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records of natural products

Triterpenoids from *Acokanthera schimperi* in Ethiopia

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Abstract: The studies on the leaves of *Acokanthera schimperi*, a traditional herb of Ethiopia, afforded eight triterpenoids, including a new triterpenoid ester, lupan-20-ol-3(β)-yl 3-hydroxyoctadecanoate (**1**), along with seven known triterpenoids, lupeol (**2**), 28-nor-urs-12-ene-3*β*,17*β*-diol (**3**), ursolic aldehyde (**4**), 3*β*-hydroxy-oleana-11,13(18)-dien-28-oic acid (**5**), alagidiol (**6**), oleanolic acid (**7**) and ursolic acid lactone (**8**). Their structures were determined by spectroscopic methods including 2D NMR techniques and X-ray diffraction analysis.

Keywords: *Acokanthera schimperi*; triterpenoids; Ethiopian medicinal plants; spectroscopic analyses; X-ray diffraction.

1. Introduction

Acokanthera schimperi (A. DC.) Schweinf, belonging to the family Apocynaceae, was called "Merenz" locally. It is a well-known East African arrow poison plant and found from Eritrea south to Tanzania and west to Uganda, Rwanda and eastern DR Congo. It is also found in southern Yemen. [1,2]. In Ethiopia, the leaves and bark of *A. schimperi* were applied to the skin to treat skin disorders, and an infusion of its leaves was gargled to treat tonsillitis [2]. Ethiopian traditional healers also used this indigenous plant for the treatment of headache, epilepsy, amnesia, elephantiasis, scabies, leprosy, etc [3]. Literature survey revealed that most of the researches were focused on the bioactivities and traditional use of this plant [4-6], and less report was found for its phytochemical studies [7]. Recently, we carried out a systematic chemical study on the leaves of *A. schimperi*, and eight triterpenoids (**1-8**), including a new triterpenoid ester, lupan-20-ol-3(β)-yl 3-hydroxyoctadecanoate (**1**), were isolated. Herein, we report the isolation, structural elucidation of these compounds.

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2. Materials and Methods

2.1. Instrumentation and Reagents

Optical rotation was measured on APVI/6W polarimeter (Rudolph Research Analytical). IR spectrum was recorded on a Nicolet 6700 FT-IR spectrometer (ThermoFisher). HRESIMS data were acquired on a Water Q-TOF Premier. NMR spectra were obtained on a Varian NMR System 600 or Bruker AV II-600 and 400, with tetramethylsilane as an internal standard. X-ray diffraction data were collected on a Bruker Xcalibur E CCD diffractometer using graphite-monochromated Mo Kα radiation. Materials for column chromatography (CC) were silica gel (Qingdao Marine Chemical Factory, Qingdao, China) or Sephadex LH-20 (GE Healthcare, USA). Analytical and preparative thin-layer chromatography (TLC) was conducted on GF254 or G plates. All the chemical reagents and solvents used for separation and purification were analytical grade and purchased from local firms (Kelong chemical reagent factory, Chengdu, China).

2.2. Plant Materials

The leaves of *A. schimperi* were collected in the Debre Libanos monastery, lying northwest of Addis Ababa, Ethiopia. It was identified by Amare Seifu Assefa, a botanist from the Ethiopian Biodiversity Institute. A voucher specimen (AS-2015-10) was deposited in the Herbarium of Ethiopian Biodiversity Institute, Ethiopia.

2.3. Extraction and Isolation

At room temperature, the powdered leaves of *A. schimperi* (3.35 kg) were extracted with EtOH:H_2O (4 × 12 L, 95:5 v/v, 7 days each time). The extracts were concentrated in vacuo to yield a residue that was suspended in H_2O (7 L) and extracted with EtOAc (3 × 7 L). The EtOAc extract (122 g) was applied to CC over silica gel (200-300 mesh, 1.8 kg) and eluted with cyclohexane-ethyl acetate (100:1-1:1, gradient system). On the basis of the TLC analysis, ten fractions A-J were obtained.

