Ryanodine Action at Calcium Release Channels. 2. Relation to Substituents of the Cyclohexane Ring

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Received September 27, 1995⊗

Ryanodine (**1**) and dehydroryanodine (**2**) are equipotent probes for the ryanodine receptor (*ryr*) of calcium release channels and differ only in 9_{eq}-methyl for **1** and 9,21-methylene for **2**. Ryanoids **1** and **2** are used here to prepare novel modifications of the cyclohexane substituents to determine their effects on *ryr* activity and selectivity. 10-Oxo-**1** when reacted with carbonyl and other reagents gave 13 C-10 derivatives including the *epi*-amine and *epi*-4-azidobenzoyl hydrazide as a candidate affinity probe. Four derivatives of **2** included the Δ^8-10-hydroxy and Δ^8-10-oxo compounds. Defunctionalization of the cyclohexane ring of **2** or its 4,6-ethylboronate was achieved in part by controlled periodate oxidation of the 9,21-diol to the 21-nor-9-oxo compounds. These in turn provided access to the 9_{ax}- and 9_{eq}-hydroxy derivatives and to the 21-nor-10-deoxy-9-oxo compound which was converted to 21-nor-10-deoxy-**1** and 10-deoxy-**2** along with the epimeric 10-deoxy-9-hydroxy compounds. Ryanoids of similar potency to **1** as inhibitors of [^{3}H]-**1** binding in mouse brain, rabbit skeletal muscle, and canine ventricle *ryr* preparations and in a rat cardiac contractility assay (inhibition of mechanical response to electrical stimulation) are *epi*-**1** and the 10-*epi*-amino, 10-*epi*-methoxyamino, and 10-*epi*-azidobenzoyl hydrazide derivatives and 10-deoxydehydroryanodine. With a few exceptions the potency of the ryanoids at the cardiac *ryr* correlates well with their inhibition of cardiac contractility, indicating that the activity is associated with stabilizing the calcium release channel in a subconducting state, thereby uncoupling the excitation−contraction process.

Introduction

Natural ryanoids contain a range of substituents at C-8, C-9, and C-10 of the cyclohexane ring. The most important ones in amount and biological activity are ryanodine (**1**)[1] and dehydroryanodine (**2**)[2] which differ only in the cyclohexane ring with 6_{ax},10_{eq}-hydroxyls in both cases and 9_{eq}-methyl for **1** and 9,21-methylene for **2** (Figure 1). The cyclohexane ring in minor and less active natural ryanoids is modified as follows: 8_{ax}-hydroxy-10-*epi*-**2** and 8_{ax}-hydroxy-10-(*O*-methyl)-10-*epi*-**1** and -**2**; 9_{ax}-hydroxy-**1** and 9_{ax}-hydroxy-10-*epi*-**1**; $\Delta^{9,10}$-8-oxo-10-deoxy-**1**.[3−5] Most of the biological activity comparisons are based on the inhibition of binding of [^{3}H]-**1** to ryanodine receptors (*ryr*) in rabbit muscle sarcoplasmic reticulum preparations.[6,7]

Chemical modifications in the cyclohexane ring of **1** and **2** generally result in reduced potency as inhibitors of [^{3}H]-**1** binding, e.g. epoxidation of the C-9/C-21 double bond[8] and further modification of the epoxide;[4] addition of thiols at this position;[9,10] oxidation or esterification of the C-10 hydroxyl to 10-oxo-**1** and 10-acetyl-**1**, respectively;[8] replacement of the C-10 substituent with a variety of other moieties mentioned in our review[11] and detailed here. In the $C\text{-}10_{eq}$ esters, a basic amino or guanidino group four or five atoms from the cyclohexane ring is very favorable for optimal binding whereas a carboxyl is not suitable.[12] Thus, the activity of **1** and **2** at the *ryr* in calcium release channels is greatly affected by the nature of the substituents on the cyclohexane ring.

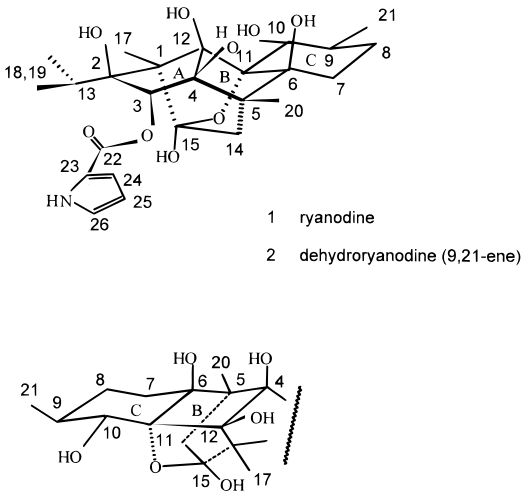

Figure 1. Structures of ryanodine (**1**) and dehydroryanodine (**2**) and partial structure showing the B and C rings viewed from the opposite side of the molecule.

The present study uses two approaches in further defining the contribution of substituents in the cyclohexane ring to the action of **1** at the *ryr*. The first starts from 10-oxo-**1** and 10-oxo-**2** to generate a variety of derivatives including oximes, hydroxyamines, amine, hydrazone, hydrazine, lactam, aromatic esters, and products from manipulation of the 9,21-double bond. The second attempts to defunctionalize the cyclohexane ring and therefore requires a more extensive series of reactions. Routes to 10-deoxy-**2** and 21-nor-10-deoxy-**1** were therefore developed via controlled periodate oxidation of the 9,21-diol, samarium iodide reduction of the 21-nor-9-oxo-10-acetoxy compound, and functionaliza-

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⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1996.

S0022-2623(95)00712-6 CCC: $12.00

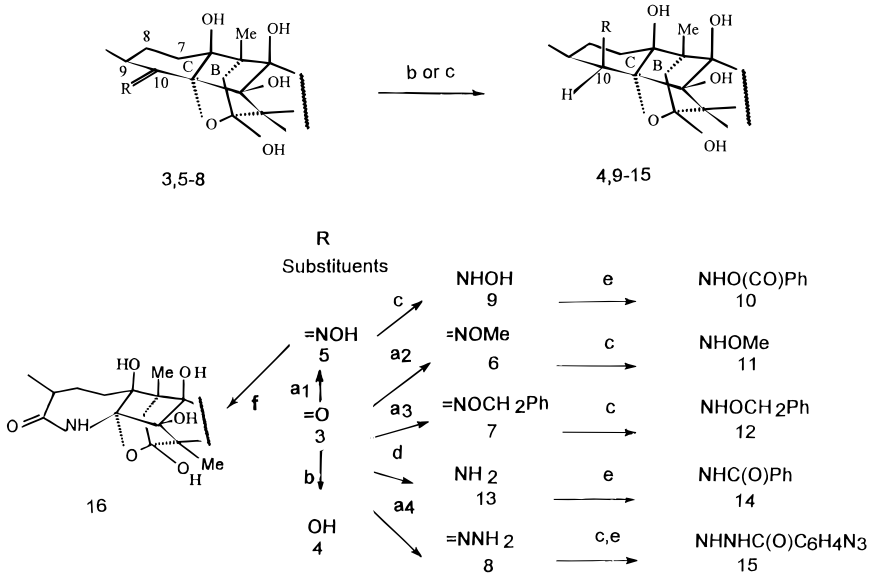

Figure 2. Modifications of the 10-hydroxyl substituent starting from 10-keto-**1** (**3**). Conditions: (a) carbonyl reagents; (a_1) NH_2-OH; (a_2) NH_2OMe; (a_3) NH_2OCH_2Ph; (a_4) NH_2NH_2; (b) $NaBH_4$; (c) $NaCNBH_3$; (d) NH_4OAc, $NaCNBH_3$; (e) ArC(O)Cl; (f) TsCl, base.

tion of the 9-oxo group with Tebbe reagent or its removal by nickel desulfuration of the thioketal.

Structural Modifications

Modifications of the 10-Hydroxyl Substituent of 1 (3−16) (Figure 2). The 10-oxo compound **3** provided easy access to derivatives of many types at C-10. We noted before[11] and detail here that borohydride reduction and recovery from the intermediate borate with methanol gave largely the 10-*epi*-hydroxy compound **4**. Carbonyl reagents using standard methods easily converted 10-oxo compound **3** to the corresponding oxime **5**, methoxime **6**, benzyl oxime **7**, and hydrazone **8**. Acidic cyanoborohydride reduction[13] then gave the *epi*-hydroxyamine **9** (characterized as its benzoyloxy derivative **10**) and the corresponding *epi*-methoxyamine **11** and *epi*-(benzyloxy)amine **12**, the latter very slowly. The products, recovered from their borates, were identified in each case as the 10_{ax} isomers from the small coupling (∼3 Hz) of H-10. Reductive amination of **3** using ammonium acetate and cyanoborohydride yielded the *epi*-amine **13** characterized as its benzamide **14**. Similarly, cyanoborohydride reduction of hydrazone **8** and methanolysis of the borate gave the hydrazine which was immediately converted to the benzoyl hydrazide (not shown) and 4-azidobenzoyl hydrazide (**15**). Reaction of oxime **5** with tosyl chloride (TsCl) in pyridine gave an intermediate which was converted gradually to lactam **16**. The oximes are assumed to have the *E*-configuration in view of the large interference between the 12-hydroxyl and the oxime substituent in the *Z* isomer. The NMR spectrum of the parent 10-oxo compound **3** has normal couplings for the protons of the cyclohexane ring whereas all of the oximes show a downfield shift (∼0.5 ppm) for the 9-methyl group and have proton couplings as average values indicating conformational change which would relieve interaction of the oxime substituent and the methyl attached to C-9.

