## 309. *The Action of Formaldehyde on Proteins.*

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It has been shown, confirming and extending the observations of Einhorn, that amides such as benzamide and phenylacetamide react with formaldehyde at pH 10 to give *N*-hydroxymethyl derivatives which condense with β-naphthol or its 6-bromo- or 3 : 6-dibromo-derivative. The 1-acylamidomethyl-2-naphthols so produced are hydrolysed by acids to 1-aminomethyl-2-naphthol or the corresponding bromine-substitution products.

Many proteins behave similarly with formaldehyde and the *N*-hydroxymethyl derivatives react in the presence of alcoholic hydrochloric acid with β-naphthol and its 6-bromo- or 3 : 6-dibromo-derivative to give condensation products which on hydrolysis yield 1-aminomethyl-2-naphthol or its mono- or its dibromo-derivative. Analysis of the condensation products for bromine provides a measure of the number of *N*-hydroxymethylamide groups and the results are roughly proportional to the number of amide groups in the protein molecule.

It is concluded that, under the alkaline conditions employed, formaldehyde converts some of the amide groups of the protein molecule into *N*-hydroxymethyl derivatives, but that under acidic conditions the formation of *N*-hydroxymethyl derivatives is reduced.

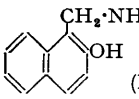
The industrial importance of the products obtained by the action of formaldehyde on proteins has led to a considerable amount of work on the nature of the reactions involved and that done up to 1945 has been reviewed by French and Edsall ("Advances in Protein Chemistry," Vol. II, 1945, p. 277). One of the first proteins to be examined was collagen and it was concluded that formaldehyde condensed with the amino-groups present, forming methylene bridges (K. H. Meyer, *Biochem. Z.*, 1929, **208**, 23). Such a conclusion could not apply to those proteins which are condensed with formaldehyde in an acid medium, in view of the instability of methylenediamines under such conditions (Knudsen, *Ber.*, 1914, **47**, 2698). Later work on other proteins has produced evidence of the interaction of groups other than amino-groups. Carpenter and Lovelace (*Ind. Eng. Chem.*, 1942, **34**, 566) pointed out that casein bound more formaldehyde than could be accounted for by the amino-groups present and suggested that hydroxyl groups, phenolic or alcoholic, also reacted. Other groups suggested as taking part in the reactions are amide (Wormall and Kaye, *J. Soc. Chem. Ind.*, 1945, **67**, 75), glyoxaline (Theis, *J. Biol. Chem.*, 1944, **154**, 87), peptide (Nitschmann and Hadorn, *Helv. Chim. Acta*, 1944, **27**, 299), indole (Frankel-Conrat, Brand, and Clark, *J. Amer. Chem. Soc.*, 1937, **69**, 200), guanidyl (Frankel-Conrat, Cooper, and Olcott, *ibid.*, 1945, **67**, 950), and disulphide (Middlebrook and Philips, *Biochem. J.*, 1942, **36**, 294). Wormall and Kaye (*loc. cit.*) found that casein, zein, and arachin (ground-nut protein) united with more formaldehyde than did the corresponding deamidated proteins and concluded that the amide groups played an important part, but, as collagen and silk fibroin, which contain no amide group, can react with formaldehyde, it is clear that groups other than the amide group participate in the reaction. Much of the evidence brought forward concerning the nature of the reaction between formaldehyde and proteins is based on the uptake of formaldehyde, as determined after liberation by acid hydrolysis and steam-distillation. It is very unlikely that all the combined formaldehyde will be determined in this way ; evidence of irreversibly bound formaldehyde, *i.e.*, formaldehyde not set free by acid hydrolysis, has been adduced (Swain, Kokes, Hipp, Wood, and Jackson, *Ind. Eng. Chem.*, 1948, **40**, 465), and it is known that formaldehyde may form stable compounds with some amino-acids (Frankel-Conrat,

Brandon, and Olcott, *loc. cit.*; Pictet and Sprengler, *Ber.*, 1911, **44**, 2030; Jacobs and Craig, *J. Biol. Chem.*, 1936, **113**, 759), tyrosine and tryptophan being converted in acid solution into *iso*quinoline and carboline derivatives.

