

ARTONINS J, K, AND L, THREE NEW ISOPRENYLATED FLAVONES  
FROM THE ROOT BARK OF ARTOCARPUS HETEROPHYLLUS LAMK.<sup>1</sup>

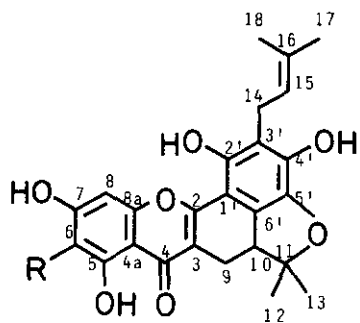
Miwa Aida, Kazuki Shinomiya, Yoshio Hano, and  
Taro Nomura\*

Faculty of Pharmaceutical Sciences, Toho University,  
2-2-1, Miyama, Funabashi, Chiba, 274, Japan

Abstract--- Three new isoprenylated flavones, artonins J (1), K (2), and L (3) were isolated from the root bark of Artocarpus heterophyllus Lamk. (Moraceae). The structures of artonins J, K, and L were shown to be 1, 2, and 3, respectively, on the basis of spectroscopic data. The model compound (5) was derived from cycloartobiloxanthone (4) by treatment with alkaline solution.

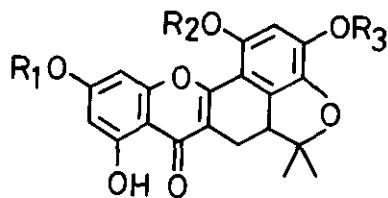
Previously we reported the structure determination of five isoprenoid-substituted phenolic compounds, artonins A (6), B, C, D, and I isolated from Indonesian moraceous plant, Artocarpus heterophyllus Lamk.<sup>2,3,4</sup> Three of them, artonins C, D, and I were regarded biogenetically natural Diels-Alder type adducts.<sup>3,4</sup> Further extensive fractionation of the extract of the root bark has now led to the isolation of new isoprenylated flavones. The root bark of A. heterophyllus, collected in Indonesia, was extracted with methanol. Three new flavones, artonins J (1), K (2), and L (3), were isolated from the extract.

Artonin J (1), yellow prisms, mp 281-282 °C,  $[\alpha]_D^{20}$  0°, C<sub>25</sub>H<sub>24</sub>O<sub>7</sub>, gave a



1 : R = H

7 : R =



2 : R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = R<sub>3</sub> = H

3 : R<sub>1</sub> = R<sub>2</sub> = CH<sub>3</sub>, R<sub>3</sub> = H

5 : R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = H

3a (=5a) : R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = CH<sub>3</sub>

3' : R<sub>1</sub> = R<sub>3</sub> = CH<sub>3</sub>, R<sub>2</sub> = H

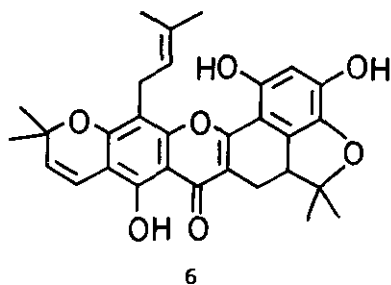
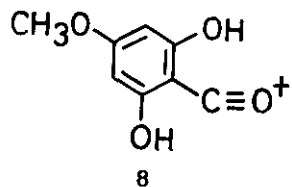
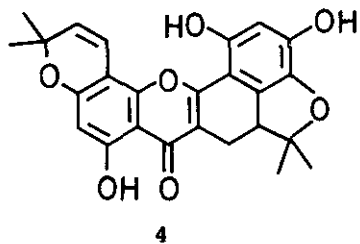


