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NEW DITERPENE ESTERS FROM *ALEURITES FORDII* FRUITS*

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Key Word Index—*Aleurites fordii*; Euphorbiaceae; tung oil tree; 12-*O*-palmityl-13-*O*-acetyl-16-hydroxyphorbol; 13-*O*-acetyl-16-hydroxyphorbol; piscicidal activity.

Abstract—A toxic diterpenoid diester and a monoester were isolated from the fruits of *Aleurites fordii*. The structure of the monoester was found to be 13-*O*-acetyl-16-hydroxyphorbol by transforming it into bisdehydrophorbol-(12,20)-diacetate. The structure of the toxic constituent was established as 12-*O*-palmityl-13-*O*-acetyl-16-hydroxyphorbol by partial synthesis from the monoester.

INTRODUCTION

Tung oil and meal, produced from the fruits of trees of the genus *Aleurites* (Euphorbiaceae) are toxic and cause diarrhoea and irritation of the skin and internal organs, etc. [1]. The toxic principle has also been the subject of a number of investigations and two amorphous toxic substances were isolated from tung press cake by Holmes *et al.* [2], who attempted to detoxify tung meal so that this protein-rich meal could be used as an animal food. Unsaturated acyclic ester structures were proposed for these substances almost entirely on the basis of UV and IR spectra [2] but the compounds were not fully characterised. We have now isolated a toxic principle **1** and a related diterpenoid **2** in a crystalline form from the fruits of *Aleurites fordii* and elucidated their structures.

RESULTS AND DISCUSSION

The seeds and the outer parts of fresh green fruits of *A. fordii* were separately extracted with MeOH. The concentrated extract from the outer parts, which showed strong toxicity to *Oryzias laptipes* (killie-fish) was extracted with EtOAc, and this extract was chromatographed on a silicic acid column using gradient elution with $CHCl_3$–Me_2CO mixtures. The fraction eluted with 10% Me_2CO was toxic and was further purified by PLC to yield **1** m.p. 177–178°, $C_{38}H_{60}O_9$, $[\alpha]_D^{12}$ +43° (*c* 0·30, MeOH), M^+ 660. The UV, (MeOH) 230 nm (log ε 3·68), and the IR, ν(KBr) 3400, 3300, 1740, 1719, 1703, 1629 and 1260 cm^{-1}, spectra show the presence of a conjugated carbonyl group. The concentrated MeOH extract of the seeds was defatted with petrol after adding water, and the residual aq soln was then extracted with EtOAc. The EtOAc solution was concentrated to give crystals of **2**, m.p. 278–282° (decomp.), $C_{22}H_{30}O_8$, $[\alpha]_D^{15}$ +65° (*c* 0·11, EtOH). The UV, λ(MeOH) 232 nm (log ε 3·70), and the IR, (KBr) 3550, 3500, 3400, 3250, 1705, 1695 (shoulder) and 1630 cm^{-1}, spectra show the similarity of **2** to **1**. The presence of an acetyl and a palmityl group in **1** is shown by the NMR (C_5D_5N–D_2O, δ 2·10, and δ 0·87, 1·24 and 2·42) and MS (M^+-60, M^+-255) spectra, while an acetyl group is shown by the NMR (δ 2·13) and the MS (M^+-60) spectra of **2**.

The NMR spectrum of **2** (Table 1) shows an acetyl group and a tertiary (δ 1·53), a secondary (δ 1·51, *d*, *J* 6 Hz) and a vinyl (δ 1·71, *dd*, *J* 2 and 1 Hz) methyl group. An olefinic proton, assignable to the β-proton of α,β-unsaturated carbonyl system, is exhibited at δ 7·86 as a multiplet coupled with a proton at δ 3·63 and also with the vinyl methyl protons, and these couplings were confirmed by the NMDR experiments (Table 2). A signal assign-

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Table 1. NMR spectral data of **1**, **2**, **4** and phorbol (δ in ppm)*

