Anal. Calcd. for $C_9H_{11}NO$: C, 72.45; H, 7.44; N, 9.40. Found: C, 72.80; H, 7.34; N, 9.54.

The acetyl derivative of VIII was prepared in the usual manner. Its m. p. was 112–113°, its mixed m. p. with III was 85–100°, and its mixed m. p. with IV was 112–113°.

2-Acetamido-6-propylphenol (XII).—To 19.1 g. (0.100 mole) of 2-acetamido-6-allylphenol (III) in 250 ml. of 95% ethanol was added 0.2 g. of platinum oxide. The mixture was reduced in twenty minutes at 25° and 2 atmospheres pressure, filtered, diluted with 800 ml. water and the product, 18 g. (94%), collected. Recrystallized from 50% ethanol, its m. p. was 112–113° and its mixed m. p. with XII prepared by the acetylation of 2-amino-6-propylphenol was 112–113°.

Anal. Calcd. for $C_{11}H_{15}NO_2$: C, 68.36; H, 7.82; N, 7.25. Found: C, 68.36; H, 7.69; N, 7.24.

2-Acetamido-4-propylphenol (XVI).—About 0.02 g. of platinum oxide in 75 ml. of 95% ethanol was reduced to platinum black. Then 1.91 g. (0.010 mole) of 2-acetamido-4-allylphenol was added and reduced in thirty minutes at 25° and 2 atmospheres pressure. The reaction mixture was filtered and evaporated to dryness and the residue was recrystallized from benzene, 1.6 g. (83%), m. p. 129–131°. Recrystallized from 60% ethanol, it yielded white needles, m. p. 130.5–132°.

Anal. Calcd. for $C_{11}H_{15}NO_2$: C, 68.36; H, 7.82; N, 7.25. Found: C, 68.42; H, 7.78; N, 7.16.

2-Amino-6-propylphenol (XI).—A mixture of 1.93 g. (0.010 mole) of 2-acetamido-6-propylphenol (XII) in 3 ml. of 6 *N* hydrochloric acid was refluxed for ninety minutes and the product worked up in the usual manner to yield 1.45 g. (77%) of pink platelets of the hydrochloride. The free base was liberated from 0.50 g. of the hydrochloride yielding 0.40 g. (99%) of white platelets, m. p. 60–61°, after recrystallization from ether and Skelly B. The product was very sensitive to air oxidation and was collected under nitrogen.

Anal. Calcd. for $C_9H_{13}NO$: C, 71.54; H, 8.67; N, 9.27. Found: C, 71.48; H, 8.50; N, 9.47.

2-Nitro-6-propylphenol (X).—A solution of 20.4 g. (0.150 mole) of *o*-propylphenol in 20.4 g. (0.34 mole) of glacial acetic acid was added to a mixture of 40.8 g. (0.46 mole) of nitric acid (d. 1.42) and 61.2 g. (1.02 mole) of glacial acetic acid at −4 to −6° with stirring over a period of two hours. The reaction mixture was immediately poured with stirring onto 300 g. of ice. It was then extracted with benzene and the extract washed with water, 5% sodium bicarbonate, and finally with water. The brown benzene solution was steam distilled and the 3 liters of distillate extracted with benzene, dried, concentrated and vacuum distilled twice. The product was an orange oil, 7.0 g. (39%), b. p. 84–89° at 1.2 mm., $n^{25}D$ 1.5542.

Anal. Calcd. for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.74. Found: C, 59.80; H, 6.03; N, 7.66.

2-Amino-6-propylphenol Hydrochloride (XIa).—A solution of 7.0 g. of 2-nitro-6-propylphenol in 150 ml. of absolute ethanol was hydrogenated over 1.5 g. of Raney nickel in one hour at 2 atmospheres pressure and at room temperature. The product was isolated in the usual manner. It weighed 6 g. (83%), m. p. 208° (dec.). A sample was converted in 91% yield to 2-acetamido-6-propylphenol (XII), m. p. 111–112°. The mixed m. p. with XII prepared by the reduction of 2-acetamido-6-allylphenol (III) was 111–112°.

2-Acetamido-4-propylphenol (XVI).—To a solution of 9.4 g. (0.050 mole) of 2-amino-4-propylphenol hydrochloride[4] in 15 ml. of water, 6.1 g. (0.060 mole) of acetic anhydride and a solution of 7.5 g. (0.055 mole) of sodium acetate trihydrate in 9 ml. of water, were added simultaneously over five minutes with stirring and cooling. Stirring was continued ten minutes and the product, 9.4 g. (97%), m. p. 130–133°, was collected and dried. A sample recrystallized several times from 60% ethanol melted at 131–132°. Its mixed m. p. with XVI prepared by the reduction of 2-acetamido-4-allylphenol (IV) was 130.5–132°.

