COMMUNICATIONS TO THE EDITOR

TETRACYCLINE[1]

Sir:

During catalytic reduction studies on chlorotetracycline (Aureomycin) using a platinum catalyst it was noted that the mixture of compounds obtained showed a low chlorine content. Since dechlorination was occurring under these conditions, chlorotetracycline was then subjected to catalytic reductions more favorable to selective removal of an aromatic halogen.

Chlorotetracycline was found to be reductively dehalogenated at room temperature and atmospheric pressure in the presence of 10% palladium on charcoal catalyst and one mole of triethylamine. Slightly over one mole of hydrogen was absorbed in 15–20 minutes using about 100 mg. of chlorotetracycline/cc. in methyl cellosolve. Some heat is produced during this rapid hydrogenolysis and the uptake of hydrogen practically stops after one mole is absorbed.

After the catalyst is removed the filtrate is poured into 5 volumes of water and the free base of tetracycline crystallizes. This product which occurs as a trihydrate can be recrystallized from methanol and water. The anhydrous form can be obtained by drying at 60° *in vacuo* for 8 hours. Either form begins to swell at 165–170° melting with decomposition at 170–173°.

Anal. Calcd. for $C_{22}H_{24}O_8N_2 \cdot 3H_2O$: C, 53.0; H, 6.0; N, 5.6; H_2O, 10.8. Found: C, 52.9; H, 6.2; N, 5.5; H_2O, 10.9.

Tetracycline base dissolved in *n*-butanol by adding hydrochloric acid crystallizes from this solution as a hydrochloride; m.p., darkens gradually and melts with gas at about 214°; $[\alpha]^{25}D$ − 257.9° (0.5% in 0.1 *N* hydrochloric acid).

Anal. Calcd. for $C_{22}H_{24}O_8N_2 \cdot HCl$: C, 55.0; H, 5.2; N, 5.8; Cl, 7.4. Found: C, 54.9; H, 5.3; N, 5.8; Cl, 7.3.

The ultraviolet absorption spectrum in 0.1 *N* hydrochloric acid shows maxima at 220 mμ (ϵ, 13,000), 268 mμ (ϵ, 18,040), and 355 mμ (ϵ, 13,320).

Treatment of tetracycline with hydrochloric acid gives anhydrotetracycline completely identical with that prepared from chlorotetracycline by treatment with hydriodic acid.[2] This would indicate that no structural changes other than removal of the halogen took place during the reduction.

Tetracycline is a potent antibiotic having an antibiotic spectrum very similar to chlorotetracycline. The former compound exhibits increased stability in neutral or alkaline solution.

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Received August 6, 1953

(1) The use of this name as a generic term is discussed in This Journal, **74**, 4976 (1952).

(2) C. W. Waller, *et al.*, *ibid.*, **74**, 4981 (1952).

IDENTIFICATION OF AN ANTIBIOTIC POLYACETYLENE FROM *CLITOCYBE DIATRETA* AS A SUBERAMIC ACID ENE-DIYNE*

Sir:

The presence of two antibiotic polyacetylenes in culture liquids of the Basidiomycete *Clitocybe diatreta* was reported recently.[1] For one of these, which was obtained crystalline, the tentative formula $C_{17}H_{12}N_2O_6$ was suggested. Analysis of a fresh sample, however, indicates the formula $C_8H_5NO_3$ rather than that above. The compound can be purified by recrystallization from methanol. It does not melt, but explodes at 198° (uncor.). Found: C, 59.01; H, 3.15; O, 29.34; N, 8.42; mol. wt. (ebullioscopic), 159 (neut. eq., 170, from the previous analysis). Calcd. for $C_8H_8NO_3$ (163.13): C, 58.90; H, 3.09; O, 29.42; N, 8.59; mol. wt., 163. The new formula was further supported by analysis of the catalytic reduction product, the values for which agreed with the formula $C_8H_{15}NO_3$. This compound is a colorless crystalline solid, m.p. 144–145° (uncor). Found: C, 55.51; H, 8.77; N, 8.05. Calcd. for $C_8H_{15}NO_3$ (173.21): C, 55.48; H, 8.73; N, 8.09.

The above data, taken in conjunction with the ultraviolet absorption spectrum (Fig. 1) point to an octadioic acid monoamide containing an ene-diyne grouping, as the probable structure of the polyacetylene. Thus, the ultraviolet absorption maxima are close to those exhibited by the lachnophyllum esters,[2,3,4] compounds of known structure containing an ene-diyne system conjugated to an (esterified) carboxyl group. The remaining CH_2NO indicated by the analysis is most readily accounted for as an amide group.

The reduction product of the antibiotic compound was identified as suberamic acid, the product to be expected on reduction of a polyacetylene of the proposed structure. To establish identity it was necessary to prepare an authentic sample of suberamic acid, since the only literature reference found to the compound is in a paper by Étaix,[5] who reports a melting point of 125–127°. Monomethyl suberate, obtained by the method of Hunsdiecker and Hunsdiecker[6] for the partial esterification of some dibasic acids, was converted to the amide by the method used by Jeffery and Vogel[7] for the preparation of some -amic acids (not including suberamic). The amide melted at 144–145° (uncor.) and gave no depression with the reduction product of the polyacetylene. Found: C, 55.57; H, 8.80; N, 8.02. Calcd. for suberamic acid, $C_8H_{15}NO_3$ (173.21): C, 55.48, H, 8.73; N, 8.09. Further proof of identity of the reduction product

(1) M. Anchel, This Journal, **74**, 1588 (1952).

(2) N. A. Sörensen and K. Stavholt, *Acta. Chem. Scand.*, **4**, 1575 (1950).

(3) T. Bruun, C. M. Haug and N. A. Sörensen, *ibid.*, **4**, 850 (1950).

(4) W. W. Wiljams, V. S. Smirnov and V. P. Goljmov, *J. Gen. Chem. (U.S.S.R.)*, **5**, 1195 (1935).

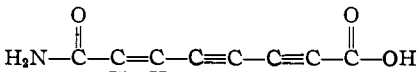
(5) L. Étaix, *Ann. chim. phys.*, [7] **9**, 356 (1896).

(6) H. Hunsdiecker and C. Hunsdiecker, *Ber.*, **75**, 291 (1942).

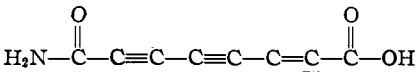
(7) G. H. Jeffery and A. I. Vogel, *J. Chem. Soc.*, 1101 (1934).

with suberamic acid was obtained by alkaline hydrolysis (of the former) to suberic acid, identified by mixed melting point.

Accordingly, the polyacetylene must have the structure I or II.



I



II

Choice between these two formulations could probably best be made on the basis of synthetic model compounds or of authentic samples of the compounds themselves.

