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Absolute configuration, conformation, and chiral properties of flavanone-(3→8″)-flavone biflavonoids from *Rheedia acuminata*

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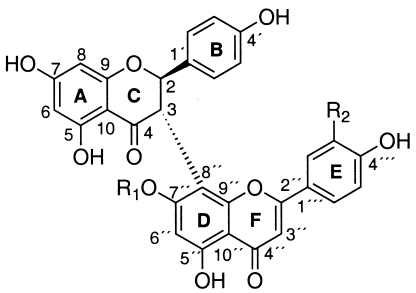
Abstract—The absolute configurations of three flavanone-(3→8″)-flavone type biflavonoids, (+)-morelloflavone (**1**), (+)-morelloflavone-7-sulfate (**2**) and (+)-volkensiflavone-7-sulfate (**3**) isolated from *Rheedia acuminata* were confirmed by circular dichroism as 2*R*,3*S*, hence clarifying the literature confusion of 2*S*,3*R* absolute configuration for (+)-morelloflavone. The conformations of this class of biflavonoids were studied for the first time by variable temperature NMR, NOESY, and computational calculations using a semi-empirical AM1 method. The coexistence of two conformers for morelloflavone (**1**) in solution at room temperature was confirmed by variable temperature NMR experiments. NOESY and computational calculations indicated that in the major and minor conformers **1a** and **1b**, respectively, the flavone DEF moiety is extended above and below the plane of the A/C-ring of the flavanone ABC unit, respectively. The C-3 proton of the C-ring of morelloflavone (**1**) is exchangeable by deuterium in acetone-d_6/D_2O at ambient temperature with retention of configuration, indicating considerable chiral stability of this class of biflavonoids.

1. Introduction

The first flavanone-(3→8″)-flavone type biflavonoid, morelloflavone (**1**), was isolated and identified from the seeds of *Garcinia morella* in 1967.[1] Optically active *dextro* rotatory,[2] racemic,[2–4] partially racemic[5] morelloflavone and its racemic derivatives[6–8] have since been reported. However, the absolute stereochemistry at C-2 and C-3 of this class of biflavonoids remains undefined, although (+)-morelloflavone is arbitrarily assigned a 2*S*,3*R*-absolute configuration by Chemical Abstract (registry number: 16851-21-1). It is also conspicuous that morelloflavone and its derivatives, e.g. volkensiflavone (**4**),[3,6] garcininanin (**5**)[7] and pancibiflavonol (**6**),[8] are often isolated as racemates. The biflavonoids represent a biosynthetically important group of natural products with significant biological activities,[9–14] hence the importance of establishing their absolute configurations and exploring their susceptibility to racemization at enolizable stereocenters.

As part of our search for fatty acid synthase (FAS) inhibitors[15] from plants (vide infra), we have isolated three flavanone-(3→8″)-flavone type biflavonoids, (+)-morelloflavone (**1**), (+)-morelloflavone-7-sulfate (**2**) and the new compound, (+)-volkensiflavone-7-sulfate (**3**) from the ethanol extract of the twigs and leaves of *Rheedia acuminata*, along with the biflavone type amentoflavone. Here we describe the structural identification and assessment of the absolute configurations of compounds **1**–**3**, as well as our findings regarding their conformations and chiral stability.



1 R_1 = H, R_2 = OH
2 R_1 = SO_3H, R_2 = OH
3 R_1 = SO_3H, R_2 = H
4 R_1 = H, R_2 = H

Keywords: *Rheedia acuminata*; biflavonoid; morelloflavone; volkensiflavone; conformation; circular dichroism; absolute configuration.
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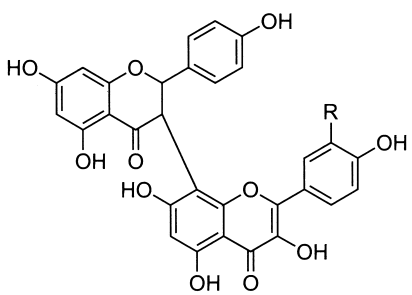
Table 1. ^{1}H NMR data of compounds **1**–**3** DMSO-d_6 (δ, ppm; J, Hz)

	1a (25°C)	**1b** (25°C)	**1** (80°C)	**2a** (25°C)	**2b** (25°C)	**3a** (25°C)	**3b** (25°C)	**3** (80°C)
H-2	5.71 d (12)	5.59 d (12.3)	5.75 d (12.0)	5.83 d (12)	5.65 d (12.4)	5.79 d (11.9)	5.58 d (12.3)	5.86 br[a]
H-3	4.89 d (12)	4.99 d (12.7)	4.89 d (12.1)	4.87 d (12)	5.09 d (12.4)	4.96 d (11.9)	5.05 d (12.3)	4.94 br[a]
H-6	5.97 br s	5.99 br s[b]	5.99 d (2.1)[b]	5.97 d (1.8)[b]	6.00 br s[b]	5.92 br s	6.00 br s	5.98 br s[b]
H-8	5.97 br s	6.02 br s[b]	6.00 d (2.1)[b]	5.95 d (1.8)[b]	6.03 br s[b]	5.92 br s	6.00 br s	5.99 br s[b]
H-2′,6′	7.15 d (8.3)	7.09 d (7.7)	7.10 d (8.5)	7.20 d (8.4)	7.10 d (8.5)	7.15 d (8.4)	NI[c]	7.16 d (8.3)
H-3′,5′	6.39 d (8.3)	6.61 d (7.9)	6.52 br d (7.1)	6.33 d (8.4)	6.60 d (8.5)	6.29 d (8.2)	6.57 d (8.9)	6.47 br d (6)[a]
HO-5	12.25 s	12.14 s	12.05 br s	12.22 s	12.04 s	12.24 s	12.10 s	12.04 br[a]
HO-3″	6.58 s	6.62 s	6.46 s	6.63 s	NI[c]	6.71 s	NI[c]	6.59 s
HO-6″	6.23 s	6.06 brs	6.22 s	7.07 s	NI[c]	7.06 s	NI[c]	7.08 br[a]
HO-2‴	7.42 br s	7.25 br s	7.31 br s	7.42 d (2.1)	7.26 br s	7.94 d (8.6)	7.65 d (8.5)	7.75 d (7.6)
HO-3‴	–	–	–	–	–	6.94 d (8.6)	6.64 d (8.5)	6.91 br[a]
HO-5‴	6.91 d (8.1)	6.50 d (8.4)	6.83 br s	6.91 d (8.2)	6.56 d (8.6)	6.94 d (8.6)	6.64 d (8.5)	6.91 br[a]
HO-6‴	7.43 br d (8.0)	6.97 br d (8)	7.22 br s	7.43 dd (8.2,2.1)	7.04 br d (9,2.0)	7.94 d (8.6)	7.65 d (8.5)	7.75 d (7.6)
HO-5″	13.07 s	12.97 s	12.86 br s	12.97 s	12.83 s	12.96 s	12.80 s	12.69 br[a]

a Series, e.g. **1a**, represents major conformer at 25°C; **b** series, e.g. **1b**, represents minor conformer at 25°C.
[a] Broadened signals, impossible to define multiplicity.
[b] Signals may be interchangeable.
[c] Not identified due to overlapping.



