Ryania Insecticide: Structural Assignments of Four Natural 8_{ax}-Hydroxy-10-epiryanoids†

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Four 8_{ax}-hydroxy-10-epiryanoids isolated from ryania, the botanical insecticide, were established by ^1H NMR spectroscopy to consist of three known compounds (A, D, and F) and a new ryanoid (E). Earlier structural assignments of A and D with a methoxy substituent at C-4 are not consistent with their ^1H and ^{13}C NMR spectra or with the conversion of A to the corresponding diketone on oxidation with periodate, a known reaction of the C-4,C-12 diol group of ryanodine. The revised structures are 8_{ax}-hydroxy-10-(*O*-methyl)-10-epiryanodine for A and 8_{ax}-hydroxy-10-(*O*-methyl)-10-epidehydroryanodine for D. NOESY experiments in chloroform but not in other solvents indicate that the pyrrole-2-carboxylate group of A lies in a different spatial orientation than that of ryanodine relative to the rest of the molecule. The new ryanoid (E) is characterized as 18-hydroxy-A, i.e., 8_{ax},18-dihydroxy-10-(*O*-methyl)-10-epiryanoidine. Evidence is detailed for the assignment of F as 8_{ax}-hydroxy-10-epidehydroryanodine, and more complete spectral data support the previously assigned structures of C_1 and C_2 in the ryanodine and anhydroryanodine series, respectively.

INTRODUCTION

Structural assignments of the ryania constituents and related compounds are an essential step in expanding the knowledge on the relation of ryanoid structure to insecticidal activity, mammalian toxicity, and action at the calcium-activated calcium channel. Current information on the structural aspects of ryanoid action is based on 10 natural ryanoids (Jefferies et al., 1992) and about 20 analogues prepared by minor chemical modifications of the natural products (Waterhouse et al., 1987).

We recently isolated most of the known natural ryanoids and one new compound for quantitation of their content in ryania insecticide and determination of their biological activity (Jefferies et al., 1992). Three of these compounds were found to be known ryanoids (A, D, and F), and a fourth (E) was a new but closely related compound, each with the 8_{ax}-hydroxy-10-epiryanodine structure (Figure 1). The ^1H and ^{13}C NMR spectra and oxidation chemistry of A and the spectra of D are not consistent with the earlier structural assignments (Ruest et al., 1985), and they are accordingly revised in the present study, which includes observations on the spatial orientation of the pyrrole-2-carboxylate group relative to the rest of the molecule. The new ryanoid E is reported with details of the structural determination relating it to A. The assignment of ryanoid F, previously reported in abstract form only without experimental details (Humerickhouse et al., 1989), is now fully documented, and more complete spectral data are provided supporting the structures of C_1 and C_2 in the ryanodine and anhydroryanodine series, respectively (Ruest et al., 1985).

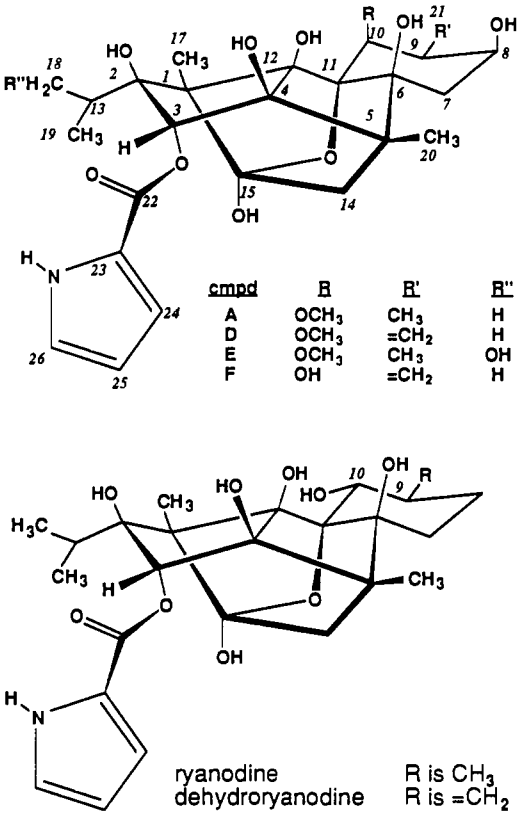

Figure 1. Structures of four 8_{ax}-hydroxy-10-epiryanoids in comparison to those of ryanodine and dehydroryanodine.

