2 g. of phosgene in 20 cc. of toluene and 1 g. of pyridine. The mixture was heated at 90° for one hour, cooled and filtered. The filtrate was washed successively with dilute acid and water. By concentration of the toluene solution, there was obtained 1 g. (45%) of a brownish solid, m.p. 183–186°. Crystallization from Skelly B gave material of m.p. 188–190°.

Anal. Calcd. for $C_{14}H_{11}ClN_2O$: N, 10.83. Found: N, 10.76.

N-(β-Diethylaminoethyl)-N-methylphenothiazine-10-carboxamide.—A solution of 5.6 g. (0.021 mole) of phenothiazine-10-carboxylic acid chloride and 5.6 g. (0.042 mole) of N-(β-diethylaminoethyl)-methylamine in 50 cc. of dry benzene was refluxed overnight. The reaction mixture was washed with water and the benzene layer separated and extracted with dilute hydrochloric acid. Addition of alkali to the acid extracts liberated the free base which was taken up in ether. Concentration of the ether solution yielded a thick oil which solidified on standing. The weight of material melting at 69–71° was 5.7 g. (77%). Crystallization from Skelly B gave 4.5 g. of product, m.p. 70–71°.

Anal. Calcd. for $C_{20}H_{25}N_3OS$: C, 67.57; H, 7.08; N, 11.82. Found: C, 67.75; H, 6.84; N, 11.55.

The base dissolved in dry ether was treated with hydrogen bromide gas. The hydrobromide salt which separated melted at 160–161°, after crystallization from isopropyl alcohol.

β-Dimethylaminoethyl Phenothiazine-10-carboxylate.—A solution of 12.3 g. (0.05 mole) of phenothiazine-10-carboxylic acid chloride and 8.9 g. (0.10 mole) of β-dimethylaminoethanol in 100 cc. of dry benzene was refluxed overnight. After the reaction mixture was washed with water, the benzene was separated and the solvent removed. The residue was dissolved in ether and the solution treated with hydrogen chloride gas. The salt, collected by filtration, weighed 13.3 g. (76%), m.p. 211–213° (dec.). Purification from absolute alcohol gave material, m.p. 215–216° (dec.).

β-Diethylaminoethyl Phenothiazine-10-carboxylate Methiodide and Methobromide.—The addition of excess methyl iodide to a dry ether solution of β-diethylaminoethyl phenothiazine-10-carboxylate resulted in the separation of the quaternary salt, m.p. 200–205° (dec.). After two crystallizations from absolute alcohol, the product melted at 210–211° (dec.).

Anal. Calcd. for $C_{20}H_{25}IN_2O_2S$: C, 49.59; H, 5.20; N, 5.78. Found: C, 49.87; H, 5.31; N, 5.79.

The methobromide obtained by addition of methyl bromide to an ether solution of the free base melted at 207–208° (dec.) after crystallization from absolute alcohol.

Anal. Calcd. for $C_{20}H_{25}BrN_2O_2S$: C, 54.91; H, 5.76. Found: C, 54.59; H, 5.82.

β-Diethylaminoethyl Phenothiazine-10-thiocarboxylate Methiodide and Methobromide.—The methiodide prepared in the foregoing manner melted at 230–231° (dec.) after crystallization from absolute alcohol.

Anal. Calcd. for $C_{20}H_{25}IN_2OS_2$: C, 48.00; H, 5.03; N, 5.59. Found: C, 47.93; H, 4.94; N, 5.70.

The methobromide, similarly prepared, crystallized from absolute alcohol, m.p. 228° (dec.).

Anal. Calcd. for $C_{20}H_{25}BrN_2OS_2$: C, 52.96; H, 5.56; N, 6.18. Found: C, 53.17; H, 5.75; N, 5.96.

N-(β-Diethylaminoethyl)-phenothiazine-10-carboxamide Methobromide.—This quaternary salt, after crystallization from absolute alcohol, melted at 225–226° (dec.).

Anal. Calcd. for $C_{20}H_{26}BrN_3OS \cdot {}^1/_2H_2O$: C, 53.93; H, 6.11; N, 9.43. Found: C, 54.04; H, 5.95; N, 9.57.

Acknowledgment.—The authors are indebted to Mr. E. F. Shelberg and staff of the Department of Microchemical Analyses for the analyses reported in this paper.

