328. *The Constitution of the Purine Nucleosides. Part III. Potentiometric Determination of the Dissociation Constants of Methylated Xanthines.*

By Alexander G. Ogston.

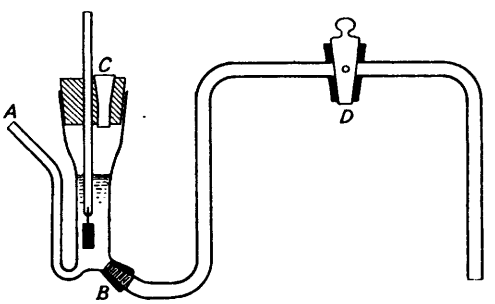
The measurement of the dissociation constants of methylated xanthines was undertaken at the suggestion of Dr. J. M. Gulland and Mr. E. R. Holiday. It was hoped to combine this evidence with that of organic chemical methods and that of ultra-violet absorption spectra, in order to throw light on their constitution. The previous work on the dissociation constants of this class of substance is not comprehensive; the constants of a few xanthines were determined by Wood (J., 1903, **83**, 568; 1906, **89**, 1831, 1839). The figures now obtained differ in some cases considerably from those of the earlier workers.

The fundamental difficulties in determining dissociation constants by the method of potentiometric titration have been discussed by Ogston and Brown (*Trans. Faraday Soc.*, 1935, **31**, 166). If the liquid junction potential is not affected by the composition of the titrated liquid, the *E.M.F.* of the cell is proportional to the mean activity of the acid solution which it contains; if this solution is sufficiently dilute, the activity coefficients in it will not differ greatly from unity, the *E.M.F.* can be taken as proportional to the concentration of hydrogen ions, and the dissociation constant can be written without great error in its classical form $K = [H][A]/[HA]$, where A and HA are the basic and the acid form of the titrand. For this reason and because of the scarcity and insolubility of some of the materials, the solutions titrated were rarely more concentrated than $M/1000$ and sometimes as dilute as $M/3000$.

Experimental.

The materials used were supplied in a pure form by Dr. J. M. Gulland.

The cell was of the form shown in the figure. Its capacity was 3 c.c.; hydrogen was introduced through the side tube and the bubbles stirred the liquid in the cell very efficiently. The liquid junction was made at the end of the bridge tube, which was closed by a porous plug of sintered glass and ground into a joint at the base of the cell; the bridge was filled with the bridge solution (3·5*N*-potassium chloride) by removing it from the cell and applying suction at the closed end; superfluous liquid was removed from the sintered end before the bridge was replaced in the cell. This type of liquid junction gave results reproducible to 1 mv. when freshly made, and constant to within 3 mv. during several hours; it also allowed the titrand solution to be completely stirred during titration with a minimum disturbance of the junction. Flow of liquid was prevented by the closed unlubricated tap on the bridge tube, which had a resistance so low that it could be kept closed during measurements.



A. *Hydrogen tube.*
B. *Sintered glass plug.*
C. *Hydrogen exit and entrance for micro-burette.*
D. *Closed ungreased tap.*

The reference electrode was 3·5*N*-calomel; the platinum electrodes were prepared and used as described by Ogston and Brown (*loc. cit.*). The p_H potential scale was standardised with $N/10$-hydrochloric acid, its activity coefficient being taken into account. Measurements were made at 18° ± 0·5° in a constant-temperature room.

Titrant $N/10$-solutions were added from a capillary microburette: this had an arbitrary scale and was calibrated by weighing water delivered by it. The total capacity was 0·3 c.c. and the accuracy $\pm 10^{-4}$ c.c.

The constants were calculated from the experimental points by the method of Ogston and Brown; the values in water are the mean in each case of two or more concordant runs and are probably accurate to $\pm 0{\cdot}05$ p_H unit; those in alcoholic solutions are less accurate owing to the

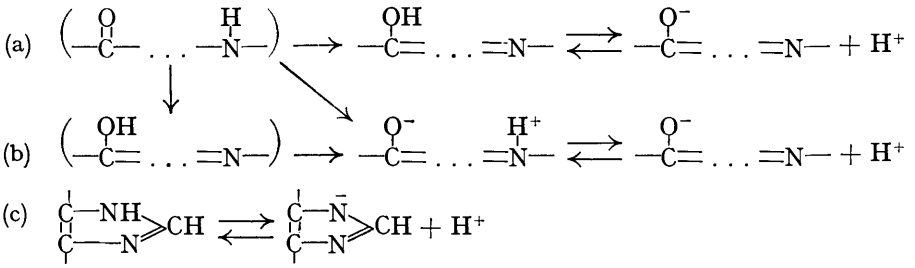
less reproducible behaviour of the electrodes in this solvent, but are probably accurate to $\pm 0{\cdot}1$ p_H unit.

