Ryanodyl 3-(pyridine-3-carboxylate): A Novel Ryanoid from Rynia Insecticide

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J. Nat. Prod., **1991**, 54 (4), 1147-1149• DOI:
10.1021/np50076a043 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 4, pp. 1147-1149, Jul-Aug 1991

RYANODYL 3-(PYRIDINE-3-CARBOXYLATE): A NOVEL RYANOID FROM *RYANIA* INSECTICIDE

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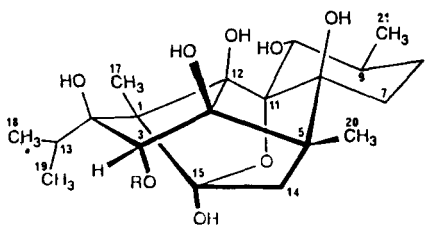
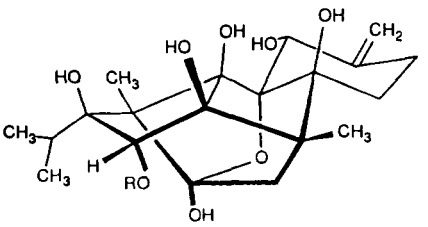
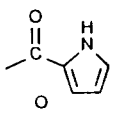
ABSTRACT.—Ryanodyl 3-(pyridine-3-carboxylate) [**3**] was isolated as a minor component from the wet $CHCl_3$ extract of *Ryania* insecticide, and its structure was assigned by chemical and spectroscopic methods. This compound is essentially inactive compared with ryanodine for insecticidal activity against *Musca domestica* adults and *Tribolium castaneum* larvae, for toxicity to mice, and for competition with [^{3}H]ryanodine at the Ca^{2+}-ryanodine receptor complex of skeletal muscle.

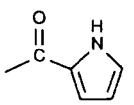
Ryania insecticide is the powdered stemwood of *Ryania speciosa* Vahl. (Flacourtiaceae), a small tree or shrub growing extensively in South America and noted for its pest control properties (1). The major insecticidal and toxic constituents are ryanodine [**1**] (2,3) and didehydroryanodine [**2**] (4), which have attracted much attention as natural but expensive insecticides and for their action at the Ca^{2+}-ryanodine receptor complex of muscle (5). Variations in the extractives of different batches of commercial *Ryania* are known to occur, and these appear to contain additional minor constituents that also warrant attention from both of these points of view (6,7). We therefore extracted a quantity of *R. speciosa* powder using published methods (2,7) and by a combination of chromatographic procedures have isolated a new crystalline ryanoid **3**.

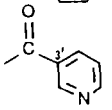
The ^{1}H- and ^{13}C-nmr spectra of **3** closely corresponded to those of **1** (8–10) with the exception of the aromatic resonances. This suggested that the alcohol portion, ryanodol [**4**], of **1** was retained but with a different esterifying group. More specifically, the signals characteristic of the pyrrole moiety were absent and instead, resonances were present whose ^{13}C shifts and ^{1}H shifts and couplings were readily assignable to a pyridine-3-carboxylate. The hrms of **3** requires $C_{26}H_{35}NO_9$ and is consistent with this proposal. The placement of the ester functionality at C-3 is corroborated by comparison of the chemical shift for the H-3 of **3** (δ 5.86) with that of **1** (δ 5.35). To verify the structure of **3**, a





1 R= 

2 R= 

3 R= 

4 R=H

small sample was hydrolyzed using aqueous base. Following workup, ryanodol and pyridine-3-carboxylic acid were isolated and structurally confirmed by direct comparisons with authentic standards.

The isolation of the new ester **3** of ryanodol [**4**] is particularly interesting in relation to biosynthetic considerations, since esterification at this position cannot be effected under normal conditions; indeed, the total synthesis of ryanodine had to be curtailed to that of ryanodol (11). Because the introduction of the pyrrole ester moiety in ryanodine probably occurs at a late stage in the biosynthesis, an alternative to direct esterification at the 3 position could perhaps involve esterification at the contiguous and relatively accessible 4-hydroxyl with later acyl migration.

In view of the interest in *Ryania* as a natural insecticide, the new ester **3** was tested for toxicity to *Musca domestica* L. adults, *Tribolium castaneum* (Herbst) larvae, and mice, and for displacement of [^{3}H]ryanodine from the Ca^{2+}-ryanodine receptor complex. In each case, the level of activity was much less than that of ryanodine (8), and this compound is therefore considered to contribute neither to the insecticidal activity nor to the mammalian toxicity of the commercial mixture. Spatial considerations alone are unlikely to account for the profound effect that replacing the pyrrole ring by the pyridine ring has on the biological activity. Other differences include the electron-rich nature of the pyrrole and its ability to provide a hydrogen bond.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc separation involved a Beckman 344-2 pump gradient system using a 10 mm × 250 mm Ultrasphere-ODS C-18 reversed-phase Si column and MeOH/H_2O mixture at 4 ml/min with detection at 265 nm. ^{1}H- and ^{13}C-nmr spectra were recorded with a Bruker WM-300 spectrometer; the solvent peaks were used as internal references. Hydroxyl peaks were confirmed by exchange with D_2O. Mass spectra were obtained using a Kratos MS-50 instrument. Radial chromatography was carried out using a model 8924 Chromatotron supplied by Harrison Research, Palo Alto, California, with one or two mm plates coated with Si gel 60 PF_{254} (Merck). Workup solutions were dried with anhydrous Na_2SO_4 and evaporated with a rotary evaporator.

