Ceriopsin E, a New Epoxy *ent*-Kaurene Diterpenoid from *Ceriops decandra*[1]

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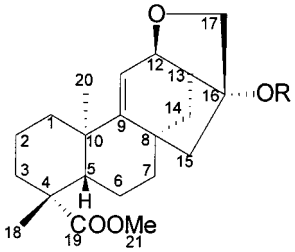
A new epoxy *ent*-kaurene diterpenoid, ceriopsin E (**1**), has been isolated from the mangrove plant *Ceriops decandra* and its structure elucidated by spectral data and single-crystal X-ray analysis.

In our continuing interest in the chemical constituents of Indian mangrove plants,[2−6] we have recently examined the plant *Ceriops decandra* (Griff.) Ding Hua (family Rhizophoraceae) collected from the Kauvery Estuary and reported the isolation of four new diterpenoids, ceriopsins A−D, from the ethyl acetate solubles of the MeOH−CH_2Cl_2 (1:1) extract of the roots.[7] We now report from the same extract a new epoxy *ent*-kaurene diterpenoid, ceriopsin E, as a minor constituent, and its structure has been elucidated as methyl *ent*-12,17-epoxy-16β-hydroxy-9(11)-kauren-19-oate (**1**) through spectral and X-ray analysis.

Ceriopsin E, colorless needles from *n*-hexane−EtOAc, mp 133−142 °C, analyzed for $C_{21}H_{30}O_4$. Its molecular ion could not be observed either in the EI or FAB (+) mass spectrum. However, its monoacetate $C_{23}H_{32}O_5$, mp 110−112 °C, gave a molecular ion at *m*/*z* 389 $[M + H]^+$ and a significant fragment peak at *m*/*z* 329 $[M + H - 60]^+$ in the positive-ion FABMS. Its IR spectrum showed hydroxyl (3420 cm^{-1} br), carbonyl (1700 cm^{-1}), trisubstituted double bond (910 cm^{-1}), and ether (1140 cm^{-1}) absorptions, with no conjugation observed in the UV spectrum.

The ^1H and ^{13}C NMR spectral data of **1** were suggestive of a kaurane diterpenoid. The ^1H NMR spectrum (Table 1) showed a carbomethoxyl, two tertiary methyl groups, two oxymethylene protons, one oxymethine proton, and one trisubstituted olefinic proton. Its ^{13}C NMR spectrum showed a signal at δ 177.7, accounting for a carbomethoxyl, and three oxygenated carbons at δ 89.7 (s), 80.1 (d), and 79.4 (t). The ^1H NMR spectrum of the monoacetate remained almost the same as that of the parent compound except for the presence of an acetate methyl group signal (δ 2.02). This suggests that the oxymethylene protons and oxymethine protons do not have free hydroxyls but are ether linkages and that the acetate is formed at a tertiary hydroxyl, which is unusual within this compound class. In support of this, the carbon (C-16, δ 89.7) connected to tertiary hydroxyl appeared deshielded at δ 95.8 (s) in the ^{13}C NMR spectrum of the acetate (**1a**). The olefinic carbons of the trisubstituted double bond observed at δ 114.3 (d) and 162.2 (s) were typical for 9(11)-kaurenes.[8] Ceriopsin E (**1**), requiring seven double-bond equivalents, should be pentacyclic with only two degrees of unsaturation contained in one double bond and a methyl ester.

Biogenetically, it might be expected that the 17-hydroxymethyl group of 16,17-dihydroxy kauranoid might form a four-membered ether with C-13 or a five-membered ether with C-12 or C-14. From the appearance of the olefinic proton H-11 as a doublet and the chemical shifts of the oxygenated carbons C-12 and C-17 being closer to the values of α-carbons of a tetrahydrofuran than those of an oxetane,[9] ceriopsin E (**1**) was formulated as a 17,12-epoxy derivative. The carbomethoxyl (C-19) was located at C-4 by comparing the chemical shifts of the A-ring carbons and C-18 with those of similar compounds.[8] The structure of ceriopsin E (**1**) could thus be derived as methyl 12,17-epoxy-16-hydroxy-9(11)-kauren-19-oate.



