A New Eudesmenolide and 2-Hydroxycostic Acid from *Sphaeranthus indicus* Linn. *X*-Ray Molecular Structure of 4α,5α-Epoxy-7α-hydroxyeudesmanolide

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A new sesquiterpene lactone, 7α-hydroxyeudesm-4-en-6,12-olide (**1**), and a new sesquiterpene acid, 2-hydroxycostic acid (**2**), along with the known compounds β-eudesmol (**3**) and ilicic acid (**4**) have been isolated from the acetone extract of *Sphaeranthus indicus* L. The structure and relative stereochemistry of compound (**1**) has been established by a combination of chemical and spectral data including the *X*-ray crystallographic studies of its derivative (**9**). The structure of compound (**2**) has been deduced mainly by spectral studies.

Sphaeranthus indicus is a medicinally important plant found abundantly growing as a weed in the rice fields all over India.[1] From this plant, some volatile compounds,[2,3] cadinene,[4] aromatic compounds,[4] and an alkaloid[5] have been reported. We have now isolated from the less polar fraction of the acetone extract a new sesquiterpene lactone (**1**) and β-eudesmol (**3**), and from the more polar fraction 2-hydroxycostic acid (**2**) along with the known ilicic acid (**4**).[6]

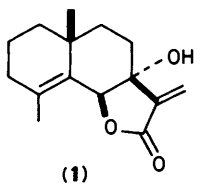
Results

Compound (**1**) was obtained as a viscous oil, molecular formula $C_{15}H_{20}O_3$ (M^+, 248), and showed characteristic bands in its i.r. spectrum (3 400, 1 760 cm^{-1}) and signals in its ^1H n.m.r. spectrum (δ 5.75 and 6.31) which revealed the presence of an α-methylene-γ-lactone and a hydroxy group. In support of this, the above signals were absent in the ^1H n.m.r. spectra of its piperidine (**5**) and dihydropyrazole (**6**) derivatives. The absence of a signal assignable to a primary or a secondary hydroxy group in the ^1H n.m.r. spectrum of (**1**) and its failure to undergo acetylation and oxidation suggested the tertiary nature of the hydroxy group. The presence of an angular methyl group and a methyl on the double bond was evident from the two singlets at δ 1.04 and 1.73 respectively. As the ^1H n.m.r. spectrum did not exhibit any signal assignable to an olefinic proton it was inferred that the double bond is tetrasubstituted, which was supported by the singlets at δ_C 145.25 and 140.50 in the ^{13}C n.m.r. spectrum of (**1**). All the above data suggested compound (**1**) to be a sesquiterpene lactone with a tertiary hydroxy group and a tetrasubstituted double bond.

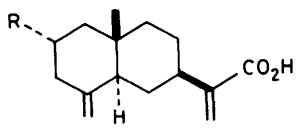
The striking feature in the ^1H n.m.r. spectrum of (**1**) was the appearance of the signals due to *exo*-methylene protons (13-H_a and 13-H_b) as two singlets which clearly revealed the absence of a proton at C-7.[7] In addition, the lactonic proton (6-H) also appeared as a singlet, at δ 5.0, inferring the quaternary nature of C-5. This enabled us to place the hydroxy group at C-7 and the double bond at C-4–C-5. Further confirmation of the double bond at C-4 was provided by the upfield shift of the C-4 methyl in the tetrahydro derivative (**8**) and in the 4,5-epoxide (**9**), and of the lactonic proton (6-H) in (**9**).

The presence of a 12,6-lactone was supported by the ^1H n.m.r. spectrum of the 11,13-dihydro derivative (**7**), which showed a little broadening of a signal due to 6-H (*J* 0.8 Hz) characteristic of 11,13-dihydroeudesmen-6,12-olide.[7] All the above data along with the ^{13}C n.m.r. data (Table 1) indicated that compound (**1**) is 7-hydroxyeudesm-4-en-6,12-olide.

