Chemical Studies on the Philippine Crude Drug Calumbibit (Seeds of *Caesalpinia bonduc*): The Isolation of New Cassane Diterpenes Fused with α,β-Butenolide

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New cassane diterpenes named neocaesalpins C and D were isolated from the Philippine crude drug calumbibit botanically originating from the seeds of *Caesalpinia bonduc* (Fabaceae), and their structures were elucidated on the basis of the spectroscopic evidence. These compounds are characterized by the presence of the α,β-butenolide moiety. Although a number of cassane furanoditerpenes have been known to occur in the same plant species, such constituents could not be isolated from the crude drug of Philippine origin in this study. It is presumed that the chemical difference resulted from chemical differentiation of the species.

Key words *Caesalpinia bonduc*; Fabaceae; cassane diterpene; Philippine folk medicine; calumbibit

Caesalpinia bonduc (L.) ROXB. (Fabaceae) is a stout prickly climber distributed throughout the tropical and subtropical regions. This plant has been recognized as medicinal in traditional medicine of various parts of the world where it occurs. Calumbibit is one of the most esteemed crude drugs in the Philippines, and botanically originates from the seeds of this plant. It is regarded as a febrifuge and purgative and is also considered to be effective for the treatment of malaria.[1] Although the seeds of this plant are chemically predominated by oil and fats, early investigations, which were carried out under its synonymous name *C. bonducella*, revealed the presence of the characteristic cassane furanoditerpenes, α-, β-, γ-, δ-, ε-caesalpin and caesalpin F.[2—8] The author also investigated the seeds of *C. bonduc* collected in Indonesia and reported earlier on the isolation and structure elucidation of neocaesalpins A and B, which are characterized by the presence of α,β-butenolide instead of the furan in caesalpins.[9] The subsequent investigation of the Philippine crude drug calumbibit has furnished two new cassane diterpenes, and their isolation and structure elucidation are described in this paper.

The chloroform extract of crushed calumbibit was subjected to the same separation procedure described previously,[9] which led to the isolation of two new compounds for which the names neocaesalpins C and D are proposed. Neocaesalpin C (**1**) was obtained as colorless prisms and its molecular formula was calculated as $C_{24}H_{36}O_9$ based on the high-resolution fast atom bombardment (HR-FAB) mass spectral analysis. The infrared (IR) spectrum had an absorption band at 3584 cm^{-1} indicating the presence of hydroxyl groups in the molecule. The IR bands at 1736 and *ca.* 1720 cm^{-1} (overlapping with ester groups) are assignable to the α,β-butenolide, and the presence of this moiety was further substantiated by the ultraviolet (UV) absorption maximum at 214 nm ($\log \varepsilon = 4.13$). The ^{1}H-nuclear magnetic resonance (NMR) spectrum (Table 1) was very similar to that of neocaesalpin B (**2**), one of the cassane diterpenes which were obtained from the seeds of *C. bonduc* of Indonesian origin, except for proton signals at H-6, H-7, H-8, and H-14. These signals were observed more than 0.3 ppm downfield due to the presence of a hydroxyl group at the 7-position. A proton signal at the 7-position was observed at δ 4.68 ppm as ddd with the following coupling constants: $J_{7\alpha\text{-}6\beta} = 5.7$, $J_{7\alpha\text{-}6\beta} = J_{7\alpha\text{-}8} = 10.8$ Hz. This finding unequivocally assigned the configuration of the 7-hydroxyl group as β. Therefore the structure of neocaesalpin C was elucidated as **1**. Comparative analysis of the ^{13}C-NMR spectra between neocaesalpins B and C (Table 2) was also consistent with the structure **1** for neocaesalpin C, with the outstanding difference being the presence of a secondary alcoholic methine carbon (δ 66.0) in place of a methylene carbon (δ 23.8).[9]

Neocaesalpin D (**3**) was obtained in crystalline forms, mp 209—213 °C. It had the molecular formula of $C_{24}H_{32}O_7$ according to HR-FAB mass spectral analysis. It is apparent from its ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) that this compound is a diterpene closely related to neocaesalpin B. The UV absorption maximum at 278 ($\log = 4.23$) indicated that this compound has an α,β-butenolide ring conjugated with one extra double bond. The presence of this conjugate system was also supported by the ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2), as mentioned below. Proton signals assigned to H-11α and H-11β in **2** were missing in the ^{1}H-NMR spectrum of **3**, and a new broad olefinic proton signal was observed at δ 5.92, which was assigned to H-11. The ^{13}C-NMR spectrum of **3** lacked the characteristic hemiketal sp^3 carbon found in that of **1**, and instead two quarternary sp^2 carbons were observed at δ 111.1 and 151.2 which were assigned to C-11 and C-12, respectively. The IR bands at 1788 and 1767 cm^{-1} will be assigned to this conjugate system. The spectral findings stated above assign the structure of neocaesalpin D to **2**. This structure is apparently derived from dehydration of the hemiketal hydroxyl group, but attempts at chemical dehydration from **2** to **3** failed.

