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CLERODANE DITERPENOIDS FROM *CYATHOCALYX ZEYLANICA*

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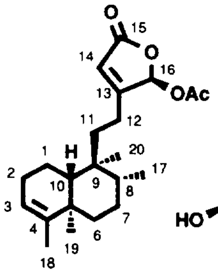
Key Word Index—*Cyathocalyx zeylanica*; Annonaceae; clerodane diterpenoids; 16-acetoxy-cleroda-3,13-dien-15,16-olide; 3-hydroxy-cleroda-4(18),13*Z*-dien-15-oic acid.

Abstract—A new clerodane diterpene, 3-hydroxy-cleroda-4(18), 13*Z*-dien-15-oic acid, has been isolated from the stem bark of *Cyathocalyx zeylanica* along with a known clerodane diterpenoid, 16-acetoxy-cleroda-3,13-dien-15,16-olide. Their structures were determined on the basis of spectral data including 2D NMR spectra and some previous ^{13}C NMR assignments of the latter compound were revised.

INTRODUCTION

Cyathocalyx zeylanica champ. is a pioneer wet zone tree growing in Sri Lanka and southern Deccan Peninsula [1]. In our previous paper, we reported on the isolation and structure elucidation of a new azafluorenone alkaloid from this species [2]. In a continuation of our studies on the isolation of biologically active constituents from Annonaceae plants in Sri Lanka, the hot methanol extract of the stem bark of *C. zeylanica* was shown to be toxic towards larvae of the crustacean *Artemia salina* (brine shrimp) and of the mosquito *Aedes aegypti*. This paper reports on the isolation and characterization of two clerodane diterpenoids (**1** and **2**), one (**2**) of which is new.



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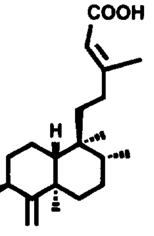

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RESULTS AND DISCUSSION

Compound **1** was isolated as an acetate and determined to be an *ent*-type clerodane diterpenoid, 16-acetoxy-cleroda-3,13-dien-15,16-olide. Previously, both (16*S*) and (16*R*) compounds were reported from *Polyalthia longifolia* [3] and *P. viridis* [4], respectively. By comparing the ^{1}H and ^{13}C NMR data of **1** with those in the literature, **1** was determined to be the (16*R*) isomer. However, our 2D NMR (COSY, HETCOR, and long-range ^{1}H–^{13}C COSY) data suggested that the previous assignments of some ^{13}C NMR signals [4] should be revised. The revised assignments are presented in Table 1.

Compound **2** was obtained as an amorphous solid, and on acetylation (Ac_2O–pyridine), it gave a mono acetate (δ_H 2.12). The mass spectrum of **2** showed a molecular ion at m/z 320, which on high-resolution MS measurement corresponded to the formula $C_{20}H_{32}O_3$. The IR spectrum of **2** showed the presence of hydroxyl group (3255 cm^{-1}) and an α,β-unsaturated carboxyl group (1692, 1634 cm^{-1}).

Examination of the ^{1}H NMR spectrum of **2** indicated the presence of two tertiary methyls (δ0.70 H_3-20; δ1.06, H_3-19), a secondary methyl (δ0.77, J = 6.5 Hz, H_3-17), and a vinyl methyl (δ2.36, *d*, J = 12 Hz, H_3-16) coupled with the olefinic proton at δ6.09 (*q*, J = 1.2 Hz, H-14). The ^{1}H NMR spectrum of **2** in deuterated pyridine also showed signals due to a proton attached to a hydroxy-bearing carbon (δ4.58, *m*, H-3) and an *exo*-olefin (δ4.95, *t*, J = 1.5 Hz and 5.63, *t*, J = 1.8 Hz, H_2-18). These data and the results of COSY and HETCOR experiments suggested the presence of the following connectivities: C(10)H-C(1)H_2-C(2)H_2-C(3)H-OH, C(19)H_3-C(5)-C(6)H_2-C(7)H_2-C(8)H-C(17)H_3, C(11)H_2-C(12)H_2, and C(16)H_3-C(13) =C(14)H-C(15)(=O)-OH. The partial structures were connected by the long-range correlations observed in the long-range ^{1}H–^{13}C COSY spectrum (Table 1).

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for compounds **1** and **2**