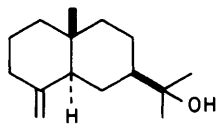
Biogenetically the C-10 methyl and C-7–C-11 bond are β-oriented and therefore the hydroxy group at C-7 must be α-oriented. The c.d. studies to establish the stereochemistry of



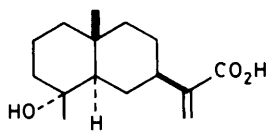
(**1**)



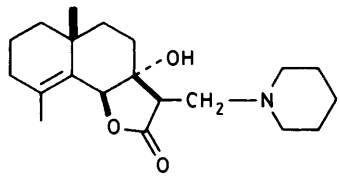
(**2**) R = OH
(**11**) R = OAc
(**12**) R = H
(**13**) R = OH; $\Delta^{3,4}$ (instead of $\Delta^{4,14}$)



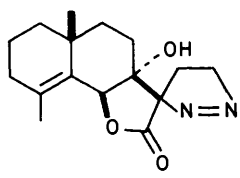
(**3**)



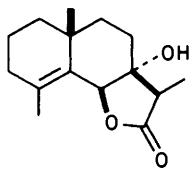
(**4**)



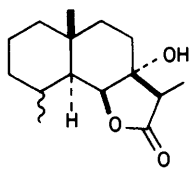
(**5**)



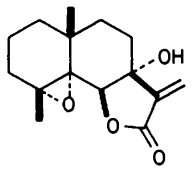
(**6**)



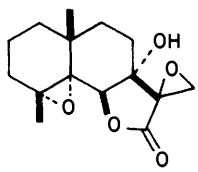
(**7**)



(**8**)



(**9**)



(**10**)

Table 1. ^{13}C N.m.r. data of compounds (**1**) and (**4**)

Carbon	Compound (**1**)	Compound (**4**)
C-1	32.885 (t)[a]	27.49 (t)[a]
C-2	31.780 (t)[a]	20.34 (t)
C-3	38.99 (t)[b]	41.26 (t)
C-4	145.25 (s)	72.78 (s)
C-5	140.50 (s)	40.42 (d)
C-6	81.75 (d)	44.77 (t)[b]
C-7	76.10 (s)	55.24 (d)
C-8	35.09 (t)[b]	43.60 (t)[b]
C-9	26.32 (t)[a]	26.90 (t)[a]
C-10	33.27 (s)	34.88 (s)
C-11	127.31 (s)	145.77 (s)
C-12	169.75 (s)	171.83 (s)
C-13	121.27 (t)	124.39 (t)
C-15	18.39 (q)	18.91 (q)
C-14	19.49 (q)	22.68 (q)

[a,b] Assignments interchangeable within a column.

Table 2. Atomic co-ordinates ($\times 10^4$) for non-hydrogen atoms with e.s.d.s in parentheses

	x	*y*	*z*
C(1)	12 368(5)	4 105(6)	6 061(3)
C(2)	12 094(10)	5 440(11)	5 565(4)
C(3)	10 411(11)	6 362(9)	5 605(3)
C(4)	9 014(11)	5 625(8)	6 002(3)
C(5)	9 246(7)	3 938(7)	6 281(3)
C(6)	7 696(7)	2 991(8)	6 519(3)
C(7)	8 027(8)	1 524(8)	6 965(3)
C(8)	9 649(7)	537(8)	6 800(3)
C(9)	11 165(8)	1 686(9)	6 675(3)
C(10)	10 838(8)	2 904(8)	6 135(3)
C(11)	6 454(5)	483(5)	6 846(2)
C(12)	5 921(4)	828(4)	6 213(1)
C(13)	5 598(5)	−560(6)	7 208(2)
C(15)	10 549(4)	1 911(5)	5 536(1)
C(14)	7 258(5)	6 344(4)	5 891(2)
O(1)	9 507(5)	5 458(5)	6 645(2)
O(2)	6 763(5)	2 231(5)	6 013(2)
O(3)	8 135(6)	2 201(5)	7 564(2)
O(4)	4 916(6)	97(6)	5 890(2)

the lactone ring were inconclusive and hence it was decided to establish the relative stereochemistry by *X*-ray crystallography. As compound (**1**) was not obtained in crystalline form its derivative (**9**) was used for *X*-ray studies.

