[CONTRIBUTION FROM THE RESEARCH LABORATORIES, PARKE, DAVIS & COMPANY]

The Chemistry of Viomycin[1]

BY THEODORE H. HASKELL, SALVATORE A. FUSARI, ROGER P. FROHARDT AND QUENTIN R. BARTZ

Viomycin, a new tuberculostatic antibiotic, is a strongly basic polypeptide with a tentative empirical formula $C_{18}H_{31-33}N_9O_8$. Acid hydrolysis liberated carbon dioxide, ammonia and urea in addition to amino acids. The acids isolated were L-serine, α,β-diaminopropionic acid, and a basic amino acid isomeric with lysine. A guanidino compound was also isolated but not identified.

Viomycin, a new tuberculostatic antibiotic which was obtained independently by two different laboratories from actinomycetes designated as *Streptomyces puniceus* and *Streptomyces floridae*,[2,3] has been assigned a tentative empirical formula $C_{18}H_{31-33}N_9O_8$ as indicated by the analyses of the crystalline sulfate, hydrochloride, picrate and reineckate.[4]

The antibiotic is a strong base which gave positive Sakaguchi,[2,3] biuret[3] and ninhydrin[3] tests. The maltol,[3] Molisch and Benedict[3] tests were negative. The antibiotic possesses one primary amino group as indicated by the Van Slyke nitrous acid determination and is devoid of free α-amino carboxy groups as shown by the ninhydrin-carbon dioxide technique. Table I gives the four-minute Van Slyke nitrous acid values on hydrolyzed and unhydrolyzed viomycin sulfate. The hydrolysis was carried out in 6 *N* hydrochloric acid on a steam-bath for 22 hours.

TABLE I

	N, %	Amino N, %	% of total
Unhydrolyzed	20.99	2.76	13.15
Hydrolyzed	20.99	10.20	48.59

The molecule resembled a polypeptide in structure since vigorous acid hydrolytic conditions were required for complete microbiological inactivation. Approximately 25% of the microbiological activity remained after incubation of viomycin sulfate in 1.0 *N* hydrochloric acid for 11 days at 37°. No appreciable amount of amino acids was liberated during this time, however, with vigorous acid hydrolysis in 6 *N* hydrochloric or 6 *N* sulfuric acids at 100° free α-amino acids were liberated as indicated by the ninhydrin–carbon dioxide procedure. These values are given in Table II.

TABLE II

NINHYDRIN–CARBON DIOXIDE VALUES ON HYDROLYZED VIOMYCIN SULFATE

6 *N* HCl		6 *N* H_2SO_4	
Time, hr.	Carboxyl N, %	Time, hr.	Carboxyl N, %
1	3.17	0	0.18
7	6.12	1	1.84
28	6.14	3	3.17
48	5.74	5	4.44
		13	5.62
		24	5.50

Calcd. for one carboxyl N group: 2.33%.

Carbon dioxide, ammonia and urea were also liberated during the hydrolysis. Values of 0.63, 0.66 and 0.29 mole, respectively, per mole of viomycin sulfate were obtained. The urea and ammonia values were obtained by aerating aliquots of the hydrolysis mixture, with and without the addition of crystalline urease, into a boric acid solution followed by titration with standard hydrochloric acid. The characteristic ultraviolet absorption band at 268 mμ[3] was completely destroyed by the hydrolysis.

Periodate oxidation studies on intact viomycin sulfate were conclusive only insofar as they indicated the absence of a sugar or an inositol moiety. The periodate uptake depended on the *p*H of the solution. 0.5 mole of HIO_4 was reduced per mole of viomycin sulfate at *p*H<1.0 after 19 hours at room temperature. At *p*H 4.4, 0.5 mole of periodate was reduced after 3 hours, and 1.45 moles after two days.

Two dimensional paper chromatography using *t*-butanol (50 parts), acetic acid (25 parts), and water (25 parts) in the first direction and phenol–water in the second, showed the presence of eight ninhydrin spots when 400 γ of hydrolysate was used. Figure 1 gives the relative positions of the ninhydrin spots on the paper sheet. Spots 6, 7 and 8 did not appear when 200 γ of hydrolysate was used.

