and the filtrate was evaporated to a crystalline mass. Recrystallization from toluene afforded the title compound (1.8 g, 59%): mp 85-87 °C.

Cinnamyl 6-Deoxy-6-lipoamido-1-thio-α-D-mannopyranoside (17). A solution of **13** (70 mg, 0.22 mmol) and *N*-(Lipoyloxy)succinimide (75 mg, 0.25 mmol) in THF (5 mL) containing Et_3N (50 μL) was kept overnight at room temperature and evaporated to dryness. The residue (100 mg) was purified by chromatography ($CHCl_3$-MeOH-H_2O, 95:5:0.5, v/v/v) to give **17** (34 mg, 30%): mp 189-190 °C (MeOH); $[\alpha]_D$ +143° (*c* 1.0, DMF); MS, *m/z* 499 ($M^{\bullet+}$), 382 ($M^{\bullet+}$ - $CH_2CH{=}CHC_6H_5$), 358 ($M^{\bullet+}$ - $SCH_2CH{=}CHC_6H_5$). Anal. ($C_{23}H_{33}NO_5S_3$) C, H, N, S.

1,2,3,4-Tetra-*O*-acetyl-6-*O*-methyl-β-D-mannopyranose. A solution of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose[16] (2.94 g, 8.4 mmol) in CH_2Cl_2 (30 mL) at 0 °C was methylated with excess CH_2N_2 in CH_2Cl_2 containing boron trifluoride etherate (0.12 mL, 0.98 mmol). After 2 h, excess CH_2N_2 was destroyed with glacial HOAc and the mixture was filtered. The filtrate was washed with aqueous $NaHCO_3$ and H_2O, dried, and evaporated to a syrup (3.0 g, 98%). An analytical sample was crystallized from EtOH: mp 102-103 °C; $[\alpha]_D$ -16° (*c* 1.02, $CHCl_3$). Anal. ($C_{15}H_{22}O_{10}$) C, H.

Cinnamyl 2,3,4-Tri-*O*-acetyl-6-*O*-methyl-1-thio-α-D-mannopyranoside (18). Compound **18** was prepared from 1,2,3,4-tetra-*O*-acetyl-6-*O*-methyl-β-D-mannopyranose in 33% overall yield similarly as **1** and the peracetate of **7**. The product was purified by flash column chromatography on silica gel with $CHCl_3$-EtOAc (98:2, v/v) as the eluant. Compound **18** was isolated as an oil: $[\alpha]_D$ +147° (*c* 2.08, $CHCl_3$); MS, *m/z* 452 ($M^{\bullet+}$), 392 ($M^{\bullet+}$ - HOAc), 332 ($M^{\bullet+}$ - 2HOAc), 303 ($M^{\bullet+}$ - $SCH_2CH{=}CHC_6H_5$); NMR ($CDCl_3$) δ 1.98, 2.06, and 2.12 (9 H, 3 OAc), 3.38 (s, OCH_3), 4.34 (m, H-5), 5.27 (br, H-1). Anal. ($C_{22}H_{28}O_8S{\cdot}H_2O$) C, H, S.

Cinnamyl 6-*O*-Methyl-1-thio-α-D-mannopyranoside (19). Compound **18** was de-O-acetylated with NaOMe in MeOH similarly as **2** and the product was purified by preparative TLC with $CHCl_3$-MeOH (90:10, v/v) as an irrigant. Compound **19** was isolated as an oil: $[\alpha]_D$ +287° (*c* 1.17, MeOH). Anal. ($C_{16}H_{22}O_5S{\cdot}H_2O$) C, H, S.

Cinnamyl 2,3,4-Tri-*O*-acetyl-6-deoxy-1-thio-α-D-mannopyranoside (20). Compound **20** was prepared from 1,2,3,4-tetra-*O*-acetyl-6-deoxy-α-D-mannopyranose in 46% overall yield similarly as **1** and the peracetate of **7**. The product was purified by flash column chromatography on silica gel with $CHCl_3$-EtOAc (99:1, v/v) as the eluant: MS, *m/z* 422 ($M^{\bullet+}$), 362 ($M^{\bullet+}$ - HOAc), 302 ($M^{\bullet+}$ - 2HOAc), 273 ($M^{\bullet+}$ - $SCH_2CH{=}CHC_6H_5$).

Cinnamyl 6-Deoxy-1-thio-α-D-mannopyranoside (21). Compound **20** was de-O-acetylated with NaOMe in MeOH similarly as **2** to give **21** in near-quantitative yield: $[\alpha]_D$ +354° (*c* 1.0, $CHCl_3$); MS, *m/z* 296 ($M^{\bullet+}$), 278 ($M^{\bullet+}$ - H_2O), 147 ($M^{\bullet+}$ - $SCH_2CH{=}CHC_6H_5$). Anal. ($C_{15}H_{20}O_4S{\cdot}0.5H_2O$) C, H, S.

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Structural Aspects of Ryanodine Action and Selectivity

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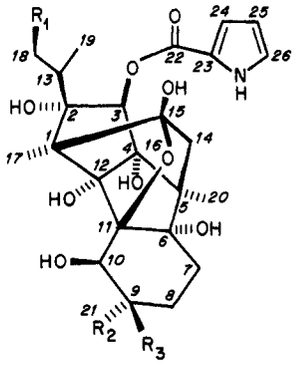
The topography and toxicological relevance of the Ca^{2+}-ryanodine receptor complex are evaluated with ryanodine and two natural analogues (9,21-didehydro and the new 18-hydroxy), 13 ryanoid derivatives (prepared from ryanodine and didehydroryanodine by functionalizing the available pyrrole, olefin, and hydroxyl substituents), and four degradation products. The potency of ryanoids at the skeletal muscle sarcoplasmic reticulum specific binding site generally parallels their toxicity to mice, supporting the toxicological relevance of the Ca^{2+}-ryanodine receptor. The optimal receptor potency of ryanodine and didehydroryanodine is reduced 3-14-fold by hydroxylation at an isopropyl methyl substituent, epimerization at C_9, oxidation or acetylation of the C_{10}-hydroxyl, or epoxidation at the 9,21-position; other ryanoids are less active. Ryanodol and didehydroryanodol, in contrast to ryanodine and didehydroryanodine, have low toxicity to mice and little activity at the mammalian receptor, yet they are potent knockdown agents for injected houseflies or cockroaches, suggesting a possible difference in the target sites of mammals and insects.

Ryanodine (**1**)[1,2] and 9,21-didehydroryanodine (**2**)[3-5] are the active principles of the botanical insecticide ryania, which is the ground stem wood of the shrub *Ryania speciosa* (family Flacourtiaceae).[6,7] Many degradation products and derivatives are known from studies on the characterization of **1**,[2] and other minor ryanoids are identified from *R. speciosa*,[5] but no information is available on their biological activity.

