Ryania Insecticide: Analysis and Biological Activity of 10 Natural Ryanoids†

Phillip R. Jefferies,‡ Robert F. Toia,‡ Brian Brannigan,‡ Isaac Pessah,§ and John E. Casida*,‡

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720, and Department of Veterinary Pharmacology and Toxicology, University of California, Davis, California 95616

Ryania insecticide, the powdered stemwood of *Ryania speciosa*, was analyzed by HPLC and ^{1}H NMR for the content (ppm, w/w) of 10 ryanoids. The compounds were isolated and their potencies compared for toxicity to housefly adults (injection, piperonyl butoxide as a synergist), flour beetle larvae (dietary exposure), and mice (intraperitoneal) and for competition with [^{3}H]ryanodine for binding to the Ca^{2+} release channel complex. Ryanodine (range 450–600 ppm) and 9,21-dehydroryanodine (650–700 ppm) are essentially equipotent and account for almost all of the biological activity of ryania in each of these systems. 9-Hydroxy-10-epiryanodine (7–17 ppm) and three 8_{ax}-hydroxy-10-epiryanodine derivatives [10-(*O*-methyl) (28–33 ppm), dehydro (30–64 ppm), and 10-(*O*-methyl)dehydro (22–32 ppm)] are very effective with houseflies but not in the other assays. 18-Hydroxyryanodine (<10 ppm), ryanodyl 3-(pyridine-3-carboxylate) (42–58 ppm), 8_{ax},18-dihydroxy-10-(*O*-methyl)-10-epiryanodine (5–10 ppm), and 9-hydroxyanhydroryanodine (12–17 ppm) are all of low activity. The potency of a ryanoid at the Ca^{2+} release channel is a better predictor of toxicity to mice and beetle larvae than to housefly adults.

INTRODUCTION

The botanical insecticide ryania is the ground stemwood of *Ryania speciosa* Vahl. (Flacourtiaceae) (Pepper and Carruth, 1945; Crosby, 1971; Casida et al., 1987). Eleven ryanoids have been obtained from various ryania preparations by a combination of wet chloroform extraction and solvent partitioning, followed by crystallization (Rogers et al., 1948) or chromatography (Jefferies et al., 1991; Ruest et al., 1985; this paper). Ten of these are considered in the present study as shown in Figure 1. One of them is a new compound (ryanoid F) and the structures of two others (A and D) have been revised (Jefferies et al., 1992). The 11th compound, ryanoid B (8-oxo-9,10-dehydroryanodine) (Ruest et al., 1985), was not obtained from the lots of ryania used for the current work.

The insecticidal activity of ryania extract was originally attributed to ryanodine (Rogers et al., 1948; Wiesner, 1972). It was later shown that the toxicity of ryania to insects and mammals and its action at the Ca^{2+}-activated Ca^{2+} channel were best accounted for by both ryanodine and the equipotent and more abundant 9,21-dehydroryanodine (Waterhouse et al., 1984, 1985, 1987). Little information was available on the toxicology and amounts of the other ryanoids or their contribution to the overall activity of ryania. The present investigation fills these gaps by describing an analytical method suitable for quantitating the ryanoids in ryania extract and by isolating them and determining their insecticidal activity, mammalian toxicity, and potency at the Ca^{2+} release channel complex.

MATERIALS AND METHODS

Chromatography. Radial TLC was carried out with a Model 8924 Chromatotron (Harrison Research, Palo Alto, CA) using plates coated with 1- or 2-mm layers of silica gel 60 PF_{254} (E. Merck, Darmstadt, Germany). HPLC utilized a Beckman 344 two-pump gradient system, a UV detector set at 254 nm, and either a semipreparative Ultrasphere ODS (5 μm) reverse-phase column (10 × 250 mm) (Beckman Instruments Inc., San Ramon, CA) or an analytical reverse-phase LichroCART column (4 × 125 mm) containing Lichrospher 100 RP-18 (5 μM) (E. Merck, Darmstadt, Germany). Separations were effected with methanol/water gradients.

