NEW CASSANE DITERPENES FROM *CAESALPINIA BONDUC* (FABACEAE)

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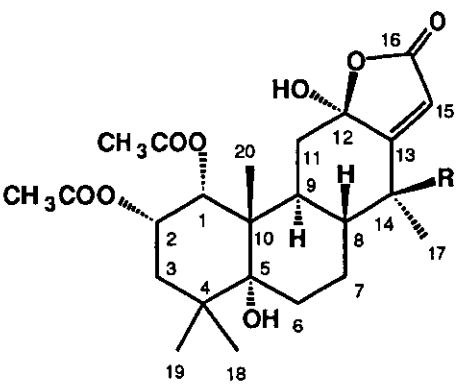
Abstract—New cassane diterpenes, neocaesalpins A and B, were isolated from the seeds of *Caesalpinia bonduc* (Fabaceae) and their structures were deduced on the basis of the spectroscopic evidence. These compounds are characterized as having an α,β–butenolide moiety whereas caesalpins, a group of diterpenes previously known from the same plant and related species, possess the furan ring. The possible biogenetic scheme for formation of an α,β–butenolide was postulated as the furan ring undergoing peroxidation followed by isomerization to give rise to the hemiketal lactone.

Caesalpinia bonduc (L.) Roxb. is a leguminous climber of pantropic occurrence, and is known as medicinal in various regions of the tropics. For example, the seeds of this plant, which are called '*Mata hyang*' in Java, Indonesia, are used as emetic or for the treatment of dysentery, and are also found as one of the ingredients in traditional Indonesian mixed herbal medicines (*jamu*). There have been several reports of chemical investigation on the seeds of this plant under its synonymous name *Caesalpinia bonducella*,[1-5] and a number of characteristic furanoditerpenes (caesalpins) have been isolated. In this report we describe the isolation and characterization of new cassane diterpenes from the seeds of this plant collected in Flores Island, Indonesia.



(1) R=OH neocaesalpin A
(2) R=H neocaesalpin B

The chloroform extract of the seeds of this plant was partitioned between methanol and hexane, and oily substances including glycerides were mostly removed by this procedure. The residue obtained from evaporation of the methanol layer was subjected to a silica gel column chromatography to give rise to two new compounds, for which the names of neocaesalpins A and B are proposed.

Neocaesalpin A was obtained as optically active colorless prisms, mp 267-270°, $[\alpha]_D$ -37.2°. It had a composition of $C_{24}H_{34}O_9$ as determined by the high resolution mass spectrometry. The structure of

Table I. ^{1}H-Nmr Spectral Data for Neocaesalpins A (**1**) and B (**2**).

Protons	neocaesalpin A (1)			neocaesalpin B (2)		
	Chemical Shifts[a)]	Multiplicity	Coupling Constants (*J* in Hz)	Chemical Shifts[a)]	Multiplicity	Coupling Constants (*J* in Hz)
H-1	5.58	*d*	2.9	5.54	*d*	2.4
H-2	5.54	*d d d*	2.9, 4.1, 13.0	5.52	*d d d*	2.4, 4.1, 13.1
H-3α	2.33	*d d*	13.0, 13.0	2.30	*d d*	13.1, 13.1
H-3β	1.40	*d d*	4.1, 13.0	1.40	*d d*	4.1, 13.1
H-6α	1.77	*d d*	2.1, 13.4	1.69	*d d*	2.7, 13.3
H-6β	1.59	*d d d*	4.0, 13.4, 13.4	*ca* 1.58[b)]	-	-
H-7α	2.27	*d d d*	12.2, 12.8, 13.4	2.15-2.20[c)]	-	-
H-7β	2.12	*d d d d*	2.1, 4.0, 4.5, 12.8	*ca* 1.24[c)]	-	-
H-8	1.94	*d d d*	4.5, 12.2, 12.2	*ca* 1.58[b)]	-	-
H-9	3.07	*d d d*	3.0, 12.2, 12.5	3.10	*d d d*	2.7, 12.6, 12.6
H-11α	2.50	*d d*	3.0, 12.8	2.42	*d d*	2.7, 12.6
H-11β	1.52	*d d*	12.5, 12.8	1.33	*d d*	12.6, 12.6
H-14	-	-	-	2.88	*d q*	4.6, 7.2
H-15	6.39	*s*	-	5.82	*s*	-
17-Me	1.87	*s*	-	1.35	*d*	7.2
18-Me	1.19	*s*	-	1.17	*s*	-
19-Me	1.10	*s*	-	1.10	*s*	-
20-Me	1.10	*s*	-	1.05	*s*	-
OAc	2.04	*s*	-	2.01	*s*	-
	2.20	*s*	-	2.15	*s*	-

a) measured in pyridine-d_5 with TMS as an internal standard (500 MHz). b) Two signals are overlapped.
c) Coupling constants are not observable because of obscure coupling patterns.

