Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [Russian original No. 3, May–June, 2011]

A NEW ACYLATED TRITERPENE WITH ANTIMICROBIAL ACTIVITY FROM THE LEAVES OF *Rauvolfia vomitoria*

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UDC 547.918

*A new acylated tritrepene, 3β-hexadecanoyloxy-lup-20(29)-en-21-ol (**1**), along with seven known compounds, lupeol (**2**), betulinic acid (**3**), ursonic acid (**4**),* β*-sitosterol (**5**),* β*-stigmasterol (**6**), 3-*O*-*β*-D-glucopyranosyl-*β*-stigmasterol (7), and palmitic acid (**8**), were isolated from the leaves of* Rauvolfia vomitoria *(Apocynaceae). Their structures were established on the basis of spectroscopic analysis and chemical evidence. The new acylated triterpene exhibited interesting antimicrobial activity against* Candida albicans *(a yeast) with the MIC value 64 μg/mL.*

Keywords: *Rauvolfia vomitoria,* Apocynaceae, leaves, acylated triterpene, antimicrobial activity.

Rauvolfia (also spelled *Rauwolfia*) is a genus of evergreen trees and shrubs in the milkweed family, Apocynaceae. The approximately 85 species in the genus are found in tropical regions. Plants of this genus are used medicinally, both in conventional western medicine and folk medicine. Alkaloids in the plants reduce blood pressure, depress the activity of the central nervous system, and act as hypnotics. *Rauvolfia vomitoria* is an important medicinal plant used in many illnesses such as venereal disease, neuropsychiatry disorders, jaundice, gastro-intestinal diseases, sexual complaints, measles, and malaria [1]. A number of studies have reported the isolation of indole alkaloids and other compounds with interesting biological activities from *Rauvolfia vomitoria* [2–6]. In continuation of the search in this medicinal species, the leaves of *Rauvolfia vomitoria*, found in the Central Region of Cameroon, were studied, which afforded a new compound and seven known compounds. In this paper we present the structural determination and the antimicrobial activity of the new acylated triterpene, 3*β*-hexadecanoyloxy-lup-20(29)-en-21-ol (**1**).

Compound **1** was obtained as reddish amorphous crystals. The molecular formula was determined to be $C_{46}H_{80}O_3$ based on the HR-TOF-ES$^+$-MS ion peak *m/z* 680.6135 (calcd for $C_{46}H_{80}O_3$ 680.6146). Its IR spectrum showed absorption bands at 3415 (OH) and 1725 (C=O) cm^{-1}. The ^{1}H NMR spectrum of **1** (Table 1) showed seven methyl singlets at δ 0.79, 0.87, 0.88, 0.95, 0.97, 1.03, and 1.68 and two doublets at δ 4.57 and 4.69, suggesting a lup-20(29)-ene type triterpene. This was confirmed by the ^{13}C NMR spectrum of **1** (Table 1), which showed signals at δ 150.8 and 109.4 assigned to carbons C-20 and C-29, respectively [7]. The ^{1}H and ^{13}C NMR spectra also showed signals at (δ_H 4.56, δ_C 81.3) and (δ_H 4.00, δ_C 68.2), suggesting the presence of two oxymethine groups in the molecule. The presence of a long chain acyl was deduced from the signals observed at δ_C 172.8 (C=O ester), δ_C 29.7 ($(CH_2)_n$), and δ_C 14.2 (CH_3). The DEPT, COSY, and HMBC spectra allowed fixing the ester group at C-3 (δ_H 4.56, δ_C 81.3) and the hydroxyl function at C-21 (δ_H 4.00, δ_C 68.2). The HMBC spectrum of **1** (Fig. 1) showed correlation spots between proton H-3 (δ_H 4.56) and carbons C-1′ (δ_C 172.8) and C-4 (δ_C 37.8); it also displayed interactions between proton H-21 (δ_H 4.00) and carbons C-18 (δ_C 48.2), C-19 (δ_C 47.9), C-20 (δ_C 150.8), and C-22 (δ_C 41.6), confirming the location of the hydroxyl group at the C-21 position.