MATERIALS AND METHODS

The ryanoids used were available from a concurrent study (Jefferies et al., 1992). Mass spectra (FAB) were recorded using a Kratos MS-50 instrument. ^1H and ^{13}C NMR spectra were acquired at 300 and 75 MHz, respectively, using a Bruker WM-300 spectrometer. Spectra were obtained at ambient temperature using concentrations from 10 to 50 mM. The solvents used were CD_3OD, CD_3COCD_3, C_5D_5N, and $CDCl_3$ with tetramethylsilane as the chemical shift reference. Fully ^1H-coupled ^{13}C spectra were obtained with the decoupler off during acquisition to enhance the nuclear Overhauser effect (NOE). All of 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. The 2D spectra used for these studies were ^1H-^1H correlation spectroscopy (COSY), homonuclear nuclear Overhauser effect (NOESY) spectra, long-range heteronuclear C-H correlation (Bax and Morris, 1981), and inverse C-H correlation (INVXCOR) (Bax et al., 1983). Both COSY and NOESY spectra were acquired with a spectral width

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Table I. Complete ^{13}C NMR Assignment for 10-Epiryanoid A

	ryanodine		A			
					long-range coupling	
position	δ (^{13}C) C_5D_5N[a]	δ (^{13}C) CD_3COCD_3	δ (^{13}C) CD_3COCD_3	δ (^1H) INVXCOR CD_3COCD_3	$J \sim 10$ Hz[b] CD_3COCD_3	$J \sim 6.5$ Hz CD_3OD
1	65.8	65.2	65.2		H-3, H_3-17, H_b-14	H_3-17
2	83.6	83.5	83.5		H-3, H_3-17, H_3-18, H_3-19	
3	91.0	91.4	90.3	5.72		
4	92.2	91.9	91.7		H-3, H_b-14, H_3-20	H_3-20
5	49.4	49.3	48.4		H-3, H_2-14, H_3-20	H_3-20
6	85.5	85.9	85.4		H_{eq}-7, H_a-14, H_3-20	H_3-20
7	26.9	27.0	33.9	2.23, 1.70		
8	29.2	29.0	71.9	4.03		
9	35.3	34.9	37.9	2.1	H_3-21, H_{eq}-7	
10	72.6	72.3	85.4	3.44		
11	87.6	87.1	83.7		H_{eq}-7, H-10	
12	97.0	96.3	95.3		H_3-17	H_3-17
13	30.5	30.4	30.0	2.22		
14	42.4	41.8	41.3	2.65, 1.90	H_3-20	
15	102.8	102.4	102.6		H_b-14, H_3-17	H_b-14, H_3-17
17	10.9	10.2	11.2	1.39		
18	19.1	18.8	18.5	0.73	H_3-19	
19	19.8	19.5	19.4	1.06	H_3-18	
20	13.2	12.6	12.3	0.94		
21	19.3	18.8	14.3	1.20		
22	161.1	160.9	160.1			H-3
23	123.8	123.2	123.4		H-24, H-26	
24	116.2	116.5	115.9	6.87	H-26	
25	110.5	110.9	110.5	6.24		
26	125.0	125.1	124.8	7.10	H-24	
OCH_3			62.2	3.50		H-10

[a] Values from Krishnamurthy et al. (1987). [b] H_a-14 is downfield and cis to the isopropyl group and H_b-14 is upfield and trans.

of 2400 Hz by using a 1024 × 256 data set, zero filling by a factor of 2 and multiplied by an unshifted sine-bell function prior to Fourier transformation. In the long-range C–H correlation and INVXCOR experiments, the spectral widths varied from 9000 to 12 000 Hz (^{13}C) and from 2160 to 2700 Hz (^1H). These experiments were acquired using a 2048 × 256 data set, zero filling to 4096 × 256, and transforming after multiplication by an exponential function (line-broadening factor of 6 Hz) in the ^{13}C dimension and an unshifted sine-bell in the ^1H dimension. The number of scans in each block of the 2D acquisitions varied with sample concentration.

