Ryanodine Action at Calcium Release Channels. 1. Importance of Hydroxyl Substituents

Phillip R. Jefferies,*,† Todd A. Blumenkopf,‡ Peter J. Gengo,§ Loretta C. Cole,† and John E. Casida*,†

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy, and Management, University of California, Berkeley, California 94720-3112, and Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709-2700

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Ryanodine (**1**) and dehydroryanodine (**2**) have a polar face formed by *cis*-hydroxyls at C-2, C-4, C-6, and C-12. The importance of the hydroxyls to the action of **1** and **2** at the ryanodine receptor (*ryr*) of calcium release channels is examined at [^{3}H]-**1** binding sites in brain and skeletal muscle and in heart membranes relative to cardiac contractility, a pharmacologic response which appears to be mediated by the *ryr*. Five types of changes are considered: blocking the 4- and 6-hydroxyls as cyclic borates and boronates; blocking the 10- and 12-hydroxyls as cyclic phosphates, phosphonates, and phosphoramidates; methylation at nitrogen or hydroxyls at C-4 and C-10; dehydration of the C-2 hydroxyl; additional data for a 4,12-oxygen-bridged series. The first change has little effect on potency possibly due to the lability of the boron protective groups whereas the cyclic phosphorus compounds have reduced activity. Methylation reduces potency the least at nitrogen and the C-4 hydroxyl. Dehydration of **1** to 2-deoxy-2(13)-dehydro-**1** allows the restoration of oxygen at C-2 by conversion to epoxides or a diol. One of the epoxides and 2-deoxy-2(13)-dehydro-**2** retain 8−31% of ryanodine's potency in the *ryr* assays and 81% in the cardiac contractility system. In the 4,12-oxygen-bridged series, high potency at the receptor and cardiac muscle is retained in the 4-hydroxy ketal.

Introduction

Ryanodine (**1**) is a potent regulator of the calcium-release channel of mammalian muscle which, depending on concentration, either opens the channel (nanomolar level), locks it in the open state, or closes the channel.[1,2] The chemistry and action of ryanodine and the equipotent dehydroryanodine (**2**) at calcium release channels are greatly influenced by the *cis*-hydroxyls at C-2, C-4, C-6, and C-12 which, along with the pyrrolecarboxylate NH and C═O, present a hydrophilic face (Figure 1) important in polarity influences on distribution and in fit at the calcium release channel (**1** binding site).[3,4] Additional hydroxyl substituents are at C-10 and the hemiketal at C-15. One approach that has been used to determine the importance of the hydroxyl substituents in conferring biological activity is to cap them by methylation[5] and another is to replace the C-4, C-12 bond with an oxygen bridge allowing the substituents at C-4 and C-12 to be modified (e.g. 4- and 12-hydroxy ketals) with a wide range of polarities.[6]

This report considers four new types of modifications in the hydroxyl substituents and their influence on activity at calcium release channels: blocking the 4- and 6-hydroxyls as cyclic borates and boronates (**3**−**7**); blocking the 10- and 12-hydroxyls as cyclic phosphates, phosphonates, and phosphoramidates (**8**−**17**); further studies on methylation at nitrogen and the C-4 and C-10 hydroxyls and combinations thereof (**18**−**22**); dehydration of the C-2 hydroxyl and related chemistry (**23**−**28**).

Cyclic boron and phosphorus investigations started with our borohydride reduction of 10-oxo-**1** to give 10-*epi*-**1** as its borate.[3] Earlier esterifications of ryanoids at C-10 relied on the use of dicyclohexylcarbodiimide, a method which gives high yields with unhindered acids[5,7] but not aromatic pyrrolecarboxylic acid.[8] We find that the ethylboronate (EtB) group[9] allows the use of more reactive acylating agents with good selectivity in esterification. Continuing studies from our laboratory describe below a range of cyclic borates and boronates, their structural assignments, and some applications as protecting groups. A series of 10,12-bridged phosphates, phosphonates, and phosphoramidates has also been prepared from **1** and **2** and their ethylboronates [**1**(EtB) and **2**(EtB)].

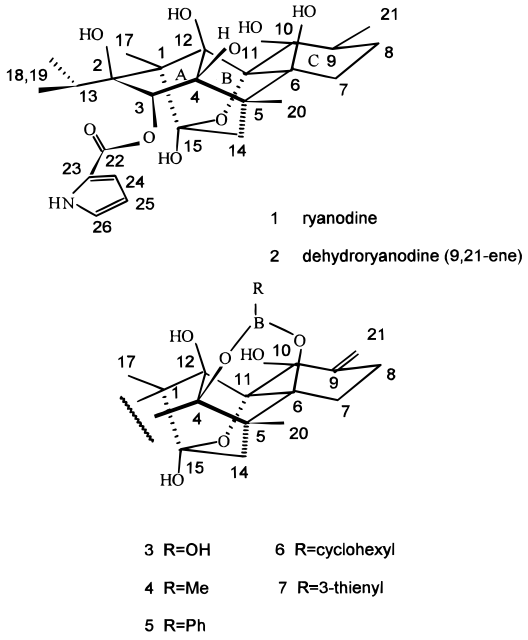

Figure 1. Structure of ryanodine (**1**) and dehydroryanodine (**2**) and partial structure of the 4,6-cyclic borate (**3**) and boronates **4**−**7** of dehydroryanodine.

† University of California.
‡ Present address: Pfizer Central Research, Groton, CT 06340.
§ Division of Pharmacology, Burroughs Wellcome Co.
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Table 1. NMR Shifts for Environmentally Sensitive Nuclei of Selected 4,6-Cyclic Boron and 10,12-Cyclic Phosphorus Derivatives of Dehydroryanodine (**2**−**4**, **8**, **14**, and **15**) and Ryanodine (**16** and **17**)[a]

position	**2**	**3**	**4**	**8**[b]	**14**	**15**	**16**	**17**
cyclic B	none	HOB	MeB	none	EtB	EtB	none	none
cyclic P	none	none	none	MeNHP	$Ph_{eq}P$	$Ph_{ax}P$	$Me_{eq}P$	$Me_{ax}P$
				^{13}C Spectral Data				
C-1	65.8	65.0	64.9	67.9	67.1	67	68.7	67.0
C-2	84.2	84.9	84.9	83.6	83.6	84.4	83.4	83.6
C-3	90.9	87.9[c]	88.0	88.8	86.5	86.9	89.6	89.5
C-4	92.4	92.2	91.6	93.8[d]	93.0[d]	93.2[d]	93.9[d]	93.7[d]
C-5	49.4	47.0[c]	46.4	51.5	47.0	48.3	50.9	51.4
C-6	86.4	88.0[c]	87.7	85.0	86.1	86.6	85.5	85.4
C-9	148.7	148.1	148.3	143.1[d]	141.7[d]	141.3[d]	32.7[d]	33.0[d]
C-10	69.7	69.1	69.2	73.4	70.8	74.2	75.1	78.7
C-11	88.3	88.1	87.7	84.5[d]	85.1[d]	84.2[d]	84.4[d]	84.3[d]
C-12	96.5	97.0[c]	97.4	102.6[d]	97.1[d]	104.5[d]	102.0	obsc
				^{1}H Spectral Data				
H-3	5.65	5.80	5.85	5.66	5.90	5.95	5.67	5.66
H-10	4.74	4.54	4.53	5.40[e]	5.83[e]	4.82[e]	4.97[f]	4.42[f]
H-17	1.39	1.37	1.36	1.49	0.95	1.63	1.41	1.49
H-20	0.91	0.86	0.84	0.99	0.94	0.87	0.87	0.98

[a] Spectral data for CD_3OD solution. [b] Data for **9**−**11** do not differ significantly from those tabulated for **8**. [c] The only significant changes (>1ppm) on adding base are for C-3, C-5, C-6, and C-12 with shifts of 84.7, 46.0, 90.2, and 95.1 ppm, respectively. [d] Doublets due to ^{31}P couplings 4−7 Hz for C-4, 6−9 Hz for C-9, 4−7 Hz for C-11, and 7−9 Hz for C-12. [e] $J = \sim 1$, 4.5 Hz. [f] $J = 5$, 10.5 Hz.

