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Novel norcassane-type diterpene from the seed kernels of *Caesalpinia crista*

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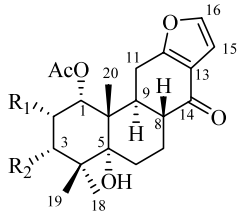
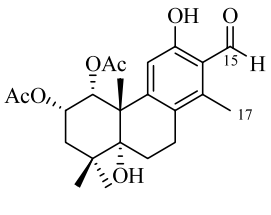
Abstract—Three novel norcassane-type diterpenes were isolated from a CH_2Cl_2 extract of the seed kernels of *Caesalpinia crista* together with four known cassane-type diterpenes. All the new compounds represent unprecedented carbon framework. Norcaesalpinin A (**1**) and B (**2**) had 17-norcassane skeleton, while norcaesalpinin C (**3**) had 16-norcassane skeleton. Their structures were elucidated on the basis of spectral analysis.

Caesalpinia crista (Fabaceae), commonly known as Bagore, is a famous medicinal plant of South Sulawesi of Indonesia. The seed kernel of the plant has been used by the people of local communities as anthelmenthic and antimalarial drug.[1] In our preliminary screening program of antimalarial agents from plant resources, we observed significant inhibition of parasitemia by the CH_2Cl_2 extract of the seed kernels of *C. crista* in mice infected with *Plasmodium berghei*.[2–4] Thus, we performed the separation of the CH_2Cl_2 extract (151 g) and isolated three novel norcassane-type diterpenes norcaesalpinin A (**1**, 32.9 mg), B (**2**, 19.7 mg) and C (**3**, 5.9 mg) together with four known cassane-type diterpenes, 2-acetoxy-3-deacetoxycaesaldekarin e (8.0 mg),[5] caesalmin B (3.5 mg),[6] caesaldekarin e (17.3 mg)[7] and 14(17)-dehydrocaesalpin F (3.5 mg),[8] by silica gel column chromatography and preparative TLC. In this paper, we will report the structure elucidation of the novel norcassane-type diterpenes.[9]



1 R_1 = OAc, R_2 = H
2 R_1 = H, R_2 = OAc



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Norcaesalpinin A (**1**)[10] was isolated as colorless amorphous solid with $[\alpha]_D^{25}$ −20.1° (*c* 0.15, $CHCl_3$). The absorption bands at 3625 and 1735 cm^{-1} in its IR spectrum indicated the presence of hydroxyl and carbonyl groups, respectively. The molecular formula of **1** was determined to be $C_{23}H_{30}O_7$ by HRFABMS. The ^1H NMR spectrum of **1** (Table 1) displayed signals corresponding to three tertiary methyls, two oxygen-substituted methines, two aliphatic methines together with two protons of a 1,2-disubstituted furan ring (δ 7.29, 6.63) and two acetyl methyls. Moreover, the ^{13}C NMR spectrum of **1** showed a ketone carbonyl carbon (δ 195.2), four olefinic carbons (δ 165.4, 142.2, 119.9, 106.5) and three oxygen-substituted carbons (δ 74.5, 67.3, 76.4) together with two ester carbonyl carbons. Except for two acetyl groups (δ 169.3, 21.1; 170.3, 20.9), the carbon framework of **1** had 19 carbon signals indicating **1** to be a norditerpene.

The partial structures deduced by the COSY and HMQC spectra (bold line) were connected based on the long-range correlations (arrows) observed in the HMBC spectrum (Fig. 1a). The methyl protons at δ 1.30 (H_3-20) showed HMBC correlations with two methine carbons at δ 74.5 (C-1) and 40.0 (C-9) and two quaternary carbons at δ 45.0 (C-10) and 76.4 (C-5), indicating that the carbons, C-1, C-5 and C-9, and the methyl group (C-20) should be connected with the quaternary carbon C-10. Furthermore, the methyl protons at δ 1.10 (H_3-18) showed the long-range correlations with the carbons at δ 28.0 (C-19), 76.4 (C-5), 40.1

