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SAPATOXINS, ALIPHATIC ESTER TIGLIANE DITERPENES FROM *SAPIUM INDICUM*

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Abstract—From the unripe fruits of *Sapium indicum*, three aliphatic esters of the tigliane nucleus were isolated. These compounds were derivatives of 4-deoxyphorbol. Sapatoxin A was identified as 12-*O*-[*n*-deca-2,4,6-trienoyl]-4-deoxyphorbol-13-acetate, B as 12-*O*-[*n*-deca-2,4,6-trienoyl]-4-deoxy-5-hydroxyphorbol-13-acetate and C as 12-*O*-[*n*-deca-2,4,6-trienoyl]-4,20-dideoxy-5-hydroxyphorbol-13-acetate, by spectroscopic analysis and hydrolysis reactions.

INTRODUCTION

Sapium indicum is a toxic species of the family Euphorbiaceae [1] and extracts induce erythema of skin *in vivo* using established methods [2]. From this plant it has recently been shown that the major toxic constituents are the novel nitrogen-containing phorbol esters known as the sapintoxins [3, 4]. These are distinctive blue UV-fluorescent substances but they were isolated together with a number of non-fluorescent compounds. The two groups of compounds could be separated by partition chromatographic methods [5]. This communication describes the separation and structure of the non-fluorescent biologically active substances known as sapatoxins, aliphatic esters of deoxyphorbols.

RESULTS AND DISCUSSION

Three aliphatic tigliane esters were isolated from the fruit extract of *S. indicum*. The major compound, sapatoxin A, had a similar ^{1}H NMR and mass spectrum to ester Ti$_1$ isolated from *Euphorbia tirucalli* [6]. Alkali-catalysed hydrolysis of **1** produced two products. The first after conversion to its acetate was identical to 4α-deoxyphorbol triacetate [6, 7], whilst the second product was identical to *n* - deca - 2,4,6 - trienoic acid methyl ester [6, 8]. The 2′*Z* and the 4′*E* configuration of this acid was evident from the ^{1}H NMR spectrum [6]. Sapatoxin A was therefore the previously known 12 - *O* - [*n* - deca - 2,4,6 - trienoyl] - 4 - deoxyphorbol - 13 - acetate **1** (Fig. 1).

Two further esters, sapatoxin B **2** and sapatoxin C **5**, were also isolated from the extract. From the ^{1}H NMR and mass spectra both **2** and **5** exhibited acetate and *n* - deca - 2,4,6 trienoate groups in their structure. However, the nuclei of **2** and **5** differed from that of **1**. Compound **2** was a diester of 4 - deoxy - 5 - hydroxyphorbol confirmed by decoupling of the 1H-4 and 1H-8 signals in its ^{1}H NMR spectrum. Mild alkali-catalysed hydrolysis of **2** produced **3**, 12 - *O* - [*n* - deca - 2,4,6 - trienoyl] - 4α - deoxy - 5 - hydroxyphorbol, by removal of the reactive tertiary acetyl group. Compound **3** was converted to its triacetate **4**. The characteristic signs of the Cotton effects in the CD spectrum of **4** when compared to those of **2**, together with the shift in the signals for 1H-1, 1H-4 and 1H-10 in their ^{1}H NMR spectra indicated that the AB *trans* junction of **2** had converted to the *cis* configuration during alkaline hydrolysis. This conversion is typical of phorbol esters which do not possess an OH group at C-4 of the nucleus [6, 7]. Sapatoxin B, **2**, was assigned as the new phorbol ester 12 - *O* - [*n* - deca - 2,4,6 - trienoyl] - 4 - deoxy - 5 - hydroxyphorbol - 13 - acetate (Fig. 1). The third ester, sapatoxin C, **5**, was isolated in only small quantities

