Di-*n*-butyl-*n*-heptylamine.—In a similar way di-*n*-butylamine and heptyl bromide gave this amine, which boils at 119–120° at 7 mm.; n_D^{15}, 1.4389; d_0^0, 0.8088.

Anal. Subs., 1.0587: 47.5 cc. of 0.0929 *N* H_2SO_4. Calcd. for $C_{15}H_{33}N$: neut. equiv., 227.3. Found: 239.

Treatment with *n*-butyl iodide gave tri-*n*-butyl-*n*-heptylammonium iodide which was analyzed, giving the calculated results.

Summary

1. A modified and more satisfactory technique for the preparation of lithium alkyls has been developed.

2. Attempts have been made to prepare penta-alkyl nitrogen compounds from quaternary ammonium halides and lithium alkyls. These products, if formed, are very unstable and at once yield tertiary amines and hydrocarbons.

3. The evidence obtained indicates that the fifth valence of nitrogen in ammonium compounds retains its unique character even under conditions most favorable for its being otherwise, and at no time does it become equivalent to or is there any exchange of groups between it and any of the other four valences.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NEW YORK UNIVERSITY]

THE BASIS FOR THE PHYSIOLOGICAL ACTIVITY OF -ONIUM COMPOUNDS. VII. DERIVATIVES OF BETAINES[1]

BY R. R. RENSHAW AND H. T. HOTCHKISS, JR.

RECEIVED JULY 27, 1926 PUBLISHED OCTOBER 6, 1926

Several phases of the problem of determining the basis for the physiological activity of the -onium compounds have been discussed in earlier papers.[2]

A number of years ago Hunt and Taveau[3] in a study of over seventy derivatives and homologs of choline showed that the muscarine effect (stimulation of the inhibitory nerves to the heart and other organs with a production of a lowering of the blood pressure) is most marked in those compounds that depart least from the choline type of structure, that is, have the grouping $(CH_3)_3NCH_2CH_2O-$ Betaine salts such as the

$$\begin{array}{c} \\ | \\ X \end{array}$$

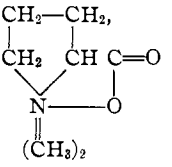
[1] This problem is being carried out in coöperation with Dr. Reid Hunt of the Harvard Medical School. The physiological data are the basis of another series of papers published elsewhere by him.

[2] Renshaw, *Science*, **62**, 384 (1925). Bencowitz and Renshaw, THIS JOURNAL, **47**, 1904 (1925). Renshaw and Ware, *ibid.*, **47**, 2989 (1925). Renshaw, Bacon and Roblyer, *ibid.*, **48**, 517 (1926). Renshaw and Bacon, *ibid.*, **48**, 1726 (1926). Bencowitz and Renshaw, *ibid.*, **48**, 2146 (1926).

[3] Hunt and Taveau, *U. S. Pub. Health Hyg. Bull.*, **73** (1911).

chloride, $(CH_3)_3N(Cl)CH_2COOH$, are, however, inert,[4] yet there is present a grouping of elements resembling closely that in the physiologically active substance. It is natural to attribute this lack of activity to the acidic character given to the molecule by the presence of the carboxyl group, for many cases are known where the introduction of a carboxyl or sulfonic acid group has altered the physiological action or the staining or dyeing properties of the substance.

The production of inert substances by the introduction of strongly acidic groups in phenol, morphine and cocaine are classical examples of such inactivations. Probably less well known is the reported non-toxic character of benzobetaine,[5] $(CH_3)_3NC_6H_4COO$, taurobetaine,[6] $(CH_3)_3NC_2H_4SO_2O$, and stachydrine,[7] substances containing groups which unmodified would be expected to show considerable activity. It is probable, too, that the carboxyl group in the aliphatic amino acids is responsible for the fact that they do not show the typical action of ammonia or the amines on the central nervous system. Plans have been made to investigate this question.