Ryanodine is a muscle poison that specifically uncouples the electrical signal of the transverse tubule from the Ca^{2+}-release mechanism of the sacroplasmic reticulum (SR).[8,9] The toxic action of ryanodine is attributed to binding to the Ca^{2+}-activated open state of the channel involved in the release of contractile Ca^{2+} from the SR, resulting in a change of channel structure possibly preventing its complete closing.[10-13] This "Ca^{2+}-ryanodine receptor complex", as assayed with [^{3}H]ryanodine[3] in skeletal and cardiac preparations, is highly specific, Ca^{2+}-activated, biochemically relevant, and pharmacologically unique.[10,11] The [^{3}H]ryanodine binding site elutes from sepharose as a high-molecular-weight oligomer made



1: $R_1 = R_3 = H$, $R_2 = CH_3$
2: $R_1 = H$, $R_2 = R_3 = {=}CH_2$
3: $R_1 = OH$, $R_2 = CH_3$, $R_3 = H$

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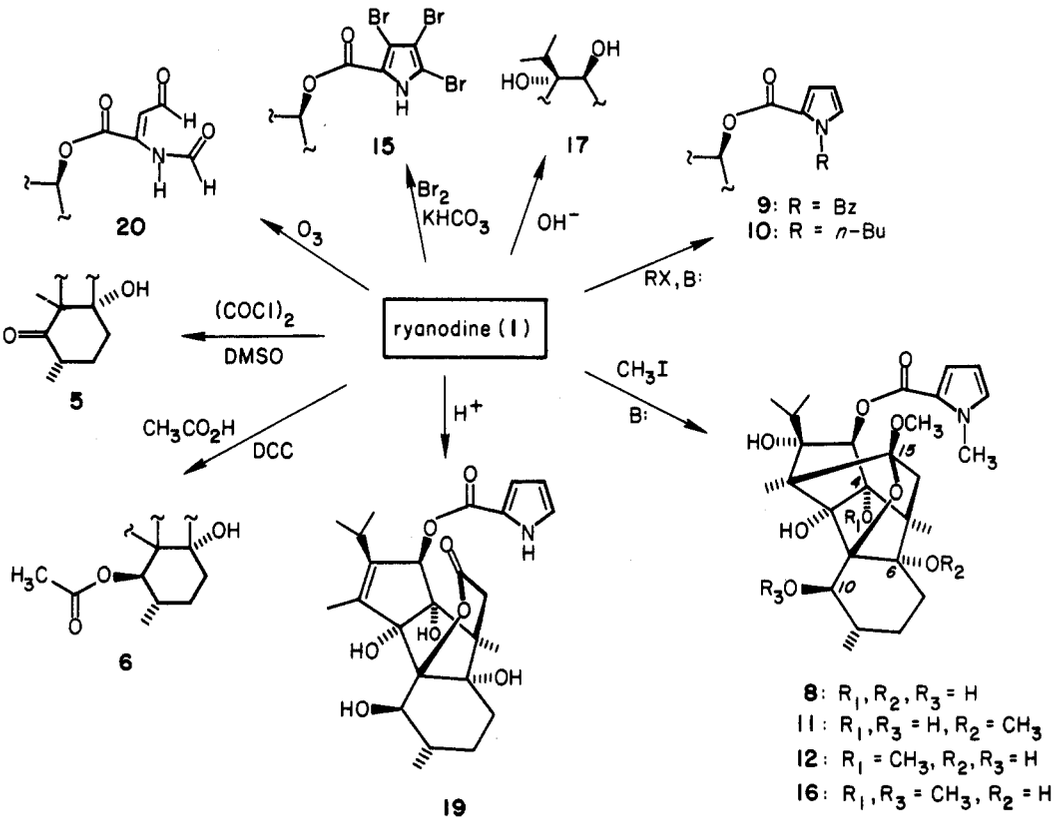
Scheme I



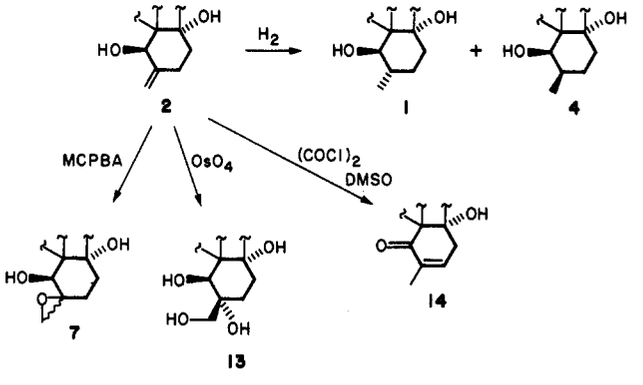
up of Ca^{2+}-ATPase and other proteins of 165 and >220 kilodaltons.[11,13]

Ryanoid structure–activity studies were undertaken earlier[10] to evaluate the topography and toxicological relevance of the Ca^{2+}-ryanodine receptor complex by comparing seven compounds for inhibitory potency at the receptor and intraperitoneal (ip) toxicity to mice. The present paper expands this series to 20 ryanoids, describes the chemical studies, and introduces insecticidal activity as a third criterion of potency. It details some of the functional groups critical for activity and shows a surprising selective toxicity of the ryanodol series between mammals and insects.

Ryanoids and Related Compounds

Ryanoids **1–3** were isolated from *R. speciosa* and **4–20** were prepared by derivatization or degradation of **1** (Scheme I) or **2** (Scheme II).

Scheme II



Natural Ryanoids. The new natural product **3** was isolated from a commercial extract of *R. speciosa*.[4] It has a hydroxyl group on one isopropyl methyl substituent (arbitrarily designated as C_{18}) without further modification of the ryanodine structure. An analogous hydroxyl substituent is present in many cincassiols, which have a ring structure related to that of the ryanoids.[14]

Ryanoid Derivatives. Attempted alkylation of **1** generally gives no reaction without a base to deprotonate the pyrrole NH or the alcohol groups. Alkylation occurs first at the pyrrole nitrogen (**9** and **10**) and then at the 15-hemiketal; these two sites react with a limited amount of methylating agent to give **8**. Somewhat surprisingly, the next group to react is the tertiary hydroxyl at C_4 or C_6, giving **11** and **12**, in preference to the secondary alcohol at C_{10}. The other hydroxyls suffer from extreme steric crowding such that even with a carbohydrate per-

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Table I. ^{13}C NMR Assignments (ppm, $CDCl_3/CD_3OD$) for Ryanodine and Four Methylated Derivatives

^{13}C position	ryanodine,[a] 1	dimethyl, *N*,15-*O*, 8	trimethyls		tetramethyl, *N*,4-*O*,10-*O*,15-*O*, 16
			N,6-*O*,15-*O*, 11	*N*,4-*O*,15-*O*, 12	
1	64.4	65.6	66.0	65.5	67.0
2	83.0	83.0	83.3	83.1	83.2
3	90.9	90.8	88.3	80.7	82.8
4	90.7	90.8	91.4	95.1	97.5
5	obsc	obsc	obsc	50.4	obsc
6	85.0	84.8	86.4	83.8	88.3
7	25.6	25.9	20.4	26.3	26.6
8	27.8	27.9	28.1	28.2	28.0
9	33.8	34.1	33.5	34.6	36.0
10	71.4	71.7	71.4	72.3	73.1
11	85.9	86.7	90.9	87.4	89.1
12	95.2	95.3	95.5	98.2	102.7
13	29.4	28.7	29.8	29.7	29.6
14	40.0	34.5	36.0	35.0	34.6
15	101.3	103.9	104.1	104.0	103.5
17	9.0	9.3	9.5	9.1	9.7
18	18.5	18.1	18.2	17.9	18.5
19	18.0	18.4	18.6	18.8	18.7
20	11.7	12.0	14.9	12.5	12.7
21	18.0	17.9	18.1	17.9	18.0
22	161.5	161.2	159.8	159.6	159.3
23	121.3	121.2	122.0	121.5	121.7
24	116.5	118.8	118.0	118.2	118.2
25	110.2	108.2	108.0	108.1	108.2
26	124.7	130.8	130.3	130.4	130.4
N-Me		36.5	36.8	36.7	36.6
4-*O*-Me				54.4	54.7
6-*O*-Me			52.2		
10-*O*-Me					56.8
15-*O*-Me		50.9	51.1	51.2	51.2

[a] The basis for each assignment is given in ref 4.

methylating procedure[15] only three of the six react to yield **16**.

