REVISED STRUCTURE OF SANGGENON A

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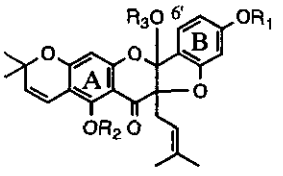
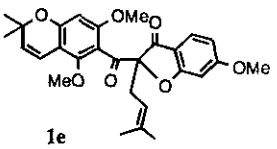
Abstract - The structure of sanggenon A, which has been isolated from the Chinese crude drug "Sang-Bai-Pi", was revised on the basis of two-dimentional NMR spectroscopic and chemical evidence. Sanggenon A is a unique 3-hydroxy-flavanone derivative bearing an isoprenyl group at the C-2 position of the skeleton. The other flavanones having sanggenon A-type structure, including sanggenons M and C, also required the revision of the structure.

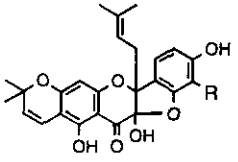
We have reported structures of a series of isoprenylated flavonoids isolated from the plants of genus *Morus* (Moraceae). Among them, sanggenon A (**1**) is a unique flavanone derivative which has been isolated as one of the major phenolic components from the root bark of the Chinese *Morus* sp.[1] Recently, Messana *et al.* reported some interesting 2-isoprenylated 3-hydroxyflavanone derivatives named soroceins, such as sorocein D (**6**), from Brazilian moraceous plants, *Sorocea bonplandii* and *S. ilicifolia*.[2,3] The structures of soroceins have been confirmed by the two-dimentional (2D) NMR techniques. The structural feature of the flavanones as well as the NMR data of the flavanone part are closely similar to those of **1**. Once two possible formulae (**1** and **2**) have been estimated for the structure of sanggenon A before formula (**1**) was concluded.[1] A large difference (Δ + 0.24 ppm) of the chemical shift values at the C-6'-H of the B ring between the diacetate (**1b**) and triacetate (**1c**) led to a conclusion that an alcoholic hydroxyl group should be located at the C-2 position. This paper deals with re-examination of the structure of sanggenon A with the aid of 2D NMR techniques and chemical methods.



1 : R_1=R_2=R_3=H
1a : R_1=Ac, R_2=R_3=H
1b : R_1=R_2=Ac, R_3=H
1c : R_1=R_2=R_3=Ac
1d : R_1=R_2=R_3=Me



1e



2 : R=H
6 : R=

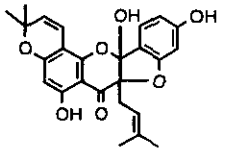


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Figure 1

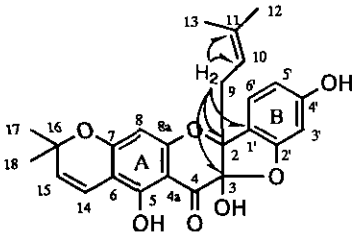


Figure 2a HMBC spectrum of **2** (correlations with C-9-H)

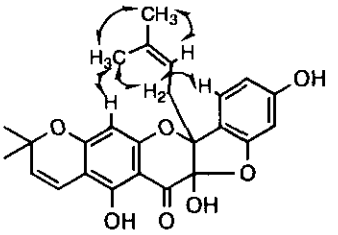


Figure 2b NOESY spectrum of **2** (correlations with isoprenyl group)

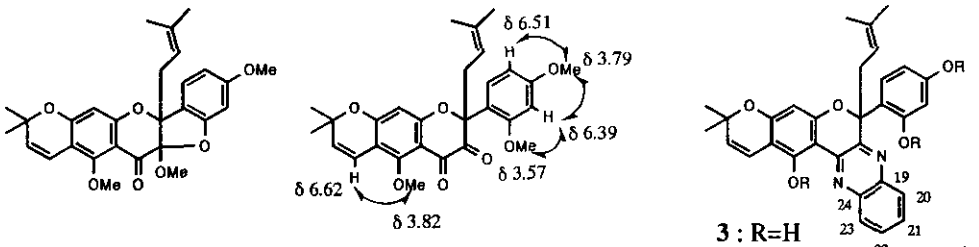


Figure 3 Revised structures of two trimethyl ethers and the formation of phenazine derivative Arrows (↷) in **2e** denote the NOE correlations with methoxyl groups