Acknowledgments.—This investigation was supported in part by a research grant (E-226) from the National Microbiological Institute of the National Institutes of Health, Public Health Service. The author is indebted to Dr. Julian Wolff for the preparation of Figure 1.

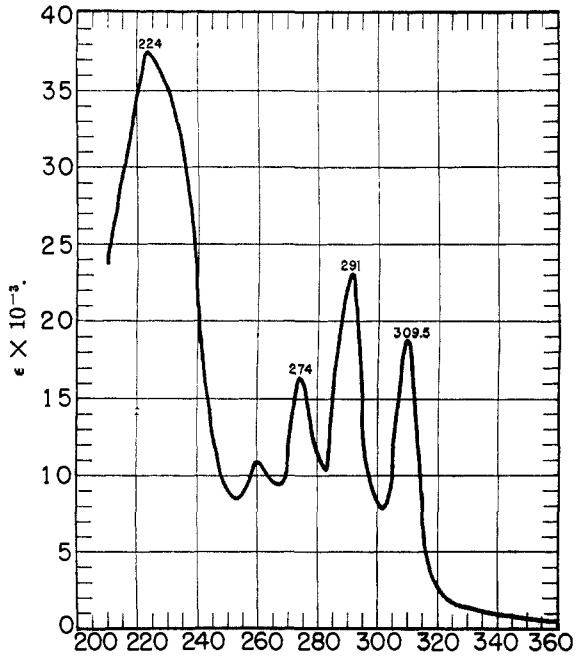


Fig. 1.—Ultraviolet absorption spectrum of the suberamic acid ene-diyne, 0.0005% in 95% ethanol. The readings were made on a Beckmann DU spectrophotometer.

THE NEW YORK BOTANICAL GARDEN MARJORIE ANCHEL
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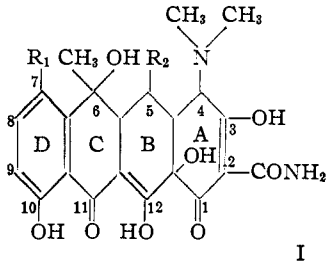
RECEIVED JULY 6, 1953

TERRAMYCIN. XI. TETRACYCLINE

Sir:

In a previous communication,[1] it has been indicated that the structure I, designated tetracycline, is common to the broad spectrum antibiotics, Terramycin (II) and Aureomycin (III).[2] At this time, we wish to report the preparation and antibiotic activity of tetracycline, I (4-dimethylamino - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,-12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo-2-naphthacenecarboxamide).[2a]



I, R_1 = H, R_2 = H
II, R_1 = H, R_2 = OH
III, R_1 = Cl, R_2 = H

I

Treatment of a dioxane–methanol solution of chlorotetracycline with hydrogen in the presence of palladized carbon resulted in the ready hydrogenolysis of the aromatic halogen atom to give the hydrochloride of tetracycline. The latter was converted to the crystalline base, I; m.p. 170–175° dec., $[\alpha]^{25}D$ − 239° (*c* 1% in methanol), pK_a 8.3, 10.2 (50% aqueous dimethylformamide). *Anal.* Calcd. for $C_{22}H_{24}N_2O_8$: C, 59.45; H, 5.44; N, 6.31; mol. wt., 444. Found: C, 59.35; H, 5.41; N, 6.15; equiv. wt. (titration), 227.

On treatment with methanolic hydrogen chloride, tetracycline was readily converted to the previously reported deschloroanhydroaureomycin.[3]

The ultraviolet absorption spectrum of I exhibits maxima at 268 mμ, log ε 4.27, and 363 mμ, log ε 4.14, in 0.01 *M* methanolic hydrogen chloride, and at 246 mμ, log ε 4.24, and 372 mμ, log ε 4.20, in 0.01 *M* methanolic sodium hydroxide.

The ultraviolet spectra of tetracycline in acidic and basic solution are nearly identical with the corresponding spectra[4] for oxytetracycline and provide further confirmation of the structure assigned to tetracycline. The spectra of tetracycline and chlorotetracycline[5] in acid solution are very similar. A slight hypsochromic shift of the long wave length tetracycline peak is attributed to the removal of the aromatic chlorine.[6] In contrast to chlorotetracycline,[7] tetracycline is quite stable in alkaline solution, and its spectrum in this medium is very similar to that of oxytetracycline. This observation demonstrates the profound influence of the aromatic chlorine on the stability of the C ring in chlorotetracycline[8] and is in agreement with

(1) C. R. Stephens, L. H. Conover, F. A. Hochstein, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **74**, 4976 (1952).

(2) Terramycin is the registered trade name of Chas. Pfizer and Company for the antibiotic whose generic name is oxytetracycline. Aureomycin is the registered trade name of Lederle Laboratories for the antibiotic whose generic name is chlorotetracycline.

(2a) This name and numbering system follows suggestions kindly made by the Editors of *Chemical Abstracts*.

(3) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952).

(4) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, *ibid.*, **73**, 4211 (1951).

(5) R. Broschard, A. Dornbush, S. Gordon, B. Hutchings, A. Kohler, G. Krupka, S. Kushner, D. Lefemine and C. Pidacks, *Science*, **109**, 199 (1949).

(6) The ultraviolet spectra of oxytetracycline and chlorotetracycline are discussed in reference 1.

(7) *Cf.* M. S. Bryer, E. B. Schoenbach, C. A. Chandler, E. A. Bliss and P. H. Long, *J. Am. Med. Assoc.*, **138**, 117 (1948).

(8) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952).

observed differences in the course of the alkaline degradation of chlorotetracycline and oxytetracycline.

Although tetracycline lacks both the 5-hydroxyl group of oxytetracycline and the 7-chloro group of chlorotetracycline, it possesses *in vitro* activity (Table I) against a variety of microörganisms which parallels the broad antimicrobial spectra of these two antibiotics.[9,10]

TABLE I

ACTIVITY[11] IN VITRO OF TETRACYCLINE

Species	Minimum inhibitory concentration, mcg./ml.
Aerobacter aerogenes	50.0
Klebsiella pneumoniae	12.5
Escherichia coli	1.56
Salmonella typhosa	0.78
S. paratyphi	0.78
Staphylococcus aureus	<0.19
Proteus sp.	50.0
Pseudomonas sp.	12.5
Brucella bronchisepticae	0.39
Mycobacterium ranae	<0.19
Streptococcus faecalis	<0.19

(9) A. C. Finlay, G. L. Hobby, S. Y. P'an, P. P. Regna, J. B. Routien, D. B. Seeley, G. M. Shull, B. A. Sobin, I. A. Solomons, J. W. Vinson and J. H. Kane, *Science*, **111**, 85 (1950).

(10) T. F. Paine, Jr., H. S. Collins and M. Finland, *J. Bact.*, **56**, 489 (1948).

(11) A serial dilution procedure was employed to determine the minimum concentration of tetracycline at which growth was completely inhibited.

