THE INSECTICIDAL ACTIVITY OF FLAVENSOMYCINOIC ACID AND SOME ANALOGUES

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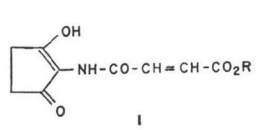
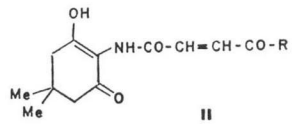
Flavensomycinoic acid, the N-containing moiety common to the *Streptomyces* sp. antibiotics flavensomycin and prasinons A and B, shows only weak insecticidal activity compared with the parent substances.

Although the complete structures of the insecticidal natural products flavensomycin, from *Streptomyces cavourensis*, and prasinons A and B, from *S. prasinus*, are unknown, the N-containing moiety (**I**) is a structural unit common to all three compounds[1]. To assess the contribution of the enolised



I



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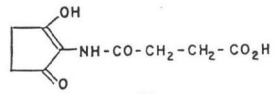

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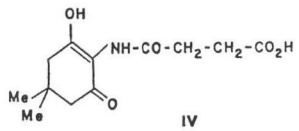

IV

1,3-dioxocyclopentylfumaramic acid residue (**I**) to insecticidal activity, a number of analogues have been synthesised and tested for toxicity against adults of the blowfly *Calliphora erythrocephala* (Table 1) and larvae of the mosquito *Aedes aegypti* (Table 2).

The synthesis of flavensomycinic acid (**I**; R=Me), flavensomycinoic acid (**I**; R=H) and dihydroflavensomycinoic acid (**III**) has already been described.[1,2] The analogues (**II**; R=Me) and (**IV**), derived from dimedone, were prepared by CORBELLA *et al.*[3] In making these compounds we used the method of the Italian workers, in which isonitrosodimedone was reduced with zinc and acetic acid, and the resulting amine was allowed to react *in situ* with an acid anhydride or chloride, but we obtained acceptable yields with a relatively short reaction time at ambient temperature. In the reaction between one equivalent of 2-aminodimedone and fumaryl chloride, some diamide (**II**; R=$C_8H_{11}O_2$·NH–) was obtained in addition to the fumaramic acid (**II**; R=OH).

Although prasinon B showed[4] activity only against larval forms of the sheep blowfly *Lucilia servicata* and was inactive by topical application to adults of this insect, we found it toxic to *C. erythrocephala* adults by injection. With both prasinon B and flavensomycin death followed recovery from a slowly developing knockdown. Flavensomycinoic acid (**I**; R=H) showed moderate activity in this test but did not approach that of flavensomycin and prasinon B which, on a molar basis, were two orders of magnitude more active. The activity of the acid (**I**; R=H) was lost on reduction of the ethylenic

Table 1. Insecticidal activity of flavensomycinoic acid analogues to *Calliphora erythrocephala*

Compound	Dose (μg/fly)	Knockdown (% Flies down)						Toxicity (% mortality)	
		0 hr	1 hr	2 hrs	3 hrs	4 hrs	18 hrs	1 Day	2 Days
Flavensomycin	0.1	7	78	75	50	48	32	55	65
Prasinon B	0.125	14	92	85	82	68	32	55	57
(I; R=H)	4.5	0	12	17	30	30		50	57
	2.5	2	0	0	0	0		7	20
(I; R=Me)	2.0*	0	0	0	0	0		0	0
(III)	5.0	0	0	0	0	0	0	0	0
(II; R=OH)	4.5	0	0	0	0	0	0	7	17
(II; R=OMe)	2.0*	7	2	0	0	0	2	7	10
(IV)	5.2	10	7	2	5	2	2	7	15
Control		0	0	0	0	0	0	0	5

* saturated solution.

double bond (to give **III**) and a saturated solution of the methyl ester (**I**; R=Me) was also inactive. The ester-linkages in the natural compounds readily undergo fission[1] to liberate the acid (**I**; R=H). The absence of this property in the methyl ester could be responsible, in part, for its inactivity; but poor solubility, giving a saturated solution of concentration lower than the toxic dose, is a more likely explanation.

Table 2. Larvicidal activity of flavensomycin, prasinon B, and some analogues against *Aedes aegypti*

Compound	Concentration (μg/ml)	Mortality (%)		
		18 hrs	48 hrs	72 hrs
Flavensomycin	2	20	77	91
Prasinon B	10	26	87	100
Control		0	0	0

The following compounds showed no toxicity at 72 hours at the concentration (μg/ml) indicated: (**I**; R=H), 11; (**I**; R=Me), 14; (**III**), 20; (**II**; R=OH), 56; (**II**; R=OMe), 14; (**IV**), 56.

None of the analogues derived from dimedone showed activity comparable to that of flavensomycinoic acid and the cyclopentandione residue is therefore of some significance, but only in conjunction with a fumaramic acid residue.

None of the synthetic compounds showed activity against mosquito larvae at concentrations equal to or greater than those concentrations of flavensomycin and prasinon B which gave a good kill.

It is clear from these results that the partial structure (**I**) makes only a minor contribution to the insecticidal activity of the natural products.

Experimental

Melting points were taken on a Kofler hot-stage apparatus and are corrected. IR spectra were determined for mulls in Nujol. Molecular weights were taken from the mass spectra. Mass spectra at high resolution were recorded at 70 eV with a Varian CH5 double focusing instrument interfaced with a Varian 620L computer. The RINGER's solution used contained 0.45 M sodium chloride (330 ml), 0.4 M potassium chloride (100 ml), 0.3 M magnesium chloride (30 ml) and water (to 500 ml).