All diterpenes obtained by other research groups from *C.*

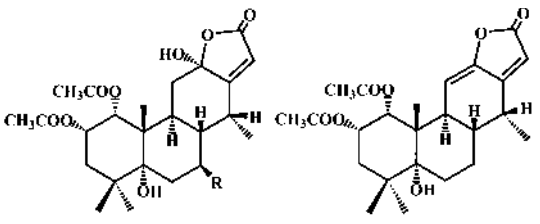

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Table 1. ^{1}H-NMR[a] Spectral Data for Neocaesalpins C and D

	Neocaesalpin C		Neocaesalpin D	
	δ ppm[b]	Multiplicity (J)[c]	δ ppm[b]	Multiplicity (J)[c]
H-1	5.67	d (2.9)	5.69	d (3.0)
H-2	5.55	ddd (2.9, 4.0, 13.2)	5.61	ddd (3.0, 4.7, 13.0)
H-3α	2.31	dd (13.2, 13.2)	2.35	dd (13.0, 13.0)
H-3β	1.41	dd (4.0, 13.2)	1.39	dd (4.7, 13.0)
H-6α	2.42	dd (5.7, 13.2)	1.69	ddd (2.0, 2.4, 12.8)
H-6β	1.87—1.93	m (10.8, 13.2)	1.59	ddd (4.3, 12.8, 12.8)
H-7	4.68	ddd (5.7, 10.8, 10.8)	α 2.00—2.08	m
			β 1.17	m
H-8	1.93—1.96	m (10.8, 12.8)	1.76	ddd (4.3, 10.4, 10.4)
H-9	3.29	ddd (2.8, 12.8, 12.8)	3.44	br d (10.4)
H-11	α 2.51	dd (2.6, 12.8)	5.92	br s
	β 1.46	dd (12.8, 12.8)		
H-14	3.87	dq (4.8, 7.2)	2.67	dq (4.4, 7.2)
H-15	5.83	s	5.88	d (0.9)
17-Me	1.57	d (7.2)	0.91	d (7.2)
18-Me	1.21	s	1.12	s
19-Me	1.12	s	1.03	s
20-Me	1.16	s	1.07	s
CH_3COO	2.01	s	2.03	s
	2.16	s	2.11	s

a) Spectra were measured at 400 MHz in pyridine-d_5. *b*) Chemical shifts as δ ppm with TMS as the internal standard. *c*) Multiplicity and coupling constants (J Hz) in parentheses.

Table 2. ^{13}C-NMR[a] Spectral Data for Neocaesalpins C and D

C	Neocaesalpin C	Neocaesalpin D
1	74.2	73.2
2	67.8	67.6
3	35.6	36.1
4	40.3	40.4
5	78.2	76.6
6	36.4	26.4
7	66.0	23.9
8	47.6	37.5
9	32.8	36.9
10	45.3	45.4
11	38.9	111.1
12	106.6	151.2
13	171.1	161.8
14	33.4	33.5
15	113.5	110.6
16	174.7	170.5[b]
17	13.0	14.3
18	28.4	27.8
19	25.5	24.7
20	17.6	19.5
$CH_3\underline{C}O$	170.2	170.2[b]
	170.3	170.3[b]
$\underline{C}H_3CO$	20.8	20.7
	21.0	20.9

a) Spectra were measured at 100 MHz in pyridine-d_5. *b*) Assignments in the same column may be interchanged.

bonduc (syn. *C. bonducella*) are tricarbocyclic derivatives fused with a furan ring.[2—8] However, the author's group has obtained from the seeds of the same plant species not furanoditerpenes but cassane-type diterpenes fused with the butenolide ring.[9] It is of interest to note that all of the plant sources from which furanoditerpenes were isolated are derived from the Western hemisphere, and not of Asian origin. As mentioned at the beginning of this article, the phytogeographic distribution of *C. bonduc* covers almost the entire tropics and subtropics worldwide. Although there are no data available on the morphologic diversity of this species, it can be presumed that several chemotypes exist depending upon phytogeographic distribution as a result of chemical differentiation. There is growing concern over the use of traditional medicines for either the treatment or prevention of diseases worldwide, and standardization of natural medicines has become one of the most important issues to maintain quality control. The results of this study may indicate that attention should be paid not only to the botanical origin (identification of species) but also chemical variation of natural medicines in the process of standardization.

Experimental

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: ^{1}H- and ^{13}C-NMR spectra with a JEOL JNM GSX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer with tetramethylsilane (TMS) as internal standard; mass spectra (MS) with a JEOL SX-102A mass spectrometer; IR spectra with a JASCO FT/IR-8000 infrared spectrometer; optical rotations with a JASCO DIP-370 polarimeter and UV spectra with a Shimadzu UV-240 spectrometer. Column chromatography was carried out with Wakogel C-200 (eluted with hexane–ethyl acetate).