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RECEIVED AUGUST 13, 1953

LOW PRESSURE VALIDITY OF THE BET EQUATION

Sir:

As a prelude to a systematic study of the adsorption of gases on solids in the micron and submicron pressure range, we have determined the adsorption isotherms of heptane on ferric oxide at 29.55, 23.10 and 16.55° and at pressures ranging from 0.01 to 70 microns. We failed to find the first order transition reported for this system by Jura, Loeser, Basford and Harkins[1] and our results are in qualitative agreement with those reported by Smith[2] and by Young and Beebe.[3] Certain experimental difficulties associated with the use of mercury float valves were encountered; it is possible to attribute the apparent first order transition previously reported to an artifact of this sort as well as to the possible lack of equilibrium suggested by Young and Beebe. Equilibrium was attained only after two weeks at one micron pressure when the conventional adsorption bulb was employed; this time was shortened to approximately two days through use of the tray system described by Jura and Criddle.[4]

The specific surface area of the ferric oxide as calculated from a BET plot of the nitrogen adsorption data at liquid nitrogen temperature is 6.82 sq. meters per gram. The adsorption data for heptane at constant temperature may be fitted by the equation $p/v = k_1p + k_2$ for relative pressures ranging from 3×10^{-4} to the highest measured; a relative pressure of 3×10^{-4} corresponds to a surface coverage, θ, of approximately 0.4 monolayer. In the pressure range investigated the above equation is characteristic of the Langmuir and the Brunauer, Emmett and Teller isotherms. In the BET notation, v_m ranges from 0.367 cc. at 29.55° to 0.401 cc. at 16.55°, while "c" ranges from 2070 to 2470. If the nitrogen surface area be accepted as correct, the area per heptane molecule in the monolayer varies from 63 sq. angström at 16.55° to 69 sq. ångströms at 29.55°; these values may be compared with that of 65 sq. ångströms at 25° reported by Loeser and Harkins.[5] The isosteric heat changes rapidly with θ up to approximately 0.4; for higher values of θ the isosteric heat is roughly constant at 14.5 kcal. per mole. The heat of adsorption calculated from the BET "c" is 13.5 kcal. per mole.

It is customary to state the range over which the BET equation fits the experimental data in terms of relative pressure; the low point of this range is generally given as 0.05. The fact that the low point of this range in our experiments is 0.0003 indicates that this statement is incorrect. In general the BET equation is valid for θ less than unity when the heat of adsorption is roughly independent of surface coverage. It might be expected that the relative pressures corresponding to this portion of the isotherm would become lower as the heat of adsorption increases. Thus it would seem preferable to state the region in which the BET equation fits the data in terms of surface coverage rather than relative pressure.

The data at hand permit the calculation of the full set of thermodynamic functions described by Hill, Emmett and Joyner.[6] These functions as well as complete details of the experimental procedure will be presented in a future publication.

(1) G. Jura, E. H. Loeser, P. R. Basford and W. D. Harkins, *J. Chem. Phys.*, **14**, 117 (1946).

(2) L. N. Smith, THIS JOURNAL, **74**, 3477 (1952).

(3) D. M. Young and R. A. Beebe, private communication.

(4) G. Jura and D. Criddle, *J. Phys. Coll. Chem.*, **55**, 163 (1951).

(5) E. H. Loeser and W. D. Harkins, THIS JOURNAL, **72**, 3247 (1950).

(6) T. L. Hill, P. H. Emmett and L. G. Joyner, *ibid.*, **73**, 5102 (1951).

GENERAL ELECTRIC COMPANY
SCHENECTADY, NEW YORK MYRON L. CORRIN

RECEIVED AUGUST 13, 1953

OXIDATION REACTIONS WITH PERTRIFLUOROACETIC ACID

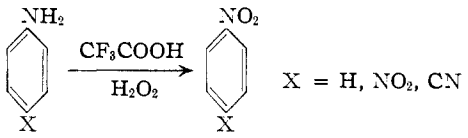
Sir:

We have recently observed that a solution of hydrogen peroxide in trifluoroacetic acid has unique properties as an oxidizing agent presumably due to the *in situ* formation of pertrifluoroacetic acid.

$$CF_3COOH + H_2O_2 \rightleftharpoons CF_3COOOH + H_2O$$

This reagent has been found to oxidize aniline and

substituted anilines to nitrobenzenes in excellent yields. Aniline, *p*-nitroaniline, and *p*-aminobenzonitrile were converted to nitrobenzene, *p*-dinitrobenzene and *p*-nitrobenzonitrile in yields of 79, 86 and 98, respectively. In contrast peracetic acid oxidation of aniline yields 11% nitrobenzene and 71% azoxybenzene.[1]



It has also been demonstrated that pertrifluoroacetic acid is an extremely active reagent for the hydroxylation of olefins. Thus oleic acid was rapidly hydroxylated in quantitative yield in chloroform solution. The hydroxylation of this olefin has been reported with performic acid, but the reaction with pertrifluoroacetic acid appears to be much faster.[2] In reactions of this type the initial product is, of course, the hydroxytrifluoroacetate of the α-glycol but this is easily hydrolyzed to the glycol.

Nitrosoamines have also been oxidized to nitramines smoothly with pertrifluoroacetic acid. Di-

$$R_2N{-}NO \xrightarrow[H_2O_2]{CF_3COOH} R_2N{-}NO_2$$

ethylnitrosamine and dibutylnitrosamine were converted to the corresponding nitramines in 76 and 77% yield, respectively. The oxidation of nitrosoamines to nitramines has been reported in a few instances, but in general the reaction has been unsatisfactory as a general preparative method.[3,4]

The experimental procedures for these oxidations have in most cases been quite simple. Excess trifluoroacetic acid was normally employed as solvent and on addition of hydrogen peroxide to this reagent no evolution of heat was observed. Hydrogen peroxide was usually used as the 90% reagent although the oxidation of oleic acid was also carried out with 30% hydrogen peroxide. The equilibrium between hydrogen peroxide and trifluoroacetic acid is apparently established very rapidly and the solution of pertrifluoroacetic acid so obtained appears to be relatively stable.

In a typical experiment 5.1 ml. (0.2 mole) of 90% hydrogen peroxide was added at 20° to 40 ml. of trifluoroacetic acid. To this solution was added 5.9 g. (0.05 mole) of *p*-aminobenzonitrile in one portion. The temperature of the resulting solution was allowed to rise to 50° and kept there by intermittent cooling for one hour. The mixture was then poured into ice water and 7.2 g. (98%) of *p*-nitrobenzonitrile obtained.

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ARTHUR F. FERRIS

RECEIVED AUGUST 21, 1953

(1) F. P. Greenspan, *Ind. Eng. Chem.*, **39**, 847 (1947).

(2) D. Swern, G. N. Billen, T. Findley and J. T. Scanlan, THIS JOURNAL, **67**, 1786 (1945).