this compound was established as **1** on the basis of the spectroscopic evidence as mentioned below. It exhibited an ultraviolet absorption maximum at 213 nm, and infrared bands at 1649 and 1715 cm^{-1}, suggesting the presence of an α,β–butenolide moiety. The ^{1}H-nmr spectrum indicated the presence of four tertiary methyls, two acetyls, and sets of complicated coupling systems, which were finally attributed to the tricarbocyclic system. With the aid of ^{1}H-^{1}H COSY those complex resonances were analyzed as shown in Table I. In the ^{13}C-nmr spectrum five quarternary, four tertiary, four secondary and six primary sp^3 carbons were recognized along with one quarternary and one tertiary sp^2 carbons, and three carbonyl carbons as shown in Table II. A combination of 2D nmr techniques, ^{13}C-^{1}H COSY and HSBC,[6] helped to combine those carbon units to construct the structure (**1**) for neocaesalpin A except its stereochemistry (Figure 1). Two carbon signals (δ 76.4 and 74.3) in the downfield region of the ^{13}C-nmr spectrum were typical of the oxygenated sp^3 quarternary carbons and were assigned to C-5 and C-14 respectively. A carbon signal at δ 106.5 in the further downfield region was assigned to a characteristic sp^3 carbon of the hemiketal. The stereochemistry for the structure (**1**) was determined as follows. The coupling constant between H-1 and H-2 was 2.9 Hz, and hence the configuration of vicinal acetoxy groups at C-1 and C-2 was suggested to be *syn*. The value of 12.2 Hz for the coupling constant between H-8 and H-9 corresponded to the *trans* junction of B- and C-rings. These stereochemical features were further substantiated by phase-sensitive NOESY correlations of the corresponding protons (Figure 1). The configuration of the hemiketal hydroxyl was unequivocally determined to be α by the presence of significant NOESY correlations between 17-methyl and H-7α/H-9 and the absence between 17-methyl and H-7β, which indicated that

the C-ring is in chair form. These correlations were also confirmed by the irradiation experiments. Thus the relative stereostructure of neocaesalpin A was established as formula (**1**).

Table II. ^{13}C-Nmr Spectral Data for Neocaesalpins A (**1**) and B (**2**).

carbons	neocaesalpin A (**1**) Multiplicity*	Chemical Shifts**	neocaesalpin B (**2**) Multiplicity*	Chemical Shifts**
1	*d*	74.4	*d*	74.3
2	*d*	67.9	*d*	67.8
3	*t*	36.0	*t*	36.0
4	*s*	40.3	*s*	40.3
5	*s*	76.4	*s*	76.4
6	*t*	26.0	*t*	26.0
7	*t*	20.0	*t*	23.8
8	*d*	47.7	*d*	39.5
9	*d*	35.2	*d*	33.1
10	*s*	45.4	*s*	45.2
11	*t*	39.1	*t*	38.9
12	*s*	106.3	*s*	106.5
13	*s*	170.6	*s*	171.0
14	*s*	74.3	*d*	36.9
15	*d*	112.8	*d*	113.2
16	*s*	178.8	*s*	174.4
17	*q*	21.6	*q*	13.2
18	*q*	28.4	*q*	28.4
19	*q*	25.4	*q*	25.4
20	*q*	17.3	*q*	17.2
$CH_3\underline{C}O$	*s*	170.3	*s*	170.2
	s	170.6	*s*	170.3
$\underline{C}H_3CO$	*q*	20.9	*q*	20.8
	q	21.0	*q*	20.9

* based on DEPT ; ** measured in pyridine-d_5 (125 MHz) with TMS as internal standard.