Summary

1. The thermal rearrangement of *o*-acetamidophenyl allyl ether has been shown to yield 2-acetamido-6-allylphenol and 2-acetamido-4-allylphenol in the ratio of 6.5 to 1.

2. The thermal rearrangement of *o*-aminophenyl allyl ether has been shown to yield 2-amino-6-allylphenol and 2-amino-4-allylphenol in the ratio of 2 to 1.

3. Several *o*-amino and *o*-acetamido alkyl phenols have been prepared and their structures proved by synthesis.

(4) Baranger, *Bull. soc. chim.*, [4] **49**, 1213 (1931).

NORTH CHICAGO, ILLINOIS RECEIVED SEPTEMBER 8, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Synthesis of D-Fructomethylose by Biochemical Oxidation[1]

BY LAURENS ANDERSON[2] AND HENRY A. LARDY

Two organisms of the genus *Acetobacter*, *A. xylinum* and *A. suboxydans*, have been widely used in the carbohydrate field for the oxidation of the grouping —CHOHCHOHCH$_2$OH to —CHOHCOCH$_2$OH. By this method a considerable number of ketose sugars have been prepared from the corresponding alcohols. As is the case with many biochemical procedures the application of the method is limited to compounds of proper steric makeup. According to "Bertrand's rule,"[3] *A. xylinum* will attack those alcohols in which the configurations about carbons two and three are the same. Hann, Tilden and Hudson[4] suggested that *A. suboxydans* is specific in its ability to oxidize polyalcohols of D-configuration. While these generalizations appear to hold for "normal" sugar alcohols ($C_nH_{n+2}(OH)_n$) of five or more carbon atoms, the results obtained with alcohols having a terminal methyl group have been anomalous. Thus Votoček, Valentin and Rác[5] were unable to oxidize L-rhamnitol (I) with *A. xylinum*, and Müller and Reichstein[6] obtained only slight oxi-

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(2) Wisconsin Alumni Research Foundation Fellow, 1946–1947.

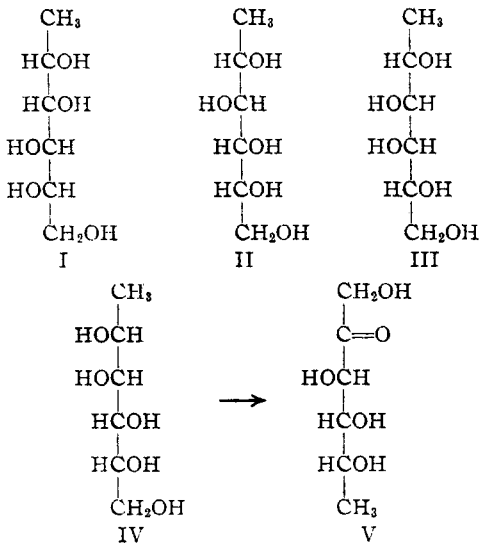
(3) Bertrand, *Compt. rend.*, **126**, 762 (1898); *Ann. chim. phys.*, [8] **8**, 181 (1904).

(4) Hann, Tilden and Hudson, THIS JOURNAL, **60**, 1201 (1938).

(5) Votoček, Valentin and Rác, *Coll. Czech. Chem. Commun.*, **2**, 402 (1930).

(6) Müller and Reichstein, *Helv. Chim. Acta*, **21**, 271 (1938).

dation of L-gulomethylitol (II), while Hann, Tilden and Hudson unexpectedly found that L-fucitol (III) was attacked by *A. suboxydans*.



An opportunity to obtain further information about the action of *A. suboxydans* on this type of alcohol was afforded when in connection with research being carried out in this Laboratory on intermediates in glycolysis it was decided to study the properties of D-fructomethylose (V). This sugar was prepared by Morgan and Reichstein[7] in 12% over-all yield from D-fructose by a procedure involving a Freudenberg–Raschig desoxydation. Since it is the expected product of an *Acetobacter* oxidation of D-rhamnitol (IV) which recently became readily available through a synthesis worked out by Haskins, Hann and Hudson,[8] the possibility of preparing it in this way was investigated. It was found that in contrast to the desoxyhexitols previously cited D-rhamnitol is oxidized by *A. suboxydans* according to the "rules." The yield of ketose in this process is 80%; D-rhamnitol is obtained in 28% yield from methyl α-D-mannoside, the starting material, making the over-all yield 22%. The product has not been obtained in a state of purity comparable to that of the chemically prepared material but could be purified by standard methods. The chief difficulty stems from the fact that the sugar is a sirup which offers little hope of crystallization. The two processes are of about equal length (six steps) and difficulty.

