ABSTRACT

A new triterpene alcohol was isolated from shea butter and its structure was shown to be 24-methylenelanost-9(11)-en-3β-ol. Gas chromatographic correlations between this triterpene alcohol and other related compounds are discussed.

INTRODUCTION

The presence of lanosta-9(11),24-dien-3β-ol (parkeol, Fig. 1, II; R1 = OH, R2 = iii), a biogenetically interesting triterpene alcohol, in shea butter from the kernels of Butyrospermum parkii (Sapotaceae) has already been known (1,2). α-Amyrin (2), β-amyrin (2,3), lupeol (2,3) and butyrospermol (2,4,5) also have been isolated from shea butter. We report here the isolation and structure of a new Δ9(11)-triterpene alcohol (II; R1 = OH, R2 = iv) from shea butter. Gas chromatographic correlations between this and related triterpene alcohols also are discussed.

EXPERIMENTAL PROCEDURES

Melting points were determined with a Micro mp apparatus (Yanagimoto Seisakusho Ltd., Kyoto, Japan) and uncorrected. All recrystallizations were performed in acetone-methanol. IR spectra (KBr) were obtained with a Type IRA-2, IR spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan). Optical rotations were measured in CHCl3 using an Automatic Polarimeter, Model MP-1T (Applied Electric Lab. Ltd., Tokyo, Japan) or a Carl Zeiss Circle Polarimeter 0.01° (Carl Zeiss, Oberkochen, Germany). Concentrations used are indicated in parentheses as g/100 ml. NMR spectra in CDCl3 were recorded on a JNM-C-60-HL (60 MHz, Japan Electron Optics Laboratory Co., Tokyo, Japan) and calibrated against internal tetramethysilane as 0 ppm. Mass spectra were recorded on a Hitachi RMU-7M mass spectrometer (Hitachi Ltd., Tokyo, Japan) by probe injection, or on a Shimadzu LKB-9000 gas chromatograph-mass spectrometer (Shimadzu Seisakusho Ltd., Kyoto, Japan).

Preparative argentation thin layer chromatography (TLC) for the fractionation of triterpene acetates was carried out on the plates of Wakogel B-10 (Wako Pure Chemical Industries Ltd., Osaka, Japan) impregnated with 10% silver nitrate, using a Toyoo continuous flow development preparative TLC (Toyoo Roshi Kaisha Ltd., Tokyo, Japan). Separated zones were observed under UV light (3600 A) after spraying a rhodamine-6G solution in ethanol on the developed plate, and were cut off and quantitatively extracted with ether. The alumina (about 300 mesh) for column chromatography was purchased from Wako Pure Chemical Industries Ltd. Gas liquid chromatography (GLC) was performed on a Shimadzu GC-5A gas chromatograph (Shimadzu Seisakusho Ltd.) equipped with a flame ionization detector and a 2 m x 3 mm internal diameter (ID) glass column packed with 3% OV-17 on Gas Chrom-Z, 80-100 mesh. The column was operated generally at 255 C with nitrogen at 50 ml/min as carrier gas. Detector temperature was 280 C. Relative retention time (RRT) was expressed by the ratio of the retention time for the substance under examination to the retention time (30 min) for β-sitosterol. Hydrolysis of triterpene acetates was performed by refluxing for 2 hr with an alcoholic 0.5 N potassium hydroxide, and then the reaction mixture was diluted with an excess of water and extracted several times with ether. The ether extract was washed 3 times with water and dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator.

Isolation of 24-Methylenelanost-9(11)-en-3β-ol from Shea Butter: Unsaponifiable material (69.6 g) was separated from shea butter (1.4 kg) in the same manner as described previously (2). It was treated with acetone and the acetone soluble portion (55 g) was acetylated by refluxing for 3 hr with acetic anhydride (300 ml). The reaction mixture then was poured onto 1.5 liter of ice cooled water. The brown solid mass obtained (50 g) was fractionated by preparative argentation TLC (hexane:benzene [7:3]) for 60 min to give three principal zones. The zone closest to the starting line (1109 mg) was further fractionated by TLC (hexane:benzene [3:2]) for 70 min to give eventually a fraction (52 mg) which con-
sisted of 24-methylenelanost-9(11)-en-3β-yl acetate (RRT = 1.62; GLC purity = 95%).

24-Methylenelanost-9(11)-en-3β-yl acetate (II; R₁ = OAc, R₂ = iv), its free alcohol (II, R₁ = OH, R₂ = iv), and its 3-keto derivative (II; R₁ = O, R₂ = iv): The acetate crystallized as plates. The mp was 159.5-160.5 C, [α] D +78 (c, 1.00); the IR spectrum was 1723, 1642, 1421, 1386, 1112, 1075, and 792 cm⁻¹; and the NMR spectrum was 0.87, 0.89, 0.98, 1.09, 2.05, 4.54, 4.69, and 5.21 ppm. Hydrolysis of the acetate gave free alcohol with RRT = 1.35; mp = 178.5-179.5 C (fine needles); [α] D +60 (c, 0.92); NMR = 0.66, 0.75, 0.83, 0.99, 1.06, 1.08, 3.28, 4.70, and 5.25 ppm; and mass spectrum (MS) = m/e 440.4021 (molecular ion [M+]), 425.3802, 407.3712, 397.3449, 356.3078, 341.2820, 323.2727, 313.2538, 273.2249, 259.2057, and 255.2114. The new alcohol was oxidized with CrO₃ as follows (6). A solution of 24-methylcycloartanol (50 mg) in pyridine (1 ml) was added to CrO₃ (55 mg) in pyridine (1 ml), and the mixture was allowed to stand at room temperature for 16 hr. Water (30 ml) was added and the product was extracted with ether. The ether extract was worked up in the usual way. The final product obtained was dissolved in hexane and chromatographed on alumina (6 g). The fractions eluted with 50 ml hexane:benzene (1:1) gave 24-methylenelanost-9(11)-en-3β-ol (29 mg) with RRT = 1.26; mp = 121-122 C (plates); IR = 3400, 3080, 1730, 1640, 1375, 1210, and 1020 cm⁻¹; and NMR = 0.65, 0.75, 0.82, 0.91, 1.09, 1.06, 1.08, 3.28, 4.70, and 5.30 ppm.

