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STRUCTURE OF SANGGENON O, A DIELS-ALDER TYPE ADDUCT DERIVED FROM A CHALCONE AND A DEHYDROPRENYLATED SANGGENON-TYPE FLAVANONE FROM *MORUS CATHAYANA*[1]

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Abstract - The structure of sanggenon O, which was isolated from the Chinese crude drug "Sag-bai-pi" and the root bark of Chinese mulberry tree (*Morus* cathayana), was revised based on its spectral and chemical evidences. The compound is regarded as a Diels-Alder type adduct produced with a chalcone and a 6-dehydroprenylflavanone forming an ether linkage between the 2′- and 3-positions and its absolute configuration was 2*S*,3*R*,3″*S*,4″*R*,5″*S*.

A lot of Diels-Alder type adducts produced from a chalcone and a dehydroprenylated phenol (flavonoids and stilbenes) have been isolated from Moraceous plants. Sanggenon O was isolated from the Chinese crude drug "Sang-bai-pi" and the root bark of Chinese mulberry tree, *Morus cathayana*,[2, 3] and the structure of the compound has been assigned as structure (**1**) (Scheme 1), i.e., a Diels-Alder type adduct derived from a chalcone derivative (A′-ring–C-8″–C-4″–C-5″–B′-ring of **1**) and an 8-dehydroprenylated sanggenon-type flavanone (3-hydroxy-2-prenylflavanone with an ether linkage between the 2′- and 3-positions).[3b] The cytotoxic activity of the compound against human oral tumor cell line (HSC-2, CC_{50}: 0.073 mM/mL) was higher than against normal human gingival fibroblast (CC_{50}: 0.14 mM/mL).[4] Recently, we determined the absolute configurations at the C-2 and C-3 positions of eight sanggenon-type flavanones, i.e., sanggenons A (**3**), C (**2**), M (**4**) (Schemes 1 and 2), and L, sanggenols F and G, and soroceins D and F to be (2*R*,3*S*)-flavanones.[5] In the continuous study of the stereochemistry of Diels-Alder type adducts with a sanggenon-type flavanone moiety, we found that the structure (**1**) postulated

