This technique greatly increases the specificity of the fermentation method and an investigation of the conditions necessary for its application to substances like animal tissue and excreta is under way.

THE FLEISCHMANN LABORATORIES
STANDARD BRANDS INCORPORATED
810 GRAND CONCOURSE
NEW YORK, N. Y.

RECEIVED SEPTEMBER 16, 1938

COMMUNICATIONS TO THE EDITOR

CRYSTALLINE COPPER-PROTEIN POSSESSING TYROSINASE ACTIVITY

Sir:

A crystalline material has been obtained from the aqueous extract from the wild mushroom, *Lactarius piperatus*, which may be phenol oxidase, or closely related to it. The crystals were six-sided plates and undoubtedly belonged to the hexagonal system. They were insoluble in water, dilute acids and salt solutions, but soluble in an aqueous solution of secondary sodium phosphate. Analysis showed a copper content of 0.25 and 13.6% nitrogen. Their phosphate solution was active in promoting the aerobic oxidation of *p*-cresol and catechol.

The procedure followed in obtaining the crystals can be described briefly as follows. The aqueous extract of the ground mushrooms was precipitated with 0.6 saturated ammonium sulfate, redissolved in water, the latter made 0.2 saturated with ammonium sulfate and the precipitate discarded. The filtrate obtained in the last operation was reprecipitated with 0.6 saturated ammonium sulfate, the precipitate formed redissolved in water and the solution treated with three volumes of cold acetone. The precipitate thus obtained was dissolved in water and treated with alumina. The liquid separated from the alumina contained about 50% of the active oxidase. This liquid was treated with boneblack and after filtering the filtrate was again precipitated with 0.6 saturated ammonium sulfate. The precipitate from the last operation was taken up in water and had an activity of 7000 units per cc. when determined according to the Graubard and Nelson method as modified by Adams and Nelson [THIS JOURNAL, **60**, 2472 (1938)]. When this liquid was gradually acidified by acetic acid, changing the *p*H from 6.5 to 5, and allowed to stand in the ice box, crystals separated.

DEPARTMENT OF CHEMISTRY
COLUMBIA UNIVERSITY
NEW YORK CITY
HAROLD R. DALTON
J. M. NELSON

RECEIVED NOVEMBER 25, 1938

TETRAMETHYLPLATINUM AND HEXAMETHYLDIPLATINUM

Sir:

Tetramethylplatinum has been prepared in 46% yield from trimethylplatinum iodide and methylsodium. It is the most soluble organoplatinum compound so far prepared, being readily soluble in the cold in benzene, acetone, ether and petroleum ether (b. p. 60–68°). The compound crystallizes from petroleum ether as large hexagonal crystals which decompose but do not melt at elevated temperatures. *Anal.* Pt, 76.84; C, 18.32; H, 4.31. We have found that the compound is one of several by-products of the Pope and Peachey [*J. Chem. Soc.*, **95**, 571 (1909)] reaction for the preparation of trimethylplatinum iodide from platinic chloride and methylmagnesium iodide. Hydrogen chloride converts tetramethylplatinum to trimethylplatinum chloride. *Anal.* Pt, 70.20; Cl, 13.10.

Hexamethyldiplatinum has been synthesized in 60% yield by heating trimethylplatinum iodide with powdered potassium in dry benzene. *Anal.* Pt, 81.13; C, 14.55; H, 3.92. The compound is very soluble in benzene, acetone and ether, but only slightly soluble in cold petroleum ether. It is best crystallized from a benzene–petroleum ether solution. Molecular weight determinations show that hexamethyldiplatinum is not dissociated at the freezing point of benzene [mol. wt.: calcd., 480.4; found, 482]. Iodine in ether converts the compound to the known trimethylplatinum iodide.

These two organoplatinum compounds are interesting because they demonstrate for the first time that true organoplatinum compounds not having acid radicals can be prepared. A detailed account of these and other organoplatinum compounds will be published shortly.

THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE
AMES, IOWA
HENRY GILMAN
M. LICHTENWALTER

RECEIVED OCTOBER 31, 1938

THE IDENTIFICATION OF METHYLCHAVICOL IN AMERICAN GUM SPIRITS OF TURPENTINE

Sir:

Recent investigations have shown that gum spirits of turpentine of slash and longleaf pine contain about 95% of pinenes [Dupont, *Ann. chim.*, [10] **1**, 184 (1924); Aschan, "Naftenföreningar, Terpener och Kamferarter," Helsingfors, 289 (1926); Palkin, Technical Bulletin 596, U. S. Department of Agriculture (1932)]. We have been able to obtain physical and chemical evidence, including the fact that homoanisic acid is obtained on oxidation, that the fractions boiling above those of the pinenes contain considerable amounts of methylchavicol (4-methoxyallylbenzene).