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Table 1. ^1H and ^{13}C NMR data for compounds **1–3** in $CDCl_3$ (J values in parentheses)[a]

	1		**2**		**3**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	5.29 d (2.2)	74.5	4.92 t (3.2)	73.5	5.91 d (2.4)	74.2
2α		67.3	2.14 m	26.7		67.5
2β	5.35 ddd (13, 4.8, 2.2)		2.33 m		5.45 ddd (13, 4.6, 2.4)	
3α	2.03 m	35.7		76.9	2.14 br t (13.3)	35.7
3β	1.41 dd (13, 4.8)		4.95 t (2.8)		1.47 dd (12.5, 4.6)	
4		40.1		41.5		39.9
5		76.4		76.5		75.2
6α	1.75 m	25.7	1.79 m	26.3	2.07 m	23.6[c]
6β	1.64 ddd (14.1, 4.4, 2.6)		1.71 m		2.79 dd (10.2, 7.5)	
7α	2.15 m	20.5	2.15 m	20.4	2.66 dd (17.5, 7.5)	23.2[c]
7β	1.84 dd (13.4, 4.4)		1.82 m		1.98 m	
8	2.37 td (12, 4.8)	43.7	2.36 m	43.9		125.7
9	2.97 td (12, 5.3)	40.0	3.29 td (12, 5.3)	39.6		153.4
10		45.0		43.9		48.7
11α	2.58 dd (17, 5.3)	22.9	2.44 dd (17, 5.3)	22.7	6.55 s	110.9
11β	2.70 dd (17, 12)		2.75 dd (17, 12)			
12		165.4		165.5		161.3
13		119.9		119.9		117.2
14		195.2		195.4		140.2
15	6.63 d (2)	106.5	6.65 d (1.7)	106.5	10.38 s	195.1
16	7.29 d (2)	142.9	7.31 d (1.7)	142.9		
17					2.46 s	13.4
18	1.10 s	25.4	1.11 s	23.1	1.17 s	27.7
19	1.19 s	28.0	1.14 s	25.1	1.21 s	25.6
20	1.30 s	17.7	1.20 s	18.4	1.43 s	29.1
1-OAc	2.15 s	21.1	2.06 s	21.4	2.00 s	21.0
		169.3		169.5		169.0
2-OAc	1.99 s	20.9			2.03 s	21.0
		170.3				170.5
3-OAc			2.05 s	21.1		
				169.2		
5-OH[b]	2.90 d (2.4)		2.28 d (2.9)		3.07 d (1.9)	
12-OH[b]					11.75 s	

[a] The ^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively.
[b] These signals were disappeared by D_2O treatment.
[c] Assignments may be interchanged within each column.

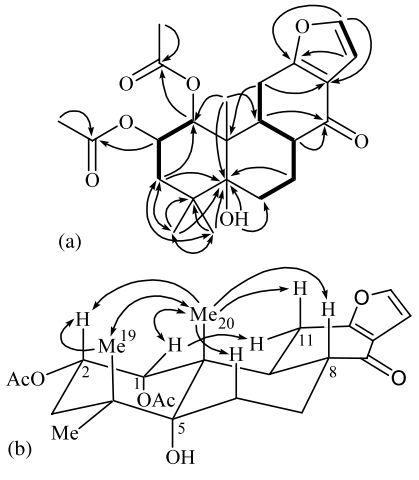

Figure 1. Connectivities (bold line) deduced by the COSY and HMQC spectra and significant long-range correlations (arrows) observed in the HMBC spectrum of **1** (a) and NOE correlations observed in the difference NOE experiments (b).