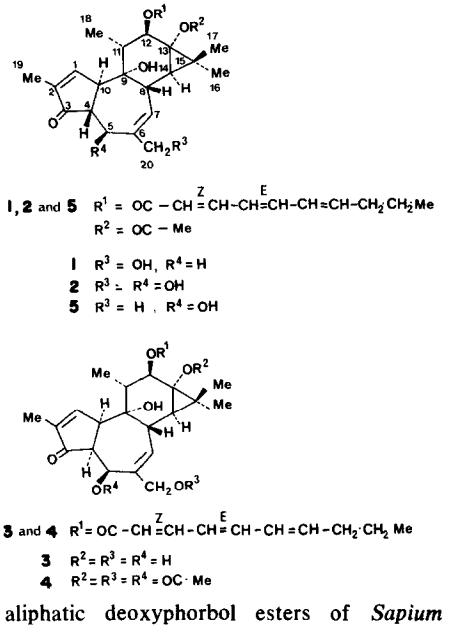

Fig. 1. The aliphatic deoxyphorbol esters of *Sapium indicum* L.

from the fruit extract, occurring in a yield of 3.5 mg/kg of dried fruits. The ^{1}H NMR spectrum of **5** was similar to that of **2**. However, the absence of the 2H AB quartet at 4.24 ppm in the spectrum of **5** and the appearence of a new 3H signal at 1.85 ppm suggested that this minor product was a new diester of 4,20 - dideoxy - 5 - hydroxy - phorbol. The *n* - deca - 2,4,6 - trienoate of **5** was tentatively assigned to C-12 and the acetate to C-13 on the basis of the IR and mass spectra. It has been shown by synthetic methods [9] that phorbol esters with a long-chain acyl group at C-12 exhibit a broad band in their IR spectra at 1700 cm^{-1} as is the case for **5**. Furthermore, the mass spectra of such esters exhibit a characteristic $M^{+\cdot}$–RCOO$^{\cdot}$ ion due to elimination of the acid as the acyloxy radical. In the case of **5** this ion was evident at *m/z* 373. Sapatoxin C was therefore assigned as 12 - *O* - [*n* - deca - 2,4,6 - trienoyl] - 4,20 - dideoxy - 5 - hydroxyphorbol - 13 - acetate (Fig. 1).

S. indicum contains two series of toxic principles based upon deoxyphorbol diterpenes. The major toxins were the nitrogen-containing sapintoxins [3, 4] whilst the aliphatic analogues the sapatoxins occurred in smaller quantities from the unripe fruits.

EXPERIMENTAL

An Et_2O-soluble resin was prepared from 2 kg of powdered *S. indicum* L. fruits. This resin was separated by centrifugal-liquid chromatography (CLC) using a 4-min Si gel disc and a gradient of toluene to EtOAc as previously described [4]. Sapatoxin A was eluted with EtOAc–toluene (7:3), B with EtOAc–toluene (9:1) and C with EtOAc–toluene (1:1).

Sapatoxin A **1**. 12 - O - [n - *deca* - 2,4,6 - *trienoyl*] - 4 - *deoxy phorbol* - 13 - *acetate*. Yield 100 mg. **1** was purified by TLC on Si gel G and EtOAc–cyclohexane (7:3) as solvent (R_f 0.25). Final purification was achieved by partition TLC on kieselguhr G coated with 20% digol and developing twice with cyclohexane–butanone (4:1) (R_f 0.79). EI/MS (250°, 70 eV), *m/z* 373 (7), 312 (22), 294 (24), 149 (100), 107 (65), 91 (87). In the CIMS (195°, *iso*-butane) an $M^{+\cdot}$ ion was exhibited at *m/z* 538 (2). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.563 (*s*, 1H-1), 7.38 (*dd*, *J* = 15 Hz, H-4'), 6.67–5.65 (4H, olefinic), 5.58 (*d*, *J* = 10.4 Hz, H-2'), 5.658 (*s*, 1H exchangeable with D_2O), 5.545 (*d*, *J* = 1.29 Hz, 1H-7), 5.468 (*d*, *J* = 9.74 Hz, 1H-12), 4.024 (*s*, 2H-20), 3.256 (*m*, 1H-10), 2.829 (*dd*, *J* = 9.01 Hz, 2H-5), 2.587 (*m*, 1H-4), 2.379 (*m*, 1H-8), 2.130 (5H, $CH_3CO^{\cdot}$, 2H-8'), 1.732 (*s*,3H-19), 1.624 (*m*, 1H-11), 1.446 (*q*, *J* = 7.35 Hz, 2H-9'), 1.258 (*s*, 3H-16), 1.203 (*s*, 3H-17), 1.113 (*d*, *J* = 7.17 Hz, H-14), 0.941 (*m*, 6H, 3H-18, 3H-10') ppm. **1** was hydrolysed with 1% NaOMe in MeOH. The parent alcohol after acetylation was identical to 4α-deoxy phorbol - 12,13,20 - triacetate [6, 7], and the acyl moiety, isolated as its Me ester was *n* - deca - 2,4,6 - trienoic acid [6, 8].

