NEW FLAVONES FROM *ARTOCARPUS COMMUNIS* FORST.

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Three new prenylflavones, KB-1 (**1**), KB-2 (**2**) and KB-3(**3**) have been isolated from Indonesian *Artocarpus communis* FORST. The structures of these flavones were determined by analyses of their ^{1}H- and ^{13}C-NMR spectra including HMBC technique. All these compounds exhibited strong cytotoxic activities against leukemia cells (L-1210) in tissue culture

KEYWORDS *Artocarpus communis*; prenylflavone; cytotoxicity; HMBC

Artocarpus communis Forst. is widely distributed throughout the tropical area in South-East Asia. There are two species[1] in Indonesia, one is named KULUR (bread fruit tree) and the other is called SUKUN (seedless bread fruit tree). During the course of our studies on the constituents of Indonesian plants, we reported the isolation and structural elucidation of five new prenylphenols, two flavones and three chalcones, from the flower of KULUR.[2] All of the phenols strongly inhibited 5-lipoxygenase.[3] Here we describe the isolation and structural elucidation of three new prenylflavones along with a known flavone, morusin.[4]

The dried bark (500 g) of KULUR collected in a suburb of Jakarta, Indonesia, was extracted with ethanol under ultrasonication. The ethanol extracts were concentrated and then chromatographed on a HP-20 resin (Nippon Rensui) column eluted successively with 40% MeOH, 80% MeOH and MeOH. Purification of the 80% MeOH fraction by silica gel column chromatography followed by high-performance liquid chromatography (HPLC) (Senshu Pack ODS-4251D, 10 x 200 mm, MeOH : H_2O = 5 : 2) gave KB-1 (**1**, 120 mg), KB-2 (**2**, 100 mg) and KB-3 (**3**, 20 mg). Similar treatment of the MeOH fraction gave morusin (**4**, 60 mg).

KB-1 (**1**),[5] mp 160-162°C, $[\alpha]_D$0. The high resolution mass spectrum (HR-MS) of **1** indicated the molecular formula $C_{25}H_{22}O_7$ (M^+ 434.1389, calcd. 434.1364). The ^{13}C-NMR spectra including the DEPT technique exhibited the signals due to a conjugated carbonyl carbon (δ179.6) and eighteen sp^2 carbons (δ95-165) besides six sp^3 carbons [an oxygen-bearing quarternary carbon (δ78.0), a methine carbon (δ36.7), a methylene carbon (δ21.3) and three methyl carbons (δ21.5, 28.0 and 27.6)]. The ^{1}H-NMR (400 MHz, d6-DMSO, δ) spectrum of 1 indicated the presence of *gem*- dimethyl (δ1.38 and 1.42, s each), a vinyl methyl (δ1.68, brs), a non-equivalent methylene [δ2.34 (dd, J = 6.6, 16.0 Hz) and 3.21 (brd, J =16.0 Hz)], a methine [δ3.83 (brd, J = 6.6 Hz)], an exomethylene [δ4.14 and 4.59 (brs each)], four hydroxyls (δ8.07, 9.75, 10.30 and 13.30), two vinyl protons [δ5.69 and 6.84 (ABq, J = 10.2 Hz)] and two aromatic protons [δ6.14 and 6.45 (s each)]. These spectral data and the ^{1}H-NMR decoupling experiments on **1** revealed the presence of the partial structures (a), (b) and (c) in the molecule (Fig. 1). Considering the empirical formula and ^{13}C-NMR data, **1** may be a pentacyclic prenylflavone derivative. To clarify the substitution pattern on the flavone framework, the HMBC spectrum of **1** was measured and the connectivities of quarternary carbons were investigated by detection of ^{1}H-^{13}C long-range correlation as shown in Fig. 2. In the HMBC spectrum of **1**, the methylene protons at δ2.34 and 3.21 (H-9) showed the cross peaks due to long-range coupling with the carbons at δ109.6 (C-6'), 128.7 (C-3), 144.4 (C-11), 161.1 (C-2) and 179.6 (C-4), and the methine proton at δ3.83 (H-10) exhibited long-range correlations with the carbons at δ100.1 (C-1'), C-6' and 135.4 (C-5'). Thus, the partial structure (a) should be connected at C-3 and C-6'. The proton signal at δ6.45 showed cross peaks with the carbon signals due to C-1' and C-5', indicating that the proton should be assigned as the H-3'. In the ^{1}H-NMR spectrum of **1**, one (δ13.33) of four hydroxyl protons appeared in a rather low field compared with the others (δ8.07, 9.75 and 10.30), suggesting the formation of an intramolecular hydrogen bond between the hydroxyl proton at δ13.33 and the carbonyl group at C-4 in the flavone molecule. Furthermore, this hydroxyl proton showed long-range correlations with the carbons at δ104.1 (s) and 98.7 (d) in the HMBC spectrum. Therefore, the hydroxyl group should be present on the C-5, and the carbon signals at δ98.7 and 104.1 could be assigned to C-6 and C-4a, respectively. The proton at δ6.14 assigned as H-6 by analysis of the C-H COSY spectrum exhibited long-range correlations with the carbons at δ100.8 (C-8) and 157.9 (C-7) besides C-5 and C-4a. In addition, the C-8 signal showed cross peaks with the protons at δ5.69 and 6.84 in the partial structure (b), and the proton at δ6.84 exhibited long-range correlations with the carbons at δ150.7 (C-8a) and the oxygen-bearing quarternary carbon at δ78.0 in the partial structure (c) as well as C-7 and C-8. Thus, the partial structure (b) should be connected with

