activity, Olivier's explanation[4] of association of the aluminum chloride complex itself would seem tenable in accounting for the higher reaction velocity at higher concentrations. Except for this variation, in regard to which independent evidence would be desirable, our method of calculation leads to results in good agreement with a unimolecular reaction order, and may be taken as supporting the mechanism of this Friedel–Crafts reaction previously advanced by Steele.

Summary

1. A correct method of calculating velocity constants from data obtained by Steele's method has been applied to experiments on the Friedel–Crafts reactions of benzoyl and benzyl chlorides with toluene.

2. The relative order of activity of various catalysts depends upon the type of reaction, and in the case of benzoyl chloride the activity increases with increasing percentage of ferric chloride.

3. Mixed catalysts covering a wide range of composition of aluminum and ferric chlorides have been investigated in respect to the reaction of benzoyl chloride with toluene. At comparable concentrations the activity increases with increasing mole percentage of ferric chloride and reaches a maximum at percentages in excess of 50 mole per cent.

4. The mixed catalysts have been shown to produce less than one mole of product for each mole of total metal chlorides present. Evidence is given in support of the explanation that this is due to the removal of the catalyst in the form of a bimetal complex with the reaction product, such as $C_6H_5COC_6H_4CH_3 \cdot AlCl_3 \cdot FeCl_3$.

5. Variation of the reaction velocity over a ten-fold range of concentration, the toluene being always in large excess, has been determined, and is of the same order of magnitude as that reported in a similar reaction by Olivier. The present work furnishes evidence in support of the explanation of this effect as being due to association of the reacting complex.

6. The new experimental data and method of calculation lead to results in satisfactory agreement with a unimolecular order for the reaction, and thus lend support to the mechanism proposed by Steele.

NEW ORLEANS, LOUISIANA RECEIVED JULY 29, 1935

[CONTRIBUTION FROM THE DEPARTMENTS OF AGRICULTURAL CHEMISTRY AND AGRICULTURAL BACTERIOLOGY, UNIVERSITY OF WISCONSIN]

The Chemistry of Mold Tissue. X. The Phospholipides of Aspergillus Sydowi[1]

BY D. W. WOOLLEY, F. M. STRONG, W. H. PETERSON AND E. A. PRILL

While several papers have been published dealing with the simple lipides of molds, no report has been found regarding the characterization of the compound lipides. A few workers have reported preliminary analyses of the phospholipides,[2] but no detailed study of these compounds has been reported. In a previous paper[3] the simple lipides of *A. sydowi* have been described; the examination of the phospholipides of this same organism is herein described.

Experimental Part

Extraction and Purification.—The mold was grown for ten days on glucose–inorganic salt medium in large sterilized incubators.[4] While still growing it was removed from the medium, washed with cold water, squeezed out by hand and dried rapidly at 30–60° in air. Two batches of mycelium were grown at intervals of eight months and the phospholipides of each batch examined separately. The phospholipides from the first batch will be designated as A, and those from the second batch as B. A was extracted from 7.27 kg., and B from 3 kg. of dry mold.

The mold was extracted continuously for about a week in a Lloyd type extractor. A was obtained by extraction with alcohol, and B by extraction with alcohol–ether (1:1). The solution from the extractor was evaporated, and the residue redissolved by adding water and ether. (In this and subsequent operations air was excluded by a stream of carbon dioxide.) The two layers were separated, filtered and the water layer discarded. A very small amount of matter remained undissolved in both preparations. The water layer contained only traces of nitrogen and phosphorus, and contained mannitol.

The ether layer was concentrated, and poured into a

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[1] This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] (a) Aso, *J. Tokyo Chem. Soc.*, **20**, 921 (1900); (b) Takata, *J. Soc. Chem. Ind.*, Japan, **32**, 171B (1929); (c) Thomas, *Am. J. Botany*, **17**, 779 (1930).

[3] Strong and Peterson, THIS JOURNAL, **56**, 952 (1934).

[4] Peterson, Pruess, Gorcica and Green, *Ind. Eng. Chem.*, **25**, 213 (1933).

large volume of acetone. In the preparation of A, alcoholic magnesium chloride was added, but by concentrating the ether solution sufficiently precipitation was effected in the case of preparation B without the use of any inorganic salt. After standing for a few days in the cold the precipitate was filtered, washed with acetone and redissolved in ether. After centrifuging,[5] the clear solution was precipitated by pouring into cold acetone. A was precipitated six times and B five times. The purified phospholipides were stored in ether solution under carbon dioxide. 54.26 g. of A (0.73% of the mold, and 9.2% of the crude lipides) was obtained and 12.74 g. of B (0.43% of the mold and 6.15% of the crude lipides). The remaining simple lipides contained only a trace of phosphorus.

Anal. Preparation A: N, 1.71; P, 3.65; N/P, 1.03. Preparation B: N, 1.68; P, 3.78; N/P, 0.99; ash, 2.8.