Sapatoxin B **2**. 12 - O - [n - *deca* - 2,4,6 - *trienoyl*] - 4 - *deoxy* - 5 - *hydroxyphorbol* - 13 - *acetate*. Yield 30 mg. **2** was purified by TLC on Si gel G using cyclohexane–toluene–EtOAC–Et_2O (4:3:8:6) and developing ×3 (R_f 0.22). Final purification was partition TLC as before using cyclohexane–butanone (7:3) (R_f 0.6). UV λ_{max}^{MeOH} nm (log ϵ): 304 (4.41). IR (KBr disc) ν_{max} cm^{-1}: 3400, 1720, 1705, 1685, 1610 and 1575 cm^{-1}. EI/MS (250°, 70 eV), *m/z* 536 (1), 494 (3), 389 (6), 371 (9), 353 (7), 311 (18), 293 (11), 149 (100), 107 (58), 91 (25). By means of CI (195°, *iso*-butane) an $M^{+\cdot}$ ion was observed at *m/z* 554 (5). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.689 (*s*, 1H-1), 7.38 (*dd*, *J* = 15 Hz, H-4'), 6.67–5.65 (4H, olefinic), 5.58 (*d*, *J* = 10.4 Hz, H-2'), 5.664 (*s*, 1H, exchangeable with D_2O), 5.584 (*d*, *J* = 1.29 Hz, 1H-7), 5.451 (*d*, *J* = 9.56 Hz, 1H-12), 5.171 (*d*, *J* = 3.31 Hz, 1H-5), 4.245 (AB *q*, *J* A/B = 27.44 Hz, 2H-20), 3.66 (*s*, 1H, exchangeable with D_2O), 3.557 (*m*, H-10), 2.583 (*m*, 1H-4), 2.277 (*m*, 1H-8), 2.13 (5H, $CH_3CO^{\cdot}$ 2H-8'), 1.757 (*s*, 3H-19), 1.624 (*m*, 1H-11), 1.446 (*q*, *J* = 7.35 Hz, 2H-9'), 1.254 (*s*, 3H-16), 1.207 (*s*, 3H-17), 1.083 (*d*, *J* = 5.15 Hz, H-14), 0.917 (*m*, 6H, 3H-18, 3H-10') ppm. Irradiation at 2.58 ppm (1H-4) induced the doublet at 5.171 ppm (1H-5) to form a sharp singlet and the multiplet at 3.557 ppm (1H-10) to form a broad singlet, whilst irradiation at 2.28 ppm (1H-8) induced the doublets at 5.584 ppm (1H-7) and 1.083 (1H-14) to become singlets. CD (MeOH), nm: 208 (θ − 17886), 230 (θ + 26103), 305 (θ − 14949). Compound **2** was hydrolysed with 0.1 M KOH in MeOH for 20 min. The 4α-mono-ester **3** was isolated from the reaction by TLC on Si gel G using $CHCl_3$–Me_2CO–EtOAc (1:1:1) (R_f 0.33). In the EIMS significant ions were exhibited at *m/z* 512 ($M^{+\cdot}$, 2), 484 (1), 368 (6), 329 (10), 310 (31), 292 (13), 149 (30), 107 (50), 91 (100). The ^{1}H NMR (60 MHz, $CDCl_3$) exhibited signals at δ 7.52–5.55 (6H, olefinic), 7.12 (*s*, 1H-1), 5.37 (*br s*, 1H-7), 5.1 (*d*, 1H-12), 4.77 (*br s*, 1H-5), 4.1 (*br s*, 2H-20), 3.69 (*m*, 1H-10), 3.21 (*m*, 1H-4), 2.3 (*m*, 1H-8), 2.1 (*m*, 2H-8'), 1.78 (*s*, 3H-19), 1.45 (*m*, 2H-9'), 1.26 (*s*, 3H-16), 1.17 (*s*, 3H-17), 0.97 (*m*, 3H-18, 3H-10', 1H-14), 3.73, 4.25, 5.54 (3 × 1H, *s*, exchangeable with D_2O) ppm. Compound **3** was acetylated with Ac_2O–pyridine (2:1) for 12 hr at 4°. A single triacetate **4** was isolated by TLC on Si gel G as before (R_f 0.6). The ^{1}H NMR of **4** was similar to **3** with the following exceptions, δ 6.03 (*br s*, 1H-5), 5.6 (*d*, 1H-12), 4.58 (*br s*, 2H-20), 2.2 (9H, $CH_3CO^{\cdot}$ × 3) ppm. The D_2O exchangeable signals at 4.25 and 3.73 in **3** were absent in **4**. The CD spectrum (MeOH) exhibited Cotton effects at 256 (θ − 4521), 321 (θ + 1386), 380 (θ + 396) nm.