Table 1  $^{13}\text{C}$  nmr chemical shifts of 1, 3, 5, 6, and 7

	1 <sup>a</sup>	3 <sup>a</sup>	5 <sup>a</sup>	6 <sup>b</sup>	7 <sup>b</sup>
C-2	161.2	161.1	161.3	161.6	161.2
C-3	111.3	112.0	111.9	112.6	112.7
C-4	179.6	179.7	179.6	181.7	181.1
C-4a	103.2	104.5	103.2	105.0	104.7
C-5	160.4	160.2	160.4	154.5	160.1
C-6	98.7	97.7	98.6	105.9	112.5
C-7	163.4	164.6	163.5	157.0	161.9
C-8	94.3	92.0	93.6	108.3	94.3
C-8a	156.2	156.2	156.4	155.3	155.1
C-1'	104.2	104.2	104.4	105.3	104.2
C-2'	146.9	152.7	150.6	151.5	148.1
C-3'	118.6	101.4	103.2	105.4	118.4
C-4'	143.8	146.2	146.2	147.0	144.7
C-5'	137.1	137.0	136.3	138.0	138.3
C-6'	128.2	133.4	132.2	133.7	129.0
C-9	19.5	19.3	19.4	20.5	20.7
C-10	46.0	46.0	46.2	47.6	47.2
C-11	92.7	92.6	92.3	93.7	94.0
C-12	27.8	27.8	27.8	28.4	28.3
C-13	22.4	22.4	22.4	22.9	22.9
C-14	22.5			116.4	23.2
C-15	123.2			128.9	123.9
C-16	129.9			78.4	131.3
C-17	25.4			28.4	25.8
C-18	17.7			28.4	17.9
C-19				22.0	22.1
C-20				123.7	123.6
C-21				131.7	131.5
C-22				25.9	25.8
C-23				18.2	17.9
-OCH <sub>3</sub>		55.8			
		56.6			

solvent; a: DMSO-d<sub>6</sub> b: acetone-d<sub>6</sub>Table 2  $^1\text{H}$  nmr chemical shifts (ppm)  
of C-6-H, C-8-H, and C-3'-H

	C-6-H	C-8-H	C-3'-H		C-6-H	C-8-H	C-3'-H
2	6.32	6.68	6.35	3	6.30	6.51	6.46
5a	6.32	6.53	6.61	3a	6.32	6.53	6.61
Δ	0.00	+0.15	-0.26	Δ	-0.02	-0.02	-0.15

measured in acetone-d<sub>6</sub>

greenish brown color with methanolic ferric chloride, and exhibited positive reaction to magnesium-hydrochloric acid. The uv spectrum was similar to those of cycloartobiloxanthone (4)<sup>5</sup>, artonins A (6)<sup>2</sup> and G (7).<sup>6</sup> The <sup>1</sup>H nmr spectrum of 1 showed the signals of the following protons: 1) protons in a 3,3-dimethylallyl (prenyl) group,  $\delta$  1.65, 1.78 (each 3H, br s), 3.40 (2H, br d,  $\underline{J}$  = 7 Hz), 5.28 (1H, m); 2) two aromatic protons,  $\delta$  6.26, 6.70 (each 1H, d,  $\underline{J}$  = 2 Hz); 3) a proton in a hydrogen-bonded hydroxyl group,  $\delta$  13.27 (1H, s); 4) protons in two methyl groups,  $\delta$  1.31, 1.65 (each 3H, s); and 5) ABX type protons,  $\delta$  2.35 (1H, t,  $\underline{J}$  = 15 Hz), 3.20 (1H, dd,  $\underline{J}$  = 15 and 7 Hz), 3.40 (1H, dd,  $\underline{J}$  = 15 and 7 Hz). In the spectrum, the chemical shift values of the two methyl groups and ABX type protons were similar to those of the relevant protons of 4, 6, and 7. The above results suggest that 1 is a flavone derivative having a skeletal structure analogous with 4, 6, and 7. The <sup>13</sup>C nmr spectrum of 1 was analysed by the gated decoupling with NOE as well as comparison with those of 6, 7 and the model compound (5) derived from 4 by treatment with an alkaline solution.<sup>7</sup> In the spectrum of 1, the chemical shifts of all the carbon atoms were similar to those of the relevant carbon atoms of 7<sup>6</sup> with the exception of the shift of the C-6 (Table 1). On the other hand, the chemical shifts of all the carbon atoms in the skeletal structure of 1 were similar to those of the relevant carbon atoms of 5 with the exception of the shift of the C-3' (Table 1). From the above results, formula (1) was proposed for the structure of artonin J.