ISOLATION OF RYANOIDS.—Powdered *R. speciosa* wood supplied by Agrisystems International, Wind Gap, Pennsylvania, was extracted using the wet $CHCl_3$ procedure (2,7) modified by using a larger quantity of water (800 ml/500 g) for more efficient extraction. The extractives were partitioned into H_2O and the aqueous phase was extracted with EtOAc. The EtOAc extract was separated from polar impurities by rapid filtration through Si gel in $CHCl_3$/MeOH (6:1) with 2% aqueous $MeNH_2$. The eluted material, in batches (0.4 g), was separated using $CHCl_3$-MeOH (20:6 to 6:1) with 2% aqueous $MeNH_2$ on a Chromatotron plate (2 mm Si gel). Ryanodyl 3-(pyridine-3-carboxylate) was present in a mixed fraction and was separated by hplc. The yield was ca. 50 mg/kg relative to the *Ryania* powder. Crystallization from Me_2CO/hexane gave **3** as prisms: mp 200–205°; ^{1}H nmr (Me_2CO-d_6) δ 9.21 (dd, 2.1, 0.9 Hz; H-2′), 8.85 (dd, 1.8, 4.5 Hz, H-6′), 8.41 (ddd, 1.8, 2.1, 7.8 Hz; H-4′), 7.61 (ddd, 1.0, 4.9, 7.8 Hz, H-5′), 5.86 (s, H-3), 3.85 (m, H-10), 2.60 and 2.08 (AB, 13.8, H_2-14), 2.35 (m, H-13), 1.82 (m, H-9), 2.12 (m, H_{ax}-7), 1.58 (m, H_{eq}-7), 1.50 (m, H_{ax}-8), 1.26 (m, H_{eq}-8), 1.47 (s, H_3-17), 1.12 (d, 6.6 Hz, H_3-21), 0.88 and 0.78 (each d, 6.5 and 6.6 Hz, H_3-18 and H_3-19); ^{13}C nmr (Me_2CO-d_6) δ 164.7 (C=O), 154.6 (C-6′), 151.1 (C-2′), 137.8 (C-4′), 126.6 (C-3′), 124.7 (C-5′), 65.3 (C-1), 83.4 (C-2), 92.2 (C-3), 91.7 (C-4), 49.1 (C-5), 85.9 (C-6), 26.7 (C-7), 28.9 (C-8), 34.8 (C-9), 72.1 (C-10), 86.9 (C-11), 96.3 (C-12), 30.4 (C-13), 41.8 (C-14), 102.8 (C-15), 10.1 (C-17), 18.7 and 18.6 (C-18, C-19), 12.5 (C-20), 19.3 (C-21). Protonated carbons were assigned by the INVCOR technique (12).

HYDROLYSIS OF **3**.—The ester (21 mg) was hydrolyzed by refluxing for 5 h in 5% KOH/MeOH. After addition of aqueous HCl to pH 4, the solution was evaporated and KCl separated with MeOH. The organic residue was filtered through Si gel in $CHCl_3$-MeOH (9:1) with 2% aqueous $MeNH_2$ to give ryanodol (10 mg), identified by R_f and ^{1}H-nmr spectrum. Later chromatographic fractions contained pyridine-3-carboxylic acid (3 mg) identified by tlc and ^{1}H nmr (D_2O).

BIOLOGICAL TESTING.—Compounds **1** and **3** were compared for toxicity to housefly adults (*M. domestica*) treated by injection following topical application of the synergist piperonyl butoxide,

for toxicity to mice treated intraperitoneally, and for inhibition of [^{3}H]ryanodine binding in a rabbit skeletal muscle preparation of the Ca^{2+}-ryanodine receptor complex (8). An additional test involved the inhibition of growth and development of first instar flour beetle larvae (*T. castaneum*) by dietary incorporation (13). In all of the above tests the new ester was essentially inactive compared with ryanodine.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grant PO1 ES00049. The biological assays were made by Richard Grendell and Judith Engel of this laboratory and Isaac Pessah of the University of California at Davis. We thank our laboratory colleague, Neil Jacobsen, for assistance with the nmr measurements. Mass spectra were recorded at the Chemistry Department, University of California, Berkeley.

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Received 17 December 1990