Cassane Furanoditerpenoids from the Seed Kernels of *Caesalpinia bonduc* from Thailand

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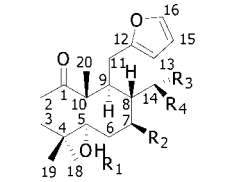
Three new cassane furanoditerpenoids (**1**–**3**), together with 13 known cassane diterpenes (**4**–**16**), were isolated from the EtOAc extract of the seed kernels of *Caesalpinia bonduc*. Their structures were elucidated on the basis of spectroscopic analysis, mainly NMR and MS. Compounds **1**–**3** exhibited good antimalarial activity against the multidrug-resistant K1 strain of *Plasmodium falciparum*, while compound **4** was inactive. None of the compounds were cytotoxic against any of the tumor cell lines tested.

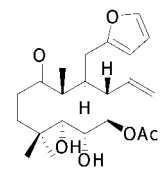
Plants belonging to the genus *Caesalpinia* (Leguminosae) have proven to be a rich source of cassane furanoditerpenes, some of which display interesting biological activities such as antiviral,[1,2] antimalarial,[3,4] antibacterial,[5] and antioxidant.[5] *Caesalpinia bonduc* (L.) Roxb is a climber distributed throughout Southeast Asia, commonly called "Sa-Wad" in Thailand. It is used as a medicinal plant in various regions of the tropics. In the herbal medicine of Thailand, seeds of this plant are used as an expectorant and antitussive agent. Previous chemical investigations of the seed kernels revealed the presence of several cassane-type diterpenoids.[6–9] We report herein the isolation of three new cassane-type diterpenes (**1**–**3**) together with 13 known diterpenes (**4**–**16**) from the seed kernels of *C. bonduc* collected in Thailand. Antimalarial and cytotoxic activities of these compounds are also reported.

Air-dried seed kernels of *C. bonduc* were successively extracted with hexane, EtOAc, and MeOH. The crude EtOAc extract was purified by normal- and reversed-phase column chromatography to yield three new compounds, bonducellpins E–G (**1**–**3**), and 13 known compounds identified as α-caesalpin (**4**),[10] bonducellpins B (**5**) and C (**6**),[7] caesalmins D (**7**) and E (**8**),[1] ε-caesalpin (**9**),[11] caesalpinins K (**10**),[12] P (**11**),[12] and C (**12**),[13] 14(17)-dehydrocaesalpin F (**13**),[14] caesalpinin I (**14**),[12] caesalmin B (**15**),[15] and 2-acetoxycaesaldekarin e (**16**)[14] by comparison of their physical and spectroscopic data with those reported in the literature.

Bonducellpin E (**1**) was isolated as a colorless, amorphous solid, and its molecular formula was assigned to be $C_{23}H_{30}O_8$ by HRFABMS, indicating nine degrees of unsaturation. The ^{1}H NMR spectrum displayed signals of three tertiary methyls, two oxygen-substituted methines, three aliphatic methines, one acetyl methyl, and one methoxy methyl, and a 1,2-disubstituted furan ring was suggested by a pair of aromatic proton doublets at δ_H 6.11 and 7.21. The ^{13}C NMR and HSQC spectra revealed the presence of 23 nonequivalent carbons including three methyl groups, three carbons bearing oxygen, three methylenes, two methines, three quaternary carbons, four aromatic carbons of the furan ring, and a ketone carbonyl carbon, together with the signals of acetyl and methyl ester groups. These ^{1}H and ^{13}C NMR spectra were similar to those of bonducellpin B (**5**),[7] except for the location of the acetyl group. The correlation between the acetate carbonyl signal at δ_C 173.6 and the oxymethine proton H-7 at δ_H 5.09 in the HMBC spectrum indicated that the acetoxyl group was attached at C-7. The full assignments and connectivity were determined by ^{1}H–^{1}H COSY correlations as indicated by bold lines and HMBC correlations shown by arrows (Figure 1). The location of a ketone at C-1 was confirmed because it showed HMBC correlations to H_2-3 and H_3-20. In addition, correlation of a proton at δ_H 3.40 (H-14) with the ester carbonyl carbon at δ_C 174.6 indicated that the carboxymethyl group must connect to C-14. The relative stereochemistry of **1** was assigned on the basis of a NOESY experiment, as depicted in Figure 2. The compound exhibited NOEs between Me-20 and Me-19, between Me-20 and H-6, and between H-6 and H-8, indicating that all these protons are located on the β-face, while the NOE response between H-7 and H-9 and between H-7 and H-14 would then allocate these proton to the α-face, suggesting that the COOMe group was β-oriented.



