139. *Attempts to Find New Chemotherapeutic Amidines. Part VIII. Some Aliphatic Monoamidines.*

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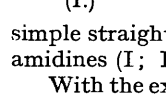
A number of aliphatic monoamidines of varying molecular weight and structure have been prepared for testing as bactericides against *M. tuberculosum*. Most of these were obtained by the sodamide method from the corresponding cyanides, which in turn were made mainly by the sodamide method of alkylation of aliphatic cyanides. Some of the limitations of these methods are discussed.

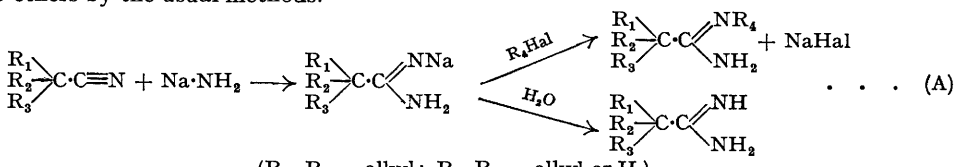
Adams, Stanley, and their co-workers (*J. Pharm. Exp. Ther.*, 1932, **45**, 121) have shown that certain dialkylacetic acids possess appreciable bactericidal activity against *M. lepræ* (at that time thought to be the infective agent causative of leprosy). This activity is most marked with acids which contain 16 to 18 carbon atoms, particularly when the carboxyl group is situated at or near the centre of the molecule, and does not appear to be confined to the carboxylic acids, as similar compounds containing the grouping $CH_2 \cdot NEt_2$ in place of the carboxyl group are only slightly less active. These authors also found that some of these acids are active against the

closely related organism *M. tuberculosum*. The preparation of a series of aliphatic monoamidines for testing as possible tuberculocidal agents is described in the present work. These have the general formula (I), where R_1 = alkyl and R_2, R_3, R_4 = alkyl or H, and include compounds varying in molecular weight from amidines containing 7 to 18 carbon atoms, and in molucular structure from unsubstituted simple straight-chain amidines (I; R_1 = *n*-alkyl, $R_2 = R_3 = R_4$ = H) to *N*-alkyl trialkylacetamidines (I; R_1, R_2, R_3, R_4 = alkyl).



(I.)

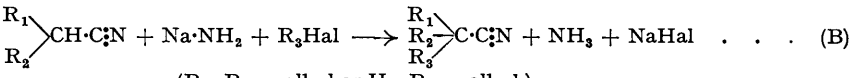
With the exception of stearamidine (Pinner, " Die Imido Äther ", p. 130), all these compounds were prepared from the corresponding cyanides by Ziegler's sodamide method (U.S.P., 2,049,582) as represented in scheme (A). This method proved particularly successful in the case of the higher di- and tri-alkylacetonitriles, which could not be converted into the corresponding imino-ethers by the usual methods.



. . . (A)

(R_1, R_4 = alkyl; R_2, R_3 = alkyl or H.)

Most of the cyanides were also prepared by the sodamide method of alkylating aliphatic cyanides as described by Ziegler and Ohlinger (*Annalen*, **1932**, **495**, 84) and represented in scheme (B). It provided a particularly convenient route to the higher molecular weight trialkylacetonitriles whose synthesis by other methods would have proved difficult and tedious.



. . . (B)

(R_1, R_2 = alkyl or H; R_3 = alkyl.)

Ziegler and Ohlinger (*loc. cit.*) showed that in most cases the alkylation process (B) is much more rapid than is amidine formation (A), and on the basis of this observation devised methods whereby, from the reaction between a cyanide and sodamide there could be obtained nearly theoretical yields of amidine or alkylated cyanide respectively depending on the absence or presence of alkyl halide. In one or two cases lower yields resulted with sterically hindered molecules.

