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PLECTRANTHOIC ACID, ACETYLPLECTRANTHOIC ACID AND PLECTRANTHADIOL, THREE TRITERPENOIDS FROM *PLECTRANTHUS RUGOSUS*

T. K. RAZDAN,* V. KACHROO, S. HARKAR, G. L. KOUL and K. L. DHAR†

Department of Chemistry, Regional Engineering College, Hazratbal, Srinagar; †Regional Research Lab., Canal Road, Jammu, India

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Key Word Index—*P. rugosus*; Labiatae; pentacyclic triterpenoids; plectranthoic acid; acetylplectranthoic acid; plectranthadiol.

Abstract—Sitosterol and three new pentacyclic triterpenoids, plectranthoic acid, acetylplectranthoic acid and plectranthadiol, have been isolated from leaves of *P. rugosus*. From spectroscopic evidence and chemical behaviour the structure of plectranthoic acid was established as (19S)-3α-hydroxy-18α-urs-12-en-29β-oic acid and acetylplectranthoic acid is the 3α-acetyl derivative of this compound. Plectranthadiol is (19*S*)- 3α-hydroxy-18α-urs-12-en-29β-ol.

INTRODUCTION

Several species of the genus *Plectranthus* have been documented for the presence of diterpene quinones, coleones and royleanones, of the abietane series [1–4]. Chrysosplenetin has been reported from *P. marrubioides* [5] and dopaldehyde caffeic ester has been isolated from *P. caninus* [6].

P. rugosus is a wild shrub, distributed widely throughout northern temperate regions of the Himalayas (altitude 5000–8000 ft). It emanates a characteristic aroma and enjoys a reputation as an antiseptic, germicidal and cardiac stimulant in local medicine. Prakash *et al.* [7] studied the composition of fixed oil derived from its seeds.

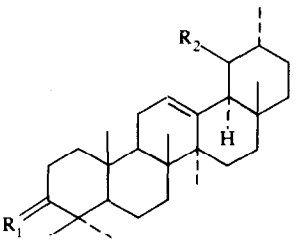
The lack of substantial data on the chemistry of this plant prompted us to examine its chemical composition. In this paper we report the presence of three new triterpenoids and sitosterol in the leaves of the plant.

RESULTS AND DISCUSSION

Compounds **1–3** respond positively to the Liebermann–Burchard, TCA and TNM tests, suggesting that they are pentacyclic triterpenoids. Compounds **1**, mp 296° (from MeOH–H_2O), M^+ at *m/z* 456.3626, $C_{30}H_{48}O_3$, $[\alpha]_D^{25}$ +42° (MeOH; *c* 1.0), gives a positive $NaHCO_3$ test. Its IR spectrum exhibits absorptions at ν_{max}^{KBr} cm^{-1} 3420 (–OH); 1695, 3200–2500, broad (–COOH); 1360, 1380 (gemdimethyl) and 1670, 830 (tri-substituted double bond). The ^1H NMR spectrum of **1** accounts for six tertiary methyls at δ 0.66–1.04, one secondary methyl at 1.14, one C-3 proton at 4.20 (*t*, *J* = 9, 6 Hz) and a proton on a tri-substituted double bond at 5.16 (1H, *br s*, *J* = 5.2 Hz).

Compound **1** on prolonged hydrogenation over 10% Pd–C is converted into a dihydro derivative **6**, M^+ at *m/z* 458.3780, $C_{30}H_{50}O_3$, whose IR and ^1H NMR spectra show the absence of a double bond.

Compound **1**, on heating with acetic anhydride and pyridine for 7 hr, forms a monoacetyl derivative **2**, M^+ at *m/z* 498.0178, $C_{30}H_{50}O_4$. Its IR spectrum reveals absorptions due to acetate (ν_{max}^{KBr} 1730, 1245 cm^{-1}). The ^1H NMR signal due to the acetoxyl methyl protons appears at δ 2.03 (3H, *s*). The C-3 proton is shifted downfield to δ 4.43 (1H, *t*, *J* = 9, 6 Hz) in **2**. Compound **1** undergoes facile Sarett oxidation and yields the keto acid **4**, M^+ at *m/z*



1 R_1 = α-OH, β-H; R_2 = COOH

2 R_1 = α-OAc, β-H; R_2 = COOH

3 R_1 = α-OH, β-H; R_2 = CH_2OH

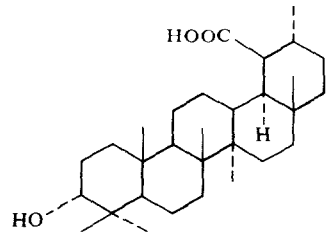
4 R_1 = O; R_2 = COOH

5 R_1 = α-OAc, β-H; R_2 = COOMe

8 R_1 = α-OH, β-H; R_2 = CH_2OAc

9 R_1 = α-OAc, β-H; R_2 = CH_2OAc

*To whom correspondence should be addressed.



