STRUCTURES OF SANGGENONS E AND P, TWO NEW DIELS-ALDER TYPE ADDUCTS FROM THE CHINESE CRUDE DRUG "SANG-BAI-PI" (MORUS ROOT BARK)[1]

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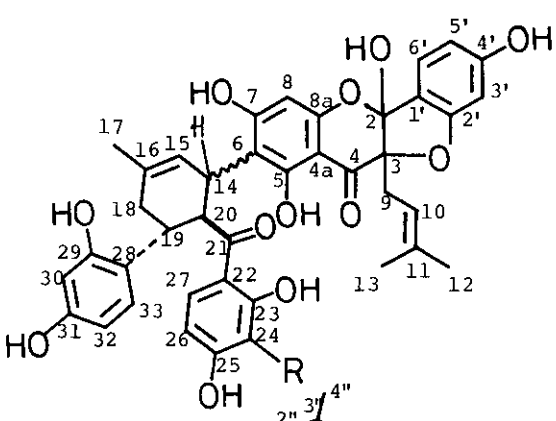
Abstract —— Two new flavanone derivatives named sanggenons E and P were isolated from the extract of the Chinese crude drug "Sang-Bai-Pi" (Japanese name "Sōhakuhi"), the root bark of Morus sp. (Moraceae). The structures of sanggenons E and P were shown to be 1 and 2, respectively, on the basis of spectral evidence. Sanggenons E and P are optically active and can be regarded biogenetically as Diels-Alder type adducts of a chalcone derivative and a dehydroprenylflavanone derivative. Sanggenon P is a stereoisomer at the C-14 position on the cyclohexene ring of sanggenon E. Furthermore, it is interesting that a pair of isomers on the methylcyclohexene ring were isolated from the same crude drug.

In the previous papers,[1c,2,3] we reported the structure determination of a series of natural Diels-Alder type adducts, isoprenylated flavonoid derivatives, and of 2-arylbenzofuran derivatives obtained from the Chinese crude drug "Sang-Bai-Pi" (Japanese name "Sōhakuhi") imported from the People's Republic of China. In the course of our studies, two new flavanone derivatives, sanggenons E (1) and P (2) were isolated as minor components from the crude drug as described in the experimental section. This paper deals with the structure elucidation of the two new components.

Sanggenon E (1), amorphous powder, $[\alpha]_D^{28}$ -86° (MeOH) gave the FD-MS showing a molecular ion peak at m/z 776, and ^{13}C nmr spectrum indicating the presence of forty-five carbon atoms (Table 1). These results indicate the composition of sanggenon E to be $C_{45}H_{44}O_{12}$. The compound 1 was positive to ferric chloride reaction, magnesium-hydrochloric acid test, and sodium borohydride test.[4] Its ir spectrum showed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties.

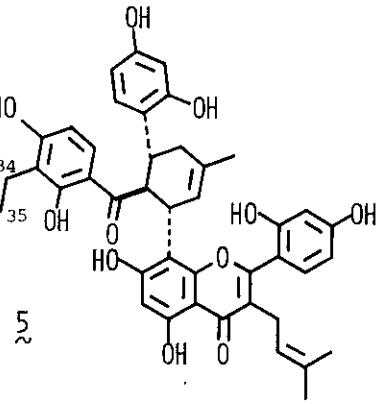


1: 14-H= β, R= (prenyl)

2: 14-H= α, R=prenyl

3: 14-H= α, R=H

4: 14-H= β, R=H



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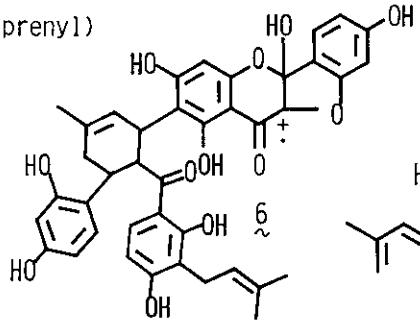

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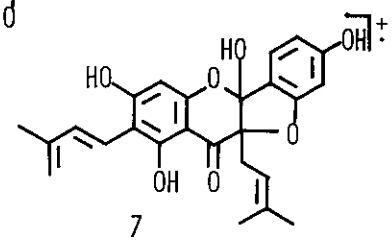

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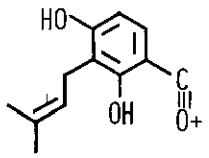