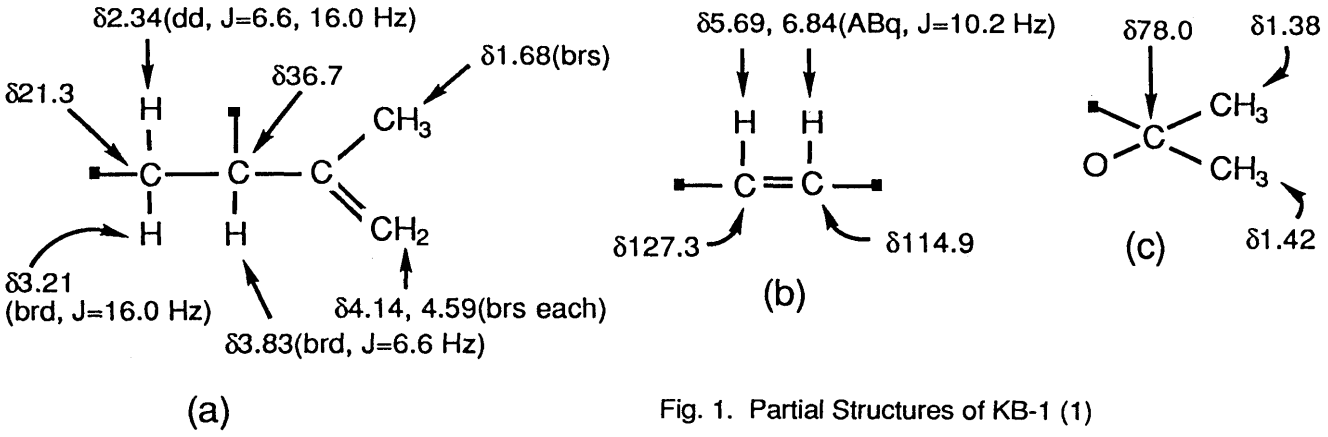

Fig. 1. Partial Structures of KB-1 (1)

the partial structure (c) and the C-8 on either side, and ring A must be formed between C-7 and C-8 because **1** was a pentacyclic compound. Finally, the remaining three hydroxyl groups should be arranged on the carbons at δ150.9 (C-2'), 160.4 (C-4') and 135.4 (C-5') in the ring E. Thus, the structure of KB-1 was represented by formula **1**.

