13. *The Interaction of Betaine with Primary Aromatic Amines, Organic Disulphides, and Sodium Sulphite.*

By Frederick Challenger, Philip Taylor, and (in part) Bernard Taylor.

When betaine (free from hydrochloride) is heated with primary aromatic amines, the corresponding *N*-monomethyl derivatives are formed. Much trimethylamine is also produced, but ammonia and mono- or di-methylamine appear to be absent. The arylaminoacetoarylamide $R\cdot NH\cdot CH_2\cdot CO\cdot NHR$ is formed in most cases and isolated as the hydrochloride. Glycine ethyl betaine and aniline similarly give ethylaniline. Diphenyl, di-*n*-butyl, and di-*n*-amyl disulphides also react with betaine at their boiling points, undergoing fission to give phenyl methyl sulphide, methyl *n*-butyl sulphide, and methyl *n*-amyl sulphide respectively. These reactions are clearly related to the isomerisation of betaine by heat, giving methyl dimethylaminoacetate (Willstätter), and to the formation of dimethyl sulphide, selenide, and telluride observed when betaine is heated with sodium sulphite, selenite, or tellurite.

Willstatter (*Ber.*, 1902, **35**, 584) and Willstätter and Kahn (*ibid.*, 1904, **37**, 401, 1853) showed that at 300° a methyl group in betaine is eliminated from the nitrogen atom and transferred to the $-CO\cdot O$ group, giving the isomeric methyl dimethylaminoacetate in a minimal yield of 50%. Straw and Cranfield (*J. Soc. Chem. Ind.*, 1936, **55**, 40T) obtained only a 30% yield. The reaction is reversible:

$$\overset{+}{N}Me_3\cdot CH_2\cdot CO\cdot \overset{-}{O} \rightleftarrows NMe_2\cdot CH_2\cdot CO_2Me$$

Glycine ethyl betaine similarly gives ethyl diethylaminoacetate, and the dimethylethyl and methyldiethyl betaines of glycine give the methyl esters of methylethylglycine and diethylglycine in yields of 50, 50, and 35%. In alkylmethyl betaines, therefore, only the methyl group appears to migrate.

Riesser (*Z. physiol. Chem.*, 1913, **86**, 440) suggested that a similar elimination of a methyl group from betaine or choline in the animal body might account for the formation of the garlic odour, resembling that of dimethyl telluride, which is exhaled by animals after ingestion of potassium tellurite.

It has never been proved that this odour is due to dimethyl telluride, although one of us with Bird (J., 1939, 163) and North (J., 1934, 68) has shown that cultures of *Scopulariopsis brevicaulis* and other moulds on media containing tellurite and selenite evolve dimethyl telluride and selenide respectively. For further references to the methylating action of the animal body, see Challenger and Higginbottom, *Biochem. J.*, 1935, **29**, 1760; Challenger, *J. Soc. Chem. Ind.*, 1935, **54** (*C. and I.*), 657; Bird and Challenger, *loc. cit.*

Riesser (*loc. cit.*) found support for his suggestion in the observation that a mixture of betaine hydrochloride or choline chloride with sodium formate and tellurite, when heated, evolved an odour resembling that of dimethyl telluride. Challenger and Higginbottom (*loc. cit.*), using betaine free from hydrochloride, confirmed the formation of this substance. Moreover, betaine and sodium selenite, with or without formate, gave dimethyl selenide on heating. This was also obtained by heating a mixture of sodium selenite, sodium formate and tetramethylammonium hydroxide. Stanek (*Z. Zuckerind.*, 1903, **27**, 479) states that in a sealed tube at 270—280° betaine gives trimethylamine, carbon dioxide, glycollic acid, and tetramethylammonium hydroxide.

The reaction has now been extended to sulphur, dimethyl sulphide being obtained by using sodium sulphite and betaine. In view of the relation of these, admittedly pyrogenic, reactions to theories of the mechanism of biological methylation, which will be fully discussed in a forthcoming publication (see also Riesser, *loc. cit.*; Challenger and Higginbottom, *loc. cit.*; du Vigneaud *et al.*, *J. Biol. Chem.*, 1940, **134**, 787; **135**, 223), it seemed of interest to study the purely chemical reactions somewhat more closely and to find other acceptors for the methyl group of betaine. It should be emphasised, however, that in some biological systems both methylation and demethylation probably involve formaldehyde (Hess, Eichel, and Uibrig, *Ber.*, 1917, **50**, 351; Robinson, J., 1917, **111**, 877; Guggenheim, "Die Biogenen Amine," Basel, 1940, 31, 192).

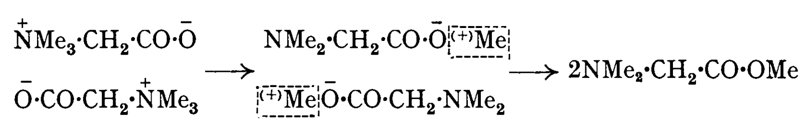
It has now been shown that when betaine (carefully freed from hydrochloride in order to avoid formation of methyl chloride) is heated with aniline, *p*-toluidine, *p*-anisidine, *p*-phenetidine or β-naphthylamine, the corresponding monomethyl derivative is formed. Much trimethylamine is also produced, but ammonia, and mono- or di-methylamine appear to be absent. An important product of the reaction in almost every case is the arylaminoacetoarylamide $R\cdot NH\cdot CH_2\cdot CO\cdot NHR$, isolated as the hydrochloride. This probably arises thus: $\overset{+}{N}Me_3\cdot CH_2\cdot CO\cdot \overset{-}{O} + 2NH_2Ph = NMe_3 + H_2O + NHPh\cdot CH_2\cdot CO\cdot NHPh$. When glycine ethyl betaine is heated with aniline, ethylaniline is produced;

the yield is much smaller than that of methylaniline in the corresponding experiment with ordinary betaine.