Preparation of Ryania Extract. Two lots (designated 1712 and 81193) of powdered *R. speciosa* stemwood were obtained in 1990 from Agrisystems International (Windgap, PA). The extraction procedure was modified from that of Rogers et al. (1948). Thus, the powder (500 g) was thoroughly blended with water (800 mL) in a plastic bag, and the friable mixture was extracted with chloroform in a Soxhlet for 112 h. The recovered chloroform was concentrated to ~200 mL and then extracted with water (4 × 300 mL). The water was saturated with sodium chloride and extracted with ethyl acetate (5 × 200 mL) which was dried (sodium sulfate) and evaporated to give ryania extract (~1.5 g). **Caution:** Care should be taken to minimize exposure to ryania and fractions thereof because of their potential toxicity.

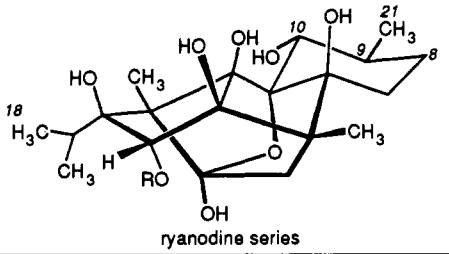
Isolation of Ryanoids. Ryania extract as above was dissolved in chloroform/methanol/40% aqueous methylamine (85:15:2), and the solution was filtered through silica gel and evaporated to dryness. The crude ryanoids were fractionated in batches (0.5 g) by radial TLC (2-mm silica gel plate) using a chloroform/methanol/40% aqueous methylamine (Ruest et al., 1985) gradient (from 95:5:1 to 80:20:3). Ester A, recovered as the first eluting fluorescent band, was purified by either rechromatography (radial TLC, 1-mm plate) or preparative HPLC (water/methanol gradient from 60:40 to 50:50). The second fluorescent zone contained residual A plus ester G, and these were subsequently readily separated by preparative HPLC under the aforementioned conditions or by extraction of G but not A from ethyl acetate into 0.1 M aqueous HCl. A multicomponent fluorescent zone recovered next from radial TLC was further purified by one or more of three procedures: preparative HPLC with water/methanol (7:3 to 1:1 gradient); radial TLC developed with ether/ethyl acetate (4:1 to 1:1 gradient); radial TLC in which the silica gel layer contained 15% PEG 8000 (Aldrich Chemical Co., Milwaukee, WI) and was developed with ether/ethyl acetate (5:1 to 1:1 gradient; under these conditions the ryanoids eluted, in order, were C_1, G, D, C_2, and E). The later eluates from radial TLC contained, in order, ryanodine, dehydroryanodine, and ester

† This study was supported in part by National Institute of Environmental Health Sciences Grant PO1 ES00049.

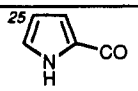
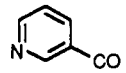
‡ University of California, Berkeley.

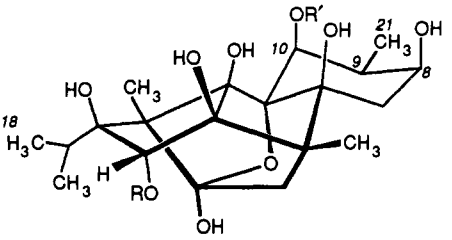
§ University of California, Davis.

0021-8561/92/1440-0142$03.00/0

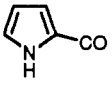


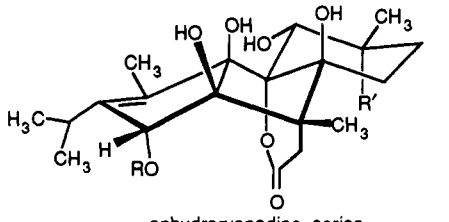
ryanodine series

designation	R	other substitution
ryanodine		
dehydroryanodine	"	9–21 = CH_2
18-hydroxyryanodine	"	18–HO
C_1	"	9–OH, 10-epi
G		
ryanodol	H	



8_{ax}–hydroxy–10–epiryanodine series

designation	R	R′	other substitution
A		CH_3	
D	"	CH_3	9–21 = CH_2
F	"	H	9–21 = CH_2
E	"	CH_3	18–HO



anhydroryanodine series

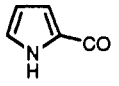
designation	R	R′
anhydroryanodine		H
C_2	"	OH

Figure 1. Structures of 10 natural ryanoids and 2 comparison compounds (ryanodol and anhydroryanodine). References to the isolation and structural assignment of each natural ryanoid are as follows: ryanodine series, ryanodine (Srivastava and Przybylska, 1970; Wiesner, 1972), 9,21-dehydroryanodine (Waterhouse et al., 1984, 1985), 18-hydroxyryanodine (Waterhouse et al., 1987), C_1 (Ruest et al., 1985), G (Jefferies et al., 1991); 8_{ax}-hydroxy-10-epiryanodine series, A and D (Ruest et al., 1985; Jefferies et al., 1992), E (Jefferies et al., 1992), F (Humerickhouse et al., 1989; Jefferies et al., 1992); anhydroryanodine series, C_2 (Ruest et al., 1985). Two comparison compounds are also shown, i.e., ryanodol and anhydroryanodine.

