(*R*)Mandelonitrile and Prunasin, the Sources of Hydrogen Cyanide in All Stages of *Paropsis atomaria* (Coleoptera: Chysomelidae)

A. Nahrstedt*

Institut für Pharmazeutische Biologie, Technische Universität, D-3000 Braunschweig, Bundesrepublik Deutschland

R. H. Davis

Wye College, University of London, Wye, Ashford, Kent, England

Z. Naturforsch. **41c,** 928–934 (1986); received May 26, 1986

Paropsis atomaria, Coleoptera, Cyanogenesis, Cyanogenic Glucoside, (*R*)Mandelonitrile, Prunasin

(*R*)Mandelonitrile and the cyanogenic glucoside prunasin have been established as the source of HCN in all stages of the Australian beetle *Paropsis atomaria.* Quantitative data for both compounds and all life stages are presented. The larvae contain a β-glucosidase activity capable of hydrolysing prunasin and also β-cyanoalanine synthase activity enabling the disposal of free cyanide.

Introduction

The larvae and imagines of *Paropsis atomaria* Ol. (Coleoptera: Chrysomelidae) are a serious pest of *Eucalyptus* trees in south east Australia and their ecological characteristics have been extensively studied by Carne [1]. When crushed the larvae smell strongly like bitter almonds and Moore [2] has shown that the milky fluid that is produced by the larvae in abdominal vesicles and which is secreted when the larvae are agitated, contains HCN (hydrocyanic acid), benzaldehyde and glucose. The secretion possesses defensive properties in that ants *(Iridomyrmex detectus)* which came into contact with the fluid were knocked down within 20 seconds and dead within 2 minutes [2]. Moore concluded that the three components detected probably "were at some stage combined in the form of a glucoside".

Cyanogenic glucosides are, indeed, known to occur in insects, having been found in recent years in several Lepidoptera; *i.e.* in all Zygaenidae, Heliconiini (Nymphalidae) and Acraeidae tested [3] and recently in two species of the Lycaenidae (*Polyommatus, Brephidium;* Nahrstedt and Davis, unpubl.). In all species the two aliphatic cyanoglucosides linamarin and (*R*)lotaustralin were the source of hydrogen cyanide. However, cyanogenic glycosides have not been isolated from cyanogenic centipedes (Chilopoda) and millipedes (Diplopoda) so far but in several species the presence of mandelonitrile (the cyanohydrin of benzaldehyde) and mandelonitrile benzoate, besides other non-cyanogenic nitriles have been established [4]. Mandelonitrile was also detected as a constituent of the pygidial gland of the tiger beetle, *Megacephala virginica* (Coleoptera: Cicindelinae) [5].

Mandelonitrile is a fairly unstable compound and decomposes into benzaldehyde and HCN (Fig. 1, arrow 2) more rapidly as the pH becomes more alkaline [15]. On the other hand cyanogenic glycosides and mandelonitrile esters represent stable cyanogenic compounds, the former being cyanogenic after hydrolysis catalysed by particular β-glycosidases [6]. Thus, the question arises as to whether HCN, benzaldehyde and glucose in the larval secretion of *P. atomaria* [2] arise from decomposition of a glucoside or a cyanohydrin (mandelonitrile) or whether they exist as free compounds in the secretion fluid. In addition, other stages of the life-cycle of *P. atomaria* needed to be investigated for cyanogenesis.

Methods and Materials

Insects

Eggs of *P. atomaria* were obtained by courtesy of staff of the Division of Entomology, C.S.I.R.O., Canberra City, Australia. On receipt the eggs were

* *Present address:* Institut für Pharm. Biol. und Phytochemie, Westf.-Wilhelms-Universität, D-4400 Münster.

