Caesaldecan, a Cassane Diterpenoid from the Leaves of *Caesalpinia decapetala*

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A new cassane diterpenoid, caesaldecan, was isolated from *Caesalpinia decapetala* with eight known compounds, spathulenol, 4,5-epoxy-8(14)-caryophyllene, squalene, lupeol, *trans*-resveratrol, quercetin, astragalin, and stigmasterol. The ^{1}H- and ^{13}C-NMR spectra of the new compound were completely assigned by using a combination of 2D NMR techniques, namely, ^{1}H–^{1}H COSY, HMQC, HMBC, and ROESY.

Key words *Caesalpinia decapetala*; Fabaceae; diterpenoid; cassane; caesaldecan

Caesalpinia decapetala ROTH (ALSTON) (Fabaceae) is a medicinal plant with a wide distribution throughout Northern Vietnam, and continues to be used in traditional Vietnamese medicine as an immunomodulatory and anti-inflammatory agent.[1)] The genus *Caesalpinia* is well known for its cassane diterpenoid content, and many different diterpenes have been isolated from this genus.[2—11)] A previous investigation of the roots of *C. decapetala* have isolated caesaljapin, lup-20(29)-en-3β-ol, betulinic acid, 3-deoxysappanchalcone, sappanchalcone, catechin, methyl gallate, and 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone.[12)] We report herein upon the isolation and structural elucidation of a new cassane diterpenoid, caesaldecan (**1**), and of eight known compounds, spathulenol (**2**), 4,5-epoxy-8(14)-caryophyllene (**3**), squalene (**4**), lupeol (**5**), *trans*-resveratrol (**6**), quercetin (**7**), astragalin (**8**), and stigmasterol (**9**) from the leaves of *C. decapetala*. This is the first report of compounds **2**—**4** and **6**—**8** in *C. decapetala*.

Caesaldecan (**1**) was isolated as white crystals, mp 150—153 °C, its molecular formula, $C_{25}H_{38}O_5$, was established by high resolution FAB-MS (Found *m*/*z*: 441.2620 $[M+Na]^+$; Calcd for $C_{25}H_{38}O_5Na$: 441.2617). Its IR spectrum had absorptions typical of hydroxyl (3450 cm^{-1}), carboxyl (1710, 1730 cm^{-1}), and ether (1020 cm^{-1}) functionalities. The ^{1}H-NMR spectrum had resonances due to the presence of three tertiary methyl groups at δ 1.18, 1.52, and 1.72, and two secondary methyl groups at δ 0.90 and 0.91. Signals typical of oxymethine protons were evident at δ 4.77 (1H, t, J=2.5 Hz, H-3) and 4.38 (1H, m, H-6), and olefinic proton signals typical of a vinyl moiety were observed at δ 6.80 (1H, dd, J=11.0, 17.5 Hz), 5.09 (1H, dd, J=17.5, 2.3 Hz) and 4.95 (1H, dd, J=11.0, 2.3 Hz).

The ^{13}C-NMR spectrum of **1** revealed the presence of 25 carbons including 6 quaternary, 7 methine, 7 methylene, and 5 methyl carbons. Signals were observed for one carboxyl group at δ 176.6, one carboxylated group at δ 171.4, olefinic carbons at δ 137.2, 135.8, 128.8, and 111.3, and two oxymethine carbons at δ 76.3 and 67.6. In the heteronuclear multiple quantum coherence (HMQC) spectrum, protons at δ 5.09 and 4.95 showed direct connectivity to a carbon at δ 111.3, while the proton at δ 6.80 had direct connectivity to a carbon at δ 135.8. Based on the cassane skeleton, which is typical for genus *Caesalpinia*,[2—11)] the partial structures, including an isoprenyl moiety and a cassane skeleton, were deduced from the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) and HMQC spectra (Fig. 2, bold line), and were connected based on long-range correlations in the heteronuclear multiple-bond correlation (HMBC) spectrum (Fig. 2, Table 1). In the ^{1}H–^{1}H COSY spectrum, the multiplets at δ 1.60 and 1.95 showed cross peaks to a triplet at δ 4.77 (H-3), and to signals at δ 1.20 and 1.42 (H-1) attributed to the H-2 proton. The methine carbinol proton at δ 4.38 (multiplet) showed coupling with the doublet at δ 1.86, and with the multiplets at δ 1.10 and 2.09, assigned to the H-6 proton. A multiplet of the methine proton appearing at δ 2.46 showed cross peaks to both the multiplet at δ 0.95 and the protons H-7 (δ 1.10,