5 R = H
6 R = OH

2. Results and discussion

Reversed-phase column chromatography of the ethanol extract of the twigs and leaves of *R. acuminata* afforded column fractions that show fatty acid synthase inhibitory activity. Further purification of the active column fractions yielded three flavanone-(3⟶8″)-flavone type biflavonoids (**1**–**3**), along with amentoflavone that was identified by spectral data.[16]

Table 2. ^{13}C NMR data of compounds **1**–**3** in DMSO-d_6 at 25°C (δ, ppm)

	1	**2**	**3**		**1**	**2**	**3**
C-2	81.0	80.9	81.1	C-2″	163.8	164.1	164.2[a]
C-3	48.4	48.5	48.7	C-3″	102.3	102.5	102.6[b]
C-4	196.3	195.5	195.6	C-4″	181.7	182.1	182.1
C-5	161.8	162.9	163.0	C-5″	160.6	159.8	160.0
C-6	95.4	95.2	95.4	C-6″	98.7	102.5	102.5[b]
C-7	163.6	163.9	164.0[a]	C-7″	162.9	157.7	157.7
C-8	96.3	96.2	96.3	C-8″	100.6	104.4	104.3
C-9	166.6	166.8	166.6	C-9″	155.3	154.5	154.5
C-10	101.6	101.5	101.6	C-10″	103.2	105.2	105.3
C-1′	128.2	128.6	128.5	C-1‴	121.1	120.6	121.0
C-2′	128.6	128.5	128.7	C-2‴	113.4	129.9	113.6
C-3′	114.5	114.2	114.4	C-3‴	145.7	116.1	145.8
C-4′	157.4	157.1	157.3	C-4‴	149.8	161.5	150.0
C-5′	114.5	114.2	114.4	C-5‴	116.2	116.1	116.3
C-6′	128.6	128.5	128.7	C-6‴	119.4	129.0	119.7

The carbon signals for each compound are contributed by its major conformer.
[ab] Signals may be interchangeable.

Compound **1** was obtained as yellow crystals, mp 280°C, $[\alpha]_D^{25}$=+188° (*c* 0.1, MeOH). The homogeneity of **1** was revealed by TLC and MS data. However, in the ^{1}H NMR spectrum recorded in DMSO-d_6 at room temperature, major peaks were accompanied by corresponding less intense peaks with close chemical shifts. The ^{13}C NMR spectrum in DMSO-d_6 at room temperature was also not well resolved. A comparison of its UV,[1–4] IR,[1,2] major ^{1}H NMR signals in acetone-d_6[1,2,5,16] and major ^{13}C NMR signals in DMSO-d_6[17] with those reported in the literature could identify the structure of **1** as morelloflavone (*syn*.fukugetin). This compound was isolated from several *Garcinia* plants with different optical activity: $[\alpha]_D^{29}$=+170° (MeOH),[2] $[\alpha]_D^{29}$=+17° (MeOH),[5] and $[\alpha]_D^{29}$=0° (solvent not reported).[2–4] The structural assignment of morelloflavone in early years was largely based on its permethyl ether and its absolute configuration was not determined.[1–4]

Compound **2** was identified as morelloflavone-7-sulfate by comparison of the $[\alpha]_D$, UV, IR, and ^{1}H NMR (Table 1) with literature data.[14]

Compound **3** is optically active, $[\alpha]_D^{25}$=+113° (MeOH), and its molecular formula was established as $C_{30}H_{20}O_{13}S$ by high resolution ESIMS and ^{13}C NMR spectra (Table 2). The ^{1}H NMR spectrum (Table 1) of **3** resembled that of volkensiflavone (**4**).[5] Further comparison of its ^{13}C NMR signals with those of **2** and **4**[18] readily concluded that **3** is volkensiflavone-7-sulfate. The significant shifts of the carbon signals of the *ipso*, *ortho* and *para* positions (D-ring) as well as the chemical shift of H-6″ caused by the sulphation at C-7″ are consistent with literature data for flavonoid sulphates.[19,20]

In 1978, Duddeck et al.[17] examined the ^{13}C NMR and CD spectra of some biflavonoids from *Garcinia* species and proposed a 2*R*,3*S*-absolute stereochemistry for morelloflavone based on comparison of its CD spectrum with those of GB-1a heptamethyl ether (**7**), GB-2a octamethyl ether (**8**) and dihydromorin (**9**). Unfortunately, the source and optical

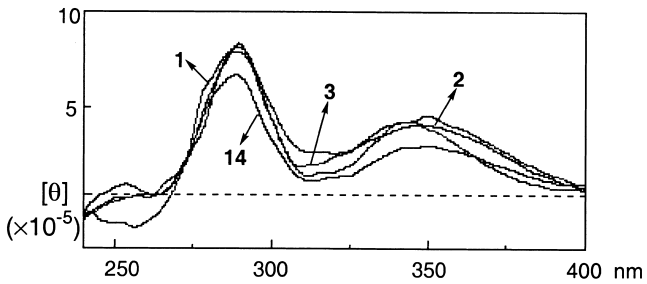
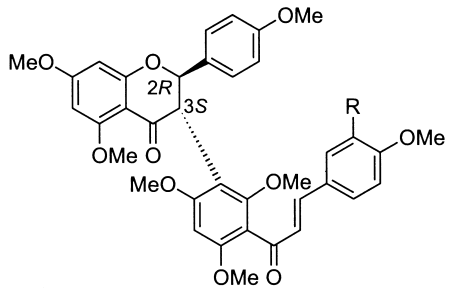


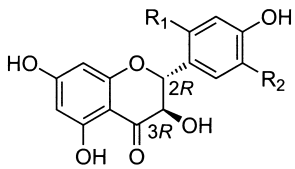
Figure 1. CD spectra of compounds **1**–**3** and **14**.

rotation of the morelloflavone examined was not clearly indicated, although we assume it should be the *dextro* diastereomer. In addition, Chemical Abstracts adopts assignments of 2*S*,3*R* and 2*R*,3*S* absolute configurations for (+) and (−)-morelloflavone (Registry #: 21945-33-5), respectively, without substantiating evidence.