F; each was collected separately and purified either by crystallization (for ryanodine and dehydroryanodine) or by recycling on radial TLC, as above, followed by preparative HPLC (water/methanol gradient from 7:3 to 3:2) (ester F).

Analysis of Ryania Extract. A sample of the crude ryanoids, as above, was dissolved in tetrahydrofuran and analyzed directly by HPLC (analytical column; water/methanol gradient from 75:25 to 60:40 over 30 or 40 min at 0.85 mL/min and then maintained isocratically at the same flow rate until A was eluted). The amounts of ryanodine and/or dehydroryanodine were determined by weight; other pyrrolecarboxylate-containing components were determined relative to these absolute values by assuming the same specific absorbance for each compound and comparing peak areas directly. For the pyridinecarboxylate G, which has a specific absorbance at 254 nm of 0.38 relative to the other ryanoids, the peak area was multiplied by 2.6.

The above HPLC analysis procedure lacks precision for components that are not adequately resolved or are present in small amount. However, this can be overcome by using the "fractionation plus HPLC" procedure in which the mixture is initially separated into two fractions by radial TLC: the first contains esters E, G, D, C_2, C_1, and A, which are then analyzed by analytical HPLC; the second contains ryanodine, dehydroryanodine, and F which are analyzed by weight and analytical HPLC.

As an adjunct to the above techniques the ^1H NMR spectrum of ryania extract, or of any of its fractions, recorded in methanol-d_4 provides semiquantitative analytical information. Specifically, it permits determination of the content of the pyridine esters (almost entirely G) relative to the pyrrole components (except ester C_2) since the signals for the 3-H acyloxymethine protons for the two types of ester are well resolved (δ 5.91 and ~5.7, respectively) and the integrals can be compared directly. An alternative reference point is available in the C-25 pyrrole proton signal which appears at δ ~6.2. With the NMR method a value of 4.1% is obtained for the pyridinecarboxylate, a result that is in good agreement with that determined by HPLC.

Toxicity to Insects and Mice. Adult female houseflies (*Musca domestica*, SCR susceptible strain) were individually treated topically on the ventrum of the abdomen with piperonyl butoxide (PB) (5 μg) applied in acetone (0.5 μL). The test compound was administered 1–2 h later by intrathoracic injection (0.22 μL) as a solution in dimethyl sulfoxide (DMSO) or water. Knockdown (KD) was determined as the number of flies immobilized 4 h posttreatment and the lethal dose (LD) as the mortality at 24 h. KD_{50} and LD_{50} values are based on two or more independent experiments with a dose differential of 1.5–2-fold and 10 flies for each dose.

First instar flour beetle (*Tribolium castaneum*) larvae of an insecticide susceptible colony were placed on a diet containing the test compound and maintained for 28 days to determine the number of larvae, pupae, and adults (Ishaaya et al., 1983). The diet was prepared by adding an acetone solution (10 mL) of the test compound, or adding acetone alone (10 mL) as the control, to a mixture of wheat flour (9.5 g) and dried yeast (0.5 g) and then evaporating the solvent and thoroughly mixing. The diet was distributed in portions (2 g) into test vials (2 cm in diameter). Ten to 12 first instar (from 0 to 3 h old, white in color) larvae were placed in each vial and held at 28 °C for 28 days. The LD_{50}s (i.e., the dietary concentration causing 50% inhibition of adult emergence) reported are the means from five replicates of 10–12 larvae at each concentration.

Mouse toxicity (male albino Swiss-Webster, 18–22 g) was determined by intraperitoneal (ip) administration of the ryanoid in 50% aqueous ethanol or methoxytriglycol (10–100 μL) as the carrier vehicle (with appropriate controls). LD_{50}s are based on mortality data after 24 h. These data are approximations since only small amounts of some test compounds were available; however, they are estimated to be accurate within a factor of 1.5-fold.

