Two Novel Long-Chain Alkanoic Acid Esters of Lupeol from Alecrim-Propolis

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Two new long-chain alkanoic acid esters of lupeol were isolated together with known triterpenoids, α-amyrin, β-amyrin, cycloartenol, lanosta-7,24-diene-3β-ol and lupeol from Alecrim-propolis collected in Brazil. The structures were characterized by spectroscopic means.

Key words Alecrim-propolis; alkanoic acid ester of lupeol; pentacyclic triterpenoid ester; procrim a; procrim b

Propolis is a resinous material that honeybees produce from exudates of various plants and beeswax in a beehive, and has been used for folk medicines and foods since ancient times in many parts of the world.[1)] Yagi and co-workers.[2)] described the bioactivity and chemical constituents of the compounds so far reported. Although many aromatic compounds were isolated from Propolis and the occurrence of triterpenoid was suggested only from the evidence of the GC/MS,[3)] no report concerning the isolation of triterpenoid has been found. In the present paper, we describe the isolation and structural characterization of two new long-chain alkanoic acid esters of lupeol, named procrims a (**1**) and b (**2**), together with known triterpenoids, α-amyrin, β-amyrin, cycloartenol, lanosta-7,24-diene-3β-ol and lupeol, from Alecrim-propolis.

Procrim a (**1**) was obtained as a resinous substance showing $[\alpha]_D$ +3.5° ($CHCl_3$). It showed a molecular peak at *m/z* 680 and 409 $[C_{30}H_{49}]^+$ in the electron impact (EI)-MS. The molecular formula $C_{46}H_{80}O_3Na$ was determined by high-resolution positive FAB-MS measurement. The ^{1}H-NMR spectrum exhibited signals due to seven tertiary methyl groups at δ 0.83, 0.84, 2×0.99, 1.01, 1.03 and 1.75, each broad singlet signal at δ 4.75 and 4.89, a hydroxyl-bearing methine proton at δ 4.85 (dd, *J*=4.9, 11.6 Hz) and a methine proton at δ 2.47 (dt, *J*=6.1, 11.0 Hz), which are reminiscent of a lupeol-type triterpene. Additionally, a terminal methyl signal at δ 0.88 and strong methylene proton signals around δ 1.30 were indicative of the presence of a fatty acid, which was also supported by the appearance of ^{13}C-signals due to a ester carbonyl group at δ 172.2, a long-chain of methylene groups at δ 25.9—32.0 and a terminal methyl group at δ 14.3. The fatty acid was estimated to be composed of C16 by high-resolution FAB-MS. The ^{13}C-NMR signals (Experimental section) showed the presence of a hydroxy group at C-3′ in the fatty acid part by the proton–proton chemical shift correlation spectroscopy (^{1}H–^{1}H COSY), heteromolecular multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC). Furthermore, the proton signals at δ 2.83 (1H, dd, *J*=4.9, 14.7 Hz), 2.85 (1H, dd, *J*=6.7, 14.7 Hz) and 4.54 (1H, br s) were also assigned as H-2′a, H-2′b and H-3′, respectively, in a part of the fatty acid, and a signal at δ 4.85 (1H, dd, *J*=4.9, 11.6 Hz) was ascribable to H-3 in the triterpene moiety by the ^{1}H–^{1}H COSY, HMQC and HMBC. The configuration at C-3′ of the fatty acid moiety was defined to be *R* according to the Mosher method[4)]; the respective differences at H-2′a, H-2′b and H-3′ of **1**-(+)-2-methoxy-2-trifluoromethyl-phenyl acetic acid (MTPA) ester and **1**-(−)-MTPA ester in the chemical shifts on the ^{1}H-NMR signals showed +0.044, +0.049 and −0.003 ppm. Therefore, the chemical structure of **1** was determined to be lupeol 3-(3′*R*-hydroxy)-hexadecanoate.[5)]

Procrim b (**2**), was obtained as a resinous substance showing $[\alpha]_D$ −146.6° ($CHCl_3$). It showed a molecular peak at *m/z* 708 and 409 $[C_{30}H_{49}]^+$ in the EI-MS. The ^{1}H- and ^{13}C-NMR spectra closely resembled those of **1**. Based on the molecular ion peak in the EI-MS, **2** was deduced to be lupeol 3-(3′*R*-hydroxy)-octadecanoate.