SPECTRAL DATA AND REACTIONS

8_{ax}-Hydroxy-10-epiryanoids. *Epiryanoid A.* ^1H NMR ($CDCl_3$) as reported (Ruest et al., 1985); ^1H NMR (CD_3OD) 7.03 (d, d, H-26), 6.86 (d, d, H-24), 6.23 (d, d, H-25), 5.66 (s, H-3), 3.94 (m, H-8), 3.51 (s, OCH_3), 3.44 (d, 3.0 Hz, H-10), 2.59 (d, 13.7 Hz) and 1.85 (d, 13.7 Hz), AB H_2-14, 2.24 (m, H-13), 2.15 (m, H-9), 2.25 (d, d, 3.5, 13.5 Hz, H_{ax}-7) and 1.71 (d, d, 2.3, 13.5 Hz, H_{eq}-7), 1.36 (s, H_3-17), 1.18 (d, 7.4 Hz, H_3-21), 1.08 and 0.73 (2d, 6.6, 6.4 Hz, H_3-18, H_3-19), 0.93 (s, H_3-20); (CD_3COCD_3) (after exchange with D_2O) 7.10 (m, H-26), 6.87 (m, H-24), 6.24 (m, H-25), 5.72 (s, H-3), 4.03 (m, H-8), 3.50 (s, OCH_3), 3.44 (d, 3.4 Hz, H-10), 2.65 (d, 13.8 Hz) and 1.90 (d, 13.8 Hz), AB H_2-14, 2.23 (d, d, 3.3, 13.5 Hz, H_{ax}-7), 2.22 (m, H-13), 2.1 (m, H-9), 1.70 (d, d, 2.4, 13.5 Hz, H_{eq}-7), 1.39 (s, H_3-17), 1.20 (d, 7.4 Hz, H_3-21), 1.06 and 0.73 (2d, 6.7, 6.4 Hz, H_3-18, H_3-19), 0.94 (s, H_3-20). ^{13}C NMR data are given in Table I.

Epiryanoid D. ^1H NMR ($CDCl_3$) identical with that published (Ruest et al., 1985); (CD_3COCD_3) 7.08 (m, H-26), 6.86 (m, H-24), 6.22 (m, H-25), 5.72 (s, H-3), 5.32 (d, 1.8 Hz) and 5.17 (d, 1.8 Hz, H-21), 4.55 (m, H-8), 3.95 (s, H-10), 3.27 (s, OCH_3), 2.64 (d, 13.8 Hz) and 1.85 (d, 13.8 Hz), AB H_2-14, 2.26 (d, d, 3.8, 13.5 Hz, H_{ax}-7), 1.75 (d, d, 2.0, 13.5 Hz, H_{eq}-7), 1.35 (s, H_3-17), 1.06 and 0.72 (2d, 6.7, 6.4 Hz, H_3-18, H_3-19), 0.96 (s, H_3-20); ^{13}C NMR (CD_3COCD_3) 160.1 (C-22), 143.8 (C-9), 124.6 (C-26), 123.4 (C-23), 121.2 (C-21), 115.8 (C-24), 110.4 (C-25), 102.8 (C-15), 96.5 (C-12), 91.8 (C-4), 90.2 (C-3), 85.05, 83.3, and 82.9 (C-2, -11, and -6), 85.4 (C-10), 72.9 (C-8), 65.6 (C-1), 56.2 (s, OCH_3), 48.3 (C-5), 41.3 (C-14), 34.0 (C-7), 29.5 (C-13), 19.4 and 18.4 (C-18, -19), 12.3 (C-20), 11.1 (C-17).

Epiryanoid E. ^1H NMR (CD_3COCD_3) 7.10 (m, H-26), 6.88 (m, H-24), 6.24 (m, H-25), 5.69 (s, H-3), 4.20 (m, H-8), 3.82 (m, H-18), 2.58 (d, 13.5 Hz) and 1.88 (d, 13.5 Hz), AB H_2-14, 3.50 (s, OCH_3), 3.44 (d, 3.3, H-10), 2.23 (d, d, 13.2, 3.6 Hz, H_{ax}-7), 2.20 (m, H-13), 2.13 (m, H-9), 1.70 (d, d, 2.4, 13.2 Hz, H_{eq}-7), 1.39 (s, H_3-17), 1.2 (d, 7.3 Hz, H_3-21), 0.93 (s, H_3-20), 0.86 (d, 6.4 Hz, H_3-19); ^{13}C NMR (CD_3COCD_3) 160.0 (C-22), 124.7 (C-26), 123.4 (C-23), 115.9 (C-24), 110.4 (C-25), 102.1 (C-15), 95.3 (C-12), 91.6 (C-4), 89.9 (C-3), 85.3 (C-10), 85.1, 84.1, and 83.8 (C-2, -6, and -11), 71.8 (C-8), 64.7 (C-18), 65.4 (C-1), 62.2 (OCH_3), 48.3 (C-5), 41.7 (C-14), 37.8 (C-9), 33.9 (C-7), 36.6 (C-13), 14.2 (C-21), 13.3 (C-19), 12.2 (C-20), 10.6 (C-17). HRMS (FAB) found m/z 540.2443. $C_{26}H_{37}NO_{11}H^+$ requires 540.2444.