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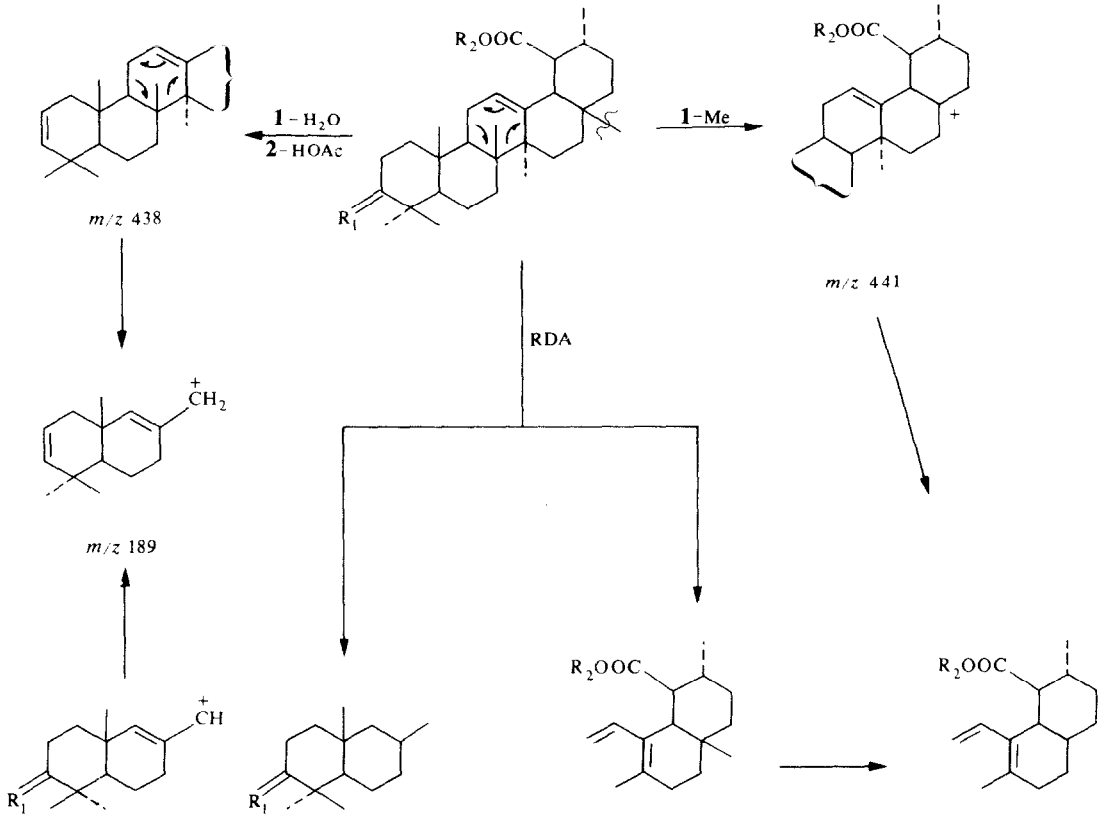
454.3440, $C_{30}H_{46}O_3$; the –C=O absorbs at 1680, 1700 cm^{-1}. Compound **4** gives a positive Zimmermann test confirming the presence of a secondary hydroxyl group at C-3. In its ^1H NMR spectrum the compound displays a two-proton multiplet at δ 2.20 due to the C-2 protons. The downfield position of the C-3 proton, its multiplicity and coupling constant, supplemented by the ease of oxidation [8–10] and difficulty in the formation of the acetate (**2**) indicates that the C-3 hydroxyl group is axial and α-oriented.

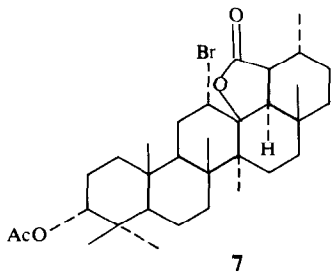
The monoacetate **2** on treatment with CH_2N_2 gives its monomethyl ester **5**, M^+ at *m/z* 512.3850, $C_{33}H_{52}O_4$. The IR spectrum of this compound shows absorptions due to ester carbonyls at ν_{max}^{KBr} cm^{-1} 1730, 1720 and 1245. The ^1H NMR spectrum of the compound reveals resonance signals due to an acetoxyl methyl at δ 2.0 and a methyl ester methyl at 3.40.