Methylation of **1** with methyl iodide and potassium *tert*-butoxide in tetrahydrofuran (THF) gives the 15-methoxy-*N*-methyl (N,15-Me_2), 4,15-dimethoxy-*N*-methyl (N,4,15-Me_3), and 6,15-dimethoxy-*N*-methyl (N,6,15-Me_3) derivatives whereas in dimethyl sulfoxide (DMSO) with sodium hydride it yields a compound formulated as the 4,10,15-trimethoxy-*N*-methyl derivative (N,4,10,-15-Me_4-**1**); these structures were assigned largely from the shifts near the alkylated positions in the carbon NMR spectra.[5] Although none of these compounds retained the high affinity of **1** for its receptor and there was a general reduction in binding with increasing methylation, the results did not allow the separation of the effect of methylation at the different centers. We recently observed that the 4-methoxy derivative of **2** retains up to 29% of the binding activity of **1** at the calcium release channel.[3] With the goal of obtaining a large number of mono- and dimethylation sites, we now report that slower and more selective methylation using the classical methods gives N-Me, 4-Me, and N,4-Me_2 derivatives, and reaction of the borate of **2** with diazomethane gives 4-Me-**2** and 4,10-Me_2-**2**.

Dehydration of **1** at the C-2 hydroxyl was reported by Wiesner in his classical study[10] involving thionyl chloride/pyridine to give a "chlorocompound" which was converted by vigorous base hydrolysis to "isoryanodol" (reassigned as a dehydroryanodol).[11] On further examination of this reaction, we have been able to effect elimination while retaining the pyrrole ester, allowing restoration of oxygen at C-2 by conversion either to the epoxides with *m*-chloroperoxybenzoic acid (MCPBA) or to a diol with osmium tetroxide.

Modification of Hydroxyl Substituents

4,6-Cyclic Boron Derivatives (Figure 1). Borate **3** is obtained on treatment of **2** with borohydride, boron trifluoride etherate, or alkaline borate solution. The formation of borates with polyols in basic solution has wide applications as a stereochemical tool.[12] It is rare for the complexes to survive acidification, and thus borate **3** is unusual in that it can be recovered from aqueous acidic solutions by solvent extraction. In an analogous manner, methylboronate **4** is prepared with **2** and lithium dimethyl borohydride or methylboronic acid, cyclohexylboronate **5** with cyclohexylborane, and phenylboronate **6** and 3-thienylboronate **7** with the corresponding boronic acids. Boronates are considered to be in equilibrium with their components in aqueous solution, and so these groups are thought ineffective for diol protection in such media. In practice **1**(EtB) is oxidized slowly in aqueous periodic acid under conditions which rapidly rupture the 4,12-diol group in **1**.[6] Both borates and boronates undergo methanolysis by slow distillation with methanol or more rapidly in the presence of methylamine.

Structural assignments of the borate and boronates as the 4,6-cyclic derivatives, **3** and for example, **4**, respectively, are based on NMR signals for environmentally sensitive nuclei compared with the parent ryanoid, showing significant upfield shifts for C-3, C-5, and H-10 and deshielding for C-6 and H-3 (Table 1). The differences are increased in the tetrahedral borate formed on addition of base relative to the shifts for **3**. In addition, the nuclear Overhauser effects (NOE's) observed for **4** show comparable interactions for the boronate methyl and both protons of C-20 and that of C-10 (Figure 1).

Several reactions confirm the location of the boron bridge between C-4 and C-6. In general six-membered cyclic borates are more stable than five,[13] but the 6,12 combination is precluded by strain. Other evidence for the 4,6 boron link is the failure of 4-Me-**2** to form a stable boronate and, conclusively, **2**(EtB) forms cyclic phosphonates which are shown below to link the 10- and 12-positions.

10,12-Cyclic Phosphorus Derivatives (Figure 2). Compound **2** was treated briefly with phosphoryl chloride in pyridine which gave the chloridate. To confirm this assignment the chloridate was treated with a range of bases and a series of amides [methyl (**8**), 2-methoxyethyl (**9**), 2-aminoethyl (**10**), and 4-aminobutyl (**11**)] was obtained. The chloridate from **2** was also converted to the phosphoric acid **12** in aqueous solution and the methyl phosphate **13** by reaction with methanol. Treatment of **2**(EtB) with phenylphosphonodichloridate in pyridine gave a mixture of isomeric phenylphosphonates (**14** and **15**) which were separated on silica. Similarly

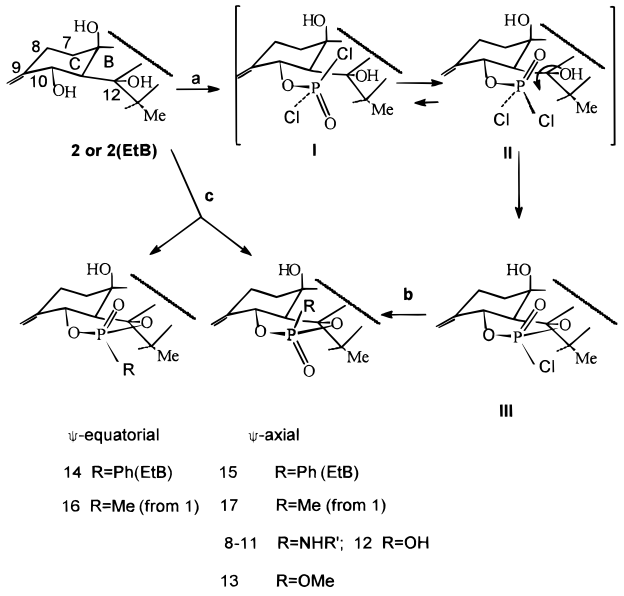

Figure 2. Formation of 10,12-cyclic phosphorus derivatives showing B and C rings viewed from the opposite side of the molecule of that shown in Figure 1. Conformations I and II and intermediate III illustrate a possible mechanism leading to the phosphorus derivatives **8**−**13**. R′ = $(CH_2)_2OMe$ (**9**), $(CH_2)_2NH_2$ (**10**), and $(CH_2)_4NH_2$ (**11**). Methylphosphonates **16** and **17** are derived from **1** and the other compounds from **2**. Conditions: (a) $POCl_3$, C_5H_5N; (b) MeOH or R′NH_2 in H_2O or C_5H_5N; (c) $PhP(O)Cl_2$ or $MeP(O)Cl_2$, C_5H_5N.

the isomeric methylphosphonates (**16** and **17**) were prepared from **1**(EtB) and separated after methanolysis of the boronate group.

Evidence for phosphorylation of the 10- and 12-hydroxyls is given by the NMR data (Table 1). The amides **8**−**11** have closely comparable NMR shifts for the ryanoid nucleus and are obtained as essentially one isomer. There is a downfield shift in **8** versus **2** of 3.7 ppm for C-10 and 0.66 ppm for H-10 and a coupling constant for H-10 to phosphorus of 4.5 Hz consistent with a twist boat conformation;[14] in addition there is a downfield shift of 6.1 ppm for C-12. Phosphorus to carbon couplings over two and three bonds are observed for C-4, C-9, C-11, and C-12. Configurations of phenylphosphonates **14** and **15** are assigned from the greater shielding of the 17-methyl group by the phenyl group in **14**; consistently isomer **15** shows greater shielding for H-10. Configurations of methylphosphonates **16** and **17** are assigned from the greater deshielding of H-10 by the P═O for isomer **16**.

The configuration of **8** is assigned with a ψ-axial amide group on the basis of an NOE between the amide methyl group and H-10. Since substitution of chloride on phosphorus is known to follow an SN_2 mechanism, the chloride is ψ-equatorial (**III**). The chloridate arises by initial phosphorylation of the relatively unhindered 10-hydroxyl. Cyclization to give the ψ-equatorial chloride requires a transition state from conformation **II** which shows a steric interaction between the 17-methyl and chloride, whereas the alternative isomer would arise from transition state **I** which is less favorable in that the P−Cl is replaced by the larger P═O. The stereochemistry of the isomeric phosphonates is determined by the initial phosphorylation of the 10-hydroxyl which shows little selectivity.