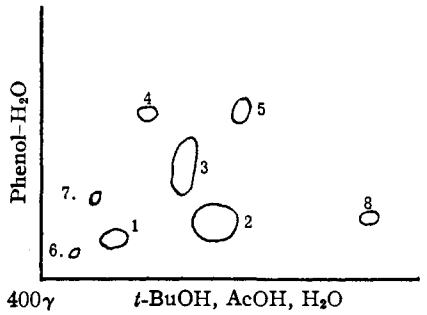

Fig. 1.—Two dimensional paper chromatogram of viomycin sulfate hydrolysate using *t*-butanol (50 parts), acetic acid (25 parts) and water (25 parts) in the first direction and phenol–water in the second; concn., 400 γ.

Four of these ninhydrin positive components have been isolated by using cation exchange chromatography. Zeo Rex,[5] a phenolic methylenesulfonic acid type exchanger was found to give better resolution of the amino acids than Dowex 50. However, when the basic constituents were adsorbed on Dowex 50, crystalline urea was isolated from the aqueous effluents. When Zeo Rex was employed, the urea had to be isolated as the dixanthydryl derivative.

(1) The substance of this paper was presented before the Section of Medicinal Chemistry at the XIIth International Congress of Pure and Applied Chemistry, New York City, September 10, 1951.

(2) A. C. Finlay, G. L. Hobby, F. Hochstein, T. M. Lees, T. F. Lenert, J. A. Means, S. Y. P'An, P. P. Regna, J. B. Routien, B. A. Sobin, K. B. Tate and J. H. Kane, *Am. Rev. Tuberc.*, **63**, 1 (1951).

(3) Q. R. Bartz, J. Ehrlich, J. D. Mold, M. A. Penner and R. M. Smith, *ibid.*, **63**, 4 (1951).

(4) J. D. Mold and Q. R. Bartz, unpublished results.

(5) Permutit Company, New York City, New York.

The basic components adsorbed on the cation exchanger were displaced with 0.1 *N* ammonia solution. Four main fractions were isolated which in the order of their elution are: urea, which was not adsorbed, L-serine, a middle fraction representing a distinct yellow band which followed the serine band, and a basic amino acid which was eluted with 1.0 *N* ammonia.

The L-serine, which corresponds to spot #2 in Fig. 1, was characterized by microbiological assay, elementary analysis, optical rotation, infrared analysis, ninhydrin–carbon dioxide determination and N-2,4-dinitrophenyl derivative. The middle fraction was further purified by passage through a triple chambered Zeo Rex column which is shown in Fig. 2. The purpose of this is twofold. In the first place it accomplishes a triple fractionation of the adsorbed solutes. Also by using progressively smaller amounts of resin in the lower chambers one can obtain perfectly horizontal boundaries for the solute bands as they leave the column.[6] An almost quantitative removal of the remaining L-serine from the middle fraction was accomplished in this manner.