This is the first report for the isolation of two new long-chain alkanoic acid esters of lupeol along with known triterpenoids, α-amyrin, β-amyrin, cycloartenol, lanosta-7,24-diene-3β-ol and lupeol from Propolis.

Experimental

General Procedures Optical rotations were determined on a JASCO DIP-1000 polarimeter (*l*=0.5). FAB-MS were obtained in a glycerol matrix in the positive ion mode using a JEOL JMS-DX300 and JMS-DX 303HF. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer (500 MHz) and chemical shifts were referenced to tetramethylsilane (TMS). Column chromatography was carried out on silica gel 60 (230—400 mesh, Merck) and Sephadex LH-20 (25—100 nm, Pharmacia Fine Chemicals), and

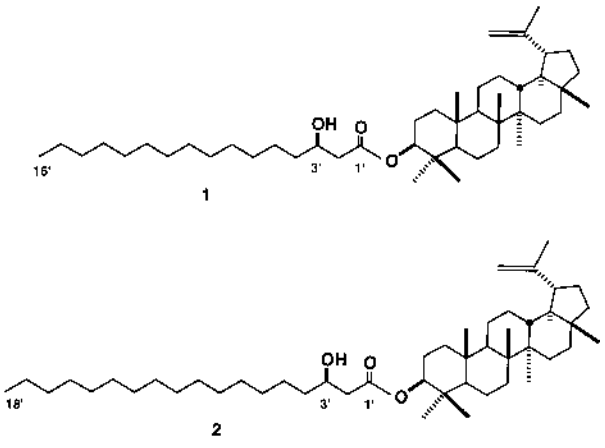

Fig. 1. Structures of Procrims a (**1**) and b (**2**)

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TLC was performed on precoated silica gel $60F_{254}$ (0.2 mm, Merck).

Materials Alecrim-propolis was purchased in Brazil in September 1999 after an examination on the spot. A voucher specimen (Lot. 000223) is deposited in Yamada Apiculture Center, Inc.

Isolation of Triterpenoids Alecrim-propolis (1377.75 g) was refluxed with hot MeOH and subsequently filtered using celite. The filtrate was evaporated under reduced pressure to give a residue (694.43 g). It was then extracted with hexane, AcOEt and MeOH, succesively. A part (10 g) of the AcOEt soluble portion (316.71 g) was subjected to Sephadex LH-20 with MeOH : AcOEt=9 : 1 as eluent to give seven fractions (Fr. 1—7). Fraction 3 (3.95 g) was chromatographed on silica gel using hexane–AcOEt=20 : 1→3 : 2, gradiently, to yield Fr. 3-2 (38 mg) along with Fr. 3-1, 3-3-14. Fraction 3-2 was subsequently separated using HPLC [solv., hexane–AcOEt=30 : 1; column, YMC-Pack SIL5A-06 II (20×250 mm)] to afford long-chain alkanoic esters, named procrim a (**1**, 10.8 mg) and procrim b (**2**, 5.9 mg). Other known constituents, α-amyrin (4.9 mg), β-amyrin (7.4 mg), cycloartenol (7.2 mg), and lanosta-7,24-diene-3β-ol (4.6 mg) and lupeol (16.1 mg) were also obtained by further separation of Fr. 3-5 (155.1 mg) with silica gel (hexane–acetone=12 : 1) and HPLC (column, COSMOSIL 5C18-AR-2, solv. MeOH; YMC-Pack SIL 5A-06 II, solv. hexane–AcOEt=10 : 1, 5 : 1).