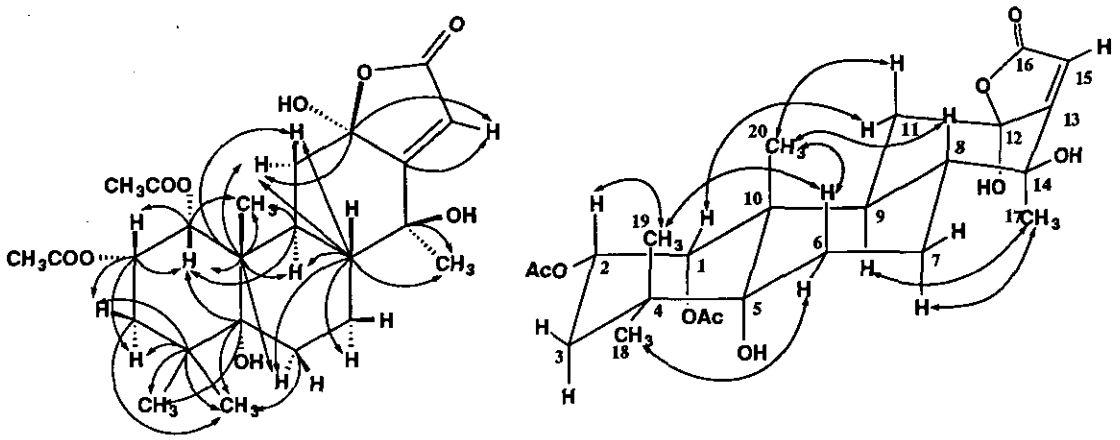
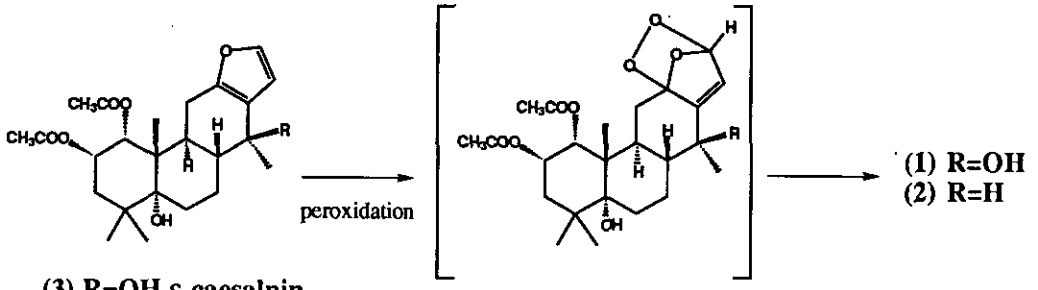


Figure 1. ^2J and ^3J Interactions (left) and NOE interactions (right) of **1** obtained from HSBC and phase-sensitive NOESY respectively.

Neocaesalpin B was obtained as optically active colorless prisms, mp 150-152°, $[\alpha]_D$ -26.5°. The molecular formula was calculated to be $C_{24}H_{34}O_8$, one oxygen short of that of neocaesalpin A, based on the high

resolution mass spectrometry. Its spectroscopic properties were very similar to those of neocaesapin A. In comparison of the ^{1}H-nmr resonances of neocaesalpin B with those of neocaesalpin A as shown in Table I, a tertiary methyl signal (δ 1.87) assigned to 17-methyl of neocaesalpin A shifted upfield as a doublet to δ 1.35 in the spectrum of neocaesalpin B. It was also observed that protons in the proximity of C-14 , *e.g.*, H-7β, H-8 and H-15, underwent significant upfield shifts. This finding suggested neocaesalpin B to be a C-14 deoxy form (**2**) of neocaesalpin A. Results of the ^{13}C-nmr spectrum (Table II) were also consistent with the structure (**2**) for neocaesalpin B: the outstanding difference being the presence of secondary sp^3 carbon (δ 36.9) in place of a qurternary oxygenated sp^3 carbon (δ 74.3). The configuration of 14-methyl was determined as α based on the magnitude of a coupling constant between H-8 and H-14 (4.6 Hz).

All diterpenes known from *Caesalpinia bonduc*[1-5] and other related species[7-9] are furan-fused tricarbocyclic derivatives. Thus the presence of a α,β–butenolide would characterize neocaesalpins A and B. It will be presumed that the butenolide moiety would arise from peroxidation of the corresponding furan followed by isomerization through either ionic or radical-based mechanisms as illustrated in Figure 2. The precursor for neocaesalpin A (**1**) would be ε-caesalpin (**3**),[2] a known component from the same plant. However, that for neocaesalpin B (**2**) remains to be known from nature. There have been several reports that in certain furanoterpenes the furan ring undergoes rapid autoxidation, even on standing in air, to give rise to the corresponding hemiketal lactones.[10] Though the chemistry of caesalpins was extensively investigated during the course of their sturucture elucidation,[1] there has been no indication that caesalpins are susceptible to air-oxidation. Hence neocaesalpins A and B are considered to be natural products arising from enzyme-catalyzed process.



(3) R=OH ε-caesalpin
(4) R=H unknown

Figure 2. A Possible Biogenetic Scheme for Formation of Neocaesalpins A (**1**) and B (**2**) from Corresponding Precursors.