1 R = H
1a R = Ac

The location of the functional groups was supported by the HMBC data (Table 1), the important correlations being the correlations between C-12 (δ 80.1) and H_2-17, H-13, H_2-14, and H-11; between C-17 (δ 79.4) and H-12, H_2-14, and H_2-15; between C-11 (δ 114.3) and H-12 and H-13, and between C-19 (δ 177.7) and H_3-18, H-5, H_2-3 and H_3-21. The relative stereochemistry was also arrived at by the important NOESY correlations (Table 1) between H-5 and H-1 and H-3, along with those between H_3-20 and H-14 and H-2, as observed in other kaurenoids. In particular, the correlations between H-15 and H-17 and H-11 revealed the cis orientation of the five-membered cyclopentane and tetrahydrofuran rings. The X-ray analysis of ceriopsin E (**1**) was undertaken, and the X-ray diagram (Figure 1) fully supported the structure and relative stereochemistry proposed from spectral analysis. The absolute configuration of ceriopsin E (**1**) was not established, but was taken to be an *ent*-derivative in view of its positive specific rotation as for *ent*-16,17-dihydroxy-9(11)-kauren-19-oic acid[10] with positive rotation and also based on the observation that most known kauranoids are *ent*-derivatives.[11] Several epoxykauranes have been reported in nature, with, for example, linkages between C-20 and C-7,[12] C-20 and C-3,[12] and C-16 and C-11.[13] Ceriopsin E is the first member of the C-17,C-12-epoxy kauranes, although 16,17-epoxykauranes have been reported.[10]

Experimental Section

General Experimental Procedures. Melting points were determined on a VEB-Analytic Dreader HMK hot plate and

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Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) Assignments and HMBC, NOESY, and COSY Correlations of Ceriopsin E (**1**)[a]

carbon	^{1}H (δ)	^{13}C (δ)	HMBC	NOESY	COSY
C-1	1.22 m 1.92 m	40.5	H_3-20, H-5, H_2-3, H_2-2	H_3-20	H_a-2
C-2	1.50 m 1.83 m	20.0	H_2-1, H_2-3	H_3-20	H_a-1, H_a-3
C-3	1.02 m 2.14 m	38.1	H_2-2, H_2-1, H-5, H_3-18		H_2-2, H_a-3
C-4		44.8	H_2-6, H_2-2, H_2-3, H_3-18, H-5		
C-5	1.52 m	46.0	H_3-18, H_3-20	H_a-1, H_a-3	
C-6	1.82 m 2.46 m	18.5	H_2-7, H-5	H_3-20 H_3-18	H_2-6, H-5
C-7	1.50 m 2.00 m	29.6	H_2-6, H_2-15, H_2-14	H_a-14	H_a-7
C-8		43.5	H-11, H_2-15, H_2-7, H-13		
C-9		162.2	H-11, H-12, H_2-15, H_2-14, H_2-7, H_2-1, H_3-20		
C-10		38.7	H-11, H-5, H_2-2		
C-11	5.18 (1H, d, J = 3.6)	114.3	H-12, H-13	H_b-1, H-12, H_a-15	H-12
C-12	4.76 (1H, dd, J = 6.6, 3.6)	80.1	H-11, H_2-17, H-13, H_2-14		H-13
C-13	2.48 m	53.1	H-11, H-12, H_2-15, H_2-14	H_a-14, H_a-6, H-12	H_2-14
C-14	1.48 m 1.75 m	42.3	H_2-15, H_2-7	H_3-20	H_a-14
C-15	1.70 m 2.20 m	55.4	H_2-17, H-13, H_2-7, H_2-14	H_b-14	H_a-15
C-16		89.7	H-12, H_2-17, H-13, H_2-15, H_2-14		
C-17	3.77 (1H, d, J = 9.9) 3.89 (1H, d, J = 9.9)	79.4	H-12, H_2-15	H_b-15 H_a-15	H_a-17
C-18	1.18 (3H, s)	27.9	H-5, H_2-3		
C-19		177.7	H_3-21, H-5, H_3-18, H_2-3		
C-20	0.96 (3H, s)	23.3	H-5, H_2-1		
C-21	3.66 (3H, s)	51.3			

[a] Chemical shifts in δ from TMS (multiplicity, J in Hz) in $CDCl_3$.

