showed the presence of several forms of protogen. Most of the activity had a distribution coefficient of 0.4 (C_{H_2O}/C_{CHCl_3}) between chloroform and 0.5 *M* aqueous phosphate buffer at *p*H 7.2. As purification proceeded this form, called protogen-A, changed into another form, protogen-B, which had a distribution coefficient of 20 in this system. On Whatman No. 1 paper strip chromatograms developed with *n*-butanol saturated with 0.5 *N* ammonium hydroxide, protogen-A had an R_f of 0.7 and protogen-B an R_f of 0.33.

Counter-current extractions were continued in a 200-tube extraction apparatus until the distribution of organic solids as measured by wet combustion,[9] the biological activity, and the theoretical distribution curve of a single substance coincided. This highly purified material was then chromatographed on silicic acid to yield an acidic oil which gave a crystalline S-benzylthiuronium salt which could be crystallized from acetone–alcohol mixtures as small needles or rosettes melting at 132–134° (uncor.). Samples of this salt gave the following analyses: C, 48.66, 47.33, 48.68; H, 6.49, 5.62, 6.33; S, 23.84, 23.82; N, 7.57, 6.60.

The sulfur–nitrogen ratios show that protogen-B itself contains sulfur, and analyses of the free acid confirmed the presence of about 30% sulfur. The S-benzylthiuronium salt of protogen-B gave half-maximum growth for *Tetrahymena geleii* at 0.3 millimicrogram per ml. of culture medium; the potency of the salt thus corresponded to about 2.8 millimicrograms per "unit."[2] The salt also had biological activity corresponding to that of the "acetate factor" and reversed the inhibitory effect of propionate for *S. faecalis*.[10]

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[9] M. J. Johnson, *J. Biol. Chem.*, **181**, 707 (1949).

[10] F. P. Day, et al., *Bacteriological Proceedings*, Soc. of Am. Bacteriologists, p. 136 (1951).

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RECEIVED OCTOBER 22, 1951

CHEMICAL NATURE OF α-LIPOIC ACID

Sir:

α-Lipoic acid, which has been obtained in crystalline form from processed liver, will in catalytic amount replace the growth-stimulating activity of acetate for certain lactic acid bacteria, and is required for the oxidative decarboxylation of pyruvate by these bacteria.[1]

Analytical data on the α-lipoic acid obtained by one of us (L.J.R.) follows. Sample 1 (3 mg.): monocarboxylic acid, *pKa* 4.7, equivalent weight 224; m.p. 47.5–48.5°. Ultraviolet spectrum indicated absence of conjugated double bonds. X-Ray characterization has been reported.[1] Sample 2 (10 mg.): m.p. 47.5°; *pKa* 4.8; equivalent weight 220. X-Ray diffraction pattern identical with that of first sample. Spot tests[2] for sulfur positive, for nitrogen negative. Nitroprusside test for thiol negative, but positive after treatment with sodium cyanide, suggesting a disulfide linkage. *Anal.* C, 46.35; H, 6.79; S, 31.21. Calcd. for $C_8H_{14}S_2O_2$: C, 46.57; H, 6.84; S, 31.08. Polarographic determination revealed presence of easily reducible group which undergoes two electron change on reduction. The half wave potential at *p*H 6.7 was −0.59 v. *versus* saturated calomel electrode and −0.31 v. at *p*H 1.3.

Bioautographic[3] and countercurrent distribution[4] studies showed that several distinct compounds possessing acetate-replacing and pyruvate oxidation factor activity, in addition to α-lipoic acid, exist in biological preparations. That one or more of these compounds may be mixed disulfides formed from reduced α-lipoic acid and naturally occurring thiols has been demonstrated. Treatment of α-lipoic acid with glutathione, cysteine or several other thiols, followed by oxidation with iodine,[5] resulted in formation of a series of new compounds, as demonstrated by bioautographs,[3] possessing acetate-replacing factor activity.

Acid hydrolysates of natural materials contain, in addition to α-lipoic acid, a chemically related acidic substance which migrates less readily than α-lipoic acid both on paper chromatograms and from a buffered aqueous phase in countercurrent distributions.[3,4,6] In the preparation of bioautographs α-lipoic acid always is partially converted to this more polar substance, but the reverse transformation does not occur. This suggested that the more polar acid is an oxidation product of α-lipoic acid. It has been demonstrated that α-lipoic acid is converted to the former acid by means of hydrogen peroxide under conditions which would convert a sulfide to a sulfoxide.[7] Furthermore, the more polar acid is converted to α-lipoic acid by treatment with hydriodic acid under conditions used for conversion of a sulfoxide to a sulfide.[8]

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[2] F. Feigl, "Manual of Spot Tests," Academic Press, Inc., New York, N. Y., 1943, pp. 163, 172.

