Cytotoxic Sesquiterpene Lactones of *Eremanthus incanus* and *Heterocoma albida*. Crystal Structure and Stereochemistry of Eregoyazin[1]

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Isolation of the sesquiterpene lactones eremanthin, eregoyazin, eregoyazidin, eremantholide A, and eremantholide B from *Eremanthus incanus* Less. and eremanthin from *Heterocoma albida* DC is reported. The structure of eregoyazin was clarified by X-ray crystallography as **2a** and the stereochemistry of eregoyazidin is discussed.

Extracts of various *Eremanthus* species (Compositae, Vernonieae) possess schistosomicidal properties.[2-4] The active constituent of the wood oil of *E. elaeagnus* Sch.-Bip. is the guaianolide eremanthin (1)[2,4] while the herbaceous parts of *E. goyazensis* Sch.-Bip. yielded the schistosomicidal and cytotoxic heliangolide goyazensolide (4a)[5] which is closely related to the cytotoxic lactones eremantholides A, B, and C (5a–c Chart I) from the wood of *E. elaeagnus.*[6,7]

In a search for other biologically active lactones we have now investigated *Eremanthus incanus* Less. and *Heterocoma albida* DC, the latter the sole representative of a monotypic genus within Vernonieae. Both species yielded eremanthin, while *E. incanus* also gave eremantholide A, eremantholide B, and small amounts of eregoyazin (2) and eregoyazidin (3).

2 and 3 were originally isolated in very small quantity from the wood of *E. goyazensis.*[11] While the two compounds were correlated with each other and with isoeremanthin (6) of known absolute configuration,[12] their stereochemistry at C(4) remained questionable because of the seemingly enantiomeric nature of their CD curves in

Table V. Torsion Angles (Deg) in Eregoyazin[a]

C(5)-C(1)-C(2)-C(3)	-31.7 (4)
C(1)-C(2)-C(3)-C(4)	13.6 (4)
C(2)-C(3)-C(4)-C(5)	10.1 (4)
C(3)-C(4)-C(5)-C(1)	-29.8 (4)
C(4)-C(5)-C(1)-C(2)	38.1 (4)
C(1)-C(5)-C(6)-C(7)	-72.1 (4)
C(5)-C(6)-C(7)-C(8)	87.6 (4)
C(6)-C(7)-C(8)-C(9)	-64.5 (5)
C(7)-C(8)-C(9)-C(10)	29.8 (7)
C(8)-C(9)-C(10)-C(1)	2.0 (8)
C(9)-C(10)-C(1)-C(5)	-26.0 (4)
C(10)-C(1)-C(5)-C(6)	45.7 (5)
O(2)-C(6)-C(7)-C(11)	-26.0 (4)
C(6)-C(7)-C(11)-C(12)	24.5 (4)
C(7)-C(11)-C(12)-O(2)	-14.8 (5)
C(11)-C(12)-O(2)-C(6)	-2.3 (4)
C(12)-O(2)-C(6)-C(7)	18.4 (4)
O(3)-C(12)-C(11)-C(13)	-1.0 (8)

[a] Standard deviations in parentheses.

Table VI. Cycloheptene Torsion Angles (Deg)

	1	8[17]		1	8[17]
ω_1	87.6	-93	ω_5	-26.0	29
ω_2	-64.5	69	ω_6	45.7	-52
ω_3	29.8	-30	ω_7	-72.1	74
ω_4	2.0	-1			

the ketone n,π* region.[11] The availability of a new small sample of eregoyazin in suitable crystalline form has now provided us with an opportunity to reinvestigate the problem by X-ray crystallography. This showed that eregoyazin was **2a**, with the C(4) methyl group α as in 4βH,5αH-dihydroisophotosantonic acid lactone acetate (**7a**) which had earlier served as a model for the CD curve of eregoyazin.[11] We also suggest that the structure of eregoyazidin is **3a**.

Crystal data of **2a** are listed in the Experimental Section. Figure 1 is a stereoscopic drawing of the molecule which also represents the absolute configuration because of the earlier correlation with isoeremanthin. Tables I–IV listing final atomic and final anisotropic thermal parameters, bond lengths and bond angles of eregoyazin are available as supplementary material. Table V lists selected torsion angles.

