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Cassane diterpenoid from *Caesalpinia major*

Sophon Roengsumran[a,*], Supassara Limsuwankesorn[a], Nattaya Ngamrojnavanich[a], Amorn Petsom[a], Chaiyo Chaichantipyuth[b], Tsutomu Ishikawa[c]

[a]*Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[b]*Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[c]*Faculty of Pharmaceutical Sciences, Chiba University, Chiba 263, Japan*

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Abstract

A new cassane diterpenoid, 14-deoxy-ε-caesalpin was isolated from the seed kernels of *Caesalpinia major* and its structure was determined by spectroscopic data.

Keywords: *Caesalpinia major*; Caesalpiniaceae; Diterpenoid; Cassane

1. Introduction

Caesalpinia major (Medik) Dandy & Exell. (Caesalpiniaceae) is a tropical plant widely distributed in Southeast Asia (Smittinand & Larsen, 1984). It is used as a medicinal plant in various regions of the tropics. For example, the decoction of the roots has been used as a tonic and an anthelmintic as well as for treatment of rheumatism and back-ache (Kitagawa et al., 1994). In the herbal medicine of Thailand, the seeds of this plant are used as an expectorant and antitussive agent. Recently, Kitagawa et al. (Kitagawa et al., 1994, 1996) isolated caesaldekarins a, b, c, d and e from the roots of *C. major* collected in Indonesia, but there is no report on the constituents of the seeds. We now report the isolation and characterization of 14-deoxy-ε-caesalpin, a new cassane diterpenoid, from the seed kernels of *C. major* collected in Thailand.

2. Results and discussion

The methanolic extract of the peeled and crushed seed kernels of *C. major* was concentrated under reduced pressure. The white precipitate was filtered and purified by chromatography on silica gel using a chloroform–methanol gradient system to obtain a new cassane diterpenoid **1** as colorless crystals from methanol.

Elemental analysis of **1** suggested the molecular formula $C_{24}H_{34}O_6$ which agreed well with the m/z 418 molecular ion obtained from FAB and EIMS. The molecular formula of **1** suggested eight degrees of unsaturation. The presence of a 2,3-disubstituted furan ring was suggested by ^{1}H-NMR signals (Table 1) for a pair of doublets of aromatic protons [δ_H 6.17 (^{1}H-15, *d*, $J = 1.8$), 7.21 (^{1}H-16, *d*, $J = 1.8$)] and four sp^2 carbon [δ_C 109.5 (C-15, *d*), 122.6 (C-13, *s*), 140.6 (C-16, *d*) and 148.6 (C-12, *s*). An infrared peak at 1738 cm^{-1} and ^{13}C-NMR resonances at δ_C 169.1 and 170.4 (CH_3–$\underline{C}O$) indicated the presence of two carboxyl groups. Thus, the three remaining degrees of unsaturation indicated a tricarbocyclic system.

The DEPT NMR spectra indicated the presence of 18 sp^3 carbons [6 methyl carbons, 4 methylene carbons, 5 methine carbons and 3 quaternary carbons], 4 sp^2 carbons [2 methine and 2 quaternary carbons] and 2 carboxyl groups. There are three C–O groups [δ_C 67.5 (C-2, *d*), 75.0 (C-1, *d*) and 76.8 (C-5, *s*)]. Thus,

* Corresponding author.

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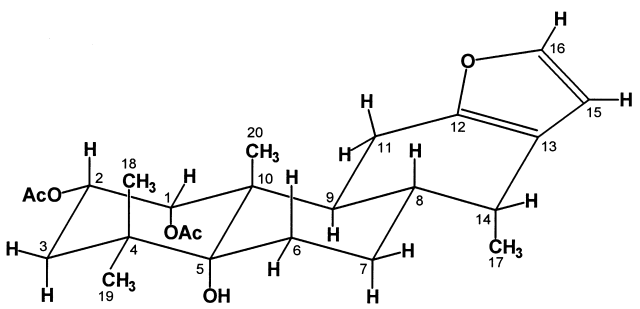
Table 1
^{1}H - and ^{13}C-NMR spectral data of **1**

