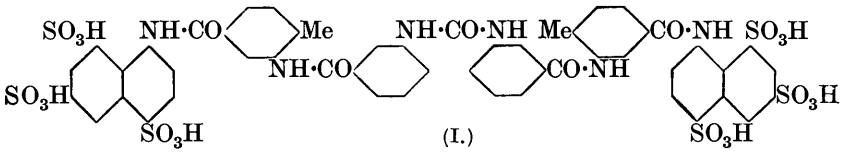
CCCCVII.—*Trypanocidal Action and Chemical Constitution. Part VII.* s-*Carbamides and Arylamides of Naphthylamine-di- and -tri-sulphonic Acids with Some Observations on the Mesomorphic State.*

By ISIDORE ELKANAH BALABAN and HAROLD KING.

THE discovery of the substance known as Bayer 205 marks an epoch both in the development of chemotherapy and in the treatment of trypanosomiasis. The chemical identity of this substance remained for long undivulged owing to war conditions, but the group of substances of which it is a member was plainly covered by a series of patents taken out by the firm of Bayer from 1914 onwards (D.R.-P. 278122, etc.). The structure was elucidated by Fourneau, Tréfouel, and Vallée (*Ann. Inst. Pasteur*, 1924, **38**, 81), who showed that Bayer 205 was in all probability the *s*-carbamide of *m'*-aminobenzoyl-*m*-aminotoluoyl-1-naphthylamine-4 : 6 : 8-trisulphonic acid (I).



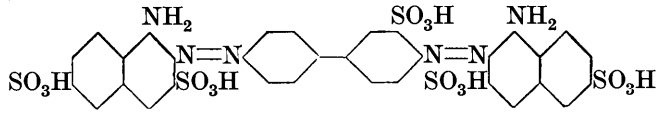
(I.)

It is natural that the discovery of an intensely trypanocidal agent like Bayer 205, colourless and containing no metallic group to which its toxic properties might be referred, should stimulate enquiry into the general principles governing its mode of action. On this point the patents are not informative, but Heymann (*Z. angew. Chem.*, 1924, **37**, 585) and Röhl (*Arch. Schiff. Trop. Hyg.*, 1926, **30**, *Beiheft* **1**, 103), both of the firm of Bayer and representing respectively the chemical and the pharmacological side, have contributed considerably to an insight into the general principles involved.

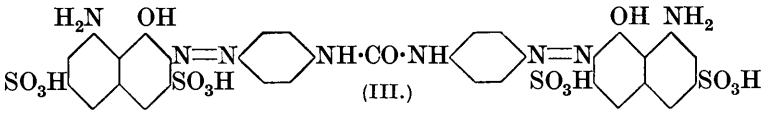
As early as 1904 Ehrlich and Shiga made the fundamental dis-

covery that Trypan-red (II), a member of the Congo-red series of cotton dyes, can cure an experimental infection of trypanosomiasis in mice.

(II.)



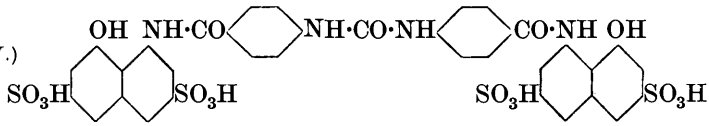
Following on this, Nicolle and Mesnil, in a classic paper (*Ann. Inst. Pasteur*, 1906, **20**, 417), examined a long range of cotton dyes all belonging to the Congo-red type and found the substance having the best curative action on infection with *Trypanosoma gambiense* in rats and monkeys to be the *s*-carbamide (III), a derivative of 1 : 8-aminonaphthol-3 : 6-disulphonic acid (H-acid) called Afridol Violet.



(III.)

There are obvious disadvantages in dyes as therapeutic agents when applied to man or cattle, and if the therapeutic action of dyes is due to fixation by tissues it should be possible to prepare colourless substances of similar build and similar properties. The chemists of the firm of Bayer therefore chose as their starting point H-acid and prepared (IV), the colourless analogue of (III).

(IV.)