Substance.	p_K. Water.	p_K. 90% EtOH.	Δp_K.	Substance.	p_K. Water.	p_K. 90% EtOH.	Δp_K.
Xanthine.....	7·7	9·3	1·6	7-Methylxanthine	8·5	8·8	0·3
1-Methyl-	7·7	9·2	1·5	1 : 7-Dimethyl- ...	8·5	8·7	0·2
3 : 7-Dimethyl-	9·9	11·3	1·4	9-Methyl-	6·3	6·8	0·5
3-Methyl-	8·5	8·6	0·1	1 : 9-Dimethyl-	6·3	6·6	0·3
1 : 3-Dimethyl-	8·6	8·7	0·1	Xanthosine.....	6·0	6·6	0·6

Discussion.

Most of the xanthines are both acids and bases; the acid dissociation constants only have been measured (Table). The basic functions are in all cases very weak, so the amount of buffering produced by the dilute solutions used was small; in terms of potential, the buffering produced was of the same order of magnitude as the reproducibility of the liquid junction potential, so no accurate estimate of their constants could be made. The acid dissociation constants are high enough to be accurately measurable.

Xanthines can conceivably function as acids in three ways: (a) as hydroxy-acids, following enolisation of one of the carbonyl groups; (b) as charged amino-acids, by "Zwitter" ion formation from (a); (c) as glyoxaline acids. These may be represented by the schemes:



The number of possible ways in which xanthine itself might function as an acid is thus considerable, though the number will be smaller for its methyl substituted derivatives. A consideration of the values obtained for the dissociation constants of deoxyxanthines by Tafel and Dodt (*Ber.*, 1907, **40**, 3757) also limits the number of possibilities which need be considered. Their results can be summarised as follows.

(1) Those compounds showing acid properties all have the glyoxaline ring unsubstituted: their dissociation constants vary from 3×10^{-12} to 8×10^{-12}. This class includes the 1 : 3-dimethyl compound.

(2) All the compounds examined which were substituted in the 7-position, and only these, showed no acid properties.

It must follow from these results that the deoxy-compounds owe their acidity only to ionisation of the –NH– in the glyoxaline ring; the 2-carbonyl group seems not to be capable of enolisation. It is therefore probably safe to infer that in the xanthines, acid properties must be due either to ionisation of the glyoxaline –NH– or to enolisation of the 6-carbonyl group; but the great difference between the values of the dissociation constants of the xanthines here obtained and those of the deoxyxanthines, as well as the failure of 7-substitution to destroy the acid properties of xanthines, makes it seem improbable that the acidity of the xanthines is due to ionisation of the glyoxaline –NH– group. It follows that the acid properties of the xanthines may be ascribed to simple enolisation or "Zwitter" ion formation involving the 6-carbonyl group. On this basis we may proceed to discuss the significance of the present results.

It is possible to distinguish between acids of types (a) and (b) by observing the change of p_K between water and 90% alcoholic solutions (see Linderstrøm-Lang, *Compt. rend. Lab. Carlsberg*, 1927, **4**, 6; Neuberger, *Proc. Roy. Soc.*, 1934, *B*, **115**, 180; and Ogston and Brown, *loc. cit.*). Uncharged acids of type (a) give a comparatively large positive

change, charged acids of type (b) only a small positive change of p_K. The results show that the xanthines fall into three classes. 3-, 7-, 9-Methyl-, 1 : 3-, 1 : 7-, 1 : 9-dimethyl-xanthines and xanthosine are " Zwitter " ions of type (b); xanthine, 1-methyl- and 3 : 7-dimethyl-xanthines are normal acids of type (a); caffeine (1 : 3 : 7-trimethylxanthine) and *iso*caffeine (1 : 3 : 9-) are not acids at all.

Systematic relationships are found in the values of the dissociation constants. The following groups have constants of sensibly the same values: 3-, 7-methyl-, 1 : 3-, 1 : 7-dimethyl-xanthines; xanthine and 1-methylxanthine; 9-methyl- and 1 : 9-dimethyl-xanthines, and xanthosine is but little, though definitely different. An explanation of these facts is to be sought in their constitutions; the steps in the argument have been numbered to ensure clarity.

(1) Substitution of the 1-position produces no change in the dissociation constant in most cases; therefore in the pairs xanthine and 1-methylxanthine, 3-methyl- and 1 : 3-dimethyl-xanthine, 7-methyl- and 1 : 7-dimethyl-xanthine, 9-methyl- and 1 : 9-dimethyl-xanthine, enolisation involving the 1-position can be excluded.

(2) It is probable that where the dissociation constants of different xanthines have the same values, the structures are similar; in those having " Zwitter " ion structures the distribution of the charge in the molecule will have the controlling effect on the dissociation constant, since this is determined largely by the electrical work of removal of the proton, and an identity of the values of the dissociation constants argues an identity of charge distribution.