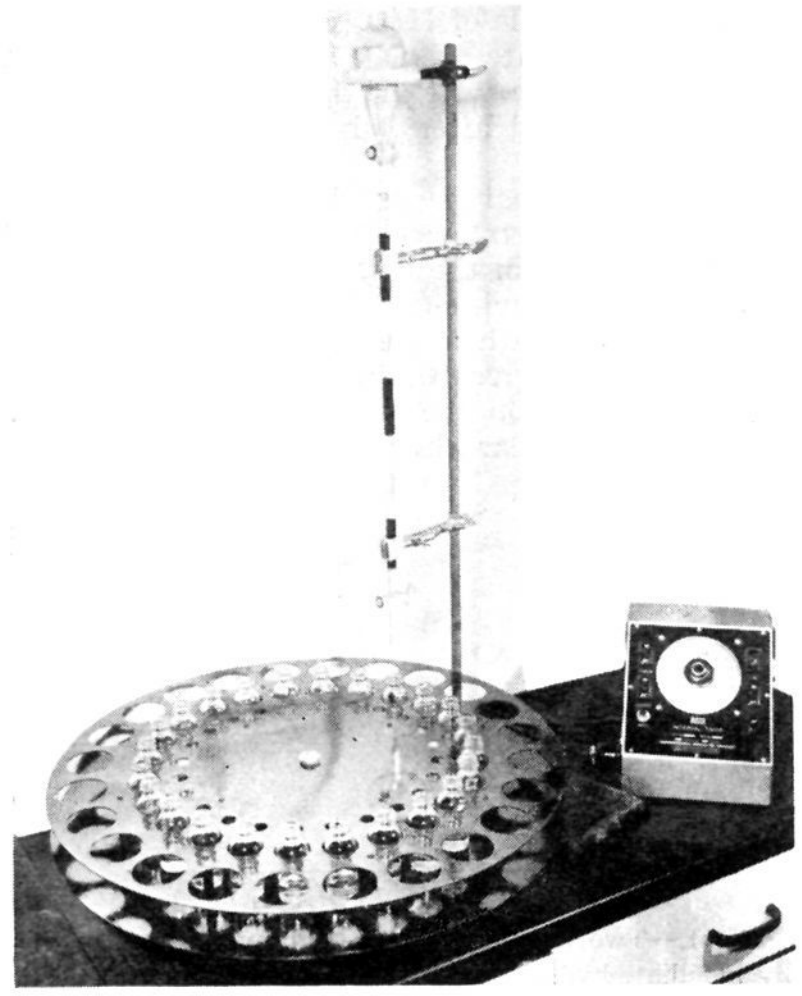

Fig. 2.—Triple chambered chromatographic column. Each chamber has 10/30 ⅀ ground glass joints with a coarse sintered glass disc. Approximately 2 cm. below the sintered disc, the inside diameter of the tube is constricted to 1 mm. Column dimensions from disc to top joint 8 × 82 mm.

The middle fraction was then passed through a strong anion exchange column (Amberlite IRA-400) to separate the amino acids from any non-amphoteric base. From the aqueous percolate there was isolated an intensely positive Sakaguchi component corresponding to spot 4 in Fig. 1. The material gave a crystalline *p*-hydroxyazobenzene *p*-sulfonate derivative. However, the regenerated free base has not as yet been obtained in crystalline form.

The resin column was eluted with either hydrochloric acid or ammonium carbonate from which a crystalline hydrochloride was obtained which analyzed correctly for α,β-diaminopropionic acid (spot 1 in Fig. 1).

The basic amino acid, corresponding to spot 3 in Fig. 1, which was obtained by eluting the Zeo Rex column with 1.0 *N* ammonia, was further purified by partition chromatography on powdered cellulose. Analyses of the crystalline hydrochloride, sulfate, picrate and *p*-hydroxyazobenzene *p*-sulfonate indicated that the compound was isomeric with lysine and identical with the basic amino acid isolated from streptothricin hydrolysates by Carter and his associates.[7]

The degradation products corresponding to the ninhydrin spots 5, 6, 7 and 8 in Fig. 1 were not isolated, the latter three evidently being present in trace amounts.

Experimental

Hydrolysis of Viomycin Sulfate.—A solution of 5.0 g. of viomycin sulfate in 250 cc. of 6 *N* hydrochloric acid was refluxed for 16 hours. The hydrolysate was freed from excess hydrochloric acid by repeated distillation *in vacuo* followed by passage through an Amberlite IR-4B column. The *p*H of the resulting solution was about 8. Elution of the Amberlite column with either acid or base produced ninhydrin negative eluates.

Chromatography on Zeo Rex (H^+ Form).—Thirty grams of Zeo Rex of 80–100 mesh size was allowed to swell overnight in 5 *N* hydrochloric acid and then rinsed with deionized water. The resin was packed in a chromatographic tube (2 × 45 cm.) according to the method of Partridge.[8] The resin depth was 33 cm. (55 cc.). After conditioning the resin by three alternate washings with 1 *N* ammonia and 2 *N* hydrochloric acid (the final washing with acid), the column was rinsed with deionized water until the effluent had a *p*H of 5.0.

The hydrolysate was adjusted to 800 cc. (*ca.* 0.01 *M*) and applied to the column at a rate of 30 cc./hr. A sharp horizontal band formed which occupied about one-half the column length. The column was then washed with 500 cc. of deionized water. The aqueous washing was ninhydrin-negative but gave a positive urease test. After evaporating the aqueous percolate to near dryness, urea was isolated as its dixanthydryl derivative,[9] m.p. 289° (dec.).