Crystallographic Structure Determination of the Epoxide (**9**).—$C_{15}H_{20}O_4$, M = 264.3, orthorhombic, space group $P2_12_12_1$ (D_2^4 No. 19), a = 7.812(1), b = 7.893(1), c = 21.840(3) Å, V = 1 346.7 Å^3, D_c = 1.304 g cm^{-3}, Z = 4, F(000) = 568.0. Monochromatic Mo-K_α radiation, λ = 0.7107 Å, μ = 1.007 cm^{-1}. Crystal dimensions were 0.5 × 0.25 × 0.6 mm.

Structure determination. A unique data set was measured within the limit $2\theta_{max.}$ = 47° using a CAD4F-11M four-circle diffractometer in the conventional $\omega/2\theta$ scan. 1 196 Independent reflections were obtained, 1 008 of which with $I > 3\sigma(I)$ were considered 'observed' and were used in the least-squares refinement without absorption correction. The structure was solved by direct methods using MULTAN-78.[8] A full-matrix least-squares refinement was used with anisotropic temperature factors for the non-hydrogen atoms. The hydrogen atoms were located on the basis of stereochemical considerations and were included in the refinement[9] with temperature factors held fixed. The final R value at convergence was 0.034.

A Cruickshank-type[10] weighting scheme was employed with a = 2.0, b = 1.0, and c = 0.025. Tables of thermal parameters and hydrogen parameters have been deposited at the Cambridge Crystallographic Data Centre.* Atomic scattering factors were taken from International Tables for *X*-ray crystallography.[11] The *X*-ray molecular structure is shown in the Figure.

Table 3. Bond distances (Å) and bond angles (°) with e.s.d.s in parentheses

Bond distance			
C(1)–C(2)	1.526(10)	C(6)–O(2)	1.454(8)
C(1)–C(10)	1.534(8)	C(7)–C(8)	1.530(9)
C(2)–C(3)	1.505(12)	C(7)–C(11)	1.501(7)
C(3)–C(4)	1.510(11)	C(7)–O(3)	1.416(8)
C(4)–C(5)	1.476(9)	C(8)–C(9)	1.517(9)
C(4)–C(14)	1.505(9)	C(9)–C(10)	1.542(9)
C(4)–O(1)	1.461(8)	C(10)–C(15)	1.543(7)
C(5)–C(6)	1.515(8)	C(11)–C(12)	1.469(5)
C(5)–C(10)	1.521(8)	C(11)–C(13)	1.322(6)
C(5)–O(1)	1.453(7)	C(12)–O(2)	1.361(5)
C(6)–C(7)	1.535(9)	C(12)–O(4)	1.204(5)
Bond angle			
C(2)–C(1)–C(10)	113.1(5)	C(6)–C(7)–O(3)	108.2(5)
C(1)–C(2)–C(3)	114.5(6)	C(8)–C(7)–C(11)	111.0(5)
C(2)–C(3)–C(4)	118.5(6)	C(8)–C(7)–O(3)	111.2(5)
C(3)–C(4)–C(5)	119.7(6)	C(11)–C(7)–O(3)	114.5(5)
C(3)–C(4)–C(14)	114.9(6)	C(7)–C(8)–C(9)	112.6(5)
C(3)–C(4)–O(1)	113.3(6)	C(8)–C(9)–C(10)	112.4(5)
C(5)–C(4)–C(14)	121.3(6)	C(1)–C(10)–C(5)	109.2(5)
C(5)–C(4)–O(1)	59.3(4)	C(1)–C(10)–C(9)	109.7(5)
C(14)–C(4)–O(1)	115.4(5)	C(1)–C(10)–C(15)	109.7(4)
C(4)–C(5)–C(6)	119.2(5)	C(5)–C(10)–C(9)	108.1(5)
C(4)–C(5)–C(10)	119.9(5)	C(5)–C(10)–C(15)	109.3(5)
C(4)–C(5)–O(1)	59.8(4)	C(9)–C(10)–C(15)	110.8(5)
C(6)–C(5)–C(10)	117.4(5)	C(7)–C(11)–C(12)	107.0(4)
C(6)–C(5)–O(1)	109.4(5)	C(7)–C(11)–C(13)	130.7(4)
C(10)–C(5)–O(1)	116.3(5)	C(12)–C(11)–C(13)	122.2(4)
C(5)–C(6)–C(7)	117.0(5)	C(11)–C(12)–O(2)	108.5(3)
C(5)–C(6)–O(2)	110.1(5)	C(11)–C(12)–O(4)	130.4(4)
C(7)–C(6)–O(2)	104.8(5)	O(2)–C(12)–O(4)	121.1(4)
C(6)–C(7)–C(8)	112.0(5)	C(4)–O(1)–C(5)	60.9(4)
C(6)–C(7)–C(11)	99.5(5)	C(6)–O(2)–C(12)	109.4(4)