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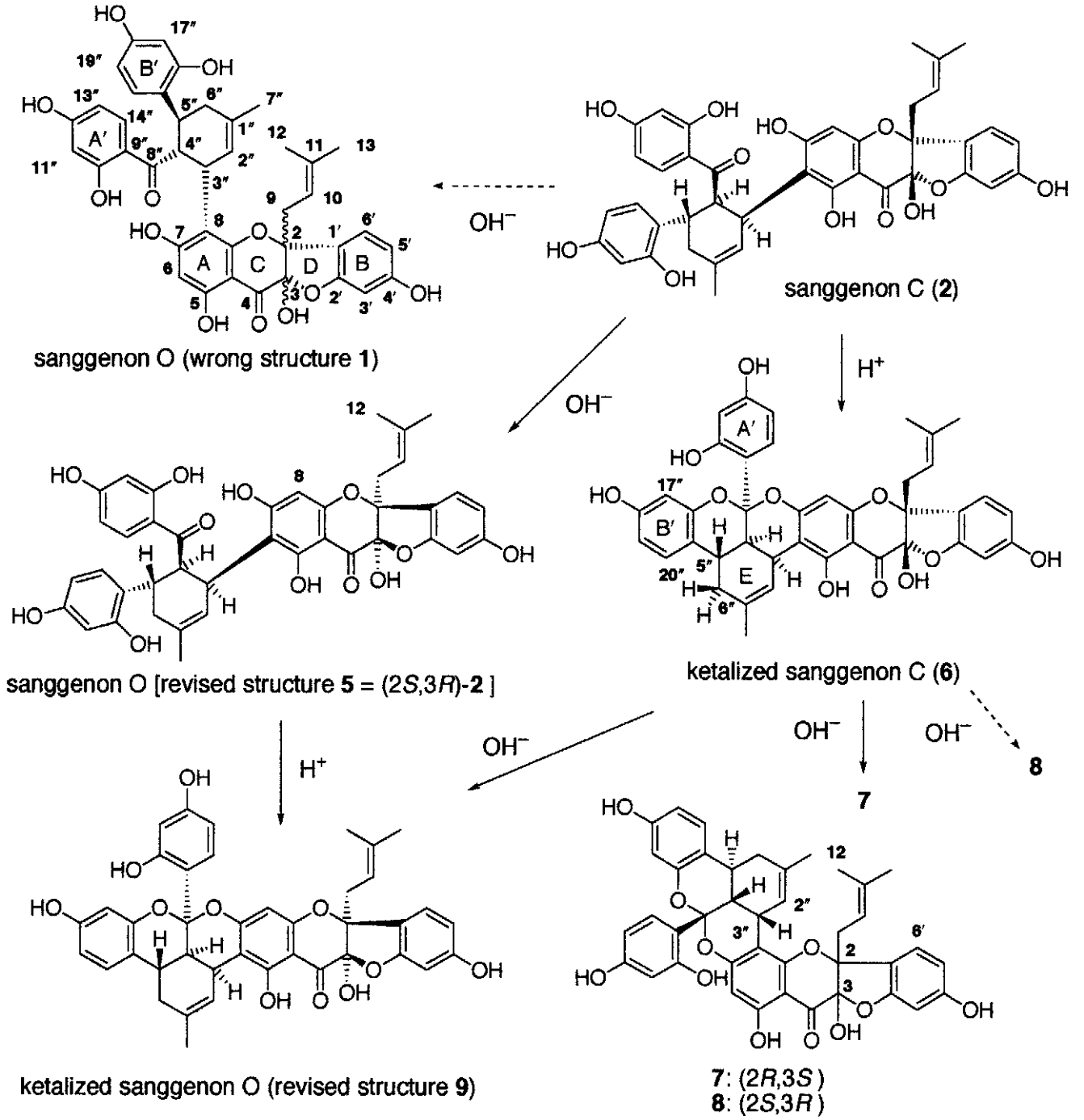
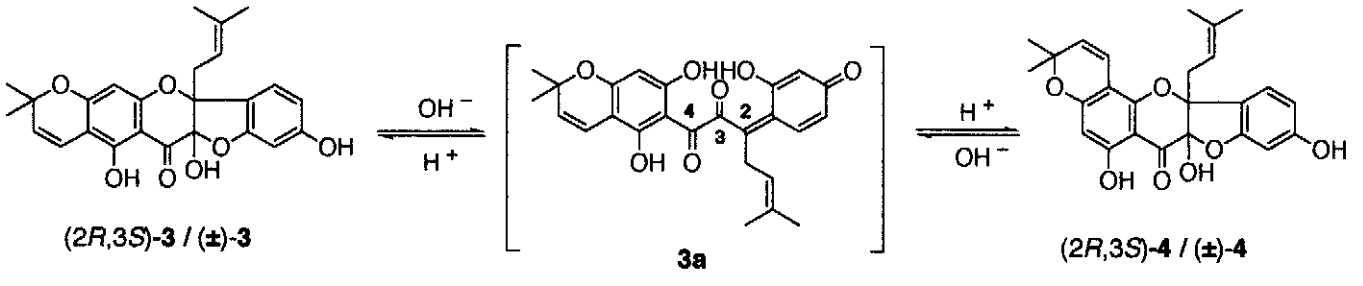
Dedicated to Professor Shô Itô on the occasion of his 77th birthday.



Scheme 1. Structures of sanggenons C (**2**), O (**5**), and their derivatives.



Scheme 2. Mechanism of simultaneous epimerization of sanggenons A (**3**) and M (**4**) at the C-2 and C-3 positions.

previously for sanggenon O is not correct. In this communication, we report the revised structure (**5**) and the absolute configurations of sanggenon O.

