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9,21-Didehydroryanodine: a New Principal Toxic Constituent of the Botanical Insecticide Ryania

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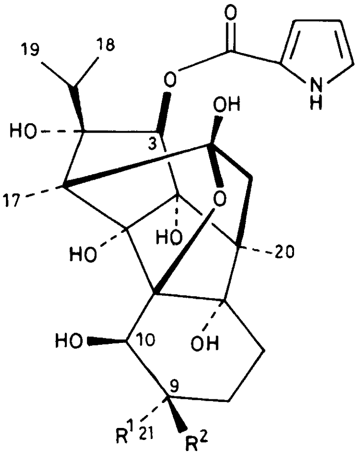
A major insecticidal constituent of *Ryania speciosa* is isolated and characterized by n.m.r. and mass spectroscopy as the new alkaloid 9,21-didehydroryanodine (**2**); reduction of (**2**) with hydrogen (or tritium) gas yields both ryanodine (**1**) and 9-epiryanodine (**3**) (or their 9,21-^{3}H analogues) in a 1 : 9 ratio.

Ryanodine (**1**), an insecticidal and toxic alkaloid from *Ryania speciosa*, was structurally defined about 15 years ago by chemical degradation[1] and *X*-ray crystallography.[2] Compound (**1**) has been considered to be the principal active constituent of the ground stemwood of this plant, which is used as ryania powder to control agricultural and garden pests.[3] Alkaloid (**1**) causes muscle contraction at very low concentration, possibly by dissociating the transverse tubules from the terminal cisternae of the sarcoplasmic reticulum.[4] Further investigation of ryanodine mode of action is presently hampered by (i) the presence of an unknown major contaminant in commercial ryanodine[5] and (ii) the lack of ryanodine radiolabelled to *high* specific activity. The present study identifies the contaminant (**2**) and uses this new natural product to prepare [^{3}H] (**1**).

Preparative h.p.l.c.† of commercial ryanodine‡ yielded two major components, (**1**) m.p. 235—237 °C (lit.[6] 229 °C) and (**2**) m.p. 175—179 °C. A fresh sample of powdered *R. speciosa* wood‡ was extracted with wet chloroform by partitioning with water[7] and the aqueous extract was directly purified by h.p.l.c.† This established the presence of (**2**) and (**1**) in a 3 : 1 ratio totalling about 0.2% (w/w) in content. Bioassays revealed that (**1**) and (**2**) are almost equitoxic on injection into houseflies and mice. On this basis (**2**) rather than (**1**) appears to be the principal toxic constituent in the ryania sample examined.



(**1**) R^1 = Me, R^2 = H
(**2**) R^1, R^2 = $=CH_2$
(**3**) R^1 = H, R^2 = Me

Analysis of the ^{1}H (Table 1) and ^{13}C n.m.r. spectra of (**1**) and (**2**) using one- and two-dimensional methods (details to be given elsewhere) established the identity of the new compound as 9,21-didehydroryanodine. In the ^{1}H n.m.r. spectrum of (**1**), there are five methyl resonances (17-H to

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† Altex Ultrasphere-ODS 4.6 × 250 mm; H_2O : MeOH, 1 : 1, 1 ml/min; 268 nm detection. Retention time (min) (**2**) 7.6, (**1**) 9.6, and (**3**) 11.4.

‡ Ryanodine was obtained from Penick Corp., Lyndhurst, New Jersey, and a fresh sample of powdered *R. speciosa* wood (ryania insecticide) was a generous gift from Progressive Agri-Systems, Inc., Stockertown, Pennsylvania 18083. In both cases the *R. speciosa* source was Trinidad.

Table 1. Selected ^{1}H n.m.r. spectroscopy δ values (300 MHz, CD_3OD).

	3-H	10-H	17-H	18/19-H		20-H	21-H	21-H_2
(**1**)	5.64s	3.80d	1.40s	0.76d	1.12d	0.90s	1.01d	
(**2**)	5.63s	5.48s	1.40s	0.75d	1.10d	0.90s		4.74d, 5.04d
(**3**)	5.63s	4.32d	1.41s	0.76d	1.13d	0.91s	1.12d	

21-H) and two distinctive methine signals: a doublet for 10-H and a singlet for 3-H. In the case of (**2**), one of the methyl doublets and the 10-H methine resonance near δ 3.8 are missing. These resonances are replaced by three new peaks in the δ 4.7—5.5 region, two of which exhibit a 2 Hz coupling. The rest of the spectrum is largely unchanged. The coupled peaks were assigned as the terminal methylene protons, and the new downfield singlet as the now allylic 10-H. The ^{13}C n.m.r. spectra support this assignment; two new low field resonances appear at δ 149.5 and 107.5, and in a DEPT[8] experiment ($\theta = 3\pi/4$) the δ 149.5 resonance is nulled and the δ 107.5 resonance becomes negative. Additionally fast atom bombardment mass spectroscopy shows a parent ion two mass units less than for (**1**). The i.r. spectrum for (**2**) is similar to that of (**1**), but is characterized by an additional C=C absorbance at 1545 cm^{-1} (KBr).

Compound (**2**) was reduced (H_2/Pd on carbon, tetrahydrofuran) to yield the expected (**1**) and 9-epiryanodine (**3**) m.p. 232—234 °C in a 1 : 9 ratio. Similar reduction with tritium gas enabled us to prepare [9,21-^{3}H] (**1**) at high specific activity (60 Ci mM^{-1}) complementing an earlier synthesis[9] of [pyrrole-^{3}H] (**1**) (3 Ci mM^{-1}).

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