Sapatoxin C **5**. 12 - O - [n - *deca* - 2,4,6 - *trienoyl*] - 4,20 - *dideoxy* - 5 - *hydroxy phorbol* - 13 - *acetate*. Yield 7 mg. **5** was purified by TLC on Si gel G using EtOAc–cyclohexane (7:3) (R_f 0.43) and finally purified by partition TLC using cyclohexane–butanone (4:1) (R_f 0.75). UV λ_{max}^{MeOH} (log ϵ) 304 (3.98) nm. IR (KBr disc) ν_{max} cm^{-1}: 3400, 1720, 1695, 1685, 1610, 1570. EI/MS (70 ev, 185°) *m/z* 538 ($M^{+\cdot}$, 2), 520 (2), 478 (3), 460 (4), 373 (27), 355 (10), 329 (6), 312 (14), 294 (12), 149 (100), 107 (43), 91 (17). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.692 (*s*, 1H-1), 7.38 (*dd*, *J* = 15 Hz, 1H-4'), 6.67–5.65 (4H, olefinic). 5.58 (*d*, *J* = 10.4 Hz, 1H-2'), 5.571 (*s*, 1H, exchangeable with D_2O), 5.449 (*d*, *J* = 9,56 Hz, 1H-12), 5.286 (*d*, *J* = 1.3 Hz, 1H-7), 4.839 (*br s*, 1H-5), 3.512 (*m*, H-10), 2.592 (*t*, *J* = 4.41 Hz, 1H-4), 2.181 (*m*, 1H-8), 2.126 (5H, $CH_3CO^{\cdot}$, 2H-8'), 1.857 (*s*, 3H-20), 1.754 (*s*, 3H-19), 1.617 (*m*, 1H-11), 1.446 (*q*, *J* = 7.72 Hz, 2H-9'), 1.208 (*s*, 3H-16), 1.72 (*s*, 3H-17), 1.036 (*d*, *J* = 5.15 Hz, 1H-14), 0.916 (*m*, 6H, 3H-18, 3H-10') ppm. Irradiation of the signal at 2.59 ppm induced the signal at 4.839 (1H-5) to become a sharp singlet and the multiplet at 3.512 ppm to become a singlet. CD (MeOH) nm 204 (θ − 37587), 228 (θ + 36795), 268 (θ − 8019), 307 (θ − 6666).

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