[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF LAFAYETTE COLLEGE AND OF WASHINGTON SQUARE COLLEGE, NEW YORK UNIVERSITY]

Thiamorpholines, Oxides and Dioxides[1]

BY WILLIAM F. HART AND JOSEPH B. NIEDERL

Short chain N-alkyl thiamorpholines were first prepared by H. T. Clarke[2] and by Lawson and Reid,[3] by the condensation of mustard gas with primary amines. The preparation of N-cetylthiamorpholine and a number of quaternary ammonium salts ("invert soaps") derived from it were described in a recent publication.[4]

This work has now been extended to include N-alkyl-thiamorpholines having alkyl chains containing 12, 14, 16 and 18 carbons, together with the corresponding N-alkyl-thiamorpholine-1-oxides and 1-dioxides. These were prepared by condensation of the appropriate primary amine with mustard gas, mustard gas sulfoxide and mustard gas sulfone, in alcoholic solution, using anhydrous sodium carbonate as the condensing agent.

$$R{-}NH_2 + (ClCH_2CH_2)_2S \xrightarrow[C_2H_5OH]{Na_2CO_3} S<(CH_2CH_2)_2>N{-}R$$

$$R{-}NH_2 + (ClCH_2CH_2)_2SO \xrightarrow[C_2H_5OH]{Na_2CO_3} OS<(CH_2CH_2)_2>N{-}R$$

$$R{-}NH_2 + (ClCH_2CH_2)_2SO_2 \xrightarrow[C_2H_5OH]{Na_2CO_3} O_2S<(CH_2CH_2)_2>N{-}R$$

R = $C_{12}H_{25}$-, $C_{14}H_{29}$-, $C_{16}H_{33}$- and $C_{18}H_{37}$-

Of these compounds suitable derivatives such as hydrochlorides and picrates were prepared. By the action of dimethyl sulfate on these tertiary amines there were obtained water soluble, capillary active quaternary ammonium salts ("invert soaps"), which suggest various industrial uses.

Experimental

N-Alkylthiamorpholines.[4]—Equivalent amounts of amine (lauryl-, myristyl-, cetyl- and octadecyl amines), mustard gas and anhydrous sodium carbonate were refluxed in alcoholic solution for eight hours. The inorganic salts were removed by filtration of the warm solution, and the free base obtained by removing the solvent *in vacuo*. It was found most convenient to purify the products as the hydrochlorides, which were prepared by taking up the free bases in dry ether and saturating the solution with dry hydrogen chloride. After filtering, these were purified by washing with dry ether and with dry acetone. The free bases are conveniently prepared by refluxing an alcoholic solution of the hydrochloride with an equivalent amount of anhydrous sodium carbonate or sodium methylate, filtering off the sodium chloride, and then crystallizing from the alcoholic solution.

N-Alkylthiamorpholines-1-oxides and Dioxides.—For the preparation of these compounds mustard gas was first oxidized to the corresponding sulfoxide and sulfone utilizing the method of Helfrich and Reid.[5] Equimolar amounts of amine and oxidized mustard gas then reacted as given above, except that the reaction time may be reduced to three hours and one hour, respectively.

Derivatives.—The picrates were prepared by adding an equal volume of saturated aqueous solution of picric acid to an aqueous solution of the hydrochloride, to which a little alcohol had been added to facilitate solution. The precipitate was filtered, air dried and recrystallized from ethyl alcohol, or in some cases from a mixture of methyl

