COMPONENTS OF THE BARK OF ARTOCARPUS RIGIDA BL. 1. STRUCTURES OF TWO NEW ISOPRENYLATED FLAVONES, ARTONINS G AND H[1]

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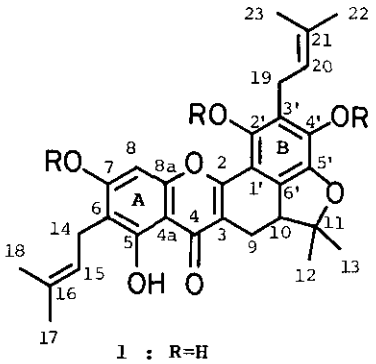
Abstract ——From the bark of Artocarpus rigida Bl. (Moraceae), collected in Indonesia, two new isoprenylated flavones, artonins G (**1**) and H (**2**) were isolated along with three known isoprenylated flavones, artonin E (**3**), cycloartobiloxanthone (**4**), and artobiloxanthone (**5**). The structures of artonins G and H were shown to be **1** and **2**, respectively, on the basis of spectroscopic data.

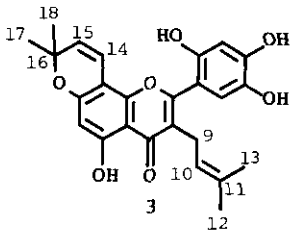
Previously, we reported the structure determination of isoprenoid-substituted phenolic compounds isolated from Indonesian moraceous plants, such as Artocarpus heterophyllus Lamk.,[2,3] Artocarpus communis Forst.,[4] and Antiaris toxicaria Lesch.[5,6] In the course of our studies on the constituents of the moraceous plants, we examined the constituents of Artocarpus rigida Bl. collected in Bogor, Indonesia. This paper deals with the characterization of two new isoprenoid-substituted flavones, artonins G (**1**) and H (**2**) as well as the isolation of three known compounds, artonin E (**3**),[4] cycloartobiloxanthone (**4**),[7] and artobiloxanthone (**5**).[7]

Artonin G (**1**), yellow needles, mp 198-203 °C, $[\alpha]_D^{22}$ 0°, $C_{30}H_{32}O_7$, gave a greenish brown color with methanolic ferric chloride, and exhibited positive reaction to magnesium-hydrochloric acid and Gibbs tests. The uv spectrum was similar to those of cycloartobiloxanthone (**4**)[4a,7] and artonin A (**6**).[2] Treatment of **1** with dimethyl sulfate gave the trimethyl ether (**1a**), of which ^{1}H nmr spectrum showed a signal of hydrogen-bonded hydroxyl group at δ 13.35. The ^{1}H nmr spectrum of **1** showed the signals of the following protons: 1) protons in two 3,3-dimethylallyl (prenyl) groups, δ 1.64, 1.77 (each 6H, br s), 3.34, 3.39 (each 2H, br d, J=7 Hz), 5.27



1 : R=H

1a : R=CH_3



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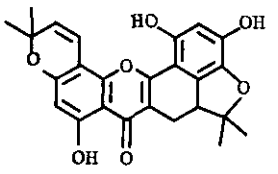

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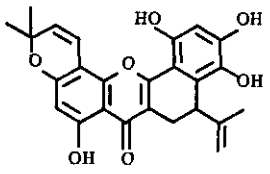

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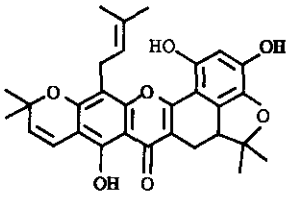

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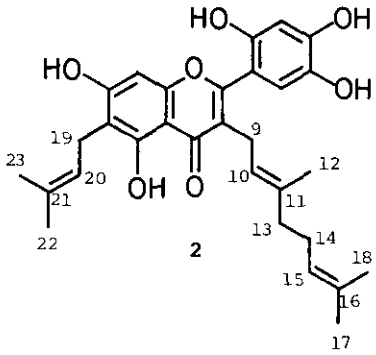

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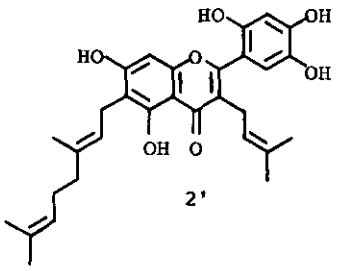
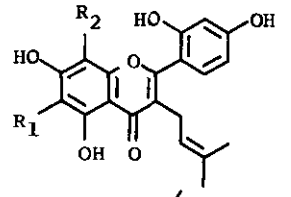