NORTH CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES]

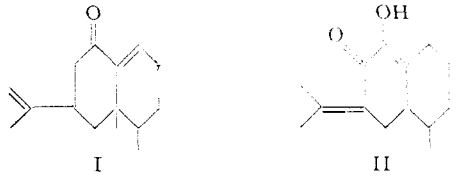
On the Structure of Eremophilone

BY T. A. GEISSMAN

RECEIVED MARCH 30, 1953

A re-examination of the oxidation of hydroxyeremophilone has disclosed that the product of the oxidation formerly regarded as having the composition $C_{12}H_{18}O_3$ is really a compound $C_{15}H_{22}O_4$. The structure advanced for this substance is in full accord with and constitutes additional evidence in support of the "unnatural" (non-isoprenoid) skeleton for hydroxyeremophilone.

The structures advanced for eremophilone (I) and hydroxyeremophilone (II) by Simonsen and his co-workers[1a-e] are of particular interest because their carbon skeletons cannot be constructed of isoprene units. While many of the experimental observations adduced in support of the structures



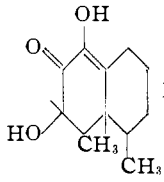
I II

I and II were satisfactorily interpreted[1] in terms of these formulas, there remain in the articles cited a number of findings and provisional conclusions which remained unexplained or unaltered when the final structure assignments were made and a summing up of the evidence was presented. Because the direct evidence of the unusual structure of hydroxyeremophilone, so far as the arrangement of the carbon skeleton is concerned, rests chiefly upon the degradation of the hydroxy ketone to 1,2-dimethylcyclohexane-2-acetic acid, it was of particular interest that an oxidation product isolated in the course of these same degradative experiments could not be satisfactorily accounted for on the basis of the structure II. This compound, described as a "phenol," $C_{12}H_{18}O_3$, was formed[1b] when hydroxyeremophilone, its benzoate and its methyl ether were oxidized, ozone and chromic acid being used in the several experiments performed.[2] The "phenol," which was soluble in alkali but not in sodium bicarbonate solution, formed an acetate and a methyl ether, both of which

(1) (a) A. E. Bradfield, A. R. Penfold and J. L. Simonsen, *J. Chem. Soc.*, 1744 (1932); (b) A. E. Bradfield, N. Hellström, A. R. Penfold and J. L. Simonsen, *ibid.*, 767 (1938); (c) A. R. Penfold and J. L. Simonsen, *ibid.*, 87 (1939); (d) F. C. Copp and J. L. Simonsen, *ibid.*, 415 (1940); (e) A. E. Gillam, J. I. Lynas-Gray, A. R. Penfold and J. L. Simonsen, *ibid.*, 60 (1941).

(2) The term "phenol" was (and is here) used simply with reference to its solubility in alkali and insolubility in sodium bicarbonate solution.

had compositions in agreement with the corresponding derivatives of $C_{12}H_{18}O_3$. It would appear that if hydroxyeremophilone were indeed II, a C_{12}-oxidation product would most probably be derived by the loss of the three carbon atoms of the isopropylidene group, and that it would be represented by III



II, $C_{12}H_{18}O_3$

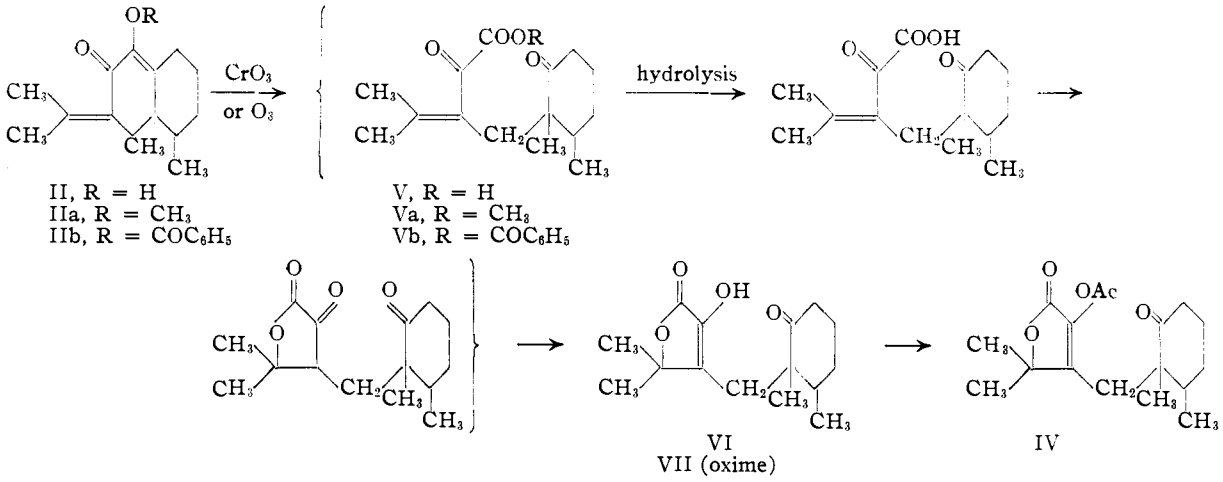
Although the oxidation of II in this manner would appear unlikely, the composition of the "phenol" seemed amply supported by the analytical data, and no more reasonable course for an oxidation to a weakly acidic product of the reported composition can be adduced.

