tures of fatty acids of interest and offers the advantage that small samples can be used. The usefulness of the method is dependent upon the carriers employed. Only greatly differing substances can be separated if the carriers differ greatly in adsorption. With a system of carriers containing numerous components differing only slightly in adsorbability, the resolution is greater. The carrier hydrocarbon system used by Weitkamp[10] in amplified distillation is an example of the latter. Unfortunately, the hydrocarbon system was unsuitable for the present use. However, it seems probable that some highly complex natural or synthetic mixture having suitable solubility and adsorbability characteristics could be found for use as a more adequate carrier system.

The suggestion of group separation of saturated and unsaturated fatty acids by displacement from silica gel has been made by Claesson.[11] Unfortunately the experiments reported here cannot be closely compared to Claesson's, for the chromatographic systems were dissimilar, the effect of increasing unsaturation generally increasing adsorption on silica gel. However, it seems unlikely that all saturated acids can be separated groupwise from all unsaturated acids. It is more probable that the "group separation" reported was the segregation of saturated from unsaturated acids of similar chain lengths which was observed in the present investigation.

Acknowledgment.—Opportunity is taken to express gratitude to Betty Gibson and Myrtis Schrode for valuable technical assistance.

(10) Weitkamp, *J. Am. Oil Chem. Soc.*, **24**, 236 (1947).

(11) Claesson, *Rec. trav. chim.*, **T65**, 9 (1946).

COLLEGE STATION, TEXAS RECEIVED MAY 3, 1951

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, DEPARTMENT OF SURGERY OF BETH ISRAEL HOSPITAL AND HARVARD MEDICAL SCHOOL]

Half-Salts of Naphthyl Acid Phosphates[1]

BY ORRIE M. FRIEDMAN AND ARNOLD M. SELIGMAN

A half-salt of β-naphthyl dihydrogen phosphate has been obtained (1) by treatment of an alkaline solution of the dihydro acid with excess mineral acid, (2) by precipitation of the acid from solution with sodium chloride or sodium sulfate and (3) by coprecipitation of the acid with its mono sodium salt. The precipitated complex has been shown to be a combination of the dihydrophosphoric acid ester and the mono sodium salt in equimolar amount. Half-salts of α-naphthyl dihydrogen phosphate and 6-bromo-2-naphthyl dihydrogen phosphate have also been obtained. Previous formulation of half-salts of this type as eight-membered ring structures is discussed and an alternate six-membered ring structure suggested.

When β-naphthyl dihydrogen phosphate,[2] m.p. 178–179°, dissolved in aqueous sodium hydroxide, was treated with an excess of mineral acid, the expected β-naphthyl dihydrogen phosphate as such was not recovered, but there was obtained a substance of higher melting point. The same higher melting substance was apparently also obtained when β-naphthyl dihydrogen phosphate was crystallized from aqueous sodium chloride or aqueous sodium sulfate. The substance melted at 203–205°, partially resolidified almost immediately and remelted completely at 250°. The potentiometric titration curve (Fig. 1) suggested that this material was an unsymmetrical dimer with a molecular weight of about 470. Although the melting point of this substance remained practically unchanged after repeated recrystallization from water–ethanol, the potentiometric titration curve of the recrystallized product showed a significant change in the relative position of the breaks in the titration curve, indicating that the dimeric product consisted of two substances. This was in fact proven to be the case by the isolation of β-naphthyl dihydrogen phosphate and sodium β-naphthyl monohydrogen phosphate in apparently equimolar amounts by extraction of the complex with boiling ethyl acetate–alcohol. Since β-naphthyl dihydrogen phosphate is a weaker acid than hydrochloric, the removal of sodium ion from sodium chloride in solution is apparently a solubility phenomenon.

The addition of separate solutions of equimolar amounts of the free acid and of the monosodium salt to one another gave an immediate precipitate of the identical complex. The same solubility phenomenon was observed when a relatively concentrated solution of β-naphthyl dihydrogen phosphate was titrated with alkali. Just before the half-molar point in the titration a precipitate appeared and then disappeared slowly with further addition of alkali.

