**Preparation of 5-Carbamyluracils.**—The 5-carbethoxyuracil obtained by either method A, B or C was mixed with an equal molar equivalent plus 10% excess of the desired amine and the two heated at 110° for 24 to 48 hours. The amide usually crystallized upon cooling and was recrystallized from hot ethyl acetate. These amides are listed in Table II.

**Hydrolysis of the 5-Carbethoxyuracils to the Corresponding Acids.**—The 5-carbethoxyuracil obtained by either method A, B or C was added to 5% sodium hydroxide solution (20 ml. for each gram of ester) and heated to reflux temperature. The solution was cooled, filtered through hardened filter paper and acidified with dilute hydrochloric acid. The acid was collected on a filter paper, dried and recrystallized from hot ethanol. The water soluble acids were recovered by evaporating the water solution to dryness under reduced pressure and then extracting with hot alcohol. The 5-carboxyuracils are listed in Table III.

INDIANAPOLIS, INDIANA

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[CONTRIBUTION FROM THE RESARCH LABORATORIES OF FLINT, EATON AND CO.]

# Isothioureas as Germicides

BY F. J. BANDELIN AND J. V. TUSCHHOFF

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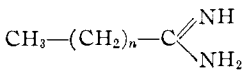
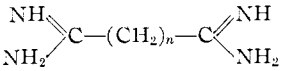
A series of homologous S-alkyl isothioureas and their 1,3-dialkyl derivatives were prepared and investigated for germicidal activity. Several members of the series were found to exhibit marked germicidal activity. Maximum activity against *Staphylococcus aureus* and *Eberthella typhi* occurred when the S-alkyl was either dodecyl or tetradecyl and when the nitrogens were substituted with either methyl or ethyl groups. These compounds exhibited foaming and detergent properties. Data indicate that these compounds have some properties similar to the quaternary ammonium salts.

The antiprotozoal properties of a number of amidines and diamidines have been observed and reported. These compounds were investigated chiefly by British workers for trypanocidal activity. Blaschko and Duthie[1,2] examined a large series of amidines as inhibitors of amine oxidase. Monoamidines of the general type

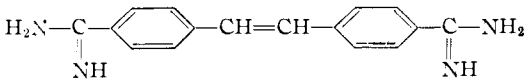


exhibited an increase in inhibition with an increase in the length of the carbon chain up to $n = 10$ with a decrease thereafter. With alkylene diamidines of the general type

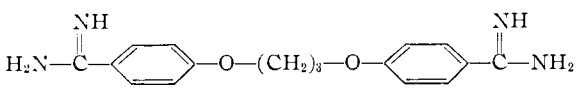


inhibition was found to have reached a maximum at $n = 12$. King, Lourie and Yorke[3,4] showed that alkyl diamidines of the latter type exhibited powerful trypanocidal action both *in vitro* and *in vivo*, the most active in this respect being undecanediamidine which produced almost 100% cures in mice and rabbits infected with *T. rhodesiense*.

Ashley, *et al.*,[5] reported the antibacterial activity of a series of symmetrical aromatic diamidines. Outstanding among these compounds were



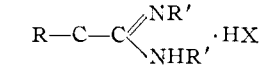
stilbamidine (4,4′-diguanylstilbene), and



propamidine 1,3-bis-(4-guanylphenoxy)-propane. These proved to be valuable chemotherapeutic agents in trypanosomiasis and in leishmanial infections.

More recently Brooks, *et al.*,[6] reported a number of S-alkylisothioureas in a comprehensive study of antitubercular compounds. Because of the similarity and structural relationship of the active moiety with the above compounds we have prepared a series of substituted isothioureas with a large aliphatic residue attached through the sulfur and with monosubstitution of each of the nitrogens with shorter aliphatic chains in order to study the influence of chemical structure upon the antibacterial properties. Since no systematic study of these compounds has appeared we wish to record here the work done in this Laboratory on this problem.

The compounds under investigation have the general type formula:



in which R is used to represent a straight chain alkyl group of 10 to 16 carbon atoms and R′ ranges from hydrogen to *n*-butyl. Hydrochlorides, hydrobromides and hydriodides were prepared from each base. These compounds, when pure, are obtained as fine white, odorless crystals. They are generally insoluble in diethyl ether and benzene, sparingly soluble in acetone and freely soluble in water and in ethanol. They are stable in acid solution and in all but strongly alkaline solutions. Aqueous solutions are nearly tasteless. Table I shows the effect of variation in the chain length of the R and R′ groups upon the germicidal activity.

