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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF *AVICENNIA OFFICINALIS* OF KRISHNA ESTUARY

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Abstract:
Phytochemical investigation on the aerial roots of *Avicennia officinalis* of Krishna estuary, India resulted in the isolation of three pentacyclic triterpenoids, betulin aldehyde (1), betulinic acid (2) and betulin (3). These are characterized by physical and spectral data. All are known compounds but we are reporting first time from the Krishna estuary species. Compound (2) was also possess Cytotoxicity.

Keywords: *Avicennia officinalis*, Mangrove, Krishna estuary, Betulin, Cytotoxicity.

Introduction

Avicennia officinalis, belonging to the family Avicenniaceae, is known to be a type of mangrove tree and grows in the tidal forests at the mouth of rivers. The plant distributes along the coast of Southern Asia [5]. In India three species of *Avicennia* are found[3] and of these *Avicennia officinalis* is of wide occurrence. According to Hartwell, the fruits are plastered onto tumors in India [6]. Indian species are used as folk remedies for boils and tumors[4], Kirtikar and Basu suggest that the roots are aphrodisiac[8]. The leaf extract of *Avicennia officinalis* showed significant diuretic activity [10]. Indochinese use the bark for skin afflictions. The resinous substance extruded from the bark acts as a contraceptive and apparently can be taken all year long without ill effects [9].

Avicennia officinalis yielded several secondary metabolites from different regions. Compounds isolated included iridoides, pentacyclic triterpenoids, sterols,waxes and oxygen-hetero cycles.A new naphthofuran, avicennol C was reported from the roots of *A. officinalis* in addition to triterpenoids and steroids [1]. Further from the roots of the plant, collected from the coast of Ongole, India has yielded *ent*-16-hydroxy -3-oxo-13-*epi*-manoyloxide, rhizophorin-β , *ent*-15-hydroxy – labda -8(17), 13-Edien-3-one , *ent* –3α, 15-dihydroxy – labda -8, 13 E-diene and *ent*-(135)-2, 3-seco-14-labden-2, 8-olide-3-oic acid , a new diterpenoid [2]. As part of our investigations on the mangroves of Indian coast, we have examined the aerial roots of *A. officinalis* collected from the Krishna estuary, Andhra Pradesh and the results are presented in this paper.

MATERIALS AND METHODS

Plant Material:

The roots of Avicennia alba were collected from the Thummalapalli, a village in Krishna estuary (Geographically located between 15 053 1N and 80 034 1E), Guntur district, Andhra Pradesh, India during January 2010. The plant was identified by one of the author (PSR) and a voucher specimen has been deposited at the Applied Chemistry Labs, Andhra University.

Instrumentation:

Column chromatography was carried out using silica gel (100-200 mesh, Merck) and TLC analysis was performed on precoated Si Gel plates (Kiesegel 60F254, Merck). The melting points were measured on VEB analytic Dreader HMK hot plate and are uncorrected. IR spectrum was measured with FT-IR Perkin Elmer 1650. NMR spectra were recorded on JEOL JNM EX 90, at 90 MHz (^{1}H); 22.5 MHz (^{13}C) and Bruker Av III, at 400 MHz (^{1}H); 100 MHz (^{13}C). MS spectra (EI-MS) were obtained on Finnigan LCQ-Deca 70 eV.

Procedure:

Extraction and Isolation: The air dried and powdered roots (1.5 kg) were extracted with dichloromethane (10 x 8L). Removal of the solvent from the combined extracts under reduced pressure gave a residue (9.0g). This residue was subjected to column chromatography over silica gel using solvents of increasing polarity from n-hexane through ethyl acetate to get 3 fractions. These are F1(from 5% hexane in EtOAc) and F2,F3 (from 10% hexane in EtOAc) and are further purified by passing over a small column of silica gel or by repeated crystallization to afford three pure compounds 1-3. These were characterized by a comparative study of their physical and spectral data with those reported for the compounds in the literature and by direct comparison with authentic samples wherever possible.

