

HETEROCYCLES, Vol. 77, No. 2, 2009, pp. 793 - 800. © The Japan Institute of Heterocyclic Chemistry
Received, 29th July, 2008, Accepted, 9th September, 2008, Published online, 11th September, 2008
DOI: 10.3987/COM-08-S(F)73

UNCARIAGAMBIRIINE AND GAMBIRCATECHOL: NOVEL CONSTITUENTS OF *UNCARIA GAMBIR* LEAVES[†]

Naomi Yoshikado,^a Shoko Taniguchi,^a Naoki Kasajima,^b Fumiaki Ohashi,^c
Kou-Ichi Doi,^c Takashi Shibata,^c Takashi Yoshida,^{a,d} and Tsutomu Hatano^{a,*}

^a Okayama University Graduate School of Medicine, Dentistry and
Pharmaceutical Sciences, Tsushima, Okayama 700-8530, Japan

^b School of Pharmaceutical Sciences, Shujitsu University, Nishigawara, Okayama
703-8516, Japan

^c Taiko Pharmaceutical Co., Ltd., 3-34-14 Uchihonmachi, Suita 564-0032, Japan

^d Present address: College of Pharmaceutical Sciences, Matsuyama University,
Bunkyo-cho, Matsuyama 790-8578, Japan

Abstract – Two new polyphenolic compounds, uncariagambirine (**1**) and gambircatechol (**2**), were isolated from *Uncaria gambir* leaves, along with dehydrodicatechin A (**3**), catechin-(8→6′)-catechin (**4**), catechin-(6→6′)-catechin (**5**), and (+)-catechin (**6**). The structures of the new compounds, one of which is composed of an indole alkaloid and (+)-catechin, were established based on spectroscopic data.

Continued investigation on the constituents of *Uncaria gambir* Roxb. (Rubiaceae) revealed the presence of two new polyphenolic compounds in the leaves. This paper deals with the structures of these new compounds. One has a complex structure composed of dihydrogambirtannine and catechin.

This plant source has been used for preparing gambir, an herbal medicinal astringent in various Asian countries. Previously, we isolated several dimeric proanthocyanidins and chalcane–flavan dimers from gambir^{1–3} and established their structures. Further investigation on the leaves of this plant led to the isolation of new compounds **1** and **2**.

The new compounds were isolated from an aqueous acetone homogenate of the leaves, which were collected in Indonesia. The concentrated filtrate from the aqueous acetone homogenate was extracted with

[†] Dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday.

diethyl ether, and then with ethyl acetate. Countercurrent distribution of the diethyl ether extract with CHCl_3 -MeOH-*n*-propanol- H_2O (45:60:10:40, $n = 3$, $r = 3$) gave two fractions containing several polyphenolic compounds. Crystallization of one of the fractions gave dehydrodicatechin A (**3**).⁴ Purification of another fraction by column chromatography using Sephadex LH-20, in combination with preparative HPLC and/or TLC, afforded the new compounds named uncariagambiriine (**1**) and gambircatechol (**2**). Column chromatography of the ethyl acetate-soluble portion using Toyopearl HW-40 followed by YMC-gel ODS-A and preparative HPLC gave catechin-(8→6')-catechin (**4**) and catechin-(6→6')-catechin (**5**),⁵ along with (+)-catechin (**6**).⁶