When boiled with betaine, diphenyl, di-*n*-butyl, and di-*n*-amyl disulphides are converted into phenyl methyl sulphide, methyl *n*-butyl sulphide, and methyl *n*-amyl sulphide respectively. This reaction is of interest in view of the fission and methylation which the two aliphatic disulphides and their lower homologues undergo in bread cultures of *S. brevicaulis* (Challenger and Rawlings, J., 1937, 868; Blackburn and Challenger, J., 1938, 1872).

Willstätter (*Ber.*, 1902, **35**, 584) points out that the conversion of betaine into the isomeric ester does not lend itself to kinetic study. Kuhn and Giral (*Ber.*, 1934, **67**, 1130; 1935, **68**, 387) conclude that the reaction is almost certainly not intramolecular, at any rate in the case of the long-chain ξ-trimethylpentadeca and π-trimethylheptadeca-betaines, which have a very high dipole moment in alcoholic solution and also undergo this isomerisation on heating. This is in agreement with modern views on several similar reactions, and is supported by our work, the mobile methyl group being intercepted by the selenite, tellurite or primary amine.

Willstätter's results and our own can be conveniently explained on the assumption that a positively charged methyl group is liberated from the betaine molecule (see, however, p. 50). In the betaine–ester conversion this attaches itself to the negative oxygen of a second molecule, which thus acquires a positive charge. This then expels a positive methyl group, which attaches itself to the negative oxygen of the first molecule :



$$\begin{matrix}\overset{+}{N}Me_3{\cdot}CH_2{\cdot}CO{\cdot}\overset{-}{O} & & NMe_2{\cdot}CH_2{\cdot}CO{\cdot}\overset{-}{O}\,\boxed{\overset{(+)}{}Me} & & \\ & \longrightarrow & & \longrightarrow & 2NMe_2{\cdot}CH_2{\cdot}CO{\cdot}OMe \\ \overset{-}{O}{\cdot}CO{\cdot}CH_2{\cdot}\overset{+}{N}Me_3 & & \boxed{\overset{(+)}{}Me}\,\overset{-}{O}{\cdot}CO{\cdot}CH_2{\cdot}NMe_2 & & \end{matrix}$$

In the case of the primary amines the positive methyl ion is presumably trapped by the unshared electrons of the nitrogen atom, a proton being expelled which gives dimethylglycine with the $NMe_2{\cdot}CH_2{\cdot}CO{\cdot}\overset{-}{O}$ ion, $NH_2Ph + \overset{+}{Me} \longrightarrow \overset{+}{N}H_2PhMe \longrightarrow NHPhMe + H^+$.

The selenite, tellurite, and sulphite ions could similarly co-ordinate the methyl group, giving neutral $CH_3{\cdot}SeO_3Na$ (etc.), which on thermal decomposition would presumably yield dimethyl selenide, telluride, or sulphide.

In this connection it should be mentioned that, when potassium methaneselenonate $CH_3{\cdot}SeO_3K$ (Bird and Challenger, forthcoming publication) is heated, it decomposes explosively, giving a strong odour of an organic selenium compound. Potassium propane 1-selenonate gives a similar odour without exploding. The lead salts of methane- and ethane-seleninic acids also give volatile organic selenium compounds on heating. Sodium benzenesulphonate is stated by Stenhouse (*Annalen*, 1866, **140**, 287; 1869, **149**, 247) to give thiophenol, diphenyl sulphide, benzene, and thianthren on heating. On the other hand, when potassium methanesulphonate was heated in a metal-bath in a stream of nitrogen and the volatile products were absorbed in mercuric cyanide and chloride solutions, the precipitate in the cyanide was mostly mercuric sulphide, acidification giving only a faint odour of methylthiol. The mercuric chloride solution smelled slightly of dimethyl sulphide.

There would, however, appear to be sufficient evidence for the suggestion that the later stages in these betaine reactions involve the decomposition of $CH_3{\cdot}SeO_3Na$, etc.

The behaviour of the disulphides is doubtless connected with the tendency of these compounds to suffer fission with numerous reagents (see Challenger and Rawlings, *loc. cit.*, for references). The dissociation of diphenyl disulphide observed by Schönberg, Rupp, and Gumlich (*Ber.*, 1933, **66**, 1932) gave *neutral* free radicals. The reaction with betaine may be represented as the co-ordination of a positive methyl group by the disulphide, followed by expulsion of a positively charged $\overset{+}{S}R$ radical, thus :

$$R:\underset{\cdot\cdot}{\overset{\cdot\cdot}{S}}:\underset{\cdot\cdot}{\overset{\cdot\cdot}{S}}:R + \overset{+}{Me} = \left[\begin{matrix}R:\overset{\cdot\cdot}{S}:\underset{\cdot\cdot}{\overset{\cdot\cdot}{S}}:R\\ \;\;Me\end{matrix}\right]^+ = \begin{matrix}R:\overset{\cdot\cdot}{S}:\\ \;\;Me\end{matrix} + \left[\underset{\cdot\cdot}{\overset{\cdot\cdot}{S}}:R\right]^+$$

The $R\overset{+}{S}$ ion would presumably react with the $NMe_2{\cdot}CH_2{\cdot}CO{\cdot}\overset{-}{O}$ ion, but the reaction mixture has not been examined for products other than methyl sulphides, R·SMe.

