The Structure of Euphorianin, an Ingol Diterpenoid from *Euphorbia poisonii*

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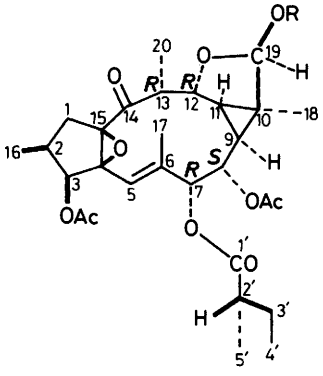
The residual uncertainty in the structure of euphorianin, an ingol diterpenoid from the latex of *Euphorbia poisonii*, has been resolved by nuclear Overhauser enhancement difference experiments which revealed the configuration at C-19 to be *S*. The ^{1}H and ^{13}C NMR spectroscopic properties of euphorianin and its transformation products are described.

Euphorianin, $C_{31}H_{42}O_{11}$, an ingol diterpenoid hemiacetal acetate[1] from the latex of *Euphorbia poisonii*, was isolated some twenty years ago. Its structure (**1**), except for the stereochemistry at C-19, is based on a crystal structure analysis of the derived lactone (**2**).[2] Euphorianin is assumed to have the same absolute configuration as other ingol derivatives.[3] In this paper we present the ^{1}H and ^{13}C NMR spectroscopic properties of euphorianin and some transformation products. The configuration at C-19 in (**1**) has been determined by nuclear Overhauser enhancement (NOE) difference experiments. The latex of *E. poisonii* contains a range of toxic and highly irritant esters of the diterpenoids 12-deoxyphorbol and resiniferonol.[3] Ingol derivatives generally lack irritant properties. Recently the 19-hydroxyingol derivative (**3**) has been isolated[4] from *E. poisonii*.

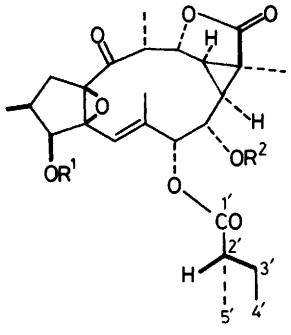
The ^{1}H NMR spectrum of euphorianin (Table 1) can be assigned by analogy with that of ingol tetra-acetate. The ^{13}C NMR spectrum (Table 2) showed some conformational broadening at ambient temperature but gave sharp signals at 60 °C. The protonated carbons were assigned by two-dimensional δ_C/δ_H correlation.

The residual uncertainty of the configuration at C-19 was readily resolved by NOE difference experiments. Irradiation of 19-H afforded NOEs at Me-18 (2%) and Me-20 (1%) consistent with the *S*(β)-configuration at C-19 as in (**1**). Hydrolysis of euphorianin followed by reacetylation gave a mixture (*ca.* 3:1) of C-19 epimers in which the epieuphorianin predominated. Irradiation of 19-H of the major epimer gave a strong NOE at 8-H (10%) together with the expected NOE at Me-18 (1%), consistent with the opposite (*R*) configuration at C-19.

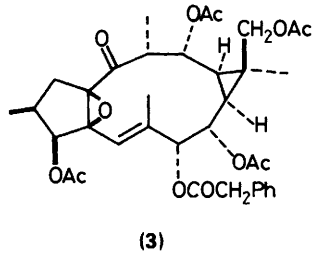
Treatment of euphorianin (**1**) with acidified MeOH afforded



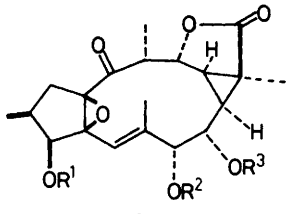
(**1**) R = Ac
(**4**) R = Me (C-19 configuration undetermined)



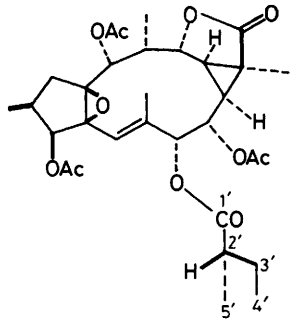
(**2**) $R^1 = R^2 = Ac$
(**5**) $R^1 = H$, $R^2 = Ac$
(**6**) $R^1 = Ac$, $R^2 = H$
(**7**) $R^1 = R^2 = H$



(**3**)



(**8**) $R^1 = R^2 = R^3 = H$
(**9**) $R^1 = R^2 = R^3 = Ac$



(**10**)

Table 1. ^{1}H NMR data (δ values; *J* in Hz) of euphorianin derivatives.[a]