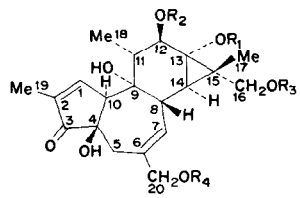
Compound solvent	**1** (a)	**2** (a)	**4** (b)	**4** (c)	Phorbol [3(b)] (b)	Phorbol [3(b)] (c)
H-1	7·74, *m* *J* 2,1	7·86, *m* *J* 2,1	7·86, *m* *J* 2,1	7·59, *m* *J* 2,1	7·81, *m* *J* 2,1	7·52, *m* *J* 2,1
H-5	2·96, *s*	2·91, 3·07 AB*q*, *J* 19†	3·03, *s*	2·28, *s*	3·06, *s*	2·27, *s*
H-7	6·13‡	6·10, *d* *J* 6	6·18, *d* *J* 6	5·46, *d* *J* 6	6·10, *d* *J* 6	5·47, *d* *J* 6
H-8	4·05, *m*	3·97, *m*	4·02, *m*§	2·90, *m*	3·90, *t*§ *J* 6, 5	2·90, *t* *J* 6, 5
H-10	3·64, *m*	3·63, *m*	3·66, *m*	2·90, *m*	3·66, *t* *J* 2, 1	2·90, *m*
H-11	*ca* 2·90	2·72, *dq* *J* 10, 6	2·75, *dq* *J* 10, 6	*ca* 1·65	2·75, *dq* *J* 10, 6	*ca* 1·70
H-12	6·04, *d* *J* 10	4·49, *d* *J* 10	5·04, *d* *J* 10	3·79, *d* *J* 10	4·98, *d* *J* 10	3·84, *d* *J* 10
H-14	Obscured	Obscured	1·52, *d*§ *J* 5	0·70, *d* *J* 5	1·30, *d*§ *J* 5	0·52, *d* *J* 5
H-16(Me)					1·57, *s*	1·14, *s*
H-17	1·58, *s*	1·53, *s*	1·82, *s*	1·19, *s*	1·65, *s*	1·02, *s*
H-18	1·63, *d* *J* 6	1·51, *d* *J* 6	1·59, *d* *J* 6	0·95, *d* *J* 6	1·60, *d* *J* 6	0·92, *d* *J* 6
H-19	1·69, *dd* *J* 2, 1	1·71, *dd* *J* 2, 1	1·69, *dd* *J* 2, 1	1·65, *dd* *J* 2, 1	1·68, *dd* *J* 2, 1	1·65, *dd* *J* 2, 1
H-20	4·36, *s*	4·24, *s*	4·25, *s*	3·73, *s*	4·26, *s*	3·75, *s*
H-16 (CH_2)	4·16, *s*	4·42, 4·20 AB*q*, *J* 12	4·57, 4·17 AB*q*, *J* 12	3·48, *s*		
OAc	2·10, *s*	2·13, *s*				
Me	0·87, *m*					
13 CH_2	1·24, *s*					
$COCH_2$	2·42, *t*					

* Coupling constants are given in Hz. Spectra were taken in C_5D_5N–D_2O (a), C_5D_5N (b) and $(CD_3)_2SO$ (c) at 90 MHz.
† δ 3·04, *s*, in C_5D_5N.
‡ Overlapped by H-12.
§ Appeared in C_5D_5N–D_2O at δ 3·95 (H-8) and 1·51 (H-14) in **4**, and at δ 3·93 (H-8) and 1·34 (H-14) in phorbol [4].

able to a cyclopropane proton, which is overlapped by the secondary and tertiary methyl signals in the NMR spectrum obtained in C_5D_5N–D_2O, is shown at δ 1·13 (*d*, *J* 5 Hz) in $(CD_3)_2SO$. The presence of two tertiary, a secondary and two primary hydroxyl groups in **2** is also shown by two singlets at δ 5·00 and 5·67, a doublet at δ 5·20, and two triplets at δ 4·50 and 4·73 ($(CD_3)_2SO$), which were all substituted by deuterium on treatment with D_2O. These NMR spectra which indicate the similarity of **2** with phorbol [3], also show that one of the geminal methyl singlets on the cyclopropane ring in phorbol is replaced in **2** by an AB quartet (*J* 12 Hz) centered at δ 4·20 and 4·42 (C_5D_5N–D_2O), which is assignable to a hydroxymethyl group. These data lead to the assumption that **2** is an acetate of 16-hydroxyphorbol.

Acetylation of the monoacetate **2** with Ac_2O–C_5H_5N gave a tetraacetate **3**, $C_{28}H_{36}O_{11}$. The NMR spectrum (C_5D_5N) shows three newly formed acetyl groups, among which two are on the primary (δ 4·31 → 4·66 or 4·77; δ 4·51 and 4·30 (AB*q*) → 4·77 (*s*) or 4·66 (*s*)), and one is on the secondary (δ 4·56 → 6·05) hydroxyl group. Upon methanolysis with NaOMe, **2** afforded the parent diterpene alcohol **4**, $C_{20}H_{28}O_7$. A tetraacetate was produced from **4**, and was identical with **3** derived from **2**. The tertiary hydroxyl group at C-13 in phorbol is also acetyled with Ac_2O–C_5H_5N [4]. Therefore, the acetyl group in **2** would be at C-13,

since if the acetyl group is on one of the other tertiary hydroxyl groups, a penta-acetate would have been formed upon acetylation. 13-*O*-Acetyl-16-hydroxyphorbol is then regarded as the most plausible structure for **2**, and this structure is supported by the NMDR data shown in Table 2.



