SYNERGISTIC EFFECTS OF SOME COMPOUNDS RELATED TO 2-DIETHYLAMINOETHYL 2,2-DIPHENYL-n-PENTANOATE (SKF 525A) ON THE INSECTICIDAL ACTIVITY OF PYRETHRINS

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A number of compounds related to SKF 525A were tested for synergism with pyrethrins, using Lesser Mealworm beetles, *Alphitobius laevigatus*, and houseflies, *Musca domestica*, as test insects. SKF 525A had previously been shown to be active, whereas 2,2-diphenyl-n-pentanoic acid and 2-diethylaminoethanol were inactive. Several active compounds resulted from esterification of 2-diethylaminoethanol with diphenylacetic acid and 1-substituted diphenylacetic acids. Most modifications of the alcoholic moiety of active 2-diethylaminoethyl esters destroyed activity. Active compounds resulted when a 2-diethylamino group was joined to a diphenylmethyl group through an ester, ketone or ether linkage, but the analogous amide was inactive. 2-Diethylaminoethyl piperonylate probably owed its activity to its piperonyl group. None of the compounds investigated approached piperonyl butoxide in synergistic activity.

Introduction

The ester 2-diethylaminoethyl 2,2-diphenyl-n-pentanoate (SKF 525A, see Table I) is well known to increase the effects on mammals of drugs of a number of different types.[1] Moreover, it has been shown to synergise the action of pyrethrins on certain insects.[2] In the work described here, a number of compounds related to SKF 525A have been tested for action as pyrethrin synergists on insects, in order to relate chemical structure to biological activity. Variation of the acid and alcohol components established sufficient conditions for synergism in esters. The effects of different linkages between the terminal moieties of the molecule were then examined.

Experimental

Methods of biological test

The test insects were Lesser Mealworm beetles, *Alphitobius laevigatus* (F.), and houseflies, *Musca domestica* L. They were reared by published methods,[3,4] except that the flies were fed on a sucrose solution after emergence. When dosed the beetles were 1–3 weeks old and the flies 3–5 days old.

The insects were dosed topically. A beetle was treated, without anaesthetisation, with 0·07 μl. of a solution of candidate synergist applied between the hind coxae by means of a micro-capillary tube;[5] 5–10 min. later it was treated with 0·025 μl. of a solution of pyrethrins applied dorsally on the neck by means of an air-pulse micro-drop applicator.[3] The pyrethrins were dissolved in Shell Risella oil 17 at a concentration of 0·5% w/v. Most of the candidate synergists were dissolved in odourless kerosene at 10% w/v. However, owing to low solubility in kerosene, four compounds (2,2-diphenyl-n-pentanoic acid, diphenylacetic acid, lignocaine and compound III of Table I) were dissolved at 6% w/v in odourless kerosene containing 40% v/v of cyclohexanone, and three compounds (Marsilid, JB 516 and compound XIII of Table I) at 10% w/v in cyclohexanone alone. Dosage with pyrethrins without synergist was done comparably, with appropriate solvent on the hind coxae and pyrethrin solution on the neck.

A fly was dosed, under brief carbon dioxide anaesthesia, with a solution containing both pyrethrins and the candidate synergist by means of a micro-capillary tube;[5] it was treated dorsally between the scutum and scutellum, a male with 0·05 μl. and a female with 0·10 μl. In most of the experiments with flies, the solvent was odourless kerosene; the concentration of pyrethrins varied from 1·0 to 1·8% w/v according to the test, and the concentration of the candidate synergist was 10 times that of the pyrethrins. However, compounds III, XIII and lignocaine were used, with pyrethrins, in solution in cyclohexanone, the concentration of the compound being 10% w/v. Dosage with pyrethrins without synergist was done comparably.

The beetles were kept at 25° after dosage, the flies at 27°. Knock-down in the groups of beetles was determined at 4 days after dosage, and mortality at 6 and 9 days. Knock-down

and mortality of flies were determined 24 h. after dosage. At the doses used the solvents alone were non-toxic to the insects, as were all the candidate synergists.

Chemical preparations

The physical properties of the compounds prepared are listed in Table II, together with details of microanalyses.

Esters (Compounds II-V, VII-XI).—All compounds containing an ester linkage were prepared by condensation of the appropriate acid chloride and alcohol. Neutral esters were purified by distillation of the reaction products *in vacuo*, and basic esters by precipitation and recrystallisation of the hydrochlorides.

Ethyl 2,2-diphenyl-n-pentanoate (XII).—Ethyl diphenylacetate (6·6 g.) and sodamide (1 g.) in anhydrous benzene (30 ml.) were heated under reflux with stirring for 22 h. After the reaction mixture had been cooled, propyl iodide (2·7 ml.) was added and the mixture was again heated under reflux with stirring for a further 24 h. The solution was filtered and fractionated *in vacuo*. Ethyl 2,2-diphenyl-n-pentanoate (2 g.) was obtained as a pale green oil.