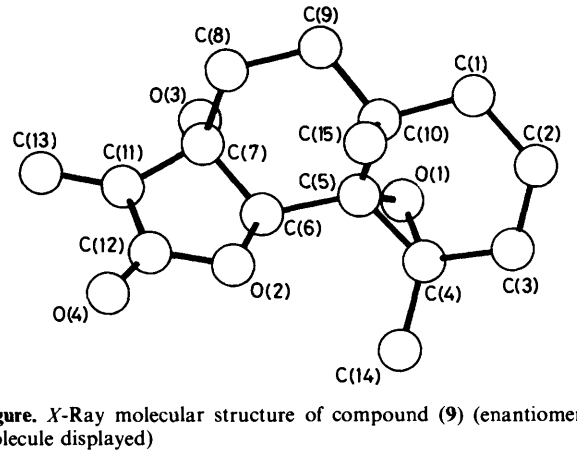


Figure. *X*-Ray molecular structure of compound (**9**) (enantiomer of molecule displayed)

Discussion

The atomic co-ordinates for non-hydrogen atoms are given in Table 2. The bond lengths and angles are given in Table 3. The

* See section 5.6.3 of Instructions for Authors, in the January issue.

bond lengths are normal and similar to those in podolide.[12] The mean C(sp^3)–C(sp^3) bond distance is 1.525(5) Å while the mean C(sp^3)–C(sp^2) bond length is 1.501(5) Å. The bonds C(6)–O(2) bond is of the C(sp^2)–O type and its value is 1.454(8) Å, the corresponding value in podolide being 1.467(3) Å. The bond length of the C(sp^2)–O and C(12)–O(2) is 1.361(5) Å, the corresponding value in podolide being 1.359(3) Å. This suggests resonance forms of the type O^+=C–O^- in the lactone. The epoxide ring produces a pseudo-olefinic effect with a significant contraction in the length of the C(4)–C(5) bond [1.467(9) Å] very near to the value of 1.468(3) Å in podolide, while the flanking bonds are longer with values of C(3)–C(4) 1.510(11) and C(5)–C(10) 1.521(8) Å. The endocyclic (ring A) valence angles at C(4) and C(5) are 119.7(6)° and 119.9(5)° considerably more than the tetrahedral value of 109°. This shows the enormous strain generated in the ring due to the epoxide function.

Most intermolecular contacts between non-hydrogen atoms are of the van der Waals type. The molecules are held together in the crystal by O–H • • • O-type hydrogen bonds between O(1) (x, y, z) and O(3) $1 - x, \frac{1}{2} + y, z$) of length 2.877 Å.

Ring A has a 1,2-diplanar sofa conformation while ring B has a chair conformation. The γ-lactone ring is a C(7) envelope. The three bonds of the epoxide ring have lengths which are fairly close to those in inuolide.[13]

From all the *X*-ray crystallographic data the *cis* relationship of the C-10 methyl, the C-7–C-11 bond, and the C-6–O bond is quite evident. The perspective view of the molecule (Figure) depicts the antipodal structure of (**9**).