Epiryanoid F. ^1H NMR (CD_3COCD_3) 7.11 (m, H-26), 6.89 (m, H-24), 6.25 (m, H-25), 5.74 (s, H-3), 5.16 (d, 1.8 Hz) and 5.18 (d, 1.8 Hz), AB H_2-21, 4.55 (d, d, 2.2, 3.6 Hz, H-8), 4.44 (s, H-10), 2.65 (d, 13.8 Hz) and 1.87 (d, 13.8 Hz), AB H_2-14, 2.26 (d, d, 3.6, 13.5 Hz, H_{ax}-7), 2.25 (m, H-13), 1.77 (d, d, 2.2, 13.5 Hz, H_{eq}-7), 1.37 (s, H_3-17), 1.08 and 0.73 (2d, 6.4, 6.7 Hz, H_3-18, H_3-19), 0.97 (H_3-20); ^{13}C NMR (CD_3COCD_3) 160.2 (C-22), 147.9 (C-9), 124.8 (C-26), 123.1 (C-23), 119.6 (C-21), 115.8 (C-24), 110.4 (C-25), 102.8 (C-15), 95.6 (C-12), 91.9 (C-4), 89.9 (C-3), 82.8, 83.4, and 85.4 (C-2, -6, and -11), 76.8 (C-10), 72.7 (C-8), 65.9 (C-1), 48.7 (C-5), 41.3 (C-14), 33.8 (C-7), 30.1 (C-13), 18.4, 19.2 (C-18 and -19), 12.1 (C-20), 11.0 (C-17). HRMS (FAB) found m/z 508.2198. $C_{25}H_{33}NO_{10}H^+$ requires 508.2182.

Reactions of Epiryanoid A and Ryanodine with Periodic Acid. Epiryanoid A (26 mg) in methanol (0.5 mL) was treated with aqueous HIO_4 (0.5 M, 0.6 mL) and set aside for 26 h. The precipitate (15 mg) was filtered and washed with water. ^1H NMR (C_5D_5N) 7.41 (m, H-26), 7.25 (m, H-24), 6.47 (s, H-3), 6.40 (m, H-25), 4.01 (m, H-8), 3.62 (d, 14.5 Hz) and 2.72 (d, 14.5 Hz), AB H_2-14, 3.58 (d, 2.5 Hz, H-10), 3.52 (s, OCH_3), 2.70 (m, H-9), 2.66 (m, H-13), 2.30 (2 H, m, H_2-7), 1.91 (s, H_3-17), 1.63, 1.35, and 1.30 (H_3 -18, -19, and -21), 1.24 (s, H_3-20); ^{13}C NMR (C_5D_5N) 220.1, 208.2 (C-4, C-12), 160.4 (C-22), 126.1 (C-26), 122.8 (C-23), 116.9 (C-24), 110.9 (C-25), 103.9 (C-15), 87.2, 86.6, 84.2, 80.2, 76.2, 71.4 63.3, 61.6, 54.1, 41.4, 36.7 (2 × C), 36.0, 22.2, 19.8, 19.7, 16.4, 14.6. HRMS (FAB) found m/z 522.2324. $C_{26}H_{35}NO_{10}H^+$ requires 522.2339.