The mass spectrum of **1** indicates that the double bond triggers the typical retro-Diel–Alder (RDA) fragmentation of ring C resulting in a fragment *a* at *m/z* 248 as the base peak and a fragment *b* at *m/z* 208. The base peak fragment is also derived by RDA fragmentation from **2** and **4** and is observed at *m/z* 250 and 206, respectively, in these derivatives. The high-resolution mass spectra of the base fragment *a* shows it to be $C_{16}H_{24}O_2$ while fragment *b* is $C_{14}H_{24}O$. These observations support the presence of the C-3 hydroxyl group and C-12 double bond [11] and substantiate the location of –COOH on ring D or E. This fact, in addition to the presence of only seven methyl groups suggests that **1** has either the urs-12-ene skeleton or its rearranged skeleton with one methyl group transformed into a carboxylic group at C-28, C-29 or C-30.

In the ^1H NMR spectrum of **1**, **2**, **4** and **6** the resonance signal due to the proton on the carbon carrying the COOH group is observed at δ 2.80 (1H, *d*, J = 16.3 Hz). The signal due to the C-18 proton is displayed at δ 2.60 (1H, *d*, J = 5 Hz). The C-18 proton and C-19 proton are shifted to δ 2.66 and 2.90, respectively, in the ^1H NMR spectrum of **5**.

The nature of the double bond and the position of the –COOH in **1** is ascertained by the formation of





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the bromo-γ-lactone **7**, M^+ at *m/z* 576.2631 and 578.2642, $C_{32}H_{49}O_4Br$. The lactone can be derived either from a C-28β COOH and C-13 hydroxyl or a C-29β COOH and C-13 hydroxyl of the intermediate bromohydrin of **2**. The ^1H NMR spectrum of the lactone displays a single-proton signal at δ 2.85 (*d*, $J = 15.5$ Hz) indicating that the carbonyl function of the lactone, hence the carboxylic group in the parent compound, is attached to a secondary carbon atom. The C-12 proton of the lactone resonates at δ 4.55. Thus the presence of a C-28 carboxyl group is ruled out. Its absence is also indicated by the mass spectrum of **1** in which fragments *a* and *e* appear as strong peaks due to the easy loss of the C-17 methyl group.

The stereochemistry at C-19 and C-20 is settled on the basis of the ^1H NMR spectra of **1** and its derivatives. The coupling constant, $J = 16.3$ Hz, due to the C-19 proton suggests *trans*-diaxial coupling between the C-19 and C-20 protons ($\vartheta = 180°$) and that due to the C-18α proton ($J = 5$ Hz) suggests axial-equatorial coupling between the C-18α proton and the C-19α proton.

An examination of models reveals that the C-19 hydrogen is α-oriented and couples strongly with the C-20β hydrogen. The formation of a bromo-γ-lactone substantiates the stereochemistry of the carboxyl group as β(S). Based on these reasonings plectranthoic acid (**1**) is assigned the structure (19*S*)-3α-hydroxy-18α-urs-12-en-29β-oic acid.

Compound **2**, mp 258°, M^+ at *m/z* 498.0178, $C_{32}H_{50}O_4$, $[\alpha]_D^{25} + 58°$, forms monomethyl ester **5** with CH_2N_2 and on treatment with BF_3-etherate undergoes hydrolysis to give a hydroxy acid whose IR, ^1H NMR and mass spectra are superimposable with those of compound **1**. Compound **2** is, therefore, identified as (19*S*) - 3α - acetyl - 18α - urs - 12 - en - 29β - oic acid.

Compound **3**, mp 220°, M^+ at *m/z* 442.3820, $C_{30}H_{50}O_2$, $[\alpha]_D^{20} + 26°$ (*c* 0.1 in MeOH), contains absorption bands in its IR spectrum at ν_{max}^{KBr} cm^{-1} 3390, 3380 (–OH); 1370, 1390 (–C(Me)(Me)); 1660 (*w*), 820 (–CH_2–CH=C). The ^1H NMR spectrum revealed resonance signals at δ 0.76 (6H, *s*), 0.83 (3H, *s*), 0.90 (6H, *s*), 0.97 (3H, *s*), 1.06 (3H, *s*), 2.25 (1H, *d*, $J = 7$ Hz), 3.10 (1H, *br s*), 4.50 (1H, *s*), 4.63 (1H, *m*, $W_{1/2} = 18.2$ Hz), 5.20 (1H, *t*, $J = 6, 3$ Hz).