Reprint requests to Prof. Dr. A. Nahrstedt.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0900–0928 $ 01.30/0

placed on plants of *Eucalyptus nitens*. When larvae had become established, second instar, they were transferred in groups of up to 100 to plastic boxes, 175 × 115 × 60 mm, with absorbent paper in the bottom, numbers per box being reduced to about 30 at the final instar. Fresh leaf material of *E. nitens* was provided twice daily in sufficient quantity to allow appetite to be satisfied and larvae were transferred to clean boxes every 1 or 2 days. After feeding had ceased, larvae positioned themselves under the paper and remained there for pupation and throughout the pupal phase. After emergence, imagines were placed on potted plants of either *E. nitens* or *E. viminalis*, the latter being preferred for egg laying. This cycle could then be repeated. All rearing took place in a growth room maintained at a temperature of 26–28 °C and with a 16 h:8 h light:dark cycle, conditions under which diapause does not occur. Specimens required for analysis were frozen, ground and lyophilized.

Chemicals

β-glucosidase from almonds was obtained from Serva (D-6900 Heidelberg), prunasin from Roth (D-7500 Karlsruhe), rac. mandelonitrile from Aldridge (D-7926 Steinheim), L-cysteine and N,N-dimethyl-p-phenylenediamine HCl from Sigma (Poole) and all others from Merck (D-6100 Darmstadt). Dimethylaminopyridine was a gift from Prof. Dr. G. Höfle (Braunschweig). Mandelonitrile lyase (nitrilase) and β-glucosidase free of nitrilase were prepared from the almond β-glucosidase by the method of Gross *et al.* [7] on DEAE-Cellulose; however, 50 µl nitrilase as used still contained 0.3 nkat β-glucosidase activity with prunasin as substrate.

HCN estimations

Either the lyophilized material directly or extracts made with MeOH or MeOH/CH_2Cl_2 and taken to dryness, were incubated with phosphate buffer, pH 6.0, at 38 °C with β-glucosidase from almonds. The incubation vial was aerated with air and the air passed through a trap containing 0.1 M NaOH. After usually 18 h the amount of HCN was estimated colourimetrically using the anthranilic acid method [8]. For estimations that involved the use of the "Wissing" apparatus [9] incubations were carried out at 27 °C with a flow of 250 ml/min nitrogen through the apparatus. Cold prepared MeOH extracts were taken to dryness at c. 10 °C and 1 ml phosphate buffer pH 6.0 and 50 µl nitrilase (c. 5 nkat) were added. After 15 min, 100 µl of β-glucosidase free of nitrilase (c. 7.5 nkat) were added and incubation continued for a further 15 min. The trap for HCN was changed at intervals of 1.5, 2, 3 and/or 4 min. Aliquots of 28 mg d.m. of larvae, 70 mg of pupae, 167 mg of imagines and 180 mg of eggs were used for incubation.

Isolation of mandelonitrile as its acetate

3.5 g lyophilized larvae were extracted with cold MeOH/CH_2Cl_2, 2:1 and the solvent evaporated at 10 °C to 1 ml. The extract was subsequently chromatographed on 5 analytical silica gel TLC plates (Merck) with benzene/$CHCl_3$/MeOH, 5:1:0.3. The zone of the cyanogen was detected with Feigl-Anger paper [10], scraped off immediately and eluted with 15 ml EtOAc/$CHCl_3$, 1:1. The eluant was concentrated to 2 ml at 10 °C and 0.1 g dimethylaminopyridine, 0.5 ml acetic anhydride and 0.2 ml pyridine were added [11]. After 18 h 0.5 ml MeOH were added and the solution was shaken twice with 20 ml 0.5 N HCl and once with 20 ml 0.5 N $NaHCO_3$. The organic phase was concentrated and chromatographed on 2 silica gel TLC plates with benzene. The mandelonitrile acetate, which was detected at 254 nm against synthesized racemic mandelonitrile acetate, was scraped off, eluted with CH_2Cl_2, evaporated and the residue taken for ^1H NMR analysis in CD_2Cl_2 in a Bruker WM400 spectrometer. $[\alpha]_{20}^{D}$ was determined on a microscale with 400 µl ($c = 0.2$) in CH_2Cl_2.

