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SYNTHESIS OF BIOLOGICALLY ACTIVE FLUORESCENT PHORBOL ESTERS

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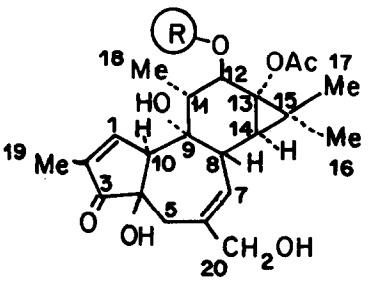
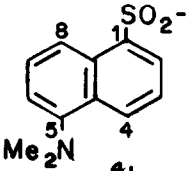
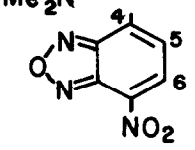
Pure fluorescent derivatives of 12-O-tetradecanoyl phorbol-13-O-acetate (TPA), labeled in the tetradecanoyl chain, are synthesized by two ways: 1) from both enantiomers of β-N-dansylaminotetradecanoic acid which requires the resolution of the (±) β-aminoacid precursor; 2) from an achiral fluorescent chain derived from 3-azatetradecanoic acid. The new phorbol derivatives retain the main biological activity of TPA.

Phorbol esters such as 12-O-tetradecanoyl phorbol-13-O-acetate (TPA) are potent tumor promoters in mouse skin [1] and display a variety of biological and biochemical effects *in vivo* and *in vitro* [2]. We have recently demonstrated that the fluorescent TPA derivative **1**, labeled by a dansylamino group linked to the tetradecanoyl chain at the β position, retains the main activity of TPA itself and thus is a suitable tool for the study of the mechanism of action of phorbol esters [3]. This



dansyl = 

NBD = 

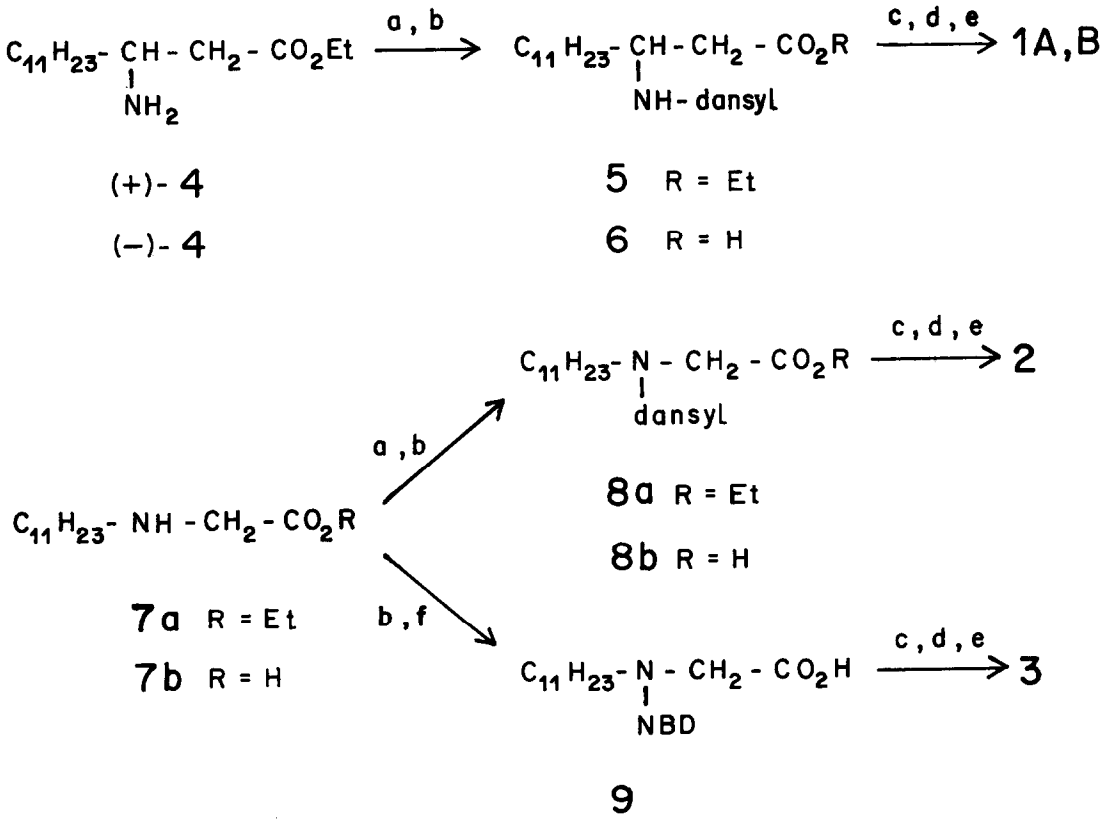
R		
H	phorbol 13-O-acetate	
$C_{13}H_{27}$-CO	TPA	
$C_{11}H_{23}$-CH(NH-dansyl)-CH_2-CO	**1A**	[from (+)-4]
	1B	[from (−)-4]
$C_{11}H_{23}$-N(Fl)-CH_2-CO	**2**	Fl = dansyl
	3	Fl = NBD

compound **1** has been first synthesized as an unresolvable mixture of the two diastereomers **1A** and **1B** and the question could be raised whether the diastereomers have different biological activities.