	1 (in $CDCl_3$)^{a}			**2** (in pyridine-d_5)^{b}		
C	δ_H	δ_C	1_H L.r. coupled^{a}	δ_H	δ_C	1_H L.r. coupled^{a}
1	1.48 *m* (2H)	18.3^{b} *t*	3, 10	1.56 *m* 1.63 *m*	21.0 *t*	10
2	1.95^{c} *m* 2.07^{d} *br d* (15)	26.8^{b} *t*	3, 10	1.58 *m* 2.40 *m*	38.3 *t*	
3	5.13^{e} *br s*	120.3 *d*	18	4.58 *m*	68.9 *d*	
4		144.3 *s*	18, 19		163.7 *s*	19
5		38.2^{b} *s*	1, 3, 6, 10, 18, 19		40.4 *s*	18, 19
6	1.18^{f} *td* (12.5, 6) 1.73 *dt* (12.5, 3)	36.7 *t*	19	1.66^{g} *m* (2H)	38.0 *t*	19
7	1.45 *m* (2H)	27.3 *t*	6, 17	1.42 *m* 1.47 *m*	27.6 *t*	17
8	1.45 *m*	36.4 *d*	6, 11, 17, 20	1.41 *m*	36.8 *d*	12, 17, 20
9		38.7^{b} *s*	1, 11, 10, 17, 20		39.5 *s*	8, 17, 20
10	1.30^{h} *dd* (10, 4)	46.5 *d*	2, 6, 8, 11, 19, 20	1.20 *dd* (11, 2)	48.8 *d*	11, 19, 20
11	1.53 *ddd* (14.5, 13, 4) 1.64 *ddd* (14.5, 13, 5)	35.0 *t*	12, 20	1.36 *td* (13, 4.5) 1.47 *td* (13, 5)	36.7 *t*	12, 20
12	2.09^{i} *dddd* (16, 13, 5, 1) 2.29 *dddd* (16, 13, 4, 1)	21.1 *t*	11	1.86 *td* (13, 5) 1.97 *td* (13, 4.5)	34.5 *t*	11, 14, 16
13		167.9^{b} *s*	12, 14		159.5 *s*	12, 16
14	$5.94^{i,j}$ *q* (1)	118.1 *s*		6.09^{k} *q* (1.2)	117.4 *d*	12, 16
15		169.8 *s*	14, 16		169.2 *s*	
16	6.84^{j} *s*	93.8 *d*	14	2.36^{k} *d* (1.2)	19.0 *q*	14
17	0.82 *d* (6.5)	16.0 *q*		0.77 *d* (6.5)	16.0 *q*	
18	$1.59^{c,d,e}$ *dt* (2, 1.5)	17.9 *q*	3	4.95 *t* (1.5) 5.63 *t* (1.8)	100.1 *t*	
19	1.01^{f} *s*	19.9 *q*	6, 10	1.06^{g} *s*	21.6 *q*	6, 10
20	0.77^{h} *s*	18.2 *q*	10	0.70 *s*	18.2 *q*	10
CO$\underline{\mathrm{Me}}$	2.18 *s*	20.6 *q*				
$\underline{\mathrm{C}}$OMe		169.0 *s*	16, CO$\underline{\mathrm{Me}}$			

[a] Long-range coupled protons observed in the long-range $^{1}H-^{13}C$ COSY spectrum.

[b] Previous assignments [4] were revised.

[c-k] Long-range couplings were observed between each other, respectively, in the $^{1}H-^{1}H$ COSY spectrum.

The relative stereochemistry of **2** was elucidated by the use of difference NOE spectra. Irradiation of H_3-20 (δ0.70) increased the intensities of the methyl protons at δ 1.06 (H_3-19) and at δ0.77 (H_3-17), while irradiation of H_3-19 caused NOE increases of H_3-20 and H-3 (δ4.58). Thus three methyl groups (H_3-17, H_3-19, and H_3-20) and H-3 have *cis* relations. On the other hand, irradiation of H-10 (δ1.20) and H-14 (δ6.09) both caused NOE increases of both protons at C-12. Thus the side chain at C-9 and H-10 are *cis* (H_3-20 and H-10 are *trans*) and the configuration of the 13,14-double bond is *Z*. From these data, **2** was concluded to be 3-hydroxy-cleroda-4(18),13*Z*-dien-15-oic acid.

EXPERIMENTAL

General. Mp: uncorr.; Optical rotations: 26°; IR: KBr; ^{1}H and ^{13}C NMR: 400 and 100 MHz, respectively, with

TMS as int. standard; EIMS and HRMS: 70 eV. Plant material was collected at Kanneliya in the Southern province of Sri Lanka.

Extraction and fractionation. Dried and powdered stem bark of *C. zeylanica* (3.5 kg) was exhaustively extracted with hot MeOH. The MeOH extract was concd and the residue was partitioned between CH_2Cl_2 and H_2O. After concentration, the CH_2Cl_2-soluble fraction was partitioned between hexane and a 10% aq. MeOH to yield a hexane extract and a 10% aq. MeOH extract (70 g). A part of the 10% aq. MeOH extract (50 g) was chromatographed over silica gel (mesh 70–230, 700 g) with CH_2Cl_2 containing increasing amounts of MeOH.

The fraction (750 mg) eluted with 1% MeOH in CH_2Cl_2, on acetylation (Ac_2O–C_5H_5N), gave **1** (560 mg) as needles after recrystallization from MeOH, mp 174–176° $[\alpha]_D$ $-24.6°$ (MeOH; *c* 0.082); ^1H and ^{13}C NMR: Table 1.

The fraction (600 mg) eluted with 5% MeOH in CH_2Cl_2 was subjected to MPLC over silica gel (G60, 30% EtOAc in hexane) to give **2** (43 mg) as an amorphous solid, $[\alpha]_D$ $-57.6°$ (MeOH; *c* 0.04); HRMS $[M]^+$ 320.2370, $C_{20}H_{32}O_3$ requires *m/z* 320.2352; EIMS *m/z* (rel. int.): 320 $[M]^+$ (3), 302 (100), 287 (21), 207 (27), 189 (82); IR ν_{max} cm^{-1}: 3255, 1692, 1634, 1423, 1251, 1180, 1059, 916; ^1H and ^{13}C NMR: Table 1.

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