also supported this formula. Compound **1** showed $\lambda_{max}^{CHCl_3}$ 652(sh), 600, 377, 350, 338(sh), 305(sh), 292, 286 nm; ν_{max}^{film} 3040, 2930, 2840, 1900, 1550, 1520, 1455, 1420, 1385, 1360, 1280, 1220, 1190, 1160, 1045, 1015, 985, 955, 915, 810, 770, 705, 645 cm^{-1}; ^{1}H-NMR (100 MHz, δ, $CDCl_3$) 1.35(d, 7 Hz, 6 H), 2.66(s, 3 H), 2.81(s, 3 H), 3.07(m, 1 H), 6.97(d, 11 Hz, 1 H), 7.21(d, 4 Hz, 1 H), 7.38(dd, 1.5, 11 Hz, 1 H), 7.61(d, 4 Hz, 1 H), 8.18(d, 1.5 Hz, 1 H); ^{13}C-NMR(25 MHz, δ, $CDCl_3$) 12.9(q), 24.0(q), 24.8(q, q), 38.2(d), 112.7(d), 125.0(d), 125.1(s), 133.2(d), 134.8(d), 136.1(d), 136.2(s), 137.3(s), 139.8(s), 144.2(s). These spectral data coincide well with the reported data[5,6] for guaiazulene as does its mass spectrum; m/z 198(M^+, 17%), 183(15), 168(10), 165(8), 155(7), 153(10), 141(9), 128(11), 115(12), 69(14), 28(100). Furthermore, our azulene was identical with an authentic sample (Aldrich Chem. Co.) in every respect.

Guaiazulene has so far been found in the essential oils of terrestrial plants[7,8] and in a marine red alga[9]. To our knowledge this is the first isolation of guaiazulene from an animal, though some sesquiterpenes possessing the guaiane skeleton have been reported from gorgonians[2]. *E. erecta* has brilliant blue polyps, which are exceptional among related gorgonians. Apparently this blue color is due to the occurrence of guaiazulene. It is also likely that guaiazulene plays a defensive role in this coral. It is interesting to speculate on the origin and the mode of concentration of the pigment.

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Sapintoxin A, a new biologically active nitrogen containing phorbol ester

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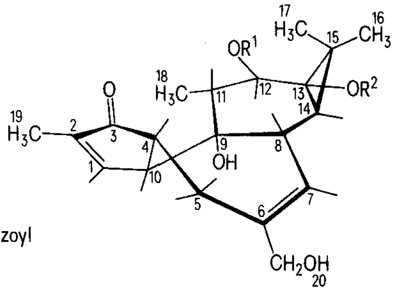
Summary. From the unripe fruits of *Sapium indicum*, an irritant compound, sapintoxin, was isolated. Spectroscopic data together with selective hydrolysis and partial synthesis confirmed sapintoxin as 12-O-[N-methylaminobenzoyl]-13-O-acetyl-4-deoxyphorbol.

Sapium indicum, a well-known Indian poisonous plant and piscicidal agent[2] has previously been investigated for its toxic principles[3]. However only the nonbiologically active substance, sapinine, was isolated. Using a new method of purification which involves highspeed centrifugal liquid chromatography (CLC) followed by neutral adsorption TLC and partition chromatography[4] we have been able to isolate a nitrogen containing phorbol ester from this plant. This compound, present in high yield from the unripe fruits, was termed sapintoxin A and represents the first nitrogen containing phorbol derivative to be isolated which demonstrates biological activity in vivo using an erythema skin test[5].

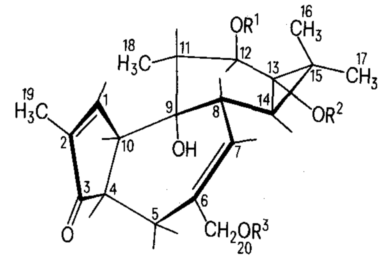
Unripe fruits were powdered and macerated for 2 weeks with acetone at room temperature. The residue left after removal of acetone below 45 °C was dissolved in 40% methanol and the steroids and lipids removed by partition with hexane. Sapintoxin was obtained as an impure resin after extraction of the methanol-phase with ether. The ether soluble resin was fractionated by CLC using a 4-mm porous silica gel disc at a flow rate of 4 ml/min and eluting in a gradient of hexane-toluene-ethylacetate. Fractions were collected at 2-min intervals and monitored by a UV flow-cell, analytical TLC and a biological test[5]. Fractions 54-59 contained sapintoxin together with yellow pigment and were bulked for further purification. Final purification was achieved using 1stly preparativelayer TLC (silica gel G, 500-μm layers, buffered at pH 7.0, eluant, cyclohexane-toluene-ethylacetate-ether 20-15-40-30) (R_f 0.1) and 2ndly partition chromatography (kieselguhr, 500 μm, buffered pH 7.0, coated with 20% diethylene glycol, eluant, cyclohexane-butanone 70-30) (R_f 0.48). Compound **1**, sapintoxin, yield 250 mg, was shown to be a single substance on the basis of its TLC and mass-spectral characteristics. The spectral data for **1** was as follows:

MS: (electron impact, 70 eV, 210°), m/z 523 (M^+, 4%, $C_{30}H_{37}O_7N$), 373 (5%), 313 (6%), 295 (3%), 151 (100%), 81 (56%); UV: λ_{max} (MeOH), 207 (shoulder, $\varepsilon = 46{,}443$), 222 ($\varepsilon = 49{,}791$), 252 ($\varepsilon = 17{,}155$), 356 ($\varepsilon = 9205$) nm. IR: (solvent chloroform), ν_{max} 3480, 1720, 1685, 1580 cm^{-1}; PMR: (250 MHz, $CDCl_3$, TMS = 0.000 ppm), δ 7.842 (d.d., J = 1.8 and 7.8 Hz, 1 H-aromatic), 7.704 (d, J = 5.05 Hz, *H*N– exchangeable with D_2O), 7.572 (s, 1 H-1), 7.420 (t, J = 7.3 Hz, 1 H-aromatic), 6.695 (d, J = 7.8 Hz, 1 H-aromatic), 6.595 (t, J = 7.8 Hz, 1 H-aromatic), 5.685 (s, 1 H-9, exchangeable with D_2O), 5.643 (d, J = 9.6 Hz, 1 H-12), 5.559 (d, J = 4.13 Hz, 1 H-7), 4.037 (s, 2 H-20), 3.275 (m, 1 H-10), 2.932 (d, J = 5.05 Hz, CH_3N–), 2.814 (m, 1 H-4), 2.441 (m, 2 H-5), 2.131 (s, 3 H-acetyl), 1.739 (m, 3 H-19), 1.32 and 1.254 (6 H-16,17), 1.224 (d, J = 5.15 Hz, 1 H-14), 0.958 (d, J = 6.43 Hz, 3 H-18). Irradiation at 7.704 ppm caused the 3 H doublet at 2.932 ppm to collapse to a sharp singlet. A similar change in the doublet at 2.932 ppm occurred when the signal at 7.704 ppm was exchanged with D_2O. **1** was hydrolysed by means of methanolic KOH (0.1 M) at room temperature for 20 min. A single mono-ester **2** was isolated using preparative partition TLC (eluant cyclohexane: Butanone 6:4, R_f 0.36). Compound **2** like **1** produced a bright blue fluorescence under long-wave UV-light.