So varied is the physiological effect of these substances inactivated by the entrance of these acid groups that one might feel some justification in believing that the inactivations are due to the acid groups themselves or to some common property induced by their presence in the molecule. The authors after considering many of the facts incline, however, to the idea that not all of these inactivations may be due to a single mechanism. The problem is, of course, very complex and much more information is needed.

Nevertheless, with regard to the action of the -onium compounds on the nervous system it is worthy of note that this takes place at great dilution, that the action is a stoppage or a stimulation of a nerve impulse and that the substances are ionogens with the active group in the cation, and of course this action is independent of the anion. This suggests that at some stage in the train of circumstances culminating in their action an electrical effect is involved. It follows from this thought that if one could neutralize or change the sign of the active ion the electrical effect would either be destroyed or reversed and one might expect this to give a corresponding change in the physiological action. Such results would be brought about by introducing acidic groups into the substances which would give them

[4] Hunt (private communication) has recently shown that even large injections of betaine hydrochloride give neither the muscarine nor nicotine effects. This confirms earlier findings; Ref. 3, p. 18.

[5] Hildebrandt, *Beitr. physiol. path. Chem.*, **9**, 470 (1907).

[6] Brieger, *Z. physiol. Chem.*, **7**, 35 (1882–1883).

[7] Ackerman, *Z. Biol.*, **64**, 44 (1914).

iso-electric points approximately that of water. If the iso-electric point were of the same order as that of water, the substance would exist in that liquid almost wholly as the electrically neutral inner salt or bipolar ion. Even a wider range of iso-electric points would make possible the existence of the bipolar ions in the blood on account of the relatively large volume and the highly buffered condition of that fluid.

The iso-electric point of betaine, calculated from the acid and basic dissociation constants cited by Bjerrum,[8] is approximately at $P_H = 7.8$ and, therefore, this substance probably exists in solution largely as the bipolar ion $^{+}[(CH_3)_3NCH_2COO]^{-}$. The electrically neutral character of the physiologically active grouping of the betaine may be assumed, as a working hypothesis, to be the cause of its inactivity. Some evidence that this is in part the explanation is given in the present paper. We have prepared a number of esters and derivatives of betaine having the trimethylamine group. These exist in solution as cations. For example, in the case of the ester the active group would be present in the blood as the cation $[(CH_3)_3NCH_2COOC_2H_5]^{+}$. Hunt[9] has found that they have, qualitatively, the typical physiological action of choline. A number of them are quantitatively much more active than that substance. The amide of betaine, $H_2NOCCH_2N(CH_3)_3Cl$, was found to be physiologically active also, but its effect was less intense than that of some of the esters.

Work is now being done on the preparation of a series of compounds of the betaine type which will afford a gradual variation of iso-electric points over a wide range. Various analogs of betaine and its ester are also being prepared.

The betaine esters were prepared by the condensation of trimethylamine with esters of the halogen acids. Great variation was found in the readiness with which the reactions took place and also in the extent to which the trimethylamine eliminated hydrohalide instead of forming the -onium compound. In certain cases (with ethyl α-bromovalerate) experiments indicated that the presence of powdered coconut charcoal was advantageous in lowering the temperature at which the condensation would take place readily and in lessening the elimination of hydrohalide. This latter effect may have been due to the lower temperature. In other cases (with ethyl phenylbromo-acetate and ethyl α-bromo-*iso*valerate) the charcoal seemed to have no effect; we were unable to prepare an -onium compound from the latter substance under several conditions tried. In all cases trimethylammonium bromide was obtained by the elimination of hydrohalide from the halogen ester. As was expected, this elimination of hydrohalide was the only reaction that occurred with the β-halogen esters.

[8] Bjerrum, *Z. physik. Chem.*, **104**, 152 (1923).

[9] (a) Hunt and Renshaw, *J. Pharmacol.*, **25**, 320 (1924). (b) Hunt and Renshaw, "The Abel Number," *ibid.* (in press).

