A New Cyclopropyl-Triterpenoid from *Ochradenus arabicus*

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One new cyclopropyl-triterpenoid (**1**), along with four known constituents including octacosan-1-ol (**2**), pentacosanoic acid (**3**), β-sitosterol (**4**), and β-sitosterol 3-*O*-β-D-glucopyranoside (**5**) were isolated from the aerial parts of *Ochradenus arabicus* for the first time. These compounds were isolated by repeated column chromatography followed by further purification through recycling HPLC. The structure of the new secondary metabolite **1** was established on the basis of UV, IR, 1D- (^{1}H- and ^{13}C-) and 2D-NMR (HMBC and HSQC), and MS spectral data. The molecular mass was determined by HR-MS, and hence the molecular formula was deduced. The configurations of stereogenic centers in the molecule were assigned by NOESY experiments, along with biogenetic considerations. The structures of the known compounds were confirmed by comparison of their physical and spectroscopic data with those reported in literature.

Introduction. – *Ochradenus* is a small genus of about eight species distributed in the desert and arid regions of Southwest Asia and Northeast Africa, with a center of diversity in the South Arabian Peninsula [1]. The taxonomy of the genus is very complex whilst only one species (*O. baccatus* DELILE) is widely distributed. The other species are restricted as endemic in dispurse parts of the Arabian Peninsula, especially in Oman and the horn of Africa [2]. *Ochradenus arabicus* CHAUDHARY, HILLC. & A. G.MILL. (Resedaceae) mostly grows in sandy, desert, and arid regions of Saudi Arabia, United Arab Emirates, Oman, and Yemen [3]. *O. arabicus* has a multitude of medicinal properties, because of which it is widely used locally for curing different ailments in many countries including Saudi Arabia [3][4]. Previous literature revealed that *O. baccatus* possesses antitumor activity by exhibiting significant cytotoxicity (*ca.* 97%) against cultured melanoma cell lines. Plant fractions also showed antimalarial (protozoa) and growth inhibitory to wheat rootlet [5]. The genus *Ochradenus* has not been extensively studied for its metabolomic potentials, however, a few natural products, including quercetin 3-gentiobioside, quercetin glycosides, 7-*O*-α-L-rhamnopyranosylquercetin 3-*O*-β-D-glucopyrannosyl-(1 → 2)-α-L-rhamnopyrannoside, 7-*O*-α-L-rhamnopyranosylquercetin 3-*O*-(6-*O*-*p*-coumaroyl-β-D-glucopyrannosyl)-(1 → 6)-β-D-glucopyrannoside, isoquercitrin, kaempferol glycosides, afzelin, and astragalin have

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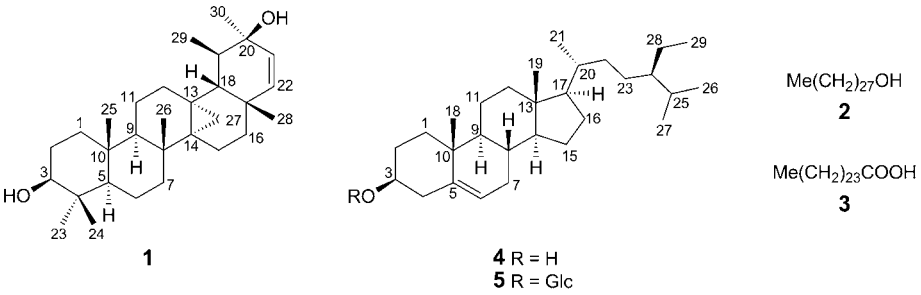

Fig. 1. *Structures of compounds* **1**–**5**

been isolated [6]. Very little is known about the potential biological activities of *O. arabicus*. In a previous study, we carried out various screening experiments to understand its potential role and biological function. The AcOEt-soluble fraction of this plant was found to be significantly rich in antioxidants, total phenolics, and total flavonoids [4]. Based on this potential, the plants could possess a diverse array of metabolites, which can cater for broader biological and pharmacological trails. Henceforward, the present study was carried out in search for its chemical constituents, and as a result, one new cyclopropyl-triterpenoid **1**, along with four known constituents, octacosan-1-ol (**2**), pentacosanoic acid (**3**), β-sitosterol (**4**), and β-sitosterol 3-*O*-β-D-glucopyranoside (**5**) (*Fig. 1*) were isolated for the first time from *O. arabicus*.

Results and Discussion. – The chromatographic separations of the MeOH extract of *O. arabicus* afforded five compounds including one new cyclopropyl-triterpenoid **1**, and four known compounds **2**–**5**. All of the known compounds were isolated for the first time from *O. arabicus*, and their structures were confirmed by comparison of the data with those reported in the literature [3][5].