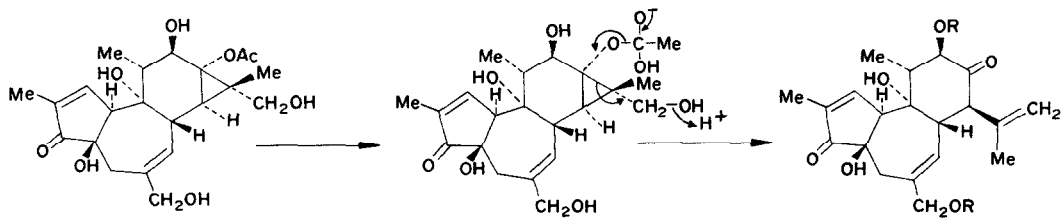
(**1**) R_1 = COMe; R_2 = $CO(CH_2)_{14}Me$; R_3 = R_4 = H
(**2**) R_1 = COMe; R_2 = R_3 = R_4 = H
(**3**) R_1 = R_2 = R_3 = R_4 = COMe
(**4**) R_1 = R_2 = R_3 = R_4 = H
(**7**) R_1 = R_3 = R_4 = COMe; R_2 = $CO(CH_2)_{14}Me$
(**8**) R_1 = COMe; R_2 = R_3 = R_4 = $CO(CH_2)_{14}Me$
(**9**) R_1 = COMe; R_2 = H; R_3 = R_4 = $CO(CH_2)_{14}Me$
(**10**) R_1 = COMe; R_2 = $CO(CH_2)_{14}Me$; R_3 = H or $CO(CH_2)_{14}Me$; R_4 = $CO(CH_2)_{14}Me$ or H

Table 2. NMDR data of **2** at 90 MHz

Irradiated at ppm		Observed at ppm		Change in multiplicity	Solvent
H-1	7·86	H-10	3·63	$m \rightarrow q$ (J 2)	C_5D_5N–D_2O
		H-19	1·71	$dd \rightarrow d$ (J 2)	
H-19	1·71	H-10	3·63	$m \rightarrow d$ (J 2)	
		H-1	7·86	$m \rightarrow d$ (J 2)	
H-10	3·63	H-1	7·86	$m \rightarrow q$ (J 1)	
		H-19	1·71	$dd \rightarrow d$ (J 1)	
H-18	1·51	H-11	2·72	$dq \rightarrow d$ (J 10)	
H-11	2·72	H-12	4·49	$d \rightarrow s$	
H-12	4·49	H-11	2·72	$dq \rightarrow q$ (J 6)	
H-7	5·56	H-8	3·11	$t \rightarrow d$ (J 5)	$(CD_3)_2SO$–D_2O
H-8	3·11	H-7	5·56	$d \rightarrow s$	
		H-14	1·18	$d \rightarrow s$	
H-14	1·18	H-8	3·11	$t \rightarrow d$ (J 6)	

The structure and the absolute configurations of **2** were confirmed by transforming it into bisdehydrophorbol-(12,20)-diacetate [5]. On treatment of **2** with 0·02 N H_2SO_4 in aq. MeOH, a less polar compound **5**, m.p. 244–248° (decomp.), $C_{20}H_{26}O_6$, λ(MeOH) 245 nm (log ϵ 3·65), was produced. The NMR spectrum showed that the methyl group on the cyclopropane ring, and C_{16}-methylene (ABq) in **2** are replaced in **5** by an isopropenyl group (δ 1·54, 4·75 and 4·92 in $(CD_3)_2SO$–D_2O). Elimination of the acetyl group upon this transformation is also observed. This acid catalyzed reaction, which can be depicted as shown is analogous to the alkali catalyzed transformation of 16-hydroxy-12-deoxyphorbol-(13)-tiglate into crotophorbolone [6], although **2** was hydrolyzed to yield **4** upon the treatment with NaOMe. The identity of the diacetate **6**, m.p. 191–192°, $C_{24}H_{30}O_8$, obtained by acetylation of **5** with Ac_2O–C_5H_5N, with bisdehydrophorbol-(12,20)-diacetate was established by m.m.p., and NMR, IR, ORD and CD spectra.

The NMR spectrum of **1** (Table 1) is almost identical with that of **2** except the presence of a palmityl signal in the former. An acetyl group (δ 2·10, s) is also exhibited by **1**. One of these two acyl groups in **1** would be at C-12 as H-12 (d, J 10 Hz) in **1** shifts downfield by 1·55 ppm from that in **2**. Upon methanolysis with NaOMe, **1** yielded methyl palmitate which was identified by GC-MS, and the parent alcohol which was converted to a tetraacetate and found to be identical with **3**. Acetylation of **1** with Ac_2O–C_5H_5N resulted in the introduction of two additional acetyl groups to yield **7**, $C_{42}H_{64}O_{11}$, whose NMR spectrum (C_5D_5N–D_2O) showed that the newly formed acetyl groups are on the primary hydroxyl groups whose methylene protons are at δ 4·62 (s) and 4·77 (s), indicating that the tertiary hydroxyl group at C-13 in **1** is masked by an acyl group. The two acyl groups in **1** are therefore at C-12 and C-13. The MS of **1** exhibits m/e 405 (M^+-255) and 600 (M^+-60) peaks which arise by the loss of a palmityl radical and an acetic acid molecule respectively, indicating the



