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THERMAL ANALYSIS OF TETRACYCLINE HYDRATES

I. PREPARATION AND IDENTIFICATION

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ABSTRACT

The known hydrates of tetracycline base have been studied, and their intermediates, true hydrates themselves, have been identified. Differences in physical and chemical properties of these hydrates have been studied and will be reported in a later paper.

INTRODUCTION

Tetracycline is a broad spectrum antibiotic, produced commercially by fermentation. After extraction and purification, the finished material may be marketed as the free base, the salt, or the multi-component complex. In the free base form, two hydrates have been reported, the trihydrate and the hexahydrate[1]. This paper reports on their thermal dehydration and on the existence of additional hydrated forms of the base.

EXPERIMENTAL

Gas Evolution Analysis (GEA) by the transpiration method was used to study the rate of evolution of water vapor of each known hydrate at fixed temperatures. This method has been used successfully to study simple gas–solid reactions involving decomposition or dehydration[2–7]. The instrument used was an Aerograph Autoprep Model A-200, with a Gow-Mac, gold-filament, thermal conductivity detector. The detector response was plotted on a strip chart recorder at a speed of 1 inch/hour. (See Fig. 1.) A glass column, approximately 3 mm i.d. by 1 foot in length, was packed loosely with about 1 g of the material to be studied. The column was fitted with a bypass valve and glass tube, so that the detector response could be zeroed on the recorder. The flow rate of dry helium through the detector was 30 cc/min, and the column temperature was set isothermally at a convenient temperature between 60 and 80°C. Moisture determinations on all starting materials were performed according to the Karl Fischer Method (see Table I).

Isothermal Thermogravimetry (TG) was also used to study the dehydration process[8–10]. The temperature was kept constant and the water vapor pressure of the

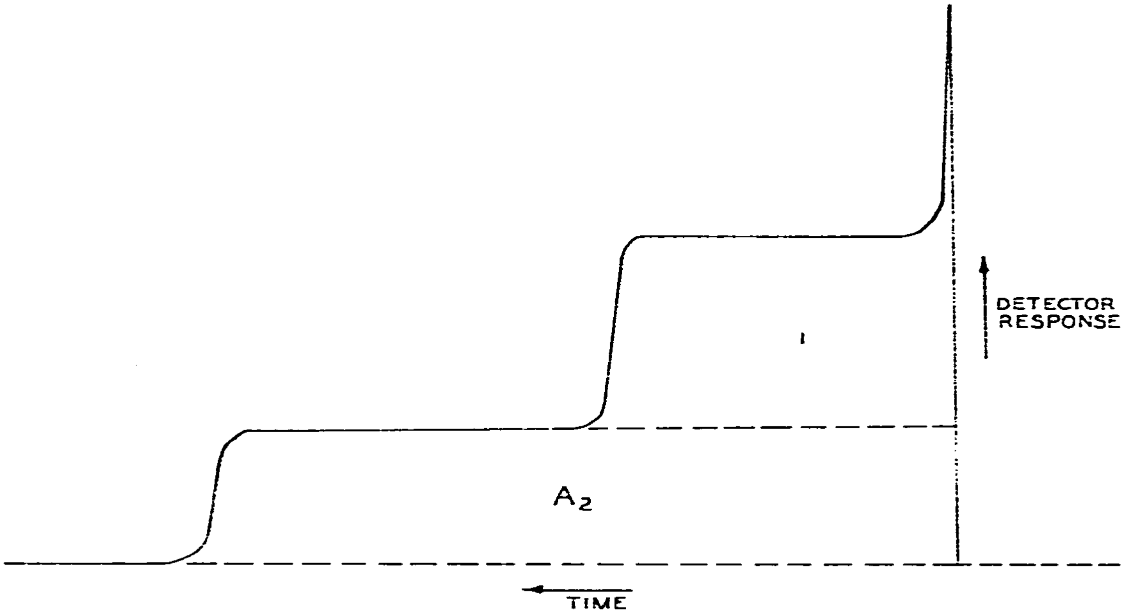

Fig. 1. General form of the GEA curve for the dehydration of a hydrate having one stable intermediate.

carrier gas was gradually lowered to give a controlled dehydration of the tetracycline hydrate. Presumably, by this method a point would be reached at which the original hydrate becomes unstable and it would then dehydrate to the next stable form. The apparatus is diagrammed in Fig. 2. A Cahn Model RG Electrobalance is positioned over a vertical pyrex chamber which is flushed with moist helium at a flow rate of 30 cc/min. Water vapor is supplied by a sparging chamber in a controlled temperature water bath. By controlling the temperature of this bath, one can control the vapor pressure of water in the carrier gas. A second inlet flushes with dry helium at 30 cc/min to prevent water vapor from reaching the balance. This chamber is surrounded by a water jacket with water criculated from a temperature controlled bath to maintain the chamber at 75 °C. Heating tape is wrapped around any part of the carrier gas plumbing in which water vapor might condense. The sample is placed in an aluminium pan suspended from the balance beam into the chamber. The weight of the sample is recorded initially and at intervals during the experiment.