EXPERIMENTAL

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-nmr) spectra with a JEOL JNM GSX-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard; mass spectra (ms) with a JEOL JMS-SX-102A mass spectrometer; infrared (ir) spectra with a JASCO FT/IR-8000 infrared spectrophotometer; ultraviolet

(uv) spectra with a Shimadzu UV-240 UV spectrometer; optical rotations with a JASCO DIP-370 polarimeter. Column chromatography was carried out with the following materials: Wakogel C-200 (eluted with hexane-ethyl acetate). Thin-layer chromatography (tlc) was conducted on a 0.25 mm pre-coated silica gel plate ($60GF_{254}$, Merck), and spots were detected by inspection under short (254 nm) or long (360 nm) wavelength uv lights, or by the colors developed with 10% H_2SO_4 spraying followed by heating on a hot plate.

Plant material The seeds of *C. bonduc* were collected in August, 1988 near Maumere in Flores Island during the Indonesia-Japan joint field survey on medicinal plants in Nusa Tenggara Timur, Indonesia. This survey was undertaken under Monbusho International Scientific Research Program: Field Research entitled "Investigation of Naturally Occurring Drug Materials in Indonesia-2" (headed by Prof. Isao Kitagawa, Osaka University, Osaka, Japan), and was endorsed by Pusat Penelitian dan Pengembangan Biologi (National Institute of Biology, Bogor, Indonesia). The first author (T. K.) participated as a regular member in this field survey. Plant samples collected in this survey were identified by Dr. Harry Wiriadinata and Mr. Tahan Uji of Bogor Botanical Garden, Bogor, Indonesia, who were also regular members of this research team. Voucher specimens of *C. bonduc* are on deposit at Faculty of Pharmaceutical Sciences, Osaka University and at Herbarium Bogoriense, Bogor, Indonesia (code name: IK 071).

Extraction and Isolation The crushed seeds (2.9 Kg) of *C. bonduc* were extracted three times with $CHCl_3$ (3 *l*) at room temperature for 3 days, and the combined extracts were evaporated to dryness under reduced pressure to yield a greenish brown extract (306 g). The extract separated into solid (46 g) and oil on cooling at room temperature, and the oily part (260 g) was partitioned between MeOH (1 *l*) and hexane (2 *l*). The MeOH layer was taken and evaporated to dryness under reduced pressure to give the residue (93 g). The residue was subjected to a silica gel column chromatography on elution with the following solvent system: *n*-hexane (2 *l*), *n*-hexane-AcOEt 97:3 (2 *l*), 94:6 (2 *l*), 9:1 (2 *l*), 85:15 (2 *l*), 4:1 (2 *l*), 3:1 (2 *l*), 2:1 (2 *l*), 1:1 (2 *l*), 1:3 (4.5 *l*) and acetone. Fractions of 500 m*l* each were taken and forty-eight fractions collected. Neocaesalpin A (1) was obtained as crystalline precipitates from the acetone solution of Fr. 33 and 34. Yield: 547 mg. The acetone solution of Fr. 39 afforded 415 mg of neocaesalpin B (2) as colorless crystals.

Neocaesalpin A (1) Colorless prisms from acetone, mp 267-270°C, $[\alpha]_D^{22}$ -37.2° (*c*=0.175, $CHCl_3$). Ir ν_{max}^{KBr} cm^{-1}: 3576, 3501, 2992, 2938, 1738, 1715, 1649, 1269, 1242, 1173, 1036, 862. Uv λ_{max}^{MeOH} nm (log ε): 213 (4.06). ^1H Nmr (500 MHz, d_6-acetone): see Table I. ^{13}C Nmr (125 MHz, d_6-acetone): see Table II. EIms *m/z* (rel. int.): 466 (M^+, 0.2), 406 (21), 346 (24), 291 (12), 135 (100). HRms: $[M]^+$ 466.2208 ($C_{24}H_{34}O_9$ requires 466.2203). *Anal.* Calcd for $C_{24}H_{34}O_9$: C, 61.79; H, 7.35. Found: C, 61.95; H, 7.40.

Neocaesalpin B (2) Colorless prisms from acetone, mp 150-152°C, $[\alpha]_D^{22}$ -26.5° (*c*=0.227, $CHCl_3$). Ir ν_{max}^{KBr} cm^{-1}: 3573, 3507, 2988, 2943, 1736, 1715, 1651, 1375, 1269, 1242, 1175, 1032, 930. Uv λ_{max}^{MeOH} nm (log ε): 213 (4.11). ^1H Nmr (500 MHz, d_6-acetone): see Table I. ^{13}C Nmr (125 MHz, d_6-acetone): see Table II. EIms *m/z* (rel. int.): 450 (M^+, 0.4), 390 (34), 330 (100), 315 (30), 275 (27), 161 (40). HRms: $[M]^+$ 450.2258 ($C_{24}H_{34}O_8$ requires 450.2253). *Anal.* Calcd for $C_{24}H_{34}O_8$: C, 63.98; H, 7.61. Found: C, 64.22; H, 7.66.

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