Previously, it was reported that sanggenon-type flavanones with a substituent at the 6- or 8-position were isomerized under basic conditions as shown in Scheme 2.[6, 7] Hano and Nomura reported that compound (**2**) also isomerized to sanggenon O (**1**) under the similar condition.[3b] More recently, we described that the 8-substituted flavanones (**1**) could not be obtained with the alkaline treatment of **2**. Instead, (2*S*,3*R*)-isomer (**5**) of sanggenon C was isolated as the sole product.[5] We reinvestigated the reaction for obtaining of **1** under the following condition: Compound (**2**) was dissolved in 0.5% sodium hydroxide solution and allowed to stand for 24 h at room temperature. The solution was then neutralized with 0.5% hydrochloric acid. By the ^{1}H NMR spectral analysis of the reaction mixture, the main compounds (**2** and **5**) and some unidentified minor compounds were detected. The latter compounds were considered to be the 8-substituted Diels-Alder type adduct isomers [(2*R*,3*S*)-**1**, (2*S*,3*R*)-**1**, (2*R*,3*R*)-**1**, or (2*S*,3*S*)-**1**].[8] The 5-OH signals of the unidentified compounds appeared at d 11.36, 11.39, 11.47, and 11.60 (in acetone-d_6) indicating their 6-positions being unsubstituted.[9] These chemical shifts differed from that of naturally occurring sanggenon O (d 12.07). These reaction products (**1**), however, could not be isolated because of their low production. The protons of these unidentified compounds were observed as broad signals. Similar broad signals were reported for the NMR spectra of all-*trans* Diels-Alder type adducts such as kuwanons G, K, and sanggenon D isolated from Moraceous plants.[2, 3a, 10] The broadening of the spectra may be induced by slow exchange of the conformations of these compounds on NMR time scale.[3a] The conformational change of the 8-substituted flavanones (**1**) was slow down presumably by the steric hindrance between B ring (or 2-prenyl group) and the substituents at the C-8 position. The resistance of transformation of **2** into **1** could be explained by the steric hindrance in the 8-substituted flavanones. This speculation was supported by the examination of molecular models of (2*R*,3*S*)-**1**, (2*S*,3*R*)-**1**, **2**, and (2*S*,3*R*)-**2** (**5**) calculated by MOPAC 97 (PM3 method) (Figure 1). The minimized total steric energies of the former two models were larger than those of the latter two models by calculated with MM2 (Table 1).[11]

In a similar examination of steric energies of the ketalized compounds (**6** – **9**), the transformation of **6** into 8-substituted flavanones (**7** and **8**) was calculated to occur (Table 1). The compound (**7**) must be the ketalized sanggenon O reported previously[3b] if the absolute configurations at the C-2 and C-3 positions of sanggenon O are 2*R* and 3*S* as the other sanggenon-type flavanones.[5] Therefore, we investigated the isomerization of **6** under basic conditions. Compound (**6**) was dissolved in 0.5% sodium hydroxide