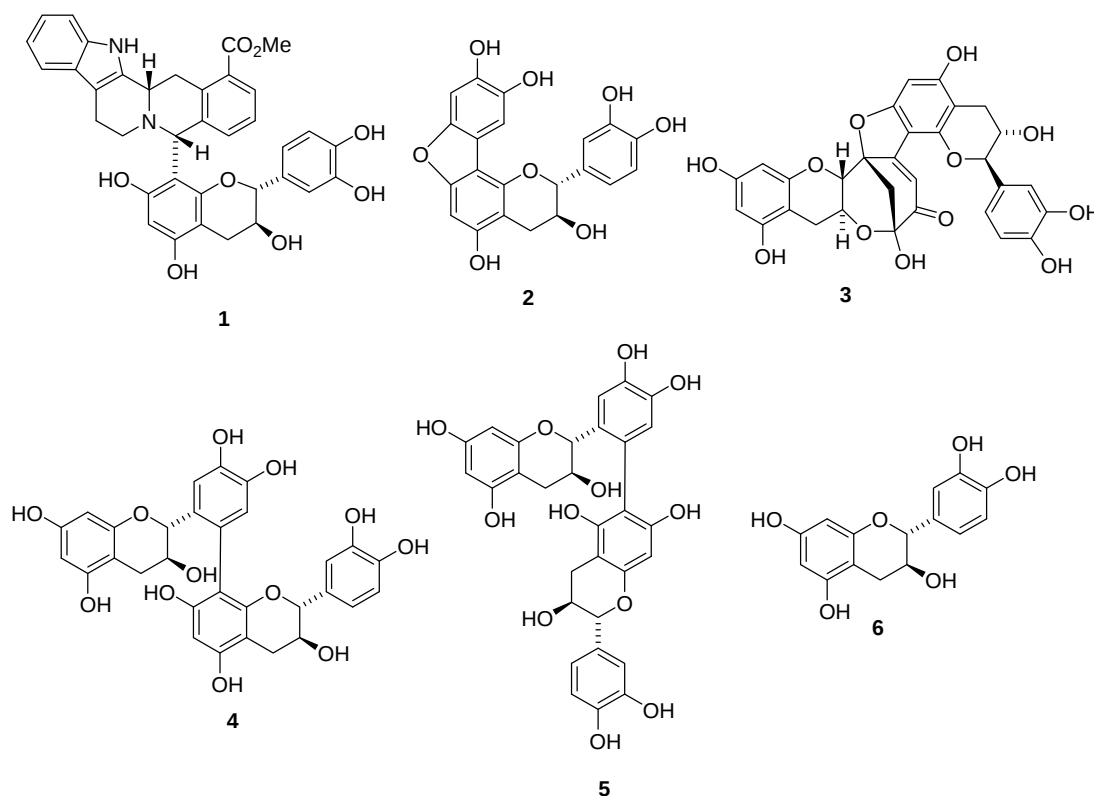


Figure 1. Structures of **1-6** isolated from *Uncaria gambir* leaves

Uncariagambiriine (**1**), $[\alpha]_{\text{D}}^{32} -32.0^\circ$ ($c = 0.1$, MeOH), was obtained as a light-brown amorphous powder. The high-resolution electrospray ionization mass spectrum (HR-ESI-MS) showed the $[\text{M}+\text{H}]^+$ ion peak at m/z 621.2225, indicating the molecular formula of $\text{C}_{36}\text{H}_{33}\text{N}_2\text{O}_8$ for this compound (calculated for $\text{C}_{36}\text{H}_{33}\text{N}_2\text{O}_8 + \text{H}$, 621.2231). The ^1H NMR spectrum (600 MHz) displayed protons that could be assigned to the A-ring [δ 5.93 (s, H-6 or H-8)], B-ring [δ 7.01 (d, $J = 2$ Hz, H-2'), 6.78 (d, $J = 8.5$ Hz, H-5'), and 6.85 (dd, $J = 2, 8.5$ Hz, H-6')], and C-ring [δ 4.62 (d, $J = 9$ Hz, H-2), 4.07 (ddd, $J = 5.5, 9, 9.5$ Hz, H-3), and 3.13 (dd, $J = 5.5, 16$ Hz, H-4a), 2.62 (dd, $J = 9.5, 16$ Hz, H-4b)] of the 6- or 8-substituted catechin residue. The presence of the catechin residue was substantiated by the following signals in the ^{13}C NMR

spectrum (150.8 MHz): δ 97.2, 100.6, 107.2 (C-6, C-8, C-10), 154.3, 156.5, and 157.1 (C-5, C-7, C-9) (A-ring carbons); δ 115.6 (2C), 120.2 (C-2', C-5', C-6'), 131.7 (C-1'), 145.6, and 145.8 (C-3', C-4') (B-ring carbons); δ 29.9 (C-4), 68.4 (C-3), and 83.7 (C-2) (C-ring carbons).