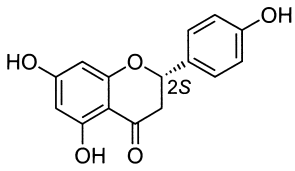


7 R = H
8 R = OH

There is considerable confusion in the literature regarding interpretation of the CD data of biflavonoids possessing one or both stereogenic constituent units.[17,21] One of the reasons for such ambiguity stems from changes in assigned absolute configuration when going from flavanone, e.g. (2*S*)-naringenin (**10**) to dihydroflavonol, e.g. (2*R*,3*R*)-dihydrokaempferol (**11**). Both (2*S*)-naringenin and (2*R*,3*R*)-dihydrokaempferol gave the same Cotton effects for their $\pi\rightarrow\pi^*$ (negative around 290 nm) and $n\rightarrow\pi^*$ (positive near 330 nm) transitions.[22] This indicates the same chirality at C-2, i.e. the B-ring is in an α-position, but not the same absolute configuration since the latter is an arbitrary designation based on the Cahn–Ingold–Prelog sequence rules. Note the change in priority going form **10** to **11**, i.e. once C-3 is hydroxylated. In addition, the Cahn–Ingold–Prelog sequence rules are often incorrectly applied when assigning absolute configuration to 3-substituted flavanones (dihydroflavonols), e.g. dihydromorin **9** with its 2*R*,3*R* configured C-ring was erroneously assigned a 2*S*,3*R* configuration.[17] Gaffield has also demonstrated that the Cotton effect due to the $\pi\rightarrow\pi^*$ transition near 290 nm is more reliable for the determination of the C-2 stereochemistry than the Cotton effect of the $n\rightarrow\pi^*$ transition at longer wavelength since the latter tends to diminish with increasing amounts of the opposite enantiomers.[22] Unequivocal evidence again come from the CD data published for the four diastereomers of taxifolin, (+)-(2*R*,3*R*)-taxifolin (**12**) (2,3-*trans*) and (+)-(2*R*,3*S*)-epitaxifolin (2,3-*cis*) exhibiting a stronger negative Cotton effect for the $\pi\rightarrow\pi^*$ transition, while (−)-(2*S*,3*S*)-taxifolin (2,3-*trans*) and (−)-(2*S*,3*R*)-epitaxifolin (2,3-*cis*) showing a stronger positive effect for the same transition near 290 nm.[23]

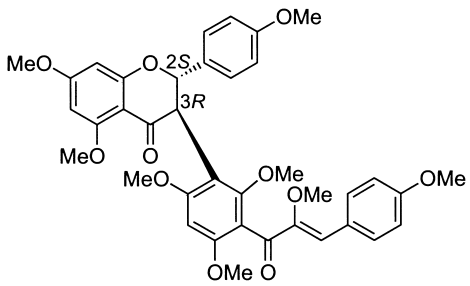


9 R_1 = OH, R_2 = H
11 R_1 = R_2 = H
12 R_1 = H, R_2 = OH



10

Inspection of the CD curves (Fig. 1) of compounds **1**–**3** indicates positive Cotton effects for both the $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions near 340 and 290 nm, respectively. The high-amplitude positive Cotton effects near 290 nm indicates 2β-orientation of the B-ring and hence 2*R* absolute configuration for all three compounds. Such an allocation is strongly supported by the opposite Cotton effects near 290 nm in the CD spectra of (+)-taxifolin (**12**), (+)-epitaxifolin[23] and compound **13**[24] all possessing 2α-positioned B-rings. It should be emphasized that although the achiral flavone DEF unit in compounds **1**–**3** is chirally perturbed by the flavanone ABC unit and may contribute to the longer wavelength $n\rightarrow\pi^*$ transition near 340 nm,[17] the diagnostic $\pi\rightarrow\pi^*$ transitions near 290 nm originate mainly from the flavanone-type chromophore when particularly compared with the CD data of compounds **7**, **8**, and **13**. When taken in conjunction with the CD data, the ^{1}H NMR coupling constants of compounds **1**–**3** ($^3J_{2,3}$, ca. 12 Hz) then facilitate assignment of 2,3-*trans* relative configuration and thus 3*S* absolute configuration. Thus, optically active (+)-volkensiflavone (**4**) which was designated 2*S*,3*R*-absolute stereochemistry by Chemical Abstracts (Registry #: 27542-37-6) should also possess 2*R*,3*S*-absolute configuration.



13

Poor resolution in the ^{1}H NMR spectra of the flavanone-(3→8″)-flavanones at room temperature was first noted by Jackson et al.[21] Such a phenomenon was attributed to the existence of various preferred conformations in the molecules at ambient or lower temperature, resulting from strong intra and inter-molecular hydrogen bonding of the various hydroxy