As an alternative, preliminary dissociation into the ions $R\overset{-}{S}$ and $R\overset{+}{S}$ may be assumed.

The reactions with sulphite, selenite, etc., may also be expressed in a somewhat different manner. The oxy-acids of sulphur, selenium and tellurium, and presumably their salts, will doubtless form compounds with betaine analogous to the hydrochloride, *e.g.*, $\overset{+}{N}Me_3\cdot CH_2\cdot CO\cdot OH\}\overset{-}{S}eO_3H$. Potassium iodide and betaine form a compound $(C_5H_{11}O_2N)_2,KI,2H_2O$ (Willstätter, *Ber.*, 1902, **35**, 598; see also Körner and Menozzi, *Gazzetta*, 1883, **13**, 351). With primary aromatic amines, analogy would suggest that a complex such as $\overset{+}{N}Me_3\cdot CH_2\cdot CO\cdot OH\}\overset{-}{N}HAr$ might possibly be formed, but no mention of compounds of this type appears in the literature. Although aniline and betaine in molecular proportion form a hard mass, suggesting the possible formation of a compound (see p. 51), the whole of the aniline is removable on extraction with cold benzene. No such formation of solid is observed with *m*-toluidine or *p*-phenetidine.

The formation of an -onium compound from betaine and a disulphide is impossible, but a compound such as $\overset{+}{N}Me_3\cdot CH_2\cdot CO\cdot OH\}\overset{-}{S}R$ might possibly exist; the type, however, is not mentioned in Beilstein's "Handbuch." No evidence of compound formation is observed on mixing betaine and thiophenol.

The kinetics of the elimination of the alkyl group from substituted ammonium and sulphonium compounds in aqueous solution at about 100° has been studied by Ingold and his collaborators (see especially J., 1933, 526, 1571; 1935, 236, 244, 255). They have shown that the reactions $R\cdot\overset{+}{N}R'R''R'''\}\overset{-}{X} = RX + NR'R''R'''$ and $R\cdot\overset{+}{S}R'R''\}\overset{-}{X} = RX + R'R''S$ may be unimolecular or bimolecular according to the polar character of R and X. The unimolecular reaction proceeds by the separation of an ion $\overset{+}{R}$, which then unites with $\overset{-}{X}$, but in the bimolecular type of reaction no free ion is eliminated.

In the absence of any evidence as to the kinetics of the various betaine decompositions described in this communication—they occur at high temperatures—it is impossible to say whether a free methyl ion is concerned in the reactions with sulphite, selenite and tellurite, where formation of an -onium compound might be expected. In the case of those with amines and disulphides, where this appears less likely, the same doubt exists, since it would be possible for a methyl group to be transferred from betaine to the amine or disulphide by Ingold's bimolecular mechanism, followed by expulsion of $\overset{+}{H}$ or $\overset{+}{RS}$. The second of these possibilities would represent a reversal of the reaction $NH_3 + Alk\overset{+}{S}R_2 \rightleftarrows Alk\overset{+}{N}R_3 + R_2S$ envisaged by Hughes and Ingold (J., 1935, 251).

Hickinbottom (J., 1934, 1700; 1937, 1119) has suggested that a positive alkyl group may be concerned in the conversion of *N*-alkylaniline hydrogen halides into nuclear alkylated anilines by heating at 300° (Hofmann–Martius reaction). See also Watson, *Ann. Reports*, 1939, 204. The temperature of our betaine–amine experiments—about 200°—is lower than that of the Hofmann–Martius reaction, but as a check the unchanged aniline in Expt. I*a* was converted through the diazonium salt into chlorobenzene. This was pure and showed no trace of homologues on distillation.

Straw and Cranfield (*J. Soc. Chem. Ind.*, 1936, **55**, 40T) state that small quantities of formaldehyde are present in the distillate obtained on heating betaine at 280—300° in a stream of air. This conclusion was based on odour, reducing properties, and action on decolourised magenta, but the authors state that no formaldehyde could be detected by absorption of the volatile decomposition products in aqueous sodium bisulphite. Compare also Dechamps (*Bull. Soc. Chim. Belg.*, 1930, **39**, 67), who fused betaine and detected formaldehyde colorimetrically.

It seemed improbable that the methyl derivatives described in this communication, particularly those obtained from the disulphides, could be produced by traces of formaldehyde arising from the betaine. In view of the methylation of ammonium chloride by formaldehyde (Werner, J., 1917, **111**, 844) and the work of Clarke, Gillespie, and Weisshaus (*J. Amer. Chem. Soc.*, 1933, **55**, 4571) on the use of formaldehyde and formic acid in the methylation of primary amines (see also Frankland, Challenger, and Nicholls, J., 1919, **115**, 198) experiments were, however, carried out to test this possibility.

When paraformaldehyde was boiled with aniline for 10 hours, the amounts of monomethylaniline, as judged by the weights of crude *N*-nitroso-*N*-methylaniline obtained in three experiments, were less than those obtained from betaine and aniline (Expts. I and I*a*) in spite of the fact that considerable quantities of paraformaldehyde were used.

No odour of dimethyl selenide or dimethyl telluride was observed on heating sodium selenite or potassium tellurite with paraformaldehyde at 220—230° and 200—210°, respectively, or at 270°.

The work of du Vigneaud and his colleagues (*J. Biol. Chem.*, 1939, **131**, 57; 1940, **134**, 787; **135**, 223) has established the conversion of trideuteromethionine, $CD_3\cdot S\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot CO_2H$, into isotopic choline in the rat, over 56% of the CD_3 group being transferred to nitrogen. This reaction is reversible (du Vigneaud, *ibid.*, 1941, **140**, 638). It therefore became of interest to determine whether the methyl group of ordinary methionine could be transferred to aniline at its boiling point. In the single experiment which was carried out, no trace of methylaniline could be detected.