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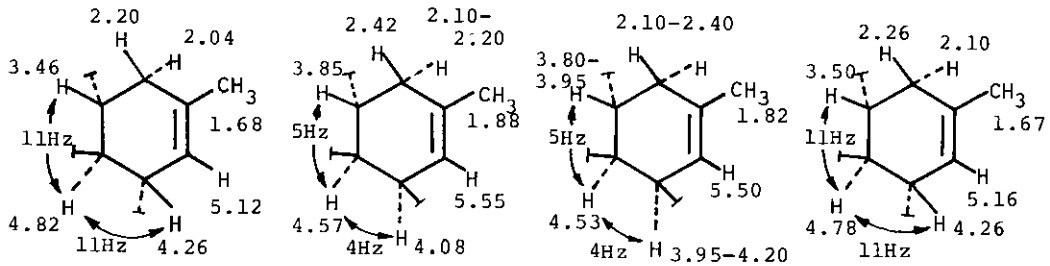
Fig. 1



1 (dmso-d_6, 100°C)

2 (acetone-d_6)

3 (acetone-d_6)

4 (dmso-d_6, 120°C)

Fig.2 ^{1}H nmr chemical shifts(ppm) and coupling constants in the methylcyclohexene rings of 1, 2, 3, and 4

Table 1 ^{13}C nmr chemical shifts (ppm) of 1, 2, 3, 4, and 5

	1[a]	4[b]		5[c]	2[d]	3[d]
C-2	103.0	102.1			102.0	102.4
C-3	90.7	90.7			91.5	92.0
C-4	186.2	187.0			187.5	188.4
C-4a	98.9	99.0			99.5	99.9
C-5	162.3	164.3			161.1	163.9
C-6	109.0	109.1			108.6	109.0
C-7	167.0	167.0			166.8	167.6
C-8	94.9	94.8			96.2	96.5
C-8a	160.3	160.2			162.4	162.0
C-1'	120.6	120.5			122.3	122.2
C-2'	160.0	159.7			160.4	161.2
C-3'	98.6	98.7			99.0	99.5
C-4'	160.2	160.2			160.4	161.2
C-5'	109.1	109.1			109.2	109.7
C-6'	124.8	124.9			125.0	125.6
C-9	31.2	31.1			31.7	32.0
C-10	117.6	117.5			118.0	118.6
C-11	135.3	135.5			136.1	136.2
C-12	25.3	25.1			25.8	25.9
C-13	17.5	17.6			18.0	18.1
C-14	37.3	37.8		39.8	33.3	33.1
C-15	124.6	124.3		123.4	121.6	121.4
C-16	129.8	131.9		132.9	131.2	132.6
C-17	22.8	22.8		22.6	23.6	23.3
C-18	37.3	37.8		39.2	31.9	32.8
C-19	37.6	37.8		39.8	32.5	33.1
C-20	45.0	45.2		45.7	48.0	47.2
C-21	208.7	208.6		208.4	208.6	206.2
C-22	114.3	114.3		114.0	113.2	113.8
C-23	162.0	164.3		162.1	163.7	164.0
C-24	113.6	102.1		113.7	115.3	102.6
C-25	162.1	164.3		161.6	163.1	164.3
C-26	106.5	107.3		106.9	107.2	105.9
C-27	130.0	132.5		131.3	128.3	128.2
C-28	119.7	119.7		121.1	120.8	119.5
C-29	155.7	155.7		155.9	155.7	155.5
C-30	103.1	103.1		102.7	103.3	102.2
C-31	156.2	156.1		155.9	157.1	155.8
C-32	106.6	106.2		106.9	107.6	107.5
C-33	129.6	129.0		131.3	134.3	132.6
C-1"	21.3		(C-34)	21.4	22.1	
C-2"	122.7		(C-35)	122.4	122.6	
C-3"	130.0		(C-36)	130.5	130.8	
C-4"	25.4		(C-37)	25.5	25.7	
C-5"	17.5		(C-38)	17.5	17.8	

solvent; a: dmso-d_6 at 90°C, b: dmso-d_6 at 80°C, c: dmso-d_6 at r.t., d: acetone-d_6 at r.t.

The uv spectrum of 1 exhibited maxima at 218(sh), 288, and 302 nm, and was similar to those of sanggenons C (3)[5] and D (4).[6] In the EI-MS of 1, the fragmentation pattern was similar to those of 3, 4, and kuwanon H (5),[7a] and showed the following characteristic fragments: m/z 708 (M^+-C_5H_8, 6, 57%), 436 (7, 22%), 205 (8, 78%). The above results suggest that sanggenon E (1) can be a Diels-Alder type adduct such as sanggenons C (3) and D (4) regarded as a cycloaddition product with the chalcone and the dehydroprenylflavanone derivative. It was also suggested that 1