(3) F. S. Brockman, D. C. Downing and G. F. Wright, *Can. J. Research*, **27B**, 469 (1949).

(4) R. Stuermer, *Ber.*, **31**, 2690 (1898).

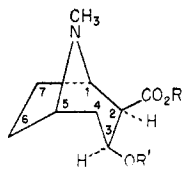
THE THREE-DIMENSIONAL STRUCTURE OF COCAINE

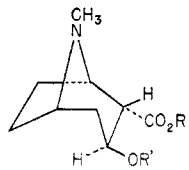
Sir:

Ecgonine is converted by the prolonged action of 33% aqueous potassium hydroxide at 100° to pseudoecgonine.[1] On the other hand, cocaine (benzoylecgonine methyl ester) has now been found to yield pseudoecgonine methyl ester by the action of as little as a tenth of a molar proportion of sodium methoxide in hot absolute methanol. The yield of pure pseudo ester is 75%: m.p. 114–116°, $[\alpha]^{20}D$ +22.8° (*c* 1.7, H_2O).

The reaction of ecgonine methyl ester with methyl iodide has been reported to yield a number of products according to the conditions employed.[2] Although somewhat different results have been obtained in attempting to reproduce this work, the rather remarkable discovery of Willstätter that both ecgonine methyl ester and the pseudo isomer react with methyl iodide to give pseudoecgonine methyl ester methiodide has been confirmed (calcd. for $C_{11}H_{20}INO_3$: C, 38.72; H, 5.91; I, 37.2. Found: C, 38.72; H, 5.88; I, 37.4). Both derivatives melted at 216–216.5° and had $[\alpha]^{20}D$ +11.3° (*c*, 2.0, methanol). The melting point of a mixture of the two products was undepressed.

These two isomerizations indicate that the ecgonine-pseudoecgonine transformation involves epimerization at the α-carbon atom (C_2, structure I). It is well known that the α-hydrogen of carboxylate ions is less labile than that of esters thereof,[3] and the lability of hydrogen in analogous quaternary ammonium compounds has also been established.[3,4] It is difficult to account for these two reactions by means of Willstätter's opinion that this transformation involves the epimerization of the β-carbon atom (C_3, structure I),[2,5] and it appears that he did not consider the possibility of epimerization at C_2.





I. R = R′ = H
II. R = CH_3, R′ = H
III, R = CH_3, R′ = COC_6H_5
IV. R = R′ = H
V. R = CH_3, R′ = H
VI. R = CH_3, R′ = COC_6H_5

That the transformation does not affect both the α- and β-carbon atoms is evident from G. Fodor's demonstration that the carboxyl and hydroxyl groups are *cis* to one another in ecgonine but *trans* in pseudoecgonine.[6] Fodor has found also that the ease of isomerization of N-acetylnorpseudoecgonine ethyl ester (to the O-acetyl isomer) is comparable to that of N-benzoyl- and N-acetylnorpseudotropine.[6,7] It is therefore concluded that

(1) A. Einhorn and A. Marquardt, *Ber.*, **23**, 468 (1890).

(2) R. Willstätter, O. Wolfes and R. Mader, *Ann.*, **434**, 111 (1923).

(3) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Company, New York, N. Y., 1940, pp. 243–244.

(4) E. D. Hughes and C. K. Ingold, *J. Chem. Soc.*, 523 (1933).

(5) (a) R. Willstätter and A. Bode, *Ann.*, **326**, 42 (1903); (b) R. Willstätter and M. Bommer, *ibid.*, **422**, 15 (1921).

(6) G. Fodor, *Nature*, **170**, 278 (1952).

(7) G. Fodor and K. Nádor, *ibid.*, **169**, 462 (1952); see also, A. Nickon and L. Fieser, THIS JOURNAL, **74**, 5568 (1952).

in pseudoecgonine the hydroxyl is *cis* to the nitrogen, as in pseudotropine, and that the carboxyl is *trans* to both the nitrogen and the hydroxyl group (IV).[6] Therefore also, the carboxyl group of ecgonine itself is *cis* to these two functions. Ecgonine may accordingly be called, the nitrogen atom being used as the point of reference, 2-*cis*-carboxy-3-*cis*-hydroxytropane (I).

The failure of N-acetylnorecgonine ethyl ester to rearrange to the O-acetyl isomer was considered by Fodor to favor Willstätter's opinion. This failure is, however, negative evidence, and it has been found here that O-benzoylnorecgonine [*Anal.* Calcd. for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.24; N, 5.09. Found: C, 65.30; H, 6.24; N, 5.20], m.p. 250° (hydrochloride, m p. 219–221°[8]) rearranges in dilute aqueous potassium carbonate to N-benzoylnorecgonine [*Anal.* Calcd. for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.24; N, 5.09. Found: C, 65.67; H, 6.19; N, 4.87], m.p. 163.5°. The neutral O-benzoyl isomer (Nujol mull) has broad weak absorption from *ca.* 3.65 to 5.5 μ attributable to NH_2^+ of a zwitterion[9] and maxima at 5.80 μ and 6.45 μ ascribable to benzoate and carboxylate ion,[9] respectively. The acidic N-benzoyl isomer (Nujol mull) has absorption maxima at 3.12 and 5.76 μ assignable to bonded hydroxyl and the carboxyl group, respectively, and a double maximum at 6.21 and 6.26 μ attributable to the disubstituted amide linkage.

Ecgonine methyl ester, cocaine, pseudoecgonine methyl ester, and pseudococaine are, in view of the foregoing considerations, to be represented by II, III, V and VI. I shall present a more detailed account of this investigation in the near future.

(8) A. Einhorn, *Ber.*, **21**, 3029 (1888).
(9) L. Larsson, *Acta Chem. Scand.*, **4**, 27 (1950).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE STEPHEN P. FINDLAY
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RECEIVED JULY 13, 1953

OXIDATION-REDUCTION POTENTIALS OF HORSERADISH PEROXIDASE

Sir:

A systematic, potentiometric study of horseradish peroxidase (HRP), organized as a joint project of the Department of Biochemistry, Medical Nobel Institute, and the Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, has now been carried to a first point of general interest.

Our studies to date indicate that the oxidation–reduction potentials of the ferri HRP/ferro HRP system are much more negative than the corresponding potentials that have been determined for other hemoproteins. Detailed data over a large range of *p*H are not yet available, but measurements made between *p*H 6 and 8 indicate that here the values of E_0' are more negative even than those reported for free iron protoporphyrin IX. The contrasts are shown in the table.