(**2**) (**5**) R = H (**6**) R = COMe

location of the palmityl and acetyl groups at C-12 and C-13 respectively rather than the reverse. Fragmentation of phorbol-12,13-diesters give M^+-RCOO· ions by elimination at C-12, and M^+-RCOOH ions by elimination at C-13 [7]. Partial methanolysis of **1** was carried out, and the product which migrated slower than **1** on TLC was purified by PLC. This product failed to show the M^+ peak in the MS spectrum, but exhibited the presence of the palmityl group by the ion peaks produced by the successive loss of methylenes in the palmityl group, and was acetylated to give a product which was identical with **7** by TLC and MS. These results show that the palmityl group is retained in the partial methanolyzate, and that its location is at C-12 since it is known that the acyl group at C-13 on the phorbol skeleton is methanolyzed before that at C-12 [7]. The structure of **1**, therefore, is 12-*O*-palmityl-13-*O*-acetyl-16-hydroxyphorbol.

Conversion of **2** to **1** was achieved by reaction with palmityl chloride and C_5H_5N, **2** yielding a viscous oily tripalmitate **8**, $C_{70}H_{120}O_{11}$, and a dipalmitate **9**, m.p. 84–85°, $C_{54}H_{90}O_{10}$, which was shown to be 16, 20-dipalmitate by the shifts of the methylene signals in the NMR spectrum (C_5D_5N–D_2O, δ 4·71 and 4·84). The tripalmitate was partially hydrolyzed with 60% $HClO_4$ in MeOH, and the hydrolyzate was purified by PLC to afford another dipalmitate **10**, $C_{54}H_{90}O_{10}$, whose spectra were different from those of **9**, along with a monopalmitate, $C_{38}H_{60}O_9$, which was identical with **1** (m.m.p., IR, and ORD spectra). The C-16 and C-20 methylene signals (C_5D_5N–D_2O), at δ 4·68 and 4·72 in **8**, at δ 4·79 and 4·28 in **10**, and at δ 4·16 and 4·36 in **1**, and the essentially equal chemical shifts of H-12 in **8**, **10** and **1** are in accord with the location of the palmityl group at C-12 in **1**. The synthesis of **1** from **2** establishes the structure and the absolute configurations of **1** to be 12-*O*-palmityl-13-*O*-acetyl-16-hydroxyphorbol. The attempts of NOE measurement to determine the configurations at C-15 failed to detect the effect between C-16, 17 and C-8, 11 protons. It is possible that the C-16 hydroxymethyl group is exo-oriented if an acetonide is formed between C-16 and C-13 hydroxyl groups, and that C-16 is endo-oriented if an acetonide is formed between C-16 and C-12 hydroxyl groups. But the attempts of acid catalyzed acetonide formation from **4** failed because of facile cleavage of the cyclopropane ring in **4** to produce **5**, and the reactions without addition of acid resulted in recovery of **4**. Although further experimental evidence would be required for the determination of the configurations at C-15, the hydroxymethyl group is probably exo-oriented by comparison of the NMR spectra of **4** and phorbol (Table 1), as all of the protons in these two compounds except those of C-17 methyl and C-14 methine show almost identical chemical shifts in C_5D_5N and also in $(CD_3)_2SO$. The C-14 proton signal of **4** (δ 1·52 in C_5D_5N and δ 0·70 in $(CD_3)_2SO$) in both of these solvents is about 0·2 ppm lower than that of phorbol (δ 1·30 in C_5D_5N and δ 0·52 in $(CD_3)_2SO$). If the C-16 hydroxymethyl group is endo oriented, effects would be observed at the protons at C-8 and C-11.

Table 3. Toxicity of **1** and rotenone to killie-fish

Compound (org. solvent)	Conc. (ppm)	Original number of test fish	Number of test fish survived after 24 hr	48 hr
1 (MeOH)	0·034	10	1	0
	0·030	10	3	1
	0·025	10	6	4
	0·021	10	8	7
	0·018	10	9	9
Control		10	10	10
Rotenone (Me_2CO)	0·035	10	1	1
	0·024	10	1	1
	0·016	10	4	3
	0·010	10	6	6
	0·007	10	8	8
Control		10	10	10

The toxicity of **1** to killie-fish, as shown by the TLm values after 24 and 48 hr (Tables 3 and 4), was found to be almost equivalent to that of rotenone.

Table 4. Median tolerance limits (TLm) of **1** and rotenone

Compound	TLm (24 hr) ppm	TLm (24 hr) μM	TLm (48 hr) ppm	TLm (48 hr) μM
1	0·026	0·039	0·024	0·036
Rotenone	0·013	0·033	0·012	0·030

EXPERIMENTAL

NMR spectra were determined at 90 MHz, and chemical shifts (δ) are given in ppm relative to TMS as internal standard.

MS were obtained with a GC-MS combination using 2 m × 3 mm i.d. glass columns containing 1% OV-1 on 60–80 mesh Chromosorb W and also using a direct inlet system (ion source temp. 270°, separator temp. 250°, electron energy 70 eV). GLC was carried out with 2 m × 3 mm glass columns packed with 1·5% SE-30 on Chromosorb W. Kieselgel G and PF_{254} were used for TLC, and silicic acid (100 mesh) was used for column chromatography. The organic solns were dried over $MgSO_4$ and evaporated in rotary evaporator below 50°. The isolation procedure was governed by the killie-fish bioassay [8].