Ca^{2+} Release Channel Potency (Pessah et al., 1985). *Preparation of Sarcoplasmic Reticulum (SR) Membranes.* Junctional SR membranes from rabbit back (fast) skeletal muscle were isolated according to the method of Saito et al. (1984). Junctional SR vesicles were rehomogenized in 0.3 M sucrose, 5 mM imidazole buffer (pH 7.4) at 3–5 mg/mL of protein, and aliquots were rapidly frozen in liquid N_2.

Measurement of [^3H]Ryanodine Equilibrium Binding. Specific binding of [^3H]ryanodine (0.6 ● 0.1 nM) to muscle preparations was measured by incubating SR vesicles (30–40 μg

of protein) for 2 h at 37 °C in the presence and absence of varying concentrations of competing ryanoid derivative in a final volume of 1 mL of 250 mM KCl, 15 mM NaCl, 50 μM $CaCl_2$, and 20 mM HEPES (pH 7.1). The assays were terminated by filtration through Whatman GF/B glass fiber filters using a Brandel (Gaithersburg, MD) cell harvester and washed twice with 2.5 mL of ice-cold wash medium, consisting of 250 mM KCl, 15 mM NaCl, 50 μM $CaCl_2$, and 20 mM Tris-HCl (pH 7.1). Radioactivity was measured by scintillation spectroscopy with an efficiency of approximately 43%. In all cases nonspecific binding of [^{3}H]-ryanodine was defined with addition of a 100-fold excess of unlabeled ryanodine. The average value of nonspecific binding to the high-affinity site amounted to 5–10% of the total binding at 0.6 nM [^{3}H]ryanodine. Filter binding of [^{3}H]ryanodine in the absence of vesicular preparation was negligible.

Analysis of Binding Data. Inhibition data were analyzed by logit transformation. Plots of log (P - (1 - P)) vs log inhibitor concentration gave straight lines, which were analyzed by linear regression analysis to obtain IC_{50} values (concentration of compound resulting in 50% of maximal inhibition). SD values averaged 13% of the IC_{50} values.

RESULTS

Analysis of Ryania for Ryanoids. Quantitative analytical data were obtained for nine ryanoids which make up about 1400 ppm (w/w) of lots 81193 and 1712 of ryania insecticide. The ryanoids are separable by HPLC on a C_{18} reverse-phase silica column using water/methanol mixtures as solvent. The resolution achieved is illustrated in Figure 2, and Rt values are given in Table I. Analysis by the "direct HPLC" method is adequately reproducible. However, for minor components, or for those that are inadequately resolved, i.e., G, C_2, C_1, and A, it is less reliable than the "fractionation and HPLC" procedure.

Ryanodine and dehydroryanodine account for 34–43 and 47–54% (w/w), respectively, of the total ryanoids determined. Esters A, D, F, and G are next in amount ranging from ~3–5% with smaller quantities of C_1, C_2, and E (~1% each). Two ryanoids reported from other lots of ryania, i.e., ester B (Ruest et al., 1985) and 18-hydroxyryanodine (Waterhouse et al., 1987), were not evident in the current lots at the discriminating level of 10 ppm.

Structure–Insecticidal Activity Relationships (Table II). Ryanodine and dehydroryanodine are essentially equipotent in all assays. Their potency is reduced or destroyed by introduction of an 18-hydroxy substituent (e.g., ryanodine vs 18-hydroxyryanodine and A vs E), by rupture of the C_1–C_{15} bond leading to opening of the cage-type region of the molecule [e.g., 9-hydroxyanhydroryanodine (C_2)], or by replacement of the pyrrole-2-carboxylate [e.g., the pyridine-3-carboxylate (G)]. The ryanoids other than ryanodine and dehydroryanodine show a wide range in their knockdown and lethal action toward houseflies but not in their retardation of development for flour beetle larvae. For example, compounds A, D, and F in the 8_{ax}-hydroxy-10-epiryanodine series and 9-hydroxy-10-epiryanodine (C_1) are close to ryanodine in their toxicity to houseflies, whereas compounds G and E are of substantially lower potency. In the beetle larvae assay, these six ryanoids are essentially inactive. Ryanodol, although apparently not a naturally occurring ryania component, has moderate activity in the housefly assays but is also inactive toward flour beetle larvae.