2'



7 : R_1=  , R_2=H

8 : R_1=H, R_2= 

Table 1 ^{13}C Nmr chemical shifts (ppm) of **1**, **2**, **3**, **4**, **6**, and **7**

	1	**4**[4a]	**6**[2]	**7**[5]	**2**	**3**[4a]		**1**	**4**[4a]	**6**[2]	**7**[5]	**2**	**3**[4a]
C-2	161.2	161.5	161.6	162.0	161.8	162.2	C-9	20.7	20.3	20.5	24.6	24.4	24.7
C-3	112.7	112.7	112.6	121.5	121.5	121.7	C-10	47.2	47.5	47.6	122.7	122.6	122.5
C-4	181.1	181.4	181.7	183.0	183.0	183.3	C-11	94.0	93.7	93.7	132.0	135.8	132.3
C-4a	104.7	104.9	105.0	105.0	105.1	105.6	C-12	28.3	28.4	28.4	25.8	16.0	25.8
C-5	160.1	162.6	154.5	160.0	160.1	162.8	C-13	22.9	22.8	22.9	17.9	40.4	17.7
C-6	112.5	99.9	105.9	111.8	111.9	99.7	C-14	22.1	116.0	116.4	22.0	27.3	115.5
C-7	161.9	159.5	157.0	162.3	162.3	160.0	C-15	123.6[a]	127.8	128.9	123.3	125.1[a]	128.0
C-8	94.3	101.9	108.3	93.5	93.5	101.6	C-16	131.5[b]	78.7	78.4	131.4	131.5[b]	78.8
C-8a	155.1	152.0	155.3	157.0	157.0	153.3	C-17	25.8	28.3	28.4	25.9	25.7[c]	28.3
C-1'	104.2	105.4	105.3	113.1	111.7	111.6	C-18	17.9	28.3	28.4	17.6	17.9[c]	28.3
C-2'	148.1	151.5	151.5	157.1	149.5	149.8	C-19	23.2		22.0		22.0	
C-3'	118.4	105.5	105.4	103.9	104.6	104.8	C-20	123.9[a]		123.7		123.4[a]	
C-4'	144.7	147.0	147.0	161.3	149.1	149.5	C-21	131.3[b]		131.7		131.4[b]	
C-5'	138.3	137.9	138.0	108.1	139.0	139.1	C-22	25.8		25.9		25.8[c]	
C-6'	129.0	133.6	133.7	132.2	117.1	117.1	C-23	17.9		18.2		17.7[c]	

Solvent: acetone-d_6

a-c: Assignments may be interchangeable in each column.

(2H, m); 2) an aromatic proton, δ 6.71 (1H, s); 3) a proton in a hydrogen-bonded hydroxyl group, δ 13.51 (1H, s); 4) protons in two methyl groups, δ 1.30, 1.63 (each 3H, s); and 5) ABX type protons, δ 2.33 (1H, t, J=15 Hz), 3.19 (1H, dd, J=7 and 15 Hz), 3.37 (1H, dd, J=7 and 15 Hz). In the spectrum, the chemical shift values of the two methyl groups (δ 1.30, 1.63) and of ABX type protons were similar to those of the relevant protons of artonin A (**6**)[2] and cycloartobiloxanthone (**4**).[7] The above results suggest that **1** is a flavone derivative having a skeletal structure analogous with **4** and **6**.[2,7] The ^{13}C nmr spectrum of **1** was analysed by the gated decoupling with NOE and long-range selective ^{1}H decoupling (LSPD) experiments as well as by comparison of the spectrum with the spectra of **4** and **6**. In the spectrum of **1**, the chemical shift values of all the carbon atoms were similar to those of the relevant carbon atoms of **4** and **6** with the exception of the values of the A ring carbon atoms and of the C-3' carbon atom in **1** (Table 1). These results suggest that one of the prenyl groups is located at the C-3' position and the other at the C-6 or C-8 position. The location of the prenyl group in the A ring was confirmed from the fact that the chemical shift values of the A ring carbon atoms observed in the ^{13}C nmr spectrum of **1** were similar to those of the relevant atoms of cudraflavone C (**7**)[8] (Table 1). Furthermore the following LSPD experiment was carried out. When the signal at δ 6.71 was irradiated, the multiplet signal at δ 161.9 (C-7), the doublet signal at δ 155.1 (C-8a, $^{2}J_{8H-C8a}$= 4.4 Hz), and the triplet signal at δ 104.7 (C-4a, $^{3}J_{8H-C4a}$=$^{3}J_{5OH-C4a}$= 4.4 Hz) changed to triplet ($^{3}J_{14H-C7}$=4.4 Hz), singlet, and doublet ($^{3}J_{5OH-C4a}$=4.4 Hz), respectively. When the signal at δ 13.51 was irradiated, the double triplet signal at δ 160.1 (C-5, $^{2}J_{5OH-C5}$=5.1 Hz, $^{3}J_{14H-C5}$=4.4 Hz) and the triplet signal at δ 104.7 (C-4a, $^{3}J_{5OH-C4a}$=$^{3}J_{8H-C4a}$=4.4 Hz) changed to triplet ($^{3}J_{14H-C5}$=4.4 Hz) and doublet ($^{3}J_{8H-C4a}$=4.4 Hz), respectively. These results support the conclusion that the signal at δ 6.71 is assigned to the proton signal at the C-8 position. From the above results, formula **1** was proposed for the structure of artonin G.