## Experimental

Since these isothiourea alkyl ethers were made by means of well known reactions it is not necessary to give preparational detail for each compound. The reactions employed were those of alkyl halides with thiourea or 1,3-dialkylthioureas to give isothiourea alkyl ether hydrohalides. Temperature and solvent both materially affected the speed of the reaction and the yield. Reactions were carried out

(1) H. Blaschko and R. Duthie, *Biochem. J.*, **39**, 347 (1945a).

(2) H. Blaschko and R. Duthie, *ibid.*, **39**, 478 (1945b).

(3) H. King, E. M. Lourie and W. Yorke, *Ann. Trop. Med. Parasit.*, **32**, 177 (1938).

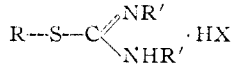
(4) H. King, E. M. Lourie and W. Yorke, *Lancet*, II, 1360 (1937).

(5) J. N. Ashley, H. J. Barber, A. J. Ewins, G. Newbery and A. D. H. Self, *J. Chem. Soc.*, 103 (1942).

(6) J. D. Brooks, P. T. Charlton, P. E. Macy, D. A. Peak and W. F. Short, *ibid.*, 452 (1950).

TABLE 1

EFFECT OF VARIOUS ALKYL SUBSTITUENTS ON THE ANTIBACTERIAL ACTIVITY OF SUBSTITUTED ISOTHIOUREA ALKYL ETHERS



| | Formula | M.p., °C. | Halogen, % Calcd. | Halogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found | C.K.D.[a] against *Staph. aureus* | C.K.D.[a] against *E. typhi* |
|---|---|---|---|---|---|---|---|---|
| Decyl- HCl | $C_{11}H_{25}N_2SCl$ | 114–115 | 14.05 | 14.00 | 11.06 | 11.11 | 4 | 15 |
| Decyl- HBr | $C_{11}H_{25}N_2SBr$ | 96–97 | 26.90 | 26.83 | 9.44 | 9.35 | 4 | 15 |
| Decyl- HI | $C_{11}H_{25}N_2SI$ | 67–68 | 36.86 | 36.79 | 8.14 | 8.03 | 4 | 15 |
| Dodecyl- HCl | $C_{13}H_{29}N_2SCl$ | 104–105 | 12.65 | 12.41 | 9.98 | 9.95 | 5 | 20 |
| Dodecyl- HBr | $C_{13}H_{29}N_2SBr$ | 90–91 | 24.61 | 24.48 | 8.62 | 8.29 | 5 | 20 |
| Dodecyl- HI | $C_{13}H_{29}N_2SI$ | 73–74 | 34.10 | 34.11 | 7.53 | 7.62 | 5 | 20 |
| Tetradecyl- HCl | $C_{15}H_{33}N_2SCl$ | 110–112 | 11.50 | 11.28 | 7.07 | 9.01 | 5 | 15 |
| Tetradecyl- HBr | $C_{15}H_{33}N_2SBr$ | 105–106 | 22.62 | 22.51 | 7.94 | 7.90 | 5 | 10 |
| Tetradecyl- HI | $C_{15}H_{33}N_2SI$ | 90–91 | 31.68 | 31.47 | 7.01 | 6.97 | 4 | 10 |
| Hexadecyl- HCl[b] | $C_{17}H_{37}N_2SCl$ | 120–122 | 10.52 | 10.53 | 8.32 | 8.22 | 1 | 5 |
| Hexadecyl- HBr | $C_{17}H_{37}N_2SBr$ | 104–105 | 21.01 | 20.96 | 7.35 | 7.35 | 1 | 5 |
| Hexadecyl- HI | $C_{17}H_{37}N_2SI$ | 89–90 | 29.68 | 29.38 | 6.55 | 6.48 | 1 | 4 |