Assignments of the methyl group positions for **8**, **11**, **12**, and **16** were made by using both ^{13}C (Table I) and ^1H NMR data (see Experimental Section). The diagnostic changes in the ^{13}C spectra are primarily at signals for carbons adjacent to the modified positions, although some shift changes are clearly due to factors other than substituent effects, such as changes in the steric environment. Thus, methylation at the C_{15} hemiketal shifts C_{14} upfield by ~5 ppm, and N-methylation shifts the C_{26} position of the pyrrole downfield by ~5 ppm. Methylation at the C_6 hydroxyl (**11**) results in a 5-ppm upfield shift for both C_7 and C_{11}, two carbons adjacent to C_6. In **12**, C_4 is shifted 4 ppm downfield and C_3 10 ppm upfield. The *N*,4-*O*,10-*O*,15-*O*-tetramethyl derivative (**16**) has additional effects including a strong downfield shift at C_{12}.

The tribromopyrrole **15** is cleanly obtained when the literature procedure[16] is modified to include an acid scavenger, which prevents rearrangement to the anhydro series. Compound **15** is easily converted to **1** by reduction in tetrahydrofuran (THF) with K_2CO_3 as the acid scavenger, thereby providing significant improvements over a reported procedure[16] to prepare [*pyrrole*-^{3}H]ryanodine.

Swern oxidation[17] on **1** leads to the expected conversion of the one secondary alcohol to a ketone (**5**), whereas this oxidation on **2** also causes the 9,21-double bond to rearrange to the 8,9-endocyclic position (**14**). Acetylation with dicyclohexylcarbodiimide (DCC) involves this same hydroxyl, giving **6**.

The double bond of **2** provides convenient access to additional compounds modified on the six-membered ring. Reduction of **2** produces a mixture of **1** and its epimer (**4**)[4,18] with varying ratios and contaminants depending on the solvent used. Oxidation with *m*-chloroperbenzoic acid (MCPBA) yields the 9,21-epoxy derivative (**7**) as an isomer mixture for which no satisfactory separation method was found in the present investigation. Diol **13** was formed in high conversion with osmium tetraoxide, but the recovered yield was poor due to the polarity of the product. The stereochemistry of **13** is not established, but attack from the least hindered side is predicted[19] and should yield the 9α-hydroxy derivative. Attempts to form the 9-keto product from **2** with ozone led instead to apparent opening of the pyrrole ring (see below).

Degradation Products. Ryanodol (**17**) was prepared by using the literature method,[20] but stronger conditions were required to hydrolyze **2** to **18**. Purification of these compounds is complicated by their sensitivity to acid. Anhydroryanodine (**19**) was also prepared by a literature procedure.[20] Ozonolysis opens the pyrrole, yielding the 25-aldehyde 26-formamide derivative (**20**).

Structure–Activity Relationships

Topography of Ca^{2+}-Ryanodine Receptor of Rabbit Skeletal Muscle SR (Table II). The natural ryanoids are at or near the optimal inhibitory structure for the available compounds. Ryanoids **1** and **2** have equivalent activity despite the substituent change. It is of interest that *R. speciosa* has two major principles of comparable activity acting at the same site. It also contains several minor ryanoids, of which the new 18-hydroxy derivative (**3**) is only 7–9-fold less potent in the receptor assay, indicating that the isopropyl group at C_2 may be involved

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Table II. Structure–Activity Relationships for Natural Ryanoids and Ryanodine Derivatives and Degradation Products as Inhibitors of the Calcium-Ryanodine Receptor and Toxicants for Mice and Houseflies

no.	compound	receptor IC_{50},[a] μM	mouse LD_{50},[a] mg/kg	housefly KD_{50},[a] μg/g
	Natural Ryanoids			
1	ryanodine	0.010	0.10[c]	0.09
2	9,21-didehydroryanodine	0.013[b]	0.10[c]	0.03
3	18-hydroxyryanodine	0.088	0.90	
	Ryanodine Derivatives			
4	9-epi	0.041[b]	0.47[c]	
5	10-keto	0.10	0.55[c]	>20
6	10-acetyl	0.14		0.5
7	9,21-epoxy	0.14	1.5	0.3
8	*N*,15-*O*-dimethyl	0.28[b]	17[c]	>20
9	*N*-benzyl	0.31	6.3	
10	*N*-butyl	0.86		
11	*N*,6-*O*,15-*O*-trimethyl	1.3		12
12	*N*,4-*O*,15-*O*-trimethyl	4.5	>20	>20
13	9,21-dihydroxy	4.9		10
14	8,9-didehydro-10-keto	7.3	>5	
15	24,25,26-tribromo	>10	>20	
16	*N*,4-*O*,10-*O*,15-*O*-tetramethyl	>10	>20	>20
	Degradation Products			
17	ryanodol	35	>20[c]	0.34
18	9,21-didehydroryanodol	10	>20	0.36
19	anhydroryanodine	>10[b]	>20[c]	
20	25-aldehyde 26-formamide			

[a] See Experimental Section for methodology. [b] Data from ref 11. [c] Data from ref 10.

in a minor hydrophobic interaction.

The equivalent potency of **1** and **2** led Sutko et al.[18] to speculate that the six-membered ring is relatively unimportant for ryanodine's activity. However, the 9-methyl group in **1** is equatorial and the 9,21-methylene of **2** is pseudoequatorial, making them similar in conformation. In epiryanodine (**4**), not only is the methyl group in the axial position, but being axial it can cause transannular interactions, distorting the shape of the ring. Here these effects are relatively minor, and **4** is 4-fold less potent than **1**. The epoxide mixture (**7**) has a 14-fold reduction in potency, consistent with small effects on the conformation and hydrophobicity of the ring. The 9,21-dihydroxy derivative **13** with a 490-fold reduction in potency clearly illustrates the importance of hydrophobic interactions at the six-membered ring. The simple change of the 9-hydroxyl group to a ketone (**5**) reduces the activity 10-fold. Compound **14** provides further indication that the ring conformation is critical since its activity is reduced 73 times relative to that of **5**. Thus, while **14** is slightly different from **5** in hydrophobicity, these isomers differ significantly in ring conformation due to the two additional sp^2 centers making the ring of **14** nearly planar. The 14-fold potency loss on acetylating the 10-position (**6**), which has relatively little effect on the ring conformation since it is an equatorial group, may be due to precluding hydrogen bonding at the 10-hydroxyl. These relationships indicate the importance of the stereochemistry and hydrophobicity of the six-membered ring and its substituents.

