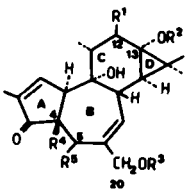
TUMOUR-PROMOTING AND NON-PROMOTING PRO-INFLAMMATORY PHORBOL ESTERS AS HUMAN LYMPHOCYTE MITOGENS

Mary Edwards, S.E. Taylor, F.J. Evans, A.M.E. Nouri and D. Gordon,
Department of Pharmacognosy, The School of Pharmacy, Brunswick Square,
London, WC1N 1AX, and the Institute of Basic Medical Sciences,
Royal College of Surgeons, London, WC2A.

Tetradecanoylphorbolacetate (TPA) a potent tumour-promoting and pro-inflammatory agent (Hecker 1971) is the only T-lymphocyte mitogen known which can dispense with the macrophage requirement for proliferative responses (Rosenstreich & Mizel 1979). We examined the effects of nine related compounds as lymphocyte mitogens by monitoring 3H-thymidine incorporation by Ficoll-Paque separated human blood mononuclear cells (Gordon & Nouri 1981). The co-mitogenic effects of the compounds on Mixed Lymphocyte Responses (MLR) using lymphocytes from 2 unrelated donors were studied in the same way.

Compound	R^1	R^2	R^3	R^4	R^5	mitogenic ED_{40} ng/ml	co-mitogenic ED_{200} ng/ml
T.P.A.	Tetradecanoate	Acetate	H	OH	H	17	3.5
12 DPPA	H	Phenylacetate	H	OH	H	45	0.53
12 DPPAA	H	—"—	Acetate	OH	H	564	0.9
12 DPA	H	Angelate	H	OH	H	81	0.8
12 DPAA	H	—"—	Acetate	OH	H	693	1.0
Phorbol	OH	H	H	OH	H	$>10^4$	$>10^3$
Sapintoxin A	N-methylamino-benzoate	Acetate	H	H	H	35	8.9
Sapintoxin B	—"—	—"—	H	H	OH	560	405



The pro-inflammatory but non-tumour promoting diesters 12DPPAA and 12DPAA (Fig. 1) were only weakly active as mitogens. The corresponding C-13 mono-esters had similar activities to TPA, suggesting that the polarity of C-20 is important for hydrogen-bonding to cell membrane proteins. As the parent alcohol phorbol was inactive, this suggested that esterification at C-13 is necessary for activity, possibly by increasing lipid solubility. Sapintoxin A had a similar activity to TPA but its 5-OH analogue, Sapintoxin B, had reduced activity. The possibility of intermolecular hydrogen bonding between the C-5 and C-20 OH groups could reduce the availability of the C-20 position for binding. The 4α-analogue of Sapintoxin A was inactive as a lymphocyte mitogen demonstrating the importance of the ring AB-trans configuration for activity. All of the 12-deoxyphorbol esters, including the non-promoting diesters acted as co-mitogens in the MLR system, being more potent than TPA. These results suggest that either a different mechanism is involved in co-mitogenicity or, more likely, that mitogenicity involves a two-stage signal, only one of which is required in co-mitogenicity. The non-promoting esters may be capable of fulfilling one signal but only partially triggering the second signal.

Hecker, E. (1971) Methods in Cancer Res. Vol. 6, 439-484, Academic Press, New York.
Rosenstreich, D.L. & Mizel, S.B. (1979) J. Immunol. 123: 1749-1754.
Gordon, D. & Nouri, A.M.E. (1981) Clin. Exp. Immunol. 44: 287-294.

0022-3573/81/120055P-01$02.50/0