Fr. C (1.1 g) was isolated by silica gel chromatography using a solvent system cyclehexane-acetone (60:1) to get two subfractions (Fr. C-1 and C-2). Fr. C-1 was separated by slica gel chromatography (chloroform-cyclohexane-acetone, 60:80:1) and purified on Sephadex LH-20 (chloroform-methanol, 2:1) to yield **4** (16 mg). Fr. C-2 was separated using preparative TLC (cyclohexane-acetone, 5:1) and purified by Sephadex LH 20 (chloroform-methanol, 2:1) to get **2** (22 mg). Fr. D (0.9 g) was isolated by silica gel chromatography (chloroform-cyclohexane-acetone, 30:50:1) and preparative TLC (cyclohexane-acetone, 6:1), and then purified by Sephadex LH-20 (chloroform-methanol, 2:1) to afford **3** (18 mg). Fr. E (0.6 g) was separated by silica gel chromatography (chloroform-cyclohexane-acetone, 30:50:1) to afford two subfractions (Fr. E-1 and E-2). Fr. E-1 was separated by silica gel chromatography (cyclohexane-acetone, 10:1) and purified with Sephadex LH-20 (chloroform-methanol, 2:1) to afford **5** (8 mg). Fr. E-2 was isolated by silica gel chromatography (cyclohexane-acetone, 70:1) to give two subfractions (Fr. E-2-1 and E-2-2). Fr. E-2-1 was separated by silica gel chromatography (cyclohexane-acetone, 10:1) and purified using Sephadex LH-20 to afford **1** (9 mg). Fr. E-2-2 was separated by silica gel chromatography (cyclohexane-acetone, 13:1) and purified with Sephadex LH-20 to give **6** (41 mg). Fr. F (0.57 g) was subjected to silica gel CC (chloroform-acetone, 150:1), and then purified by Sephadex LH-20 to give the **7** (7 mg). Fr. G (1.47 g) was isolated by silica gel chromatography (chloroform-acetone, 140:1; chloroform-ethyl acetate, 40:1) and purified with Sephadex LH-20 to afford **8** (8 mg).

Lupan-20-ol-3(β)-yl 3-hydroxyoctadecanoate (**1**): white amorphous powder; $[\alpha]_D^{20}$: + 5.97 (c 0.067, CH_2Cl_2); IR (KBr) υ_{max} 3455, 2923, 2853, 1714, 1665, 1381, 1359, 1172, 979 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$) spectral data, Table 1; HRESIMS (positive) *m*/*z* 749.6432 $[M+Na]^+$ (calcd for $C_{48}H_{86}O_4Na$, 749.6424).

Alagidiol (**6**): colorless crystal (CH_2Cl_2); ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 3.20 (1H, dd, J = 11.5, 4.7 Hz, H-3), 1.11, 0.99, 0.97, 0.84, 0.84, 0.76 (each 3H, s, 6 × CH_3), 0.97 (3H, d, J = 7.0 Hz, CH_3), 0.85 (3H, d, J = 6.6 Hz, CH_3). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 83.6 (C-18), 78.9 (C-3), 55.3 (C-5), 50.7 (C-9), 45.9 (C-21), 45.8 (C-17), 41.4 (C-14), 41.1 (C-8), 39.4 (C-13), 38.8 (C-4), 38.7 (C-1), 37.1 (C-10), 35.0 (C-16), 32.9 (C-7), 30.8 (C-22), 28.2 (C-20), 27.9 (C-23), 27.3 (C-2), 25.7 (C-15 or C-19), 24.8 (C-12), 22.7 (C-29), 22.7 (C-30), 21.9 (C-15 or C-19), 21.4 (C-11), 18.3 (C-6), 17.8 (C-28), 16.3 (C-24), 16.0 (C-27), 15.9 (C-25), 15.3 (C-26). HRESIMS (positive) m/z 467.3860 $[M+Na]^+$ (calcd for $C_{30}H_{52}O_2Na$, 467.3865).