Alkylation of the hydrogen-bonding substituents establishes their contribution to binding. Progressive methylation reduces activity, showing that both the pyrrole and hydroxyl groups at each of C_4, C_6, C_{10}, and C_{15} play some role in binding. Dimethylation at the *N*-pyrrole and 15-hydroxyl diminishes activity 28-fold. The influence of additional methyls in **11** and **12** indicates that the hydroxyl group at C_4 is more important than that at C_6. With four methyl groups (**16**), there is negligible interaction with the ryanodine receptor site.

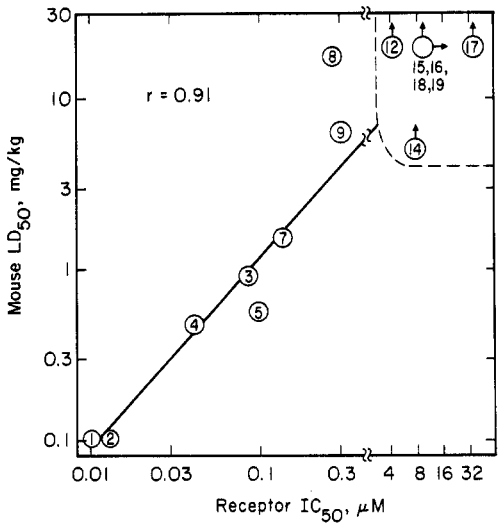

Figure 1. Relation of potency for decreasing [^{3}H]ryanodine binding to the Ca^{2+}-ryanodine receptor and for toxicity to mice of natural ryanoids (**1–3**) and ryanodine derivatives (**4, 5, 7–9, 12,** and **14–16**) and degradation products (**17–19**). Compound designations and data are given in Table II. An arrow indicates <50% inhibition or toxicity at the highest dose tested, and the direction of the arrow refers to the receptor or mouse assay. Least-squares correlation coefficient *r* is based on the eight compounds with actual values for both IC_{50} and LD_{50}.

Table III. Selective Toxicity of Ryanodine and Ryanodol in Mammalian and Insect Systems

system	ryanodine, **1**	ryanodol, **17**	potency ratio
	Mammalian Systems		
receptor IC_{50}, μM	0.010	35	3500
mouse LD_{50}, mg/kg	0.10	>20	>200
	Insect Systems[a]		
housefly KD_{50}, μg/g			
alone	0.30	1.1	3.6
+PB	0.09	0.34	3.8
cockroach KD_{50}, μg/g	0.5	1.0	2.0

[a] LD_{50} values (24 h) for injected **1** and **17** are >20 μg/g without PB whereas with PB the values are 0.24 and 1.1 μg/g injected and topical, respectively.

Medium-sized *N*-alkyl groups such as in **9** and **10** diminish activity by 31–86-fold while tribromination of the pyrrole (**15**) destroys the activity, thus indicating the importance for receptor fit of hydrogen bonding at the nitrogen and the conformation of the pyrrolecarboxylate group. This is further exemplified by the 220-fold activity loss on oxidative opening of the pyrrole ring (**20**).

Removal of the pyrrolecarboxylate to give **17** or **18** practically destroys activity. Anhydroryanodine (**19**), with a different overall shape due to opening of one bridging ring, has negligible activity as well.

Relation of Potency for Inhibiting the Ca^{2+}-Ryanodine Receptor of Rabbit Skeletal Muscle SR and for Toxicity to Mice (Table II, Figure 1). Poisoning by **1** involves sequential labored breathing, a brief episode of violent convulsions, and rigidity of the peripheral musculature culminating in death. Comparative receptor inhibition and mouse toxicity data are available on 15 ryanoids, i.e., three natural products (**1–3**), nine ryanoid derivatives (**4, 5, 7–9, 12,** and **14–16**), and three degradation products (**17–19**). Correlation of the potency at the re-

ceptor with the toxicity to mice (correlation coefficient of 0.91) establishes the toxicological relevance of the receptor in poisoning.

Insecticidal Activity and Selective Toxicity (Tables II and III). The structure–activity relationships of injected ryanoids are considerably different for LD_{50} to mice and KD_{50} for houseflies pretreated with piperonyl butoxide (PB) (an inhibitor of oxidative metabolism). Both **1** and **2** are relatively nonselective toxicants while **5** is selectively toxic to mice and **7** to houseflies. Most interestingly, the ryanodol series (**17**, **18**) shows high selective toxicity for houseflies and cockroaches compared with mice.

Housefly Metabolites of [^{3}H]Ryanodine. [^{3}H]Ryanodine is metabolized in houseflies to at least two products, on the basis of analyses of methanol extracts of the excreta at 1 h and the flies at 4 h after injection. In both cases the labeled products chromatographed on TLC in the region of **1**, in the region of an unidentified metabolite of intermediate R_f, and at the origin (the position of **17**), corresponding to 14–21%, 21–24%, and 52–58% of the tritium recovered, respectively. HPLC of a comparable excreta extract gave 14% and 76% tritium recovery in the chromatographic positions of **1** and **17**, respectively. These results show metabolism of **1** in houseflies and suggest but are not sufficient to establish conversion to **17**.

Conclusion. Ryanodine is the best probe available for a major unsolved problem of muscle biology, i.e., how signal transmission occurs at the transverse tubule–SR junction.[21] [^{3}H]Ryanodine, in turn, is the key to identifying SR proteins associated with the Ca^{2+}-release channel(s).[13] The present investigation establishes the toxicological relevance of the Ca^{2+}-ryanodine receptor and the topography of the specific binding site in skeletal muscle heavy SR preparations. It also reveals that some ryanoid derivatives are selective toxicants between insects and mammals, possibly due to species differences at the receptor level.

Experimental Section

Chemistry. General. All ryanoid derivatives were prepared from a few hundred milligrams of each of **1** and **2** purified by HPLC from commercial ryanodine. Reagents and methods were as follows unless noted otherwise: all reactions were stirred magnetically under a nitrogen atmosphere; THF was dried by distillation from sodium/benzophenone, and other solvents were dried by standing over 3-Å molecular sieves; workup solutions were dried with saturated NaCl and anhydrous Na_2SO_4 and evaporated with a rotary evaporator. Analyses: analytical TLC was carried out on 3 × 7 cm 0.2-mm aluminum-backed silica plates and preparative TLC on 20 × 20 cm 0.5-mm glass silica plates; HPLC separations involved a Beckman 344 two-pump gradient system using a 4.6-mm (for analytical) or 10-mm (for preparative) × 250-mm Ultrasphere-ODS C-18 reverse-phase silica column with MeOH/water mixtures at 1 mL/min or 4 mL/min, respectively, detecting at 268 nm. Purified material was >95% pure on the basis of HPLC analysis, except possibly for **20**, which has no chromophore. Due to the small scale involved, most compounds were obtained as oils or films. Spectroscopy: ^{1}H and ^{13}C NMR spectra were recorded with a Bruker WM-300 spectrometer using $CDCl_3/CD_3OD$ (the CD_3OD obscures (obsc) C_1 of ryanoids at ~50 ppm) and internal tetramethylsilane as reference, and key proton assignments are indicated; mass spectra required fast-atom-bombardment ionization and were obtained by using a Hewlett-Packard 8450 instrument and a Phrasor ion source, a Kratos MS-50 (at the Chemistry Department, University of California, Berkeley, CA), or a modified Kratos MS-50 with a liquid secondary ion MS source (at the Mass Spectrometry Facility, University of California, San Francisco, CA, supported by NIH Division of Resources, Grant RR01614).