Extraction and isolation of **1** *and* **2**. *Aleurites fordii* Hemsl. fruits were collected in September and October at Okayama University campus and Handayama Botanical Garden. The seeds were removed, and the outer parts (29 kg) of the fruits were soaked in MeOH (70 l.) twice for a week at 20°. The combined MeOH solns were concn to 7 l. and were extracted × 7 with EtOAc (5 l.). The EtOAc extracts were dried and evaporated to afford a dark green residue (150 g). A portion of the residue (10 g) was redissolved in EtOAc (3 l.) and washed successively with 0·5 N NaOH and H_2O. A dark green residue (3 g) which showed strong toxicity to killie-fish was obtained upon evaporation of the EtOAc soln. This residue was dissolved in MeOH, silicic acid (5 g) was added to the soln, and the solvent evaporated. The silicic acid which had adsorbed the extract was placed on the top of a silicic acid column (250 g), and eluted with $CHCl_3$–Me_2CO (100:0 → 50:50). The active fractions ($CHCl_3$–Me_2CO, 9:1) were combined and purified further by PLC on silica gel PF_{254} and development with $CHCl_3$–Me_2CO (3:2) to yield crude crystals of **1**. Recrystallization from MeOH–H_2O afforded pure toxic principle **1** (27 mg), m.p. 177–178°, $[\alpha]_D^{12}$ +43° (*c* 0·3, MeOH). ν_{max}^{KBr} 3400, 3300, 1740, 1719, 1703, 1629, 1260 cm^{-1}. λ_{max}^{MeOH} 230 nm (log ε 3·68). MS *m/e* 660 (M^+), 642 (M^+-H_2O), 600 (M^+-HOAc), 582 (M^+-HOAc–H_2O), 405 (M^+-Me$(CH_2)_{14}$COO·), 387 (M^+-Me$(CH_2)_{14}$COO·–H_2O). NMR Table 1. (Found: C, 67·93; H, 9·09. $C_{38}H_{60}O_9$·MeOH requires: C, 67·60; H, 9·31%). The powdered seeds (12 kg) were defatted with petrol (5 l.), and soaked × 5 in MeOH (10 l. each) for 5 days. The combined solns were concentrated to *ca* 1·5 l., and extracted × 5 with *n*-hexane, and then with EtOAc (1·5 l.). The combined EtOAc solns were dried and evaporated to give a crystalline residue (0·8 g). Recrystallization from MeOH gave **2** as colourless needles, m.p. 278–282° (decomp.). $[\alpha]_D^{15}$ +65° (*c* 0·11, EtOH). ν_{max}^{KBr} 3550, 3500, 3400, 3250, 1705, 1695 (shoulder), 1630 cm^{-1}. λ_{max}^{MeOH} 232 nm (log ε 3·70). MS *m/e* 422 (M^+), 404 (M^+-H_2O), 362 (M-HOAc). NMR Table 1. (Found: C, 62·25; H, 7·37. $C_{22}H_{30}O_8$ requires: C, 62·54; H, 7·16%).

Tetra-acetate **3**. A mixture of **2** (105 mg), Ac_2O (3 ml) and C_5H_5N (3 ml) was allowed to stand 18 hr at 20°, and then poured into ice–H_2O. The resultant soln was extracted with EtOAc, the EtOAc washed successively with 10% HCl, 5% $KHCO_3$ and H_2O, dried and concentrated to give a pale yellow residue. The product was purified by passing through an Al_2O_3 (10 g) column and eluting with Et_2O which afforded a syrup of acetate **3** (72 mg) which showed one spot on TLC (silica gel, Et_2O). $[\alpha]_D^{12}$ +80° (*c* 0·15, MeOH). ν_{max}^{KBr} 3550, 3380, 1730, 1710 (shoulder), 1630, 1230–1210 cm^{-1}. MS 548 (M^+). NMR (C_5D_5N) 7·75 (1H, *m*, H-1), 2·93 (2H, *s*, H-5), 6·10 (1H, H-7, overlapped by H-12), 3·89 (1H, *t*, *J* 5 and 7 Hz, H-8), 3·60 (1H, *m*, H-10), 2·77 (1H, *dq*, *J* 10 and 6 Hz, H-11), 6·05 (1H, *d*, *J* 10 Hz, H-12), 4·77 (2H, *s*, H-16), 1·44 (3H, *s*, H-17), 1·20 (4H, *d*, *J* 6 Hz, H-18 and H-14), 1·73 (3H, *dd*, *J* 2 and 1 Hz, H-19), 4·66 (2H, *s*, H-20), 1·87, 1·98, 2·03, 2·05 (4 OAc). (Found: C, 61·52; H, 6·73. $C_{28}H_{36}O_{11}$ requires: C, 61·35; H, 6·62%).