This substance delayed the death of mice suffering from trypanosomiasis by one day. Now from contemporaneous work on *dyes* it was known that substantive character to cotton was favoured by the presence of chains of aminobenzoyl groups and by the linking of these by carbonyl chloride. When, therefore, the aminobenzoyl groups in (IV) were duplicated in chain formation there was an increase of activity, but a falling off when they were triplicated. By chemical evolution from this striking discovery there resulted the gradual elaboration of the substance Bayer 205. It is quite clear from Heymann's account that the discovery of Bayer 205 arose from the recognition of the fact that it is possible to prepare colourless substances of substantive properties, which, being of similar molecular build to trypanocidally active dyes, also exhibit trypanocidal properties.

Röhl (*loc. cit.*), while also recognising the substantive properties

of these colourless substances, made the significant observation that these colourless complex carbamides form precipitates at very high dilutions with some of the simplest basic proteins, *e.g.*, clupeine, salmine, thymine, and sturine—constituents of the spermatozoa of fish. As a consequence, he supposes that the action of Bayer 205 on trypanosomes is due to a combination of the sulphonic acid groups with basic cell constituents of the parasites forming difficultly soluble salts.

Although the outline of a plausible picture of the principles underlying the action of Bayer 205 has been presented, no experimental evidence has been adduced in support of it. The work detailed in this communication was therefore undertaken in order to familiarise ourselves with this important field and to test the views which have been advanced.

For this purpose six readily accessible naphthylaminedisulphonic acids have been used as a starting point, *viz.*, Freund's acid (1-naphthylamine-3 : 6-disulphonic acid), amino-G-acid (2-naphthylamine-6 : 8-disulphonic acid), C-acid (2-naphthylamine-4 : 8-disulphonic acid), amino-J-acid (2-naphthylamine-5 : 7-disulphonic acid), H-acid (8-hydroxy-1-naphthylamine-3 : 6-disulphonic acid), and 2R-acid (8-hydroxy-2-naphthylamine-3 : 6-disulphonic acid).

These with the exception of H-acid have been converted into their *s*-carbamides, H-acid under the same conditions yielding the internal *ON*-carbonyl derivative, 1 : 8-ON-*carbonylaminonaphthol*-3 : 6-*disulphonic acid*. They have also been converted into their *m*-nitrobenzoyl derivatives and these in turn into the *m*-aminobenzoyl derivatives. The latter have been phosgenated to form the *s*-carbamides and have also been converted into the *m'*-nitrobenzoyl-*m*-aminobenzoyl derivatives. The latter have finally been reduced to the *m'*-aminobenzoyl-*m*-aminobenzoyl derivatives, which on phosgenation have given the *s*-carbamides. The general scheme of synthesis is shown below :

$$\begin{array}{rl} & NH_2R \longrightarrow RNH\cdot CO\cdot NHR \\ & NO_2\cdot Bz\cdot NHR \quad (\textit{First carbamide}) \\ \text{(1st)} & NH_2\cdot Bz\cdot NHR \longrightarrow RNH\cdot Bz\cdot NH\cdot CO\cdot NH\cdot Bz\cdot NHR \\ \downarrow & NO_2\cdot Bz\cdot NH\cdot Bz\cdot NHR \quad (\textit{Second carbamide}) \\ \text{(2nd)} & NH_2\cdot Bz\cdot NHBz\cdot NHR \longrightarrow RNH\cdot BzNH\cdot BzNH\cdot CO\cdot NHBz\cdot NHBz\cdot NHR \\ & \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad (\textit{Third carbamide}) \end{array}$$

$$(Bz = \cdot C_6H_4\cdot CO\cdot)$$

These have all been isolated in a state of purity free from sodium chloride and, excluding the nitro-compounds, have been tested for toxicity on normal mice and for trypanocidal action on mice infected experimentally with *T. equiperdum*. The maximum doses

tolerated by mice expressed in milligrams per gram of mouse are given in the following table :

	Aminobenzoyl derivatives.		Carbamides.		
	First.	Second.	First.	Second.	Third.
Freund's Acid	4·0	3·25	>2·0	1·5	0·4
Amino-G-Acid	1·5	1·0	1·75	**0·5**	**0·2**
C-Acid	3·0	1·0	2·0	**1·0**	**0·3**
Amino-J-Acid	3·0	0·75	1·75	**1·0**	0·3
H-Acid	2·25	1·0	3·0 *	**0·3**	**0·3**
2R-Acid	>2·5	0·7	2·0	0·1	0·2

* *ON*-Carbonyl derivative.

