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FINAL REPORT

Covering the Period

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Prepared By

Henry Rapoport

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Final Report, 1 Mar 63, pp. 8
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I. Summary

Oxidation of dihydrosaxitoxin with periodate proceeds at alkaline pH's with the consumption of one mole of oxidant. The product is a weakly basic amine; no formaldehyde was found.

Oxidation of saxitoxin with concentrated hydrogen peroxide gives guanidine and β -guanidinopropionic acid. With dilute hydrogen peroxide, a new compound, $C_9H_{11}O_2N_6Cl$, is obtained.

The action of phosphorus and hydrogen iodide on ryanodol leads to a γ -lactone as the main product plus several other crystalline products. The γ -lactone is most likely a substituted indan on the basis of spectrophotometric studies.

Oxidation of ryanodol with periodate proceeds to an oxidation product from which formic and isobutyric acids can be cleaved.

II. Discussion

A. Chemistry of Saxitoxin

During this period, our entire emphasis was on the oxidative degradation of saxitoxin in order to obtain breakdown products of value in the structural determination. Three procedures were examined, viz., oxidation with periodate, drastic oxidation with hydrogen peroxide, and mild oxidation with hydrogen peroxide.

1. Oxidation with Periodate

Since previous work indicated a rather indiscriminate and continuous oxidation of saxitoxin by periodate, dihydrosaxitoxin was used instead. Dihydrosaxitoxin, obtained by catalytic hydrogenation of pure saxitoxin, was treated with periodic acid at several pH levels. Contrary to previous reports, we found that no appreciable oxidation took place between pH 1 to 5. At pH 7.5, consumption reached one mole and optimal conditions were established as pH 8 where 1 to 1.2 moles were consumed in a forty-eight hour period, after which there was no further consumption.

After a preparative-scale oxidation, the reaction mixture was rectified by ion exchange procedures. No formaldehyde was found, and the reaction product appeared to be mostly a weakly basic amine which could not be obtained crystalline.

2. Drastic Oxidation with Hydrogen Peroxide

Guanidinopropionic acid has been reported as resulting from the action of hydrogen peroxide on saxitoxin. Since this is an important degradation product, we have repeated this reaction, looking for other products as well.

Saxitoxin was oxidized with 12% hydrogen peroxide at pH 5 for one hour at 90°. By chromatographic procedures, the reaction product could be separated into guanidine and β -guanidinopropionic acid in estimated yields of 80 and 30%, respectively.

3. Mild Oxidation with Hydrogen Peroxide

One of the most interesting reactions which saxitoxin undergoes is oxidation by oxygen in the presence of alkali. This reaction is of particular interest since the reaction mixture soon develops an ultraviolet absorption at 334 μ , indicating the possible formation of an aromatic heterocycle. Repeated attempts to isolate specific compounds from such oxidation mixtures have failed. Therefore, we made a detailed examination of the oxidation of saxitoxin by dilute hydrogen peroxide over the pH range 1 to 13 to see if a similarly absorbing substance could be obtained.

From very carefully controlled oxidations, both on the acid and alkaline side, a crystalline compound can be isolated in about 35% yield. This material

$[C_9H_{11}O_2N_6]^+Cl^-$, appears to be an aromatic heterocycle on the basis of its ultraviolet spectrum: $\lambda_{max}^{H^+}$ 211 μ (ϵ 14,200), 237 (11,100), 263 (6140), 324 (21,000); $\lambda_{max}^{OH^-}$ 218 μ (ϵ 10,400), 254 (14,000), 347 (17,400).

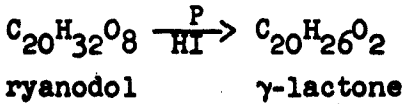
B. Chemistry of Ryanodine

During this period, most of our effort was spent on a detailed investigation of the aromatic products resulting from the action of phosphorus and hydrogen iodide on ryanodol. In addition to the major product, the γ -lactone, several other aromatic compounds also have been isolated. Continued work with the periodate product from ryanodol has resulted in a new C_{13} compound.

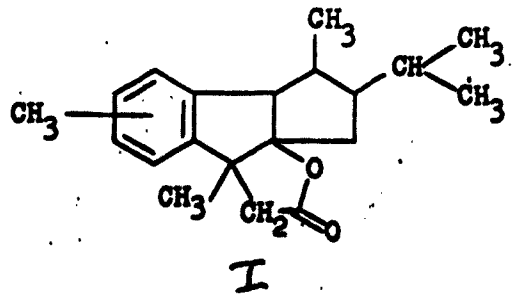
1. The Action of Phosphorus and Hydrogen

Iodide on Ryanodol

When ryanodol is heated with phosphorus and hydrogen iodide in glacial acetic acid, a number of products are obtained, the chief of which is a γ -lactone.



This γ -lactone has been tentatively assigned the structure I, mostly on the basis of spectro-



photometric evidence. Its ultraviolet absorption is consistent with a substituted indan structure and in the infrared it clearly shows γ -lactone absorption. The other assignments have been made on the basis of nuclear magnetic resonance studies. These studies and assignments are consistent with those published by Valenta et al., *Experientia*, 15, 111 (1962).

2. Oxidation of Ryanodol

On treatment with periodate, ryanodol consumes three moles of oxidant to give a "3 mole product." This oxidation product, on mild hydrolysis, gives a C_{19} -acid and formic acid. More drastic hydrolysis of the C_{19} -acid gives isobutyric acid and a new, C_{15} -compound.