18-Hydroxyryanodine (3). Commercial ryanodine[4] was purified by HPLC with a Partisil ODS-3 Magnum 20 column using 42% MeOH at 14 mL/min introducing 250 mg in 5 mL of 50% aqueous MeOH. Retention times (t_R) for **1**, **2**, and **3** were 22.5, 19.5, and 14.0 min, respectively. The commercial sample contained 0.1% **3**. NMR: δ 7.04 (br s, 1 H), 7.00 (d, J = 4, 1 H), 6.29 (d, J = 2, 1 H), 5.50 (s, 1 H), 3.90 (d, J = 10, 1 H), 3.64 (AB q, 2 H, H-18), 2.31 (d, J = 14, 1 H), 2.27 (m, 1 H), 2.05 (p, J = 7, 1 H), 1.93 (d, J = 14, 1 H), 1.84 (m, 1 H), 1.54 (m, 2 H), 1.42 (s, 3 H), 1.34 (d, J = 7, 3 H), 1.25 (m, 1 H), 1.03 (d, J = 7, 3 H), 0.87 (s, 3 H). MS (MS-50, glycerol/thioglycerol/NaCl): m/z 532 (M + Na)$^+$.

10-Ketoryanodine (5). Compound **1** (25 mg, 50 μmol) was treated according to Omura and Swern,[17] and the quenched reaction was extracted four times with ethyl acetate. HPLC [45–60% MeOH (15 min)] gave 5 mg (20%) of **5** (t_R = 11 min) and 15 mg (60%) of recovered **1** (t_R = 13 min). NMR: δ 7.04 (br s, 1 H), 6.91 (dd, J = 1, 2, 1 H), 6.28 (dd, J = 2, 3, 1 H), 5.63 (s, 1 H), 3.00 (m, J = 6, 1 H, H-8), 2.53 (d, J = 14, 1 H), 2.38 (td, J = 5, 12, 1 H), 2.21 (p, J = 7, 1 H), 2.06 (d, J = 14, 1 H), 1.95 (m, 1 H), 1.84 (dd, J = 4, 12, 1 H), 1.48 (dd, J = 6, 13, 1 H), 1.30 (s, 3 H), 1.09 (d, J = 7, 3 H), 1.00 (d, J = 7, 3 H), 0.95 (s, 3 H), 0.81 (d, J = 6, 3 H). MS (modified MS-50, KCl/glycerol): m/z 530 (M + K)$^+$.

10-Acetylryanodine (6). A solution of **1** (20 mg, 40 μmol), dicyclohexylcarbodiimide (100 mg, 480 μmol), and acetic acid (50 mg, 830 μmol) in 5 mL of dry THF was stirred for 48 h. Methanol was added and the solvent evaporated. The residue was dissolved in aqueous $NaHCO_3$ and extracted twice with ethyl acetate. TLC of the organic extract gave 2 mg (9%) of **6**. NMR: δ 7.07 (br s, 1 H), 6.98 (m, 1 H), 6.33 (m, 1 H), 5.31 (d, J = 11, 1 H, H-9), 5.30 (s, 1 H), 2.37 (d, J = 14, 1 H), 2.31 (m, 1 H), 2.13 (s, 3 H, H_3-Ac-10), 2.03 (m, 2 H), 1.93 (d, J = 14, 1 H), 1.61 (m, 2 H), 1.44 (s, 3 H), 1.28 (m, 1 H), 1.14 (d, J = 6, 3 H), 0.97 (d, J = 6, 3 H), 0.89 (d, J = 6, 3 H), 0.86 (s, 3 H). MS (HP 8450, glycerol): m/z 558 (M + 1)$^+$, 580 (M + Na)$^+$.

9,21-Epoxyryanodines (7), an epimeric mixture. After **2** (27 mg, 54 μmol) was dissolved in 5 mL of 40% $MeCN/CH_2Cl_2$, MCPBA (25 mg, 140 μmol) was added and the solution stirred for 4 h. Aqueous $NaHSO_3$ and $NaHCO_3$ were added, and the solution was partially evaporated to remove the organic solvents. This aqueous solution was extracted three times with ethyl acetate. HPLC [38–48% MeOH (20 min)] gave 7 mg (26%) of **7** (t_R = 15 min). NMR: δ 7.05 (br s, 1 H), 6.97 (m, 1 H), 6.29 (m, 1 H), 5.50 (s), 5.51 (s), (area 5.50–5.51 = 1 H), 4.59 (s, 0.4 H, H-21), 4.45 (s, 0.6 H, H-21), 3.18 (d, J = 4, 0.4 H, H-8), 2.96 (d, J = 5, 0.6 H, H-8), 2.46 (m, 2 H), 2.30 (m, 2 H), 1.95 (m, 1 H), 1.40 (s, 1.2 H), 1.36 (s, 1.8 H), 1.35 (m, 2 H), 1.13 (m, 3 H), 0.91 (s, 3 H), 0.86 (d, J = 6, 3 H). MS (HP 8450, glycerol/NaCl): m/z 530 (M + Na)$^+$.

Di- and Trimethylryanodines (8, 11, and 12). Compound **1** (0.103 g, 0.21 mmol) was dissolved in 10 mL of dry THF, and potassium *tert*-butoxide (70 mg, 0.63 mmol) was added to give a suspension. Methyl iodide (88 mg, 0.63 mmol) was added, and the mixture was heated to 60 °C, to attain solution, for 18 h. The reaction was quenched with aqueous $NaHCO_3$ and evaporated to remove the THF, and the aqueous phase was saturated with NaCl and extracted four times with ethyl acetate. HPLC [60–75% MeOH (20 min)] gave three major fractions, **8**, **12**, and **11**, t_R = 17, 19, and 21 min, respectively.

The yield of dimethyl derivative **8** was 7.8 mg (7%). NMR: δ 6.97 (dd, J = 2, 4, 1 H), 6.88 (dd, J = 2, 2, 1 H), 6.16 (dd, J = 2, 4, 1 H), 5.41 (s, 1 H), 3.94 (s, 3 H, MeN), 3.88 (d, J = 10, 1 H), 3.42 (s, 3 H, Me-15), 2.30 (m, 1 H), 2.25 (d, J = 14, 1 H, H-14), 2.05 (d, J = 14, 1 H, H-14), 1.92 (dt, J = 5, 12, 1 H), 1.83 (m, 2 H), 1.38 (s, 3 H), 1.27 (m, 1 H), 1.10 (d, J = 6, 3 H), 1.04 (d, J = 6, 3 H), 0.91 (s, 3 H), 0.87 (d, J = 7, 3 H). See Table I for ^{13}C NMR. MS (MS-50, glycerol/thioglycerol/NaCl): m/z 544 (M + Na)$^+$.