Proton	(1)	(2)	(4)	(5)	(6)	(7)	(9)	(10)
1	2.64 (dd, *J* 8.2, 14.5)	2.68 (dd, *J* 9, 15)	2.69 (dd, *J* 9, 15)	2.64 (dd, *J* 9, 15)	2.80 (dd, *J* 9, 15)	2.57 (dd, *J* 9, 15)	2.69 (dd, *J* 9, 14.4)	Obsc.
	1.73 (d, *J* 14.5)	1.77 (d, *J* 15)	1.74 (d, *J* 15)	1.89 (d, *J* 15)	1.8 (d, *J* 15)	1.71 (d, *J* 15)	1.83 (d, *J* 14.4)	Obsc.
2	2.44 (br sext, *J* 8)	2.45 (m)	2.45 (m)	2.45 (m)	2.47 (m)	2.45 (m)	2.52 (sext, *J* 7.5)	2.40 (m)
3	5.07 (d, *J* 8.5)	5.12 (d, *J* 8)	5.11 (d, *J* 8)	4.22 (d, *J* 7)	5.15 (d, *J* 8)	4.13 (d, *J* 8)	5.15 (d, *J* 8.4)	5.15 (d, *J* 15)
5	5.48 (quint, *J* 1.3)	5.58 (br s)	5.55 (br s)	5.68 (br s)	5.60 (br s)	5.50 (br s)	5.61 (s)	5.87 (br s)
7	5.34 (br s)	5.27 (br s)	5.33 (br s)	5.38 (br s)	5.20 (br s)	5.30 (br s)	5.28 (br s)	5.30 (br s)
8	4.98 (dd, *J* 10.9, 1.3)	4.49 (dd, *J* 1, 8)	4.67 (dd, *J* 1, 10)	4.56 (m)	3.41 (m)	3.34 (d, *J* 10)	4.73 (dd, *J* 4.4, 9.0)	4.57 (d, *J* 12)
9	1.39 (br dd, *J* 9, 11)	Obsc.	Obsc.	Obsc.	Obsc.	Obsc.	1.66 (dd, *J* 10.2, 14.9)	Obsc.
11	1.19 (dd, *J* 1.6, 8.9)	Obsc.	1.15 (d, *J* 8)	Obsc.	1.30 (dd, *J* 2, 8)	Obsc.	Obsc.	Obsc.
12	4.60 (dd, *J* 1.6, 4.8)	4.70 (d, *J* 5)	4.43 (d, *J* 4)	4.75 (d, *J* 6)	4.70 (d, *J* 4)	4.69 (d, *J* 4)	4.49 (dd, *J* 1.0, 10.2)	4.73 (br d, *J* 2)
13	3.43 (dq, *J* 4.8, 6.6)	3.60 (dq, *J* 4, 7)	3.36 (m)	3.65 (dq, *J* 4, 7)	3.55 (m)	3.57 (m)	3.64 (dq, *J* 4.5, 6.6)	Obsc.
14	—	—	—	—	—	—	—	5.64 (d, *J* 2)
16	0.89 (d, *J* 7.4)	0.90 (d, *J* 7)	0.91 (d, *J* 7)	1.00 (d, *J* 6)	0.90 (d, *J* 7)	0.94 (d, *J* 7)	0.94 (d, *J* 7.3)	0.90 (d, *J* 7)
17	2.04 (d, *J* 1.3)	*ca.* 2.04 Nr	*ca.* 2.04 Nr	2.12 (d, *J* 4)	2.05 (d, *J* 2)	1.99 (br s)	2.10 (br s)	*ca.* 2.04 Nr
18	1.26 (s)	1.31 (s)	1.14 (s)	1.36 (s)	1.38 (s)	1.34 (s)	1.37 (s)	1.39 (s)
19	6.16 (dd, *J* 0.4, 0.8)	4.60 (s)						
20	1.00 (d, *J* 6.6)	0.96 (d, *J* 6)	0.99 (d, *J* 7)	1.04 (d, *J* 6)	0.98 (d, *J* 7)	1.04 (d, *J* 6)	1.01 (d, *J* 6.6)	0.98 (d, *J* 7)
OMe			3.33 (s)					
2′	2.41 (sext, *J* 7)	2.42 (sext, *J* 7)	2.44 (sext, *J* 7)	*ca.* 2.35 (m)	*ca.* 2.40	Obsc.	—	Obsc.
3′(a)	1.66 (ddq, *J* 13.6, 7.5, 7.3)	Obsc.	Obsc.	Obsc.	Obsc.	Obsc.	—	Obsc.
3′(b)	1.46 (ddq, *J* 13.6, 6.5, 7.3)	Obsc.	Obsc.	Obsc.	Obsc.	Obsc.	—	Obsc.
4′	0.82 (t, *J* 7.3)	0.85 (t, *J* 7)	0.86 (t, *J* 7)	0.91 (t, *J* 6)	0.88 (t, *J* 7)	0.86 (t, *J* 8)	—	0.88 (t, *J* 4)
5′	1.14 (d, *J* 7)	1.12 (d, *J* 7)	1.14 (d, *J* 7)	1.17 (d, *J* 8)	1.15 (d, *J* 8)	1.13 (d, *J* 7)	—	1.20 (d, *J* 8)
Ac	2.02, 2.00, 1.90 (all s)	2.04, 1.99 (both s)	2.04, 2.20 (both s)	2.01 (s)	2.08 (s)	—	2.05, 2.08, 2.15 (all s)	2.16, 2.05, 2.07 (all s)

