569. *The Action of Formaldehyde on Proteins. Part II.* Some Reactions of N-Hydroxymethylamides.*

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The equilibria between formaldehyde, amides, and *N*-hydroxymethylamides have been studied under alkaline conditions. In acid solution the methylenebisamides are formed (Einhorn, *Annalen*, 1905, **343**, 207; 1908, **361**, 113). It has been found that *N*-hydroxymethylamides condense with primary alcohols to form *O*-ethers, with thiols to form sulphides, and with carboxylic acids to form esters; with primary amines two molecules of the *N*-hydroxymethyl compound combine to form tertiary amino-derivatives. The reaction with secondary amines (Einhorn, *loc. cit.*) has been confirmed. 1 : 4-Bishydroxymethyl-2 : 5-diketopiperazine is not formed in acid solution, and under acid conditions does not condense with amides but loses formaldehyde which forms a methylenebisamide. *N*-Hydroxymethylamides do not condense with peptides. These results are applied to a study of the nature of the products formed by the action of formaldehyde upon proteins.

Haworth, MacGillivray, and Peacock (Part I *) showed that several proteins when treated with formaldehyde in alkaline solution gave products which condensed with β-naphthol and with 6-bromo- and 3 : 6-dibromo-2-naphthol to form compounds yielding 1-aminomethyl-2-naphthol or its derivatives on hydrolysis. Ground-nut protein insolubilised with formaldehyde under acid conditions (Traill, *Chem. and Ind.*, 1945, 58; 1950, 23) condensed with a much smaller amount of 6-bromo-2-naphthol and, if the acid-treated protein had been previously boiled with water, the amount taken up was negligible. Evidence was adduced that amongst the first products of the action of formaldehyde on alkaline solutions were *N*-hydroxymethylamides or labile derivatives, and it seemed feasible that more complicated reactions took place during treatment with formaldehyde under acid conditions, as a result of the condensation of the *N*-hydroxymethylamides with other groupings present in the protein molecules. The object of the present work was to examine the reactions of *N*-hydroxymethylamides with model substances containing groupings such as hydroxyl, thiol, amino-, carboxyl, amide, or peptide, which are present in protein molecules, and to compare the stability of the products with those of acid–formaldehyde-treated proteins. The *N*-hydroxymethylamides (I) were prepared by the action of formaldehyde on a wide variety of amides in the presence of aqueous potassium carbonate, and included those from benzamide (I; R = Ph), phenylacetamide, β-phenyl-

(I) $R{\cdot}CO{\cdot}NH{\cdot}CH_2OH$ $R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}R$ (II)

propionamide, hippuramide, phenylacetylglycine amide, *N*-phenylacetyl-DL-alanine amide, and succinamide. All these *N*-hydroxymethylamides condensed with β-naphthol in alcoholic hydrochloric acid, and the 1-acylaminomethyl-2-naphthol derivative gave 1-aminomethyl-2-naphthol on hydrolysis with hydrochloric acid. The *N*-hydroxymethylamides are stable in neutral aqueous solution at room temperature but the reaction between the original amides and formaldehyde in presence of potassium carbonate is reversible. The formation and dissociation of a number of substances of type (I) have been examined by determination of the liberated formaldehyde with 2 : 4-dinitrophenylhydrazine (Lamberton, Lindley, Owston, and Speakman, *J.*, 1949, 1641), with the results given in Table 1. When formaldehyde in aqueous potassium carbonate was mixed with benzamide, equilibrium was reached in 48 hours and the reciprocal of the equilibrium constant was $1{\cdot}6 \times 10^{-5}$; for *N*-phenylacetylglycine amide, the time was 70 hours and the above reciprocal was $4{\cdot}5 \times 10^{-5}$. These figures were of the same order as the dissociation constants, and better agreement could not be expected. The hydroxymethyl derivatives of acylated amino-amides such as phenylacetylglycine amide are somwhat less stable than those of benzamide and similar amides, and the stability of the bishydroxymethyl derivatives of succinamide

* *J.*, 1950, 1493, is considered to be Part I of this series.

TABLE 1. *Dissociation of* N-*hydroxymethylamides.*

0·5 G. of hydroxymethyl derivative and 0·02 g. of potassium carbonate in 50 c.c. of water at room temperature.

R in (I)	Percentage of total formaldehyde liberated after: 5 hr.	17 hr.	51 hr.	120 hr.	212 hr.	15 days	10^5K
Ph	23·6	38·1	43·3	44·5	—	44·8	2·8
,, (K_2CO_3, 0·04 g.)	33·5	39·9	40·4	42·3	—	42·2	—
CH_2Ph	—	37·7	—	42·5	42·2	—	2·3
	54 hr.	98 hr.	142 hr.	21 days			
$CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2$	49·5	53·3	53·8	53·2	—	—	7·3
$CH_2Ph{\cdot}CO{\cdot}NMe{\cdot}CH_2$	26 hr.	68 hr.	115 hr.	21 days			
Succinamide	6·8	9·4	9·9	10·0			

is noteworthy. In acid solution the *N*-hydroxymethylamides are converted into methylenebisamides of type (II), and these compounds are also obtained by condensing formaldehyde and amides at pH 0. In Table 2 the figures relating to the decomposition of *N*-hydroxymethylamides in acid solution are given.

TABLE 2. *Decomposition of* N-*hydroxymethylamides in acid.*

0·5 G. of hydroxymethyl derivative in 50 c.c. of 2N-hydrochloric acid.

R in (I)	Percentage of formaldehyde split off: 20 hr.	50 hr.	112 hr.	250 hr.	16 days	21 days
Ph	18·3	35·4	41·6	43·1	—	—
$CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2$	8·8	—	30·4	40·7	—	—
Succinamide	10·1	19·1	24·5	—	—	37·8
Acetate of (I; R = CH_2Ph)	13·8	24·3	44·8	65·0	73·0	—

Methylenebisamides (II) are stable compounds; they are not attacked by N-sodium hydroxide during 1 hour at 90° and are relatively stable towards acids, but in 10 hours' boiling with 4% alcoholic hydrochloric acid and β-naphthol the formaldehyde is removed as methylenebis-β-naphthol.