Methanolysis of **2**. A soln (0·2 ml) of NaOMe (250 mg of Na in 50 ml of dry MeOH) was added to a soln of **2** (56 mg) in dry MeOH (20 ml), and the mixed soln was stirred at 20° for 5 hr. Amberlite IR-120 (H form) was added to neutralize the soln. After filtration the filtrate was evaporated to give a syrupy residue which was purified by PLC on silica gel PF_{254} using $CHCl_3$–Me_2CO (1:3) and subsequent elution from the adsorbent with MeOH. The MeOH soln was evaporated to yield amorphous **4** (36 mg). $[\alpha]_D^{12}$ +103° (*c* 0·51, MeOH). λ_{max}^{MeOH} 230 nm (log ε 3·75). ν_{max}^{KBr} 3370 (broad), 1697, 1622 cm^{-1}. NMR(CD_3OD) 7·63 (1H, *m*, H-1), 2·43 (1H, *s*, H-5), 5·61 (1H, *d*, J 6 Hz, H-7), 3·09 (2H, *m*, H-8 and H-10), 1·87 (1H, *m*, H-11), 4·03 (1H, *d*, J 10 Hz, H-12), 0·92 (1H, *d*, J 5 Hz, H-14), 3·60 and 3·73 (2H, AB*q*, *J* 12 Hz, H-16), 1·32 (3H, *s*, H-17), 1·07 (3H, *d*, *J* 7 Hz, H-18), 1·72 (3H, *dd*, *J* 2 and 1, H-19), 3·91 (2H, *s*, H-20). (Found: C, 58·69; H, 7·64. $C_{20}H_{28}O_7$·3/2H_2O requires: C, 58·90; H, 7·60%). A soln of **4** (16 mg) in Ac_2O (0·2 ml) and C_5H_5N (0·2 ml) was left 18 hr at 20° and concn. The residue was purified by PLC (silica gel PF_{254}) with Et_2O to afford a syrupy acetate (12 mg) which was identical with **3** by comparison of IR (KBr) and NMR spectra.

Transformation of **2** *into bisdehydrophorbol* **5** *and its diacetate* **6**. A suspension of **2** (80 mg) in a mixture of MeOH–H_2O (7:2, 9 ml) and 0·1 N H_2SO_4 (2 ml) was refluxed in N_2 for 1·5 hr, and kept at 20° for 18 hr. The resulting soln was neutralized with Amberlite IRA-410 (OH form), filtered and evaporated. The residual crystalline mass was suspended in $CHCl_3$–Me_2CO (7:3), the insoluble crystals filtered and recrystallized from MeOH to afford the starting material (35 mg). The mother liquor was concn to give colourless plates, which were recrystallized from $CHCl_3$–Me_2CO (7:3) to afford **5** as colourless needles (15 mg), m.p. 244–248° (decomp.). ν_{max}^{KBr} 3520, 3450, 3330, 1693, 1650, 1620 cm^{-1}. λ_{max}^{MeOH} 245 nm (log ε 3·65). CD(dioxane, Δε) 284 nm (+2·21), 345 nm (−1·29) (*c* 0·125). NMR ($(CD_3)_2SO$–D_2O) 7·62 (1H, *m*, H-1), 2·34 (2H, *s*, H-5), 5·31 (1H, *d*, J 5 Hz, H-7), 2·98 (1H, *m*, H-10), 2·15 (obscured, H-11), 3·93 (1H, *d*, J 11 Hz, H-12), 4·92 and 4·75 (2H, H-16), 1·54 (3H, *s*, H-17), 1·07 (3H, *d*, *J* 7 Hz, H-18), 1·66 (3H, *dd*, *J* 2 and 1 Hz, H-19), 3·71 (2H, *s*, H-20). NMR [$(CD_3)_2SO$] 4·68 (1H, *t*, *J* 5 Hz), 4·52 (1H, *d*, J 5·5 Hz), 5·00 (1H, *s*), 5·76 (1H, *s*). These signals disappeared upon addition of D_2O. MS *m/e* 362 (M^+). (Found: C, 65·78; H, 7·21. Calc. for $C_{20}H_{26}O_6$: C, 66·28; H, 7·23%). A mixture of **5** (20 mg), Ac_2O (1 ml) and C_5H_5N (1 ml) was allowed to stand 18 hr at 20°, poured into ice-H_2O, and the resultant soln was exhaustively extracted with EtOAc. The EtOAc was washed successively with dil. HCl, satd $NaHCO_3$ soln and H_2O, dried and concentrated to give a pale brown syrup which was crystallized from $CHCl_3$-petrol. Recrystallization from the same solvent mixture afforded fine colourless needles (19 mg), m.p. 123–125°. This product was shown to be a $CHCl_3$ solvate by the IR spectrum (760 cm^{-1}) and elemental analysis. Found: C, 53·04; H, 5·51. $C_{24}H_{30}O_8$·$CHCl_3$ requires: C, 53·09; H, 5·30%). After drying at 110° for 20 hr, solvent-free crystals of **6**, m.p. 191–192°, (Found: C, 64·26; H, 6·74. Calc. for $C_{24}H_{30}O_8$: C, 64·56; H, 6·77%), were obtained and identified with authentic bisdehydrophorbol-(12,20)-diacetate by m.m.p. and IR, NMR, ORD and CD spectra.

