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THE LARVICIDAL ACTIVITY OF SOME 12,13-EPOXYTRICHOTHEC-9-ENES

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Abstract—Seventeen naturally-occurring 12,13-epoxytrichothec-9-enes and 19 of their derivatives and transformation products have been tested for larvicidal activity against *Aedes aegypti* and the majority have been examined for toxicity to mammalian cells in tissue culture. The principal structural requirements for high biological activity were the same in both tests, and only a small number of examples of selectivity of action were encountered. Of these trichodermin alone was larvicidal but non-toxic to mammals by oral administration and only mildly dermatitic.

The naturally-occurring 12,13-epoxytrichothec-9-enes show remarkable selectivity and specificity of biological action towards higher plants and microorganisms [1]. Although most members of the group inhibit, in low concentration, the growth and development of mammalian cells in tissue culture, some, notably trichodermin (antibiotic WG 696) (I; R^1=R^3=H, R^2=OAc) and crotocin (antibiotic T) (X) are non-toxic, with LD_{50} > 1000 mg/kg when administered orally to mice [2, 3], and these compounds have been considered [2, 4, 5] for possible practical application as antifungal antibiotics.

Only three members of the group, verrucarin A (antibiotic 379Y) [6] (I; R^1R^2=R^5, R^3=H), roridin A (antibiotic 379X) [6] (I; R^1R^2=R^7, R^3=H) and, more recently, before this work had been completed, diacetoxyscirpenol (I; R^1=R^2=OAc, R^3=OH) have been reported [7, 8] to show insecticidal activity. Since many of the epoxytrichothecene-producing fungi are known to be pathogenic to insects [9], we have extended our studies of structure–activity relationships within this class of compound [1] with the object of revealing more examples of selectivity of biological action of possible practical value. Judged solely from this viewpoint the results of tests for larvicidal activity against mosquito, a rapid and convenient bioassay for insecticidal activity, have been somewhat disappointing. In general, those compounds possessing relatively high larvicidal activity are also highly toxic to mammalian cells and are therefore potentially hazardous. Some exceptions to this general rule were revealed, however. Additionally, some examples of high cytotoxicity without larvicidal activity were observed.

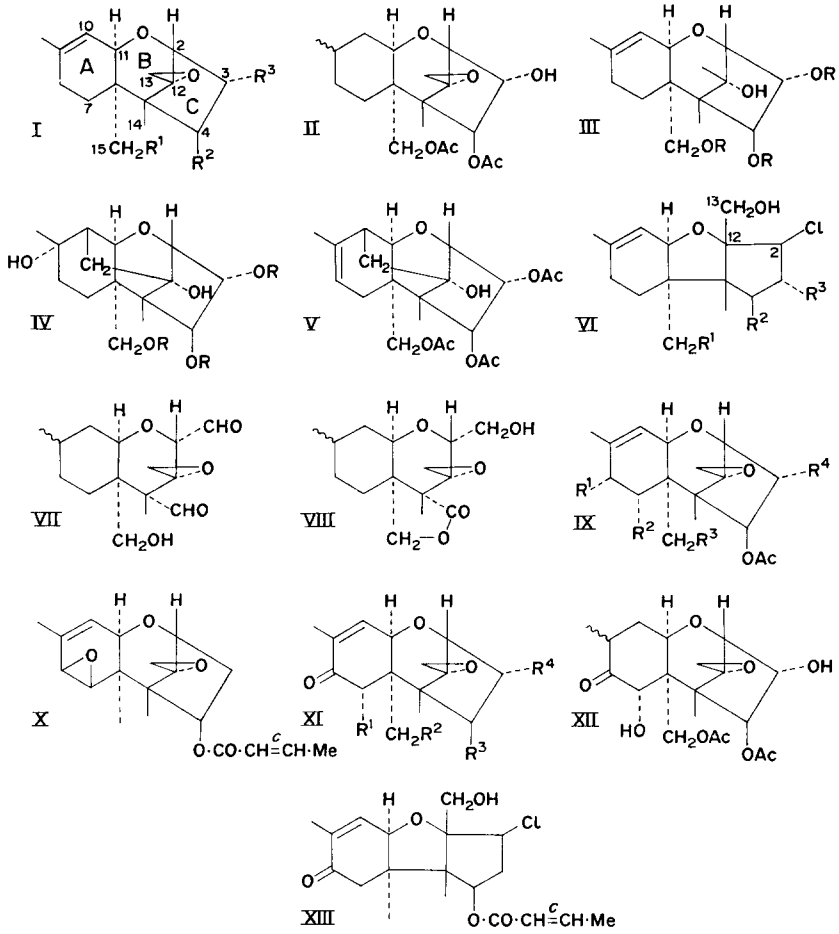
Grove and Mortimer [1] studied structure–activity relationships in the trichothecanes, and particularly in transformation products of diacetoxyscirpenol, using two cell lines, one (HEp2) of human origin. Their results were consistent with the hypothesis that cytotoxicity was associated with the 12,13-epoxytrichothecane nucleus, but other structural features, notably the presence of a 9-ene and esterification of some, though not all, of the hydroxyl substituents were important factors in the manifestation of high toxicity.