Compound **1** was obtained as colorless powder from the AcOEt-soluble fraction of the MeOH extract. The appearance of a pink spot on TLC after spraying with an aqueous $Ce(SO_4)_2$ solution indicated a terpenoid skeleton, and the presence of OH and olefinic groups was evident through IR absorption bands at 3510, 3042, 1645, and 816 cm^{-1}. The EI-MS exhibited a molecular-ion peak at *m*/*z* 440, corresponding to the molecular formula $C_{30}H_{48}O_2$ for compound **1**. The molecular formula was further confirmed by HR-EI-MS (*m*/*z* 440.3650, $C_{30}H_{48}O_2^+$; calc. 440.3654) and ^{13}C-NMR data (*Table*).

The analysis of the ^{1}H- and ^{13}C-NMR data along with DEPT experiments and 2D-HMQC correlations revealed the presence of seven Me, nine CH_2, two olefinic CH, five CH, and seven sp^3 quaternary C-atoms in compound **1**. H–C(3), the H-atom geminal to an OH group, was observed at δ(H) 3.25–3.27 as a *multiplet*, whereas the olefinic H-atoms H–C(21) and H–C(22) appeared at δ(H) 5.50 (*d*, *J* = 16.2, 1 H) and 5.67 (*d*, *J* = 16.2), respectively. The ^{1}H-NMR data (*Table*) of **1** showed the presence of six tertiary Me signals at δ(H) 0.94, 0.79, 0.95, 0.86, 1.32, and 1.32, and one secondary Me signal at δ(H) 0.85 (*d*, *J* = 6.6, Me(29)). The high-field signals at δ(H) 0.31 (*d*, *J* = 4.0) and 0.54 (*d*, *J* = 4.0) were assigned to CH_2(27) H-atoms of the cyclopropyl moiety.

Table. *^{1}H- and ^{13}C-NMR data* (600 and 150 MHz, resp.; $CDCl_3$) *and HMBCs of* **1**. δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC
CH_2(1)	1.53–1.54 (*m*)	32.2	–
CH_2(2)	1.84–1.85 (*m*)	28.1	–
H–C(3)	3.25–3.27 (*m*)	78.8	C(2), C(4), C(23)
C(4)	–	40.5	–
H–C(5)	1.55–1.56 (*m*)	52.1	C(4), C(10)
CH_2(6)	1.02–1.04 (*m*), 1.28–1.29 (*m*)	25.9	–
CH_2(7)	1.54–1.55 (*m*)	30.4	–
C(8)	–	26.1	–
H–C(9)	1.26–1.27 (*m*)	31.9	C(5), C(8), C(11)
C(10)	–	36.3	–
CH_2(11)	1.06–1.08 (*m*)	26.4	–
CH_2(12)	1.56–1.58 (*m*)	21.1	–
C(13)	–	47.1	–
C(14)	–	45.3	–
CH_2(15)	1.22–1.25 (*m*)	29.7	–
CH_2(16)	1.82–1.83 (*m*)	29.3	–
C(17)	–	48.8	–
H–C(18)	1.46–1.50 (*m*)	47.9	C(13), C(17), C(19), C(22)
H–C(19)	1.61–1.64 (*m*)	36.3	–
C(20)	–	82.3	–
H–C(21)	5.50 (*d*, J = 16.2)	134.1	C(19), C(20), C(22)
H–C(22)	5.67 (*d*, J = 16.2)	130.8	C(16), C(17), C(21)
Me(23)	0.94 (*s*)	25.4	C(3), C(4), C(5)
Me(24)	0.79 (*s*)	13.9	C(3), C(4), C(5)
Me(25)	0.95 (*s*)	18.1	C(9), C(10)
Me(26)	0.86 (*s*)	19.9	C(8), C(9), C(14)
CH_2(27)	0.31, 0.54 (2*d*, J = 4.0)	29.9	C(13), C(14), C(18)
Me(28)	1.32 (*s*)	24.4	C(17), C(22)
Me(29)	0.85 (*d*, J = 6.6)	18.3	C(18), C(19), C(20)
Me(30)	1.32 (*s*)	24.3	C(20), C(21)

The ^{13}C-NMR and DEPT data showed a disubstituted C=C bond at δ(C) 134.1 and 130.8, one O-bearing CH at δ(C) 78.8, six tertiary Me at δ(C) 13.9, 18.1, 19.9, 24.3, 24.4, and 25.4, one secondary Me at δ(C) 18.3, and one O-bearing quaternary C-atom at δ(C) 82.3. The CH_2(27) C-atom of the cyclopropyl moiety appeared at δ(C) 29.9. On account of the molecular formula $C_{30}H_{48}O_2$, the index of H-atom deficiency (IHD) of **1** was found to be seven, which was assigned to cyclopropyl containing pentacyclic triterpenoid including one olefinic functionality. The position of various functional groups including the cyclopropyl ring was established through HMBC experiments (*Fig. 2*). The HMBCs showed cross-peaks between H–C(21) (δ(H) 5.50 (*d*, J = 16.2)) and the quaternary C-atoms C(20) (δ(C) 82.3) and C(17) (δ(C) 48.8), and the CH C-atom C(22) (δ(C) 130.8). Similarly, the HMBC experiment showed correlation between H–C(22) (δ(H) 5.67 (*d*, J = 16.2) and the quaternary C-atom C(17) (δ(C) 48.8) and CH C-atom C(21) (δ(C) 134.1). The CH_2(27) H-atoms (δ(H) 0.31 (*d*, J = 4.0) and 0.54 (*d*, J = 4.0)) showed correlations with the quaternary C-atoms C(13) (δ(C)