Modifications of the Cyclohexane Ring of 2 (17−20) (Figure 3). Protection of **2** as the 4,6-ethylboronate[14] [**2**(EtB)] (prepared with lithium triethylborohydride) allowed direct esterification at C-10 with either

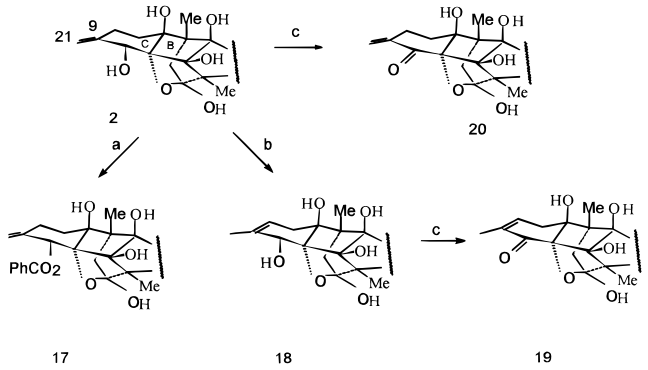

Figure 3. Modifications of the cyclohexane ring of **2**. Conditions: (a) (1) $LiBEt_3H$, (2) PhCOCl, C_5H_5N, (3) MeOH, $MeNH_2$; (b) Pd/C; (c) Swern oxidation.

TsCl or benzoyl chloride to obtain **17**. Oxidation of the Δ^8-10-hydroxy compound **18** gave the Δ^8-10-oxo derivative **19**, as previously noted in our review,[11] and analogously of **2** to the methylene ketone **20**, which unfortunately proved to be unstable.

Defunctionalization of the Cyclohexane Ring of 2 (21−33) (Figure 4). The initial goal was to deoxygenate **2** at C-10, i.e. to prepare 10-deoxy-**2** (**30**). The synthesis started with the 9,21-diol (produced from **2** by osmium tetroxide oxidation)[8] initially protected as the ethylboronate [**2**(EtB)] to block the 4,12-diol from periodate oxidation and to reduce water solubility of the intermediates. Oxidation with 1 molar equiv of sodium periodate then gave the 21-nor-9-oxo derivative [**21**(EtB)] which was reduced with superhydride to the 21-nor-9_{ax}-hydroxy compound [**22**(EtB)] [assigned from the small coupling (∼4 Hz) of H-10], from which the parent (**24**) was recovered by methanolysis with methanol/methylamine. Treatment of **22** with samarium iodide[15] gave the 21-nor-10-deoxy-9-oxo derivative (**27**) as its ethylboronate in 30% yield. Mild acetylation of **21**(EtB) yielded exclusively the 10-acetate which on treatment with samarium iodide gave the 21-nor-10-deoxy-9-oxo compound (**27**) as its ethylboronate in high yield. Reduction of the latter ethylboronate with sodium borohydride in methanol and removal of the boronate

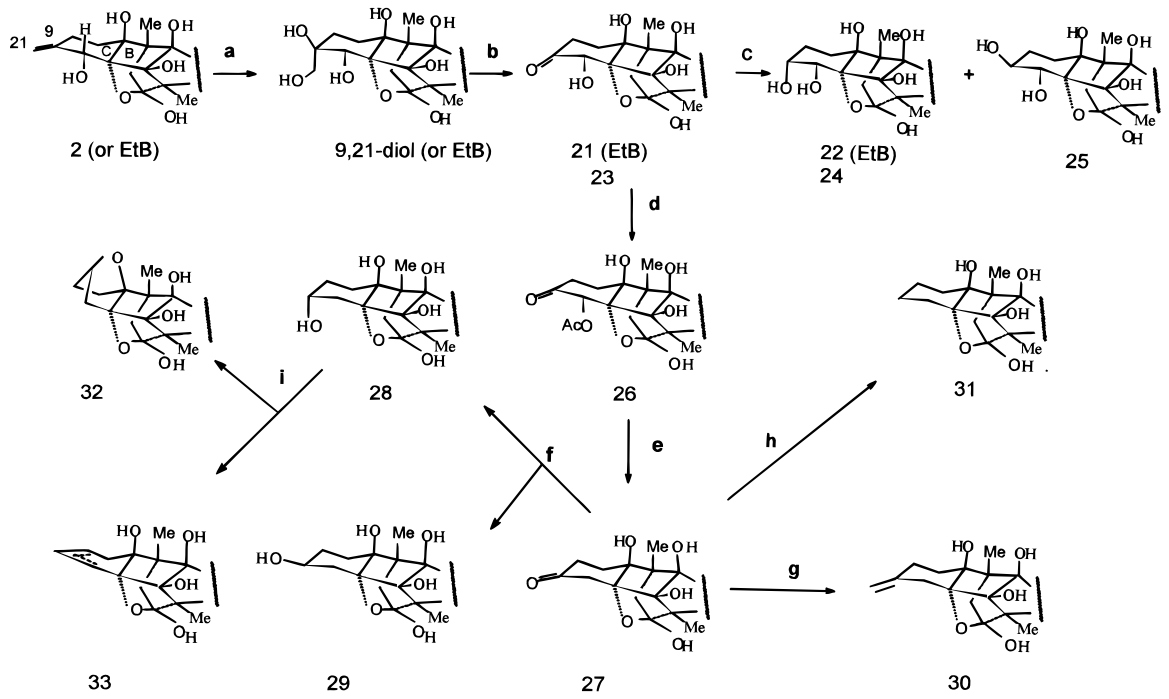

Figure 4. Defunctionalization of the cyclohexane ring of **2**. (a) OsO_4/C_5H_5N; (b) $NaIO_4$; (c) $LiEt_3BH$ or LAH; (d) Ac_2O/C_5H_5N; (e) SmI_2; (f) LAH; (g) Tebbe reagent; (h) (1) $(CH_2SH)_2/BF_3{\cdot}Et_2O$, (2) $MeOH/MeNH_2$, (3) Ni; (i) $POCl_3/C_5H_5N$.

group gave the 21-nor-10-deoxy-9_{ax}-hydroxy compound **28** with a smaller amount of the 9_{eq} epimer **29**, and these were assigned from the line shapes of the NMR signals for the C-9 protons.

An alternative method was developed for conversion of **2** to the 21-nor-9-oxo compound **23** without use of the boronate group. Osmylation of **2** in pyridine and purification as for the boronate gave the known 9,21-diol.[8] Although the 4,12-diol group is oxidized rapidly by periodic acid,[16] reactions with periodate are slower at pH >4,[17] and in practice the more exposed 9,21-diol oxidized much faster at neutral pH. Reduction of the 21-nor-9-oxo compound **23** with samarium iodide gave its 10-deoxy derivative **27** in poor yield, not improved by inclusion of pivalic acid,[18] the major byproduct being the 21-nor-9_{eq}-hydroxy derivative **25** assigned from the large coupling (9.8 Hz) for the diaxial protons at C-9 and C-10. Brief reduction of **23** with lithium aluminum hydride gave a mixture of the 21-nor-9_{ax}-hydroxy compound with its 9_{eq} epimer (**24** and **25**). Mild acetylation of **23** gave in high yield the 21-nor-9-oxo-10-acetoxy compound **26** which was efficiently reduced with samarium iodide to the 10-deoxy derivative **27**. Brief reduction of **27** with lithium aluminum hydride gave both epimeric alcohols (**28** and **29**). Reaction of oxo compound **27** with the Wittig reagent in dimethyl sulfoxide (DMSO)[19] was unsuccessful. On the other hand methylenation with Tebbe reagent[19] gave a good yield of 10-deoxy-**2** (**30**).

Another goal was to obtain 21-nor-10-deoxy-**1** (**31**). Although this is an expected product of the 9-tosylhydrazone and borohydride in acetic acid,[20] no deoxygenation was observed. However, **31** was obtained from **27** by conversion to the ethylene thioketal[21] using boron trifluoride etherate, methanolysis of the borate ester and then brief desulfurization with nickel. Deoxygenation of the 9-position was also achieved by treatment of the 9_{ax}-hydroxy compound **28** with phosphoryl chloride/pyridine which gave a small alkene fraction containing the 21-nor-10-deoxy-Δ^8/Δ^9 compounds **33**. The main and less polar product showed NMR data characteristic of the 1,4-oxygen-bridged cyclohexane ring of the 21-nor-10-deoxy-6,9-oxido compound **32** which would arise by internal S_N2 reaction with the intermediate chlorophosphate or derived ion pair.

Structure−Activity Relationships

Rabbit skeletal muscle, mouse brain, and canine left ventricular membranes were used for assays of [^{3}H]-**1** binding and inhibition thereof by the test compounds.[14] A pharmacologic response mediated by the cardiac *ryr* was also examined involving contraction on electrical stimulation of cardiac muscle using rat right ventricular muscle strips.[14] Conditions specifically considered in the companion paper[14] are not detailed here. The data in Tables 1 and 2 are generally for compounds with >10% of the activity of **1** in at least one of the assays. Compounds of lower activity or those closely related to tabulated compounds (oxime, hydrazone, and substituted hydroxyamines) are given in the Supporting Information.