Anal. Calcd. for $C_{27}H_{20}N_2O_3$: C, 77.14; H, 4.76; N, 6.67. Found: C, 76.77; H, 4.57; N, 6.44.

The column was then developed with 0.1 *N* ammonia at a flow rate of 10 cc./hr. Fractions were collected at hourly intervals by an automatic fraction collector. Two visual bands formed on the column. The lowest band, which was black and quite difficult to see, represented pure L-serine. This was followed by a yellow band which was completely displaced by tube 70. At tube 82, 1 *N* ammonia was applied to the column and fractions collected until the *p*H of the effluent was around 11 (tube 95). Then tube 96 was collected as one large fraction (350 cc.) at a much faster rate.

The progress of the fractionation was followed by one dimensional paper chromatography using a mixture of *t*-butanol, acetic acid and water (50:25:25) as the mobile phase

(6) L. Hagdahl, *Acta Chem. Scand.* **2**, 574 (1948).

(7) (a) Second National Medicinal Chemistry Symposium of the American Chemical Society, June 15–17, 1950, Notre Dame, Ind. W. R. Taylor, pp. 33–38. (b) H. E. Carter, W. R. Taylor, R. L. Clark, W. R. Hearn, P. Kohn and J. R. Rothrock, "Abstracts of Papers," 118th Meeting, American Chemical Society, Chicago, Illinois, September, 1950, p. 16A–17A. (c) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers" 119th Meeting, American Chemical Society, Cleveland, Ohio, April, 1951, p. 25A.

(8) S. M. Partridge and R. G. Westfall, *Biochem. J.*, **44**, 418 (1949).

(9) F. Allen and J. Luck, *J. Biol. Chem.*, **82**, 693 (1929).

and 0.1% ninhydrin in *n*-butanol as the spotting reagent. Whatman #4 paper was employed. The fractionation data are summarized in Table III.

TABLE III

Tube No.	Number and character of zones	R_F value(s)
1–25	Ninhydrin negative	.....
26–49	1 strong, compact zone	0.40–0.42
50–63	2 strong, compact zones	0.25; 0.42
64–70	3 strong zones	0.06[a]; 0.12[a]; 0.25
71–81	Ninhydrin negative	.....
82–96	1 strong compact zone	0.32–0.35[b]

[a] Sometimes showed as one zone R_F 0.10. [b] A weak ninhydrin zone of R_F 0.55 appeared occasionally.

Isolation of L-Serine.—Eight grams of 100–200 mesh Zeo Rex was packed into a triple chambered chromatographic column (see Fig. 2). Approximately 3 g. was added to the top chamber, 2.7 g. to the middle and 2.3 g. to the bottom chamber. A glass float placed over each bed of resin minimized the disturbance from incoming liquid. Tubes 50–63 from Table III were applied to the column at a flow rate of 5 cc./hr. After rinsing with deionized water, the column was eluted with 0.1 *N* ammonia and 22 fractions were collected at hourly intervals. Tubes 1–5 on paper strips showed but one zone R_F 0.40–0.42. Tube 6 showed 2 zones of R_F 0.25 and 0.42. Tubes 7–22 showed one zone of R_F 0.25.

Tubes 1–5 were combined with tubes 26–49 from Table III and were concentrated to dryness *in vacuo*. The resulting white residue after recrystallization from 25 cc. of 80% ethanol yielded 0.822 g. (16.4%) of white needles, $[\alpha]^{26}D$ −6.21° (*c* 10.0% in water); $[\alpha]^{26}D$ +16.3° (*c* 10.0% in 2 *N* HCl): lit.[10]: −6.83° and +14.8°, respectively. The infrared absorption spectrum (in nujol mull) was identical with authentic L-serine.

Anal. Calcd. for $C_3H_7O_3N$: C, 34.28; H, 6.71; N, 13.33; carboxyl-nitrogen, 13.34; equiv. wt., 105.1. Found: C, 34.46; H, 6.67; N, 13.34; carboxyl-nitrogen, 13.30 (ninhydrin–carbon dioxide procedure); equiv. wt., 107.9 (perchloric acid titration); microbiological assay, 1045 mg./g.

The 2,4-dinitrophenyl derivative,[11] m.p. 178° (uncor.), λ_{max} 361 mμ, ε 17480 (in *p*H 7.0 phosphate buffer) was further characterized by analysis and by a mixed melting point with authentic L-N-2,4-dinitrophenylserine, which showed no depression.