It is evident that a compound of the structure III should show the highly distinctive ultraviolet absorption characteristic of an enolizable cyclic α-diketone, and that an examination of the absorption spectrum of the "C_{12}-phenol" would give valuable information. To this end, a sample of the compound was prepared in the manner described.[1b] The product obtained was clearly the "C_{12}-phenol" previously described: it was formed in about the reported yield, it had the proper solubility characteristics and melting point, and formed an acetate of the expected melting point. Analyses of the "phenol" and its acetate (and later its oxime) disclosed, however, that the "C_{12}-phenol" actually has the composition $C_{15}H_{22}O_4$. From considerations of its absorption spectra (Fig. 1) in neutral and alkaline solution, and of the absorption spectra of its acetate and its oxime, it is now possible to assign to this compound the structure IV—one which is in accord with the structure II for eremophilone, and which permits a rational interpretation of the oxidation reaction in which it is formed.

The "phenol" (IV) shows an absorption maximum at 238 mμ (log ϵ 3.88) in alcohol. The maximum is shifted to 277 mμ (3.93) in alkaline solution.[3] The acetate (VI) shows end absorption only, rising to a plateau at about 215 mμ (4.07), with a well-defined low-intensity maximum at 295 mμ (1.64), indicative of an isolated carbonyl group. The oxime (VII) has a spectrum substantially identical with that of the parent "phenol" an indication that the carbonyl group is isolated with respect to the chromophore responsible for the 238 mμ maximum.

These data lead to the formulation of the oxidation shown in the diagram.

This interpretation accounts for the hitherto puzzling observation that the same product is obtained from hydroxyeremophilone (II), its methyl ether (IIa) or its benzoate (IIb). The immediate intermediate products of the oxidations of these three compounds would be the acid (V), the ester (Va) and the anhydride (Vb), the last two of which under the conditions of the various experiments would suffer hydrolysis and thus lead to IV.



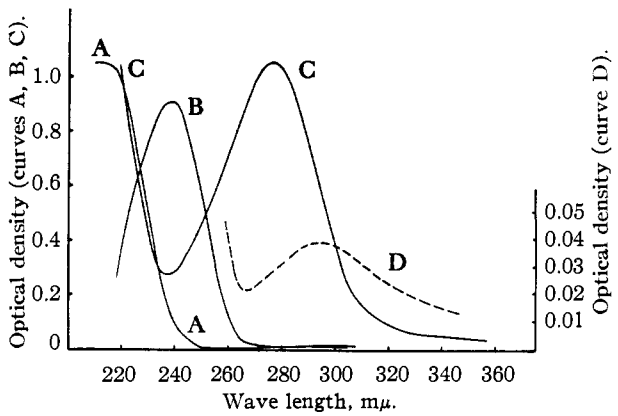


Fig. 1.—A, acetate (VI), 1.10 × 10^{-4} M in ethanol; B, "C_{15}-phenol" 1.15 × 10^{-4} M in ethanol; C, "C_{15}-phenol" 1.15 × 10^{-4} M in 0.1 N KOH–ethanol (oxime essentially identical with B); D, acetate (VI) 1.10 × 10^{-3} M in ethanol.

