The Isolation and Structure Elucidation of New Cassane Diterpene-Acids from *Caesalpinia crista* L. (Fabaceae), and Review on the Nomenclature of some *Caesalpinia* Species

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New cassane diterpene-acids, neocaesalpins H and I, were isolated from the leaves of *Caesalpinia crista* (Fabaceae), and their structures were deduced on the basis of the spectroscopic and chemical basis. These compounds were characterized as having an α,β-butenolide hemiacetal ring that is rare in nature. The lacking of 5-hydroxy group also distinguished neocaesalpins H and I from cassane diterpenes (caesalpins) occurring in other *Caesalpinia* species from the phytochemical viewpoint. The nomenclature of three *Caesalpinia* species was also reviewed, and it was found that some species belonging to the genus *Caesalpinia* are improperly named and should be changed to valid names.

Key words *Caesalpinia crista*; Fabaceae; cassane diterpene; neocaesalpin; nomenclature; scientific name

Plants belonging to the genus *Caesalpinia* (Fabaceae) occur mainly in the tropics and subtropics, and a few species among them have been utilized as crude drugs in folk medicine throughout the regions where they occur botanically.[1)] Extensive chemical investigation has been undertaken exclusively on *C. bonduc* (L.) ROXB.,[1—4)] *C. major* (MEDIC.) DANDY *et* EXELL (also under the name of its synonymous name *C. bonducella*),[5—9)] and *C. pulcherrima* (L.) SWARTZ[10—15)] of medicinal value among the genus, which revealed the presence of characteristic furano-diterpenes phytochemically classified as cassane diterpenes and otherwise known as caesalpins. We also undertook chemical studies on *C. bonduc* seeds that have been reputed in both Indonesian and Philippine traditional medicine, and reported the isolation of a new type of hemiacetal diterpenes fused with an α,β-butenolide ring for which the general skeletal name of neocaesalpins was proposed.[1,2)] Though the genus *Caesalpinia* consists of more than 100 species, most plant species belonging to this genus have long remained uninvestigated chemically. This fact has thus instigated us to further engage in the chemistry of *Caesalpinia* species. *Caesalpinia crista* L. ("nantenkadsura" in Japanese) is, the one selected by us for phytochemical study under the concept stated above, a prickly climber that occurs mainly in the mangrobe marsh in the tropics or subtropics including the Ryukyu Islands south of Yakushima Island, Japan. We started chemical study on this species in order to obtain phytochemical accounts of this species on which no chemical information under its name with the solid evidence is available, and herewith report the isolation and structure elucidation of two novel cassane diterpene-acids named neocaesalpins H (**1**) and I (**2**). During a search of literatures concerning the chemistry of *Caesalpinia* species, we have encountered confused use of botanical names in some *Caesalpinia* species. Therefore, the botanical nomenclature of some *Caesalpinia* species was also reviewed, which led to the suggestion that some may require correction of names.

Crushed dry leaves of *Caesalpinia crista* were extracted with acetone, and the extract was subjected to a series of column chromatography over silica gel, Sephadex LH-20 and reversed-phase silica gel. Further purification of chromatographic fractions by preparative thin layer chromatography led to the isolation of neocaesalpins H (**1**) and I (**2**) in pure forms.