In the present work the influence of the steric structure was particularly noticeable in amidine formation. For instance, reaction of sodamide with α-ethyl-α-*n*-butylacetonitrile occurred with evolution of heat and was complete in a matter of minutes, giving an almost theoretical yield of α-*ethyl*-α-n-*butylacetamidine*, while the corresponding reaction with α-*ethyl*-α-n-*butyl*-α-sec.-*octylacetonitrile*, required heating for 48—72 hours on the steam-bath and even then the maximum yield of α-*ethyl*-α-n-*butyl*-α-sec.-*octylacetamidine* was only 32%. In contrast, the monoalkyl-acetonitriles reacted almost violently with sodamide and in these cases simple amidine formation did not appear to be the sole reaction as the expected amidines were only isolated in very poor yield. *Decoamidine*, for example, was obtained only in 10% yield, and no stearamidine was obtained by this method. Moreover, no *N*-*n*-octyldecoamidine was isolated after treating the reaction product of sodamide and deconitrile with *n*-octyl bromide. However, these results are inconclusive, as these particular compounds can only be isolated from the reaction mixture as crystalline salts, *e.g.*, hydrochlorides, and it is difficult to crystallise long-chain aliphatic compounds of this type in the presence of any appreciable amounts of by-products. In general, it seems that simple straight-chain aliphatic amidines are obtained more satisfactorily by the imino-ether than by the sodamide method.

The dependence of the rate of reaction on the steric configuration is less marked in alkylation reactions. For instance, di- and tri-alkylation of acetonitrile can be effected in one stage, but at the same time there is sufficient difference in the rates of alkylation of unsubstituted and substituted acetonitriles to allow the preparation of any desired product in satisfactory yield. In a typical experiment the monoalkylation of acetonitrile with *n*-octyl bromide and sodamide gave deconitrile in 52% yield together with a 10% yield of *di*-n-*octylacetonitrile*. Similarly *di*-n-*hexylacetonitrile* was formed in about 10% yield during monoalkylation of acetonitrile, in 42% yield by dialkylation of acetonitrile, and in 45% yield by the partial alkylation of octonitrile.

Of more significance in alkylation is the varying reactivity of the halogen atom of the alkyl halide according to the nature of the alkyl groups involved. Ziegler and Ohlinger (*loc. cit.*)

instance the difficulty of introducing the *cyclo*hexyl group, and in the present work similar trouble was experienced in alkylation with *sec.*-octyl bromide. The alkylation of ethylbutylacetonitrile with *n*-octyl bromide gives *α-ethyl-α-butyl-α*-n-*octylacetonitrile* in 80% yield, while with *sec.*-octyl bromide the yield of α-ethyl-α-*n*-butyl-α-*sec.*-octylacetonitrile was only 33%. Moreover, the latter nitrile was accompanied by appreciable quantities of *α-ethyl-α*-n-*butylacet*-N′-sec.-*octylamidine hydrobromide*, indicating that the rate of alkylation with the less reactive *sec.*-octyl bromide is of the same order as that of amidine formation. Again, in the attempted alkylation of acetonitrile with *sec.*-octyl bromide, only traces of material corresponding to the mono- and di-*sec.*-octylacetonitriles were obtained. In this case polymerisation of the acetonitrile seemed to take place much more rapidly than did alkylation and diacetonitrile was the main reaction product.

The formation of *N*-substituted amidines by alkylation of sodio-amidines also appears to be considerably influenced by the structure of the alkyl halide employed; *e.g.*, from the sodium salt of *α-ethyl-α*-n-*butylacetamidine*, *α-ethyl-α*-n-*butylacet*-N-n-*octylamidine* was obtained in 40% yield but *α-ethyl-α*-n-*butylacet*-N-sec.-*octylamidine* in only 8% yield.

N-(p-*Aminobenzenesulphonyl*)-*α-ethyl-α*-n-*butyl-α*-sec.-*octylacetamidine* was obtained by reaction of the sodium salt of the corresponding amidine with *p*-acetamidobenzenesulphonyl chloride followed by hydrolysis. It was soluble in the usual organic solvents but completely insoluble in water and, similarly to other sulphanilylamidines (cf. Northey, Pierce, and Kertesz, *J. Amer. Chem. Soc.*, 1942, **64**, 2763), it was also insoluble in alkali.

Biological data on these compounds will be published elsewhere, but preliminary investigation indicated that, while some of these amidines possess bactericidal properties and show marked *in vitro* activity against *M. tuberculosum*, they were considered too toxic for the prolonged administration likely to be required to demonstrate activity against guinea pig infections.