Modifications of the 10-Hydroxyl Substituent of 1 (3−16) (Table 1, Figure 2). This series derived from 10-oxo-**1** (**3**) generally retains moderate to high activity at *ryrs* with fair correspondence for the different tissues where such data are available. The *epi*-hydroxy compound **4** is 34−97% as active as **1** in the 4 assays, i.e. the normal and *epi* stereochemistry are equally favorable. Oximes **5**−**7** and hydrazone **8** are similar in potency to oxo compound **3**. The hydroxyamines and amine approximate the activity of **1** when small substituents are present (**9**, **11**, and **13**) but are considerably less potent with aryl moieties (**10** and **12**). Although the *epi*-benzamide **14** is less active than the *epi*-amine **13**, the azidobenzoyl hydrazide **15** is 67% as active as **1** at the skeletal muscle *ryr*. In this candidate photoprobe, in contrast with earlier candidates,[22] the photoreactive substituent lies near the cage structure. Lactam **16**

Table 1. Modifications of the 10-Hydroxyl Substituent of Ryanodine and the Cyclohexane Ring of Dehydroryanodine in Relation to Action at Calcium Release Channels

		activity relative to ryanodine[a]			
		ryanodine receptor			
no.	10-substituent or modification of cyclohexane ring	rabbit muscle	mouse brain	canine ventricle	rat ventricular strip assay
1	ryanodine	100	100	100	100
	Selected Modifications of the 10-Hydroxy Substituent of **1**[b]				
3	oxo	30			
4	10-*epi*-hydroxy	97	69	78	34
5	oxime	26			
6	methoxime	13	13	17	34
9	*epi*-hydroxyamine	32			
11	*epi*-methoxyamine	68		36	31
13	*epi*-amine	68	65		
14	*epi*-benzamide	9.5			
15	*epi*-4-azidobenzoylhydrazide	67			
16	lactam	42		7.4	11
	Selected Modifications of the Cyclohexane Ring of **2**[b]				
17	10-benzoyloxy	3.9	17		
18	Δ^8-10-hydroxy	26		27	11
19	Δ^8-10-oxo	12			

[a] Values for **1** are as follows: IC_{50}s of 3.5 and 3.3 nM for rabbit muscle and mouse brain; K_i of 1.8 nM for cardiac ventricle preparation; and IC_{50} of 34 nM for rat ventricular muscle strip assay. Standard error values as percent of the mean of three to five experiments (each in duplicate for receptor assays) were independent of the compound and averaged 10, 12, 4, and 6 for the *ryr* assays (rabbit muscle, mouse brain, and canine ventricle) and ventricular strip assay, respectively. [b] Data for **7**, **8**, **10**, **12**, and **20** are given in the Supporting Information.

Table 2. Defunctionalization of the Cyclohexane Ring of Dehydroryanodine in Relation to Action at Calcium Release Channels

		activity relative to ryanodine[b] = 100			
		ryanodine receptor			
no.[a]	selected modifications of cyclohexane ring	rabbit muscle	mouse brain	canine ventricle	rat ventricular strip assay
21	21-*nor*-9-oxo (EtB)	13	8.3		
23	21-*nor*-9-oxo	10	7.1	6.5	5.9
24	21-*nor*-9_{ax}-hydroxy	0.62	0.50	2.7	26
28	21-*nor*-10-deoxy-9_{ax}-hydroxy	0.20	0.40	0.42	28
30	10-deoxydehydro	37	77	45	121
31	21-*nor*-10-deoxy	6.5	18		

[a] Data for **22**, **25**−**27**, **29**, **32**, and **33** are given in the Supporting Information. [b] Standard values for **1** are given in Table 1.

appears to be less active on cardiac preparations relative to skeletal muscle *ryr* than the other 10-hydroxy variants.

Modifications of the Cyclohexane Ring of 2 (17−**20) (Table 1, Figure 3).** The 10-benzoyloxy compound **17** is 4−17% as active as **1**, falling in the same range as 10-acetoxy-**1**.[8] Introduction of unsaturation at C-8 reduces potency to about one-third, i.e. alcohols **18** versus **1** and ketones **19** versus **3**.

Defunctionalization of the Cyclohexane Ring of 2 (21−**33) (Table 2, Figure 4).** The target compounds 10-deoxy-**2** (**30**) and 21-nor-10-deoxy-**1** (**31**) were compared with intermediates in their preparation from **2**. Replacing the 9-methylene of **2** with 9-oxo as in **21** and **23** reduces potency to about 10% and the epimeric alcohols **24** and **25** from reduction of **23** are even less active (except for **24** in the functional assay). 9_{ax}-Hydroxy-**1** and 9_{ax}-hydroxy-10-*epi*-**1** occur naturally and have low activity[3,4] similar to that of compound **24**, which is a 21-nor analog. The 9_{ax}-hydroxy compounds **24** and **28** show high potency in the functional assay relative to their 9_{eq} isomers **25** and **29**, but this isomer difference is not evident in the *ryr* assay where they are relatively less effective. Deoxygenation at C-10 affects the activity differently depending on the other substituents and the bioassay system, i.e. greatly reduces *ryr* activity in the 21-nor-9-oxo series (**27** versus **23**) and confers similar potency in the 21-nor-9_{ax}- and -9_{eq}-hydroxy series (**28** and **29** versus **24** and **25**). On the same basis, **25** and **29** show similar low values in all assays whereas **24** and **28** appear to differ significantly in the canine ventricle compared with the *ryr* assays. There is little potency loss in any of the assay systems on removing the 10-hydroxyl substituent (**30** versus **2**), somewhat more for the 21-nor-10-deoxy compound **31**, and the deoxy-Δ^8/Δ^9 compound **33** is of only weak activity as is the 6,9-oxido compound **32**.

Correlation between Relative Potencies of Ryanodine Derivatives as Inhibitors of [^{3}H]-1 Specific Binding in Three *ryr* Preparations and of Cardiac Contractility of Rat Muscle Strips (Figure 5). The data in this study and a companion paper[14] indicate little if any structural specificity of **1** and its analogs in differentiating the [^{3}H]-**1** binding site of cardiac *ryr* versus either rabbit skeletal muscle *ryr* ($r = 0.88$, $n = 29$) or mouse brain *ryr* ($r = 0.93$, $n = 23$). These systems contain mixed receptor types: skeletal muscle, two isoforms of *ryr*-1; brain, *ryr*-2 as the main component with *ryr*-1 in parts of this organ; cardiac, mainly *ryr*-2 making this organ source perhaps the most homogeneous.[7] Bound **1** is retained with a 76 kDa fragment of peptide bearing the carboxyl terminus after hydrolysis both with and without photoaffinity attachment.[22,23] Sequence studies show that this part of the protein is largely conserved across the various receptors so that it is unlikely that very large differences would be found in the selectivity of ryanodine analogs acting on *ryr* preparations from different organs.

The cardiac contractility assay[14] is a physiological function test with intact tissue and is therefore dependent on both penetration to the site and potency at the *ryr*. With the exception of five numbered compounds and the 4,12-oxygen-bridged derivatives (see Figure legend) which are at least 5-fold more potent on a relative basis in the cardiac contractility than in the cardiac *ryr* assay, there was a good correlation between these two assays ($r = 0.85$, $n = 15$), consistent with interaction of the ryanoids at the high-affinity **1** receptor on sarcoplasmic reticulum calcium release channels rendering them unable to enter normal open or closed gating configurations. The negative inotropic effect was slow in onset and progression consistent with the intrinsic activity being associated with stabilizing the calcium release channel in a subconducting state[24] and uncoupling the excitation−contraction process. Also consistent with this mode of action was the ability of these derivatives to increase internal [Ca^{2+}] in electrically stimulated cardiomyocytes isolated from adult rats.[25]

Experimental Section

Chemistry. General. The companion paper[14] gives sources of **1** and **2**, the general methods of product isolation, and the conditions for ^{1}H and ^{13}C NMR and HRMS. Reactions were carried out on a semimicroscale with product isolation by preparative TLC (0.25− or 0.5-mm silica gel F_{254}) or rotary

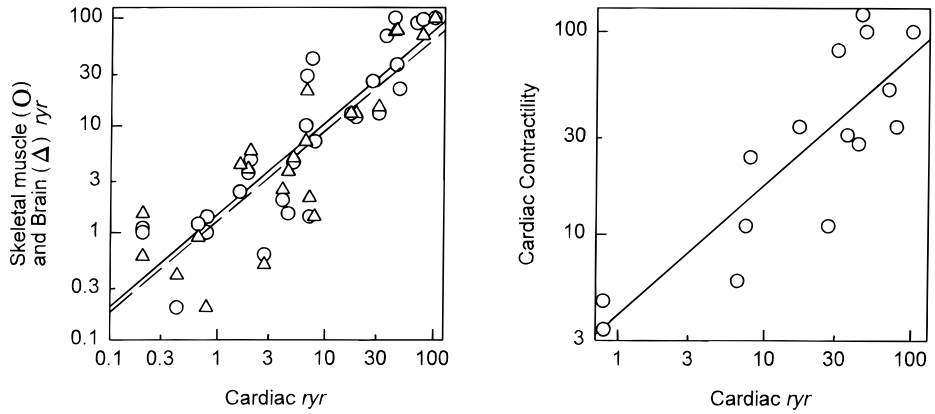

Figure 5. Correlation between relative potencies of ryanodine derivatives (**1** is rated as 100) as inhibitors of [^{3}H]-**1** specific binding at cardiac *ryr* versus either skeletal muscle and brain *ryr* (left) or cardiac contractility of rat muscle strips (right). All data involving discrete potency values in this study and a companion paper[14] are plotted with the exception of the following: **24** and **28** in this study; **18**, **23**, **27**, and the 4,12-oxygen-bridged derivatives in the companion paper. Compounds without discrete potency values had little or no activity in both the cardiac *ryr* and contractility assays. Correlation coefficients and *n* are as follows: canine left ventricle cardiac *ryr* versus rabbit skeletal muscle *ryr* (left figure, upper correlation line) $r = 0.88$ and $n = 29$; cardiac *ryr* versus mouse brain *ryr* (left figure, lower correlation line) $r = 0.93$ and $n = 23$; cardiac *ryr* versus rat strip contractility assay (right figure) $r = 0.85$ and $n = 15$.

