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TOXIC PHORBOL ESTERS FROM VARIETIES OF THE CHINESE TALLOW TREE

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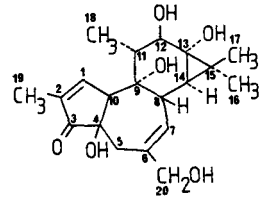
New sources of vegetable oils from plants of the family Euphorbiaceae may be implicated as causative agents in human cancers. Ito et al (1983) have demonstrated positive Epstein-Barr viral expression assays with extracts of these plants. Such oils are used in the manufacture of soaps, lipsticks, nail varnish, wood varnish, paints, candles and foodstuffs. The tallow tree (Sapium sebiferum (L) Roxb.), indigenous to China is one such commercial source of oil. This oil is generally considered to be non-toxic and our preliminary investigations seemed to confirm this traditional belief in that the extract was inactive when tested in vivo for pro-inflammatory activity. However Seip et al (1983) detected trace quantities of 12-deoxyphorbol esters from the plant. We have been able to obtain four varieties of tallow tree from the Jiangxi Province and this communication describes their examination for toxic phorbol esters. The seeds of these four varieties were essentially similar in that they possessed a white outer tallow layer. They differed slightly in their shape and size but not sufficiently so as to be confused with other species of Sapium (Radcliffe-Smith 1986).

An ether extract of each of these varieties was prepared by established techniques (Evans and Taylor 1983). These extracts were examined for their pro-inflammatory activity on mouse skin by the method of Evans and Schmidt (1979), and also analysed chemically for the presence of phorbol derivatives by TLC (Evans 1986) and HPLC (Zorbax Gold series C8, water/methanol gradient). Varieties 2, 3 and 4 (Table 1) induced a pronounced erythema of mouse skin and phorbol derivatives based upon the tigliane nuclei 4-deoxyphorbol, 4,20-deoxy-5-hydroxy-phorbol and 12-deoxyphorbol were detected in the extracts.

Table 1



Compound	Max. yield % w/w of seeds	Irritant activity 10ug/mouse ear	Variety*
Sapintoxin A	0.0007	+++	2,3,4
Sapintoxin C	0.0005	-	2,4
12-deoxyphorbol-benzoate	0.0004	++	2

*Variety: 1, non toxic; 2, known in China as Grape sapium; 3, known as Chicken Foot sapium; 4,known as Wooden Club sapium.

Following a larger scale extraction of samples 2 and 3, phorbol esters were isolated by chromatographic methods. The pure compounds were identified by spectroscopic methods (^{1}H-NMR, MS, UV, IR). The yields obtained and biological activities of these compounds are given in Table 1. Variety 1 did not induce inflammation of mouse skin and no phorbol derivatives were detected in this extract. Two of the pro-inflammatory phorbol esters isolated from the seeds of varieties 2 and 4 may be tumour-promoting agents in vivo in that we have previously shown that they activate the enzyme protein kinase C (Ellis et al 1985). This enzyme is believed to be the tumour-promoting phorbol ester receptor site (Aitken 1986). This work is supported by an SERC project grant and a Pharmaceutical Society postgraduate studentship.

Aitken A. (1986) Chap. 10 in "Naturally occurring phorbol esters", CRC press Inc. Boca Raton, USA

Ellis C. et al (1985) J. Pharm. Pharmacol. 37: 23P

Evans F.J. (1986) Chap. 1 in "Naturally occurring phorbol esters", ibid.

Evans F.J. and Schmidt R.J. (1979) Inflammation 3(3) 215-223

Evans F.J. and Taylor S.E. (1983) Prog. Chem. Org. Nat. Prod. 44 1-99

Ito Y. et al (1983) Cancer Letters 18: 87-95

Radcliffe-Smith A. (1986) Chap. 3 in "Naturally occurring phorbol esters", ibid.

Seip E.H. et al (1983) Planta Medica 49: 199-203