MS: m/z 481 (M^+, 7% $C_{28}H_{35}O_6N$), 330 (10%), 312 (20%), 151 (100%), 81 (60%); UV: 207 λ_{max} (MeOH), (shoulder, $\varepsilon = 21{,}164$), 222 ($\varepsilon = 32{,}708$), 252 ($\varepsilon = 13{,}708$), 356 ($\varepsilon = 7215$) nm; PMR: (60 MHz, $CDCl_3-D_2O$), δ 7.4–8.0 (2 H-aromatic), 7.18 (s, 1 H-1), 6.40–6.90 (2 H-aromatic), 5.24 (d, 1 H-7), 5.21 (d, 1 H-12), 3.99 (s, 2 H-20), 3.66 (m, 1 H-10), 3.55 (m, 1 H-5α), 2.94 (s, CH_3–N), 2.83 (m, 1 H-4), 2.55 (d.d., 1 H-5β), 1.88 (m, 1 H-11), 1.82 (m, 3 H-19), 1.28 and 1.18 (6 H-16, 17), 1.15 (d, 3 H-18), 0.78 (d, 1 H-14). Compound **1** was converted to the cis orientated mono-ester 12-O-[N-methylamino benzoyl]-4α-deoxyphorbol **2** by treatment with weak alkali. This conversion was evident from comparison of the PMR spectra of **1** and **2** where the following characteristic shifts in signals were recorded[6,7], 7.18 ppm in **2** from 7.572 ppm in **1** (1 H-1), 5.24 ppm from 5.56 ppm (1 H-7), 2.55 and 3.55 ppm from 2.44 ppm (2 H-5). Furthermore the disappearance of a 3 H-acetyl signal together with an upfield shift of the 1 H-12 signal from 5.64 ppm in **1** to 5.21 ppm in **2** indicated that the acetyl group in **1** was located at C-13 of the nucleus. The signal at 5.21 ppm was confirmed as being due to the 1 H-12 because irradiation at 1.88 ppm (1 H-11) caused the doublet at 5.21 ppm to collapse to a sharp singlet. The upfield shift of the 1 H-12 signal due to hydrolysis of the reactive tertiary C-13 ester group has been confirmed previously using synthetic methods[8]. Treatment of compound **2** with pyridine/acetic anhydride (2/1) yielded the tri-ester **3** purified by partition TLC (solvent system cyclohexane: butanone 90:10, R_f 0.9). A single bright blue fluorescent reaction product was obtained; UV: λ_{max} (MeOH), 207 (shoulder, $\varepsilon = 132{,}775$), 222 ($\varepsilon = 193{,}512$), 252 ($\varepsilon = 81{,}925$), 356 ($\varepsilon = 45{,}200$) nm; MS: m/z 565 (M^+, 22%, $C_{32}H_{39}O_8N$), 415 (18%), 355 (4%), 313 (7%), 295 (9%), 151 (100%), 134 (85%); PMR: (60 MHz, $CDCl_3-D_2O$), δ 7.4–8.0 (2 H-aromatic), 7.18 (s, 1 H-1), 6.40–6.90 (2 H-aromatic), 5.71 (d, 1 H-12), 5.24 (d, 1 H-7), 4.44 (bs, 2 H-20), 3.66 (m, 1 H-10), 3.55 (m, 1 H-5α), 2.94 (s, CH_3–N), 2.83 (m, 1 H-4), 2.55 (d.d., 1 H-5β), 2.09 and 2.21 (6 H-acetyls), 1.88 (m, 1 H-11), 1.80 (m, 3 H-19), 1.28 and 1.18 (6 H-16, 17), 1.15 (d, 3 H-18), 0.81 (d, 1 H-14). The downfield shift in the PMR-spectrum of **3** of the 1 H signal at 5.71 ppm from 5.21 ppm in **2** and the downfield shift of the 2 H signal at 4.44 ppm from 3.99 ppm in **2** together with additional 3 H-acetyl signals at 2.09 and 2.21 ppm confirmed **3** as 12-O-[N-methylamino benzoyl]-13-20-diacetyl-4 α-deoxyphorbol.



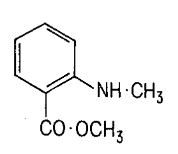
1 R^1 = N-Methylaminobenzoyl
R^2 = Acetyl



2 R^2 = R^3 = H
R^1 = N-Methylaminobenzoyl
3 R^2 = R^3 = Acetyl
R^1 = N-Methylaminobenzoyl
4 R^1 = R^2 = R^3 = H
5 R^1 = R^2 = R^3 = Acetyl

The mono-ester **2** was resistant to hydrolysis with both perchloric acid and potassium hydroxide under mild conditions, as is the established case for 12-O-acyl tigliane esters[8]. Transesterification of **2** was achieved with sodium methoxide in methanol under nitrogen over a period of 16 h. This reaction produced a brown solution from which the polyol **4** and the blue fluorescent methyl ester **6** were isolated by means of TLC on silica gel using the solvent system $CHCl_3$: acetone 5:4 (R_f 0.07 and 0.75, respectively). **4** was obtained in only a 50% yield from this reaction. It was converted to its stable triacetate **5** by reaction with acetic anhydride: pyridine 2:1. Compound **5** was identical to the previously known 4α-deoxyphorbol-12-13-20-triacetate[6] (UV, IR, MS, PMR, TLC). The methyl ester **6** was identical to the methyl ester synthesised from commercially available N-methylaminobenzoic acid (Aldrich Chemicals Ltd.; UV, MS, TLC, PMR).

6



Sapintoxin **1** was therefore confirmed as being 12-O-[N-methylaminobenzoyl]-13-O-acetyl-4-deoxyphorbol (fig.). This compound was found to be a rapidly acting pro-inflammatory agent on mammalian skin, but was considerably less potent than the well-known tumour-promoter and pro-inflammatory ester tetradecanoylphorbol-acetate[9] from *Croton* seed oil.

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