The assignment to IV of the structure of an α-hydroxy-α,β-unsaturated-δ-lactone on the basis of the spectral behavior described is made without direct analogy with comparable models, but from the following considerations. The bathochromic shift of 39 mμ in the position of the high-intensity maximum upon ionization finds close parallels in comparable systems. Enolic α-diketones, β-dike-

(3) The original spectrum is observed after reacidification of the alkaline solution.

tones and β-keto esters all show similar behavior. In Table I are listed a number of recorded examples.

TABLE I

Compound[a]	λmax, mμ Neutral	Alkaline	Δλ	Ref.
3,3-Diphenylcyclopentan-1,2-dione	260	305	45	4
3-Methylcyclohexan-1,2-dione	268.5	312	43.5	7
2,3-Cholestadienone	(α) 272	320	48	8
	(β) 270		50	
Cyclohexan-1,3-dione	255	280	25	6
5,5-Dimethylcyclohexan-1,3-dione	255	282	27	5
2-Ethyl-4-*n*-propylcyclopentan-1,3-dione	252	272[b]	20	5
Acetylacetone	275	290	15	5
Ethyl acetoacetate	244	277	33	9

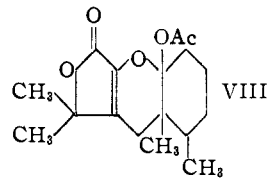
[a] The compounds are named as the diketones although they exist as enols in the alcohol solutions studied. [b] Not in alkali: ionized by dilution.

The spectra of α,β-unsaturated lactones have been studied by Jones and his co-workers, who have found that the unsaturated δ-lactones show high-intensity ($\epsilon \sim 10^4$) maxima at about 213–214 mμ, and that γ-lactones have maxima at about 211–214 mμ.[10] α,β-Unsaturated acids behave similarly, having maxima in the neighborhood of 210–215 mμ. β-Hydroxy- and β-alkoxycrotonic acids show maxima at about 230–240 mμ, the corresponding α-substituted acids having maxima at about 225 mμ.[11,12] Although data are lacking on the properties of β-hydroxy lactones[13] in alkaline solution (with the hydroxyl group ionized), data on dialkylamino lactones support the view that the conversion of the β-hydroxyl group to the ionic form would cause a large bathochromic shift: β-dialkylamino-δ-lactones show maxima at 265–268 mμ, -γ-lactones at 285–290 mμ.[14]

In view of these data, the spectral behavior of the "C_{15}-phenol" is entirely consonant with the structure IV, a view which is further substantiated by the absorption spectrum of the acetate (VI). It is well known that acetylation of an enolic or phenolic hydroxyl group nearly or completely cancels its contribution to a chromophore upon which it is found. Polyhydroxy flavone acetates absorb very nearly at the same wave lengths as flavone itself; phenyl acetate resembles benzene in its ultraviolet absorption; and numerous other examples of this are recognized. The absorption maximum of VI is substantially that of the α,β-unsaturated-δ-lactones described by Jones[10] and shows clearly that the hydroxyl group is a part of the system responsible for the 238-mμ maximum exhibited by IV.

That the lactone IV contains a carbonyl group is demonstrated by the formation of an oxime (VII); and that the carbonyl group is not involved in the 238-mμ chromophore is shown by the fact that the oxime has an absorption spectrum very similar to that of IV, and by the 295 mμ (log ε 1.63) peak in the spectrum of VI, characteristic of the isolated aliphatic carbonyl group.

A further comment may be made regarding the structure of VI. It is evident from IV that a monoacetate could have the structure VI or VIII.



VIII

That VIII does not represent the acetate of IV is shown by the spectrum: VIII should show absorption characteristic of an α-alkoxy-α,β-unsaturated-δ-lactone, and should show a maximum of about 235 mμ (*cf.* IV, the hydroxy compound). The spectrum of the acetate shows clearly that its structure is VI.

These observations offer additional support for the "unnatural" structure (II) proposed for hydroxyeremophilone. It should be noted that neither the present work nor earlier studies on these constituents of *Eremophila Mitchelli* oil support by more than inference the same "unnatural" carbon skeleton for eremophilone (I) itself. Indeed, on the basis of the structures I and II, the rearrangement of eremophilone oxide to hydroxyeremophilone is most unusual; and although a proposal has been put forward to account for this transformation it lacks conviction, and the rearrangement deserves careful re-examination. This study is now being pursued.