Anal. Calcd. for $C_9H_9O_7N_3$: C, 39.83; H, 3.35; N, 15.49. Found: C, 40.00; H, 3.34; N, 15.33.

Isolation of α,β-Diaminopropionic Acid.—This compound was isolated by two different procedures depending on the starting material. In those cases where it was contaminated only with the Sakaguchi component, passage over Amberlite IRA-400 (OH^- Form) effectively freed it from the guanidino component which was not retained by the resin (Procedure (a)). When the starting material contained in addition the components of R_F 0.06 and 0.12 fractionation in the triple chambered column was impractical and recourse to crystallization of the phosphotungstate salts was necessary.

Procedure (a).—Tubes 7–22, obtained from the triple chambered column, were combined and concentrated to dryness *in vacuo;* wt. *ca.* 0.5 g.

This was dissolved in 125 cc. of water and passed through a column containing 6 g. of 80–100 mesh Amberlite IRA-400 (in hydroxyl form) at a flow rate of 10 cc./hr. The column was washed with water until the washings gave a negative Sakaguchi and ninhydrin reaction. The water effluent was set aside for the isolation of the Sakaguchi component as given below.

The Amberlite column was then eluted with 0.1 *M* ammonium carbonate solution. The eluate was concentrated to dryness *in vacuo* several times to remove the ammonium carbonate. The residue was dried *in vacuo;* wt. 0.356 g. This was dissolved in a slight excess of 5 *N* hydrochloric acid and concentrated to dryness *in vacuo*. Trituration of the residue with methanol gave a granular precipitate which on three recrystallizations from methanol–water yielded 0.075 g. of white needles.

Anal. Calcd. for $C_3H_8O_2N_2 \cdot HCl$: C, 25.65; H, 6.43; N, 19.92; Cl (ionic), 25.23. Found: C, 25.87; H, 6.78; N, 20.06; Van Slyke amino nitrogen, 19.0 (5 min.); carboxyl-nitrogen, 10.10; Cl (ionic), 24.69.

Procedure (b).—Tubes 64–70 from Table III were combined and passed through an Amberlite IRA-400 to remove the guanidino component as described in procedure (a). The water effluent was combined with the corresponding fraction from procedure (a).

The Amberlite column was eluted with 0.1 *N* hydrochloric acid and the eluant concentrated to dryness *in vacuo*. The resulting brown gum (wt. 0.52 g.) showed four ninhydrin spots by one dimensional paper chromatography (R_F, 0.06, 0.12, 0.25, and 0.55).

Thirty grams of purified 12-phosphotungstic acid was dissolved in 225 cc. of 0.25 *N* hydrochloric acid and to this was added 0.52 g. of the brown gum dissolved in 50 cc. of 0.25 *N* hydrochloric acid. Fractional recrystallization of phosphotungstate salts which formed gave a crystalline fraction, which, when converted to the hydrochloride[12] and chromatographed on paper, showed but two zones (R_F, 0.10 (weak) and 0.25). Fractional recrystallization from aqueous methanol gave 0.044 g. of α,β-diaminopropionic acid (R_F 0.25).

Anal. Found: C, 26.15; H, 6.58; N, 19.44; Cl (ionic), 24.77.

Isolation of the Lysine Isomer.—Tubes 82–96 from Table III were combined and concentrated to dryness *in vacuo*. The weight of hygroscopic brown gum obtained was about 0.700 g. One dimensional paper chromatography showed the presence of two other faint ninhydrin spots (R_F 0.10, 0.55) in addition to the main component of R_F 0.33.[13] This gum was converted to the hydrochloride and concentrated to dryness *in vacuo*.

The resulting product was partitioned on a cellulose chromatographic column (2.5 × 75 cm.) using 50 g. of purified Solka Floc BW 200.[14] The solvent employed was *t*-butanol, acetic acid and water (40:40:20). The column was packed according to the directions of Peterson and Reineke.[15] A flow rate of 5 cc./hr. was used and 117 fractions were collected at hourly intervals. The progress of the fractionation was followed by one dimensional paper chromatography and the results are summarized in Table IV.