Ryanodine was treated with HIO_4 (Kelly et al., 1951) to give "oxoryanodine", mp 224–226 °C, after crystallization from

methanol. ^{1}H NMR (C_5D_5N) 7.49 (m, H-26), 7.26 (m, H-24), 6.46 (m, H-25), 6.40 (s, H-3), 4.45 (d, 10.6 Hz, H-10), 4.08 (d, 14.9 Hz) and 2.76 (d, 14.9 Hz), AB H_2-14, 2.58 (m, 13-H), 1.80 (s, H_3-17), 1.57, 1.34, and 1.24 (d, 7.1, 6.8, 6.4 Hz, H_3-18, -19, and -21), 1.14 (s, H_3-20); ^{13}C NMR (C_5D_5N) 221, 215 (C-4, C-12), 160.1 (C-22), 126.3 (C-26), 122.3 (C-23), 116.9 (C-24), 111.0 (C-25), 103.7 (C-15), 87.5 (C-3), 90.7, 80.5, 77.1, 71.0, 60.3, 52.5, 41.5, 37.0, 35.0, 28.5, 28.0, 21.7, 19.1, 18.5, 18.3, 13.8.

Ryanoid C_1 and Anhydroryanoid C_2. The following ^{1}H and ^{13}C NMR data confirm the structures of C_1 and C_2 and provide additional spectroscopic data on these compounds.

Ryanoid C_1. mp 186–190 °C, after crystallization from chloroform [lit. mp 188–190 °C (Ruest et al., 1985)]; ^{1}H NMR ($CDCl_3/C_5D_5N$ 9:1) as reported; ^{13}C NMR (CD_3COCD_3) 160.4 (C-22), 125.0 (C-26), 123.0 (C-23), 117.2 (C-24), 110.7 (C-25), 104.1 (C-15), 96.4, 91.8, 86.2, 85.3, and 83.2 (C-2, -4, -6, -11, and -12), 90.3 (C-3), 77.0 (C-10), 71.0 (C-9), 65.8 (C-1), 55.1 (C-5), 41.1 (C-14), 31.5, 30.7, 26.7, 24.0, 19.3, 18.6, 11.9, 11.1.

Anhydroryanoid C_2. mp 276–278 °C, after crystallization from acetone [lit. mp 274–278 °C (Ruest et al., 1985)]; ^{1}H NMR (CD_3OD) 7.06 (m, H-26), 6.88 (m, H-24), 6.26 (m, H-25), 6.16 (s, 2.3 Hz, H-3), 4.2 (s, H-10), 3.43 (d, 19.2 Hz) and 2.57 (d, 19.7 Hz), AB H_2-14, 2.77 (m, H-13), 2.02 (m, H_{ax}-7), 1.88 (d, 2.2 Hz, H_3-17), 1.78 (H_{ax}-8), 1.72 (H_{eq}-7), 1.44 (H_{eq}-8), 1.28 (s, H_3-21), 1.08, 0.99 (d, 7.0, 7.3, H_3-18 and -19), 0.98 (s, H_3-20); ^{13}C NMR (CD_3OD) 172.1 (C-15), 162.7 (C-22), 145.5 (C-1), 139.1 (C-2), 126.9 (C-26), 124.0 (C-23), 117.9 (C-24), 112.0 (C-25), 94.3, 93.0, 90.0, and 84.1 (C-4, -6, -11, and -12), 85.2 (C-3), 74.5 (C-9), 71.3 (C-10), 49.5 (C-5), 41.0 (C-14), 35.0 (C-8), 29.1 (C-13), 23.8 (C-7), 23.8 (C-21), 22.3 (C-18), 20.4 (C-19), 15.2 (C-20), 14.3 (C-17).

RESULTS AND DISCUSSION

Structural Reassignments for 10-Epiryanoids A and D. The structure originally assigned to A (Ruest et al., 1985) was based largely on spectroscopic data and on analogy with ryanodine along with the molecular formula $C_{26}H_{37}O_{10}N$, which requires the addition of OCH_2 to ryanodine. ^{1}H NMR identifies the methoxyl, pyrrole, and methyl groups characteristic of a ryanoid. Double-resonance studies indicate the substitution and stereochemistry of the cyclohexane ring protons. Thus, the sharp δ 3.44 doublet (J = 3.0 Hz) for a hydroxymethine is assigned to H_{eq}-10 inverted from its position in ryanodine, and the small couplings of the other oxymethine suggest that the 8-hydroxyl is also axial. The methoxyl group of A is placed at C-4 on the basis of an IR assignment suggesting reduced H-bonding of the ester C=O, a shift (δ 0.33) for the 3-H from its position in ryanodine, and resistance to periodate oxidation.