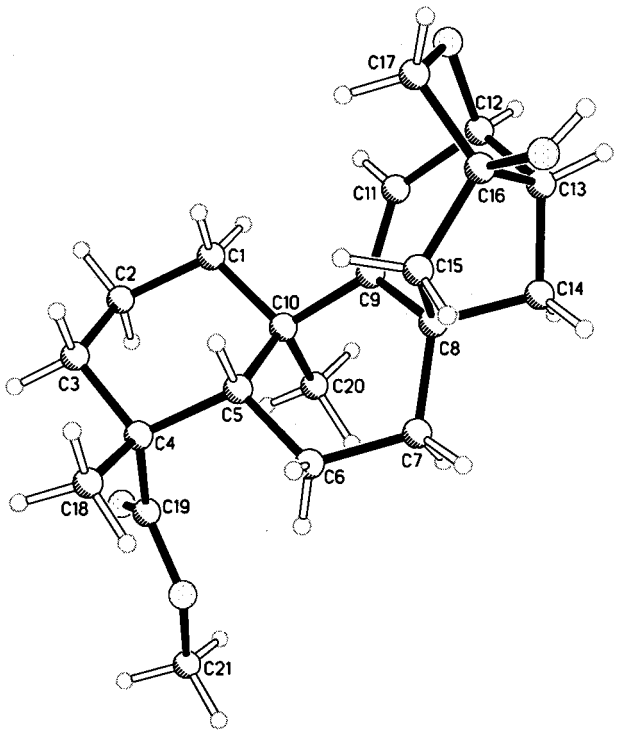

Figure 1. X-ray structure of compound **1**.

are uncorrected. Optical rotations were determined on a Rudolph Autopol-III polarimeter. IR spectra were recorded on a Perkin-Elmer-841 IR spectrometer in $CHCl_3$ solution. UV spectra were recorded on a Milton Roy Spectronic 1201 spectrometer in $CHCl_3$. ^{1}H NMR spectra were measured on a Bruker Advance DRX 300 and a JEOL JNM EX-90 spectrometer. ^{13}C NMR spectra were measured on a Bruker Advance DRX 300 spectrometer at 75 MHz and JEOL JNM EX-90 spectrometer at 22.5 MHz using $CDCl_3$ as a solvent and tetramethylsilane as an internal reference. Mass spectra were obtained on a JEOL JMS-300 spectrometer. Elemental analysis was performed on a Carlo Erba 1108 instrument.

Plant Material. The roots of *Ceriops decandra* were collected from the Parangipattai coast (11° 07′ N, 79° 50′ E), Kauvery Estuary, India, in March 1999. The plant material was identified by Prof. B. Kondala Rao, Department of Marine Living Sources, Andhra University, Visakhapatnam. Voucher specimens (code: AU1/182) were deposited at the Marine Museums of the School of Chemistry, Andhra University, and the National Institute of Oceanography, Goa, India.

Extraction and Isolation. The air-dried and powdered plant material (3.0 kg) was exhaustively extracted with CH_2Cl_2−MeOH (1:1). Removal of the solvent from the combined CH_2Cl_2−MeOH extracts gave a residue (60 g), which was extracted with EtOAc (5 × 600 mL). Removal of the solvent from the EtOAc extract under reduced pressure gave a residue (35 g). This residue was subjected to column chromatography over silica gel (Acme brand, 100−200 mesh, 350 g) using solvents of increasing polarity from *n*-hexane through EtOAc. In all, 240 fractions (800 mL) were collected. The fractions showing similar spots were combined and subjected to chromatography over silica gel or silver nitrate (20%)-impregnated silica gel columns to yield ceriopsins A−D[7] and now ceriopsin E (**1**) (40 mg) from the column fractions 160−166 (*n*-hexane−EtOAc, 6.75:2.25).