The ^1H NMR spectrum also indicated seven aromatic protons that form an ABCD system [δ 7.36 (br d, $J = 8$ Hz), 7.06 (br t, $J = 8$ Hz), 6.98 (br t, $J = 8$ Hz), and 7.43 (br d, $J = 8$ Hz) (H-9, H-10, H-11, H-12 of the alkaloid residue)] and an ABC system [δ 7.71 (br d, $J = 8$ Hz), 7.26 (br t, $J = 8$ Hz), and 7.54 (br d, $J = 8$ Hz) (H-17, H-18, H-19)]. In addition, signals were observed for two methine protons [δ 5.39 (s) (H-21) and 3.94 (br d, $J = 13$ Hz) (H-3)], three sets of methylene protons [δ 3.97 (dd, $J = 2.5, 16.5$ Hz) and 3.15 (br dd, $J = 13, 16.5$ Hz) (H-14a, H-14b); δ 3.42 (br dd, $J = 5, 12$ Hz) and 2.56 (dt, $J = 3, 12$ Hz) (H-5a, H-5b); δ 2.85 (br m) and 2.75 (br d, $J = 15$ Hz) (H-6a, H-6b)], and a methyl group [δ 3.86 (3H, s) (COOCH_3 at C-16)], aside from the signals attributable to the catechin residue. Among the aliphatic proton signals, one of the methine signals at δ 3.94 indicated vicinal couplings with the methylene protons at δ 3.97 and δ 3.15 (H-3–H-14a/14b), while the methylene signals at δ 3.42 and δ 2.56 showed couplings with the remaining methylene signals at δ 2.85 and δ 2.75 (H-5a/5b–H-6a/6b). These signals, in conjunction with the mass spectral data indicating the presence of the two nitrogen atoms, suggested the presence of a gambirtannine–yohimbine-type indole alkaloid⁷ moiety in the molecule.

The presence of the dihydrogambirtannine^{8,9} (DHGT) structure in the molecule and the assignment of the ^1H signals described above were substantiated by the ^{13}C NMR spectral data and the ^1H – ^{13}C heteronuclear single-quantum correlation (HSQC) spectroscopy/heteronuclear multiple-bond correlation (HMBC) data, as shown in Table 1 and Figure 2.

The HMBC spectrum also revealed connectivity, indicating the location of the linkage between the DHGT and the catechin moieties. The connectivity of catechin H-2 with the ^{13}C signal at δ 154.3 led to the assignment of this carbon signal to C-9 of the A-ring carbons. The C-9 signal was also correlated with H-4, and the connectivity H-4–C-5–H-6–C-7 verified the assignment of these signals. The ^{13}C signal at δ 107.2, which showed a correlation with the H-6 signal, was thus assigned to C-8, while the H-21 signal of the DHGT residue exhibited couplings with C-7, C-8, and C-9 of the catechin residue. The location of the alkaloid–catechin linkage was therefore assigned to C-21 (DHGT) and C-8 (catechin).

The circular dichroism (CD) spectrum (in MeOH)¹⁰ displayed a negative Cotton effect at around 270 nm ($[\theta]_{273} -8.6 \times 10^3$), and the sign was the same as the corresponding Cotton effect for (+)-catechin, which is indicative of the *R*-configuration at C-2 of the catechin residue.^{11,12} The Cotton effects in the short wavelength region, $[\theta]_{214} +5.5 \times 10^4$ and $[\theta]_{200} -2.4 \times 10^4$ (at the lowest wavelength measured), were attributable to the interaction between the E'-ring (DHGT) and the A-ring (catechin), which corresponded to the couplet centered at around 205 nm for the A-ring–A-ring interaction in proanthocyanidins

[exemplified by procyanidin B1 in Figure 3 (b)].^{13,14} Thus, the *R*-configuration of C-21 of the DHGT was assigned.

Table 1. NMR spectral data for **1** (acetone-*d*₆, δ in ppm)