System	Temp., °C.	*p*H	E_0', volt	Ref.
ferri HRP/ferro HRP	30	6.1	−0.21	
		7.3	−0.27	
ferri protoporphyrin IX/ ferro protoporphyrin IX	30	7.0	−0.14[a]	1
metmyoglobin/myoglobin	30	7.0	+0.05	2
methemoglobin/hemoglobin	30	7.0	+0.14	3
ferri cytochrome c/ ferro cytochrome c	30	7.0	+0.25	4, 5

[a] Value found by extrapolation of experimental data on the basis of an estimated pK_a' value.

It would appear that the different ferri hemoprotein/ferro hemoprotein systems range from among the most positive biological oxidation–reduction systems known to among the most negative systems known. It seems reasonable to ask now whether the well-known resistance to reduction displayed by free catalase might not be at least in part the result of a very negative oxidation–reduction potential for the ferri catalase/ferro catalase system.

The author wishes to acknowledge the great aid of Dr. Hugo Theorell and Dr. Karl-Gustav Paul, who directed the preparation of HRP in crystalline form. Dr. W. Mansfield Clark has lent invaluable advice, and has supplied the equipment for the potentiometric measurements.

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RECEIVED AUGUST 7, 1953

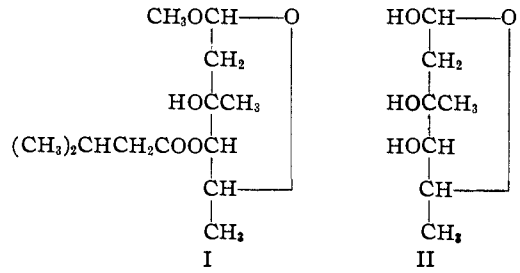
(1) J. Shack and W. M. Clark, *J. Biol. Chem.*, **171**, 143 (1947).
(2) J. F. Taylor and V. J. Morgan, *ibid.*, **144**, 15 (1942).
(3) J. F. Taylor and A. B. Hastings, *ibid.*, **131**, 649 (1939).
(4) F. L. Rodkey and E. G. Ball, *ibid.*, **182**, 17 (1950).
(5) K. G. Paul, *Arch. Biochem.*, **12**, 441 (1947).
(6) Public Health Service Research Fellow of the National Institutes of Health, 1951–1953. These studies supported in part by a grant from Eli Lilly and Company.

MAGNAMYCIN.[1] II. MYCAROSE, AN UNUSUAL BRANCHED-CHAIN DESOXYSUGAR FROM MAGNAMYCIN

Sir:

Methanolysis of the antibiotic Magnamycin[2,3] by 1 *N* methanolic hydrochloric acid yields a crystalline *base* of the formula $C_{29-30}H_{47-49}NO_{12}$ and an oily *neutral substance*, $C_{13}H_{24}O_5$ [b.p. 116° (1.1 mm.), $n^{25}D$ 1.4493, $[\alpha]^{25}D$ − 10.7° (*c* 9, $CHCl_3$), *Anal.* Calcd. for $C_{13}H_{24}O_5$: C, 59.98; H, 9.29; OCH_3, 11.90; mol. wt., 260. Found: C, 60.04; H, 9.40; OCH_3, 11.70; sap. eq., 263]. We wish to record evidence which proves that the neutral substance is the 4-isovaleryl methyl glycoside (I)[4] of a new sugar, *mycarose*, of the structure (II).[4]

(1) Magnamycin is the registered trade name of Chas. Pfizer and Company for the antibiotic carbomycin.
(2) F. W. Tanner, A. R. English, T. M. Lees and J. B. Routien, *Antibiotics and Chemotherapy*, **2**, 441 (1952).
(3) R. L. Wagner, F. A. Hochstein, K. Murai, H. Messina and P. P. Regna, THIS JOURNAL, in press.
(4) These formulas should be regarded as devoid of configurational implications. The stereochemistry of mycarose is now under investigation.



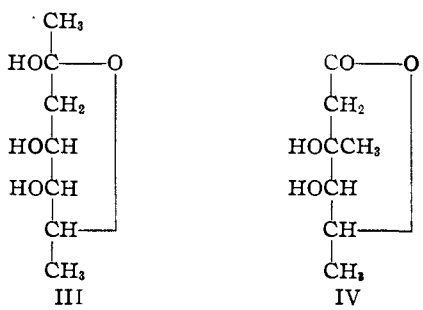
I II

Alkaline hydrolysis of the cleavage product yields isovaleric acid (*p*-nitrobenzyl ester, infrared spectrum, R_F value) and a mixture of anomeric methyl mycarosides, which is separable by fractionation *in vacuo* into a crystalline isomer, m.p. 60.5–61° [b.p. 65° (1.1 mm.), $[\alpha]^{25}D$ − 141° (*c*, 1, $CHCl_3$), *Anal.* Calcd. for $C_8H_{16}O_4$: C, 54.63; H, 9.15; OCH_3, 17.62. Found: C, 54.74; H, 9.18; OCH_3, 17.94], and an oily isomer [b.p. 107° (1.1 mm.), $n^{25}D$ 1.4649, $[\alpha]^{25}D$ + 54° (*c*, 2.3, $CHCl_3$), *Anal.* Found: C, 54.71; H, 9.01; OCH_3, 17.82].

Aqueous acid hydrolysis of the methyl mycarosides yields mycarose as a crystalline solid, m.p. 128–129° $[[\alpha]^{25}D$ − 31.1° (*c*, 4, H_2O), *Anal.* Calcd. for $C_7H_{14}O_4$: C, 51.84; H, 8.70; CCH_3 (2), 18.58. Found: C, 52.07; H, 8.72; CCH_3, 12.69]. Mycarose reduces hot Fehling solution very slowly, contains two methyl groups bound to carbon, shows three active hydrogen atoms in the Zerewitinoff determination, and exhibits only end absorption in the ultraviolet.

Mycarose consumes *two* moles of periodate, and yields one mole each of acetaldehyde and formic acid, as well as lesser amounts of 1,3,5-triacetylbenzene, m.p. 162–163° [mixture melting point with an authentic sample[5] not depressed]. When the reaction mixture from the oxidation of mycarose with *one* mole of periodate is treated with 2,4-dinitrophenylhydrazine, 1-(2,4-dinitrophenyl)-3(*or* 5)-methylpyrazole,[6] m.p. 139–140° [*Anal.* Calcd. for $C_{10}H_8N_4O_4$: C, 48.39; H, 3.25; N, 22.57; CCH_3 (1), 6.05. Found: C, 48.75; H, 3.21; H, 22.65; CCH_3, 3.02], identical with a sample prepared from synthetic acetoacetaldehyde and 2,4-dinitrophenylhydrazine, is produced.

The formation of acetoacetaldehyde, acetaldehyde, and formic acid from mycarose on periodate oxidation, taken with the characterization data, requires that the sugar be formulated as (II) or (III).



III IV

(5) L. Claisen and N. Stylos, *Ber.*, **21**, 1145 (1888).

(6) L. Claisen and P. Roosen, *Ber.*, **24**, 1888 (1891), describe the formation of both possible methylphenylpyrazoles from acetoacetaldehyde and phenylhydrazine.

