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PARTIAL SYNTHESIS OF THE FLUORESCENT PHORBOL ESTER PROBE, SAPINTOXIN D

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Abstract—The partial synthesis of the novel fluorescent phorbol ester Sapintoxin D (12-*O*-[2-methylaminobenzoyl]-phorbol-13-acetate), and its previously unreported C-20 acetate analogue, from phorbol is described. Sapintoxin D is a convenient fluorescent probe for the study of the mechanisms of action of phorbol esters in mammalian cells

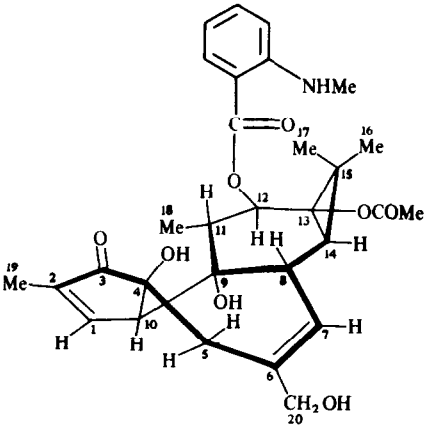
Sapintoxin D is one of a novel group of phorbol esters known as the sapintoxins, originally isolated from the dried fruits of *Sapium indicum* (Euphorbiaceae) [1] and is one of the most potent and complete in its spectrum of biological activities comparable to 12-*O*-tetradecanoylphorbol-13-acetate. This compound exhibits a 2-methylaminobenzoate ester moiety at C-12 of the phorbol nucleus, which conveys its fluorescent properties. This characteristic has been valuable in the study of *in vivo* binding of phorbol esters to Swiss 3T3 fibroblasts and GH_3 pituitary cells using fluorescence microscopy [2]

The yields of sapintoxin D obtained from plant sources were very low (5 mg/kg dried fruits) [3] and in this communication we describe the partial synthesis of this phorbol ester from readily available starting materials. Previous attempts to synthesize this compound were unsuccessful due to the instability of the amino group under reaction conditions. Our method involves the use of the α-amino protecting 9-fluorenylmethyloxycarbonyl group (FMOC) which is stable toward acids and catalytic hydrogenation but is readily cleaved under mildly basic, nonhydrolytic conditions [4]. FMOC-Cl was initially reacted with 2-*N*-methylanthranilic acid to produce 2-(9-fluorenylmethyloxycarbonylamino)benzoic acid using the method of ref. [5].

EXPERIMENTAL

Compound **1**; ^{1}H NMR (80 MHz, $CDCl_3$, TMS = 0 ppm), δ = 8.08 *dd* (*J* = 7.27, 2.34 Hz 2H anthranilate aromatic), 7 80–7.05 *m* (8H FMOC aromatic, 2H anthranilate aromatic), 4 33 *m* (–CH–CH_2 of FMOC), 2 95 *m* (N–Me). Phorbol 13,20-diacetate was produced from phorbol by standard acetylation reactions [6] and was coupled to **1** using dicyclohexylcarbodiimide (DCC) to produce 12-*O*-[*N*-(9-fluorenylmethyloxycarbonyl)-benzoate]-phorbol 13,20-diacetate (**2**) Compound **2**, ^{1}H NMR (80 MHz, $CDCl_3$, TMS = 0 ppm), δ 8 11 *dd* (*J* = 8 73, 2 18 Hz), 7 91–6 95 *m* 8H FMOC aromatic, 2H anthranilate aromatic, H-1, 5 65 *d* (*J* = 2 91 Hz, H-7), 5 58 *d* (*J* = 10 18 Hz H-12), 5 52 (exchangeable with D_2O, 1 × OH), 4 47 *s* (2H-20), 4 09 *m* (CH-CH_2 of FMOC), 3 32 *m* (N-Me, H-8, H-10), 2.47 *s* (2H-5), 2 07 *s* (2 × Ac), 1 78 *br s* (3H-19), 1 26 *m* (9H-16, 17, 18), 0 91 *d* (5 82 Hz 14) Dimethylformamide (DMF) was used as solvent and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) as catalyst

C-20 deacetylation of **2** was carried out using 1% $HClO_4$–MeOH for 24 hr under N_2 This was followed by FMOC-deprotection by stirring with piperidine in dichloromethane Final purification by prep. TLC [6] (cyclohexane–toluene–EtOAc–Et_2O, 4·3·8:6) afforded sapintoxin D (R_f 0 18) as a clear glassy resin Spectral (^{1}H NMR mass, UV, IR) and biological data (ID_{50}, ED_{50}, K_a) of the synthetic compound were identical with those for authentic sapintoxin D from natural sources [3] The previously unreported ^{13}C NMR data are illustrated in Fig 1. Sapintoxin D 20-acetate (**3**), ^{1}H NMR (80 MHz, $CDCl_3$, TMS = 0 ppm), δ 7 85 *dd* (*J* = 7.28, 2 18, Hz, 1H aromatic) 7 73 *s* (exchangeable with D_2O, N-H), 7.59 *s* (H-1), 7 38 *m* (1H-aromatic), 6 77–6 40 *m* (2H-aromatic), 5 76 *d* (*J* = 4 95, H-7), 5.67 *d* (*J* = 10 18, H-12), 5 64 *s* (exchangeable with D_2O 1 × OH), 4 46 *s* (2H-20), 3 36 *m* (H-8, H-10), 2 91 *d* (*J* = 5.14 Hz exchangeable with D_2O, MeN), 2 51 *s* (2H-5), 2 12 *s* (Ac–),



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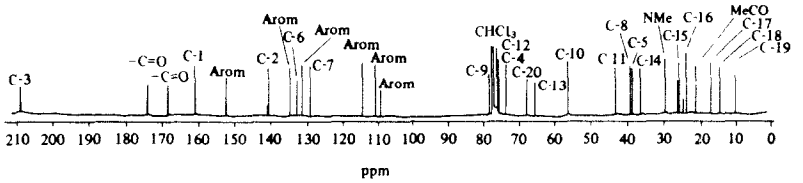

Fig 1 62 9 MHz ^{13}C NMR spectrum ($CDCl_3$) of sapintoxin D, ^1H noise decoupled

2 05 *s* (Ac–), 1.79 *m* (Me-19), 1 59 *s* (exchangeable with D_2O, 1 × OH), 1 26 *m* (Me-16) (Me-17) 0 91 *m* (Me-18, H-14) EIMS (70 eV, 200°) *m/z* (%) 581 $[M]^+$ (6 25) ($C_{32}O_9NH_{39}$), 539 (0 73), 495 (0 42), 431 (12 5), 371 (2 10), 353 (2 10), 329 (2 92), 311 (21 67), 293 (7 08), 151 (100), a previously unreported biologically active compound, was isolated during the synthesis as a result of incomplete deacetylation of (2) followed by FMOC-deprotection as above This compound was purified as for sapintoxin D (R_f 0 58) to give a clear, glassy resin

Crystallization of sapintoxin D and **3** was avoided due to the problem of ring AB conversion to the biologically inactive *cis* analogue [6] The synthesis described above is a convenient method of producing the fluorescent probe sapintoxin D, which has a wide range of biological activities, suitable for binding studies in cancer research

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