Experimental.

Suspensions of sodamide in benzene were prepared and estimated as described by Ziegler and Ohlinger (*loc. cit.*, p. 106).

Ethyl-*n*-butylacetonitrile and ethyl*iso*propylacetonitrile were prepared from the corresponding malonic ester and acetic acids respectively by the usual methods.

Alkylation of Nitriles with Sodamide and Alkyl Halide.—The apparatus consisted of a 3-necked flask carrying a sealed stirrer, a condenser, and a dropping funnel, which was fitted with a small stirrer. The nitrile (1 mol.) and alkyl halide (1 mol.) were placed in the flask with an equal volume of dry benzene and heated on the steam-bath, to 45° for the monoalkylation of acetonitrile, and to 80° for alkylation of substituted acetonitriles. The suspension of sodamide (1 mol.), mechanically stirred to ensure homogeneity, was introduced from the dropping funnel, under stirring, at such a rate that fairly brisk but not violent reaction was maintained, or in the case of monoalkylation of acetonitrile, that the temperature did not rise above 50°. When addition of sodamide was complete, heating was continued for about one hour to complete the reaction. When the mixture was cool, water was carefully added to it and the benzene layer was separated, washed with water, and dried over sodium sulphate. The benzene was removed, and the product distilled under reduced pressure, with redistillation through a column when necessary. Where dialkylation of acetonitrile was attempted the procedure was essentially the same. Two mols. of alkyl halide and sodamide were used to one of acetonitrile; the temperature was kept below 50° until one mol. of sodamide had been added, and then allowed to rise to the boiling point of benzene while the second mol. was introduced.

Amidine Formation.—A stirred sodamide suspension (1·1 mols.) was slowly added with stirring to a solution of the nitrile (1 mol.) in benzene (an equal volume in the case of the lower aliphatic nitriles and about half the volume with the higher members of the series). With nitriles of comparatively low molecular weight a fairly brisk reaction usually occurred after a few minutes, and in some cases cooling in ice was necessary to moderate this reaction. When all the sodamide was added the mixture was heated for some time on the steam-bath to complete the reaction. With the higher secondary and tertiary acetonitriles, however, no reaction occurred during the sodamide addition and the mixture had to be heated on the steam-bath with stirring for periods up to 73 hours. When reaction was complete, the product was in the form of a crystalline mass of the sodio-amidine. Water was added carefully with ice cooling until two clear layers were obtained; the benzene layer was washed with a small amount of water and dried over sodium carbonate. The subsequent isolation and purification of the product varied according to the particular properties of the amidine concerned. In some cases repeated fractionation under reduced pressure yielded a pure compound; in others the hydrochloride was purified by recrystallisation from acetone or alcohol–acetone. Generally, where it was possible, a combination of these two procedures was more satisfactory.

N-Substituted amidines were obtained by addition of an alkyl bromide (1 mol.) to the sodio-amidine obtained as above and refluxing on the steam-bath for about 6 hours, or until the separation of sodium halide was complete. The mixture was then diluted with water, and the amidine worked up as in the case of the unsubstituted derivatives. It was sometimes advantageous to isolate and purify the unsubstituted amidine, dissolve it in benzene, and convert it into the sodio-amidine by addition of the theoretical quantity of sodamide and then treat this with the alkyl bromide. The product obtained by this method was usually more readily purified.

The amidine bases were obtained as colourless viscous oils or crystalline solids of low m. p., which

tended to lose their crystalline form on standing in the atmosphere. They were all soluble in the usual organic solvents but could generally be crystallised from light petroleum (b. p. 40—60°) by cooling a solution to low temperatures. The lower members were soluble in water, but solubility decreased rapidly with increase of molecular weight, and this was also the case with their hydrochlorides. The hydrochloride of an amidine containing 16 carbon atoms was soluble only in 500 parts of water. In the hope of finding a more soluble salt suitable for biological testing, the nitrate, sulphate, isethionate, tartrate, citrate, and gluconate of stearamidine were prepared, but none was soluble in 1000 parts of water. On the other hand, the hydrochlorides were quite soluble in methyl and ethyl alcohol.