TABLE OF COMPOUNDS

Compound	Formula	M. p., °C.	Analyses, % N Calcd.	Found
N-Alkyl-thiamorpholines				
1. N-Dodecyl	$C_{16}H_{33}NS$	26	5.15	5.24
(a) Hydrochloride	$C_{16}H_{34}NSCl$	173	4.54	4.50
(b) Picrate	$C_{22}H_{36}O_7N_4S$	61	11.19	11.32
(c) Methosulfate	$C_{18}H_{39}O_4NS_2$	105	3.50	3.58
2. N-Tetradecyl	$C_{18}H_{37}NS$	82	3.67	3.67
(a) Hydrochloride	$C_{18}H_{38}NSCl$	164	4.16	4.15
(b) Picrate	$C_{24}H_{40}O_7N_4S$	63	10.60	10.96
(c) Methosulfate	$C_{20}H_{43}O_4NS_2$	147	3.30	3.40
3. N-Hexadecyl	$C_{20}H_{41}NS$	91	4.27	4.36
(a) Hydrochloride	$C_{20}H_{42}NSCl$	162	3.82	3.93
(b) Picrate	$C_{26}H_{44}O_7N_4S$	112	2.98	3.05
(c) Methosulfate	$C_{22}H_{47}O_4NS_2$	210	3.08	3.15
(d) Ethosulfate	$C_{24}H_{51}O_4NS_2$	202	2.90	2.97
4. N-Octadecyl	$C_{22}H_{45}NS$	131	3.93	4.13
(a) Hydrochloride	$C_{22}H_{46}NSCl$	173	3.57	3.57
(b) Picrate	$C_{28}H_{48}O_7N_4S$	63	9.58	9.16
(c) Methosulfate	$C_{24}H_{51}O_4NS_2$	171	2.90	3.09
N-Alkyl-thiamorpholine-1-oxides				
1. N-Dodecyl	$C_{16}H_{33}ONS$	83	4.87	4.72
(a) Hydrochloride	$C_{16}H_{34}ONSCl$	203	4.32	4.23
(b) Picrate	$C_{22}H_{36}O_8N_4S$	82	10.84	10.80
(c) Methosulfate	$C_{18}H_{39}O_5NS_2$	112	3.32	3.40
2. N-Tetradecyl	$C_{18}H_{37}ONS$	88	4.43	4.16
(a) Hydrochloride	$C_{18}H_{38}ONSCl$	183	3.97	4.44
(b) Picrate	$C_{24}H_{40}O_8N_4S$	108	10.28	10.39
(c) Methosulfate	$C_{20}H_{43}O_5NS_2$	115	3.15	3.25
3. N-Hexadecyl	$C_{20}H_{41}ONS$	89	4.07	4.09
(a) Hydrochloride	$C_{20}H_{42}ONSCl$	176	3.68	3.91
(b) Picrate	$C_{26}H_{44}O_8N_4S$	73	9.78	9.92
(c) Methosulfate	$C_{22}H_{47}O_5NS_2$	100	2.98	2.95
4. N-Octadecyl	$C_{22}H_{45}ONS$	112	3.76	4.23
(a) Hydrochloride	$C_{22}H_{46}ONSCl$	148	3.43	3.48
(b) Picrate	$C_{28}H_{48}O_8N_4S$	108	9.32	10.04
(c) Methosulfate	$C_{24}H_{51}O_5NS_2$	131	2.81	2.78
N-Alkyl-thiamorpholine-1-dioxides				
1. N-Dodecyl	$C_{16}H_{33}O_2NS$	73	4.61	4.51
(a) Hydrochloride	$C_{16}H_{34}O_2NSCl$	205	4.12	4.37
(b) Picrate	$C_{22}H_{36}O_9N_4S$	113	10.52	12.77
(c) Methosulfate	$C_{18}H_{39}O_6NS_2$	203		
2. N-Tetradecyl	$C_{18}H_{37}O_2NS$	85	4.15	4.05
(a) Hydrochloride	$C_{18}H_{38}O_2NSCl$	145	3.63	3.83
(b) Picrate	$C_{24}H_{40}O_9N_4S$	103	9.99	9.84
(c) Methosulfate	$C_{20}H_{43}O_6NS_2$	198	3.06	3.17
3. N-Hexadecyl	$C_{20}H_{41}O_2NS$	88	3.89	3.59
(a) Hydrochloride	$C_{20}H_{42}O_2NSCl$	160	3.53	3.57
(b) Picrate	$C_{26}H_{44}O_9N_4S$	100	9.51	9.36
(c) Methosulfate	$C_{22}H_{47}O_6NS_2$	173	2.88	2.91
4. N-Octadecyl	$C_{22}H_{45}O_2NS$	92	3.61	3.47
(a) Hydrochloride	$C_{22}H_{46}O_2NSCl$	151	3.30	3.40
(b) Picrate	$C_{28}H_{48}O_9N_4S$	99	9.08	8.96
(c) Methosulfate	$C_{24}H_{51}O_6NS_2$	169	2.72	2.72

(1) Presented before the Division of Organic Chemistry at the Atlantic City meeting of the American Chemical Society, April 11, 1946.

(2) H. T. Clarke, *J. Chem. Soc.*, **101**, 1583 (1912).

(3) W. E. Lawson and E. E. Reid, THIS JOURNAL, **47**, 2821 (1925).

(4) W. F. Hart and J. B. Niederl, *ibid.*, **66**, 1610 (1944).