The oxidation proceeded very rapidly, reaching completion in three days. The yield quoted is based on copper reduction analyses of the liquor. The results of these analyses were calculated in terms of glucose since no quantitative data are available for the fructomethyloses. The tagatomethyloses are said to have about 90% of the reducing power of glucose[9]; if the fructomethyloses behave similarly the calculated yield is somewhat low.

In view of the erratic behavior of other methyl hexitols in *Acetobacter* oxidations, it was necessary to obtain rigorous proof that the compound obtained was the anticipated D-fructomethylose. Although Reichstein and collaborators[7] prepared four of the eight possible 6-desoxy-2-ketohexoses, little information as to the properties of these sugars was published by this group. It was noted that they reduced Fehling solution in the cold and were otherwise very reactive.

The sirupy sugar obtained in this investigation caused the appearance of cuprous oxide in a few seconds on being treated with Fehling solution at room temperature and in general had the characteristic reactivity of its class. Thus, in contrast to other ketohexoses, it was about 50% oxidized by hypoiodite in fifteen minutes. It is interesting to note that similar results have been obtained with D-*threo*-pentulose[10] ("D-xyloketose").[11,12] It would seem that the 6-desoxyketohexoses and the ketopentoses closely resemble each other in their behavior with a variety of reagents. This behavior undoubtedly arises from a structural feature common to both types of sugars; *i. e.*, the lack of a hydroxyl more distant from the keto group than the γ carbon atom. This precludes the existence of pyranose rings, leaving as possible structures the more reactive furanose and open chain forms.

The substitution of a methyl for a hydroxymethyl group in the six position apparently alters the susceptibility of a sugar to the Selivanov test. Although definitely positive, the response of D-fructomethylose to this test was weak. Classification of the sugar as a ketose follows from the above considerations and from its reaction with α-methyl-α-phenylhydrazine which is regarded[13,13a] as a ketose reagent. Upon treatment under the prescribed conditions the sirup gave a positive reaction.

In performing the qualitative tests listed samples of D-rhamnose were treated simultaneously with the reagent being used and in all cases the results were completely negative.

The configuration of the sugar was established by converting it to 6-desoxy-D-*arabo*-hexose phenylosazone,[14] phenylosotriazole and *p*-bromophenylosazone, obtainable only from D-fructomethylose and the aldoses D-rhamnose and D-quinovose; D-quinovose would be a very unlikely result of the *Acetobacter* oxidation of D-rhamnitol;

(7) Morgan and Reichstein, *Helv. Chim. Acta*, **21**, 1023 (1938). References to preceding papers are given in this article.

(8) Haskins, Hann and Hudson, THIS JOURNAL, **68**, 628 (1946).

(9) Barnett and Reichstein, *Helv. Chim. Acta*, **20**, 1529 (1937).

(10) See Wolfrom, Thompson and Evans, THIS JOURNAL, **67**, 1794 (1945), for a discussion of the nomenclature of ketoses.

(11) Schmidt and Treiber, *Ber.*, **66**, 1765 (1933).

(12) Hassid, private communication.

(13) Van der Haar, "Nachweis, etc.," Berlin, 1920, p. 221 ff.

(13a) Neuberg, *Ber.*, **35**, 960 (1902); Neuberg and Mandl, *Arch. Biochem.*, **11**, 451 (1946).

(14) *Cf.* note 10. The recent suggestion of Sowden, THIS JOURNAL, **69**, 1047 (1947), with reference to the nomenclature of sugar derivatives with fewer asymmetric carbon atoms than the parent compound is followed in this paper.

moreover, its reactions are very similar to those of D-rhamnose which is excluded as a possibility by the results of the qualitative tests. The product must therefore be D-fructomethylose. The quantities of the precipitates obtained with all reagents giving them were much greater than could be accounted for by possible impurities in the preparation.