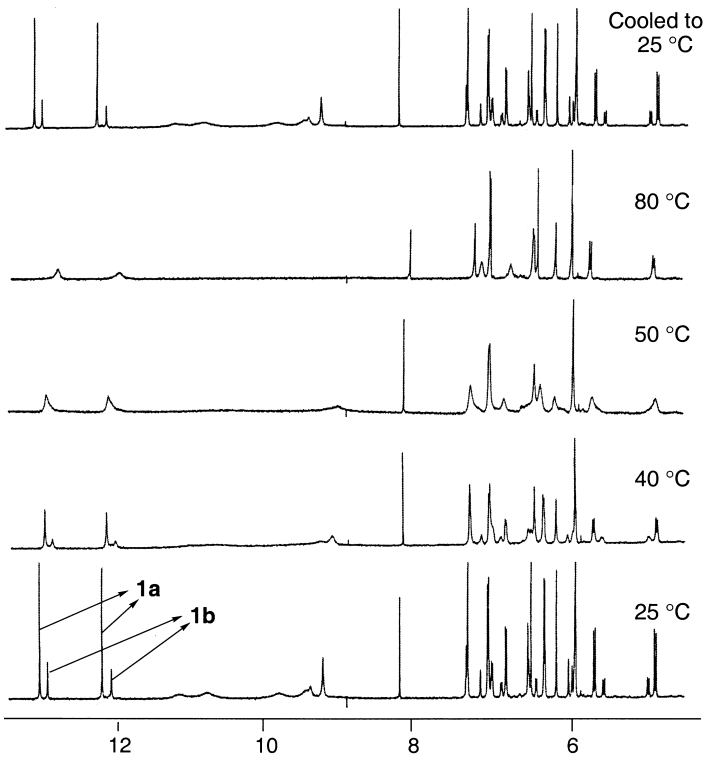


Figure 2. Variable temperature NMR experiments of **1**.

groups. A single set of signals could be obtained at elevated temperatures. This problem was also encountered in the flavanone-(3→8″)-flavone type biflavonoids, e.g. morelloflavone, whose NMR data in one reference were obtained at 120°C in DMSO-d_6.[25] Recently, Terashima et al.[7] and Ito et al.[8] reported the presence of two conformers in two flavanone-(3→8″)-flavonol type biflavonoids, garcinianin (**5**) and pancibiflavonol (**6**), respectively, but further conformational analysis of these compounds was not attempted.

Duplication of signals (in a ratio of 1:0.37) in the ^{1}H NMR spectrum of the optically active morelloflavone (**1**) in DMSO-d_6 suggested the existence of two conformers at room temperature, the major reasons leading to the existence of such conformers being rotational restrictions about the interflavanyl C(3)–C(8″) bond. Elevation of the temperature revealed changes in the spectrum as are shown in Fig. 2. At 80°C (the maximum temperature designed for the probe utilized in this experiment), a single set of signals and thus

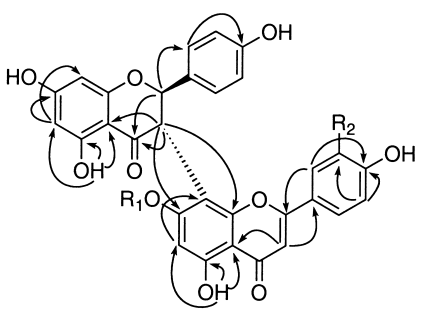


Figure 3. Key HMBC correlation patterns of **1** (R_1=H, R_2=OH), **2** (R_1=SO_3H, R_2=OH), and **3** (R_1=SO_3H, R_2=H).

only one conformer was observed. The observed chemical shifts of most signals of **1** at 80°C are the averages of the chemical shifts of the two conformers at room temperature (Fig. 2, Table 1).[26] At this temperature the $^3J_{2,3}$ (C-ring) values were very similar to those at 25°C (12.0–12.3 Hz), which indicated that the conformation of the C-ring did not change significantly. We thus assume that at this temperature the molecule has basically overcome the rotational restrictions. This was supported by the NOESY experiment at 80°C showing correlations of H-2‴ and H-6‴ (E-ring) with both H-2 and H-3 (C-ring). When the same NMR sample was cooled down to room temperature after half an hour, the ^{1}H NMR spectrum of **1** showed the same ratio of the two conformers. This variable temperature NMR experiment thus unequivocally confirmed the existence of two distinct conformers for morelloflavone (**1**) in solution at room temperature.

To establish the conformations of the two conformers (**1a**, **1b**) in solution, 2D NMR including COSY, HMQC and HMBC were used to facilitate the assignments of their ^{1}H and ^{13}C NMR signals at room temperature. The ^{1}H and ^{13}C NMR signals of the major conformer **1a** were unequivocally assigned and listed in Tables 1 and 2. Key HMBC correlations that are helpful in assigning quaternary carbons are shown in Fig. 3. For the minor conformer **1b**, we were only able to assign its ^{1}H NMR signals since the ^{13}C NMR signals were generally of low intensity.

The NOESY experiment at room temperature in DMSO-d_6 was then employed to determine the correlations of spatially close protons of the two conformers in solution. A strong cross peak was observed between H-3 of the C ring (δ 4.90) and H-2‴ and H-6‴ (E-ring) (δ 7.42 and 7.43) in the major

Table 3. Key NOE correlations of compounds **1**–**3** observed from NOESY experiments at 25°C

	1	2	3
H-2 (**a**)	H-2′,6′ (**a**), H-2 (**b**)	H-2′,6′ (**a**), H-2 (**b**)	H-2′,6′ (**a**), H-2 (**b**)
H-3 (**a**)	H-2′,6′,2‴,6‴ (**a**), H-3 (**b**)	H-2′,6′,2‴,6‴ (**a**), H-3 (**b**)	H-2′,6′,2‴,6‴ (**a**), H-3 (**b**)
H-2′,6′ (**a**)	H-2,3 (**a**)	H-2,3 (**a**)	H-2,3 (**a**)
H-3′,5′ (**a**)	H-3′,5′ (**b**)	H-3′,5′ (**b**)	H-3′,5′ (**b**)
H-2‴ (**a**)	H-3,3″ (**a**), H-2‴ (**b**)	H-3,3″ (**a**), H-2‴ (**b**)	H-3,3″ (**a**), H-2‴ (**b**)
H-5‴ (**a**)	H-5‴ (**b**)	H-5‴ (**b**)	H-3‴,5‴ (**b**)
H-6‴ (**a**)	H-3,3″ (**a**), H-6‴ (**b**)	H-3,3″ (**a**), H-6‴ (**b**)	H-3,3″ (**a**), H-6‴ (**b**)
HO-5 (**a**)	H-6 (**a**)	NI[a]	H-6 (**a**)
HO-5″ (**a**)	H-6″ (**a**), H-6″ (**b**)	NI[a]	H-6″ (**a**)

a and **b** represent major and minor conformer, respectively, for each compound at 25°C.
[a] Not identified.

rotamer. Other NOE correlations are summarized in Table 3. This clearly indicated that in the major conformer **1a** the flavone DEF moiety is extended above the plane of the A/C-ring of the flavanone moiety. Attempts to observe the key NOE correlations for the minor conformer **1b** failed. Instead, substantial cross peaks between the same proton in the minor conformer and the major conformer were observed, e.g. H-2‴ of **1b** at δ 7.25 to H-2‴ of **1a** at δ 7.42 (Table 3). This unusual NOE phenomena was first noted by Hatano et al. in two conformers of procyanidin dimers, which was caused by the 'rotational conformation exchange' between the two conformers.[27] When examining the ROESY spectrum of **1** at room temperature, the minor conformer **1b** again 'relayed' the NOE to the major conformer **1a**, and vice versa. The same NOE associations were observed at different dilutions in DMSO-d_6, and also in acetone-d_6, hence excluding the possibility that these effects resulted from self-assembly of molecules.

