Indian Journal of Chemistry
Vol. 45B, November 2006, pp. 2556-2557

Note

Bioactive diterpenes from the mangrove *Avicennia officinalis* Linn

C Subrahmanyam*, S Ratna Kumar & G Damodar Reddy
Department of Organic Chemistry, FD & W,
School of Chemistry, Andhra University
Visakhapatnam 530 003
E-mail: cvlk2000@yahoo.co.in

Received 23 August 2005, accepted (revised) 21 March 2006

The hexane extract of the roots of *A. officinalis* Linn, collected from the coast of Ongole, A.P., has yielded *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide, rhizophorin-B **1** and compound **A**, while ethyl acetate extract give triacontan-1-ol, ribenone, excoecarin A, *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide, *ent*-15-hydroxy-labda-8(17),13*E*-dien-3-one, compound **A, 1** and *ent*-3α,15-dihydroxy-labda-8(17),13*E*-diene. Compound **A** is identified as *ent*-(13*S*)-2,3-seco-14-labden-2,8-olide-3-oic acid **2**, a new diterpenoid, on the basis of its spectral data. Excoecarin A, *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide and *ent*-15-hydroxy-labda-8(17),13*E*-dien-3-one show moderate antifungal activities against *Rhizopus oryzae* and *Aspergillus niger* while rhizophorin-B **1** exhibits antibacterial activity against *Bacillus subtilis.*

Keywords: Mangrove, *Avicennia officinalis*, diterpenes, antimicrobial activity

IPC: Int.Cl.[8] A61K

The widely occurring white mangrove *A. officinalis* Linn, yielded different secondary metabolites from different regions. Compounds isolated included iridoids, pentacyclic triterpenoids, sterols, waxes and oxygen-hetrocycles[1]. As part of our investigations on the mangroves of the Indian coast, we examined the roots of *A. officinalis* collected from the Ongole coast, Andhra Pradesh, and the results are reported in this communication.

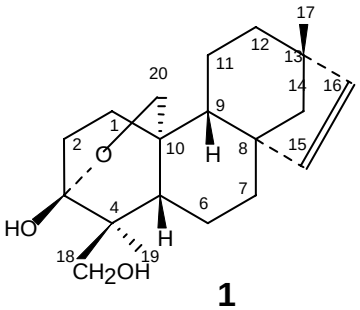
The roots of *A. officinalis* were extracted successively with hexane and ethyl acetate. From the hexane extract, which consisted mostly of waxes, the waxes were removed by treatment with hot methanol and the residue was chromatographed to yield *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide[2], compound **A**, and rhizophorin-B **1** (ref.3). The ethyl acetate extract was washed with aq. NaOH and the residue was chromatographed to yield triacontan-1-ol, *ent*-3-oxo-13-*epi*-manoyl oxide (ribenone); (13*R*,14*R*)-*ent*-8α-13,14-15-bisepoxy-13-*epi*-labdan-3-one (excoecarin A)[4]; *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide[2]; *ent*-15-hydroxy-labda-8(17)-dien-3-one[2]; compound **A, 1** and, *ent*-3α,15-dihydroxy-labda-8(17), 13E-diene[2]. The known compounds were identified by comparison of their physical and spectral data with the data reported in literature.

Compound **A**, m.p. 154°C, $[\alpha]_D$ -53.2° , analysed for $C_{20}H_{32}O_5$ from its FAB mass (m/z 353 M+1) and ^{13}C NMR spectra. It showed hydroxylic (3450); carbonyl (1765 and 1720) and vinylic (990, 985 and 940) cm^{-1}absorptions in its IR spectrum. The ^1H NMR spectrum of compound **A** showed the presence of a vinyl group [δ6.05, dd, *J*=17.0,11.0 Hz (1H); 5.10,d, *J*=17.0Hz (1H); 4.95,d, *J*=11.0 Hz (1H) and five tertiary methyl groups (1.55,s, 1.45,s, 1.32,s, 1.15s, 1.00s). The ^{13}C NMR spectrum of compound **A** showed 20 signals consisting of a carboxylic group (δ 182.1, s); a lactone carbonyl (174.0, s); a vinyl group (148.3, d, 109.8, t); two quaternary oxygen bearing carbons (75.9, s, 73.2,s), two quaternary carbons (42.5, s, 35.1, s), two CH-groups (50.8, d, 48.2, d), five CH_2-groups (46.6, t, 42.2, t, 41.8, t, 22.7, t, 17.0, t) and five methyl groups (32.5, q, 27.4, q, 24.8, q, 23.4, q, 20.0, q). These data indicated that compound **A** was a new secolabdane diterpene, *ent*-(13*S*)-2,3-seco-14-labden-2,8-olide-3-oic acid **2**. Compound **2** is represented in the *ent*-form because of its negative rotation and its stereo-chemistry at C-13 was taken to be (*S*) by analogy with rhizophorin-A **3** (ref. 4,5) and similar naturally occurring diterpenoids.