Methylbetaine (Carbomethoxymethyl-trimethylammonium Bromide), $CH_3OOCCH_2N(CH_3)_3Br$. (With E. A. Wilson.)—To a molecular equivalent of liquid trimethylamine in toluene was added at —10°, 15 g. of cold methyl bromo-acetate. A very vigorous reaction took place. After standing overnight in a pressure bottle, the product was washed with dry ether, dissolved in absolute methyl alcohol and then fractionally precipitated several times with dry ether. The product so obtained crystallizes in long, flat, rectangular needles; m. p., 182.5° (corr.).

Anal. Calcd. for $C_6H_{14}NO_2Br$: Br, 37.67. Found: 37.56, 37.68.

Ethylbetaine (Carbethoxymethyl-trimethylammonium Bromide), $C_2H_5OOCCH_2N(CH_3)_3Br$.—This ester was prepared in a manner analogous to the preparation of the methyl ester from liquefied trimethylamine and ethyl bromo-acetate in toluene at —10°. It was purified by several fractional reprecipitations of its solution in absolute ethyl alcohol by dry ether; m. p., 158.4° (corr.). This compound is considerably more active than neurine and perhaps a hundred times as active as choline in giving the muscarine effect. It has an unusual tendency to act on the vagus nerve endings to the heart as compared with its action on analogous blood vessels.[10]

Anal. Calcd. for $C_7H_{16}NO_2Br$: Br, 35.36. Found: 35.39, 35.19.

***n*-Butylbetaine (Carbo-*n*-butoxymethyl-trimethylammonium Bromide)**, $C_4H_9OOCCH_2N(CH_3)_3Br$. (With H. R. Halliday.)—This ester was prepared from *n*-butyl bromo-acetate and trimethylamine in a manner analogous to that described above. When purified by fractional precipitation from its absolute alcohol solution by dry ether it forms rosets of thin plates; m. p., 100.4° (corr.).

Anal. Calcd. for $C_9H_{20}NO_2Br$: Br, 31.45. Found: 31.31, 31.28.

Benzylbetaine (Carbobenzoxymethyl-trimethylammonium Bromide), $C_7H_7OOCCH_2N(CH_3)_3Br$. (With H. R. Halliday.)—This product was prepared and purified by the method used for the butyl ester. From an alcohol-ether mixture it crystallizes in long, thin plates; m. p., 111.5° (corr.).

Anal. Calcd. for $C_{12}H_{18}NO_2Br$: Br, 27.77. Found: 27.64, 27.70.

Methyl(Carbethoxy)Methyl-trimethylammonium Bromide, $C_2H_5OOCCH(CH_3)N(CH_3)_3Br$. (With W. K. Viertel.)—Fifteen g. of ethyl-bromopropionate was mixed with 5 g. of trimethylamine in 10 cc. of toluene cooled to —10° in a pressure bottle. The mixture was allowed to warm slowly to room temperature. It was finally heated for one and one-half hours at 50°. The product was purified from the small amount of trimethylammonium bromide formed by fractionally precipitating its absolute alcoholic solution with dry ether. It crystallizes in slender, rectangular, needle-like crystals; m. p., 146.5° (corr.).

An interesting point regarding the relationship of structure to physiological activity came up with respect to this compound. Hunt and Menge[11] had shown that a methyl derivative of acetylcholine, acetyl-α-methylcholine, $CH_3COO—CH_2C(CH_3)HN(CH_3)_3Cl$, gave an intense and a very extraordinarily persistent muscarine action. A single injection of a few tenths of a milligram of this substance per kilogram of body weight sufficed to keep the blood pressure uniformly lowered to one-half of its original value for hours. When it was found that the simple betaine ester resembled so closely choline and its esters, it seemed a safe prediction that this methyl derivative of the betaine ester might also have a very persistent action. Hunt[9b] found, however, that neither this compound nor the betaine esters with other side chains were at all conspicuous for the persistency of their action.