Position	¹³ C	¹ H	HMBC
Catechin moiety			
C-2	83.7	4.62	H-3 ^a , H-4 ^a , H-2' ^a , H-6' ^a
C-3	68.4	4.07	H-2 ^a , H-4 ^a
C-4	29.9	3.13, 2.62	H-2 ^a
C-5	156.5		H-4 ^a , H-6 ^a
C-6	97.2	5.93	H-4 ^a
C-7	157.1		H-6 ^a , H-21 ^b
C-8	107.2		H-4 ^a , H-6 ^a , H-21 ^b
C-9	154.3		H-2 ^a , H-4 ^a , H-21 ^b
C-10	100.6		H-4 ^a , H-6 ^a
C-1'	131.7		H-2 ^a , H-3 ^a , H-5' ^a
C-2'	115.6	7.01	H-2 ^a , H-6' ^a
C-3'	145.6		H-2' ^a , H-5' ^a
C-4'	145.8		H-2' ^a , H-5' ^a , H-6' ^a
C-5'	115.6	6.78	H-6' ^a
C-6'	120.2	6.85	H-2 ^a , H-2' ^a
Dihydrogambirtannine (DHGT) moiety			
C-2	135.1		H-3 ^b , H-6 ^b , H-14 ^b
C-3	56.5	3.94	H-5 ^b , H-14 ^b , H-21 ^b
C-5	50.1	3.42, 2.56	H-6 ^b , H-21 ^b
C-6	22.1	2.85, 2.75	H-5 ^b
C-7	108.4		H-5 ^b , H-6 ^b , H-9 ^b
C-8	126.7		H-9 ^b , H-10 ^b , H-12 ^b
C-9	118.6	7.43	H-11 ^b , H-12 ^b
C-10	119.6	6.98	H-11 ^b , H-12 ^b
C-11	121.9	7.06	H-9 ^b , H-10 ^b
C-12	111.9	7.36	H-10 ^b , H-11 ^b
C-13	137.7		H-9 ^b
C-14	34.6	3.97, 3.15	
C-15	135.1		H-14 ^b , H-17 ^b , H-18 ^b , H-19 ^b , H-21 ^b
C-16	129.9		H-14 ^b , H-18 ^b , H-21 ^b
C-17	129.3	7.71	H-18 ^b , H-19 ^b
C-18	127.5	7.26	
C-19	133.2	7.54	H-17 ^b , H-18 ^b , H-21 ^b
C-20	139.8		H-14 ^b , H-18 ^b , H-21 ^b
C-21	61.9	5.39	H-3 ^b , H-5 ^b , H-19 ^b
C-22	168.3		H-17 ^b , H-23 ^b
C-23	52.2	3.86	

^a ¹H signals due to the catechin residue

^b Due to the DHGT residue

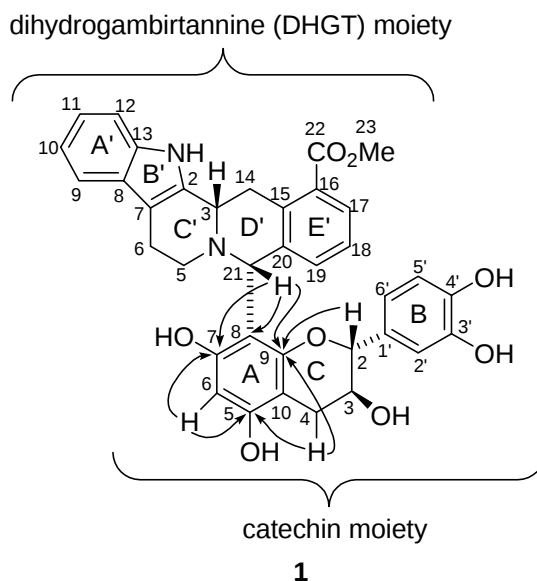


Figure 2. Important HMBC correlations observed for **1**.

The HMBC correlations (H→C) indicated the linkage C-21 (DHGT) – C-8 (catechin).

The rotating-frame Overhauser effect spectroscopy (ROESY) spectrum showed the correlation between H-21 (DHGT) and H-3 (DHGT), indicated the same orientation of these two protons. This correlation is satisfied with the *S*-configuration at C-3 and *R*-configuration at C-21 of DHGT [Figure 3 (a)].

The location of the linkage of DHGT and catechin and stereochemistry of this compound were further substantiated by the correlation between H-19 (DHGT) and H-2'/H-6'/H-3 (catechin), as well as H-21 (DHGT) and H-2'/H-6'/H-2 (catechin) in the ROESY. Based on these data, we assigned structure **1** for uncariagambiriine.

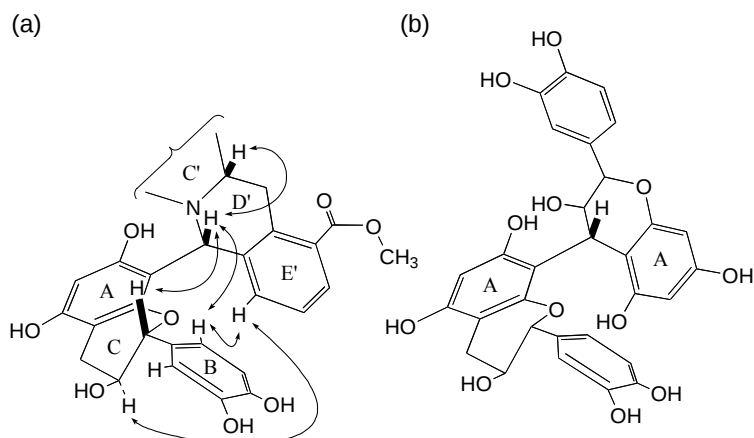


Figure 3. Important ROE correlations observed for **1**.