During some of the experiments outlined above it was found that *N*-hydroxymethylamides frequently yielded *O*-ethers when treated with alcohols in the presence of acids and in some cases the reactions occurred with considerable ease. Similar ready *O*-alkylation is familiar with carbinol bases (Robinson and McLeod, *J.*, 1921, 1470) and the alkylation of compounds of type (I) has previously been reported for a few cases (Kadowski, *Bull. Chem. Soc. Japan*, 1936, **11**, 248; Sorenson, U.S.P. 2,201,927; Allbrecht, Frei, and Sallmann, *Helv. Chim. Acta*, 1941, 233E). The *O*-ethers have now been prepared from a variety of *N*-hydroxymethylamides and primary alcohols, including *N*-benzoylserine, but reaction has not been observed with secondary or tertiary alcohols. The *O*-ethers showed reactivity resembling that of the parent *N*-hydroxymethylamide. They were more slowly decomposed to the parent alcohol, amide, and formaldehyde by boiling water or by N-sodium hydroxide at room temperature, and they were converted into methylenebisamides by warming or by long contact in the cold with aqueous hydrochloric acid. They underwent alcoholysis when warmed with another alcohol in the presence of concentrated hydrochloric acid, and with β-naphthol they condensed as readily as did the *N*-hydroxymethylamide and gave the same products. A few sulphides have also been prepared by condensation with thiols; they were decidedly more stable, and reacted with β-naphthol only after 3 hours' boiling, compared with 20 minutes for the corresponding *O*-ethers.

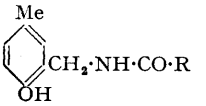
Compounds of type (III) were prepared by Einhorn (*Annalen*, 1905, **343**, 207; 1908, **361**, 113) from amides, formaldehyde and secondary amines, or from *N*-hydroxymethylamides and secondary amines. The reaction between methylenebis-*sec.*-amines and amides can also be employed, but we find the best yields are obtained by the first of the three methods. The reactions with primary amines and *N*-hydroxymethylamides were less well-known. Fraenkel-Conrat and Olcott (*J. Amer. Chem. Soc.*, 1948, **70**, 2673) described the production of an unstable compound of type (IV) by condensing formaldehyde with acetamide and alanine, and similar structures were proposed by Sallemann and Graenacher (U.S.P. 2,448,125) for the basic products obtained by condensing *N*-hydroxy-

methylamides with aliphatic amines and formaldehyde in acetic acid solution. The reaction between *N*-hydroxymethylamides and primary amines has now been investigated more thoroughly. For *N*-hydroxymethyl derivatives of benzamide, phenylacetamide, and β-phenylpropionamide and methylamine, condensation was effected in water at

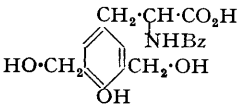
$R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NR'R''$ (III) $R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NH{\cdot}R'$ (IV) $(R{\cdot}CO{\cdot}NH{\cdot}CH_2)_2NR'$ (V)

100° for 12 hours or preferably in glacial acetic acid at 75° for 45 minutes. The analytical data and molecular weights of the crystalline products proved that *N*-hydroxymethylamides reacted with primary amines to give products of type (V), and not of type (IV) as previously suggested. Burke's observation (*ibid.*, 1947, **69**, 2136) that symmetrical bishydroxymethylurea reacted with a primary amine to form a cyclic triazine derivative is, however, quite consistent with the production of compounds of type (V). The compounds of types (III) and (V) are fairly stable in alkaline solution at room temperature, and they do not condense with β-naphthol under the conditions which are effective with *N*-hydroxymethylamides. The secondary amine condensation products of type (III) are decomposed more slowly than primary amine products of type (V) by boiling water or aqueous acid.

$R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}OAc$ (VI)



(VII)

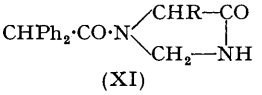


(VIII)

During the examination of the reactions of primary amines with *N*-hydroxymethylamides in acetic acid, esters of type (VI) were encountered. They were, however, more conveniently prepared by heating *N*-hydroxymethylamides with glacial acetic acid at 75°. They are not rapidly decomposed by boiling water, and towards acids and alkalis they are rather more stable than the *N*-hydroxymethylamides, but they readily react with β-naphthol under the same conditions, and to give the same products, as the corresponding compounds of type (I).

N-Hydroxymethylamides readily condense with active phenols and naphthols (Einhorn, *loc. cit.*) and it has now been shown that *N*-hydroxymethyl-benzamide and -phenylacetamide react normally with *p*-cresol in alcoholic hydrochloric acid, to give derivatives of type (VII). *N*-Benzoyltyrosine therefore represented an interesting model, but all attempts to effect its reaction with *N*-hydroxymethyl-benzamide or -phenylacetamide failed. Under the acidic conditions employed the first gave methylenebisbenzamide and the second gave a mixture of methylenebisphenylacetamide and *N*-ethoxymethylphenylacetamide; *N*-benzoyltyrosine was recovered in both cases. *N*-Benzoyltyrosine did not react with formaldehyde in aqueous or alcoholic hydrochloric acid, but in sodium hydroxide solution the 3 : 5-dihydroxymethyl derivative (VIII) was produced, similar to the compound obtained under these conditions from *p*-cresol (Ullmann and Brittner, *Ber.*, 1909, **42**, 2539).