This was shown by the isolation of methylchavicol from turpentine by the method described by Balbiano [*Ber.*, **42**, 1504 (1909)], for the separation of methylchavicol from anethole. Seventy-five grams of a fraction, b. p. 88–95° at 10 mm., obtained by fractionation of steam-distilled residues of spirits of turpentine was dissolved in 400 cc. of ether and shaken for one hour with a solution of 81 g. of mercuric acetate dissolved in 350 cc. of water. After separating the ether, the aqueous solution was heated for ten hours at 70–80° with 70 g. of sodium hydroxide and 80 g. of granulated zinc. The mixture was then distilled with steam, the distillate extracted with ether and the ether solution dried and distilled, leaving 8 g. of methylchavicol, the main fraction of which boiled at 213–215°; d^{25}_{25} 0.9600; n^{22}_{D} 1.51372. Physical constants recorded in the literature are: b. p. 214–215° [Klages, *Ber.*, **32**, 1439 (1899); d^{21} 0.9645; n_D 1.5236; (Beilstein, "Handbuch der organischen Chemie," fourth edition, **6**, 571 (1923)]. Ten grams of this product was oxidized at room temperature with 630 cc. of 4% potassium permanganate solution. After removal of manganese dioxide, the filtrate was evaporated to about one-third of its original volume and acidified with hydrochloric acid. The precipitated anisic acid was recrystallized from water and melted at 184–185° (corr.); yield, 2.5 g. Calcd. for $C_8H_8O_3$: C, 63.18; H, 5.30. Found: C, 63.32; H, 5.46. The acid did not lower the melting point in the mixed melting point test with an authentic sample of this material.

The aqueous filtrate from which the anisic acid had been removed was evaporated to a small volume, extracted with ether and dried. The ether was distilled and about 0.3 g. of a substance, apparently homoanisic acid, was obtained. It was recrystallized from water and melted at 85.5–86.5°. Homoanisic acid melts at 85–86° [Pschorr, Wolfes and Buckow, *Ber.*, **33**, 172 (1900)]. Calcd. for $C_9H_{10}O_3$: C, 65.04; H, 6.07. Found: C, 65.30; H, 6.54.

We assume that the difference in odor between highly purified turpentine and American gum spirits of turpentine can be partly attributed to the presence of phenol ethers.

G. & A. LABORATORIES, INC.
SAVANNAH, GEORGIA
TORSTEN HASSELSTROM
BURT L. HAMPTON

RECEIVED OCTOBER 28, 1938

PANTOTHENIC ACID AS A GROWTH FACTOR FOR THE DIPHTHERIA BACILLUS

Sir:

It has been shown in experiments already presented elsewhere that β-alanine and nicotinic acid are essential for the growth of certain strains of the diphtheria bacillus [Mueller, *Proc. Am. Exptl. Biol. Med.*, **36**, 706 (1937)]. β-Alanine has long been known to be a constituent of meat extract both in the free form and also combined with histidine in the compound carnosine, in which form it has also been shown to be available to the diphtheria bacillus [Mueller, *J. Biol. Chem.*, **123**, 421 (1938)]. In a personal communication, we have recently learned from Dr. R. J. Williams that pantothenic acid [Williams, *et. al.*, THIS JOURNAL, **55**, 2912 (1933); **60**, 2719 (1938)] also yields β-alanine upon hydrolysis. Dr. Williams suggests, further, that β-alanine may be effective in producing growth with *C. Diphtheriae* only insofar as it serves as a building stone for the production of pantothenic acid, which may be the material actually required by the organism, and he has supplied us with specimens of pantothenic acid and calcium pantothenate in varying degrees of purity. With this material we have been able to test out this theory and to show that it well may be correct.

Using a control medium containing the products of complete acid hydrolysis of casein, lactic, pimelic and nicotinic acids and inorganic salts, but completely deficient in β-alanine, a series of media has been prepared in which increasing quantities of β-alanine were added to one set, pantothenic acid or calcium pantothenate to a second and the latter substance hydrolyzed by autoclaving with normal hydrochloric acid for thirty minutes at 15 lb. (1 atm.) to a third. The various media were autoclaved (*p*H 7.6) for ten minutes at 10 lb. (0.67 atm.) pressure. After inoculation with the strain of the diphtheria bacillus (Allen), and incubation for three days at 35°, the resulting bacterial growth was centrifuged down, washed and the bacterial nitrogen determined by a method already described, as a measure of bacterial growth [Mueller, *J. Bact.*, **29,** 383 (1935)]. The table shows the results obtained: A = β-alanine; B = Ca pantothenate (about 50%) stated to yield 16% β-alanine on hydrolysis; C = solution B after acid hydrolysis.