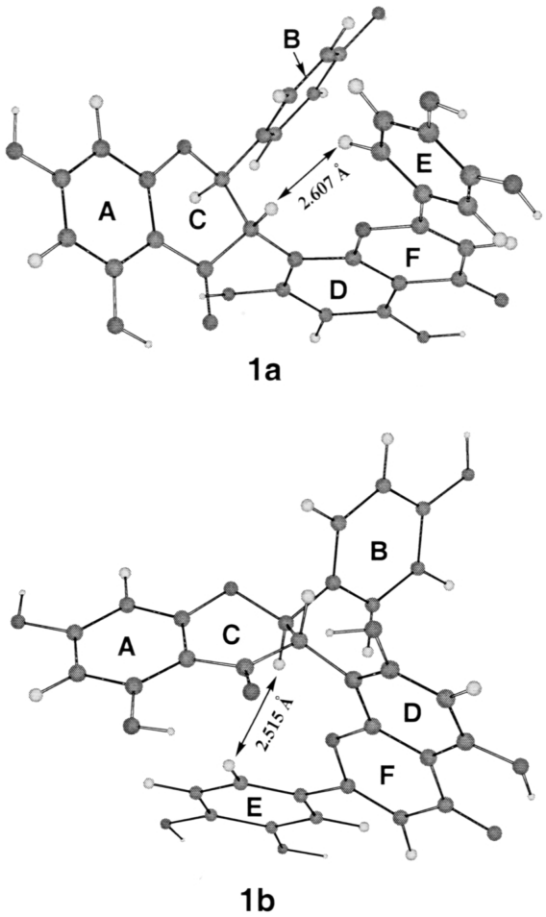


Figure 4. Steric view of conformer **1a** and **1b**.

To further explore the conformations of the two conformers of **1**, computational calculations using SYBYL 6.7 software[28] were performed. Since the rotational restriction was about the interflavonoid C-3→C-8″ bond, the dihedral angle (ϕ) of C-4–C-3–C-8″–C-7″ was set as the key variable parameter. The 'systematic search' in Sybyl was carried out with 10° increments of the dihedral angle. The results revealed two local energy minima at dihedral angles of 60 and 270°, which were consistent with the Dreiding models of **1**. The two low energy conformers were next optimized using the semi-empirical AM1 method. The final optimization results indicated that the energy of conformer **a** (ϕ=61.3°) was slightly lower than that of conformer **b** (ϕ=278.1°) by 1.65 kcal/mol. The optimized conformers **a** and **b** are shown in Fig. 4 and are most likely to correspond to the major, more extended conformer **1a** and the minor, compressed conformer **1b**, respectively, in solution. The calculated distance between H-3 and H-6‴ in the structure of conformer **a** was 2.607 Å, explaining the presence of the NOE correlation between the two protons observed for **1a** in the NOESY spectrum of **1**.

In conformer **b** (Fig. 4), the flavone DEF moiety is located under the plane of the A/C-ring of the flavanone unit to form the more 'compact' structure. The calculated distance between H-2 (C) and H-6‴ (*E*) is 2.515 Å. Although significant NOE correlations, e.g. the one between H-2 and H-6‴, were not observed within the minor conformer **1b** in solution due to the rotational conformation exchange in the timescale of the experiment, the ^{1}H NMR data of **1b** strongly supported such a structure. Comparison of the ^{1}H NMR chemical shifts between **1a** and **1b** revealed considerable differences for the chemical shifts of their H-3′, H-5′, H-6″, H-2‴, H-5‴ and H-6‴ resonances (Table 1). This could be explained by the anisotropic shielding/deshielding effects of the aromatic rings. In conformer **b**, H-3′,5′ (δ 6.61) are deshielded by the anisotropy of the D-ring; while in conformer **a**, H-3′,5′ (δ 6.39) could be shielded by the E-ring. An upfield shift was observed for H-6″ ($\Delta\delta$, −0.17 ppm) from **1a** to **1b** since the H-6″ might be shielded by the B-ring in conformer **1b**. Significant shieldings for H-2‴, H-5‴ and H-6‴ in the compact conformer **b** are probably due to the shielding effect of the A-ring. The above NMR data indicated that conformer **b** represented the minor conformer **1b** of **1** in solution. Similar

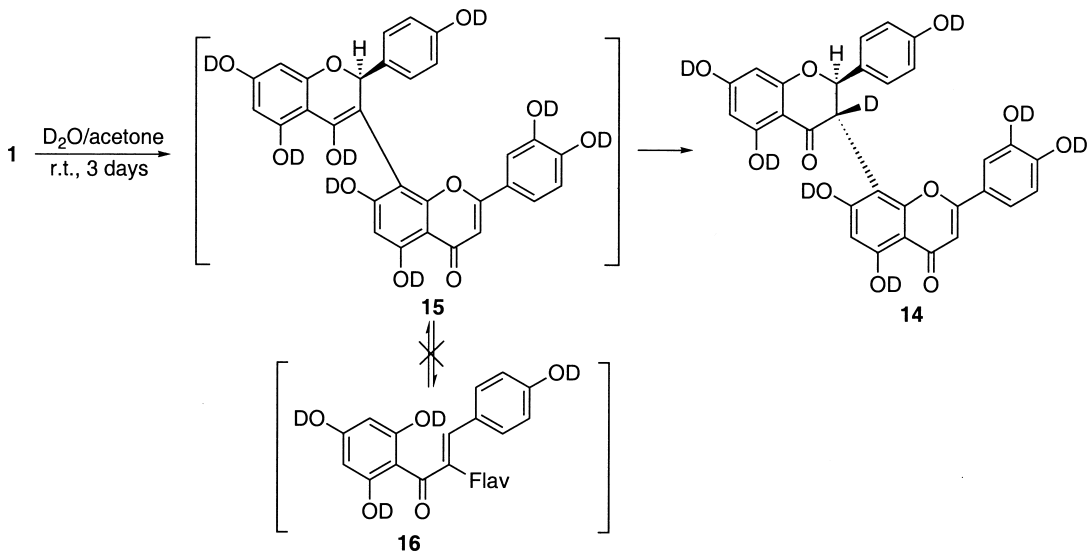


Figure 5. Deuterium exchange of compound **1**.

