sanggenon C (II),<sup>2</sup> and showed the following characteristic fragments<sup>6</sup>:  $m/e$  708 ( $\text{M}^+$ ), 640 (IV), 598 ( $\text{M}^+ - \text{C}_6\text{H}_6\text{O}_2$ ), 436 (V),<sup>2</sup> 421 ( $436 - \text{CH}_3$ ),<sup>2</sup> 353 ( $421 - \text{C}_5\text{H}_8$ ),<sup>2</sup> 218 (VI),<sup>2</sup> 137 (VII),<sup>2</sup> 110 (VIII).<sup>2</sup> This result suggests that sanggenon D (I) may be a Diels-Alder adduct such as kuwanon G<sup>7</sup> (IX) (= albanin F<sup>8</sup> = moracenin B<sup>9</sup>) regarded as a cyclo-addition product with the chalcone and the dehydroprenylflavanone derivative.

The  $^1\text{H}$  and the  $^{13}\text{C}$  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  $^1\text{H}$  and the  $^{13}\text{C}$  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  $^1\text{H}$  and the  $^{13}\text{C}$  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  $^1\text{H}$  nmr spectrum of I showed a more complex pattern than the spectrum at room temperature (Fig. 2). On the other hand, the  $^1\text{H}$  nmr spectrum of II did not show such a remarkable variation as of I (Fig. 3). In the  $^{13}\text{C}$  nmr spectrum of I (100° C,  $^{12}\text{C}$ -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)<sup>1</sup> except the signals of carbon atoms (C-6 and