(a) The ROE (H↔H) correlations around the linkage C-21 (DHGT) – C-8 (catechin) and the spatial relationship of the E'-ring–A-ring, corresponding to the Cotton effect in the short wavelength region.

(b) The spatial relationship of the A-ring–A-ring in procyanidin B1.

This is the first example of an alkaloid–catechin complex among the constituents of *U. gambir*. The stereochemistry of this compound is in agreement with the biogenesis: Compound **1** could be produced from dihydrogambirtannine or related alkaloids and (+)-catechin in plants.

Gambircatechol (**2**), $[\alpha]_D^{26} -145.5^\circ$ ($c = 1.0$, MeOH), was obtained as a light-yellow amorphous powder. The molecular formula of $C_{21}H_{16}O_8$ was determined based on the $[M+H]^+$ ion peak at m/z 397.0915 in the HR-ESI-MS (calculated for $C_{21}H_{16}O_8+H$, 397.0923). The 1H NMR spectrum showed the protons that could be assigned to the A-ring [δ 6.62 (s, H-6 or H-8)], the B-ring [δ 6.98 (d, $J = 2$ Hz, H-2'), 6.82 (d, $J = 8$ Hz, H-5'), and 6.83 (dd, $J = 2, 8$ Hz, H-6')], and the C-ring [δ 4.82 (d, $J = 8$ Hz, H-2), 4.11 (ddd, $J = 5.5, 8, 8.5$ Hz, H-3), and 3.00 (dd, $J = 5.5, 16$ Hz, H-4a), 2.68 (dd, $J = 8.5, 16$ Hz, H-4b)] of the substituted catechin residue. The spectrum also displayed two further 1H singlets in the aromatic region (δ 6.95 and 7.24), suggesting the presence of a tetra-substituted benzene ring (D-ring) in the molecule. The ^{13}C NMR spectrum, however, showed six sp^2 carbon signals (δ 98.6, 107.4, 116.0, 142.3, 144.3, and 150.2), corresponding to a tri-oxygenated benzene ring, in addition to the signals assigned to the catechin residue. Combined with the molecular formula indicated by the MS data, the D-ring contains the two hydroxyl groups and is connected to the catechin residue through one ether bond and one C–C bond.

The location of the linkage on C-8 of the catechin residue was substantiated by the correlation between H-2 and C-9, and the connectivity among H-4–C-5–H-6–C-7 on the catechin residue, as indicated by the HMBC spectrum [Figure 4 (a)]. The ^{13}C signal at δ 91.1 among the catechin residue carbons, which showed a correlation with the H-6 signal, was thus assigned to C-8. One of the two aromatic singlets on the D-ring (δ 7.24), which showed a correlation with C-8, was assigned to H-12 of the D-ring. The remaining aromatic singlet was therefore assigned to H-15 of the D-ring.

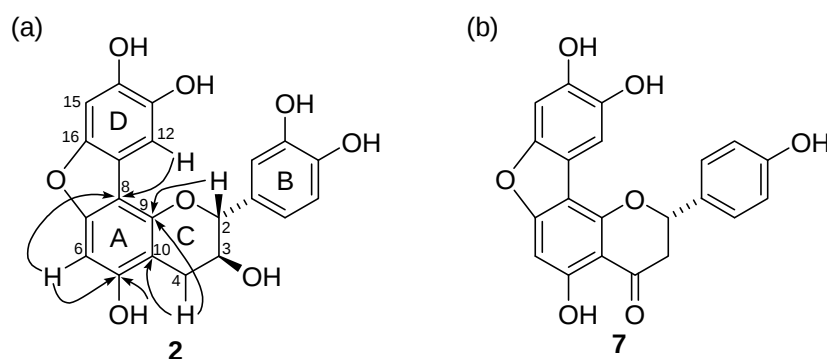


Figure 4. Important HMBC correlations observed for **2** and structure of **7**

- (a) The HMBC correlations (H→C) indicated the linkage C-8 – C-11.
 (b) Structure of anastatin B (**7**)

Table 2. NMR spectral data for **2** (acetone-*d*₆, δ in ppm)