Position	δ_C (ppm)	δ_H (ppm)	Multiple bond correlation
1	75.0 *d*	5.24 br.d ($J = 2.6$)	C-2, C-3, C-5, C-9, C-10, C-20, *CO* (169.0)
2	67.5 *d*	5.30 *ddd* ($J = 2.6$, 4.7, 13.0)	C-1, C-3, C-4, C-19
3	35.8 *t*	2.02 *dd* (J = 13.0, 13.0)	C-2, C-4, C-18, C-19
		1.38 *ddd* ($J = 1.2$, 4.7, 13.0)	C-1, C-2, C-4, C-5, C-19
4	40.3 *s*	–	–
5	76.8 *s*	–	–
6	25.6 *t*	1.67 *ddd* ($J = 2.7$, 4.9, 13.5)	C-7
		1.70 *ddd* ($J = 2.7$, 5.6, 13.5)	C-4, C-5, C-7, C-10
7	23.9 *t*	1.40 *m*	C-6, C-8
		1.97 *m*	C-6, C-14
8	34.3 *d*	1.85 *dddd* ($J = 5.5$, 5.5, 12.2, 12.2)	C-7, C-9, C-14, C-17
9	32.7 *d*	2.57 *ddd* ($J = 6.7$, 10.7, 12.2)	C-8, C-10, C-11, C-14, C-20
10	44.9 *s*		
11	22.1 *t*	2.39 *dd* ($J = 10.7$, 16.6)	C-9, C-10, C-12, C-13
		2.28 *dd* ($J = 6.7$, 16.6)	C-8, C-9, C-12, C-13
12	148.6 *s*	–	–
13	122.6 *s*	–	–
14	31.3 *d*	2.64 *dq* ($J = 5.5$, 7.0)	C-8, C-9, C-12, C-13, C-17
15	109.5 *d*	6.17 *d* ($J = 1.8$)	C-12, C-13, C-16
16	140.6 *d*	7.21 *d* ($J = 1.8$)	C-12, C-13, C-15
17	17.4 *q*	1.04 *d* ($J = 7.0$)	C-7, C-8, C-12, C-14
18	28.2 *q*	1.10 *s*	C-3, C-4, C-5, C-19
19	26.0 *q*	1.19 *s*	C-2, C-3, C-4, C-5, C-18
20	17.4 *q*	1.22 *s*	C-1, C-5, C-9, C-10
$CH_3\underline{C}O$	169.0 *s*	–	–
$CH_3\underline{C}O$	170.3 *s*	–	–
$\underline{C}H_3CO$	20.9 *q*	1.98 *s*	$\underline{C}O$ (170.3)
$\underline{C}H_3CO$	21.1 *q*	2.14 *s*	$\underline{C}O$ (169.0)

the presence of two carboxyl groups and two C–O groups were attributed to two ester groups, which was also supported by the two methyl groups at δ_C 20.9 *q* and 21.1 *q*. Thus, the remaining C–O group at δ_C 76.8 must be an alcohol, which was indicated by the presence of a free hydroxyl group in the IR spectrum (3590 cm^{-1}) and a mass fragment at *m/z* 400 $[M - H_2O]^+$. Detailed analysis of 2-D ^{1}H- and ^{13}C-NMR spectra including COSY, NOESY, HMQC and HMBC led us to presume that compound **1** is a cassane-type diterpenoid compound (see Scheme 1).

An HMQC experiment revealed the connectivity



Scheme 1.

between δ_C 140.6 (C-16)–δ_H 7.21 (H-16), and δ_C 109.5 (C-15)–δH 6.17 (H-15) of the furan ring. The HMBC indicated the correlation of a proton at δ_H 6.17 with carbons at δ_C 122.6, 140.6 and 148.6 while, a proton at δ_H 7.21 was correlated to carbons at δ_C 109.5, 122.6 and 148.6 (Table 1). This, completed the assignment of all protons and carbons of the furan moiety. A proton at δ_H 2.64 (H-14) was connected to carbon at δ_C 31.3 (C-14) and correlated to carbons at δ_C 17.4, 32.7, 34.3, 122.6 and 148.6 ppm, suggesting that C-14 must connect to the furan ring. By the same analogy, the HMBC data indicated that the carbon at δ_C 22.1 (C-11) could be assigned as the methylene carbon connected to the furan ring. Moreover, the 17-methyl appeared as a doublet (δ_H 1.04, $J = 7.0$) in the ^{1}H NMR spectrum, thus C-14 was a non-oxygenated carbon as in caesaldekarins a, b, c, and d (Kitagawa et al., 1994, 1996).

Correlations of protons and carbons at δ_H 5.24–δ_C 75.0 and δ_H 5.30–δ_C 67.5 were also seen in the HMQC experiment. A COSY experiment and the small coupling constant ($J = 2.6$) between H-1 and H-2, indicated that both protons were adjacent and in an axial-equatorial conformation. The HMBC experiment revealed correlation of a proton at δ_H 5.24 to carbons at δ_C

17.4, 32.7, 35.8, 44.9, 67.5, 76.8 and 169.0, indicating the connection of C-1 to C-2 and C-10. By the same analogy as for C-1, the C-2 carbon could be connected to C-1 and C-3, and had long range correlations to C-4 (δ_C 40.3) and C-19 (δ_C 26.0). The correlation between δ_H 1.19 (H-19) and an oxygen bearing carbon at δ_C 76.8 made possible the assignment of this part of the molecule. These proton and carbon chemical shifts of **1** agreed with those reported for neocaesalpins A and B (Kinoshita, Kaneko, Noguchi & Kitagawa, 1996).