2.4. X-ray Crystallographic Study

Alagidiol (**6**) (Figure 1): $C_{30}H_{52}O_2$, M = 444.71, T = 293 K, λ = 0.71073 Å, orthorhombic, P2(1)2(1)2, a = 19.6011 (9) Å, b = 19.5010 (7) Å, c = 7.7285 (3) Å, V = 2954.2 (2) Å^3, Z = 4. D_c = 1.000 g cm^{-3}, Mu = 0.060 mm^{-1}, F(000) = 992. Data were collected using a colorless block of size 0.35 × 0.30 × 0.25 mm in the range 2.95° ≤ θ ≤ 26.37° within the index range $-24 \leq h \leq 21$, $-24 \leq k \leq 16$, $-9 \leq l \leq 9$. 9498 reflections measured, 5461 unique reflections, R_{int} = 0.0199. Refinement by full-matrix least-squares on F^2 converged to give final R indices R_1 = 0.0522, wR_2 = 0.1175 [$I > 2\sigma(I)$] and R_1 = 0.0701, wR_2 = 0.1258 (all data). Data/restraints/parameters = 5461/8/313, goodness-of-fit on F^2 = 1.046, largest difference peak and hole are 0.14 and -0.16 e Å^{-3}. Crystallographic data for **6** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 1854896. These data can be obtained free of charge via www.ccdc.cam.ac.uk/deposit (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; deposit@ccdc.cam.ac.uk).

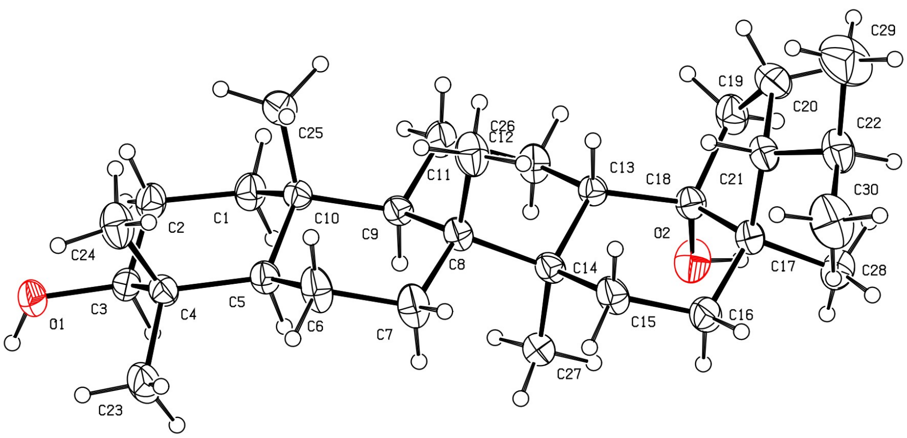

Figure 1. X-ray ctystal structure of compound **6**

3. Results and Discussion

Phytochemical research was performed for the leaves of *A. schimperi* by chromatography and afforded a new triterpenoid ester, lupan-20-ol-3(β)-yl 3-hydroxyoctadecanoate (**1**), together with seven known triterpenoids, namely lupeol (**2**) [9], 28-nor-urs-12-ene-3*β*,17*β*-diol (**3**) [10], ursolic aldehyde (**4**) [8], 3*β*-hydroxy-oleana-11,13(18)-dien-28-oic acid (**5**) [11], alagidiol (**6**) [12,13], oleanolic acid (**7**) [14] and ursolic acid lactone (**8**) [15] (Figure 2). The structures of the known compounds were identified by comparing their spctroscopic data with those reported in the literatures.

Compound **1** was isolated as white amorphous powder. Its molecular formula was determined to be $C_{48}H_{86}O_4$ on the basis of HRESIMS (m/z: $[M+Na]^+$ calcd 749.6424; found 749.6432) and its ^{13}C-NMR spectroscopic data (Table 1), indicated 6 degrees of unsaturation.

