TABLE I

5% washed wet cells was used per cup in 0.3 *M* phosphate buffer of *p*H of 7.0 and 0.6% $NaHCO_3$ under 400 mm. pressure of CO_2. Substrate was added to approximate 20 mM. of carbon in all experiments except of pyruvic where 10 mM. was added. Glycerol and pyruvic was fermented for 18 hr., glucose for 6 hr. and erythritol for 42 hr. After 0.5 hr. of fermentation 50 millimoles of formaldehyde containing 67,000 counts was added from a side arm. Total volume was 50 ml.

Substrate fermented	Fermented, mM.	Propionic acid produced, mM.	CO_2[a]	Propionic acid: Average[a] activity	Carboxyl[a] group	α and β[a] carbon	Total activity
Glycerol	3.63	3.36	274	2180	3110	1750	22,000
i-Erythritol	3.96	4.08	604	2300	3650	1630	28,200
Pyruvic acid	2.64	0.76	1230	2500	3200	2150	5,700
Glucose	1.62	2.32	308	2320	..	..	16,100

[a] Activity expressed as counts/minute/mM. of carbon.

dioxide fixation. In relation to these observations it is of interest that Wood and Werkman[6] identified formaldehyde in the fermentation medium of *Propionibacterium* when dimedon was used as a trapping agent.

It is clear from the table that the distribution of formaldehyde carbon in the propionic acid from the various fermentations is quite similar, although different substrates have been employed. These data indicate that formaldehyde participates in the formation of propionic acid in a manner common to all the fermentations with *Propionibacterium arabinosum* thus far investigated, and that it may be an essential intermediate in the reactions involved. This problem is being investigated further.

(6) H. G. Wood and C. H. Werkman, *J. of Bacteriology*, **30**, 652 (1935).

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An Improved Method for the Hydrolysis of Diazonium Salts

BY JOHN P. LAMBOOY

The great importance of the replacement of a diazonium group by a hydroxyl group is well known in the laboratory and in the chemical manufacturing industry. In the majority of cases the yields from the reaction are discouragingly small as a result of the coupling of the phenol with the undecomposed diazonium compound and the formation of tars.

Our recent need for a variety of phenols led us to investigate procedures by which the yield of the reaction could be increased. The improved procedure is based simply on the provisions that the phenol is removed immediately after its formation by the hydrolysis being performed in an actively steam distilling system and that the diazonium salt solution be dilute so that the chances of side reactions occurring are reduced. Not only does the procedure appear to be one of general usefulness in cases where the phenol is volatile with steam but the yields are considerably increased and the quality of the product is unusually high.

Experimental

A three-liter, round-bottom flask is equipped for ordinary steam distillation except that in addition to the steam inlet tube and the vapor outlet tube a cold finger type addition tube (Fig. 1) is also passed through the stopper. This tube reaches to within 1 cm. of the surface of the hydrolysis mixture. To the flask is added a solution of 200 ml. of water and 150 ml. of concentrated sulfuric acid. This solution is brought to the boiling point by a burner and then steam injection is begun. Any time after vapors begin to condense in the condenser the addition of the diazonium solution may be begun.

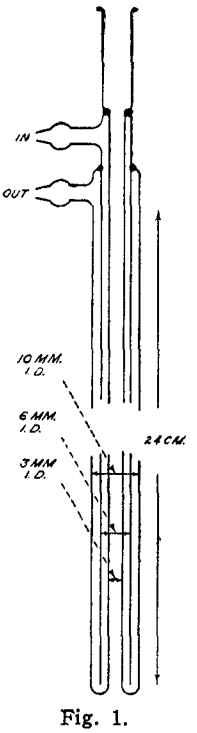

Fig. 1.

Just preceding and during the addition of from 80 to 100 ml. of the diazonium solution, the addition tube is cooled by the rapid flow of cold water. The rate of addition must be controlled or the rate of steam injection reduced to prevent excessive frothing. The distillation is continued until droplets of phenol are no longer seen in the condensate and until the volume of the hydrolyzing solution has returned almost to its starting level, before another addition is made. By controlled application of heat from the burner, the volume of the hydrolyzing solution can be maintained fairly constant.

Using the cold diazonium salt solution prepared from *o*-toluidine with 20% sulfuric acid and sodium nitrite, this modification has been used on 0.5-, 1.5-, 2.0- and 3.5-mole batches. In all cases the amine sulfate solutions were prepared in 0.5-mole lots and in all but the 0.5-mole batch, kept in the refrigerator until needed. Once started, each successive 0.5-mole lot was diazotized during the hydrolysis of the preceding one. Due to the accumulation of sulfuric acid in the boiling flask it was necessary to permit a gradual increase in volume from the original 350 ml. to approximately 1700 ml. in the case of the 3.5-mole batch. Corresponding volume increases were also permitted in the 1.5- and 2.0-mole batches. Hydrolysis of the 1.5-mole batch was done by slow and continuous addition of the diazonium solution. This was found to be less convenient than the periodic addition of portions because of the need to keep large volumes of diazonium solution cold in the vicinity of the hot distillation apparatus. The time required was somewhat increased because of the refluxing caused by the continuous flow of cold water through the addition tube.

The *o*-cresol was extracted with ether and processed in the usual fashion. After removal of the ether the material was fractionated through a saddle packed column 50 cm. long and 18 mm. i. d. equipped with a cutter head. The *o*-cresol, boiling at 190–191° (746–747 mm.), produced a pure white crystalline material melting 33–34° (cor.).