The stability of the amidine group in these compounds depends as might be expected on their structure, the order of stability of the acetamidines being tertiary > secondary > primary. In general, it was observed that (i) primary acetamidines decomposed on attempted distillation under pressures of the order of 1 mm., while secondary and tertiary amidines were stable under these conditions, and (ii) primary and secondary acetamidines were readily hydrolysed by alkali to the corresponding amides, while the tertiary amidines were remarkably stable to hydrolytic agents. For instance α-ethyl-α-*n*-butyl-α-*sec.*-octylacetamidine was recovered unchanged after boiling for 24 hours with 50% alcoholic potassium hydroxide, and similarly was not affected by the action of nitrous acid in sulphuric acid.

The nitriles were all obtained as colourless oils. They were stable at the temperatures required for their distillation under reduced pressure but their susceptibility to hydrolysis appeared to be of the same order as that of the corresponding amidines.

The following nitriles and amidines were prepared by these methods: Deconitrile, from *n*-octyl bromide, sodamide, and acetonitrile in 52% yield, b. p. 120°/19 mm., $n_D^{18°}$ 1·4310 (Found: N, 9·05. Calc. for $C_{10}H_{19}N$: N, 9·15%); *di*-n-*octylacetonitrile* was obtained in the preceding experiment in approx. 10% yield, b. p. 196—197°/10 mm., $n_D^{18°}$ 1·4468 (Found: N, 5·3. $C_{18}H_{35}N$ requires N, 5·3%); octonitrile, from *n*-hexyl bromide, sodamide, and acetonitrile in 59% yield, b. p. 111°/37 mm., $n_D^{16°}$ 1·4240 (Found: N, 11·1. Calc. for $C_8H_{15}N$: N, 11·2%); *di*-n-*hexylacetonitrile* was formed in about 10% yield in the preceeding experiment, in about 45% yield from octonitrile, sodamide, and hexyl bromide, and in 42% yield by dialkylation of acetonitrile, b. p. 154°/17 mm., $n_D^{23°}$ 1·4411 (Found: N, 6·5. $C_{14}H_{27}N$ requires N, 6·7%); αα-*diethyl*-α-n-*butylacetonitrile*, from diethylacetonitrile, sodamide, and butyl bromide in 65% yield, b. p. 92°/14 mm. (Found: N, 9·0. $C_{10}H_{19}N$ requires N, 9·15%); *ethyliso*propyl-n-*hexylacetonitrile*, from ethyl*iso*propylacetonitrile, sodamide, and hexyl bromide in 54% yield, b. p. 135°/22 mm., $n_D^{18°}$ 1·4430 (Found: N, 7·4. $C_{13}H_{25}N$ requires N, 7·2%); *ethyl*-n-*butyl*-n-*hexylacetonitrile*, from ethylbutylacetonitrile, sodamide, and *n*-hexyl bromide in 70% yield, b. p. 151—153°/17 mm., $n_D^{22°}$ 1·4439 (Found: N, 6·75. $C_{14}H_{27}N$ requires N, 6·7%); *ethyl*-n-*butylbenzylacetonitrile*, from ethylbutylacetonitrile, benzyl chloride, and sodamide in 76% yield, b. p. 126°/0·5 mm., $n_D^{23°}$ 1·5030, (Found: N, 6·55. $C_{15}H_{21}N$ requires N, 6·5%); *ethyl*-n-*butyl*-sec.-*octylacetonitrile*, from ethylbutylacetonitrile, *sec.*-octyl bromide, and sodamide in 33% yield, b. p. 138°/1 mm. (Found: N, 6·2. $C_{16}H_{31}N$ requires N, 5·9%); *ethyl*-n-*butyl*-n-*octylacetonitrile*, from ethylbutylacetonitrile, sodamide and *n*-octyl bromide in 80% yield, b. p. 129°/1 mm., $n_D^{17°}$ 1·4469 (Found: N, 5·95. $C_{16}H_{31}N$ requires N, 5·9%); *decoamidine hydrochloride*, from deconitrile and sodamide in about 10% yield, m. p. 116°, hygroscopic (Found: N, 13·65; Cl, 17·25. $C_{10}H_{22}N_2$,HCl requires N, 13·55; Cl, 17·2%) (the free base decomposed on attempted distillation under 1 