Table 1 ^{13}C NMR Chemical shifts (ppm)

	2	C-H connectivities	**3a**		**2**	C-H connectivities	**3a**
C-2	92.5		85.4	C-9	32.0	2.77 (dd, J = 6 and 15)	36.8
C-3	102.3		152.7			3.07 (dd, J = 8 and 15)	
C-4	188.8		144.1	C-10	118.5	5.18 (m)	118.9
C-4a	100.5		108.1	C-11	136.8		134.5
C-5	159.5		155.6	C-12	25.8	1.62 (3H, s)	25.9
C-6	103.1		110.4	C-13	18.0	1.56 (3H, s)	18.1
C-7	164.3		157.4	C-14	115.8	6.59 (dd, J = 0.5 and 10)	117.1
C-8	96.3	5.77 (d, J = 0.5)	101.6	C-15	127.5	5.50 (d, J = 10)	127.8
C-8a	163.2		159.4	C-16	78.4		76.9
C-1'	120.6		123.4	C-17	28.3	1.40 (3H, s)	27.8
C-2'	161.4		159.0	C-18	28.3	1.43 (3H, s)	28.5
C-3'	99.4	6.44 (d, J = 2)	100.0			C-19	142.0
C-4'	161.2		160.0			C-20	129.0
C-5'	109.8	6.50 (dd, J = 2 and 8)	103.6			C-21	129.2
C-6'	125.5	7.31 (d, J = 8)	127.8			C-22	128.2
						C-23	129.0
						C-24	140.1
						5-OMe	61.7
						2'-OMe	55.7
						4'-OMe	55.2

Complete assignments of both compounds (**2**) and (**3a**) were performed by 2D NMR methods (HHCOSY, CHCOSY, long-range CHCOSY, NOESY). Solvent; $CDCl_3$

In order to perform the complete assignments of ^{1}H and ^{13}C signals, 2D NMR spectra (^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, HMBC, NOESY) were analysed and the assignments are summarized in Table 1. In the HMBC spectrum, one of the methylene protons (δ 3.07) at the isoprenyl group exhibited the significant correlation with the carbon at C-1' (δ 120.6) of the ring B, indicating that the isoprenyl group could linked to the carbon at the C-2 position as in the formula (**2**) (Figure 2a). This suggestion was also supported in the 2D NOESY spectrum showing the significant correlations between one of the vinyl methyl group (δ 1.62) and the C-8-H (δ 5.77) as well as between one of methylene protons at the C-9 (δ 2.77) and the C-6'-H of the ring B (Figure 2b). Thus the above 2D NMR spectra suggested the structure of sanggenon A to be the formula (**2**). On the other hand, the exhaustive methylation of sanggenon A with dimethylsulfate gives two trimethyl ethers (**1d** and **1e**). One of them has been represented by the formula (**1e**) having a β-diketone structure.[4] According to the suggestion by the 2D NMR studies, both the formulae (**1d** and **1e**) for the two trimethyl ethers would be revised to the formulae (**2d**) and (**2e**), respectively. The structure of **2e** was supported by the 2D NOESY spectrum which showed a significant correlation between the C-3'-H and two methoxyl signals (Figure 3). Conclusive evidence for the α-diketone structure of **2e** was obtained by a reaction with *o*-phenylenediamine. The reaction of **2e** and *o*-phenylenediamine in a solution of acetic acid-methanol (3 : 1) at room temperature gave a phenazine derivative (**3a**) in 85 % yield. Compound (**3a**), yellow oil, M^{+} 550, exhibited poitive reaction to Dragendorff test. The ^{1}H NMR spectrum of **3a** revealed characteristic AA'BB' type of the aromatic proton signals due to a phenazine moiety at δ 8.09, 7.95 (each 1H, td, J = 8 and 1.5 Hz), 7.67, 7.60 (each 1H, d, J = 8 Hz) ppm in addition to the proton signals due to a 2,2-dimethylpyran ring [δ 1.40, 1.44 (each 3H, s), 5.54 (1H, d, J = 10 Hz), 6.70 (1H, dd, J = 0.7 and 10 Hz)], four aromatic protons in the rings A and B [δ 6.27 (1H, d, J = 0.7 Hz), 6.33 (1H, dd, J = 2 and 8 Hz), 6.34 (1H, d, J = 2 Hz), 7.01 (1H, d, J = 8 Hz)], an isoprenyl group [δ 1.54, 1.59 (each 3H, br s), 3.37 (2H, br d, J = 8 Hz), 5.28 (1H, m)], and three methoxyl groups [δ 3.43, 3.73, 3.83 (each 3H, s)]. The ^{13}C NMR spectrum also indicated **3a** to be the phenazine derivative (Table 1). The trimethyl ether having the diketone structure was thus revised to the formula (**2e**). An analogous experiment with sanggenon A was also carried out in a solution of methanol-acetic acid (9 : 1) to afford a phenazine derivative (**3**) in 35 % yield.[5] Therefore, the formula (**2**) was confirmed as the structure of sanggenon A.

