

rabbit Ab to hen ovalbumin (OA), bovine serum albumin (BSA), and bovine ribonuclease (RNase). In outline, the method is as follows. A protein Ag is treated with N-acetyl *d,l*-homocysteine thiolactone (AHT),^{2,3} which places a number of SH groups on its surface without seriously affecting its capacity to precipitate with Ab directed to the original protein. A specific precipitate is then prepared with the thiolated protein (T-Ag) and the Ab. After the precipitate is freed from non-specific proteins, it is dissolved in a glycine-sulfate buffer at pH 2.4, in which the Ag-Ab bonds are largely dissociated,^{4,5} and the appropriate amount of the bifunctional organic mercurial⁶ 3,6-bis-(acetoxymethylmercurimethyl)-dioxane (MMD) is added. The T-Ag is cross-linked by -S-Hg- bonds and precipitates, leaving much of the Ab in solution. The detailed aspects of the method are illustrated by the preparation of pure rabbit anti-OA Ab.

Thiolation of OA.⁷—To 4.5 ml. of an 8.8% solution of OA in water was added 3.0 ml. of carbonate buffer, pH 10.7 (170 g. anhyd. K₂CO₃ and 15 g. anhyd. NaHCO₃ per liter), and 0.30 g. AHT (Schwarz Laboratories) in 1.5 ml. H₂O. After 2 hr. at 0°, the mixture was diluted with 35 ml. of phosphate buffer, pH 6.8, Γ/2 0.4, to stop the reaction, and was dialyzed with agitation against phosphate buffer, pH 7.0, Γ/2 0.05 for 24 hr. at 6°. This dialysis left only an insignificant amount of hydrolyzed AHT in the protein solution, and SH analysis⁸ indicated that about 6.5 SH were coupled per OA molecule. T-OA was indistinguishable from OA in the ultracentrifuge, and specifically precipitated about 90% of the Ab precipitable by OA.

Purification of Anti-OA Ab.—To 1.0 g. of a γ-globulin fraction, containing 290 mg. of Ab precipitable by T-OA, was added 30 mg. of T-OA in a total volume of 240 ml. of phosphate buffer, pH 7.0, Γ/2 0.05. After 2 hr. the specific precipitate was centrifuged and washed thoroughly with the phosphate buffer. It then was dissolved in 25 ml. of cold glycine-H₂SO₄ buffer, pH 2.4 (32 g. glycine and 240 ml. 1 N H₂SO₄ in 1 liter) and 4 ml. of 1.0 × 10⁻³ M MMD then was added. A precipitate formed almost immediately, and was centrifuged after 1 hr. at 6°. The supernatant was brought to neutral pH by the addition of 30 ml. of phosphate buffer, pH 7.22, Γ/2 1.0. Overnight, a small amount of precipitate formed (13 mg.) and was removed. The supernatant, constituting the purified Ab preparation, contained 146 mg. of protein. Ag-Ab titration experiments showed that at least 90% of the Ab protein was precipitable

by OA after a period of 40 hr. at 6°. The yield was 50%, based on the amount precipitable by T-OA.

Essentially the same procedure was used to isolate anti-BSA and anti-RNase Ab. About 90% and 70% of the Ab precipitable by BSA and RNase, respectively, were precipitated by the corresponding thiolated proteins. Of this Ab, 83% and 35%, respectively, were obtained in essentially pure condition. A more detailed account of these and related results will be published.

(9) Contribution No. 1538 from Sterling Chemistry Laboratory. This work was supported in part by grant E-1204 to Professor S. J. Singer from the U. S. Public Health Service, and grant G-2855 to Professor J. M. Sturtevant from the National Science Foundation.