| Decyldimethyl- HCl | $C_{13}H_{29}N_2SCl$ | 36–37 | 12.65 | 12.54 | 9.98 | 9.89 | 3 | 7 |
| Decyldimethyl- HBr | $C_{13}H_{29}N_2SBr$ | 48–49 | 24.61 | 24.31 | 8.62 | 8.66 | 3 | 15 |
| Decyldimethyl- HI | $C_{13}H_{29}N_2SI$ | 56–57 | 34.10 | 34.36 | 7.53 | 7.34 | 3 | 7 |
| Dodecyldimethyl- HCl | $C_{15}H_{33}N_2SCl$ | 34–35 | 11.50 | 11.34 | 9.07 | 9.04 | 20 | 50 |
| Dodecyldimethyl- HBr | $C_{15}H_{33}N_2SBr$ | 56–57 | 22.62 | 22.61 | 7.94 | 7.83 | 20 | 50 |
| Dodecyldimethyl- HI | $C_{15}H_{33}N_2SI$ | 66–57 | 31.68 | 31.46 | 7.01 | 7.08 | 20 | 50 |
| Tetradecyldimethyl- HCl | $C_{17}H_{37}N_2SCl$ | 38–39 | 10.52 | 10.48 | 8.32 | 8.23 | 10 | 10 |
| Tetradecyldimethyl- HBr | $C_{17}H_{37}N_2SBr$ | 58–59 | 21.01 | 21.04 | 7.35 | 7.39 | 10 | 15 |
| Tetradecyldimethyl- HI | $C_{17}H_{37}N_2SI$ | 68–69 | 29.68 | 29.56 | 6.55 | 6.45 | 10 | 15 |
| Hexadecyldimethyl- HCl | $C_{19}H_{41}N_2SCl$ | 53–54 | 9.74 | 9.72 | 7.69 | 7.84 | 4 | 5 |
| Hexadecyldimethyl- HBr | $C_{19}H_{41}N_2SBr$ | 61–62 | 19.58 | 19.46 | 6.84 | 6.81 | 4 | 5 |
| Hexadecyldimethyl- HI | $C_{19}H_{41}N_2SI$ | 77–78 | 27.81 | 27.61 | 6.14 | 6.30 | 4 | 5 |
| Decyldiethyl- HCl | $C_{15}H_{33}N_2SCl$ | Oil | 11.50 | 11.48 | 9.07 | 8.94 | 8 | 7 |
| Decyldiethyl- HBr | $C_{15}H_{33}N_2SBr$ | Oil | 22.62 | 22.74 | 7.94 | 7.81 | 8 | 7 |
| Decyldiethyl- HI | $C_{15}H_{33}N_2SI$ | Oil | 31.68 | 31.41 | 7.01 | 7.09 | 8 | 7 |
| Dodecyldiethyl- HCl | $C_{17}H_{37}N_2SCl$ | 51–52 | 10.52 | 10.46 | 8.32 | 8.23 | 50 | 25 |
| Dodecyldiethyl- HBr | $C_{17}H_{37}N_2SBr$ | 44–45 | 21.01 | 19.96 | 7.35 | 7.10 | 50 | 20 |
| Dodecyldiethyl- HI | $C_{17}H_{37}N_2SI$ | 34–35 | 29.68 | 29.68 | 6.55 | 6.71 | 50 | 15 |
| Tetradecyldiethyl- HCl | $C_{19}H_{41}N_2SCl$ | 57–58 | 9.74 | 9.58 | 7.69 | 7.57 | 50 | 15 |
| Tetradecyldiethyl- HBr | $C_{19}H_{41}N_2SBr$ | 52–53 | 19.58 | 19.51 | 6.84 | 6.71 | 50 | 15 |
| Tetradecyldiethyl- HI | $C_{19}H_{41}N_2SI$ | 38–39 | 27.81 | 27.77 | 6.14 | 6.22 | 50 | 8 |
| Hexadecyldiethyl- HCl | $C_{21}H_{45}N_2SCl$ | 64–65 | 9.03 | 9.10 | 7.12 | 7.00 | 5 | 5 |
| Hexadecyldiethyl- HBr | $C_{21}H_{45}N_2SBr$ | 55–56 | 18.25 | 18.18 | 6.41 | 6.01 | 5 | 5 |
| Hexadecyldiethyl- HI | $C_{21}H_{45}N_2SI$ | 44–45 | 26.23 | 26.21 | 5.78 | 5.84 | 5 | 5 |