shielding/deshielding phenomena were also reported for the conformers of procyanidin dimers.[27]

Compound **2** has two predominant conformers (**2a** and **2b**) in DMSO-d_6 at room temperature in a ratio of approximately 1:0.20 as indicated by its ^{1}H NMR spectrum. The assignments of the ^{1}H and ^{13}C NMR signals of the major conformer **2a** were facilitated by the HMBC spectrum (Fig. 3) and are listed in Tables 1 and 2. The NOE evidence (Table 3) suggested that the conformer **2a** adopted a conformation similar to the major conformer **1a**. The identified signals for the minor conformer **2b** were similar to those of **1b** (Table 1), indicating that **2b** also adopts a conformation similar to **1b**.

In addition to the major conformer **3a** with a conformation similar to **1a** and **2a** (Tables 1–3; Fig. 3), the ^{1}H NMR spectrum of **3** in DMSO-d_6 at room temperature also displayed a minor conformer **3b**. The close chemical shifts for H-2 and H-3 of **3b** and **1b** indicated their close spatial and structural resemblance.

Variable temperature ^{1}H NMR experiments were further performed on compounds **2** and **3** in DMSO-d_6. Compound **3** showed broadening of a number of signals at 80°C (Table 1). This presumably indicated 'intermittent' rotations in the NMR timescale, i.e. the temperature was not high enough to permit free rotation. Compound **2** was converted to **1** at this temperature in about 8 min. The hydrolysis of the sulphate group in the biflavonoid **2** is apparently easier than in aliphatic compounds.[29]

Optical activity in biflavonoids is of significant biogenetic importance.[30] Although optically active morelloflavone can be racemized under relatively drastic reaction conditions, e.g. reflux with HBr in acetic acid,[2] 3,8″-type biflavonoids with flavanone and/or dihydroflavonol constituent units are often isolated in fully racemized form. During measurement of the ^{1}H NMR spectrum of **1** in acetone-d_6 with a few drops of D_2O, the C-3 proton underwent deuterium exchange on standing at room temperature for 3 days, as evidenced by the disappearance of the signal of H-3 and conversion of a doublet to a singlet for the signal of H-2. Such an H/D-exchange was also observed in DMSO-d_6/D_2O on standing at room temperature for 2 days. In both cases, deuterium exchange of the A/D-ring protons (H-6, H-8, H-6″) did not occur as observed in procyanidin dimers.[27] These observations imply that the C-3 proton is sufficiently acidic to facilitate the process of epimerization near neutral pH conditions. However, the deuterated product **14** is still optically active as indicated by its CD spectrum (Fig. 1) and specific optical rotation. The magnitude of the specific rotation of **14** compared to that of morelloflavone (**1**) indicated that the deuterium exchange probably did not change the relative 2,3-*trans* C-ring configuration, i.e. deuteration of **15** occurs with retention of configuration at C-3 (C-ring). This suggests that the enolic tautomer (**15**) does not induce the well-known flavanone (**1**)↔chalcone (**16**) interconversion leading to racemization under mild neutral condition (Fig. 5). In fact, it supports the finding that isomerization in this class of compounds occurs predominantly via formation of a B-ring quinomethane-type intermediate.[31]

The chiral stability of the optically active morelloflavone (**1**) was further established by the following experiments. After reflux of **1** in MeOH/H_2O (9:1) for 24 h, reflux of **1** in 5% AcOH/H_2O for 2 h, or allowing **1** to sit at room temperature in 1% NaOAc in MeOH/H_2O (9:1) for 3 days, the CD spectrum of **1** remained unchanged. This indicated that **1** and related biflavonoids with a flavanone ABC moiety should be stable to racemization under routine extraction and isolation processes using regular organic solvents. An exception may be EtOAc in either the extraction and/or fractionation process since this solvent consistently gives considerable amounts of HOAc via hydrolysis in the final stages of solvent evaporation. The isolation of racemic morelloflavone and its derivatives in previous studies[2–4,7,8] may then suggest that the optically active morelloflavone is racemized in plants during the ageing and/or isolation process or that the racemates are indeed biosynthesized via a non-stereospecific mechanism. Racemic morelloflavone may thus form via electrophilic radical (**18**) substitution of 2′,4′,6′,4-tetrahydroxychalcone (**17**) with luteolin (**19**). The