These compounds, and some naturally-occurring trichothecenes isolated more recently, together with their derivatives, have now been tested against larvae of the mosquito *Aedes aegypti*.

METHODS AND MATERIALS

Larvicidal tests. Mature eggs of *Aedes aegypti* kindly supplied by Dr. M. T. Gillies, M.R.C. Unit of Mosquito Behaviour, University of Sussex, were received on filter paper circles and were hatched in boiled-out water (350 ml, depth 3 cm) containing liver powder (0·5 g) at 27 ± 1°. After 4 days, the developing 4th stage larvae were counted out into groups of 100 and transferred to cylindrical glass containers containing glass-distilled water (pH 6, 50 ml) in which the test chemical, dissolved in acetone (0·25 ml) had been dispersed.

The containers were incubated at 27 ± 1° for 3 days and mortality was assessed daily by a modification of the photomigration technique of Storrs [13]. A glass migration trough 26 cm long and of semi-circular cross-section (4 cm dia), was equipped with two perforated stainless steel barriers, ground to a sleeve fit, at 2·5 and 7·5 cm from a light source (15 W) placed at one end. The test solution containing the larvae was carefully poured into the space between the end of the trough and the first barrier. When the liquid had reached a stable common level in the trough, the light was switched on and the barriers raised. Active larvae rapidly migrated to the far end of the trough. After 1 min the second barrier was dropped into place at the 7·5-cm mark and the light switched off. The number of dead or moribund larvae between the near end of the trough and the second barrier was then counted. When mortality was high it was simpler to count the number of live larvae at the far end of the trough and obtain a figure for mortality by subtraction. Two determinations of mortality were made and the mean taken. Reproducibility was excellent [13]. In controls, with 0·5% aqueous acetone only, the mortality in no case exceeded 7 per cent. Diacetoxyscirpenol was used as standard, as required; no significant change in larval viability was observed during the course of the investigation.



R^5 = —O·CO·CHOH·CHMe·CH_2·CH_2·O·CO·CH $\overset{t}{=}$ CH·CH $\overset{c}{=}$ CH·CO·O–

R^6 = —O·CO·CH(O)—CMe·CH_2·CH_2·O·CO·CH $\overset{t}{=}$ CH·CH $\overset{c}{=}$ CH·CO·O–

R^7 = —O·CO·CHOH·CHMe·CH_2·CH_2·O·CH(CHOH·Me)·CH $\overset{t}{=}$ CH·CH $\overset{c}{=}$ CH·CO·O–

R^8 = —O·CO·CH = CMe·CH_2·CH(O—CHMe)(O—CH·CH $\overset{t}{=}$ CH·CH $\overset{c}{=}$ CH·CO·O–)