$R{\cdot}CO{\cdot}NH{\cdot}R'$ (IX) $R{\cdot}CO{\cdot}NH{\cdot}CHR'{\cdot}CO{\cdot}NR''R'''$ (X)



(XI)

This failure to bring about reaction between formaldehyde or *N*-hydroxymethylamides with the amide-nitrogen atom of *N*-benzoyltyrosine illustrates the general lack of reactivity which we have observed with monoacylated primary amines of type (IX). Although diketopiperazine (Cherbuliez and Feer, *Helv. Chim. Acta*, 1922, **5**, 678) and polyamides of the Nylon type (Lewis and McCreath, B.P. 582,517; Cairns, Foster, Larcher, Schneider, and Schreiber, *J. Amer. Chem. Soc.*, 1949, **71**, 651) have been shown to give the expected *N*-hydroxymethyl derivatives, Fraenkel-Conrat, Coober, and Olcott (*J. Amer. Chem. Soc.*, 1945, **67**, 950) found that the silk fibroin and polyglutamic acid did not condense with formaldehyde at pH 4. A number of peptides of type (X; *e.g.*, R = Ph or CH_2Ph, R′ = H or Me, and NR″R‴ = morpholino, NHEt, or NEt_2) have been synthesised as described

in the Experimental section, but these substances did not react with formaldehyde under a wide variety of conditions, including the use of potassium carbonate or pyridine as basic, and hydrochloric or formic acid as acid, catalyst, with water, alcohol, or dioxan as solvent. As it was thought possible that the products might be decomposed during attempted isolation, the reaction mixtures from formaldehyde and amides of types (IX) and (X) were treated with β-naphthol in acid solution, but no derivative of a substituted 1-aminomethyl-2-naphthol could be isolated as such or as the toluene-*p*-sulphonyl derivative. In addition, analytical determination with 2 : 4-dinitrophenylhydrazine showed no formaldehyde uptake after formaldehyde and amides of types (IX) and (X) had been in contact for 21 days at room temperature. All the amides of types (IX) and (X) which we have examined fail to react with *N*-hydroxymethylamides, and in this connection it is particularly interesting that the *N*-hydroxymethyl derivatives of phenylacetylglycine amide and phenylacetyl-DL-alanine amide cannot be converted into cyclic derivatives of type (XI) by intramolecular condensation of the *N*-hydroxymethylamide group with the peptide imide group. In 1 : 4-bishydroxymethyl-2 : 5-diketopiperazine the hydroxymethyl group is already linked to a peptide group. When this was treated with phenylacetamide in acid solution the formaldehyde was split from the diketopiperazine and formed the methylenebisamide of phenylacetic acid.

For the reactions occurring during formaldehyde treatment of proteins condensation with amino-groups has frequently been postulated. Secondary amines and formaldehyde usually give methylenebisamines, but the reaction with primary amines has been variously interpreted, and methylenebisamine (Kempf, *Annalen*, 1890, **256**, 219), hydroxymethylamine, and methyleneimine structures (Henry, *Bull. Acad. Roy. Belg.*, 1894, **28**, 355) have been suggested. Our experiments with benzylamine and formaldehyde indicate that the product, m. p. 50°, is a cyclic trimer of *N*-methylenebenzylamine, and the salts, described by Kempf (*loc. cit.*) are in fact benzylamine salts. The cyclic trimers and methylenebisamines are stable to cold alkalis and are rapidly decomposed by dilute mineral acids, and it is unlikely that these compounds play any part in the acid-insolubilising reactions. On the other hand the experimental results described in Part I and in this and the following paper provide convincing evidence of the importance of reactions at amide centres of the protein molecule. Other centres may also be involved and, as a result of these experiments with model substances, deductions may be now be made concerning the reactions occurring during the insolubilising of proteins with formaldehyde in acid solution.

Macromolecules are usually more sluggish in their reactions than less complex polymers, and as it is not always possible to draw firm conclusions about the behaviour of macromolecules from experiments with simple compounds, the following conclusions must be regarded as tentative: (1) *N*-Hydroxymethylamides are not likely to persist to any appreciable extent in acid-treated proteins; and small amounts present would contribute to the reaction with β-naphthol and would be eliminated by boiling water. (2) There is no evidence for the formation of methylene bridges between amide and peptide groups, or between amide and aromatic nuclei, *e.g.*, tyrosine. (3) Methylene bridges between the amide groups and hydroxyl, thiol, carboxyl, or primary or secondary amino-groups may be present in acid-treated unboiled fibre and these linkages may be responsible for some of the formaldehyde eliminated during boiling with water, and for some of the reactions with β-naphthol. (4) Methylenebisamide linkages would probably be formed in the acid-insolubilising bath; the products would not liberate formaldehyde when boiled with water, would be relatively stable towards acids and alkalis, and would not yield 1-aminomethyl-2-naphthol derivatives.

Experimental

Equilibria between Amides, Formaldehyde, and Water.—To a solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid (Brady, *J.*, 1931, 757) was added sufficient formaldehyde solution to give a faint precipitate. The reagent was then kept at the ordinary temperature for 48 hours and filtered. It was found that 0·2% formaldehyde solution precipitated 99% of the formaldehyde as the dinitrophenylhydrazone after 15 minutes; Lamberton *et al.* (*loc. cit.*) used a reaction time of 5 hours, during which the equilibria examined in this paper would have

appreciably changed. 0·2% Formaldehyde solution showed no loss of strength after 7 days in 2N-hydrochloric acid or 0·08% potassium carbonate solution. In N-sodium hydroxide a fall in formaldehyde content was observed after 48 hours. The solubility of the dinitrophenylhydrazone in 2N-hydrochloric acid at ordinary temperature was 0·5 mg./10 c.c.

Dissociation of N-*Hydroxymethylamides in Alkaline Solution.*—In a typical experiment the following results were obtained :

N-Hydroxymethylbenzamide (0·5429 g.), potassium carbonate (0·02 g.) made up to 50 c.c. in water; 10 c.c. samples were withdrawn.

Time after mixing (hrs.)	5	17	51	120	15 days
Wt. of dinitrophenylhydrazone (g.)	0·0387	0·0624	0·0709	0·0731	0·0734
Total formaldehyde set free (%)	23·6	38·1	43·3	44·5	44·8

Whence $K = 2{\cdot}8 \times 10^{-5}$.

Combination of Formaldehyde with Amides in Alkaline Solution.—A standardised solution of formaldehyde (2·55 g./100 c.c.) was used and the equilibrium found in the usual way :

Benzamide. Time to reach equilm. ..... 48 hrs., whence $1/K = 1{\cdot}6 \times 10^{-5}$.
Phenylacetylglycine amide. Time to reach equilm. ..... 70 hrs., whence $1/K = 4{\cdot}5 \times 10^{-5}$.