EXPERIMENTAL.

I. *Betaine and Aniline.*—A mixture of aniline (80 g.; 1 mol.) and betaine (104 g.; 1·03 mols.) was heated under reflux for 8·3 hours. Dilution with water, addition of aqueous sodium hydroxide, extraction with ether, and drying over sodium sulphate (a process employed in all the analogous experiments) yielded an oil (80 g.). A portion of this (35 g.) with hydrochloric acid (98 c.c.) and water (125 c.c.) gave a solid (A). [The weight was not recorded, but in another experiment aniline (6 g.) and betaine (7·5 g.) gave 0·7 g. of A.] This was separated (see below), and the filtrate cooled in ice, treated with a slight excess of sodium nitrite, and extracted with ether. The extract was washed with sodium hydroxide solution and with water and dried over sodium sulphate, giving an oil (8 g.). This oil, containing *N*-nitroso-*N*-methylaniline, was dissolved in a mixture of anhydrous ether (16 g.) and alcoholic hydrogen chloride (32 g.) and after 2 days a yellow hydrochloride was separated. Treatment with aqueous sodium hydrogen carbonate and extraction with ether yielded a green solid, which was recrystallised several times from benzene; it then formed blue prisms, m. p. 116°. The m. p. was also 116° on recrystallisation from water (green leaflets) and in admixture with authentic *p*-nitrosomethylaniline, m. p. 116° (Fischer and Hepp, *Ber.*, 1886, **19**, 2992).

The solid (A) was a hydrochloride which with aqueous sodium hydroxide gave a base (B), m. p. 111—112°, on repeated crystallisation from aqueous alcohol [Found: C, 74·4, 74·2; H, 6·4, 6·1; N, 12·35, 12·9; *M* (camphor), 231, 231. Calc. for $C_{14}H_{14}ON_2$: C, 74·3; H, 6·25; N, 12·4%; *M*, 226].

Anilinoacetanilide was prepared by boiling aniline (4 mols.) with chloroacetyl chloride (1 mol.) for 10 mins. (Meyer, *Ber.*, 1875, **8**, 1158). On repeated crystallisation from dilute alcohol it melted at 111°, alone and in admixture with the base (B). Both specimens gave white hydrochlorides. That from (B) decomposed at 205—208° after two crystallisations from alcohol, the authentic hydrochloride at 205—212°, and the mixture at 205—212° when heated fairly rapidly. The original solid (A) after two crystallisations from alcohol decomposed at 205—207°, and at 205—212° in admixture with authentic hydrochloride.

Anilinoacetanilide hydrochloride is stated by Motylewski (*Bull. Int. Acad. Pol.*, 1926, *A*, 93) to melt at 216°.

Anilinoacetanilide (B) with sodium nitrite and dilute hydrochloric acid in ice-cold alcohol gave a solid which separated from aqueous alcohol in pale yellow crystals, m. p. 142—143° (decomp.) alone and in admixture with *N*-nitrosoanilinoacetanilide, m. p. 142—143°, prepared from the authentic base in the same manner (Motylewski, *loc. cit.*, gives m. p. 145°) [Found for the specimen from base (B): C, 66·0; H, 5·35; N, 16·6. Found for the authentic specimen: C, 66·1; H, 5·3; N, 16·7. Calc. for $C_{14}H_{13}O_2N_3$: C, 65·9; H, 5·1; N, 16·5%].

In a second experiment in which aniline (13·4 g.; 1 mol.) and betaine (16·8 g.; 1 mol.) were heated for 12 hours the evaporated ethereal extract of the basified product (15 g.) was treated with aqueous zinc chloride (16·5 g. in 37·5 c.c. of water) to remove most of the aniline (see Frankland, Challenger, and Nicholls, *loc. cit.*), and the residual bases extracted with hot light petroleum, yielding 2·9 g. of oil. This oil (1 g.) was mixed with acetic anhydride (1 g.), poured into an equal volume of water, and treated with concentrated aqueous ammonia, giving a white solid (0·8 g.), m. p. 80—90°. After three recrystallisations from light petroleum this melted at 99—100°, alone and in admixture with authentic acetomethylanilide, m. p. 99—100°.

The evolution of trimethylamine during the heating of betaine and aniline was established in two separate experiments by passing the evolved vapours through dilute hydrochloric acid. Evaporation left a deliquescent residue, soluble in chloroform and in alcohol. It was analysed without recrystallisation (Found: Cl, 37·6, 37·5, and 37·2, 37·25. Calc. for C_3H_9N,HCl: Cl, 37·1%).

I*a*. In another experiment using betaine (21 g.) and aniline (16 c.c.) it was noticed that the mixture set to a hard mass in about 2 mins., suggesting the possible formation of a compound. No rise in temperature was observed. The mixture was then heated in a paraffin bath at 205° for 9—10 hours. The bases were liberated as usual (19 g.). With hydrochloric acid (53 c.c.) and water (68 c.c.) the hydrochloride of anilinoacetanilide (3·28 g.) separated overnight. This gave no oil with sodium hydroxide and was free from the hydrochloride of aniline or methylaniline. The cooled filtrate was treated with sodium nitrite (6·0 g.) in water (15 c.c.), the *N*-nitroso-*N*-methylaniline (5 g.) extracted with ether, and the cold aqueous solution slowly added to cuprous chloride (16 g.) in hydrochloric acid (150 c.c.). The chlorobenzene so produced (2·75 g.) distilled constantly at 130°.