R^9 = —O·CO·CHOAc·CHMe·CH_2·CH_2·O·CO·CH $\overset{t}{=}$ CH·CH $\overset{c}{=}$ CH·CO·O–

R^{10} = —O·CO·CH $\overset{c}{=}$ CH·Me

Compounds were prepared by literature methods [1, 10–12] and purified to the standard used previously [1]. We are indebted to Professors Sir Ewart Jones, Ch. Tamm and F. M. Strong for specimens of trichothecin, roridins and T_2-toxin, respectively and to Drs. W. O. Godtfredsen, J. Gyimesi and J. R. Hanson for specimens of trichodermin, crotocin and calonectrin respectively.

RESULTS AND DISCUSSION

For the larvicidal tests, we used a modification of the photomigration bioassay developed by Storrs [13]. A lag period is normally observed between the administration of an epoxytrichothecene and the appearance of toxic symptoms [14]. For this reason the incubation time in this bioassay was extended to a maximum of 3 days and the concentration of the more active compounds was adjusted to give 20–70 per cent kill over this period of time. The results are shown in the Tables and are compared with cytotoxicities determined on the cell line HEp2 [1]. Some of these results for cytotoxicity have already been reported [1]: for the remainder we are indebted to Dr. P. H. Mortimer.

Considering first the transformation products of scirpentriol (Table 1), reduction of the 9-ene in diacetoxyscirpenol (I; R^1=R^2=OAc, R^3=OH) to give (II) brings about a fall in larvicidal activity. Opening of the 12,13-epoxide ring, either by reduction to give

Table 1. Larvicidal activity and mammalian toxicity of derivatives and transformation products of 12,13-epoxytrichothec-9-en-3α,4β,15-triol (scirpentriol)

Compound (Common name)	Structure	*A. aegypti* larvae Mortality (%) Concn (μg/ml)	Hours 24	48	72	HEp2 Cells Lowest toxic dose (ng/ml)	LD_{50} i.p. in rat [14] (mg/kg)
Scirpentriol	(I; R^1=R^2=R^3=OH)	25	5	15	38	75	0·8
15-Acetyl- (deacetylanguidin)	(I; R^1=OAc, R^2=R^3=OH)	25	19	24	26	2·5	
4β,15-Diacetyl- (diacetoxyscirpenol)	(I; R^1=R^2=OAc, R^3=OH)	25	10	19	49	5	
		50	42	74			0·75
3α,4β,15-Triacetyl-	(I; R^1=R^2=R^3=OAc)	25	20	43	53	25	1·1
4β,15-Diacetyl-9,10-dihydro-	(II)	25	1	4		100	
		100	7	50	62		18

The following transformation products of scirpentriol showed no cytotoxicity (inactive at 5000 ng/ml) and no larvicidal activity (less than 7% mortality at 100 μg/ml): Trichothec-9-en-3α,4β,12,15-tetraol (III; R=H), 3α,4β,15-triacetoxytrichothec-9-en-12-ol (III; R=Ac), 10→13-cyclotrichothecan-3α,4β,9α,12,15-pentaol (IV; R=H), 3α,4β,15-triacetoxy-10→13-cyclotrichothecan-9α,12-diol (IV; R=Ac), 3α,4β,15-triacetoxy-10→13-cyclotrichothec-8-en-12-ol (V), 3α,15-diacetoxy-2β-chloroapotrichothec-9-en-4β,13-diol (VI; R^1=R^3=Ac, R^2=OH), 12,13-epoxy-15-hydroxy-3,4-secotrichothecan-3,4-dione (VII), 12,13-epoxy-3,15-dihydroxy-3,4-secotrichothecan-4-oic acid 4→15 lactone (VIII).

compounds of structure (III), or by hydrolytic rearrangement to give the 10→13 cyclo- and apo- products (IV) (V) and (VI) leads to complete loss of activity. Activity is also lost in the ring C seco- products (VII) and (VIII) in which the 12,13-epoxide is retained but has become accessible to rearside nucleophilic attack. These conclusions are identical with those derived from consideration [1] of the cytotoxicities of those compounds.