Decomposition of N-*Hydroxymethylamides in Acid Solution.*—The methylenebisamide formed was removed by filtration before the liberated formaldehyde was determined. The results are given in Table 2. When the *N*-hydroxymethylbenzamide solution was heated for 15 minutes in a water-bath with 2 : 4-dinitrophenylhydrazine, 91·2% of the total formaldehyde present was precipitated as the hydrazone; *N*-hydroxymethylphenylacetylglycine amide, treated similarly, gave 91·8% of formaldehyde.

N-*Hydroxymethylamides and their Reaction with* β-*Naphthol.*—*Phenylacetyl-*DL-*alanine ethyl ester* was prepared in a manner similar to the acid (see below), by using magnesium oxide to neutralise the hydrochloric acid liberated. It crystallised from ethanol or *cyclo*hexane in needles, m. p. 162° (Found : C, 66·3; H, 7·3; N, 6·2. $C_{13}H_{17}O_3N$ requires C, 66·4; H, 7·2; N, 6·0%). The *amide* was prepared from the ester (20 g.) in ammonia (*d* 0·880; 150 c.c.), stirred for 3 hours, further saturated with ammonia, and stirred for 2 hours. The product (12·6 g.) crystallised from water in prisms, m. p. 154—155° (Found : C, 64·4; H, 6·9; N, 13·4. $C_{11}H_{14}O_2N_2$ requires C, 64·1; H, 6·8; N, 13·3%).

N-*Hydroxymethylphenylacetylglycine amide.* Phenylacetylglycine amide (Hotter, *J. pr. Chem.*, 1888, **38**, 97) (10·0 g.), anhydrous potassium carbonate (0·4 g.), water (10 c.c.), and 40% aqueous formaldehyde were warmed in a water-bath until all was in solution. The *hydroxymethyl* derivative (10·0 g.) crystallised on cooling, and, recrystallised from water, had m. p. 144—148° (Found : C, 59·4; H, 6·2; N, 12·6. $C_{11}H_{14}O_3N_2$ requires C, 59·5; H, 6·3; N, 12·6%).

N-*Hydroxymethylphenylacetyl-*DL-*alanine amide,* prepared similarly in 87% yield, crystallised from water in plates, m. p. 137—140° (Found : C, 61·2; H, 6·7; N, 11·7. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%). *N*-Hydroxymethylhippuramide was prepared similarly but decomposed on attempted recrystallisation. N-*Hydroxymethyl-*β-*phenylpropionamide* crystallised from benzene or ethyl acetate in prisms, m. p. 98° (Found : C, 67·4; H, 7·0. $C_{10}H_{13}O_2N$ requires C, 67·1; H, 7·3%).

Condensation of the *N*-hydroxymethylamides with β-naphthol in alcoholic acid was effected as described by Einhorn (*Annalen*, 1905, **343**, 207); on hydrolysis in alcohol with concentrated hydrochloric acid all of the products described below gave the hydrochloride of 1-aminomethyl-2-naphthol. The following crystallised from ethanol : 1-N-*Phenylacetylglycylamidomethyl-2-naphthol,* needles, m. p. 189° (Found : C, 72·2; H, 5·7; N, 7·9. $C_{21}H_{20}O_3N_2$ requires C, 72·4; H, 5·8; N, 8·1%). 1-*Phenylacetyl-*DL-*alanylamidomethyl-2-naphthol,* needles, m. p. 200° (Found : C, 72·6; H, 5·9; N, 7·9. $C_{22}H_{22}O_3N_2$ requires C, 72·9; H, 6·1; N, 7·7%). 1-*Hippuroylamidomethyl-2-naphthol,* prisms, m. p. 185—186° (Found : C, 71·6; H, 5·6; N, 8·1. $C_{20}H_{18}O_3N_2$ requires C, 71·9; H, 5·4; N, 8·4%). 1-β-*Phenylpropionamidomethyl-2-naphthol,* prisms, m. p. 121—122° (Found : C, 78·3; H, 6·2; N, 5·1. $C_{20}H_{19}O_2N$ requires C, 78·6; H, 6·2; N, 4·6%).

Methylenebisamides.—These were prepared by dissolving the corresponding amides in a mixture of saturated sodium sulphate solution (860 c.c.), concentrated sulphuric acid (258 c.c.), and 40% formaldehyde solution (56 c.c.). The amide (0·5 g.) was mixed with the acid formaldehyde (5 c.c.) and warmed until solution was complete. The methylenebisamide separated on cooling. The compounds were also obtained when the *N*-hydroxymethylamides were warmed with 0·5N-hydrochloric acid until solution occurred, and

separated on cooling. The following crystallised [from acetic acid: *Methylenebisphenylacetylglycylamide*, plates, m. p. 260° (Found: C, 63·5; H, 6·1; N, 14·1. $C_{21}H_{24}O_4N_4$ requires C, 63·6; H, 6·1; N, 14·2%). *Methylenebisphenylacetyl-*DL*-alanine amide*, prisms, m. p. 280° (decomp.) (Found: C, 64·9; H, 6·9; N, 13·3. $C_{23}H_{28}O_4N_4$ requires C, 65·1; H, 6·6; N, 13·2%). *Methylenebishippuramide*, plates, m. p. 258° (Found: C, 61·9; H, 5·8; N, 15·7. $C_{19}H_{20}O_4N_4$ requires C, 62·0; H, 5·5; N, 15·2%).