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TABLE 1. ^{1}H and ^{13}C NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)*

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	39.9 (CH_2)	1.23 (m)	19	47.9 (CH)	2.40 (m)
		1.43[a] (m)	20	150.8 (C)	
2	23.7 (CH_2)	1.66 (m)	21	68.2 (CH)	4.00 (dd, J = 5.3, 8.3, H_{eq})
		1.70[a] (m)	22	41.6 (CH_2)	2.43 (m, H_{eq})
3	81.3 (CH)	4.56 (dd, J = 5.4, 7.2, H_{ax})			2.50 (dd, J = 5.3, 7.5, H_{ax})
4	37.8 (C)		23	28.0 (CH_3)	0.87 (s)
5	55.4 (CH)	0.83 (m)	24	16.6 (CH_3)	0.88 (s)
6	18.2 (CH_2)	1.43[a] (m)	25	16.1 (CH_3)	1.03[a] (s)
		1.54[a] (m)	26	16.0 (CH_3)	0.97 (s)
7	35.5 (CH_2)	1.39 (m)	27	14.5 (CH_3)	0.95 (s)
		1.50 (m)	28	17.9 (CH_3)	0.79 (s)
8	40.8 (C)		29	109.4 (CH_2)	4.57 (d, J = 7.4)
9	50.3 (CH)	1.32[a] (m)			4.69 (d, J = 7.4)
10	37.1 (C)		30	19.3 (CH_3)	1.68[a] (m)
11	20.9 (CH_2)	1.42 (m)	1′	172.8 (C)	
		1.44 (m)	2′	29.4 (CH_2)	1.92 (m)
12	25.0 (CH_2)	1.15 (m)	3′	38.3 (CH_2)	1.03[a] (m)
		1.68[a] (m)	4′	31.9 (CH_2)	1.30[a] (m)
13	38.0 (CH)	1.70[a] (m)	5′–11′	29.7 (CH_2)	1.30[a] (m)
14	42.8 (C)		12′	29.5 (CH_2)	1.30[a] (m)
15	36.6 (CH_2)	1.43[a] (m)	13′	27.4 (CH_2)	1.03[a] (m)
		1.54[a] (m)	14′	25.5 (CH_2)	1.46 (m)
16	34.2 (CH_2)	1.43[a] (m)	15′	22.7 (CH_2)	1.26 (m)
17	43.0 (C)		16′	14.2 (CH_3)	0.90 (m)
18	48.2 (CH)	1.40 (m)			

*Assignments were based on DEPT, COSY, HSQC, HMBC experiments; [a]overlapping signals.

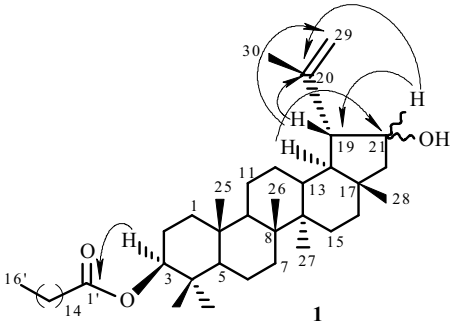

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Fig. 1. Key HMBC correlations (H→C) of **1**.

Moreover, correlations were seen between proton H-18 (δ_H 48.2) and carbons C-17 (δ_C 43.0), C-20 (δ_C 150.8), and C-21 (δ_C 81.3), and between proton H-19 (δ_H 2.40) and carbons C-20 (δ_C 150.8), C-21 (δ_C 81.3), and C-29 (δ_C 109.4). The COSY spectrum showed correlations between proton H-21 (δ_H 4.00) and H-19 (δ_H 2.40), H-22 (δ_H 2.43 and 2.50). However, from the coupling constants observed, it was not evident that the stereochemistry of the 21-OH was due to the overlapped proton chemical shifts at δ_H 2.40–2.44. The coupling constant of proton H-3 (J = 5.4; 7.2 Hz) was in agreement with the *β*-orientation of the ester group at C-3 position. From the mass spectrum fragmentation, the acyl group was established as CH_3-$(CH_2)_{14}$-C=O. Therefore, the structure of **1** was established as 3*β*-hexadecanoyloxy-lup-20(29)-en-21-ol, isolated for the first time from the natural source.

The structures of the known compounds were identified by their spectral data in comparison with literature values as lupeol (**2**) [7], betulinic acid (**3**) [7], ursonic acid (**4**) [7], *β*-sitosterol (**5**) [8], *β*-stigmasterol (**6**) [8], 3-*O*-*β*-D-glucopyranosyl-*β*-stigmasterol (**7**) [8], and palmitic acid (**8**).