Compound **3**, on heating with Ac_2O–C_5H_5N, forms a mixture of monoacetate **8**, M^+ at *m/z* 484.3917, $C_{32}H_{52}O_3$, and diacetate **9**, M^+ at *m/z* 526.4023, $C_{34}H_{54}O_4$. On Sarett oxidation it readily gives the keto-acid **4**. These observations indicate the presence of a C-3α hydroxyl group and a primary OH group in compound **3**. The primary nature of the second hydroxyl group is also borne out from the ^1H NMR spectrum of compound **3**; it contains an AB system centred at δ 3.56 with a splitting of 12 Hz indicating that the group is present at a nonhindered equatorial position.

The mass spectrum of compound **3** shows an intense peak at *m/z* 207 and prominent peaks at 234 and 208 resulting from retro-Diels–Alder fragmentation of ring C. This substantiates the presence of a C-12 double bond and a C-3 hydroxyl group and fixes the position of –CH_2OH in ring D/E. The striking feature of the mass spectrum is the presence of low intensity peaks at *m/z* 427 and 411 arising from the molecular ion by the easy loss of the C-17 methyl group or the CH_2OH group, respectively. The base peak fragment at *m/z* 203 is derived from the fragment at *m/z* 234.

The presence of only seven methyl groups together with the multiplicity of the C-18 proton suggests that compound **3** also belongs to the 18α-urs-12-ene group with one of the methyls transformed into –CH_2OH. The ^1H NMR spectrum of the compound reveals an ABX pattern; it contains a two-proton quartet at δ 3.16 with splittings of 11 and 5 Hz indicating the presence of CH_2OH–CH–CH–CH_2 in the compound. The conclusive information about the position and configuration of the –CH_2OH group at C-19 is provided by the keto-acid **4**. The structure of compound **3** is finally confirmed as (19*S*) - 3α - hydroxy - 18α - urs - 12 - en - 29β - ol by its partial synthesis from compound **2** with the help of $LiAlH_4$ reduction.

EXPERIMENTAL

IR spectra were recorded on KBr, unless otherwise stated. ^1H NMR spectra were run at 220, 90 and 60 MHz with TMS as int. standard. Mass spectra were recorded at 70 eV.

Extraction and isolation. The crushed and defatted leaves of *P. rugosus* (6.5 kg) were extracted with MeOH in a Soxhlet. The residue (79 g) left after the removal of the solvent was chromatographed over neutral Al_2O_3 (3 kg). The development of the column with petrol yielded sitosterol.

The C_6H_6 eluate from the column was a mixture of four components (TLC: C_6H_6–EtOAc, 7:3). This material on rechromatography over Si gel (1:40) yielded colourless granular compound **1**, R_f 0.03 ($CHCl_3$), M^+ at *m/z* 456.3604 (calc. for $C_{30}H_{48}O_3$, 456.3626). IR: ν_{max} cm^{-1} 3420, 2930, 2500–3200 (*br*), 1695, 1680, 1500, 1380, 1360, 1260, 1020. ^1H NMR (90, 220 MHz; DMSO-d_6): δ 0.66 (3H, *s*, H-24), 0.68 (3H, *s*, H-25), 0.83 (9H, *s*, H-23, H-26, H-27), 1.04 (3H, *s*, H-28), 1.14 (3H, *s*, H-30), 1.80–1.46 (18H, *m*), 2.60 (1H, *d*, $J = 5$ Hz), 2.80 (1H, *br d*, $J = 16.3$ Hz, H-19), 3.24 (4H, *br s*), 4.20 (1H, *t*, $J = 6, 9$ Hz, H-3), 5.16 (1H, *br s*, $J = 5.2$ Hz). MS *m/z*: 456 $[M]^+$, 441 $[M - Me]^+$, 438 $[M - H_2O]^+$, 423 $[M - Me - H_2O]^+$, 395, 300, 287, 256, 248 (100%), 233, 219, 207, 205, 189.

