Chemistry of Natural Compounds, Vol. 48, No. 1, March, 2012 [Russian original No. 1, January–February, 2012]

ISOLATION OF A LUPANE TRITERPENE FATTY ACID ESTER WITH ANTIBACTERIAL ACTIVITY FROM THE LEAVES OF *Finlaysonia obovata*

Pravat Manjari Mishra*, A. Sree, and Mallika Panigrahi

UDC 547.92

Pentacyclic triterpenes have recently attracted much attention due to their great potential as multitarget therapeutics. Structural modifications based on natural triterpenes have been extensively explored to find more potential pentacyclic triterpenes as preventive and therapeutic agents [1, 2]. Studies of structure–activity relationships (SAR) of pentacyclic triterpenes showed that A-ring functions had a significant impact on biological activities [1, 2]. Ester functionality at C-3 is essential for enhanced biological activities of pentacyclic triterpenes. Jagdeesh et al. synthesized some 3-*O*-allyl and cinnamyl esters of the lupane class of pentacyclic triterpene acids, specifically betulinic acid, and these compounds were found to exhibit antifeedant activity [3]. Fatty acid ester of triterpenoid from *Celastrus rosthornianus* showed cytotoxicity against human cervical squamous carcinoma (Hela) cells [4]. Lupeol long-chain fatty acid esters with antimalarial activity were isolated from *Holarrhena floribunda* [5].

Finlaysonia obovata is a latex-exuding mangrove plant (Periplocaceae) found in the tidal flats in India, Burma, and Malaysia, the leaves of which are reported to be eaten as salad in the Moluccas. Mangrove latex-bearing plants were found to show antibacterial, antiviral, and other activities [6]. Earlier we have studied the antibacterial activity, GC-MS analysis of extracts, lipid of leaf, isolation of a rare antibacterial triterpene and a steroid from *F. obovata* [7–9]. The present paper deals with the isolation and spectral characterization of lup-20(29)-ene-3-tetradecanoate from the hexane extract of leaves of *F. obovata*. This article presents all the spectral data for lup-20(29)-ene-3-tetradecanoate and isolation from a latex-exuding mangrove plant for the first time.

The column chromatography of hexane extracts of the leaves of *F. obovata* [fraction (hexane–EtOAc, 9.8:0.2)] yielded compound **1** on crystallization from $CHCl_3$–MeOH. The triterpene moieties of compound **1** were preliminary checked by TLC employing the Lieberman-Burchard spray reagent. Compound **1** displayed ^1H and ^{13}C NMR spectra (Table 1) exhibiting features characteristic of the lup-20(29)-ene skeleton [δ_H 4.68 (br.s, H-29a); 4.57 (br.s, H-29b); δ_C 150.9 (C-20) and 109.3 (C-29)] and signals due to seven tertiary methyl groups, which are reminiscent of a lupeol-type triterpene [10]. In addition to these, compound **1** showed characteristic features of lupeol, broadened signals of long- chain fatty acid units [10, 11]. The ^1H NMR spectrum of **1** showed a broad, intensive singlet at δ 1.25 due to methylene protons, indicating the presence of a long fatty acid ester moiety. The major differences between the NMR spectral data of lupeol and compound **1** are the chemical shifts of H-3 and C-3, which, when contrasted, show significant downfield shifts, with $\Delta\delta_H$ 1.29–1.37 ppm and $\Delta\delta_C$ 2.0–2.4 ppm respectively. The C-3 signal appeared at a higher frequency (δ 80.5) than that observed for lupeol (δ 79.3). Compound **1** is therefore a lupeol derivative possessing a long-chain fatty acid unit at C-3. This observation was supported by the presence of an additional methyl (δ 14.5) and acyl group (δ 173.7) whose carbonyl was downshifted (Δδ ca. 3.52) relative to 3*β*-acetyl-lup-20(29). The attachment of the long-chain fatty acid ester moiety to C-3 is also confirmed by the HMBC correlation between H-3 and the carbonyl carbon signal of the fatty acid ester chain. The basic skeleton of **1** was supported by the key HMBC correlations: H-3/C-1′, C-23, C-24, C-5; H-5/C-23; H-19/C-29, C-20, C-30; H-2′/C-1′ (Fig. 1). The molecular ion recorded on the MS at *m/z* 636 was conclusive of the esterified nature of the triterpene. Alkaline hydrolysis of **1** furnished the known compound lupeol and tetradecanoic acid, which was characterized by GC-MS analysis. The mass spectrum of the alkaline hydrolysis product of **1** made it possible to recognize the tetradecanoic acid esterified at the C-3 position of the triterpene.

