Alkylene-bis-isothiocyanates: Novel Insect Growth Regulators

György Matolcsy, István Ujváry

Plant Protection Institute, Hungarian Academy of Sciences,
H-1022 Budapest, Herman Ottó ut 15, Hungary

Lynn M. Riddiford and Kiyoshi Hiruma

Department of Zoology, University of Washington, Seattle, Washington 98195, USA

Z. Naturforsch. **41c,** 1069–1072 (1986); received May 30, 1986

Alkylene-bis-isothiocyanates, Insect Cuticle, Spiracles, Crochets, Insect Control

In search for selective anti-insect agents some mono- and bis-isothiocyanates were prepared and tested on third instar larvae of *Manduca sexta*. The alkylene-bis-isothiocyanates **4**, **5** and **6** had a novel effect preventing formation of the abdominal spiracles and of the hooks (crochets) at the following molt to the 4th instar. Some of these larvae died in the molt, while spiracles remained absent also in the larvae which were able to molt to the 5th instar. Histological examination indicated a selective destruction of spiracle forming cells. This novel action might serve as starting point of a new approach in selective insect control.

During recent years the search for safer, more selective insecticides has led to the discovery of various insect growth regulators which function either as the juvenile hormone (JH) of insects to prevent metamorphosis and to interfere with embryonic development [1, 2], or as anti-JHs to prevent reproductive maturation [3]. Some thiolcarbamates are known to possess JH- [4, 5] or anti-JH [6] activity. Dithiocarbamates have been found to inhibit the activity of tyrosinase [7], an enzyme participating in insect cuticle formation, a promising target for the development of selective insecticides. Based on these considerations, we prepared and bioassayed new derivatives of thiol-, thion- and dithiocarbamic acids, including isothiocyanates derived from lipophilic mono- and diamines.

Manduca sexta larvae were reared on artificial diet [8] under a 12L:12D photoperiod at 25 °C. For the assays the compound was dissolved in acetone and 1 µl applied to the dorsal midline of 3rd instar larvae within 6 h of ecdysis. The larvae were checked after ecdysis to the 4th instar, to the 5th instar and to the pupa. Doses of 25, 50 and 100 µg were applied to at least 10 larvae per dose.

During the bioassays of these compounds we discovered that alkylene-bis-isothiocyanates had a novel effect in preventing the formation of the abdominal spiracles at the following molt. The loss of a majority of the spiracles led to reduced growth and death before pupation. Moreover, these compounds prevented the formation of the hooks (crochets) on the abdominal prolegs which are necessary to the caterpillar to hang onto the plant while feeding. Thus, both of these novel actions lead to disruption of insect feeding and growth and therefore are good candidates for a new type of insect growth regulators.

Fig. 1 shows the structure of the isothiocyanates (**1**–**6**) and of the potential metabolic precursor (**7**) of **5**. Compounds **1**–**4** are known from the chemical literature, while **5**–**7** are new. Their synthesis will be described elsewhere.

The alkyl-monoisothiocyanates 3-methylbut-1-yl (**1**) and adamant-1-yl-isothiocyanate (**2**) proved to be inactive, whereas phenyl-isothiocyanate (**3**) was ineffective at 10 µg but lethal at 50 µg. By contrast, the alkylene-bis-isothiocyanates **4**, **5** and **6** and the potential precursor of **5** (**7**) all prevented abdominal spiracle formation to varying degrees during the following molt to the 4th instar (Fig. 2a). Compound **4** was clearly the most effective in this regard in that 80% of the larvae receiving only 2.5 µg had malformed spiracles. At doses of 25 µg or higher this compound was toxic and killed the larvae within 24 h of application. The cycloaliphatic 1,3-bis-isothiocyanate **5** was somewhat less effective at blocking spiracle formation, 10 µg/larva dose being needed to cause malformed spiracles in 80% of the larvae. It was not lethal since even the larvae given 100 µg grew normally and molted to the 4th instar on time.

Reprint requests to Prof. Gy. Matolcsy.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/1100–1069 $ 01.30/0