RESULTS AND DISCUSSION

It was found that both the trihydrate and the hexahydrate gave similarly-shaped GEA curves. An idealized GEA curve is illustrated in Fig. 1 and actual curves are shown in Figs. 3 and 4. The apparent noise is in reality a response to the oven heating cycle. At the beginning of the experiment a sharp spike is seen in the response curve, presumably due to an initial overrun on the heating cycle and the evolution of

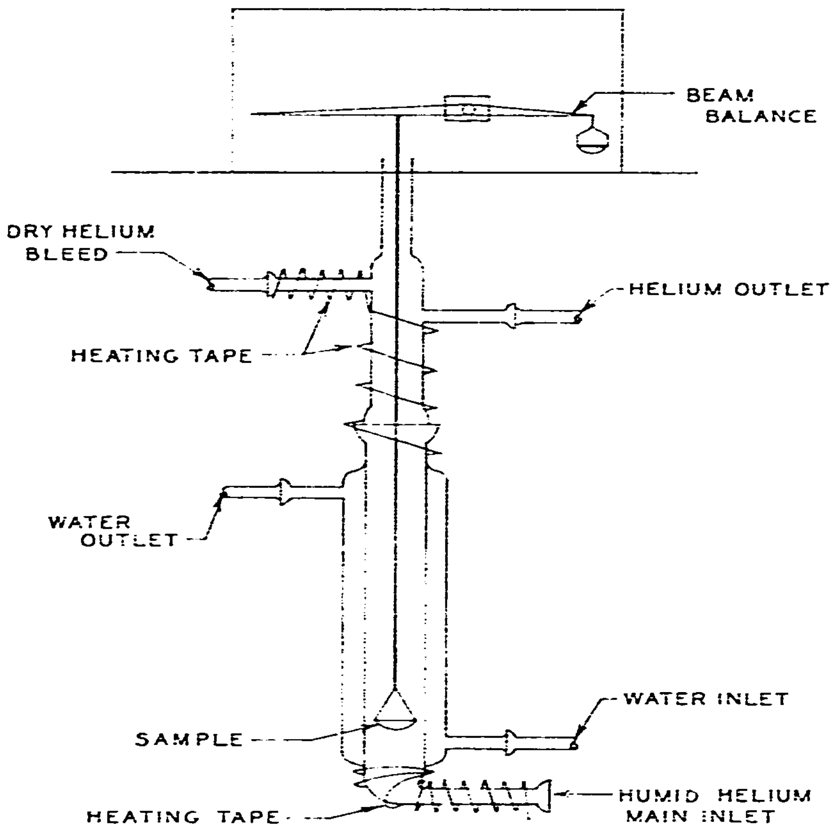

Fig. 2. TGA apparatus.

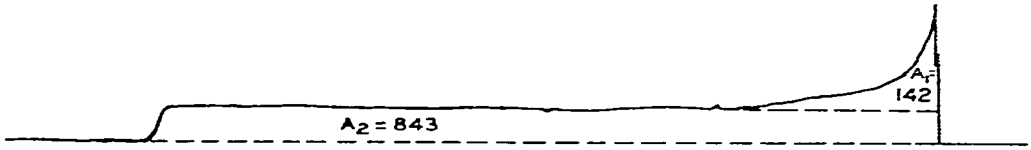

Fig. 3. Dehydration of TC·$6H_2O$ by GEA, 60 °C, 1°/hr., att.-8.

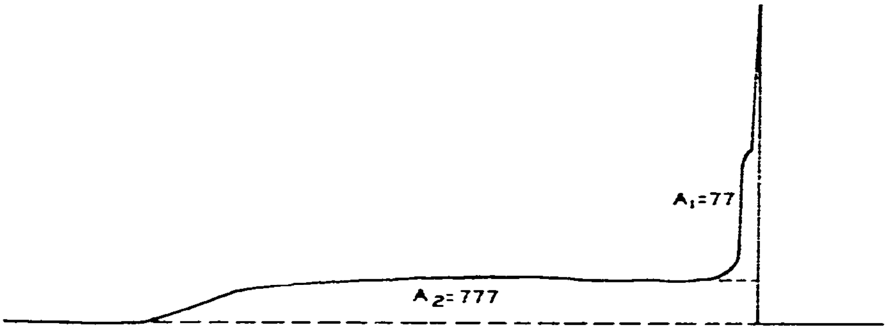

Fig. 4. Dehydration of TC·$3H_2O$ by GEA, 80 °C, 1°/hr., att.-8.