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THE LOWER OXIDATION STATES OF ALUMINUM[1]

Sir:

Evidence for the existence of the +2 and +1 oxidation states of aluminum includes demonstration of the existence of certain compounds prepared

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in the absence of solvents,[2] spectroscopic evidence,[3] and data relating to the anodic oxidation of aluminum in liquid ammonia and other solvents.[4] Thermochemical considerations[5] also indicate that these oxidation states should exhibit appreciable stability even in the form of the crystalline halides.

We wish to make a preliminary report on what we believe to be conclusive evidence for the existence of these oxidation states in solution, based upon potentiometric titrations of liquid ammonia solutions of aluminum(III) iodide with liquid ammonia solutions of potassium using the equipment and procedures described previously.[6]

In a typical experiment, 8.31×10^{-4} g. eq. wt. of pure aluminum(III) iodide dissolved in *ca.* 45 ml. of anhydrous liquid ammonia was titrated with 8.56×10^{-2} *M* potassium solution. The titration curve shows two quite distinct end-points which correspond to the addition of 3.25 and 6.60 ml. of the potassium solution; the calculated volumes required for reduction of Al^{+3} to Al^{+2} and Al^{+1} are 3.24 and 6.49 ml., respectively.

Following the end-point corresponding to completion of reduction to Al^{+2}, a trace of white crystalline solid appears, the potential decreases gradually, then increases until the end-point corresponding to Al^{+1} is reached. A similar trend is observed following reduction to Al^{+1}. In view of the known chemistry of Al^{+3} in liquid ammonia,[7,8] it seems reasonable to attribute this behavior in both instances to the occurrence of slow ammonolytic reactions resulting in the separation of ammonobasic salts. Finally, when a slight excess of potassium over that required for reduction to Al^0 is added, the potential increases *ca.* 1200 mv. and this is coincident with the appearance of a permanent blue coloration which is too intense to be attributable to an ammonia solution of aluminum.[8]

These and related experiments will be described in more detail in a later communication. We are presently extending this method to the study of the intermediate oxidation state problem with other Group III elements, and those of both the lanthanide and actinide series.

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BOOK REVIEWS

Méthodes Modernes d'Analyse Quantitative Minérale. By GASTON CHARLOT, Professeur, and DENISE BÉZIER, Sous-chef de travaux, l'École de Physique et de Chimie Industrielle. Masson et Cie., Éditeurs, 120 Boulevard Saint-Germain, Paris VI^e, France, 1949. vii + 685 pp. Illustrated. 16 × 23.5 cm. Price, 2000 fr.

The title indicates the volume to deal with mineral analysis, but it is considerably more than merely this. It comprises two sections, on General Considerations (25 chapters, 285 pages) and on Analysis of the Principal Elements (54 short chapters, 368 pages). The first section presents well-written and readable discussion of volumetric analysis, acidimetry, oxidimetry, oxidation and reduction materials, acidimetry and *p*H change on oxidation-reduction titrations, titration with complexes, precipitation titration, nonaqueous acidimetry, extraction methods, gravimetric apparatus and techniques, physical analytical methods, colorimetry, absorption and emission spectroscopy, potentiometric titration, electroanalysis, polarography, conductimetry, miscellaneous methods, organic reagents, properties of precipitates, separations, analysis for traces, semi-micro and micro analysis, dissolving of materials, removal of organic matter during analysis, and gas analysis. This section alone is a good coverage of general quantitative analysis in up-to-date fashion.

The second part of the book deals with the analysis of the principal elements, from aluminum to zirconium, sixty-five of them, including the rare earths as one "element." For each the treatment follows the pattern of: Separations (general and special cases and treatments, footnoted and cross-referenced), Gravimetric Methods, Volumetric, Colorimetric, Electrolytic, Potentiometric, Physical, Special Cases, as these methods may variously apply. References to the original literature are plentiful and well-chosen, including post-war publications. The format and typography are good, and proof-reading errors very few. The book will be a good reference source for general inorganic and mineral analysis.

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Fundamental Mechanisms of Photographic Sensitivity. Edited by J. W. MITCHELL. Academic Press Inc., Publishers, 125 East 23rd Street, New York 10, N. Y. 1951. viii + 347 pp. 19.5 × 25.5 cm. Price, $9.50.

The papers which make up this book were presented at a conference held at the University of Bristol in March, 1950, and represent contributions from workers in Europe, Great Britain and the United States. As will be seen from the list of contents given at the end of this review, the scope of the conference was broader than the title of the book suggests. Some of the papers contained in the book have already appeared in scientific journals, but many have not. A few appear in the book in abridged form. No discussion of the papers is recorded, but Dr. Mitchell has written an able review of the status of the theory of sensitivity and latent image formation as it appeared to him at the conclusion of the conference.