Hydrogenation of **1**. To a soln of **1** (100 mg) in EtOAc (50 ml) was added 10% Pd–C (0.5 g) and H_2 passed for 60 hr. After usual work-up and purification on a Si gel column, colourless shining crystals of **6** (85 mg), mp 246–248°, M^+ at *m/z* 458.3780 (calc. for $C_{30}H_{50}O_3$, 458.3761) were recovered. IR ν_{max} cm^{-1}: 3495, 2500–3200 (*br*), 1690, 1360, 1380. ^1H NMR (60 MHz, DMSO-d_6): δ 0.69–1.10 (6 × tertiary Me's), 1.16 (3H, secondary Me), 2.60 (1H, *d*, $J = 5$ Hz, H-18), 2.63 (1H, *br s*), 2.80 (1H, *d*, $J = 16$ Hz), 4.20 (1H, *t*, $J = 6$, 8 Hz).

Acetylation of **1**. Compound **1** (80 mg) in C_5H_5N (1.8 ml) was refluxed with Ac_2O (3 ml) for 7 hr. After usual work-up the product **2** was purified on a Si gel column to give 60 mg of **2**, mp 258°, M^+ at *m/z* 498.0178 (calc. for $C_{32}H_{50}O_4$, 498.0138). IR ν_{max} cm^{-1}: 1730, 1680, 1460, 1365, 1360, 1245, 1020, 820. ^1H NMR (90 MHz; $CDCl_3$): δ 0.70–1.13 (6 ×tertiary Me's), 1.23 (3H, *s*, secondary Me), 2.80 (1H, *d*, *J* = 16 Hz, H-19), 2.03 (3H, *s*, $-OCOCH_3$), 2.60 (1H, *d*, *J* = 5.5 Hz, H-18), 4.43 (1H, *t*, *J* = 6, 9 Hz, H-3), 5.26 (1H, *br s*, H-12). MS *m/z*: 498 $[M]^+$, 438 $[M-HOAc]^+$, 423 $[M-HOAc-Me]^+$, 300, 250, 248 (100%), 205, 203, 189.

Sarett oxidation of **1**. The soln of **1** (0.1 g) in C_5H_5N (1.5 ml) was added to a well-stirred ice-cold suspension of CrO_3–C_5H_5N complex, prepared from 0.6 g CrO_3 and 6 ml C_5H_5N. The mixture was left overnight and after usual work-up colourless needles of **4** (0.83 g), mp 218°, M^+ at *m/z* 454.3440 (calc. for $C_{30}H_{46}O_3$, 454.3447) were recovered. IR ν_{max}^{nujol} cm^{-1}: 1680, 1700, 2500–3200 (*br*). ^1H NMR (60 MHz; $CDCl_3$): δ 0.69–1.04 (6 ×tertiary Me's), 1.16 (3H, *s*, secondary Me), 2.20 (2H, *m*, H-2), 2.60 (1H, *d*, *J* = 16 Hz), 5.16 (1H, *br s*, *J* = 6 Hz). MS *m/z*: 454 $[M]^+$, 439 $[M-Me]^+$, 396 $[M-CH_3-CO_2]^+$, 380, 355, 248 (100%), 206, 203, 189.

Methylation of **2**. Compound **2** (150 mg) in Et_2O was methylated with freshly prepared CH_2N_2 to give **5**. It was purified by crystallization from C_6H_6–petrol, mp 217°, M^+ at *m/z* 512.3850 (calc. for $C_{33}H_{52}O_4$, 512.3865). IR ν_{max} cm^{-1}: 1730, 1720, 1245, 1200, 1640, 820. ^1H NMR (60 MHz; $CDCl_3$): δ 0.63–1.10 (6 ×tertiary Me's), 1.16 (3H, *s*, secondary Me), 2.00 (3H, *s*, $-OCOCH_3$), 2.60 (1H, *d*, *J* = 5 Hz), 2.90 (1H, *br d*, *J* = 16.5 Hz), 3.40 (3H, *s*, $-COOCH_3$), 4.43 (1H, *t*, *J* = 6, 8 Hz), 5.23 (1H, *br s*). MS *m/z*: 512 $[M]^+$, 497 $[M-Me]^+$, 482 $[M-2\times Me]^+$, 438 $[M-2\times Me-CO_2]^+$, 420 $[M-2\times Me-CO_2-H_2O]^+$, 262 (100%), 250, 247, 232, 191.