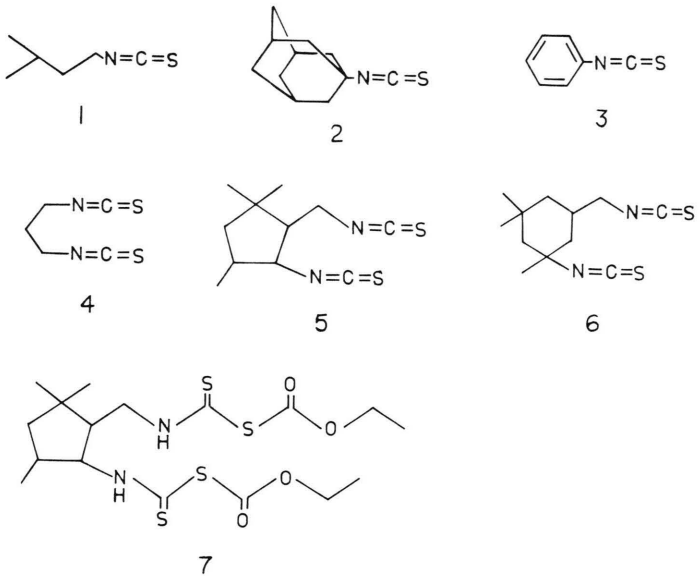

Fig. 1. Chemical structure of the isothiocyanate derivatives tested. Compounds **5**, **6** and **7** are mixtures of optical isomers.

The 1,4-bis-isothiocyanate **6** and the possible precursor of **5** (**7**) were still less effective with 25–50 μg necessary to cause spiracular anomalities. Although the small number of compounds studied so far does not permit unambiguous conclusion about the structure-activity relationships, the data on these seven compounds suggest that the effect on spiracular formation is linked to the bis-isothiocyanate structure, the 1,3-structure being favored over the 1,4-substitution.

To examine the spiracular effect in more detail, we used compound **5**, 1-isothiocyanato-2-isothiocyanatomethyl-3,3,5-trimethyl-cyclopentane. Spiracular malformations seen after application of 10–100 μg ranged from partial to complete loss of the spiracle (Fig. 2a).

In the latter case histological sections showed that the new cuticle completely covered spiracular opening (Fig. 2b). Apparently the spiracle forming cells had been selectively damaged by this compound. No other cuticular defects were seen after the 10 μg dose, but at 50 and 100 μg the dorsal cuticle near the site of application was deformed and the marking pattern distorted indicating damage to some of the epidermal cells. In a few cases no cuticle formed so that the larva died during ecdysis when the unprotected epidermis ruptured. Therefore, the spiracular epidermis appears to be exquisitely sensitive to these compounds. This sensitivity apparently does not require direct contact with the compound although we cannot exclude the possibility that some run-off from the 1 μl acetone solution may have reached the spiracle due to the small size of the larva (70 ± 16 mg, $N = 10$) at the time of application.

The 4th instar larvae with the malformed or missing spiracles grew more slowly, but 72% were able to molt to the 5th (final) larval instar. The spiracles remained abnormal or absent in these larvae. If more than 70% of the spiracles were severely affected, the larvae died before pupation. Thus the toxic effect of these compounds on the spiracular epidermis is permanent. Respiration apparently is sufficient for normal growth and pupation if only a few spiracles are blocked since the tracheal system is interconnecting [9, 10], but rapidly becomes limiting when the majority are partially or totally blocked.

Many of the treated larvae were missing crochets on one or several prolegs. The crochet epidermis is normally free from the overlying cuticle during the feeding stage [11] and is quite distant from the site of application. Therefore, it too seems peculiarly sensitive to the effects of these bis-isothiocyanates. Under field conditions the lack of these crochets would make feeding on leaves difficult, if not impossible, since the presence of the crochets enable the prolegs to grip the plant.

