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TRITERPENES FROM *EUONYMUS REVOLUTUS*

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Key Word Index—*Euonymus revolutus*; Celastraceae; pentacyclic triterpenes; 2α,3α-dihydroxyolean-12-en-28-oic acid.

Abstract—Fourteen pentacyclic triterpenes, which included ten D:A-friedooleananes, three lup-20(29)-enes and 2α,3α-dihydroxy-olean-12-en-28-oic acid, were isolated from the stem bark extract of *Euonymus revolutus*.

INTRODUCTION

The genus *Euonymus* consists of about 176 species distributed in the Himalayas, China, Japan and Sri Lanka [1]. All three species of *Euonymus* found in Sri Lanka, *E. revolutus*, *E. thwaitesii* and *E. walkeri* are endemic [2]. *Euonymus revolutus* Wight is a small or moderately sized tree found in the upper montane forests of Sri Lanka. We have recently reported the isolation of three new D:A-friedooleananes [3] and a new lupene diol [4] from the dichloromethane extract of the stem bark of *E. revolutus*. In this paper we report the isolation of 14 pentacyclic triterpenes including 2α,3α-dihydroxy-olean-12-en-28-oic acid and sitosterol from the same extract.

RESULTS AND DISCUSSION

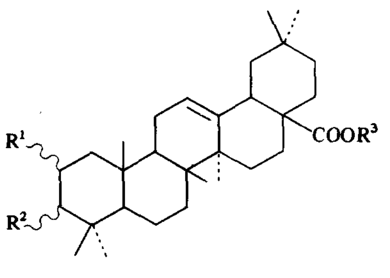
Plant material used in our study was collected at Hakgala in the Nuwara Eliya district. Combined column chromatography and preparative layer chromatography of the cold dichloromethane extract of the stem bark yielded D:A-friedooleanan-3-one (friedelin), 3-oxo-D:A-friedooleanan-28-al (canophyllal), 28-hydroxy-D:A-friedooleanan-3-one (canophyllol), 3α-hydroxy-lup-20(29)-en-28-oic acid (*epi*-betulinic acid), 3-oxo-lup-20(29)-en-28-oic acid (betulonic acid), 3-oxo-D:A-friedooleanan-28-oic acid (oxocanophyllic acid), 3β-hydroxy-D:A-friedooleanan-28-oic acid (canophyllic acid), 29-hydroxy-D:A-friedooleanan-3-one, sitosterol, 3α-hydroxy-2-oxo-D:A-friedooleanan-28-oic acid [3], 2α-hydroxy-3-oxo-D:A-friedooleanan-28-oic acid [3], 2α,3α-dihydroxy-lup-20(29)-en-28-oic acid [4], 28,29-dihydroxy-D:A-friedooleanan-3-one [5], 29-hydroxy-3-oxo-D:A-friedooleanan-28-oic acid [3], and an acid (**1**).

The IR spectrum of the most polar acid (**1**) indicated the presence of hydroxy and carboxylic acid groups. High resolution mass spectroscopy ($C_{30}H_{48}O_4$) suggested the presence of a pentacyclic triterpene with two hydroxy groups and a carboxylic acid group. The ^{1}H NMR spectrum showed signals due to an equatorial carbinol methine proton at $\delta 3.43$ (1H, *d*, $J = 3$ Hz), an axial carbinol methine proton at $\delta 3.92$ (1H, *m*, $W_{1/2} = 22$ Hz), an olefinic proton at $\delta 5.29$ (1H, *m*) and seven angular methyl groups. The mass spectrum of compound **1** indicated it to be an olean-12-ene [6]. Treatment of the acid (**1**) with diazomethane yielded a methyl ester (**2**). Comparison of physical constants and spectroscopic data indicated the ester **2** to be methyl 2α,3α-olean-12-en-28-oate [7, 8]. Additional evidence for the structure of the acid (**1**) was obtained by the formation of 3-oxoolean-12-en-28-oic acid (**3**) when the acid **1** was refluxed in benzene with *p*-toluenesulphonic acid. The acid **3** was found to be identical with the acid obtained by oxidation of an authentic sample of 3β-hydroxy-olean-12-en-28-oic acid. Hence compound **1** was assigned the structure 2α,3α-dihydroxy-olean-12-en-28-oic acid. The free acid **1** has not been isolated previously from natural sources although it has been isolated as its methyl ester from *Pseudopanax arboreum* (Araliaceae) [7] and two species of *Shorea* (Dipterocarpaceae) [8].

EXPERIMENTAL

Mps were determined on a Kofler hot stage apparatus and are uncorr. Identities of compounds were established by mmp, co-TLC, IR and NMR comparison. Petrol refers to the fraction having boiling range 60–80° and preparative TLC was carried out on Merck Kieselgel 60 $PF_{254+365}$. Optical rotations were measured at 25° in $CHCl_3$. IR spectra were recorded using KBr discs. ^{1}H NMR spectra were recorded at 60 MHz with TMS as internal standard. Low and high resolution EI mass spectra were recorded at 70 eV using the direct insertion probe.

Extraction and isolation of compounds. The dried and powdered stem bark (5 kg) of *E. revolutus* was extracted with cold



1 $R^1 = R^2 = \alpha\text{-OH}, \beta\text{-H}; R^3 = H$

2 $R^1 = R^2 = \alpha\text{-OH}, \beta\text{-H}; R^3 = Me$

3 $R^1 = H; R^2 = O; R^3 = H$

CH_2Cl_2. Evaporation of the CH_2Cl_2 extract yielded a dark brown solid which was re-extracted with MeOH. The dry MeOH extract (70 g) was chromatographed on silica gel using C_6H_6–EtOAc–MeOH.