Methylation of the complex with diazomethane gave in equimolar amounts a white, crystalline, ether-insoluble solid, which proved to be sodium methyl β-naphthyl phosphate, and an ether-soluble oil, which proved to be dimethyl β-naphthyl phosphate. The identical solid was obtained by treatment of the monosodium salt of β-naphthyl dihydrogen phosphate with diazomethane. The identical oil was obtained both by treatment of β-naphthyl dihydrogen phosphate with diazomethane and by reaction of β-naphthyl phosphoryl dichloride with methanol.

Comparison of infrared absorption spectrum of the complex with that of a finely ground mixture of equimolar portions of the free acid and of the monosodium salt in suspension in mineral oil revealed differences in the 3.0, 6.4, 9.3–9.6, 11.2, 11.5 and 15.5 micron regions (Fig. 2). The specific significance of these differences is obscure but the

(1) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **72**, 624 (1950).

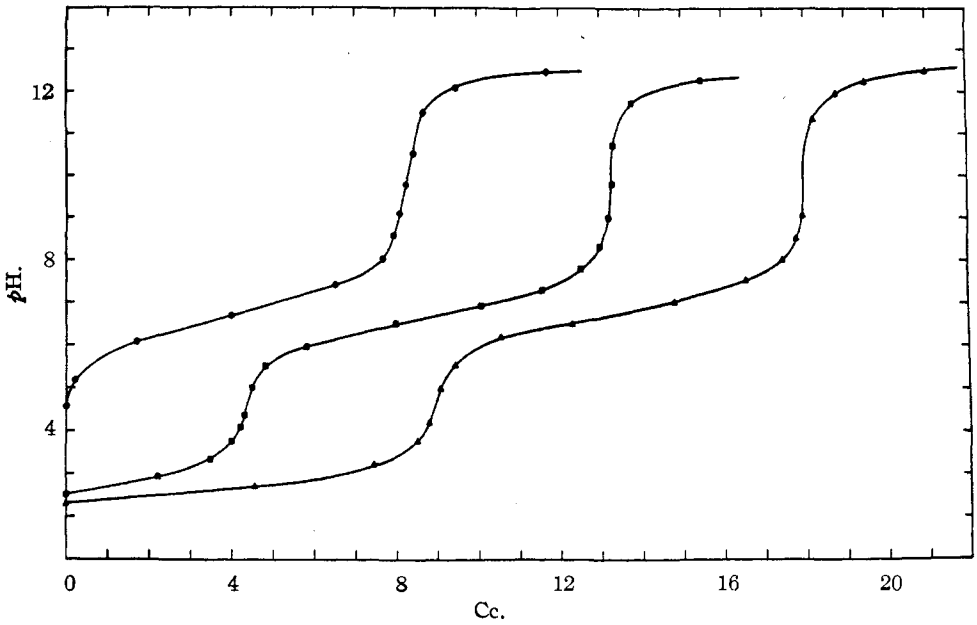


Fig. 1.—Potentiometric titration of β-naphthyl dihydrogen phosphate ▲, sodium half-salt of β-naphthyl dihydrogen phosphate ■, and sodium β-naphthyl monohydrogen phosphate ●, 0.2 g. in 15 cc. of water with 0.0971 *N* sodium hydroxide.