Our earlier studies (Waterhouse et al., 1985, 1987; Krishnamurthy et al., 1987) assigned shifts for all carbons of ryanodine and used these to identify the positions of methoxyl groups arising from methylation of ryanodine. For example, methylation of the C-4 hydroxyl was identified by a downfield shift for C-4 of 4 ppm and an upfield shift for C-3 of 10 ppm, effects expected qualitatively from model compounds. For A no such shifts are observed (Table I). Indeed, the only carbon shifts differing significantly from those of ryanodine are C-7, -8, -9, -10, -11, and -21 of the cyclohexane ring, suggesting that the methoxyl is positioned at C-8 or C-10. Epiryanoid D, whose structure follows by analogy with A, similarly shows differences in its ^{13}C spectra only in the six-membered ring and significantly shows the methoxyl group well shifted from its position in A, consistent with substitution at C-8 or C-10 (Table II). In the fully coupled spectrum of A the methoxyl carbon appears as a doublet of quartets with a small coupling (6.5 Hz), which is only likely for proton coupling of two or three bonds. The origin of this splitting was traced to H-10 by long-range coupling correlation optimized for 6.5 Hz. Several other correlations were observed (Table I) at this frequency. Optimization at 10 Hz gave additional correlations, and all of the results allow the full assignment of the quaternary carbons C-1, -2, -4, -5, -6, -11, -12, and -15 using the same arguments applied before to ryanodine with additional support for C-11 arising from the presence of coupling to H-7 in the case of A. The IR (carbonyl frequency, $CHCl_3$) and shift for the 3-H in $CDCl_3$ reported by Ruest et al. (1985) evidently are a result of long-range disturbance of the pyrrolecarboxylic side chain by changes in the cyclohexane ring, which now carries four axial substituents for a chair conformation.

Table II. Selected ^{13}C NMR Shifts for Ryanodine and Four 10-Epiryanoids

	δ (^{13}C) CD_3COCD_3				
		10-epiryanoids			
position	ryanodine	A	D	F	E
3	91.4	90.3	90.2	89.9	89.9
7	27.0	33.9	34.0	33.8	33.9
8	29.0	71.9	72.9	72.7	71.8
9	34.9	37.9	143.8	147.9	37.8
10	72.3	85.4	85.4	76.8	85.3
OCH_3		62.2	56.2		62.2

The reported resistance of A to periodate oxidation (Ruest et al., 1985) is clearly anomalous. Although we have not measured relative rates, we found that, under conditions similar to those which cleave ryanodine, A reacts to give a less polar product with the expected spectral properties of the diketone from cleavage of the C-4,C-12 diol. Thus, the ^{13}C NMR of the diketone lacks two quaternary carbons, which are replaced by two carbonyl groups (220 and 208 ppm) near their positions in the corresponding diketone from ryanodine (221 and 215 ppm) (referred to as oxoryanodine) (Kelly et al., 1951). We have not carried out similar treatment of the less abundant D, but the correspondence of its ^{13}C spectrum with that of A (Table II) suggests that it also carries an axial methoxyl at C-10.

Structural Assignments for 10-Epiryanoids E and F. Epiryanoid F, the most polar pyrrolecarboxylate encountered, follows dehydroryanodine on radial chromatography and may be purified by HPLC (Jefferies et al., 1992). The HRMS corresponds to $C_{25}H_{33}O_{10}N$, consistent with a hydroxydehydroryanodine or an isomer thereof. The ^{1}H NMR spectrum shows the general features of epiryanoid D lacking the methoxyl group. Thus, the multiplet at δ 4.55 for H-8 corresponds closely to ester D (δ 4.55), although the corresponding H-10 singlets are separated by ~0.5 ppm, reflecting the methoxyl substitution or perhaps a different stereochemistry at C-10. Although strong allylic coupling is not present, COSY does show that H-10 and H-8 are each coupled to separate vinyl protons. To assign the stereochemistry of C-10 unequivocally, the NOESY spectrum was measured, and this shows strong interactions of the downfield vinyl proton with H-10 and of the upfield vinyl proton with H-8. Both protons are evidently equatorial and associated with a chair conformation, which should be most stable. The only alternative configuration that could justify these interactions would require a twist-boat conformation which should also show larger couplings of H-8 and H_2-7. The carbon shifts for F and D are consistent with methylation of C-10 in D (Table II), showing the expected upfield shift for C-9 and downfield shift for C-10. Clearly epiryanoid F isolated here is the same as that of Humerickhouse et al. (1989).