We have now synthesized these diastereomers **1A** and **1B**, starting from (+)- and (-)-ethyl β-aminotetradecanoate **4** respectively. On the other hand, to avoid the formation of diastereomer mixtures, we have developped a more direct approach to pure labeled derivatives using an achiral fluorescent tetradecanoyl chain. We have chosen to insert a nitrogen atom at the 3 position of the tetradecanoyl chain to which a fluorescent group, dansyl in compound **2** or 4-nitrobenzo-2-oxa-1,3-diazole (NBD) in compound **3**, is linked.

The key step in the synthesis of **1A** and **1B** is the resolution of (+)-ethyl β-aminotetradecanoate **4** [4], which was conveniently achieved in reasonable yield as follows. After extensive search for an acidic resolving agent, (-)-dibenzoyl-L-tartaric acid (DBTA) was found to give a 1:1 salt which, after three to five recrystallizations [5] from EtOH/i-PrOH (1:20 v/v) yielded partially resolved (+)-**4**, ee 50-65 %. Obviously, complete purification would be extremely laborious in this way, due to extensive co-crystallization of the two diastereomeric salts. By contrast, recrystallization of a suitable

Scheme



a) Dansyl-Cl, NEt_3, CH_2Cl_2, RT, 15h b) NaOH, EtOH, reflux, 1h c) ClCO-COCl, benzene, RT, 15h d) Phorbol-13,20-O-diacetate, DMAP, CH_2Cl_2, RT, 2-3 weeks e) $HClO_4$, MeOH, RT, 24h f) NBD-Cl, NaOAc, EtOH, 50 °C, 2h.

enantiomeric derivative or salt should be a valuable alternative since, as a rule, the formation of solid solutions is less common with enantiomer mixtures [6a]. To this end, the 3,5-dinitrobenzoate (DNB) salt of **4** was found particularly appropriate due to the lowest solubility of the enantiomers. Thus, starting from partially resolved (+)-**4**, DNB salt (ee > 50%), two or three recrystallizations from diethyl ether provided (+)-**4**, DNB salt, mp 93°C, $[\alpha]_{546}^{25}$= +6.8° (EtOH, c=5), ee > 98 %, as determined by differential scanning calorimetry [6b]. Finally, aminoester (+)-**4**, oil, $[\alpha]_{546}^{25}$ = +12.5° ($CHCl_3$, c=5) was readily obtained in 30 % overall yield. The other enantiomer (-)-**4**, oil, $[\alpha]_{546}^{25}$= -12.4° ($CHCl_3$, c=5), ee > 98 %, was similarly prepared using (+)-DBTA as a resolving agent.

Both (+)- and (-)-**4** were converted into pure oily **1A** and **1B** respectively, by following the route previously described with (±)-**4** [3] (Scheme). The spectral characteristics (absorption, fluorescence emission, ^{1}H-NMR) [7] of **1A** and **1B** are entirely consistent with those of their mixture published earlier [3].

The synthesis of the fluorescent derivatives **2** and **3** (Scheme) begins with the common precursor 3-azatetradecanoic (or N-undecylglycine) ethyl ester **7a** (oil), which was readily prepared by alkylation of glycine ethyl ester with 1-bromoundecane in MeCN in the presence of $NaHCO_3$ (90°C, 20 h, 33 % yield).

The preparation of **2** parallels that of **1** described above: i) dansylation of **7a** followed by saponification gave acid **8b** (oil, 90 %), ii) esterification of (+)-phorbol 13,20-O-diacetate using a large excess of the crude acid chloride of **8b** followed by the cleavage of the 20-OAc protective group afforded the desired fluorescent product **2** (oil, TLC on SiO_2 with AcOEt/cyclohexane 60:40 as an eluent, 60% yield). In the preparation of **3**, the saponification of **7a** to **7b** (mp 210 °C, dec, 90%) was performed prior to the introduction of the NBD group, which is extremely sensitive to alkalis. Thus, the condensation of NBD chloride, according to Fager et al. [8] afforded **9** (SiO_2 column chromatography with acetone/MeOH 70:30 as an eluent, 13% yield). The subsequent steps performed as above provided **3** (oil, TLC on SiO_2 with AcOEt:cyclohexane 60:40 as an eluent, 24% yield). The compounds **2** and **3** were characterized by mass, ^{1}H-NMR and fluorescence spectrometry [9].

Biological studies [10,11] indicate that the fluorescent derivatives **1-3** retain potent activity in competing the binding of (^{3}H)-PDBu to C3H/10T1/2 cells. They are also equipotent with TPA as activators of purified protein kinase C and phospholipid metabolism. These data substantiate our first results concerning the potentiality of such probes to characterize the receptor sites of phorbol esters.

REFERENCES AND NOTES

1) I. Berenblum, in "A Comprehensive Treatise", ed. F.F. Becker, Plenum Press, New york, 323 (1975).