TABLE IV

Tube no.	No. of zones	R_F
1–30	1—very weak	0.55
31–76	1—strong	.33
77–116	1—weak	.13

In order to check on the purity of fractions 31–75, they were rechromatographed on paper after first concentrating in lots of 5, *i.e.* 31–35, 36–40, etc. Fractions 31–70 still showed only one zone, R_F 0.32–0.35, whereas fractions 71–75 showed two zones, R_F 0.13 (weak) and 0.35 (strong).

Tubes 31–70 from Table IV were combined and concentrated to dryness, *in vacuo*, yielding a light brown very hygroscopic gum. This was converted to the *p*-hydroxyazobenzene *p*-sulfonic acid salt which crystallized as orange sheaths from water. The yield was 2.4178 g., m.p. 240–241° (uncor.).

Anal. Calcd. for $C_6H_{14}N_2O_2(C_{12}H_{10}N_2O_4S)_2$: C, 51.26; H, 4.87; N, 11.91; S, 9.12. Found: C, 50.89; H, 5.02; N, 11.62; S, 9.18.

The sulfonate salt was regenerated to the free base by a modification of Moore and Stein's procedure,[10] the excess sulfuric acid being removed by passage through an Amberlite IR-4B column. On lyophilization of the aqueous eluate, 0.650 g. of light tan very hygroscopic gum was obtained.

In order to determine the nature of the nitrogen linkages, a Van Slyke amino nitrogen analysis was performed. The analyst reported that during the weighing of the base the product picked up considerable moisture and became tacky. Therefore, it is to be noted that although the value given

(10) "Biochemical Preparations," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1949, p. 13.

(11) F. Sanger, *Biochem. J.*, **42**, 288 (1948).

(12) D. D. Van Slyke, *J. Biol. Chem.*, **22**, 281 (1915).

(13) Chromatographed as the hydrochlorides.

(14) Brown Company, 500 Fifth Avenue, New York 18, N. Y.

(15) D. H. Peterson and L. M. Reineke, *J. Biol. Chem.* **181**, 95 (1949).

in this analysis are not "true" values, the relative values of the total nitrogen (Kjeldahl) and the amino nitrogen (Van Slyke) are a reliable index of the disposition of the nitrogens in the molecule.

Anal. Total nitrogen (Kjeldahl): 13.3; Van Slyke amino nitrogen: 12.2 (5 min.); 12.7 (30 min.).

This analysis thus indicates that all of the nitrogen in the molecule is in the form of primary amino nitrogen.

Owing to its extremely hygroscopic nature the compound could only be analyzed satisfactorily in the form of its derivatives. The picrate was obtained as yellow needles from water, m.p. 205–206° (uncor.), λ_{max} 356, $E^{1\%}_{1\,cm.}$ 475 (in *p*H 7.0 phosphate buffer).

Anal. Calcd. for $C_6H_{14}N_2O_2(C_6H_3N_3O_7)_2$: C, 35.76; H, 3.31; N, 18.54; mol. wt., 146. Found: C, 36.12; H, 3.55; N, 18.62; mol. wt., 144 (calcd. from ultraviolet absorption).

The hydrochloride was obtained as a white amorphous powder from methanol–ether, m.p. 153–155° (uncor.).

Anal. Calcd. for $C_6H_{14}N_2O_2 \cdot 2HCl$: C, 32.88; H, 7.36; N, 12.79; Cl, 32.36; carboxyl-nitrogen, 6.39. Found: C, 32.91; H, 7.40; N, 12.58; Cl, 32.50; carboxyl-nitrogen, 0.70.

The sulfate was obtained as white needles from methanol–water, m.p. 220–224° (dec.).

Anal. Calcd. for $C_6H_{14}N_2O_2 \cdot H_2SO_4$: C, 29.51; H, 6.55; N, 11.47. Found: C, 29.97; H, 6.60; N, 11.85.

It is evident from the analytical data given above that the compound is a diamino acid which is isomeric with lysine. Further proof was demonstrated by infrared analysis of its salts. Strong absorption in the region of the carboxyl group (5.76–5.90 μ) was found in each case. Furthermore, Dr. H. E. Carter and associates, at the 119th Meeting of the American Chemical Society, April, 1951, reported the demonstration, from paper chromatographic studies, of the presence of the same diamino acid in viomycin as that found in streptothricin.