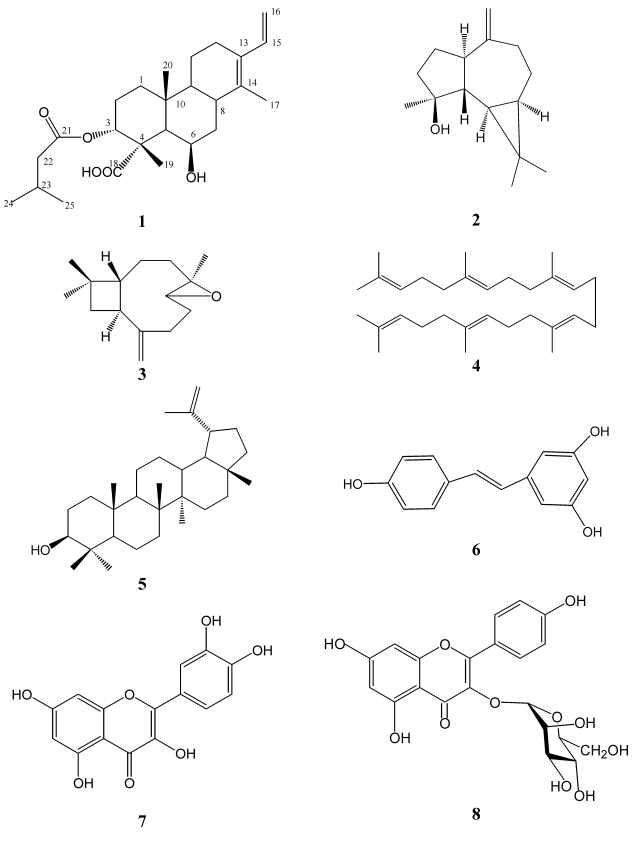

Fig. 1. Structures of Compounds **1**—**8**

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2.09), assigned to the H-8 proton. The multiplets at δ 1.08 and 1.79 showed couplings with the H-9 proton (δ 0.95), and with protons at δ 1.97 (multiplet) and 2.30 (broad doublet) attributed to the H-11 proton. The assignments of the A, B and C rings were established from detail long-range correlations in the HMBC spectrum as shown in Table 1. Furthermore, in the HMBC spectrum, the proton at δ 6.80 (H-15) showed long-range correlations to carbons at δ 128.8 (C-13)/137.2 (C-14), while the vinyl proton at δ 5.09/4.95 (H-16) correlated with the carbon at 128.8 (C-13), and the methyl protons at δ 1.72 (H-17) correlated with carbons at δ 36.5 (C-8)/128.8 (C-13)/137.2 (C-14). The methine proton at δ 4.77 (H-3) showed long-range correlations to carbons at δ 176.6 (C-18)/171.4 (C-21)/19.6 (C-19), whereas the proton at δ 2.13 (H-23) correlated with carbons at δ 171.4 (C-21)/22.6 (C-24, C-25). In addition, H–C long-range correlations between the proton at δ 4.38 (H-6) and the carbon at δ 46.0 (C-5), and between the hydroxyl proton at δ 4.30 (6-OH) and the carbon at 46.0 (C-5) were observed in the HMBC. The above data indicated that the vinyl moiety was located at C-13, the methyl group at C-14, the carboxyl group at C-18, the carboxylated moiety at C-3 by an ester linkage, and the hydroxyl group was at C-6. The stereochemistry of **1** was determined from the coupling pattern in the ^{1}H-NMR spectrum and detailed analysis of rotation frame Overhauser effect spectroscopy (ROESY) data as shown in Table 1. In the ^{1}H-NMR spectrum, the H-3 proton was confirmed as equatorial by spin-coupling constants (J_{eq-ax} = J_{eq-eq} = 2.5 Hz). In the ROESY spectrum, H-20 (δ 1.18) had cross-peaks with H-19 (δ 1.52) and 6-OH (δ 4.30), indicating that they were all in the same plane and β-oriented, while H-5 (δ 1.86) had cross-peaks with H-6 (δ 4.38) indicating that they were α-oriented. Similarly, H-19 had cross-peaks with H-3 and 6-OH. These findings also confirmed that the carboxyl group must be located at C-18. Based on the above data and the NMR results summarized in Table 1, the structure of **1** was proposed for caesaldecan, its first identification in nature. Cassane diterpenoids in which the C-13 methyl group has migrated to C-14 are common.[7,10,12] However, the isoprene moiety has not been found previously in *Caesalpinia* species. The eight known compounds were identified as spathulenol (**2**),[13] 4,5-epoxy-8(14)-caryophyllene