Artonin K (2), yellow prisms, mp 312-314 °C,  $[\alpha]_D^{20}$  0°, C<sub>21</sub>H<sub>18</sub>O<sub>7</sub>, gave a greenish brown color with methanolic ferric chloride, and exhibited positive reaction to magnesium-hydrochloric acid. The uv spectrum was similar to that of 1. The <sup>1</sup>H nmr spectrum of 2 showed the signals for the following protons: 1) three aromatic protons,  $\delta$  6.35 (1H, s), 6.32, 6.68 (each 1H, d,  $\underline{J}$  = 2 Hz); 2) a proton in a hydrogen-bonded hydroxyl group,  $\delta$  13.28 (1H, s); 3) protons in two methyl groups,  $\delta$  1.33, 1.66 (each 3H,

s); 4) ABX type protons,  $\delta$  2.37 (1H, t,  $J = 15$  Hz), 3.24 (1H, dd,  $J = 15$  and 7 Hz), 3.42 (1H, dd,  $J = 15$  and 7 Hz); and 5) protons in a methoxyl group,  $\delta$  3.90 (3H, s). The chemical shifts and coupling patterns of 2 were similar to those of the relevant signals of 1 and 5. These results suggest that 2 is a monomethyl ether of 5. The location of the methoxyl group was supported from the following results. The mass spectrum of 2 showed the fragment ion at  $m/z$  167 (8) and the uv spectrum showed no bathochromic shift of band II (263 nm) upon addition of sodium acetate.<sup>10</sup> From the above results, formula(2) was proposed for the structure of artonin K.

Artonin L (3), yellow prisms, mp 249-250 °C,  $[\alpha]_D^{20} 0^\circ$ ,  $C_{22}H_{20}O_7$ , showed the similar color reactions with those of 2. The uv spectrum of 3 was similar to those of 1 and 2. The  $^1H$  nmr spectrum of 3 showed the signals for the following protons: 1) three aromatic protons,  $\delta$  6.46 (1H, s), 6.30, 6.51 (each 1H, d,  $J = 2$  Hz); 2) a proton in a hydrogen-bonded hydroxyl group,  $\delta$  13.28 (1H, s); 3) protons in two methyl groups,  $\delta$  1.33, 1.66 (each 3H, s); 4) ABX type protons,  $\delta$  2.35 (1H, t,  $J = 15$  Hz), 3.21 (1H, dd,  $J = 15$  and 7 Hz), 3.42 (1H, dd,  $J = 15$  and 7 Hz); and 5) protons in two methoxyl groups,  $\delta$  3.88, 3.91 (each 3H, s). The mass spectrum of 3 showed the fragment ion at  $m/z$  167 (8) and uv spectrum showed no bathochromic shift of band II (263 nm) upon addition of sodium acetate.<sup>10</sup> From the above results, artonin L is a monomethyl ether of 2 and two possible structures (3 and 3') were suggested. Discrimination between the structures was carried out as follows. The  $^{13}C$  nmr spectrum of 3 was analysed by gated decoupling with NOE as well as by comparison with the spectrum of 5. The chemical shifts of all the carbons were similar to those of the relevant carbons of 5 with the exception of the shifts of the five carbons (C-6, 7, 8, 2', and 3') (Table 1). Furthermore comparison of the  $^1H$  nmr spectra of 2 and 5a indicates that methylation of the hydroxyl group at C-2' position caused higher field shift ( $\Delta +0.15$  ppm) of the proton at

the C-8 position (Table 2). On the other hand, comparison of the  $^1\text{H}$  nmr spectra of 3 and its monomethyl ether (3a = 5a) indicates that no high field shift ( $\Delta -0.02$  ppm) of the proton at the C-8 position was observed by methylation of the hydroxyl group in the B ring (Table 2). From the above results, formula (3) was proposed for the structure of artonin L.

#### EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, sh = shoulder. The general procedures followed and the instruments used are described in our previous paper.

Plant material: Root bark of *A. heterophyllum* was collected in the Botanical Garden of Bogor, Indonesia, in March 1988, and was identified by the members of Botanical Garden of Bogor.