has an isoprenylated dihydroxybenzoyl moiety in the structure.[7a] The ^{1}H nmr spectrum of sanggenon E (1) observed at room temperature showed the complex patterns, and the signals appeared broad. At higher temperature, the spectrum showed the simple patterns, and the signals appeared more sharply. The similar results were reported in the case of sanggenon D (4).[6] The ^{1}H nmr spectrum of 1 (200 MHz, dmso-d_6, 100 °C), analysed by a decoupling experiment and by comparison with the spectra of 3 and 4, showed the signals of the following protons: 1) aromatic protons in a flavanone moiety, δ 5.64 (1H, s, C-8-H), 6.32 (1H, d, J=2, C-3'-H), 6.40 (1H, dd, J=2 and 8, C-5'-H), 7.22 (1H, d, J=8, C-6'-H), 2) aromatic protons in a 2,4-dihydroxyphenyl moiety, δ 5.96 (1H, dd, J=2 and 8, C-32-H), 6.12 (1H, d, J=2, C-30-H), 6.76 (1H, d, J=8, C-33-H), 3) aromatic protons in a 3-(γ,γ-dimethylallyl)-2,4-dihydroxybenzoyl moiety, δ 6.16 (1H, d, J=8, C-26-H), 7.54 (1H, d, J=8, C-27-H), and 4) protons in two γ,γ-dimethylallyl moieties, δ 1.26, 1.44 (each 3H, s, C-3"-CH_3), 1.54, 1.60 (each 3H, s, C-11-CH_3), 2.56, 2.89 (each 1H, m, C-9-H), 2.90 (2H, m, C-1"-H x 2), 5.03 (2H, m, C-10-H and C-2"-H), while the chemical shifts and coupling constants of protons of the relevant methylcyclohexene ring are shown in Fig. 2. Comparative examination of the ^{1}H nmr spectra of 1, 3, and 4 revealed that the chemical shifts and coupling constants of protons of the methylcyclohexene ring of 1 resembled those of 4 better than those of 3 (Fig. 2).

The ^{13}C nmr spectrum of 1 was analysed as shown in Table 1. Assignments of the carbon atoms in 1 were performed by off-resonance decoupling technique as well as by comparison of the ^{13}C nmr spectra of sanggenons C (3), D (4), and kuwanon H (5).[7a] In the ^{13}C nmr spectrum of 1, the signal of C-24 appeared at a lower applied magnetic field than that of C-24 of 4, whereas the chemical shift values of all the carbon atoms except of the C-24 were essentially the same as those of the relevant carbon atoms of 4. The chemical shift values of the carbon atoms of the 3-(γ,γ-dimethylallyl)-2,4-dihydroxybenzoyl moiety of 1 were similar to those of the relevant carbon atoms of 5 (Table 1). These results suggest that sanggenon E is C-24-γ,γ-dimethylallylsanggenon D. The location of the γ,γ-dimethylallyl group in the 2,4-dihydroxybenzoyl moiety was also supported by the following result. In the uv spectrum of 1 no bathochromic shift was observed in the presence of aluminum chloride. Taking the report on the aluminum chloride-induced shift into account, the γ,γ-dimethylallyl group is suggested to be located ortho to a chelated hydroxyl group.[8] From these results, we propose the formula (1) for the structure of sanggenon E.

Sanggenon P (2), amorphous powder, $[\alpha]_D^{22}$ +215° (MeOH) gave the FD-MS showing a molecular ion peak at m/z 776, and the ^{13}C nmr spectrum indicating the presence of forty-five carbon atoms (Table 1). These results indicated the composition of sanggenon P to be $C_{45}H_{44}O_{12}$. The compound 2 was positive to ferric chloride reaction, magnesium-hydrochloric acid test, and sodium borohydride test,[4] and its ir spectrum showed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties. The uv spectrum of 2 exhibited maxima at 218(sh), 236(infl.), 287, and 296 nm, and was similar to those of sanggenons C (3),[5] D (4),[6] and E (1). No bathochromic shift was observed in the presence of alumunum chloride.[8] The ^1H nmr spectrum of 2 (400 MHz, acetone-d_6), analysed by a decoupling experiment and comparison with the spectra of 3, 4, and 1, showed the signals of the following protons: 1) aromatic protons in a flavanone moiety, δ 5.74 (1H, s, C-8-H), 6.47 (1H, d, J=2, C-3'-H), 6.48 (1H, dd, J=2 and 8, C-5'-H), 7.32 (1H, d, J=8, C-6'-H), 2) aromatic protons in a 2,4-dihydroxyphenyl moiety, δ 6.28 (1H, dd, J=2 and 8, C-32-H), 6.32 (1H, d, J=2, C-30-H), 6.94 (1H, d, J=8, C-33-H), 3) aromatic protons in a 3-(γ,γ-dimethylallyl)-2,4-dihydroxybenzoyl moiety, δ 6.43 (1H, d, J=8, C-26-H), 8.28 (1H, d, J=8, C-27-H), 4) protons in two γ,γ-dimethylallyl moieties, δ 1.52, 1.58, 1.60, 1.70 (each 3H, s, C-11-CH_3 and C-3"-CH_3), 2.73 (1H, dd, J=7 and 14, C-9-H), 3.10 (1H, dd, J=9 and 14, C-9-H), 3.27 (2H, d, J=7, C-1"-H x 2), 5.17 (2H, m, C-10-H, and C-2"-H), and 5) protons in two hydrogen-bonded hydroxyl groups, δ 12.10, 12.80 (each 1H, s, C-5-OH and C-23-OH), while the chemical shifts and coupling constants of protons of the methylcyclohexene ring are shown in Fig. 2. Comparative examination of the ^1H nmr spectra of 1, 2, and 3 revealed that the chemical shifts and coupling constants of protons of the methylcyclohexene ring of 2 resembled those of 3 better than those of 1 (Fig. 2).

