types of bonding, and some other reason must account for the lack of solubility of the magnesium salt in chloroform.

Experimental

Bismuth 8-quinolinolate was precipitated by the method of Berg[6] and air-dried to yield the monohydrate. The anhydrous bismuth salt was prepared by drying the hydrate at 145° to constant weight. The loss was 3.41% compared to 2.78% calculated.

Anal.,[10] Calcd. for $C_{27}H_{20}O_4N_3Bi$: C, 49.18; H, 3.06; N, 6.37; Bi_2O_3, 35.34. Found, C, 48.93, 48.88; H, 3.11, 3.10; N, 6.33, 6.30; Bi_2O_3, 35.13, 35.00. Calcd. for $C_{27}H_{18}O_3N_3Bi$: C, 50.56; H, 2.83; N, 6.55; Bi_2O_3, 36.33. Found: C, 50.69, 50.47; H, 3.09, 2.93; N, 6.84, 6.83; Bi_2O_3, 35.70, 35.73.

Magnesium 8-quinolinolate was precipitated by the method of Berg[7] and dried to the dihydrate at 80° as recommended by the Duvals[8] and more strongly by Duval.[9] The anhydrous magnesium salt was prepared by drying the dihydrate at 145° as recommended by Duval.[9] The loss was 10.73% compared to 10.33% calculated. There was some decomposition as shown by elemental analysis.

Anal.,[10] Calcd. for $C_{18}H_{16}O_4N_2Mg$: C, 62.01; H, 4.63; N, 8.04;, MgO, 11.83. Found: C, 61.71, 61.73; H, 4.72, 4.84; N, 8.00, 8.07; MgO, 12.16, 12.16. Calcd. for $C_{18}H_{12}O_2N_2Mg$: C, 69.15; H, 3.87; N, 8.96; MgO, 12.90. Found: C, 67.71, 67.52; H, 4.11, 3.87; N, 8.83, 8.73; MgO, 14.26, 14.14.

8-Quinolinol was recrystallized from aqueous ethyl alcohol and melted at 73°.

Infrared spectra of Nujol mulls of the samples were taken on a Baird Associates Infrared spectrophotometer using both a sodium chloride prism and a calcium fluoride prism.

Acknowledgment.—The author expresses appreciation to Dr. A. W. Baker of the Dow Chemical Company for recording the infrared spectra.

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(8) T. Duval and C. Duval, *Anal. Chim. Acta*, **2**, 45 (1948).
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(10) Micro-Tech Laboratories, Skokie, Ill.

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Some Observations on the Mechanism of the Reimer–Tiemann Reaction

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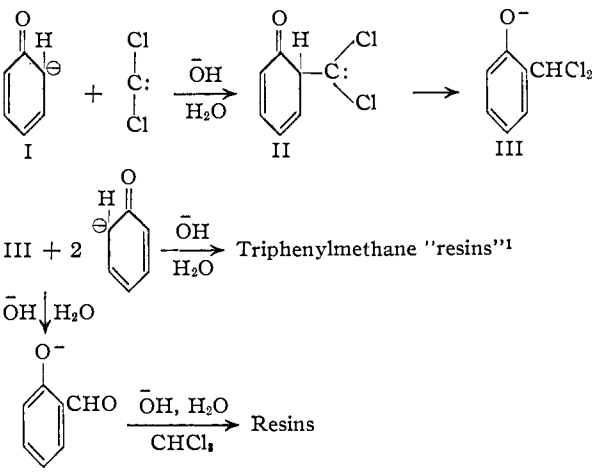
The relatively low yields obtained in Reimer–Tiemann reactions, as well as the fact that starting phenols are frequently recovered, have led many authors to follow Armstrong and Richardson's[1] original suggestion that a diaryl acetal intermediate is formed.

A re-examination of existing experimental data as well as new evidence have led us to the conclusion that a diaryl acetal, if formed at all, does not appear to be the main path by which the aldehyde is formed: (1) Armstrong and Richardson's[1] evidence for the existence of a diaryl acetal rests upon the isolation in 3–6% yield of unstable and unanalyzed oils. (2) The work of Pauly[2] has shown that dialkyl acetals are unstable in the presence of aqueous alkali; Armstrong also notes the instability of his oils in the presence of aqueous sodium bicarbonate. (3) It has now been established that 2-hydroxy-3,6-dimethylbenzaldehyde can be isolated directly from the *alkaline* reaction mixture resulting from the reaction between 2,5-dimethylphenol, aqueous alkali and chloroform. (4) By increasing the amount of alkali, and chloroform in a Reimer–Tiemann reaction (which according to Armstrong's optimum conditions yielded 15% aldehyde and 80% recovered phenol), the amount of recovered phenol was nearly halved, while resin formation increased accordingly.