It will be observed that there is a very rough parallelism between the corresponding derivatives of different acids. The only compounds showing any trypanocidal action, and that of a low order as indicated below, are those carbamides the tolerated doses of which are expressed in heavy type. For these, the curative action is shown in the following table, each substance as a rule being tested on 4 or 5 infected mice, and r signifying the number of days before relapse occurred in the most favourable cases :

	Second carbamide.	Third carbamide.
Amino-G-Acid	0·3, $r = 4$	0·2, $r = 7$
C-Acid	0·5, $r = 5$	0·3, $r = 12$
Amino-J-Acid	0·5, $r = 1$	No action
H-Acid	0·2, $r = 5$	0·175, $r = 5$

The striking fact emerges from these results that trypanocidal activity only appears at the *s*-carbamide stage of combination of the aminobenzoyl and aminobenzoylaminobenzoyl derivatives and that there is no trace of activity in any of the aminobenzoyl compounds. All the derivatives, moreover, of Freund's acid and of 2R-acid examined were inactive. This is contrary to the claim made in D.R.-P. 288273, where the *s*-carbamide of *m'*-aminobenzoyl-*m*-aminobenzoyl-1-naphthylamine-3 : 6-disulphonic acid is said to cause the disappearance of trypanosomes from the blood of infected animals at high dilutions. This particular substance had not the slightest detectable action on the course of infection of *T. equiperdum* in mice, and trypanosomes exposed to 1% concentration of it for 24 hours *in vitro* were not rendered non-infective by it.

As the efficacy of the above-mentioned active carbamides was of a very low order, it was deemed of interest to investigate the corresponding series of compounds from the parent 1-naphthylamine-4 : 6 : 8-trisulphonic acid * contained in Bayer 205, particularly as Fourneau *et alii* (*loc. cit.*) have recorded a chemotherapeutic index, C/T, of 1/12 for the *s*-carbamide of *m'*-aminobenzoyl-

* For brevity of nomenclature, this acid is hereinafter referred to as B-Acid, following Fourneau (*loc. cit.*).

m-aminobenzoyl-1-naphthylamine-4 : 6 : 8-trisulphonic acid, and Röhl (*loc. cit.*) states that the simple *s*-carbamide of B-acid is weakly active.

The following table gives the tolerated and the curative doses observed for the parallel series of derivatives of B-acid.

		Aminobenzoyl derivatives.		Carbamides.		
	Acid.	First.	Second.	First.	Second.	Third.
Dosis tolerata ...	3·0	2·0	1·5	>4·0	1·0	0·3
Dosis curativa ...	None	None	None	4·0, $r > 30$ 2·5, $r = 15$	None	0·15, $r > 30$

This table supports the main conclusion drawn from the previous tables that trypanocidal activity is only found in the *s*-carbamides and is absent from the parent acids and their aminobenzoylated derivatives.

Substantive Properties and Trypanocidal Action.

Although the recognition of the substantivity of colourless substances, *i.e.*, their affinity for natural fibres, was in the minds of the discoverers of Bayer 205 and led to its development, the question arises whether the therapeutic action of Bayer 205 or any of its analogues is due to this substantive property. Fortunately, this can, at least to some extent, be submitted to the test of experiment.

As the result of preliminary trials on the dyeing at 100° of pure wool, silk, and cotton by two members of the Congo-red series of dyes, namely, Trypan-red and Trypan-blue, with and without the use of " assistants," it was found that cotton was the most suitable fibre and sodium chloride the best " assistant." The same conditions could now be applied to the invisible dyeing of cotton by the colourless series of substances synthesised and tested for trypanocidal activity as related above. A limitation is, however, imposed in that it is only possible to " develop " the invisibly dyed cotton in those instances where there is a free naphthol group present. This limits the applicability of this mode of detection of the degree of substantivity of these colourless substances to the derivatives of H-acid and 2R-acid. Accordingly each substance (0·01 g.) mentioned in the table below was dissolved in water (10 c.c.), sodium chloride (0·5 g.) added, and a cotton skein (0·5 g.) immersed in the solution. The solution was heated at 100° for 1½ hours; the cotton was then removed and washed five times by decantation with water, 50 c.c. each time, and the colour was developed on the cotton by immersion in neutral diazotised *p*-nitroaniline at 12° as recommended for developing colour on cotton in