Bioassay for Cytotoxicity:

Cytotoxicitywas evaluated by Mitotic Index (MI) Assay and Cell Proliferation Kinetics. Cell population growth occurs as cells pass through interphase and mitosis to complete the cell cycle. Many cells lose the capacity to divide as they mature or divide only rarely. Other cells are capable of rapid cell division. Mitotic index is a measure for the proliferation status of a cell population. It is defined as the ratio between the number of cells in mitosis and the total number of cells. The mitotic index can be worked out from a slide, even with light microscopy and is a measure of Cytotoxicity [7]. It is the number of cells containing visible chromosomes divided by the total number of cells in the field of view.The most convenient andreadily available tissue for cytogenetic study is peripheral blood.Few drops of whole blood (0.5mL),collected from the left hand vein of one of the author(MVVR) ,are cultured in medium RPMI 1640 and a mitogen phytohemagglutinin (PHA) 100µL was added. For positive control 0.3µg of Mytomycin C (cross-linking agent) was to added to the culture . For compound induced cultures five different doses of test compound (5mg, 10mg 15mg, 20mg and 25mg per culture) were added. And for negative control group we didn't add any additive. Peripheral blood cultures were placed in a 37°C incubator for 72 hrs.and blood cultures exposed to test compounds.

RESULTS AND DISCUSION:

Compound 1:

Compound 1 was obtained as colourless needles from methanol, m.p. 192-193^0 C, $[\alpha]_D$+ 19.3^0 (c1.2, $CHCl_3$). Its molecular ion was assigned as $C_{30}H_{48}O_2$ from its mass ion at m/z 440 in its mass spectrum. It gave positive Lieberman –Burchard test for terpenoids. Its IR absorptions showed hydroxyl (3480 cm^{-1}) and aldehyde carbonyl (1725 cm^{-1}) functional groups. The ^{1}H NMR spectral data indicated the presence of six tertiary methyls (δ 0.75, 0.82, 0.95, 0.97, 1.03 and 1.70), two exocyclic methylene protons at δ 4.81 and 4.61 (1 H each as broad singlet), hydroxymethine proton (3α -H) at δ 3.4 (1H, dd, J=12.2, 8.2 H_Z) and aldehyde proton at δ 9.71 (1H, S). Its ^{13}CNMR (Table 1) spectral data revealed the presence of 30 carbons. The ^{13}C NMR and DEPT experiments confirmed the presence of olefinic carbons at δ149.6 (s), δ 110.1 (t) and oxygenated carbon at δ 79.0.Further the spectra also displayed aldehyde carbon signal at δ 206.5. A search in literature showed that the above Physical and spectral data is identical to the data reported for triterpene betulin aldehyde (1) [11]. This is the first report from mangrove species of Krishna estuary.

Compound 2:

Compound 2 obtained as colourless needles from methanol-chloroform, m.p. 307-309^{0}C, [α]$_D$ + 15.2^0 (c1.0, pyridine) and analysed for $C_{30}H_{48}O_3$ from its molecular ion at m/z 456 in its mass spectrum. It gave a positive Liebermann-Burchard test for triterpenoids. Its IR spectrum showed bands at 3445 (hydroxyl), 1685 (carbonyl) and 885 cm^{-1} (methylene). The 1H NMR spectrum showed five tertiary methyls at δ 99.0, 98.0 95.0, 83.0, 76.0 and one methyl group on double bond at δ 1.70. The spectrum also showed peaks for a pair of olefinic protons at δ 4.75 and 4.62 (each one H, br-s; exomethylene group), a carbinolic proton at δ 3.19 (dd, J=12.0, 6.4 Hz) referring to its α–orientation and a peak at δ 3.0 (1H, m).

The 13 C NMR data (Table 1) showed olefinic carbons at 150.6 (s) and δ 109.3 (t), hydroxy methine carbon at δ 79.0(d) and carbonyl carbon at δ 179.5(s).