(5) O. B. Helfrich and E. E. Reid, *ibid.*, **42**, 1208 (1920).

and ethyl alcohols. In a few cases where the hydrochloride was only very slightly soluble in water, the picrate was prepared by adding an excess of alcoholic picric acid solution to an alcoholic solution of the hydrochloride. The picrate was allowed to crystallize, then was washed with water and recrystallized from ethyl alcohol after air drying.

Invert Soaps.—The quaternary methosulfates were prepared by refluxing equivalent quantities of the free base and di-methyl sulfate and half the total volume of dry benzene for four hours. They were finally crystallized from methyl alcohol or ethyl acetate.

The melting points were determined on Fisher–Johns electrical melting point apparatus, and the point of complete liquefaction was determined.

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Summary

Studies in the utilization of mustard gas for peace time industrial purposes have now been extended also to its oxidation products. Thus far, these compounds were used in the preparation of a series of "invert soaps" containing a thiamorpholine nucleus and possessing rather promising properties.

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NOTES

The Reversible Inactivation of Gliotoxin by Thiols

BY CHESTER J. CAVALLITO, JOHN HAYS BAILEY AND WILLIAM F. WARNER

In a recent publication, Dutcher, Johnson and Bruce[1] reported results at variance with the observation[2] that cysteine inactivates gliotoxin. The inactivation of gliotoxin has been investigated with a number of thiols at several *p*H values and is readily observable when antibacterial activity is tested by both the dilution and the cylinder-plate method. In any inactivation studies of this type one obviously includes control tests which would determine the inactivating action of *p*H alone.

Dilute solutions of gliotoxin buffered at *p*H values of 6, 7 or 8 rapidly lost their antibacterial activity when treated with an excess of cysteine, N-acetylcysteine or thioglycolate, but not with S-methylcysteine. Longer standing than ten minutes prior to testing produced no further inactivation. When the reaction mixture was allowed to stand in air rather than under nitrogen, antibacterial activity as measured by the plate method, was slowly regenerated. Addition of more thiol again eliminated this activity. It therefore appeared that the thiol inactivation of gliotoxin was reversible by oxidation. This could be shown by treating gliotoxin with cysteine to produce an inactive mixture which after titration with iodine solution (to the starch–iodine end-point) showed complete regeneration of antibacterial activity.

The observed reversible inactivation of gliotoxin by means of reactive thiol compounds favors the dithio structures for gliotoxin rather than the thiosulfinate structure, which latter should not be capable of reversible reduction–oxidation. Whether gliotoxin is merely reduced to the dithiol structure or forms an intermediate product with the inactivating thiol was not shown, as a result of limited quantities of the antibiotic available. However, it would appear that the reaction represents an equilibrium between active (oxidized or dithio-) gliotoxin and inactive (reduced or dithiol-) gliotoxin and the thiol and dithio forms of the inactivator. This would be in agreement with the observed reaction[1] of gliotoxin with alkaline thioglycolate.

The reaction of gliotoxin with thiol groups is in agreement with our postulated mode of action for a large group of antibiotics and would be an example of method 1 (oxidation) discussed in an earlier paper,[3] in which an antibiotic disulfide could oxidize —SH groups essential to certain enzymes to enzyme —S—S— groups.

The failure of Dutcher, Johnson and Bruce to observe the reaction of cysteine with gliotoxin might result from testing for antibacterial action under conditions which would allow reoxidation of reduced gliotoxin to the active dithio-form.

Experimental

Gliotoxin was dissolved in a minimum of ethanol, and diluted with 0.5 *M* potassium phosphate buffer of the *p*H desired. The thiol compound was also dissolved in 0.5 *M* phosphate buffer of corresponding *p*H values. The two solutions were mixed so that each cc. of mixture contained 0.1 mg. of gliotoxin, not more than 5% ethanol and variable quantities of the thiol. The mixture was allowed to stand at room temperature for various periods of time, then tested for antibacterial activity against *Staphylococcus aureus* by the usual dilution and cylinder-plate methods. Buffer alone at *p*H of 6, 7 or 8 produced no loss of antibacterial activity in twenty-four hours; 0.1 mg. per cc. of cysteine produced a noticeable loss, 0.4 mg. per cc., nearly complete loss and 1.0 mg. per cc., total loss of antibacterial activity after ten minutes reaction time.

(1) Dutcher, Johnson and Bruce, THIS JOURNAL, **67**, 1736 (1945).
(2) Cavallito and Bailey, *Science*, **100**, 390 (1944).
(3) Cavallito, Bailey, Haskell, McCormick and Warner, *J. Bact.*, **50**, 61 (1945).