dyeing practice. After thorough washing, the skeins of cotton were dried, and the colour intensities compared by means of a Lovibond tintometer. The same conditions were used with derivatives of 2R-acid except that 2·5% sodium chloride was used, higher concentrations having a salting-out effect even at 100°. The following table gives the relative colour intensities of the dyed skeins of cotton expressed in terms of the content of naphthol, the assumption being made that the azo-compounds formed from a given acid and its derivatives are all analogously constituted and of equal tinctorial value per unit naphthol molecule. The free acids, their first carbamides, and their first nitrobenzoyl derivatives are omitted from the table, as they were under these conditions devoid of substantive property.

	Aminobenzoyl derivatives.		Nitrobenzoyl derivatives.	Carbamides.	
	First.	Second.	Second.	Second.	Third.
H-Acid	1	2	1	4	6
2R-Acid	4	5	2	9	12

The salient feature of this table is the pronounced maximum of substantivity to cotton at the *s*-carbamide stage of combination exhibited by these colourless substances, which are built up on the type or model of the Congo-red series of cotton dyes. Now it is precisely at the *s*-carbamide stage of combination that trypanocidal activity appears, so that there seems to be a parallelism between the substantive property of these carbamides to cotton and their trypanocidal activity. When the same conditions were applied to wool there was no selective substantivity exhibited.

In further support of this parallelism, it has been shown that the most active of all trypanocidal agents, Bayer 205, is also substantive to cotton. This cannot be demonstrated by development on the fibre, since Bayer 205 will not couple with diazotised amines, but it can be effected by making use of the observations of Röhl that these complex carbamides form precipitates with some of the simplest basic proteins at high dilutions. For this purpose, clupeine sulphate was chosen, as it is readily obtained from herrings' roe. The sensitiveness of the test for the detection of Bayer 205 by clupeine sulphate is not very great, but it may be increased by addition of 20% sodium chloride solution, as is shown in the following table :

Dilutions of Bayer 205	1 in 5,000	1 in 10,000	1 in 50,000	1 in 100,000
1 C.c. + 2 drops 1% clupeine sulphate	Ppt.	Turbid	Clear	Clear
Bayer 205 + 20% NaCl	1 in 5,000	1 in 10,000	1 in 50,000	1 in 100,000
1 C.c. + 2 drops clupeine sulphate...	Ppt.	Ppt.	Turbid	Faint turbidity

Higher concentrations of sodium chloride have a salting-out effect on the clupeine sulphate.

The absorption of Bayer 205 by cotton was investigated by means of this reaction in the following way : Skeins of cotton (0·1 g.) were extracted in succession with pure ether, absolute alcohol, and several changes of boiling water and then dried. They were added to a solution of Bayer 205 (1·5 c.c.) containing sodium chloride (0·3 g.) and heated at 100° in stoppered tubes for an hour. When cool, 1 c.c. was removed and treated with 2 drops of 1% clupeine sulphate. The results of a series of experiments with controls are in the following table :

Tube.	Contents.	Reagent 2 drops clupeine sulphate.
1, 2	1·5 c.c. of 1 in 50,000 Bayer 205 + 0·3 g. NaCl + 0·1 g. cotton.	Solution clear.
3, 4	1·5 c.c. of 1 in 50,000 Bayer 205 + 0·3 g. NaCl.	Standard turbidity for this dilution.
5, 6	1·5 c.c. of water + 0·1 g. cotton + 0·3 g. NaCl.	Solution clear.

It therefore follows that Bayer 205 at a dilution of 1 in 50,000 is removed by cotton in the presence of sodium chloride, the concentration of Bayer 205 falling below 1 in 100,000. It would be of great interest to apply this method of detecting the substantive property to cotton of these colourless substances to the trypanocidally active series of B-acid described above. Unfortunately, it is extremely difficult, especially with limited supplies of material, to free them from sodium chloride, the uncontrolled presence of which would interfere seriously with the tests.

It was naturally of interest to determine whether the variation of the substantive property with structure was paralleled by the insolubility of the complexes formed between the protamines and the sulphonic acids. For this purpose, the *m*-aminobenzoyl and *m'*-aminobenzoyl-*m*-aminobenzoyl derivatives of H-acid and 2R-acid and the corresponding *s*-carbamides were compared in respect of their precipitability at high dilutions by clupeine sulphate. It was found that the *s*-carbamides did not form complexes of outstanding insolubility, but rather that they were very slightly more soluble than the complexes with the corresponding amines.