Most closely related to compound **1** is ε-caesalpin, for which the absolute configuration of the *p*-bromobenzoate derivative was confirmed by X-ray crystallography (Balmain, Bjamer, Connolly & Ferguson, 1967). The structure of compound **1** differed from ε-caesalpin only at C-14. Therefore, compound **1** was proposed to be 14-deoxy-ε-caesalpin. The stereochemistry of **1** was adopted from the stereochemistry of ε-caesalpin. The NOESY experiment clearly exhibited a cross peak between H-9 and the 17-methyl group. Moreover, the magnitude of the coupling constants between H-8_{ax} and H-14 (5.5 Hz), and between H-8_{ax} and H-9_{ax} (12.2 Hz) also supported the proposed configuration of C-14 (McPherson, Che, Cordell, Soejarto & Pezzuto, 1986).

The known compound neocaesalpin B was also isolated from the crude hexane extract. Its spectroscopic properties were in agreement with those reported by Kinoshita et al. (1996). Conversion of previously unknown **1** to neocaesalpin B by enzymatic oxidation followed by isomerization was also postulated by Kinoshita et al. The isolation of both **1** and neocaesalpin B in the same plant makes this suggestion very plausible, and it seems that cassane-type furanoditerpenes are characteristic chemical constituents in Caesalpinia species (Qudrat-I-Khuda & Ali, 1963; Canonica, Jommi, Manitto & Pelizzoni, 1963; Purushothaman, Kalyani, Subramanian & Shanmuganathan, 1981; Pascoe, Burke & Chan, 1986; Sengupta & Roy, 1970; Ogawa, Aoki & Sashida, 1992).

3. Experimental

Mps uncorr: on Fisher-John melting point apparatus; Optical rotations: on a JASCO DIP-370 digital polarimeter; UV spectra: on a Hewlett Packard 8452A; IR spectra: on a Perkin-Elmer 1760X FT-IR Spectrophotometer. ^{1}H, ^{13}C HMQC and HMBC NMR experiments were recorded on a JEOL JNM-A500 spectrometer. Microanalyses were determined on a Perkin-Elmer PE 2400 Series II.

3.1. Plant material

The seeds of *C. major* were collected from Amphur Sanamchaikate, Chachoengsao Province, Thailand in November 1996. Botanical identification of the plant specimen was achieved through comparison with specimen no. 55398 in the herbarium of the Royal Forest Department of Thailand, Bangkok, Thailand.

3.2. Extraction and isolation

The fresh seeds of *C. major* were dehulled and the seed kernels (3 kg) were crushed in a blender and macerated with MeOH (3 × 4 l). The methanol extract was filtered and evaporated *in vacuo* until the volume was 1/5 of the original volume, at which point a white precipitate (23 g) appeared. This was removed by filtration and subsequently applied on silica gel column eluted with $CHCl_3$–MeOH. Compound **1** (0.5 g, 0.017% w/w) was obtained as colorless crystals from methanol.

The mother liquor was concentrated to dryness and redissolved in 90% aq. MeOH which was re-extracted with hexane. This hexane extract (10.9 g) was chromatographed on silica gel and eluted with hexane–$CHCl_3$ and $CHCl_3$–EtOAc. Neocaesalpin B (10 mg, 3 × 10^{-4}% w/w) was obtained as colorless crystals, mp 149–151°C (lit. 150–152 °C).

3.3. 14-Deoxy-ε-caesalpin (*1*)

Colourless needle-crystals, mp 202–203 °C (MeOH), (Found C, 69.07; H, 8.28. $C_{24}H_{34}O_6$ requires: C, 68.90; H, 8.13%); $[\alpha]_D^{25}$ + 34.1° ($CHCl_3$; C 0.4840); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 220 (3.4); IR ν_{max}^{KBr} cm^{-1}: 3590, 2970, 2945, 2879, 2865, 1738, 1369, 1254, 1228, 1035; ^{1}H- and ^{13}C-NMR spectral data: Table 1. FABMS *m/z*: 419 [M + H$^+$]; EIMS (30 ev) *m/z* (rel. int.): 418 [M$^+$] (12), 400 (16), 358 (10), 340 (37), 298 (12), 280 (34), 265 (31), 158 (35), 146 (100).

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