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PHORBOL DERIVATIVES FROM *SAPIUM INSIGNE*

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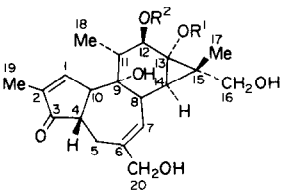
Abstract—From an ether extract of the twigs and leaves of *Sapium insigne* four new diterpene esters were isolated. They were identified as 12-*O*-(2′*E*, 4′*E*-decadienoyl)-4-deoxy-16-hydroxyphorbol-13-acetate, 12-*O*-hexanoyl-4α-deoxyphorbol-13-acetate, 12-*O*-hexanoyl-4α-deoxy-16-hydroxyphorbo-1-13-acetate and 12-*O*-dodecanoyl-4α-deoxy-16-hydroxyphorbol-13-acetate by spectroscopic and chemical methods.

INTRODUCTION

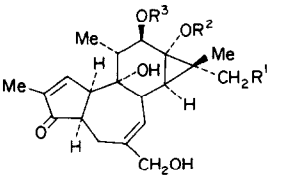
Sapium insigne is a small Indian tree which yields an acrid milky juice [1]. This juice acts as a vesicant and has been reported to cause dermatitis of the skin [1], although the nature of the toxic principles has not been elucidated. A related species from this genus, *Sapium japonicum*, has been found to contain a poisonous phorbol-ester [2] and, more recently, the toxic principles of *Sapium indicum* were identified as phorbol and deoxyphorbol esters, known as the sapintoxins [3, 4] and the sapatoxins [5]. A resin produced by ether extraction of the leaves and twigs of *Sapium insigne* demonstrated pronounced pro-inflammatory activity *in vivo* [6]. This communication describes the isolation and structure elucidation of four new phorbol derivatives from this resin.

RESULTS AND DISCUSSION

Four aliphatic tigliane diesters were isolated from *Sapium insigne*. The major compound, **1**, was responsible for the pro-inflammatory activity of the ether soluble resin produced from this plant. Compound **1** had an irritant dose 50% (ID_{50}) on mouse skin of 0.037 μg/per ear. From the mass spectrum of **1** it was evident that this biologically active compound was a diester of a polyhydroxylated tigliane diterpene and that the acyl substituents were acetate and decadienoate, respectively. Decadienoic acid can exist as one of four geometric isomers. The ^{1}H NMR spectrum of **1** assisted in the assignment of this acyl substituent as the all *trans* isomer 2′*E*, 4′*E*-decadienoic acid. The H-6′ signal in this spectrum appeared at δ 2.31 and irradiation of this signal induced the doublet of triplets at 5.87 to change to a doublet (J = 12 Hz) confirming the latter signal as the H-5′. It has been shown by synthetic methods and planar projection diagrams [7] that there is a loss of conjugation in isomers during the *trans–trans* to the *cis–trans* transition. This would be exhibited in the ^{1}H NMR spectrum by a downfield shift in the olefinic proton signals for the all-*trans* isomer due to the greater inductive effect of the carbonyl groups of the ester function This effect was exhibited in the ^{1}H NMR spectrum of **1** where H-6′, H-4′ and H-2′ were recorded at δ 2.31 (*m*), 7.64 (*dd*, J = 12, 15 Hz), and 5.94 (*d*, J = 15 Hz), respectively, when compared with the chemical shifts of a similar *cis–trans* isomer of 2.1, 7.35 and 5.50 [8]. Furthermore the IR spectrum of **1** exhibited a maximum at 1000 cm^{-1} which is characteristic of *trans–trans*-decadienoic acid methyl ester [7]. The parent diterpene of **1** was also identified from its ^{1}H NMR spectrum. In this spectrum the assignments for H-4, H-5, H-7 and H-11 were obtained by double resonance experiments thereby confirming that the nucleus of **1** belonged to the 4-deoxyphorbol rather than the phorbol series of esters. However, the 3H singlet for the Me-16 position of 4-deoxyphorbol was absent from this spectrum, whilst an extra 2H AB quartet was exhibited at δ 4.17. Compound **1** was accordingly a diester of 4-deoxy-16-hydroxyphorbol. The acetate function was assigned to C-13 and the decadienoate to C-12 on the basis of selective hydrolysis reactions. The ester was resistant to acid catalysed hydrolysis whilst alkaline catalysed hydrolysis produced the monoester, **5**, in which the acetate moiety was absent. In