Points 1 and 2 establish the instability of acetals of hydroxybenzaldehydes in aqueous alkaline media. It appears highly improbable that any such intermediate would exist for any appreciable time under the normal Reimer–Tiemann reaction conditions. Point 3 confirms this observation for one particular reaction, even though the yield of aldehyde is low (see Experimental). Point 4 allows the general conclusion that, if any acetal is formed at all, its importance in tying up starting material becomes a function of the relative concentrations of the reactants.

A slight modification of the exact mechanism of the remaining steps in the over-all reaction is furthermore considered desirable in view of the following considerations. Hine[3] has presented reasonable kinetic evidence that carbon dichloride is the reactive species rather than chloroform itself. This viewpoint is somewhat strengthened by the steric requirements of a nucleophilic attack upon chloroform; certain highly hindered abnormal Reimer–Tiemann products[4] could hardly be formed unless carbon dichloride were the reacting species. The fate of *o*- and *p*-hydroxybenzaldehydes in the presence of aqueous alkali and chloroform remains to be elucidated. Although no Cannizzaro reaction appears to take place with *o*- and *p*-hydroxybenzaldehydes[5] and as a consequence it might be expected that the carbonyl group in these compounds would be too unreactive to undergo base-catalyzed addition of chloroform, nevertheless salicylaldehyde reacts rapidly with alkali and chloroform under normal Reimer–Tiemann reaction conditions with the formation of black resins.

The following scheme is therefore proposed



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(2) H. Pauly and R. F. von Buttler, *Ann.*, **383**, 280 (1911).
(3) J. Hine, THIS JOURNAL, **72**, 2438 (1950).
(4) Hans Wynberg, Ph.D. Thesis, University of Wisconsin, 1952. An account of this and related work will be the subject of another communication.
(5) G. Lock, *Ber.*, **62**, 1177 (1929).

Experimental[6]

The Reimer-Tiemann Reaction with 2,5-Dimethylphenol.—Ten grams of 2,5-dimethylphenol, m.p. 74.5–75.5°, was stirred, while heated under reflux with 150 ml. of chloroform, 150 g. of potassium hydroxide and 120 ml. of water for 1.5 hours. The mixture was titrated and found to be 0.16 *N* in base. The solution was then steam distilled and 240 mg. of pale yellow needles, m.p. 56.6–60.5°, was isolated from the distillate. Evaporative sublimation at 55° (0.01 mm.) yielded the analytical sample of 2-hydroxy-3,6-dimethylbenzaldehyde as pale yellow needles, m.p. 60.5–61.5° (reported 62–63° [7]). *Anal.* Calcd. for $C_9H_{10}O_2$: C, 71.98; H, 6.71; Found: C, 71.78; H, 6.73.

The Reimer-Tiemann Reaction with *p*-Hydroxybenzoic Acid.—When 13.8 g. (0.1 mole) of *p*-hydroxybenzoic acid was refluxed for one hour with 90 ml. of water, 65 g. of 85% potassium hydroxide (1.0 mole) and 16.0 g. (0.125 mole) of chloroform, there was obtained after acidification and ether extraction according to the directions of Armstrong, 14.2 g. of brown solid. The aldehyde, determined as the phenylhydrazone and 2,4-dinitrophenylhydrazone amounted to 12.5 and 11.5%, respectively. No water or ether-insoluble material was found; the remainder, 12.0 g. (87%) is considered recovered starting material (see reference 2).

The experiment was repeated, using 13.8 g. (0.1 mole) of the phenol, 81.0 g. (1.25 moles) of potassium hydroxide and 35.4 g. (0.3 mole) of chloroform, followed after a reaction period of 30 minutes with another 1.25 moles of potassium hydroxide and 0.3 mole of chloroform. Separation of the reaction product into ether-soluble and ether-insoluble material after acidification, yielded 3.77 g. (27.5%) of brown powder, molecular weight 400–425 (Rast), insoluble in water and ether, soluble in sodium bicarbonate. Calculated molecular weight of the triphenylmethane derivative is 410. The ether-soluble material, 9.21 g. (62%), was estimated to contain 16.2% aldehydic material and 51% recovered phenol by the method described above.

(6) All melting points are corrected.

(7) K. Auwers and F. Winternitz, *Ber.*, **35**, 465 (1902).

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Desthiobiotin in the Biosynthesis of Biotin

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Desthiobiotin refers to a compound derivable from biotin by Raney nickel reduction.[1] Desthiobiotin satisfies the biotin requirement of some biotin-requiring microörganisms, is inactive as a biotin source for others, and is an antimetabolite of biotin for a third group.[2] Dittmer, *et al.*,[3] have presented microbiological evidence for the conversion of desthiobiotin to biotin by growing yeast. Tatum has reported[4] evidence that desthiobiotin is the biotin derivative that accumulates during growth in the presence of pimelic acid of a biotin-requiring mutant that does not utilize desthiobiotin. Thus it is commonly accepted that desthiobiotin as well as pimelic acid is a normal precursor of biotin.