Neocaesalpin H (**1**) was isolated as optically active colorless needles, mp 255—256 °C, $[\alpha]_D^{25}$ −73.2°. The molecular formula of **1** was deduced as $C_{20}H_{28}O_5$ from the high-resolution mass spectrometry. The IR spectrum showed carbonyl absorptions at 1738 (lactone) and 1717 cm^{-1} (carboxyl). The UV absorption maximum at 213 nm along with the IR absorption band at 1738 cm^{-1} attested to the presence of an α,β-butenolide ring. This was further substantiated by the presence of the following signals in its ^{1}H- and ^{13}C-NMR spectra: an olefinic proton at δ 5.87 (s), a carbonyl carbon at δ 171.38, a quaternary olefin carbon at δ 174.46 and an olefinic methine carbon at δ 113.22. These ^{1}H- and ^{13}C-NMR signals along with one sp^3 quaternary carbon at δ 106.86 in its ^{13}C-NMR spectrum were in agreement with those assigned to an α,β-butenolide hemiacetal occurring in neocaesalpins A—C.[1,2)] The intense fragment ion at *m*/*z* 330 in the mass spectrometry of **1** corresponded to the dehydration of the hemiacetal hydroxyl from the molecular ion. In addition, the ^{13}C-NMR spectrum of **1**, which is summarized in Table 1, revealed the presence of twenty carbon signals accounting for three methyl carbons, six methylene carbons, four sp^3 methine carbons, one olefinic methine carbon, three sp^3 quaternary carbons, one quaternary olefinic carbon as well as two carbonyl carbons. The classification of these carbon species was based on DEPT. Many of proton signals occurred as multiplet peaks reflecting that the structure of **1** was composed of complex spin systems due to the lacking of substituents in rings. However, the signals at δ 1.79, δ 2.13 and δ 2.62 were observed as either clear dd or ddd coupled signals. The HMBC ^2J and/or ^3J correlation (Fig. 1) between a proton signal at δ 2.62 and three carbon signals (C-8, C-12, C-13), and between a proton signal at δ 2.13 and six carbon signals (C-4, C-6, C-7, C-10, C-19, C-20) unequivocally assigned these proton signals to H_α-11 and H-5, respectively. The assignment of H_α-11 and H-5 along with H-9 that had no appreciable HMBC correlation was confirmed by HMQC.

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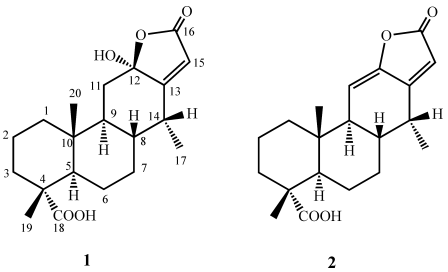
Table 1. ^{13}C-NMR[a)] Data of Neocaesalpins H (**1**) and I (**2**)

Carbon	**1**	**2**
C-1	38.79[b)]	37.61[c)]
C-2	18.44	18.20
C-3	37.48	37.51[c)]
C-4	47.44	47.46
C-5	49.62	49.25
C-6	24.47	24.44
C-7	30.71	30.04
C-8	41.09	38.83
C-9	45.87	48.89
C-10	36.66	37.00
C-11	38.84[b)]	111.37
C-12	106.86	150.60
C-13	174.46	162.17
C-14	36.97	33.56
C-15	113.22	110.40
C-16	171.38	170.38
C-17	13.19	14.37
C-18	181.17	180.93
C-19	17.61	17.02
C-20	14.60	15.68

a) Spectra were measured at 150 MHz with TMS as internal standard. Assignments were based on HMQC and HMBC correlations. *b*, *c*) Assignments may be interchangable.



1 **2**

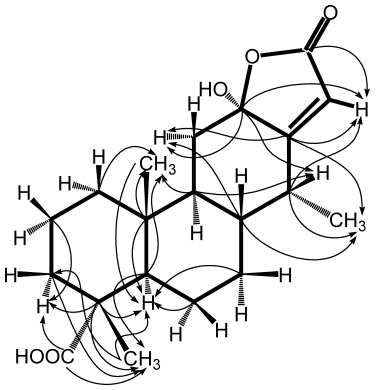


Fig. 1. HMBC Correlations in Neocaesalpin H (**1**)

The assignment of the corresponding carbon signals were in part based on their comparison with those in literatures.[1,2,16)] The further HMBC correlation analysis coupled with HMQC led to the assignment of the rest of signals. Coupling constants of such key protons as H-5 and H-9, which are essential for establishing the stereochemistry of ring fusions, were also obtained. H-9 was observed as ddd with coupling constants of 10.2, 10.2 and 3.0 Hz. Though protons (H_β-11 and H-8) adjacent to H-9 were either overlapped with other signals or observed as multiplets, it is apparent that H-9 shared