Artonin H (**2**), an amorphous powder, $C_{30}H_{34}O_7$, gave a brown color with methanolic ferric chloride, and exhibited positive reaction to magnesium-hydrochloric acid and Gibbs tests. The uv spectrum was similar to the spectra of artonin E (**3**),[4] cudraflavone C (**7**),[8] and kuwanon C (**8**).[9] These results suggest that **2** is a flavone derivative having an isoprenoid substituent at the C-3 position.[10] The ^{1}H nmr spectrum of **2** showed the signals of the following protons: 1) protons in a

prenyl group as well as a geranyl or neryl group, δ 1.46, 1.54, 1.60, 1.64, 1.77 (each 3H, br s), 1.88, 1.99 (each 2H, m), 3.15, 3.36 (each 2H, br d, J=7 Hz), 5.04, 5.15, 5.28 (each 1H, br t, J=7 Hz); 2) three aromatic protons, δ 6.38, 6.58, 6.81 (each 1H, s); and 3) a proton in a hydrogen-bonded hydroxyl group, δ 13.45 (1H, s). The ^{13}C nmr spectrum of **2** was analysed by the gated decoupling with NOE experiment as well as by comparison of the spectrum with the spectra of **3**[4] and **7**.[8] In the spectrum of **2**, the chemical shift values of the A and C ring carbon atoms were similar to those of the relevant atoms of **7**, while the chemical shift values of the B ring carbon atoms were similar to those of the relevant atoms of **3** (Table 1). The C_{10} side chain in the structure of **2** was confirmed to be a geranyl group by comparing the chemical shifts of the ten carbon atoms (C-9 - C-18) of **2** with those of the relevant atoms of geraniol and nerol.[11] From the above results, two possible structures (**2** and **2'**) were suggested. Discrimination between the structures was carried out on following results. The assignments of methylene proton signals of the prenyl group and the C-1 methylene proton signals of the geranyl group were confirmed by the decoupling experiment. When the proton signal at δ 3.36 was irradiated, the broad triplet signal at δ 5.28 changed to broad singlet as well as the broad singlet methyl proton signals at δ 1.64 and 1.77 to two sharp doublets (J=0.6 Hz). When the signal at δ 3.15 was irradiated, the broad triplet signal at δ 5.15 changed to broad singlet as well as the broad singlet methyl proton signal at δ 1.46 to sharp doublet (J=0.6 Hz). In latter experiment other methyl signals showed no change. These results support the conclusion that the signal at δ 3.36 is assigned to the methylene proton signal of the prenyl group while the signal at δ 3.15 to the C-1 methylene proton signal of the geranyl group. The location of the geranyl group was confirmed by the LSPD experiment. When the methylene proton signal at δ 3.15 was weakly irradiated, the carbonyl carbon atom signal at δ 183.0 (C-4) changed from triplet ($^3J_{9H-C4}$ = 4.4 Hz) to singlet. This result confirmed the geranyl group to be located at the C-3 position. From the above results, formula **2** was proposed for the structure of artonin H. The four flavone derivatives having a geranyl group at the C-3 position have been isolated from Morus rubra (Moraceae),[12] and artonin H is the first example of the compound of similar structure isolated from Artocarpus sp.

EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, sh = shoulder, infl = inflection. The general procedures and the instruments used are described in our previous papers.[3,13]

Isolation of Artonins G (1) and H (2) from the Bark of A. rigida Bl.