On the other hand, alkaline treatment of sanggenon A gives an equilibrium mixture of (±) - sanggenon A and a Wessely-Moser rearrangement-type product at the ratio of 2 : 5 (Figure 4), and the rearrangement product has been identified as (±) - sanggenon M.[4] From the revision of the structure of **2**, the formula (**4**) formerly proposed for the structure of sanggenon M must be turned into the formula (**5**). Furthermore, the alkaline treatment of **2** gave an interesting result that the both recovered products showed no optical rotation.[4] Sanggenon A has a large optical rotatory value ($[\alpha]_D$ + 176°) due to two chiral centers at the C-2 and C-3 positions in the molecule. Disappearance of the optical activity by the alkaline treatment suggested that epimerization reaction takes place not only at the C-2 position, but also simultaneously at the C-3 position. The revised formula (**2**) for sanggenon A gave a satisfying mechanism for the simultaneous epimerization at the C-2 and C-3 positions as depicted in Figure 4.

As described above, the conversion of the diacetate (**2b**) into the triacetate (**2c**) induced a large high-field shift (Δ + 0.24 ppm) at the C-6'-H of the ring B (Table 2). A possibility of the structural change of **2c** was ruled out from the fact that an alkaline hydrolysis of **2c** gave an equilibrium mixture of (±)-**2** and (±)-**5** as

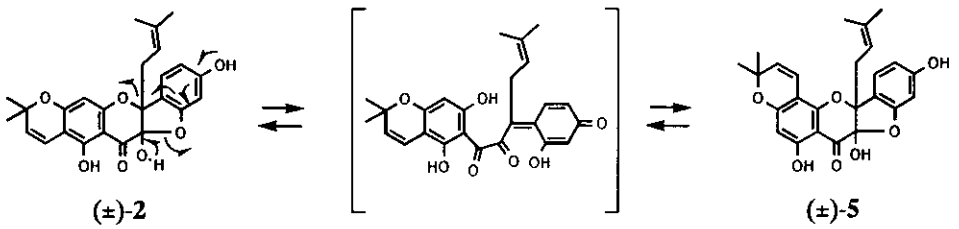
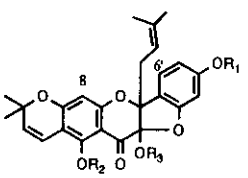


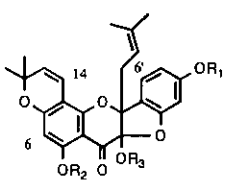
Figure 4 A mechanism of simultaneous epimerization at the C-2 and C-3

Table 2 Acetylation shifts (ppm in $CDCl_3$) in sanggenons A (**2**) and M (**5**)

	sanggenon A				sanggenon M		
	C-8-H	C-9-H	C-6'-H		C-6-H	C-14-H	C-6'-H
diacetate (**2b**)	6.12	3.07	7.42	diacetate (**5b**)	6.14	6.47	7.43
triacetate (**2c**)	6.28	2.85	7.18	triacetate (**5c**)	6.14	6.58	7.11
	Δ -0.16	Δ +0.22	Δ +0.24		Δ ±0.00	Δ -0.11	Δ +0.32