Natural Product Department, Institute of Minerals & Materials Technology, Bhubaneswar-751013, Orissa, India, fax: 91 674 2581637, e-mail: pravatmanjari@yahoo.co.in; pravatmanjari@immt.res.in. Published in *Khimiya Prirodnykh Soedinenii*, No. 1, pp. 144–146, January–February, 2012. Original article submitted September 06, 2010.

0009-3130/12/4801-0161

TABLE 1. ^{1}H (400 MHz, δ, ppm, J/Hz) and ^{13}C NMR (100 MHz) Spectral Data of Compound **1**

C atom	HMQC		C atom	HMQC	
	δ_C	δ_H		δ_C	δ_H
1	38.3	1.64 (m), 1.2 (m)	19	47.9	2.25 91H, td, J = 11.5, 9.0)
2	23.7	1.58 (m), 1.31 (m)	20	150.9	
3	80.5	4.45 (1H, dd, J = 9.6, 8.0)	21	29.2	1.38 (m), 2.10 (m)
4	37.8		22	39.9	1.41 (m), 1.31 (m)
5	55.3	1.45 (1H, m)	23	27.9	0.89 (3H, s)
6	17.9	1.81 (m), 1.61 (m)	24	16.5	0.74 (3H, s)
7	34.1	2.20 (m), 1.40 (m)	25	15.9	0.85 (3H, s)
8	40.8		26	16.1	1.05 (3H, s)
9	50.3	1.35 (1H, d, J = 11.5)	27	22.6	0.98 (3H, s)
10	37.0		28	18.1	0.80 (3H, s)
11	20.9	1.50 (m), 1.31 (m)	29	109.3	4.57 (1H, br.s, J = 2.5)
12	25.0	1.32 (m), 1.60 (m)			4.68 (1H, br.s, J = 2.5)
13	38.0	1.64 (1H, m)	30	19.7	1.68 (3H, s)
14	42.9		Ester		
15	27.4	1.30 (m), 1.62 (m)	1′	173.7	
16	35.5	2.30 (m), 1.42 (m)	2′	29.1	2.35 (2H, t, J = 7.4)
17	42.8		3′–13′	29.2–29.7	1.25 (br.s)
18	48.2	1.44 (m)	14′	14.5	1.15 (3H, t, J = 6.7)

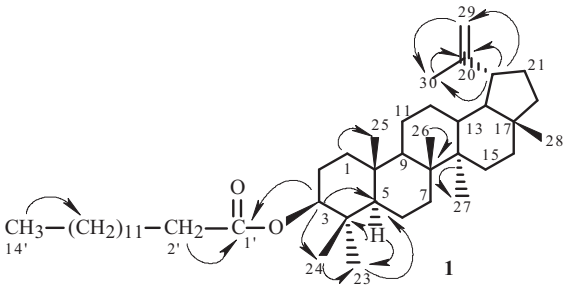

Fig. 1. Important HMBC correlations of compound **1**.

On the basis of the above spectral data and chemical conversion studies, the structure of compound **1** was assigned as lup-20(29)-ene-3-tetradecanoate. This compound was first isolated from *Koelpinia linearis* [12]. There are several reports on the isolation of triterpene esters from mangrove plants [13, 14], but this is the first report of all spectral characterization of lup-20(29)-ene-3-tetradecanoate and the first report from a latex-exuding mangrove plant.