[a] Sext = sextet; Obsc. = obscured; Nr = not resolved; quint = quintet.

the methyl acetal (**4**) as a single isomer (whose C-19 configuration was not determined). Mild acid hydrolysis of (**1**) followed by oxidation yielded the γ-lactone (**2**) which was used for the X-ray analysis.[2] Partial hydrolysis of (**2**) with methanolic K_2CO_3 gave the 3-deacetyl derivative (**5**) and the 8-deacetyl derivative (**6**) (see Table 1). The 7-H resonance is unchanged in (**2**), (**5**), and (**6**) and since the 2-methylbutanoate unit is present in all three compounds it follows that it must be attached to C-7.

Attempted de-epoxidation of the lactone (**2**) with Zn–Cu couple did not give the expected $\Delta^{4(15)}$-derivative but yielded the 8-deacetyl derivative (**6**), the 3,8-dideacetyl derivative (**7**), and the 3,7,8-trideacyl derivative (**8**). Acetylation of (**8**) afforded the triacetate (**9**) while acetylation of (**7**) gave back the original lactone (**2**), thus eliminating any possibility of transesterification[5] during the reaction.

Treatment of euphorianin with $NaBH_4$ resulted in reduction of the C-14 ketone and hydrolysis of the hemiacetal acetate. Acetylation of the product gave a mixture of C-19 epimers. Mild acid hydrolysis[2] of this mixture followed by oxidation yielded the lactone triacetate (**10**) which was isolated as a single compound. Reduction of the C-14 carbonyl appears to be stereospecific, presumably from the β-face to give the 14α-OH. The small coupling (*J* 2 Hz) of 14-H with 13-H is consistent with this suggestion.

Euphorianin (**1**) represents the second structure found to be a 19-hydroxyingol derivative.[4] Not surprisingly it has the same stereochemistry as ingol tetra-acetate although this is not always apparent when the ingol nucleus is drawn with re-entrant angles.[2]

Experimental

M.p.s were determined on a Kofler hot-stage microscope and are uncorrected. IR spectra were run (in CCl_4 solution) on a Perkin-Elmer 137 instrument. Specific rotations were obtained on an AA-100 automatic digital polarimeter. ^{1}H NMR spectra (Table 1) were determined at 200 MHz on a Bruker WP 200 SY instrument, shifts in ppm relative to $CHCl_3$ at δ 7.25, for compounds (**1**) and (**9**), and at 90 MHz on a Perkin-Elmer R32 instrument, shifts relative to Me_4Si, for all others. Spectra were run in $CDCl_3$ + CD_3OD for (**7**) and in $CDCl_3$ for all others. ^{13}C NMR spectra (Table 2) were determined on a Bruker WP 200 SY instrument at 50 MHz, shifts in ppm relative to $CDCl_3$ at δ 77.0. Mass spectra were determined using MS 12 (low resolution) and MS 902S (high resolution) instruments. Preparative TLC utilized silica gel GF 254 coated to 0.5 mm thickness and viewed under a UV lamp. Analytical TLC utilized silica gel GF 254 of 0.25 mm thickness and the components were either located by iodine vapour, viewed under UV lamp, or sprayed with a solution of cerium(IV) sulphate in conc. sulphuric acid–water (50:50). Plates were eluted with methylene chloride–methanol or light petroleum–ethyl acetate mixtures. Light petroleum refers to the fraction boiling in the range 60–80 °C. Ether refers to diethyl ether.