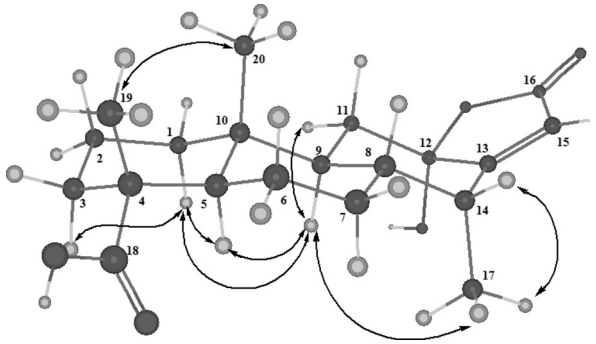


Fig. 2. Molecular Model of Neocaesalpin H (**1**) with Arrows Representing Main ROESY Correlations

coupling constants of 10.2 Hz and 10.2 Hz with both H_β-11 and H-8, since a coupling constant of 3.0 Hz was shared between H-9 and H_α-11. The large coupling constants of 10.2 Hz displayed that H_β-11, H-8 and H-9 were in axial position depicting H-9 transplanar to both 11-H_β and H-8. H-5 proton was also found axial to H_β-6 as reflected by the large coupling constant of 11.2 Hz. The small coupling constant of 1.2 Hz between H-5 and H_α-6 indicated that both protons occurred almost perpendicular to each other. A dq signal at δ 2.77 was assignable to a H-14 methine, which was coupled with both CH_3-17 and an axial H-8 methine at the coupling constants of 7.1 and 4.1 Hz, respectively. H-14 methine proton was depicted equatorial based on the relatively small coupling constant (4.1 Hz) which H-14 shared with H-8. These spectral data stated above suggested that three hexagonal rings are *trans*-fused, and also that a hemiacetal hydroxyl at C-12 is α-configuration in view of its energy-minimized conformation. However, the spectroscopic analyses alone have still left the stereochemistry at the 4-position of **1** undetermined. The ROESY data not only enabled the determination of the stereochemistry at the 4-position but also further substantiated the relative stereochemistry of the whole structure as summarized in Fig. 2 by arrows in the perspective structure of **1**. The presence of correlation from CH_3-19 to CH_3-20 and absence of correlation from CH_3-19 to H-5 and H_α-3 supported that CH_3-19 was β-axial whereas COOH-18 was α-equatorial. The rest of main correlations observed in the ROESY were as follows: H-5 correlated to H-9, H_α-3 and H_α-1, and H-9 correlated to H_α-11 and CH_3-17. The absolute stereochemistry of **1** has not determined yet. It should be noted that only a few caesalpins having the diol functionality have the absolute stereochemistry determined.

Neocaesalpin I (**2**) was obtained in optically active colorless fine needles, mp >260 °C, $[\alpha]_D^{25}$ +27.7°. It had the molecular formula of $C_{20}H_{26}O_4$ according to the high-resolution mass spectrometry. The UV absorption maximum at 277 nm indicated that it had an α,β-butenolide ring conjugated with one extra double bond. This type of a conjugate α,β-butenolide ring was found only in neocaesalpin D[1)] among cassane diterpenes obtained from the genus *Caesalpinia*. Since the proton and carbon resonances of **2** referred to above were in agreement with those assigned to a conjugate α,β-butenolide ring of neocaesalpin D,[1)] the structure of neocaesalpin I was readily presumed to be a dehydrated form of **1**. Treatment of **1** with acid smoothly caused a dehydration to yield one prod-

Table 2. A List of Confusing Scientific Names of Some *Caesalpinia* Species

Synonymous or erroneous (invalid) names	Correct (valid) names	Names in Japanese
C. bonduc Roxb., non L. *C. jajabo* Maza. *C. globulorum* Bakh. *et* v. Royen	*C. major* (Medic.) Dandy *et* Exell	Hasu-no-mi-kadsura
C. bonducella (L.) Flem. *C. crista* sensu auct., non L. *C. crista* L.	*C. bonduc* (L.) Roxb. emend. Dandy *et* Exell	Shirotsubu
C. nuga (L.) Ait. f.	*C. crista* L. emend. Dandy *et* Exell	Nanten-kadsura

uct, which was identical in all respects with **2**.

