[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF STANFORD UNIVERSITY]

Some Complex Sodium Bismuth Salts of Triethanolamine and Triisopropanolamine

BY WILLIAM T. MILLER

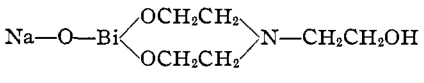
The synthetic work reported in this paper was undertaken because of the development of a bismuth preparation by Dr. P. J. Hanzlik of the Stanford Medical School for the treatment of human syphilis.[1,2] Dr. Hanzlik's material was compounded empirically from sodium bismuthate, triisopropanolamine and propylene glycol. Its pharmacological action was found to be dependent upon the presence of the amine rather than the glycol.

No pure complex bismuth salts with the trihydroxyamines have been previously reported.

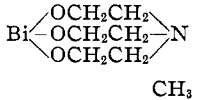
Discussion and Results

Since reduction of the bismuthate was indicated as the initial reaction in Hanzlik's preparation, attempts were made to prepare complex bismuth salts from sodium hydroxide, bismuth hydroxide and the free amine. These were successful and widely different experimental conditions were found to yield mixtures of sodium bismuth salts with good conversion of the bismuth hydroxide. However, considerable experimental trial was necessary before analytical data indicating the isolation of single substances could be obtained. Presumably equilibrium mixtures were formed in solution from which various solid phases might be obtained depending upon the conditions used. Conditions are described for the preparation of sodium-bismuthyl-triethanolamine I, bismuth triethanolamine II and sodium-bismuthyl-triisopropanolamine III, as pure crystalline compounds. These salts may be represented as follows.

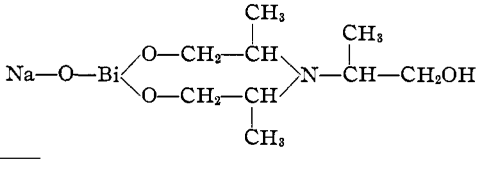
I



II



III



I and II were found to form a series of hydrates. With diethanolamine indications of compound formation were secured although no pure salts were isolated, but in the case of ethanolamine there appeared to be no reaction.

The sodium bismuth trihydroxyamine salts were very soluble in water and in the lower alcohols, but were insoluble in acetone, ether, higher ketones and other inert organic liquids tested. Their aqueous solutions slowly hydrolyzed to form some insoluble material but were stabilized by the addition of small amounts of excess amine or glucose even at high dilution.[3] However, aqueous solutions of bismuth triethanolamine could not be stabilized in this way. All the salts absorbed carbon dioxide slowly from the air and darkened somewhat on continued exposure to daylight.

No pure products could be isolated from the reaction between sodium bismuthate and triisopropanolamine in propylene glycol solution, nor could sodium-bismuthyl-triisopropanolamine be recovered as a pure substance from its solution in propylene glycol. However, by carrying out the reaction between the amine and sodium bismuthate in the absence of the glycol in xylene solution it was demonstrated that this salt was formed.

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Experimental Results

Analytical Procedures

Bismuth.—Samples of approximately 0.2 g. of the amine salts were dissolved in 30 to 40 cc. of water with the addition of 2 cc. of concd. nitric acid. The bismuth was then precipitated directly as the phosphate using the procedure of Schoeller and Waterhouse.[4]

Nitrogen.—A semi-micro modification of the Kjeldahl method was found to give excellent results.[5] Samples were chosen so as to require about 10 cc. of 0.04 *N* acid, and boric acid was used to collect the evolved ammonia.

Sodium.—Acidified aqueous solutions containing 0.3 to 0.4 g. samples of the salts were saturated with hydrogen

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(1) Hanzlik, Lehman and Richardson, *Am. J. Syphilis Gonorrhea and Venereal Diseases*, **21**, 1 (1937).

(2) Report by the Council on Pharmacy and Chemistry, *J. Am. Med. Assoc.*, **113**, 2214, 2219 (1939) (see also references).

(3) For pharmacological experiments mixtures of the sodium salts and excess amine are suggested.

(4) Schoeller and Waterhouse, *Analyst*, **45**, 435 (1920); compare Blasdale and Parle, *Ind. Eng. Chem., Anal. Ed.*, **8**, 352 (1936).

(5) Allen, *ibid.*, **3**, 239 (1931).

sulfide and the precipitated bismuth sulfide filtered off. The filtrates were evaporated to dryness; the residues were treated with concd. nitric acid and re-evaporated to oxidize organic matter. Finally, the salt residues were converted into sodium chloride by evaporating twice with hydrochloric acid and weighed after heating to constant weight. Results tended to run slightly high with known compounds.

Water.—Crystal water was determined by heating samples protected from direct daylight in an evacuated drying pistol with refluxing benzene using concentrated sulfuric acid as the drying agent.