2a : R_1=Ac R_2=R_3=H
2b : R_1=R_2=Ac R_3=H
2c : R_1=R_2=R_3=Ac



5a : R_1=Ac R_2=R_3=H
5b : R_1=R_2=Ac R_3=H
5c : R_1=R_2=R_3=Ac

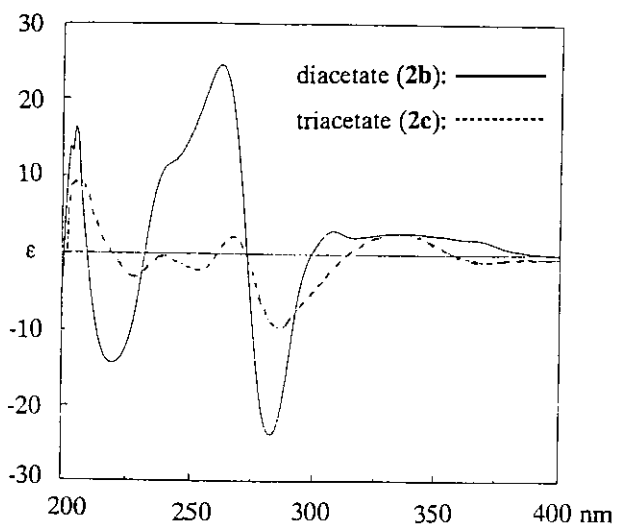
Table 3 The C=O stretching of the C-4 carbonyl group in the IR spectra

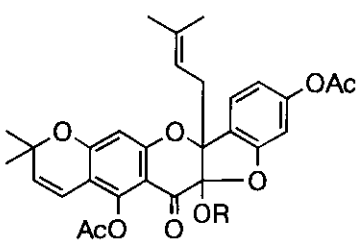
2	**2a**	**2b**	**2c**
1645 cm^{-1}	1645 cm^{-1}	1674 cm^{-1}	1686 cm^{-1}

measured in a chloroform solution

described above. The high-field shift in **2c**, which led to the incorrect structure of sanggenon A, could not be due to an anisotropic effect of 3-*O*-acetyl group, but due to any other effect. While the C-6'-H showed the high-field shift in the triacetate (**2c**), the C-8-H of the ring A showed a large low-field shift (Δ - 0.16 ppm) as compared with that of diacetate (**2b**) (Table 2). The similar phenomenon was also observed in sanggenon M triacetate (**5c**) (Table 2). The C-6'-H of the triacetate (**5c**) showed a large high-field shift (Δ + 0.32 ppm) as compared with the diacetate (**5b**), as in the case of **2c**. However, low-field shift was not observed at the C-6-H of the ring A, but at the C-14-H of a 2,2-dimethylpyran ring (Table 2). This fact led to the assumption that the low-field shift at C-8-H in the case of **2** as well as at the C-14-H in the case of **5** was caused by disappearance of a diamagnetic effect of the ring B. Namely, the conversion of the diacetate into the triacetate might induce a conformational change of the molecule. In addition, a large-high field shift (Δ + 0.22 ppm) at one of methylene protons (C-9-H) was observed in sanggenon A triacetate (**2c**) as compared with the diacetate (**2b**) (Table 2). Regarding that the high-field shift is due to an anisotropic