The present paper describes attempts to produce evidence of the condensation of formaldehyde with protein-amide groups based on the isolation of compounds, which, it is reasonable to suppose, could only have been formed from such amide–formaldehyde condensation products. Einhorn (*Annalen*, 1905, **343**, 207; 1908, **361**, 113) showed that formaldehyde reacted with amides under acid conditions to give *N*-methylenebisamides of type (I), and under more alkaline conditions to give *N*-hydroxymethylamides of type (II). Compounds of types (I) and (II) were more stable than the corresponding amine derivatives, and several *N*-hydroxy

$$\underset{\text{(I.)}}{R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}R} \qquad \underset{\text{(II.)}}{R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}OH}$$

methylamides were condensed with β-naphthol in cold dilute alcoholic hydrochloric acid to give 1-acylamidomethyl-2-naphthols (III). In the presence of concentrated sulphuric acid, condensation was also effected with anisole, *p*-nitrophenol, quinol, and catechol, but such reaction condition appeared too drastic for use with proteins. We have prepared in good yields con-



(III.)

$$R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}N{<}[CH_2]_5 \quad \text{(IV.)}$$

$$C_6H_5{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}C_6H_5 \quad \text{(V.)}$$

densation products from β-naphthol, with *N*-hydroxymethylbenzamide (II; R = Ph) and N-*hydroxymethylphenylacetamide* (II; R = $CH_2Ph$), and hydrolysed the products, also in good yields, by warm alcoholic hydrochloric acid to 1-aminomethyl-2-naphthol.

The 1-aminomethyl-2-naphthol was not produced when β-naphthol, formaldehyde, and ammonium chloride were allowed to react in cold or warm hydrochloric acid solution, and consequently the 1-amino-2-naphthol cannot be derived from loosely bound formaldehyde and ammonia liberated by hydrolysis of the amides. Similar products prepared from 6-bromo- and 3 : 6-dibromo-2-naphthol yielded 6-*bromo-* and 3 : 6-*dibromo*-1-*aminomethyl*-2-*naphthol* respectively after hydrolysis. Dimethylaniline also reacts with (II; R = Ph) and (II; R = $CH_2Ph$) to yield p-*benz-* and p-*phenylacet-amidomethyldimethylaniline* respectively, and, although these were hydrolysed to *p*-aminomethyldimethylaniline, the yields were not so good as with β-naphthol and its bromo-derivatives. On the other hand, bisbenzanilido- (I; R = Ph) and bisphenylacetamido-methane (I; R = $CH_2Ph$), benzamidomethyl- (IV; R = Ph) and 1-*phenylacetamidomethyl-piperidine* (IV; R = $CH_2Ph$), and *phenylacetamidomethyl benzyl ether* (V) did not react with β-naphthol under the conditions employed for condensation with *N*-hydroxymethylamides, and at higher reaction temperature compounds (I; R = Ph) and (I; R = $CH_2Ph$) gave methylenebis-β-naphthol.

As a result of these model experiments it is obvious that if a protein reacts with formaldehyde to give *N*-hydroxymethylamides (I) then such compounds should condense with β-naphthol and similar phenols to give products capable of hydrolysis to 1-aminomethyl-2-naphthol and related bases. Conversely, the isolation of 1-aminomethyl-2-naphthol from the product of condensation of β-naphthol with a formaldehyde-treated protein, would be strong evidence that the latter contained the group $-CO{\cdot}NH{\cdot}CH_2{\cdot}OH$ or $-CO{\cdot}NH{\cdot}CH_2R$ where R was a group readily removed during condensation under the conditions employed. When arachin (ground-nut protein) was treated with formaldehyde under alkaline conditions and then condensed with β-naphthol or its 6-bromo-derivative, it yielded products which on hydrolysis gave 1-aminomethyl- and 6-bromo-1-aminomethyl-2-naphthol respectively. Similarly, 3 : 6-dibromo-1-aminomethyl-2-naphthol was obtained by hydrolysis of the product prepared from formaldehyde-treated gluten and 3 : 6-dibromo-2-naphthol. These observations are regarded as direct evidence of the formation of *N*-hydroxymethylamide derivatives of the proteins under these conditions. The hydrolysis of the condensation products of β-naphthol, 6-bromo- and 3 : 6-dibromo-2-naphthol with formaldehyde-treated protein produced a complicated mixture from which the corresponding aminomethyl-2-naphthol is only isolated in small yield after a somewhat tedious process. Determination of bromine in the products obtained from formaldehyde-treated protein and 6-bromo-2-naphthol, however, provides a measure of the extent of condensation with *N*-hydroxymethylamides or similar reactive groups and Table I records the result obtained when the bromine determinations were made by Robertson's method (*J.*, 1915, **107**, 902). It was shown in model experiments that alcoholic sulphuric acid could be used instead of hydrochloric acid in