The dried bark of A. rigida Bl. (1 Kg) collected in Botanical Garden of Bogor, Indonesia in February 1988, and identified by members of the Botanical Garden, was extracted with n-hexane (3 l) at room temperature for 3 days, and such was repeated two more times. The residue was extracted, successively, with benzene (3 l x 3), acetone (3 l x 3), and methanol (3 l x 3). Evaporation of the n-hexane, benzene, acetone, and methanol solutions to dryness yielded 23 g, 10 g, 29 g, and 18 g of the residue, respectively. The acetone extract (29 g) was chromatographed on silica gel (250 g) with benzene : acetone = 10 : 1 (fraction 1), benzene : acetone = 6 : 1 (fr. 2), benzene : acetone = 2 : 1 (fr. 3), benzene : acetone = 1 : 1 (fr. 4), acetone (fr. 5), each fraction (eluted volume of 1 l) being monitored by tlc (silica gel). The fraction 2 (2.0 g) was rechromatographed on silica gel (200 g) with n-hexane : ethyl acetate = 6 : 1 (frs. 1'-6'), n-hexane : ethyl acetate = 3 : 1 (frs. 7'-11'), n-hexane : ethyl acetate = 1 : 1 (frs. 12'-18'), and ethyl acetate (frs. 19'-20'), each fraction (eluted volume of 300 ml) being monitored by tlc. The fraction 9' (0.2 g) was purified by flash chromatography (*silica gel, solvent, n-hexane* : ethyl acetate = 1 : 2) followed by preparative tlc (silica gel, n-hexane : ethyl acetate = 3 : 1) to give artonin G (**1**, 25 mg). The fraction 4 (18.8 g) was rechromatographed on silica gel (200 g) with benzene : acetone = 6 : 1 (frs. 1"-2"), benzene : acetone = 5 : 1 (frs. 3"-5"), benzene : acetone = 4 : 1 (frs. 6"-9"), benzene : acetone = 3 : 1 (frs. 10"-12"), benzene : acetone = 2 : 1 (frs. 13"-16"), and benzene : acetone = 1 : 1 (frs. 17"-22"), each fraction (eluted volume of 300 ml) being monitored by tlc. The combined fraction (3"-5") was evaporated to leave the residue (1.5 g), which was extracted with benzene, and 1.2 g of the insoluble residue remained. The benzene solution was concentrated to afford the residue (0.3 g), which was purified by preparative tlc (silica gel, chloroform : methanol = 10 : 1) to give cycloartobiloxanthone (**4**, mp 280-283 °C, 104 mg).[7] The insoluble residue (1.2 g) in benzene was purified by repeated recrystallization from benzene-acetone to give artonin E (**3**, mp 245-250 °C, 1.1 g).[4] The combined fraction (7"-8", 1.0 g) was fractionated by gel filtration (TOYOPEARL HW-40, solvent system, methanol) followed by preparative hplc (solvent, n-hexane : ethyl acetate = 4 : 3, column, Senshu Pak SSC-Silica 4251-N, 1 cm ϕ x 25 cm, detector uv 280 nm) to give artonin H (**2**, 13 mg). The benzene extract (10 g) was chromatographed on silica gel (200 g) with n-hexane : ethyl acetate = 3 : 2 (frs. 1"'-3"'), n-hexane : ethyl acetate = 1 : 1 (frs. 4"'-5"'), and ethyl acetate (fr. 6"'), each fraction (1 l) being monitored by tlc. The fraction 4"' (3.8 g) was rechromatographed on silica gel (50 g) with *n-hexane : ethyl acetate = 4 : 1* followed by crystallization from chloroform to give artobiloxanthone (**5**, mp 163-167 °C, 38 mg).[7] The identification of the known compounds (**3** and **4**) was carried out by direct comparisons with the relevant authentic samples. The identification of the known compound **5** was carried out by comparing the physical and spectral data of **5** with the published data.[7]

Artonin G (1)

Compound **1** was recrystallized from n-hexane-ether to give yellow needles, mp 198-203 °C, $[\alpha]_D^{20}$ 0° (MeOH, c=0.31). $FeCl_3$ test: positive (greenish brown). Mg-HCl test: positive (red). Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 210 (4.64), 234 (4.37), 271 (4.29), 330 (infl 4.11), 376 (4.29). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 235 (sh 4.36), 280 (4.28), 310 (sh 3.90), 365 (sh 4.21), 390 (4.24). Ir ν_{max}^{KBr} cm^{-1} : 3550, 3400 (br), 1645, 1605, 1550, 1450. EI-Ms (i.v. 70 eV), m/z (rel. int.):

