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Novel Nonterpenoid Insect Growth Regulators

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A novel series of aliphatic bisthiolcarbamate insect growth regulators (IGR's) was synthesized in accordance with a generalized molecular model for activity proposed in this paper. These compounds differ significantly in structure from the well-known terpenoid IGR's. One of the new compounds, *N*-ethyl-1,2-bis(isobutylthiolcarbamoyl)ethane, compound XX, which adheres closely to the proposed template showed outstanding activity in the yellow mealworm, *Tenebrio molitor* morphogenetic assay. It was nontoxic to several other insect species and to other nontarget higher organisms including the rat, rabbit, and fish.

Most of the insect growth regulators (IGR's) with juvenile hormone activity which have been synthesized and reported to date are derived from terpenes and sesquiterpenes (Wigglesworth, 1970; Bowers, 1971; Pfiffner, 1971; Menn and Beroza, 1972; Sláma et al., 1974; and Menn and Pallos, 1975).

More recently, several investigators described IGR's which did not retain the integrity of the terpenoid skeleton. These include a report by Zaoral and Sláma (1970) who described several peptide derivatives, more specifically, the ethyl ester of L-isoleucyl-L-alanyl-*p*-aminobenzoic acid which showed a much greater morphogenetic effect on last

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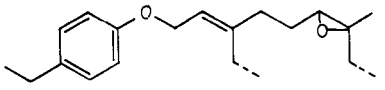
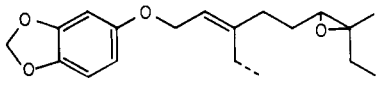
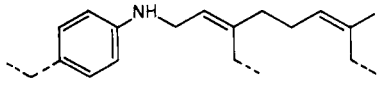
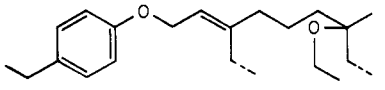
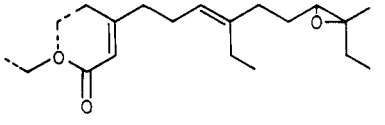
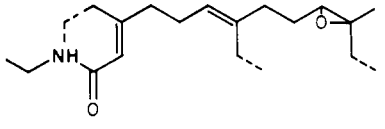
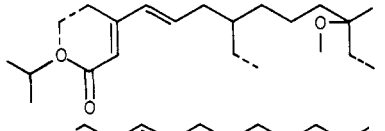
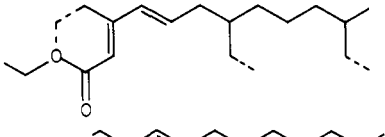
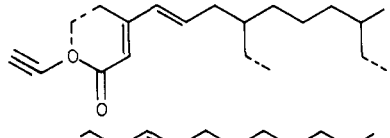
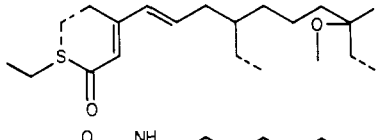
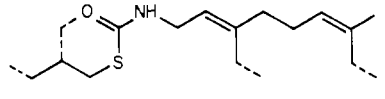
Table I. Structural Features of Selected Aromatic and Nonaromatic IGR's

No.	Structure	Reference
I		Pallos et al. (1969)
II		Bagley and Bauernfeind (1972)
III		Wakabayashi et al. (1971)
IV		Sarmiento et al. (1973)
V		Roller et al. (1967)
VI		Cruikshank and Palmere (1971)
VII		Henrick et al. (1973)
VIII		Staal et al. (1973)
IX		Staal et al. (1973)
X		Staal et al. (1973)
XI		Pallos and Menn (1974)

instar larvae or newly moulted pupae of the European linden bug, *Pyrrhocoris apterus*, and the cotton stainer bug, *Dysdercus cingulatus*, than known terpenoid IGR's. Babu and Slama (1972) have shown that the peptide derivative, ethyl pivalolyl-L-alanyl-*p*-aminobenzoate, showed a high order of systemic activity in sunflower plants as measured morphogenetically on last instar larvae of *Dysdercus cingulatus*.

A further departure from the terpenoid skeleton was recently reported by Punja et al. (1973). These investigators prepared several chrysanthemic acid esters which showed significant morphogenetic activity on the barred stainer bug, *Dysdercus fasciatus*, in comparison to several known terpenoid IGR's.

It is striking that while geranyl and farnesyl IGR's usually show high morphogenetic activity in coleopterous and/or dipterous species, the nonterpenoid IGR's described thus far appear to have activity linked primarily to hemipterous species.