	R_1	R_2	R_3	R_4
1	OH	OAc	H	CO_2Me
4	OAc	OAc	Me	OH
5	OAc	OH	H	CO_2Me



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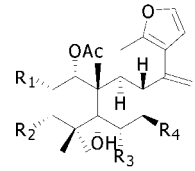
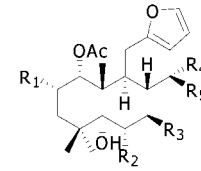
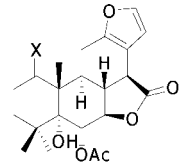


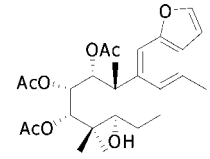
	R_1	R_2	R_3	R_4
3	H	H	OAc	OH
11	OAc	H	H	H
12	H	OAc	H	H
13	OAc	OAc	H	H



	R_1	R_2	R_3	R_4	R_5
6	H	H	OH	H	CO_2Me
7	H	OAc	OAc	OH	Me
8	H	OAc	OAc	Me	OH
9	OAc	H	H	Me	OH
10	H	H	OH	H	Me



14 X = O
15 X = H, α-OAc



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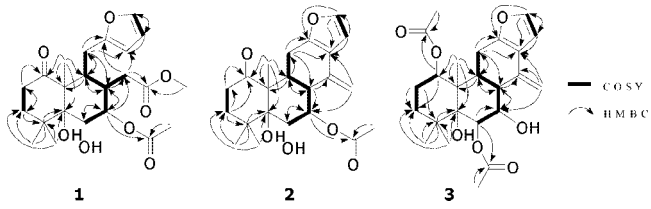

Figure 1. Key HMBC and COSY correlations for compounds **1**, **2**, and **3**.

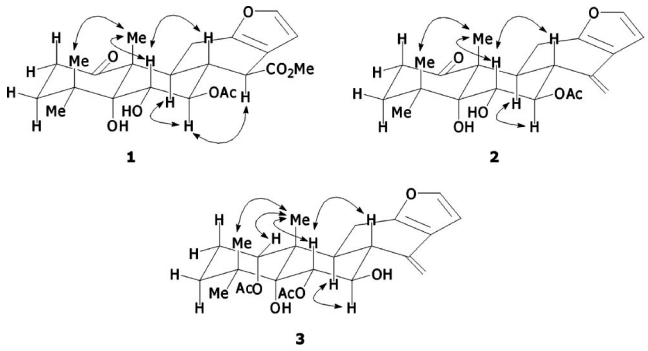

Figure 2. Selected NOESY correlations of compounds **1**, **2**, and **3**.

Bonducellpin F (**2**) was isolated as a colorless, amorphous solid and had the molecular formula $C_{22}H_{28}O_6$, as established by HRFABMS. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** revealed them to be very similar, with the only difference being the appearance of a pair of doublets due to an exomethylene at δ_H 5.06 and 4.57 in the ^{1}H NMR spectrum, coupled in the HSQC spectrum to a newly appearing olefinic carbon resonance at δ_C 104.2, while the carbomethoxy signal at δ_H 3.72 and at δ_C 174.6 and 52.1 had disappeared. Assignment of the relative stereochemistry of **2** was also accomplished by analysis of the NOESY spectrum as depicted.

