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THE STRUCTURE OF RUBRATOXIN A, A TOXIC METABOLITE OF PENICILLIUM RUBRUM

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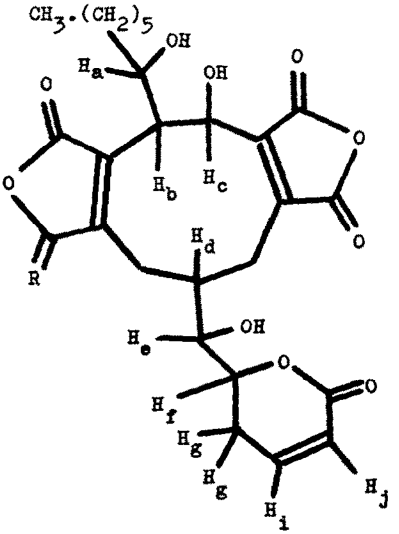
Strains of *Penicillium rubrum* liberate into the culture solution at least two toxic metabolites which have been isolated as crystalline compounds (1, 2). The structure of Rubratoxin B, $C_{26}H_{30}O_{11}$ **(I), has recently been described (3) and the** arguments for proposing that Rubratoxin A, $C_{26}H_{32}O_{11}$, **has structure (II) are outlined in this paper.**



(I) R = =O

(II) R = H, OH

The stereochemistry is not implied.

The highest peak in the mass spectrum of Rubratoxin A **occurs at m/e** 458 **and corresponds to** $C_{25}H_{30}O_8^+$ **(requires** 458.194, **found** 458.193 **). The elemental analysis, however, suggests that this ion represents a loss of carbon dioxide and water from the molecular ion and that the empirical formula of Rubratoxin A should be**

$C_{26}H_{32}O_{11}$ (found C 59.7, H 6.1, requires C 60.0, H 6.2). Although it has not been possible to prepare a pure crystalline acetate, the impure product from the reaction with excess acetic anhydride in pyridine gives a peak in the mass spectrum at m/e 688 corresponding to $C_{34}H_{40}O_{15}$ (found 688.237, requires 688.237) which is required for the tetra-acetate of Rubratoxin A.

A detailed comparison of the nuclear magnetic resonance spectra of Rubratoxins A and B (Table I) shows that the molecules have many features in common. The most striking difference is the presence of a broad one proton singlet at τ 4.3 in the spectrum of Rubratoxin A which is absent in that of Rubratoxin B. This signal is sharpened after the sample has been treated with D_2O and can be assigned to an isolated hemi-acetal derivative.

TABLE I

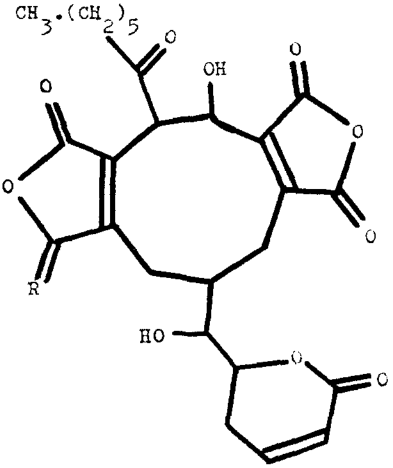
Nmr signals (τ) obtained from spectra run in deuterioacetone on a Varian HA-100 spectrometer

Proton	Rubratoxin A	Rubratoxin B
H_a	5.70	5.60
H_b	6.75	6.57
H_c	4.46	4.38
H_d	7.91	7.90
H_e	6.30	6.23
H_f	5.26	5.35
H_g	7.4	7.55
H_i	2.91	3.00
H_j	4.08	4.12

A peak at 250 mμ(ϵ 9,700) in the ultra violet spectrum of Rubratoxin B has been assigned to two disubstituted maleic anhydride groups (3). The UV spectrum of Rubratoxin A has a shoulder at 252 mμ (ϵ 4,430) and an inflection at 225 mμ as well as a peak at 204 mμ (ϵ 31,900). The infra red spectrum gives further confirmation of the presence in Rubratoxin A of one disubstituted maleic anhydride function, an $\alpha\beta$ -unsaturated γ-lactone and an $\alpha\beta$ -unsaturated δ-lactone. (ν^{nujol} 1852, 1815, 1770, 1728, 1712 and 1700 cm^{-1}). The integrated intensity of the whole carbonyl region in acetonitrile solution agrees closely with the value obtained by summing the intensities of the individual chromophores measured using model compounds.

Unlike Rubratoxin B, Rubratoxin A decomposes on heating in sodium bicarbonate solution, the only volatile product being heptaldehyde. Heating Rubratoxin A in 2N sodium hydroxide gave a mixture of volatile aldehydes, the main constituents of which are heptaldehyde, acetaldehyde and the αβ-unsaturated C_{14} aldehyde formed from two molecules of heptaldehyde. There are four possible structures containing an anhydride and the corresponding αβ-unsaturated γ-lactone function in a structure analogous to that of Rubratoxin B, but only one (structure II) is a vinylogous β-hydroxy aldehyde derivative which could give heptaldehyde by a facile retro aldol reaction.

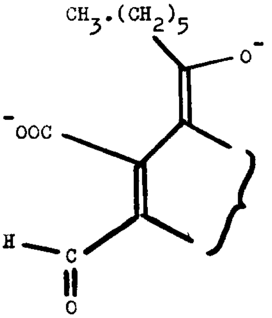
Treatment of Rubratoxin B with Jones' reagent at 0° for five minutes gives the monoketone (III). Similar treatment of Rubratoxin A gives the corresponding product (IV), but on prolonged reaction (ca one hour) it also gives the bis anhydride monoketone (III) identical in every respect with that formed from Rubratoxin B.



(III) R = =O

(IV) R = H, OH

The mono anhydride ketone (IV) shows the expected $\lambda_{max}^{acetonitrile}$ 250 mμ (ε 4,700) but in alkali gives a new chromophore at 402 mμ (ε 23,300) which is consistent with the formation of the conjugated enolate ion (V).



(V)

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