α-Amyrin[6] $[\alpha]_D^{28}$ +66.4° (c=0.19, $CHCl_3$), EI-MS (m/z): 426 $[C_{30}H_{50}O]^+$, ^{1}H-NMR (in pyridine-d_5) δ: 0.91, 0.99, 1.06, 1.08, 1.17, 1.27 (each 3H, s, H_3-23, 24, 25, 26, 27, 28), 0.92 (3H, d, J=6.1 Hz, H_3-29), 0.95 (3H, d, J=6.7 Hz, H_3-30), 3.48 (1H, dd, J=4.9, 11.0 Hz, H-3), 5.23 (1H, t-like, J=3.7 Hz, H-12). ^{13}C-NMR (in pyridine-d_5) δ: 39.2, 28.1, 78.1, 39.4, 55.8, 18.8, 33.3, 40.3, 48.1, 37.2, 23.7, 124.9, 139.9, 42.3, 28.5, 26.9, 34.0, 59.2, 39.9, 39.8, 31.5, 41.8, 29.0, 16.6, 15.9, 17.1, 23.5, 28.8, 21.6, 17.8 (C-1—30, respectively).

β-Amyrin[6] $[\alpha]_D^{28}$ +68.6° (c=0.19, $CHCl_3$), EI-MS (m/z): 426 $[C_{30}H_{50}O]^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.92, 0.93, 0.94, 0.99, 1.08, 1.11, 1.24, 1.27 (each 3H, s, H_3-23, 24, 25, 26, 27, 28, 29, 30), 3.47 (1H, dd, J=4.9, 11.0 Hz, H-3), 5.27 (1H, t-like, J=3.7 Hz, H-12). ^{13}C-NMR (in pyridine-d_5) δ: 39.1, 27.2, 78.1, 39.4, 55.7, 18.8, 33.0, 40.1, 48.0, 37.3, 23.9, 122.4, 145.2, 41.9, 28.1, 26.5, 32.8, 47.5, 47.1, 31.2, 34.9, 37.4, 28.8, 15.8, 16.6, 17.1, 26.2, 28.6, 33.5, 23.8 (C-1—30, respectively).

Cycloartenol[7] $[\alpha]_D^{29}$ +30.5° (c=0.18, $CHCl_3$), EI-MS (m/z): 426 $[C_{30}H_{50}O]^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.32, 0.57 (each 1H, d, J=4.0 Hz, H_2-19), 0.98 (3H, d, J=6.1 Hz, H_3-21), 0.95, 1.15, 1.25 (each 3H, s, H_3-18, 28, 29, 30), 1.67 (3H, s, H_3-26), 1.72 (3H, s, H_3-27), 3.56 (1H, dd, J=4.3, 11.6 Hz, H-3), 5.27 (1H, m, H-24). ^{13}C-NMR (in pyridine-d_5) δ: 31.9, 30.3, 78.5, 40.3, 47.0, 21.0, 28.0, 47.8, 20.0, 26.0 26.0, 35.5, 45.1, 48.7, 32.8, 26.5, 55.5, 17.9, 29.8, 36.1, 18.8, 35.5, 25.7, 125.8, 130.9, 17.8, 25.9, 25.4, 14.0, 19.3 (C-1—30, respectively).

Lanosta-7,24-dien-3β-ol[8] $[\alpha]_D^{29}$ −7.5° (c=0.17, $CHCl_3$), EI-MS (m/z): 426 $[C_{30}H_{50}O]^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.84 (3H, s, H_3-19), 0.92 (3H, d, J=6.1 Hz, H_3-21), 0.93, 1.05, 1.15, 1.20, 1.67, 1.73 (each 3H, s, H_3-18, 30, 29, 28, 26, 27), 3.50 (1H, m, H-3), 5.30 (1H, m, H-24), 5.36 (1H, m, H-7). ^{13}C-NMR (in pyridine-d_5) δ: 37.7, 28.7, 78.4, 39.6, 51.2, 24.5, 118.5, 146.1, 49.3, 35.3, 18.5, 34.2, 43.9, 51.6, 34.3, 25.6, 53.6, 22.3, 13.5, 36.1, 18.8, 35.5, 28.8, 125.8, 130.9, 17.8, 25.9, 28.3, 15.6, 27.5 (C-1—30, respectively).