STERLING CHEMISTRY LABORATORY⁹
YALE UNIVERSITY
NEW HAVEN, CONNECTICUT

S. J. SINGER
JOHN E. FOTHERGILL
JOHN R. SHAINOFF

RECEIVED MARCH 19, 1959

SOLUTION OF METALS IN PEROXYTRIFLUOROACETIC ACID

Sir:

A solution prepared by the addition of 90% hydrogen peroxide to concentrated trifluoroacetic acid has been found to dissolve very readily several metals which fall below hydrogen in the electromotive series: Cu, Bi, Ag, Hg, Tl. In general, the rate of solution increases with an increase in temperature, in concentration of the trifluoroacetic acid and hydrogen peroxide, and in the surface area of metal exposed. In the case of bulk silver, the reaction with anhydrous or very concentrated acid and 90% hydrogen peroxide produces a dark film on the surface of the silver metal. Conceivably, the unusual dissolving property of the trifluoroacetic acid-hydrogen peroxide mixture is due to the *in situ* formation of peroxytrifluoroacetic acid which has been found¹ to have remarkable oxidizing properties in the case of certain organic substances. Silver metal also dissolves in perfluorobutyric and perfluoroöctanic acids upon addition of hydrogen peroxide, the rate of solution in these mixtures appearing to increase with the number of carbon atoms in the acid.

An attempt has been made to exploit the reaction of silver with the hydrogen peroxide-trifluoroacetic acid mixture as a possible method for determining ratios of interest to atomic weight problems. Only carefully purified materials were used in these experiments. As the first step, a weighed sample (approximately 1 g.) of fused silver was converted to silver trifluoroacetate by the action of concentrated trifluoroacetic acid and 90% hydrogen peroxide. The reaction system was warmed during the dissolution of the silver metal. The product trifluoroacetate was dried and weighed to yield the ratio AgO₂C₂F₃:Ag. Next, by treatment with dry hydrogen chloride gas at 150°, the trifluoroacetate was transformed to AgCl which, after fusion, also was weighed. From the latter weight and the weight of silver metal, one can calculate the ratio AgCl:Ag. All of these operations were carried out in the same reaction vessel of fused quartz.

(1) W. D. Emmons and A. F. Ferris, *THIS JOURNAL*, **75**, 4623 (1953).

(2) R. Benesch and R. E. Benesch, *THIS JOURNAL*, **78**, 1597 (1956).

(3) R. Benesch and R. E. Benesch, *Proc. Nat. Acad. Sci. U. S.*, **44**, 848 (1958).

(4) S. J. Singer and D. H. Campbell, *THIS JOURNAL*, **77**, 3504 (1955).

(5) S. J. Singer, L. Eggman and D. H. Campbell, *ibid.*, **77**, 4855 (1955).

(6) J. T. Edsall, R. H. Maybury, R. B. Simpson and R. Straessle, *ibid.*, **76**, 3131 (1954).

(7) The thiolation procedure herein described was developed before the method of Benesch and Benesch³ was available. The latter method involves milder thiolation conditions, but we have not yet determined whether such preparations are satisfactory for our Ab purification procedure.

(8) F. A. Pepe and S. J. Singer, *ibid.*, **78**, 4583 (1956).

Our experiments revealed that the quartz was attacked very slightly during the dissolution of Ag metal in the warm trifluoroacetic acid-hydrogen peroxide mixture. After making suitable corrections for the loss in weight of the quartz vessel, the calculated ratio AgCl:Ag was low by 0.6 to 1.2 parts per thousand. The attack on the quartz vessel and the low AgCl:Ag ratio make it fairly certain that, under the conditions of the experiment, the trifluoroacetic acid breaks down to a slight extent to yield hydrogen fluoride.

The trifluoroacetic acid-hydrogen peroxide reaction has been tested also with a number of other metals in addition to those mentioned above with these results (asterisk indicates clear solution was obtained):

Metal dissolves rapidly: Mg*, Mn, In*, Fe, Pb*
Metal dissolves moderately rapidly: Be*, Al*, Ni*, Sn, W, Sb*

Metal inert to reagent: Cr, Ta, Nb, Au

Elements tested but results indecisive: Si, Zr, Ti, Ga, P, Os, Ir, Pt.

The authors are indebted to Dan S. Kemp for valuable help with part of these experiments. The assistance of a research grant from the National Science Foundation is gratefully acknowledged.

CHEMISTRY DEPARTMENT
REED COLLEGE
PORTLAND, OREGON

ARTHUR F. SCOTT
JANE G. SHELL

RECEIVED FEBRUARY 10, 1959

BOOK REVIEWS

The Infra-red Spectra of Complex Molecules. By L. J. BELLAMY, B.Sc., Ph.D., Senior Principal Scientific Officer, Ministry of Supply. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1958. xvii + 425 pp. 14.5 × 22 cm. Price, \$8.00.