Hydrolysis.—The phospholipides were hydrolyzed in 5 to 6 g. lots by refluxing for about six hours with 5% sulfuric acid. Ether was added, and the layers separated and filtered. The ether layer contained only traces of nitrogen and phosphorus.

For the separation of the water-soluble constituents the scheme of Newman and Anderson[6] was employed, with a few slight modifications. The crude barium glycerophosphate was purified by precipitating it as the lead salt, decomposing the latter with hydrogen sulfide in the presence of excess barium hydroxide, passing in carbon dioxide to remove excess barium, and reprecipitating the barium glycerophosphate with alcohol. A pure white product was obtained.

Anal.:[7] Calcd. for $C_3H_7O_6PBa \cdot {}^1/_2H_2O$: Ba, 43.4; P, 9.8. Found: Preparation A, Ba, 43.7; P, 9.8. Preparation B, Ba, 43.3; P, 9.8.

The presence of glycerol was shown by the specific test proposed by Eegruive.[8] The intensity of the test as compared to that given by an equal quantity of barium α-glycerophosphate indicated that a mixture of α- and β-glycerophosphates was present.

Choline was separated as the chloroplatinate, after removing barium glycerophosphate.[6] Choline chloroplatinate from A melted at 241–243° with decomposition, and from B at 242–243° with decomposition. *Anal.* Calcd. for $(C_5H_{14}ON)_2PtCl_6$: Pt, 31.42. Found: Pt, 31.64.

Cholamine was precipitated by picrolonic acid after removing barium glycerophosphate and choline chloroplatinate.[6] Cholamine picrolonate from A melted at 223–225° with decomposition, and from B at 222–226° with decomposition.[6]

Anal.: Calcd. for $C_{12}H_{15}O_6N_5$: NH_2-N, 4.31. Found: NH_2-N, 4.3.

By continued steam distillation of another portion of the aqueous hydrolysate made alkaline with sodium hydroxide, a small amount of cholamine was separated and was identified as the picrolonate. This afforded additiona evidence for the presence of cholamine in the hydrolysate

Nitrogen Distribution in the Aqueous Hydrolysate

	Total water soluble N, %	
	In A	In B
Ammonia N[9]	13.8	7.86
Amino N (Van Slyke)	50	57.8

By the method of Lintzel[10] all the N other than ammonia-N and primary amino N was approximately accounted for as choline.

The Ether Solution.—This made up 62% of the phospholipides in A, and 56.2% in B. Of this material, 14.4% in A was insoluble in petroleum ether. This insoluble material was acidic, and black in color, and was perhaps a decomposition product, since with rapidly prepared phospholipides (preparation B) it amounted to only 0.44%. Also, the amount of petroleum ether insoluble material seemed to increase with the age of the phospholipides.

The ether-soluble fatty acids of B were separated by the lead soap–ether method[11] into 13.8% saturated acids having an iodine number of 6[12] and 86.2% unsaturated acids having an iodine number of 95.7.

The saturated acids melted at 55.5–56.5° and had a neut. eq. of 276. Hence, they were probably a mixture of about equal parts of palmitic and stearic acids.[13]

On hydrogenation of the unsaturated acids in ethanol with a Raney nickel catalyst under about 100 atmospheres at 25–125°,[14] followed by one recrystallization from acetone of the hydrogenated acids, an acid melting at 69.5°, showing no depression of the melting point with pure stearic acid, and with a neut. eq. of 284.2 (calcd. for stearic acid, 284.3) was obtained.

On oxidation[15] of the unsaturated acids with cold permanganate only a trace of acids insoluble in chloroform was obtained. The chloroform soluble product melted at 130°[16] and showed no depression of the melting point with pure 9,10-dihydroxystearic acid.

On bromination of the unsaturated acids from A only traces of petroleum ether-insoluble acids were produced. The main product was a dark oil, presumably dibromostearic acid.

On fractionation of the ethyl esters of the unsaturated acids from 17.6 g. of A, a main fraction boiling at 175–177° (3 mm.) having an iodine number of 83.5, and a smaller fraction boiling at 175–180° (3 mm.) having an

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(5) The solid matter was a light-colored solid, insoluble in ether. It contained nitrogen and phosphorus, and preliminary work indicated that it consisted of two compounds of very peculiar composition. The study of these compounds is being continued.

(6) Newman and Anderson, *J. Biol. Chem.*, **102**, 229 (1933).

(7) Barium was determined according to King, *Biochem. J.*, **26**, 586 (1932).

(8) Eegruive, *Z. anal. Chem.*, **100**, 31 (1935).

(9) Characterized as ammonia by the formation of hexamethylenetetramine tetraiodide according to Mulliken, "Identification of Pure Organic Compounds," John Wiley and Sons, Inc., New York City, 1916, Vol. II, pp. 20–21.