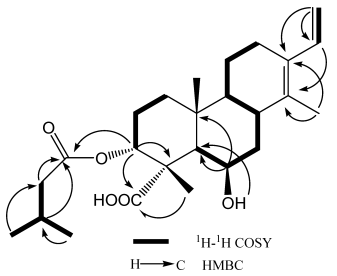

Fig. 2. Selected H–C Long-Range Correlations in HMBC and H–H Correlations in ^{1}H–^{1}H COSY Spectra of **1**

Table 1. NMR Assignments of Compound **1**

C	δ_C [a]	δ_C [b]	δ_H [c]	HMBC (H to C)	ROESY
1α	33.7 t	33.4 t	1.42 br d (12.0)		
1β			1.20 dd (2.5, 12.0)	3, 20	
2α	22.8 t	22.7 t	1.95 m		
2β			1.60 m		
3	76.6 d	76.3 d	4.77 t (2.5)	1, 4, 5, 18	H-19
4	50.6 s	49.6 s	—		
5	46.1 d	46.0 d	1.86 d (2.1)	3, 6, 9	H-6
6	70.1 d	67.6 d	4.38 m	5, 8, 10	H-5
			4.30 br s (6-OH)	5, 6	H-19, H-20
7α	40.7 t	40.7 t	2.09 m	8	
7β			1.10 m		
8	36.7 d	36.5 d	2.46 m		
9	54.5 d	54.5 d	0.95 m	10, 20	
10	37.0 s	36.7 s	—		
11α	21.6 t	21.4 t	1.79 m	9, 10	
11β			1.08 m	10	
12α	26.8 t	26.4 t	1.97 m		
12β			2.30 br d (14.5)	13	
13	129.6 s	128.8 s	—		
14	136.4 s	137.2 s	—		
15	135.7 d	135.8 d	6.80 dd (11.0, 17.5)	12, 13, 14	
16α	111.2 t	111.3 t	4.95 dd (11.0, 2.3)	13	
16β			5.09 dd (17.5, 2.3)	13, 15	
17	16.3 q	16.1 q	1.72 s	8, 13, 14	
18	181.0 s	176.6 s	12.13 br s (18-OH)	4	
19	19.8 q	19.6 q	1.52 s	3, 4, 5, 19	H-3, H-20, 6-OH
20	17.5 q	17.0 q	1.18 s	1, 5, 9, 10	H-19, 6-OH
21	172.3 s	171.4 s	—		
22	44.0 t	43.5 t	2.07 d (3.5)	23, 24	
23	26.0 d	25.5 d	2.13 m	21, 22	
24	22.7 q	22.6 q	0.90 d (2.0) [d]	22, 23, 25	
25	22.7 q	22.6 q	0.91 d (2.0) [d]	22, 23, 24	

a) 75 MHz, $CDCl_3$, *b*) 150 MHz, DMSO, *c*) 600 MHz, DMSO. Chemical shifts are given in ppm; multiplicities and coupling constant *J* (in parentheses) in Hz. *d*) Assignments may be interchanged.