Ethyl 2,2-diphenyl-n-pentanoate was hydrolysed with ethanolic potash for 72 hours under reflux. The solution was acidified and extracted with diethyl ether. The ethereal extract yielded colourless crystals, m.p. 153–155°, of 2,2-diphenyl-n-pentanoic acid.[6] (Found: C, 80·0; H, 7·1. $C_{17}H_{18}O_2$ requires C, 80·3; H, 7·1%.)

N-(2-*diethylaminoethyl*) *diphenylacetamide* (XIII) was prepared by condensation of diphenylacetyl chloride with diethylamino ethylamine, according to the method of Mndzhoyan.[7]

2-*Diethylaminoethyl diphenylmethyl ketone* (XIV) was prepared by the condensation of 1,1-diphenylacetone, formaldehyde and diethylamine in acid medium, according to the method of Sprague & Schultz.[8]

2-*Diethylaminoethyl diphenylmethyl ether* (XV) was prepared by the condensation of 2-diethylaminoethyl chloride with benzhydrol in alkaline medium, according to the method of Forberg.[9]

4-*Diethylamino*-1,1-*diphenylbutane* (XVI) was prepared by the condensation of 3-chloropropyl-*N*-diethylamine with the sodium derivative of diphenylmethane in an atmosphere of nitrogen, according to the method of Benoit *et al.*[10]

Results

Table I lists the more important of the compounds tested and indicates their effects on the toxicity of pyrethrins. Other compounds, less closely related chemically to SKF 525A, were also tested, namely: the alcohol and acid components of SKF 525A, 2-diethylaminoethanol and 2,2-diphenyl-n-pentanoic acid; diphenyl acetic acid; diphenylpropylacetonitrile; (±)-6-dimethylamino-4,4-diphenylheptan-3-one (methadone); and ω-diethylamino-2,6-dimethylacetanilide (lignocaine). Of these, diphenylpropyl-acetonitrile was feebly synergistic for houseflies, and lignocaine feebly synergistic for both species; otherwise these compounds were inactive. Two inhibitors of mammalian monoamine oxidase, *N*-isonicotinoyl-*N'*-isopropylhydrazine (Marsilid) and 2-phenyl isopropylhydrazine (JB 516), were tested on the beetles only, and the former was feebly active.

Discussion

Table I gives results for the presence or absence of synergism, with a minor gradation provided by distinguishing between marked and slight synergism. However, a few additional comparisons were made, the results of which are indicated in the course of this discussion. In the Table there are relatively few discrepancies between the results for beetles and those for flies. Where the results differed, the compound was active on the beetles but not on the flies.

Compounds I-VII in Table I were different esters of 2-diethylaminoethanol. The activity of compound III shows that two unsubstituted phenyl groups in an acetic acid moiety were sufficient to give a synergistic ester, although compound III was less active than SKF 525A. The inactivity of compounds IV and V indicates that a single unsubstituted phenyl group in the

Table I

Synergistic activity of compounds related to SKF 525A

Number	Candidate synergist* Name	Formula	Synergistic activity† *A. laevigatus*	*M. domestica*
I	2-Diethylaminoethyl 2,2-diphenyl-n-pentanoate (SKF 525A)	$C_3H_7 \cdot C(C_6H_5)_2 \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	+	+
II	2-Diethylaminoethyl 2,2-diphenylbutyrate	$C_2H_5 \cdot C(C_6H_5)_2 \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	+	+
III	2-Diethylaminoethyl diphenylacetate (Trasentin)	$(C_6H_5)_2CH \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	+	+
IV	2-Diethylaminoethyl phenylacetate	$C_6H_5 \cdot CH_2 \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	o	o
V	2-Diethylaminoethyl benzoate	$C_6H_5 \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	o	o
VI	2-Diethylaminoethyl 2,2,3-triphenylpropionate	$C_6H_5 \cdot CH_2 \cdot C(C_6H_5)_2 \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	+	o
VII	2-Diethylaminoethyl piperonylate	$CH_2O_2C_6H_3 \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	+	+
VIII	2-Di-n-butylaminoethyl diphenylacetate	$(C_6H_5)_2CH \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_4H_9)_2$	+	o
IX	2-Dimethylaminoethyl diphenylacetate	$(C_6H_5)_2CH \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot N(CH_3)_2$	o	o
X	Isopentyl diphenylacetate	$(C_6H_5)_2CH \cdot CO \cdot OC_5H_{11}$	o	o
XI	Isopentyl 2,2-diphenylbutyrate	$C_2H_5 \cdot C(C_6H_5)_2 \cdot CO \cdot OC_5H_{11}$	o	o
XII	Ethyl 2,2-diphenyl-n-pentanoate	$C_3H_7 \cdot C(C_6H_5)_2 \cdot CO \cdot OC_2H_5$	o	(not tested)
XIII	*N*-(2-Diethylaminoethyl)-diphenylacetamide	$(C_6H_5)_2CH \cdot CO \cdot NH \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	o	o
XIV	2-Diethylaminoethyl diphenylmethyl ketone	$(C_6H_5)_2CH \cdot CO \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	+	(+)
XV	2-Diethylaminoethyl diphenylmethyl ether	$(C_6H_5)_2CH \cdot O \cdot CH_2 \cdot CH_2 \cdot N(C_2H_5)_2$	+	+
XVI	4-Diethylamino-1,1-diphenylbutane	$(C_6H_5)_2CH \cdot (CH_2)_2 \cdot N(C_2H_5)_2$	+	