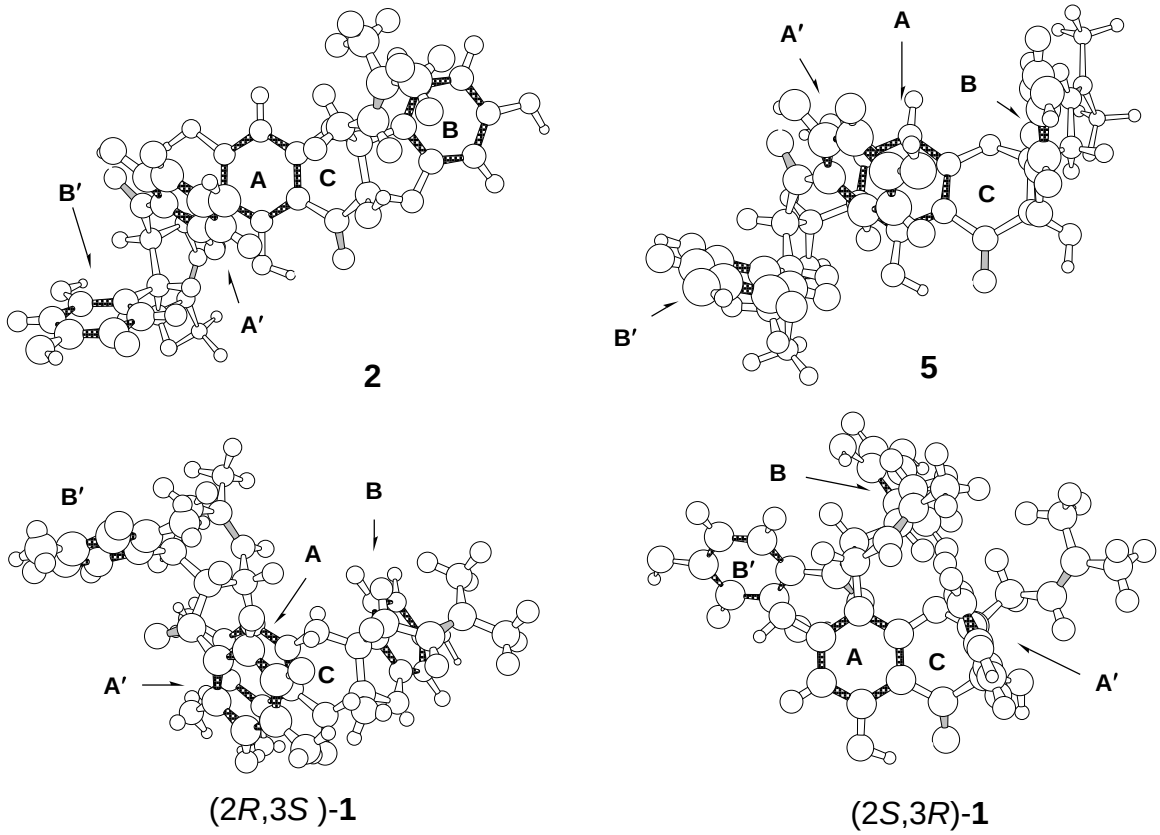


Figure 1. Molecular models calculated with MOPAC 97.

Table 1. Steric energies (kJ/mol) of the molecular models (2*R*,3*S*)-**1**, (2*S*,3*R*)-**1**, **2**, and **5** – **9**

Model	(2*R*,3*S*)-**1**	(2*S*,3*R*)-**1**	**2**	**5** [(2*S*,3*R*)-**2**]	**6**	**7**	**8**	**9**
Stretch	13.5	17.9	13.4	13.6	15.4	15.5	15.4	16.2
Bend	88.0	112.3	99.2	84.4	94.9	96.7	86.2	95.3
Stretch-Bend	0.8	1.5	1.1	1.3	1.6	1.2	1.2	1.3
Torsion	– 31.8	– 26.4	– 46.0	– 32.6	– 75.1	– 77.9	– 73.4	– 71.1
Non 1,4 van der Waals	– 159.3	– 151.3	– 160.8	– 195.0	– 84.7	– 90.4	– 92.9	– 98.5
1,4 van der Waals	89.5	89.4	84.6	85.3	111.8	113.7	110.5	112.7
Dipole/Dipole	24.6	6.1	– 6.7	18.0	23.8	24.0	30.0	36.0
Total	25.3	49.5	– 15.2	– 22.0	87.7	82.2	77.0	91.9

solution and allowed to stand for 45 min at room temperature. The solution was then neutralized with 0. 5% HCl to yield **9**[5] and 8-substituted flavanone (**7**).[12] The structure of **7** was confirmed by its HMBC spectrum in which the cross peaks between 5-OH (d 11.39) and C-4a (d 100.9)/C-6 (d 96.9 correlated with H-6 by its HMQC spectrum) were observed. The absolute configurations at the C-2 and C-3 positions were assigned as 2*R* and 3*S* with the differential CD spectrum as described previously, the CD spectrum of **7** minus that of the 1:1 mixture of **6** and **9** gave the (2*R*,3*S*)-sanggenon-type CD spectrum;