The osotriazole of this series had not been reported in the literature at the time that this work was done. Both antipodes have since been prepared, the D-form by Hardegger and El Khadem[15] and the L-form by Haskins, Hann and Hudson.[16] The results published by these investigators are in good agreement with those obtained in this work as regards both melting point and specific rotation.

Attempts to prepare derivatives characteristic of D-fructomethylose only were not successful. The *o*-nitrophenylhydrazone described in the original paper of Morgan and Reichstein was not obtained, possibly because of impurities in the sugar. It is believed that crystals of the *p*-bromophenylhydrazone appeared at one stage of the reaction with *p*-bromophenylhydrazine but that further reaction to give the osazone occurred during the working up of the reaction mixture. The hydrazone, if it could be prepared, would serve for the preparation of the pure sugar. Acetonation and acetylation of the sugar were both tried. The former gave negative results but the sirupy acetate might be subject to purification, perhaps by vacuum distillation, after which the pure sugar could be obtained by catalytic saponification. Sufficient material to test this possibility was not obtained.

Experimental

D-Rhamnitol (IV) was synthesized from methyl α-D-mannoside according to Haskins, Hann and Hudson.[8] It was found convenient to use refluxing methyl isobutyl ketone in place of acetone in a pressure bottle as the solvent in the tosyl–iodine exchange reaction. Excess sodium iodide is not required when the higher boiling ketone is used. In the last step the D-rhamnose solution obtained from the hydrolysis of the methyl α-D-rhamnoside was hydrogenated directly after treatment with silver carbonate and concentration to a smaller volume.

D-Fructomethylose (V).—The bacterial oxidation was carried out in a cotton stoppered 2-liter Erlenmeyer flask containing 4.5 g. of D-rhamnitol, 0.5 g. of Difco yeast extract, 0.075 g. of potassium acid phosphate and 0.1 g. of glycerol dissolved in 100 ml. of water. After sterilization for fifteen minutes at 15 pounds the medium was inoculated with 1 ml. of a culture of *A. suboxydans* from an original stock maintained by the Department of Agricultural Bacteriology.[17] After incubation for three days at 30° the medium was well covered with pellicle. An aliquot withdrawn at this time and analyzed by the Shaffer–Hartmann method assayed 40.6 mg. reducing sugar per ml., calculated as glucose. One day later the value was 38.4 mg. per ml. After an initial filtration to remove the bacterial cells the liquor was treated with Norite, filtered and evaporated under reduced pressure to a volume of 15 ml. The Norite treatment was repeated twice and the final filtrate was concentrated to a brown sirup. This was taken up in absolute ethanol; the insoluble material was centrifuged off and washed twice with ethanol and the washings combined with the original solution. Samples of the sugar for further experiments were obtained by evaporating the ethanol from aliquots of this solution.

Reaction with Hypoiodite (Willstätter–Schudel Titration).—A sample (0.099 g.) of the sirup was dissolved in a mixture of 40 ml. of 0.05 *N* iodine, 35 ml. of 0.1 *N* sodium hydroxide and 10 ml. of water and allowed to stand fifteen minutes after which it was acidified and the remaining iodine was titrated with standard thiosulfate. The consumption of iodine was 0.662 g. per g. of sugar or 47% of the amount consumed by an equal weight of glucose under identical conditions.

The Selivanov Test was carried out according to Roe[18] except that only qualitative comparisons were made. D-Fructomethylose gave about one-tenth of the amount of color given by an equal quantity of D-fructose. The rhamnose control remained colorless.

6-Desoxy-D-*arabo*-hexose Phenylosazone from D-Fructomethylose.—The osazone was prepared according to directions given by Fieser.[19] After two recrystallizations from ethanol–water the compound melted at 183° (rapid heating) and showed no depression when mixed with 6-desoxy-D-*arabo*-hexose phenylosazone prepared from D-rhamnose. Fischer and Zach[20] found m. p. 185° for "d-rhamnosazon," while Van der Haar[21] gives 182° for the L-isomer and Freudenberg and Raschig[22] reported melting points from 187 to 191° for these osazones depending on the sugar from which they were made.

Anal. Calcd. for $C_{18}H_{22}N_4O_3$ (342.39): N, 16.37. Found: N, 16.17.

It was noted that the addition of the phenylhydrazine reagent to a solution of the crude sirup caused the immediate formation of a small amount of a bright yellow precipitate which did not increase on standing. The material had the same nitrogen content as that obtained by heating. This observation suggests the possibility that a small amount of D-fructomethylosone or other dicarbonyl compound was formed by the bacteria.