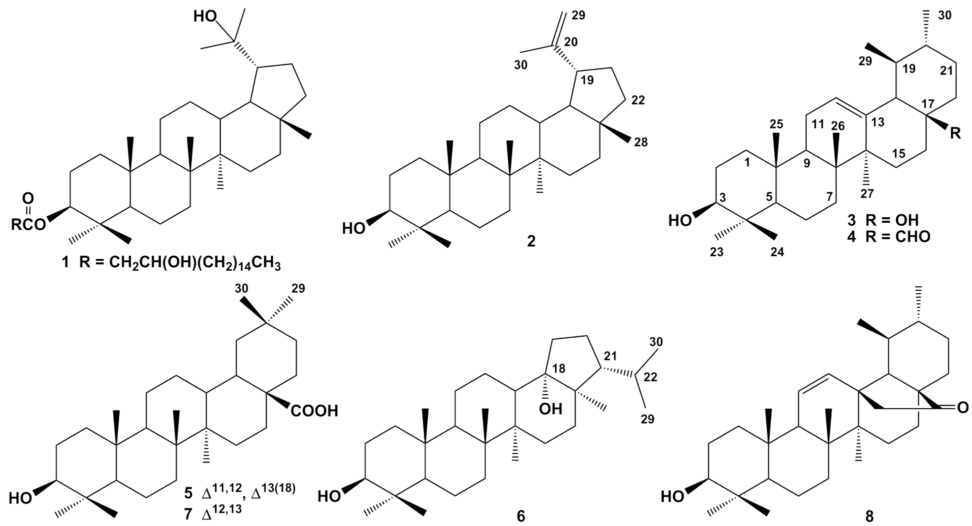

Figure 2. Structures of compounds **1-8**

The IR absorptions indicated the presence of hydroxyl group (3455 cm^{-1}) and carbonyl group (1714 cm^{-1}). Analysis of the ^{1}H and ^{13}C NMR spectra of **1** (Table 1) showed a set of triterpenoid signals, eight tertiary methyl groups [δ_H 1.22 (s), δ_C 31.9; δ_H 1.12 (s), δ_C 24.7; δ_H 1.05 (s), δ_C 16.1; δ_H 0.95 (s), δ_C 14.8; δ_H 0.86 (s), δ_C 16.2; δ_H 0.85 (s), δ_C 28.0; δ_H 0.84 (s), δ_C 16.6; δ_H 0.80 (s), δ_C 19.2;], ten methylene groups [δ_H 1.00(m), 1.68 (m), δ_C 38.3; δ_H 1.27 (m), 1.63 (m), δ_C 23.7; δ_H 1.40 (m), 1.51 (m), δ_C 18.2; δ_H 1.40 (m), δ_C 34.4; δ_H 1.26 (m), 1.47 (m), δ_C 21.4; δ_H 1.11 (m), 1.76 (m), δ_C 27.5; δ_H 1.32 (m), 1.62 (m), δ_C 28.7; δ_H 1.34 (m), 1.50 (m), δ_C 35.5; δ_H 1.60 (m), 1.87 (m), δ_C 28.9; δ_H 1.10 (m), 1.31 (m), δ_C 40.1], six methine groups [δ_H 4.54 (dd, 10.0, 6.24), δ_C 81.4; δ_H 0.79 (m), δ_C 55.2; δ_H 1.27 (m), δ_C 50.1; δ_H 1.71 (m), δ_C 37.4; δ_H 1.33 (m), δ_C 48.2; δ_H 1.79 (m), δ_C 49.9] and six quaternary carbons (δ_C 73.4, 44.6, 43.5, 41.3, 37.7, 36.9). These NMR data were matching with the triterpenoid skeleton of lupan-20-ol-3(β)-yl hexadecanoate [16], which indicated the presence of a 3β,20-dihydroxylupane skeleton for **1**. In addition to the signals for triterpenoid, signals for a saturated fatty acid were also found, one CH_3 [δ_H 0.88 (t, 6.8), δ_C 14.1], one oxygen-bearing CH [δ_H 3.99 (m), δ_C 68.2], fifteen CH_2 [δ_H 2.50 (dd, 16.2, 2.9), 2.39 (dd, 16.2, 9.0), δ_C 41.6; δ_H 1.25~1.50 (m, 28H), δ_C 36.5, 25.4, 29.3~31.5 (11C), 22.7] and one carbonyl quaternary carbon (δ_C 172.7), which was similar with the NMR signals of the fatty acid moiety of methyl 3-hydroxyoctadecanoate and 2-*O*-[(*R*)-3-hydroxyhexadecanoyl]glycerol [17,18]. Compared with 3β,20-dihydroxylupane, the downfield of H-3 (+1.34), C-3 (+2.4) and upfield of C-2 (-3.9) of **1** indicated that the fatty acid was esterified with 3-OH [19,20], which was confirmed by the correlation of δ_H 4.54 (H-3) and δ_C 172.7 (C-1') in the HMBC spectrum (Figure 3). Thus, the structure of compound **1** was elucidated and named as lupan-20-ol-3(β)-yl 3-hydroxyoctadecanoate.

