show identical temperature dependences (indicated by circle and triangles in Fig. 2, open for UpUpU, filled for UpUpC). The third U resonance of UpUpU has a temperature dependence very similar to that of the C resonance in UpUpC; we assign this third U resonance (indicated by open squares in Fig. 2) to the uracil at the 3′ wobble end of UpUpU. This assignment is valuable for a study of the relative binding constants of the three bases of UpUpU and UpUpC to the anticodon region of $tRNA^{Phe}$.

In order to elucidate the source of these temperature-dependent chemical shifts in terms of molecular structure, we have examined the n.m.r. spectra of the relevant 5′-nucleotides, nucleosides, and dinucleoside phosphates under the same conditions. For each of U, C, 5′-UMP, and 5′-CMP the resonances due to the protons at position-6 moved to higher magnetic field (lower chemical shift) with increasing temperature, at rates comparable to those shown in Fig. 2, whereas the resonances due to the protons at position-5 did not vary appreciably with temperature. Similar behavior was observed for both bases of each of UpU and CpU. Schweizer *et al.* have demonstrated that substitution of a charged phosphate group at position-5′ in U or C causes a specific downfield shift (deshielding) of the resonance due to the proton at position-6, while causing no appreciable change in the resonance due to the proton at position-5 (3). They presented strong evidence that this was due to the bases being in the *anti* conformation relative to the 5′-position—that is, with the base rotated about the glycosyl bond so that the 5,6 side of the pyrimidine ring is directed towards the 5′-position. In this conformation the proton at position-6 of the pyrimidine ring is much nearer the 5′-position than is the proton at position-5 of the pyrimidine ring. A comparison of earlier 30 °C nuclear magnetic resonance spectra of pUpU and UpU (4) suggests that the effect of a monocharged 5′-phosphodiester linkage is similar to that of a 5′-hydroxyl group. The observed temperature dependences reported here for 5′-UMP and 5′-CMP are consistent with the removal of a specific deshielding effect by a gradual conversion of the conformations from *anti* to *syn*. Our observation that U, C, UpU, and CpU all exhibit behavior similar to that of 5′-UMP and 5′-CMP indicates that the 5′-hydroxyl group also has a specific deshielding effect on the protons at position-6, which is considerably weaker than that due to a doubly-charged 5′-phosphate but comparable to that due to a singly-charged 5′-phosphodiester group. Thus, the temperature dependences of the resonances due to the U and C moieties of UpUpU and UpUpC suggest that in the trinucleoside diphosphates at physiological temperatures all the bases are predominantly in the *anti* conformation.

Acknowledgment

We are indebted to Dr. R. G. Shulman and Dr. M. P. Schweizer for helpful discussions.

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Synthetic approach to ryanodine; the use of *O*-spirodienone lactone in organic synthesis

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The key steps for this synthetic approach are the preparation of *o*-spirodienone lactone **15**, its reaction with methylvinyl ketone which gave **16** and **17**, and the conversion of **16** and **17** into compound **19** by base. Finally ozonolysis of **26** followed by internal condensation gave compound **28**.

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The insecticide ryanodine **1** isolated from *Ryana speciosa* Vahl is the ester of pyrrole-α-carboxylic acid with ryanodol **2**. The structure elucidation of this complex insecticide and one of its key degradation products, anhydroryanodol **3** was solved by Valenta and Wiesner and their

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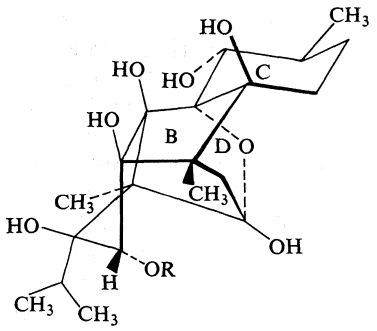
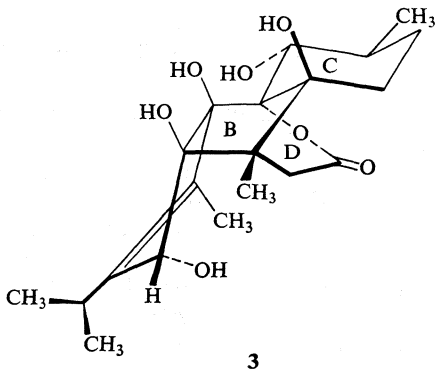


2 R = H



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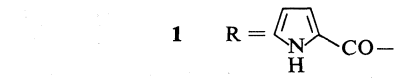

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co-workers (1). Recently, an X-ray analysis on ryanodol-*p*-bromo benzyl ether achieved by Srivastava and Przybylska (2) has confirmed the proposed structure for ryanodine except that the configuration of the isopropyl side chain was found to be the opposite.