1 R^1 = –COMe R^2 = –CO–CH$^{1'}$=$^{2'}$CH–CH$^{3'}$=$^{4'}$CH–CH$^{5'}$=CH–$(CH_2)_4$Me



2 R^1 = H, R^2 = –COMe, R^3 = –CO$(CH_2)_4$Me

3 R^1 = OH, R^2 = –COMe, R^3 = –CO$(CH_2)_4$Me

4 R^1 = OH, R^2 = –COMe, R^3 = –CO$(CH_2)_{10}$Me

5 R^1 = OH, R^2 = H, R^3 = –CO–CH^{E}=CH–CH^{E}=CH–$(CH_2)_4$Me

6 R^1 = H, R^2 = H, R^3 = –CO$(CH_2)_4$Me

the ^{1}H NMR spectrum of **5** there was a corresponding upfield shift in the signal for H-12 from δ 5.42 in **1** to 5.04 in **5**. This shift is known to be characteristic of esters which exhibit a free C-13 tertiary hydroxy group [9]. Furthermore, there were characteristic shifts in the signals for H-1, H-4, H-7 and H-10 in the spectrum of **5** due to alkaline catalysed conversion of the AB ring *trans* junction of **1** to the *cis*-orientated configuration in **5** [10]

Compound **2** was identified as a diester of 4α-deoxyphorbol from its ^{1}H NMR and mass spectra [10]. The acyl moieties were acetate and hexanoate, respectively. Alkaline catalysed hydrolysis of **2** produced the monoester **6** The mass spectrum of **6** exhibited a $[M]^+$ ion at *m/z* 446, confirming that the hexanoate group was located at C-12 of the 4α-deoxyphorbol nucleus

Compounds **3** and **4** were isolated as a mixture and differed only in the chain length of their C-12 acyl group They were identified by their ^{1}H NMR spectra, which demonstrated the characteristic signals of a 4α-deoxy-16-hydroxyphorbol diester [11] with acetate and straight chain aliphatic ester moieties Multiple development TLC showed the mixture to consist of two compounds, and in the mass spectrum $[M]^+$ ions were observed at *m/z* 588 and 504 In both compounds the aliphatic acid was eliminated as an acyloxy ion, RCOO , which occurs in phorbol-type 12,13-diesters where the longer acyl group is positioned at C-12 [12] Accordingly, although paucity of material precluded hydrolysis reactions, the acetate moiety could be assigned to C-13 and the aliphatic ester group to C-12 in both cases

4-Deoxy-16-hydroxyphorbol esters are rare in the Euphorbiaceae, having been previously isolated only from *Croton flavens* [11] It is interesting to note that, although the major compound from *Sapium insigne* is a 4-deoxy-16-hydroxyphorbol ester, several 4α-deoxyphorbol derivatives were isolated as minor products. It is possible that these AB ring *cis* compounds are artefacts of isolation, having been formed by epimerization of the corresponding naturally occurring AB *trans* analogues [13] However, no evidence was found for the presence of the 4α-epimer of the major compound (**1**) which is known to be susceptible to epimerization

EXPERIMENTAL

Dried leaves and twigs (1 kg) of *Sapium insigne* Benth were powdered and extracted by cold maceration with Me_2CO After removal of Me_2CO under red. pres below 45°, the residue was dissolved in aq MeOH and extracted with C_6H_{14} to remove lipid material and steroids The MeOH phase was partitioned with Et_2O and the Et_2O phase washed with H_2O Evaporation of the Et_2O produced a resinous material (2 g) which was separated by CC on Florosil, heated at 110° for 60 min and partially deactivated with 5% H_2O The following solvent gradient was employed toluene–C_6H_{14} (3 2, 100 ml), toluene (100 ml), toluene–EtOAc, 9 1 (200 ml), 8 1 (270 ml), 7 1 (160 ml), 6 1 (175 ml), 5 1 (180 ml), 4 1 (200 ml), 3 1 (200 ml), 2 1 (210 ml), 1 1 (200 ml), 1 2 (210 ml), 1 3 (200 ml), 1 4 (200 ml), 1 5 (180 ml), 1 6 (210 ml), 1 7 (160 ml), 1 8 (180 ml), 1 9 (200 ml), EtOAc (200 ml); Me_2CO (500 ml)

