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THE STRUCTURE OF RYANODINE. I[1]

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Abstract

Ryanodine consumes one mole of periodic acid and gives oxoryanodine $C_{25}H_{33}O_9N$. Splitting off water from ryanodine gives anhydroryanodine $C_{25}H_{33}O_8N$. The analyses of these two derivatives are therefore in agreement with the formula for ryanodine $C_{25}H_{35}O_9N$. Alkaline hydrolysis of ryanodine gives pyrrole-α-carboxylic acid and an alcohol $C_{20}H_{32}O_8$. This alcohol splits off water to give a compound $C_{20}H_{30}O_7$. Alkaline hydrolysis of oxoryanodine consumes three moles of alkali and gives a mixture of low molecular acidic products. Alkaline fusion of oxoryanodine gives a neutral aromatic nitrogen-free crystalline substance.

Folkers and associates (1) have isolated from the flacourtiaceous plant *Ryania speciosa* Vahl. the insecticidal principle ryanodine. They formulated it tentatively as $C_{25}H_{35}NO_9$ or $C_{26}H_{37}NO_9$. It melts at 219-220°C., is neutral to litmus, and shows six or seven active hydrogens. The ultraviolet absorption maximum is at 268.5 $m\mu$.

We have repeated the isolation of ryanodine and the substance, purified by a nine-funnel countercurrent distribution between ether and water, analyzed reasonably well for $C_{25}H_{35}NO_9$. This formula is substantiated by two derivatives which are much more easily purified and analyzed.

Upon oxidation with periodic acid or lead tetraacetate, ryanodine consumes one mole of each reagent, respectively, and gives a compound which we call oxoryanodine $C_{25}H_{33}NO_9$, m.p. 227°C. No volatile carbonyl compound could be detected.

Oxoryanodine can be differentiated from ryanodine by its sparing solubility in alcohol, from which it can be obtained in high purity. Treatment of ryanodine with acids in ether, for instance sulphuric acid, gives a beautiful crystalline compound, anhydroryanodine $C_{25}H_{33}NO_8$, m.p. 275°C., which can be sublimed without decomposition for analysis.

Both oxoryanodine and anhydroryanodine have ultraviolet spectra identical with ryanodine. The infrared spectrum of ryanodine shows a single carbonyl peak at 1684 cm.$^{-1}$. Oxoryanodine, in addition to this, shows a second carbonyl

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peak at 1719 cm.$^{-1}$. Anhydroryanodine shows in addition to the original carbonyl peak a second one at 1735 cm.$^{-1}$. The splitting off of water is therefore connected with the appearance of a new carbonyl group.

However, the group splitting off water is different from the grouping which is oxidized by periodic acid since anhydroryanodine consumes, as does ryanodine, one mole of periodic acid or lead tetraacetate.

The ultraviolet spectrum of ryanodine is, as can be seen from Fig. 1, closely related to the spectrum of pyrrole-α-carboxylic ester. Also, the infrared spectra of both compounds show several parallel bands. Alkaline saponification of ryanodine results in the consumption of one mole of alkali and gives pyrrole-α-carboxylic acid, identified by the melting point of the ester and its ultraviolet spectrum.

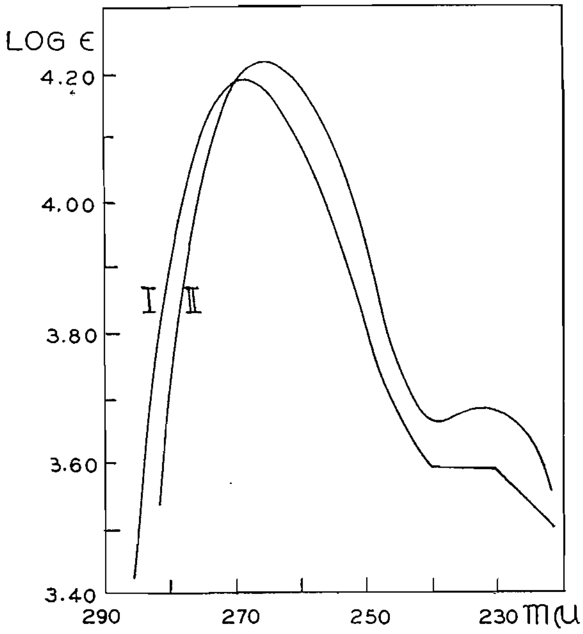

FIG. 1. *Ultraviolet spectra in alcohols. I. Ryanodine. II. Pyrrole-α-carboxylic methyl ester.*

Prolonged continuous extraction of the alkaline hydrolysis mixture gives a beautifully crystalline compound, m.p. 252°C., which analyzes well for $C_{20}H_{32}O_8$. Active hydrogen determinations on this compound show a content of five or possibly six active hydrogens. A microhydrogenation failed to show the presence of double bonds. The compound has no ultraviolet absorption. The infrared spectrum of the compound suggests, in spite of the results of the microhydrogenation, the presence of double bonds, possibly of the $R_2-C=CH-R$ and $RHC=CHR$ type and besides this, of course, a strong peak due to bonded —OH is observed. The compound does not show carbonyl absorption.