#### Isolation of Artonin J (1), K (2), and L (3)

The dried root bark of *A. heterophyllum* (23 Kg) was extracted with methanol (200 l x 3) at room temperature for 3 days. Evaporation of the methanol under reduced pressure yielded 1.4 Kg of residue. The residue (1 Kg) was extracted with benzene (3 l) and acetone (3 l) at room temperature. The benzene and acetone solutions were evaporated to give 150 g and 370 g of residues, respectively. The benzene extract (150 g) was chromatographed on silica gel (1200 g) using *n*-hexane, *n*-hexane-benzene (1 : 1), benzene, benzene-ethyl acetate (99 : 1, 97 : 3, 9 : 1, and 4 : 1), and then acetone as an eluent (frs. 1-200), each fraction (500 ml) monitored by tlc. The fraction eluted with benzene-ethyl acetate (97 : 3, 6.0 g) was rechromatographed on silica gel (200 g) with *n*-hexane containing increasing amount of acetone as an eluent (frs. 1'-30', eluted volume 300 ml each). The fraction eluted with *n*-hexane-acetone (3 : 1, frs. 22'-29', 0.7 g) was fractionated by preparative tlc [silica gel, chloroform-acetone (10 : 1), *n*-hexane-ethyl acetate (1 : 1)] to give artonin J (1, 9 mg). The fraction eluted with *n*-hexane-acetone (3 : 1, frs. 19'-21', 0.2 g) was fractionated by preparative tlc [*n*-hexane-ethyl acetate (2 : 1), chloroform-acetone (20 : 1)] to give artonin L (3, 12 mg). The fraction eluted with benzene-ethyl acetate (9 : 1, frs. 163-192, 5.1 g) was rechromatographed on silica gel (200 g) with *n*-hexane containing increasing amount of acetone as an eluent (frs. 1"-30"). The fraction eluted with *n*-hexane-acetone (7 : 3, frs. 20"-21", 0.1 g) was fractionated by preparative tlc [chloroform-acetone (5 : 1), *n*-hexane-acetone (3 : 2), benzene-ethyl acetate (1 : 1)] to give artonin K (2, 1 mg).

#### Artonin J (1)

Compound (1) was recrystallized from methanol to give yellow prisms, mp 281-282 °C.

$\text{FeCl}_3$  test; positive (greenish brown).  $\text{Mg-HCl}$  test; positive (red).  $[\alpha]_{\text{D}}^{20}$  0° ( $c = 0.18$ , MeOH). EI-MS:  $m/z$  (rel. int.) 436 ( $\text{M}^+$ , 100%), 393 (66), 380 (83), 337 (44), 309 (16),

255 (9), 190 (5), 153 (9). HR-Ms:  $m/z$  436.1540 ( $M^+$ ,  $C_{25}H_{24}O_7$  requires 436.1522),  $m/z$  393.0987 ( $C_{22}H_{17}O_7$  requires 393.0974),  $m/z$  380.0889 ( $C_{21}H_{16}O_7$  requires 380.0896). Ir  $\nu_{\max}^{KBr} cm^{-1}$ : 3570, 3400 (br), 1650 1610, 1550, 1500, 1490, 1460 (sh), 1450, 1430. Uv  $\lambda_{\max}^{EtOH} nm$  (log  $\epsilon$ ): 380 (4.01), 320 (infl 3.68), 264 (4.03), 232 (4.05), 210 (4.35). Uv  $\lambda_{\max}^{EtOH+AlCl_3}$ : 408 (4.02), 350 (infl 3.94), 310 (sh 3.62), 278 (4.02), 232 (sh 4.07), 210 (4.37).

#### Artonin K (2)

Compound (2) was recrystallized from acetone to give yellow prisms, mp 312-314 °C.  $FeCl_3$  test; positive (greenish brown). Mg-HCl test; positive (red).  $[\alpha]_D^{20} 0^\circ$  ( $c = 0.10$ , MeOH). EI-Ms:  $m/z$  (rel. int.) 382 ( $M^+$ , 75), 367 (14), 339 (100), 311 (15), 167 (4). HR-Ms:  $m/z$  382.1039 ( $M^+$ ,  $C_{21}H_{18}O_7$  requires 382.1053),  $m/z$  367.0814 ( $C_{20}H_{15}O_7$  requires 367.0818),  $m/z$  339.0490 ( $C_{18}H_{11}O_7$  requires 339.0505), Ir  $\nu_{\max}^{KBr} cm^{-1}$ : 3450 (br), 3100 (br), 1660 (sh), 1650, 1610, 1580, 1550, 1510, 1490, 1450, 1430. Uv  $\lambda_{\max}^{EtOH} nm$  (log  $\epsilon$ ): 382 (4.02), 320 (sh 3.55), 263 (4.16), 232 (4.05), 207 (4.36). Uv  $\lambda_{\max}^{EtOH+AlCl_3}$ : 416 (4.08), 343 (sh 3.85), 305 (sh 3.78), 277 (4.14), 232 (sh 4.06), 203 (4.40). Uv  $\lambda_{\max}^{EtOH+AcONa}$ : 426 (infl 3.73), 384 (4.02), 320 (sh 3.50), 263 (4.19), 232 (sh 4.06), 205 (4.46).