Bonducellpin G (**3**) was obtained as a colorless, amorphous solid with the molecular formula $C_{24}H_{32}O_7$, as determined by HRFABMS. The ^{1}H and ^{13}C signals of **3** were virtually identical to those of **2**. The absence of a ketone carbonyl signal, along with resonances indicating an additional acetoxyl group (δ_H 2.22 and δ_C 169.3, 21.7) and an oxymethine (δ_H 4.89 and δ_C 76.0), indicated that the ketone carbonyl had been replaced by an acetoxyl group at C-1. This was confirmed by the HMBC cross-peak of a proton at δ_H 4.89 to the acetate carbonyl carbon at δ_C 169.3. Another acetoxyl group was determined to be at C-6 on the basis of long-range correlation of the ester carbonyl carbon at δ_C 172.2 with the protons at δ_H 2.22 and 5.44 (H-6). The relative configurations of the acetoxyl groups at C-1 and C-6 were assigned to be δ-oriented by NOESY data that showed key cross-peaks between H-1 and Me-20 and between H-6 and Me-20.

Compounds **1**–**4** were evaluated for antiplasmodal activity against the multidrug-resistant K1 strain of *Plasmodium falciparum*. Compounds **1**–**3** exhibited good activity, with IC_{50} values of 1.6, 5.8, and 3.8 μM, respectively, while compound **4** was inactive at a concentration of 10 μM. All compounds were also tested for cytotoxicity against five human tumor cell lines: BT474, CHAGO, Hep-G2, KATO-3, and SW-620. None were cytotoxic at a concentration of 10 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Shimadzu FTIR-8300. Mass spectra were obtained using a JEOL JMS DX-303 mass spectrometer. The NMR spectra were recorded on a Varian YH400 NMR spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR.

Plant Material. Seed kernels of *Caesalpinia bonduc* (L.) Roxb. were collected from Amphur Sanamchaikate, Chachoengsao Province, Thailand, in June 2005. Botanical identification of the plant specimen was achieved through comparison with specimen No. BKF 55398 in the herbarium of the Royal Forest Department of Thailand, Bangkok, Thailand.

Extraction and Isolation. The air-dried, powdered seed kernels of *C. bonduc* (2.5 kg) were extracted successively with *n*-hexane, EtOAc, and MeOH at room temperature (each for 3 days). The crude EtOAc extract (34 g) was chromatographed over a silica gel column using a hexane–EtOAc gradient followed by a MeOH–CH_2Cl_2 gradient of increasing polarity to afford six fractions (I–VI). Fraction III was recrystalized from MeOH to give 14(17)-dehydrocaesalpin F (**13**) (148.2 mg), and the mother liquor was then evaporated and rechromatographed on silica gel eluted with hexane–EtOAc (8:2) to furnish 2-acetoxycaesaldekarin e (**16**) (12.7 mg). Fraction IV was subjected to flash column chromatography eluted with a gradient system of hexane–EtOAC, and five subfractions were (VI 1–5) collected. Subraction VI 1 was further purified by reversed-phase column chromatography on ODS C18 (63–212 μm) eluted with MeOH–H_2O (4:6) to afford bonducellpin C (**12**) (10.4 mg). Subfraction VI 2 was rechromatographed on a silica gel column (hexane–EtOAc, 7.5:2.5) to give caesalpinin I (**14**) (6.3 mg), while subfraction VI 3 eluted with MeOH–CH_2Cl_2 (2:98) gave α-caesalpin (**4**) (26.7 mg) and caesalpinin P (**11**) (13.6 mg). Subfraction VI 4 was subjected to a silica gel column with a gradient solvent system of EtOAc–benzene (from 5:95 to 20:80) to yield ε-caesalpin (**9**) (21.4 mg), ceasalpinin K (**10**) (12.2 mg), and caesalpinin C (**12**) (14.3 mg). Subfraction VI 5 was purified on a silica gel column (EtOAc–benzene, 2:8) to afford caesalmin B (**15**) (7.5 mg).