Methylated Derivatives. Treatment of **2** in acetone/K_2CO_3 with methyl iodide at ambient temperature slowly gives a mixture containing mainly the *N*-Me derivative **18** with a small amount of the 4-Me compound **20**. These are then replaced by N,4-Me_2-**2** (**21**) followed by more complex mixtures. Exclusive monomethylation at nitrogen is obtained in the same way using **2**(EtB). The 4-Me derivative **20** is obtained by reaction of **3** with diazomethane[3] or, on a larger scale, there is also a small quantity of the 4,10-Me_2 derivative (**22**) and trace levels of the *N*-Me compound (**18**) and probably the 4,15-Me_2 derivative (not obtained pure). Treatment of borate **3** with diazomethane gives 4-Me-**2** by an insertion reaction which appears to be unique. Structural assignments for the new methylated derivatives are based on ^1H and ^{13}C NMR shifts which have been established for N- and 4-O-methylation.[5] In the 4,10-Me_2 derivative **22** the methoxyl group promotes a strong downfield shift of the attached C-10 and shielding for the adjacent C-9. As expected the H-10 signal is shielded by the 10-Me group.

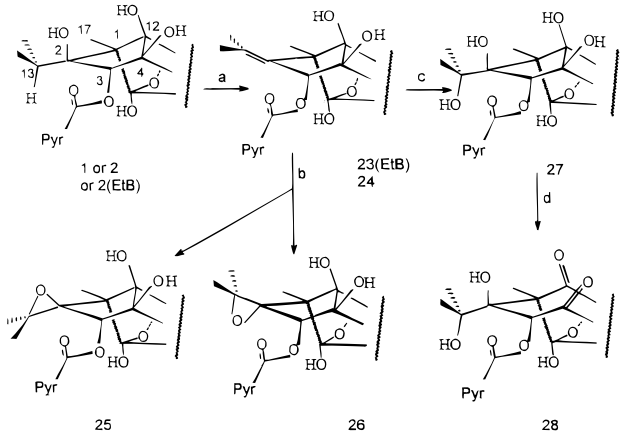

Figure 3. Products from dehydration of the 2-hydroxyl of the A ring. Conditions: (a) (1) $SOCl_2$, C_5H_5N, (2) 5% aqueous NaOH; (b) MCPBA; (c) OsO_4, C_5H_5N; (d) $NaIO_4$ (Pyr = 2-pyrrole).

Products from Dehydration of the 2-Hydroxyl of the A Ring (Figure 3). Treatment of **1** or **2** or **2**(EtB) with thionyl chloride/pyridine at −10 °C[10] leads to dehydration at the 2-position. The initial product may be the 10,12-cyclic sulfite which hydrolyzes rapidly in cold 5% NaOH solution to give 2-deoxy-2(13)-dehydro-**2**(EtB) (**23**) retaining the pyrrole ester. Longer base treatment results in accumulation of pyrrole-2-carboxylic acid and Wiesner's dehydroryanodol.[10,11] The dehydration reaction is equally successful with unprotected **1** or **2**. Epoxidation of **24** derived from **1** gives major (**25**) and minor (**26**) epoxides. Reaction of **24** (from **1**) with osmium tetroxide gives 13-hydroxy-**1** (**27**) which resists oxidation of the 2,13-diol group even when a large excess of periodic acid is used. The 4,12-dioxo compound **28** was not further oxidized by the reagent; evidently the diol is locked in a *trans* conformation unfavorable for oxidation. Attempted dehydration of the 13-hydroxyl of the diol (**27**) with thionyl chloride/pyridine results in rearrangement and leads to a product which shows signals in the proton NMR expected for 13-hydroxyanhydroryanodine.

Structural assignments of the dehydration products are based on ^1H and ^{13}C NMR. In the 2,13-enes (**23** and **24**), reflecting its allylic nature, H-3 moves downfield (0.6 and 1.0 ppm, respectively) and appears as a multiplet through coupling to the side chain methyls.

Table 2. Potency at Calcium Release Channels of Selected 4,6-Cyclic Boron, 10,12-Cyclic Phosphorus, and Methylated Derivatives of Ryanodine or Dehydroryanodine

		activity relative to ryanodine as 100[a]			
		ryanodine receptor			rat
no.	name or substituent	rabbit muscle	mouse brain	canine ventricle	ventricular strip assay
1	ryanodine	100	100	100	100
	4,6-Cyclic Boron Derivatives				
3	borate[b]	100	75	43	28
4	methylboronate[b]	22		48	100
5	cyclohexylboronate[b]	71			
6	phenylboronate[b]	47			
7	3-thienylboronate[b]	90		69	52
	10,12-Cyclic Phosphorus Derivatives[c]				
8	P(O)NHMe[b]	4.5[d]	5.0[d]	5.0	<3.5
16	$P(O)Me_{eq}$	4.2	3.0		
17	$P(O)Me_{ax}$	3.6	3.9	1.9	<3.5
	Methylated Derivatives[c]				
18	N-Me[b]	12	13	19	100
20	4-Me[b]	29	21	6.6	<3.5
22	4,10-Me_2[b]	3.5	1.8		

[a] Values for **1** are as follows: IC_{50}s of 3.5 and 3.3 nM for rabbit muscle and mouse brain preparations; K_i of 1.8 nM for canine ventricle receptor; and IC_{50} 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] Derived from **2**. Others are derived from **1**. [c] Data for **9**−**15**, **19**, and **21** are given in the Supporting Information. [d] The $P(O)NH_2$ analog (not detailed here) is of similar potency.

Epoxides **25** and **26** show H-3 singlets at 5.7 and 5.4; this difference is probably due to the anisotropy of the ester carbonyl since in **25** the position of the isopropyl side chain limits the orientation of the pyrrole ester to a plane about parallel that of the epoxide ring whereas in **26** the epoxy oxygen and pyrrole ring are less encumbered which allows some level of rotation toward the 4-hydroxyl as in **1**.[15]

Structure−Activity Relationships

Assay Systems. The test compounds are compared for potency in competing with [^{3}H]-**1** for binding in rabbit skeletal muscle, mouse brain, and canine left ventricular membranes. They are also examined for inhibition of the contraction on electrical stimulation of cardiac muscle using rat right ventricular muscle strips, a pharmacologic response which appears to be mediated by the cardiac *ryr*.[9]

The potency values in Tables 2−4 are generally for compounds with ≥3% of the activity of **1** in the same experiment whereas data for the remaining compounds are given in the Supporting Information. The IC_{50} or affinity (K_i) is normalized relative to **1** as 100, as in our earlier report.[3] On this basis a compound of IC_{50} or K_i value 20-fold greater than that of **1** has a relative activity of 5.

4,6-Cyclic Boron and 10,12-Cyclic Phosphorus Derivatives (Table 2, Figures 1 and 2). Despite large differences in the size and polarity of the boron substituents, derivatives **3**−**7** are similar to the parent **1** or **2** in potency at the receptor, suggesting that they may undergo dissociation under the assay conditions, i.e. incubation at pH 7.4 for 1 to 2 h and 37 °C. The intrinsic activity of **3** and **7** is less than that of **1** and **4** in eliciting a negative inotropic effect in cardiac muscle strips.

Table 3. Potency at Calcium Release Channels of Selected Products from Dehydration of the 2-Hydroxyl of the A Ring of Ryanodine and Dehydroryanodine

		activity relative to ryanodine as 100[a]			
		ryanodine receptor			rat
no.	name or substituent	rabbit muscle	mouse brain	canine ventricle	ventricular strip assay
1	ryanodine	100	100	100	100
23	2-deoxy-2(13)-dehydro-**2**(EtB)	4.8	5.8	2	26
24	2-deoxy-2(13)-dehydro-**2**	13	15	31	81
25	epoxide A of 2-deoxy-2(13)-dehydro-**1**	7.1	1.4	7.9	24
26	epoxide B of 2-deoxy-2(13)-dehydro-**1**	1.4		0.8	3.4
27	13-hydroxy-**1**	2.0	2.5	4.0	26

[a] Standard values for **1** are given in Table 2. Data for **28** are given in the Supporting Information.

