The Mechanism of the Biological Formation of Anthraquinones

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It has been well established that anthraquinones produced by moulds are derived from acetate [1]. However, the experiments performed with isotopic acetate as an added substrate could not distinguish between the two proposed mechanisms for the formation of the anthraquinones.

As early as 1944 Tatum [2] suggested, on the basis of structural interpretation of monocyclic mould metabolites, that the anthraquinones arise by a condensation of a phthalic acid with a monocylic phenolic carboxylic acid. The other hypothesis [3] involves an overall condensation of acetate throughout the anthraquinone molecule, the most attractive idea being an enzyme-bound linear condensation.

The recent finding of the participation of malonate in fatty acid synthesis has brought about a new trend of thinking about the biosynthesis of acetate-derived compounds. From the experimentally confirmed examples of 6-methylsalicylic acid [4], orsellinic acid [5], oxytetracycline [6], and in the course of this work of rugulosin [7]; the generalization that the so-called acetate-derived compounds are actually made up from an acyl-coenzyme A derivative acting as a primer on the condensation of malonyl units, can be made with great confidence.

On the basis of this generalization the two different mechanisms for the anthraquinone formation can be subjected to experimental investigation. In the first mechanism two monocyclic compounds are individually formed prior to the condensation of the anthraquinone, *i.e.* each of the monocyclic compounds are built up from one acetate unit and three malonate units. The obtained anthraquinone molecule will thus have two acetate units, the other carbon atoms coming from malonate. The positions of the acetate units will be as indicated in Fig. 1.

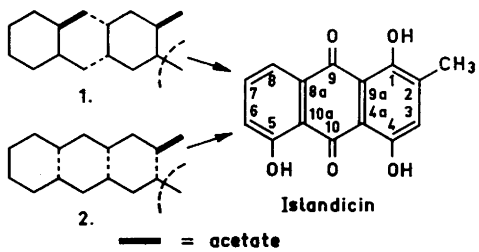

Fig. 1.

On the other hand, if the second mechanism is valid the resulting anthraquinone will carry only one acetate unit as shown in Fig. 1.

In an experiment performed with a culture of *Penicillium islandicum* Sopp (N.R.R.L. 1036) growing on a Czapek-Dox medium, 0.1 mC of malonic acid-2-^{14}C was added. The anthraquinone islandicin was isolated, diluted with nonradioactive carrier and subjected to the following degradations as described in detail elsewhere [1,8]: (*a*) total combustion, (*b*) Kuhn-Roth oxidation and Schmidt degradation of the formed acetic acid, and (*c*) oxidation to 3-hydroxyphthalic acid with subsequent decarboxylation to *m*-hydroxybenzoic acid. Nitration of the latter yielded trinitro-*m*-hydroxybenzoic acid with the carboxyl group representing one of the carbon atoms of special interest in the present problem. The picric acid obtained after decarboxylation was further degraded by a bromopicrin reaction.

The values of the radioactivities listed in Table 1 show that the methyl group of islandicin has a significantly lower specific radioactivity than the position 9 which, on the other hand, has the same specific activity as the positions theoretically directly labelled from malonate in both of the proposed mechanisms.

These results are only compatible with the second of the proposed mechanisms

Table 1.

Substance	*Counts/min/mmole*
Islandicin	108×10^4
Kuhn-Roth acetic acid:	
Carboxyl group (C(2) of islandicin)	0.26×10^4
Methyl group	9×10^4
Trinitro-*m*-hydroxybenzoic acid (C(5, 6, 7, 8, 8a, 9, 10a) of islandicin)	56.56×10^4
Carboxyl group of trinitro-*m*-hydroxy-benzoic acid (C(9) of islandicin)	13.9×10^4
Bromopicrin (C (6, 8, 10a) of islandicin)	13.96×10^4
Carbondioxide from bromopicrin reaction (C (5, 7, 8a) of islandicin)	0.3×10^4

with the modification that one activated acetate unit initiates the condensation of seven malonate units to form the anthraquinone in an over-all reaction.

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An Attempt to Evaluate a Proton Activity Function from the H_R-Function

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Recently a method was developed for the evalution of ion activity functions with the aid of the Hammett acidity function, H_0[1]. It was found by Wyatt [2], that H_0, *i.e.* the proton activity function, is a unique function of the water activity in several strong acids. It was now of interest to learn if other acidity functions give analogous results. The most intensively studied function besides H_0 is H_R defined by the reaction:

$$ROH + H^+ \rightleftharpoons R^+ + H_2O \quad (1)$$

$$H_R = -pK_1 - \log([R^+]/[ROH]) = -\log(y_{ROH}/y_{R+})a_{H+} + \log a_{H_2O} \quad (2)$$

where K_1 is the thermodynamic equilibrium constant of reaction (1) and y_{ROH} and y_{R+} are the activity coefficients of ROH and R^+. ROH is an alcohol of the triphenylcarbinol type and R^+ the corresponding substituted triphenylmethyl cation. For the sake of simplicity of notation we define:

$$y_{ROH}/y_{R+} = \varphi_R \quad (3)$$

From (2) and (3):

$$\log\varphi_R \cdot a_{H+} = -H_R + \log a_{H_2O} \quad (4)$$

If $\varphi_R a_{H+}$ can be used as a measure of the proton activity it should be a unique function of the water activity as found for $\varphi_0 a_{H+}$ where φ_0 is defined by:

$$\varphi_0 = y_B/y_{BH+} \quad (5)$$

i.e. the ratio of the activity coefficients of the base and acid forms of the indicators used in the evaluation of H_0. According to definition:

$$\log\varphi_0 a_{H+} = -H_0 \quad (6)$$

(In preceding papers [1,3] φ_0 has been denoted by φ. By calling it φ_0 the relation to H_0 is indicated in the same manner as with φ_R and H_R). The indicators used in the evaluation of H_0 are mostly substituted anilines.

H_R has recently been measured in the systems $H_2SO_4-H_2O$, $HClO_4-H_2O$ and HNO_3-H_2O by Deno and coworkers [4,5].

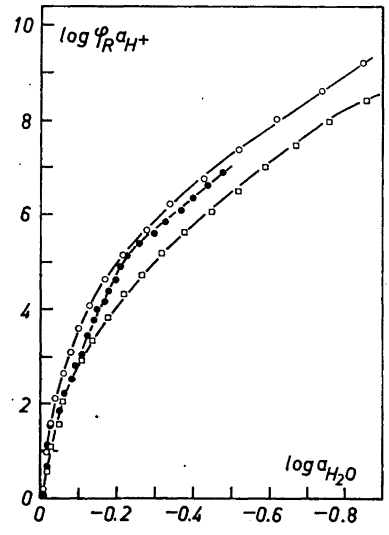

Fig. 1. log $\varphi_R a_{H+}$ plotted against log a_{H_2O} for the three systems:

○ $HClO_4-H_2O$
□ $H_2SO_4-H_2O$
● HNO_3-H_2O