Triterpenoids from *Acokanthera schimperi* in Ethiopia

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(Received July 11, 2018; Revised August 21, 2018; Accepted September 18, 2018)

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-3β,17β-diol (3), ursolic aldehyde (4), 3β-hydroxyoleana-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. © 2018 ACG Publications. All rights reserved.

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 an 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-60 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:H₂O (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 H₂O (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 silica 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; [α]_D^{20} + 5.97 (c 0.067, CHCl₃); IR (KBr) νmax 3455, 2923, 2853, 1714, 1665, 1381, 1359, 1172, 979 cm⁻¹; ¹H-NMR (600 MHz, CDCl₃) and ¹³C-NMR (150 MHz, CDCl₃) spectral data, Table 1; HRESIMS (positive) m/z 749.6432 [M+Na]⁺ (calcd for C₄₈H₇₆O₄Na, 749.6424).
Alagidiol (6): colorless crystal (CH₂Cl₂); ¹H-NMR (600 MHz, CDCl₃) δH: 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 × CH₃), 0.97 (3H, d, J = 7.0 Hz, CH₃), 0.85 (3H, d, J = 6.6 Hz, CH₃). ¹³C-NMR (150 MHz, CDCl₃) δC: 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 C₃₀H₅₂O₂Na, 467.3865).

2.4. X-ray Crystallographic Study

Alagidiol (6) (Figure 1): C₃₀H₅₂O₂, M = 444.71, T = 293 K, λ = 0.71073 Å, orthorhombic, P2₁2₁2₁, a = 19.6011 (9) Å, b = 19.5010 (7) Å, c = 7.7285 (3) Å, V = 2954.2 (2) Å³, Z = 4. Dc = 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 ≤ h ≤ 21, -24 ≤ k ≤ 16, -9 ≤ l ≤ 9. 9498 reflections measured, 5461 unique reflections, Rint = 0.0199. Refinement by full-matrix least-squares on F² converged to give final R indices R₁ = 0.0522, wR₂ = 0.1175 [I > 2σ(I)] and R₁ = 0.0701, wR₂ = 0.1258 (all data). Data/restraints/parameters = 5461/8/313, goodness-of-fit on F² = 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 crystal 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-3β,17β-diol (3) [10], ursolic aldehyde (4) [8], 3β-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 spectroscopic data with those reported in the literatures.

Compound 1 was isolated as white amorphous powder. Its molecular formula was determined to be C₃₈H₆₆O₂ on the basis of HRESIMS (m/z: [M+Na]⁺ calcd 749.6424; found 749.6432) and its ¹³C-NMR spectroscopic data (Table 1), indicated 6 degrees of unsaturation.
Triterpenoids from *Acokanthera schimperi*

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 ¹H and ¹³C 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, 6.8, 2.9), 2.39 (dd, 16.2, 6.4), δ_C 41.6; δ_H 1.25~1.50 (m, 15 H), 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-dihydroxyxypenate, 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$_3$, $\delta$ in ppm, $J$ in Hz).

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<th>Position</th>
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<th>$\delta_C$</th>
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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 pentaacyclic triterpenoids, friedelin, lupeol and β-amyrin, from A. spectabilis (also known as A. oblongifolia [23]) [24]. From A. oppositifolia, a pentaacyclic 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 pentaacyclic 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 triterpene in the chemotaxonomy of genus Acokanthera need further studied.
Acknowledgments

This work was financially supported by the Fundamental Research Funds for the Central Universities (ZYGX2016J120). The authors thanked Dr. Haokun Yuan, School of Life Science and Technology, University of Electronic Science and Technology of China, for his kind help in crystallization of compound 6.

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