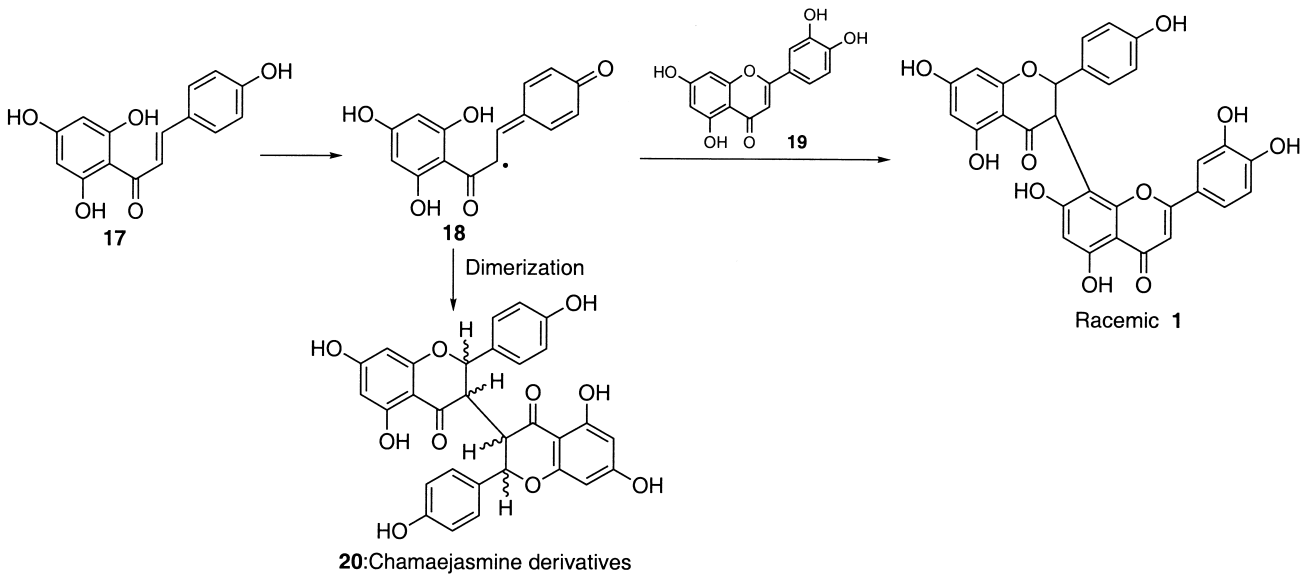


Figure 6. Proposed mechanism for the formation of racemic **1** and chamaejasmine derivatives.

intermediate may then undergo non-stereoselective cyclization. Isolation of naturally occurring chamaejasmine and its derivatives (**20**) with different configurations at C-2 and C-3 seems to support this hypothesis (Fig. 6).[32–35] EtOAc was, however, extensively utilized during isolation of this group of compounds.

This study demonstrates that the CD method can be effectively utilized to assess the absolute stereochemistry of flavanone-(3→8″)-flavone type biflavonoids and related analogues. This class of compounds furthermore possesses two preferred conformations in solution at room temperature, of which the minor one could be easily regarded as 'impurities'. Our results also suggest that racemization of the biflavonoids probably occur in the plants, not during routine extraction and isolation processes.

3. Experimental

3.1. General

Optical rotations were determined on an AutoPol® IV automatic polarimeter. CD spectra were recorded on a JASCO J-715 Spectrometer. UV spectra were measured on a Hewlett Packard 8453 spectrometer. IR spectra were recorded on an ATI Mattson Genesis Series FTIR Spectrometer. NMR spectra were recorded on Bruker Avance DPX-300 (300 MHz), DRX-400 (400 MHz) or DRX-500 (500 MHz) NMR spectrometers. Chemical shifts are expressed relative to the deuterated solvent (DMSO-d_6: δ_H/δ_C, 2.49/39.5; acetone-d_6: δ_H, 2.04). Variable temperature NMR experiments were run on the DRX-500 NMR spectrometer. COSY, HMQC, HMBC (J=10 Hz), NOESY (mixing time, 800 ms) and ROESY NMR spectra were performed with standard pulse programs. To exclude the possibility of self-assembly in conformational studies, the ^{1}H NMR was run at different concentrations (c=0.006, 0.018, and 0.12 M) in DMSO-d_6 or acetone-d_6. The chemical shifts in different sample concentrations remained same and the NOESY and ROESY experiments were thus performed at these concentrations, primarily at 0.018 M in DMSO-d_6. ESI-FTMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. Column chromatography was run using reversed-phase silica gel (RP-18, 40 μm, J. T. Baker) and silica gel (40 μm, J. T. Baker). Analytical and preparative TLC was performed on silica gel sheets (Alugram® Sil G/UV$_{254}$, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F$_{254S}$, Merck, Germany). All the computational calculations were performed on a SGI Octane 2 R12000 workstation with the MOPAC package in SYBYL 6.7 software.[28] The geometry optimizations of all the structures leading to energy minima and the conformational analysis were achieved by using semiemperical method of Austin Model 1 (AM1). During the geometry optimization the key word PRECISE was chosen for convergence. This work was part of a multidisciplinary project aimed at identifying natural products with FAS activity, detailed results of which will be published elsewhere.

3.2. Plant material

The twigs and leaves of *R. acuminata* Tr. & PI. (Clusiaceae) were collected by Mr Manuel Rimachi in Maynas, Lorento, Peru in June, 1998, and identified by Mr M. Rimachi and Professor Sidney McDaniel. A voucher specimen of this plant is deposited at the Herbarium of Mississippi State University (Voucher # IBE 12306).

3.3. Extraction and isolation

The dried powdered twigs and leaves (328 g) were extracted with 95% EtOH (0.5 L×4) at room temperature. Removal of the solvent in vacuo under 45°C yielded an EtOH extract (39.1 g, IC$_{50}$=20 μg/mL for FAS). The EtOH extract was dissolved in MeOH/H$_2$O (9:1, 400 mL) and defatted with hexane (200 mL×3). The MeOH/H$_2$O layer was concentrated to dryness to give a residue (23 g, IC$_{50}$=40 μg/mL). The residue was subjected to column chromatography on reversed phase silica gel C-18 using aqueous MeOH system

(0⟶100% MeOH in water) to give 30 pooled fractions. Column fractions showing activity (IC_{50}, 3–30 μg/mL) were further purified as follows. Part of fraction 6 (150 mg) was chromatographed on silica gel eluting with $CHCl_3$–acetone (9:1–4:1) to afford compound **2** (36.1 mg). Part of fraction 9 (60 mg) was separated on preparative TLC using $CHCl_3$–MeOH (4:1) as mobile phase to yield compound **3** (18 mg). Part of fraction 15 (250 mg) was chromatographed on silica gel eluting with $CHCl_3$/MeOH (20:1) to give compound **1** (50.2 mg). Part of fraction 21 (15 mg) was purified on preparative TLC with $CHCl_3$/MeOH (9:1) to furnish amentoflavone (5.1 mg).