O-Ethers R·CO·NH·CH_2·OR′.—The ethers were prepared by warming a mixture of *N*-hydroxymethylamide (0·5 g.) with the appropriate alcohol (5 c.c.) and concentrated hydrochloric acid (0·1 c.c.) until solution occurred. The product usually separated on cooling, in some cases in a few minutes, in others only after several hours; in a few cases it was necessary to concentrate the solutions under reduced pressure. The ethers were also prepared by setting the mixed reactants aside for 18—20 hours at ordinary temperature. The following were prepared: N-*Methoxymethylphenylacetylglyine amide*, plates (from water), m. p. 146° (Found: C, 60·9; H, 6·7; N, 11·8. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%). N-*Ethoxymethylphenylacetylglycine amide*, plates (from water), m. p. 159° (Found: C, 62·2; H, 7·2; N, 11·7. $C_{13}H_{18}O_3N_2$ requires C, 62·4; H, 7·2; N, 11·2%), soluble in ethanol, hot acetone, benzene, chloroform, hot ethyl acetate, or pyridine, fairly soluble in dioxan, sparingly soluble in hot, light petroleum, insoluble in carbon tetrachloride and ether. [This ether (0·2 g.), when warmed with 0·5N-hydrochloric acid (2 c.c.), deposited the methylenebisamide on cooling. In N-sodium hydroxide at room temperature the ether set free the following percentages of total formaldehyde: 48 hours, 14·9; 9 days, 55·8.] N-n-*Propoxy-*, plates (from water), m. p. 145° (Found: C, 63·4; H, 7·3; N, 10·8. $C_{14}H_{20}O_3N_2$ requires C, 63·6; H, 7·6; N, 10·6%), N-iso-*butoxy-*, plates (from water), m. p. 120—121° (Found: C, 64·9; H, 7·9; N, 9·9. $C_{15}H_{22}O_3N_2$ requires C, 64·8; H, 7·9; N, 10·1%), and N-*benzyloxy-methylphenylacetylglycine amide*, leaflets (from aqueous ethanol), m. p. 138° (Found: C, 68·9; H, 6·2; N, 8·6. $C_{18}H_{20}O_3N_2$ requires C, 69·2; H, 6·4; N, 9·0%). [The benzyl ether (0·2 g.), ethyl alcohol (2 c.c.), and concentrated hydrochloric acid (0·1 c.c.) were warmed until all was in solution. The solid which separated on cooling melted at 157°, raised to 158° by crystallisation from water and unchanged after admixture with an authentic specimen of the ethyl ether.] N-*Ethoxy-*, prisms (from water), m. p. 128° (Found: C, 63·7; H, 7·5; N, 10·6. $C_{14}H_{12}O_3N_2$ requires C, 63·6; H, 7·6; N, 10·6%), and N-*benzyloxy-methylphenylacetyl-*DL*-alanine amide*, leaflets (from alcohol), m. p. 101° (Found: C, 69·8; H, 6·8; N, 8·3. $C_{19}H_{22}O_3N_2$ requires C, 69·9; H, 6·8; N, 8·6%) (this compound was obtained by neutralising the reaction mixture with potassium carbonate and removing the excess of benzyl alcohol by distillation under reduced pressure). N-*Methoxy-*, plates (from water), m. p. 138—139° (Found: C, 59·1; H, 6·1; N, 12·8. $C_{11}H_{14}O_3N_2$ requires C, 59·4; H, 6·3; N, 12·6%), N-*ethoxy-*, leaflets (from water), m. p. 166° (Found: C, 60·7; H, 7·0; N, 11·8. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%), and N-*benzyloxymethylhippuramide*, leaflets (from alcohol), m. p. 133° (Found: C, 68·3; H, 6·1; N, 9·8. $C_{17}H_{18}O_3N_2$ requires C, 68·5; H, 6·0; N, 9·4%). *N*-Ethoxymethylbenzamide could not be isolated; it was rapidly transformed into methylenebisbenzamide in ethanolic 0·2N-hydrochloric acid. *N-n*-Butoxymethylbenzamide, isolated as a liquid, b. p. 180°/3 mm., was converted into *methylenebisbenzamide* during several weeks (Found: C, 69·3; H, 7·9; N, 7·2. $C_{12}H_{17}O_2N$ requires C, 69·6; H, 8·2; N, 6·8%). N-*Ethoxy-*, leaflets (from light petroleum), m. p. 55—56° (Found: C, 68·8; H, 7·6; N, 7·3. $C_{11}H_{15}O_2N$ requires C, 68·4; H, 7·3; N, 7·3%), and N-n-*butoxymethylphenylacetamide* leaflets (from light petroleum), m. p. 56° (Found: C, 70·7; H, 8·4; N, 6·4. $C_{13}H_{19}O_2N$ requires C, 70·6; H, 8·6; N, 6·4%), were obtained normally.

Ether of N-*Benzoylserine Ethyl Ester and* N-*Hydroxymethylphenylacetylglycine amide* [CH_2Ph·CO·NH·CH_2·CO·NH·CH_2·O·CH_2·CH(NHBz)·CO_2Et].—*N*-Benzoylserine ethyl ester (Erlenmeyer, *Annalen*, 1904, **337**, 251) (1·0 g.), *N*-hydroxymethylphenylacetylglycine amide (0·8 g.), acetone (4·0 c.c.), and concentrated hydrochloric acid (0·05 c.c.) were warmed in a water-bath until solution was completed. By next morning a solid *ether* (0·43 g.) had crystallised. This was fractionally crystallised from aqueous alcohol and gave leaflets, m. p. 182—183° (Found: C, 62·7; H, 6·0. $C_{23}H_{27}O_6N_3$ requires C, 62·6; H, 6·1%).

The ether of N-*benzoylserine ethyl ester and* N-*hydroxymethylphenylacetamide*

[CH_2Ph·CO·NH·CH_2·O·CH_2·CH(NHBz)·CO_2Et]

prepared similarly, separated from dilute alcohol in needles, m. p. 110° (Found: C, 65·3; H, 6·3; N, 6·9. $C_{21}H_{24}O_5N_2$ requires C, 65·6; H, 6·3; N, 7·3%).

The foregoing ethers, like the parent hydroxy-compounds, when boiled with β-naphthol, ethanol, and hydrochloric acid under reflux for 20 minutes, gave the corresponding condensation product of β-naphthol.

N-*Benzyloxymethylphenylacetamide.* (*a*) This was prepared in the usual way. (*b*) *N*-Hydroxymethylphenylacetamide (3·0 g.) was heated for 6 hours 105—110° (oil-bath) with benzyl alcohol (10 c.c.) and 80% formic acid (0·4 c.c.). The excess of benzyl alcohol was removed under reduced pressure; the *product* separated from benzene in leaflets, m. p. 69—70° (Found: C, 75·0; H, 7·0; N, 5·5. $C_{16}H_{17}O_2N$ requires C, 75·3; H, 6·9; N, 5·5%). It was converted into the methylenebisamide by warming its solution in alcoholic hydrochloric acid for 20 minutes. It did not condense with β-naphthol in 14 days at room temperature in alcoholic N-hydrochloric acid.