chromatography (1-mm silica gel GF_{254}) as crystalline solids or resins. HPLC (C-18 reverse phase silica column developed with discriminating MeOH−water mixtures) was used to verify the absence of **1** or **2** in the final samples. Sample purities were estimated to be >98% based on NMR, TLC, and HPLC. NMR data which differ little from the fully assigned reference spectra[26,27] are given only in abbreviated form.[28]

Modifications of the 10-Hydroxyl Substituent of 1 (3−16). 10-Oxo-1 (3). Ryanodine (**1**, 30 mg, 0.061 mmol) in DMSO (0.2 mL) and CH_2Cl_2 (2 mL) was added gradually at −60 °C to a solution from addition of DMSO (0.2 mL, 2.6 mmol) to CH_2Cl_2 (2 mL) and oxalyl chloride (25 mg, 0.20 mmol) in CH_2Cl_2 (0.2 mL). After 10 min Et_3N (0.2 mL, 1.45 mmol) was added, and after a further 10 min the solution was allowed to warm to ambient temperature. Saturated $NaHCO_3$ aqueous solution was added and the product isolated with ethyl acetate and purified by rotary chromatography in $CHCl_3$/MeOH (49:1) to give **3**[8] (20 mg, 67%) and **1** (7 mg, 23%) with $CHCl_3$/MeOH (9:1): ^{1}H NMR (CD_3OD) δ 7.04 (m, H-26), 6.86 (m, H-24), 6.24 (m, H-25), 5.69 (s, H-3), 2.62 (d, $J = 13.9$ Hz) and 2.08 (d, $J = 13.9$ Hz) AB H_2-14, 2.21 (m, H-13), 2.99 (m, H-9), 2.43 (dt, $J =$ 13, 13, and 6 Hz, H-7_{ax}), 1.44 (dd, $J = 13$ and 6 Hz, H-7_{eq}), 1.95 (m, H-8_{eq}), 1.75 (dq, $J = 13$, 13, 13, and 5 Hz, H-8_{ax}), 1.25 (s, H_3-17), 1.08 (d, $J = 6.8$ Hz) and 0.74 (d, $J = 7.0$ Hz) (H_3-18 and -19), 0.93 (s, H_3-20), 1.00 (d, $J = 7.0$ Hz, H_3-21); ^{13}C NMR (CD_3OD) δ 66.0 (C-1), 82.9 (C-2), 90.8 (C-3), 91.5 (C-4), 49.5 (C-5), 84.0 (C-6), 26.7 (C-7), 31.9 (C-8), 41.4 (C-9), 215.0 (C-10), 89.7 (C-11), 97.0 (C-12), 30.8 (C-13), 42.6 (C-14), 103.6 (C-15), 11.0 (C-17), 18.7 and 19.3 (C-18 and -19), 12.5 (C-20), 13.7 (C-21), 161.0 (C-22), 123.2 (C-23), 116.9 (C-24), 110.9 (C-25), 125.6 (C-26).

10-*epi*-Hydroxy-1 (4). 10-Oxo-**1** (**3**, 13 mg, 0.026 mmol) was reduced with $NaBH_4$ (20 mg, 0.48 mmol) in MeOH at −18 °C during 45 min. Acidification (HCl), extraction with ethyl acetate, and methanolysis of the borate by distillation with MeOH during 3 h gave a mixture, separated by rotary chromatography with $CHCl_3$/MeOH (19:1) to give 10-*epi*-**1** (**4**, 10 mg, 77%): ^{1}H NMR ($CD_3OD/CDCl_3$) δ 6.99 (m, H-26), 6.85 (m, H-24), 6.20 (m, H-25), 5.54 (s, H-3), 3.81 (d, $J = 3.0$ Hz, H-10), 2.0 (m, H-9), 2.47 (d, $J = 14.0$ Hz) and 1.85 (d, $J = 14$ Hz) AB H_2-14, 2.17 (m, H-13), 2.04 (m, H-7_{ax}), 2.50 (m, H-8_{ax}), 2.35 (m, H-8_{eq}), 1.29 (s, H_3-17), 1.03 (d, $J = 6.8$ Hz) and 0.73 (d, $J = 7.0$ Hz) (H_3-18 and -19), 0.85 (s, H_3-20), 0.91 (d, $J =$ 6.9 Hz, H_3-21); ^{13}C NMR (CD_3OD) δ 65.2 (C-1), 83.4 (C-2), 89.7 (C-3), 91.3 (C-4), 49.5 (C-5), 85.2 (C-6), 26.6 and 23.6 (C-7 and -8), 31.7 (C-9), 74.7 (C-10), 80.9 (C-11), 95.1 (C-12), 29.9 (C-13), 40.2 (C-14), 102.0 (C-15), 10.4 (C-17), 18.8 and 18.2 (C-18 and -19), 11.5 (C-20), 17.1 (C-21), 161.5 (C-22), 121.9 (C-23), 116.5 (C-24), 110.4 (C-25), 124.9 (C-26). A later fraction of **1** (1 mg, 8%) was eluted with $CHCl_3$/MeOH (17:3).

Oxime and Hydrazone Derivatives of 10-Oxo-1 (5−8). The oximes (**5**−**7**) were prepared by heating **3** in pyridine solution with the hydroxyamine hydrochloride at 50 °C for 1−2 h and purification by rotary chromatography in $CHCl_3$/MeOH mixtures. Oxime **5** had R_f 0.50 ($CHCl_3$/MeOH, 9:1): NMR (CD_3OD) as for **3** except ^{1}H δ 2.15 (m), 1.90 (m), 1.66 (m) and 1.46 (m) (H_2-7 and H_2-8), 2.93 (sext, $J = 6.5$ Hz, H-9), 1.31 (s, H_3-17), 0.93 (s, H_3-20), 1.42 (d, $J = 7.2$ Hz, H_3-21); ^{13}C δ 84.1 and 85.6 (C-6 and -11), 28.9 (C-8), 33.9 (C-9), 161.6 (C-10), 85.6 (C-11), 19.1 (C-21). The methoxime (**6**) had R_f 0.37 ($CHCl_3$/MeOH, 19:1): NMR spectra as for oxime **5** and ^{1}H δ 1.36 (d, $J = 7$ Hz, H_3-21), 3.84 (s, OMe); ^{13}C NMR (CD_3OD) δ 163.4 (C-10), 62.6 (OMe); HRMS (FAB) *m/z* $C_{27}H_{36}N_2O_9H^+$ 521.2499, found 521.2506. The benzyl oxime **7** had R_f 0.39 in $CHCl_3$/MeOH (19:1): NMR spectra as for oxime **5** except ^{1}H δ 5.04 (d, $J = 12.5$ Hz) and 5.08 (d, $J = 12.5$ Hz) AB (CH_2Ar), 1.39 (d, $J = 6.9$ Hz, H_3-21), 1.16 (s, H_3-17), 0.73 (phenyl); ^{13}C NMR (CD_3OD) δ 77.5, 129.0, 129.3, 129.5, and 138.7 (Ar). The hydrazone **8** prepared with hydrazine at pH 8 was purified by TLC using a silanized plate with $CHCl_3$/MeOH (19:1): NMR (CD_3OD) as for oxime **5** except ^{13}C δ 30.6 (C-9), 156.3 (C-10), 16.1 (C-21).

***epi*-Hydroxyamines 9−12.** Oxime **5** (28 mg, 0.055 mmol) in MeOH (2 mL) was acidified (0.1% HCl) and kept at pH 3−4 (pH paper) while $NaCNBH_3$ (30 mg, 0.48 mmol) was added during 1 h. After 12 h, aqueous HCl was added and the product isolated by extraction into ethyl acetate and distillation with MeOH (3 h). Hydroxyamine **9** (8.7 mg, 31%) was isolated by TLC with $CHCl_3$/MeOH/$MeNH_2$ (83:14:3): R_f 0.28 in $CHCl_3$/MeOH (9:1); NMR (CD_3OD) as for **4** except ^{1}H δ 2.97 (d, $J = 2.5$ Hz, H-10), 2.20 (m, H-9), 1.03 (d, $J = 7.0$ Hz, H_3-21); ^{13}C δ 25.8 and 27.6 (C-7 and −8), 69.0 (C-10), 84.8 (C-11), 19.4 (C-21); HRMS (FAB) *m/z* $C_{25}H_{36}N_2O_9Na^+$ 531.2319, found 531.2308. (Benzoyloxy)amine **10** was prepared in high yield by reaction either with benzoyl chloride in THF/Et_3N or with *N*-(benzoyloxy)succinimide: HRMS (FAB) *m/z* $C_{32}H_{40}N_2O_{10}Na^+$ 635.2581, found 635.2590. Methoxyamine **11** (30%), R_f 0.31 in $CHCl_3$/MeOH (19:1), was obtained in the same way from the methoxime: NMR (CD_3OD) as for hydroxyamine **9** except ^{1}H δ 3.51 (s, OMe) 3.34 (d, $J = 3.9$ Hz, H-10); ^{13}C δ 66.6 (C-10), 61.5 (OMe); HRMS (FAB) *m/z* $C_{26}H_{38}N_2O_9H^+$ 523.2656, found 523.2660. (Benzyloxy)amine **12** (R_f 0.27), prepared as above (15%), was separated from the benzyl oxime (R_f 0.22) by TLC with $CHCl_3$/MeOH/$MeNH_2$ (94:5:1): NMR (CD_3OD) as for hydroxyamine **9** except ^{1}H δ 4.63 (d, $J = 14$ Hz) and 4.70 (d, $J = 14$ Hz) CH_2Ar, 3.40 (d, $J = 3.7$ Hz, H-10), 2.39 (d, $J = 13.8$ Hz) and 1.79 (d, $J = 13.8$ Hz) AB H_2-14, 7.28 (Ar); HRMS (FAB) *m/z* $C_{32}H_{42}N_2O_9H^+$ 599.2969, found 599.2979.