#### Artonin L (3)

Compound (3) was recrystallized from methanol to give yellow prisms, mp 249-250 °C.  $FeCl_3$  test; positive (greenish brown). Mg-HCl test; positive (red).  $[\alpha]_D^{20} 0^\circ$  ( $c = 0.24$ , MeOH). EI-Ms:  $m/z$  (rel. int.) 396 ( $M^+$ , 79), 381 (24), 353 (100), 325 (18), 311 (6), 167 (20). HR-Ms:  $m/z$  396.1259 ( $M^+$ ,  $C_{22}H_{20}O_7$  requires 396.1209),  $m/z$  381.0966 ( $C_{21}H_{17}O_7$  requires 381.0974),  $m/z$  353.0670 ( $C_{19}H_{13}O_7$  requires 353.0661). Ir  $\nu_{\max}^{KBr} cm^{-1}$ : 3450 (br), 3100 (br), 1660 (sh), 1650, 1600, 1580, 1550, 1540, 1510 (sh), 1500 (sh), 1490, 1470, 1450, 1430. Uv  $\lambda_{\max}^{EtOH} nm$  (log  $\epsilon$ ): 381 (4.09), 320 (sh 3.55), 263 (4.16), 232 (sh 3.77), 210 (4.30). Uv  $\lambda_{\max}^{EtOH+AlCl_3}$ : 417 (4.15), 341 (3.76), 305 (sh 3.46), 278 (4.16), 240 (sh 3.68), 212 (4.27). Uv  $\lambda_{\max}^{EtOH+AcONa}$ : 450 (infl 3.55), 383 (4.09), 320 (sh 3.50), 263 (4.19), 232 (sh 3.85), 210 (4.36).

#### Artonin L Monomethyl Ether (3a)

A mixture of 3 (2 mg,  $5 \times 10^{-3}$  mmol),  $Me_2SO_4$  (0.05 ml, 0.5 mmol), and  $K_2CO_3$  (1 g,  $7 \times 10^{-3}$  mmol) in acetone (5 ml) was kept at room temperature for 2 h and treated as usual. The product was purified by preparative tlc [ $n$ -hexane-acetone (3 : 1)] to give artonin L monomethyl ether (3a, 1 mg, 5% yield). Compound (3a) was recrystallized from benzene to give yellow needles, mp 249-250 °C.  $FeCl_3$  test; positive (greenish brown). EI-Ms:  $m/z$  (rel. int.) 410 ( $M^+$ , 100), 395 (39), 367 (55).  $^1H$  Nmr (acetone- $d_6$ ):  $\delta$  1.35, 1.68 (each 3H, s), 2.37 (1H, t,  $J = 15$  Hz), 3.24 (1H, dd,  $J = 7$  and 15 Hz), 3.44 (1H, dd,  $J = 7$  and 15 Hz), 3.92 (3H, s), 3.96 (6H, s), 6.32, 6.54 (each 1H, d,  $J = 2$  Hz), 6.61 (1H, s).

Formation of Compound 5 from Cycloartobiloxanthone (4)

Cycloartobiloxanthone (4, 30 mg,  $7 \times 10^{-2}$  mmol) was dissolved in 25% aqueous NaOH solution (15 ml, 0.09 mol) and refluxed for 1 h. The reaction mixture was acidified by 6N-HCl solution and extracted with ether. The ether extract was treated as usual and purified by preparative tlc [ $n$ -hexane-acetone (4 : 3)] to give 5 (15 mg, 5% yield). Compound (5) was recrystallized from acetone to give yellow needles, mp 303-304°C. FeCl<sub>3</sub> test; positive (green). Mg-HCl test; positive (red). EI-MS:  $m/z$  (rel. int.) 368 (M<sup>+</sup>, 67), 353 (13), 310 (5), 297 (18), 255 (6), 184 (5), 153 (7). HR-MS:  $m/z$  368.0891 (M<sup>+</sup>, C<sub>20</sub>H<sub>16</sub>O<sub>7</sub> requires 368.0897). Ir  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3450 (br), 3200 (br), 1690, 1660 (sh), 1650, 1640, 1630, 1610, 1570, 1560, 1530, 1510, 1470, 1460, 1450. Uv  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 382 (4.06), 320 (infl 3.74), 264 (4.16), 232 (sh 3.96), 207 (4.37). Uv  $\lambda_{\max}^{\text{EtOH+AlCl}_3}$ : 415 (4.13), 343 (3.88), 305 (sh 3.74), 277 (4.16), 230 (sh 4.04), 204 (4.40). <sup>1</sup>H Nmr (acetone-d<sub>6</sub>):  $\delta$  1.32, 1.65 (each 3H, s), 2.35 (1H, t,  $J$  = 15 Hz), 3.20 (1H, dd,  $J$  = 7 and 15 Hz), 3.40 (1H, dd,  $J$  = 7 and 15 Hz), 6.24 (1H, d,  $J$  = 2 Hz), 6.34 (1H, s), 6.51 (1H, d,  $J$  = 2 Hz), 13.30 (1H, s).