2-Hydroxycostic Acid (**2**).—This was isolated as a crystalline solid, m.p. 187—189 °C, molecular formula $C_{15}H_{22}O_3$ (M^+, 250) from the more polar fraction of the acetone extract. The i.r. and ^{1}H n.m.r. spectra suggested it to be a hydroxy acid. The presence of a secondary OH group was supported by the conversion of compound (**2**) into its monoacetate (**11**) and as expected the signal assignable to C*H*OH at δ_H 3.98 in the ^{1}H n.m.r. spectrum of (**2**) moved downfield to δ_H 5.02 in that of compound (**11**). A critical comparison of the ^{1}H n.m.r. spectrum of compound (**2**) with those of costic acid (**12**), 2α-hydroxyisocostic acid (**13**),[14] and ivaline[15] revealed that compound (**2**) is 2α-hydroxycostic acid. The following spin-decoupling results were in support of the structure proposed.

Irradiation of the four-fold doublet centred at δ_H 3.98 2-H affected the multiplicity of two two-proton multiplets centred at δ_H 2.75 (3-H_2) and 2.15 (1-H_2). Furthermore, irradiation at δ_H 4.51 (br s, 15-H_a) sharpened the multiplet at δ_H 2.75, indicating its allylic nature and hence the signal at δ_H 2.75 must be assigned to 3-H_2.

Experimental

Optical rotations were taken for solutions in chloroform. I.r. spectra were measured for Nujol mulls, and ^{1}H and ^{13}C n.m.r. spectra for solutions in $CDCl_3$ with $SiMe_4$ as internal standard. Mass spectra were determined at 70 eV using a direct inlet system.

Extraction of S. indicus.—The whole plant collected near Lonavala, Maharashtra, India during November 1980 was shade-dried and powdered, and the powder (3 kg) was extracted with acetone to give an extract (150 g, 5%).

Isolation of Compound (**1**).—The acetone extract (100 g) was fractionated over t.l.c.-grade silica gel and eight broad fractions were collected with acetone–light petroleum as eluant. The fifth fraction on repeated chromatography yielded compound (**1**) as a yellowish oil (8.5 g), $[\alpha]_D$ −56.81° (*c* 0.43); $\nu_{max.}$ 3 400, 1 760, and 1 650 cm^{-1}; δ_H(90 MHz) 1.04 (3 H, s), 1.73 (3 H, s), 5.04 (1 H, s), 5.75 (1 H, s), and 6.31 (1 H, s); *m/z* 248 (M^+, 20%), 233 (18), 215 (43), 204 (13), and 117 (100).

2α-Hydroxycostic Acid (**2**).—The seventh fraction, after repeated column chromatography [acetone–light petroleum (2:3) as eluant] followed by preparative t.l.c. [acetone–light petroleum (2:3) as developer], gave compound (**2**) as a crystalline solid (0.06g), m.p. 187—189 °C; $\nu_{max.}$ 3 350, 2 700, 1 690, 1 630, and 890 cm^{-1}; δ_H(100 MHz; C_5D_5N) 0.80 (3 H, s), 2.15 (2 H, m), 2.75 (2 H, m), 3.98 (1 H, m) 4.51 (1 H, br s), 4.80 (1 H, br s), 5.57 (1 H, s), and 6.30 (1 H, s); *m/z* 250 (M^+, 18%), 232 (100), 217 (95), 191 (72), 171 (65), 145 (70), 119 (72), 105 (52), 91 (55), and 69 (40).

β-Eudesmol (**3**).—This was obtained from the fourth fraction as a viscous liquid (0.2 g). Comparison of its spectral data (i.r., ^{1}H n.m.r., and mass) with those of an authentic sample[16] established its identity.

Ilicic Acid (**4**).—Obtained as a crystalline solid (1.5 g), from the seventh fraction, m.p. 177—179 °C; $[\alpha]_D$ −32.8° (*c* 0.05), it was identified by comparison of its physical constants with those reported.[6]

Piperidine Derivative of (**1**).—To a solution of compound (**1**) (25 mg) in ethanol (5 ml) was added piperidine (2.5 ml) at room temperature, and the mixture was kept at room temperature for 12 h. Excess of piperidine and ethanol was removed under reduced pressure to afford compound (**5**) as a viscous oil (2.5 mg), δ_H(60 MHz) 1.06 (3 H, s), 1.8 (3 H, s), 3.1 (3 H, d), and 5.03 (1 H, s).