The latter is excluded by the smooth formation from mycarose, by hypobromite oxidation, of a *lactone*, $C_7H_{12}O_4$, m.p. 108–109° $[[\alpha]^{25}D$ − 35.0° (*c*, 1.86, H_2O), *Anal.* Calcd. for $C_7H_{12}O_4$: C, 52.49; H, 7.55; mol. wt., 160. Found: C, 52.35; H, 7.46; sap. eq., 154], clearly of the structure (IV).[4]

Since the methyl isovalerylmycaroside obtained from magnamycin is not attacked by periodic acid, the isovaleryl residue must be attached at position 4, as in (I).

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RECEIVED AUGUST 14, 1953

MANGANESE REVERSAL OF AUREOMYCIN INHIBITION OF BACTERIAL CELL-FREE NITRO-REDUCTASE

Sir:

There have been several reports indicating that aureomycin inhibits oxidations and/or coupled phosphorylations mediated by various mammalian tissues.[1–3] Van Meter, *et al.*,[4] have reported the inhibition of the respiration of rat liver mitochondria by aureomycin and the reversal of this inhibition by added magnesium. Until recently, there have been no reports of cell-free bacterial systems inhibited by aureomycin. It has been reported from this laboratory[5] that cell-free extracts of *Escherichia coli* (E-26) reduce the nitro groups of chloramphenicol and *p*-nitrobenzoic acid to the corresponding arylamines. Aureomycin in low concentrations markedly inhibited these reductions. The present communication shows that in the partially resolved nitro reductase system, Mn^{++} significantly reversed the inhibitory activity of aureomycin on the reduction.

Cells of *E. coli* (E-26) were grown and harvested as previously described. Arylamine formation was determined by the Bratton–Marshall technique. Cell-free extracts were prepared by suspending 4 g. wet weight of *E. coli* in 20 ml. of cold, distilled water. The suspension was placed in the 9KC Raytheon sonic oscillator for 60 minutes. The extract was then centrifuged in the cold at 27,000 × g. Untreated extracts were capable of reducing nitro groups actively and aureomycin markedly inhibited the reduction. The extracts were dialyzed with stirring for 96 hours at 5° *versus* 4 liters of frequently changed distilled water. After dialysis the extracts were completely inactive in reducing nitro groups. The activity could be almost completely restored by adding to the reaction mixture L-cysteine, diphosphopyridine nucleotide (DPN) and L-malic acid. In this system 1.2×10^{-4} *M* Mn^{++} further stimulated the formation of arylamine. Higher concentrations were

(1) W. F. Loomis, *Science*, **111**, 474 (1950).

(2) T. M. Brady and J. A. Bain, Trans. Fall Meeting, Am. Soc. Pharmacol. and Exp. Therap., 5 (1951).

(3) J. C. Van Meter and J. J. Oleson, *Science*, **113**, 273 (1951).

(4) J. C. Van Meter, A. Spector, J. J. Oleson and J. H. Williams, *Proc. Soc. Exp. Biol. Med.*, **81**, 215 (1952).

(5) A. K. Saz and J. Marmur, *Proc. Soc. Exp. Biol. Med.*, **82**, 783 (1953).

inhibitory. The degree of inhibition of arylamine formation in the fortified system, by low concentrations of aureomycin, was similar to the inhibition in original untreated extracts. It was found that dihydrodiphosphopyridine nucleotide (DPNH) could replace the requirement of the system for L-malate and DPN. In this system, aureomycin was considerably less effective in inhibiting arylamine formation. When 10^{-3} *M* L-malate, 2×10^{-4} *M* DPN and 0.05 ml. dialyzed extract were incubated together in 0.05 *M* tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5 and the formation of DPNH determined by absorption at 340 mμ in the Beckman DU spectrophotometer, it was observed that no formation of DPNH occurred unless 6×10^{-3} *M* Mn^{++} was added to the reaction mixture. These results indicated that aureomycin inhibited arylamine formation by preventing the formation of DPNH and consequently the transfer of hydrogen to nitro groups. Since Mn^{++} was essential for the formation of DPNH, it seemed possible that aureomycin could prevent the formation of DPNH by binding Mn^{++}.[6] If such were the case, excess Mn^{++} added to the reaction might be expected to reverse the inhibitory activity of aureomycin. Table I shows that Mn^{++} reverses aureomycin inhibition of arylamine formation.

TABLE I

The tubes were incubated at 37° for 120 minutes. Each tube contained (final concentration) 0.05 *M* tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5; 3×10^{-4} *M* chloramphenicol, 1×10^{-3} *M* L-malate, 1×10^{-5} *M* DPN; 5×10^{-3} *M* L-cysteine; 0.30 ml. dialyzed extract, final volume 1.5 ml.

Aureomycin concn., micrograms/ml.	Micrograms arylamine formed/ml. No Mn^{++}	6×10^{-3} *M* Mn^{++}
0	17.0	14.6
90	1.1	6.3
45	1.7	8.3
18	2.8	9.4
9	4.0	10.8

In the present system, it would seem that aureomycin inhibits by binding Mn^{++}; possibly this effect is due to the formation of a chelate.[7] Other biological reactions with a need for metal activators are being studied in order to determine whether the inhibition by aureomycin is a general phenomenon of cation-requiring reactions. The implication of the findings in terms of antibiotic activity of aureomycin are under investigation.

(6) Due to the high absorption of aureomycin in the spectrophotometer at 340 mμ, it has not been possible to show directly the inhibition of DPNH formation by aureomycin.

(7) The binding of metallic cations by aureomycin and terramycin was reported very recently by A. Albert, *Nature*, **172**, 201 (1953).

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BETHESDA, MARYLAND RITA B. SLIE

RECEIVED JULY 29, 1953

CORRELATION OF RATES OF SOLVOLYSIS

Sir:

We wish to point out the usefulness of the two-parameter equation

$$\log (k/k^0)_A - \log (k/k^0)_{A_0} = ab$$

where A and A^0 refer to any compound and to a standard compound (*e.g.*, methyl bromide) respectively, k is the first-order rate constant for solvolysis of A or A^0 in any solvent, k^0 is the corresponding rate constant in a standard solvent (*e.g.*, 80% ethanol), a is a constant characteristic of only the compound, and b is a constant characteristic of only the solvent.

Those data on the solvolysis of organic bromides and chlorides in which at least three solvents have been investigated for each compound were used to test the equation; these are for 124 reactions of 15 compounds and 19 solvents, including 18 reactions which form substituted amines or quaternary ammonium salts. After assigning $a = 0.00$ for methyl bromide (A^0), $a = 1.00$ for *t*-butyl chloride, and $b = 0.00$ for 80% ethanol–20% water by volume, the best values of a and b for other compounds and solvents may be found without any complicated methods or equipment.