any small amount of unbound water present. The detector response then stabilizes at a fixed level followed by a drop to a lower level, and finally a second drop to another level. In each case there were three plateaus separated by two sharp drops in the detector response. This seems reasonable, in light of the previous findings that simple reactions of the form

$$\text{solid}_1 \rightleftarrows \text{solid}_2 + \text{gas}$$

are heterogeneous and proceed along an interface at a constant rate[2,4,7,11-13]. As the interface moves down the column and the carrier gas reaches a constant water-vapor pressure this will give rise to a constant detector response and thus a plateau. As the interface reaches the end of the column, the response will drop as the concentration of water-vapor drops and will stabilize at a plateau corresponding to the rate of evolution of water for the next species. All interfaces, of course, begin at the same time and proceed down the column at different rates, in the same manner as a gas chromatographic separation. As a drop in detector response is reached on the dehydration curve, indicating the arrival of the first interface at the effluent end of the column, the second interface has moved a finite distance down the column. This being the case, it is a safe assumption that a sample taken at the effluent end of the column just after a response drop and the stabilization of a new plateau will have the composition of the intermediate hydrate. Samples were taken in this manner and Karl Fischer moisture determinations run to determine the water concentration of the intermediate hydrate (see Table I).

Another method of determining the composition of the intermediate was used, making use of relative areas under the dehydration curve. The second plateau in the dehydration curve is assumed to be the dehydration of the intermediate, this beginning with initiation of the experiment, simultaneously with the first dehydration step. If we extend the second plateau back to the origin of the experiment (see Fig. 1), the total area (A_t) under the curve will then be divided into two portions with A_1 representing the amount of water evolved in the first step of the dehydration, and A_2 the amount evolved in the second step. By determining the weight of water evolved (W_1) and the weight of sample remaining (W_2) by weighing the tared vessel before and after the experiment, the water content of the intermediate can be determined by the equation:

$$\frac{A_2/A_t(W_1)\times 100}{A_2/A_t(W_1)+W_2} = \%\ \mathrm{H_2O}\ \text{of the intermediate hydrate}$$

The results are summarized in Table I. Calculated moisture contents for tetracycline hydrates are given in Table II.

Samples taken from the effluent end of the column after the second detector response drop were found to contain 0.5% or less water in all cases.

An interesting dissimilarity in the dehydration curves of these two hydrates is that for $\mathrm{TC\cdot 6H_2O}$ the first detector response drop is slow while the second drop is

TABLE I

MOISTURE CONTENT OF INTERMEDIATE HYDRATES

Starting material	*Temp. (°C)*	A_2	A_t	W_1 *(g)*	W_2 *(g)*	*% Moisture*	
						Graphic method	*K.F. method*
$TC \cdot 3H_2O$ (K.F. 10.7%)	80	777	854	0.1870	1.7901	8.7	8.6
$TC \cdot 6H_2O$ (K.F. 20.0%)	60	843	985	0.2473	1.0405	16.9	16.2

TABLE II

THEORETICAL MOISTURE CONTENT OF POSSIBLE TETRACYCLINE HYDRATES

Hydrate	*% moisture*
$TC \cdot 2H_2O$	7.5
$TC \cdot 2\frac{1}{2}H_2O$	9.2
$TC \cdot 3H_2O$	10.8
$TC \cdot 4H_2O$	13.9
$TC \cdot 5H_2O$	16.8
$TC \cdot 6H_2O$	19.5

quite rapid (Fig. 3). In the case of $TC \cdot 3H_2O$ (Fig. 4), the situation is exactly the opposite. The rate of drop of the detector response depends on the width of the interface for that particular reaction. This difference in interfacial width might be an indication of differences in mechanism or in the bonding force.

It seems evident that the intermediate in the dehydration of $TC \cdot 6H_2O$ is $TC \cdot 5H_2O$, and the intermediate in the dehydration of $TC \cdot 3H_2O$ is $TC \cdot 2\frac{1}{2}H_2O$. It is interesting to note that the water concentrations of the intermediates are in the same ratio as those of their respective starting materials.

The thermogravimetric results are shown graphically in Figs. 5 and 6. The weight of the sample (in milligrams) is plotted against the time, with changes in vapor pressure noted at the times at which they were changed. In the case of the hexahydrate, $TC \cdot 5H_2O$ is clearly the intermediate, with the hexahydrate being unstable at 102 mm Hg, and $TC \cdot 5H_2O$ being stable down to 55 mm Hg. This agrees well with the results of the gas evolution analysis experiment. The transition from $TC \cdot 3H_2O$ to $TC \cdot 2\frac{1}{2}H_2O$ is obscured because of the variability of the weight data.