Methyl *ent*-12,17-epoxy-16*β*-hydroxy-9(11)-kauren-19-oate (ceriopsin E) (1): colorless needles (*n*-hexane−EtOAc); mp 133−142 °C; $[\alpha]_D^{25}$ −59.2° (*c* 1.0, $CHCl_3$); IR (Nujol) ν_{max} 3420, 1700, 1140, 910 cm^{-1}; UV transperent; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; *anal.* C 72.63%, H 8.49%; calcd for $C_{21}H_{30}O_4$, C 72.83%, H 8.67%.

Acetylation of Ceriopsin E (1). Ceriopsin E (**1**) (25 mg) was acetylated with a mixture of acetic anhydride (1.5 mL) and pyridine (1.5 mL) at room temperature for 24 h. After the usual workup, it yielded a monoacetyl derivative (**1a**) (22 mg): colorless oil; $[\alpha]_D^{25}$ −41.3° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.98 (3H, s, H_3-20), 1.18 (3H, s, H_3-18), 2.02 (3H, s, acetate methyl), 3.63 (3H, s, CO*OMe*), 4.0 (2H, brs, H_2-17), 4.78 (1H, dd, J = 6.0, 4.0 Hz, H-12), 5.25 (1H, d, J = 4.0 Hz, H-11); ^{13}C NMR ($CDCl_3$, 22.4 MHz) δ 40.6 (C-1), 20.1 (C-2), 38.2 (C-3), 44.9 (C-4), 45.8 (C-5), 18.4 (C-6), 29.6 (C-7), 43.4

(C-8), 162.1 (C-9), 38.8 (C-10), 114.8 (C-11), 78.7 (C-12), 50.8 (C-13), 42.1 (C-14), 55.0 (C-15), 95.8 (C-16), 77.3 (C-17), 27.9 (C-18), 177.6 (C-19), 23.4 (C-20), 171.2 (*OCO*CH3), 51.2 (CO*OMe*), 21.2 (OCO*CH*$_3$); FABMS (+) *m*/*z* 389 $[M + H]^+$ (88), 345 (16), 329 (100), 307 (22), 289 (22).

Single-Crystal X-ray Structure Analysis of 1.[14] Data were acquired with a Bruker SMART diffractometer with Mo Ka radiation ($\lambda = 0.71073$ Å) and a graphite monochromator, $C_{21}H_{30}O_4$ (346.45), crystal size 0.50 × 0.40 × 0.06 mm^3, monoclinic, space group $P2_1$, 173(2) K, $a = 8.1450(5)$ Å, $b = 20.7221(14)$ Å, $c = 21.9174(15)$ Å, $\beta = 100.168(2)°$, $V = 3641.2(4)$ Å^3, $D_c = 1.264$ Mg/m^3, $Z = 8$, $F(000) = 1504$, $\mu = 0.086$ mm^{-1}. A total of 23 966 reflections were collected in the range $0.94° < \theta < 26.37°$, independent reflections 14 024 [R(int) = 0.0358], completeness to $\theta = 26.37°$, 99.9%; absorption correction, SADABS; max. and min. transmission, 0.9949 and 0.838063, refinement method (Sheldrik's SHELX-9), full-matrix least-squares on F^2, data/restraints/parameters, 14024/1/1337; goodness-of-fit on F^2, 0.998; final R indices [$I > 2\sigma(I)$], $R_1 = 0.0446$, $wR_2 = 0.0817$; R indices (all data), $R_1 = 0.0701$, $wR_2 = 0.0905$, absolute structure parameter, −0.1(6); largest difference peak and hole, 0.174 and −0.196 e/Å^3.

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(14) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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