II. *Betaine and* p-*Toluidine.*—A mixture of betaine (2 g.; 0·91 mol.) and the amine (2 g.; 1 mol.) was heated for 8 hours; the product was cooled, treated with aqueous sodium hydroxide, and extracted with ether, yielding 2·5 g. of oil. With hydrochloric acid (5 c.c.) and water (5 c.c.) the oil gave a solid (C) (0·3 g.). This was the hydrochloride of a base which was liberated by trituration with aqueous sodium hydroxide. When recrystallised from aqueous alcohol and then from benzene, it melted at 132—133° alone, and at 133—134° in admixture with authentic *p*-toluidinoaceto-*p*-toluidide, m. p. 133—134° (Meyer, *Ber.*, 1875, **8**, 1161, gives m. p. 136°). This base gave, with sodium nitrite and hydrochloric acid in ice-cold alcohol, a solid (D), which when recrystallised from aqueous alcohol and then from benzene had m. p. 156—159° (decomp.). In admixture with authentic *N*-nitroso-*p*-toluidinoaceto-*p*-toluidide (see p. 54), m. p. 156—159° (decomp.), another sample of (D) melted at 157—159° (decomp.) [Found for this sample of (D) : C, 68·15, 68·0; H, 6·2, 6·1; N, 14·55, 14·6. $C_{16}H_{17}O_2N_3$ requires C, 67·8; H, 6·05; N, 14·85%].

With tin and hydrochloric acid, (D) gave a white solid containing no nitroso-group. On recrystallisation from methyl alcohol it melted at 134—135° and was probably *p*-toluidinoaceto-*p*-toluidide (the m. p. varied with the rate of heating).

In a further experiment betaine (6 g.; 1·03 mols.) and *p*-toluidine (5·3 g.; 1 mol.) were heated for 8 hours, and the product (4 g.) treated with hydrochloric acid (8 c.c.) and water (8 c.c.). Removal of the insoluble hydrochloride (C) and treatment of the filtrate with sodium nitrite (3 g.), extraction with ether, and washing of the extract with aqueous sodium hydroxide gave a solid, which on repeated crystallisation from light petroleum (b. p. 40—60°) gave crystals (E), m. p. 49° [Found for a sample of (E) obtained in an earlier experiment: C, 63·9; H, 7·0; N, 18·5. Calc. for $C_8H_{10}ON_2$: C, 63·95; H, 6·7; N, 18·7%]. In admixture with authentic *N*-nitroso-*N*-methyl-*p*-toluidine of m. p. 49° (Ullmann, *Annalen*, 1903, **327**, 108, gives m. p. 53°) this sample of (E) melted at 48—49°.

In the earliest experiments in which betaine and *p*-toluidine were heated together a hydrochloride other than (C) was precipitated on addition of dilute hydrochloric acid to the product of the reaction obtained as before. This hydrochloride (X) on trituration with sodium hydroxide solution gave the free *base*, m. p. 141—142°, and 143—144° after repeated crystallisation from aqueous alcohol [Found: C, 70·3, 70·45; H, 6·95, 7·0; N, 12·8, 12·95; *M* (camphor), 338. $C_{19}H_{23}O_2N_3$ requires C, 70·1; H, 7·15; N, 12·9%; *M*, 325]. The structure $(CH_3{\cdot}C_6H_4{\cdot}NH{\cdot}CO{\cdot}CH_2)_2N{\cdot}CH_3$ is in agreement with this molecular formula.

The reason for the formation of this compound in some experiments and not in others has not been decided. It was accompanied by some *p*-toluidinoaceto-*p*-toluidide, which was isolated as the nitroso-compound (D) along with (E), since in some experiments the hydrochlorides (C) and (X) were not removed before nitrosation.

III. *Betaine and* p-*Anisidine*.—The amine (7·6 g.; 1 mol.) and betaine (7·25 g.; 1 mol.) were boiled for 8 hours, and the product (8·5 g.) separated as before. With dilute hydrochloric acid (1 : 1; 30 c.c.) it gave a solid (F), which was separated [when *p*-anisidine (3 g.; 1 mol.) and betaine (2 g.; 0·7 mol.) were used, 0·4 g. of (F) was obtained].

The filtrate with sodium nitrite (7 g.) gave after the usual treatment a red solid (1·7 g.), which after two crystallisations from light petroleum formed yellow crystals, m. p. 44—45°, alone and in admixture with authentic *N*-nitroso-*N*-methyl-*p*-anisidine of the same m. p., prepared by the method of Hodgson and Crook (J., 1932, 1814) (Found: C, 58·1; H, 6·1; N, 16·9. Calc. for $C_8H_{10}O_2N_2$: C, 57·8; H, 6·1; N, 16·9%). (F) was a hydrochloride; the base, recrystallised from benzene and then from absolute alcohol, was white and had m. p. and mixed m. p. 131—132° with authentic *p*-anisidinoaceto-*p*-anisidide, m. p. 131—132° (see p. 54). A portion of the base was suspended in acetone, cooled in ice and salt, and treated with hydrochloric acid and sodium nitrite. The precipitate, after recrystallisation from benzene, decomposed at 153—156° when heated rapidly, giving a red liquid, and at 155—159° in admixture with authentic *N*-nitroso-*p*-anisidinoaceto-*p*-anisidide, m. p. 155—159° (decomp.).