The foregoing discoveries clearly showing that active compounds exist which are decidedly nonterpenoid chemical structures encouraged us also to search for simple compounds not containing terpene chains and yet retaining some of the important biological manifestations so characteristic of the terpenoid IGR's.

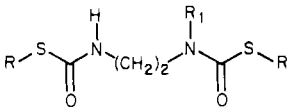
Figure 1. Generalized template for certain IGR's.

In our search we were guided by a simple working hypothesis: that the chemical skeleton shown in Figure 1 represents a template to which certain active IGR's should conform.

Table I lists several aromatic and nonaromatic IGR's and the cecropia moth, *Hyalophora cecropia*, *Cecropia* juvenile hormone 1 (compound V). The structures were drawn in conformity with the proposed template.

Compounds I–IV are terpenoids containing aromatic moieties bridged to the aryl group via an ether linkage (I, II, and IV) or a secondary amine moiety (III). Compounds V–XI embody nonaromatic configurations containing a variety of functional groups such as carboxylic acid esters (V, VII, VIII, and IX), an amide (VI), a thioester (X), and a thiocarbamate (XI). As shown here, these compounds contain a variety of functional groups which, when opti-

Table II. Physical Properties and Morphogenetic and Ovistatic Activity of Several Bisthiolcarbamate IGR's



No.	R	R_1	n_D^{30} or mp, °C	ir, cm^{-1} NH	ir, cm^{-1} C=O	ED_{50} values[a] Morphogenetic *T. molitor* pupa, μg/pupa	ED_{50} values[a] Ovicidal *E. acrea* eggs, % dip
XII	C_2H_5	C_2H_5	1.5235	3320	1640	0.5	0.07
XIII	*n*-C_3H_7	CH_3	1.5200	3320	1650	0.5	0.07
XIV	*n*-C_3H_7	C_2H_5	1.5110	3310	1640	0.1	0.06
XV	*n*-C_3H_7	*i*-C_3H_7	1.5145	3320	1650	0.5	
XVI	*i*-C_3H_7	CH_3		3320	1670, 1635	3.0	0.1
XVII	*i*-C_3H_7	C_2H_5	47-52	3300	1668, 1630	0.1	0.1
XVIII	*i*-C_3H_7	*i*-C_3H_7	65-68	3320	1670, 1630	2.0	>0.1
XIX	*i*-C_4H_9	CH_3	38-40	3330	1675, 1630	0.05	0.005
XX	*i*-C_4H_9	C_2H_5	1.5100	3320	1650	0.003	0.005
XXI	*i*-C_4H_9	*i*-C_3H_7	42-46	3320	1667, 1630	5.0	0.1
I[b]						0.0025	>0.1

[a] ED_{50} effective dose resulting in morphogenetic or ovicidal effect in 50% of treated pupae or eggs, respectively. [b] Included for comparison.

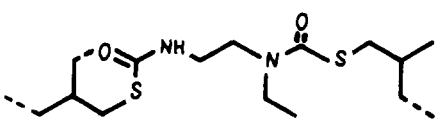


Figure 2. Bisthiolcarbamate IGR, compound XX.

mally placed in an IGR molecule, impart a high order of biological activity. By critical evaluation of published structure–activity relationships, and by adherence to the template shown in Figure 1, we have designed the structure shown in Figure 2.

MATERIALS AND METHODS

Chemicals and Apparatus. The *N*-alkylethylenediamines were purchased from Aldrich Chemical Co., Milwaukee, Wis. The chlorothiolformates were synthesized by H. Tilles, Stauffer Chemical Co. (Tilles, 1959, 1965). The NMR spectra were obtained on a Varian HA-60-IL spectrometer in deuteriochloroform solution with tetramethylsilane as an internal reference. The mass spectra were measured on a Varian MAT CH-5 spectrophotometer. Elemental analyses were performed on a Perkin-Elmer 240 Elemental Analyzer. Melting points are uncorrected.

Synthesis. The process, described by Batty (1948) for making certain bisthiolcarbamates, was modified, and the following general synthesis scheme was applied: 0.05 mol of the *N*-alkylethylenediamine and 5.0 g of sodium hydroxide were dissolved in 50 ml of water. The chlorothiolformate (0.1 mol) was slowly added, under ice–water bath cooling, at a rate which maintained an internal temperature of 10–20 °C. The mixture was stirred for 0.5 hr at room temperature and extracted twice with methylene chloride; the organic phase was separated, washed with water, dried over anhydrous $MgSO_4$, filtered, and the solvent stripped off.