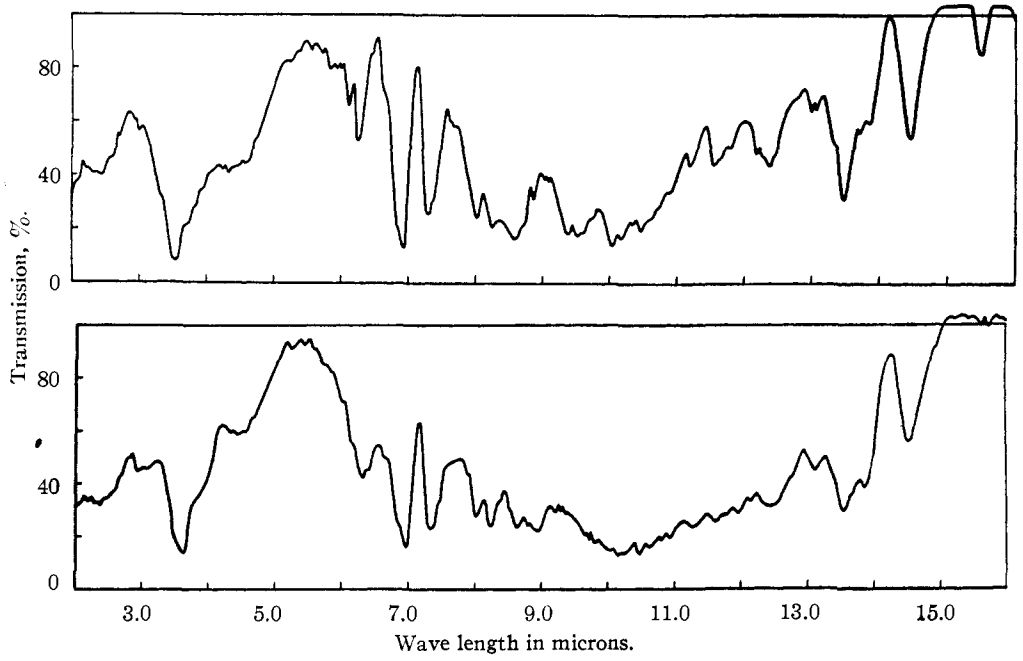
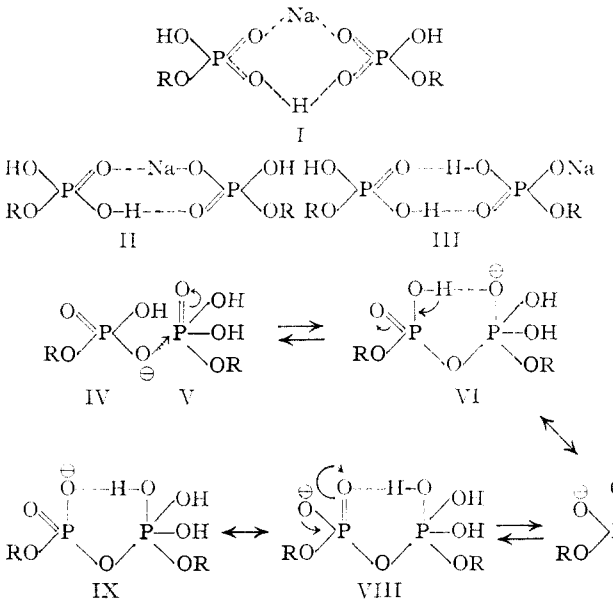


Fig. 2.—Infrared absorption spectra of an equimolar mixture of the dihydro acid and the monosodium salt (upper) and the sodium half-salt of β-naphthyl dihydrogen phosphate (lower) ground in mineral oil.

fact of the differences indicates that in the complex either (1) these two substances are bound chemically, loose and readily reversible though the combination might be, or (2) they are bound physically in a crystal lattice in a way that affects their absorption characteristics. The physical bonding in the crystal would presumably be one for which the acid and monobasic salt have a special predisposition and one which would result in the solubility behavior observed. That this half-salt formation is uniquely a solubility phenomenon does not seem likely from the fact that neither the nature of the cation nor the form of the monoester group appears concerned with the complex formation other than determining the solubility of the product. Complexes of apparently identical type containing potassium or ammonium ion were obtained when solutions of β-naphthyl dihydrogen phosphate in solution in the appropriate alkali were treated with excess mineral acid. Products corresponding to sodium half-salts were obtained when either 6-bromo-2-naphthyl dihydrogen phosphate or α-naphthyl dihydrogen phosphate was crystallized from aqueous sodium chloride. Even more gener-



ally, acridine half-salts of yeast and muscle adenylic acid,[3] sodium half-salts of cetyl[4] and cholesteryl[4] monoesters of phosphoric acid and a quinoline half-salt of dimyristin phosphatidic acid[5] have been obtained.

An eight-membered ring structure (I) containing a hydrogen and a sodium bridge has been proposed by Wagner-Jauregg[4] for half-salts of this type. The bonds represented by broken lines are designated as half-bonds and the structure (I) exists, presumably, as two equivalent resonating forms. Resonance in hydrogen bonds in general and in this case in particular is unlikely on theoretical grounds from evidence obtained by electron diffraction studies in analogous cases with carboxylic acid dimers having eight-membered hydrogen-bonded structures.[6,7,8] Such data indicate that neither are the two carboxyl carbon–oxygen bonds equivalent nor is hydrogen symmetrically situated between the two oxygen atoms. Resonance in the Wagner-Jauregg structure would also appear unlikely on steric grounds by analogy with eight-membered rings in the carboxylic acid dimers which are apparently non-planar.[7,8] Calculations indicate that the carboxyl groups are rotated 146° with respect to one another.