2) "A Comprehensive Survey, Cocarcinogenesis and Biological Effects of Tumor Promoters", eds E. Hecker et al., Raven Press New York, Vol.7 (1982); P.M. Blumberg, CRC Crit. Rev. Toxicol. 8, 153, 199 (1980-1981).

3) P.L. Tran, J. Malthête, L. Lacombe and M.L. Capmau, Nouv. J. Chim. **8**, 751 (1984); P.L. Tran and M.A. Deugnier, Carcinogenesis **8**, 433 (1985).

4) In the synthesis of (±)-**4**, the yield of ethyl 3-ketotetradecanoate was greatly improved (50% instead of 26%) by using the Meldrum's acid method described by Y. Oikawa, K. Sugano and O. Yonemitsu, J. Org. Chem., **43**, 2087 (1978); Org. Synth., **63**, 198 (1978).

5) The DSC thermograms of the DBTA salts show a single peak which gives evidence of the formation of solid solutions between the two diastereomers. The purification was carried out up to mp ≈ 173-175 °C, corresponding to 50-65 % diastereomeric excess.

6) J. Jacques, A. Collet and S.H. Wilen, in "Enantiomers, Racemates and Resolutions", J. Wiley, New York (1981), a) p. 427; b) pp. 151-159.

7) In ref. 3, the major component of the mixture is **1A** and the minor component is **1B**.

8) R.S. Fager, C.B. Kutina and E.W. Abrahamson, Anal. Biochem. **53**, 290 (1973).

9) ^{1}H-NMR spectra (200 MHz, $CDCl_3$) (Chain = azatetradecanoyl moiety).
2 δ (ppm) 0.81 (d, 3H, J 6.5 Hz, 18-Me), 0.88 (t, 3H, J 7 Hz, Me-chain), 1.05-1.3 (m, CH_2-chain and 14-H), 1.13, 1.18 (2s, 6H, 16- and 17-Me), 1.42 (m, 2H, CH_2-chain), 1.7 (m, 2H, CH_2-chain), 1.78 (d, 3H, J 2 Hz, 19-Me), 2.0 (m, 1H, 11-H), 2.05 (s, 3H, 13-Ac), 2.5, 2.53 (AB, 2H, J 18 Hz, 5-CH_2), 2.67 (m, 1H, OH), 2.90 (br.s, 6H, NMe_2), 3.25 (m, 2H, 8- and 10-H), 3.37 (m, 2H, CH_2-chain), 4.01 (s, 2H, 20-CH_2), 4.15, 4.27 (AB, 2H, J 18 Hz, N-CH_2-CO), 5.41 (br.s, 1H, OH), 5.43 (d, 1H, J 10.5 Hz, 12-H), 5.65 (d, 1H, J 5 Hz, 7-H), 7.18 (d, 1H, J 7 Hz, dansyl 6-H), 7.5 (m, 3H, 1-H and dansyl 3- and 7-H), 8.26 (d, 1H, J 7 Hz, dansyl 4-H), 8.30 (d, 1H, J 8 Hz, dansyl 8-H), 8.53 (br.d, 1H, J 8 Hz, dansyl 2-H).
3 δ (ppm) 0.88 (t, 3H, J 6.5 Hz, Me-chain), 0.93 (d, 3H, J 7 Hz, 18-Me), 1.10 (d, 1H, J 5 Hz, 14-H), 1.15, 1.17 (s, 6H, 16- and 17-Me), 1.26 (m, chain), 1.38 (m, 2H, chain), 1.79 (d+m, 5H, J 3 Hz, 19-Me and CH_2-chain), 1.96 (s, 3H, 13-Ac), 2.17 (m, 1H, 11-H), 2.50, 2.53 (AB, 2H, 5-CH_2), 2.51 (br.s, 1H, OH), 3.26 (m, 2H, 8 and 10-H), 3.74 (m, 2H, CH_2-N-), 4.02 (AB A_2, 2H, 20-CH_2), 4.75, 4.84 (AB, 2H, J 17 Hz, N-CH_2-CO), 5.35 (m, 1H, OH), 5.45 (d, 1H, J 10 Hz, 12-H), 5.66 (br.d, 1H, J 5.5 Hz, 7-H), 6.23 (br.d, 1H, J 9 Hz, NBD 5-H), 7.57 (br.t, 1H, 1-H), 8.48 (d, 1H, J 9 Hz, NBD 6-H).
Mass spectrometry: **2** $C_{47}H_{66}N_2O_{10}S$, m/e 851 (M^+), **3** $C_{41}H_{56}N_4O_{11}$, m/e 781 (M^+).
UV (EtOH): **2** λ max (nm) 250, 330, 395. **3** λ max (nm) 230, 330, 470.
Fluorescence spectra (EtOH): **2** λ max 525 nm (excitation at λ 330 nm), **3** λ max 530 (excitation at λ 470 nm).

10) Results to be published.

11) A similar behaviour has been recently reported for a TPA derivative which carries a dansyl group in the terminal position of a 12-dodecanoyl chain. R.M.J. Liskamp, A.R. Brothman, J.P. Arcoleo, O.J. Miller and I.B. Weinstein, Biochem. Biophys. Res. Commun. **131**, 920 (1985).

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