(C-4) and 35.7 (C-3), and the protons at δ 1.19 (H_3-19) with the carbons at δ 25.4 (C-18), C-3, C-4 and C-5. Thus, the carbons C-3, C-5, C-18 and C-19 were connected with the quaternary carbon C-4. Moreover, the connectivity of C-13 (δ 119.9) and C-11 (δ 22.9) with the oxygen-substituted olefinic carbon C-12 (δ 165.4) was established on the basis of HMBC correlation between the methylene protons (H_2-11) and the carbon C-13. Likewise, the connectivity between C-15 (δ 106.5) and C-13 was established based on the HMBC correlation between H-15 and C-13. The ether linkage between C-16 (δ 142.9) and C-12 (δ 165.4), i.e. presence of furan ring, was confirmed by the long-range correlation observed between H-16 and C-12. The HMBC correlations between two methine protons at δ 2.37 (H-8) and δ 2.97 (H-9) with the ketone carbonyl carbon at δ 195.2 indicated that the ketone carbonyl should be C-14. Accordingly, all the nineteen carbons in the main skeleton have been accounted, which have one carbon (C-17 attached at C-14) less than cassane-type diterpene.[5–8]

Thus, **1** should be 17-norcassane-type diterpene. The locations of the two acetyl groups were determined to be C-1 and C-2, because of the long-range correlations of the ester carbonyl carbon at δ 169.3 (1-OCO-) with the protons at δ 2.15 (1-OCOCH_3) and 5.29 (H-1) and of the ester carbonyl carbon at δ 170.3 (2-OCO-) with the protons at δ 1.99 (2-OCOCH_3) and 5.35 (H-2)

The relative stereochemistry of **1** was determined on the basis of coupling constants and the results of difference NOE experiments (Fig. 1b). The large J value for H-2 ($J_{2,3ax}=13$ Hz) indicated that H-2 should be *axial*, while small J value for H-1 (2.2 Hz) suggested that H-1 should be *equatorial*. In the difference NOE experiments irradiation of the methyl protons at δ 1.30 (H_3-20) caused NOE increases at H-1, H-2, H-6_{ax}, H-8 and H_3-19. These correlations indicated that all the protons H-1, H-2, H-6_{ax}, H-8, H_3-19 and H_3-20 should be β-oriented in rings A and B in chair conformation. The NOE correlations between H-1 and H-11_{eq}, between H_3-20 and H-11_{ax}, and large J value for H-9 ($J_{8,9}=J_{9,11ax}=12$ Hz) suggested that H-9 should be α-*axial* oriented. Moreover, the absolute configuration of C-9 was concluded to be *S* based on the positive cotton effect ($[\theta]_{302}$ +6993) at R-band in CD spectrum of **1**.[11,12] Thus, the stereostructure of norcaesalpinin A was determined as **1**.

Norcaesalpinin B (**2**),[13] having the same molecular formula $C_{23}H_{30}O_7$ as **1**, was isolated as colorless amorphous solid. The ^{1}H and ^{13}C NMR spectra (Table 1) of **2** were identical to those of **1** except for the difference in the position of the acetyl groups. Both oxygen-substituted methine protons of **2** resonated at δ 4.95 and 4.92 instead of δ 5.35 and 5.29 in **1**. The COSY correlations of these two methine protons with the same methylene group (H_2-2) suggested that the acetyl group in **2** were at C-1 and C-3 instead of C-1 and C-2 in **1**. The small J value of both methine protons (H-1: t, $J=3.2$ Hz; H-3: t, $J=2.8$ Hz) indicated them to be *equatorial* and the remaining part of the molecule was the same as in **1**. Based on these facts and a positive cotton effect ($[\theta]_{300}$ +5337) at R-band similar to that of **1** observed in CD spectrum, the structure of norcaesalpinin B was determined to be **2**.