Sodium-bismuthyl-triethanolamine.—A solution of 2.5 g. of sodium in 50 cc. of ethyl alcohol (95%) was mixed with 15 g. of pure triethanolamine and 10 g. of bismuth hydroxide (Merck purified hydrated bismuth oxide) added. The resulting mixture was heated to 65° in a flask protected by a soda-lime tube for four hours with occasional shaking. The reaction mixture was then cooled, filtered and the residue washed with 85 cc. of alcohol; yield 5.5 g., residue. The clear filtrate was diluted with 150 cc. of acetone and then with three additional 25-cc. portions at one-hour intervals and let stand overnight. Filtration yielded 7 g. of white crystalline alcohol washed product. Calcd. for $[(C_2H_5O)N(C_2H_4O)_2 \cdot BiO]Na \cdot 3H_2O$: H_2O, 12.03. Found: H_2O, 11.87. Calcd. for the anhydrous salt: Bi, 52.9; N, 3.54; Na, 5.82. Found: Bi, 53.0; N, 3.61; Na, 6.00.

Additional product was obtained by further dilution of the filtrate with acetone. This material slowly absorbed carbon dioxide from the air and darkened on continued exposure to light. Its solutions in propylene glycol appeared to be perfectly stable for thirty days.

The anhydrous salt was obtained directly from 4 g. of sodium, 30 g. of amine and 10 g. of bismuth hydroxide in 100 cc. of alcohol. The reaction mixture was heated at 65° for two hours, filtered to yield 6.8 g. of residue and then refiltered with a little charcoal. The clear filtrate deposited 2.9 g. of crystals on standing overnight at 65°. Found: Bi, 52.9.

The insoluble residues from these reactions were found by microscopical examination to be composed largely of diamond-shaped crystals mixed with some bismuth oxide. Longer periods of reaction increased the amounts of insoluble residue.

Bismuth Triethanolamine.—Thirty grams of amine and 10 g. of bismuth hydroxide were added to a solution prepared from 4.6 g. of sodium and 100 cc. of ethyl alcohol and the mixture heated overnight at 65°. Filtration yielded 7.8 g. of residue which was shown to be largely crystalline by microscopical examination. Filtration with charcoal then yielded a perfectly clear filtrate. Two volumes of absolute methyl alcohol were added and the solution let stand. Beautiful needle-like crystals slowly formed; yield 4.2 g. after washing with alcohol and acetone. The crystals exhibited parallel extinction and were strongly double refracting. They were anhydrous and did not contain sufficient sodium to determine analytically. Calcd. for $Bi(C_2H_4O)_3N$: Bi, 58.9; N, 3.94. Found: Bi, 58.5; N, 3.96. Triethanolamine was shown to be reformed when this salt was hydrolyzed; hydrochloride, m. p. 178–179°.[6]

This substance dissolved readily in water to form a solution which slowly hydrolyzed and was not stabilized by the addition of excess amine. It was slightly soluble in ethyl alcohol; less soluble in methyl alcohol but fairly soluble in propylene glycol.

Sodium-bismuthyl-triisopropanolamine.—A solution prepared from 7.6 g. of sodium and 125 cc. of methyl alcohol was mixed with 42 g. of triisopropanolamine and 30 g. of bismuth hydroxide. The resulting mixture was heated at 65° overnight. Filtration yielded 4.5 g. of methyl alcohol washed residue. The filtrate was treated with charcoal and refiltered to yield 150 cc. of perfectly clear light amber colored solution. No immediate precipitation was caused by adding an equal volume of water. However, the addition of 25 cc. of acetone to the aqueous alcoholic solution precipitated some solid. This was redissolved by heating and the hot solution allowed to cool slowly; yield after washing with three 25-cc. portions of 1 to 3 alcohol–acetone and vacuum drying, 18.5 g. of white crystalline material. Acetone (50 cc.) was added to the filtrate plus washings and the solution again heated to dissolve the first formed precipitate; yield 12.6 g. The combined product (31.1 g.) was let stand over 40% concd. sulfuric acid at room temperature for seven days. Calcd. for $[(C_3H_7O)N(C_3H_6O)_2 \cdot BiO]Na \cdot 3.5\ H_2O$: H_2O, 12.60. Found: H_2O, 12.55. Calcd. for the anhydrous salt: Bi, 47.8; N, 3.20; Na, 5.26. Found: Bi, 48.1; N, 3.19; Na, 5.39.

This substance was very soluble in water with some hydrolysis and in methyl and ethyl alcohols. It absorbed carbon dioxide slowly from the air and exhibited parallel extinction between crossed Nicol prisms. Solutions in propylene glycol (up to 15%) appeared to be perfectly stable for thirty days.