Table 4. Potency at Calcium Release Channels of 4,12-Oxygen-Bridged Derivatives of Ryanodine or Dehydroryanodine

		activity relative to ryanodine as 100[a]			
		ryanodine receptor			rat
substituents		rabbit	mouse	canine	ventricular
4	12	muscle	brain	ventricle	strip assay
OH	H	64	54	17	64
H	OH	19	21	9.6	69
$PhCH_2ONH$	OH	21	8.3	16	<3.5
NHOH	H	67	43	6.9	4.9
NHOMe	H	34	28	7.3	<1.1

[a] Standard values for **1** are given in Table 3.

The 10,12-cyclic phosphorus derivatives **8**−**17** are the first analogs evaluated with a C-12 substituent other than in the ketals where only the H or OH group is easily accommodated.[6] In general the cyclic phosphorus substituent is not favorable possibly due more to blocking of the 12- than the 10-hydroxyl. On the other hand attachment of β-aminopropionate to C-10 is highly favorable,[7] and it may be that this effect would be found in the ψ-equatorial rather than the ψ-axial isomer of the 4-aminoethyl- or -butylamide (**10** or **11**). The axial isomer available from this work does, however, interfere with the polar face of **2**. Both isomers are available from phosphorylation with phenyl- and methylphosphonodichloridates. Phenyl derivatives **14** and **15** have negligible activity whereas methylphosphonates **16** and **17** have reduced activity with little preference between the isomers. In general, the data reported here from three different tissues agree in that formation of 10,12-cyclic phosphorus derivatives practically eliminates the potency of **1** and **2** at the level of recognition and function.

Methylated Derivatives (Table 2). In the monomethyl series the cardiac strip system is quite sensitive to the *N*-Me compound **18** whereas the receptor assays show relatively weaker binding. Methylation at the 4-OH shows the opposite trend favoring binding to the skeletal muscle and brain. These effects seem to be additive for two or three methyl groups which are remote from each other as in the N,15-Me_2[5] and 4,10-Me_2 derivatives which retain 2−4% of the activity

relative to the parent. On the other hand the N,4-Me_2 compound with proximate methyls is lower in potency (0.2−1.0%), and this is also reflected in the trimethyl series.[5]

Products from Dehydration of the 2-Hydroxyl of the A Ring (Table 3, Figure 3). On the basis of molecular modeling it has been suggested that effective binding for ryanoids in skeletal muscle *ryr* requires the fixed arrangement of the substituents of **1** at C-2 in relation to the pyrrole ester function.[4] The data available for this region are limited to the effect of radical change of the ester[5] and its removal or replacement with pyridine-3-carboxylate.[16,17] Alteration of the substituents at C-2 is represented by the 1-hydroxy-2-propyl substituent in the natural 18-hydroxy-**1**,[5] and dehydration to anhydroryanodine[11] with the ketal bridge replaced with a lactone, which is also thought to strongly reduce binding. However, 2-deoxy-2(13)-dehydro-**2** (**24**) (in contrast to its ethylboronate **23**) retains much of the activity of **1** in the ventricle contractility assay (although less so in the binding assays) so no special role can be assigned to the 2-hydroxyl group and the isopropyl has greater freedom of movement than previously thought. In epoxide **25**, oxygen is restored to C-2, the methyls shift down, and the position of C-13 reverts to near that in **1** but binding is only slightly reduced from that for the parent alkene (**24**). On the other hand the arrangement of these groups in the isomeric epoxide **26** is unfavorable. 13-Hydroxy-**1** (**27**) is compelled to adopt the conformation with the sterically less demanding 13-hydroxyl on the underface in this structure which has resemblance to epoxide **26** and also binds very weakly. 4,12-Seco compound **28**, like other 4,12-dioxo compounds,[6] has low activity.

4,12-Oxygen-Bridged Series (Table 4). This table summarizes the potencies in the rabbit skeletal muscle and mouse brain systems for a range of ryanoids modified by replacing the C-4, C-12 bond with an oxygen bridge from our earlier study[6] and adds here values in cardiac muscle. Similar results are obtained with skeletal muscle and brain for the various 4,12-oxygen-bridged derivatives which are generally more active than with the cardiac receptor. The two hydroxy ketals are particularly active in the ventricular strip assay as in the case of N-Me-**2** discussed above.

Experimental Section

Chemistry. General. Starting compounds **1** and **2** of >95% purity were isolated as crystalline materials as previously described.[6] Solutions of boranes and complex hydrides were supplied by Aldrich. Reactions were carried out on a semimicroscale with product isolation, as crystalline solids or resins, by TLC (0.25- or −0.5 mm silica gel F_{254} from E. Merck, Darmstadt, Germany) and/or rotary chromatography (1-mm silica gel GF_{254}) using a Chromatron Model 8924 (Harrison Research, Palo Alto, CA). Determination of product purity and structure involved TLC, ^{1}H NMR, ^{13}C NMR, and HRMS. 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. Purities were estimated to be >98%. Solvents used were HPLC grade. Mass spectra were recorded with a Kratos MS-50 instrument.

^{1}H, ^{31}P, and ^{13}C NMR spectra were acquired at 300, 121.5, and 75 MHz, respectively, using a Bruker AM-300 spectrometer and CD_3OD or CD_3OD−$CDCl_3$ as the solvent. The chemical shift reference was tetramethylsilane for ^{1}H and ^{13}C and external trimethyl phosphate in CD_3OD-$CDCl_3$ for all ^{31}P spectra. The ^{13}C spectra, acquired using a 60° pulse (4.1 μs) and 1.5 s interpulse delay, had a digital resolution of 2.3 Hz. 2D spectra included in these studies were ^{1}H−^{1}H correlation spectroscopy (COSY) and homonuclear nuclear Overhauser effect (NOESY) spectra. Both COSY and NOESY spectra were acquired with spectral widths varied from 2100 to 2400 Hz by using a 1024 × 256 data set, zero filling by a factor of 2, and multiplication by an unshifted sine-bell function prior to Fourier transformation. NMR data which differ little from the fully assigned spectra[18,19] are given in abbreviated form.[20]

4,6-Cyclic Boron Derivatives (3−7). Cyclic Borate 3. A solution of **2** (45 mg, 0.092 mmol) in MeOH (1 mL) and $B(OH)_3$ (100 mg, 1.6 mmol) in water (3 mL) was basified to pH 12 with 1 M NaOH. After 10 min the solution was acidified and extracted with ethyl acetate. TLC with $CHCl_3$−MeOH−acetic acid, 89:10:1, gave **3** (35 mg, 74%) (R_f 0.54 vs 0.39 for **2**; **3** is retained at the origin on replacing acetic acid with $MeNH_2$): ^{1}H NMR (CD_3OD−$CDCl_3$) δ 7.04 (m, H-26), 6.97 (m, H-24), 6.24 (m, H-25), 5.80 (s, H-3), 5.06 (br s) and 4.98 (br s) (H_2-21), 4.54 (br s, H-10), 2.62 (d, J = 13.7 Hz) and 2.00 (d, obsc) (H_2-14), 2.58 (m, H-8_{ax}), 2.30 (m, H-8_{eq}), 2.30 (m, H-13), 1.96 (dt, 13, 5 Hz, H-7_{ax}), 1.40 (m, H-7_{eq}), 1.37 (s, H_3-17), 0.75 (d, 6.3 Hz) and 1.11 (d, J = 6.7 Hz) (H_3-18 and -19), 0.86 (s, H_3-20); HRMS (FAB) m/z $C_{25}H_{32}NO_{10}BNa^+$ 540.2017, found 540.2012. Borate **3** was also obtained from **2** by treatment with BF_3·Et_2O in CH_2Cl_2 (60%) and was formed exclusively by reaction with $NaBH_4$. **1** reacted similarly with the above reagents and also gave a high yield (80%) of the borate with BH_3·THF. When $(EtO)_3BF_4$ was added to a THF solution of **2**, the anhydro borate was recovered (65%): ^{1}H NMR (CD_3OD−$CDCl_3$) δ 7.01 (H-26), 6.86 (H-24), 6.22 (m, H-25 and H-3), 5.13 and 4.98 (br s, H_2-21), 3.30 (d, J = 19.5 Hz) and 2.47 (d, J = 19.5 Hz) (H_2-14), 2.70 (m, H-13), 1.84 (br s, H_3-17), 0.94 (d, J = 6.1 Hz) and 1.06 (d, J = 6.9 Hz) (H_3-18 and -19), 0.91 (H_3-20).