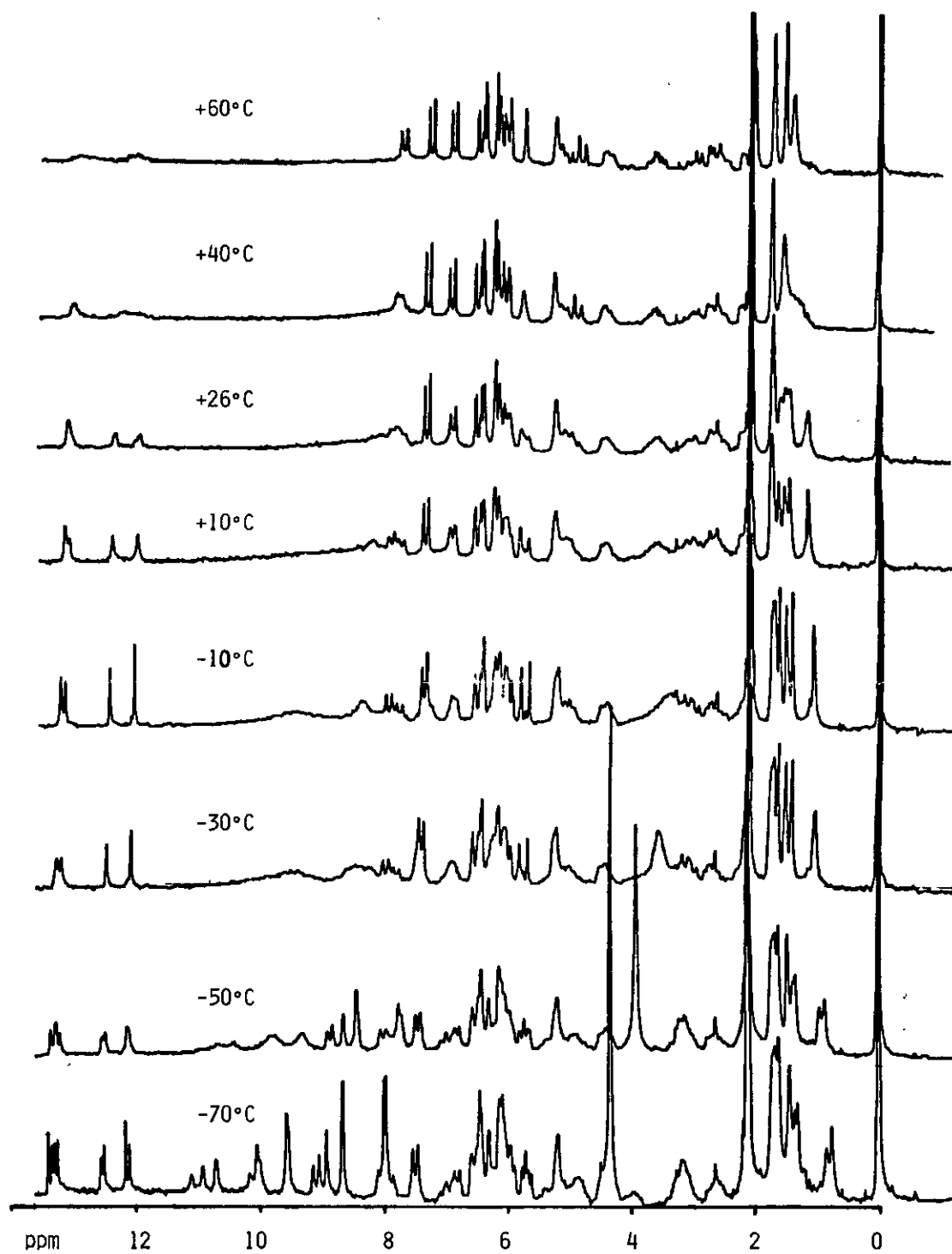


Fig. 2  $^1\text{H}$  nmr spectra of I in acetone- $\text{d}_6$

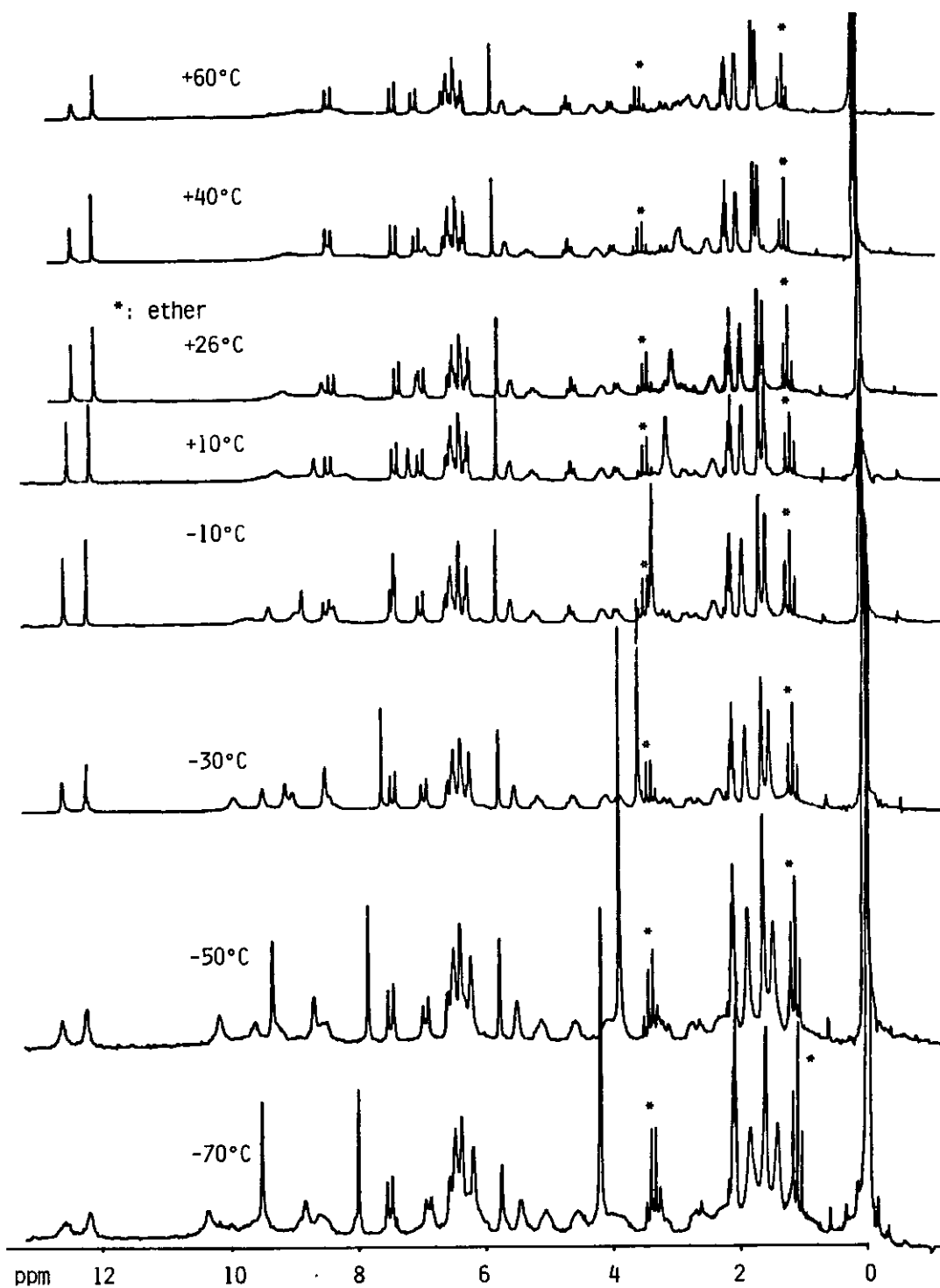


Fig. 3  $^1\text{H}$  nmr spectra of 11 in acetone- $\text{d}_6$

Table 1  $^{13}\text{C}$  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* <sup>1</sup> 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* <sup>1</sup> 164.2
C-4'	159.9	160.2	159.5	159.8	C-26	107.0	107.4	105.9* <sup>3</sup> 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* <sup>2</sup> 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* <sup>2</sup> 155.8
C-12	25.3, 24.8	25.3	25.9	25.5	C-32	105.7	106.5	107.5* <sup>3</sup> 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:  $^{12}\text{C}$ -DMSO- $\text{d}_6$  at 26°C, b:  $^{12}\text{C}$ -DMSO- $\text{d}_6$  at 100°C, c: acetone- $\text{d}_6$  at 25°C, d: DMSO- $\text{d}_6$  at 35°C,

[ ]:  $\text{CD}_3\text{CN}$ , \*: 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).<sup>7a</sup> 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).<sup>2</sup> 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  $^{13}\text{C}$  nmr spectrum of I with that of sanggenon C (II)<sup>2</sup> and kuwanon G (IX).<sup>7a</sup> 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  $^1\text{H}$  nmr spectrum (120° C, DMSO- $\text{d}_6$ ) using sequential decoupling and by comparison of

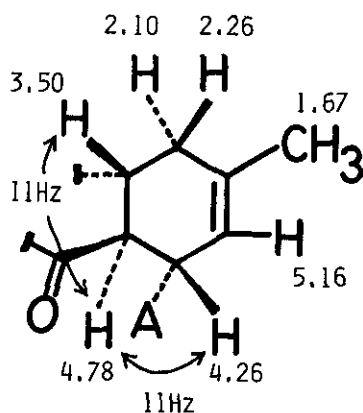

 I (DMSO- $d_6$  at 120°C)

Fig. 4

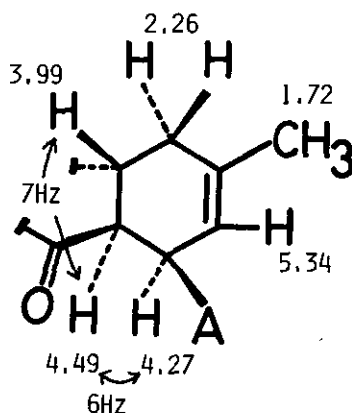

 II (DMSO- $d_6$  at 120°C)