Lupeol[9] $[\alpha]_D^{27}$ +20.7° (c=0.23, $CHCl_3$), EI-MS (m/z): 426 $[C_{30}H_{50}O]^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.84, 0.90, 0.99, 1.05, 1.06, 1.25 (each 3H, s, H_3-23, 24, 25, 26, 27, 28), 1.75 (3H, s, H_3-30), 2.50 (1H, dt, J=5.5, 11.0 Hz, H-19), 3.47 (1H, m, H-3), 4.74, 4.90 (each 1H, d, J=2.4 Hz, H_2-29). ^{13}C-NMR (in pyridine-d_5) δ: 40.3, 27.8, 78.2, 39.6, 55.9, 18.8, 34.7, 41.2, 50.8, 37.5, 21.2, 28.3, 38.3, 43.1, 30.0, 35.8, 43.2, 48.6, 48.3, 151.1, 30.2, 39.3, 28.7, 16.5, 16.4, 14.8, 16.2, 18.2, 110.0, 19.5 (C-1—30, respectively).

Procrim a (1) High-resolution positive-ion FAB-MS (m/z): 703.6027 (Calcd for $C_{46}H_{80}O_3Na$: 703.6005). ^{1}H-NMR (in pyridine-d_5) δ: 0.83, 0.84, 2×0.99, 1.01, 1.03 (each 3H, s, H_3-23, 24, 25, 26, 27, 28), 0.88 (3H, m, H_3-16′), 1.75 (3H, s, H_3-30), 2.47 (1H, m, H-19), 2.83 (1H, dd, J=4.9, 14.7 Hz, H-2′a), 2.85 (1H, dd, J=6.7, 14.7 Hz, H-2′b), 4.54 (1H, br s, H-3′), 4.75, 4.89 (each 1H, br s, H_2-29), 4.85 (1H, dd, J=4.9, 11.6 Hz, H-3). ^{13}C-NMR (in pyridine-d_5) δ: 40.2, 24.3, 80.6, 38.2, 55.7, 18.5, 34.5, 41.1, 50.5, 37.3, 21.1, 27.8, 38.3, 43.1, 30.0, 35.8, 43.2, 48.6, 48.3, 151.0, 30.1, 38.6, 28.2, 17.0, 16.3, 14.3, 16.2, 18.2, 110.0, 19.5 (lupeol C-1—30, respectively), 172.2, 44.0, 68.4, 38.2 (fatty acid C-1′—4′, respectively), 25.0—32.0 (fatty acid C-5′—13′, respectively), 32.1, 23.0, 14.3 (fatty acid C-14′—16′, respectively).

Procrim b (2) EI-MS (m/z): 708 and 409 $[C_{30}H_{49}]$. ^{1}H-NMR (in pyridine-d_5) δ: 0.83, 0.84, 2×0.99, 1.01, 1.04 (each 3H, s, H_3-23, 24, 25, 26, 27, 28), 0.88 (3H, m, H_3-18′), 1.76 (3H, s, H_3-30), 2.48 (1H, m, H-19), 2.84 (2H, m, H_2-2′), 4.54 (1H, br s, H-3′), 4.75, 4.90 (each 1H, br s, H_2-29), 4.85 (1H, dd, J=4.9, 11.6 Hz, H-3). ^{13}C-NMR (in pyridine-d_5) δ: 40.1, 24.2, 80.5, 38.1, 55.5, 18.4, 34.4, 41.0, 50.4, 37.2, 21.0, 27.6, 38.2, 42.9, 29.9, 35.7, 43.1, 48.5, 48.2, 151.1, 29.9, 38.5, 28.1, 17.1, 16.2, 14.6, 16.0, 18.1, 110.0, 19.5 (lupeol C-1—30, respectively), 172.1, 43.9, 68.3, 38.1 (fatty acid C-1′—4′, respectively), 25.0—32.0 (fatty acid C-5′—15′, respectively), 32.0, 22.9, 14.2 (fatty acid C-16′—18′, respectively).

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References and Notes

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