The yield of the 6-*O*-methyl trimethyl derivative **11** was 12 mg (11%). NMR: δ 6.90 (dd, J = 2, 4, 1 H), 6.85 (m, 1 H), 6.14 (dd, J = 2, 4, 1 H), 5.66 (s, 1 H), 3.97 (s, 3 H, MeN), 3.78 (d, J = 10, 1 H), 3.44 (s, 3 H, Me-15), 3.36 (s, 3 H, Me-6), 2.25 (d, J = 14, 1 H, H-14), 2.12 (d, J = 14, 1 H, H-14), 2.10 (m, 1 H), 2.05 (m, 1 H), 1.92 (m, 1 H), 1.74 (m, 2 H, H-7,8), 1.38 (s, 3 H), 1.15 (m, H-7), 1.09 (s), 1.06 (d), 1.04 (d), (area 1.15–1.04 = 10 H), 0.75 (d, J = 6, 3 H). See Table I for ^{13}C NMR. MS (HP 8450, glycerol/NaCl): m/z 558 (M + Na)$^+$.

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The yield of the 4-*O*-methyl trimethyl derivative **12** was 13 mg (12%). NMR: δ 6.90 (dd, *J* = 2, 4, 1 H), 6.86 (dd, *J* = 2, 2, 1 H), 6.15 (dd, *J* = 2, 4, 1 H), 6.13 (s, 1 H), 3.96 (s, 3 H, MeN), 3.92 (d, *J* = 8, 1 H), 3.84 (s, 3 H, Me-4), 3.44 (s, 3 H, Me-15), 2.28 (s, 2 H, H-14), 2.12 (p, *J* = 7, 1 H), 1.89 (m, 2 H), 1.58 (m, 2 H), 1.31 (s + m, 4 H), 1.05 (overlapping doublets, 6 H), 0.98 (s, 3 H), 0.78 (d, *J* = 7, 3 H). See Table I for ^{13}C NMR. MS (modified MS-50, glycerol/KCl): *m/z* 574 $(M + K)^+$.

***N*-Benzylryanodine (9).** Potassium hydride (5 mg, 40 μmol) was suspended in 5 mL of dry THF to which was added 1 mL of dry Me_2SO. Compound **1** (10 mg, 20 μmol) was added after 5 min, and benzyl bromide (8.5 mg, 50 μmol) was added 30 min later. After 3 h, the reaction was quenched with aqueous $NaHCO_3$ and the pH was adjusted to 8 with 2 M H_3PO_4. After the THF was evaporated off, the aqueous solution was extracted four times with ethyl acetate. TLC (10% MeOH/$CHCl_3$) gave two bands. The lower band at R_f 0.60 was extracted to yield 3 mg of **9** (25%). HPLC [75–100% MeOH (25 min)]: t_R (**9**) = 8 min. NMR: V 7.26 (m, 3 H), 7.1–7.0 (m, 4 H), 6.25 (dd, *J* = 2, 4, 1 H), 5.62 (d, *J* = 16, 1 H, H-Bz), 5.48 (d, *J* = 16, 1 H, H-Bz), 5.28 (s, 1 H), 3.89 (d, *J* = 10, 1 H), 2.32 (m + d, *J* = 14, 2 H), 1.96 (m, 1 H), 1.87 (d, *J* = 14, 1 H), 1.81 (m, 1 H), 1.52 (m, 1 H), 1.40 (s + m, 5 H), 1.13 (d, *J* = 7, 3 H), 1.03 (d, *J* = 6, 3 H), 0.90 (d, *J* = 6, 3 H), 0.64 (s, 3 H). MS (MS-50, glycerol/thioglycerol/NaCl): *m/z* 606 $(M + Na)^+$.

***N*-Butylryanodine (10).** Ryanoid **1** (25 mg, 50 μmol) was dissolved in 30 mL of dry THF, and potassium *tert*-butoxide (17 mg, 150 μmol) was added. After 30 min, *n*-butyl iodide (13.8 mg, 150 μmol) was added and the solution stirred for 18 h. TLC analysis (10% MeOH/$CHCl_3$) indicated no reaction, so 1.5 mL of dimethyl sulfoxide (Me_2SO) was added and the solution heated to reflux. After 18 h, 5 mL of aqueous $NaHCO_3$ was added and the THF evaporated. The aqueous solution was extracted three times with ethyl acetate. HPLC [60–100% MeOH (60 min)] gave 3.7 mg of **10** (t_R = 15.5 min). NMR: δ 6.99 (dd, *J* = 2, 4, 1 H, H-24), 6.93 (dd, *J* = 2, 2, 1 H, H-26), 6.16 (dd, *J* = 2, 4, 1 H, H-25), 5.38 (s, 1 H, H-3), 4.32 (m, 2 H, H_2-1-Bu), 3.89 (d, *J* = 10, 1 H), 2.39 (d, *J* = 14, 1 H), 2.31 (m, 1 H), 2.03 (dt, *J* = 5, 12, 1 H), 1.92 (d, *J* = 14, 1 H), 1.83 (m, 1 H), 1.71 (m, 2 H, H_2-2-Bu), 1.54 (m, 2 H), 1.38 (s, 3 H), 1.33 (m, 3 H), 1.14 (d, *J* = 7, 3 H), 1.04 (d, *J* = 6, 3 H), 0.93–0.88 (overlapping methyls and methylene, 8 H). MS (MS-50, glycerol/thioglycerol/NaCl): *m/z* 572 $(M + Na)^+$.

9,21-Dihydroxyryanodine (13). Compound **2** (5 mg, 10 μmol) was dissolved in 5 mL of 50% aqueous THF. Osmium tetraoxide (<1 mg) was added and then *N*-methylmorpholine *N*-oxide (1.2 mg, 10 μmol). After 24 h, TLC (10% MeOH/$CHCl_3$) indicated that **2** had been replaced by a more polar product. The reaction was quenched with aqueous $NaHSO_3$ and $NaHCO_3$ and the aqueous solution extracted four times with ethyl acetate. HPLC (50% MeOH) gave 1 mg (10%) of **13** (t_R = 9 min). NMR: δ 7.04 (br s, 1 H), 6.94 (d, *J* = 3, 5, 1 H), 6.28 (dd, *J* = 2, 3, 1 H), 5.52 (s, 1 H), 4.41 (s, 1 H, H-9), 4.24 (d, *J* = 12, 1 H, H-21), 3.58 (d, *J* = 12, 1 H, H-21), 2.49 (d, *J* = 14, 1 H), 2.27 (p, *J* = 6, 1 H), 1.99 (dt, *J* = 6, 14, 1 H), 1.89 (d, *J* = 14, 1 H), 1.84 (m, 1 H), 1.64 (m, 1 H), 1.39 (s, 3 H), 1.32 (m, 1 H), 1.12 (d, *J* = 7, 3 H), 0.90 (s, 3 H), 0.83 (d, *J* = 6, 3 H). MS (HP 8450, glycerol/NaCl): *m/z* 548 $(M + Na)^+$.