Plant Material Calumbibit, the seeds of *C. bonduc*, was purchased at the Quiapo crude drug market in Metro Manila, the Philippines, in March 1998, when the collection of Philippine crude drugs was undertaken as part of the Monbusho International Scientific Research Program: Field Research entitled "Ethnobotanical Survey in Philippine Tropical Rain Forest," headed by the author. The sample specimen of calumbibit was deposited as a voucher at the Medicinal Plant Research Station, Faculty of Pharmaceutical Sciences, Teikyo University.

Extraction and Isolation The crushed calumbibit (502 g) was extracted three times with chloroform at room temperature, and the combined extracts were evaporated to dryness under reduced pressure to yield a brown extract (49 g). The whole extract was partitioned between methanol (200 ml) and hexane (400 ml) and the methanol layer was evaporated to dryness under reduced pressure to give the residue (13 g). The residue was subjected to silica gel column chromatography on elution with a mixed solvent system of hexane and ethyl acetate, increasing the amount of the latter gradually. Each fraction collected was evaporated to dryness under reduced pressure, and the residue was dissolved in a small amount of acetone. Certain fractions fur-

nished crystalline precipitates, which were collected and recrystallized from acetone to give neocaesalpins C (16 mg) and D (95 mg).

Neocaesalpin C (1) Colorless prisms, mp >260 °C. $[\alpha]_D^{25}$ −50° (c=0.034). IR (KBr) cm^{-1}: 3584, 2946, 1736, 1368, 1252, 1227, 1034. UV λ_{max} (MeOH) nm (log ε): 214 (4.13). ^{1}H-NMR (pyridine-d_5) δ: Table 1. ^{13}C-NMR (pyridine-d_5) δ: Table 2. EI-MS m/z (int. %): 448 (M^+-H_2O, 1), 430 ($M^+-2\times H_2O$, 11), 406 (M^+-CH_3COOH, 25), 388 ($M^+-CH_3COOH-H_2O$, 17), 370 ($M^+-CH_3COOH-2\times H_2O$, 53), 346 ($M^+-2\times CH_3COOH$, 44), 328 ($M^+-2\times CH_3COOH-H_2O$, 89), 310 ($M^+-2\times CH_3COOH-2\times H_2O$, 100). FAB-MS m/z: 489.2116 (Calcd for $C_{24}H_{36}O_9\cdot Na^+$: 489.2100).

Neocaesalpin D (2) Colorless prisms, mp 209—213 °C. $[\alpha]_D^{25}$ +71.9° (c=0.089). IR (KBr) cm^{-1}: 2944, 1788, 1767, 1730, 1373, 1256, 1231. UV λ_{max} (MeOH) nm (log ε): 278 (4.23). ^{1}H-NMR (pyridine-d_5) δ: Table 1. ^{13}C-NMR (pyridine-d_5) δ: Table 2. EI-MS m/z (int. %): 414 (M^+-H_2O, 7), 372 (M^+-CH_3COOH, 10), 354 ($M^+-CH_3COOH-H_2O$, 42), 312 ($M^+-2\times CH_3COOH$, 59), 294 ($M^+-2\times CH_3COOH-H_2O$, 100. FAB-MS m/z: 433.2243 (Calcd for $C_{24}H_{32}O_7\cdot H^+$: 433.2226).

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References and Notes

1) Quisumbing E., "Medicinal Plants of the Philippines," Katha Publishing Co., Quezon, Philippines, 1978, pp. 369—372. In this literature the origin of calumbibit was described as *C. crista* L., while *C. bonduc* and *C. bonducella* were treated as its synonyms.
2) Ali M. E., Khuda M. Q., *Chem. Ind.* (London), **1960**, 463—464.
3) Canonica L., Jommi G., Manitto P., Pagononi U. M., Pelizzoni F., *Gazz. Chim. Ital*., **96**, 662—686 (1966).
4) Canonica L., Jommi G., Manitto P., Pagononi U. M., Pelizzoni F., *Gazz. Chim. Ital*., **96**, 687—697 (1966).
5) Canonica L., Jommi G., Manitto P., Pagononi U. M., Pelizzoni F., *Gazz. Chim. Ital*., **96**, 698—715 (1966).
6) Balmain A., Connolly J. D., Ferrari M., Ghisalberti E. L., Pagononi U. M., Pelizzoni F., *Chem. Commun*., **1970**, 1244—1245.
7) Pascoe K. O., Burke B. A., Chan W. R., *J. Nat. Prod*., **49**, 913—915 (1986).
8) Balmain A., Bjamer K., Connolly J. D., Ferguson G., *Tetrahedron Lett*., **49**, 5027—5031 (1967).
9) Kinoshita T., Kaneko M., Noguchi H., Kitagawa I., *Heterocycles*, **43**, 409—414 (1996).