Cyclic Methylboronate 4. The cyclic ethylboronates **1**(EtB) and **2**(EtB) are described in the companion paper.[9] To prepare the methylboronate (**4**), compound **2** (20 mg, 0.041 mmol) in THF (3 mL) was treated with a 0.5 M hexane solution of $LiMe_2BH_2$ (0.2 mmol) at 0 °C. After 10 min water was added and the product isolated by acidification (HCl) and extraction with ethyl acetate as above. Evaporation of the solvent, storage in MeOH for 10 min and TLC first in hexane−ethyl acetate (1:1) and then in $CHCl_3$−MeOH (9:1) (R_fs 0.44 and 0.55, respectively) gave boronate **4** (11 mg, 53%). ^{1}H NMR (CD_3OD−$CDCl_3$) as the borate (Table 1), 0.20 (MeB); ^{13}C NMR (CD_3OD−$CDCl_3$) as for **3** (Table 1), −2.73 (MeB); HRMS (FAB) m/z $C_{26}H_{34}NO_9BLi^+$ 522.2487, found 522.2482. The same product was obtained in 60% yield by reaction with methylboronic acid in THF.

Cyclic Cyclohexylboronate 5. Cyclohexene (0.8 g, 10 mmol) was added to BH_3·THF (0.5 mL, 0.5 mmol). After 20 min **2** (20 mg, 0.041 mmol) was added and the mixture left for 4 h. Isolation as for **4** and TLC sequentially with (1) $CHCl_3$−MeOH (9:1) and (2) hexane−ethyl acetate−MeOH (67:32:1) (R_fs 0.66 and 0.40, respectively) gave **5** (8.2 mg, 35%): ^{1}H and ^{13}C NMR (CD_3OD−$CDCl_3$) as for **4** (Table 1); ^{1}H 1.55 (m) and 1.20 (m) (cyclohexyl); ^{13}C NMR δ 27.2, 27.3, 28.0 (cyclohexyl).

Cyclic Phenylboronate 6. Reaction of **2** and $PhB(OH)_2$ in THF and purification by TLC in $CHCl_3$−MeOH (9:1) gave **6** (45%) (R_f 0.65): ^{1}H and ^{13}C NMR (CD_3OD−$CDCl_3$) as for **4** (Table 1); ^{1}H δ 7.72 and 7.24 (phenyl); ^{13}C δ 132.8 (w), 134.3, 131.1, 127.6 (phenyl).

Cyclic 3-Thienylboronate 7. This compound was prepared as for **6** with 3-thienylboronic acid (R_f 0.65): ^{1}H and ^{13}C NMR (CD_3OD−$CDCl_3$) as for **4** (Table 1); ^{1}H δ 7.80, 7.33, and 7.22 (thienyl); ^{13}C δ 124.7, 125.0, and 132.0 (thienyl).

10,12-Cyclic Phosphorus Derivatives (Cyclic Chlorophosphate and 8−17). Cyclic Chlorophosphate. Dehydroryanodine (**2**) (30 mg, 0.061 mmol) was dissolved in pyridine (2 mL), and phosphoryl chloride (0.2 mL, 0.22 mmol) was added slowly at −10 °C. After 10 min the mixture was warmed to room temperature (15 min) and then added to ice. Workup involved acidification (HCl), saturation with salt, and extraction twice with an equal volume of ethyl acetate to give the crude chloridate (22 mg) (63%). ^{1}H NMR (CD_3OD) δ 6.99

(m, H-26), 6.85 (m, H-24), 6.21 (m, H-25), 5.55 (s, H-3), 5.67 (d, J = 6.4, H-10), 4.91 and 4.93 (H_2-21), 2.43 (d, J = 14.5 Hz) and 1.98 (d, J = 14.5 Hz) AB H_2-14, 2.25 (m, H-13), 2.15 (m, H-7_{ax}), 2.50 (m, H-8_{ax}), 2.33 (m, H-8_{eq}), 1.43 (s, H_3-17), 1.05 (d, J = 6.8 Hz), and 0.72 (d, J = 7.0 Hz) (H_3-18 and -19), 0.96 (s, H_3-20).

Cyclic *N*-Methylphosphoramidate 8. The chloridate above (22 mg) was treated with 40% $MeNH_2$, and after 10 min the solution was adjusted to pH 7 and extracted with ethyl acetate. The product was purified by TLC using first ethyl acetate−EtOH (19:1) and rechromatography with $CHCl_3$−MeOH (85:15) (R_f 0.41) to give **8** (7.6 mg, 35%): ^{1}H NMR (CD_3OD) (Table 1); ^{13}C NMR (Table 1); δ 28.1 (*N*-Me); ^{31}P NMR δ 5.0; HRMS (FAB) m/z $C_{26}H_{35}N_2O_{10}PNa^+$ 589.1927, found 589.1926.

Cyclic *N*-(2-Methoxyethyl)phosphoramidate 9. The chloridate fraction as above (17 mg, 0.03 mmol) was dissolved in pyridine (0.75 mL) with $MeOCH_2CH_2NH_2$ (0.15 g, 0.2 mmol). After 30 min the solution was added to ice and acidified (HCl) and the neutral product isolated with ethyl actetate. TLC using ethyl acetate−EtOH (19:1) gave **9** (8.0 mg, 44%) (R_f 0.27); ^{1}H and ^{13}C NMR (CD_3OD) as for **8** (Table 1); ^{1}H δ 3.40 (CH_2O), 3.15 (m, NCH_2), 3.30 (OMe); ^{13}C δ 73.4 (CH_2O), 59.0 (OMe), 42.3 (NCH_2); ^{31}P NMR δ 2.5; HRMS (FAB) m/z $C_{28}H_{39}N_2O_{11}PH^+$ 611.2370, found 611.2361.

Cyclic *N*-(2-Aminoethyl)phosphoramidate 10. The chloridate from **2** (30 mg, 0.061 mmol) was treated with ethylenediamine (0.2 g, 3.3 mmol). After 40 min the excess amine was evaporated and the product purified by TLC with $CHCl_3$−MeOH−$MeNH_2$, 82:15:3 (R_f 0.30). The product contained a little diamine which was removed by washing an ethyl acetate solution with water to give **10** (13 mg) (36%): ^{1}H and ^{13}C NMR (CD_3OD) as for **8** (Table 1); ^{1}H δ 3.14 (m) ($PNCH_2$) and 2.70 (m) (CH_2NH_2); ^{13}C δ 43.7 and 44.2 for aminoethyl carbons; ^{31}P NMR δ 2.3; HRMS (FAB) m/z $C_{27}H_{38}N_3O_{10}PH^+$ 596.2373, found 596.2382.

Cyclic *N*-(4-Aminobutyl)phosphoramidate 11. The chloridate from **2** (20 mg, 0.041 mmol) and $NH_2(CH_2)_4NH_2$ (0.15 g, 1.7 mmol) in pyridine (0.5 mL) were set aside for 1 h when the solvent was removed in vacuo, and the residue was recovered from water (pH 8) with ethyl acetate. Purification by TLC using $CHCl_3$−MeOH−$MeNH_2$ (77:20:3) gave **11** (7.5 mg, 30%) (R_f 0.25): ^{1}H and ^{13}C NMR (CD_3OD) as for **8** (Table 1); ^{13}C δ 42.1, 42.5 ($PNCH_2$, NH_2CH_2), 30.6, 31.3 (CH_2CH_2); ^{31}P NMR δ 2.8.

Cyclic Phosphate 12. The chloridate above from **2** (20 mg, 0.041 mmol) was dissolved in 1 M NaOH and after 5 min acidified (HCl) to pH 1, saturated with salt, and extracted exhaustively with ethyl acetate. Evaporation gave **12** (10 mg, 44%) which was purified by filtration through a C-18 reversed phase silica column with MeOH−water (1:1): ^{1}H NMR (CD_3OD) δ 7.03 (m, H-26), 6.87 (m, H-24), 6.23 (m, H-25), 5.68 (br s, H-3), 5.55 (br, H-10), 2.55 (d, J = 14.0 Hz) and 2.05 (d, obsc) (H_2-14), 1.49 (s, H_3-17), 0.74 (d, J = 6.8 Hz) and 1.10 (d, J = 7.0 Hz) (H_3-18 and -19), 0.99 (s, H_3-20); ^{31}P NMR δ −2.8; HRMS (FAB) m/z $C_{25}H_{32}NO_{11}PNa^+$ 576.1611, found 576.1600.