Acetylation of **1**. A mixture of **1** (12 mg), Ac_2O (0·3 ml) and C_5H_5N (0·3 ml) was kept 18 hr at 20° and concn. The residue was chromatographed over Al_2O_3 (1 g) with $CHCl_3$–Me_2CO (9:1) to give diacetate **7** (9 mg) as a colourless syrup $[\alpha]_D^{18}$ +52° (*c* 0·365, dioxane). ν_{max}^{Nujol} 3350, 1737, 1725, 1610, 1265, 1235 cm^{-1}. NMR (C_5D_5N–D_2O), 7·76 (1H, *m*, H-1), 2·98, 2·82 (2H, AB*q*, *J* 19 Hz, H-5), 6·06 (1H, H-7, overlapped by H-12), 3·83 (1H, *m*, H-8), 3·62 (1H, *m*, H-10), 6·04 (1H, *d*, *J* 10 Hz, H-12), 4·77 (2H, *s*, H-16 or H-20), 4·62 (2H, *s*, H-20 or H-16), 1·48 (3H, *s*, H-17), 1·74 (3H, *dd*, J 2 and 1 Hz, H-19), 2·11, 2·03, 1·90 (3 OAc), 0·88, 1·27, 2·43 (palmityl). MS *m/e* 744 (M^+), 684 (M^+-

HOAc), 624 (684–HOAc), 564 (624–HOAc), 489 (M^+-$Me(CH_2)_{14}CO_2$.), 429 (M^+-HOAc-$Me(CH_2)_{14}CO_2$.).

Methanolysis of **1**. A soln of **1** (3 mg) in 0·1% NaOMe in MeOH (1·1 ml) was kept at 20° for 14 hr, and treated with Amberlite IR-120 (H form). The resin was filtered with suction and the filtrate was concn. The residue was taken up in H_2O, and extracted with $CHCl_3$. The $CHCl_3$ was washed with H_2O, dried and evaporated to give a colourless syrup, which had an identical GLC Rt and MS to authentic methyl palmitate.

The aq. layer and the washing were combined and evaporated to dryness, and the residue was treated with Ac_2O (0·5 ml) and C_5H_5N (0·5 ml) at 20° for 15 hr. The reaction mixture was evaporated to afford the acetate (1·3 mg) as a colourless syrup. This acetate was identified with **3** by TLC (Et_2O and also $CHCl_3$–Me_2CO (3:1)), and by MS and ORD spectra.

Partial methanolysis of **1**. A soln of **1** (3 mg) in 0·1% NaOMe in MeOH (1·1 ml) was allowed to stand at 20°, and the reaction monitored by TLC ($CHCl_3$–Me_2CO, 1:1). The reaction was stopped after 2 hr by addition of HOAc (1 drop), and the solvent was distilled *in vacuo* to give a mixture which was fractionated by PLC using $CHCl_3$–Me_2CO (1:1). Recovery was carried out with MeOH, and the syrupy partial methanolyzate (0·2 mg) which had a lower R_f than **1** on TLC was obtained by evaporation of the eluant. Upon acetylation with Ac_2O and C_5H_5N (0·05 ml each), an acetate which was identical with **7** by MS was obtained.