3.3.1. Morelloflavone (1). Yellow crystals from MeOH, mp 280°C, $[\alpha]_D^{25}$=+188° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.56), 225 (sh, 4.54), 278 (sh, 4.30), 290 (4.35), 344 (4.16) nm; IR (KBr) ν_{max} 3400 (br), 1642, 1610, 1515, 1366, 1260, 1160, 1088, 838, 744 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ: major conformer **1a**, 13.15 (1H, s, HO-5″), 12.31 (1H, s, HO-5), 7.49 (2H, m, H-2‴,6‴), 7.23 (2H, d, *J*=7.7 Hz, H-2′,6′), 7.01 (1H, d, *J*=7.7 Hz, H-5‴), 6.52 (2H, d, *J*=7.7 Hz, H-3′,5′), 6.46 (1H, s, H-3″), 6.29 (1H, s, H-6″), 6.01 (2H, br s, H-6,8), 5.86 (1H, d, *J*=12 Hz, H-2), 4.98 (1H, d, *J*=12 Hz, H-3); minor conformer **1b**, identifiable signals:13.05 (0.3H, s, HO-5″), 7.34 (0.3H, br s, H-2‴), 6.67 (1H, m, H-3′,5′,3″), 6.10 and 6.08 (0.3H each, s, H-6 or 8, 6″), 5.78 (0.3H, d, *J*=12 Hz, H-2), 5.08 (0.3H, d, *J*=12 Hz, H-3); ^1H and ^{13}C NMR in DMSO-d_6, see Tables 1–2; ESIMS *m/z* 555 $[M-H]^-$; CD (*c* 4.5×10^{-5} M, MeOH) λ ([θ]) 288 (+8.4×10^5), 350 (+4.0×10^5) nm.

3.3.2. Morelloflavone-7-sulfate (2). Yellow powder, $[\alpha]_D^{25}$=+227° (*c* 0.09, MeOH) {lit.[14] $[\alpha]_D^{24}$=+262° (*c* 1, MeOH)}; UV (MeOH) λ_{max} (log ε) 212 (4.48), 225 (sh, 4.41), 275 (sh, 4.17), 288 (4.20), 348 (4.05) nm; IR (KBr) ν_{max} 3420 (br), 1643, 1603, 1518, 1259, 1161, 1038, 838, 777, 633 cm^{-1}; NMR: see Tables 1–2; ESIMS *m/z* 635 $[M-H]^-$; CD (*c* 4.7×10^{-5} M, MeOH) λ ([θ]) 290 (+8.3×10^5), 348 (+4.0×10^5) nm.

3.3.3. Volkensiflavone-7-sulfate (3). Yellow powder, $[\alpha]_D^{25}$=+113° (*c* 1.32, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.90), 225 (sh, 4.83), 275 (sh, 4.58), 288 (4.62), 328 (4.46) nm; IR (KBr) ν_{max} 3240 (br), 1641, 1602, 1250, 1167, 1039, 837, 630 cm^{-1}; NMR: see Tables 1 and 2; HRESIMS *m/z* 619.0582 (calcd for $[M(C_{30}H_{20}O_{13}S)-H]$, 619.0551). CD (*c* 5×$10^{-5}$ M, MeOH) λ ([θ]) 290 (+8.2×10^5), 344 (+4.1×10^5) nm.

3.4. Hydrogen/deuterium exchange of 1

To a solution of **1** (3 mg) in acetone-d_6 (0.2 mL) in the 3 mm NMR tube was added four drops of D_2O. After standing at room temperature for 3 days, the sample was found to be converted to **14**.

3.4.1. 3-[^{2}H]-Morelloflavone (14). Yellow powder, $[\alpha]_D^{25}$=+203° (*c* 0.24, MeOH); ^1H NMR (acetone-d_6/D_2O, 300 MHz) δ: major conformer **14a**, 7.40 (1H, d, *J*=8.3 Hz, H-6‴), 7.36 (1H, br s, H-2‴), 7.14 (2H, d, *J*=8 Hz, H-2′,6′), 6.94 (1H, d, *J*=8.3 Hz, H-5‴), 6.46 (2H, d, *J*=7.7 Hz, H-3′,5′), 6.45 (1H, s, H-3″), 6.31 (1H, s, H-6″), 5.94 (2H, br s, H-6,8), 5.79 (1H, s, H-2); minor conformer **14b**, 7.25 (0.3H, br s, H-2‴), 7.09 (0.6H, d, *J*=7.7 Hz, H-2′,6′), 7.02 (03H, br d, *J*=8 Hz, H-6‴), 6.61 (0.6H, d, *J*=7.7 Hz, H-3′,5′), 6.56–6.54 (m, H-3″,5‴), 6.13, 6.03 (0.3H each, s, H-6 or 8, 6′), 5.94 (overlapped, H-6 or 8), 5.67 (0.3H, s, H-2); ESIMS *m/z*: 562 $[M(C_{30}H_{12}D_8O_{11})-D]^-$; CD (*c* 2.5×$10^{-5}$ M, MeOH), λ ([θ]) 289 (+7.9×10^5), 350 (+3.2×10^5) nm.

Similarly, compound **1** was converted to **14** in the DMSO-d_6/D_2O solution in 2 days. ^1H NMR (DMSO-d_6/D_2O, 300 MHz) δ: major conformer **14a**, 7.35 (2H, m, H-2‴,6‴), 7.10 (2H, d, *J*=8.1 Hz, H-2′,6′), 6.91 (1H, d, *J*=8.9 Hz, H-5‴), 6.53 (1H, s, H-3″), 6.36 (2H, d, *J*=8.1 Hz, H-3′,5′), 6.25 (1H, s, H-6″), 5.96 (2H, br s, H-6,8), 5.66 (1H, s, H-2); minor conformer **14b**, 7.18 (0.3H, br s, H-2‴), 7.06 (0.6H, d, *J*=8.1 Hz, H-2′,6′), 6.94 (0.3H, br d, *J*=8.3 Hz, H-6‴), 6.61 (0.3H, s, H-3″), 6.59 (0.6H, d, *J*=8.1 Hz, H-3′,5′), 6.48 (0.3H, d, *J*=8.3 Hz, H-5‴), 6.01 (0.6H) and 6.00 (0.3H) (each br s, H-6,8,6″), 5.55 (0.3H, s, H-2).

3.5. Chiral stability of 1

A solution of compound **1** (~1.5 mg) in MeOH/H_2O (9:1, 10 mL) was refluxed at 90°C. The reaction process was monitored by measuring the CD spectrum of the solution every 6 h. After 24 h, the CD spectrum of the solution showed Cotton effects similar to that of fresh **1**. Similarly, the CD spectrum of the solution of **1** (~1.5 mg) in 5% AcOH/H_2O (10 mL) which was refluxed at 100°C for 2 h remained unchanged.

A solution of compound **1** (~1.5 mg) in 1% NaOAc in MeOH/H_2O (9:1, 10 mL) was stirred at room temperature for 3 days. The CD spectrum of the solution showed Cotton effects similar to those of fresh **1**.

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