504 (M^+, 100%), 461 (91), 449 (95), 405 (30), 393 (18), 363 (11), 350 (6), 165 (4). High-resolution ms (HR-ms): m/z 504.2143 ($C_{30}H_{32}O_7$ requires 504.2148), 461.1585 ($C_{27}H_{25}O_7$ requires 461.1600), 449.1579 ($C_{26}H_{25}O_7$ requires 449.1600). ^{1}H Nmr (acetone-d_6): δ 1.30, 1.63 (each 3H, s, C-11-CH_3), 1.64 (6H, br s, C-16-CH_3 and C-21-CH_3), 1.77 (6H, br s, C-16-CH_3 and C-21-CH_3), 2.33 (1H, t, J=15 Hz, C-9-H), 3.19 (1H, dd, J=7 and 15 Hz, C-10-H), 3.34 (2H, br d, J=7 Hz, C-14-H x 2), 3.37 (1H, dd, J=7 and 15 Hz, C-9-H), 3.39 (2H, br d, J=7 Hz, C-19-H x 2), 5.27 (2H, m, C-15-H and C-20-H), 6.71 (1H, s, C-8-H), 13.51 (1H, s, C-5-OH).

Artonin G Trimethyl Ether (**1a**)

A mixture of **1** (14 mg), Me_2SO_4 (0.1 ml), and K_2CO_3 (3 g) in acetone (30 ml) was refluxed for 15 h and treated as usual. The product was purified by crystallization from n-hexane-acetone to give artonin G trimethyl ether (**1a**, 3 mg), yellow needles, mp 220 °C. $FeCl_3$ test: positive (brown). EI-Ms, m/z 546 (M^+), 531, 503, 491 (base peak). ^{1}H Nmr (acetone-d_6): δ 1.39 (3H, s, C-11-CH_3), 1.64, 1.67 (each 3H, br s, C-16-CH_3 and C-21-CH_3), 1.71 (3H, s, C-11-CH_3), 1.78, 1.79 (each 3H, br s, C-16-CH_3 and C-21-CH_3), 2.38 (1H, t, J=15 Hz, C-9-H), 3.25 (1H, dd, J=7 and 15 Hz, C-10-H), 3.35 (4H, m, C-14-H x 2 and C-19-H x 2), 3.42 (1H, dd, J=7 and 15 Hz, C-9-H), 3.85, 4.00, 4.03 (each 3H, s, OCH_3), 5.17, 5.22 (each 1H, m, C-15-H and C-20-H), 6.71 (1H, s, C-8-H), 13.35 (1H, s, C-5-OH).

Artonin H (**2**)

The compound **2** was obtained as an amorphous powder. $FeCl_3$: positive (brown). Mg-HCl test: positive (reddish orange). Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 206 (4.69), 230 (sh 4.39), 258 (4.44), 302 (4.05), 325 (sh 3.98). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 235 (infl 4.34), 263 (4.40), 315 (4.04), 395 (infl 3.66). Ir ν_{max}^{KBr} cm^{-1} : 3400 (br), 1660, 1630, 1595, 1530, 1460. EI-Ms (i.v. 70 eV), m/z (rel. int.): 506 (M^+, 73), 463 (12), 451 (10), 437 (58), 395 (11), 384 (20), 381 (100), 339 (11), 328 (20), 325 (22), 165 (20). HR-Ms: m/z 506.2325 ($C_{30}H_{34}O_7$ requires 506.2305), 437.1617 ($C_{25}H_{25}O_7$ requires 437.1600), 381.0968 ($C_{21}H_{17}O_7$ requires 381.0974). ^{1}H Nmr (acetone-d_6): δ 1.46 (3H, br s, C-11-CH_3), 1.54, 1.60 (each 3H, br s, C-16-CH_3), 1.64, 1.77 (each 3H, br s, C-21-CH_3), 1.88 (2H, m, C-13-H x 2), 1.99 (2H, m, C-14-H x 2), 3.15 (2H, br d, J=7 Hz, C-9-H x 2), 3.36 (2H, br d, J=7 Hz, C-19-H x 2), 5.04 (1H, br t, J=7 Hz, C-15-H), 5.15 (1H, br t, J=7 Hz, C-10-H), 5.28 (1H, br t, J=7 Hz, C-20-H), 6.38 (1H, s, C-8-H), 6.58 (1H, s, C-3'-H), 6.81 (1H, s, C-6'-H), 13.45 (1H, s, C-5-OH).

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