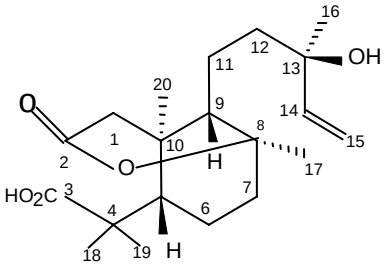
The above compounds were tested for their antimicrobial activities using the cup diffusion method[6]. Excoecarin A, *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl-oxide and *ent*-16-hydroxy-labda-8(17),13*E*-dien-3-one exhibited moderate antifungal activity at 500 μg mL^{-1} level against *Rhizopus oryzae* and *Aspergillus niger* and **1** exhibited antibacterial activity against *Bacillus subtilis* at 200 μg mL^{-1} level.

Experimental Section

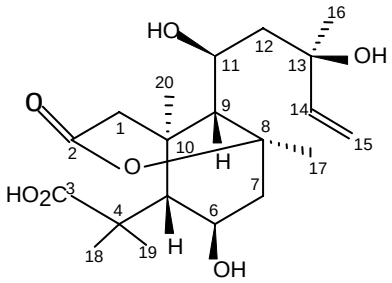
The dried root powder of *A. officinalis* (1.7 kg) was extracted successively with *n*-hexane (15 L) and ethyl acetate (15 L) in an aspirator bottle. From the resulting extracts solvents were removed and the residues were worked up as follows. The residue from the hexane extract (5.6 g) was boiled with MeOH



1



2



3

(100 mL), cooled and the separated waxes were filtered. Solvent was removed from the mother liquor and the residue (2.0 g) was chromatographed on a column of SiO_2 using solvents of increasing polarities from hexane through ethyl acetate. Further purification of some of the resulting fractions yielded *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide (25 mg); compound **A** (25 mg) and **1** (5mg) from the 2-15% ethyl acetate in hexane eluants.

The greenish brown gum (14.2 g) from the ethyl acetate extract was dissolved in ether (200 mL) and the ether layer was washed with 10% aq. NaOH (3 × 100 mL), water (100 mL) and solvent was removed. The resulting gum (7.0 g) was then subjected to chromatography on a SiO_2 gel column using solvents of increasing polarity from hexane through ethyl acetate. Further purification of some of the resulting fractions gave triacontan-1-ol (90 mg); ribenone (60 mg); excoecarin A (100 mg); *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide (30 mg); *ent*-15-hydroxylabda-8(17),13*E*-diene (30 mg); compound **A** (30 mg), **1** (10 mg) and *ent*-3α,15-dihydroxy-labda-8(17), 13*E*-diene (30 mg) from the 2-15% ethyl acetate in hexane eluants.

Compound A: [*ent*-(13*S*)-2,3-seco-14-labden-2,8-olide-3-oic acid, **2**]: Colourless needles from MeOH, m.p.154°C, $[\alpha]_D$ -53.2° (*c*, 0.52, $CHCl_3$); R_f 0.40 (hexane acetone 8:2). IR (KBr): 3450 br, 1765, 1720, 990, 985, 940 cm^{-1}. FABMS (NBA matrix): m/z 353 [(M+1) of $C_{20}H_{32}O_5$; 10], 335 [(M+1)-H_2O;70], 256 (100%); ^1H NMR (90 MHz, d_5-pyridine, TMS): δ 6.05,dd; *J*=17.0, 11.0, Hz (1H, H-16); 5.10, d, *J*=17.0 Hz (1H, H-15); 4.95, d, *J*=11.0 Hz (1H, H-15); 1.55, s (3H,H-17); 1.45, s (3H,H-16), 1.32, s (3H,H-18), 1.15, s (3H,H-20),1.00s (2H;H-19); ^{13}C NMR (15.04 MHz, d_5-pyridine, TMS): δ 46.6, 174.0, 182.1, 42.5, 48.2, 22.7, 42.2, 75.9, 50.8, 35.1, 17.0, 41.8, 73.2, 148.3, 109.8, 32.5, 23.4, 27.4, 24.8, 20.0, (C-1 to C-20).

Acknowledgement

We thank Departments of Botany and Pharmaceutical Sciences, Andhra University for their help in the identification of the plant material and antimicrobial studies, respectively; UGC, New Delhi for the COSIST and DRS facilities at AU and a Project Fellowship to G Damodar Reddy and to CSIR for a SRF to S Ratna Kumar.

References

1 Anjaneyulu A S R, Murthy Y L N, Lakshmana Rao V & Sreedhar K, *Indian J Chem*, 42B, **2003**, 3117, and references cited therein.

2 Konishi T, Azuma M, Itoga R, Kiyosawa S, Fujiwara & Shimada Y, *Chem Pharm Bull*, 44, **1996**, 229.

3 Anjaneyulu A S R, Anjaneyulu V & Lakshmana Rao V, *Indian J Chem*,39B, **2000**, 803

4 Garcia-Granados A, Linan E, Martinez A, Rivas F & Arias J M, *Phytochem*, 38, **1995**, 1237.

5 Anjaneyulu A S R & Lakshmana Rao V, *Nat Prod Lett*, 15, **2001,**13.

6 Cruickshan R, Duguid J P & Swin R H A, In the *Practice of Medical Microbiology*, 12th Edn (Churchill, Livingstone, London), 2, **1975**, p.110.