(3) The previous argument depends for its validity on the assumption that in neutral solution the compounds are almost entirely present in their acid form. The typical keto-enol-ionic equilibrium may be expressed

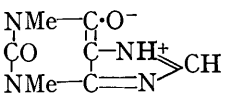
$$\text{Keto} \underset{}{\overset{k_1}{\rightleftharpoons}} \text{Enol} \overset{k_2}{\rightleftharpoons} \text{Ions}$$

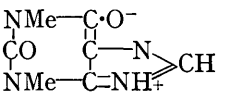
whether the " enol " form is a normal acid or a " Zwitter " ion, and the overall dissociation constant is given by

$$K = k_1k_2/(1 + k_1)$$

Argument (2) applies essentially to k_2. That k_1 and k_2 should mutually balance, or that the values of both k_1 and k_2 should be identical in a number of compounds, to produce identical values of K, can be regarded as improbable. However, if k_1 be very large (*i.e.*, the substance be present chiefly in its enol or acid form), K approaches the value of k_2, and the identical values of K obtained for different compounds makes the fulfilment of this condition most probable.

(4) Two structures (I and II) are possible for the neutral form of 1 : 3-dimethylxanthine. Of these, (I) is the more probable, since the opposite charges are closer together than in (II) and will have a lower electrical free energy. In the light of the previous arguments the structures (III), (IV), and (V) may be assigned to the neutral forms of 3-methyl-, 7-methyl-, and 1 : 7-dimethyl-xanthine respectively.


(I.)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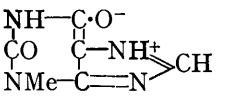
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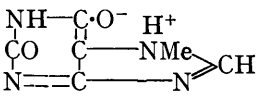
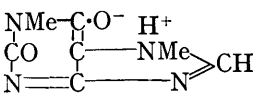
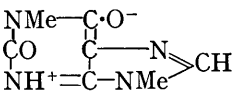

(IV.)


(V.)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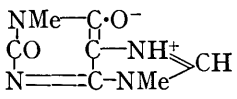
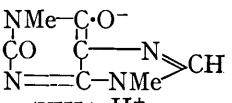
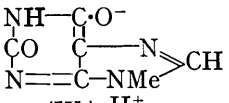

(VII.)

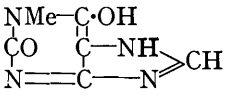

(VIII.)

(5) From its similarity to 9-methyl- and 1 : 9-dimethyl-xanthine, it seems probable that xanthosine is substituted in the 9-position. This supports the results of the absorption spectra measurements of Gulland, Holiday, and Macrae (J., 1934, 1639). For 1 : 9-dimethylxanthine there are three possible structures (VI, VII, and VIII). Of these,

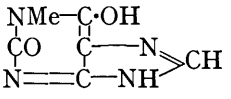
(VII) may be excluded, since such a charge distribution should give a dissociation constant not very different from those of the 1 : 3-dimethylxanthine group. The greater value of the constant indicates a greater distance between the charges. The difference between the properties of 1-methyl- and 1 : 9-dimethyl-xanthine shows that in one of the two the glyoxaline ring must be directly involved, since otherwise it is doubtful if substitution in it could produce so profound an effect. In view of the structure assigned to 1-methylxanthine (*vide infra*) it seems most likely that 1 : 9-dimethylxanthine has structure (VIII). It follows that 9-methylxanthine may be represented as (IX).



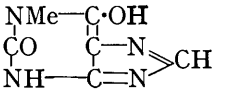
(IX.) H^+



(X.)



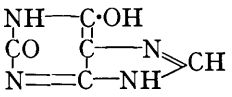
(XI.)

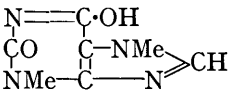


(XII.)

(6) 1-Methylxanthine might have one of three structures (X), (XI), and (XII). (XII) may at once be excluded on the grounds of the difference of behaviour from 1 : 3-dimethylxanthine and because there seems to be no reason why it should not pass over into a " Zwitter " ion form similar to (I). Of (X) and (XI), the latter seems more likely on the somewhat doubtful ground that (X) might be expected to form a " Zwitter " ion similar to (IV). The neutral form of xanthine may therefore be provisionally written as (XIII).

(XIII.)





(XIV.)

(7) Only one structure seems possible for 3 : 7-dimethylxanthine; enolisation of the 2-carbonyl is excluded, but in this case the 1-position must be involved with the 6-carbonyl group. The neutral form is represented by (XIV). In this case the absence of any xanthine having the same dissociation constant makes it unnecessary that the enol form should preponderate largely in neutral solution.

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