Elution of the column with C_6H_6 yielded friedelin (38 mg), mp 263–264°, $[\alpha]_D$ −18.0° (lit. [9, 10] mp 264°, $[\alpha]_D$ −22.1°); canophyllal (50 mg), mp 268–269°, $[\alpha]_D$ −17.0° (lit. [11], mp 268–270°, $[\alpha]_D$ −18.1°); canophyllol (40 mg), mp 281–282°, $[\alpha]_D$ −21.2° (lit. [11] mp 280–282°, $[\alpha]_D$ −20.0°); C_6H_6 and 12% EtOAc gave *epi*-betulinic acid (40 mg), mp 280–283°, $[\alpha]_D$ −8.0° (lit. [12] mp 279–283°, $[\alpha]_D$ −11.0°); C_6H_6 and 25% EtOAc afforded betulonic acid (80 mg), mp 258–259°, $[\alpha]_D$ +36.0° (lit. [13] mp 252°, $[\alpha]_D$ +31.0°); oxocanophyllic acid (256 mg), mp 310°, $[\alpha]_D$ −32.0° (identical with that obtained by oxidation of an authentic sample of canophyllic acid); C_6H_6 and 40% EtOAc yielded canophyllic acid (35 mg), mp 312° (lit. [14] mp 312°); C_6H_6 and 80% EtOAc gave 29-hydroxy-D:A-friedooleanan-3-one (109 mg), mp 271–272°, $[\alpha]_D$ −20° (lit. [15] mp 270–272°, $[\alpha]_D$ −24.0°); EtOAc yielded sitosterol (18 mg), mp 136–137°, $[\alpha]_D$ −35.0° (lit. [16] mp 136–137°, $[\alpha]_D$ −35.0°); EtOAc and 5% MeOH gave 3α-hydroxy-2-oxo-D:A-friedooleanan-28-oic acid (30 mg), mp 288–290° [3]; 2α-hydroxy-3-oxo-D:A-freidooleanan-28-oic acid (114 mg), mp 258–260° [3]; 2α,3α-dihydroxylup-20(29)-en-28-oic acid (120 mg), mp 298–300° [4]; 28,29-dihydroxy-D:A-friedooleanan-3-one (50 mg), mp 286–288° (lit. [5] mp 286–288°); 29-hydroxy-3-oxo-D:A-friedooleanan-28-oic acid (100 mg), mp 288–290° [3]; EtOAc and 10% MeOH afforded 2α,3α-dihydroxyolean-12-en-28-oic acid (**1**) (150 mg), mp 300–302° (Found $[M]^+$ 472.3565, calc. for $C_{30}H_{48}O_4$, $[M]^+$, 472.3552); IR ν_{max}^{KBr} cm^{-1}: 3640–3100, 3400, 1690; ^{1}H NMR ($CDCl_3$–CD_3OD): δ5.29 (1H, *m*, H-12), 3.92 (1H, *m*, $W_{1/2}$ = 22.0 Hz, H-2β), 3.43 (1H, *d*, *J* = 3.0 Hz, H-3β), 1.00 (3H, *s*), 0.95 (12H, *s*), 0.86 (6H, *s*) (7 × *t*-Me); MS *m/z* (rel. int.): 472 $[M]^+$ (1), 454 (1), 436 (1), 462 (2), 408 (1), 393 (1), 302 (1), 248 (100), 235 (4), 223 (9), 203 (47), 189 (10), 175 (4), 163 (5), 147 (5), 133 (21), 119 (10). The methyl ester (**2**) obtained by treatment of compound **1** with CH_2N_2 as colourless crystals (14 mg), mp 280–283° $[\alpha]_D$ +58° (*c* 1.0) (lit. [7] mp 277–280°, $[\alpha]_D$ +55.0°; lit. [8] mp 296–299°, $[\alpha]_D$ +58.0°); IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1230, ^{1}H NMR: δ5.29 (1H, *m*, H-12), 4.13 (1H, *m*, $W_{1/2}$ = 22 Hz, H-2β), 3.63 (3H, *s*, OMe), 3.43 (1H, *d*, *J* = 3.0 Hz, H-3β), 1.09 (3H, *s*), 1.03 (3H, *s*), 1.00 (6H, *s*), 0.92 (3H, *s*), 0.89 (3H, *s*), 0.75 (3H, *s*), (7 × *t*-Me).

Reaction of compound **1** *with* p-TsOH. Compound **1** (30 mg) was refluxed with *p*-TsOH (1 mg) in dry C_6H_6 (1 ml) for 10 min. The mixture was cooled and diluted with Et_2O. The Et_2O fraction was washed with 10% Na_2CO_3, dried and evaporated to dryness. The residue was purified by preparative TLC ($CHCl_3$) and crystallized from C_6H_6–MeOH to give 3-oxo-olean-12-en-28-oic acid (**3**) (11 mg) mp 297°, $[\alpha]_D$ +65.0° (*c* 1.0) (lit. [17] mp 299–300°).

Oxidation of 3β-hydroxyolean-12-en-28-oic acid. An authentic sample of 3β-hydroxyolean-12-en-28-oic acid (50 mg) mp 309–310°, $[\alpha]_D$ +78.0° (lit. [18] mp 305–310°, $[\alpha]_D$ +80.0°) was treated with CrO_3 (60 mg) in pyridine (1 ml). The usual work up afforded 3-oxo-olean-12-en-28-oic acid (38 mg) mp 297°, $[\alpha]_D$ +65.7°, identical with the acid **3** obtained above.

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