The effect of daphnane esters on platelet aggregation and erythema of the mouse ear

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Esters of phorbol (Zucker et al 1974) and monoesters of 12-deoxyphorbol with a C-13 ester group and a free C-20 hydroxy group (Westwick et al 1979) are potent platelet-aggregating agents as well as inducers of long-lasting erythema on the mouse ear (Evans 1978). The aggregation produced with platelets at low concentration (0·3 μM) is slow and irreversible while at higher concentrations (1 μM) it is rapid, irreversible and involves the release of platelet constituents. The diesters of 12-deoxyphorbol which have an acetyl group at C-20 do not aggregate platelets at concentrations as high as 30 μM (Williamson et al 1979).

The daphnane ester, resiniferonol-9-13-14-orthophenyl acetate (ROP), and the C20 daphnane esters resiniferatoxin and tinyatoxin (Fig. 1) have structural similarities with 12-deoxyphorbol esters. They occur in the same plants of the genus *Euphorbia* (Evans & Schmidt 1976) and while like the 12-deoxyphorbol esters (Evans 1978) they are potent inducers of erythema of the mouse ear, the redness lasts for only a few hours.

We now report that while the two C-20 acyl daphnanes have no aggregating effects on human blood platelets, ROP induces the first stage of platelet aggregation only.

The esters resiniferatoxin and tinyatoxin were isolated from the latex of *Euphorbia poissonii* (Evans & Schmidt 1976) and ROP was produced from resiniferatoxin by acid-catalysed trans-esterification.

Male human blood was collected with forearm venipuncture from healthy donors who had received no medication within the previous 14 days. Nine volumes of blood were mixed with one volume of trisodium citrate (3·24%) and platelet rich plasma (PRP), was produced by centrifugation at 160 *g* for 20 min at 25 °C. The platelet count was adjusted to 300 000 ml^{-1} with plasma and the PRP was stored at 37 °C for up to

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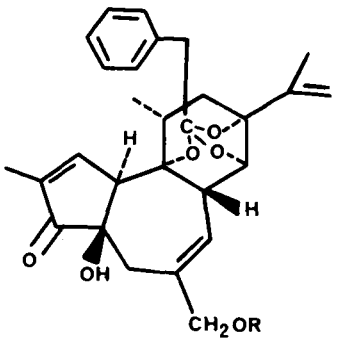
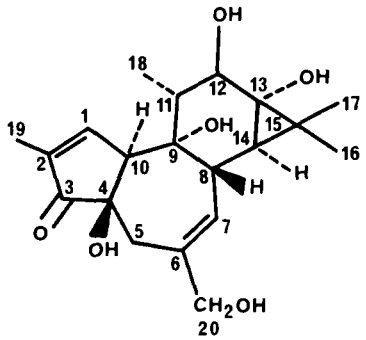


DAPHNANES



PHORBOL

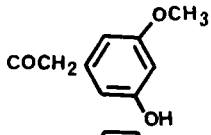
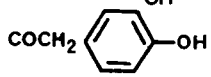
	R	ID50(nm)	PLATELET AGGREGATION (um)
RESINIFERATOXIN		0.00021	>70
TINYATOXIN		0.0012	> 70
ROP	H	1.50	7 - 70 (27% maximum)

Fig. 1.

4 h and was not used within 30 min of collection. Aggregation was followed with a Born Mk III aggregometer and the viability of the platelets was tested by constructing a dose-response curve to ADP-induced aggregation at the beginning and the end of each experiment. Acetone was used as the vehicle for the daphnane esters and at a maximum concentration of 0·5% v/v in PRP it did not induce aggregation or modify the ADP-induced aggregation. The esters were tested in a concentration range of 0·01 to 70 μM. The 50% irritant dose (ID50) of the esters in the mouse ear erythema test was also determined as previously described (Evans & Schmidt 1979).