12-O-(2E,4E-*decadienoyl*)-4-*deoxy*-16-*hydroxyphorbol*-13-*acetate* (**1**) Compound **1** (18 mg colourless resin) was present in the fractions eluted from the column with toluene–EtOAc, 3 1, 2 1, 1 1 and 1 2 It was initially isolated by TLC on Si gel G developing twice with EtOAc–cyclohexane (7 3) (R_f 0 39) Compound **1** gave a dark brown colour when sprayed with H_2SO_4 and heated It was finally purified by partition TLC on Kieselguhr G coated with 20% diethylene glycol, eluting twice with cyclohexane–butanone (95 5) (R_f 0.33) Compound **1** gave a single spot in several TLC systems and exhibited the following spectral characteristics EIMS (190°, 70 eV, measured values within 10 ppm calculated values) *m/z* (rel int) 556 $[M]^+$ (1, $C_{32}O_8H_{44}$), 538 (1), 520 (1), 496 (3), 478 (4), 460 (4), 388 (2), 370 (3), 328 (18), 310 (35), 292 (20), 208 (30), 180 (40), 151 (100), 121 (83), 81 (67) UV λ_{max}^{MeOH} (log ε) nm 204 (4 09), 262 (4 13) IR ν_{max}^{KBr} cm^{-1} 3410, 1720, 1705, 1625, 1000 ^{1}H NMR (250 MHz, $CDCl_3$) δ 7 64 (*dd*, *J* = 11 8, 15 1 Hz, 1H-olefinic), 7 59 (*s*, H-1), 6 15 (*t*, *J* = 11 8 Hz, 1H-olefinic), 5 94 (*d*, *J* = 15 1 Hz, 1H-olefinic), 5 87 (*t*, *J* = 18 8 Hz, 1H-olefinic), 5 65 (*d*, *J* = 5 5 Hz, H-7), 5 61 (*s*, 1H exchangeable with D_2O), 5 48 (*s*, 1H exchangeable with D_2O), 5 42 (*d*, *J* = 9 9 Hz, H-12), 4 17 (AB*q*, J_{AB} = 23 9 Hz, 2H-16), 4 02 (AB*q*, J_{AB} = 16 3 Hz, 2H-20), 3 29 (*m*, H-10), 3 08 (*m*, H-8), 2 51 (*br s*, 2H-5), 2 31 (*dd*, *J* = 15 1, 7 4 Hz, $-CH_2-$, H-4), 2 05 (*s*, MeCO–), 1 77 (*d*, *J* = 1 5 Hz, 3H-19), 1 73 (superimposed on δ 1 77, *m*, H-11), 1 25 (*s*, $-(CH_2)_n-$), 1 18 (*s*, 3H-17), 1 15 (*br s*, H-14), 0 89 (*m*, 3H-18, Me–) Irradiation of the signal at δ 3 08 (H-8) induced the doublet at 5 65 (H-7) to form a singlet, and irradiation of the doublet at 5 42 (H-12) induced the multiplet at 1 73 (H-11) to form a broad singlet Furthermore, irradiation of the signal at δ 2 31 (H-4, $-CH_2$) caused the broad singlet at 2 51 (2H-5) to sharpen and the triplet at 5 87 (1H-olefinic) to change from a triplet to a doublet

Compound **1** was unaffected by incubation with 10% perchloric acid–MeOH at room temp for 24 hr, but with 0 1 M KOH–MeOH at room temp it was readily hydrolysed to yield the monoester, 12-*O*-(2*E*,4*E*,decadienoyl)-4α-deoxy-16-hydroxyphorbol (**5**) EIMS *m/z* (rel int) 514 $[M]^+$ (1, $C_{30}O_7H_{42}$), ^{1}H NMR (similar to that of **1**, with the following exceptions) δ 7 08 (H-1), 5 14 (H-7), 5 04 (H-12), 3 54 (H-10), 3 42 (*m*, H-5α), 2.80 (H-4), 2 58 (H-5β) The acetate signal at δ 2 05 was absent in the spectrum of **5**