In this communication, we wish to describe a synthetic approach which appears to be an attractive scheme for the syntheses of ryanodine and its closely related degradation product **3**.

5,6-Dimethoxyindane **4** which is readily available from veratraldehyde (3) was treated with bromine in carbon tetrachloride at room temperature and gave bromoindane **5**. Reaction of **5** with magnesium in ether in presence of methyl iodide gave the expected Grignard derivative of **5** which by reaction with triethyl orthoformate followed by acid hydrolysis gave methoxyphenolaldehyde **6**[2,3,4] (m.p. 118–119 °C, 75% yield from 4).

Reaction of **6** with boron tribromide in dichloromethane (4) gave the *o*-phenol **9** (m.p. 178 °C, 80% yield). Treatment of **9** with bromoacetyl bromide in benzene and pyridine gave bromoacetate **10** (m.p. 110 °C, 70% yield) which was transformed into lactone aldehyde **11** (m.p. 131–132 °C, 75% yield) in boiling tetrahydrofuran containing potassium carbonate.[5]

Wolff–Kischener reduction of lactone aldehyde **11** gave lactone **14** (m.p. 74–75 °C; 80% yield). Treatment of lactone **14** with one mole of sodium hydroxyde in acetonitrile–water followed by the addition of one mole of *N*-bromosuccinimide yielded the stable *o*-spirodienone lactone **15** (m.p. 97–98 °C; 90% yield; mol. wt. (mass spectroscopy) 220; ν_{max} 1790, 1665, 1640, and 1600 cm^{-1}; λ_{max} (EtOH) 355 mμ (3100); τ 3.97 (1H, singlet, H-1), 5.25 and 5.60 (2H, double doublet, J = 15 c.p.s., H-2 and H-3) and 8.10 (3H, singlet, CH_3)).

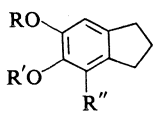
Compound **15** was refluxed for 5 min in methylvinyl ketone and gave the tricyclic compounds **16** and **17** (ratio 1 ~ 1) which could be separated by preparative thin-layer chromatography. **16** (oil; mol. wt. (mass spectroscopy) 290; ν_{max} 1810, 1740, and 1720 cm^{-1}; τ 5.34 and 5.74 (2H, double doublet, J = 14 c.p.s., H-3 and H-4), 6.44 (1H, doublet, J = 2 c.p.s., H-1), 6.93 (1H, octuplet, J = 2, 6, and 9 c.p.s., H-2), 7.87 (3H,

[2]All new crystalline compounds gave satisfactory analytical and mass spectral (mol. wt.) data. Infrared (dichloromethane), ultraviolet, and nuclear magnetic resonance (deuteriochloroform with tetramethylsilane as internal standard) spectra are consistent with the structures proposed. All yields reported have been calculated after purification by crystallization.

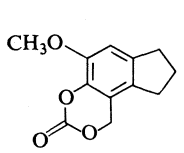
[3]A specific cleavage of one methoxyl group has occurred during the Grignard reaction. Discussion concerning this unexpected result will be included in our article.

[4]Reduction of **6** with lithium aluminium hydride gave phenol alcool **7** (m.p. 100 °C) which was transformed into the carbonate **8** (m.p. 146 °C) by reaction with phosgene in benzene containing pyridine. This result shows that the methoxyl group remaining in **6** is at C-6.

[5]A rigorous chemical proof for lactone aldehyde **11** was obtained in the following way. Lactone aldehyde **11** was transformed into compound **12** (m.p. 99–100 °C) by methylation with dimethyl sulfate in aqueous base followed by esterification with diazomethane. Alkylation of the known methoxy phenol aldehyde **6** with ethyl bromoacetate in tetrahydrofuran containing potassium carbonate gave compound **13** (m.p. 83–84 °C) which by hydrolysis in aqueous base followed by diazomethane esterification gave a compound which was found identical with compound **12**.