Comparison of the ^{13}C nmr spectra of 2 and 3 indicated that the chemical shifts of the carbon atoms of 2 were similar to those of the relevant carbon atoms of 3, except the carbon atom at C-24 position which was affected by additional substituent. From above results, we propose the formula (2) for the structure of sanggenon P.

Sanggenon P (2) is a stereoisomer at the C-14 position on the methylcyclohexene ring of sanggenon E (1). It is interesting that two pair of stereoisomers, sanggenons C (3)[5] and D (4),[6] and sanggenons E (1) and P (2), coexist in the Morus root bark. Taking into account these results and the previous papers[2,7] on the synthesis of model compounds, it is suggested that sanggenons E (1) and P (2) are

regarded biogenetically as Diels-Alder type adducts of a chalcone derivative and a dehydroprenylflavanone derivative.

EXPERIMENTAL

Abbreviations: s=singlet, d=doublet, dd=double doublet, m=multiplet, br=broad, sh=shoulder, infl.=inflection. The general experimental procedures used are described in the previous papers.[1c,3] The following instruments were used: uv spectra; Hitachi 340 UV spectrometer, ir spectra; Hitachi 260-30 IR spectrometer, optical rotation; JASCO DIP-4, ms; JEOL JMS 01SG-2, Hitachi RMU-7M, ^{1}H nmr spectra; JEOL GX-400 and Varian XL-200 FT NMR spectrometers, ^{13}C nmr spectra; JEOL GX-400 and Hitachi R-900 FT NMR spectrometers.

Isolation of Sanggenons E (1) and P (2)

The crude drug "Sang-Bai-Pi" (Japanese name "Sōhakuhi", 56 Kg), a species of Morus (Moraceae), imported from the People's Republic of China, was finely cut and then extracted with n-hexane, benzene, and acetone successively. Evaporation of the acetone solution to dryness yielded 740 g of residue. The residue (330 g) was chromatographed on silica gel (1200 g), benzene-MeOH being used as an eluent, and each fraction was checked by tlc. The fractions eluted with benzene containing 5% MeOH were evaporated to give the residue (33 g). The residue (15 g) was rechromatographed on silica gel (250 g) with benzene-ethyl acetate as an eluent. The fractions eluted with benzene containing 5% ethyl acetate were evaporated to give the residue, which was fractionated by preparative tlc (solvent system: n-hexane:acetone =1:1, chloroform:MeOH=5:1, benzene:ethyl acetate=1:1, chloroform:acetone=3:2, ethyl ether only) to give sanggenons E (1, 168 mg) and P (2, 15 mg).

Sanggenon E (1)

Compound 1 was obtained as an amorphous powder. FD-MS m/z: 776 (M^+). $FeCl_3$ test: reddish violet. Mg-HCl test: violet. $NaBH_4$ test: orange. $[\alpha]_D^{28}$ -86° (c=0.056 in MeOH). uv λ_{max}^{EtOH} nm (log ε): 218 (sh 4.93), 288 (4.60), 302 (4.57); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 220 (sh 4.93), 288 (4.58), 307 (4.58). ir ν_{max}^{Nujol} cm^{-1}: 3350 (br), 1660 (sh),1650 (sh), 1635 (sh), 1630, 1610 (sh), 1570.

Sanggenon P (2)

Compound 2 was obtained as an amorphous powder. FD-MS m/z: 776 (M^+). $FeCl_3$ test: reddish violet. Mg-HCl test: orange. $NaBH_4$ test: violet. $[\alpha]_D^{22}$ +215° (c=0.052, in MeOH). uv λ_{max}^{EtOH} nm (log ε): 218 (sh 4.47), 236 (infl. 4.29), 287 (4.21), 296 (4.19); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 218 (sh 4.39), 237 (infl. 4.16), 291 (4.12), 300

(4.14). ir ν_{max}^{KBr} cm^{-1}: 3400 (br), 1660 (sh), 1640 (sh), 1620, 1600.

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