6-Desoxy-D-*arabo*-hexose Phenylosotriazole.—The methods described by Haskins, Hann and Hudson[23,24] were followed in preparing this compound from samples of 6-desoxy-D-*arabo*-hexose phenylosazone prepared from D-rhamnose and D-fructomethylose, respectively. For detailed directions the reader is referred to the recent paper[16] by the same authors. Our compound had the following constants: From D-rhamnose, m. p. 137–138°, $[\alpha]^{24}D$ −99.4° (in pyridine, *c* 0.805). *Anal.* Calcd. for $C_{12}H_{15}N_3O_3$ (249.26): N, 16.86. Found: N, 16.80, 16.71. From D-fructomethylose, m. p. 137–138°, $[\alpha]^{26}D$ 98.5° (in pyridine, *c* 0.63). *Anal.* Found: N, 16.79, 16.90.

A mixture of the two samples showed no depression in melting point. Haskins, Hann and Hudson[16] give m. p. 136–137° and $[\alpha]^{20}D$ + 101.5° for 6-desoxy-L-*arabo* hexose phenylosotriazole ("L-rhamnose phenylosotriazole"), and Hardegger and El Khadem[15] give m. p. 140° for the D-isomer ("*d*-chinovose-phenylosotriazole"). The latter workers distilled their material in high vacuum for analysis.

Attempted Preparation of D-Fructomethylose *p*-Bromophenylhydrazone.[25] **6-Desoxy-D-*arabo*-hexose *p*-Bromophenylosazone.**—A solution of 0.5 g. of sirup in 1 ml.

(15) Hardegger and El Khadem, *Helv. Chim. Acta*, **30**, 900 (1947).

(16) Haskins, Hann and Hudson, THIS JOURNAL, **69**, 1461 (1947).

(17) Thanks are due Dr. Elizabeth McCoy who kindly supplied his culture.

(18) Roe, *J. Biol. Chem.*, **107**, 15 (1934).

(19) Fieser, "Experiments in Organic Chemistry," 2nd ed., New York, N. Y., 1941, p. 126. The quantity of water recommended by Fieser for dissolving the solid reagents was found insufficient. Two and a half times the amount given was required.

(20) Fischer and Zach, *Ber.*, **45**, 3770 (1912).

(21) Van der Haar, *op. cit.*, p. 212.

(22) Freudenberg and Raschig, *Ber.*, **62**, 373 (1929).

(23) Haskins, Hann and Hudson, THIS JOURNAL, **67**, 939 (1945).

(24) Hann and Hudson, *ibid.*, **66**, 735 (1944).

(25) The procedure was adapted from that used by Schmidt and Treiber to prepare the *p*-bromophenylhydrazone of D-*threo*-pentulose.

of water and 2 ml. of ethanol was warmed with 0.6 g. of *p*-bromophenylhydrazine until the reagent dissolved, then placed in a vacuum desiccator over sulfuric acid and potassium hydroxide. After three days apparently colorless crystals which may have been the hydrazone were deposited. After an unsuccessful attempt to dissolve the crystals in warm ethanol the adhering gum was washed from them with liberal quantities of cold ethanol. The product which was now yellow melted at 224° after two recrystallizations from ethyl acetate. Votoček and Valentin[26] give 222–223° as the melting point of "*d*-rhamnose *p*-bromophenylosazone" and Freudenberg and Raschig[22] quote 225° for "*d*-chinovose *p*-bromophenylosazon."

Anal. Calcd. for $C_{18}H_{20}N_4O_3Br_2$ (500.21): N, 11.20. Found: N, 11.14, 11.22.

Acetylation of a small sample of the sugar with acetic anhydride in pyridine at 0° furnished only a water-insoluble oil which has so far not yielded crystalline material.

(26) Votoček and Valentin, *Compt. rend.*, **183**, 62 (1926).

We are indebted to Prof. Homer Adkins for providing facilities for the high pressure hydrogenation of the D-rhamnose.

Summary

The oxidation of D-rhamnitol by *Acetobacter suboxydans* was found to proceed normally to give crude D-fructomethylose sirup in 80% yield.

The behavior of the sugar with some standard carbohydrate reagents is described.

Recently reported physical constants of 6-desoxy-D-*arabo*-hexose phenylosotriazole are confirmed.

Methods are indicated by which purification of the crude sugar might be effected.