mm. pressure); α-*ethyl*-α-iso*propylacetamidine* was obtained in about 80% yield as its water-soluble *hydrochloride*, m. p. 208—209° (Found: N, 17·3; Cl, 21·6. $C_7H_{16}N_2$,HCl requires N, 17·0; Cl, 21·6%); the free base, recovered from the hydrochloride had b. p. 74—76°/0·05 mm., $n_D^{19°}$ 1·4768 (Found: N, 21·5. $C_7H_{16}N_2$ requires N, 21·9%); α-*ethyl*-α-n-*butylacetamidine*, yield up to 90%, b. p. 113°/2 mm. (Found: N, 19·4. $C_8H_{18}N_2$ requires N, 19·7%), did not yield a crystalline hydrochloride; *picrate*, m. p. 110° (Found: N, 18·9. $C_8H_{18}N_2$,$C_6H_3O_7N_3$ requires N, 18·9%); *monobenzoyl* derivative, m. p. 125—127° (Found: N, 11·9. $C_{15}H_{22}ON_2$ requires N, 11·4%); *dibenzoyl* derivative, m. p. 167—168° (Found: N, 7·9. $C_{22}H_{26}O_2N_2$ requires N, 8·0%); αα-*di*-n-*hexylacetamidine*, b. p. 125°/0·08 mm., m. p. 32—34° (Found: N, 12·5. $C_{14}H_{30}N_2$ requires N, 12·4%), the yield was only about 20% pure product because of difficulty in purifying the crude reaction product which contained an appreciable amount of amide; *picrate*, m. p. 85—86° (Found: N, 15·5. $C_{14}H_{30}N_2$,$C_6H_3O_7N_3$ requires N, 15·4%); αα-di-*n*-octylacetamidine, isolated in about 30% yield as its *hydrochloride*, m. p. 99—100° (Found: N, 9·0. Cl, 11·05. $C_{18}H_{38}N_2$,HCl requires N, 8·8; Cl, 11·1%); the free base recovered from the hydrochloride had m. p. 48—50°; α-*ethyl*-α-n-*butylacet*-N-sec.-*octylamidine hydrochloride*, by alkylation of the sodium salt of ethylbutylacetamidine, m. p. 214—216° (Found: N, 9·6; Cl, 12·2. $C_{16}H_{34}N_2$,HCl requires N, 9·6; Cl, 12·2%); *hydrobromide*, m. p. 180—181° (Found: N, 8·25; Br, 23·9. $C_{16}H_{34}N_2$,HBr requires N, 8·35; Br, 23·8%); α-*ethyl*-α-n-*butylacet*-N-n-*octylamidine*, isolated in about 40% yield of pure base, b. p. 162—165°/1 m., $n_D^{14°}$ 1·4695 (Found: N, 10·7. $C_{16}H_{34}N_2$ requires N, 11·0%), did not form a crystalline hydrochloride; αα-*diethyl*-α-n-*butylacetamidine* distilled between 120° and 130°/2 mm. and recrystallised from light petroleum (b. p. 40—60°), m. p. 56—58° (Found: N, 16·1. $C_{10}H_{22}N_2$ requires N, 16·5%) (yield, *ca.* 80%, did not form a crystalline hydrochloride); *picrate*, m. p. 113—114·5° (Found: N, 17·6. $C_{10}H_{22}N_2$·$C_6H_3O_7N_3$ requires N, 17·5%); α-*ethyl*-α-iso*propyl*-α-n-*hexylacetamidine*, b. p. 122°/15 mm., $n_D^{18°}$ 1·4822 (Found: N, 13·15. $C_{13}H_{28}N_2$ requires N, 13·2%) (yield, 40%), did not form a crystalline hydrochloride; α-*ethyl*-α-n-*butyl*-α-n-*hexylacetamidine*, b. p. 149°/1 mm., $n_D^{21°}$ 1·4789 (Found: N, 12·1. $C_{14}H_{30}N_2$ requires N, 12·4%) (yield, 75%), did not form a crystalline hydrochloride; α-*benzyl*-α-*ethyl*-α-n-*butylacetamidine*, a glass-like gum, b. p. 178°/1 mm. (Found: N, 11·8. $C_{15}H_{24}N_2$ requires N, 12·1%) (yield, 70%); α-*ethyl*-α-n-*butyl*-α-sec.-*octylacetamidine hydrochloride*, m. p. 146—147° (Found: N, 9·5; Cl, 12·3. $C_{16}H_{34}N_2$,HCl requires N, 9·6; Cl, 12·2%) (yield, 32%); α-*ethyl*-α-n-*butyl*-α-n-*octylacetamidine*, b. p. 165°/0·8 mm., $n_D^{21°}$ 1·4757 (Found: N, 11·0. $C_{16}H_{34}N_2$ requires N, 11·0%) (yield about 50%), did not form a crystalline hydrochloride; αα-*diethyl*-α-n-*butylacet*-N-sec.-*octylamidine*, b. p. 156°/2 mm. (Found: N, 10·1. $C_{18}H_{38}N_2$ requires N, 9·9%), did not form a crystalline hydrochloride or picrate.