The antibacterial screening of compound **1** (zone 11 mm) showed activity against *Aeromonas hydrophila* and trace activity against four pathogens, viz. *Micrococcus* sp., *Vibrio alginolyticus*, *Edwardsiella tarda*, and *Staphylococcus aureus* at 100 μg disc^{-1}. At 50 μg disc^{-1} it showed trace activity against *A. hydrophila*. The inhibitory activity of this lupeol triterpene may be due to the presence of long-chain fatty acid ester [4, 5].

Melting points were determined on a Buchi melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded at 400 MHz, and the ^{13}C NMR spectra were recorded at 100 MHz on an AL-400 MHz FT-NMR spectrometer (JEOL, Japan). GC-MS analysis was carried out on the Shimadzu QP-5000 system equipped with a mass selective detector. $[\alpha]_D$ was measured by a Jasco digital polarimeter. EI-MS analysis was carried out on the GC-MS-Shimadzu QP-5000 system, and elemental analysis was performed on a Carlo Elba, EA-1108 analyzer.

Plant Material. The leave of *F. obovata* was collected from Bhitarkanika mangrove forest of Orissa (during late winter) and identified by K. S. Murthy, I/C SMP unit, Central Research Unit (AY), Bhubaneswar. The specimen (accession No. 12550) has been deposited at the herbarium (RRL-B) of IMMT, Bhubaneswar.

Extraction and Isolation. The hexane extract (0.2 g) of leaves of *F. obovata* was chromatographed on a silica gel (100–200 mesh) column. The fraction (hexane–EtOAc, 9.8:0.2) of the eluate, on crystallization from $CHCl_3$–MeOH, afforded a white wax, compound **1**.

Lup-20(29)-ene-3-tetradecanoate (1). White wax; crystallized from $CHCl_3$–MeOH (50 mg); MF: $C_{44}H_{76}O_2$; mp 103–104°C; $[\alpha]_D^{25}$ +34.4° (0.1 g/100 mL, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCL_3$) spectral data see Table 1. EI-MS *m/z*: 636 $[M]^+$ (2), 621 (10), 593 (5), 468 (2), 429 (10), 413 $[M - C_{14}H_{28}O_3]^+$, 409 (10), 393 (7), 365 (5), 298 (5), 257 (10), 204 (2), 191 (5), 189 (60), 175 (5), 147 (10), 121 (10), 81 (20), 43 (100).

Alkaline Hydrolysis of Lup-20(29)-ene-3-tetradecanoate. To a solution of compound **1** (10 mg) in $CHCl_3$ (10 mL), 10 mL of 2 N NaOH was added. The reaction mixture allowed to cool to room temperature and the organic layer was separated. The aqueous layer was extracted two more times with 10 mL portions of $CHCl_3$. The combined organic layer, which was concentrated and purified by preparative TLC ($CHCl_3$), furnished a colorless solid that was identified as lupeol (5 mg) from spectral data (^1H and ^{13}C NMR) [15]. The aqueous layer was acidified to pH 2.0 with 1 N HCl and extracted with 10 mL portions of diethyl ether three times to yield tetradecanoic acid (1 mg), which was identified by GC-MS analysis.

Tetradecanoic Acid. GC-MS, EI-MS *m/z* (rel. int.): 228 $[M]^+$ (10), 201 (5), 171 (6), 155 (40), 127 (45), 99 (20), 57 (85), 43 (100).

Antibiotic Activity Testing of Compound 1. Earlier, the antibacterial assay of the hexane extract of *F. obovata* was studied by us against seven freshwater fish pathogenic bacteria [8]. The hexane extract was found active against five tested pathogens. The antibacterial screening of pure compound **1** isolated from hexane extract (100 and 50 μg /10 mm disc) was carried out by the same method as described earlier.

ACKNOWLEDGMENT

The authors thank the Director, IMMT, Bhubaneswar for facilities, and K. S. Murthy, I/C SMP Unit, Central Research Institute (AY), Bhubaneswar for identification of the plant. The authors thank DOD/MoES for funding the project.

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