condensing the formaldehyde-treated protein with the bromonaphthols, and this avoided errors caused by occluded hydrochloric acid. Formaldehyde-treated silk fibroin condensed with a negligible amount of 6-bromo-2-naphthol and this is consistent with the absence of amide groups in this protein. The observation that proteins treated with formaldehyde at pH 0 contain only a very low percentage of groups which react with the naphthol was to be expected, as under these acid conditions amides form *N*-methylenebisamides (I) which, as shown above, do not react with β-naphthol. Arachin, glycinin, gliadin, and gluten, treated with formaldehyde in 1% potassium carbonate solution (pH 10) and then condensed with 6-bromo-1-naphthol, show a bromine content corresponding to reaction with approximately 13% of the amide groups present in the protein (see Table I). The discrepancy may be caused by steric factors preventing reactions between formaldehyde and hindered amide groups, or alternatively the reaction between *N*-hydroxymethylamide groups and 6-bromonaphthol may be retarded. This steric explanation finds some support from the experiments, summarised in Table II, with 3 : 6-dibromo-2-naphthol

TABLE I.

*Reactions of formaldehyde-treated proteins with 6-bromo-2-naphthol.*

| Protein. | pH of $CH_2O$ solution. | Found : Br, %. | No. of amide groups/$10^5$ g. of protein which reacted. | No. of amide groups/$10^5$ g. of protein (see Traill, *Chem. & Ind.*, 1950, 23). | % of amide groups which reacted. |
|---|---|---|---|---|---|
| Silk fibroin ..... | 10 | 0·05; 0·08 | — | 0 | — |
| Arachin (ground nut)... | 10 | 1·23; 1·30 | 16·5 | 127 | 13·0 |
| | 0 | 0·40; 0·45 | 5·2 | — | 4·1 |
| Glycinin (soya bean) ... | 10 | 1·21; 1·26 | 15·5 | 119 | 13·0 |
| | 0 | 0·25; 0·28 | 3·2 | — | 2·7 |
| Zein (maize) ..... | 10 | 3·25; 3·30 | 46 | 214 | 21·6 |
| | 0 | 0·22; 0·30 | 3·5 | — | 1·6 |
| Gliadin ..... | 10 | 2·84; 2·78 | 36 | 307 | 11·7 |
| | 0 | 0·49; 0·52 | 5·8 | — | 2·0 |
| Gluten ..... | 10 | 1·92; 2·21 | 31 | 235 | 13·2 |
| Casein ..... | 10 | 0·65; 0·71 | 8·2 | 83 | 9·8 |

TABLE II.

*Reactions of formaldehyde-treated proteins with 3 : 6-dibromo-2-naphthol.*

| Protein. | pH of $CH_2O$ solution. | Found : Br, %. | No. of amide groups/$10^5$ g. of protein treated. | No. of amide groups/$10^5$ g. of protein. | % of amide groups which reacted. |
|---|---|---|---|---|---|
| Arachin (ground nut)... | 10 | 1·8 | 12·5 | 127 | 9·8 |
| | 0 | 0·4 | 3·2 | — | — |
| Gliadin ..... | 10 | 4·1 | 28 | 307 | 9·1 |
| | 0 | 0·9 | 6·5 | — | 2·1 |
| Zein ..... | 10 | 3·5 | 23 | 214 | 10·1 |
| | 0 | 0·41 | 3·3 | — | 1·6 |
| Gluten ..... | 10 | 3·3 | 22·5 | 235 | 9·6 |

TABLE III.