Structure–Channel Potency and Mouse Toxicity Relationships (Table II). The Ca^{2+} release channel in rabbit skeletal muscle accepts ryanodine and dehydroryanodine equally well. The activity is reduced 3–10-fold by the presence of the 8_{ax}-hydroxy and 10-(*O*-methyl) substituents in the epiryanodine and epidehydroryanodine series (A and D, respectively). Hydroxylation at the 18-position also reduces potency to a similar extent. All other modifications in the natural material essentially destroyed the activity.

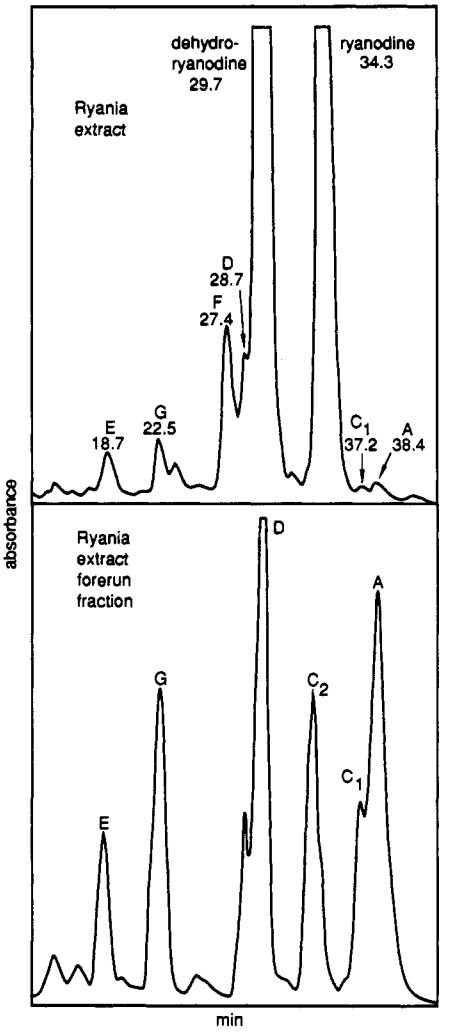


Figure 2. HPLC analysis of ryanoid constituents in ryania. The ryania extract is analyzed directly (top) and for a different sample as a forerun fraction from radial HPLC which removes all of the ryanodine, dehydroryanodine, and F. Ryanoids are identified in Figure 1. Analytical C_{18} reverse-phase silica column developed with water/methanol gradient (see Materials and Methods) was used. The specific absorbance of G is 0.38 relative to the other compounds.

Ryanodine and dehydroryanodine are much more toxic than the other ryanoids to mice, and the relative toxicities of the ryanoids generally parallel their potency order at the Ca^{2+} release channel (except A and D). This parallel relationship extends to the potencies with beetle larvae but not to those with houseflies.

DISCUSSION

The two major ryanoids of ryania insecticide are highly toxic on injection into either houseflies or mice, providing an initial indication of relatively nonselective action (Waterhouse et al., 1984, 1987). Favorable selective toxicity was first evident between ryanodine and ryanodol in their toxic action to houseflies and cockroaches compared with mice or with the Ca^{2+} release channel (Waterhouse et al., 1987). This selectivity is now also seen for A, C_1, D, and F with houseflies vs the mammalian systems. The potency orders established for the compounds in their toxicities to beetle larvae and mice are remarkably similar to those in the Ca^{2+} release channel assay. This suggests that the

Table I. Analysis of Ryanoid Constituents in Ryania

compound[a]	desig	HPLC Rt,[b] min	ppm (w/w) HPLC[c] 81193[e]	ppm (w/w) fractionation and HPLC[d] 81193[e]	ppm (w/w) fractionation and HPLC[d] 1712[e]
normal ryanoids[f]					
ryanodine		20.3	480 ± 19[g]	450[g]	600[g]
dehydroryanodine		15.9	700 ± 13[g]	700[g]	650[g]
ryanodyl 3-(pyridine-3-carboxylate)	G	11.1	58 ● 6	53	42
9-hydroxy-10-epiryanodine	C_1	22.5	17 ± 3	11	7
8_{ax}-hydroxy-10-epiryanodine					
10-(*O*-methyl)	A	23.0	33 ± 2	28	28
10-(*O*-methyl)dehydro	D	14.8	32 ± 3	30	22
dehydro	F	13.4	64 ± 4		30[g]
18-hydroxy-10-(*O*-methyl)	E	8.4	10 ± 3	10	5
anhydroryanodine					
9-hydroxy	C_2	18.3		17	12