| Decyldiisopropyl- HCl | $C_{17}H_{37}N_2SCl$ | Oil | 10.52 | 10.31 | 8.32 | 8.11 | 5 | 5 |
| Decyldiisopropyl- HBr | $C_{17}H_{37}N_2SBr$ | Oil | 21.01 | 20.09 | 7.35 | 7.42 | 5 | 5 |
| Decyldiisopropyl- HI | $C_{17}H_{37}N_2SI$ | Oil | 29.68 | 29.68 | 6.55 | 6.38 | 5 | 5 |
| Dodecyldiisopropyl- HCl | $C_{19}H_{41}N_2SCl$ | Oil | 9.74 | 9.51 | 7.69 | 7.79 | 5 | 4 |
| Dodecyldiisopropyl- HBr | $C_{19}H_{41}N_2SBr$ | Oil | 19.58 | 19.46 | 6.84 | 6.76 | 5 | 4 |
| Dodecyldiisopropyl- HI | $C_{19}H_{41}N_2SI$ | Oil | 27.81 | 27.76 | 6.14 | 6.46 | 5 | 4 |
| Tetradecyldiisopropyl- HCl | $C_{21}H_{45}N_2SCl$ | Oil | 9.03 | 9.10 | 7.12 | 7.10 | 1 | 5 |
| Tetradecyldiisopropyl- HBr | $C_{21}H_{45}N_2SBr$ | Oil | 18.35 | 18.28 | 6.41 | 6.40 | 1 | 5 |
| Tetradecyldiisopropyl- HI | $C_{21}H_{45}N_2SI$ | 40–41 | 26.22 | 26.17 | 5.78 | 5.82 | 1 | 5 |
| Hexadecyldiisopropyl- HCl | $C_{23}H_{49}N_2SCl$ | Oil | 8.44 | 8.56 | 6.65 | 6.43 | Insoluble | |
| Hexadecyldiisopropyl- HBr | $C_{23}H_{49}N_2SBr$ | 45–46 | 17.18 | 17.09 | 6.01 | 6.05 | Insoluble | |
| Hexadecyldiisopropyl- HI | $C_{23}H_{49}N_2SI$ | 50–51 | 24.82 | 24.68 | 5.47 | 5.82 | Insoluble | |
| Decyldibutyl- HCl | $C_{19}H_{41}N_2SCl$ | Oil | 9.74 | 9.74 | 7.69 | 7.70 | 8 | 10 |
| Decyldibutyl- HBr | $C_{19}H_{41}N_2SBr$ | Oil | 19.58 | 19.31 | 6.84 | 6.52 | 8 | 10 |
| Decyldibutyl- HI | $C_{19}H_{41}N_2SI$ | Oil | 27.81 | 27.71 | 6.14 | 5.96 | 15 | 10 |
| Dodecyldibutyl- HCl | $C_{21}H_{45}N_2SCl$ | Oil | 9.03 | 8.99 | 7.12 | 7.16 | 5 | 2 |
| Dodecyldibutyl- HBr | $C_{21}H_{45}N_2SBr$ | Oil | 18.25 | 18.54 | 6.41 | 6.34 | 5 | 2 |
| Dodecyldibutyl- HI | $C_{21}H_{45}N_2SI$ | Oil | 26.22 | 26.16 | 5.78 | 5.76 | Insoluble | |
| Tetradecyldibutyl- HCl | $C_{23}H_{49}N_2SCl$ | Oil | 8.44 | 8.41 | 6.65 | 6.52 | Insoluble | |
| Tetradecyldibutyl- HBr | $C_{23}H_{49}N_2SBr$ | 33–34 | 17.18 | 17.24 | 6.01 | 6.06 | Insoluble | |
| Tetradecyldibutyl- HI | $C_{23}H_{49}N_2SI$ | Oil | 24.82 | 24.62 | 5.47 | 5.39 | Insoluble | |
| Hexadecyldibutyl- HCl | $C_{25}H_{53}N_2SCl$ | 37–38 | 7.91 | 7.81 | 6.24 | 6.18 | Insoluble | |
| Hexadecyldibutyl- HBr | $C_{25}H_{53}N_2SBr$ | 38–39 | 16.21 | 16.16 | 5.68 | 5.74 | Insoluble | |
| Hexadecyldibutyl- HI | $C_{25}H_{53}N_2SI$ | 44–45 | 23.53 | 23.51 | 5.17 | 5.01 | Insoluble | |