Dihydropyrazole Derivative of Compound (**1**).—To a solution of compound (**1**) (100 mg) in dry methanol (1 ml) was added excess of an ethereal solution of diazomethane and the mixture was kept at room temperature for 12 h. The solvent was removed to yield the dihydropyrazole derivative (**6**) as a yellowish crystalline solid (105 mg), m.p. 145—147 °C (decomp.); $\nu_{max.}$ 3 540 and 1 750 cm^{-1}; δ_H(60 MHz) 1.06 (3 H, s), 1.83 (3 H, s), 2.1 (m), 4.51 (2 H, dd, *J* 10 Hz), and 5.83 (1 H, s).

Dihydro Derivative of Compound (**1**).—Compound (**1**) (100 mg) was hydrogenated using $Pd(OH)_2/CaCO_3$ as catalyst to give, after the usual work-up, the dihydro derivative (**7**) as a crystalline solid (95 mg), m.p. 143—145 °C; $\nu_{max.}$ 3 300, 1 760, and 1 650 cm^{-1}; δ_H(90 MHz), 1.04 (3 H, s), 1.17 (3 H, d, *J* 6 Hz), 1.8 (3 H, s), and 4.95 (1 H, d, *J* 0.8 Hz); *m/z* 250 (M^+, 10%), 235 (50), 217 (25), 189 (20), 149 (50), 104 (40), and 44 (100).

Tetrahydro Derivative.—Compound (**1**) (100 mg) was hydrogenated using Pd/C (10%) in ethanol to give the tetrahydro derivative (**8**) as a viscous oil (94 mg); $\nu_{max.}$ 3 500 and 1 765 cm^{-1}; δ_H(60 MHz) 0.84 (3 H, d), 0.88 (3 H, s), and 1.13 (3 H, d).

Epoxide of Compound (**1**).—To a solution of compound (**1**) (450 mg) in chloroform (25 ml) was added a solution of *m*-chloroperbenzoic acid (1.2 g) in chloroform (10 ml) and the mixture was kept at 0 °C for 48 h. The usual work-up followed by chromatography gave the epoxide (**9**) as the major compound (220 mg), m.p. 155—157 °C; $\nu_{max.}$ 3 300 and 1 750 cm^{-1}; δ_H(90 MHz) 1.12 (3 H, s), 1.33 (3 H, s), 3.93 (1 H, s), 5.84 (1 H, s), and 6.25 (1 H, s); *m/z* 264 (M^+, 5%), 246 (5), 231 (5), and 221 (100).

Diepoxide of Compound (**1**).—The minor compound obtained during chromatography of the epoxidation product of compound (**1**) was further purified by preparative t.l.c. to afford

the diepoxide (**10**) as a viscous oil (100 mg); $\nu_{max.}$ 3 300 and 1 795 cm^{-1}; δ_H(90 MHz) 1.1 (3 H, s), 1.38 (3 H, s), 3.07 (1 H, d, *J* 5 Hz), 3.45 (1 H, d, *J* 5 Hz), and 4.12 (1 H, br s); *m/z* 280 (M^+, 20%), 237 (100), 195 (55), 149 (60), and 123 (90).

Acetylation of 2α-Hydroxycostic Acid (**2**).—Compound (**2**) (25 mg) was acetylated using pyridine and acetic anhydride and the usual work-up gave the acetate (**11**) as a crystalline solid, m.p. 145—146 °C; $[\alpha]_D$ +12° (*c* 0.15); $\nu_{max.}$ 1 750, 1 700, 1 650, and 1 240 cm^{-1}; δ_H(90 MHz) 0.80 (3 H, s), 2.04 (3 H, s), 4.57 (1 H, s), 5.02 (1 H, m), 5.73 (1 H, s), and 6.37 (1 H, s); *m/z* 232 (M^+ − 60, 96%), 217 (22), 171 (50), 145 (55), 119 (98), 105 (87), 91 (100), 79 (82), and 69 (65).

Acknowledgements

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