4 R = CH_3, R′ = CH_3, R″ = H
5 R = CH_3, R′ = CH_3, R″ = Br
6 R = CH_3, R′ = H, R″ = CHO
7 R = CH_3, R′ = H, R″ = CH_2OH
9 R = H, R′ = H, R″ = CHO
10 R = $BrCH_2CO$, R′ = H, R″ = CHO
12 R = CH_3, R′ = CH_3OOCCH_2, R″ = CHO
13 R = CH_3, R′ = $C_2H_5OOCCH_2$, R″ = CHO



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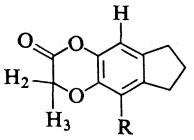
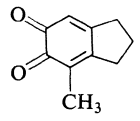


11 R = CHO
14 R = CH_3



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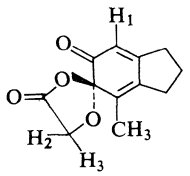

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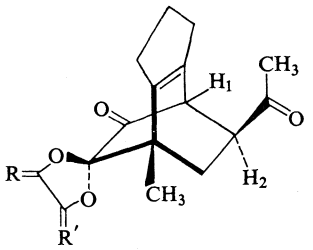


16 R = H_3, H_4, R′ = O
17 R = O, R′ = H_3, H_4

singlet, CH_3CO) and 8.75 (3H, singlet, CH_3)), and **17** (m.p. 113–115 °C; mol. wt. (mass spectroscopy) 290; ν_{max} 1810, 1740, and 1720 cm^{-1}; τ 5.34 and 5.68 (2H, double doublet, J = 14 c.p.s., H-3 and H-4), 6.36 (1H, doublet, J = 2 c.p.s., H-1), 6.83 (1H, octuplet, J = 2, 6, and 9 c.p.s., H-2), 7.83 (3H, singlet, CH_3CO) and 8.74 (3H, singlet, CH_3)).

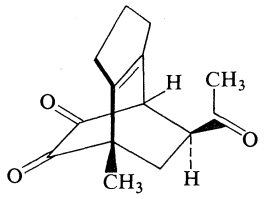
Treatment of an ethereal solution of the mixture **16** and **17** with aqueous base gave the liquid triketone **18**.[6] Treatment of compound **18** with aqueous tetrahydrofuran containing sodium hydroxide at room temperature gave the tetracyclic hydroxydiketone **19** (m.p. 69–70 °C; mol. wt. (mass spectroscopy) 232; ν_{max} 3520, 1745, and 1720 cm^{-1}; τ 6.66 (1H, doublet, J = 4 c.p.s., H-1), 7.50 (2H, singlet, H-2, and H-3)[7] and 8.73 (3H, singlet, CH_3)). Compound **19** was best prepared in 70% overall yield by treating directly the crude mixture **16** and **17** with the basic treatment described above. Consequently, by a simple three-step procedure (**14** → **15** → **16** and **17** → **19**), a high yield of the complex tetracyclic hydroxydiketone **19** can be obtained from the simple aromatic compound **14**.[8] Compound **19** was transformed into the keto-acetate **21** (m.p. 128–130 °C, 90% yield) by a 15 min reflux in pyridine – acetic anhydride and was selectively ketalized with ethylene glycol, benzene, and *p*-toluenesulfonic acid to give ketal acetate **22** (m.p. 149–152 °C; 90% yield). Reaction of ketal acetate **22** with lithium borohydride in tetrahydrofuran at room temperature followed by addition of lithium aluminium hydride and reflux gave the *trans*-diol **23**[9] (m.p. 145–147 °C; 80% yield). Reaction of *trans*-diol **23** with pyridine in acetic anhydride at room temperature for 3 h gave the liquid acetate **26**.

[6]The formation of **18** from **16** and **17** combined with the spectral data confirms the position of the methyl ketone side chain in **16** and **17** as shown but does not determine the configuration of this side chain. The methyl ketone side chain has been shown to be *syn* to the double bond by several deuterium exchange experiments. Those results will be included in our article.

[7]The singlet at 7.50 τ was shown to correspond to H-2 and H-3 by deuterium exchange experiments.