Conversion of Euphorianin (**1**) *to the Methyl Acetal* (**4**).—Euphorianin[2] (30 mg) was dissolved in MeOH (3 ml) and a drop of 0.1M H_2SO_4 added. The mixture was set aside at 29 °C for 1 h. It was then mixed with ice-cold water and extracted with ether (2 × 20 ml). The ether extract was washed to neutrality,

Table 2. ^{13}C NMR shifts[a] for euphorianin and some derivatives.

Carbon	Euphorianin (1) (60 °C)	Lactone (2) (room temp.)	Methyl acetal (4) (room temp.)
1	34.7t	34.6t	34.8t
2	29.5d	29.2d	29.1d
3	77.0d	76.6d	76.9d
4	73.1*s	73.1*s	73.0*s
5	116.3d	115.9d	116.1d
6	142.9s	143.1s	142.7s
7	76.5d	76.5d	77.2d
8	70.3d	68.7d	68.7d
9	27.7d	28.4d	25.5d
10	33.5s	29.0s	33.3s
11	31.3d	30.2d	29.1d
12	77.9d	72.5d	76.4d
13	48.7d	47.5d	48.8d
14	207.3s	206.0s	207.9s
15	72.9*s	73.1*s	73.0*s
16	16.8q	16.8q	16.8q
17	17.4q	17.5q	17.5q
18	18.5q	16.0q	16.9q
19	103.0d	174.3s	106.1s
20	7.6q	6.6q	7.9q
1′	174.9s	174.8s	175.1s
2′	41.1d	40.9d	41.0d
3′	26.6t	26.6t	26.7t
4′	11.2q	11.3q	11.3q
5′	16.5q	16.5q	16.5q
CH_3CO	170.3s	170.1s	170.2s
	170.0s	169.7s	169.9s
	169.8s		
CH_3CO	20.9q	20.7q	21.0q
	20.7q	20.3q	20.4q
	20.2q		
OCH_3	—	—	55.0q

[a] Multiplicities from DEPT spectra. Proton-bearing carbon atoms of (1) assigned using two-dimensional δ_C/δ_H correlation. * Values can be interchanged along the vertical column.

dried (Na_2SO_4), and evaporated to dryness to give a slightly impure solid (25 mg). Purification by preparative TLC followed by crystallization from ether–light petroleum afforded the *methyl acetal* (**4**) (20 mg, 70%) as needles m.p. 148–151 °C (from ether–light petroleum), $[\alpha]_D^{20}$ +22.12° (*c* 0.55 in MeOH) (Found: C, 64.1; H, 7.6%; M^+, 562.2786. $C_{30}H_{42}O_{11}$ requires C, 64.1; H, 7.5%; M^+, 562.2778); ν_{max} 1 744, 1 700, 1 370, and 1 235 cm^{-1}; *m/z* (EI MS; 180 °C; 70 eV) 562.2786 $(M)^+$ (16.32%), 502.2571 ($M^+ - CH_3CO_2H$) (1.07), and 418.1995 [$M^+ - CH_2CO - CH_3CH_2CH(CH_3)CO_2H$] (3.51).

Base-catalysed Hydrolysis of (**2**).—The lactone (**2**) (70 mg) in methanol (4 ml) was mixed with 0.1M K_2CO_3 in MeOH (0.3 ml) and left at room temperature for 12 h. The two hydrolysis products were isolated by preparative TLC. The less polar, 3-*deacetyl derivative* (**5**) (10 mg, 15%) crystallized as needles from $CHCl_3$–light petroleum, m.p. 220–222 °C; ν_{max} 3 620, 1 780, 1 750, 1 703, 1 457, and 1 220 cm^{-1}; *m/z* (EI MS; 180 °C; 70 eV) 504.2342 (M^+) (2.34%) ($C_{27}H_{36}O_9$ requires M^+ 504.2359) and 402.1628 [$M^+ - CH_3CH_2CH(CH_3)CO_2H$] (2.86). The more polar product, the 8-*deacetyl derivative* (**6**) (20 mg, 31%), was obtained as an amorphous solid, ν_{max} 1 234, 1 456, 1 700, 1 746, 1 774, and 3 435 cm^{-1}; *m/z* (EI MS; 190 °C, 70 eV) 504.2382 (M^+) (0.77%) ($C_{27}H_{36}O_9$ requires 504.2359), 420.1788 [$M^+ - CH_3CH_2C(CH_3)CO$] (1.60), and 360.1598, [$M^+ - CH_3CH_2C(CH_3)CO - CH_3CO_2H$] (15.44).

