SAPINTOXIN D, A NEW PHORBOL ESTER FROM *SAPIUM INDICUM*

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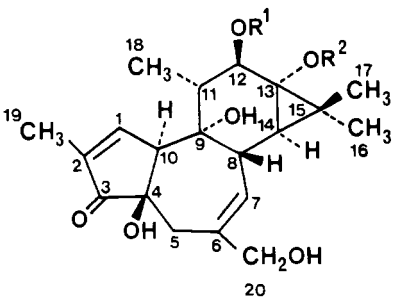
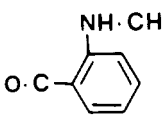
ABSTRACT.—From the unripe fruits of *Sapium indicum* L. (Euphorbiaceae) a biologically active nitrogen-containing phorbol ester was isolated by a combination of centrifugal liquid chromatography and thin-layer chromatography. This compound was identified as 12-O-[*N*-methylaminobenzoyl]-phorbol-13-acetate (sapintoxin D) by spectroscopic and hydrolysis methods. Sapintoxin D occurred in a yield of 5 mgm per kilogram of dried fruits.

The phorbol esters have been isolated as the toxic constituents of several genera of the family Euphorbiaceae. In particular, the genus *Euphorbia* has been shown to produce a variety of related diterpenes (1). The genus *Sapium*, also of the family Euphorbiaceae, has been less well investigated phytochemically despite the fact that these plants are a known toxicological hazard (2). Phorbol traditionally occurs in plants as a series of long chain aliphatic diesters, as in the case of tetradecanoylphorbol-acetate (TPA), the tumor-promoting constituent of *Croton tiglium* (3). A similar long chain aliphatic phorbol diester has previously been isolated from *Sapium japonicum* (4). Our previous investigations of *S. indicum* have shown that the major toxic constituents of this plant are 4-deoxyphorbol and 4-deoxy-5-hydroxyphorbol esters (5, 6) in contrast to the phorbol esters of *S. japonicum*. Furthermore, these esters all exhibited an *N*-methylamino-benzoate moiety and represent the first biologically active tigliane derivatives to be obtained containing a nitrogen atom in their structure. Re-examination of *S. indicum* fruits demonstrated the presence of a minor bright blue uv fluorescent compound in a yield of 5 mg per kilogram of dried plant material. We report here the isolation of this new nitrogen-containing phorbol ester.

FIGURE 1



20

R^1	R^2	Compound
	C·O·CH$_3$	1
H	H	2

The ether soluble fraction of the fruit oil was separated by gradient elution centrifugal liquid chromatography (clc). Sapintoxin D (**1**) was present in the toluene-ethylacetate (1:9) fraction from this separation. Compound **1** was finally purified by adsorption thin-layer-chromatography (tlc) on silica gel G as adsorbent followed by partition tlc with 20% digol coated on kieselguhr G. A resin was produced which was shown to be a single entity on the basis of analytical tlc and mass-spectrometry (ms). Compound **1** induced both a pronounced erythema of mammalian skin (7) and a dose-dependent aggregation of human and rabbit blood platelets (8).

Hydrolysis of **1** with 0.5M sodium methoxide afforded two products. The first was the polyol **2**, which was identified as phorbol (ms, ^{1}H-nmr), and the second was a uv blue fluorescent methyl ester which was identical to the methyl ester synthesized from commercially available *N*-methylamino benzoic acid (ms, ^{1}H-nmr). The ^{1}H-nmr spectrum of **1** was typical of a phorbol diester (1) with a free C-20 primary hydroxy group and *N*-methylaminobenzoate and acetate moieties at the C-12 and C-13 positions (5, 6). This was confirmed by the electron impact ms where a 5% relative abundance $M^{+\cdot}$ ion was evident at *m/z* 539, and a base peak corresponding to *N*-methylaminobenzoic acid was exhibited at *m/z* 151. It has been shown by synthetic methods that in the ms of phorbol diesters (3) where the higher molecular weight acyl derivative is at C-12 of the nucleus this group is eliminated as the acyloxy ion RCOO·, with the consequent formation of a prominent $M^{+\cdot}$–RCOO· ion. In the ms of **1**, this ion was observed at *m/z* 389. Furthermore, it has also been shown (3) that phorbol diesters with a high molecular weight acyl group at C-12 exhibit a rounded band in their infra-red spectrum (ir) at 1700 cm^{-1} as is the case for compound **1**. Sapintoxin D (**1**) was accordingly assigned as 12-*O*-[*N*-methylaminobenzoyl]-phorbol-13-acetate (fig. 1). Although nitrogen-containing 4-deoxyphorbol esters have been obtained from natural sources,sapintoxin D is the first phorbol ester to be isolated which contains an *N*-methylaminobenzoate group.