8,9-Didehydro-10-ketoryanodine (14). Oxalyl chloride (29 mg, 23 μmol) and Me_2SO (100 μL) were added to 2 mL of CH_2Cl_2 chilled on dry ice/acetone. Two minutes later, **2** (5 mg, 10 μmol) in 3 mL of CH_2Cl_2 and 0.5 mL of Me_2SO was added dropwise over 0.5 min. After the mixture was stirred for 30 min at 0 °C, 0.5 mL of triethylamine was added and the solution warmed to room temperature. Following evaporation, the residue was dissolved in aqueous $NaHCO_3$, which was extracted three times with ethyl acetate. HPLC (70% MeOH) gave 4 mg (80%) of **14** (t_R = 8 min). NMR: δ 7.05 (dd, *J* = 1, 1, 1 H), 6.93 (dd, *J* = 1, 3, 1 H), 6.31 (dd, *J* = 3, 1, 1 H), 5.54 (s, 1 H), 4.72 (s, 1 H, H-8), 2.93 (m, 1 H, H-7), 2.63 (s, 3 H, H_3-21), 2.51 (d, *J* = 14, 1 H), 2.36 (m, 1 H, H-7), 2.22 (m, 1 H), 2.11 (s, 3 H, H_3-17), 2.05 (d, *J* = 14, 1 H), 1.09 (d, *J* = 7, 1 H), 0.95 (s, 3 H), 0.86 (d, *J* = 6, 3 H).

24,25,26-Tribromoryanodine (15). Compound **1** (50 mg, 0.10 mmol) and $KHCO_3$ (100 mg, 1.0 mmol) were added to 20 mL of MeOH, and bromine (81 mg, 0.5 mmol) in 5 mL of MeOH was introduced dropwise. A yellow color persisted at the end of the addition. After 3 h, the solution was evaporated and the residue dissolved in water and extracted three times with ethyl acetate. TLC (10% MeOH/$CHCl_3$) gave 48 mg (66%) of **15** (R_f 0.5). HPLC [80% MeOH]: t_R (**15**) = 15 min. NMR: δ 6.29 (s, 1 H, H-3), 4.07 (d, *J* = 11, 1 H), 3.59 (d, *J* = 20, 1 H), 2.74 (p, *J* = 7, 1 H), 2.46 (d, *J* = 20, 1 H), 1.83 (s + m, 4 H), 1.61 (m, 2 H), 1.41 (m, 2 H), 1.15 (d, *J* = 7, 3 H), 1.11 (d, *J* = 6, 3 H), 0.99 (s, 3 H), 0.96 (d, *J* = 7, 3 H). MS (MS-50, glycerol/thioglycerol/NaCl): *m/z* 750, 752, 754, 756 $(M + Na)^+$. Reduction of **15** by a reported procedure[16] but using THF as the solvent and K_2CO_3 as an acid scavenger gave **1** in high yield.

***N*,4-*O*,10-*O*,15-*O*-Tetramethylryanodine (16).** Sodium hydride (24 mg, 0.5 mmol) was slowly dissolved in dry Me_2SO at room temperature followed by the quick addition of methyl iodide (40 μL, 0.67 mmol). Then **1** (25 mg, 50 μmol) was added, and after the mixture was stirred for 15 min, 100 mL of saturated $NaHCO_3$ was added and the product recovered by extracting three times with 2,2,4-trimethylpentane. TLC (10% EtOH/$CHCl_3$) gave 20.6 mg (76%) of **16** (R_f 0.5). NMR: δ 6.88 (dd, *J* = 2, 4, 1 H), 6.85 (br s, 1 H), 6.16 (s, 1 H), 6.14 (m, 1 H), 3.95 (s + d, 4 H, MeN), 3.84 (s, 3 H, Me-4), 3.77 (s, 3 H, Me-10), 3.43 (s, 3 H, Me-15), 2.31 (d, *J* = 14, 1 H), 2.26 (d, *J* = 14, 1 H), 2.03 (p, *J* = 7, 1 H), 1.85 (m, 2 H), 1.55 (m, 2 H), 1.32 (s, 3 H), 1.27 (m, 1 H), 1.06–1.03 (2 d + s, 9 H), 0.76 (d, *J* = 6, 3 H). See Table I for ^{13}C NMR. MS (MS-50, glycerol/thioglycerol/NaCl): *m/z* 572 $(M + Na)^+$.

Ryanodol (17). Following hydrolysis by the literature procedure,[20] the product was purified by HPLC using a Hamilton PRP-1 column (4.6 × 250 nm) [40–100% MeOH (25 min)] (both solvents containing 0.1% 40% NH_4OH) to give **17** (t_R = 4–8 min). NMR: δ 4.14 (s, 1 H, H-3), 3.80 (d, *J* = 10, 1 H), 2.50 (d, *J* = 14, 1 H), 2.17 (p, *J* = 7, 1 H), 2.07 (dt, *J* = 5, 12, 1 H), 1.83 (m, 1 H), 1.77 (d, *J* = 14, 1 H), 1.54 (m, 1 H), 1.44 (m, 1 H), 1.32 (s, 3 H), 1.29 (m, 1 H), 1.13 (s, 3 H), 1.09 (d, *J* = 7, 3 H), 1.02 (d, *J* = 6, 3 H), 1.01 (d, *J* = 6, 3 H). ^{13}C NMR: δ 101.3, 94.4, 90.8, 89.9, 85.4, 84.8, 83.2, 71.2, 63.6, 39.6, 33.6, 28.8, 27.6, 25.0, 18.1, 17.7, 17.5, 11.7, 8.6, one peak obsc.

Didehydroryanodol (18). A solution of **2** (76.8 mg, 0.16 μmol) in 10 mL of MeOH containing 10% KOH was refluxed for 18 h. Water was added, the MeOH evaporated, the pH adjusted to 8 with 2 M H_3PO_4, NaCl added to saturation, and the solution extracted four times with ethyl acetate. HPLC (as for **17**) gave 35.2 mg (55%) of **18**. NMR: δ 5.04 (d, *J* = 2, 1 H, H-21), 4.93 (d, *J* = 2, 1 H, H-10), 4.76 (s, 1 H, H-21), 4.16 (s, 1 H), 2.50 (d, *J* = 14, 1 H), 2.31 (dd, *J* = 4, 15, 1 H, H-8), 2.16 (p, *J* = 7, 1 H), 2.04 (dt, *J* = 5, 13, 1 H, H-8), 1.76 (d, *J* = 14, 1 H), 1.37 (m, 1 H), 1.32 (s, 3 H), 1.15 (s, 3 H), 1.07 (d, *J* = 7, 3 H), 1.01 (d, *J* = 7, 3 H), one signal near δ 3 obsc.

Anhydroryanodine (19). This compound was prepared by literature procedures.[20] HPLC [30% MeOH (2 min), 30–100% MeOH (25 min)] gave **19** (t_R = 18 min). NMR (CD_3OD, CD_2HOD = δ 3.30): δ 7.05 (dd, *J* = 2, 3, 1 H), 6.87 (dd, *J* = 2, 4, 1 H), 6.25 (dd, *J* = 3, 4, 1 H), 6.17 (d, *J* = 2, 1 H, H-3), 4.00 (d, *J* = 10, 1 H), 3.42 (d, *J* = 20, 1 H, H-14), 2.77 (p, *J* = 7, 1 H, H-13), 2.53 (d, *J* = 20, 1 H, H-14), 1.86 (d, *J* = 2, 3 H, Me-17), 1.80 (m, 1 H), 1.58 (m, 2 H), 1.50 (m, 2 H), 1.09 (d, *J* = 7, 3 H), 1.08 (d, *J* = 6, 3 H), 0.98 (d, *J* = 7, 3 H), 0.96 (s, 3 H).