10-*epi*-Amine 13 and Its Benzamide 14. 10-Oxo-**1** (**3**, 20 mg, 0.041 mmol) and ammonium acetate (0.17 g, 2.2 mmol)

in MeOH (3 mL) were set aside with $NaCNBH_3$ (12 mg, 0.19 mmol) for 24 h. Excess borohydride was destroyed by acidification (HCl), and the amine borate was recovered from the neutral solution with ethyl acetate, distilled with methanol for 4 h, and purified by rotary chromatography with $CHCl_3$/MeOH/$MeNH_2$ (83:14:3) to give **13** (10 mg, 53%): NMR (CD_3OD/$CDCl_3$) as for hydroxyamine **9** except ^1H δ 3.21 (d, $J = 3.7$ Hz, H-10), 2.49 (d, $J = 13.7$ Hz) and 1.84 (d, $J = 13.7$ Hz) AB H_2-14, 0.94 (d, $J = 7.2$ Hz, H_3-21); ^{13}C δ 30.8 (C-9), 57.5 (C-10), 19.3 (C-21). Benzamide **14** prepared in THF with benzoyl chloride/Et_3N was purified by TLC with $CHCl_3$/MeOH (19:1): NMR (CD_3OD) as for hydroxyamine **9** except ^1H δ 7.71, 7.47 and 7.37 (Ar), 4.38 (d, $J = 3.70$ Hz, H-10), 1.35 (s, H_3-17), 0.82 (d, $J = 6.8$ Hz, H_3-21); ^{13}C δ 29.6 (C-9), 53.3 (C-10), 131.8, 130.0, 128.7 and 126.9 (Ar); HRMS (FAB) *m/z* $C_{32}H_{40}N_2O_9H^+$ 597.2812, found 597.2827.

10-*epi*-Hydrazine and Its 4-Azidobenzoyl hydrazide (15). 10-Oxo-**1** (**3**, 15 mg, 0.031 mmol) was added to hydrazine (50 mg, 1.8 mmol) in MeOH at pH 7. After formation of the hydrazone was complete (2 h), $NaCNBH_3$ (25 mg, 0.40 mmol) was added and the solution maintained at pH 5−6 for 12 h. The solution was acidified (pH 1−2) and left for 3 h, basified ($NaHCO_3$), extracted with ethyl acetate, and deborated with methanol as above to give crude hydrazine (R_f 0.49 in $CHCl_3$/MeOH/$MeNH_2$, 78:19:3): NMR (CD_3OD) as for hydroxyamine **9** except ^1H δ 2.97 (d, $J = 3.8$ Hz, H-10), 1.03 (d, $J = 7.0$ Hz, H_3-21); ^{13}C δ 67.2 (C-10). The benzoyl hydrazide, prepared as for the benzamide **14**, was purified by TLC with $CHCl_3$/MeOH (19:1), R_f 0.18: NMR (CD_3OD/$CDCl_3$) as for benzamide **14** except ^1H δ 3.11 (d, $J = 3.5$ Hz, H-10), 1.12 (d, $J = 7.2$ Hz, H_3-21); HRMS (FAB) *m/z* $C_{32}H_{41}N_3O_9H^+$ 612.2921, found 612.2948. The 4-azidobenzoyl hydrazide **15** was prepared in the same way from the acid chloride and was purified twice by TLC in $CHCl_3$/MeOH (92:8). NMR as for the benzoylhydrazide except ^1H 7.70, 7.46, and 7.41 (phenyl).

Lactam 16. The oxime **5** (10 mg, 0.02 mmol), TsCl (30 mg, 0.16 mmol), and pyridine (30 mg, 0.38 mmol) were dissolved in THF (2 mL). An early faster spot on TLC (R_f 0.88 $CHCl_3$/MeOH (9:1)) was replaced with the lactam (R_f 0.45) overnight. Isolation by addition of water, acidification, extraction with ethyl acetate, and TLC in $CHCl_3$/MeOH (9:1) gave the lactam **16** (5.7 mg, 57%): NMR (CD_2OD) as for **3** except ^1H δ 3.02 (ddq, $J = 11.5$, 2.0, and 7.0 Hz, H-9), 1.58 (dt, $J = 13$, 13, and 6 Hz, H-7_{ax}), 1.80 (br q), 1.75 (dq, $J = 13$, 13, 13, and 5 Hz, H-8_{ax}), 0.92 (s, H_3-20), 1.09 (d, $J = 6.7$ Hz, H_3-21); ^{13}C δ 30.0 (C-8), 38.7 (C-9), 181.2 (C-10), 18.2 (C-21).

Modifications of the Cyclohexane Ring of 2 (17−20). Dehydroryanodine 4,6-Di-*O*-ethylboronate [2(EtB)]. **2** (50 mg, 0.10 mmol) in THF (2 mL) was treated with 1 M lithium triethyl borohydride in THF (1 mL, 1.0 mmol) at 0 °C for 10 min. The product isolated by addition of water, acidification (HCl), and ethyl acetate extraction was set aside in MeOH for 15 min to decompose complex boronates. TLC with $CHCl_3$/MeOH (9:1) (R_f 0.55) gave **2**(EtB) (45 mg, 85%): NMR (CD_3OD/$CDCl_3$) as for the methylboronate[14] except ^1H δ 0.91 (m) and 0.72 (m) (EtB); ^{13}C NMR δ 8.2 and 7.1 (br) (EtB); HRMS $C_{27}H_{36}O_9NBNa^+$ 552.2381, found 552.2389. The boronate was hydrolyzed by slow distillation with MeOH during 3 h. Alcoholysis could be avoided by evaporation of hydroxylic solvents at <30 °C.

Esters from 2(EtB) Including the 10-Benzoate 17. **2**(EtB) (10 mg, 0.019 mmol) was treated with benzoyl chloride (100 mg, 0.71 mmol) in pyridine (1 mL) for 24 h. The product was deborated by slow distillation with MeOH (4 h) and purified by TLC in $CHCl_3$/MeOH (92:8) to give **17** (6 mg, 53%): NMR (CD_3OD/$CDCl_3$) as for **4** except ^1H δ 5.49 (H-10), 4.71 and 4.81 (H_2-21), 8.14, 7.56, and 7.45 (Ar). The 10-tosylate (EtB) was prepared similarly during 4 days and separated by TLC using $CHCl_3$/MeOH (9:1): NMR as for **2**(EtB) except ^1H δ 5.49 (H-10), 4.88 and 4.87 (H_2-21), 1.07 (s, H_3-17), 7.76, 7.27, and 2.16 ($ArCH_2$); ^{13}C δ 145.5 (C-9), 68.6 (C-10), 110.9 (C-21), 141.6, 133.7, 129.0, 127.7, 21.1 ($ArCH_2$). Mesylation under these conditions gave the 10,15-bis(mesyloxy) compound (45%): NMR as for **2**(EtB) except ^1H δ 5.38 (H-10), 5.18 and 5.04 (H_2-21), 2.87 (d) and 2.63 (d) AB H_2-14, 1.35 (s, H_3-17), 3.14 (s) and 3.16 (s) 2 MeS; ^{13}C δ 60.8 (C-1), 143.4 (C-9), 66.4 (C-10), 41.9, 39.5, and 37.1 (C-14, 2 MeS), 108.4 (C-15), 110.9 (C-21); HRMS (FAB) *m/z* $C_{29}H_{40}NO_{13}BS_2Na^+$ 708.1959, found 708.1944.

Δ^8-10-Hydroxy-1 (18) and Δ^8-10-Oxo-1 (19). Dehydroryanodine (**2**) was isomerized by heating at 50 °C in MeOH with Pd/C for 5 h and the product[29] (**18**, 74%) separated by rotary chromatography with $CHCl_3$/MeOH (18:1−17:3). Oxidation of **18** (20 mg, 0.041 mmol) with oxalyl chloride (8 mg, 0.067 mmol) as for **1** and TLC with $CHCl_3$/MeOH (19:1) gave the ketone (**19**, 12 mg, 60%): NMR (CD_3OD) as for **3** except ^1H δ 2.72 (d, $J = 14$ Hz) and 2.07 (d, $J = 14$ Hz) AB H_2-14, 2.95 (dq, $J = 18$ and 2.5 Hz, H-7_{ax}), 2.17 (obsc, H-7_{eq}), 6.69 (m, H-8), 1.31 (s, H_3-17), 1.78 (m, H_3-21); ^{13}C NMR (CD_3OD) δ 84.0 and 86.6 (C-6 and -11), 32.9 (C-7), 148.0 (C-8), 135.4 (C-9), 199.0 (C-10), 15.4 (C-21).