(**3**),[14] squalene (**4**),[15] lupeol (**5**),[16] *trans*-resveratrol (**6**),[17] quercetin (**7**),[18] astragalin (**8**),[19] and stigmasterol (**9**)[20] by comparison of the ^{1}H-, ^{13}C-NMR, and MS data with those reported in the literature.

Experimental

Melting points were determined using a Kofler micro-hotstage. IR spectra were obtained on a Hitachi 270-30 type spectrometer using KBr discs. The optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. FAB-MS and HR-FAB-MS were obtained using a JEOL JMS-DX 300 spectrometer. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were obtained using a Bruker AM 600 FT-NMR spectrometer with TMS as the internal standard and ^{13}C-NMR (75 MHz) was performed using a Bruker DRX 300 spectrometer. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70—230 mesh and 230—400 mesh, Merck).

Plant Material The leaves from *C. decapetala* were collected at Tamdao Mountain, Vinhphuc province, in Vietnam during November 2003 and were identified by Dr. Ha Van Tue of the Institute of Ecology, Biological Resources, VAST, Vietnam. A voucher specimen (VN265) was deposited at the herbarium in the same institute.

Extraction and Isolation Air-dried and powdered leaves of *C. decapetala* (3.0 kg) were extracted three times with hot MeOH to give the methanol extract (150 g), which was then suspended in water and extracted with chloroform. The chloroform fraction (80 g) was chromatographed on a silica gel column, using $CHCl_3$–MeOH gradient (from 100 : 0 to 0 : 100) as eluent, to yield four fractions (Fr. A—D). Fraction A (40 g) was chromatographed on a silica gel column using hexane–acetone (from 100 : 0 to 0 : 100) to give five subfractions (Fr. A1, 8 g; Fr. A2, 9 g; Fr. A3, 7 g, Fr. A4, 10 g, Fr. A5, 6 g). Subfraction A1 was chromatographed on a silica gel column using hexane–ethyl acetate (100 : 1) as eluent to yield squalene (**4**) (58 mg) as colorless oils. Subfraction A2 was chromatographed on a silica gel column using hexane–ethyl acetate (50 : 1) as eluent to yield spathulenol (**2**) (18 mg) and 4,5-epoxy-8(14)-caryophyllene (**3**) (20 mg) as colorless oils. Subfraction A3 was chromatographed on a silica gel column using hexane–ethyl acetate (30 : 1) as eluent to give caesaldecan (**1**) (90 mg), lupeol (**5**) (50 mg) and *trans*-resveratrol (**6**) (12.5 mg) as white crystals. Stigmasterol (**9**) (1.5 g) was formed as white crystals from subfraction A4. The residue of the aqueous fraction was adsorbed on highly porous polymer resin (Dianion HP-20, Mitsubishi Chem. Ind. Co. Ltd., Tokyo, Japan) and eluted with water containing increasing concentrations of MeOH (100% H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, 100% MeOH). The 20% MeOH and 40% MeOH fractions were combined and chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (70 : 30 : 4) as eluent. Repeated CC on a YMC RP-18 column using a MeOH–H_2O (7 : 3) system yielded quercetin (**7**) (55.0 mg) and astragalin (**8**) (10.2 mg, as yellow powders).

Caesaldecan (**1**): White crystals. mp: 150—153 °C; $[\alpha]_D^{25}$: +75° (c=1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3450 (OH), 2953 (CH), 1710 and 1730 (C=O) and 1020 (C–O–C); positive FAB-MS m/z: 441.2 $[M+Na]^+$; HR-FAB-MS m/z: 441.2620 $[M+Na]^+$ (Calcd for $C_{25}H_{38}O_5Na$: 441.2617); ^{1}H- and ^{13}C-NMR are given in Table 1.

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