Sulphides.—N-*Ethylthiomethylphenylacetylglycine amide.* Ethanethiol (5 c.c.), the *N*-hydroxymethylamide (1·5 g.), and concentrated hydrochloric acid (0·05 c.c.) were heated to 60—70° in a sealed tube for 15 minutes. The solid did not all dissolve and after being kept for 3 hours the mixture was filtered and the residue washed with ether. The *sulphide* crystallised from water in plates, m. p. 127° (Found: C, 58·9; H, 6·6; N, 10·7; S, 11·6. $C_{13}H_{18}O_2N_2S$ requires C, 58·6; H, 6·8; N, 10·5; S, 12·0%). It was treated with sodium hydroxide at room temperature and the liberated formaldehyde estimated: N-NaOH, 48 hours 0, 9 days 19%; 2N-NaOH, 48 hours, 15·1%. The compound is more stable than the *O*-ether.

N-*Ethylthiomethylphenylacetyl*-DL-*alanine amide* formed prisms (from dilute methanol), m. p. 124—125° (Found: C, 59·8; H, 7·3; N, 9·6; S, 11·3. $C_{14}H_{20}O_2N_2S$ requires C, 60·0; H, 7·1; N, 10·0; S, 11·4%). N-*Ethylthiomethylbenzamide,* formed prisms (from dilute methanol), m. p. 74—75° (Found: C, 61·6; H, 6·7; N, 7·2; S, 16·3. $C_{10}H_{13}ONS$ requires C, 61·6; H, 6·7; N, 7·2; S, 16·4%).

The sulphides condensed with β-naphthol to give 1-acylamidomethyl-2-naphthols after 3 hours' boiling with acidified ethanol.

Formaldehyde and Benzylamine.—Formaldehyde was condensed with benzylamine as described by Henry (*Bull. Acad. Roy. Belg.*, 1895, **29**, 23). The product distilled at 100°/0·005 mm., giving a pale yellow oil, which solidified and crystallised from aqueous ethanol in colourless prisms, m. p. 50° [Found: C, 80·7; H, 7·8; N, 11·7%; *M* (in camphor), 378. Calc. for $(C_8H_9N)_3$: C, 80·7; H, 7·6; N, 11·8%; *M*, 357]. This compound which was also prepared by Kempf's method (*loc. cit.*) was not stable in acid solution and the percentages of formaldehyde set free under two conditions were: (*a*) 2N-HCl added rapidly: 5 minutes, 18·3%; 1 hour, 27·4%. (*b*) 2 Equivs. added during 30 minutes: 59·5%. For comparison the corresponding figures for methylenebisdibenzylamine are (*a*) 1 hour, 20·5%, and (*b*) 86·4%.

Condensation Products of Primary Amines and N-*Hydroxymethylamides.*—*Bisbenzamidomethylmethylamine* (V; R = Ph, R′ = Me). *N*-Hydroxymethylbenzamide (5 g.) and a 23% solution of methylamine in glacial acetic acid (5 g.) were dissolved in glacial acetic acid (50 c.c.) and kept at 75° for 45 minutes, after which the solvent was removed under reduced pressure at 40°. Water (20 c.c.) was added to the residue and the insoluble benzamide (0·3 g.) removed. The filtrate was made alkaline to litmus with sodium hydroxide and left overnight at 0°. A colourless oil separated which was taken up in ethanol, and water was added until the mixture became turbid; crystals (1·0 g.; m. p. 120—124°) were gradually deposited; recrystallisation from dilute alcohol gave long prisms, m. p. 127—128° [Found: C, 68·8; H, 7·0; N, 14·1%; *M* (in camphor), 311. $C_{17}H_{19}O_2N_3$ requires C, 68·7; H, 6·4; N, 14·1%; *M*, 297]. The compound was soluble in acetone, alcohol, benzene, ether, ethyl acetate, or hot carbon tetrachloride, and insoluble in light petroleum and water. *Bisbenzamidomethyl*-n-*butylamine,* prepared (*a*) as above from *n*-butylamine and (*b*) by heating aqueous solutions of *n*-butylamine and *N*-hydroxymethylbenzamide on a water-bath for 12 hours, separated from alcohol in prisms, m. p. 115° (Found: C, 71·1; H, 7·5; N, 12·4. $C_{20}H_{25}O_2N_3$ requires C, 70·8; H, 7·4; N, 12·4%). This compound was more stable than methylenebisdibenzylamine. After it had been dissolved rapidly in 2N-hydrochloric acid no formaldehyde was found after 5 minutes; after 5 hours 16·5% of the total formaldehyde was split off. When 2 equivs. of hydrochloric acid were added during 30 minutes no formaldehyde was found in the solution.

Methylbisphenylacetamidomethylamine, prepared by the acetic acid method in low yield, separated from aqueous alcohol in prisms, m. p. 144° (Found: C, 70·3; H, 7·1; N, 13·0. $C_{19}H_{23}O_2N_3$ requires C, 70·2; H, 7·1; N, 12·9%). *Methyldi-*(β-*phenylpropionaminomethyl*)*amine,* prepared and crystallised similarly, formed plates, m. p. 118—119° (Found: C, 71·2; H, 7·6; N, 12·1. $C_{21}H_{27}O_2N_3$ requires C, 71·4; H, 7·7; N, 11·9%).

n-Butylamine did not react under either of the above conditions with *N*-hydroxymethylphenylacetamide or *N*-hydroxymethyl-β-phenylpropionamide.