Norcaesalpinin C (**3**)[14] was also obtained as colorless amorphous solid having $[\alpha]_D^{25}$ −55.3° (c 0.10, $CHCl_3$). The IR spectrum of **3** indicated the presence of hydroxyl (3575 cm^{-1}) and carbonyl (1785 cm^{-1}) functionalities. The molecular formula of **3** was determined to be the same as **1** and **2** ($C_{23}H_{30}O_7$) by HRFABMS. The ^{1}H NMR spectrum of **3** (Table 1) displayed signals due to four tertiary methyls, two acetyl methyls, an aldehyde (δ 10.38), an olefine (δ 6.55), two hydroxyls (δ 11.75, 3.07) and three methylenes. The ^{13}C NMR spectrum of **3** had signals corresponding to twenty-three carbons including an aldehydic carbon (δ 195.1), two ester carbonyl carbons (δ 170.5, 169.0), six olefinic carbons (δ 161.3, 153.4, 140.2, 125.7, 117.2, 110.9) and three oxygen-substituted carbons (δ 74.2, 67.5, 75.2). The main carbon framework of **3** also had only 19 carbon signals indicating it also to be a norditerpene. The ^{1}H and ^{13}C NMR data of **3** were similar to those of 2-acetoxy-3-deacetoxycaesaldekarin e,[5] except for the presence of an aldehydic group (δ_H 10.38, δ_C 195.1) instead of two proton signals of 1,2-disubstituted furan. The aldehydic proton (δ 10.38) showed HMBC correlations with two olefinic carbons at δ 161.3 (C-12) and 117.2 (C-13) (Fig. 2a), indicating the aldehydic carbon to be connected with the olefinic carbon C-13. Moreover, the methyl protons at δ 2.46 (H_3-17) showed HMBC correlations with three olefinic carbons at δ 125.7 (C-8), 140.2 (C-14) and 117.2 (C-13), suggesting that the carbons C-8 and C-13 and the methyl group (C-17) should be connected with the quaternary carbon C-14. The HMBC correlations of the hydroxyl proton at δ 11.75 (12-OH) with the carbon at δ 110.9 (C-11) and C-12 and C-13 suggested that the hydroxyl group should be located at C-12. Thus, **3** was determined to be 16-norcassane-type diterpene. The α-orientation of both acetyl groups at C-1 and C-2 and the hydroxyl group at C-5 were determined from the results of difference NOE experiments (Fig. 2b) and analysis of the coupling constants of each proton, which were identical to that of **1**.

To the best of our knowledge, both **1** and **2** are the first examples of 17-norcassane-type diterpenes, which may be biosynthesized through decarboxylation of C-17 from cassane-type diterpene. Similarly, **3** represents unprecedented 16-norcassane-type diterpene, probably derived from 2-acetoxy-3-deacetoxycaesaldekarin e, isolated from the same extract, via oxidative cleavage of the C-15 double bond. Moreover, the antimalarial activity of norcaesalpinin A (**1**) was examined in mice infected with chloroquinene-resistant *P. berghei* (strain NK 65) in 6-day suppressive test.[15,16] At a dose of 10, 1 and 0.1 mg/kg (p.o.) norcaesalpinin A (**1**) significantly suppress the parasitemia by 48.0, 40.9 and 33.0%, respectively. At the same concentrations artemisinin, a known antimalarial drug used as positive control, reduced parasititemia by 65.5, 37.4 and 26.9%, respec-

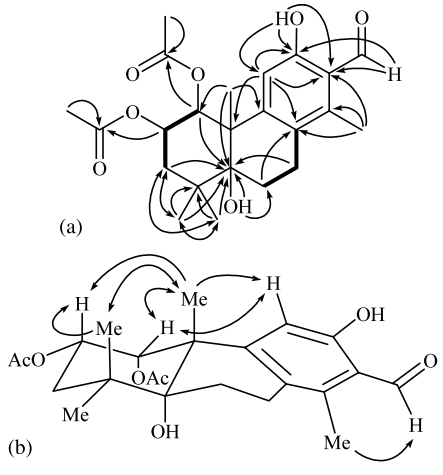

Figure 2. Connectivities (bold line) deduced by the COSY and HMQC spectra and significant long-range correlations (arrows) observed in the HMBC spectrum of **3** (a) and NOE correlations observed in the difference NOE experiments (b).

tively. These results suggested that the antimalarial activity of the CH_2Cl_2 extract of *C. crista* was possibly due to diterpenes. The mechanism of norcaesalpinin A (**1**) and the antimalarial activity of other isolated compounds were under progress and will be reported in a full paper.