A search in literature showed that the above data, physical and spectral characteristics, of compound 2 well

Table 1: ^{13}C NMR data of compounds 1, 2 and 3

Carbon	Compound **1**	Compound **2**	Compound **3**
1	38.8	38.6	38.7
2	27.3	27.0	27.1
3	78.9	79.0	79.0
4	38.8	38.7	38.7
5	55.5	55.3	55.3
6	18.2	18.2	18.3
7	34.3	34.2	34.3
8	40.8	40.5	40.9
9	50.4	50.4	50.4
10	37.1	37.0	37.2
11	20.7	20.8	20.8
12	25.5	25.4	25.2
13	37.7	38.2	37.3
14	42.5	42.3	42.7
15	29.2	30.7	28.0
16	28.2	32.0	29.2
17	259.3	56.1	47.8
18	48.0	46.1	48.8
19	47.5	49.5	47.8
20	149.7	150.6	150.2
21	29.8	29.9	29.8
22	33.2	37.2	34.0
23	27.9	27.5	27.4
24	15.4	15.2	15.3
25	15.9	15.8	16.1
26	16.1	16.0	16.0
27	14.2	14.5	14.7
28	205.6	179.5	60.2
29	110.1	109.3	109.2
30	19.0	19.3	19.1

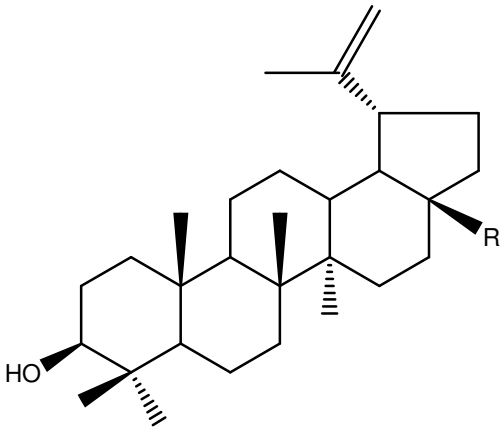
Compounds 2 and 3 were measured at 100 MHz in $CDCl_3$; Compound 1 was measured at 22.5 MHz in $CDCl_3$.

Table 2: M I and CPK studies of compounds 1,3 and 2

S. No	Conc. of Compound (mg/mL)	Compound 1		Compound 3		Compound 2	
		MI	CPK	MI	CPK	MI	CPK
01	5	5.98	0.43	5.92	0.43	4.52	0.40
02	10	5.84	0.41	5.78	0.44	3.63	0.39
03	15	5.35	0.39	5.47	0.45	2.56	0.28
04	20	5.21	0.41	5.62	0.42	1.54	0.16
05	25	4.89	0.44	4.82	0.39	--	--
	Mean± SD	5.454 ± 0.4511	0.416 ± .0194	5.522 ± 0.4272	0.426 ± 0.0230	3.0625 ± 1.2931	0.3075 ± 0.1123
06	MMC (0.3 µg /mL) Mean±SD	3.48 ± 0.214	0.43 ± 0.011				
07	Control Mean±SD	6.28 ± 0.442	0.46 ± 0.751				

Table 2 shows MI and CPK values of cultures exposed to test compounds. The selected concentrations of all test compounds are 5 mg,10 mg,15 mg,20 mg and 25 mg per culture. The MI values of control are 6.82, 5.96, 5.87 and 6.84. The MI values of cultures exposed to test compound 2 were decreased gradually in higher doses when compared to compound 1 and compound 3. There is no significant difference in compound 1 and 3 When compared to control values compound 2 does increased induced significant mitodepression. The CPK values of control group are 0.46, 0.48, 0.45, and 0.47. The CPK values of compound 2 exposed cultures, in 5mg, 10mg 15mg, 20mg and 25mg doses, decreased gradually in higher doses. Based on MI and CPK values compound 2 (betulinic acid) possess Cytotoxicity.