Reaction of N-*Hydroxymethylamides and Secondary Amines.*—1-*Phenylacetylglycylamidomethylpiperidine,* $CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NC_5H_{10}$. (*a*) Aqueous formaldehyde (0·3 c.c; 40%) was slowly added to piperidine (0·3 c.c.) cooled in water. To this solution phenylacetyl-

glycine amide (0·5 g.) and alcohol (1·5 c.c.) were added and the mixture was boiled for 4 hours under reflux. (*b*) A solution of 1 : 1′-methylenebispiperidine (Braun and Röver, *Ber.*, 1903, **36**, 1196) and phenylacetylglycine amide in ethanol was heated for 4 hours. The crystals which separated on cooling were filtered off, washed with ether, and recrystallised from water in plates, m. p. 148° (Found : C, 66·1; H, 7·8; N, 14·5. $C_{16}H_{23}O_2N_3$ requires C, 66·4; H, 8·0; N, 14·5%). *Benzamidomethyldibenzylamine*, prepared as in (*a*), separated from alcohol in long prisms, m. p. 145—146° (Found : C, 80·1; H, 6·8; N, 8·9. $C_{22}H_{22}ON_2$ requires C, 80·0; H, 6·7; N, 8·5%). This compound did not liberate formaldehyde when mixed with hydrochloric acid at the room temperature under the conditions given for the condensation products with amides and primary amines. *Dibenzylphenylacetamidomethylamine* crystallised from alcohol in prisms, m. p. 99° (Found : N, 8·0. $C_{23}H_{24}ON_2$ requires N, 8·1%).

Acetoxymethylamides.—N-*Acetoxymethylphenylacetamide.* *N*-Hydroxymethylphenylacetamide (2 g.) was heated in glacial acetic acid (20 c.c.) at 75° for 30 minutes. The solvent was removed under reduced pressure at 40° and water added to the residue. The product (1·5 g.) which separated crystallised from benzene in needles, m. p. 98° [Found : C, 63·8; H, 6·4; N, 7·0%; *M* (in camphor), 198. $C_{11}H_{13}O_3N$ requires C, 63·8; H, 6·3; N, 6·8%; *M*, 207]. N-*Acetoxymethylphenylacetamide* in N-sodium hydroxide at room temperature split off 19·1% of the total formaldehyde in 48 hours and 22·3% in 9 days. The same compound in 2N-hydrochloric acid at room temperature split off the following percentages of formaldehyde : 20 hours, 13·8; 50 hours, 24·3; 112 hours, 44·8; 250 hours, 65·0; 16 days, 73·0. Only a trace of methylenebisamide was formed.

N-*Acetoxymethyl*-β-*phenylpropionamide* was prepared similarly from *N*-hydroxymethyl-β-phenylpropionamide and separated from benzene–*cyclo*hexane in prisms, m. p. 68—69° (Found : C, 65·5; H, 6·8; N, 6·5. $C_{12}H_{15}O_3N$ requires C, 65·2; H, 6·9; N, 6·3%).

Model Peptides and their Attempted Condensation with Formaldehyde.—1 : 4-Bishydroxymethyl-2 : 5-diketopiperazine was prepared by the action of formaldehyde on diketopiperazine at pH 8·5 (Cherbuliez and Feer, *loc. cit.*), but there was no condensation between diketopiperazine and formaldehyde at pH 0 or 3·6. The dihydroxy-compound (0·5 g.) and phenylacetamide (1·1 g.) were suspended in a buffer solution (15 c.c.) at pH 0 and warmed for a few minutes until solution was obtained. After a further 2 minutes a heavy white precipitate was obtained which was collected and washed with water. Crystallisation from hot ethanol yielded needles (0·85 g.), m. p. 210°, which did not depress the melting point of an authentic specimen of bisphenylacetamidomethane.

Phthaloylglycine chloride was prepared by a modification of the method described by Sheehan and Frank (*J. Amer. Chem. Soc.*, 1949, **71**, 1856). Phthaloylglycine (20 g.) (Gabriel, *Ber.*, 1907, **40**, 2648) and phosphorus pentachloride (20·5 g.) were suspended in dry carbon tetrachloride (150 c.c.) and the mixture heated, with shaking, on a water-bath until the evolution of hydrogen chloride ceased (about 20 minutes). A little undissolved material was filtered off and the carbon tetrachloride removed under reduced pressure. The oily residue soon crystallised on cooling, and recrystallisation from dry benzene gave needles (21 g.), m. p. 82—83° (Sheehan and Frank, *loc. cit.*, give m. p. 85°).

Phthaloylglycine diethylamide. Phthaloylglycine chloride (10·0 g.) was added, with shaking and cooling, during 10 minutes to a solution of diethylamine (25 c.c.) in dry carbon tetrachloride (75 c.c.). After 30 minutes at room temperature the heavy white precipitate was filtered off, washed with carbon tetrachloride and then with water, and dried. The *amide* crystallised from alcohol in needles (11 g.), m. p. 151° (Found : C, 64·3; H, 5·9; N, 10·4. $C_{14}H_{16}O_3N_2$ requires C, 64·6; H, 6·2; N, 10·7%), soluble in toluene or benzene, insoluble in light petroleum (b. p. 60—80°) or carbon tetrachloride.

Phthaloylglycine morpholide, prepared similarly in 85% yield, crystallised from water or alcohol in plates, m. p. 200—201° (Found : C, 61·2; H, 5·3; N, 10·4. $C_{14}H_{14}O_4N_2$ requires C, 61·3; H, 5·1; N, 10·2%), soluble in hot benzene, but insoluble in ligroin or carbon tetrachloride. For preparation of glycine diethylamide, phthaloylglycine diethylamide (4·6 g.) in suspension in 0·16N-alcoholic hydrazine hydrate (120 c.c.) was heated under reflux for 4 hours, the solvent evaporated under reduced pressure, and the syrupy residue heated with 2N-hydrochloric acid (50 c.c.) at 50° for 10 minutes. The phthaloylhydrazide (3·2 g.) was filtered off and the filtrate concentrated under reduced pressure. The *amide hydrochloride* separated and crystallised from hot alcohol as hygroscopic needles, m. p. 101° (Found : Cl, 21·7. $C_6H_{15}ON_2Cl$ requires Cl, 21·4%). *Glycine morpholide hydrochloride*, prepared similarly in 68% yield, crystallised from hot ethanol in hygroscopic needles, m. p. 106° (Found : Cl, 19·4. $C_6H_{13}O_2N_2Cl$ requires Cl, 19·7%).