KB-2 (**2**),[6] mp 166-168°C. The HR-MS of **2** indicated the molecular formula $C_{25}H_{26}O_8$ (M^+454.1612, calcd. 454.1626). KB-2 was deduced to be a tetracyclic analogue of **1**, because the ^{1}H- and ^{13}C-NMR data were very similar to those of **1** except in a few variations. In the ^{1}H-NMR spectrum of **2**, two singlet methyl [δ1.09 (6H)] and two methylene [δ1.60 (2H) and 2.48 (2H)] signals appeared instead of the signals due to the partial structure (a) which appeared in the spectrum of **1**. Considering the spectral data, the structure of **2** appeared to be the formula shown in Fig. 2, and this was confirmed by analysis of the HMBC spectrum of **2** and the results of NOE experiments on its 2',4',5'- trimethyl ether.[7] In the HMBC spectrum of **2**, the proton signal at δ1.09 (H-12 and H-13) due to *gem*-dimethyl group exhibit the cross peaks with the signals due to the methylene carbon at δ42.0 (C-10) and an oxygen-bearing quarternary carbon at δ70.5 (C-11), and the methylene protons at δ2.5 (H-9) showed long-range correlations with the carbons at δ42.0 (C-10), 121.7 (C-3), 162.2 (C-2) and 183.0 (C-4). Thus, the 1,1-dimethyl propanol moiety should be connected to C-3. In the NOE experiments on the trimethyl ether of **2**, irradiation at δ6.86 (H-6') enhanced the methoxyl proton signal at δ3.88 (MeO-5', 4.1%), while irradiation at δ6.62 (H-3') increased the intensity of two methoxyl signals at δ3.82 (MeO-4', 4.0%) and 3.97 (MeO-2', 4.4%). Thus, the structure of **2** was determined as shown in Fig 2.

KB-3 (**3**),[8] mp 243-245°C. The HR-MS of **3** indicated the molecular formula $C_{25}H_{24}O_7$ (M^+ 436.1495, calcd. 436.1520). The ^{1}H- and ^{13}C-NMR data of **3** suggested that the framework of **3** should be the same as that of **2** and the structural difference in **3** and **2** must be in the side chain moiety attached to the C-3. In the ^{1}H-NMR spectrum of **3**, proton signals appeared due to the prenyl group [δ1.42 (9H, s, CH_3 x 3) and 1.57 (3H, s, CH_3), δ3.05 (brd, J = 6.6 Hz, CH_2), δ5.05 (brt, J = 6.6 Hz, vinyl proton]. Thus, the structure of **3** was represented by the formula shown in Fig. 2. All these compounds (**1**, **2** and **3**) strongly inhibited (IC_{50} = 0.2-0.5 μg/ml) the growth of leukemia cells (L-1210) in tissue culture. Details of the biological activities of these compounds will be published

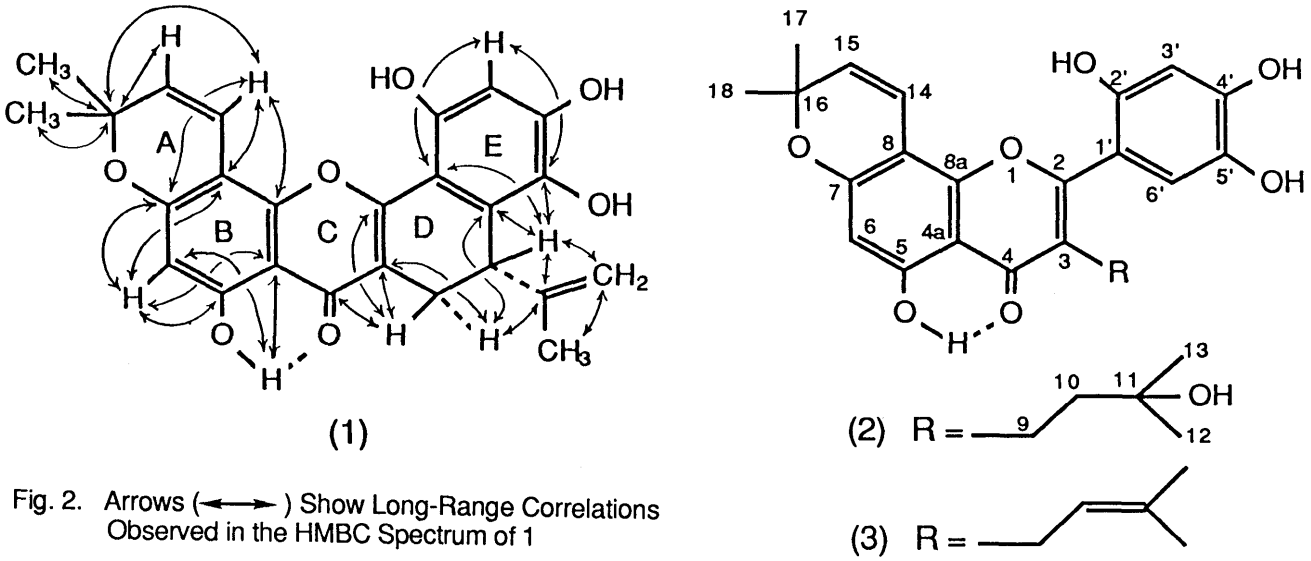

Fig. 2. Arrows (⟷) Show Long-Range Correlations Observed in the HMBC Spectrum of 1

elsewhere.