IV. *Betaine and* p-*Phenetidine*.—The amine (8 g.; 1 mol.) and betaine (6·8 g.; 1 mol.) were boiled for 8 hours. The product (10·7 g.), isolated in the usual way, was treated with hydrochloric acid (15 c.c.) and water (30 c.c.), giving a solid (G). The filtrate with sodium nitrite (6 g.) in water (30 c.c.) gave on extraction with ether a red oil (3·3 g.) which soon solidified. Light petroleum removed a solid, which when crystallised twice from the same solvent formed pale yellow needles, m. p. 46·5—47·5°, alone and in admixture with authentic *N*-nitroso-*N*-methyl-*p*-phenetidine of the same m. p. prepared by Stedman's method (J., 1924, **125**, 1375).

The base from (G) after crystallisation from benzene and then from aqueous alcohol melted at 137—138° and at 136—137° in admixture with authentic *p*-phenetidinoaceto-*p*-phenetidide, m. p. 137—138° (see p. 54).

V. *Betaine and* β-*Naphthylamine*.—The base (9 g.; 1 mol.) and betaine (7·5 g.; 1·02 mols.) were heated for 8 hours at 200—220°. The product (7 g.) with hydrochloric acid (1 : 1, 30 c.c.) gave a solid (H). It appeared to be a hydrochloride but was not identified. Nitrosation of the filtrate gave a red oil, which after the usual treatment solidified, and after four crystallisations from alcohol formed pale yellow needles, m. p. and mixed m. p. 87—88° with authentic *N*-nitroso-*N*-methyl-β-naphthylamine, m. p. 87—88° (von Pechmann, *Ber.*, 1895, **28**, 2370) (Found: C, 70·55; H, 5·65; N, 14·75. Calc. for $C_{11}H_{10}ON_2$: C, 70·95; H, 5·4; N, 15·05%).

VI. *Betaine and Diphenyl Disulphide*.—The disulphide (8·2 g.; 1 mol.) and betaine (9 g.; 2·02 mols.) were boiled under reflux for 8 hours. The brown liquid was warmed with water and extracted with ether, and the extract washed with dilute hydrochloric acid, dried, and evaporated. The dark liquid residue (10 g.) was distilled at 23 mm. and the fraction of b. p. 100° was heated for 1 hour at 100° with excess of 3% potassium permanganate solution. After decoloration with sodium formate the mixture was extracted with ether, and the extract dried over calcium chloride and evaporated, leaving a semi-solid residue. The white solid was separated and recrystallised several times from water; it then melted at 86—87°, alone and in admixture with authentic phenylmethylsulphone of the same m. p.

A second experiment yielded fractions of b. p. 60—80° and 80—100° (20 mm.). Oxidation as before gave the sulphone of m. p. 85—86° and 87—88°.

VII. *Betaine and Di-*n*-butyl Disulphide.*—The disulphide (5 g.; 1 mol.), free from *n*-butylthiol and hydrogen sulphide, was boiled with betaine (6·3 g.; 1·92 mols.) for 11 hours, during which time a further 10 g. of the disulphide were added. The product, freed from betaine and bases, as in VI, formed a red oil (12·3 g.), which was distilled at the ordinary pressure. The fraction, b. p. 37—180°, (1·1 g.) was shaken mechanically with saturated aqueous mercuric chloride (500 c.c.) for 1 hour, giving a red precipitate (3·1 g.) which gradually became brown (the formation of red mercuric chloride derivatives under similar conditions is described by Challenger and Rawlings, J., 1937, 873). This was treated with sodium hydroxide solution and warmed on the steam-bath from time to time and volatile products were aspirated through one bottle of 4% aqueous mercuric cyanide and three bottles of 4% aqueous mercuric chloride (see Blackburn and Challenger, *loc. cit.*). No precipitate formed in the mercuric cyanide at any time, but a white deposit formed in the first mercuric chloride bottle after 15—30 mins. and after 8 days 0·9 g. was obtained, m. p. 105—116°. It was recrystallised twice from benzene and after slight shrinkage at 115° had m. p. and mixed m. p. 116—118° with authentic methyl *n*-butyl sulphide dimercurichloride, m. p. 116—118° (Blackburn and Challenger, *loc. cit.*, p. 1875).

VIII. *Betaine and Di-*n*-amyl Disulphide.*—The disulphide (4 g.; 1 mol.) and betaine (3 g.; 1·32 mols.) were boiled for 8 hours. The product (4·3 g.), obtained as in VII, was distilled at the ordinary pressure, giving 1·5 g. of a yellow oil, b. p. above 60°; 1 g. with saturated aqueous mercuric chloride (300 c.c.) gave a pink solid (3·1 g.). Aspiration of this in alkaline suspension as in VII gave no precipitate in the mercuric cyanide during 21 days, but in 24 hours 0·16 g. of solid, m. p. 123—127°, formed in the first mercuric chloride bottle. After four crystallisations from benzene this had m. p. and mixed m. p. 125—126° with authentic methyl *n*-amyl sulphide mercurichloride of the same m. p. (Blackburn and Challenger, *loc. cit.*, p. 1875). During the next 20 days a further deposit (0·6 g.), m. p. 124—126°, formed in the mercuric chloride.

The remainder of the crude distillate (0·5 g.) was treated with 2·5 c.c. of perhydrol (30% H_2O_2) in glacial acetic acid (0·5 c.c.), heated at 100° for 1 hour under reflux, cooled, and extracted with chloroform. The extract, washed with sodium bicarbonate solution and dried over calcium chloride, yielded an oil which solidified in ice and salt. After three crystallisations from ether–light petroleum it had m. p. and mixed m. p. 35—36° with authentic methyl-*n*-amylsulphone of the same m. p. (see p. 54).