Experimental

Hydroxyeremophilone benzoate was isolated as previously described,[1a] from a sample of *Eremophila mitchelli* oil.[15] The compound's properties agreed with those reported by Bradfield, *et al.*

Oxidation of Hydroxyeremophilone.—Pure, crystalline hydroxyeremophilone was readily obtained by saponification of the purified benzoate; but for the oxidation experiment the crude product of the saponification of 10.1 g. of benzoate was used directly. The ice-cooled solution of this material in 60 ml. of glacial acetic acid was stirred while a solution of 7 g. of chromic acid in 50 ml. of 80% acetic acid was added dropwise over several hours. The excess oxidizing agent was destroyed with sodium bisulfite and the solution poured into water. The acetic acid was removed by steam distillation, and the oily residue removed with ether. The ether solution was extracted with six 2-ml. portions of 1 *N* sodium hydroxide. When the alkaline solution was saturated with carbon dioxide there was formed a greenish-yellow, crystalline precipitate (0.9 g.). Recrystallized from dilute methanol, the compound (IV) formed colorless prisms, m.p. 192.5–193.5° (reported[1b] 193–194.5°).

Anal. Calcd. for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.44, 67.27; H, 8.07, 8.12.

The **acetate (VI)** was prepared by dissolving 50 mg. of IV

(4) R. M. Horowitz, Ph.D. Thesis, University of California, Los Angeles, 1949.

(5) E. R. Blout, V. W. Eager and D. C. Silverman, THIS JOURNAL, **68**, 566 (1946).

(6) H. Bastron, R. E. Davis and L. Butz, *J. Org. Chem.*, **8**, 515 (1942).

(7) H. S. French and M. E. Holden, THIS JOURNAL, **67**, 1239 (1945).

(8) E. T. Stiller and O. Rosenheim, *J. Chem. Soc.*, 353 (1938).

(9) P. Grossman, *Z. physik. Chem.*, **109**, 305 (1924).

(10) J. L. Haynes and E. R. H. Jones, *J. Chem. Soc.*, 954 (1946).

(11) L. N. Owen, *ibid.*, 385 (1945).

(12) L. N. Owen and M. U. S. Sultanbawa, *ibid.*, 3089 (1949).

(13) E. R. H. Jones and M. C. Whiting, *ibid.*, 1419 (1949).

(14) E. R. H. Jones and M. C. Whiting, *ibid.*, 1423 (1949).

(15) A sample of the oil was kindly furnished by Dr. A. R. Penfold, Museum of Applied Arts and Sciences, Sydney, N.S.W., Australia.

in a mixture of 1 ml. of acetic anhydride and 0.5 ml. of dry pyridine, heating the solution to boiling and allowing it to stand overnight. The excess acetic anhydride was decomposed with ice and the crystalline product recrystallized from methanol. The acetate formed tiny, colorless, stout needles, m.p. 163–164° (reported[1b] 164–165°).

Anal. Calcd. for $C_{14}H_{20}O_4$: C, 66.64; H, 7.91. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.24, 66.11; H, 7.61, 7.88.

The oxime (VII) was prepared by refluxing for one hour a solution of equal weights of IV, hydroxylamine hydrochloride and sodium acetate in 50% aqueous ethanol. The addition of water and cooling in ice caused the oxime to crystallize in tiny, colorless prisms, m.p. 192–193°. Mixed with IV, the m.p. was 171–175°.

Anal. Calcd. for $C_{12}H_{19}O_3N$: C, 63.96; H, 8.51. Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24. Found: C, 63.65; H, 8.01.

The ultraviolet absorption spectrum of the oxime was substantially the same as that of IV, λ_{max} 239 mμ.

LOS ANGELES, CALIFORNIA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Chartreusin, a New Antibiotic Produced by *Streptomyces chartreusis*, a New Species

BY BYRON E. LEACH, KENNETH M. CALHOUN, LEROY E. JOHNSON, CHARLOTTE M. TEETERS AND WILLIAM G. JACKSON

RECEIVED APRIL 22, 1953

Chartreusin is a new antibiotic obtained from *Streptomyces chartreusis*. Analysis of the crystalline antibiotic indicates it to be a weak acid with a formula approximating $C_{18}H_{18}O_8 \cdot 2H_2O$. The ultraviolet and infrared absorption spectra and an antibacterial spectrum are given for chartreusin.