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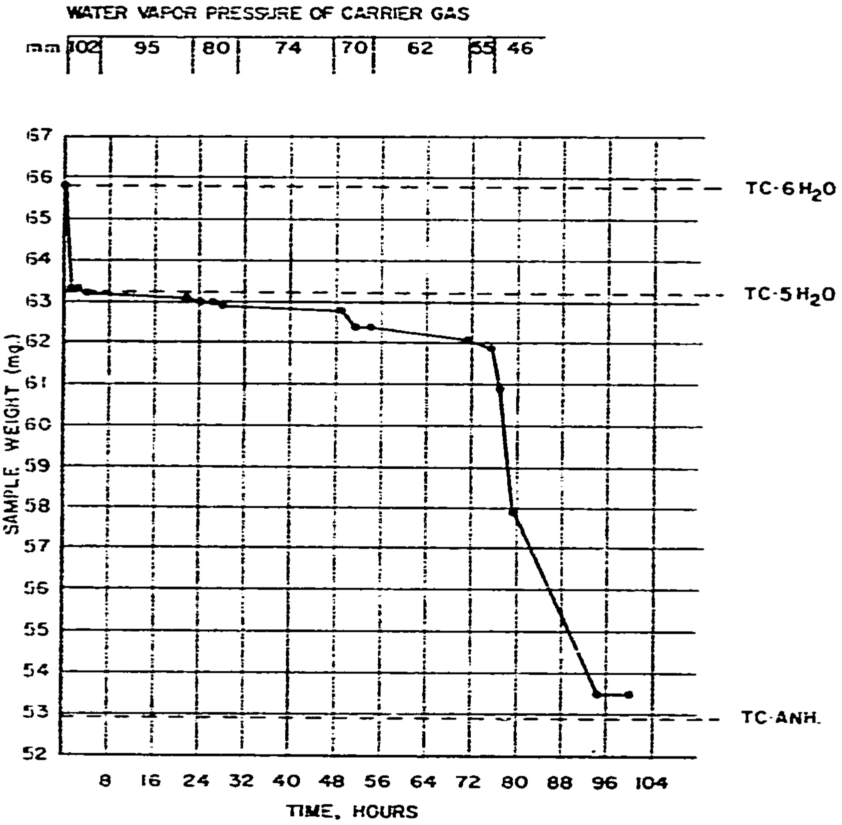

Fig. 5. Dehydration of TC·6H_2O at 75°C under varying vapor pressure of water by TG.

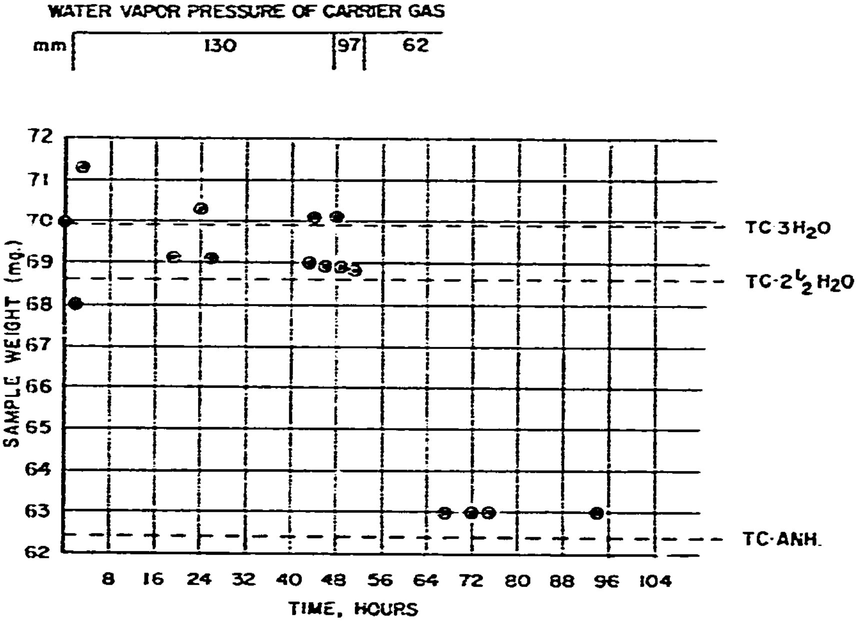

Fig. 6. Dehydration of TC·3H_2O at 75°C under varying vapor pressure of water by TG.

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