10-Oxo-2 (20). Dehydroryanodine (**2**, 30 mg, 0.061 mmol) was oxidized in the same way as **1** using oxalyl chloride (7 mg, 0.056 mmol). The product was purified by TLC with $CHCl_3$/MeOH (19:1) containing 1% quinol and was stored similarly in MeOH at −20 °C: NMR (CD_3OD) as for **3** except ^1H δ 5.30 (br s) and 5.85 (br s) H_2-21, 2.75 (m), 2.35 (m), 2.15 (m), and 1.55 (m); ^{13}C NMR (CD_3OD) δ 84.0 and 83.3 (C-6 and -11), 24.9 and 26.7 (C-7 and -8), 144.3 (C-9), 201.5 (C-10), 123.2 (C-21).

Defunctionalization of the Cyclohexane Ring of 2 (21−33). 21-Nor-9-oxo-1 (23) and Its Ethylboronate (21). The boronate (80 mg, 0.15 mmol) in pyridine (2 mL) was added to OsO_4 (60 mg, 0.24 mmol) in pyridine (1 mL). After 12 h saturated aqueous solutions of $NaHCO_3$ (2 mL) and $NaHSO_3$ (2 mL) were added, and the solution was stirred for 1 h. Acidification (HCl), extraction with ethyl acetate, and chromatography on silica (4 g) gave a trace of **1** (2 mg) with $CHCl_3$/MeOH (20:1), and then the diol(EtB) (38 mg, 45%) was recovered with $CHCl_3$/MeOH (93:7). A small amount of deborated diol (5 mg, 6%) was eluted with $CHCl_3$/MeOH (85:15). The diol(EtB) above (32 mg, 0.057 mmol) in water (4 mL) and THF (1 mL) were treated dropwise with stirring with 0.1 M $NaIO_4$ (0.6 mL, 0.06 mmol). The product isolated with ethyl acetate was purified by rotary chromatography using $CHCl_3$/MeOH mixtures to give ketol boronate **22** (26 mg, 94%): TLC R_f 0.60 for $CHCl_3$/MeOH, 9:1; ^1H NMR (CD_3OD) as for **2**(EtB) except δ 4.65 (s, H-10), 2.54 (d, $J = 13.9$ Hz) and 1.97 (d, $J = 13.9$ Hz) AB H_2-14, 2.75 (m, H-8_{ax}), 2.40 (m, H-8_{eq}), 2.20 (m, H-7_{ax}), 1.66 (ddd, $J = 1.0$, 5.9, and 13.0 Hz, H-7_{eq}), 0.87 (m) and 0.75 (m) ($EtBO_2$); ^{13}C NMR (CD_3OD) δ 44.9 (C-5), 85.2 (C-6), 25.6 (C-7), 34.3 (C-8), 209.7 (C-9), 72.5 (C-10), 90.0 (C-11), 95.8 (C-12), 7.7 and 5.9 (br) ($EtBO_2$).

Hydroxylation of **2** (50 mg, 0.096 mmol) as described above gave the known diol[8] with the modification that the pyridine was removed from the aqueous layer and the latter extracted five times with an equal volume of ethyl acetate. The organic layers were combined, evaporated, and chromatographed on silica using $CHCl_3$/MeOH (93:7) to remove traces of **1** and then $CHCl_3$/MeOH (85:15) to give the diol (45 mg, 72%). Oxidation with 1 molar equiv of 0.05 M periodate at 10 °C and TLC with $CHCl_3$/MeOH (80:20) gave the ketol **23** (R_f 0.60, 36 mg, 77%): ^1H NMR (CD_3OD) δ 7.05 (m, H-26), 6.88 (m, H-24), 6.24 (m, H-25), 5.66 (s, H-3), 4.85 (s, H-10), 2.59 (d, $J = 14.0$ Hz) and 1.94 (d, $J = 14.0$ Hz) AB H_2-14, 2.70 (m, H-8_{ax}), 2.33 (m, H-8_{eq}), 2.30 (m, H-7_{ax}), 1.65 (dd, $J = 7.2$ and 11.9 Hz, H-7_{eq}), 2.25 (m, H-13), 1.37 (s, H_3-17), 1.09 (d, $J = 6.7$ Hz) and 0.75 (d, $J = 6.3$ Hz) (H_3-18 and -19), 0.96 (s, H_3-20); ^{13}C NMR (CD_3OD) 65.5 (C-1), 83.0 (C-2), 91.0 (C-3), 91.2 (C-4), 49.8 (C-5), 85.0 (C-6), 27.5 (C-7), 35.6 (C-8), 211.1 (C-9), 74.2 (C-10), 92.3 (C-11), 96.0 (C-12), 30.9 (C-13), 41.7 (C-14), 103.5 (C-15), 9.9 and 12.8 (C-17 and -20), 18.8 and 19.4 (C-18 and -19), 161.5 (C-22), 123.2 (C-23), 117.1 (C-24), 111.0 (C-25), 125.6 (C-26); HRMS $C_{24}H_{31}NO_{10}Na^+$ 516.1846, found 516.1841.

21-Nor-9$_{ax}$-hydroxy-1 (24) and Its Ethylboronate (22). The ketol boronate **21** (10 mg, 0.019 mmol) in THF (2 mL) was treated at 0 °C with 1 M $LiEt_3BH$ in THF (0.3 mL, 0.3 mmol). After 10 min the solution was diluted with water, acidified (HCl), extracted with ethyl acetate, and purified by rotary chromatography with $CHCl_3$/MeOH mixtures to give the diol **22** (4 mg, 39%): NMR (CD_3OD) as for **2**(EtB) except ^1H δ 3.99 (d, $J = 4$ Hz, H-10), 3.96 (m, H-9), 2.65 (d, $J = 13.9$

Hz) and 1.97 (d, J = 13.9 Hz) AB H_2-14; ^{13}C NMR (CD_3OD) δ 21.1 and 28.4 (C-7 and -8), 66.8 (C-9), 72.7 (C-10), 87.0 (C-11), 8.0 and 6.7 (br) ($EtBO_2$). Methanolysis of the boronate was carried out by slow distillation with methanol containing initially 5% $MeNH_2$ (40%) during 4h. Chromatography on silica (0.5 g) and elution with $CHCl_3$/MeOH (9:1) containing 1% NH_3 gave diol **24** (8 mg): ^{1}H NMR (CD_3OD) as for ketol **23** except δ 4.19 (d, J = 3.9 Hz, H-10), 3.92 (m, H-9), 2.15 (dt, J = 13, 13, and 4.2 Hz, H-7$_{ax}$), 1.27 (ddd, J = 13.5, 3.0, and 1.0 Hz, H-7$_{eq}$), 2.01 (m, H-8$_{ax}$), 1.78 (m, H-8$_{eq}$), 1.42 (s, H_3-17); ^{13}C NMR (CD_3OD) 88.8 and 85.9 (C-6 and −11), 25.4 and 28.8 (C-7 and -8), 67.1 (C-9), 73.0 (C-10), 97.2 (C-12); HRMS (FAB) m/z $C_{24}H_{33}NO_{10}Na^+$ 518.2002, found 518.1988.

21-Nor-9$_{eq}$-hydroxy-1 (25), 21-Nor-9-oxo-10-acetoxy-1 (26), and 21-Nor-10-deoxy-9-oxo-1 (27). The ketol **23** (58 mg, 0.12 mmol) was dissolved in acetic anhydride (0.5 mL, 0.53 mmol) and pyridine (0.05 mL). Monoacetylation was complete in 70 min at ambient temperature. Excess acetic anhydride was hydrolyzed by gradual addition of MeOH (1.5 mL) at 25 °C. The product isolated after acidification (HCl) and extraction with ethyl acetate was washed with saturated $NaHCO_3$ solution and chromatographed on silica (5.0 g). Elution with $CHCl_3$/MeOH (97:3) gave the crystalline acetate **26** (54 mg, 84%): NMR (CD_3OD) as for the ketol **23** except ^{1}H δ 5.42 (s, H-3), 5.85 (br s, H-10), 2.67 (d, J = 14.1 Hz) and 1.89 (d, J = 14.5 Hz) AB H_2-14, 2.70 (m, H-8$_{ax}$), 2.35 (m, H-8$_{eq}$), 1.49 (s, H_3-17), 0.90 (s, H_3-20); ^{13}C NMR (CD_3OD) δ 47.7 (C-5), 85.0 and 88.9 (C-6 and -11), 26.2 and 35.2 (C-7 and -8), 205.0 (C-9), 74.0 (C-10), 20.7 and 170.8 (acetate). The acetate **26** (16 mg, 0.030 mmol) in THF/MeOH (4:1, 5 mL) was treated gradually at −60 °C under argon with 0.1 M SmI_2 in THF (1 ml, 0.1 mmol). Reduction was fast, and the reaction was repeated twice. Workup with saturated K_2CO_3 (2 mL) and $NaHSO_3$ (2 mL) solutions, extraction with ethyl acetate, evaporation of the solvents, and separation from salts with ethyl acetate gave the crude ketone. Purification was effected by chromatography on silica (3.5 g). Elution with $CHCl_3$/MeOH (9:1) gave the ketone **27** (38 mg, 89%): NMR (CD_3OD) as for diol **24** except ^{1}H δ 2.98 (d, J = 15.3 Hz) and 2.23 (obsc) H-10, 2.47 (d, J = 14.1 Hz) and 1.90 (d, J = 14.5 Hz) AB H_2-14, 2.33 (m, H-7$_{ax}$), 1.89 (m, H-7$_{eq}$), 2.55 (m, H-8$_{ax}$), 2.35 (m, H-7$_{ax}$), 1.49 (s, H_3-17); ^{13}C NMR (CD_3OD) δ 29.7 (C-8), 212.6 (C-9), 43.0 (C-10), 87.6 (C-11), 93.9 (C-12). Reduction of the ketol (**23**, 48 mg, 0.097 mmol) as described above and TLC using first $CHCl_3$/MeOH (85:15) and then 8:2 gave the ketone **27** (10.5 mg, 23%), unreacted ketol (12 mg, 25%), and diol **25** (7 mg, 15%): NMR (CD_3OD) as for ketol **23** except ^{1}H δ 4.00 (d, J = 8.9 Hz, H$_{eq}$-10), 3.80 (m, H$_{eq}$-9), 2.09 (dt, J = 12.5, 12.5, and 4.5 Hz, H-7$_{ax}$), 0.89 (s, H_3-20); ^{13}C NMR (CD_3OD) 86.0 and 88.0 (C-6 and -11), 25.6 and 26.8 (C-7 and -8), 72.5 and 73.0 (C-9 and -10); HRMS (FAB) m/z $C_{24}H_{33}NO_{10}Na^+$ 518.2002, found 518.1988. Similarly acetylation of 21-nor-9-oxo-**1**(EtB) (**21**) and reduction as above for **26** gave 21-nor-10-deoxy-9-oxo-**1**(EtB) in 80% yield (reduced to 30% without the acetylation step).