Fraction V was rechromatographed on silica gel flash column chromatography (a gradient solvent system of MeOH–CH_2Cl_2) to give six subfractions (V 1–6). Subfraction V 1 was further subjected to silica gel column chromatography eluted with acetone–CH_2Cl_2 (3:97 and 5:95) to yield bonducellpin F **2** (9.3 mg) and bonducellpin G **3** (11.3 mg); subfraction V 3 eluted with acetone–benzene (2:8) gave caesalmins D (**7**, 5.2 mg) and E (**8**, 4.8 mg). Subfractions V 5–6 were combined and rechromatographed on a silica gel column (acetone–CH_2Cl_2, 5:95) and then by preparative TLC (acetone–benzene, 1:4) to yield bonducellpins B (**5**, 6.7 mg) and E (**1**, 5.6 mg).

Bonducellpin E (1): colorless, amorphous solid; $[\delta]_D^{20}$ +25.4 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3522, 1750, 1714, 1244, 1044 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.21 (1H, d, J = 2.0 Hz, H-16), 6.11 (1H, d, J = 2.0 Hz, H-15), 5.09 (1H, dd, J = 10.4, 9.2 Hz, H-7), 4.06 (1H, dd, J = 9.2, 5.6 Hz, H-6), 3.72 (3H, s, COO*CH*$_3$), 3.50 (1H, dd, J = 16.0, 4.0 Hz, H-11a), 3.40 (1H, d, J = 10.0 Hz, H-14), 2.90 (1H, d, J = 5.6 Hz, 6-OH), 2.65 (1H, m, H-9), 2.54 (2H, m, H-2), 2.53 (1H, m, H-8), 2.46 (1H, m, H-11b), 2.07 (3H, s, 7-OAc), 2.02 (1H, m, H-3a), 1.66 (1H, m, H-3b), 1.45 (3H, s, CH_3-20), 1.40 (3H, s, CH_3-19), 1.27 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 212.9 (CO, C-1), 174.6 (CO, *C*OOCH$_3$), 173.6 (CO, 17-OAc), 151.4 (CH, C-12), 141.3 (CH, C-16), 111.8 (C, C-13), 108.1 (C, C-15), 81.6 (C, C-5), 80.5 (CH, C-7), 74.2 (CH, C-6), 54.9 (C, C-10), 52.1 (CH_3, COO*CH*$_3$), 45.8 (CH, C-14), 38.7 (C, C-4), 38.3 (CH, C-8), 37.6 (CH, C-9), 37.2 (CH_2, C-3), 35.3 (CH_2, C-2), 28.6 (CH_3, C-18), 27.5 (CH_3, C-19), 24.0 (CH_2, C-11), 21.0 (CH_3, AcO-17), 14.6 (CH_3, C-20); HRFABMS *m/z* 434.1988 (calcd for $C_{23}H_{30}O_8$, 434.1941).

Bonducellpin F (2): colorless, amorphous solid; $[\delta]_D^{20}$ +12.1 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} (cm^{-1}) 3450, 1730, 1715, 1193; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.23 (1H, d, J = 2.0 Hz, H-16), 6.40 (1H, d, J = 2.0 Hz, H-15), 5.36 (1H, t, J = 9.6 Hz, H-7), 5.06 (1H, d, J = 1.9 Hz, H-17a), 4.57 (1H, d, J = 1.9 Hz, H-17b), 4.17 (1H, dd, J = 8.0, 4.0 Hz, H-6), 3.43 (1H, dd, J = 16.4, 4.4 Hz, H-11a), 3.33 (1H, d, J = 4.0 Hz, 6-OH), 2.79 (1H, ddd, J = 16.4, 11.6, 4.4 Hz, H-9), 2.58 (2H, m, H-11b and H-2a), 2.55 (1H, m, H-8), 2.48 (1H, m, H-2b), 2.14 (CH_3, s, 7-OAc), 1.88 (1H, m, H-3a), 1.75 (1H, m, H-3b), 1.44 (6H, s, CH_3-19 and CH_3-20), 1.30 (CH_3, s, C-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 213.3 (CO, C-1), 174.8 (CO, *C*OOCH$_3$), 152.4 (CH, C-12), 141.6 (CH, C-16), 139.1 (C, C-14), 118.8 (C, C-13), 106.3 (C, C-15), 104.2 (CH_2, C-17), 81.6 (C, C-5), 78.3 (CH, C-7), 74.9 (CH, C-6), 54.0 (C, C-10), 41.6 (CH, C-8), 39.2 (CH, C-9), 38.8 (C, C-4), 38.0 (CH_2, C-3), 35.6 (CH_2, C-2), 28.9 (CH_3, C-18), 27.1 (CH_3, C-19), 26.0 (CH_2, C-11), 21.5 (CH_3, COO*CH*$_3$), 15.6 (CH_3, C-20); HRFABMS *m/z* 388.1822 (calcd for $C_{22}H_{28}O_6$, 388.1886).