Cyclic Methyl Phosphate 13. The chloridate from **2** (21 mg, 0.042 mmol) gave **13** on storage in MeOH. It was purified by TLC in $CHCl_3$−MeOH (85:15): ^{1}H NMR (CD_3OD) 7.04 (m, H-26), 6.87 (m, H-24), 6.23 (m, H-25), 5.68 (br s, H-3), 5.52 (br, H-10), 4.92 (obsc H_2-21), 3.87 (d, J = 11.4 Hz, OMe), 2.59 (d, J = 14.2 Hz) and 2.02 (d, J = 14.2 Hz) AB H_2-14, 2.45 (m, H-8_{ax}), 2.35 (br, H-8_{eq}), 2.35 (m, H-13), 2.25 (m, H-7_{ax}), 1.40 (m, H-7_{eq}), 1.47 (s, H_3-17), 0.74 (d, J = 6.6 Hz) and 1.11 (d, J = 6.2 Hz) (H_3-18 and -19), 0.99 (s, H_3-20); ^{31}P NMR δ −2.1. HRMS (FAB) m/z $C_{26}H_{34}NO_{11}PNa^+$ 590.1767, found 590.1778.

Cyclic Phenylphosphonates 14 and 15. Compound **2**(EtB)[9] (11 mg, 0.021 mmol) in pyridine (0.75 mL) was treated with $PhP(O)Cl_2$ (0.1 mL, 0.70 mmol) at −10 °C. After 20 min the phosphonates were isolated by addition to ice, acidification (HCl) and extraction with ethyl acetate. TLC with $CHCl_3$−MeOH (9:1) gave the less polar isomer **14** (5.2 mg) (40%) (R_f 0.41): ^{1}H NMR (CD_3OD) δ 7.04 (dd, H-26), 6.89 (dd, H-24), 6.24 (dd, H-25), 5.90 (s, H-3), 5.83 (dt, 4.2, 1, 1 Hz, H-10), 5.08 (br s) and 5.04 (br s) (H_2-21), 2.63 (d, J = 14.1 Hz) and 2.15 (d, J = 14.0 Hz) AB H_2-14, 2.32 (m, H-13), 2.65−2.45 and 2.35−2.1 (br m, H-7_{ax}, H-8_{ax}, H-8_{eq}), 1.52 (dd, J = 13.1, 5 Hz, H-7_{eq}), 1.0−0.92 (H_3-17, -19, -20), 0.84 (m, EtB), 0.69 (H_3-18), 7.90, 7.62, and 7.57 (each m, phenyl); ^{13}C NMR (CD_3OD) (Table 1); δ 8.0, 6.8 (EtB) 129.6 (d, J = 13.6 Hz), 131.0 (d, J = 9 Hz), 128 (d, obsc), and 141.7 (s, phenyl); ^{31}P NMR δ 15.9. HRMS (FAB) m/z $C_{33}H_{39}NO_{10}PBH^+$ 652.2482, found 654.2481. The more retained isomer (6.6 mg, 51%) (R_f 0.37) was **15**: ^{1}H NMR (CD_3OD) as for the isomer (**14**) and ^{1}H δ 5.06 and 4.94 (br ss, H_2-21), 4.82 (d, J = 5.4 Hz, H-10), 1.40 (br d, H-7_{eq}), 2.43 (m, H-13), 1.63 (s, H_3-17), 1.19 (d, J = 6.8 Hz) and 0.78 (d, J = 7.0 Hz) (H_3-18 and -19), 0.92 (s, H_3-20), 7.82, 7.66 and 7.55 (each m, phenyl), 0.65−0.55 (EtB); ^{13}C NMR (CD_3OD−$CDCl_3$) (Table 1); δ 7.5, 6.9 (EtB), 127.5, 130.1, 131.7, 134.2; ^{31}P NMR δ 15.3; HRMS (FAB) m/z $C_{33}H_{39}NO_{10}BPNa^+$ 674.2302, found 674.2317.

Cyclic Methylphosphonates 16 and 17. Compound **1**(EtB) (25 mg) was treated with $MeP(O)Cl_2$ as for **14** and **15**, and the products were isolated with ethyl acetate after 30 min at ambient temperature. Methanolysis of the boronate was complete after slow distillation with MeOH for 2 h. The mixture was separated by TLC using first $CHCl_3$−MeOH (9:1) and, to remove traces of **2**, ethyl acetate−EtOH (97:3). The component of R_f 0.30 and 0.43, respectively, was **16**: ^{1}H NMR (CD_3OD) δ 7.04 (m, H-26), 6.87 (m, H-24), 6.24 (m, H-25), 5.67 (s, H-3), 4.97 (dd, J = 10.6, 5.1 Hz, H-10), 2.58 (d, J = 13.5 Hz) and 2.05 (d, J = 13.5 Hz) AB H_2-14, 2.33 (m, H-13), 2.20 (dt, J = 13, 13, 5.5 Hz, H-7_{ax}), 1.50 (dd, J = 12.5, 4.5 Hz, H-7_{eq}), 1.73 (obsc, H-8_{ax}), 1.30 (m, H-8_{eq}), 2.35 (m, H-9), 1.41 (s, H_3-17), 0.74, 1.00, 1.10 (ddd, H_3-18, -19, -21), 0.97 (s, H_3-20), 1.84 (d, 18 Hz, Me-P); ^{13}C NMR (CD_3OD) (Table 1); δ 12.4 (d, J = 173 Hz, P-Me). The component of R_f 0.53 and 0.50, respectively, was **17**: ^{1}H NMR (CD_3OD) as for the isomer (**16**) except δ 4.42 (dd, J = 10.8, 4.5 Hz, H-10), 1.73 (dt, J = 14, 14, 5 Hz, H-8_{ax}), 1.86 (m, H-9), 1.49 (s, H_3-17), 1.12 (d, J = 6.7 Hz, H_3-21), 1.00 (d, J = 6.5 Hz) and 0.98 (d, J = 6.5 Hz) (H_3-18 and -19), 0.98 (s, H_3-20), 1.65 (d, J = 17 Hz Me-P); ^{13}C NMR (CD_3-OD) (Table 1); δ 10.0 (d, J = 145 Hz, P-Me); HRMS (FAB) m/z $C_{26}H_{36}NO_{10}PNa^+$ 576.1974, found 576.1963.

Methylated Derivatives (18−22). N-Me-2 (18), N-Me-(EtB)-2 (19), and N,4-Me_2-2 (21). Dehydroryanodine (**2**, 20 mg, 0.041 mmol) in acetone (4 mL) and MeI (0.5 mmol) were stirred with dry K_2CO_3 (0.5 g) overnight. Three main products were separated by TLC with $CHCl_3$−MeOH (93:7). **18** (6.4 mg, 30%) (R_f 0.25): ^{1}H NMR (CD_3OD) as for **2** (see Table 1) except δ 3.90 (N-Me); ^{13}C NMR (CD_3OD) δ 119.2 (C-24), 108.6 (C-25), 131.2 (C-26), 36.9 (N-Me); HRMS (FAB) m/z $C_{26}H_{35}NO_9Na^+$ 528.2210, found 528.2217. **20** (see below) (1.5 mg, 7.4%) (R_f 0.32). **21** (5.1 mg, 25%) (R_f 0.43): ^{1}H NMR (CD_3OD) δ 6.09 (H-3), 3.90 (N-Me), 3.81 (4-OMe); ^{13}C NMR (CD_3OD) δ 80.9 (C-3), 95.6 (C-4), 98.5 (C-12), 118.5 (C-24), 108.3 (C-25), 130.7 (C-26), 36.8 (N-Me), 54.7 (4-OMe); HRMS (FAB) m/z $C_{27}H_{37}NO_9Na^+$ 542.2366, found 542.2359. Replacement of MeI with Me_2SO_4 or methyl tosylate gave similar results. Methylation of **2**(EtB) (8.0 mg) with MeI and TLC as above gave **19** (4.2 mg, 52%) (R_f 0.4) and polymethylated products.