*Reaction of formaldehyde-treated arachin (ground-nut protein) under varying alkaline conditions with 6-bromo-2-naphthol.*

| $K_2CO_3$ in the hardening solution, %. | Found : Br, %. | No. of amide groups/$10^5$ g. of protein treated. | No. of amide groups/$10^5$ g. protein. | % of amide groups which reacted. |
|---|---|---|---|---|
| 1 | 1·2; 1·3 | 16·5 | 127 | 13·0 |
| 2·5 | 1·6; 1·7 | 23 | 127 | 18·0 |
| 5 | 2·2; 2·3 | 31 | 127 | 24·4 |
| 7·5 | 2·2; 2·5 | 31 | 127 | 24·4 |
| 10 | 2·5; 2·6 | 35 | 127 | 28·0 |

and formaldehyde-treated proteins. The results are similar to those reported above in Table I, but the heavier naphthol derivative employed in this case inhibits the reaction, and the bromine contents at pH 10 correspond to reaction with only 10% of the amide groups present in the protein. The high values for zein, particularly the value with 6-bromo-2-naphthol, are difficult to explain; formaldehyde-treated zein dissolves in the alcohol during treatment with the bromonaphthols possibly with greater exposure of *N*-hydroxymethylamide groups to attack.

When the concentration of the potassium carbonate used in condensing the protein and formaldehyde is raised from 1 to 10% the number of hydroxymethyl groups, as measured by the bromine present after condensation with 6-bromo-2-naphthol, increases. It was observed that the protein was more dispersed in more concentrated potassium carbonate solution, and this factor, which is under further consideration, may be one facilitating reaction. Table III summarises these results.

## Experimental.

*N-Hydroxymethylphenylacetamide* (II; R = $CH_2Ph$).—A suspension of phenylacetamide (50 g.) in 4% potassium carbonate solution (50 c.c.) and 40% formaldehyde solution (40 c.c.) was warmed on a water-bath for a few minutes until dissolution was complete. The *N-hydroxymethyl* compound which separated on cooling was collected after 12 hours, washed first with a little dilute sodium hydroxide solution and then with water, and dried. It crystallised from toluene in colourless plates (51 g.), m. p. 78° (Found: C, 65·8; H, 6·65; N, 8·4. $C_9H_{11}O_2N$ requires C, 65·4; H, 6·7; N, 8·5%), which were soluble in hot water, alcohol, or benzene.

*1-Phenylacetamidomethyl-2-naphthol* (III; R = $CH_2Ph$).—A solution of *N*-hydroxymethylphenylacetamide (5 g.) and β-naphthol (5 g.) in alcohol (50 c.c.) containing concentrated hydrochloric acid (1 c.c.) or 50% sulphuric acid (1 c.c.) was refluxed for 20 minutes. After cooling, the product was collected, and a further crop obtained by concentrating the liquors. Recrystallisation from hot alcohol gave *1-phenylacetamidomethyl-2-naphthol* in long colourless needles (7·4 g.), m. p. 141° (Found: C, 78·0; H, 5·8; N, 5·0. $C_{19}H_{17}O_2N$ requires C, 78·3; H, 5·8; N, 4·8%). Instead of by refluxing as described above, the reaction may be carried out by keeping the mixture for 4 days at room temperature.

*6-Bromo-1-phenylacetamidomethyl-2-naphthol*, prepared similarly in 85% yield from 6-bromo-2-naphthol (Franzen and Stauble, *J. pr. Chem.*, 1922, **103**, 352), crystallised from alcohol in long needles, m. p. 184° (Found: C, 61·7; H, 4·4; N, 3·5. $C_{19}H_{16}O_2NBr$ requires C, 61·6; H, 4·3; N, 3·8%).