* All dialkylamino derivatives were tested as the free base
† +, Marked synergism; (+), slight synergism; o, no effect

Table II

Chemical data on candidate synergists

Compound Number	Physical constants Boiling point or melting point	n_D	Analyses Carbon % Found	Carbon % Required	Hydrogen % Found	Hydrogen % Required	Nitrogen % Found	Nitrogen % Required
II*	124–126°		70·5	70·3	8·3	8·1		
III*	113–115°		69·0	69·1	7·5	7·5		
IV	104–109°/0·1 mm.	$1·4947^{22°}$	70·8	71·4	9·1	9·0	6·1	6·0
V*	122–124°		60·9	60·5	7·8	7·8		
VII*	136–138°		55·7	55·7	6·4	6·6	4·4	4·6
VIII*	83–84°		71·3	71·4	8·2	8·4		
IX*	159–161°		67·9	67·6	7·2	6·9		
X	91–93°/0·5 mm.	$1·5350^{24°}$	81·1	80·9	7·7	7·8		
XI	148°/0·3 mm.	$1·5340^{25°}$	81·5	81·3	8·6	8·4		
XII	75–82°/0·2 mm.	$1·5449^{27°}$	81·1	80·9	8·3	7·8		
XIII	93–95°		76·9	77·4	8·4	8·4	9·5	9·0
XIV*	140–142°		71·8	72·4	7·8	7·9	4·2	4·2
XV	126–127°/0·1 mm.	$1·5359^{28°}$	80·4	80·5	9·0	8·9		
XVI	163–164°/1 mm.	$1·5395^{22°}$	84·2	85·4	9·6	9·7	5·0	5·0

* Results for these compounds refer to the hydrochlorides

acid does not confer activity. Compound VI, with three phenyl groups in the acid, was active on the beetles—slightly more so, in fact, than SKF 525A—but was inactive on flies. The activity of the piperonylic ester (VII), compared with the inactivity of compound V, suggests that compound VII owes its activity to the piperonyl group, especially since many piperonyl compounds synergise the action of pyrethrins on insects; compound VII, like SKF 525A, was much less active than piperonyl butoxide, a pyrethrin synergist used in practical insect control.[11]

The results with compounds VIII–XII indicate that the alcoholic portion of an active ester can be varied but little if activity is to be retained. Whereas the 2-diethylamino ester of diphenylacetic acid (III) was active, the 2-dimethylamino ester (IX) was inactive, and the 2-di-n-butylamino ester was active only on the beetles. Comparison of the inactivity of compounds X, XI and XII with the activity of compounds III, II and I, respectively, indicates that an aliphatic alcohol does not confer activity.

Compounds III and XIII–XVI form a series in which a 2-diethylamino group was joined to a diphenylmethyl group by different linkages. Ester (III), ketone (XIV) and ether (XV) linkages gave active compounds; a simple hydrocarbon linkage (XVI) gave a compound active on beetles but inactive on flies; an amide linkage (XIII) gave an inactive compound, but this is relatively insoluble in lipophilic solvents, and may not pass readily through insect cuticle.

To sum up, active compounds were obtained when a 2-diethylamino moiety was joined to a diphenylmethyl moiety through an ester, ketone or ether linkage. For the ester linkage at least, the latter moiety could be replaced by a 1,1-diphenyl-n-butyl group or a 1,1-diphenylpropyl group (compounds I and II).

It was found previously[2] that both piperonyl butoxide and SKF 525A synergised the action of pyrethrins on insects of the species used here, but both antagonised the action of malathion. Antagonism between piperonyl butoxide and malathion has been noted by other workers using houseflies[12,13] and the mosquito *Anopheles stephensi*.[14] Recently Abdallah[15] has suggested that in the work just referred to the two compounds may have antagonised malathion merely by retarding its penetration into the insect. He observed antagonism when TOCP was applied topically on the same part of the housefly as parathion or paraoxon, but synergism when applied to a different part. However, in work with beetles[2] and in some of that on houseflies,[13] the doses of malathion and of its antagonist were put respectively on to different parts of the surface of the insect. Under these circumstances the antagonism is likely to be a biochemical phenomenon—perhaps due to depression by the antagonist of the biological oxidation of malathion to the more toxic malaoxon.[16] Similarly, synergists for pyrethrins may act by depressing the metabolism (probably oxidative, at least in part) of pyrethrins to products not yet identified.

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