If, on the other hand, several reactions must be considered simultaneously, a much larger number of experiments of this type, varying the concentrations of both the reactants, would be needed and the method would become rather laborious and time-consuming.

We would suggest, for ΔH work, a titration technique which might give similar advantages over "point measurements" as does the titration technique introduced by Leden [3] for determining equilibrium constants: a much larger number of data for a given amount of time and labor, and a better check for small analytical errors.

It would, however, not be perfectly satisfactory to keep S in a calorimeter, and T in a buret (albeit thermostated), to add T portion-wise to S and measure the temperature in S after each addition. The temperature in S would naturally change during the reactions, so that one would have to make an increasing correction for the temperature difference between T and S. Moreover, the temperature in S might finally drift several degrees from the original one, say 25.0°C. Even if one knows the equilibrium constants for the various reactions at 25.0°C, the constants would now be different, which one would have to consider in the calculations.

These considerations have led us to try another simple device which we think has not been described before. The main parts are shown in Fig. 1, and a typical temperature history during the titration in Fig. 2.

The apparatus is kept in a thermostated room of approximately the standard temperature desired, 25.0°C. The upper part of the buret with solution T is thus air-thermostated. From the buret, T passes through a bulb, which is immersed in a water thermostat which keeps the temperature more accurately, and which also contains the calorimeter with S. The calorimeter is, as usual, provided with a thermometer and an electric heater, used for calibration. The unusual feature is a cooler of thin glass tubing, through which cool air may be blown; heat is then conducted away from the solution through the tube wall to the air. The temperature of S may be adjusted with any reasonable accuracy to equal the standard temperature of the thermostat bath and T; in practice, the final adjustment is best made with the heater, so the cooler is applied to produce a temperature somewhat below the standard one, after which the heat is applied.

The temperature history of the solution S will be as is seen in Fig. 2. Before each addition, the temperature in S is made equal to that in T; the addition is made, the temperature rise is recorded, and then the temperature of S is again brought back to the standard.

In this way, there will be practically no correction for temperature difference between S and T, and the temperature in S, even after an addition, may be kept within, say, 0.1° from the standard so that the equilibrium constants for the standard temperature may be used in the calculations.

An apparatus has been constructed according to these principles by one of us (K.S.) and is now being used to study a number of equilibrium systems. The main advantage is the increased speed of working, and the accuracy is quite satisfactory. A detailed description of the apparatus will appear elsewhere [4].

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The Occurrence of Endocrocin in *Penicillium islandicum*

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Earlier investigations of the biogenesis of polyhydroxyanthraquinones have shown that the anthraquinone molecule originates from acetate through head to tail couplings of acetate units. As discussed in another paper the experiments with labelled acetate as substrate do not explain the formation of the ring system since the intramolecular

condensation of a C—16 polyketoacid and the condensation of two C—8 benzene derivatives will give rise to the same isotopic pattern [1]. In both cases one has to presume a polyhydroxyanthraquinone-carboxylic acid as an intermediate step in the formation of the polyhydroxyanthraquinone. The biological formation of the anthraquinones *via* anthraquinone-carboxylic acids is indicated by the occurrence in nature of a polyhydroxyanthraquinone-carboxylic acid with the carboxyl group in the expected position in the molecule.

Asahina and Fuzikawa [2] have found 4,5,7 - trihydroxy - 2 - methylanthraquinone-3-carboxylic acid (endocrocin) in the lichen *Nephromopsis endocrocea*. The same lichen also produces physcion, the 7-methylether of the decarboxylated endocrocin. Shibata and Natori [3] have isolated endocrocin from the mold *Aspergillus amstelodami*, which also produces catenarin. The formation of catenarin from endocrocin is easily explained by hydroxylation at the 1-position and decarboxylation of endocrocin.

Endocrocin has now been found in a UV-mutant of *Penicillium islandicum*. This strain produces in addition both emodin [4], *i. e.* decarboxylated endocrocin, and catenarin as well as islandicin. This gives further support to anthraquinone-carboxylic acids being intermediates in the formation of the anthraquinones.

In spite of the slight solubility of endocrocin in water, it was mainly found (about 3 mg/l) in the culture medium (pH 4.5) of *Penicillium islandicum*.

The isolated endocrocin decomposed slowly over the range 290—320°C. Asahina and Fuzikawo describe the melting point of endocrocin as decomposition up against 318°C.

The isolated product was soluble in 0.5 N $NaHCO_3$ with a brown red color and gave all the color reactions reported by Asahina and Fuzikawa (ethanolic ferric chloride, a red brown coloration; concentrated H_2SO_4, a red coloration; methanolic magnesium acetate, an orange red coloration). It had an absorption curve that was very close to that of emodin in appearance. The acetylated product was soluble in $NaHCO_3$ and had m. p. 212°C (Asahina and Fuzikawa 212°C).

Thermal decomposition of the isolated endocrocin yielded emodin. The emodin was identified by its absorption spectrum and its m. p. (257°C) which was not depressed on admixture with authentic emodin.

Experimental. Culture conditions. Fourteen flasks, each containing 500 ml of Czapek-Dox medium were cultivated according to Howard and Raistrick [5] for thirty days. The mycelium was filtered off and washed with distilled water.

Isolation of endocrocin. The culture medium was acidified with concentrated HCl and extracted with ether (3 l). After concentrating to about 500 ml the ether solution was treated with 0.5 N $NaHCO_3$ until no more pigment could be removed. The pigments in the aqueous solution were taken up in ether after acidification of the solution with HCl. The ether phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by developing on a thick paper chromatogram (Whatman 3 MM) in chloroform-methanol-formic acid (4 %) (10:1:1). The region containing endocrocin (R_F 0.75) was extracted with acetone. The endocrocin was with difficulty recrystallized from glacial acetic acid, m.p. 290—320°C (decomp.).

Decarboxylation of endocrocin. A small portion of the endocrocin was heated at 320°C for half an hour in a test tube. The emodin which sublimed on the walls of the test tube was rinsed out with acetone and resublimed in vacuum at 200—220°C. The emodin had m.p. 257°C.

Acetylation of endocrocin. About 20 mg of endocrocin were boiled for 2 min with 2 ml of acetic anhydride and a few drops of concentrated H_2SO_4. The reaction solution was poured onto crushed ice after cooling to room temperature. The precipitate formed was filtered off and recrystallized twice from methanol yielding small pale yellow needles with constant melting point (212°C).

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