Reaction of the Lactone (**2**) *with Zn–Cu Couple*.—Zn–Cu couple was prepared by stirring Zn dust (2 g) for 1 min with dil. HCl (3%; 1.6 ml). The resulting mixture was immediately washed (by decantation) with 3% HCl (×3), distilled water (×5), aqueous $CuSO_4$ (2%; 3 ml; ×2) distilled water (×5), and finally methanol (×5).

A solution of the lactone (**2**) (80 mg) in dry MeOH (10 ml) and was heated under reflux for 37 h with Zn–Cu couple. The mixture was cooled and filtered. The solvent was removed by distillation to give a crude product (75 mg). Analytical TLC showed three products, two major and one minor. The mixture was separated by preparative TLC.

The least polar product (25 mg, 34%) was identified as the 8-deacetyl derivative (**6**) (TLC; ^1H NMR). The second product was the non-crystalline 3,8-*dideacetyl derivative* (**7**) (21 mg, 31%); *m/z* (EI MS; 200 °C; 70 eV) 462.2242 (M^+) (4.9%) ($C_{25}H_{34}O_8$ requires 462.2253), 444.2183 ($M^+ - H_2O$) (1.19), 360.1557 [$M^+ - CH_3CH_2CH(CH_3)CO_2H$] (7.38), and 342.1464 [$M^+ - CH_3CH_2CH(CH_3)CO_2H - H_2O$] (2.46). The most polar product (4.2 mg, 7.6%) was identified as the 3,7,8-*trideacyl derivative* (**8**) (^1H NMR), which was acetylated with pyridine–Ac_2O to give the *triacetate* (**9**) (4.4 mg); ν_{max} 1 236, 1 370, 1 700, 1 750, and 1 780 cm^{-1}; *m/z* (EI MS; 190 °C; 70 eV) 504.1984 (M^+) (1.94%) ($C_{26}H_{32}O_{10}$ requires 504.1995), 444.1723 ($M^+ - CH_3CO_2H$) (1.79), 402.1678 ($M^+ - CH_3CO_2H - CH_2CO$) (7.04), and 342.1462 ($M^+ - 2CH_3CO_2H - CH_2CO$) (4.70).

Reduction of Euphorianin: the Lactone (**10**).—Euphorianin (**1**) (100 mg) was dissolved in dry methanol (10 ml) and cooled in ice. $NaBH_4$ (3.5 mg) was added slowly with stirring, the reaction being followed by TLC. Work-up gave a solid which was dissolved in pyridine (1.5 ml) and cooled in ice–salt. Cold Ac_2O (3 ml) was then added, and the mixture left overnight. The usual work-up afforded a solid (80 mg) which was dissolved in acetone (4 ml), treated with 2M H_2SO_4 (1 drop), and left for 1 h. It was then cooled in ice–water and treated with Jones' reagent.[2] The usual work-up gave a crude product which was purified by preparative TLC to yield the *lactone* (**10**) (28 mg, 28%); m.p. 208–210 °C (from $CHCl_3$–light petroleum), $[\alpha]_D^{20}$ +35.25° (*c* 0.75, MeOH) (Found: C, 63.05; H, 7.1%; $C_{31}H_{42}O_{11}$ requires C, 63.05; H, 7.1%); ν_{max} 1 220, 1 372, 1 750, and 1 775 cm^{-1}; *m/z* (EI MS; 200 °C; 70 eV) 590.2693 (M^+) (0.16%), 548.2621 ($M^+ - CH_2CO$) (1.46), and 428.1851 [$M^+ - CH_3CH_2CH(CH_3)CO_2H - CH_3CO_2H$] (2.62).

Acetylation of 3,8-*Dideacetyldehydroeuphorianin* (**7**).—The dihydroxy-lactone (**7**) (15 mg) in pyridine (40 drops) was cooled and treated with cold Ac_2O (80 drops) and the mixture left at room temperature for 36 h. Work-up gave a crude product which was purified by preparative TLC to yield a pure crystalline material identical (^1H NMR, IR, m.p., mixed m.p.) with the lactone (**2**).

Acknowledgements

C. O. F. is indebted to the Association of Commonwealth Universities for a fellowship, and to Obafemi Awolowo University, Nigeria for sabbatical leave which enabled him to work in the Chemistry Department, University of Glasgow, where this work was completed.

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Paper 9/00460B
Received 23*rd January* 1989
Accepted 21*st August* 1989