Palmitylation of **2**. To a stirred soln of **2** (330 mg) in C_5H_5N (10 ml), palmitoyl chloride (1·3 ml) was added dropwise at 0°. The mixture was allowed to stand at 20° for 25 hr, poured into ice-H_2O (60 ml), and extracted with $CHCl_3$. The $CHCl_3$ soln was washed successively with 1 N HCl, 0·5 N NaOH and satd NaCl soln, dried and concentrated to yield a pale brown oily product, which was chromatographed on silicic acid (30 g) with $CHCl_3$; 10 ml fractions were collected. Evaporation of fractions 33–83 gave the tripalmitate **8** (158 mg) as a colourless viscous oil. $[\alpha]_D^{12}$ + 34·7° (*c* 0·72, dioxane). $\nu_{max}^{CHCl_3}$ 3360, 1720, 1620, 1200, 1240 cm^{-1}. NMR (C_5D_5N–D_2O) 7·74 (1H, *m*, H-1), 2·93, 2·80 (2H, AB*q*, *J* 19, H-5. These protons appear as a singlet at δ 2·86 in C_5D_5N.), 6·07 (1H, H-7, overlapped by H-12), 3·74 (1H, *m*, H-8), 3·50 (1H, *m*, H-10), *ca.* 2·9 (1H, *m*, H-11), 5·94 (1H, *d*, *J* 10 Hz, H-12), 4·68 (2H, *s*) and 4·72 (2H, *s*, H-16 and H-20), 1·48 (3H, *s*, H-17), 1·64 (3H, *d*, *J* 6 Hz, H-18), 1·76 (3H, *dd*, *J* 2 and 1, H-19), 2·09 (3H, *s*, OAc), 0·89, 1·28, 2·36 (palmityl). (Found: C, 72·70; H, 10·21. $C_{70}H_{120}O_{11}$.MeOH requires: C, 72·84; H, 10·60%). Evaporation of fractions 84–105 afforded the dipalmitate **9** which was recrystallized from MeOH to give fine colourless needles (224 mg), m.p. 84–85°. $[\alpha]_D^{18}$ +60° (*c* 0·3, dioxane). ν_{max}^{KBr} 3400, 1730, 1710, 1695, 1625, 1260 cm^{-1}. NMR (C_5D_5N–D_2O) 7·80 (1H, *m*, H-1), 6·08 (1H, *d*, *J* 5 Hz, H-7), 4·84 (2H, *s*, H-16 or H-20), 4·71 (2H, *s*, H-20 or H-16), 4·47 (1H, *d*, *J* 10 Hz, H-12), 3·80 (1H, *t*, *J* 5 Hz, H-8), 3·54 (1H, *m*, H-10), 2·97, 2·82 (2H, AB*q*, *J* 19 Hz, H-5), 2·67 (1H, *m*, H-11), 1·71 (3H, *dd*, *J* 2 and 1 Hz, H-19), 1·62 (3H, *d*, *J* 6 Hz, H-18), 1·47 (3H, *s*, H-17), 2·06 (3H, *s*, OAc), 0·88, 1·24, 2·29 (palmityl). (Found: C, 71·72; H, 10·11. $C_{54}H_{90}O_{10}$ requires: C, 72·11; H, 10·09%).

Selective hydrolysis of tripalmitate **8**. A soln of the tripalmitate **8** (128 mg) in MeOH (12 ml) containing 60% $HClO_4$ (0·1 ml) was kept at 20° for 62 hr. The reaction was stopped by adding NaOAc (200 mg), and the mixture was evaporated to yield a colourless oily residue which was dissolved in EtOAc. The EtOAc soln was washed with 0·5 N NaOH and satd NaCl soln, dried and evaporated to afford an oily residue, which was shown by TLC ($CHCl_3$–Me_2CO, 3:2) to be a mixture of 3 products (R_f 0·68, 0·30, 0·11 (trace)) in addition to a small amount of the starting material (R_f 0·85). This mixture was fractionated by PLC ($CHCl_3$–Me_2CO, 3:2) and the band R_f 0·68 area yielded upon elution and evaporation the dipalmitate **10** (49 mg) as a colourless syrup. $[\alpha]_D^{12}$ + 42·9° (*c* 1·17, dioxane). $\nu_{max}^{CHCl_3}$ 3350, 1723, 1625, 1200–1240 cm^{-1}. NMR (C_5D_5N–D_2O) 7·73 (1H, *m*, H-1), 2·99 (2H, *s*, H-5), 6·14 (1H, H-7, overlapped by H-12), 3·81 (1H, *m*, H-8), 3·61 (1H, *m*, H-10), *ca* 2·8 (1H, *m*, H-11), 6·03 (1H, *d*, *J* 10 Hz, H-12), 4·28 (2H, *s*, H-16 or H-20), 1·49 (3H, *s*, H-17), 1·63 (3H, *d*, *J* 6 Hz, H-18), 1·67 (3H, *dd*, *J* 2 and 1 Hz, H-19), 4·79 (2H, *s*, H-20 or H-16), 2·12 (3H, *s*, OAc), 0·89, 1·28, 2·36 (palmityl).

The product obtained from band R_f 0·30 area was recrystallized from MeOH–H_2O to afford the monopalmitate as colourless needles (11 mg), m.p. 173–175°, which were identical with **1** (m.m.p., and IR and ORD spectra).

Production of **5** *upon an attempt of acid catalyzed acetonide preparation from* **4**. A soln of **4** (70 mg), camphorsulfonic acid (20 mg) and 2,2-dimethoxypropane (1·4 ml) in DMF (0·5 ml) was stirred at 0° for 3 hr and poured into sat. $NaHCO_3$ soln, and extracted with EtOAc. The EtOAc extracts were washed with sat. NaCl soln, dried and evaporated to give a colourless oily residue which was purified by PLC ($CHCl_3$–Me_2CO, 1:3) to afford a crystalline mass (42 mg). Recrystallization from $CHCl_3$–Me_2CO gave colourless prisms, m.p. 247–249° (decomp.), which were identical with **5** (m.m.p. and IR and NMR spectra).

Evaluation of toxicity of **1** *to killie-fish*. The bioassay using killie-fish was carried out essentially in the same way as described by Kawazu *et al.* [8], and the toxicity of **1** was compared with that of rotenone. A test soln was prepared by adding a Me_2CO or MeOH soln (0·5 ml) of the compounds of known concentration into aerated H_2O (200 ml). The median tolerance limits were estimated by straight-line graphical interpolation [9].

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