The α,β-unsaturated lactone ring of eregoyazin approximates an envelope form with C(7) as the flap below

(1) Work at The Florida State University was supported in part by a grant (CA-13121) from the U.S. Public Health Service through the National Cancer Institute. Work at Ribeirão Preto was supported in part by the Conselho Nacional de Desinvolvimento Cientifico Tecnologico.

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(7) Eremanthin was also isolated[2,8,9] from the schistosomicidal wood oil of *Vanillosmopsis erythropappa*, the herb of which produces the cytotoxic heliangolide 15-deoxygoyazensolide (**4b**).[10]

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Figure 1. Stereoscopic view of eregoyazin.

Chart I

1

2a, 4α-Me
b, 4β-Me

3a, 4α-Me
b, 4β-Me

4a, R = OH
b, R = H

5a, R = (isopropyl)

b, R = (sec-butyl)

c, R = (isopropenyl)

6

7a, R = H
b, R = Br

8

9

the near plane of C(11), C(12), O(2), and C(6). The C=O and the ethylenic group of the lactone barely deviate from coplanarity, the C(13)–C(11)–C(12)–O(2) torsion angle being only –1°. Hence the coincidence of the negative sign of the lactone n,π* Cotton effect[11] with the (very slight) chirality of the C=C—C=O chromophore (Beecham's generalization[13]) may be fortuitous. However, the signs of the C=C—C=O and C(11)–C(7)–C(6))–O(2) torsion angles are paired as in most other sesquiterpene lactones,[14,15] and the latter which, as has been suggested,[15b]

Figure 2. View of eregoyazin through axis of cyclopentanone bond.

may provide a better correlation with the CD is strongly negative.

The cycloheptene torsion angles (Table VI) closely resemble those of bromogaillardin (8) which has been described as a chair.[16,17] While they are not ideal for the C_s chair form of a cycloheptene where $\omega_1 = -\omega_7$, $\omega_2 = -\omega_6$, $\omega_3 = -\omega_5$, and $\omega_4 = 0$,[18] they are certainly not in keeping with a C_2 twist, a C_s boat, or distorted boat.[13,17]

The cyclopentanone ring is a half-chair with C(5) 0.249 Å above and C(1) 0.36 Å below the plane defined by C(2), C(3), and C(4). This arrangement is shown more clearly in Figure 2 which is a view of the molecule through the axis of the O=C bond and seems to differ somewhat from that in bromodihydroisophotosantonic acid lactone acetate (7b) where C(1), C(2), C(3), and C(5) are closely coplanar,[19] possibly as the result of the extra substituent on C(2).

Figure 2 becomes on octant diagram if a vertical line is drawn through C(3) directly behind O(1). On the assumption that the conformation of eregoyazin in solution approximates that in the crystalline state and that it is not significantly different from 4βH,5αH-dihydroisophotosantonic acid lactone acetate (7a), the relatively weak positive Cotton effects of both of these substances in the 300-nm region (ketone n,π* Δε ~0.5 and 0.7) qualitatively fit the analysis by Kirk[20] of the chiroptical properties of

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Table VII. ¹³C NMR Spectra (δ)
of *E. incanus* Constituents^a

	1	2a^b	3a^b	5a^c	5b
C(1)	47.01 d^d	47.45 d^g	47.81 d	205.52	205.52
C(2)	30.52 t^e	42.38 t	42.64 t	104.72 d^d	104.35 d
C(3)	29.14 t^e	218.00	218.00	187.42	186.83
C(4)	149.89	38.41 d^g	39.16^g	130.63	129.97
C(5)	52.54 d^d	52.44 d	51.72 d	135.09 d^d	134.73 d
C(6)	83.10 d	84.07 d	84.07 d	81.76 d^d	81.29 d
C(7)	45.24 d^d	43.87 d	48.32 d^e,g	64.08 d^d	63.69 d
C(8)	29.58 t^e	29.77 t	30.29 t	78.22 d^d	77.58 d
C(9)	120.79 d	122.09 d	122.61 d	44.20 t	43.93 t
C(10)	137.82	135.45	135.86	90.20	89.96
C(11)	140.08	139.86	42.33 d	61.06	60.38
C(12)	169.66	169.49	177.95	175.61	174.23
C(13)	119.08 t	120.11 t	13.00 q	20.45 q^e	20.49 q^e
C(14)	27.86 q	27.04 q	27.28 q	21.29 q^e	21.17 q^e
C(15)	110.72 t	15.31 q	15.65 q	20.23 q^e	20.29 q^e
C(1')				108.26	108.06
C(2')				32.65 d	39.28 d
C(3')				17.13 q^f	22.73 t
C(4')				16.83 q^f	12.41 q^f
C(5')					11.80 q^f