	γ added/10 cc. medium			Mg bacterial N/10 cc. medium		
	A	B	C	A	B	C
1 Control +	..	0.0	..	..	0.13	..
2 Control +	..	.31	..	..	.41	..
3 Control +	..	.62	..	..	.60	..
4 Control +	..	1.5	1.5	..	1.47	0.32
5 Control +	..	3.1	3.1	..	2.78	.16
6 Control +	1	6.2	6.2	0.39	3.67	.29
7 Control +	2.5	15.5	15.5	.24	4.70	2.05
8 Control +	5	31	31	6.12	5.35	5.76
9 Control +	10	62	62	6.78	6.60	lost[a]
10 Control +	20	125	..	7.30	7.06	..

[a] Heavy growth.

This and other similar experiments uniformly show a smooth, gradual increase in growth with the addition of pantothenic acid, whereas with β-alanine there is no definite increase over controls until a concentration of from about 2.5 γ per 10 cc. is used. At this point, initiation of growth is slow and irregular, possibly dependent on the size of the inoculum, (platinum loop of pellicle). At levels of 5, 10 and 20 γ concentration of β-alanine or its equivalent of panthothenic acid, growth is heavy and the amounts produced by the two materials are very nearly the same. Hydrolysis of pantothenic acid yields a solution with the properties of β-alanine, *i. e.*, no effect until the concentration of the latter in the hydrolytic products reaches 2.5 γ/10 cc. medium, whereas doubling this concentration regularly leads to heavy growth.

These facts tend to support the view that β-alanine must first be built up into some more complex material before it may be directly utilized by *C. Diphtheriae*, whereas with pantothenic acid no such preliminary synthesis is required.

It is interesting and significant that Snell, Brown and Peterson [THIS JOURNAL, **60,** 2825 (1938)] have shown recently that both pantothenic acid and nicotinic acid are apparently essential factors for growth of certain strains of the lactic acid bacteria. These organisms, unlike the diphtheria bacillus, seem to be unable to effect the synthesis of pantothenic acid from β-alanine, requiring the presence of the more complex material for satisfactory growth.

DEPARTMENT OF BACTERIOLOGY AND
IMMUNOLOGY J. HOWARD MUELLER
HARVARD UNIVERSITY MEDICAL SCHOOL A. W. KLOTZ
BOSTON, MASSACHUSETTS

RECEIVED NOVEMBER 23, 1938

PRESSURE-AREA RELATIONS FOR MONOMOLECULAR FILMS OF TRI-*p*-CRESYL PHOSPHATE AND RELATED COMPOUNDS

Sir:

Interesting differences in surface activity have been observed in a study of phosphorus-organic compounds closely related in structure. Monomolecular film properties of the three isomers of tricresyl phosphate (ortho, meta and para) are practically identical but quite unlike those of either tri-*p*-cresyl phosphite or tri-*p*-cresyl thiophosphate.

Pressure–area relations have been studied on a modified Cenco film-balance apparatus, using general techniques similar to those described by N. K. Adam[1] and Harkins, Ries and Carman.[2,3] The volatile solvent used for the Eastman compounds was twice-distilled benzene. Films were spread on distilled water.

Tri-*p*-cresyl phosphate forms well-behaved, compressible films, whereas the corresponding phosphite and thiophosphate show extremely poor film-forming properties. Films of the phosphite and thiophosphate require compression far beyond the minimum film area for monomolecular

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(1) N. K. Adam, "The Physics and Chemistry of Surfaces," Clarendon Press, Oxford, 1938.

(2) Harkins, Ries and Carman, THIS JOURNAL, **57**, 2224 (1935).

(3) Harkins, Carman and Ries, *J. Chem. Phys.*, **3**, 692 (1935).

thickness before an appreciable rise in pressure is observed. For example, at a pressure of only 1 dyne per cm., the tri-*p*-cresyl thiophosphate film thickness (62.9 Å.) is over five times as great as the maximum monomolecular thickness (11.6 Å.) of tri-*p*-cresyl phosphate.

An interesting correlation between polar group structure and film properties is apparent when we consider the schematic diagrams in Fig. 1.