The average error in $\log (k/k^0)_{calcd.} - \log (k/k^0)_{obs.}$ is 0.18, corresponding to a factor of 1.52 in k itself, excluding 33 standard cases where the error is zero. The maximum error, which corresponds to a factor of 7.6, occurs with benzhydryl chloride in 90% acetone. This is a more than satisfactory fit considering the wide range of rates being correlated; *e.g.*, the ratios of the fastest to the slowest rate measured are 2.8×10^6, 8.7×10^4 and 3.4×10^5 for methyl bromide, benzhydryl chloride and *t*-butyl chloride, respectively.

The 15 compounds and their a values are picryl chloride (−0.42), *p*-nitrobenzoyl chloride (− 0.37), phenacyl bromide (− 0.04), methyl bromide (0.00), benzoyl chloride (+ 0.06), ethyl bromide (+ 0.15), *i*-butyl bromide (+ 0.16), *n*-butyl bromide (+ 0.18), benzyl chloride (+ 0.19), *p*-methylbenzoyl chloride (+ 0.41), *i*-propyl bromide (+ 0.42), α-phenylethyl chloride (+ 0.64), benzhydryl chloride (+ 0.78), *t*-butyl bromide (+ 0.93) and *t*-butyl chloride (+ 1.000).

The 19 solvents and their b values are triethylamine (− 17.3), *n*-butylamine (− 10.2), pyridine (− 9.66), aniline (− 4.78), 100%, 96.7% and 69.5% methanol (−0.94, −0.51, +0.61), 100%, 90%, 80%, 60% and 50% ethanol (−0.79, −0.52, 0.00, +0.88, +1.14), 90%, 80%, 70% and 50% acetone (−0.72, +0.04, +0.42, +1.02), water (+2.95), acetic acid (+0.57) and formic acid (+4.00).

The success of this correlation is due to effective cancellation of kinetic energy and entropy terms which restrict the applicability of many linear free-energy relationships.

Strictly, this type of approach should be confined to compounds whose leaving group is the same or closely similar to the leaving group of the standard compound; furthermore, the atom which is the reaction site should be of the same species in both the standard compound (A^0) and the compound being studied (A). In all the cases above, a bromide or chloride ion is the leaving group, and the reaction is a simple displacement at a carbon atom.

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RECEIVED AUGUST 13, 1953

* National Science Foundation Fellow.

INVOLVEMENT OF THYMIDINE IN THE UTILIZATION OF 5-AMINO-4-IMIDAZOLECARBOXAMIDE

Sir:

Resting cell suspensions of *Lactobacillus arabinosus* 17-5 have been reported to convert 5-amino-4-imidazolecarboxamide to purines and to require phosphate, formate and glucose for this conversion.[1] Similar results are also obtained with broken cell suspensions. In the present investigation, aminopterin (4-amino-4-desoxyfolic acid) has been found to inhibit the utilization of the amine by disintegrated cells, and the inhibition by low but not high concentrations of aminopterin is prevented by thymidine but not by thymine or hypoxanthine desoxyriboside.

Cells from a culture of *L. arabinosus* incubated for 20 hours in a previously described medium[2] modified by omission of adenine and guanine and by addition of 1 mg. of *p*-aminobenzoic acid per l. of medium were harvested by centrifugation, washed, suspended in one-fiftieth of the original volume of *M*/15 phosphate buffer (*p*H 7) and disintegrated by sonic vibration (75 min., 10 kc., 1.0 amp.). The disintegrated cells (0.2 ml.) were added to tubes containing 9.8 mg. of solution containing 0.75 ml. of 1 *M* phosphate buffer (*p*H 7), 100 mg. of glucose, 1 mg. sodium formate, 150 γ 5-amino-4-imidazolecarboxamide, and the supplements indicated in Table I. The tubes were incubated under 1 ml. of benzene for 12 hours at 37°. After centrifugation of the reaction mixture, 1 ml. of the supernatant was used for determination of the remaining 5-amino-4-imidazolecarboxamide.[3] As indicated in Table I, the amine is completely utilized in the absence of aminopterin, but as little as 5 γ per 10 ml. of aminopterin completely inhibits the utilization of the amine. The inhibitory effect of this concentration of aminopterin is almost completely prevented by thymidine, but inhibitions by higher concentrations of the inhibitor are affected progressively less by thymidine. Folinic acid-SF has a very slight effect on the inhibition by aminopterin and is more effective in combination with thymidine.

TABLE I

EFFECT OF THYMIDINE ON AMINOPTERIN INHIBITION OF UTILIZATION OF 5-AMINO-4-IMIDAZOLECARBOXAMIDE

Aminopterin, γ per 10 ml.	5-Amino-4-imidazolecarboxamide utilized, γ per 10 ml. Thymidine, γ per 10 ml. 0	1	5	10	20	40	100
0	150						
1	71						
5	0	35	77	111	133		
10	0		69	84	84		
100	0			28	36	48	30
10[a]	12			103			

[a] Supplemented with folinic acid-SF, 1 γ per 10 ml.

The fact that thymidine stimulates the utilization of from 5 to 35 times its weight of 5-amino-4-imidazolecarboxamide in the presence of 5 γ of aminopterin per 10 ml. suggests a catalytic role of thymidine in the utilization of the amine. Similar results are also obtained with 5-amino-4-imidazolecarboxamide riboside prepared independently by a method analogous to that of Greenberg.[4]

These results as well as the synergistic effect of thymidine and folinic acid in promoting the growth of *Leuconostoc citrovorum* 8081[5] indicate that thymidine is associated with the functioning of folinic acid in these systems. The role of thymidine may involve the formation of conjugates with the substrates followed by cleavage and reutilization or involve a function of thymidine in the biosynthesis of the coenzyme form of folinic acid.

(1) W. Shive, *Fed. Proc.*, **12**, 639 (1953); J. M. Weaver and W. Shive, paper presented before Southwest Regional Meeting, American Chemical Society, Little Rock, Ark., December, 1952.

(2) E. M. Lansford, Jr., and W. Shive, *J. Biol. Chem.*, **194**, 329 (1952).

(3) M. R. Stetten and C. L. Fox, Jr., *ibid.*, **161**, 333 (1948).

(4) G. R. Greenberg, THIS JOURNAL, **74**, 6307 (1952).

(5) T. J. Bardos, T. J. Bond, J. Humphreys and W. Shive, *ibid.*, **71**, 3852 (1949).