4-Me-2 (20) and 4,10-Me_2-2 (22). Borate **3** (45 mg, 0.088 mmol) in MeOH−ether, 7:3 (3 mL), was treated with excess ethereal diazomethane at 0 °C. Evolution of nitrogen was rapid. After 24 h the product was separated by TLC in $CHCl_3$−MeOH (19:1) to give three products. The main fraction (26 mg) (R_f 0.25) was purified further by TLC with acetone−hexane, 1:1 (R_f 0.65), to give **18** (∼1 mg) and **20** (18.3 mg, 39%): ^{1}H NMR (CD_3OD) δ 6.12 (H-3) and 3.79 (4-OMe); ^{13}C NMR (CD_3OD) δ 81.7 (C-3), 95.8 (C-4), 99.6 (C-12), 54.7 (4-OMe); HRMS (FAB) m/z $C_{26}H_{35}NO_9Na^+$ 528.2210, found 528.2202. The R_f 0.51 fraction was **22** (2.5 mg, 50%): ^{1}H NMR (CD_3OD) δ 6.12 (H-3), 4.40 (H-10), 3.78 (4-OMe), 3.48 (10-OMe); ^{13}C NMR (CD_3OD) δ 144.4 (C-9), 79.0 (C-10), 54.2 (4-OMe), 58.4 (10-OMe); HRMS (FAB) m/z $C_{27}H_{37}NO_9Na^+$ 542.2366, found 542.2354. This was followed by a mixture (2 mg) containing 4,15-Me_2-**2** (∼80% pure): ^{1}H NMR ($CDCl_3$) as a mixture δ 6.21 (s, H-3), 3.78 (4-OMe), 3.34 (15-OMe),[5] 2.2 (H-14).

Dehydration at C-2 (23−28). 2-Deoxy-2(13)-dehydro-2(EtB) and -2 (23 and 24) and 2-Deoxy-2(13)-dehydro-1. **2**(EtB) (15 mg, 0.028 mmol) in pyridine (0.5 mL) at −10 °C was treated dropwise with $SOCl_2$ (0.10 mL). After 10 min the

solution was warmed to room temperature and added to ice. The product isolated by addition of HCl and extraction with ethyl acetate was a mixture of **23** (see below) and a sulfite [NMR signals at δ 5.56 (H-10), 1.42 (H_3-17), 1.80 and 1.48 (H_3-18 and -19] which hydrolyzed after stirring for 3 min with 5% NaOH solution. Extraction of the acidified solution with ethyl acetate and TLC with $CHCl_3$−MeOH (19:1) (R_f 0.41) gave **23** (7.0 mg, 53%): ^{1}H NMR (CD_3OD−$CDCl_3$) δ 7.00 (m, H-26), 6.83 (m, H-24), 6.20 (m, H-25), 6.38 (m, H-3), 4.99 and 4.89 (br s, H_2-21), 4.54 (br s, H-10), 2.40 (d, J = 13.4 Hz) and 1.83 (d, J = 14.0 Hz) (H_2-14), 1.32 (H_3-17), 1.78 (d, J = 1.3 Hz) and 1.47 (d, J = 1.78 Hz) (H_3-18 and -19), 0.86 (s, H_3-20), 0.83 and 0.69 (EtB); ^{13}C NMR (CD_3OD−$CDCl_3$) δ 60.8 (C-1), 131.1 and 133.9 (C-2 and -13), 79.6 (C-3), 89.2 (C-4), 45.7 (C-5), 85.8 (C-6), 25.7 and 28.7 (C-7 and -8), 147.0 (C-9), 68.5 (C-10), 87.6 (C-11), 95.1 (C-12), 40.2 (C-14), 103.7 (C-15), 11.8 and 12.8 (C-17 and -20), 22.1 and 22.4 (C-18 and -19), 107.7 (C-21), 160.8 (C-22), 121.8 (C-23), 116.0 (C-24), 110.2 (C-25), 124.2 (C-26), 7.7 and 5.5 (EtB). Treatment of **2** in the same way gave **24**: HRMS (FAB) m/z $C_{25}H_{31}NO_8Na^+$ 496.1947, found 496.1938. For use in reactions detailed below, **1** was similarly converted to 2-deoxy-2(13)-dehydro-**1**: ^{1}H NMR (CD_3OD−$CDCl_3$) δ 7.00 (m, H-26), 6.83 (m, H-24), 6.20 (m, H-25), 6.10 (m, H-3), 3.80 (d, J = 10.5 Hz, H-10), 2.27 (d, J = 13.5 Hz) and 1.75 (d, J = 14.0 Hz) H_2-14, 1.39 (H_3-17), 1.80 (d, J = 1.3 Hz) and 1.53 (d, J = 1.8 Hz) H_3-18 and -19, 0.99 (d, J = 6.8 Hz, H_3-21), 0.90 (s, H_3-20); ^{13}C NMR (CD_3OD−$CDCl_3$) δ 62.5 (C-1), 130.1 and 133.3 (C-2 and -13), 83.5 (C-3), 90.2 (C-4), 49.0 (C-5), 87.2 (C-6), 26.0 and 28.1 (C-7 and -8), 34.4 (C-9), 71.8 (C-10), 83.5 (C-11), 94.8 (C-12), 40.6 (C-14), 103.1 (C-15), 11.2 and 11.8 (C-17 and -20), 21.9 and 22.8 (C-18 and -19), 18.3 (C-21), 162.3 (C-22), 121.4 (C-23), 116.7 (C-24), 110.4 (C-25), 124.7 (C-26).

Epoxides A and B of 2-Deoxy-2(13)-dehydro-1 (25 and 26). A solution of 2-deoxy-2(13)-dehydro-**1** (17.0 mg, 0.036 mmol) and MCPBA (40 mg, 0.23 mmol) in 20% CD_3OD−$CDCl_3$ (0.5 mL) was monitored by NMR until most (85%) of the starting material had reacted. The product taken up into ethyl acetate (5 mL) was washed with $NaHCO_3$, $NaHSO_3$, and K_2CO_3 solutions and separated by TLC with $CHCl_3$−MeOH−$MeNH_2$, 88:10:2. The less absorbed component (R_f 0.75) was **26** (2.0 mg, 9.4%): ^{1}H NMR (CD_3OD−$CDCl_3$) δ 7.01 m (H-26), 6.97 (m, H-24), 6.21 (m, H-25), 5.42 (s, H-3), 3.83 (H-10), 2.36 (d, J = 14.0 Hz) and 1.92 (d, J = 14.0 Hz) (H_2-14), 1.50, 1.21, 1.10, and 0.87 (H_3-17, -18, -19, and -20), 0.98 (d, J = 6.8 Hz, H_3-21); ^{13}C NMR (DMSO) δ 58.6 (C-1), 67.6 (C-2), 76.6 (C-3), 88.7 (C-4), 48.0 (C-5), 84.2 (C-6), 27.8 and 28.9 (C-7 and -8), 33.7 (C-9), 70.6 (C-10), 92.3 (C-12), 40.9 (C-14), 101.9 (C-15), 10.7 and 12.6 (C-17 and -20), 25.5 and 23.9 (C-18 and -19), 18.7 (C-21), 158.8 (C-22), 121.6 (C-23), 115.2 (C-24), 109.4 (C-25), 124.4 (C-26); HRMS (FAB) m/z $C_{25}H_{33}NO_9Na^+$ 514.2053, found 514.2059. An intermediate fraction (R_f 0.70) of unreacted alkene (2 mg, 9%) was followed by **25** (8 mg, 45%, R_f 0.50): ^{1}H NMR (CD_3OD−$CDCl_3$) δ 6.97 (m, H-26), 6.84 (m, H-24), 6.25 (m, H-25), 5.73 (s, H-3), 3.81 (d, J = 10.4 Hz, H-10), 2.30 (d, J = 13.5 Hz) and 1.95 (d, J = 13.5 Hz) AB H_2-14, 1.42, 1.25, 1.06, 0.92 (H_3-17, -18, -19, and -20), 0.97 (d, J = 7.0 Hz, H_3-21); ^{13}C NMR (CD_3OD) δ 63.7 (C-1), 88.0, 85.5, 85.5 (C-2, -6, and -11), 85.6 (C-3), 91.2 (C-4), obsc (C-5), 26.8 and 29.2 (C-7 and -8), 35.6 (C-9), 71.1 and 72.8 (C-10 and -13), 94.9 (C-12), 42.3 (C-14), 102.8 (C-15), 10.0 and 13.0 (C-17 and -20), 26.4 and 26.4 (C-18 and -19), 18.9 (C-21), 161.4 (C-22), 122.9 (C-23), 117.2 (C-24), 110.9 (C-25), 125.6 (C-26); HRMS (FAB) m/z $C_{25}H_{33}NO_9Na^+$ 514.2053, found 514.2054.