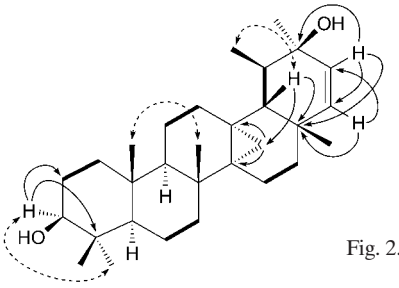

Fig. 2. *Important HMBC* (H → C), *NOESY* (H ⇠⇢ H), *and key COSY* (—) *correlations of* **1**

47.1) and C(14) (δ(C) 45.3). H–C(3) (δ(H) 3.25–3.27) showed cross-peaks with the quaternary C-atom C(4) (δ(C) 40.5) and CH_2(2) (δ(C) 28.1).

The positions of the C=C bond and of the O-bearing quaternary C-atom C(20) were thus confirmed through HMBC interactions. The assignment of ^{13}C-NMR chemical shifts was completed with the help of HMQC and DEPT experiments. All these data confirmed the structure of compound **1** to be 13,27-cycloursane-type triterpene with an OH group at C(20) and a C(21)=C(22) bond. The NOESY experiments revealed the assignment of the relative configuration at the stereogenic centers in the molecule. Based on the above evidences, the structure of compound **1** was assigned as (3β,13α)-13,27-cyclours-21-ene-3,20-diol. The ^{1}H- and ^{13}C-NMR spectra of **1** resembles those of the reported derivatives of 13,27-cycloursane, isolated from *Ficus microcarpa* [7], with the expected differences due to the nature and position of the olefinic group and other substituents, which have already been confirmed through HMBC interactions (*Fig. 2*).

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Experimental Part

General. Thin-layer chromatography (TLC): precoated silica-gel *G60 F_{254}* plates (SiO_2; *Merck*). Column chromatography (CC): SiO_2 (0.040–0.063 mm, *Merck*). Recycling prep. HPLC: *JAI*, *LC-9210 NEXT*; eluent, AcOEt/hexane 1:1 (*Sigma–Aldrich*, HPLC grade). IR Spectra: *Bruker-ATR* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance 600* spectrometer (600 (^{1}H) and 150 (^{13}C) MHz, resp.); δ in ppm rel. to residual $CDCl_3$ (δ(H) 7.24, δ(C) 77.0) as internal standard, *J* in Hz. EI- and HR-EI-MS: *JEOL JMS HX 110* mass spectrometers, in *m*/*z*.

Plant Material. The whole plant of *O. arabicus* CHAUDHARY, HILLC. & A. G.MILL. was collected from Al-Jabel Al-Akhdar (N 23°04′22.00″; E 57°40′07.00″), Oman, in 2012, and was identified by plant taxonomist at the Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa, Oman. A voucher specimen has been deposited with the Herbarium of the Department.

Extraction and Isolation. The dried and powdered plant material of *O. arabicus* (28.4 kg) was extracted with 90% MeOH at r.t. The crude extract (440 g) thus obtained was then extracted successfully with hexane, CH_2Cl_2, AcOEt, and BuOH. The AcOEt fraction (27.6 g) was subjected to CC (SiO_2; hexane, hexane/CH_2Cl_2, and CH_2Cl_2/MeOH, gradual increase in the polarity of the mobile phase) to give seven sub-fractions, *Frs. $JOAF_1$–$JOAF_7$*. *Fr. $JOAF_3$* (CH_2Cl_2/hexane 70:30) was again subjected to HPLC. Compound **1** (2 mg) was obtained as amorphous powder, along with some semi-pure compounds. The semi-pure compounds were then purified by prep. TLC plates and identified as known compounds **4** (18 mg; CH_2Cl_2/hexane 50:50) and **5** (11 mg; CH_2Cl_2/hexane 80:20), resp. *Fr. $JOAF_2$* (CH_2Cl_2/hexane

25 : 75) was separated by repeated CC (SiO_2; hexane/CH_2Cl_2 and CH_2Cl_2/MeOH) to afford compounds **2** (6 mg; CH_2Cl_2/hexane 15 : 85) and **3** (7 mg; CH_2Cl_2/hexane 30 : 70).

(3β,13α)-13,27-Cyclours-21-ene-3,20-diol (**1**). Amorphous powder. IR (MeOH): 3510, 3042, 1645, 816. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see the *Table.* HR-EI-MS: 440.3650 (M^+, $C_{30}H_{48}O_2^+$; calc. 440.3654).

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