[a] Critical killing dilution: The figures in these columns represent the greatest dilution in liters of one gram of compound which will kill organisms of standard phenol resistance in ten but not in five minutes at 37° using the technique described for determination of phenol coefficients in circular 198 of the U. S. Department of Agriculture. [b] Synthesis reported by J. M. Sprague and T. B. Johnson, THIS JOURNAL, 59, 1837 (1937).

either by refluxing the reactants in toluene or by heating at 130–140° without solvent. In all cases equimolar quantities of the reacting materials were used. The resulting isothiourea alkyl ether hydrohalides were isolated and recrystallized from petroleum ether.

As a rule the alkyl iodides reacted more rapidly and more completely than the alkyl bromides which in turn reacted more rapidly and completely than the alkyl chlorides. The three different hydrohalides were formed by treating the appropriate thiourea with an alkyl chloride, bromide or iodide according to the anion desired.

## Discussion

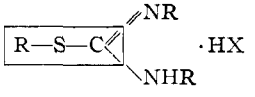
From the data in Table I it is apparent that the germicidal activity increases with the length of the S-alkyl chain reaching a maximum with the dodecyl derivatives when tested against both *Staphylococcus aureus* and *Eberthella typhi*. The tetradecyl derivatives also showed a comparable peak activity against *Staphylococcus aureus* and activity to a lesser extent against *Eberthella typhi*. Germicidal activity fell off rapidly when substitution was made with alkyls greater than tetradecyl. Monosubstitution of both of the nitrogens with identical alkyls also influenced germicidal activity to a marked extent. While the dodecyl isothioureas, unsubstituted, had relatively little or only fair activity against *Eberthella typhi* and less activity against the *Staphylococcus aureus*, the dimethyl substituted isothioureas exhibited a maximum effect against *Eberthella typhi* and the diethyl derivatives against the *Staphylococcus aureus*. In the compounds studied if the nitrogens were substituted with alkyls larger than ethyl, *i.e.*, isopropyl and butyl, a marked decrease in activity occurred.

Against the *Staphylococcus aureus*, the series of compounds showed two peaks of activity, the maximum occurring with the dodecyl diethyl derivatives and the tetradecyl diethyl derivatives with a lesser peak with the dodecyl dimethyl derivatives. Against *Eberthella typhi* three peaks of activity were encountered, the maximum with the dodecyl dimethyl compounds and two less prominent peaks occurring with the dodecyl hydrogen and with the tetradecyl diethyl compounds. Ethyl substitution upon the nitrogens gives a peak activity against *Staphylococcus aureus* with identical and maximum activity achieved with the dodecyl and tetradecyl thioethers. Dimethyl substitution of the nitrogens gives maximum activity against *Eberthella typhi*. Against the latter organism the effect of structure appears to be somewhat more specific than against the *Staphylococcus aureus*. The data indicate that substitution upon the nitrogens has a profound effect upon germicidal activity with a relatively high specificity of certain combinations. It seems therefore that greatest germicidal activity occurs when the long carbon chain of the alkyl thioether is dodecyl or tetradecyl provided that the nitrogens are both substituted with either dimethyl or diethyl groups.

Thus it seems that shorter carbon chains or compounds of slightly lower molecular weight were more effective against *Eberthella typhi* than against *Staphylococcus aureus*. This parallels somewhat the findings with the quaternary ammonium salts.

In almost all cases bacteriological results of the different hydrohalide salts of the same isothiourea alkyl ethers were the same or with few exceptions showed only slight variation which may be attributed to the difference in the base ratio caused by the difference in the molecular weight of the halide involved.

Actually, the effective *s*-alkyl chains may be considered longer than the terminology employed since the compounds are thioethers in which the addition of a sulfur and a carbon atom must be considered as part of the functional chain. They




may therefore be considered as equivalent to a carbon chain approximately two carbons longer than the actual nomenclature indicates. With this consideration the findings somewhat parallel those of certain quaternary ammonium salts where the peak activity is encountered with alkyl chains of $C_{14}$ to $C_{16}$. These compounds further resemble the quaternary ammonium salts in that they have foaming and detergent qualities and are precipitated by soaps. Also like the quaternary salts these compounds combine with lecithin and certain proteins. However, unlike the quaternary ammonium salts, the isothiourea alkyl ethers are practically tasteless in dilute solution, lacking the astringent, metallic taste peculiar to the quaternary ammonium salts.

DECATUR, ILLINOIS