[8]Compound **15** is crystalline and stable at room temperature; consequently, the *o*-spiro lactone ketal blocking group has a high stabilizing effect since the corresponding *o*-quinone **20** is unstable at room temperature. Furthermore, such blocking group on the *o*-quinone permits a very stereospecific Diels–Alder reaction with an unsymmetrical dienophile. The preparation of compound **20** will be reported in our article.

[9]A chemical reduction of ketal acetate **22** with sodium in liquid ammonia containing ethanol gave two compounds which were separated by column chromatography. The minor product was the *trans*-diol **23** and the major product, the *cis*-diol **24** (m.p. 115–117 °C). The diol **24** was shown to be the *cis* isomer in the following way: reaction of **24** with phosgene in benzene containing pyridine gave the carbonate **25** (m.p. 102–103 °C; 85% yield).



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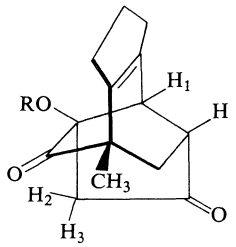
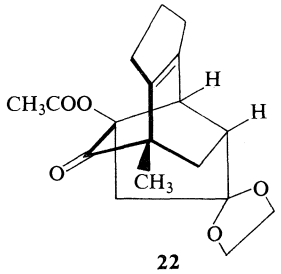


19 R = H
21 R = CH_3CO



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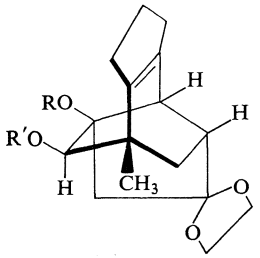
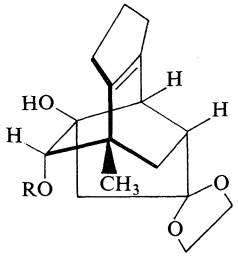
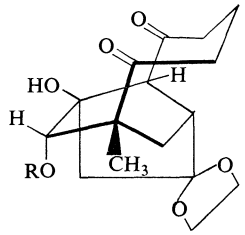


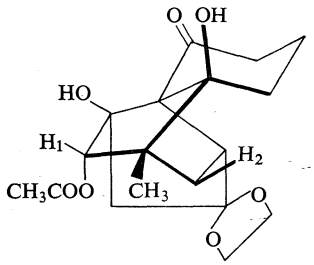
24 R = H, R′ = H
25 R, R′ = C = O



23 R = H
26 R = CH_3CO



27 R = CH_3CO



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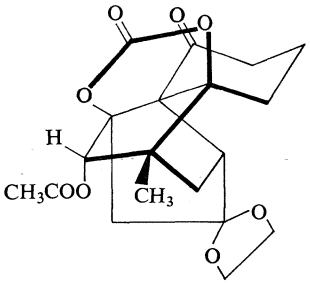

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The entry into a ring skeleton related to ryanodine and its degradation product **3** was achieved in the following way: ozonolysis of **26** in methanol at room temperature followed by catalytic reduction with palladium-on-charcoal gave crude diketone **27** which underwent internal aldol condensation by filtration through silica gel to give dihydroxyketo acetate **28** (m.p. 176–177 °C; 55% yield from **23**; ν_{max} 3450, 1735, and 1700 cm^{-1}; τ 4.75 (1H, doublet, J = 2 c.p.s., H-1)[10], 6.10 (4H, multiplet, OCH_2CH_2O), 7.78 (3H, singlet, CH_3CO) and 9.00 (3H, singlet, CH_3)).

Compound **28** gave the six-membered carbonate **29** by reaction with phosgene in benzene containing pyridine (m.p. 204–205 °C; 85% yield; ν_{max} 1755, 1740, and 1715 cm^{-1}).

Compound **28** contains in principle all the necessary functional groups for the complete elaboration of rings A, B, C, D, and E of ryanodine and its degradation product **3**. Work in this direction and in the synthesis of new polycyclic systems of the type **19** by using different dienophiles with **15** is now in progress.

Acknowledgment

Financial support by the National Research Council of Canada is gratefully acknowledged.

[10]H-1 appears as a doublet due to W coupling (5) with H-2. By irradiation at 8.2–8.4 τ, the doublet at 4.75 τ collapsed into a singlet.

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