Today's explosive growth of infra-red spectra makes this volume a welcome book. The absorption bands with which it deals constitute a kind of molecular language, telling us of the manifold events in the world of atomic partnerships; and the number of recognizable "words" in this language has now grown so large that a "dictionary" containing them all would be almost as large as the unabridged Webster's. Bellamy's book does not strive to be even an abridged dictionary, but is rather a lexicon of usage; and with respect to the language of the infra-red, one may fairly say that it is a kind of Fowler's "Modern English Usage," a uniquely valuable guide.

In the words of the introduction this book is an attempt to present a critical review of the data on which infra-red spectral correlations are based. There are twenty-three chapters; and with the exception of the first introductory and the final concluding chapters, each covers a class of compounds in which the factors influencing the frequencies and intensities of characteristic bands are discussed. These chapters are assembled together in four sets concerned, respectively, with C-C and C-H linkages, with C-O and O-H linkages, with C-N and N-H linkages and with the vibrations involving other elements and groups of inorganic origin. Each set of chapters is followed by half-a-dozen graphs to illustrate typical characteristic absorption bands; and in the introductory chapters there are five correlation charts, a total of thirty-three full-page illustrations. The final chapter (added in this second edition) is a comprehensive discussion of the origin and significance of group frequency shifts. With regard to this chapter, the author says in the preface that the reader would do well to treat small portions of it with the reserve accorded to an author's account of his own activities. This is an unmerited apology; the conclusions of this chapter are especially valuable in pointing the way in a field where guidance is particularly needed, today.

At the end of the book there is the usual subject index, a compound index and a table of reciprocals to use in converting wave-numbers. An author-index would have been helpful as there are 1681 references in the bibliographies placed at the end of each chapter.

Readers from many fields will be interested in this book. As a guide in the use of infra-red spectra for identifying compounds, it is invaluable. Those, who need help in employing infra-red spectra to interpret molecular structure, might welcome illustrations to show the exact types of

motion associated with different bands; but these usually can be found by consulting references. The relations of infra-red spectra to thermodynamic properties are largely omitted, perhaps because such relationships are outside the scope of the book; but the thermodynamic evidence for spectral interpretation is frequently important. However in sum, this volume assembles a compact and clear body of facts and conclusions which will be most helpful to workers in many fields all the way from the theoretical to the applied aspects of the subject.

CHEMISTRY DEPARTMENT
THE JOHNS HOPKINS UNIVERSITY DONALD H. ANDREWS
BALTIMORE, MARYLAND

Anleitungen für die Chemische Laboratoriumspraxis. Band VIII. Tabellen zur Röntgenstrukturanalyse. By DR. KONRAD SAGEL, Wissenschaftl. Mitarbeiter im Metall-Laboratorium der Metallgesellschaft A. G. Frankfurt (Main). Springer-Verlag, Reichpietschauer 20, Berlin W 35, Germany. 1958. viii + 204 pp. 15.5 × 23 cm. Price, DM 28.—.

This book contains a compilation of formulas, numerical tables and other information (such as a collection of crystallographic data for a large number of compounds) which will be of use to practically any person working in the field of X-ray diffraction. The information is given in four sections: (A) Tables for Indexing Diffraction Maxima; (B) Tables for Intensity Determination; (C) Tables for Analysis of Diffuse Background; and (D) Several Physical and Mathematical Tables. In each section (except D) the tables are preceded by a compilation of formulas and explanatory material.

The only other compilation comparable to this book is Vol. II of Internationale Tabellen zur Bestimmung von Kristallstrukturen (published in 1935 by Gebrüder Bornträger, lithographed in 1944 by Edwards Brothers, Ann Arbor, Mich.), which, of course, is very much out of date (a new edition is to be published soon by the Kynoch Press, Birmingham, England). X-Ray crystallographers, and other people working in the field of X-ray diffraction, will certainly want to have both books. Dr. Sagel's book often contains more information on topics covered by both books. For example, Dr. Sagel gives tables of absorption corrections for plate-like and spherical crystals as well as for cylinders; and his treatment of polarization factors also includes the case of crystal-monochromatized radiation. In those cases where the literature offers a rather wide selection of data, Dr. Sagel has clearly attempted to list information obtained by the best and most modern techniques. His compilation of