The procedure has been tried on a number of substituted anilines, and while the quantities of materials used in the

preparation of the diazonium solutions varied in the usual fashion depending on the quantities of starting material, the hydrolysis was always done in the same manner.

PER CENT. YIELD OF CORRESPONDING PHENOL.

Batch size, moles	0.15	0.5	1.0	1.5	2.0	3.5
o-Ethylaniline	88					
o-Toluidine		89		86	84	84
m-Toluidine		91				
p-Toluidine		89				
2,4-Dimethylaniline[a]			80+			

[a] Private communication from Dr. W. R. Nummy, Department of Chemistry, University of Rochester.

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The Absorption Spectrum of 3,7-Dimethylxanthine[1]

BY ERNEST E. LOCKHART AND MABEL C. MERRITT

Gulland and Holiday[2] reported that the ultraviolet absorption spectrum of 3,7-dimethylxanthine (I) showed only one band in acid or alkaline solution and, from other studies,[3] concluded that the presence of a substituent (H–, CH_3–, sugar) in position 7 of the purine nucleus indicated a one-band spectrum, whereas the presence of a substituent in position 9 indicated a spectrum containing two bands. Conversely, the character of the spectrum (one or two bands) established the position of the double bond in the imidazole ring. This hypothesis was used as a basis for distinguishing xanthines from isoxanthines and for determining the position of substituents in xanthosines.

Gulland, *et al.*, did not state in the first paper at what *p*H in the alkaline region the absorption data were obtained, but in the second paper, in which the spectra of substituted xanthines (I omitted) were discussed, they reported that the *p*H of the alkaline solutions was 10. If one may assume that the earlier work was also done at *p*H 10, then one may infer that I shows only one band in solutions the *p*H of which is not greater than 10.

We have had occasion to study the spectra of 3,7- and 1,3-dimethyl- and 1,3,7-trimethylxanthines (I, II, III) in acid and in alkaline solution and have obtained data that seem to correlate more reasonably with the structure of I than do the data of Gulland, *et al.*

Our data for II and III agree essentially with those reported by Gulland, Holiday and Macrae[3] and by Loofbourow, Stimson and Hart.[4] The spectra of I, however, although similar to those of II and III at *p*H values between 1.3 and 6.9, showed definite secondary maxima at 235 mμ at *p*H values of 11.0 and 12.9. Molecular extinction coefficients calculated for selected wave lengths at seven *p*H values are summarized in Table I.

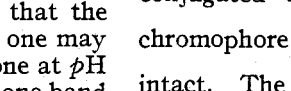
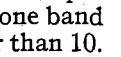
TABLE I

ABSORPTION CHARACTERISTICS OF 3,7-DIMETHYLXANTHINE[a]

	Maxima				Minima			
	I		II		I		II	
*p*H	λ	ε	λ	ε	λ	ε	λ	ε
1.3	273	9800			244	2540		
4.3	273	9950			244	2360		
6.9	273	9930			244	2320		
9.4	274	9960			246	3100		
10.6	274	10100	[b]		251	3980	[b]	
11.0	274	10000	235	6720	251	4000	230	6590
12.9	275	9930	235	7030	251	4050	230	6790

[a] λ, wave length, mμ; ε, molar absorbance; I, primary; II, secondary. [b] Shoulder.

Several conclusions may be drawn from these data: (1) the spectrum of I will show two bands in alkaline solution, (2) substitution in the 7-position of the xanthine nucleus does not inhibit the appearance of the second band, (3) the two-band spectra of xanthines do not necessarily determine the position of the substituent in the imidazole portion of the nucleus, (4) xanthines cannot be differentiated from isoxanthines by analysis of any spectral information that has been presented to date. Therefore, the Gulland hypothesis should be re-examined.

Other evidence[5] supports our belief that the imidazole portion of the purine molecule contributes little to the spectra of this group. Our data also show that the spectrum of a 3-substituted xanthine can have two bands and that enolization can occur at the 2-position, forming a second conjugated system and leaving the principal chromophore  of Cavalieri,[5] *et al.*, intact. The  in position 6 participates in both systems. The secondary band of I at 235 mμ coincides approximately with that of xanthine,[3,5] of 1-methylxanthine[3] and 9-methylxanthine.[3] We hope to examine the spectra of 7-methyl- and 3-methylxanthines to see whether these also have two bands in highly alkaline solution.

If the extinction data for each *p*H at 235 mμ are plotted against *p*H, according to the method of Stenström and Goldsmith[6] an approximate value for pK_a of 9.9 or K_a of 1.3×10^{-10} is obtained.

(1) This investigation was conducted with the assistance of grants-in-aid from the following ogranizations: American Can Company, Maywood, Illinois; Dow Chemical Company, Midland, Michigan; Nestlé Company, New York, N. Y.; Pillsbury Mills, Inc., Minneapolis, Minnesota; Standard Brands, Inc., New York, N. Y.; Wilson & Company, Chicago, Illinois.

(2) J. M. Gulland and E. R. Holiday, *Nature*, **132**, 782 (1933).

(3) J. M. Gulland, E. R. Holiday and T. F. Macrae, *J. Chem. Soc.*, 1639 (1934).

(4) J. R. Loofbourow, M. M. Stimson and M. J. Hart, THIS JOURNAL, **65**, 148 (1943).

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(6) W. Stenström and N. Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).