24-Methylenelanost-9(11)-en-3β-ol acetate (II; R₁ = OAc, R₂ = iv) and its free alcohol (II, R₁ = OH, R₂ = iv): 24-Methylenelanost-9(11)-en-3β-ol acetate (II; R₁ = OAc, R₂ = iv) and its free alcohol (II, R₁ = OH, R₂ = iv) was isolated from the acetylated unsaponifiables (37 g) of rice bran oil by HCl isomerization. Repeated recrystallization (11) of the acetylated unsaponifiables (37 g) of rice bran oil from acetone-methanol gave a triterpene acetate fraction (4.3 g) containing the acetates of cycloartenol (RRT = 1.52, ca. 32%), 24-methylenecycloartenol (RRT = 1.69, ca. 68%) and a trace amount of cycloartenol (RRT = 1.26). Preparative argentation TLC (hexane:benzene [7:3]) of the acetate fraction (3 g) for 40 min afforded three fractions, 1-3. Fraction 1 (58 mg) from the zone closest to the solvent front was rich in cycloartenyl acetate. Fraction 2 (868 mg) from the medium zone was cycloartenyl acetate. Fraction 3 (2010 mg) from the zone closest to the starting line gave 24-methylenecycloartenyl acetate.

24-Methylenecycloartenyl acetate (I; R₁ = OAc, R₂ = iv) and its free alcohol (I; R₁ = OH, R₂ = iv): 24-Methylenecycloartenyl acetate (I; R₁ = OAc, R₂ = iv) and its free alcohol (I; R₁ = OH, R₂ = iv) was synthesized from 24-Methylcycloartenyl acetate fraction (4.3 g) containing the acetates of cycloartenol (RRT = 1.52, ca. 32%), 24-methylenecycloartenol (RRT = 1.69, ca. 68%) and a trace amount of cycloartenol (RRT = 1.26). Preparative argentation TLC (hexane:benzene [7:3]) of the acetate fraction (3 g) for 40 min afforded three fractions, 1-3. Fraction 1 (58 mg) from the zone closest to the solvent front was rich in cycloartenyl acetate. Fraction 2 (868 mg) from the medium zone was cycloartenyl acetate. Fraction 3 (2010 mg) from the zone closest to the starting line gave 24-methylenecycloartenyl acetate.

The acetate crystallized as fine needles with mp = 171-172.5 C; [α] D +53 (c, 1.25); and IR = 1703, 1382, 1373, 895, 886, 810, and 790 cm⁻¹; and NMR = 0.66, 0.75, 0.82, 0.91, 1.09, 1.06, 1.08, 3.28, 4.70, and 5.30 ppm.

Isolation of 24-methylenecycloartenyl acetate from the acetylated unsaponifiables of rice bran oil: Repeated recrystallization (11) of the acetylated unsaponifiables (37 g) of rice bran oil from acetone-methanol gave a triterpene acetate fraction (4.3 g) containing the acetates of cycloartenol (RRT = 1.52, ca. 32%), 24-methylenecycloartenol (RRT = 1.69, ca. 68%) and a trace amount of cycloartenol (RRT = 1.26). Preparative argentation TLC (hexane:benzene [7:3]) of the acetate fraction (3 g) for 40 min afforded three fractions, 1-3. Fraction 1 (58 mg) from the zone closest to the solvent front was rich in cycloartenyl acetate. Fraction 2 (868 mg) from the medium zone was cycloartenyl acetate. Fraction 3 (2010 mg) from the zone closest to the starting line gave 24-methylenecycloartenyl acetate.

Synthesis of 24-Methylenolanost-9(11)-en-3β-ol from 24-Methylocycloartenol by HCl Isomerization

24-Methylcycloartenyl acetate and its isomerization: A solution of 24-methylocycloartenyl acetate (1500 mg) in ether (50 ml) was hydrogenated in the presence of platinum oxide (110 mg) for 4 hr at room temperature. After removal of the catalyst, 24-methylocycloartenyl acetate (1480 mg, RRT = 1.64) was recovered from the ether solution. It showed, on recrystallization, an mp = 125-126 C (long needles, >99% GLC purity); [α] D +53 (c, 1.25); and IR = 1730, 1370, 1248, and 1022 cm⁻¹. Hydrolysis of the acetate gave free alcohol (RRT = 1.33) with a mp = 138-139 C (fine needles). A solution of 24-methylocycloartenyl acetate (1073 mg) in CHCl₃ (30 ml) was treated with a stream of dry HCl at -30 C for 4 hr. The CHCl₃ was evaporated and the residue was taken up in ether. The ether solution was washed first with sodium carbonate aqueous solution and then with water, after which the ether layer was dried over anhydrous sodium sulfate and the ether was evaporated. Preparative argentation TLC (hexane:benzene [7:3]) of the isomerized product (1045 mg) for 50 min gave three fractions, 1-3.