Anal. Calcd. for $C_8H_{18}NO_2Br$: Br, 33.28. Found: 33.34, 33.42.

[10] Hunt and Renshaw, *J. Pharmacol.*, **25**, 351 (1925).

[11] Ref. 3, footnote p. 33.

Propyl(Carbethoxy)Methyl-trimethylammonium Bromide, $C_2H_5OOCCH(C_3H_7)N(CH_3)_3Br$.—Stoichiometric quantities of trimethylamine and ethyl α-bromovalerate were allowed to stand overnight in a pressure bottle in the presence of a small quantity of powdered coconut charcoal. The white solid product formed was purified by solution in ethyl alcohol and precipitation with ether. It forms microscopic crystals; m. p., 179.6° (corr.).

Anal. Calcd. for $C_{10}H_{22}NO_2Br$: Br, 29.81. Found: 29.82, 29.71.

***n*-Butyl(Carbethoxy)Methyl-trimethylammonium Bromide,** $C_2H_5OOCCH(C_4H_9)N(CH_3)_3Br$.—Stoichiometric quantities of trimethylamine and ethyl bromo-*n*-caproate were heated in a pressure bottle for three hours at 50°. The solid product was washed with ether and purified by reprecipitation of its acetone solution with absolute ether. So obtained, it crystallizes in thin plates melting at 144.5° (corr.).

Anal. Calcd. for $C_{11}H_{24}NO_2Br$: Br, 28.35. Found: 28.34, 28.43.

Phenyl(Carbethoxy)Methyl-trimethylammonium Bromide, $C_2H_5OOCCH(C_6H_5)N(CH_3)_3Br$.—Sixteen and one-tenth g. of ethylphenylbromo-acetate and a molecular equivalent of trimethylamine were heated a number of hours at 50° in a pressure bottle. The product was purified by fractional precipitation of its absolute alcohol solution with anhydrous ether. So obtained, the product forms rosets of small, very slender crystals; m. p., 197.5–198° (corr.). The substance appears to dissociate readily in acetone solution.

Hunt[9b] has found that the introduction of the phenyl group practically abolishes the intense "muscarine" action of the ester. The compound had a weak stimulating nicotine action and a pronounced paralyzing nicotine action.

Anal. Calcd. for $C_{13}H_{20}NO_2Br$: Br, 26.39. Found: 26.16, 26.36.

Betaine-amide(Carbamylmethyl-trimethylammonium Chloride), $H_2NOCCH_2N(CH_3)_3Cl$.—Twelve and two-tenths g. of chloro-acetamide, 30 cc. of toluene and the calculated amount of liquid trimethylamine were heated in a pressure bottle for three hours at 70°. The resulting solid product was purified by precipitation from its absolute alcoholic solution with dry ether. Recrystallized from alcohol it forms feather-shaped, crystal aggregates; m. p., 194.5° (corr.). This non-acidic derivative of betaine is distinctly active physiologically, though its action is less intense than that of some of the esters.[9b]

Anal. Calcd. for $C_5H_{13}N_2OCl$: Cl, 23.24. Found: 23.40, 23.44.

The authors wish to express their appreciation to the Directors of the Bache Fund, National Academy of Sciences, for a grant made to support this investigation.

Summary

1. The preparation of a number of esters and other derivatives of betaine is described.

2. All the derivatives of betaine studied in which the acid hydrogen atom has been replaced are, unlike betaine itself, physiologically active. It is suggested that the physiological inactivity of betaine is due to its existence in the blood stream as the electrically neutral and hence physiologically inert bipolar ion, $^{+}[(CH_3)_3NCH_2COO]^{-}$. The esters of betaine and their derivatives, as well as the amide of betaine, form electrically active cations, and all of them are physiologically active.

NEW YORK, N. Y.