The effect of esterification of the free hydroxyl groups is, however, different in the two tests. Unlike cytotoxicity which passes through a maximum at the monoacetate (I; R^1=OAc, R^2=R^3=OH), the greatest larvicidal activity is found in the completely esterified product (I; R^1=R^2=R^3=OAc). Although it is tempting to attribute this result in the larvicidal test to the influence of increasing lipoidal solubility as the number of acetate residues is increased, this is unlikely to be a complete, or indeed the correct, explanation. Firstly, in the 12,13-epoxytrichothec-9-en-8-ones (Table 3), the opposite effect is observed with a series of acetates of the tetrahydroxy-compound (XI; R^1=R^2=R^3=R^4=OH). In this series the water-soluble tetraol (nivalenol) is the most active and the fully acetylated derivative (XI; R^1=R^2=R^3=R^4=Ac) the least active: cytotoxicity again passes through a maximum as the series is ascended. Secondly, the lipoid-soluble 3α,15-diacetate calonectrin (I; R^1=R^3=OAc, R^2=H) (Table 2) is inactive at a comparable concentration. Thirdly, scirpentriol though more water-soluble than the mono- and di-hydroxyl compounds trichodermol (I; R^1=R^3=H, R^2=OH) and verrucarol (I; R^1=R^2=OH, R^3=H) is more active than either.

The naturally-occurring esters of these two alcohols and of trichothecolone (XI; R^1=R^2=R^4=H, R^3=OH) show high larvicidal activity, particularly some of the macrolide esters of verrucarol. Surpris-

Table 2. Larvicidal activity and mammalian toxicity of some naturally-occurring 12,13-epoxytrichothec-9-enes and their derivatives

Compound (Common name)	Structure	*A. aegypti* larvae Mortality (%) Concn (μg/ml)	Hours 24	48	72	HEp2 Cells Lowest toxic dose (ng/ml)	LD_{50} i.p. in mouse or rat (mg/kg)
4β-Hydroxy- (trichodermol)	(I; R^1=R^3=H, R^2=OH)	25	2	1	2	250	
4β-Acetoxy- (trichodermin)	(I; R^1=R^3=H, R^2=OAc)	25	21	55	70	75	>500* [2]
3α,15-Diacetoxy- (calonectrin)	(I; R^1=R^3=OAc, R^2=H)	25	6	6	6		
4β,15-Dihydroxy- (verrucarol)	(I; R^1=R^2=OH, R^3=H)	25	1	2	2	7000	
Verrucarin A	(I; R^1R^2=R^5, R^3=H)	3·1	30	66	81		
		6·2	46	81	94	1	0·5 [15]
Verrucarin B	(I; R^1R^3=R^6, R^3=H)	3·1	27	75	93	5	7·0† [15]
Roridin A	(I; R^1R^2=R^7, R^3=H)	25	4	3	4		1·0† [15]
Roridin H	(I; R^1R^2=R^8, R^3=H)	3·1	8	43	62	25	
Acetylverrucarin A	(I; R^1R^2=R^9, R^3=H)	6·2	40	60	78		
Verrucarin A chlorohydrin	(VI; R^1R^2=R^5, R^3=H)	25	1	1	1		
4β,8α,15-Triacetoxy-3α,7α-dihydroxy-	(IX; R^1=R^3=OAc, R^2=R^4=OH)	50	3	6		10	1·2 [14]
3α,4β,8α,15-Tetraacetoxy-7α-hydroxy-	(IX; R^1=R^3=R^4=OAc, R^2=OH)	50	0	6		250	
Crotocin	(X)	25	8	25	25	250	810 [3]
T_2-toxin	(IX; R^1=O.CO.CH_2.$CHMe_2$ R^2=H, R^3=OAc, R^4=OH)	25	27	67	81	1	3·0 [16]
1-Naphthyl methylcarbamate (carbaryl)		1	54	67	73		

* Subcutaneous.
† Intravenous.