Diethylaminoacetic Acid Ethyl Betaine.—" Triethyl betaine " was prepared by a modification of Hofmann's method (*Proc. Roy. Soc.*, 1862, **11**, 526) from triethylamine (32 g.; 1·2 mols.) and ethyl chloroacetate (33 g.; 1 mol.) by heating with absolute alcohol (40 c.c.) under reflux for 24 hours. A sealed tube is not necessary. The quaternary salt was precipitated with dry ether, giving an oil which solidified overnight. This was separated (yield, about 53 g.) and dissolved in water, adhering ethyl chloroacetate removed by three extractions with ether, and the aqueous solution evaporated twice at 100° to remove triethylamine. The aqueous solution was then shaken for 2½ hours with silver oxide (1·3 mols.) prepared from silver nitrate (57 g.) and potassium hydroxide (19 g.), filtered, and evaporated under diminished pressure, then in an open vessel at 100°, and the residue dried in a desiccator; yield, 24 g. of a brownish solid. This was dried for 56 hours at 105°. Another specimen gave with aqueous picric acid a picrate, m. p. 196—198° after two recrystallisations from water. Ingold and Rothstein (J., 1931, 1682), who prepared it from ethyl diethylaminoacetate and ethyl iodide, give m. p. 193—194°.

IX. *Diethylaminoacetic Acid Ethyl Betaine and Aniline.*—The betaine (20 g.; dried at 105°) and aniline (12 g.) were boiled for 10 hours, and the product made alkaline with sodium hydroxide and extracted with ether, yielding 19 g. of oil. This was well stirred with a solution of zinc chloride (21 g.) in water (50 c.c.), giving a viscid solid. After filtration this was well washed with light petroleum and with ether and then extracted under reflux for 4 hours with boiling light petroleum. The dried solvents were evaporated, giving 0·9 g. of an oil having an odour of ethylaniline. Treatment with acetic anhydride (0·9 g.), dilution with water, addition of a slight excess of ammonia (compare Morgan and Grist, J., 1918, **113**, 691), and leaving below 0° gave an oil. A portion of this solidified on nucleation with ethylacetanilide and a trace of the resulting solid was used to nucleate the remainder of the oil, giving a white solid (0·4 g.), m. p. 47—53°. After successive crystallisations from ethyl acetate, ether and ethyl acetate this melted at 51—53° alone and in admixture with authentic ethylacetanilide, m. p. 51—53°.

X. *Methionine and Aniline.*—Methionine (2·3 g.; 1 mol.) and aniline (8·2 g.; 5·7 mols.) were heated in an oil-bath at 190—210° for 10·5 hours. Traces of ammonia and a mercaptan were evolved but the methionine appeared unchanged. Basification, extraction with ether, evaporation of the solvent, distillation in steam, and extraction with ether finally yielded 5·7 g. of almost pure aniline, b. p. 183—185°. No oily drops of nitrosoamine were produced on diazotisation of this or of the slight residue in the distillation flask. The characteristic odour of methylaniline was absent throughout the experiment.

XI. *Aniline and Paraformaldehyde.*—(*a*) Aniline (18·6 g.; 1 mol.) and paraformaldehyde (trioxymethylene, 12·0 g.; 2 mols.) were heated on a sand-bath. Water was eliminated at about 130°. The mixture was then gradually heated in an oil-bath, under reflux; a vigorous reaction occurred at 210°, giving a dark red tar. Distillation in steam gave only 1—2 c.c. of oil still containing aniline. This was dissolved by addition of hydrochloric acid, and sodium nitrite added. Extraction with ether yielded only 0·7 g. of a dark oil containing a little *N*-nitroso-*N*-methylaniline. This result may be compared with that of Expt. I*a*, where 5 g. of a much purer nitrosoamine were obtained by the use of betaine.

(*b*) Aniline (186 g.; 10 mols.) and paraformaldehyde (12 g.; 2 mols.) were heated in an oil-bath. A clear solution was obtained at 120° and water separated at 140°. The temperature was raised to 210°, where it was maintained for 11 hours. No violent reaction occurred at any time. The product was dissolved in dilute hydrochloric acid (475 c.c. of concentrated acid and 150 c.c. of water) and diazotised. Much red resin was produced, but extraction with benzene and ether removed very little of this. Evaporation of the extract after washing with aqueous sodium bicarbonate and with water, and drying over sodium sulphate yielded 4·5 g. of the nitrosoamine almost free from phenol. Reduction with tin and hydrochloric acid, basification, and distillation in steam yielded 1·5 g. of methylaniline, b. p. 190—195°. This was characterised by heating at 100° with 2 : 4-dinitrochlorobenzene, *N*-methyl-2 : 4-dinitrodiphenylamine, m. p. 165—167°, being obtained. Reitzenstein (*J. pr. Chem.*, 1903, **68**, 255) gives m. p. 167°.

(*c*) Repetition of Expt. XI (*b*) gave 2·7 g. of crude nitrosoamine.

XII. *Betaine and Sodium Sulphite.*—Betaine (5 g.) and anhydrous sodium sulphite (5 g.) were heated on a sand-bath in a stream of carbon dioxide, which then passed through (*a*) dilute hydrochloric acid (1 : 1; three bottles) to remove trimethylamine, (*b*) saturated aqueous mercuric cyanide (3 bottles), (*c*) an aqueous suspension of mercuric iodide, and finally (*d*) two bottles containing aqueous mercuric chloride. The greenish-yellow solid which separated in (*b*) was presumably a compound of mercuric sulphide and mercury dimethylthiol (Challenger and Rawlings, J., 1937, 873). No alteration occurred in (*c*), but a white solid (0·4 g.) separated in (*d*). This with sodium hydroxide gave an odour of an alkyl sulphide. Extraction with hot benzene yielded white crystals, m. p. and mixed m. p. 155—160° with authentic dimethyl sulphide mercurichloride, m. p. 155—160°. All the specimens showed shrinkage from 150—155°. Mercurichlorides of this type tend to lose alkyl sulphide below their m. p.