positive Cotton effects at 220, 259, 301, and 340 nm and negative Cotton effects at 239 and 281 nm.[5] The 8-substituted ketalized compound (**7**), however, was different from the ketalized sanggenon O which was derived from sanggenon O under acidic conditions.[3b] But, the ketalized sanggenon O was identified as the 6-substituted ketalized compound (**9**)[5] by their ^{1}H and ^{13}C NMR, IR, and CD spectra. These data indicated that the structure of sanggenon O must be represented by the formula (**5**) but not the formula (**1**) postulated previously. Furthermore, the HMBC and NOESY spectra of sanggenon O also indicated structure (**5**) showing cross peaks between 5-OH (d 12.07) and C-4a (d 99.9)/C-6 (d 108.8, indicating the aromatic carbon substituted with a carbon-functional group) in the HMBC spectrum and the cross peak between H-8 (d 5.72) and H_3-12 (d 1.52) in the NOESY spectrum. Finally, sanggenon O was identified as compound (**5**),[5] (2*S*,3*R*)-**2**, by their ^{1}H and ^{13}C NMR, IR, and CD spectra. From the above data, the structure of sanggenon O was unambiguously revised to formula (**5**).[13] The absolute configuration of the compound (**5**) was already determined to be 2*S*,3*R*,3″*S*,4″*R*,5″*S*.[5]

Optically active flavanones are easily racemized upon heating, weak basic or acidic conditions.[14] On the other hand, all the chiral centers of **2** were stable under acidic conditions.[5] Therefore, **5** is presumably a natural product but not an artifact derived from **2** because no basic compound was isolated from the acetone extract of Sang-bai-pi and the conditions of the isolation was not basic.[2, 3, 15]

REFERENCES AND NOTES

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8. It could not be negligible that one or two compounds among them are D-ring opened flavanones, 3-oxo-2-prenylflavanones.
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10. The absolute configuration of all-*trans* Diels-Alder type adducts with a 3″,4″-*trans*-4″,5″-*trans*-3″,4″,5″-trisubstituted methylcyclohexene ring is 3″*R*,4″*R*,5″*S*.[2]
11. Chem 3D User's Guide, Version 5.0, Chambridg Soft, Chambridge, 1999, Chapter 10.
12. By the ^{1}H NMR spectral analysis (acetone-d_6) of the reaction mixture, a main compound (**7**) and three minor compounds (**6**, **9**, and an unidentified compound) were detected. The unidentified compound was considered to be **8** (d 11.50, 5-OH), but the compound could not be isolated. Compound **7**: MALDI-TOF-MS *m/z* 691 $[M + H]^+$, $[a]^{22}_D$ + 424° (*c* 0.1, MeCN), UV (EtOH) λ_{max} nm (log ε) 225 (sh 3.57), 285 (3.14), 309 (3.18), 361 (2.43); (EtOH+$AlCl_3$) λ_{max} nm (log ε) 226 (sh 3.59), 284 (3.10), 323 (3.19), 420 (2.19), ^{1}H NMR (500 MHz, acetone-d_6) δ 1.15 (3H, br s, H-12), 1.39 (3H, br s, H-13), 1.43 (3H, br s, H-7″), 1.88 (1H, br dd, *J* = 10, 14 Hz, H-6″), 2.56 (1H, br dd, *J* = 4, 14 Hz, H-6″), 2.69 (1H, dd, *J* = 5, 12 Hz, H-9), 2.74 (1H, br ddd, *J* = 4, 10, 12 Hz, H-5″), 3.16 (1H, dd, *J* = 8, 12 Hz, H-9), 3.22 (1H, br t, *J* = 4 Hz, H-3″), 3.36 (1H, dd, *J* = 4, 12 Hz, H-4″), 5.08 (1H, m, H-10), 5.84 (1H, br d, *J* = 4 Hz, H-2″), 6.02 (1H, s, H-6), 6.31 (1H, dd, *J* = 2, 8 Hz, H-13″), 6.32 (1H, d, *J* = 2 Hz, H-17″), 6.38 (1H, d, *J* = 2 Hz, H-3′), 6.45 (1H, d, *J* = 2 Hz, H-11″), 6.47 (1H, dd, *J* = 2, 8 Hz, H-19″), 6.58 (1H, dd, *J* = 2, 8 Hz, H-5′), 7.05 (1H, dd, *J* = 2, 8 Hz, H-20″), 7.11 (1H, d, *J* = 8 Hz, H-14″), 7.22 (1H, br s, 3-OH), 7.48 (1H, d, *J* = 8 Hz, H-6′), 11.39 (1H, s, 5-OH), ^{13}C NMR (125 MHz, acetone-d_6) δ 17.5 (C-12), 23.5 (C-7″), 25.8 (C-13), 28.1 (C-5″), 31.9 (C-9), 33.7 (C-3″), 36.0 (C-4″), 36.9 (C-6″), 92.5 (C-2), 96.9 (C-6), 99.5 (C-3′), 100.9 (C-4a), 101.9 (C-3), 103.5 (C-8″), 104.0 (C-17″), 104.7 (C-8), 105.0 (C-11″), 107.7 (C-13″), 109.9 (C-5′), 110.1 (C-19″), 116.7 (C-9″), 117.3 (C-15″), 117.6 (C-10), 120.9 (C-1′), 122.8 (C-2″), 125.4 (C-6′), 127.9 (C-20″), 130.0 (C-14″), 133.9 (C-1″), 138.2 (C-11), 153.1 (C-18″), 157.2 (C-16″), 157.8 (C-10″), 160.2 (C-12″), 161.0 (C-8a), 161.3, 163.1 (C-2′, C-4′), 161.8 (C-5), 163.0 (C-7), 188.9 (C-4).