Epiryanoid E shows HRMS (FAB) of $C_{26}H_{37}O_{11}N$, suggesting in conjunction with the ^{1}H NMR spectral

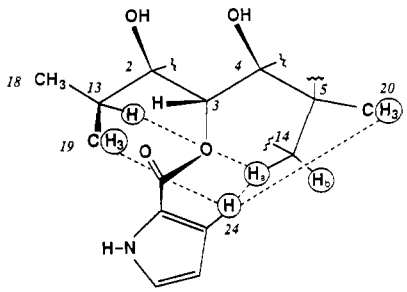

Figure 2. NOEs associated with orientation of pyrrolecarboxylate moiety for ryanodine and epiryanoid A in acetone-d_6.

features that it is a hydroxylated derivative of A. Thus, there are ^{1}H NMR signals corresponding to the methoxylated cyclohexane ring of A and the pyrrole ester, but one of the methyls of A is replaced by a two-proton signal at δ 3.82 corresponding to a hydroxymethyl. The COSY spectrum shows that these protons and one secondary methyl are both coupled to H-13. The ^{13}C spectrum of E shows a close correspondence to that of A (Table II). In addition, one methyl is replaced by an oxygenated carbon at 64.7 ppm with a consequent shift (6 ppm) for C-13. Similar hydroxylation of ryanodine and other ryanoids is proven (Nohara et al., 1981; Waterhouse et al., 1987).

Conformational Assignment of Ryanodine. The spectral differences reported by Ruest et al. (1985) for ryanodine and epiryanoid A in $CHCl_3$ that led to the erroneous methoxyl assignment for the latter evidently result from conformational changes of the pyrrole-2-carboxylate in relation to the hydroxyl at C-4. Hydrogen bonding of the ester carbonyl to the 4-OH indicated by the IR spectrum must result from a fine balance of interactions, since apart from the carbonyl the 4-OH can accept or donate a hydrogen bond in a network including the 2-, 6-, and 12-OHs. In epiryanoid A the network is altered by extension to the 8-OH and inversion of the C-10 substituent, changes which could affect the outcome for hydrogen bonding of the 4-OH. In extending the spectral study, we find that the shielding of the H-3 in ryanodine (δ 5.35) compared to epiryanoid A (δ 5.68) in $CDCl_3$ is not retained in CD_3COCD_3 or CD_3OD solution, with both appearing between δ 5.65 and 5.72, suggesting similar conformations in the more polar solvents.

For pyrrole-2-carboxylates conjugation requires planarity of the aromatic ring and carbonyl group leading to syn and anti conformations (Kaye et al., 1980). Hindered esters appear to favor the former. Further variation is possible for the ryanoids through rotation of the ester C-3 to oxygen bond, although steric interactions restrict the carbonyl to be syn with H-3. The syn conformations should show significant contacts between the pyrrole H-3 (H-24) and the polycyclic residue which are not predicted for the anti conformation. To test this proposition, we measured NOEs and find that in CD_3COCD_3 solution H-24 of both ryanodine and epiryanoid A shows NOEs to the methyl protons of C-20, to the upfield methyl of the isopropyl group, and to the downfield H-14, which is located by its NOE to H-13 of the isopropyl group. These results are interpreted to require the syn conformation (Figure 2). On the other hand, in $CDCl_3$ after D_2O exchange, although epiryanoid A retains all of the NOEs of the pyrrole proton listed above, none of these are detected for ryanodine in this solvent. The ryanodine structure can be viewed as forming two faces, i.e., a hydrophilic face bearing four hydroxyl groups and a relatively lipophilic face carrying the isopropyl and hemiketal bridge. In the syn conformation (Figure 2) the hydrocarbon part of the pyrrole ring extends the more lipophilic face as shown by the NOEs of H-24 with the protons of C-20, H-14, and one of the isopropyl methyls, whereas the carbonyl and imino groups of the pyrrolecarboxylate extend the hydrophilic face.

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