*6-Bromo-1-benzamidomethyl-2-naphthol*, prepared similarly in 90% yield from *N*-hydroxymethylbenzamide (Einhorn, *loc. cit.*), separated from alcohol in colourless needles, m. p. 219—220° (Found: C, 60·3; H, 3·8; Br, 22·8; N, 4·0. $C_{18}H_{14}O_2NBr$ requires C, 60·7; H, 3·9; Br, 22·5; N, 3·9%).

3:6-Dibromo-2-naphthol was prepared by the following modification of the method described by Franzen and Stauble (*loc. cit.*). A solution of bromine (160 g.) in acetic acid (60 c.c.) was added with shaking and cooling at 25—30° during 30 minutes to a solution of β-naphthol (72 g.) in acetic acid (220 c.c.). After 2 hours at room temperature the suspension of 1:6-dibromo-2-naphthol which had formed was warmed to 40—50°, and a solution of bromine (96 g.) in acetic acid (30 c.c.) added rapidly. After 2 hours on the water-bath, the product was cooled, collected, washed with acetic acid (250 c.c.) and then water, and dried in air. This crude 1:3:6-tribromo-2-naphthol (160 g.), m. p. 110—122° (Franzen and Stauble, *loc. cit.*, gave m. p. 133° for 1:3:6-tribromo-2-naphthol containing 1 mole of acetic acid), was refluxed with acetic anhydride (700 c.c.) for 3 hours. When the reaction mixture cooled to room temperature, 1:3:6-tribromo-2-naphthyl acetate (97 g.), m. p. 183—185°, separated; after crystallisation from benzene it had m. p. 187—188° (Franzen and Stauble, *loc. cit.*, give m. p. 188°). 1:3:6-Tribromo-2-naphthyl acetate (75 g.) was reduced by refluxing it for 6 hours with tin (70 g.), concentrated hydrochloric acid (200 c.c.), and alcohol (500 c.c.), and after decantation from unused tin the solution was concentrated under reduced pressure to half its volume and poured into water (2 l.). 3:6-Dibromo-2-naphthol (52 g.), m. p. 129—131°, separated and after crystallisation from benzene had m. p. 133—134° (Found: Br, 53·1. Calc. for $C_{10}H_6OBr_2$: Br, 52·9%) (Franzen and Stauble, *loc. cit.*, give m. p. 135°).

*3:6-Dibromo-1-benzamidomethyl-2-naphthol*, prepared in the usual way, crystallised from alcohol in stout colourless prisms, m. p. 146° (Found: C, 49·3; H, 3·2; N, 2·9. $C_{18}H_{13}O_2NBr_2$ requires C, 49·7; H, 3·0; N, 3·2%).

*3:6-Dibromo-1-phenylacetamidomethyl-2-naphthol*, prepared similarly in 80% yield, crystallised from alcohol in long colourless needles, m. p. 218° (Found: C, 50·8; H, 3·5; Br, 35·4. $C_{19}H_{15}O_2NBr_2$ requires C, 50·8; H, 3·3; Br, 35·6%).

*p-Benzamidomethyl-NN-dimethylaniline hydrochloride*, prepared from *N*-hydroxymethylbenzamide (0·5 g.) and dimethylaniline (0·4 g.) in alcohol (5 c.c.) containing concentrated hydrochloric acid (1 c.c.), separated from alcohol in long colourless needles (0·22 g.) after 20 days' storage at room temperature and had m. p. 183° (Found: C, 66·5; H, 6·8; N, 9·9. $C_{16}H_{19}ON_2Cl$ requires C, 66·1; H, 6·5; N, 9·6%).

*p-Phenylacetamidomethyl-NN-dimethylaniline hydrochloride*, prepared similarly, separated from alcohol in colourless needles, m. p. 202° (Found: C, 66·5; H, 6·5; N, 9·4. $C_{17}H_{21}ON_2Cl$ requires C, 67·0; H, 6·9; N, 9·2%).