The yield from two experiments was treated with sodium hydroxide under ether, and the extract left with aqueous potassium platinochloride overnight. Evaporation of the ether left a residue, which was crystallised twice from hot water; it formed pale yellow needles, m. p. and mixed m. p. 160°, with authentic bis(dimethyl sulphide)platinous chloride, $2(CH_3)_2S{\cdot}PtCl_2$. Enebuske (*J. pr. Chem.*, 1888, **38**, 358) gives m. p. 159°.

Preparation and Reactions of Reference Compounds.—N-*Nitroso*-p-*toluidinoaceto*-p-*toluidide*. *p*-Toluidinoaceto-*p*-toluidide was prepared from chloroacetic acid and 3 mols. of *p*-toluidine by Meyer's method (*Ber.*, 1875, **8**, 1161). With nitrous acid in ice-cold alcohol it gave a yellow solid, which after four crystallisations from benzene melted at 156—159° (decomp.) (Found: C, 67·75, 68·0; H, 5·9, 6·05; N, 14·7, 14·75. $C_{16}H_{17}O_2N_3$ requires C, 67·8; H, 6·05; N, 14·85%).

When 0·5 g. was heated at 100° with tin (2 g.) and hydrochloric acid (14 c.c.) for 80 mins., the yellow colour disappeared. Addition of excess of sodium hydroxide and extraction with ether yielded *p*-toluidinoaceto-*p*-toluidide. When the heating was continued for 92 hours, *p*-toluidine was obtained (m. p. and mixed m. p. of the free base and the acetyl derivative).

p-Anisidinoaceto-*p*-anisidide was prepared by Meyer's method—a new method of preparation in the case of this compound. Successive crystallisation from alcohol, benzene, and alcohol gave a product, m. p. 131—132° [Found: C, 67·25; H, 6·35; N, 9·85; *M* (camphor), 263. Calc. for $C_{16}H_{18}O_3N_2$: C, 67·1; H, 6·35; N, 9·8%; *M*, 286].

N-*Nitroso*-p-*anisidinoaceto*-p-*anisidide* was prepared from the parent anisidide in acetone solution, sodium nitrite and hydrochloric acid below 0° being used. Complete solution occurred, but a solid slowly separated which was recrystallised first from benzene and then from aqueous alcohol. It melted and decomposed at 155—159° when heated rapidly (Found: C, 60·65; H, 5·7; N, 13·15. $C_{16}H_{17}O_4N_3$ requires C, 60·9; H, 5·45; N, 13·35%).

p-Phenetidinoaceto-*p*-phenetidide was obtained by Meyer's method. Alternate crystallisations from aqueous alcohol and benzene gave a product, m. p. 137—138° (Found: C, 68·9; H, 7·05. Calc. for $C_{18}H_{22}O_3N_2$: C, 68·75; H, 7·05%).

Methyl-n-*butylsulphone*. Methyl *n*-butyl sulphide (1·4 g.) in glacial acetic acid (1·4 c.c.) was heated under reflux with perhydrol (7 c.c.) for 1 hour at 100°. A vigorous reaction occurred. Extraction of the mixture with chloroform, washing the extract with sodium bicarbonate solution and water, and drying over calcium chloride gave an oil (1 g.), which solidified in ice and salt, m. p. 28—31°. After one crystallisation from cooled benzene–light petroleum and four from ether–light petroleum this melted constantly at 29—30° (Found: C, 43·5; H, 8·7. $C_5H_{12}O_2S$ requires C, 44·1; H, 8·9%).

Methyl-n-*amylsulphone*. The sulphide (1 g.) in glacial acetic acid (1 c.c.) and perhydrol (5 c.c.) were treated as for the *n*-butyl derivative. Evaporation of the chloroform extract gave an oil (0·6 g.) solidifying below 0° and melting at 31—35°. Several crystallisations from ether–light petroleum gave a white solid of constant m. p. 35—36° (Found: C, 47·05; H, 9·15. $C_6H_{14}O_2S$ requires C, 47·95; H, 9·4%).

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(Addendum, January 8th, 1942.) It has now been shown by du Vigneaud *et al.* (*J. Biol. Chem.*, 1941, **141**, 625) that on administration of trideuteromethionine to rats the methyl groups of the choline isolated from the tissues contain 72·4 atoms % of deuterium. Similar figures (73·0 and 72·4) were obtained for the methyl group of the tissue creatine and the urinary creatinine.

The authors therefore regard these reactions as true transmethylations (transfer of a methyl group as such) which do not involve the elimination of dideuteroformaldehyde, CD_2O. With an amino-group, as in ethanolamine, this would give $\cdot NH\cdot CD_2\cdot OH \longrightarrow \cdot NH\cdot CD_2H$ instead of $\cdot NH\cdot CD_3$ and the atom percentage of deuterium in the methyl group could not rise above 66·6.

Furthermore, the work of du Vigneaud (*ibid.*, 1940, **135**, 223) on the comparative action of choline and betaine in effecting the replacement of methionine by homocystine in the diet of rats, and of Stettin (*ibid.*, 1941, **140**, 143), who administered isotopic (^{15}N) betaine to rats, has furnished evidence of the demethylation of betaine with (in the last-named research) formation of isotopic glycine.