A representation of the Wagner-Jauregg formulation more in accord with theoretical principle would be (II), and since there is no *a priori* reason for a sodium bridge the alternate structure (III) containing two hydrogen bridges is also possible. Although these formulations are empirically consistent with the facts, they afford no apparent explanation of half-salt formation.

A plausible formulation would derive from addition in a sense of the anion (IV) formed in base, to the polarized phosphorus–oxygen bond of an undissociated molecule (V). The six-membered ring (VI) formed by a hydrogen bridge might be expected to be in equilibrium with the tautomeric form (IX). These structures might achieve a certain stability by resonance with structures such as (VII) and (VIII). This formulation, though not that of a pyrophosphate anion, differs from it only by elements of a hydroxyl group. Structures of this type could conceivably be intermediate in pyrophosphate hydrolysis.

Experimental[9]

Sodium β-Naphthyl Hydrogen Phosphate–β-Naphthyl Dihydrogen Phosphate Complex (a) From Alkaline Solution with Excess Acid.—A solution of 2.7 g. of β-naphthyl dihydrogen phosphate[2] and 2.0 g. of sodium hydroxide in 25 cc. of water was added to 50 cc. of 10% hydrochloric acid with good agitation. The voluminous precipitate was collected on a filter and when thoroughly dry in air, weighed 2.7 g., m.p. 202–204° (partial resolidification, final m.p. 244°).

(b) From Aqueous Sodium Chloride.—When 1.0 g. of β-naphthyl dihydrogen phosphate[2] was crystallized from 10 cc. of water containing 0.4 g. of sodium chloride there was obtained 0.9 g. of product separated in a centrifuge and dried in a vacuum desiccator, m.p. 203–205° (partial resolidification, final m.p. 244°). This product gave a potentiometric titration curve and an infrared spectrum essentially identical to those given by the product obtained in (a) Figs. 1 and 2. Recrystallization with an equivalent amount of sodium sulfate gave the same product.

(c) By Coprecipitation.—A solution of 0.2 g. of β-naphthyl dihydrogen phosphate[2] in 3 cc. of water and 0.22 g. of sodium β-naphthyl monohydrogen phosphate[2] in three cc. of water when mixed gave an immediate precipitate. The product was separated on a filter, m.p. 203–205° (partial resolidification, final m.p. 244°), 0.35 g. The infrared absorption spectrum was essentially identical to that for the products from (a) and (b), Fig. 2.

Anal. Calcd. for $C_{20}H_{17}P_2O_8NaH_2O$: C, 49.2; H, 3.89; mol. wt., 488. Found: C, 49.61, 49.07; H, 3.89, 3.71; potentiometric titration (Fig. 1) indicated mol. wt., 470.

Fractionation of the Half-Salt of β-Naphthyl Dihydrogen Phosphate.—To a finely ground suspension of 2.1 g. of the half-salt, obtained in (a), in 100 cc. of boiling ethyl acetate was added 15 cc. of methanol. After boiling for five minutes the suspension was filtered, leaving a residue of a white powder, 1.3 g., m.p. 211° (with partial resolidification). From the filtrate by evaporation to dryness there was obtained 0.8 g. of a pinkish crystalline product m.p. 176–177°, which gave no depression in melting point when mixed with β-naphthyl dihydrogen phosphate. Re-extraction of the residual white powder with 50 cc. of boiling ethyl acetate, to which 10 cc. of methanol was added, left an insoluble portion 1.1 g., sintered 228°, m.p. 296°. The potentiometric titration curve proved it to be sodium β-naphthyl monohydrogen phosphate. An additional 0.2 g. of β-naphthyl dihydrogen phosphate, m.p. 178–180°, was obtained from the filtrate (total yield, 1.0 g.).