13-Hydroxy-1 (27). 2-Deoxy-2(13)-dehydro-**1** (23 mg, 0.048 mmol) in pyridine (2 mL) was treated with osmium tetroxide (45 mg, 0.18 mmol). After 2 days saturated $NaHCO_3$ (3 mL) and $NaHSO_3$ aqueous solutions (3 mL) were added, and the mixture was stirred for 24 h. Product isolation involved acidification (HCl) and extraction with ethyl acetate followed by rotary chromatography with $CHCl_3$−MeOH (93:7) to give **27** (18 mg, 74%): ^{1}H NMR (CD_3OD−$CDCl_3$) δ 6.97 (m, H-24 and -26), 6.22 (m, H-25), 5.56 (s, H-3), 3.82 (d, J = 10.2 Hz, H-10), 2.97 (d, J = 14.0 Hz) and 1.45 (d, J = 14.0 Hz) AB H_2-14, 1.42, 1.35, 1.21 (br), 0.80 (H_3-17, -18, -19, and -20), 0.97 (d, J = 6.8 Hz, H_3-21); ^{13}C NMR (CD_3OD−$CDCl_3$) δ 65.0 (C-1), 87.4, 85.2, 84.2 (C-2, -6, and -11), 91.7 (C-3), 90.9 (C-4), 49.8 (C-5), 25.8 and 28.0 (C-7 and -8), 34.2 (C-9), 71.8 and 76.3 (C-10 and -13), 95.9 (C-12), 40.7 (C-14), 102.1 (C-15), 9.9 and 11.9 (C-17 and -20), 25.3 and 29.5 (C-18 and -19), 18.2 (C-21), 161.4 (C-22), 121.6 (C-23), 117.5 (C-24), 110.7 (C-25), 124.7 (C-26); HRMS (FAB) m/z $C_{25}H_{35}NO_{10}Na^+$ 532.2159, found 532.2157.

When 13-hydroxy-**1**(EtB) [**27**(EtB)] was treated with thionyl chloride/pyridine and then 5% NaOH as for the preparation of **23**, 13-hydroxyanhydroryanodine was obtained by TLC (R_f 0.31, $CHCl_3$−MeOH, 9:1): ^{1}H NMR δ 6.20 (m, H-3 and H-25), 3.86 (d, J = 10.5 Hz, H-10), 3.34 and 2.50 (d, J = 19.7 Hz) AB H_2-14, 2.01 (d, J = 2.2 Hz, H_3-17), 1.38 (s) and 1.23 (s) (H_3-19 and -18), 1.03 (d, J = 6.6 Hz, H_3-21), 0.83 (s, H_3-20), 0.87 (m) and 0.72 (m) (EtB).

13-Hydroxy-4,12-seco-4,12-dioxo-1 (28). Oxidation of **27** (5 mg, 0.0098 mmol) with sodium periodate (0.5 mL of 0.1 M, 0.05 mmol) during 6 h gave only **28**. This sample was recovered unchanged after 18 h in 0.5 M periodic acid: ^{1}H NMR (CD_3OD−$CDCl_3$) δ 7.02 (m, H-26), 6.95 (m, H-24), 6.24 (m, H-25), 5.33 (s, H-3), 3.73 (d, J = 10.5 Hz, H-10), 3.62 (d, J = 15.3 Hz) and 2.20 (d, J = 15.3 Hz) AB H_2-14, 1.64, 1.42, 1.19, 0.90 (H_3-17, -18, -19, and -20), 0.96 (d, J = 6.8 Hz, H_3-21); ^{13}C NMR (CD_3OD−$CDCl_3$) δ 60.3 (C-1), 81.4 (C-2), 87.0 (C-3), 214.1 (C-4), 51.2 (C-5), 77.5 (C-6), 24.6 and 27.6 (C-7 and -8), 33.9 (C-9), 71.0 (C-10), 89.5 (C-11), 220.2 (C-12), 76.7 (C-13), 39.9 (C-14), 103.4 (C-15), 15.2 (C-17), 27.3, 27.3 (C-18 and -19), 21.0 (C-20), 18.2 (C-21), 159.2 (C-22), 121.6 (C-23), 117.1 (C-24), 110.9 (C-25), 125.1 (C-26); HRMS (FAB) m/z $C_{25}H_{33}NO_{10}Na^+$ 530.2002, found 530.2013.

Biology. [^{3}H]Ryanodine Binding. Rabbit Skeletal Muscle and Mouse Brain Membranes. The assay conditions are reported by Jefferies et al.[6] for skeletal muscle and Lehmberg and Casida[21] for brain membranes.

Cardiac Preparations. Cardiac membranes were prepared as reported by Jones et al.[22] with minor modification. Briefly, canine ventricles were minced in 10 mM $NaHCO_3$, and homogenized with a Polytron tissue grinder (3 × 5 s bursts, maximum speed) followed by four passes with a motor-driven glass−Teflon pestle homogenizer (nominal clearance, 0.13−0.18 mm). The homogenate was centrifuged at 100*g* (4 °C) for 20 min, and the resulting supernatant was centrifuged for 45 min at 4500*g* (4 °C). The protein pellet was resuspended to approximately 1 mg/mL in buffer containing (mM): MOPS (3-(*N*-morpholino)propanesulfonic acid), 20; KCl, 1000; AEBSF (4-(2-aminoethyl)-benzenesulfonyl fluoride, HCl), 0.1; pH 7.0. Saturation binding experiments were carried out to determine the affinity and maximum binding density of [^{3}H]-**1** binding sites. Binding was initiated by addition of membranes (100−200 μg/mL) to tubes containing [^{3}H]-**1** (0.1−70 nM) and MOPS−AEBSF buffer in a volume of 0.5 mL and incubation for 2 h at 37 °C. Nonspecific binding was defined in a duplicate set of tubes containing 10 μM unlabeled **1**. K_i values were calculated from specific binding isotherms using iterative nonlinear regression analysis (InPlot, GraphPad Software, San Diego, CA). In competition experiments, membranes (100−200 μg/mL) were incubated with unlabeled **1** or its analogs (0.1 pM to 10 μM), [^{3}H]-**1** (3 nM), and MOPS−AEBSF buffer. Binding was terminated in both types of experiments by rapid filtration through GF/C glass fiber filters with a Brandel cell harvester. Each tube was subsequently washed four times with ice-cold buffer before liquid scintillation counting. IC_{50} values were calculated using non-linear regression, and K_i values were determined using the Cheng−Prusoff equation.[23]

Ventricle Contractility Assays. Ventricular contractility was measured as described previously.[24] Right ventricular muscle strips from rats were isolated and suspended in water-jacketed glass tissue baths containing Krebs-Henseleit solution (30 °C, aerated with O_2−CO_2, 95:5) of the following composition (mM): NaCl, 118; KCl, 4.5; $CaCl_2$, 2.5; KH_2PO_4, 1.1; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 11. Muscle strips were secured to an electrode mounted at the bottom of the bath and connected to a Grass FT03 force displacement transducer. Muscles were stimulated to contract by administering square-wave pulses (20-ms duration, 1.2× threshold voltage, 2 Hz) with a Grass model S9D stimulator; resting tension was 0.5 g. Contractions were recorded on a Grass Polygraph.

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Supporting Information Available: Extended Tables 2 and 3 with potency values for the weakly active compounds (2 pages). Ordering information is given on any current masthead page.

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