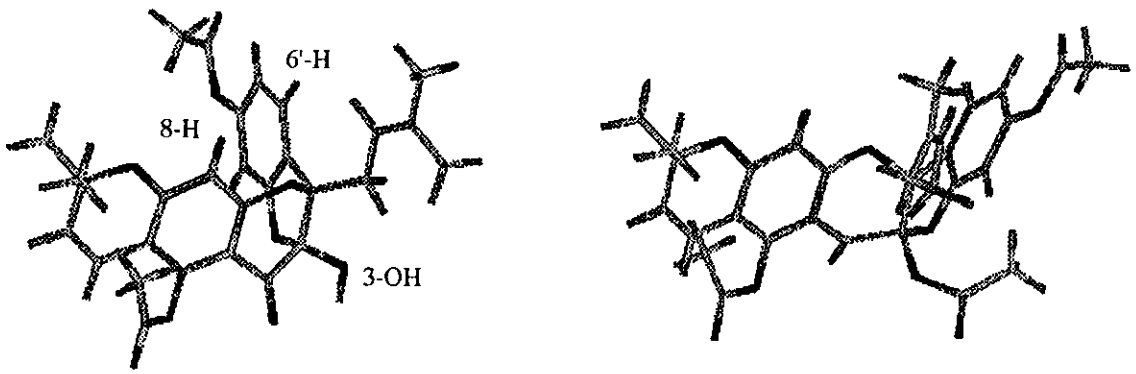
effect of the 3-*O*-acetyl group, the relationship between the isoprenyl group at the C-2 position and the hydroxy group at the C-3 position is *cis*-configuration in relative.[1] In order to examine the conformational change, the IR spectra of monoacetate (**2a**), diacetate (**2b**) and triacetate (**2c**) were analysed, especially, with a respect to C=O stretching at the C-4 position (Table 3). The C=O stretching in the diacetate (**2b**) resonates at higher wave-number (1674 cm^{-1}) than that of the monoacetate (**2a**, 1645 cm^{-1}), indicating the elimination of a hydrogen-bond between the carbonyl group at the C-4 position and the hydroxyl group at the C-5 position in the diacetate (**2b**). However, the C=O stretching in the triacetate (**2c**) resonates further at higher wave-number (1686 cm^{-1}) than that of the diacetate (**2b**). This fact suggested that the diacetate (**2b**) has still a weak hydrogen-bond between the carbonyl group at the C-4 position and the hydroxyl group at the C-3 position and that the weak hydrogen-bond might control a conformation of the molecule. This assumption was supported by comparing the circular dichroism (CD) spectrum of the diacetate (**2b**) with that of the triacetate (**2c**), showing that the CD curve of **2c** is different too far from that of **2b** (Chart 1). Finally, the optimal structures of **2b** and **2c** were calculated by PM-3 MO method in MOPAC ver. 6 program. As a result, the optimized structure of the diacetate (**2b**) keeping a hydrogen-bond was extremely different from that of the triacetate (**2c**) (Figure 5). The result of the calculation demonstrated that a sharp bend of the ring B face to the ring C in **2b** is caused by repulsion between two lone electron orbits on the two oxygen atoms at the C-3 position. It is clear that, in the diacetate (**2b**), the C-8-H lies in a surrounding being under the influence of a diamagnetic effect of the ring B, whereas the effect of the ring B could be eliminate in the triacetate (**2c**). This conformational change is reasonable for the account of the low-field shift of the C-8-H in the triacetate (**2c**). Such a conformational change is also likely in sanggenon M triacetate (**5c**) showing the similar low-field shift at the C-14-H (Table 2). On the other hand, the C-6'-H of the ring B in the conformation (**2b**) lies in a surround being under the influence of a paramagnetic effect





2b : R=H
2c : R=Ac

Chart 1 CD spectra of **2b** and **2c** (in MeOH)



diacetate (**2b**) triacetate (**2c**)

Figure 5 Optimized structures of **2b** and **2c**

of the lone electron pair on the oxygen atom at the O-1 position (Figure 5). The large high-field shift of the C-6'-H in the triacetate (**2c**) as well as in the sanggenon M triacetate (**5c**) was thus concluded to be caused by the elimination of a paramagnetic effect of the lone electron pair at the O-1 oxygen atom through the conformational change.

Some flavanone derivatives having sanggenon A type of structure have been isolated from "Sang-Bai-Pi", including a natural Diels-Alder type adduct sanggenon C (**7**) exhibiting a hypotensive effect to a rat. From the above revision of the structure of sanggenon A, the formula (**6**) for sanggenon C would be revised to new one. The exhaustive methylation of sanggenon C gave two octamethyl ethers (**7a**) and (**7b**) (Figure 6).[6] One of the octamethyl ethers has a diketone partial structure as was observed in the methylation of sanggenon A. The formation of a phenazine derivative (**8c**)[7] in the reaction of the octamethyl ether with *o*-phenylenediamine in a solution of methanol-acetic acid (1 : 3) apparantly required the revised structure (**8b**) for the octamethyl ether (Figure 6). The structure of sanggenon C has thus confirmed to be the formula (**8**). Since an alkaline treatment of **8** gave (±)-sanggenon O,[8] the structure of sanggenon O was also confirmed to be the formula (**9**) (Figure 6).