Isolation of prunasin

32 g lyophilized larvae were defatted with hot petrol and extracted for 4 h with hot MeOH. The extract was dried by evaporation under vacuum, the residue suspended in 20–30 ml H_2O and filtered. The clear solution was lyophilized and dissolved in a few ml of EtOAc/MeEtCO/H_2O/AcOH, 7:5:1.5:0.2; this solution was applied to a centrifugally accelerated TL chromatograph (Chromatotron model 7924, Harrison Research, Palo Alto, CA) equipped with 4 mm silica gel layer and chromatographed using the above mentioned solvent. 8 fractions of 30 ml were collected of which 3, 4 and 5 showed cyanogenesis when tested with β-glucosidase. They were combined, taken to dryness, dis-

solved in 1 ml MeOH and chromatographed on four 1 mm silica gel plates (Merck) with the above mentioned solvent. The zones at R_f 0.4 were cyanogenic with β-glucosidase. They were scraped off, eluted with MeOH and the eluant was evaporated. The residue was dissolved in acetone-d_6, filtered and used for ^{1}H NMR spectroscopy. After silylation the isolate was gas chromatographed on an ICN-microbore-column DB5, 180–230 °C at 1.5 °C/min, flow 5 ml N_2/min, FID.

β-Glucosidase activity in larvae

180 mg d.m. of larvae were extracted several times with acetone at −20 °C. The dry acetone powder was extracted with 3.5 ml 0.05 M phosphate buffer pH 6, filtered and the residual HCN was removed by bubbling nitrogen through. 0.2 ml of this solution was incubated with 122 µg (0.4 µmol) prunasin in 2.5 ml buffer and HCN liberation was monitored using the "Wissing" apparatus [9] as described.

Detection of β-cyanoalanine

200 mg d.m. of larvae were defatted with petrol, then extracted with MeOH and concentrated to 5 ml under reduced pressure. 10 to 50 µl were chromatographed on silica gel TLC with $CHCl_3$/MeOH/NH_4OH (25%) using β-cyanoalanine as reference. After detection with ninhydrine reagent and heating to 100 °C for 15 min a weak blue zone which is typical for β-cyanoalanine [22] was visible in the range of the reference at R_f 0.6. When compared to the intensity of blue colour of the reference the amount of β-cyanoalanine in the larvae was roughly estimated as c. 5 µg/100 mg d.m.

β-Cyanoalanine synthase activity

Activity was measured by a modification of the procedure of Miller and Conn [12]. Frozen insect material was homogenized with 0.5 M Tris-HCl buffer, pH 8.5, and centrifuged. To 1 ml of the supernatant were added 0.5 ml of 0.05 M KCN and 0.5 ml 0.1 M L-cysteine, both in 0.1 M Tris-HCl buffer, pH 8.5, and adjusted to pH 8.5 immediately before use. All solutions were brought to 30 °C before mixing and incubations were carried out for 10 min at that temperature, after which the reaction was stopped by the addition of 0.5 ml of 0.03 M $FeCl_3$ in 1.2 M HCl followed by 0.5 ml of 0.02 M N,N-dimethyl-*p*-phenylenediamine mono HCl in 7.2 M HCl. After allowing 20 min for colour development 2 ml of *n*-butanol was added, the solutions mixed with a vortex mixer and then centrifuged. The absorption of the butanol layer containing methylene blue formed from the liberated sulphide was read at 600 nm. Under these conditions 1 µmole of sulphide has an absorbance of 13.3

Results

The total HCN content measured, as described, with added almond β-glucosidase which catalyses steps (1) and (2) Fig. 1 since it also contains nitrilase,

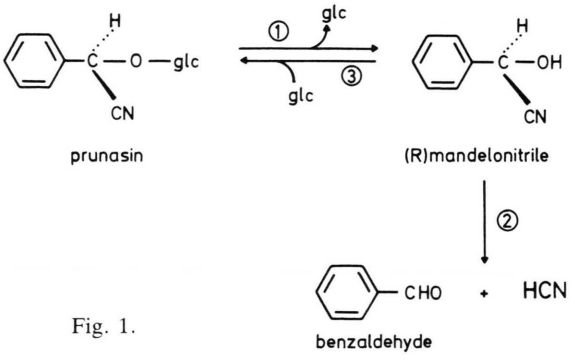

Fig. 1.

shows that all stages of the beetle are cyanogenic under these conditions but vary quantitatively, Table I; the larvae liberate about 15.5 µmol cyanide per g.d.m., the pupae less than 10%, imagines c. 1.6% and eggs less than 1% of the amount obtained from the larvae. However, the pulverized lyophilized material, smelled of bitter almonds and liberated HCN spontaneously and so some HCN may have been lost prior to analysis; thus, the values given in Table I are approximate and may be slightly less than the full amounts.