12-O-*Hexanoyl*-4α-*deoxyphorbol*-13-*acetate* (**2**) This natural compound (6 mg colourless resin) was eluted from the column by toluene–EtOAc, 4 1, 3 1 and 2 1, and was initially purified by TLC on Si gel G eluting twice with EtOAc–cyclohexane (7 3) (R_f 0 40) On spraying with H_2SO_4 and heating, **2** gave a yellow-brown colour and was finally purified by partition TLC as previously described, developing with cyclohexane–EtOAc (95 5) (R_f 0.64) EIMS (70 eV, 180°) *m/z* (rel int) 488 $[M]^+$ (< 0 5, $C_{28}O_7H_{40}$), 470 (< 0 5), 428 (< 0 5), 372 (< 0 5), 329 (1), 312 (60), 294 (100) CIMS (isobutane, 195°) *m/z* (rel int) 489 $[M+1]^+$ (5), 471 (11), 429 (5), 411 (5), 373 (35), 356 (35), 355 (100), 313 (41), 295 (43). UV λ_{max}^{EtOH} nm (log ε) 208 (3 94), 263 (3 47). IR ν_{max}^{KBr} cm^{-1} 3400, 1725, 1460, 1375, 1245, 1160 ^{1}H NMR (250 MHz, $CDCl_3$) δ 7 04 (*s*, H-1), 5 47 (*d*, *J* = 10 3 Hz, H-12), 5 15 (1H exchangeable with D_2O), 5 12 (*d*, *J* = 6 0 Hz, H-7), 3 95 (AB*q*, J_{AB} = 28 0 Hz, 2H-20), 3 49 (*m*, H-10), 3 40 (*m*, H-5α), 2 80 (*m*, H-4), 2 49 (*dd*, *J* = 4 41, 15 44 Hz, H-5β), 2 43–2 31 (*m*, H-8, OC–CH_2–), 2 06 (*s*, MeCO–), 1 79 (*m*, 3H-19), 1 68 (*m*, H-11), 1 25 (*s*, 3H-16), 1 19 (*s*, $-(CH_2)_3-$), 1 17 (*s*, 3H-17), 1 09 (*d*, *J* = 6 3 Hz, 3H-18), 0 92 (*m*, Me–), 0 79 (*d*, *J* = 5 2 Hz, H-14)

Mild alkaline hydrolysis of **2** with 0 1 M KOH–MeOH produced a monoester 12-*O*-hexanoyl-4α-deoxyphorbol (**6**) EIMS (70 eV, 190°) *m/z* (rel int) 446 $[M]^+$ (10, $C_{26}O_6H_{38}$), 428 (16), 330 (10), 312 (38), 294 (65) ^{1}H NMR (250 MHz, $CDCl_3$) δ 7 08 (*s*, H-1), 5 35 (*br s*, H-7), 4 92 (*d*, *J* = 9 9 Hz, H-12), 3 95 (AB*q*, J_{AB} = 20.0 Hz, 2H-20), 3 65 (*m*, H-10), 3 54 (*m*, H-5α), 2 80 (*m*, H-4), 2 57 (*dd*, *J* = 5, 15 Hz, H-5β), 2 45–2 36 (*m*, H-8, –OC–CH_2–), 1.80 (*m*, 3H-19), 1 25 (*s*, 3H-16, $-(CH_2)_3-$), 1 17 (*s*, 3H-17), 1 06 (*d*, *J* = 7 67 Hz, 3H-18), 0 90 (*m*, Me–), 0 65 (*d*, *J* = 5 85 Hz, H-14)

12-O-*Hexanoyl*-4α-*deoxy*-16-*hydroxyphorbol*-13-*acetate* (**3**)

and 12-O-*dodecanoyl*-4α-*deoxy*-16-*hydroxyphorbol*-13-*acetate* (4) These were isolated as an inseparable mixture (2.5 mg colourless resin) eluted from the column with toluene–EtOAc, 3 1, 2 1, 1 1 Isolation was achieved by TLC on Si gel G eluting with $CHCl_3$–Me_2CO (5 4) (R_f 0 50) followed by final purification on partition TLC as before, developing twice with cyclohexane–EtOAc (9.1) (R_f 0 40). The mixture was a single spot in these systems, giving a brown colour after spraying with H_2SO_4 and heating EIMS (70 eV, 190°) *m/z* (rel int.). 588 $[M]^+$ (< 0.5, $C_{34}O_8H_{52}$), 546 (< 0 5), 528 (2), 504 $[M]^+$ (1, $C_{28}O_8H_{40}$), 486 (3), 444 (7), 426 (11), 389 (8), 370 (10), 328 (28), 310 (67), 292 (39), 99 (100). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7 05 (*br s*, H-1), 5.46 (*d*, *J* = 10 6 Hz, H-12), 5.09 (*br s*, H-7), 3.98 (AB*q*, J_{AB} = 20.0 Hz, 2H-20), 3 95 (*q*, *J* = 19 Hz, 2H-16), 3 51 (*m*, H-10), 3.39 (*m*, H-5α), 2 81 (*m*, H-4), 2 51 (*dd*, *J* = 4.0, 14 6 Hz, H-5β), 2.34 (*m*, H-8, –CO–CH_2–), 2.07 (*s*, Me–CO), 1 79 (*br s*, 3H-19), 1.70 (*m*, H-11), 1 25 (*s*, –$(CH_2)_n$–, 3H-17), 1 00 (*d*, *J* = 7.3 Hz, 3H-18), 0 88 (*m*, Me–), 0.82 (*m*, H-14).

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