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Structure elucidation of two triterpenoids from *Ficus fistulosa*

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Abstract

From *Ficus fistulosa* two triterpenoids 3β-acetyl ursa-14:15-en-16-one and lanosterol-11-one acetate were isolated along with five known triterpenoids, 3β-acetyl-22,23,24,25,26,27-hexanordamaran-20-one, 24-methylenecycloartenol, sorghumol (isoarborinol), 11α,12α-oxidotaraxeryl acetate, and ursa-9(11):12-dien-3β-ol acetate. Their structures were elucidated on the basis of spectral data interpretation. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Ficus fistulosa*; Moraceae; 3β-acetyl ursa-14:15-en-16-one; Lanosterol-11-one acetate; Biodiversity conservation study; Cuc Phuong National Park, Vietnam

1. Introduction

As part of a biodiversity conservation study, *Ficus fistulosa* Reiw ex. Bl (Moraceae), indigenous to Vietnam, was collected from the forest of the Cuc Phuong National Park, Vietnam for study. No work on the plant has been reported. MeOH extracts of the leaves and barks exhibited antiplasmodial activity. Fractionation of the bark MeOH extract led to the isolation of seven triterpenes, two of which are new. The isolation and structure identification/elucidation of the compounds are reported herein.

2. Results and discussion

The MeOH extract was prepared from the bark materials of *F. fistulosa*. Fractionation by silica gel column chromatography, followed by HPLC purification afforded seven triterpenoids. The structures of the compounds were elucidated by using 1-D and 2-D ^{1}H and ^{13}C NMR spectroscopic techniques (APT, ^{1}H–^{1}H COSY, HMBC and HETCOR) and mass spectral analysis. (Fig. 1).

The known triterpenes 3β-acetyl-22,23,24,25,26,27-hexanordamaran-20-one (Tanaka, Matsuda & Matsunaga, 1987), 24-methylenecyclo artenol (Anjaneyulu & Raju, 1987), sorghumol (isoarborinol) (Nes, Heupel, Benson, Stafford & Haddon, 1984; Nes, Wong, Griffin & Duax, 1991), 11α,12α-oxidotaraxeryl acetate (Nes et al., 1984; Nes et al., 1991; Tanaka & Matsunaga, 1988; Matsunaga, Tanaka & Akagi, 1988) and ursa-9(11):12–dien–3β–ol acetate (Ito & Lai, 1979) were identified by comparison of their physical and spectral data with literature values.

The molecular formula of compound **1** ($C_{32}H_{52}O_3$) was established by CIMS, ^{1}H and ^{13}C NMR, and APT experiments. The ^{1}H NMR and APT experiment showed that compound **1** contains eight methyl groups (δ_H 0.65, 0.88, 0.91, 0.92, 0.93, 1.18, 1.60, and 1.68), an acetyl group (δ_H 2.06), a ketone (δ_C 189.74), nine quartenary carbons (δ_C 36.43, 37.73, 39.55, 44.87, 130.99, 139.05, 164.70, 170.83, and 189.74), an olefinic proton (δ_H 5.10), and four olefinic carbons (δ_C 125.09, 130.99, 139.05, and 164.70). Two of the methyl groups [δ_H 1.60 (singlet) and 1.68 (singlet)] were attached to a quarternary olefinic carbon at δ_C 130.99, suggesting that they are at the terminal position of a side-chain.

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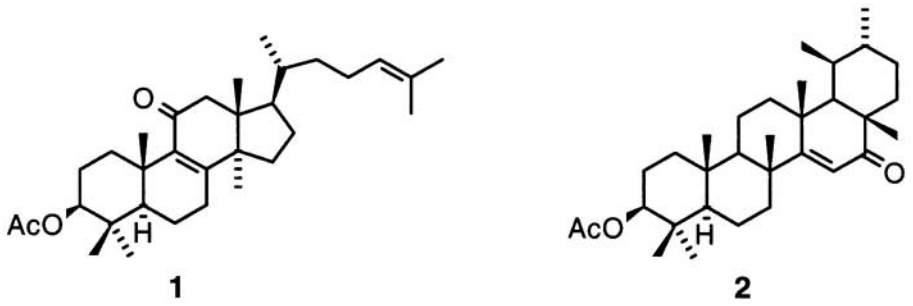

Fig. 1. Structures of lanosterol-11-one acetate (**1**) and 3β-acetyl ursa-14:15-en-16-one (2).