Table I. Enzymatically liberated total content of HCN at different stages of *Paropsis atomaria*.

	d.m. per indiv. [mg]	CN^- (µmol) per g d.m.	per indiv.
larvae	20	15.50	0.3
pupae	–	1.40	–
imagines	35	0.26	0.01
eggs	–	0.12	–

The presence of benzaldehyde in the larval secretion [2] indicated that the unstable cyanohydrin, mandelonitrile, might be the source of cyanide in the live insects. In order to test for its presence acetylation of the purified extract was carried out to stabilize the cyanogen, using dimethylaminopyridine as a catalyst [11]. The ^{1}H NMR spectrum of the lyophilized product gave resonances that unambiguously indicated the presence of mandelonitrile acetate; a multiplet at 7.5 ppm (5 aromatic protons), a singlet at 6.4 ppm (1 cyanohydrin proton) and a singlet at 2.15 ppm (3 protons of the acetyl group). The specific rotation of the acetate was found to be +12.5°. There is no specific rotation of the (*S*) or (*R*) mandelonitrile acetate reported in the literature. However, (*R*)mandelonitrile possesses an $[\alpha]_{25}^{546}$ of +46.9° in benzene and so the value of +12.5° for the acetate shows that, although some racemization occurred, the original material was (+) and so had the (*R*) configuration. Therefore, at least most of the mandelonitrile present in the sample had the (*R*) configuration (Fig. 1).

The next series of experiments was carried out to establish whether or not a cyanogenic glucoside was also present since glucose had been reported to be present in the larval secretion [2]. For these experiments the "Wissing" apparatus [9] was used because it allows the temperature of the incubation to be controlled, rapid isolation of HCN by means of a fast stream of nitrogen and addition of reagents and changing of the trap without loss of HCN. Fig. 2 shows the kinetics of HCN liberation from larvae and imagines, as examples, under the conditions described. It is obvious that little HCN was still being liberated by the added nitrilase after the first 15 min but, after addition of β-glucosidase free of nitrilase, the liberation rate was accelerated showing production of HCN due to the action of β-glucosidase. This is a strong indication of the presence of a cyanogenic β-glucoside. Table II shows the amount of HCN obtained under these conditions from all stages of *P. atomaria*. The amount of HCN liberated from a glucoside was calculated from the difference of the actual HCN value after 30 min and a hypothetical HCN value after extrapolating the nitrilase kinetic to 30 min. These values again are approximate as the nitrilase used was not completely free of β-glucosidase activity and HCN production had not ceased completely at the end of each 15 min period. Also some β-glucosidase activity was already present in the insects; activity corresponding to c. 0.6 nkat per 100 g.d.m. was measured in larvae at 27 °C. However, the results indicate that the amount of HCN fixed in a glucoside is about 10% of total HCN in larvae, c. 20% in pupae and eggs and up to c. 50% in imagines.