Compound 5 Trimethyl Ether (5a = 3a)

A mixture of 5 (7 mg, 0.02 mmol), Me<sub>2</sub>SO<sub>4</sub> (0.10 ml, 1 mmol), and K<sub>2</sub>CO<sub>3</sub> (2 g,  $1.4 \times 10^{-2}$  mmol) in acetone (10 ml) was kept at room temperature for 7 h and treated as usual. The product was purified by preparative tlc [ $n$ -hexane-acetone (3 : 1)] to give 5a (3 mg). 5a was identified with 3a derived from 3 by comparison of physical and spectral data.

## ACKNOWLEDGEMENT

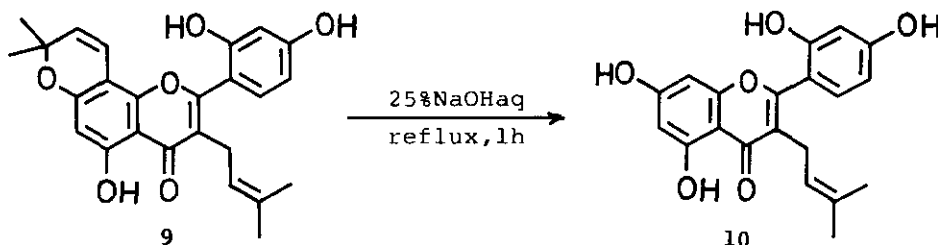
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## REFERENCES AND NOTES

1. Part 16 in the series "Constituents of the Moraceae Plants". For Part 14 see Y. Hano, T. Okamoto, and T. Nomura, *Heterocycles*, 1991, 32, 1357. For Part 15 see Y. Hano, M. Aida, T. Nomura, and S. Ueda, *J. Chem. Soc., Chem. Commun.*, 1992, 1177.
2. Y. Hano, M. Aida, M. Shiina, T. Nomura, T. Kawai, H. Ohe, and K. Kagei, *Heterocycles*, 1989, 29, 1477.
3. Y. Hano, M. Aida, and T. Nomura, *J. Nat. Prod.*, 1990, 53, 391.



4. Y. Hano, M. Aida, T. Nomura, and S. Ueda, J. Chem. Soc., Chem. Commun., 1992, 1177.
5. M. U. S. Sultanbawa and S. Surendrakumar, Phytochemistry, 1989, **28**, 599.
6. Y. Hano, R. Inami, and T. Nomura, Heterocycles, 1990, **31**, 2173.
7. The similar result was obtained in the case of morusin (9) as follows. Morusin (9, 30 mg,  $7 \times 10^{-2}$  mmol) was dissolved in 25% NaOH aqueous solution (15 ml, 0.09 mol) and refluxed for 1 h. The reaction mixture was treated as usual and purified by preparative tlc to give 10 (15 mg, 59% yield). The compound(10) was identified with albanin A by comparing the spectroscopic data of 10 with the published data.<sup>8,9</sup>



8. M. Takasugi, S. Ishikawa, T. Masamune, A. Shirata, and K. Takahashi, 22nd Symposium on the Chemistry of Natural Products, Symposium Paper, p. 275, Oct. 1979, Fukuoka, Japan.
9. F. Ferrari, I. Messana, and M. do Carmo, and M. de Araujo, Planta Med. 1989, **55**, 70.
10. T. J. Mabry, K. R. Markham, and M. B. Thomas, "The Systematic Identification of Flavonoids", Springer-Verlag, New York, 1970.

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