EXPERIMENTAL

PLANT MATERIAL.—Samples of *Sapium indicum* L. used in this study were collected and authenticated by M. A. Gafur and A. K. Choudhury in Bangladesh. Dried unripe fruits were received at the School of Pharmacy, London, and voucher specimens have been retained.

EXTRACTION.—Powdered fruits were extracted with acetone, and the oily residue left after removal of the solvent was dissolved in 40% methanol. The lipids, triterpenoids, and steroids were removed from this extract by partition with hexane. The methanolic phase was re-extracted with ether, and the ether was removed by distillation to yield a brown pro-inflammatory resin.

SEPARATION.—About 8 g of brown resin was separated into fractions by gradient elution centrifugal liquid chromatography on a 4 mm silica gel disc at a solvent flow of 4 mls/min. A gradient from toluene to ethylacetate was used as previously described (5). The toluene-ethylacetate (1:9) fraction contained compound **1** in low yield. This fraction, after removal of the solvent by distillation, was further purified by tlc on silica gel G 500 μ layers with cyclohexane-toluene-ethyl acetate-ether (20:15:40:30) as solvent (R_f 0.1) and, finally, by means of partition tlc with kieselguhr G 500 μ coated with 20% digol and developed twice with cyclohexane-butanone (6:4) (R_f 0.5).

SPECTRAL DATA FOR SAPINTOXIN D.—Ms (70 e.v. 195°C), *m/z* 539 ($C_{30}H_{37}O_8N$, 5%, $M^{+\cdot}$), 479 (1%), 389 (14%), 329 (10%), 311 (12%), 151 (100%); uv, λ max (MeOH) (log ϵ); 222 (4.57), 252 (4.19), 356 (3.88) nm; ir (KBr disc); ν max, 3380, 1720, 1685, 1580, 1520, 970, 750 cm^{-1}; ^{1}H-nmr (250 MHz, $CDCl_3$, TMS=0.000 ppm), δ 7.854 (1H, d.d., J=8.09, 1.84 Hz, aromatic), 7.706 (1H, s, exchangeable with D_2O), 7.616 (s, H-1), 7.398 (1H, t, J=8.46, 1.47 Hz, aromatic), 6.685 (1H, d, J=8.82 Hz, aromatic), 6.584 (1H, t, J=8.09 Hz, aromatic), 5.708 (1H, J=4.78 Hz, H-7), 5.667 (1H, s, exchangeable with D_2O), 5.633 (1H, d, J=10.29 Hz, H-12), 4.034 (2H, d, J=4.74 Hz, 2H-20), 3.296 (2H, m, H-8, H-10), 2.927 (d, J=5.15 Hz, N-C*H*$_3$), 2.568 (2H, s, 2H-5), 2.125 (3H, s, C*H*$_3$.CO-), 1.774 (3H, m, 3H-19), 1.254 (6H, s, 3H-16, 3H-17), 0.927 (4H, m, H-14, 3H-18) ppm.

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LITERATURE CITED

1. F. J. Evans and C. J. Soper, *Lloydia*, **41**, 193 (1978).
2. R. N. Chopra, I. C. Chopra, K. L. Handa and L. D. Kapur, "Indigenous Drugs of India", 2nd Edition, U.N. Dhur & Sons, Ltd., Calcutta-12, 1958, p. 589.
3. E. Hecker and R. Schmidt, Progress in the Chemistry of Organic Natural Products, **31**, 377 (1974).
4. H. Ohigashi, K. Kawazu, K. Koshimizu and J. Mitsui, *Agr. Biol. Chem.*, **36**, 2529 (1972).
5. S. E. Taylor, M. A. Gafur, A. K. Choudhury and F. J. Evans, *Experientia*, **37**, 681 (1981).
6. S. E. Taylor, M. A. Gafur, A. K. Choudhury and F. J. Evans, *Phytochemistry*. In press (1981).
7. F. J. Evans and R. J. Schmidt, *Inflammation*, **3**, 215 (1979).
8. J. Westwick, E. M. Williamson and F. J. Evans, *Thrombosis Res.*, **20**, 683 (1980).