Specific Example. *N*-Ethylethylenediamine (4.4 g; 0.05 mol) and 15.2 g (0.1 mol) of isobutylchlorothiolformate gave 15.3 g of compound XX; yield, 95.6%. The compound was characterized as follows: ir 3320 (NH), 1650 (C=O); NMR δ 0.95 (d, 12 H, methyl), 1.17 (t, 3 H, methyl), 1.80 (m, 2 H, methine), 2.83 (d, 4 H, methylene), 3.38 (g, 2 H, methylene), 3.50 (s, 4 H, methylene), 6.15 (br s, 1 H, NH); mass spectrum *m/e* (relative intensity) parent at 320 (0.3), 231 (9), 203 (8), 187 (9), 174 (17), 146 (23), 141 (18), 115 (56), 70 (30), 58 (49), 57 (100), 41 (60), 29 (60). Anal. Calcd for $C_{14}H_{28}N_2O_2S_2$: C, 52.47; H, 8.80; N, 8.74. Found: C, 52.05; H, 9.06; N, 8.60.

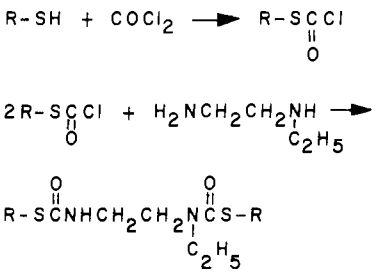


Figure 3. Generalized synthesis scheme for bisthiolcarbamates.

The synthesis of this new class of IGR's is outlined in Figure 3. Further synthetic details were given by Pallos (1974). The physical properties of the new compounds are described in Table II.

Bioassay. Candidate compounds were evaluated by topical application in 1 μl of acetone to the venter of newly moulted pupae of the yellow mealworm, *Tenebrio molitor*. Morphogenetic activity was determined by presence on the emerged adultoids of urogomphi, gin traps, pupal cuticle, or production of adult–pupal intermediates or secondary pupae. The ED_{50} values are expressed as the applied dose at which 50% of the treated pupae exhibited one or more of the aforementioned abnormalities.

Ovicidal activity was determined on the salt-marsh caterpillar, *Estigmene acrea*, by dipping newly laid egg masses in acetone solutions of the candidate compounds. Ovicidal activity was determined by failure of the treated eggs to hatch into viable larvae.

RESULTS AND DISCUSSION

The structure–activity relationships shown in Table II summarize the morphogenetic and ovicidal effects of several bisthiolcarbamates. It is apparent from these results that morphogenetic activity is enhanced in those compounds where $R_1 = C_2H_5$, namely compounds XIV, XVII, and XX. Replacing the proton of the primary amide with alkyl groups invariably reduced activity. The structure–activity data with respect to ovicidal action appear to follow the pattern shown with respect to morphogenetic activity. Again, the C_2H_5 substitution on R_1 appears to be optimal for bioactivity.

The candidate IGR of greatest interest is compound XX

Table III. Acute Toxicity Evaluation of Compound XX

Species	Route of administration	LD_{50}
Rat ♀	Oral	2710 mg/kg[a]
Mouse ♀	Oral	2330 mg/kg[a]
Rabbit	Dermal	>4640 mg/kg[a]
Mosquito fish	In water	6 ppm[b]

[a] Mammalian toxicity data were determined after 14 days holding. [b] Fish toxicity determined after 5 days holding.

[*N*-ethyl-1,2-bis(isobutylthiolcarbamoyl)ethane]. It possesses the highest morphogenetic and ovicidal activity in this series of IGR's. In morphogenetic activity it approaches the activity of compound R-20458 (compound I) (Pallos et al., 1969). In contrast, the ovicidal action of compound XX is more than 20-fold greater than that shown by compound I.

Ovicidal tests with eggs of other species of insects, including other lepidopterans, were negative. This suggests that compound XX may be highly specific in its action. Furthermore, no morphogenetic or ovicidal activity was demonstrated by compound XX when either last instar larvae or young adult *E. acrea* were treated with this compound. Additionally, evaluation of the contact and systemic activity of compound XX on the bean aphid, *Aphis fabae*, proved to be negative. This is in contrast to results obtained with several other nonterpenoid IGR's which were active on hemipterans.

Although the mode of action of these bisthiolcarbamates is presently unknown, possibly the specificity of action might be due to the requirements of an exact fit of the molecule on an active site during a sensitive stage in the development of a susceptible insect. Susceptibility, whether species-dependent or limited to a given developmental phase in the life history of an insect, may also be governed by the metabolic instability and/or penetrability and transport to the site of action of these IGR's. As shown in Table II, even slight structural modification of the proposed template markedly influences bioactivity in the target insect.

The selective action of compound XX is not limited to insects. The acute toxicity data shown in Table III clearly demonstrate the nontoxicity of this IGR to rats, mice, and rabbits and only mild toxicity to fish.

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