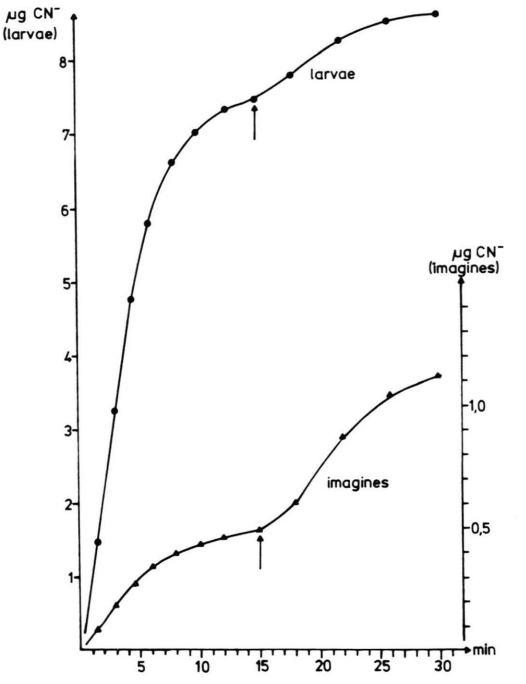

Fig. 2. Kinetics of HCN liberation during incubation of larvae (28 mg d.m.) and imagines (167 mg d.m.) with nitrilase at $t=0$ and after adding β-glucosidase (↑) at $t=15$ min.

Table II. HCN liberated from different stages of *P. atomaria* under the conditions described in the text using nitrilase for 15 min (A), followed by addition of β-glucosidase for another 15 min (B). A represents the amount of (*R*)mandelonitrile, B that of prunasin, C the total amount of HCN.

	CN^- (µmol per g d.m.)		
	A	B	C (A+B)
larvae	10.55	1.35	11.90
pupae	0.47	0.13	0.60
imagines	0.13	0.13	0.26
eggs	0.20	0.05	0.25

Although imagines had the highest percentage of glucoside, larvae were taken to isolate and characterize the cyanogenic glucoside because the absolute amount was highest in larvae. The conditions used

for extraction were chosen to eliminate most of the unstable free mandelonitrile and only one cyanogenic zone was detected on TLC by means of β-glucosidase; this zone was eluted and lyophilized. The ^{1}H NMR spectrum of this compound in acetone-d_6 showed resonances at 7.63–7.47 ppm, multiplet (5 aromatic protons), 5.95 ppm, singlet (1 cyanohydrin proton), 4.41 ppm ($J = 7.2$ Hz, doublet of the anomeric glucose proton indicating β-configuration) and 3.2–3.8 ppm (residual glucose protons). This spectrum was identical to that of prunasin taken as a reference. In addition a GLC run of the TMS ether of the isolate gave a retention time t_R of 35.6 min, identical with TMS-prunasin [13], and co-eluted with authentic TMS-prunasin. Therefore, the cyanogenic glucoside of *P. atomaria* is prunasin (2-β-D-glucopyranosyloxy-2-phenyl-2*R*-acetonitrile).

The other cyano compound investigated was β-cyanoalanine. Its presence could not be demonstrated unequivocally. A positive result was obtained by chromatographic separation and comparison but a second test was negative. However, the presence of β-cyanoalanine synthase activity was clearly shown. The activities found were 0.28 nkat per g fresh weight for actively feeding larvae, 0.18 for pupae and 0.58 for imagines; these correspond to 0.0085, 0.0095 and 0.011 nkat per mg protein respectively.

Discussion

In addition to larvae, which had been investigated by Moore [2], the present results show that all other stages of *P. atomaria* are cyanogenic although larvae contain by far the highest concentration of cyanogens (Table I). For comparison *Geophilus vittatus* (Geophilidae: Chilopoda) contain 7 μmol cyanide per g fresh weight (0.25 mol per individual) [14]; *Harpaphe haydeniana* (Xystodemidae: Diplopoda) contains 2.5 μmol per g f.w. (0.6 μmol per ind.) [4]; Lepidoptera that contain cyanogenic glucosides accumulate c. 30–300 μmol per g.d.m. (3–30 μmol per ind.) depending on the stage of development [3]. Thus, the larvae of *P. atomaria* are similar to the myriapods in their cyanogenic potential. The marked decline in cyanogenesis during the following stages may indicate that content in pupae, imagines and eggs represent residual cyanogenics from the larvae. Since the foodplant used in this study, *Eucalyptus nitens*, did not contain cyanogenic compounds, the cyanogenics must be synthesized by the larvae of *P. atomaria* as suggested by Moore [2].