Methylation of the Half-Salt.—To a cold ethereal solution of diazomethane, prepared from 6.0 g. of N-nitrosomethylurea by treatment with 12 cc. of 50% sodium hydroxide solution followed by distillation with 60 cc. of ether, was slowly added 2.0 g. of finely powdered half-salt, m.p. 202–204°, as obtained in (a). A vigorous reaction with evolution of gas occurred. The mixture was allowed to stand at room temperature for one hour. On filtration there was obtained a white crystalline residue, 1.2 g., m.p. 222–223°, which gave no depression in melting point when mixed with sodium β-naphthyl methyl phosphate. The filtrate, when evaporated to dryness, left β-naphthyl dimethyl phosphate as a pale yellow oil, 1.0 g., $n^{25}D$ 1.5610; b.p. 150–160°, 0.5 mm., $n^{25}D$ 1.5615.

(3) R. S. Tipson, *J. Biol. Chem.*, **120**, 621 (1937).

(4) T. Wagner-Jauregg and A. Wildermuth, *Ber.*, **77**, 481 (1944).

(5) I. R. Hunter, R. L. Roberts and E. B. Kester, THIS JOURNAL, **70**, 3244 (1948).

(6) J. Karle and L. O. Brockway, *ibid.*, **66**, 574 (1944).

(7) O. Bastiansen, C. Finbak and O. Hassel, *Tids. Kjemi, Bergvesen Met.*, [9] **4**, 81 (1944); *C. A.*, **40**, 3097[9] (1946).

(8) A. I. Gubanov, *J. Exptl. Theoret. Phys. (U. S. S. R.)*, **16**, 523 (1946); *C. A.*, **41**, 633[d] (1947).

(9) Microanalysis by Shirley M. Golden, all melting points are corrected.

Anal. Calcd. for $C_{12}H_{13}PO_4$: C, 57.20; H, 5.16. Found: C, 57.30; H, 5.59.

Sodium β-Naphthyl Methyl Phosphate.—Finely powdered sodium β-naphthyl monohydrogen phosphate,[2] 0.3 g., was slowly added to a cold ethereal solution of diazomethane obtained from 2.0 g. of N-nitrosomethylurea. The addition caused a vigorous reaction with evolution of gas. After one hour at room temperature the mixture was filtered. The residue was triturated with boiling ethyl acetate to which a small amount of methanol was added, leaving a small amount of high-melting residue. On concentration the filtrate gave a white crystalline precipitate, 0.2 g., m.p. 222–223°.

Anal. Calcd. for $C_{11}H_{10}PO_4Na$: C, 50.70; H, 3.87. Found: C, 51.02; H, 3.70.

β-Naphthyl Dimethyl Phosphate (a) Methylation of β-Naphthyl Dihydrogen Phosphate.—The dihydro acid, 2.3 g., was added slowly to a cold solution of diazomethane distilled with 100 cc. of ether from 10 g. of N-nitrosomethylurea. When the diazomethane and excess solvent were distilled there remained a pale yellow oil, 2.5 g., b.p. 160–165°, 0.5 mm., $n^{25}D$ 1.5612.

Anal. Calcd. for $C_{12}H_{13}PO_4$: C, 57.20; H, 5.16. Found: C, 57.71; H, 5.60.

(b) From β-Naphthyl Phosphoryl Dichloride.—The phosphoryl dichloride,[2] 12.1 g., was refluxed in 100 cc. of reagent methanol with 10 g. of potassium carbonate for one hour. After filtration the solution was distilled to remove the solvent. There was obtained an almost colorless oil, 11.58 g., b.p. 160–165°, 0.5 mm., $n^{25}D$ 1.5655.

Anal. Calcd. for $C_{12}H_{13}PO_4$: C, 57.20; H, 5.16. Found: C, 57.20; H, 5.33.

Sodium Half-Salt of α-Naphthyl Dihydrogen Phosphate.—A precipitate was obtained by the addition of aqueous sodium chloride to a solution of α-naphthyl dihydrogen phosphate.[2] Concentrated solutions were required because of the solubility of the product, a factor which also made the removal of occluded sodium chloride difficult. When titrated potentiometrically, however, the product gave the same characteristic curve as was obtained with the β-isomer. The first equivalence point at *p*H 3.5 was reached after addition of 3.55 cc. and the second at *p*H 9 after addition of 10.4 cc. of 0.1000 *N* alkali. The discrepancy in the neutral equivalence is a result of the occluded sodium chloride.