(10) Lintzel, *Biochem. Z.*, **238**, 438, 452; **241**, 273 (1931). The accuracy of this method in our hands was about ±10%.

(11) Assoc. Official Agr. Chem., "Methods of Analysis," Washington, D. C., 1930, 3 ed., p. 324.

(12) All iodine numbers were determined according to Rosenmund and Kuhnhenn, *Z. Nahr. Genussm.*, **46**, 154 (1923).

(13) "International Critical Tables," McGraw-Hill Book Co., Inc., New York City, 1928, Vol. IV, p. 165.

(14) The authors wish to express their thanks to Homer Adkins of the Department of Chemistry for hydrogenating the unsaturated acids.

(15) Lapworth and Mottram, *J. Chem. Soc.*, **127**, 1628 (1925).

(16) Crowther and Hynd, *Biochem. J.*, **11**, 139 (1917); Bosworth and Brown, *J. Biol. Chem.*, **103**, 115 (1933).

iodine number of 97 were obtained. Ethyl oleate has an iodine number of 82.

Hence, oleic acid was the chief unsaturated acid; traces of a more unsaturated acid were also present. No palmitoleic acid was found.

Summary

The ether-soluble phospholipides of *A. sydowi* were found to be a mixture of lecithin and cephalin. From the mycelium 0.4–0.7% of phospholipides was isolated. Glycerophosphoric acid, choline, cholamine and oleic acid were identified as the chief hydrolysis products. Stearic, palmitic, and a more unsaturated acid were also probably present in small amounts.

MADISON, WISCONSIN RECEIVED OCTOBER 17, 1935

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

An x-Ray Investigation of the Solid Solution Nature of Some Nitrate Contaminated Barium Sulfate Precipitates[1]

BY GEORGE H. WALDEN, JR., AND M. U. COHEN

Almost from the inception of quantitative analysis the contamination of precipitates by foreign substances has been a universal difficulty encountered by analytical chemists. The cause of the phenomenon has been assigned[2] in general to (1) inclusion of the mother liquor in the precipitate mass, (2) adsorption on the crystal faces, both "inner" and "outer," (3) compound formation and (4) solid solution. That such theories have been purely speculative in character is demonstrated by the fact that their application has varied with different workers from the assumption of one such theory to explain all cases of contamination, almost to the assumption of all such theories to explain any particular case.

The resulting confusion in a field as complex as this is easily understood when the experimental evidence is examined. This has been almost entirely confined to analytical studies of precipitate contamination as a function of the medium in which the precipitates have been formed, or even to the purely pragmatic study of the weight relations of contaminated precipitates. It is clear from a survey of the literature that such methods by themselves cannot furnish conclusive evidence for the mechanism of contamination, and that a truly critical evaluation of the various possibilities awaits the development of experimental methods of an entirely different character. It is further desirable that such experimental methods be applicable almost universally, and not limited either to certain precipitates or to certain contaminants. In these respects the method of radioactive indicators, for example, is severely limited. However, a very positive and direct experimental test is available, at least for theories (3) and (4), in the combination of chemical analysis with x-ray diffraction studies.

In the application of this method to precipitate contamination, that which is due to inclusion and adsorption will lead to no observable changes in the diffraction pattern. Compound formation will lead to the formation of a new solid phase which if present in sufficient amount will manifest itself by the appearance of new lines in the diffraction pattern. Finally, the entrance of a contaminant by the process of solid solution will cause a change in the lattice dimensions dependent on the proportion of contaminant present in the precipitate without affecting the lattice symmetry of the latter.

The great power of this method has been recognized in the case of crystals prepared from melts particularly in metallic systems,[3] and the very numerous studies in that field prove beyond question its validity and utility. There has been, however, but very meager application to crystals formed by precipitation from solution. As part of a systematic investigation by Grimm[4] and his students of solid solutions in ionic crystals, Wagner[5] studied the system $BaSO_4$–$KMnO_4$ with the help of the x-ray technique and established its solid solution nature.

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(1) This article is based upon part of a dissertation submitted by M. U. Cohen in partial fulfilment of the requirements for the degree of Doctor of Philosophy under the Faculty of Pure Science of Columbia University, May, 1935. The material was presented at the New York Meeting of the American Chemical Society, April, 1935.

(2) I. M. Kolthoff, *J. Phys. Chem.*, **36**, 860 (1932); *Chem. Weekblad*, **31**, 102 (1934), and following papers.

(3) Westgren, *Trans. Am. Inst. Min. Met. Eng.*, Inst. Metals, p. 13 (1931); Jette, *ibid.*, **111**, 75 (1934).

(4) (a) Grimm, "Handbuch der Physik," **24**, 1096 ff. (1933); (b) Dahlmann, Diss., Wurzburg, 1930; (c) Peters, Diss., Wurzburg, 1930.

(5) Wagner, *Z. physik. Chem.*, **2B**, 27 (1929).