21-Nor-10-deoxy-9$_{ax}$- and -9$_{eq}$-hydroxy-1 (28 and 29). Ketone **27** (28 mg, 0.058 mmol) in THF (2 mL) was treated with ∼1 M $LiAlH_4$ in ether (1.5 mL, ∼1.5 mmol). After 5 min the product was isolated by addition of water, acidification (HCl), and separation by TLC with $CHCl_3$/MeOH (85:15) to give the less polar **28** (R_f 0.43, 16 mg, 55%): NMR (CD_3OD) as for ketol **23** except ^{1}H δ 4.0 (br m, $W_{1/2h}$ = 9 Hz, H-9), 2.23 (m, H-13), 2.25 (dt, J = 13, 13, and 5.0 Hz, H-7$_{ax}$), 1.30 (m, H-7$_{eq}$), 1.95 (m, H-8$_{ax}$), 1.70 (m, H-8$_{eq}$), 1.32 (s, H_3-17); ^{13}C NMR (CD_3OD) 83.8 and 87.2 (C-6 and -11), 23.6 and 29.1 (C-7 and -8), 68.2 (C-9), 32.3 (C-10), 11.2 (C-17); HRMS (FAB) m/z $C_{24}H_{33}NO_9Na^+$ 502.2053, found 502.2054. The more polar fraction was the equatorial alcohol **29** (R_f 0.18, 7 mg, 15%): ^{1}H NMR as for ketol **23** except δ 4.0 (tt, J = 5.4 and 10.7 Hz, H-9), 2.11 (dt, H-7$_{ax}$), 1.33 (br d, J = 12 Hz, H-7$_{eq}$), 1.95−1.65 (m, H_2-10 and H_2-8), 0.89 (s, H_3-20); ^{13}C NMR (CD_3OD) 92.5 (C-4), 83.3 and 87.4 (C-6 and -11), 26.1 and 29.8 (C-7 and -8), 68.3 (C-9), 35.2 (C-10), 11.2 (C-17); HRMS (FAB) m/z $C_{24}H_{33}NO_9Na^+$ 502.2053, found 502.2064. Reduction of **27** as the ethylboronate with $NaBH_4$ in MeOH at −20 °C and then deboronation with MeOH/$MeNH_2$ gave a similar mixture of **28** and **29**.

10-Deoxy-2 (30). Ketone **27** (10 mg, 0.021 mmol) in THF (0.2 mL) and toluene (0.5 mL) was treated with Tebbe reagent (0.5 M in toluene) (0.2 mL, 0.10 mmol). After 15 min 0.25 M NaOH (1 mL) and ether were added. After 15 min the pH was adjusted to 10, and the ether extract was washed with saturated $NaHCO_3$, evaporated, and left for 12 h. The product was separated from inorganic material by extraction into ether and purified twice by TLC (2 × 0.5 mm plates) with $CHCl_3$/MeOH (9:1) solution to give **30** (6.4 mg, 63%): ^{1}H NMR (CD_3OD/$CDCl_3$) δ 6.99 (m, H-26), 6.87 (m, H-24), 6.21 (m, H-25), 5.46 (s, H-3), 4.71 (q, J = 1.9 Hz) and 4.64 (q, J = 1.9 Hz) (H_2-21), 2.71 (br d, J = 12.4 Hz, H-10$_{ax}$), 2.43 (d, J = 13.8 Hz) and 1.82 (d, J = 13.8 Hz) AB H_2-14, 2.40 (br t, H-8$_{ax}$), 2.20 (m, H-13), 2.13 (m, H-8$_{eq}$), 2.10 (br d, J = 12.5 Hz, H-10$_{eq}$), 2.05 (m, obsc, H-7$_{ax}$), 1.36 (br dd, J = 12.2 and 4.0 Hz, H-7$_{eq}$), 1.29 (s, H_3-17), 1.03 (d, J = 6.7 Hz) and 0.75 (d, J = 6.3 Hz) (H_3-18 and -19), 0.85 (s, H_3-20); ^{13}C NMR (CD_3OD) δ 64.9 (C-1), 83.0 (C-2), 91.3 (C-3), 91.4 (C-4), 48.3 (C-5), 86.0 (C-6), 27.5 and 29.6 (C-7 and -8), 149.5 (C-9), 35.2 (C-10), 83.8 (C-11), 94.4 (C-12), 29.8 (C-13), 40.8 (C-14), 101.5 (C-15), 10.5 (C-17), 18.3 and 18.8 (C-18 and -19), 12.3 (C-20), 110.7 and 110.6 (C-21 and -22), 122.0 (C-23), 116.8 (C-24), 110.6 (C-25), 125.1 (C-26); HRMS (FAB) m/z $C_{25}H_{33}NO_8Na^+$ 498.2104, found 498.2120. **30** was also obtained in 25% yield by using CH_2I_2/Zn/$TiCl_4$.[30] **30** was not available by treatment of **27** with the Wittig reagent in DMSO.[18]

21-Nor-10-deoxy-1 (31). Ketone **27** (3.8 mg, 0.0079 mmol) in ether (2 mL) was treated with 1,2-ethanedithiol (0.075 mL, 0.80 mmol) and BF_3·Et_2O (0.1 mL, 0.77 mmol). After 15 min water was added, and the product was extracted with ether and washed with saturated $NaHCO_3$. The ether extract was deborated by slow distillation (1 h) with MeOH/$MeNH_2$ as described above for **24** and stirred with Ni (∼100 mg) in EtOH (2 mL) for 2 h. Addition of water, acidification (HCl), extraction with ethyl acetate, separation from Ni, and purification by TLC with $CHCl_3$/MeOH (9:1) gave **31** (2.4 mg, 66%): ^{1}H NMR (CD_3OD/$CDCl_3$) δ 6.97 (m, H-26), 6.85 (m, H-24), 6.21 (m, H-25), 5.46 (s, H-3), 2.43 (d, J = 13.8 Hz) and 1.86 (d, J = 13.5 Hz) AB H_2-14, 2.20 (m, H-13), 1.28 (s, H_3-17), 1.02 (d, J = 6.6 Hz) and 0.75 (d, J = 6.3 Hz) (H_3-18 and -19), 0.82 (s, H_3-20); ^{13}C NMR (CD_3OD/$CDCl_3$) as for **30** except 25.1 and 26.7 (C-7 and -10), 19.3 and 20.6 (C-8 and -9), 83.6 (C-11), 94.7 (C-12); HRMS (FAB) m/z $C_{24}H_{33}NO_8Na^+$ 486.2104, found 486.2095. The sequence of these steps is important since the borate complexes with Ni. Longer exposure to Ni gives a poor yield of **31**.