### *Hydrolysis of 1-acylamido-2-naphthol and derivatives.*

*1-Aminomethyl-2-naphthol.*—The following is a typical experiment. 1-Phenylacetamidomethyl-2-naphthol (5 g.) was refluxed for 3 hours with alcohol (100 c.c.) and concentrated hydrochloric acid (30 c.c.). The crystalline material which separated on cooling was collected, washed with alcohol, dried, and crystallised from hot water; 1-aminomethyl-2-naphthol hydrochloride (2·0 g.) separated in long needles, m. p. 224—225° (decomp.) (Found: C, 63·1; H, 5·7; N, 6·7. Calc. for $C_{11}H_{12}ONCl$: C, 63·0; H, 5·7; N, 6·7%) [Mario Betti, *Gazzetta*, 1906, **36** (1), 388, and Einhorn, *loc. cit.*, give m. p. 224—225° (decomp.)]. The *ditoluene-p-sulphonyl* derivative, prepared by warming, for 15 minutes on the water-bath, a mixture of the hydrochloride (0·2 g.), 10% sodium hydroxide solution (5 c.c.), and toluene-*p*-sulphonyl chloride (0·5 g.), crystallised from alcohol in stout prisms, m. p. 198° (Found: C, 62·7; H, 4·8; N, 3·1. $C_{25}H_{23}O_5NS_2$ requires C, 62·4; H, 4·8; N, 2·9%).

Hydrolysis of 1-benzamidomethyl-2-naphthol and *NN'*-di-(2-hydroxy-1-naphthyl)succinamide, m. p. 218° (Einhorn, *loc. cit.*), to 1-aminomethyl-2-naphthol were effected similarly.

*6-Bromo-1-aminomethyl-2-naphthol.*—The *hydrochloride* prepared similarly in 80% yield from 6-bromo-1-benz- or 6-bromo-1-phenylacet-amido-2-naphthol, crystallised from hot water in colourless silky

needles, m. p. 248° (decomp.) (Found : C, 46·1; H, 3·8; N, 4·85. $C_{11}H_{11}ONClBr$ requires C, 45·7; H, 3·8; N, 4·85%), which gradually became pink on exposure to the air. The *base*, liberated by treatment with aqueous sodium carbonate and isolated with ether, crystallised from alcohol in colourless prisms, m. p. 153° (Found : C, 52·8; H, 4·2; N, 5·7. $C_{11}H_{10}ONBr$ requires C, 52·6; H, 4·9; N, 5·5%), which rapidly became pink. The *ditoluene*-p-*sulphonyl* derivative crystallised from alcohol in thick colourless prisms, m. p. 208° (Found : N, 2·7; Br, 14·7. $C_{25}H_{22}O_5NBrS_2$ requires N, 2·5; Br, 14·3%).

**3** : 6-*Dibromo*-1-*aminomethyl*-2-*naphthol*.—The *hydrochloride*, prepared similarly from 3 : 6-dibromo-1-benz- or 3 : 6-dibromo-1-phenylacet-amidomethyl-2-naphthol, separated from hot water in long colourless needles, m. p. 235° (Found : C, 36·3; H, 2·8; N, 3·7. $C_{11}H_{10}ONClBr_2$ requires C, 35·9; H, 2·7; N, 3·8%), which became faintly pink on exposure to air. The *ditoluene*-p-*sulphonyl* derivative crystallised from alcohol in short thick needles, m. p. 199—200° (Found : C, 47·3; H, 3·5; Br, 24·9. $C_{25}H_{21}O_5NBr_2S_2$ requires C, 46·9; H, 3·3; Br, 25·0%).

1-*Phenylacetamidomethylpiperidine* (IV; R = $CH_2Ph$), prepared by refluxing *N*-hydroxymethylphenylacetamide (2 g.) and piperidine (1·7 c.c.) in methyl alcohol (10 c.c.) for 3 hours, crystallised from alcohol in slender needles (2·2 g.), m. p. 128° (Found : C, 72·5; H, 8·4; N, 12·4. $C_{14}H_{20}ON_2$ requires C, 72·4; H, 8·6; N, 12·1%). This compound and also benzamidomethylpiperidine (IV; R = Ph), m. p. 122° (Einhorn, *loc. cit.*), were recovered after treatment with β-naphthol and 4% alcoholic hydrogen chloride at room temperature for 14 days.