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2. The antimalarial activity of extract was carried out according to the method in the literatures (Refs. 3 and 4). In brief, Balb/c mice (7- to 8-week-old, 20–25 g) were used for the present study. Parasites were collected into a heparinized syringe from tail vain of a donor mouse infected with chloroquine-sensitive *Plasmodium berghei* (strain Anka) harbouring about 20% parasitemia. The blood was diluted with mixture of 0.48% citric acid, 1.32% sodium citrate and 1.47% dextrose (ACD) to a final concentration of 1×10^6 infected erythrocytes per 1.0 ml and administrated to the fresh mice intraperitoneally. The CH_2Cl_2 extract of *C. crista* dissolve in 1% carboxymethyl cellulose (CMC) was given orally to newly inflected mice (three mice per group) at doses of 1, 5 and 10 mg/mice three time per day, when the parasitemia level of the blood became 1% (designated as day 0). Equal volume of 0.5% CMC-treated group was considered as a control. To evaluate the antimalarial activity of the extract, tail blood smears were prepared and stained with Giemsa (E. Merck, Germany) every day after treatment of the extract. The parasitemia level in the blood of the mice decreased after 2 consecutive day of the treatment by CH_2Cl_2 extract and% suppression of the parasitemia was calculated on the 4th day using the formula: [(average% of parasitemia in control–average% of parasitemia in treated mice)/average% of parasitemia in control]×100. The suppression of parasitemia at 10, 5 and 1 mg/kg doses were 98.6, 95.5 and 73.2%, respectively. The parasitemia level of the control group was 19.8% at the same time interval and considered to be 100% parasitemia.
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10. **Norcaesalpinin A (1)**: Colorless amorphous solid; $[\alpha]_D^{25}$ −20.1° (*c* 0.15, $CHCl_3$); CD λ_{max} (2.51×10^{-4} M, EtOH) nm: 302 ([θ] +6993), 263 ([θ] −8597); IR ($CHCl_3$) ν_{max} 3625, 1735, 1670 cm^{-1}; HRFABMS *m/z*: 419.2073 [calcd for $C_{23}H_{31}O_7$ $(M+H)^+$, 419.2070].
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13. **Norcaesalpinin B (2)**: Colorless amorphous solid; $[\alpha]_D^{25}$ +32.1° (*c* 0.10, $CHCl_3$); CD λ_{max} (2.87×10^{-4} M, EtOH) nm: 300 ([θ] +5337), 263 ([θ] −9757); IR ($CHCl_3$) ν_{max} 3650, 1735, 1670 cm^{-1}; FABHRMS *m/z*: 419.2056 [calcd for $C_{23}H_{31}O_7$ $(M+H)^+$, 419.2070].
14. **Norcaesalpinin C (3)**: Colorless amorphous solid; $[\alpha]_D^{25}$ −55.3° (*c* 0.10, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3575, 1785, 1640 cm^{-1}; FABHRMS *m/z*: 419.2084 [calcd for $C_{23}H_{31}O_7$ $(M+H)^+$, 419.2070].
15. The antimalarial activity of norcaesalpinin A (**1**), on the other hand, tested in the mice infected with chloroquinene-resistant *P. berghei* (strain NK 65) according to the method described by Murakami et al., 2003 (Ref. 16). The diluted blood (2.0 ml) containing 1×10^7 infected erythrocytes, collected from donor mice, were inoculated intravenously in the tail vain of mice. The test compound **1** and artemisinin suspended in 0.5% CMC (0.5 ml) were given per oral at doses of 10, 1 and 0.1 mg/kg daily (five mice per group), the first administration was made 2 h after parasite inoculation (designated as day 0). At day 6 the parasitemia levels of control group treated only with 0.5% CMC became 18.2% and the parasitemia level of compound 1 and artemisinin treated groups and suppression of parasitemia were calculated using the same equation mention above.
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