^a Run at 67.9 MHz in CDCl₃ unless specified otherwise. Unmarked signals are singlets. ^b Run on a mixture of eregoyazin and eregoyazidin. ^c Run in acetone-*d₆*. ^d Assignment made by single-frequency off-resonance decoupling. ^e,f Assignments may be interchanged. ^g See ref 22.

twisted cyclopentanones, with the quasi-axial α-methyl group on C(4) contributing considerably less than a quasi-axial C_α–H bond in the dissignate sense to the overall rotation which is based on contributions from the six chiral four-center components O=C—C_α—R.[21]

Ceteris paribus inversion of the center at C(4) should then produce a reasonably strong negative Cotton effect as the result of introducing a second quasi-axial C_α–H bond and in fact, 4αH,5αH-dihydrophotosantonic acid lactone acetate (9) has Δε ~–2. On the other hand Δε for eregoyazidin is only –0.6, a value which in view of the close correspondence between 2a and 7a would be very surprising for a substance of formula 3b. Moreover, the ¹³C NMR spectra of eregoyazin and eregoyazidin (Table VII) are strikingly similar except for those signals directly affected by saturation of the exocyclic double bond.[22] This observation also seems incompatible with the assumption that the two substances are C(4) epimers. Consequently

(21) The relevant torsional angles in 1 are O(1)–C(3)–C(2)–H(2A) = 76°, O(1)–C(3)–C(2)–H(2B) = 47°, O(1)–C(3)–C(2)–C(1) = 166°, O(1)–C(3)–C(4)–H(4) = 53°, O(1)–C(3)–C(4)–C(5) = 171°, and O(1)–C(3)–C(4)–C(15) = 68°.

(22) Signals of 2a and 3a were assigned by comparison with the spectrum of 1. However, it is possible that C(1) of 2a and 3a occur at higher field than C(1) of 1 due to shielding by the quasi-axial C(4) methyl and that therefore the assignments of C(1) and C(4) should be interchanged.

we are inclined to the belief that eregoyazidin has formula 3a, especially since it has been shown earlier that it possesses the stable configuration at C(4).

Experimental Section

Extraction of *Eremanthus incanus*. Aerial parts of *Eremanthus incanus* Less. (19.9 kg) collected by Dr. Hermogenes de Freitas Leitão Filho in Sao João Del Rei, Minas Gerais, Brazil, in October 1978 (voucher no. UEC-8485 on deposit in herbarium of UNICAMP) was extracted with CHCl₃, giving 430 g of crude extract, and was worked up in the usual manner.[23] The crude gum was chromatographed over 2.1 kg of silica gel, 600-mL fractions being eluted as follows: fractions 1–7, hexane–EtOAc (100:3); 8–17, hexane–EtOAc (50:1); 18–30, hexane–EtOAc (10:1); 31–80, hexane–EtOAc (20:1); 81–96, hexane–EtOAc (5:1); 97–112, hexane–EtOAc (4:1); 113–124, hexane EtOAc (5:2); 125–167, EtOAc; 168–212, EtOH. Fractions 19–34 contained eremanthin by TLC. The material from fraction 25 (290 mg) was purified by preparative TLC (EtOAc–hexane, 1:3) and recrystallized to give pure eremanthin, mp 70–71 °C, identical in all respects with authentic material. The ¹³C NMR spectrum is given in Table VII. Fraction 41 solidified and was identified as β-sitosterol by direct comparison, yield 0.21 g. Fractions 66–85 solidified but showed several spots on TLC, one of which corresponded to eregoyazin. Two constituents were separated by preparative TLC (EtOAc–hexane, 2:3) with considerable loss. The upper band was recrystallized from MeOH to give colorless rods of eregoyazidin (mp 185–186 °C, 15 mg) identified by comparison (TLC, ¹H NMR) with authentic material. The lower band was recrystallized from MeOH to give colorless cubes of eregoyazin (mp 178–179 °C, 20 mg) identified by comparison with authentic material (TLC, ¹H NMR). The ¹³C NMR spectrum of Table VII was determined on a mixture of eregoyazin and eregoyazidin.