[a] See Figure 1 for structures. [b] Semipreparative C_{18} reverse-phase silica column developed with 50:50 water/methanol (see Materials and Methods). [c] Analytical C_{18} reverse-phase silica column developed with water/methanol gradient (see Materials and Methods and Figure 2). Results are means and standard deviations of six determinations. [d] Fractionation by radial TLC with analysis by a combination of ^1H NMR and HPLC as in footnote c (see Materials and Methods and Figure 2). [e] Lot numbers for samples obtained in 1990. [f] 18-Hydroxyryanodine not detected (i.e., <10 ppm) and not evident by preparative radial TLC of these lots. [g] By weight.

Table II. Biological Activity of 10 Natural Ryanoids and 2 Comparison Compounds Relative to That of Ryanodine

compound[a]	desig	potency relative to that of ryanodine,[b] %: housefly injected KD_{50}	housefly injected LD_{50}	beetle larva dietary LD_{50}	mouse ip LD_{50}	Ca^{2+} release channel IC_{50}
ryanodine series						
ryanodine		100	100	100	100[c]	100
dehydroryanodine		90	136	91	100[c]	110
18-hydroxyryanodine					11[c]	11[c]
3-(pyridine-3-carboxylate)	G	<12	<12	0.2	<2	0.7
9-hydroxy-10-epiryanodine	C_1	26	68	0.5	1.1	2.0
8_{ax}-hydroxy-10-epiryanodine series						
10-(*O*-methyl)	A	53	213	1.4	3.6	27
10-(*O*-methyl)dehydro	D	57	100	0.7	3.1	11
dehydro	F	53	74	2.4	2.9	1.8
18-hydroxy-10-(*O*-methyl)	E	8	9	<0.1	<10	<0.5
anhydroryanodine series						
9-hydroxy	C_2	<0.3	<0.7	1.8	1.1	1.5
comparison compounds						
ryanodol		25	<34	0.3	<0.5[c]	0.03[c]
anhydroryanodine		0.2	0.5		<0.5[c]	<0.1[c]

[a] See Figure 1 for structures. [b] Absolute values for ryanodine: housefly (with PB) KD_{50} and LD_{50} 0.63 and 1.7 μg/g, respectively, injected in water and 0.14 and 0.36 μg/g, respectively, injected in DMSO; beetle larvae LD_{50} 0.10 ppm; mouse ip LD_{50} 0.10 mg/kg; muscle receptor IC_{50} 10 ± 2 (SD) nM. In the treatment of houseflies, ester C_2 and anhydroryanodine were administered in DMSO and the other compounds in water. [c] Data from Waterhouse et al. (1987). [d] Cincassiol relative potency <0.5 on beetle larvae.

receptor in beetle larvae is similar to that in the mouse and in rabbit skeletal muscle. It appears that selective toxicity patterns depend on the species being compared and that perhaps the fly receptor differs from that of the beetle larvae (based on toxicity) and mouse (based on toxicity and the radioligand assay).

The biological activity of the ryanoids is generally dependent on the overall structure. However, relative to ryanodine, minor structural variations in some regions of the molecule have little effect on potency, whereas in others they have a far greater impact. For example, the 9,21-dehydro derivatives are generally similar in potency to the parent ryanoids (dehydroryanodine vs ryanodine and D vs A), whereas an 18-hydroxy substituent drastically reduces potency (18-hydroxyryanodine vs ryanodine and E vs A). Oxygenation of the cyclohexane portion of ryanodine also significantly reduces activity such that the 9,21-dihydroxy derivative is virtually inactive (Waterhouse et al., 1987). On an analogous basis, ryanoids C_1 and F with 8- or 9-hydroxy substituents are also of low receptor potency, but for the latter compound much of the activity is restored when it is methylated to give ester D. It is plausible that these variations in biological activity may be associated with changes in lipophilicity. The acyl group also has a profound effect, e.g., ryanodine vs the pyridinecarboxylate G.