21-Nor-10-deoxy-6,9-oxido-1 (32) and 21-Nor-10-deoxy-Δ^8/Δ^9-1 (33). The axial alcohol **28** (16 mg, 0.033 mmol) in pyridine (2.0 mL) was treated dropwise with phosphoryl chloride (0.2 mL, 2.1 mmol) at −20 °C. The solution was warmed to ambient temperature (15 min) and the crude product isolated by addition to water, acidification (HCl), extraction with ethyl acetate and TLC with $CHCl_3$/MeOH (19:1) to give the ether **32** (5.8 mg, 38%): ^{1}H NMR (CD_3OD) as for **30** except δ 4.50 (t, J = 4.5 Hz, H-9), 2.44 (ddd, J = 13.1, 4.0, and 2.0 Hz, H-10$_{exo}$), 1.28 (m, H-10$_{endo}$), 2.54 (d, J = 13.8 Hz) and 1.77 (d, J = 13.8 Hz) AB H_2-14, 2.25 (obsc, H-7$_{endo}$), 1.53 (ddd, J = 12, 8, and 4 Hz, H-7$_{exo}$), 1.62−1.75 (obsc, H_2-8), 1.24 (s, H_3-17); ^{13}C NMR (CD_3OD) δ 46.3 (C-5), 94.6 (C-6), 22.4 (C-7), 31.3 and 34.3 (C-8 and -10), 88.6 (C-11); HRMS (FAB) m/z $C_{24}H_{31}NO_8Na^+$ 484.1947, found 484.1956. A less mobile fraction (2.7 mg) appeared to be a mixture of 8- and 9-enes (4:1) (**33**). Major component: ^{1}H NMR (CD_3OD) δ 5.64 (s, H-3), 5.55 (br m) and 5.65 (br m) H-8 and −9, 2.65 (d, J = 13.8 Hz) and 2.01 (d, J = 13.5 Hz) AB H_2-14, 2.69 (br m) H_2-7 and H_2-10, 2.23 (m, H-13), 1.30 (s, H_3-17), 1.08 (d, J = 6.8 Hz) and 0.74 (d, J = 7.0 Hz) H_3-18 and −19, 0.94 (s, H_3-20). Minor component: ^{1}H NMR (CD_3OD) δ 6.0 (br m), 5.85 (br m), 5.63 (s), 1.32 (s), 0.96 (s); ^{13}C NMR (CD_3OD) 86.2 and 89.4 (C-6 and -11), 28.6 and 29.7 (C-7 and -10), 124.8 and 123.6 (C-8 and -9), 97.0 (C-12); HRMS (FAB) m/z $C_{24}H_{31}NO_8Na^+$ 484.1947, found 484.1950.

Biology. Previously described procedures were used to assay [^{3}H]-**1** binding in the *ryr* of rabbit skeletal muscle,[3] mouse brain,[9] and canine ventricle[14] membranes. The structure−activity findings for *ryr* recognition were compared with those for mediation of a pharmacological response measured as inhibition of mechanical response to electrical stimulation of cardiac muscle.[14]

Acknowledgment. We thank our University of California laboratory colleagues Gary Quistad, Joyce James and Motohiro Tomizawa for helpful discussions and Elisabeth Lehmberg and Loretta Cole for receptor assays with skeletal muscle and brain. The research was greatly facilitated by the advice of Todd Blumenkopf of the Division of Chemistry of Burroughs Wellcome Co. (currently at Pfizer Central Research, Groton CT). This work was supported in part by National Institute of Environmental Health Sciences Grant PO1 ES00049 and by a grant from Burroughs Wellcome Co., Research Triangle Park, NC.

Supporting Information Available: Extended Tables 1 and 2 with potency values for weakly active compounds (2 pages). Ordering information is given on any current masthead page.

References

(1) Wiesner, K. The Structure of Ryanodine. *Adv. Org. Chem.* **1972**, 295−316.
(2) 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.
(3) Jefferies, P. R.; Toia, R. F.; Brannigan, B.; Pessah, I. N.; Casida, J. E. Ryania Insecticide: Analysis and Biological Activity of 10 Natural Ryanoids. *J. Agric. Food Chem.* **1992**, *40*, 142−146.
(4) Welch, W.; Ahmad, S.; Airey, J. A.; Gerzon, K.; Humerickhouse, R. A.; Besch, H. R., Jr.; Ruest, L.; Deslongchamps, P.; Sutko, J. L. Structural Determinants of High-Affinity Binding of Ryanoids to the Vertebrate Skeletal Muscle Ryanodine Receptor: A Comparative Molecular Field Analysis. *Biochemistry* **1994**, *33*, 6074−6085.
(5) Humerickhouse, R. A.; Besch, H. R., Jr.; Gerzon, K.; Ruest, L.; Sutko, J. L.; Emmick, J. T. Differential Activating and Deactivating Effects of Natural Ryanodine Congeners on the Calcium Release Channel of Sarcoplasmic Reticulum: Evidence for Separation of Effects at Functionally Distinct Sites. *Mol. Pharmacol.* **1993**, *44*, 412−421.
(6) 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.
(7) Coronado, R.; Morrissette, J.; Sukhareva, M.; Vaughan, D. M. Structure and Function of Ryanodine Receptors. *Am. J. Physiol.* **1994**, *266*, C1485−C1504.
(8) 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.
(9) Jefferies, P. R.; Lehmberg, E.; Lam, W.-W.; Casida, J. E. Bioactive Ryanoids from Nucleophilic Additions to 4,12-Seco-4,12-dioxoryanodine. *J. Med. Chem.* **1993**, *36*, 1128−1135.
(10) Kahl, S. D.; McPherson, P. S.; Lewis, T.; Bentley, P.; Mullinnix, M. J.; Windass, J. D.; Campbell, K. P. Radioimmunoassay for the Calcium Release Channel Agonist Ryanodine. *Anal. Biochem.* **1994**, *218*, 55−62.
(11) Jefferies, P. R.; Casida, J. E. Ryanoid chemistry and action. In *Natural and Engineered Pest Management Agents*; Hedin, P. A., Menn, J. J., Hollingworth, R. M., Eds.; American Chemical Society Symposium Series No. 551, American Chemical Society: Washington, DC, pp 130−144.
(12) Gerzon, K.; Humerickhouse, R. A.; Besch, H. R., Jr.; Bidasee, K. R.; Emmick, J. T.; Roeske, R. W.; Tian, Z.; Ruest, L.; Sutko, J. L. Amino- and Guanidinoacylryanodines: Basic Ryanodine Esters with Enhanced Affinity for the Sarcoplasmic Reticulum Ca^{2+}-Release Channel. *J. Med. Chem.* **1993**, *36*, 1319−1323.
(13) Hutchins, R. O.; Natale, N. R. Cyanoborohydride. Utility and Applications in Organic Synthesis. A Review. *Org. Prep. Proc. Int.* **1979**, *11*, 201−244.
(14) Jefferies, P. R.; Blumenkopf, T. A.; Gengo, P. J.; Cole, L. C.; Casida, J. E. Ryanodine Action at Calcium Release Channels. I. Importance of Hydroxyl Substituents. *J. Med. Chem.* **1996**, *39*, 2331−2338.
(15) Molander, G. A.; Hahn, G. Lanthanides in Organic Synthesis. 2. Reduction of α Hetero Substituted Ketones. *J. Org. Chem.* **1980**, *51*, 1135−1138.
(16) Kelly, R. B.; Whittingham, D. J.; Wiesner, K. The Structure of Ryanodine. I. *Can. J. Chem.* **1951**, *29*, 905−910.
(17) Buist, G. J.; Bunton, C. A. The Mechanism of Oxidation of α-Glycols by Periodic Acid. Part I. Ethylene Glycol. *J. Chem. Soc.* **1954**, 1406−1413.
(18) Kusuda, K.; Inanaga, J.; Yamaguchi, M. A Highly Efficient Deoxygenation of α-Oxygenated Esters Via SmI_2-Induced Electron Transfer Process. *Tetrahedron Lett.* **1989**, *30*, 2945−2948.
(19) Pine, S. H.; Shen, G. S.; Huang, H. Ketone Methylenation Using the Tebbe and Witting Reagents−A Comparison. *Synthesis* **1991**, 165−167.
(20) Hutchins, R. O.; Natale, N. R. Sodium Borohydride in Acetic Acid. A Convenient System for the Reductive Deoxygenation of Carbonyl Tosylhydrazones. *J. Org. Chem.* **1978**, *43*, 2299−2301.
(21) Fieser, L. F. Preparation of Ethylenethioketals. *J. Am. Chem. Soc.* **1954**, *76*, 1945−1947.
(22) Witcher, D. R.; McPherson, P. S.; Kahl, S. D.; Lewis, T.; Bentley, P.; Mullinnix, M. J.; Windass, J. D.; Campbell, K. P. Photoaffinity Labeling of the Ryanodine Receptor/Ca^{2+} Release Channel with an Azido Derivative of Ryanodine. *J. Biol. Chem.* **1994**, *269*, 13076−13079.
(23) Callaway, C.; Seryshev, A.; Wang, J.-P.; Slavik, K. J.; Needleman, D. H.; Cantu, C. III; Wu, Y.; Jayaraman, T.; Marks, A. B.; Hamilton, S. L. Localization of the High and Low Affinity [^{3}H]-Ryanodine Binding Sites on the Skeletal Muscle Ca^{2+} Release Channel. *J. Biol. Chem.* **1994**, *269*, 15876−15884.
(24) Sitsapesan, R.; Williams, A. J. Mechanisms of Caffeine Activation of Single Calcium-Release Channels of Sheep Cardiac Sarcoplasmic Reticulum. *J. Physiol.* **1990**, *423*, 425−439.
(25) Watson, M. J.; Gengo, P. J. Effects of Ryanodine on Calcium Homeostasis in Single Adult Rat Cardiomyocytes. *Biophys. J.* **1995**, *68*, A180.
(26) 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.* **1985**, 1011−1016.
(27) Krishnamurthy, V. V.; Casida, J. E. COLOC-S: a Modified COLOC Sequence for Selective Long-Range X-H Correlation 2D NMR Spectroscopy. *Magn. Reson. Chem.* **1987**, *25*, 837−842.
(28) ^{1}H and ^{13}C signals that differ <0.03 and <1 ppm, respectively, from the analogous signals of the reference compound are not given.
(29) Ruest, L.; Taylor, D. R.; Deslongchamps, P. Investigation of the constituents of *Ryania speciosa*. *Can. J. Chem.* **1985**, *63*, 2840−2843.
(30) Hibino, J.; Okazoe, T.; Takai, K.; Nozaki, H. Carbonyl Methylenation of Easily Enolizable Ketones. *Tetrahedron Lett.* **1985**, *26*, 5579−5580.

JM950712D