TLC of fractions 103–120 indicated the presence of a mixture. One of the spots corresponded to eremantholide A. Fractions 110–112 were combined and purified twice by preparative TLC (hexane–EtOAc, 7:2) to give 85 mg of eremantholide A, mp 179–180 °C. The previously unreported high-resolution NMR spectrum (270 MHz, CDCl₃) displayed signals at δ 5.59 (H-2), 6.00 (dq, J = 3, 1.5 Hz, H-5), 4.93 (ddd, J = 7.5, 3, 2 Hz, H-6), 2.78 (dd, J = 7.5, 4.5 Hz, H-7), 4.02 (ddd, J = 12.5, 4.5, 2.5 Hz, H-8), 2.29 (dd, J = 14, 2.5 Hz, H-9a), 1.98 (dd, J = 14, 2.5 Hz, H-9b), 1.31 (3 protons, H-13), 1.46 (3 protons, H-14), 2.03 (dd, J = 2, 1.5 Hz, H-15), 1.98 (septet J = 6.5 Hz, H-2¹), 1.05 and 0.94 (2 d, J = 6.5 Hz, 3 pentets each, H-3¹ and H-4¹). Multiplets were assigned by spin-decoupling experiments. The ¹³C NMR spectrum is reported in Table VII.

Fractions 113–115 were combined (815 mg) and purified by silica gel thick-layer chromatography (CHCl₃–MeOH, 4:1, four developments). The upper band gave 30 mg of eremantholide B: mp 240–241 °C; [α]_D +60° (c 0.2, EtOH). The ¹H NMR spectrum was identical with that of eremantholide A except for the presence of the 2-methylbutyryl side chain. The ¹³C NMR spectrum is reported in Table VII. The lower band yielded 100 mg of eremantholide A. The material in fractions 126–167 could not be purified satisfactorily.

X-ray Analysis of Eregoyazin. Single crystals of 2a were obtained by slow crystallization from MeOH. They were orthorhombic, space group $P2_12_12_1$, with a = 6.734 (3), b = 12.702 (3), and c = 15.445 (5) Å and d_{calcd} = 1.238 g cm⁻³ for Z = 4 ($C_{15}H_{18}O_3$, M_r = 246.31). The intensity data were collected on a Hilger-Watts diffractometer (Ni-filtered Cu Kα radiation, θ–2θ scans, pulse-height discrimination). A crystal measuring approximately 0.20 × 0.25 × 0.4 mm was used for data collection. A total of 1060 reflections were measured for θ < 57°, of which 966 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure[24] and was refined by full-matrix least-squares methods. In the final refinement anisotropic thermal parameters were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations, but their parameters were not refined. The final discrepancy indices are

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$R = 0.045$ and $R_w = 0.051$ for the 966 observed reflections. The final difference map has no peaks greater than ± 0.2 e Å^{-3}.

Extraction of *Heterocoma albida*. Aerial parts of *Heterocoma albida* DC (4 kg) were collected by Dr. de Freitas Leitão Filho in Serra do Caraca, Minas Gerais, Brazil, in December 1978, extracted with CHCl$_3$, and worked up in the usual manner.[23] The crude gum (80 g) was chromatographed over 600 g of silica gel, 400-mL fractions being collected as follows: fractions 1–8, hexane; 9–16 hexane–EtOAc (50:1); 17–46, hexane–EtOAc (20:1). Subsequent fractions contained progressively more EtOAc. Fractions 198–209 were EtOAc; subsequent fractions were EtOAc–EtOH mixtures. The only identifiable material was obtained from fractions 32–33 (98 mg), which showed one spot on TLC and were combined. Purification by PLC (hexane–EtOAc) gave 49 mg of eremanthin.

Notes

Methanolysis of 2-Bromo-3,3-dimethylbutane and Its Reaction with Ethanethiolate Ion in Methanol[1]

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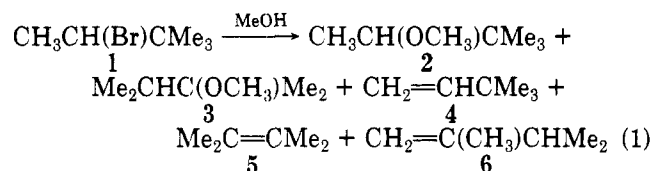*University of California, Santa Cruz, California 95064*

David L. Eck

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We have recently had occasion to repeat some experiments reported in 1973 by Bunnett and Eck.[2] In some cases our observations differ substantially from those reported earlier, enough to affect significantly the conclusions drawn from the work. In other cases we confirm earlier reports.