Bonducellpin G (3): amorphous solid; $[\delta]_D^{20}$ +7.8 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} (cm^{-1}) 3455, 1732, 1710, 1084; ^{1}H NMR ($CDCl_3$, 400

MHz) δ 7.24 (1H, d, J = 1.8 Hz, H-16), 6.45 (1H, d, J = 1.8 Hz, H-15), 5.44 (1H, d, J = 8.8 Hz, H-6), 5.28 (1H, s, H-17a), 5.25 (1H, s, H-17b), 4.90 (1H, br s, H-1), 4.35 (1H, dd, J = 16.0, 9.2 Hz, H-7), 2.93 (1H, br s, 7-OH), 2.68 (1H, ddd, J = 16.4, 11.2, 4.8 Hz, H-9), 2.56 (1H, dd, J = 15.6, 11.6 Hz, H-11a), 2.40 (1H, m, H-11b), 2.38 (1H, m, H-8), 2.22 (3H, s, 6-OAc), 2.08 (3H, s, 1-OAc), 1.91 (1H, m, H-2a), 1.77 (1H, m, H-2b), 1.27 (3H, s, H-20), 1.19 (3H, s, H-18), 1.18 (3H, s, H-18), 1.15 (CH_2, m, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.2 (CO, 6-*C*OOCH$_3$), 169.3 (CO, 1-*C*OOCH$_3$), 150.9 (C, C-12), 142.2 (CH, C-16), 139.3 (C, C-14), 120.0 (C, C-13), 107.0 (CH_2, C-17), 106.8 (CH, C-15), 79.4 (C, C-5), 77.6 (CH, C-6), 76.0 (CH, C-1), 72.6 (CH, C-7), 54.0 (C, C-10), 45.0 (CH, C-8), 38.7 (C, C-4), 38.1 (CH, C-9), 32.5 (CH_2, C-3), 30.9 (CH_3, C-18), 25.2 (CH_3, C-19), 23.2 (CH_2, C-11), 22.3 (CH_2, C-2), 22.2 (CH_3, 6-COO *CH$_3$*), 21.7 (CH_3, 1-COO *CH$_3$*),17.4 (CH_3, C-20); HRFABMS *m/z* 432.2156 (calcd for $C_{24}H_{32}O_7$, 432.2148).

Bioassays. Antimalarial activity *in vitro* was determined by means of the microculture radioisotope technique based on the method described by Desjardins et al.[16] The parasite *P. falciparum* (K1, multidrug-resistant strain) was cultured continuously according to the method of Trager and Jensen.[17] An IC_{50} value of 4.0×10^{-3} μM (n = 3) was observed for the positive control, dihydroartemisinin. Cytotoxicity was assessed against human cell cultures, BT474 (breast carcinoma), CHAGO (lung carcinoma), Hep-G2 (human hepatocarcinoma), KATO-3 (gastric carcinoma), and SW-620 (colon carcinoma), using the MTT [3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide] colorimetric method.[18,19] Three preparations were used for each experiment. Data are presented as means ± SEM. Statistical analyses were done by means of the Student's *t*-test. A *P* value of less than 0.05 was considered a significant difference.

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