Bromination of **2**. A soln of **2** (50 mg) in MeOH (8 ml) was treated with Br_2 (25 mg) in MeOH (3 ml). After 30 min the soln was cooled in an ice bath to give colourless needles of **7**, M^+ at *m/z* 576.2621; 578.2602 (calc. for $C_{32}H_{49}O_4Br$, 576.2621; 578.2662): mp 231°. IR ν_{max} cm^{-1}: 1735, 1715, 1360, 1365, 1150, 1175. ^1H NMR (60 MHz; $CDCl_3$): δ 0.70–1.06 (6 ×tertiary Me's), 1.16 (3H, *s*, secondary Me), 2.05 (3H, *s*, $OCOCH_3$), 2.55 (1H, *d*, *J* = 6 Hz), 2.85 (1H, *br d*, *J* = 16 Hz, H-19), 4.16 (1H, *t*, *J* = 4, 8 Hz, H-12), 4.55 (1H, *t*, *J* = 10, 5 Hz, H-3). MS *m/z*: 576, 578 $[M]^+$, 563, 561 $[M-Me]^+$, 519, 517 $[M-Me-HCHO]^+$, 501, 503 $[M-HOAc-Me]^+$, 473, 475 $[M-OAc-CO_2]^+$, 422 $[M-HOAc-Me-Br]^+$, 365, 347, 328, 313, 248 (100%); 231, 205, 285, 293.

The development of the column with C_6H_6–EtOAc (1:2) afforded a binary mixture which was separated by repeated CC to yield compound **3**, mp 220°, from Me_2CO–petrol. The compound shows M^+ at *m/z* 442.3820 (calc. for $C_{30}H_{50}O_2$, 442.3811). ^1H NMR (90 MHz, $CDCl_3$): δ 0.76 (6H, H-24, H-25), 0.83 (3H, H-28), 0.90 (6H, H-23, H-27), 0.97 (3H, H-26), 1.06 (3H, H-30), 2.25 (1H, *d*, H-18), 3.10 (1H, –OH), 4.50 (1H, –OH), 4.63 (1H, *m*, H-3), 5.20 (1H, *t*, H-12). MS *m/z*: 442, 427 $[M-Me]^+$, 411 $[M-CH_2OH]^+$, 393, 288, 234, 208, 207, 204, 203 (100%), 189.

Acetylation of compound **3**. Compound **3** (40 mg) in C_5H_5N (1 ml) was refluxed with Ac_2O (2 ml) for 9 hr. After usual work-up and separation by prep. TLC (Si gel G; C_6H_6–EtOAc, 9:1) compound **8** (15 mg) and compound **9** (21 mg) were recovered. IR ν_{max} cm^{-1}: (**8**) 3380, 1730, 1360, 1365, 1240, 820; (**9**) 1735, 1730, 1360, 1365, 1245, 1100, 820. MS *m/z*: (**8**) 484.3917 (calc. for $C_{32}H_{52}O_3$, 484.3916), 469 $[M-Me]^+$, 442 $[M-COCH_2]^+$, 425 $[M-OAc]^+$, 411 $[M-CH_2OAc]^+$, 276, 261, 250, 208, 207, 189; (**9**) 526.4023 (calc. for $C_{34}H_{54}O_4$, 526.4022), 511 $[M-Me]^+$, 453 $[M-CH_2OAc]^+$, 438 $[M-CH_2OAc-Me]^+$, 276, 250, 249, 248 (100%), 234, 216, 205, 203, 189.

Sarett oxidation of compound **3**. Compound **3** (30 mg) was oxidized like compound **1** to yield compound **4** (20 mg). The IR, ^1H NMR, mass spectra were found to be similar to that of compound **4**.

Partial synthesis of compound **3**. Acetylplectranthoic acid (**2**) (0.08 g), isolated from the same plant, was refluxed with $LiAlH_4$ (0.50 g) in dry Et_2O (80 ml) for 4 hr to give a colourless compound **1** (0.06 g), mp 220° (undepressed with the natural product).

The EtOAc eluant from a neutral Al_2O_3 column was a mixture of three components. On rechromatography over Si gel and development of the column which $CHCl_3$–C_6H_6 (8:2) colourless shining needles of compound **2**, were recovered, mp 258°, M^+ at *m/z* 498.0178 (calc. for $C_{32}H_{50}O_4$, 498.0188), $[\alpha]_D^{25}+58°$.

Hydrolysis of compound **2**. Compound **2** (100 mg) in Et_2O was treated with trace amounts of BF_3–etherate and left overnight. After removing the solvent in vacuum, a granular substance was obtained, it was purified by crystallization from MeOH–$CHCl_3$. The compound melted at 296° and showed identical ^1H NMR and mass spectra to those of compound **1**.

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