Position	¹³ C	¹ H	HMBC
Catechin			
C-2	82.6	4.82	H-4, H-2', H-6'
C-3	67.9	4.11	H-2, H-4
C-4	29.0	2.68, 3.00	H-2
C-5	155.4		H-4, H-6
C-6	91.1	6.62	
C-7	157.1		H-6
C-8	106.1		H-6, H-12
C-9	150.2		H-2, H-4
C-10	103.8		H-3, H-4, H-6
C-1'	131.7		H-2, H-5'
C-2'	114.9	6.98	H-2, H-6'
C-3'	145.6		H-2', H-5'
C-4'	145.7		H-2', H-5', H-6'
C-5'	115.8	6.82	
C-6'	119.5	6.83	H-2, H-2'
D-ring			
C-11	116.0		H-15
C-12	107.4	7.24	
C-13	142.3		H-12, H-15
C-14	144.3		H-12, H-15
C-15	98.6	6.95	
C-16	150.2		H-15

The signal for H-6 of the catechin residue (δ 6.62) was shifted downfield relative to the corresponding proton of (+)-catechin (**6**) (δ 5.85). Analogous downfield shift was observed in anastatin B (**7**) [Figure 4 (b)],¹⁵ a flavanone having an ether linkage on the corresponding location, where H-6 of anastatin B (δ 6.59) was shifted downfield from the corresponding proton (δ 6.0) in naringenin.¹⁶ The location of the ether linkage was thus assigned between the hydroxyl group of C-7 and C-16.

The *R*-configuration at C-2 of the catechin residue was again indicated by the negative Cotton effect [θ]₂₆₁ -6.2×10^3 in the CD spectrum.¹⁷ The structure **2** was thereby assigned to gambircatechol.

Since various alkaloids and catechins/proanthocyanidins have been reported as constituents of *Uncaria* species,⁷ other constituents that are structurally related to the compounds reported in this paper will likely be found in some of those species.

ACKNOWLEDGMENTS

The Varian INOVA 600AS NMR instrument used in this study is the property of the SC-NMR laboratory at Okayama University, Japan.

REFERENCES AND NOTES

1. S. Taniguchi, K. Kuroda, K. Doi, M. Tanabe, T. Shibata, T. Yoshida, and T. Hatano, *Heterocycles*, in press.
2. S. Taniguchi, K. Kuroda, N. Yoshikado, K. Doi, M. Tanabe, T. Shibata, T. Yoshida, and T. Hatano, *Heterocycles*, 2007, **74**, 595.
3. S. Taniguchi, K. Kuroda, K. Doi, M. Tanabe, T. Shibata, T. Yoshida, and T. Hatano, *Chem. Pharm. Bull.*, 2007, **55**, 268.
4. T. C. Soest, *Liebigs Ann. Chem.*, 1971, **754**, 137.
5. S. Sang, X. Cheng, R. E. Stark, R. T. Rosen, C. S. Yang, and C.-T. Ho, *Bioorg. Med. Chem.*, 2002, **10**, 2233.
6. Yield from leaves: **1**, 2.4×10^{-4} %; **2**, 2.0×10^{-4} %; **3**, 4.7×10^{-4} %; **4**, 4.5×10^{-3} %; **5**, 5.3×10^{-3} %; **6**, 0.11 %.
7. G. Laus, *Phytother. Res.*, 2004, **18**, 259.
8. L. Merlini, R. Mondelli, G. Nasini, and M. Hesse, *Tetrahedron*, 1967, **23**, 3145.
9. E. Wenkert, K. G. Dave, C. T. Gnewuch, and P. W. Sprague, *J. Am. Chem. Soc.*, 1968, **90**, 5251.
10. The UV data for **1**: $\lambda_{\max}^{\text{MeOH}}$ 206 (4.87), 225 (4.75), 281 (4.07).
11. O. Korver and C. K. Wilkins, *Tetrahedron*, 1971, **27**, 5459.
12. T. Hatano, H. Miyatake, M. Natsume, N. Osakabe, T. Takizawa, H. Ito, and T. Yoshida, *Phytochemistry*, 2002, **59**, 749.
13. M. A. Barret, W. Klyne, P. M. Scopes, A. C. Fletcher, L. J. Porter, and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2375.
14. W. Gaffield, L. Y. Foo, and L. J. Porter, *J. Chem. Res. (S)*, 1989, 144.
15. M. Yoshikawa, F. Xu, T. Morikawa, K. Ninomiya, and H. Matsuda, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1045.
16. Y. Shirataki, I. Yokoe, M. Endo, and M. Komatsu, *Chem. Pharm. Bull.*, 1985, **33**, 444.
17. The UV data for **2**: $\lambda_{\max}^{\text{MeOH}}$ 205 (4.66), 221 (4.44), 264 (4.11), 286 (4.02).