Tests for insecticidal activity

A. *Calliphora erythrocephala.* Batches of ten 4-day post-emergence adults (4 replicates) were injected (30 gauge needle) with a solution (1 μl) of the test substance, delivered by a micrometer syringe into the haemolymph in the dorsum of the thorax. The solvent was dimethylsulphoxide - RINGER's solution (1:1). The flies were then removed to glass cylindrical dishes inverted over filter paper and incubated at 25~26°C. Sucrose and water were provided. Knockdown paralysis was recorded for 4 hours after injection. Mortality was recorded daily and compared with a solvent control. The toxicity

data in Table 1 have been corrected for deaths in the control.

B. *Aedes aegypti*. Batches of 100 4th stage larvae were incubated with a solution (50 ml) of the test substance and mortality was assessed at intervals by the photomigration technique[5].

2-Aminodimedone

Zinc powder (AR, 1 g) was added portionwise with vigorous stirring to dioxan (3 ml) whilst isonitrosodimedone[6] (169 mg) in acetic acid (1 ml) was added dropwise during 30 minutes. The temperature rose to 40°C and, after cooling and filtration, the aminodimedone-containing filtrate was used as described below.

N-2-(5,5-dimethyl-1,3-dioxocyclohexyl)succinamic acid (IV)

Succinic anhydride (100 mg) in dioxane (2 ml) was added and the mixture was stirred at room temperature. After 30 minutes, the solution was concentrated *in vacuo* to small bulk and the residue was dissolved in sodium hydrogen carbonate and extracted with chloroform. Coloured neutral by-products were discarded. The aqueous layer was acidified to pH 3 with concentrated hydrochloric acid and re-extracted with chloroform. Recovery gave a solid which crystallized from benzene in prisms, m.p. 147~148°C, (lit.[3] 140~142°C) (63 mg) of the acid (**IV**) (Found: M 255. Calcd. for $C_{12}H_{17}NO_5$: M 255) γ_{max} 3260, 1710, 1585 cm^{-1}.

Methyl N-2-(5,5-dimethyl-1,3-dioxocyclohexyl)fumaramate (**II**; R=OMe)

After the addition of N,N-dimethylaniline (1 ml) fumaric acid methyl ester chloride (160 mg) was added dropwise to the stirred solution. After 2 hours, the solution was concentrated *in vacuo* to small bulk and added cautiously, to 2 N hydrochloric acid. The precipitated citrine solid (26 mg) was filtered off, combined with similar material obtained by extraction of the filtrate with chloroform, and crystallized from benzene giving the ester (**II**; R=OMe) as citrine prisms m.p. 192°C (lit.[3] 190~191°C) Found: M 267. Calcd. for $C_{13}H_{17}NO_5$: M 267. γ_{max} 3240, 3060, 1722, 1602, 1585 cm^{-1}.

N-2-(5,5-dimethyl-1,3-dioxocyclohexyl) fumaramic acid (**II**; R=OH)

The above preparation was repeated using fumaryl chloride (160 mg). The precipitate (12 mg) crystallized from chloroform in citrine prisms, m.p. 289°C of NN'-bis(5,5-dimethyl-1,3-dioxocyclohexyl)fumaramide (**II**; $R=C_8H_{11}O_2\cdot NH$) (Found: M 390.1781. $C_{20}H_{26}N_2O_6$ requires M 390.1791), γ_{max} 3245, 3070, 1585 cm^{-1}.

Extraction of the filtrate with chloroform furnished a red gum which was purified by preparative t.l.c. on Merck G_{254} silica gel (20×20×0.075 cm) in diisopropylether - formic acid - water (90: 7: 3). Recovery, in the usual way, of material from a yellow band Rf 0.51 gave the acid (**II**; R=OH) as citrine prisms, m.p. 200~203°C, (Found: M 253.0966. $C_{12}H_{15}NO_5$ requires M 253.0950), γ_{max} 3240, 3045, 1700, 1635, 1590 cm^{-1}.

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References

1) GROVE, J. F.; M. FUKUOKA, A. W. JOHNSON & J. N. C. LOPES: The chemistry of prasinons A and B. Identification of the N-containing moiety. J. Chem. Soc. Perkin Trans. I. in press.
2) CANONICA, L.; A. CORBELLA, G. JOMMI, F. PELIZZONI & G. SCOLASTICO: The synthesis of flavensomycinic acid. Tetrahedron Letters 1966: 3031~3034, 1966
3) CORBELLA, A.; G. JOMMI, G. RICCA & G. RUSSO: On cyclic 2-amino-1,3-diketones. Gazz. 95: 948~965, 1965
4) BOX, S. J.; M. COLE & G. H. YEOMAN: Prasinons A and B: Potent insecticides from *Streptomyces prasinus*. Appl. Microbiol. 26: 699~704, 1973
5) GROVE, J. F. & M. HOSKEN: The larvicidal activity of some 12,13-epoxytrichothec-9-enes. Biochem. Pharmacol. 24: 959~962, 1975
6) HAAS, P.: Isonitroso- and nitrodimethyldihydroresorcin. J. Chem. Soc. 91: 1433~1448, 1907