Stearamidine was prepared by the method of Pinner (*loc. cit.*), and the hydroochloride obtained as a colourless crystalline product which melted partly at 80° and completely at 236° (lit. m. p. 220°) (Found: N, 8·9; Cl, 11·0. Calc. for $C_{18}H_{38}N_2$,HCl: N, 8·8; Cl, 11·1%). The free base recovered from the

hydrochloride had m. p. 80—83° (Found: N, 9·8. Calc. for $C_{18}H_{38}N_2$: N, 9·9%). The *isethionate*, melted partly at 60° and completely at 195° (Found: N, 6·95. $C_{18}H_{38}N_2,C_2H_6O_4S$ requires N, 6·9%).

N-(p-*Acetamidobenzenesulphonyl*)-α-*ethyl*-α-n-*butyl*-α-sec.-*octylacetamidine*.—To α-ethyl-α-*n*-butyl-α-*sec.*-octylacetamidine (6·4 g.) dissolved in dry ether (10 c.c.) was added gradually and with stirring finely ground sodamide (1 g.) in benzene. The mixture was warmed on the steam-bath for 10 minutes, cooled, and *p*-acetamidobenzenesulphonyl chloride (4·8 g.) added. A smooth reaction took place with separation of sodium chloride. The mixture was heated on the steam-bath for 30 minutes, cooled, treated with water, and the ethereal layer separated. On addition of light petroleum (b. p. 40—60°), the required *sulphonylamidine* separated and was filtered off. It recrystallised from benzene in rhombohedral plates, m. p. 137° (Found: N, 9·5. $C_{24}H_{41}O_3N_3S$ requires N, 9·3%) (yield, 2·8 g.). It was soluble in ethanol, methanol, acetone, and chloroform, insoluble in light petroleum, water, dilute mineral acids, and alkali.

N-(p-*Aminobenzenesulphonyl*)-α-*ethyl*-α-n-*butyl*-α-sec.-*octylacetamidine*.—The above acetyl compound (2·5 g.) was refluxed for 6 hours with 1·5N-aqueous-alcoholic sodium hydroxide (30 c.c.). On cooling, an oil separated which readily crystallised. It was filtered off and recrystallised from aqueous alcohol and from benzene-light petroleum in laminæ; m. p. 74—76° (Found: C, 64·8; H, 10·3; N, 10·6; S, 7·4. $C_{22}H_{39}O_2N_3S$ requires C, 64·5; H, 9·5; N, 10·3; S, 7·8%). It was soluble in ethanol, methanol, acetone, chloroform and benzene, and insoluble in water, dilute acids and alkali.

The above project was an extension of attempts in these laboratories to find new chemotherapeutic amidines, and at the same time part of a scheme of pooled research directed towards the discovery of new tuberculocides under The Therapeutic Research Corporation of Great Britain Ltd., to the Directors of which we express our thanks for permission to publish the above results. Our thanks are also due to Mr. S. Bance, B.Sc., A.R.I.C., who was responsible for the semi-microanalyses recorded.

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