Sodium-bismuthyl-triisopropanolamine from Sodium Bismuthate.—A solution of 20 g. of triisopropanolamine in 75 cc. of xylene was added to 20 g. sodium bismuthate and the mixture warmed with stirring until a vigorous reaction began. After the initial reaction subsided the mixture was heated to the boiling point for about one minute and then at 65° for an hour. Some gaseous ammonia was evolved and considerable crystalline material precipitated as clusters of fine needles; yield 21.3 g. of crystals mixed with some apparently unreacted sodium bismuthate. This product was extracted with 100 cc. of one to one ethyl and methyl alcohols; residue 6.8 g. After clarification with charcoal, the filtrate was evaporated below 50° to a moist solid. This was treated with 50 cc. of acetone and 10.8 g. of slightly colored product obtained on filtration. A pure white purified sample was obtained by treating this product with charcoal in methyl alcohol solution followed by partial evaporation and the addition of acetone; yield 4.2 g. Found: 12.5 H_2O. For the anhydrous salt, found: Bi, 47.9; N, 3.16. Triisopropanolamine was recovered from this salt as the hydrochloride, m. p. 196–198°, when inserted into the melting point bath at 195°; *i. e.*, salt slowly decomposed near its melting point.

Summary

The preparation and properties of sodium-bismuthyl-triisopropanolamine, sodium-bismuthyl-

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[6] Germann and Knight, This Journal, 55, 4150 (1933).

triethanolamine and bismuth triethanolamine are reported. These compounds represent new types of complex bismuth salts, and their pharmaceutical action is being investigated.

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Studies in the Composition of Human Hair

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It seems well established that there are differences in the chemical composition of various keratins. Eukeratins, *e. g.*, wool and hair, are distinguished from pseudokeratins in that their basic amino acids (histidine, lysine, and arginine) are found in a relatively constant molecular ratio of 1:4:12.[1] On the other hand, the proportions of their non-basic amino acids show wide variations, particularly with respect to cystine, a high content of which is characteristic of keratins. Whether secondary factors, such as age and sex, give rise to further variations is less definitely established.

As early as 1806[2] an attempt was made to correlate the pigmentation of human hair with variations in its sulfur content. Rutherford and Hawk[3] studied the relation of age, sex, pigmentation, and race to the chemical composition of hair. Conflicting results have since been reported by many investigators. For example, from the analyses of twenty-three samples of hair from children (thirteen years and under) and six adults, Wilson and Lewis[4] could deduce no consistent relation between the cystine content and color, age, or sex, although there was an "apparent tendency" for the values to be larger in adult hair. With reference to the many previous studies, the general statement seems valid that actual relations were not definitely established because of the small number of samples, the relatively wide dispersion of the data, and the small obtained differences. At times deviations from the mean were almost as great within the groups as between them.

This paper represents an extended investigation of the cystine, cysteine,[5] nitrogen, and sulfur content of human hair.

Experimental

The hair was cleaned and defatted by washing in benzene, dried in a vacuum oven for three to five hours at 65–70°, and stored in a vacuum desiccator over phosphorus pentoxide until analyzed. Total nitrogen was determined by the macro-Kjeldahl method. Total sulfur was determined gravimetrically by the Benedict–Denis method after preliminary oxidation with concentrated nitric acid, as suggested by Wilson and Lewis.[4]

Cystine was determined by the Sullivan[6] and Shinohara[7] methods, and cysteine by the methods of Shinohara[8] and Lavine.[9] The hair was hyrolyzed with 20% hydrochloric acid for ten hours in an electric oven at 124–127°. The hydrolysates were decolorized with kaolin.

Discussion

The results of analyses of 120 samples of human hair are summarized in the table; they are averages of determinations on at least two portions of each hair sample. Uniform methods of analysis were used throughout and, insofar as was possible, the variables were limited to age, sex, and pigmentation. The range of values for each of the constituents is given in the table. There was considerable variation within the groups, this being particularly true of the cystine and cysteine values.

The presence of cysteine as shown by the Shinohara method is not in agreement with the observations of Wilson and Lewis,[4] who did not obtain a reduction of the uric acid reagent without the addition of sodium sulfite. Perhaps the explanation of this discrepancy lies in variations in procedure. Their more thorough extraction (total of ninety-six hours) and drying (several days at 100°) may have destroyed the small amount of cysteine present. Further, in agreement with the observations of previous workers,[10,11] we have found that the cysteine content of the hydroly-

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[1] R. J. Block, *J. Biol. Chem.*, **121**, 761 (1937).

[2] L. N. Vauquelin, *Ann. chim. phys.*, **58**, 41 (1806).

[3] T. A. Rutherford and P. B. Hawk, *J. Biol. Chem.*, **3**, 459 (1907).

[4] R. H. Wilson and H. B. Lewis, *ibid.*, **73**, 543 (1927).

[5] Some doubt exists that cysteine is a constituent of the protein molecule. However, work now in progress in this Laboratory indicates that cysteine is a primary hydrolysis product of keratins. The values reported here are to be regarded as minimal.

[6] W. C. Hess and M. X. Sullivan, *J. Biol. Chem.*, **117**, 423 (1937).

[7] K. Shinohara, *ibid.*, **112**, 683 (1936).

[8] K. Shinohara, *ibid.*, **109**, 665 (1935).

[9] T. F. Lavine, *ibid.*, **109**, 141 (1935).

[10] Y. Okuda, *J. Dept. Agr. Kyushu Imp. Univ.*, **1**, 4 (1925).

[11] B. Kassel and E. Brand, *J. Biol. Chem.*, **125**, 435 (1938).