In summary, this study confirms that the toxicity of ryania insecticide to insects and mammals is primarily attributable to the two major constituents, ryanodine and dehydroryanodine. In addition, the analytical method reported here provides a procedure for monitoring different lots of ryania that may have substantial variations in their relative proportions of the naturally occurring ryanoids, depending on the source of the plant material. Although some of the minor components have appreciable toxicity toward houseflies, their relative concentrations in the lots of ryania examined are too low for them to be significant contributors to the overall insecticidal activity. However, they provide a range of structural variations not easily accessible synthetically, and these are useful probes for further understanding the relationship between structure and toxicity or receptor potency.

LITERATURE CITED

Casida, J. E.; Pessah, I. N.; Seifert, J.; Waterhouse, A. L. Ryania Insecticide: Chemistry, Biochemistry and Toxicology. In *Pesticide Science and Biotechnology*; Greenhalgh, R., Roberts, T. R., Eds.; Blackwell: Oxford, England, 1987; pp 177–182.

Crosby, D. G. Minor Insecticides of Plant Origin. In *Naturally Occurring Insecticides*; Jacobson, M., Crosby, D. G., Eds.; Dekker: New York, 1971; pp 198–205.

Humerickhouse, R. A.; Paschal, J.; Elvey, T.; Berry, D.; Besch, H. R., Jr. Atypical Pharmacology of Commercial "Ryanodine": Characterization of a Novel Ryania Constituent. *Pharmacologist* **1989**, *31*, 185 (Program and Abstracts ASPET, Abstract 363).

Ishaaya, I.; Elsner, A.; Ascher, K. R. S.; Casida, J. E. Synthetic Pyrethroids: Toxicity and Synergism on Dietary Exposure of *Tribolium castaneum* (Herbst) Larvae. *Pestic. Sci.* **1983**, *14*, 367–372.

Jefferies, P. R.; Toia, R. F.; Casida, J. E. Ryanodyl 3-(Pyridine-3-carboxylate): A Novel Ryanoid from *Ryania* Insecticide. *J. Nat. Prod.* **1991**, *54*, 1147–1149.

Jefferies, P. R.; Lam, W.-W.; Toia, R. F.; Casida, J. E. Ryania Insecticide: Structural Assignments of Four Natural 8_{ax}-Hydroxy-10-epiryanoids. *J. Agric. Food Chem.* **1992**, submitted for publication.

Pepper, B. P.; Carruth, L. A. A New Plant Insecticide for Control of the European Corn Borer. *J. Econ. Entomol.* **1945**, *38*, 59–66.

Pessah, I. N.; Waterhouse, A. L.; Casida, J. E. The Calcium-Ryanodine Receptor Complex of Skeletal and Cardiac Muscle. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 449–456.

Rogers, E. F.; Koniuszy, F. R.; Shavel, J., Jr.; Folkers, K. Plant Insecticides. I. Ryanodine, A New Alkaloid from *Ryania speciosa* Vahl. *J. Am. Chem. Soc.* **1948**, *70*, 3068–3088.

Ruest, L.; Taylor, D. R.; Deslongchamps, P. Investigation of the Constituents of *Ryania speciosa*. *Can. J. Chem.* **1985**, *63*, 2840–2843.

Saito, A.; Seiler, S.; Chu, A.; Fleischer, S. Preparation and Morphology of Sarcoplasmic Reticulum Terminal Cisternae from Rabbit Skeletal Muscle. *J. Cell. Biol.* **1984**, *99*, 875–885.

Srivastava, S. N.; Przybylska, M. The Crystal Structure of Ryanodol *p*-Bromobenzyl Ether. *Acta Crystallogr.* **1970**, *B26*, 707–715.

Waterhouse, A. L.; Holden, I.; Casida, J. E. 9,21-Didehydroryanodine: A New Principal Toxic Constituent of the Botanical Insecticide Ryania. *J. Chem. Soc., Chem. Commun.* **1984**, 1265–1266.

Waterhouse, A. L.; Holden, I.; Casida, J. E. Ryanoid Insecticides: Structural Examination by Fully Coupled Two-dimensional ^{1}H–^{13}C Shift Correlation Nuclear Magnetic Resonance Spectroscopy. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1011–1016.

Waterhouse, A. L.; Pessah, I. N.; Francini, A. O.; Casida, J. E. Structural Aspects of Ryanodine Action and Selectivity. *J. Med. Chem.* **1987**, *30*, 710–716.

Wiesner, K. The Structure of Ryanodine. *Adv. Org. Chem.* **1972**, 295–316.

Received for review August 14, 1991. Accepted October 28, 1991.