The nature of the biotin derivative that accumulates when *Aspergillus niger* is grown with aeration in the presence of pimelic acid has been investigated by Wright, *et al.*[5–7] The predominant biotin derivative is biotin L-sulfoxide.[8] Biotin itself also is converted by the mold to biotin L-sulfoxide. It was established that biotin L-sulfoxide originates enzymatically since biotin is unaffected by the medium or by an autoclaved culture of *Aspergillus niger* under conditions simulating growth of the mold.

Experiments similar to those carried out with added pimelic acid have now been completed in which *Aspergillus niger* was grown in the presence of desthiobiotin. It is demonstrated in this paper by paper chromatographic procedures that, under the conditions previously described, desthiobiotin is converted to biotin L-sulfoxide, presumably through biotin as an intermediate. This finding is clear evidence that desthiobiotin is not utilized in an anomalous manner, a point not established by previous studies, since for sulfoxide formation to occur it is *a priori* established that a thiophane ring must exist. These experiments do not necessarily prove of course that desthiobiotin is an *obligate* intermediate in the biosynthesis of biotin.

Experimental

Aspergillus niger was grown in shaker flasks on the basal medium and under conditions described previously in detail.[5] One 500-ml. lot of medium was unsupplemented and served as a control. A second flask was supplemented with 50 γ of DL-desthiobiotin and a third was supplemented with 1 mg. of DL-desthiobiotin. After growth for 5 days the mycelia were filtered off and the culture filtrates paper chromatographed (0.02-ml. culture filtrate applied 10 times, Whatman No. 1 paper, ascending technique, development time 18 hours, room temperature in butanol (40), water (50), acetic acid (10)). This solvent system readily separates a number of biotin derivatives (biocytin, R_F = 0.37, biotin L-sulfoxide, R_F = 0.46, biotin D-sulfoxide, R_F = 0.57), but biotin (R_F = 0.83) and desthiobiotin (R_F = 0.89) are not well separated. R_F values in this solvent system are not significantly influenced by salts and other extraneous materials contained in the culture filtrates studied. Areas

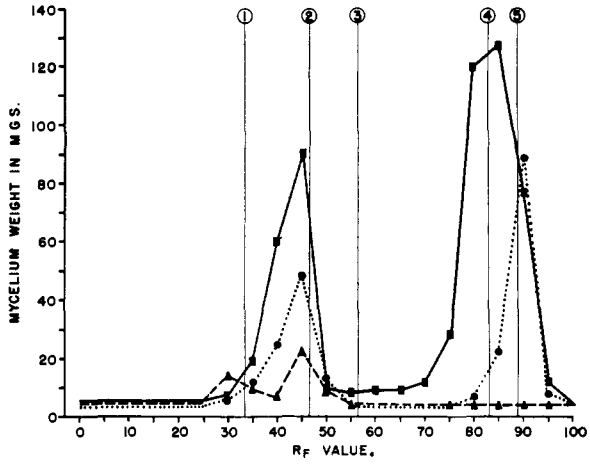

Fig. 1.—Bioautography of *Aspergillus niger* culture filtrates: —■—■—■—, medium supplemented with 1 mg. of DL-desthiobiotin/500 ml.; . .●. .●. .●. ., medium supplemented with 50 γ of DL-desthiobiotin/500 ml.; ---▲---▲---▲---, medium unsupplemented. Vertical lines designated by circled figures represent reference R_F values for: 1, biocytin; 2, biotin L-sulfoxide; 3, biotin D-sulfoxide; 4, biotin; 5, desthiobiotin.

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(2) V. G. Lilly and L. H. Leonian, *Science*, **99**, 205 (1944).

(3) K. Dittmer, D. B. Melville and V. du Vigneaud, *ibid.*, **99**, 203 (1944).

(4) E. L. Tatum, *J. Biol. Chem.*, **160**, 455 (1945).

(5) L. D. Wright and E. L. Cresson, THIS JOURNAL, **76**, 4156 (1954).

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(7) L. D. Wright, E. L. Cresson, J. Valiant, D. E. Wolf and K. Folkers, *ibid.*, **76**, 4163 (1954).

(8) This nomenclature refers to the optical rotation of the sulfoxide and not to its spacial relationship to any reference compound. Since asymmetry is present in biotin itself biotin D-sulfoxide and biotin L-sulfoxide are diamers and not enantiomorphs.