Most of the cyanogens of *P. atomaria* are represented by (*R*)mandelonitrile. Since crushed material of all stages liberates HCN over a long period it is unlikely that free HCN is present, at least in large amounts. Even lyophilized material liberates an appreciable amount of HCN for several hours; a cyanogenic glucoside cannot be responsible for this HCN production as such a process needs water. The configuration of mandelonitrile and its derivatives has only been established in a few organism: *H. haydeniana* and the millipede *Gomphodermus pavanii* contain (*R*)mandelonitrile, whereas *Polydesmus pallaris* contains (*R*)mandelonitrile benzoate [4]. The (*R*)configuration of mandelonitrile from *P. atomaria* is, therefore, in agreement with the results obtained for other arthropods. It has been pointed out [5] that centipedes, millipedes and carabid beetles have independently evolved a common cyanogen. From this view it is of interest that in all cases in which it has been established ([14] and this paper) the configuration of mandelonitrile (or its derivative at C-2) was (*R*).

In addition to (*R*)mandelonitrile a stabilized cyanogenic glucoside, prunasin, is present at c. 10% of total cyanogens in the larvae and it seems likely that prunasin also accounts for 20–50% of total cyanogens in the pupae, eggs and imagines. Although the presence of a cyanogenic glucoside has often been discussed for Myriapoda and Chrysomelidae this is the first instance in which it has been established with certainty in these arthropods. Duffey and Towers [15] worked on the structure elucidation of a putative cyanoglucoside in *H. haydeniana* but without success; existence of a cyanogenic glucoside was excluded for the polydesmid *Oxidus gracilis* which produces benzaldehyde and HCN from phenylalanine [16]. The presence of an unidentified cyanogenic glucoside has been reported in the millipede *Jonospeltus splendidus*, which also contains mandelonitrile in its defensive secretion [17]. The (*R*)configuration of prunasin at C-2 fits well with that of (*R*)mandelonitrile; it seems probably that prunasin stems from transglucosylation of (*R*)mandelonitrile by a glucosyltransferase, as indicated by arrow (3) in Fig. 1. In this respect the biogenetic potency of *P. atomaria* resembles that of the Lepidoptera which have been shown to synthesize their cyanogenic glucosides themselves [3, 18]. The only other exam-

ple currently known of an insect that contains a cyanogenic glucoside is the Neo-Guinean bug, *Leptocoris isolata,* which forms its cyanoglucoside, cardiospermin, from a cyanohydrin that is obtained from precursors in its host plant [19].

Nothing is known about the localization of prunasin in the body of *P. atomaria.* According to Moore [2] mandelonitrile should be stored in the abdominal vesicles of the larvae, the fluid possessing a pH 3.4 at which mandelonitrile is fairly stable. Thus, the glucoside may be localized elsewhere in the body where stabilization is necessary in order to protect the organism from its own HCN, *e.g.* the haemolymph as in Lepidoptera [3]. The production of a stable glucosylated mandelonitrile may be an advantage to the animal because it allows distribution of the cyanogenic compound into other compartments of the body and need not be stored in special glands or vesicles. A β-glucosidase is then necessary for mandelonitrile and HCN production as required and such activity was demonstrated to be present in the acetone powder of larvae of *P. atomaria.* Thus, besides mandelonitrile, which is able to liberate HCN by decomposition, a HCN generating system using prunasin and a β-glucosidase is also present. It may be significant that prunasin represents a higher proportion of the total cyanogens in pupae, eggs and imagines, stages that are immobile and/or lack a defensive secretion; this basis for HCN production may then assume greater importance. Another possibility, although higher speculative, is that *P. atomaria* is positioned at an evolutionary step from an unstable cyanohydrin to a stabilized cyanoglucoside.

Alternatively, prunasin may be involved as an intermediate in mandelonitrile production. The defensive secretion of the millipede *J. splendidus* was reported to contain a β-glucosidase together with mandelonitrile, glucose and the unidentified cyanoglucoside [17]. Thus, one may argue that mandelonitrile is produced from a cyanogenic glucoside as precursor by a β-glucosidase in the secretion chamber, whereas the glucoside itself is produced elsewhere in that organism. A similar more detailed view in which a putative cyanoglucoside is involved in mandelonitrile production is given by Duffey [14] using *H. haydeniana* as a model.