Isolation of Guanidino Component.—The water eluates, from procedures (a) and (b) from the α,β-diaminopropionic acid isolation, were combined and concentrated to dryness *in vacuo*. The residue was dissolved in ethanol, and upon addition of ether, 0.200 g. of an amorphous white powder was obtained. The material gave a strong Sakaguchi test and a light pink ninhydrin color, R_F 0.25 in *t*-butanol, acetic acid, and water (50:25:25). Conversion to the *p*-hydroxyazobenzene *p*-sulfonic acid salt gave yellow needles from water, m.p. 212–215° (uncor.).

Anal. Found: C, 47.78; H, 5.06; N, 16.89; S, 7.52.

Acknowledgment.—We wish to express our appreciation to Mr. Albert Ryder for his technical assistance; to Dr. H. B. Devlin for the Van Slyke amino acid determinations; to Dr. O. D. Bird for the microbiological assays; to Mr. C. E. Childs and his associates for the microanalyses; to Dr. J. M. Vandenbelt for the ultraviolet and infrared absorption measurements and to Dr. A. J. Glazko for his helpful suggestions on paper chromatography.

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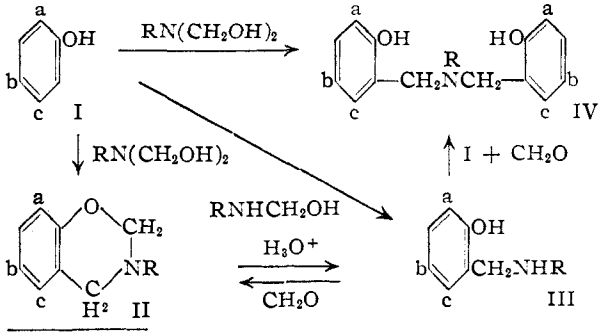
N,N-Bis-(hydroxybenzyl)-amines: Synthesis from Phenols, Formaldehyde and Primary Amines[1]

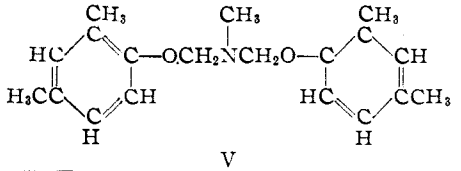
BY W. J. BURKE, RICHARD P. SMITH AND CARL WEATHERBEE

Condensation of phenols with formaldehyde and primary amines is shown to yield N,N-bis-(hydroxybenzyl)-amines directly in certain instances. Evidence is presented which indicates that the nature of the substituent ortho to the phenolic hydroxyl plays an important role in determining the course of the reaction.

Recent work[2] has shown that reaction of para-substituted phenols with formaldehyde and primary aliphatic amines offers a convenient route to either 3,4 - dihydro - (3,6 - disubstituted) - 1,3,2H - benzoxazines (II) or *o*-alkylaminomethylphenols (III), depending upon reaction conditions. Continuation of this study has demonstrated that a third type of product, N,N-bis-(2-hydroxybenzyl)-alkylamines (IV), can also be obtained directly in high yield from such systems in certain instances.



Auwers[3] has described the synthesis of substituted N,N-bis-(hydroxybenzyl)-alkylamines by a rather complicated procedure involving condensation of primary amines with bromohydroxybenzyl bromides, obtained by the bromination of various methylphenols. The method of preparation used in the present work is much less involved and in addition permits the synthesis of a greater variety of products. For example, simple refluxing of a methanol solution containing 2,4-dimethylphenol, formaldehyde and methylamine, in a molar ratio of 2:2.1, respectively, resulted in an 85% yield of N,N-bis-(2-hydroxy-3,5-dimethylbenzyl)-methylamine, IVa (a, b, R = CH_3; c = H). This structure was assigned rather than the isomeric N,N-bis-(2,4-dimethylphenoxymethyl)-methylamine (V), since a Zerewitinoff determination indicated the presence of two active hydrogens in



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(1) Presented in part before the Organic Division of the American Chemical Society in San Francisco in March, 1949.

(2) W. J. Burke, THIS JOURNAL, 71, 609 (1949).

(3) K. Auwers, *Ann.*, 344, 93 (1906).