Introduction

A new antibiotic has been obtained from culture filtrates and also from the mycelial mat of two hitherto undescribed *Streptomyces* sp. isolated from the soil. *Streptomyces chartreusis* was isolated from an African soil while another *Streptomyces* sp. which produces this antibiotic was obtained from a Michigan soil. The generic name, chartreusin, has been assigned to this antibiotic because of the characteristic color of the crystalline material.

Chartreusin has been crystallized from concentrates of the culture filtrates as greenish-yellow crystals. The sodium salt also has been crystallized. Elemental analyses and molecular weight determination indicate an empirical formula of $C_{18}H_{18}O_8 \cdot 2H_2O$. At room temperature, the antibiotic is stable for several hours over the range of *p*H 2 to *p*H 10. However, prolonged heating at *p*H 2 or 10 destroys the antibiotic activity.

The ultraviolet and visible spectra, Fig. 1, and the infrared spectrum, Fig. 2, afford further characterization of the antibiotic. In general appearance the ultraviolet spectrum resembles that of substituted 1,2-naphthoquinones.[1,2]

Chartreusin is active against certain Gram-positive organisms and mycobacteria. It also is active against the *Micrococcus pyogenes* v. *aureus* phage.[3]

Table I shows its antibacterial properties.

The acute LD_0 subcutaneously in mice for chartreusin is 2500 mg. per kg.; however, the sodium salt has an acute LD_{50} intravenously in mice of 250 mg. per kg. In chronic studies the antibiotic seems to have a cumulative toxicity, particularly when the sodium salt is used.

Acknowledgments.—The authors are grateful to Dr. J. L. Johnson for the infrared spectrum, Mr. L. Scholten for the ultraviolet studies, Mr. W. A. Struck for the microanalytical data and Mr. O. F. Swoap for the toxicity studies. We wish also to acknowledge the assistance of Dr. H. A. Nelson and Mr. W. H. DeVries for preparation of material.

TABLE I

CONCENTRATION OF CHARTREUSIN REQUIRED TO INHIBIT GROWTH OF MICROÖRGANISMS IN NUTRIENT BROTH

Organism	Mcg./ml.
Bacillus subtilis	1.25
Bacillus subtilis (M.R.-1280)[a]	1.00
Salmonella typhosa	>30
Escherichia coli	>30
Salmonella gallinarum	>30
Salmonella schottmuelleri	>30
Brucella bronchiseptica	>25
Proteus vulgaris	>30
Micrococcus pyogenes v. *aureus*	5.0
Mycobacterium tuberculosis (607)	1.7
Mycobacterium tuberculosis (H37Rv)	2.0

[a] Streptomycin resistant.

Experimental

Assay.—Chartreusin was assayed by the paper-disc method outlined for the assay of fumagillin.[3,4] Fumagillin was used as the assay standard for the isolation work. A unit of activity is defined as being equivalent to one microgram of fumagillin.

Identification of Culture.—This culture which we have named *Streptomyces chartreusis* could not be identified by following the key in Bergey's Manual of Determinative Bacteriology.[5]

The culture is a mesophilic saprophyte with characteristic powdery blue-gray to blue-green spores produced on various media. The sporulating hyphae formed open spirals on some media. White filamentous aerial mycelia were produced, approximately one micron in diameter.

Fermentation.—The organism, *Streptomyces chartreusis*, is carried in culture on agar slants. The surface growth was used to inoculate shaken flasks (500-ml. erlenmeyer flasks each containing 100 ml. of culture broth). The flasks, after about three days of fermentation, were used to inoculate 5-gallon fermenters. These fermenters were

(1) A. K. Macbeth, J. R. Price and F. L. Winzor, *J. Chem. Soc.*, 325 (1935).

(2) R. G. Cooke, A. K. Macbeth and F. L. Winzor, *ibid.*, 878 (1939).

(3) F. R. Hanson and T. E. Eble, *J. Bact.*, **58**, 527 (1949).

(4) F. R. Hanson and T. E. Eble, *Antibiotics and Chemotherapy*, **1**, 54 (1951).

(5) R. S. Breed, E. G. D. Murray and A. P. Hitchens, "Bergey's Manual of Determinative Bacteriology," 6th Ed., The Williams and Wilkins Company, Baltimore, Md., 1948, pp. 929–933.