Resiniferatoxin and tinyatoxin in doses of up to 70 μM did not cause aggregation of human blood platelets (Fig. 1). ROP, which differs from the other compounds in that it has a free C-20 hydroxy group, caused a slow irreversible aggregation of human platelets in doses of from 7 to 70 μM. A maximum aggregation of only 27% was produced which could not be inhibited by indomethacin, indicating that the characteristic platelet release reaction did not occur. In the erythema test, resiniferatoxin and tinyatoxin were potent inflammatory agents with ID50's of 0·00021 and 0·0012 nM respectively. ROP was about 7×10^3 times less potent than resiniferatoxin and 1×10^3 times less potent than tinyatoxin (Fig. 1) in this test.

These findings add further support to our previous conclusions (Westwick et al 1979) that a C-20 primary hydroxy group on the nucleus is necessary for aggregating activity on human platelets, whereas erythema of the mouse ear is only quantitatively affected with the structural change in the molecule.

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β-Adrenoceptor mediated actions of RO363 and (−)-isoprenaline in anaesthetized cats, rats and rabbits

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RO363 [(±)-1-(3,4-dimethoxyphenethylamino)-3-(3,4-dihydroxyphenoxy)-2-propanol)] is a recently developed directly acting β-adrenoceptor stimulant, which, on the basis of in vitro studies shows a high degree of selectivity for β_1-adrenoceptors (Iakovidis et al 1979). Preliminary studies in anaesthetized cats (Raper et al 1978) showed that RO363 was approximately half as potent as (−)-isoprenaline in eliciting increases in heart rate and more than 100 times less potent as a vasodilator in the hindlimb vasculature. In the present experiments the in vivo activity of RO363 has been further investigated in anaesthetized cats, rats and rabbits.

Arterial blood pressure and heart rate were recorded in artificially respired, bilaterally vagotomized cats (bethanidine not given) and rats anaesthetized with chloralose (80 mg kg^{-1} + pentobarbitone sodium 6 mg kg^{-1}) and urethane (1·25 g kg^{-1}) respectively, and in spontaneously breathing sodium pentobarbitone (35 mg kg^{-1}) anaesthetized rabbits. Constant dose-response curves to intravenous (−)-isoprenaline were first obtained and thereafter responses to RO363 were monitored.

In all three species RO363 and (−)-isoprenaline produced positive chronotropic effects and vasodepressor responses that were antagonized by propranolol (0·5 mg kg^{-1}). Dose-response curves to the two catecholamines were parallel and similar maximal responses were obtained. The duration of the responses to RO363 were 2–3 times longer than those to (−)-isoprenaline (Fig. 1). In cats, RO363 produced small, propranolol sensitive, increases in blood pressure at low doses, while higher doses produced depressor responses

Table 1. In vivo comparisons of the activities of RO363 and (−)-isoprenaline. Mean i.v. ED50 doses ($\times 10^{-10}$ mol kg^{-1}) ± s.e.m. are shown for the production of (−)-isoprenaline (ISO) and RO363-induced increases in heart rate (HR), decreases in diastolic blood pressure (DBP) and soleus muscle contractility (Soleus), and inhibition of 5-HT-induced bronchoconstriction (BC). Doses quoted for decreases in hindlimb perfusion pressure (HLPP) are those required to produce a reduction of 30 mmHg following intra-arterial administration of the compounds. Mean relative potencies from individual experiments (± s.e.m.) are also shown (ED50 RO363: ED50 (−)-isoprenaline).

	n	ED50 (ISO)	ED50 (RO363)	Relative Potency
Cat, HR	6	2·3±0·7	5·9±0·8	2·8±0·7
Rat, HR	4	0·7±0·3	1·0±0·4	1·7±0·4
Rabbit, HR	4	3·4±1·0	24±3	8·5±1·4
Cat, DBP	7	3·3±1·7	75±31	28±6
Rat, DBP	3	5·6±3·2	141±8	24±2
Rabbit, DBP	4	3·0±1·2	96±39	34±10
Cat, BC	7	0·8±0·1	30±5	42±10
Cat, Soleus	6	1·7±0·2	157±30	95±17
Cat, HLPP	8	0·12±0·03	35±22	225±88

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