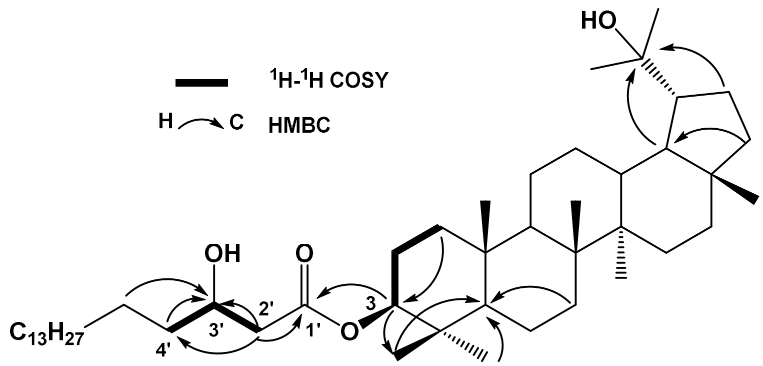

Figure 3. Selected ^{1}H-^{1}H COSY and HMBC correlation of compound **1**

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

Position	δ_H (mult., J in Hz)	δ_C	Position	δ_H (mult., J in Hz)	δ_C
1	1.00 (1H, m); 1.68 (1H, m)	38.3	**20**		73.4
2	1.27 (1H, m); 1.63 (1H, m)	23.7	**21**	1.60 (1H, m); 1.87 (1H, m)	28.9
3	4.54 (1H, dd, 6.24, 10.0)	81.4	**22**	1.10 (1H, m); 1.31 (1H, m)	40.1
4		37.7	**23**	0.85 (3H, s)	28.0
5	0.79 (1H, m)	55.2	**24**	0.84 (3H, s)	16.6
6	1.40 (1H, m); 1.51 (1H, m)	18.2	**25**	0.86 (3H, s)	16.2
7	1.40 (2H, m)	34.4	**26**	1.05 (3H, s)	16.1
8		41.3	**27**	0.95 (3H, s)	14.8
9	1.27 (1H, m)	50.1	**28**	0.80 (3H, s)	19.2
10		36.9	**29**	1.12 (3H, s)	24.7
11	1.47 (1H, m); 1.26 (1H, m)	21.4	**30**	1.22 (3H, s)	31.9
12	1.11 (1H, m); 1.76 (1H, m)	27.5	**1'**		172.7
13	1.71 (1H, m)	37.4	**2'**	2.39 (1H, dd, 9.0, 16.2) 2.50 (1H, dd, 2.9, 16.2)	41.6
14		43.5	**3'**	3.99 (1H, m)	68.2
15	1.32 (1H, m); 1.62 (1H, m)	28.7	**4'**		36.5
16	1.34 (1H, m); 1.50 (1H, m)	35.5	**5'**	1.25~1.50 (28H, m)	25.4
17		44.6	**6'-16'**		29.3~31.5
18	1.33 (1H, m)	48.2	**17'**		22.7
19	1.79 (1H, m)	49.9	**18'**	0.88 (3H, t, 6.8)	14.1

Acokanthera belongs to the family Apocynaceae and it comprises 5 species, *A. laevigata*, *A. oblongifolia*, *A. oppositifolia*, *A. rotundata* and *A. schimperi*. Previous phytochemical studies on this genus were focused on the toxic cardiac glycosides, which were correlated with its usage as arrow poisons [21, 22]. But, besides cardenolides, less other type natural products were identified from genus *Acokanthera*. Karawya reported the isolation of three pentacyclic triterpenoids, friedelin, lupeol and β-amyrin, from *A. spectabilis* (also known as *A. oblongifolia* [23]) [24]. From *A. oppositifolia*, a pentacyclic triterpenoid, lupeol, and its ester, lup-20(29)-en-3β-*O*-(3′-β-hydroxy) palmitate, together with β-sitosterol, have been isolated [25]. In our present study on *A. schimperi*, eight pentacyclic triterpenoids have been isolated, and they can be divided into four triterpene skeleton types, oleanane, ursane, lupane and hopane, which indicated the high structural diversity of triterpene in *A. schimperi*. Compound **1**, a new triterpenoid ester isolated in this study, was similar with lup-20(29)-en-3β-*O*-(3′-β-hydroxy) palmitate, a lupane ester from *A. spectabilis* [25]. Due to the lack of phytochemical data of this genus, the importance of triterpeoid in the chemotaxonomy of genus *Acokanthera* need further studied.

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