Phenylacetylglycine diethylamide. Phenylacetyl chloride (2·1 c.c.) was added during 15 minutes to a cooled, stirred solution of glycine diethylamide hydrochloride (1·5 g.) in water (10 c.c.) to which was added magnesium oxide (1·5 g.). After 30 minutes at room temperature the mixture was made acid to Congo-red, and the solid collected, washed with dilute sodium hydroxide solution, and dried. The *phenylacetyl* derivative crystallised from ethanol as plates (1·4 g.), m. p. 84—85° (Found: C, 67·4; H, 8·0; N, 11·6. $C_{14}H_{20}O_2N_2$ requires C, 67·7; H, 8·1; N, 11·3%), soluble in hot benzene or chloroform. The *morpholide*, prepared similarly (75% yield), crystallised from ethanol in stout prisms, m. p. 175° (Found: C, 64·5; H, 6·9; N, 10·5. $C_{14}H_{18}O_3N_2$ requires C, 64·8; H, 6·9; N, 10·7%).

Phenylacetyl-DL-alanine was prepared by a modification of Shiple and Sherwin's method (*J. Biol. Chem.*, 1922, **53**, 472). Phenylacetyl chloride (7·7 c.c.) was added with vigorous stirring during 30 minutes to an ice-cold solution of DL-alanine (5·0 g.) in water (100 c.c.) in which was suspended magnesium oxide (6·0 g.). After a further 15 minutes' stirring the mixture was acidified and the precipitate filtered off, washed with water, and dried. The product was washed with benzene to remove phenylacetic acid and crystallised from hot ethanol, giving plates (10·2 g.), m. p. 150° (Shiple and Sherwin, *loc. cit.*, give m. p. 149—150°). Phenylacetylglycine ethyl ester, prepared (*a*) similarly from glycine ethyl ester hydrochloride or (*b*) by addition of pyridine to a chloroform solution of phenylacetyl chloride and glycine ethyl ester hydrochloride, crystallised from ethanol in needles, m. p. 82° (Found: N, 6·2. Calc. for $C_{12}H_{15}O_3N$: N, 6·3%). Hotter (*loc. cit.*) gives m. p. 79°. When the ester (1 g.) was boiled under reflux for 10 hours with morpholine (2·5 c.c.) and the excess of morpholine removed under reduced pressure only unchanged ester (0·9 g.), m. p. 81°, was obtained.

The following were also prepared: *Phthaloylglycine ethylamide*, colourless needles (from alcohol), m. p. 240° (Found: C, 62·2; H, 5·0; N, 12·0. $C_{12}H_{12}O_3N_2$ requires C, 62·1; H, 5·2; N, 12·1%). *Phthaloyl-DL-alanine morpholide*, colourless prisms (from alcohol), m. p. 172° (Found: C, 62·3; H, 5·5; N, 9·9. $C_{15}H_{16}O_4N_2$ requires C, 62·5; H, 5·6; N, 9·7%), and *diethylamide*, hexagonal prisms (from alcohol), m. p. 85° (Found: C, 65·5; H, 6·2; N, 10·2. $C_{15}H_{18}O_3N_2$ requires C, 65·7; H, 6·5; N, 10·2%). Glycine ethylamide hydrochloride, prisms (from ethanol), m. p. 128—130° (von Braun and Münch, *Ber.*, 1927, **60**, 350, give m. p. 134°) (Found: Cl, 26·0. Calc. for $C_4H_{11}ON_2Cl$: Cl, 25·6%). *DL-Alanine diethylamide hydrochloride*, needles (from ether–alcohol), m. p. 136° (Found: Cl, 20·5. $C_7H_{17}ON_2Cl$ requires Cl, 19·7%). *Phenylacetylglycine ethylamide*, plates (from aqueous methanol), m. p. 170° (Found: C, 65·2; H, 7·3; N, 12·5. $C_{12}H_{16}O_2N_2$ requires C, 65·5; H, 7·3; N, 12·7%). *Phenylacetyl-DL-alanine diethylamide*, prisms (from dilute methanol), m. p. 116° (Found: C, 68·7; H, 8·5; N, 10·3. $C_{15}H_{22}O_2N_2$ requires C, 68·7; H, 8·4; N, 10·7%). *Hippuroylmorpholide*, needles (from aqueous methanol), m. p. 150° (Found: C, 63·2; H, 6·6; N, 10·9. $C_{13}H_{16}O_3N_2$ requires C, 62·9; H, 6·5; N, 11·3%).

Condensations with p-*Cresol and Tyrosine.*—*3-Benzamidomethyl-*p-*cresol* (Me = 1). *N*-Hydroxymethylbenzamide (0·5 g.) and *p*-cresol (0·5 g.) in ethanol (2 c.c.) and concentrated hydrochloric acid (0·05 c.c.) were either (*a*) boiled under reflux for 20 minutes, or (*b*) dissolved and kept overnight. The *product* separated from alcohol in prisms, m. p. 160° (Found: C, 74·5; H, 6·0; N, 6·1. $C_{15}H_{15}O_2N$ requires C, 74·7; H, 6·2; N, 5·8%).

*3-Phenylacetylglycylamidomethyl-*p-*cresol*, similarly prepared, separated from alcohol as prisms, m. p. 154—155° (Found: C, 68·9; H, 6·3; N, 8·6. $C_{18}H_{20}O_3N_2$ requires C, 69·2; H, 6·4; N, 8·9%).

3 : 5-Bishydroxymethyl-N-benzoyltyrosine. N-Benzoyltyrosine (Erlenmeyer and Halsey, *Annalen*, 1899, **307**, 138) (1·0 g.), sodium hydroxide (0·2 g.), 40% formaldehyde (1·0 c.c.), and water (1·0 c.c.) were mixed and set aside for 14 days. The water was then evaporated *in vacuo* over sulphuric acid, leaving a sticky residue which was redissolved in a little water, filtered from a trace of solid, and made just acid to Congo-red. The precipitated *product* (0·6 g.) was crystallised several times from water, and gave colourless plates, m. p. 175—176° (decomp.) (Found: C, 62·8; H, 5·5; N, 4·2. $C_{18}H_{19}O_6N$ requires C, 62·6; H, 5·5; N, 4·1%).

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