Quite recently, chemical reports on plant materials under the name of "*C. crista*" have been published.[17,18] However, all compounds but one isolated from this plant species have a tertially hydroxyl group at the 5-position of cassane skeleton and also a few acetyloxy groups,[17] the feature of which characterizes those of *C. bonduc*. There is evidence, as mentioned hereinafter, to suspect that "*C. crista*", which is literally the same as the one we worked on, might be a botanical synonym to *C. bonduc*. One reason is that types of constituents are closely related to those isolated from *C. bonduc* from the chemotaxonomical viewpoint. The other is that the scientific names of "*C. crista* sensu auct., non L." and "*C. crista* L." were often used as either a synonym or invalid name to *C. bonduc*, which cannot help but be very confusing even to botanists. There was an article[19] referred to "*C. crista*" dealing with phytochemical analysis, which clearly regarded it as a synonym to *C. bonduc*. These facts indicate that old (invalid) names are still in use. There are many references to plants under the names of *C. bonduc* Roxb., *C. crista* L., *C. bonducella* (L.) Flem., and a few under *C. nuga* (L.) Ait. f. Dandy and Exell did a study on the typification of these species and found it necessary to change the application of the names.[20] Plants for a long time erroneously named *C. crista* and *C. bonducella* are now named *C. bonduc* (L.) Roxb. The species widely known as *C. bonduc* Roxb. is now *C. major* (Medic.) Dandy *et* Exell; another synonym of the new name is *C. jajabo* Maza. *C. nuga* is the present *C. crista* L. Confusion of names will be derived from the fact that there are enormous morphologic variations (and possibly chemical variations as well) at the infra-specific level in some *Caesalpinia* species. The nomenclature of three *Caesalpinia* species that have long confused not only phytochemists but also botanists is regularized in Table 2, in which both valid and invalid names along with Japanese names are shown.[21] We suggest that all phytochemists take notice of abiding by the valid nomenclature in order not to disturb a search of literatures in chemical databases.

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 ECP-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer with tetramethylsilane (TMS) as internal standard; mass spectra (MS) and high-resolution mass spectra (HR-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 or Merck Kieselgel 60 (eluted with hexane–ethyl acetate or benzene–acetone), Sephadex LH-20 (Pharmacia, eluted with MeOH–$CHCl_3$) and RP-8 reversed-phase silica gel (eluted with MeOH–H_2O), and thin-layer chromatography (TLC) was conducted on a 0.25 mm pre-coated silica-gel plate ($60F_{254}$, Merck), and spots were detected by inspection under short (254 nm) or long (365 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 leaves of *C. crista* were collected in Iriomote Island, Okinawa, Japan, in March 2001. The processing of plant materials was carried out at Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus. A voucher specimen in complete form with flowers was on deposit at the Herbarium of Medicinal Plant Garden, Teikyo University.

Extraction and Isolation The dried leaves (2.1 kg) of *C. crista* were extracted two times with distilled acetone at room temperature, and the combined extracts were evaporated to dryness under reduced pressure to yield greenish viscous syrup (127.4 g). The extract (120 g) was dissolved in acetone and adsorbed on silica gel (120 g). The adsorbed material was transferred to a silica gel column (600 g) packed in hexane. The column was eluted with the following solvent system: hexane–ethyl acetate 9 : 1 (1000 ml), 4 : 1 (2000 ml), 3 : 1 (3000 ml), 2 : 1 (4000 ml), 1 : 1 (4000 ml), and acetone (2000 ml). Fractions of 500 ml each were taken and 26 fractions (fr. 1—fr. 26) were collected. Fr. 17 was evaporated and the residue was subjected to a series of chromatographic separation by use of Sephadex LH-20 and RP-8 reversed-phase silica gel to afford semicrystalline crude materials. The crude materials were finally purified by preparative thin layer chromatography followed by recrystallization from MeOH–H_2O to furnish neocaesalpin I (**2**; 127 mg) in pure forms. The crystalline precipitates deposited in fr. 22 was recrystallized from MeOH–H_2O to give neocaesalpin H (**1**) as colorless needles (268 mg).