Furthermore, the structures of sanggenons B,[9] D,[10] E ,[11] L ,[4] P,[11] and S[12] have been proposed on the basis of the comparisons of the NMR data with those of sanggenon A or sanggenon C. Therefore, the formulae of the flavanones would be revised to new ones **10**, **11**, **12**, **13**, **14**,[13] and **15**, respectively (Figure 6).

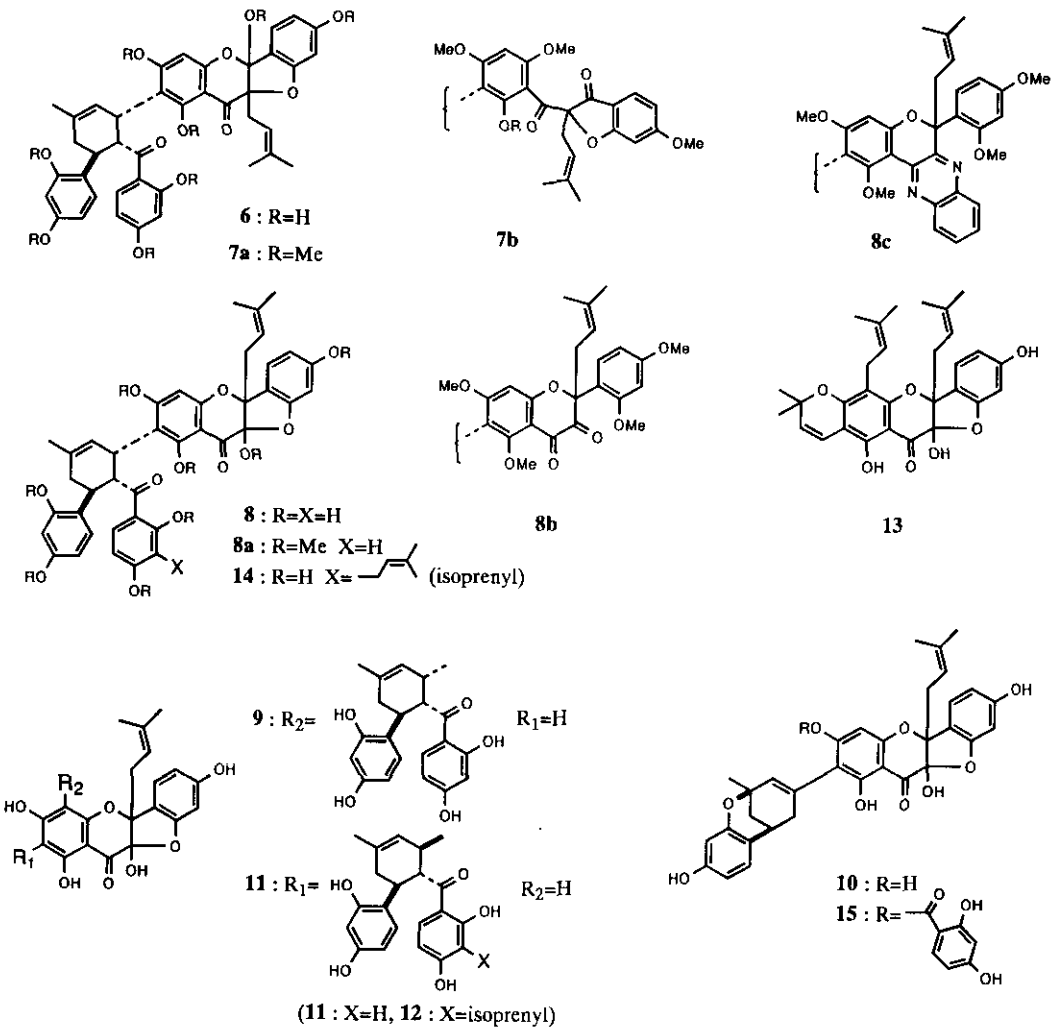


Figure 6 Revised structures of sanggenon derivatives

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4. Y. Hano, M. Itoh, N. Koyama, and T. Nomura, *Heterocycles*, 1984, **22**, 1791.
5. The phenazine derivative (**3**), orange powder. EI-MS: *m/z* 408 (M^+). ^{1}H-NMR ($CDCl_3$): δ 1.48, 1.50 (each 3H, s, C-16-CH_3), 1.57, 1.65 (each 3H, br s, C-11-CH_3), 3.10, 3.66 (each 1H, br dd, J = 9 and 14 Hz, C-9-H), 5.14 (1H, m, C-10-H), 5.54 (1H, d, J =10 Hz, C-15-H), 6.22 (1H, d, J = 0.7 Hz, C-8-H), 6.29 (1H, dd, J = 2 and 8 Hz, C-5'-H), 6.39 (1H, d, J =2 Hz, C-3'-H), 6.71 (1H, dd, J = 0.7 and 10 Hz, C-14-H), 7.14 (1H, d, J = 8 Hz, C-6'-H), 7.69, 7.75 (each 1H, td, J = 2 and 8 Hz, C-21 and 22-H), 7.92, 8.01 (each 1H, dd, J = 2 and 8 Hz, C-20 and 23-H). ^{13}C-NMR

($CDCl_3$): δ 18.3 (C-13), 25.9 (C-12), 28.3, 28.5 (C-17 and C-18), 37.6 (C-9), 86.7 (C-2), 96.7 (C-3'), 98.6 (C-8), 104.4 (C-6), 106.0 (C-5'), 107.2 (C-4a), 115.8 (C-14), 117.8 (C-10), 120.0 (C-1'), 125.2 (C-23), 126.4 (C-15), 127.0 (C-20), 128.0 (C-6'), 129.2 (C-21), 131.2 (C-22), 135.1 (C-11), 138.2 (C-19), 139.1 (C-24), 145.4 (C-4), 151.5 (C-3), 155.3 (C-4'), 156.4 (C-5), 156.6 (C-8a), 157.0 (C-2'), 159.3 (C-7).