Compound **1** was analyzed for antimicrobial activity using the XTT colorimetric assay performed according to Pettit et al. [9] as modified by Kuete et al. [10]. The antibacterial activity studies against *Staphylococcus aureus* (ATCC25922), *Salmonella typhi* (ATCC6539), *Pseudomonas aeruginosa* (ATCC27853), and *Escherichia coli* (ATCC 10536), and the antifungal activity against *Candida albicans* (ATCC 9002), were carried out with gentamicin and nystatin, respectively, as positive control. Compound **1** showed moderate inhibitory activities against growth of *E. coli* (MIC = 256 μg/mL), *S. typhi* (MIC = 512 μg/mL), *S. aureus* (MIC = 512 μg/mL), and *C. albicans* (MIC = 64 μg/mL), and no inhibitory activity against growth of *P. aeruginosa* (up to 512 μg/mL). However, the activity recorded on *C. albicans* can be considered interesting, as the MIC value recorded (64 μg/mL) was only 4-fold greater than that of nystatin.

EXPERIMENTAL

The mps were determined using a Kofler microhot stage apparatus. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. IR spectra (ν_{max}, cm^{-1}) were obtained from potassium pellets on a Nicolet 510 FT instrument. Mass spectra were recorded on a Micromass Q-Tof instrument, a Negmag R10-10C spectrometer, and an HP-5973 Mass Selective Detector. ^1H NMR (δ, ppm, J/Hz) and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, using a Bruker AC 400 spectrometer. Multi-impulsional 2D NMR experiments (^1H–^1H COSY, ^1H–^1H NOESY, ^{13}C–^1H HSQC, ^{13}C–^1H HMBC) were performed using standard Bruker micro-programs. Silica gel 60 (70–230 mesh) was used for column chromatography at normal pressure, while silica gel 60 H (5–40 μm) and 60 AC (20–40 μm) were used for column chromatography under compressed air (300 mbar). Precoated silica gel 60 F_{254} aluminum plates were used for thin layer chromatography and eluted with mixtures of solvents such as hexane–CH_2Cl_2 (9:1), CH_2Cl_2–MeOH (19:1), and CH_2Cl_2–MeOH (9:1).

Plant Material. The leaves of *R. vomitoria* Afzel (Apocynaceae) were collected from Mbalmayo (Central region of Cameroon) in February 2007 and identified by M. Victor NANA, a botanist at the National Herbarium of Yaounde, Cameroon where the voucher specimens are deposited (Ref 1959/SRFK).

Extraction and Isolation. The leaves of *R. vomitoria* were sun-dried, ground into powder form (5.0 kg), and macerated at room temperature with MeOH (3 × 10 L) for 10 days. The solvent was evaporated under reduced pressure to yield the total crude extract (60 g), which was subjected to column chromatography over silica gel (70–230 mesh), 450 g, and eluted with *n*-hexane, *n*-hexane–EtOAc, and EtOAc–MeOH in increasing polarity to give a total of 130 fractions (300 mL each). TLC permitted the combination of the resulting fractions into five series designated A–E. Further column chromatography over silica gel 60 C (40–70 μm) of series B, eluting with hexane–EtOAc in increasing polarity, afforded 3*β*-hexadecanoyloxy-lup-20(29)-en-21-ol (**1**, 25 mg).

3*β*-Hexadecanoyloxy-lup-20(29)-en-21-ol (1). Reddish amorphous crystals; mp 78°C (hexane–EtOAc); $[\alpha]_D^{20}$ +14° (*c* 0.34, $CHCl_3$). IR spectrum (KBr, ν_{max}, cm^{-1}): 3415 (OH), 3015, 1725 (C=O), 1260, 1180, 895. GC/MS, *m/z*: 680 $[M]^+$, 440, 437, 413, 283, 264, 208, 171, 157, 115. HR-TOF-ES$^+$ MS *m/z* 680.6135 (calcd for $C_{46}H_{80}O_3$ 680.6146). For ^1H and ^{13}C NMR spectral data (400 and 100 MHz, $CDCl_3$/MeOH-d_4), see Table 1.

ACKNOWLEDGMENT

One of the authors (J. Wandji) is grateful for a grant (No. F/2624-3F) from the International Foundation for Science (Sweden), and to the sponsorship of the "Universite Paris Descartes, France" during his multiple research visits in the "Laboratoire de Pharmacognosie, Faculte des Sciences Biologiques et Pharmaceutiques de Paris".

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