The ^{1}H NMR of the olefinic proton at δ 5.10 (t, $J = 6.62$ Hz) indicated that it is not a part of an α,β-conjugated system which further suggested that the α,β-conjugated system is endocyclic and tetrasubstituted. The ^{1}H and ^{13}C NMR spectra showed an acetyl group attached to a β-hydroxy group at the C-3 position (δ_H 2.06) and a dimethyl group at C-4 position (δ_H 0.88 and 0.91), typical of a triterpenoid. Comparison of these data with those of structurally related compounds, 3β,26-dihydroxy-5α-lanosta-8,24-dien-11-one and 3β-hydroxy-26-oxo-5α-lanosta-8,24-dien-11-one (Lin, Tome & Won, 1990; Parker & Nes, 1992) led to the elucidation of the structure of compound **1** as lanosterol-11-one acetate.

The molecular formula of compound **2** ($C_{32}H_{50}O_2$) was established by CIMS, ^{1}H and ^{13}C NMR, and APT experiment. The ^{1}H NMR and APT experiment showed that compound **2** contains eight methyl groups (δ_H 0.88, 0.90, 0.98, 0.99, 1.09, 1.14, 1.17, and 1.30), one acetyl group (δ_H 2.08), and one α,β-unsaturated ketone (δ_C 208). The ^{13}C NMR and APT experiment showed 32 carbons with seven quartenary carbons (δ_C 37.57, 37.98, 40.38, 43.03, 171.0, 181.0, 208.8). The ^{1}H NMR and ^{13}C NMR chemical shift (C-15, δ_C 181, quarternary and C-16, δ_H 5.84 (singlet) and δ_C 119) suggested that the α,β-unsaturated ketone is exocyclic with respect to the adjacent fused ring system. The ^{1}H NMR chemical shifts of C-26 (CH_3, d, $J = 6.3$ Hz) and C-27 (CH_3, d, $J = 6.6$ Hz) were indicative of the two methyl groups attached at C-19 and C-20 in ursane core structure (Matsunaga et al., 1988; Ito & Lai, 1979; Gonzalez, Andres, Ravelo, Luis, Bazzochi & West, 1990). The ^{1}H and ^{13}C NMR indicated an acetyl derivative of a β-hydroxyl group at the C-3 position (δ_H 2.08) and a dimethyl group at C-4 (δ_H 0.88 and 0.90), typical of a C-3 acetylated triterpenoid (Tanaka et al., 1987; Anjaneyulu & Raju, 1987; Nes et al., 1984; Nes et al., 1991; Tanaka & Matsunaga, 1988; Matsunaga et al., 1988; Ito & Lai, 1979; Lin et al., 1990; Parker & Nes, 1992; Gonzalez et al., 1990). The ^{1}H and ^{13}C NMR chemical shifts of the compound were assigned using a combination of 2D homonuclear correlation experiments (^{1}H–^{1}H COSY, HETCOR) and long-range proton-detected heteronuclear correlation experiment (HMBC). On the basis of spectral data analysis and by comparison of these data with those of the structurally related ursa-9(11):12-dien-3β-ol and 3,11-dioxo-ursan-12-ene (Matsunaga et al., 1988; Ito & Lai, 1979; Gonzalez et al., 1990) the structure of compound **2** was deduced as 3β-acetyl ursa-14:15-en-16-one.

3. Experimental

3.1. General

Melting points were determined on a Fisher-John hot stage apparatus and were uncorrected. ^{1}H-NMR spectra were recorded using a Varian XL-300 spectrometer operating at 300 MHz. ^{13}C-NMR spectra were recorded on the same instrument (75.4 MHz). NMR spectra were obtained in $CDCl_3$ with TMS as internal standard. CIMS (isobutane gas) were obtained on a Varian MAT-112S spectrometer. Column chromatography was performed on Merck Si gel 60 (60–200 mesh). The separation of the pure compounds was performed on a Waters' HPLC system, equipped with a Waters' 996 photodiode array detector, Waters' 717 plus autosampler, Waters' 600 Controller, Digital Venturis 466 computer system, and Hewlett Packard DeskJet 600 printer. Analytical HPLC separation of the constituents was performed over *YMC* C-18 ODS-AQ column [4.6 mm×250 mm] (YMC, Inc., Wilmington, NC) and the semi-preparative HPLC separation was performed over *YMC* C-18 ODS-AQ column [50 mm×250 mm] using acetonitrile/water gradient solvent system.

3.2. Plant material

The bark of *Ficus fistulosa* (4 kg) was collected from conservation plot #I of the rain forest of the Cuc Phuong National Park, Cuc Phuong, Vietnam. The identity of the plant was made by T.-C. Khan and Tran van On. Voucher herbarium specimens (KO-1029A, KO-1092A and KO-1378A) have been deposited at the Hanoi College of Pharmacy. The material was dried, milled, extracted with methanol and concentrated *in vaccuo* at 40°C to afford 220 g of residue.