Table 3. Larvicidal activity and mammalian toxicity of some naturally-occurring 12,13-epoxytrichothec-9-en-8-ones and their derivatives

Compound (Common name)	Structure	*A. aegypti* larvae Mortality (%) Concn (μg/ml)	Hours 24	48	72	HEp2 Cells Lowest toxic dose (ng/ml)	LD_{50} i.p. in mouse or rat (mg/kg)
4β-Hydroxy-(trichothecolone)	(XI; R^1=R^2=R^4=H, R^3=OH)	25	4	4	4	5000	
Trichothecin	(XI; R^1=R^2=R^4=H, R^3=R^{10})	25	21	55	80	75	250*† [20]
Trichothecin chlorohydrin	(XIII)	25	2	3	3	>10,000	
3α,4β,7α,15-Tetrahydroxy-(nivalenol)	(XI; R^1=R^2=R^3=R^4=OH) [10]	25	12	22	58	225	4·0 [17]
4β-Acetoxy-3α,7α,15-trihydroxy-(fusarenone)	(XI; R^1=R^2=R^4=OH, R^3=OAc) [10]	25	8	7	20		<10 [18]
4β,15-Diacetoxy-3α,7α-dihydroxy-	(XI; R^1=R^4=OH, R^2=R^3=OAc) [10]	25	5	8	22	30	1·2 [14]
3α,4β,7α,15-Tetraacetoxy-	(XI; R^1=R^2=R^3=R^4=OAc) [10]	25	0	5	6	250	
3α,7α,15-Trihydroxy-(vomitoxin)	(XI; R^1=R^2=R^4=OH, R^3=H) [12]	100	3	4	4		70 [19]
3α-Acetoxy-7α,15-dihydroxy-	(XI; R^1=OH, R^2=R^4=OAc, R^3=H) [12]	100	3	6			46 [19]
3α,7α,15-Triacetoxy-	(XI; R^1=R^2=R^4=OAc, R^3=H) [12]	25	1	3	5		
4β,15-Diacetoxy-9-10-dihydro-3α,7α-dihydroxy-	(XII) [10]	50	2	2			

* Subcutaneous.
† Intravenous.

ingly, in this group, roridin A was inactive against *A. aegypti*; although it was not tested against HEp2 cells, it was reported [15] to be as active as verrucarin A against P815 tumour cells. T_2-toxin and crotocin show moderate larvicidal activity but the tri- and tetra-acetates [11] of structure (IX) and the mono- and tri-acetates of structure (XI; R^1=R^2=R^4=OH, R^3=H) [12] are inactive. The parent triol (XI; R^1=R^2=R^4=OH, R^3=H) is also inactive high-lighting the importance in an active epoxytrichothecene larvicide of an electronegative substituent at the 4-position.

As with the transformation products of scirpentriol, the biological activity of these naturally-occurring esters is diminished, in compound (XII) by reduction of the 9-ene, and eliminated, in the chlorohydrins (VI; R^1R^2=R^5, R^3=H) and (XIII), by molecular rearrangement involving the epoxide ring.

The most active larvicidal compounds, verrucarins A and B, roridin H, T_2-toxin and the mono- and di-acetates of scirpentriol are also the most cytotoxic (25 ng/ml or less) suggesting a similar or possibly identical mode of action. Scirpentriol and its triacetate, nivalenol and its diacetate, trichothecin, crotocin and trichodermin had reasonably good larvicidal action at 25 μg/ml and only moderate mammalian cytotoxicity. Nevertheless, the intraperitoneal toxicities to rat or mouse of the scirpentriol and nivalenol relatives are high with LD_{50} in the range 1–5 mg/kg. Although crotocin and trichothecin are much less toxic they are markedly dermatitic [3, 20]. Of the compounds tested, only trichodermin appeared to have the desired selectivity coupled with weak dermatitic properties. Carbaryl showed larvicidal activity of the same order at 1 μg/ml.

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