*Benzyl Phenylacetamidomethyl Ether* (V).—*N*-Hydroxymethylphenylacetamide (3 g.) was heated for 6 hours in an oil-bath at 105—110° with benzyl alcohol (10 c.c.) containing 80% formic acid (0·5 c.c.). After removal of excess of benzyl alcohol under reduced pressure, the residue solidified on cooling and crystallised from alcohol in colourless leaflets (3·4 g.), 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, 6·7; N, 5·5%). This ether was recovered after treatment with β-naphthol and 4% alcoholic hydrochloric acid for 14 days at room temperature.

*Bisphenylacetamidomethane* (I; R = $CH_2Ph$).—Phenylacetamide (5 g.), suspended in a solution at pH 0 containing saturated sodium sulphate (37·5 c.c.), concentrated sulphuric acid (11 c.c.), and 40% formaldehyde (2·5 c.c.), was heated on a water-bath. Dissolution was rapid and after a few minutes' heating the heavy white precipitate produced was collected, washed, and crystallised from alcohol; it formed long colourless needles (4·6 g.), m. p. 210° (Found : C, 72·2; H, 6·2; N, 10·4. Calc. for $C_{17}H_{18}O_2N_2$ : C, 72·3; H, 6·4; N, 10·1%). Hepp (*Ber.*, 1877, **10**, 1650), who prepared this compound from benzyl cyanide and methylal in presence of acetic–sulphuric acid, gives m. p. 205°. The compound did not react with β-naphthol in presence of cold 4% alcoholic hydrochloric acid during 14 days at room temperature, but after the mixture had been refluxed methylenebis-β-naphthol, m. p. 197°, was obtained.

*Bisbenzamidomethane* (I; R = Ph), m. p. 216° (Einhorn, *loc. cit.*, gives 216°), prepared similarly, gave methylenebis-β-naphthol after 10 hours' refluxing with β-naphthol in alcoholic hydrochloric acid.

*Isolation of* 1-*Aminomethyl*-2-*naphthol and its Bromo-derivatives from the* 2-*Naphthol and the Bromo*-2-*naphthol compounds of Formaldehyde-treated Arachin (Ground-nut Protein) and Gluten.—Arachin (ground-nut) protein.* This was supplied by Imperial Chemical Industries Limited, Nobel Division, and had been prepared in the following way. The oil was removed from crushed blanched ground-nuts by solvent extraction, and the protein extracted from the resultant meal by means of 0·15% aqueous sodium hydroxide. The clarified extract was brought to pH 5·0 with sulphur dioxide, and the precipitated protein washed with water and spray-dried.

*Reaction.* Arachin (50 g.) was stirred for 90 minutes at 60—70° with 40% formaldehyde solution (200 c.c.), potassium carbonate (2·5 g.), and water (200 c.c.). The slime was collected, drained, and suspended in water (400 c.c.) containing a little sulphuric acid to coagulate the protein and facilitate filtration. After the protein had been washed with water and alcohol, the alcohol-wet product was suspended in alcohol (500 c.c.) containing concentrated hydrochloric acid (50 c.c.) and either (*a*) β-naphthol (25 g.) or (*b*) 6-bromo-2-naphthol (25 g.).