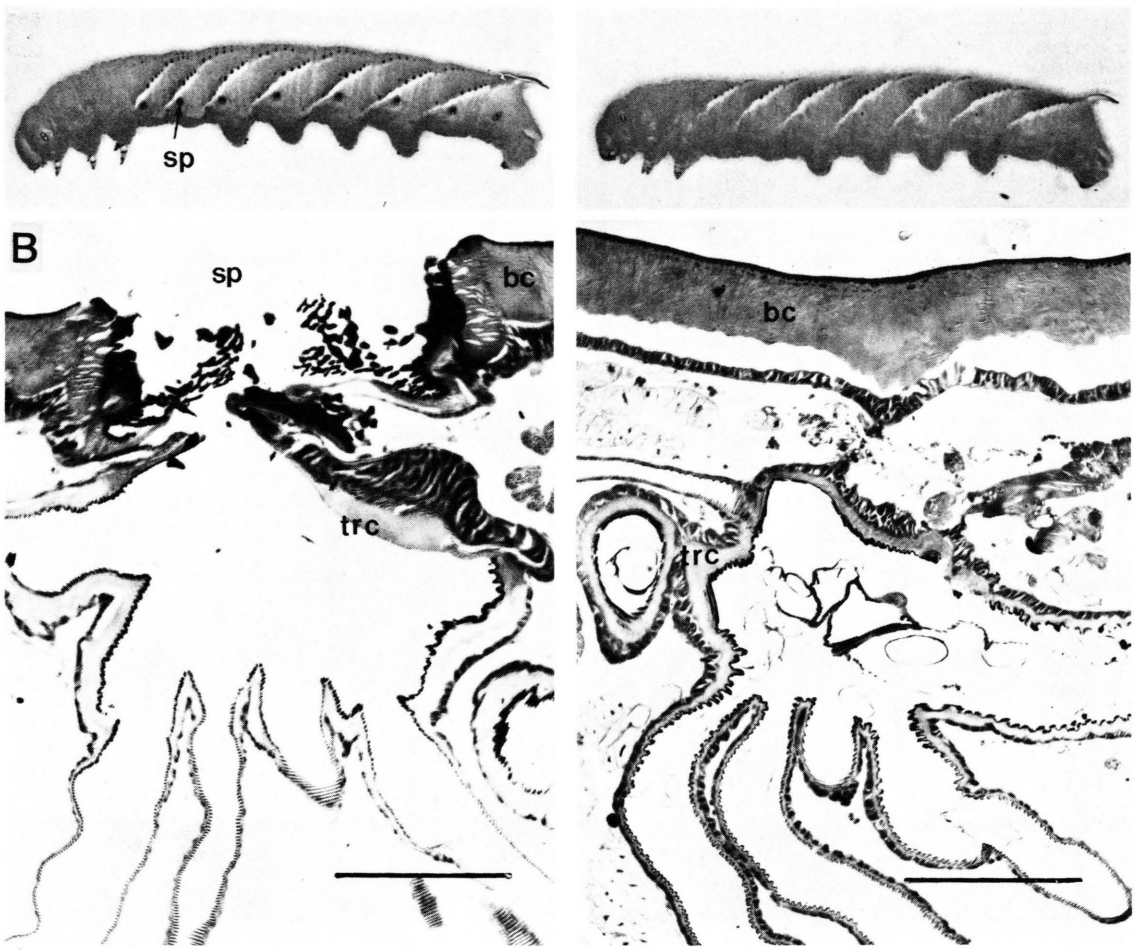

Fig. 2. a) Fifth instar *Manduca* larvae: left, control; right, treated with 100 μg **5**, 1-isothiocyanato-2-isothiocyanatomethyl-3,3,5-trimethyl-cyclopentane 6 h after ecdysis to the third larval instar. Note the missing abdominal spiracles (sp).
b) Paraffin sections (5 μm) stained with Mallory's trichrome stain of the spiracular region in the normal and the treated larvae above. Note the complete absence of the spiracular opening in the treated larva. The detachment of the epidermis from the overlying body cuticle was an artifact introduced during tissue preparation. Bar = 0.3 mm. bc, body cuticle; trc, tracheal cuticle; sp, spiracle.

Although the mechanism of this toxic action on specific epidermal cell types is not known, its potential use in selective insect control is high since it attacks key cells necessary for insect life. The synthesis of further derivatives within this class, aimed at the optimization of activity and elucidation of structural requirements for this activity are in progress.

We thank Dr. Shirley Reiss for the histological sections and Dr. James W. Truman for helpful comments on the manuscript. These studies were supported by UNDP/UNIDO/FAO project HUN/82/006 to GM and University of Washington Graduate School Research Fund and NSF DCB 80-11152 to LMR.

[1] G. B. Staal, Ann. Rev. Entom. **20,** 417–460 (1975).
[2] F. Sehnal, in: Endocrinology of Insects (R. G. H. Downer and H. Laufer, eds.), pp. 657–672, Alan R. Liss, Inc., New York 1983.
[3] W. S. Bowers, in: Endocrinology of Insects (R. G. H. Downer and H. Laufer, eds.), pp. 517–523, Alan R. Liss, Inc., New York 1983.
[4] F. M. Pallos, P. E. Letchworth, and J. J. Menn, J. Agric. Food Chem. **24,** 218–221 (1976).
[5] H. Kisida, M. Hatakoshi, N. Itaya, and I. Nakayama, Agric. Biol. Chem. **48,** 2889–2891 (1984).
[6] S. J. Kramer, L. W. Tsai, S. F. Lee, and J. J. Menn, Pestic. Biochem. Physiol. **17,** 134–141 (1982).
[7] Sh. Pomerantz, J. Biol. Chem. **238,** 2351–2357 (1963).
[8] R. A. Bell and F. G. Joachim, Ann. Entom. Soc. Am. **69,** 365–373 (1976).
[9] V. B. Wigglesworth, Adv. Insect Physiol. **17,** 85–148 (1983).
[10] P. J. Mill, in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 3 (G. Kerkut and L. I. Gilbert, eds.), pp. 517–593, Pergamon Press, Oxford 1985.
[11] M. J. Fain and L. M. Riddiford, Wilh. Roux Arch. dev. Biol. **181,** 285–307 (1977).