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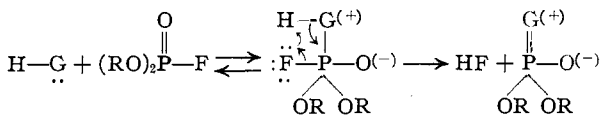
WILLIAM SHIVE

RECEIVED JULY 31, 1953

REACTIVATION OF ACETYLCHOLINESTERASE[1] INHIBITED BY ALKYLPHOSPHATES

Sir:

Certain phosphate esters such as tetraalkyl pyrophosphates, dialkyl *p*-nitrophenyl phosphates, and dialkyl fluorophosphates are potent irreversible inhibitors of acetylcholinesterase (and esterases in general). These compounds are of general interest because the most potent chemical warfare gases and some powerful insecticides belong to this class and owe their lethal action to their inactivation of cholinesterase.[2] The theory of the inhibitory process[3-5] has been developed in accordance with the theory of enzymatic hydrolysis.[6] The inhibitory reaction (here illustrated with a fluorophosphate)



yields a phosphorylated enzyme. Here H—G is the active site (esteratic site) of the enzyme and contains an acidic group (H) and a basic group (. .). The phosphorylated enzyme is analogous to the acylated enzyme which is an intermediate in the enzymic hydrolysis of esters of carboxylic acids. But whereas the acylated enzyme reacts rapidly with water to produce the corresponding acid and

(1) This work was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract No. DA-49-007-MD-37 and (in part) by the Division of Research Grants and Fellowships of the National Institutes of Health, Grant No. RG-1463, United States Public Health Service.

(2) D. Nachmansohn and I. B. Wilson, *Advances in Enzymology*, Vol. XII, New York, 1951, p. 259.

(3) I. B. Wilson and F. Bergmann, *J. Biol. Chem.*, **185**, 479 (1950).

(4) I. B. Wilson, *ibid.*, **190**, 111 (1951).

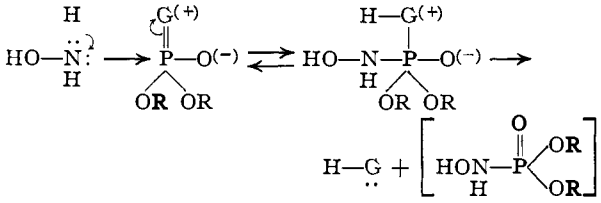
(5) I. B. Wilson, *ibid.*, **199**, 113 (1952).

(6) I. B. Wilson, F. Bergmann and D. Nachmansohn, *ibid.*, **186**, 781 (1950).

regenerate the enzyme the phosphorylated enzyme reacts only very slowly with water. It is the slowness of this reaction which makes these compounds inhibitors rather than substrates.[4]

Theory predicts that nucleophilic reagents should dephosphorylate the enzyme and thus restore its activity. When R = ethyl (inhibitor = tetraethyl pyrophosphate or diethyl fluorophosphate) reactivation is readily accomplished by a large number of compounds containing amino, hydroxyl, mercaptyl, guanidino, amidino, pyridyl or hydroxylamine groups.[4] When R = isopropyl (inhibitor = diisopropyl fluorophosphate) reactivation is far more difficult.

The reactivation process occurs as follows (illustrated with hydroxylamine)



Acetylcholinesterase contains an activation anionic site which binds alkylated cationic amino groups. Experiments show that this site survives the inhibition of the enzyme and can contribute to the reactivation process. It is, therefore, to be expected that a very good reactivator could be produced by combining an intrinsically good reactivating group such as hydroxylamino with a suitably placed quaternary nitrogen structure in the same molecule. We have therefore synthesized nicotinhydroxamic acid methiodide and compared it to hydroxylamine as a reactivating agent.

R	Inhibitor	Reactivator at 24°, 0.1 *M*	Time in hours	% reactivation
Ethyl	Tetraethyl-pyrophosphate (24–48 hrs. exposure)	Hydroxylamine	0.5	40[a]
		Nicotinhydroxamic acid methiodide	0.25	63
			1	91
Isopropyl	Diisopropyl fluorophosphate (1 hr. exposure)	Hydroxylamine	4	17
			24	19
		Nicotinhydroxamic acid methiodide	4	50
			24	96

[a] Taken from ref. 5.

With this new compound we have for the first time obtained large and indeed even complete reactivations of diisopropyl fluorophosphate inhibition. The practical significance of this theory and of the new compound are self-evident.

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NEW YORK, N. Y. ESTELLE K. MEISLICH

RECEIVED AUGUST 3, 1953

BOOK REVIEWS

Non-Aqueous Solvents—Applications as Media for Chemical Reactions. By LUDWIG F. AUDRIETH, Professor of Chemistry, University of Illinois, and JACOB KLEINBERG, Professor of Chemistry, University of Kansas. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1953. xii + 284 pp. 16 × 23.5 cm. Price $6.75.

The present volume on "Non-Aqueous Solvents" is devoted to a topic which should be much more widely studied and understood by organic, inorganic and analytical chemists. The subject is vitally important to the industrial chemist who is constantly seeking more rapid and cheaper methods for the production of his products. Our students are all taught about metathetical and solvation reactions where water is the solvent, but little is said about the same reactions when solvents such as ammonia, alcohol, *etc.*, are used. The universal availability and use of water as a solvent has thus at times partially blinded us and restricted our perspective.

Very appropriately the volume is dedicated to Edward Curtis Franklin, Charles A. Kraus, and Paul Walden, pioneers in the field of "Non-Aqueous Solvent Chemistry." The first chapter is devoted to the properties of solvents, starting with the special characteristics which have been largely responsible for the outstanding position which water occupies. This is followed by a discussion of the nature of differentiating and leveling solvents and type reactions in non-aqueous solvents.

The second chapter is devoted to the historical development of acid–base concepts, stressing the solvent system of Franklin–Kraus, the protonic concept of Brönsted–Lowry, and the electronic theory of Lewis. Succeeding chapters deal with *liquid ammonia as a dispersion medium, the nitrogen system of compounds, reactions in liquid ammonia, metal-ammonia solutions, and nitrogen-containing solvents.* Special chapters are devoted to acetic acid, sulfuric acid, hydrogen fluoride and liquid sulfur dioxide. A separate chapter devoted to acid chlorides deals with selenium(IV) oxychloride, carbonyl chloride (phosgene), nitrosyl chloride and phosphorus(V) oxychloride. The use of halogens and interhalogens as solvents is summarized with tables showing the preparation of nitrosyl and nitronium complexes in bromine trifluoride, the behavior of elements in the solvents I_2, ICl and IBr and the solubility of halides in I_2, ICl, IBr and BrF_3.

A concluding chapter takes up the very interesting, but much less explored field of high temperature solvent systems. These include phenomena involved in ceramics and geochemical phenomena such as no doubt occurred in nature in the formation and deposition of our minerals and ore deposits.

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Cytochemistry—A Critical Approach. By J. F. DANIELLI, Professor of Zoology, King's College, London, W. C. 2. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1953. v + 139 pp. 14.5 × 22 cm. Price, $4.00.

Danielli presents in seven chapters the subject matter of a series of lectures designed to guide workers entering the "almost undeveloped" field of cytochemistry. He gives