6. T. Nomura, T. Fukai, Y. Hano, and J. Uzawa, *Heterocycles*, 1981, **16**, 2141.
7. The phenazine derivative (**8c**). Orange powder. FAB-MS: *m/z* 893 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 1.58, 1.60 (each 3H, br s, C-11-CH_3), 1.75 (3H, br s, C-16-CH_3), 2.42 (2H, m, C-18-H x 2), 3.37 (2H, br d, J = 8 Hz, C-9-H x 2), 3.20, 3.44, 3.61, 3.64, 3.71, 3.73, 3.74, 3.77 (each 3H, s OCH_3), 4.27 (1H, m, C-19-H), 4.65 (1H, br, C-14-H), 4.67 (1H, t, J = 8 Hz), 5.33, (1H, m, C-10-H), 5.38 (1H, br s, C-15-H), 5.87 (1H, br, C-26-H), 6.21 (1H, s, C-8-H, C-20-H), 6.23 (1H, d, J = 2 Hz, C-24-H), 6.31 (1H, dd, J = 2 and 8 Hz, C-5'-H), 6.34 (1H, d, J = 2 Hz, C-3'-H), 6.35 (1H, dd, J = 2 and 8 Hz, C-32-H), 6.37 (1H, d, J = 2 Hz, C-30-H), 6.73 (1H, br, C-27-H), 6.93 (1H, d, J = 8 Hz, C-6'-H), 7.08 (1H, d, J = 8 Hz, C-33-H), 7.60, 7.67 (each 1H, td, J = 1.5 and 7.6 Hz, phenazine moiety), 7.95, 8.09 (each 1H, dd, J = 1.5 and 7.6 Hz, phenazine moiety).
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12. Y. Hano, K. Ichikawa, M. Okuyama, J. Yamanaka, T. Miyoshi, and T. Nomura, *Heterocycles*, 1995, **40**, 953.
13. The revised formula (**14**) for the structure of sanggenon P was coincided with that of sorocein H isolated from *Sorocea bonplandii* by I. Messana *et al.* (see Ref. 2). The spectral data of sanggenon P were agreed with those of sorocein H.

Received, 10th March, 1997