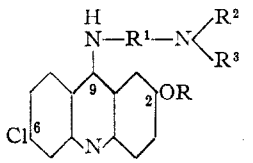
MADISON 6, WISCONSIN RECEIVED OCTOBER 6, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF RESEARCH IN PURE CHEMISTRY, MELLON INSTITUTE]

The Hydroxyethyl Analog of Quinacrine

BY WARNER W. CARLSON AND LEONARD H. CRETCHER

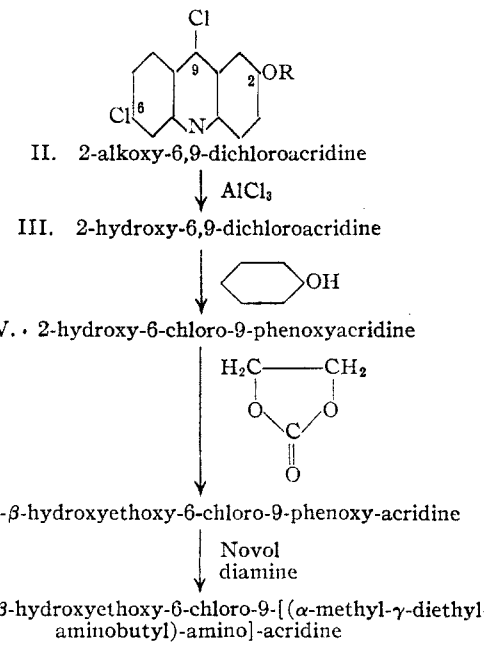
Modification of the host toxicity of quinacrine (I) was attempted by hydroxyalkylation at the



I. For quinacrine, R = CH_3—; $R^1{}_2$ = —$CH(CH_3)(CH_2)_3$—; R^2 = R^3 = C_2H_5—

2-position of the acridine ring. Literature reports were found of the introduction of the hydroxyl group into (a) the R_1 residue of the aliphatic side-chain,[1] (b) the terminal dialkylamino grouping,[2] and (c) one instance of hydroxylation of both R_1 and R_2 or R_3.[3] Introduction of a hydroxyl grouping into the R_1 residue was somewhat variable in effect, although generally there was a lowering of antimalarial activity unaccompanied by a compensating decrease in host toxicity.[1a,c] The presence of a hydroxyl group in the terminal dialkylamino grouping, either alone or in conjunction with another such radical in the R_1 residue, uniformly lowered antimalarial activity.[2b,3] No previous study was found of the effect of a hydroxyalkyl group at position 2.

The synthesis of the hydroxyethyl analog of quinacrine (VI) was accomplished as outlined in the accompanying diagram. The necessary intermediate II customarily is obtained by ring closure



II. 2-alkoxy-6,9-dichloroacridine

↓ $AlCl_3$

III. 2-hydroxy-6,9-dichloroacridine

↓ OH

IV. 2-hydroxy-6-chloro-9-phenoxyacridine

↓

V. 2-β-hydroxyethoxy-6-chloro-9-phenoxy-acridine

↓ Novol diamine

VI. 2-β-hydroxyethoxy-6-chloro-9-[(α-methyl-γ-diethylaminobutyl)-amino]-acridine

of an appropriately substituted diphenylamine carboxylic acid by means of phosphorus oxychloride,[4] this procedure precluding the presence of a hydroxyalkyl radical at this stage. Direct synthesis of the phenol III was unsatisfactory, while preparation of this compound by the action of various hydrolytic agents on II failed because of the lability of the 9-chloro substituent. Dealkylation of II by anhydrous aluminum chloride afforded the phenol III in good yield. Hydroxyethylation of III, or the 9-phenoxy derivative IV, was difficult because of the ease with which the 9-substituent split off to form an acridone

(1) (a) Cherntsov and Drozdov, *J. Gen. Chem. (U. S. S. R.)* **9**, 1435 (1939); (b) Magidson and Grigorovskii, *Ber.*, **69B**, 396 (1936); (c) Wiselogle, "Survey of Antimalarial Drugs," Vol. II, Part 2, J. W. Edwards, Ann Arbor, 1946, Compounds SN186, 5557, 5559, 5578 and 5545.

(2) (a) Burckhalter, Jones, Holcomb, and Sweet, THIS JOURNAL, **65**, 2012 (1943); (b) ref. 1(c), Compounds SN189, 845, 856, and 9616.

(3) Ref. 1(c), Compound SN5588.

(4) Mietzsch and Mauss, *Angew. Chem.*, **47**, 633 (1934).