13. Sanggenon O (**5**), $[\alpha]^{22}_{D}$, – 42° (*c* 0.05, MeOH), lit.,[3b] – 64° (*c* 0.13, MeOH), investigated here was isolated from ethanol extract of the root bark of *M. cathayana* collected in Sichuan Province, the People's Republic of China, in 1995.[4] From the semi-dried root bark (4.2 kg), 900 mg of **2** and 80 mg of **5** were isolated. There is not clear answer for the question "Why was the wrong structure (**1**) proposed to sanggenon O ?" It could be considered that the former sanggenon O[3b] contained the 8-substituted compounds (**1**), because the UV spectrum of **5** recorded here [UV (EtOH) λ_{max} nm (log ε) 220 (sh 3.62), 230 (sh 3.51), 288 (3.36), 309 (3.31), 340 (infl. 2.99); (EtOH+$AlCl_3$) λ_{max} nm; no shift] was slightly different from the spectrum reported previously, UV (EtOH) λ_{max} nm (log ε) 222 (sh 4.68), 284 (4.48), 287 (sh 4.47), 305 (sh 4.39); (EtOH+$AlCl_3$) λ_{max} nm (log ε) 287 (4.46), 318 (4.45), 360 (sh 3.80).[3b] A bulky group at the *ortho*-position of intramolecular hydrogen-bonded hydroxyl group (5- OH) resists to the aluminum induced UV shift due to chelation.[16] Although C-11″ position of **5** was unsubstituted, it was indicated by the molecular dynamics (MM2) of the model (Figure 2) that the 8″-carbonyl and the 10″-OH groups were surrounded by the A, B, and B′ rings on three sides. Attempt to isolate the 8-substituted flavanone (**1**) from *M. cathayana* is in progress.

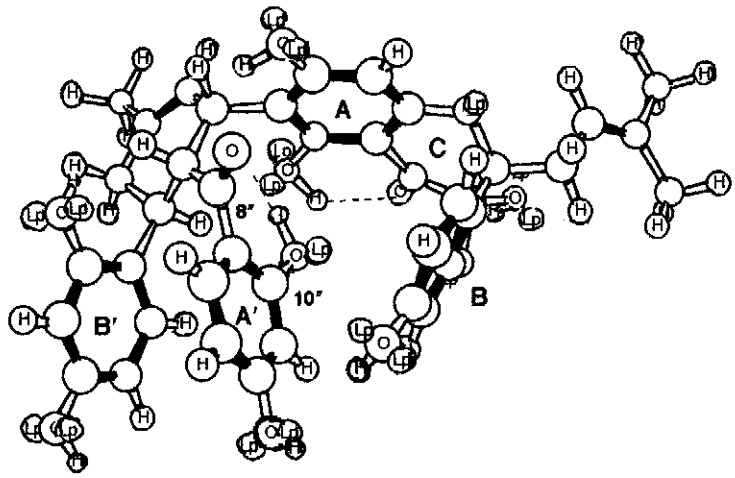

Figure 2. Molecular models of sanggenon O (**5**).

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15. A part of **5** isolated here[13] may be the artifact from **2** because the ethanol extract contains basic compounds such as 1-deoxynojirimycin.[17]
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