Fig. 5

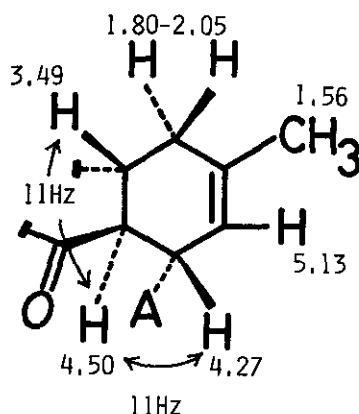

 IX (DMSO- $d_6$  at 100°C)

Fig. 6

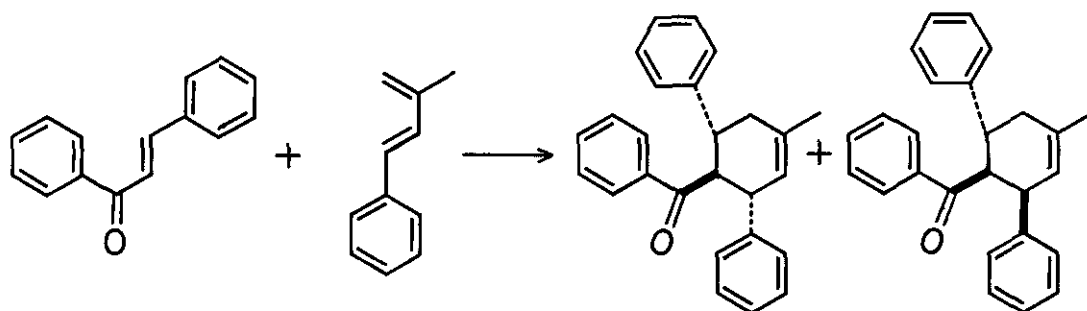


Fig. 7

X

XI

the  $^1\text{H}$  nmr spectra of sanggenon C (II)<sup>2</sup> and other Diels-Alder adducts obtained from *Morus* species.<sup>7-12</sup> The chemical shifts ( $\delta$ ) 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,  $\text{C}_8\text{-H}$ ), 6.41 (1H, dd,  $J = 2$  and 8,  $\text{C}_5\text{-H}$ ), 6.33 (1H, d,  $J = 2$ ,  $\text{C}_3\text{-H}$ ), 7.21 (1H, d,  $J = 8$ ,  $\text{C}_6\text{-H}$ ); aromatic protons in a 2,4-dihydroxyphenyl moiety, 5.95 or 6.14 (1H, d,  $J = 2$ ,  $\text{C}_{30}\text{-H}$ ), 5.98 (1H, dd,  $J = 2$  and 8,  $\text{C}_{32}\text{-H}$ ), 6.76 (1H, d,  $J = 8$ ,  $\text{C}_{33}\text{-H}$ ); aromatic protons in a 2,4-dihydroxybenzoyl moiety, 5.95 or 6.14 (1H, d,  $J = 2$ ,  $\text{C}_{24}\text{-H}$ ), 6.06 (1H, dd,  $J = 2$  and 8,  $\text{C}_{26}\text{-H}$ ), 7.60 (1H, d,  $J = 8$ ,  $\text{C}_{27}\text{-H}$ );  $\gamma,\gamma$ -dimethylallyl moiety, 1.46, 1.37 (each 3H, s,  $\text{C}_{11}\text{-CH}_3$ ), 2.50-2.70 (1H, m,  $\text{C}_9\text{-H}$ ), 2.89 (1H, dd,  $J = 8$  and 17,  $\text{C}_9\text{-H}$ ), 5.10 (1H, m,  $\text{C}_{10}\text{-H}$ ). As the methylene protons of  $\gamma,\gamma$ -dimethylallyl group appeared to be nonequivalent, it is suggested that the

$\gamma,\gamma$ -dimethylallyl group is located at the asymmetric center.<sup>1</sup> Detailed comparative examination of the  $^1\text{H}$  nmr spectra of sanggenon D (I), C (II)<sup>2</sup> and kuwanon G (IX)<sup>7a</sup> 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 dehydroprenyl-flavanone derivative. In the previous communication,<sup>7b</sup> 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.

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