If the aqueous solution of the C_{20} alcohol is acidified, the compound can be extracted in a much shorter time on a continuous extractor. The compound thus obtained is difficult to crystallize but crystallizes on sublimation in high vacuum and melts then at 220°C. It analyzes for $C_{20}H_{30}O_7$. The infrared spectrum of this compound shows a carbonyl peak at 1720 cm.$^{-1}$. Although this is not the exact value of the second carbonyl peak of anhydroryanodine, it seems reasonable to assume that the two compounds are in the same relationship as ryanodine and anhydroryanodine.

Alkaline saponification of oxoryanodine was also investigated. Somewhat surprisingly, the compound consumes three moles of alkali and gives a mixture of low molecular acidic products.

Alkali fusion of oxoryanodine gives besides pyrrole-α-carboxylic acid a neutral substance, m.p. 158°C. (in a low yield), which can be obtained in beautiful crystals and sublimed for analysis.

The ultraviolet (λ_{max} 258, 280 log ϵ 4.00, 3.90) and infrared spectrum point to an aromatic compound.

The analysis checks best to $C_{12}H_{14}O_2$ although the formula $C_{18}H_{20}O_3$ has still to be considered.

Experimental Part

Ryanodine

The amyl acetate residue (6 gm.), prepared from 2400 gm. of *Ryania* powder according to the procedure of Folkers *et al.* (1), was dissolved in 300 ml. of ether and placed in a separatory funnel. Into a second and a third separatory funnel were placed 300 ml. of ether. After shaking the first funnel with 300 ml. of water, the water layer was transferred to the second funnel and a new 300 ml. portion of water was added to the first funnel. The process was repeated as in an ordinary countercurrent distribution until six portions of water had passed through the three funnels. The combined water layers were then evaporated *in vacuo* to a smaller volume and extracted six times with ether. The ether solution was evaporated to dryness, yielding 3 gm. of pure white foam. This material crystallized easily from ether, and after four recrystallizations attained a constant melting point of 228-229°C. It was dried at 80°C. for 24 hr. in high vacuum. Calc. for $C_{25}H_{35}NO_9$: C, 60.84; H, 7.15; N, 2.84%. Found: C, 60.44; H, 7.28; N, 2.88; active H, 1.36%.

Titration with lead terraacetate or periodic acid gave a consumption of 0.83 mole of either reagent in 15 min. An extended reaction period did not alter this consumption.

Anhydroryanodine

Pure ryanodine (310 mgm.) was dissolved in 300 ml. of absolute ether and 0.5 ml. of concentrated sulphuric acid and 9 gm. of anhydrous copper sulphate were then added. The mixture was allowed to stand for three days. At the end of this period the acid was neutralized by adding powdered sodium carbonate, and the ether solution was washed with a 5% sodium carbonate solution. The aqueous washings were extracted several times with ether, and the combined and dried ether extracts were taken to dryness. In this way 245 mgm. of a substance was obtained, which, after four crystallizations from methanol–chloroform, melted at 275°C.; further recrystallization did not alter the melting point. For analysis the compound was sublimed in high vacuum at 190°C. The melting point of the sublimate was 275°C. Calc. for $C_{25}H_{33}NO_8$: C, 63.14; H, 7.00; N, 2.95%. Found: C, 62.63, 62.65, 63.12; H, 6.96, 6.82, 7.17; N, 2.99, 2.89; active H, 1.26%.

Oxoryanodine

Ryanodine (250 mgm.) was dissolved in 5 ml. of methanol and to this solution was added 5 ml. of 0.5 *M* periodic acid and 2 ml. of water. After approximately ten minutes, oxoryanodine started to crystallize in shining rodlike crystals. In two hours the crystals were collected on a filter and washed with water. Upon drying the yield was 243 mgm. and the melting point 205-207°C. After six recrystallizations from methanol the compound melted constantly at 227°C. and gave a strong depression with ryanodine. For analysis it was dried at 110°C. for 24 hr. in high vacuum. Calc. for $C_{25}H_{33}NO_9$: C, 61.09; H, 6.77; N, 2.85%. Found: C, 61.26, 60.76, 60.69; H, 6.76, 6.64, 6.58; N, 3.21, 2.99, 3.33, 2.82; active H, 1.21%.