25-Aldehyde 26-Formamide 20. Compound **1** (25 mg, 50 μmol) was dissolved in MeCN and chilled on ice. Ozone was introduced at 1.5 mmol/min for 2 min, followed by flushing with nitrogen. Triphenylphosphine (30 mg) was added, and after the mixture was stirred for 1 h, the solvent was evaporated and the residue dissolved in chloroform and extracted three times with water. The combined aqueous solution was extracted four times with ethyl acetate to yield 10 mg (40%) of **20**. NMR: δ 9.16 (s, 1 H, H-25), 7.7–7.3 obsc, 5.65 (s, 1 H), 3.81 (d, *J* = 10, 1 H), 2.48 (d, *J* = 14, 1 H), 2.23 (m, 1 H), 2.09 (m, 1 H), 1.96 (d, *J* = 14, 1 H), 1.83 (m, 1 H), 1.58 (m, 1 H), 1.43 (m, 1 H), 1.37 (s, 3 H), 1.28 (m, 1 H), 1.10 (d, *J* = 7, 3 H), 1.02 (d, *J* = 7, 3 H), 0.96 (s, 3 H), 0.75 (d, *J* = 6, 3 H).

Biology. Inhibition of Ca^{2+}-Ryanodine Receptor. Rabbit fast skeletal muscle was homogenized and subjected to differential centrifugation as previously described[10,11] to obtain the heavy SR fraction enriched with elements from the terminal cisternae (TC). The TC SR membranes were treated with 1 M NaCl, 1 mg of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)/mg of protein, and 40 mM Tris/maleate, pH 7.1, to release the receptor complex to the 110000*g* (1 h) supernatant.[11]

The protein content of the solubilized receptor was determined,[22] and it was then divided into 1-mL fractions, which were rapidly frozen in liquid nitrogen and stored at −70 °C until needed. Inhibition experiments were performed under competitive conditions where 50 μg of soluble receptor protein (10 μL) was added to 1 mL of assay medium containing 5 nM [^{3}H]ryanodine (60 Ci/mmol), a desired concentration of test ryanoid, 0.5 M NaCl, 0.1% CHAPS, 300 μM $CaCl_2$, and 40 mM Tris/maleate, pH 7.1. Samples were allowed to equilibrate for 80 min at 37 °C followed by rapid filtration through Whatman GF/C glass fiber filters.[10,11]

Specific binding (>95% in each case) was defined as the difference between total binding (with [^{3}H]ryanodine alone) and nonspecific binding (with [^{3}H]ryanodine fortified with 10 mM unlabeled ryanodine). Concentrations for 50% inhibition (IC_{50} values) were determined from regression analyses of Hill data.[23] The 95% confidence limits had an average range of ±3% of the IC_{50} values.

Toxicity to Mice. LD_{50} values were determined from mortality data 24 h after ip administration of the ryanoid to male albino Swiss-Webster mice (18–22 g) with 50% aqueous ethanol or methoxytriglycol (10–100 μL) as the carrier vehicle compared with appropriate controls. The reported LD_{50}'s are based on plots of logarithmic dose vs. probit percent mortality. They are approximations, due to the small amounts of test compounds, but are estimated to fall within 1.5-fold of the actual values.

Toxicity to Insects. The standard knockdown (KD) assay used adult female houseflies (*Musca domestica*, SCR susceptible strain) treated by intrathoracic injection of a 50% aqueous ethanol solution (1 μL) of the test compound 2 h after topical treatment on the abdomen with 7.5 μg of PB applied in 0.5 μL of acetone. The KD endpoint was the number of flies immobilized 4 h posttreatment, the KD_{50} being determined from logarithmic dose–probit KD plots. In other studies, houseflies (some pretreated topically with 7.5 μg of PB and others not) were administered a ryanoid topically or by injection and adult male American cockroaches (*Periplaneta americana*) were treated by intracoxal injection with 1 or 17 in 50% aqueous ethanol (10 μL) as above. KD_{50} and LD_{50} values are based on two independent experiments with a dose differential of 1.5–2-fold and 10 houseflies or six cockroaches for each dose.

Metabolism of [^{3}H]Ryanodine in Houseflies. Ten adult female houseflies were injected as above with [^{3}H]ryanodine (1.1×10^6 dpm, 160 μg/g). Each batch of treated flies was held in a 100-mL glass beaker for 1 or 4 h, at which time the flies were removed and the flies and their excreta were separately extracted with MeOH, with tritium recovery values of 55%, 21%, and 4% at 1 h and 38%, 40%, and 6% at 4 h, for the flies, excreta, and unextracted residue, respectively. Methanol extracts of the 1-h excreta and the flies 4 h after treatment were subjected to TLC on 0.20-mm silica gel plates (15% MeOH/$CHCl_3$) with metabolite detection by liquid scintillation counting. These TLC conditions gave R_f values of 0.46 and 0.00 for **1** and **17**, respectively. A comparable excreta extract was subjected to analytical HPLC [0% MeOH (1 min), 0–100% MeOH (10 min); R_T = 12.5 min (**1**), 10.5 min (**17**)], comparing the standard compounds and the ^{3}H-labeled metabolites.

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Comparative Antitumor Studies on Platinum(II) and Platinum(IV) Complexes Containing 1,2-Diaminocyclohexane

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The synthesis and characterization of a group of platinum(IV) compounds containing the various isomeric forms of 1,2-diaminocyclohexane (DACH) are described. Antitumor tests with the new complexes, as well as with other platinum(II) compounds containing the DACH ligand, revealed that *trans,cis*-Pt^{IV}(*SS*-DACH)$(OH)_2Cl_2$, **7**, is more active than its mirror image, *trans,cis*-Pt^{IV}(*RR*-DACH)$(OH)_2Cl_2$, **6**, against L1210 leukemia implanted in mice. However, activity is dependent on the tumor model, and against B16 melanoma implanted in mice, the activities of the two enantiomers are reversed, with **6** being more active than **7**. The results of the tests are discussed in light of the mechanism by which Pt(IV) compounds are believed to express their antitumor effects.

Since the first report of the anticancer activity of *cis*-dichlorodiammineplatinum(II), **1**, by Rosenberg et al.,[1] a wide variety of platinum compounds have been synthesized and examined for their antitumor activity.[2] Extensive study of the structure–activity relationships of the platinum-based anticancer agents has revealed that the most active compounds are those having cis-coordination sites occupied by mono- or bidentate amine ligands (or ammonia) and two sites occupied by weak trans-directing groups, e.g., Cl^-, NO_3^-, SO_4^{2-}, etc. On the molecular level, the compounds are believed to express their antitumor effects by loss of the weak trans-directing groups and subsequent binding of the platinum nucleus to DNA.[3-5]

In addition to antitumor active Pt(II) complexes, oxidation of a variety of divalent compounds with H_2O_2, Cl_2, or Br_2 yields 6-coordinate complexes of Pt(IV), which are themselves generally active as anticancer agents.[1,6-10] In light of the fact that substitution reactions on Pt(IV) are

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