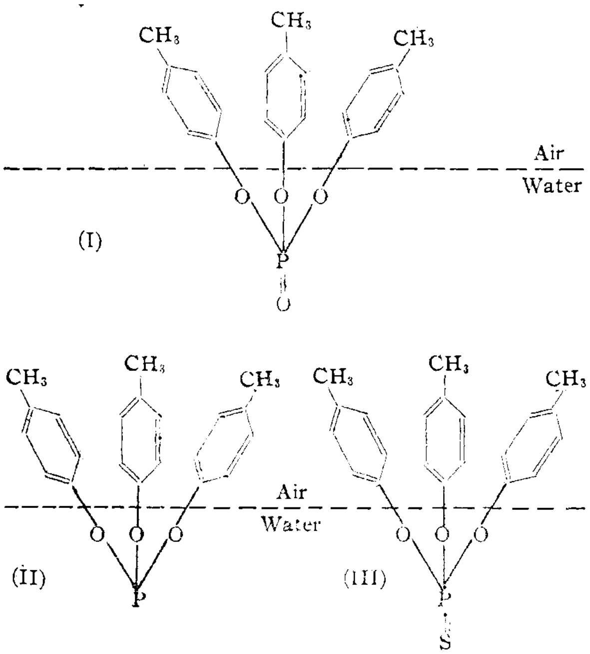

Fig. 1.—Schematic diagrams of molecular orientation at an air–water interface: (I) tri-*p*-cresyl phosphate; (II) tri-*p*-cresyl phosphite; (III) tri-*p*-cresyl thiophosphate. At expanded areas the rings are probably drawn close to the water.

The two poor film-forming compounds (II and III) differ from the tri-*p*-cresyl phosphate (I) in that they lack the P=O group. We must, therefore tentatively conclude that the important contribution to the polarity of the molecule is made by the P=O group as indicated by the superior film properties of tri-*p*-cresyl phosphate.

The most important pressure–area characteristics of a typical tri-*p*-cresyl phosphate film (27.9°) may be briefly summarized. The molecular area values at film pressures of zero (extrapolated), 5 and 8.76 dynes per cm. (the maximum) are, respectively, 99.4, 69.3 and 46.5 sq. Å. Using a density of 1.1284 g. per cc. the film thickness values at the same pressures are, respectively, 5.42, 7.78 and 11.6 Å. Partial overlapping of the molecules may perhaps account for the small areas in the final stages of the compression. The compressibility at $f = 0$, $[(a_0 - a_1)/a_0]/(f_1 - f_0)$ is 0.0607.

The above-mentioned compounds with each of the three rings attached directly to the polar group are much more compressible than the polycyclic compounds in which only one ring is attached to the principal polar group.[4] The molecular configurations of the latter type permit an approach toward vertical orientation and allow closer packing. It has been shown that films of horizontally oriented long chain molecules (polymers of ω-hydroxydecanoic acid) are much more compressible than those of vertically oriented molecules.[3] This present work indicates that perhaps the same general relationship applies to ring systems. The film compressibilities of a number of multiple ring compounds which are capable of vertical orientation range from one-sixth to about one-eighteenth that of tri-*p*-cresyl phosphate. In both types of orientation the films formed by ring compounds are, in general, more compressible than those composed of straight chain molecules. An increase in maximum film pressure with increasing vertical length of the ring system is also noted.

[4] Pressure–area relations for such a series of three-ring and five-ring compounds have been investigated and the data tabulated.[2,5]

[5] Harkins, Carman and Ries, THIS JOURNAL, **58**, 1377 (1936).

THE RESEARCH AND DEVELOPMENT DEPARTMENT
SINCLAIR REFINING COMPANY
EAST CHICAGO, INDIANA HERMAN E. RIES, JR.

RECEIVED NOVEMBER 17, 1938

THE STRUCTURE OF LUMISTEROL

Sir:

In a memoir entitled "Photochemical Interaction between Ketones and Alcohols," Weizmann, Bergmann and Hirshberg[1] in a discussion of the mechanism of ergosterol irradiation state that "at variance with the assumption of Spring[2] that this important reaction involves primarily epimerization at C_3, Windaus and Dimroth[3] were able to demonstrate that the secondary hydroxyl group at C_3 is actually not influenced by irradiation. . . ."

In the interest of accuracy it is necessary to note that in the paper cited I state that "the reactions of lumisterol, the primary photoisomeride of ergosterol, establish that it is a stereoisomer of the latter," no suggestion being made that it is the

[1] Weizmann, Bergmann and Hirshberg, THIS JOURNAL, **60**, 1530 (1938).

[2] Spring, *Chemistry and Industry*, **55**, 837 (1936).