Neocaesalpin H (**1**): Colorless needles, mp 255—256 °C. $[\alpha]_D^{25}$ −73.2° (c=0.101, MeOH). IR (KBr) cm^{-1}: 3364, 2934, 2361, 1738, 1684, 1474, 1458, 1248, 1132. UV λ_{max} nm (log ε): 213 (4.25). ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 0.81 (3H, s, 20-CH_3), 1.07 (1H, ddd, J=13.2, 12.8, 3.3 Hz, H_α-1), 1.16 (3H, d, J=7.1 Hz, 17-CH_3), 1.38—1.43 (3H, m, H_β-2, H_β-7, H_β-11), 1.41 (3H, s, 19-CH_3), 1.43—1.53 (4H, m, H_α-2, H_β-6, H_α-7, 8-H), 1.61 (2H, m, H_β-1, H_α-6), 1.73 (1H, br ddd, J=13.0, 1.0, 0.9 Hz, H_β-3), 1.79 (1H, br ddd, J=10.2, 10.2, 3.0 Hz, H-9), 2.04 (1H, ddd, J=13.0, 13.2, 3.8 Hz, H_α-3), 2.13 (1H, dd, J=11.3, 1.2 Hz, H-5), 2.62 (1H, dd, J=12.6, 3.0 Hz, H_α-11), 2.77 (1H, dq, J=7.1, 4.1 Hz, H-14), 5.87 (1H, s, H-15). ^{13}C-NMR (150 MHz, pyridine-d_5) δ: see Table 1. EI-MS m/z (rel. int.): 348 (M^+, 13), 330 (M^+-H_2O, 53), 304 (75), 207 (70), 161 (100). HR-MS: m/z 348.1942 (Calcd for $C_{20}H_{28}O_5$: 348.1937).

Neocaesalpin I (**2**): Colorless fine needles, mp >260 °C. $[\alpha]_D^{25}$ +27.7° (c=0.098, MeOH). IR (KBr) cm^{-1}: 2926, 2361, 1748, 1717, 1456, 1391, 1240. UV λ_{max} nm (log ε): 277 (4.26). ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 0.79 (3H, s, 20-CH_3), 0.81 (3H, d, J=7.4 Hz, 17-CH_3), 1.03 (1H, ddd, J=13.2, 12.9, 3.7 Hz, H_α-1), 1.31 (1H, m, H_β-7), 1.34 (3H, s, 19-CH_3), 1.37 (1H, m, H_α-7), 1.44 (1H, m, H_β-6), 1.49 (1H, m, H_β-2), 1.58 (2H, m, H_α-2, H_α-6), 1.65 (1H, dddd, J=11.8, 11.8, 4.1, 4.1 Hz, H-8), 1.74 (2H, m, H_β-1, H_β-3), 1.95 (1H, dd, J=10.4, 0.8 Hz, H-9), 2.07 (1H, ddd, J=13.5, 13.5, 4.1 Hz, H_α-3), 2.08 (1H, dd, J=11.9, 2.3 Hz, H-5), 2.57 (1H, dq, J=7.4, 4.2 Hz, H-14), 5.85 (1H, br s, H-11), 5.90 (1H, d, J=0.9 Hz, H-15). ^{13}C-NMR (150 MHz, pyridine-d_5) δ: see Table 1. EI-MS m/z (rel. int.): 330 (M^+, 46), 284 (18), 207 (78), 161 (100). HR-MS: m/z 330.1836 (Calcd for $C_{20}H_{26}O_4$: 330.1831).

Dehydration of Neocaesalpin H (1) A mixture of neocaesalpin H (**1**; 30 mg), *p*-toluenesulfonic acid (0.5 mg) and 30 ml of dry benzene was refluxed for 10 h. The reaction mixture was evaporated *in vacuo*, and the residue was subjected to preparative TLC to afford the dehydrated product. The product was recrystallized from MeOH–H_2O to furnish colorless needles (12 mg), which were identical to neocaesalpin I (**2**) in the IR and ^{1}H-NMR spectra.

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