MI = Mitotic Index (No. of dividing cells / Total No. of cells X 100)
CPK = Cell Proliferation Kinetics [(M1+2M2+3M3)/100]
(--) = Cytotoxic (lethal dose)



Betulin aldehyde (**1**) : R = CHO
Betulinic acid (**2**) : R= COOH
Betulin (**3**) : R = CH_2OH

agreed with the data reported for Betulinic acid [11]. Thus the compound 2 was identified as Betulinic acid (2).

Compound 3:

This was obtained as colourless needles from methanol, m.p. 250-251 0C, [$\alpha]_D$ + 20.0^0 (c1.2, $CHCl_3$) and analysed for $C_{30}H_{50}O_2$ from its molecular on at m/z 442 in its EI mass spectrum. It gave a positive Liebermann-Burchard test for triterpenoids and IR spectrum showed hydroxyl (3450 cm^{-1}) and methylene (880 cm^{-1}) absorptions. The 1H NMR spectra showed five tertiary methyls and one methyl on double bond and two exocyclic methylene protons at δ 4.58 and δ 4.68 (1H each as singlet), a hydroxy methine proton (3 α-H) at 3.19 (dd, J = 11.2,4.8 Hz). Further Two hydroxy methylene protons appeared at δ 3.4 (1H, d, J=10.4 Hz) and δ 3.8 (1H, d, J = 10.4 Hz). The 13 C NMR data (Table 1) showed olefinic carbons at δ 150.2(s) and δ 109.2 (t), hydroxy methine carbon at δ 79.0(d) and hydroxy methylene carbon at δ 60.2 (t). All the physical and spectral characteristics (IR, 1H NMR and 13 C NMR) were in full agreement with the data of pentacyclic triterpene, Betulin (3) to show their identity [11].

CONCLUSION:

Chemical examination of white mangrove *Avicennia officinalis* of Krishna estuary, India afforded three pentacyclic triterpernoids, betulin aldehyde, betulinic acid and betulin. All are known compounds but we are reporting first time from the Krishna estuaryspecies. Betulinic acid was showed Cytotoxicity.

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REFERENCES

1. ASR.Anjaneyulu, YLN Murthy, V.Laskhmana Rao and K.Sreedhar, 2003.Indian J. Chem, 42B, 3117-3119.
2. C.Subrahmanyam, S.Ratnakumar and G.Damodar Reddy, 2003. Bioactive diterpenes from the mangrove *Avicennia officinalis* Linn Indian J chem., 45B, 2556-2557.
3. E.L.Little ,1983. Common fuel crops, A Hand book of their identification, M/S McClain Printing Co, 1983, Parsons, WV.354 pp.
4. J.A.Duke and K.K.Wain, 1981. Medicinal plants of the world, 3 Vols,1654 pp.
5. J.D. Hooker ,1982. The Flora of British India, Vol. II, Saibiaceae to Goruacea, M/s. Birhen Singh Mehendra pal Singh, Dehra Dun, 1982
6. J.L.Hartwell, 1967-1971,Plants used against cancer, A Survey,Lloydia 30-34.
7. Kannan TP, Quah BB and Ahmad Azlina. 2006. Mitotic Index and Chromosomal Analyses for Hydroxyapatite Implantation in Rabbits. Archives of Orofacial Sciences. 1: 15-20.
8. KR.Kirtikar and B.D.Basu, 1975. Indian Medicinal Plants, 4 Vol., 2nd ed, Jayyed Press, New Delhi
9. L.M.Pery, 1980. Medicinal plants of East and South East Asia, MIT Press,Cambridge,
10. M. Sharma and H.S.Garg , 1996. Indian J Cherm, 35 B,459.
11. Mahato SB and AP Kundu (1994). 13C NMR spectra of pentacyclic triterpenoids-a complilation and some salient features. Phytochemistry, 37, 1517-1575.