It is important that cyanogenic organisms should themselves have protection against HCN in order that they do not succumb to their own toxin. It has been suggested [3] that conversion of cyanide to β-cyanoalanine is the major pathway for its detoxication in arthropods, rather than to thiocyanate; the presence of β-cyanoalanine synthase activity supports this suggestion. This could not be confirmed by unequivocal identification of β-cyanoalanine in the insects. However, its existence need only be temporary since conversion to aspartic acid could follow, as in plants [20]. This would not only result in increased economy of nitrogen utilization but also avoid the build up of a compound which could itself have toxic properties [21].

Acknowledgements

Prof. E. E. Conn (Davis, Ca) kindly provided the contact to Australian research workers of whom we particularly thank Dr. P. B. Carne and Dr. P. B. Moore (C.S.I.R.O., Canberra) for providing insect material. Dr. V. Wray (Braunschweig) monitored the NMR spectra and Dr. G. Höfle (Braunschweig) provided information on the acetylation procedure and 4-DMAP. Technical assistance in analyses and rearing of insects was given by C. Forth, J. Craston, A. Fermor and C. Ladley. The work was carried out under MAFF licence number PHF 64/75 issued under the Tree Pests (Great Britain) Order 1980.

[1] P. B. Carne, Austr. J. Zool. **14,** 647 (1966).
[2] B. P. Moore, J. Austr. Ent. Soc. **6,** 36 (1967).
[3] R. H. Davis and A. Nahrstedt, in: Comprehensive Insect Physiology, Biochemistry and Pharmacology (Edit. G. A. Kerkut and L. I. Gilbert), **Vol. 11,** p. 635, Pergamon Press, Oxford 1985.
[4] S. S. Duffey, in: Cyanide in Biology (Edit. B. Vennesland *et al.*), p. 385, Academic Press, London 1981.
[5] M. S. Blum, T. H. Jones, G. J. House, and W. R. Tschinkel, Comp. Biochem. Physiol. **69B,** 903 (1981).
[6] W. Hösel, in: Cyanide in Biology (Edit. B. Vennesland *et al.*), p. 217, Academic Press, London 1981.
[7] M. Gross, G. M. Jacobs, and J. E. Poulton, Anal. Biochem. **119,** 25 (1982).
[8] A. Nahrstedt, Dtsch. Apoth. Z. **117,** 1357 (1977).
[9] F. Wissing, in: Cyanide in Biology (Edit. B. Vennesland *et al.*), p. 473, Academic Press, London 1981.
[10] B. Tantisewie, H. W. L. Ruijgrok, and R. Hegnauer, Pharm. Weekbl. Ned. **104,** 1341 (1969).
[11] G. Höfle, W. Steglich, and H. Vorbrüggen, Angew. Chemie **90,** 602 (1978).
[12] J. M. Miller and E. E. Conn, Plant Physiol. **65,** 1199 (1980).
[13] A. Nahrstedt, in: Cyanide in Biology (Edit. B. Vennesland *et al.*), p. 146, Academic Press, London 1981.
[14] T. H. Jones, W. E. Conner, J. Meinwald, H. Eisner, and T. Eisner, J. Chem. Ecol. **2,** 421 (1976).
[15] S. S. Duffey and G. N. H. Towers, Can. J. Zool. **56,** 7 (1978).
[16] G. N. H. Towers, S. S. Duffey, and S. M. Siegel, Can. J. Zool. **50,** 1047 (1972).
[17] K. Bano and R. V. Krishnamoorthy, Curr. Sci. **47,** 387 (1978).
[18] A. Nahrstedt and R. H. Davis, Comp. Biochem. Physiol. **82B,** 745 (1985).
[19] J. C. Braekman, D. Daloze, and J. M. Pasteels, Biochem. Syst. Ecol. **10,** 355 (1982).
[20] P. A. Castric, K. J. F. Farnden, and E. E. Conn, Arch. Biochem. Biophys. **152,** 62 (1972).
[21] H. M. Schlesinger, S. W. Applebaum, and Y. Birk, J. Insect Physiol. **22,** 1421 (1976).
[22] K. Schwarzmeier, Chem. Ber. **109,** 3379 (1976).