[3] Windaus and Dimroth, *Ber.*, **70**, 376 (1937); *cf.* Dimroth, *ibid.*, **69**, 1123 (1936).

C_3 epimer of ergosterol. In the paper (Heilbron, Moffet and Spring[4]) of which the note cited is an abstract, the conclusion reached is that ergosterol and lumisterol differ in configuration around C_{10} and that "no information is available concerning the relative orientation of the hydroxyl groups and the C_9-hydrogen atoms of the two sterols."

Again, contrary to the statement of Weizmann, Bergmann and Hirshberg, Windaus and Dimroth did not *demonstrate* that the secondary hydroxyl group is not influenced by irradiation but include in their paper the following statement: "Einen Anhaltspunkt dafür dass sterische Änderungen an der Hydroxyl-gruppe an C_3 vor sich gehen, haben wir nicht gefunden; wir halten diese Annahme für unwahrscheinlich."[5]

NOTE BY THE EDITOR.—Dr. Bergmann has written to the Editor that he regrets that the statement of Dr. Spring relative to the photo-isomerization of ergosterol was misunderstood and therefore inaccurately quoted in the article referred to.

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[4] Heilbron, Moffet and Spring, *J. Chem. Soc.*, 411 (1937).

[5] "We have found no experimental evidence that rearrangement of the hydroxyl group on C_3 occurs spontaneously, and we believe that such a change is unlikely."

THE UNIVERSITY OF MANCHESTER F. S. SPRING
MANCHESTER, ENGLAND

RECEIVED AUGUST 9, 1938

THE REDUCTION OF α-HALOGENATED KETONES: THE SYNTHESIS OF *dl*-PSEUDOEPHEDRINE

Sir:

The great advantage of aluminum isopropylate in the reduction of aldehydes and ketones lies in its specificity for the carbonyl group, side reactions such as condensation and the like being either absent or negligible. Lund [*Ber.*, **70,** 1520 (1937)] has shown this with certain ketones with primary bromine in the α-position. This reduction without removal of the α-halogen has now been tested with α-bromo ketones which have β-hydrogen available for a loss of halogen acid. The results with open chain ketones containing secondary α-bromine show that removal of bromine, and reduction to the bromohydrin, occur in about equal amounts. Thus α-bromopropiophenone with aluminum isopropylate forms the bromohydrin in about 35% yield (b. p. 73–75° (0.1 mm.)), which in turn with methylamine yields a mixture of isomeric hydroxyamines, one of which is *dl*-pseudoephedrine, m. p. 116.5–117.0°; the hydrochloride, m. p. 162–163° (over-all yield from the bromo ketone, 10%). This was identified by comparison with an authentic sample, m. p. 116.5–117.2°, mixed m. p. 116.5–117.0°, prepared from *dl*-ephedrine kindly supplied by Dr. E. H. Volwiler, Abbott Laboratories, Chicago, Illinois, and by Dr. R. H. Manske, the Canadian National Research Council, Ottawa. Contrary to expectations, no *dl*-ephedrine was found.

Cyclic secondary α-bromo ketones, and open chain tertiary α-bromo ketones, such as 2 bromocholestanone, and α-bromoisobutyrophenone, respectively, yield products almost entirely free of bromine. This work is being continued with other types of halogenated ketones.

DEPARTMENT OF CHEMISTRY PHILIP G. STEVENS
McGILL UNIVERSITY
MONTREAL, CANADA

RECEIVED NOVEMBER 21, 1938

NEW BOOKS

Tabellen und Vorschriften zur quantitativen Analyse. Gravimetrie, Elektroanalyse, Probierkunde der Edelmetalle und Gasanalyse. (Tables and Directions for Quantitative Analysis, Gravimetric, Electroanalysis, Assay of the Noble Metals, and Gas Analysis.) By W. D. TREADWELL, Professor in the United Technical Institute in Zurich. Verlagsbuchhandlung Franz Deuticke, Helferstorferstrasse 4, Wien, Germany, 1938. xii + 284 pp. 126 figs. 17.5 × 25 cm. Price, RM. 9.

The author states in the preface that his object is to present in the shortest possible form a selection of proved methods of analysis in order to make it possible for the analyst already acquainted with the fundamentals to do his work more easily. The reviewer cannot imagine any publication in which could be found more information concerning analytical chemistry contained in less than 300 pages. Here is evidence showing extreme energy on the part of the author in collecting valuable data concerning, and proved directions for determining, more than the usual list of cations and anions. It also shows his skill in collating such material in a very logical manner. About 275 references to original literature are given.

In the interest of economy of space the author has collected in one chapter the description of the common oper-