A nine-funnel countercurrent distribution of oxoryanodine between ether and water (200 ml. of each phase) gave the following result:

Funnel No.	Weight of substance
1	868 mgm.
2	67
3	1
4, 5, 6, 7, 8, 9	0

Alkali Fusion

Oxoryanodine (4.5 gm.) was added in small portions with stirring to 30 gm. of fused potassium hydroxide at 320-330°C. in a nickel crucible. After cooling, the melt was dissolved in 250 ml. of water and ice, and the solution was continuously extracted with ether for 24 hr. The ether extract was dried over anhydrous sodium sulphate, the greater portion of the ether was removed by distillation through a 12-in. Vigreaux column, and the final traces of solvent were removed *in vacuo* at 10°C. The residue (167 mgm.) was dissolved in ether and this solution was chromatographed in ether on alumina. The residue from the eluate (98 mgm.) was sublimed at 40-60°C./0.15 mm. Part of the oily sublimate crystallized from petroleum ether in red-coated warts. This material (20 mgm.) was recrystallized twice and sublimed at 110°C./0.015 mm. to give white needles which melted at 158°C. Calc. for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42%; calc. for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09%. Found: C, 75.35; H, 7.18%.

The aqueous solution, which had been continuously extracted with ether, was acidified to Congo red with sulphuric acid and then extracted continuously with ether for 24 hr. After removal of the ether, 2.5 gm. of a dark resinous material was obtained. This material was sublimed at 80°C./0.15 mm. to give a white solid. Three recrystallizations from ether – petroleum ether and resublimation at 95°C./0.015 mm. gave a pure substance which sublimed at 175-180°C. when subjected to a melting point determination. A positive test was obtained with alcoholic ferric chloride solution. Calc. for $C_5H_5NO_2$: C, 54.05; H, 4.53; N, 12.61%, Found: C, 54.15, 54.31; H, 4.40, 4.28; N, 12.54, 12.72%. The ultraviolet spectrum in alkaline alcohol showed λ_{max} 253 mμ; log ϵ 4.14 and in acidic alcohol showed λ_{max} 264 mμ; log ϵ 4.23.

The acidic substance (124 mgm.) was converted to the methyl ester with methanol and an ethereal solution of diazomethane. The product was recrystallized once from ether – petroleum ether, and then sublimed at 50°C./0.015 mm. to give beautifully crystalline rods, m.p. 73-74°C. alone and in admixture with an authentic specimen of the methyl ester of α-pyrrole carboxylic acid. Calc. for $C_6H_7NO_2$: C, 57.60; H, 5.64; N, 11.19%. Found: C, 57.59, 57.33; H, 5.31, 5.20; N, 10.87, 10.71%.

Saponification of Ryanodine

Ryanodine (2.7 gm.) was refluxed with 50 ml. of 5% alcoholic potassium hydroxide for six hours. After evaporation of the alcohol *in vacuo,* 50 ml. of water was added and the solution extracted continuously with ether for a week. Evaporation of the ether gave a white foam which crystallized in beautiful prisms from ether. The yield was 1.4 gm. After six crystallizations the m.p. was sharp and constant at 252°C. It was dried for analysis at 80°C. in high vacuum. Calc. for $C_{20}H_{32}O_8$; C, 59.97; H, 8.07%. Found: C, 59.97, 59.90; H, 7.95, 7.99%; active H, 1.27%; N, 0.00%.

Microhydrogenation: no uptake in glacial acetic acid with Adams catalyst.

From the exhausted residue pyrrole-α-carboxylic was extracted after acidification and identified by spectrum and mixed melting point of the methyl ester.

In another experiment performed analogously the hydrolysis mixture was immediately acidified, and extracted six times with ether which removed the pyrrole-α-carboxylic acid.

After this an 18-hr. continuous extraction gave a quantitative yield of a white foam which could only be precipitated as a powder by petroleum ether from ether and after a few reprecipitations crystallized on sublimation (m.p. 220°C.) Calc. for $C_{20}H_{30}O_7$: C, 62.80; H, 7.92%. Found: C, 62.53; H, 7.87%; N, 0.00%.

The analyses were performed in the microanalytical laboratory of Dr. R. Dietrich, Zurich.

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Reference

1. Rogers, E., Koniuszy, F. R., Shavel, J., and Folkers, K. J. Am. Chem. Soc. 70: 3086. 1948.