3.3. Isolation

The methanol extract of *Ficus fistulosa* was reconstituted with MeOH/water (3:2) solution and succesively partitioned with petroleum ether/water, chloroform/water, and ethyl acetate/water. The chloroform fraction (10.4 g) was subjected to a column chromatography over silica gel (60–200 mesh) using petroleum ether/ethyl acetate (gradient) to obtain six fractions (fraction *F1–F6*). Fraction *F3* (2.17 g) was column chromatographed over silica gel (60–200 mesh) using petroleum/ethyl acetate (gradient) to obtain five fractions (*F7–F11*). Fraction *F11* (500 mg) was subjected to HPLC separation to afford seven terpenoids, two of which are new: 3β-acetyl-22,23,24,25,26,27-hexanordamaran-20-one (Tanaka et al., 1987) (29 mg), 24-methylenecyclo-artenol (Anjaneyulu & Raju, 1987) (17 mg), sorghumol (Nes et al., 1984; Nes et al., 1991) (8 mg), 11α,12α-oxidotaraxeryl acetate (Nes et al., 1984; Nes et al., 1991; Tanaka & Matsunaga, 1988; Matsunaga et al., 1988) (23 mg) and ursa-9(11):12-dien-3β-ol acetate (Ito & Lai, 1979) (20 mg).

3.4. 3-β-Acetyl-5α-lanosta-8,24-diene-11-one (**1**)

31 mg; mp 144-147°C; ^{1}H NMR ($CDCl_3$, 299.9 MHz) δ 0.65 (3H, s, CH_3-18), 0.88 (3H, s, CH_3-29), 0.91 (3H, s, CH_3-30), 0.92 (3H, d, $J = 6.5$ Hz, CH_3-21), 0.93 (3H, s, CH_3-19), 1.18 (3H, s, CH_3-28), 1.60 (3H, s, CH_3-27), 1.68 (3H, s, CH_3-26), 2.06 (3H, s, acetyl CH_3), 4.52 (1H, dd, CH-3), 5.09 (1H, t, CH-24); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 15.76 (C-30), 16.63 (C-18), 17.69 (C-21), 18.43 (C-19), 18.69 (C-28), 21.24 (C-32), 23.69 (C-6), 23.82 (C-23), 24.86 (C-27), 24.86 (C-15), 25.72 (C-26), 27.35 (C-29), 28.74 (C-2), 30.06 (C-7), 31.98 (C-16), 34.46 (C-22), 36.13 (C-20), 36.26 (C-1), 36.43 (C-10), 37.73 (C-4), 39.55 (C-13), 44.87 (C-14), 47.75 (C-12), 48.92 (C-5), 49.82 (C-17), 79.60 (C-3), 125.09 (C-24), 130.99 (C-25), 139.05 (C-9), 164.70 (C-8), 170.83 (C-31), 189.74 (C-11); CIMS (70 eV) $m/z + 1$ 485 (4), 484 (30), 467 (18), 423 (41).

3.5. 3β-acetyl ursa-14:15-en-16-one (**2**)

22 mg; mp 167–169°C; ^{1}H NMR ($CDCl_3$, 299.9 MHz) δ 0.883 (3H, s CH_3-29), 0.902 (3H, s, CH_3-30), 0.981 (3H, d, CH_3-27, $J = 6.3$ Hz), 0.992 (3H, s, CH_3-24), 1.092 (3H, d, CH_3-26, $J = 6.63$), 1.138 (3H, s, CH_3-23), 1.174 (3H, s, CH_3-25), 1.298 (3H, s, CH_3-28), 4.493 (1H, dd), 5.84 (1H, s); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 15.5 (C-24), 16.50 (C-6), 16.50 (C-29), 18.60 (C-11), 21.27 (C-32), 21.96 (C-27), 22.64 (C-23), 23.31 (C-2), 24.94 (C-26), 25.81 (C-25), 27.86 (C-30), 28.62 (C-12), 28.86 (C-1), 30.68 (C-22), 32.26 (C-19), 34.51 (C-28), 36.70 (C-20), 37.28 (C-21), 37.57 (C-4), 37.98 (C-10), 39.61 (C-13), 40.38 (C-8), 40.88 (C-7), 43.03 (C-17), 47.56 (C-9), 55.25 (C-5), 58.56 (C-18), 80.60 (C-3), 119.0 (C-15), 171.0 (C-31), 181.0 (C-14), 208.0 (C-16); CIMS $m/z + 1$ 467 (10), 423 (53), 407 (2.65), 358 (25).

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