6-Bromo-2-naphthyl Dihydrogen Phosphate.—6-Bromo-2-naphthol[10] was converted to the phosphoryl dichloride with phosphorus oxychloride and pyridine and then hydrolyzed to the dihydrogen phosphate by exposure to moisture by the method previously described.[2] A nicely crystalline product was obtained by recrystallization from acetic acid, m.p. 207–209°.

Anal. Calcd. for $C_{10}H_8PO_4Br$: C, 39.58; H, 2.66. Found: C, 39.74; H, 2.93.

Sodium Half-Salt of 6-Bromo-2-naphthyl Dihydrogen Phosphate.—The half-salt was obtained by addition of aqueous sodium chloride to an aqueous solution of the phosphate. Potentiometric titration of the product had to be carried out in the presence of methanol because of the limited solubility of the half-salt in water. The first equivalence point at *p*H 4 was reached after addition of 1.6 cc. and the second at *p*H 9 after the addition of 4.6 cc. of standard alkali.

(10) C. F. Koelsch, *Org. Syntheses*, **20**, 18 (1940).

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE INC.]

The Isolation of Three New Crystalline Antibiotics from Streptomyces[1]

BY JULIUS BERGER, A. I. RACHLIN, W. E. SCOTT, L. H. STERNBACH AND M. W. GOLDBERG

Three new crystalline antibiotics were isolated from cultures of three unidentified streptomyces. Their most likely empirical formulas are: $C_{46-47}H_{80-82}O_{13}$ (X-206), $C_{25}H_{40}O_7$ (X-464) and $C_{34}H_{52}O_8$ (X-537A). The rather toxic antibiotics are active *in vitro* against certain gram-positive bacteria and mycobacteria, and inactive *in vivo* against bacterial and protozoan infections, when tested at tolerated dose levels.

In the course of our search for new antibiotics, three Streptomyces were isolated from soil samples of Montclair, N. J., Salem, Va., and Hyde Park, Mass. These unidentified organisms were referred to as X-206, X-464 and X-537A, respectively. When grown on a variety of media in aerated submerged culture, all three produced antimicrobially active substances. Three antibiotics were obtained in crystalline form, one from each of the organisms, and in each case from the cells of the cultures, which contained 5–10 times as much active substance as the filtered broth. Although the new antibiotics are chemically different, their biological activities and certain chemical properties are so similar that they are being reported here as a group.

All three antibiotics are colorless, optically active, organic acids, containing C, H and O. Antibiotics X-206 and X-464 were isolated by alcohol extraction of the cells and purified by chromatography of their alkali salts. Antibiotic X-537A was obtained in form of the sodium salt by extraction of the cells with butyl acetate. The salt was soluble in benzene and hot petroleum ether, and insoluble in water, which facilitated its separation from other products. Similar unusual solubility properties were also observed for the alkali salts of the X-206 and X-464 antibiotics, possibly indicating the presence of some common structural feature in all three of them.

The analytical results so far obtained can best be correlated with the following empirical formulas for the three antibiotics: X-206, $C_{46-47}H_{80-82}O_{13}$; X-464, $C_{25}H_{40}O_7$ and X-537A, $C_{34}H_{52}O_8$. Since all three compounds, and particularly antibiotic X-206, are of rather high molecular weight, related empirical formulas are, of course, not excluded.

Only antibiotic X-537A has a characteristic ultraviolet absorption spectrum, with maxima at 317 mμ (ε 3700) and 249 mμ (ε 6400) for the free acid in isopropyl alcohol. It is also the only one of the three compounds to give a positive ferric chloride reaction. An absorption spectrum of this type, together with the observed ferric chloride reaction, would be consistent with the presence in the X-537A antibiotic of an aromatic ring, substituted by a hydroxyl and a carboxyl group. However, this is not yet supported by any other experimental evidence.

All three antibiotics are active *in vitro* against certain gram-positive bacteria and mycobacteria, but they are inactive against gram-negative

(1) Presented before the Division of Agricultural and Food Chemistry (Fermentation Subdivision) of the American Chemical Society, 119th Meeting, Cleveland, Ohio, April, 1951.