After being shaken at room temperature for 17 days, the protein was collected, washed with alcohol, dried, and refluxed, with stirring, for 5 hours with alcohol (400 c.c.) and concentrated hydrochloric acid (100 c.c.) (*A*). The solid (partly hydrolysed protein) was collected, the filtrate neutralised with solid sodium hydrogen carbonate, and the sodium chloride removed by filtration and the alcohol under reduced pressure. The residue was dissolved in hot ether (350 c.c.), dried ($Na_2SO_4$), recovered (0·5 g.), dissolved in dilute hydrochloric acid (40 c.c.), and filtered. The filtrate, was extracted with ether (3 × 25 c.c.) to remove non-basic compounds, and neutralised with sodium carbonate, and the liberated base extracted with ether (*B*). In case (*a*) removal of the ether (*B*) gave a brown viscous oil, which solidified on cooling and was identified as 1-aminomethyl-2-naphthol by conversion into the hydrochloride (35 mg.), m. p. 220—222° (decomp.), and the ditoluene-*p*-sulphonyl derivative, m. p. 198°, identical with the derivatives described on p. 1496. In case (*b*) removal of the ether (*B*) yielded a brown solid (20 mg.), m. p. 135—145°, raised to 155° by crystallisation from alcohol, which was identified as 6-bromo-1-aminomethyl-2-naphthol by comparison with the specimen prepared as described above and by conversion into the ditoluene-*p*-sulphonyl derivative, m. p. 208°. Gluten (40 g.) was similarly treated with 3 : 6-dibromo-2-naphthol (10 g.) to stage (*A*). The solution which was obtained on hydrolysis was filtered to remove a small amount of insoluble material and neutralised with solid sodium hydrogen carbonate. The precipitate was collected and the sodium chloride dissolved in water, leaving a residue (1·5 g.). This insoluble material was dissolved in aqueous sodium hydroxide and treated with toluene-*p*-sulphonyl chloride in the usual way, and the product separating from the alkaline solution was collected (30 mg.). Several crystallisations from alcohol gave the ditoluene-*p*-sulphonyl derivative of 3 : 6-dibromo-1-aminomethyl-2 naphthol, m. p. 198—199°, identical with the specimen prepared as above.

*Reactions of formaldehyde-treated proteins with* 6-*bromo- and* 3 : 6-*dibromo*-2-*naphthol.*

*Reactions of Formaldehyde with Proteins at* pH 10.—The protein (10 g.) was stirred for 30 minutes at 60—70° with a solution (pH 10) composed of 40% formaldehyde (10 c.c.), potassium carbonate (1 g.), and water (90 c.c.). The resulting slime was filtered off, poured into 0·5% sulphuric acid, and collected. The

precipitate was well washed with water, dried for several days, and powdered. Arachin was also condensed with formaldehyde in the presence of 2·5, 5·0, 7·5, and 10·0% of potassium carbonate. The products were worked up in the usual way and condensed with 6-bromo-2-naphthol, and the condensation product was purified and analysed for bromine.

*Reactions of Formaldehyde with Proteins at* pH 0.—Arachin, glycinin, gliadin, and zein (10 g.) were stirred as above at 60—70° with a solution at pH 0 composed of saturated sodium sulphate solution (73 c.c.), concentrated sulphuric acid (22 c.c.), and 40% formaldehyde (5 c.c.). The powdery products were easily collected, washed with water, and dried in a vacuum desiccator.

*Reactions of Hardened Proteins with* (i) 6-*Bromo*-2-*naphthol and* (ii) 3 : 6-*Dibromo*-2-*naphthol.*—Powdered and hardened protein (2 g.) was suspended in alcohol (10 c.c.) containing 50% sulphuric acid (1 c.c.) and 6-bromo-2-naphthol (1 g.) or 3 : 6-dibromo-2-naphthol (1·5 g.). After 14 days at room temperature, the product was collected and washed with alcohol, and unchanged naphthol removed by extraction for 8 hours with ether in a Soxhlet apparatus. The protein was finally washed well with water, dried for 3—4 hours at 110—120°, and powdered before the bromine determination. Zein, hardened at pH 0, was soluble in alcohol and the following procedure was adopted. Zein (2 g.) (hardened at pH 0), 6-bromo- or 3 : 6-dibromo-2-naphthol (1 g.), and alcohol (20 c.c.) containing 50% sulphuric acid were allowed to react for 18 days at room temperature. The protein was precipitated by dropping the solution with rapid stirring into ether, and the white resinous product was collected, washed well with ether, and dried at 80° for 1 hour. The clear hard resin was redissolved in alcohol, recovered by pouring the solution into ether, and dried first at 80° for 1 hour and finally at 110—120° for 4 hours before analysis.

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