Results

From solvolysis of 2-bromo-3,3-dimethylbutane (**1**) in methanol in the presence of 2,6-lutidine (eq 1), we obtained

$$CH_3CH(Br)CMe_3 \xrightarrow{\text{MeOH}} CH_3CH(OCH_3)CMe_3 + \underset{\textbf{1}}{} \underset{\textbf{2}}{}$$

$$\underset{\textbf{3}}{Me_2CHC(OCH_3)Me_2} + \underset{\textbf{4}}{CH_2=CHCMe_3} +$$

$$\underset{\textbf{5}}{Me_2C=CMe_2} + \underset{\textbf{6}}{CH_2=C(CH_3)CHMe_2} \quad (1)$$

much (41.3%) rearranged ether **3** and a little (4.4%) of unrearranged ether **2**, as well as substantial amounts of rearranged olefins **5** (27.8%) and **6** (25.0%) and a mere 1.5% of olefin **4** with an unrearranged skeleton. These figures are normalized to total 100%; the measured product proportions (Table I) in samples taken at various times during the solvolysis reaction showed little variation, although it may be noted that **6** exceeded **5** in early samples while **5** exceeded **6** later on. Bunnett and Eck reported only the olefin products, as 83.2% **5**, 16.8% **6**, and a mere trace of **4**.[2] Our experience differs from that reported earlier both in our observation of ether products and in the relative amounts of **5** and **6** found.

We also repeated the determination of the products of reaction of **1** with ca. 0.8 M methanolic NaOCH$_3$. Our product composition, 92.1% **4**, 1.4% **5**, 0.8% **6**, 0.5% **2**, and 0.9% **3**, is very similar to that reported by Bunnett and

Eck.[2] Likewise our evaluation of the pseudo-first-order rate constant for the reaction of **1** with 0.80 M NaOCH$_3$ in methanol at 69.9 °C as 1.33×10^{-5} s^{-1} is close to their value of 1.44×10^{-5} s^{-1}.

Bunnett and Eck[2] reported the reaction of **1** with methanolic sodium ethanethiolate containing also ethanethiol to form mainly olefin **4**, very small amounts of olefins **5** and **6**, and no ether or thioether product. In contrast, we find ethyl 3,3-dimethyl-2-butyl sulfide (**7**) to be the predominant product (46%), accompanied by olefins **4**, **5**, and **6** in proportions somewhat as given earlier (eq 2; see Experimental Section).

$$1 + EtS^- \xrightarrow[\text{EtSH}]{\text{CH}_3\text{OH}} 4 + 5 + 6 + \underset{\textbf{7}}{CH_3CH(SEt)CMe_3} \quad (2)$$
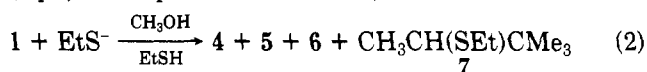
Our determinations of the pseudo-first-order rate constant for reaction of **1** with 0.756 M EtS$^-$Na$^+$ and 0.378 M EtSH in methanol at 70.0 °C gave a value, 2.07×10^{-5} s^{-1}, close to the 2.40×10^{-5} s^{-1} with 0.800 M EtS$^-$Na$^+$ and 0.400 M EtSH published earlier.[2]

We have confirmed the report of Bunnett and Eck[3] that the reaction of the tertiary alkyl bromide 2-bromo-2,3,3-trimethylbutane with methanolic sodium ethanethiolate furnishes no thioether product.

Of interest is the method we used for the preparation of **1**, namely, the S$_N$2 reaction of 3,3-dimethyl-2-butyl benzenesulfonate with tetrabutylammonium bromide in dry acetone. We obtained **1** in 62% yield (by GLC) or 41% yield (isolated), accompanied by olefins. This represents preparative employment of earlier kinetic/product observations of other workers;[4] our estimate of 62% substitution is similar to their estimates regarding reactions of other 3,3-dimethyl-2-butyl arenesulfonates.

Discussion

Our observation that the reaction of **1** with methanolic EtS$^-$Na$^+$ gives nearly equal amounts of substitution product **7** and olefin **4** is significant with regard to the mechanism of the elimination component of this reaction. It has been proposed[5] that eliminations induced by thiolate ions occur by the "E2C" mechanism in which the base attacks simultaneously the carbon (usually designated C$_\alpha$) from which the nucleofugal group is to depart and a hy-

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