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5) ^{13}C-NMR (100 MHz, d6-DMSO, δ): 21.3 (C-9), 21.5(C-12), 27.6, 28.0(C-17, C-18), 36.7(C-10), 78.0(C-16), 98.7(C-6), 100.8(C-8), 102.7(C-3'), 104.1(C-4a), 105.1(C-1'), 109.6(C-6'), 111.1(C-13), 114.9(C-14), 127.3(C-15), 128.7(C-3), 135.4(C-5'), 144.4(C-11), 150.7(C-8a), 150.9(C-2'), 157.9(C-7),160.4(C-4')160.7(C-5), 161.1(C-2), 179.6(C-4).

6) ^{1}H-NMR(400 MHz, CD_3OD, δ): 1.09(6H, s, H-12 and H-13), 1.42(6H, s, H-17 and H-18), 1.60(2H, m, H-10), 2.48(2H, m, H-9), 5.58(1H, d, J = 9.5 Hz, H-15), 6.14(1H, s, H-6), 6.46(1H, s, H-3'), 6.60(1H, d, J = 9.5 Hz, H-14), 6.73(1H, s, H-6'), ^{13}C-NMR(100 MHz, CD_3OD, d): 20.3(C-9), 27.8(C-12 and C-13), 27.4(C-17 and C-18), 42.0(C-10), 70.5(C-11), 78.1(C-16), 99.1(C-6), 101.2(C-8),103.8(C-3'), 104.9(C-4a), 110.7(C-1'), 114.8(C-14), 115.9(C-6'), 121.7(C-3), 127.2(C-15), 138.5(C-5'), 148.9(C-4'), 149.0(C-2'), 152.8(C-8a), 159.5(C-7), 161.7(C-5), 162.2(C-2), 183.0(C-4).

7) This compound was prepared by the action of diazomethane in MeOH. ^{1}H-NMR(400 MHz, $CDCl_3$, δ): 1.15(6H, s, H-12 and H-13), 1.45(6H, s, H-17 and H-18), 1.63(2H, m, H-10), 2.43(2H, m, H-9), 3.81(3H, s, MeO-2'), 3.87(3H, s, MeO-5'), 3.93(3H, s, MeO-4'), 5.48(1H, d, J = 10.2 Hz, H-15), 6.26(1H, s, H-6), 6.58(1H, d, J =10.2 Hz, H-14), 6.62(1H, s, H-3'), 6.86(1H, s, H-6').

8) ^{1}H-NMR(400 MHz, d6-DMSO, δ): 1.42(9H, s, H-12, H-17 and H-18), 1.57(3H, brs, H-13), 3.05(2H, d, J =6.6 Hz, H-9), 5.05(1H brt, J =6.6 Hz, H-10), 5.70(1H, d, J =10.2 Hz, H-15), 6.22(1H, s, H-6), 6.47(1H, s, H-3'), 6.53(1H, d, J =10.2 Hz, H-14), 6.70(1H s, H-6'), 8.63, 9.32, 9.51(OH x 3), 13.20(HO-5). ^{13}C-NMR(100 MHz, d6-DMSO, δ): 17.4(C-13), 23.8(C-9), 25.5(C-12), 27.7(C-17 and C-18), 78.2(C-16), 98.9(C-6), 100.5(C-8), 104.0(C-3'), 104.3(C-4a), 109.4(C-1'), 114.2(C-14), 116.1(C-6'), 120.0(C-3), 121.5(C-10),127.8(C-15), 131.5(C-11), 138.1(C-5'), 148.6(C-4'), 148.8(C-2'), 151.8(C-8a), 158.6(C-7), 161.0(C-5), 161.7(C-2), 181.9(C-4).

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