It may therefore be suggested on the basis of the limited number of examples examined by us that there appears to be a rough parallelism between the substantivity to cotton and trypanocidal action *in vivo*, in that salient peaks of substantivity occur at the *s*-carbamide stage of combination and trypanocidal action is only found in the *s*-carbamides. The interpretation of this is probably that substantivity to cotton is paralleled by the substantivity either

to the tissues of the mammalian host or to the trypanosomes or to both.

The Mesomorphic State of Derivatives of Naphthylaminedisulphonic Acids.

The recognition of the mesomorphic or liquid crystalline state of matter is largely due to the pioneering labours from 1877 onwards of O. Lehmann. The development of the subject has been mainly confined to pure crystalline substances which when melted showed anisotropy or which, when converted into the isotropic liquid state by heat, developed the anisotropic fluid phase on cooling. This field has been especially enriched by Vörlander's syntheses of numerous organic compounds showing this property. Within the last few years an extension of the subject has taken place in that it has been shown that anisotropic fluids may separate from aqueous media and the richest field in this respect is that of the soaps.

It is historically of interest that the first anisotropic fluid ever observed belonged to the latter class. Virchow in 1854 (*Arch. path. Anat. Physiol.*, **6**, 562) showed that the material separating from a hot alcoholic extract of brain tissue or spinal cord in contact with water swelled up with production of the so-called " myelin-forms " and in 1858 Mettenheimer made the important observation that the " myelin-forms " were anisotropic. There is little doubt that it is the combined fatty acids present in the phosphatides (lecithin, cephalin, phrenosin, etc.) which are primarily responsible for this property. In 1866, Neubauer (*ibid.*, **36**, 303) obtained " myelin-forms " by exposure of oleic acid to ammonia, but Lehmann first recognised the crystalline fluid phase of the oleic acid salts (*Ann. Physik*, 1895, **56**, 771). During subsequent years there are occasional references to be found to the anisotropy of soap solutions or to the anisotropy of " myelin-forms " produced from soaps (Krafft, *Z. physiol. Chem.*, 1902, **35**, 364, 376; Adami and Aschoff, *Proc. Roy. Soc.*, 1906, **78**, *B*, 30; Friedel, *Ann. Physique*, 1922, **18**, 273). In 1923, however, Maclennan (*J. Soc. Chem. Ind.*, **42**, 393) revealed the rich field of anisotropic fluids from aqueous solutions covered by the soaps and following on this McBain and Langdon (J., 1925, **127**, 852) and McBain and Elford (J., 1926, 421) have given a full account of the set of equilibria which govern such systems.

The compounds described in this paper are rich in examples of anisotropic fluids from aqueous media and provide a wonderful wealth of material for quantitative study. So far they have only been examined qualitatively, and incidentally to the main theme of this communication, but the qualitative observations lead one

to believe that the whole group of phenomena are subject to the limitations imposed by the phase rule. Structurally the compounds exhibiting this property are far removed from the fatty-acid type and are more akin to the isolated cases of mesomorphy of 10-bromo- and 10-chloro-phenanthrene-3(or 6)-sulphonic acid of Sandqvist (*Ber.*, 1915, **48**, 2054; *Kolloid Z.*, 1916, **19**, 113; *Arkiv Kemi*, 1916, **7**, No. 2).

Of the compounds described in this paper, the phenomenon of mesomorphy is restricted to those derived from disulphonic acids of the naphthylamines and has been observed in derivatives of each of the disulphonic acids mentioned on p. 3070, but is particularly pronounced in derivatives of C-acid, amino-J-acid, and amino-G-acid. Among these derivatives, it may occur as early as the first *m*-nitrobenzoyl derivative and be found in all the subsequent derivatives up to the *s*-carbamide of the *m'*-aminobenzoyl-*m*-aminobenzoyl derivative. As a rule it seems to be the least pronounced in the *s*-carbamides.

Derivatives of the naphthylaminedisulphonic acids show a general resemblance in behaviour. They are soluble in hot water and on being allowed to cool usually give, not crystals which are amenable to filtration, but, rather, highly hydrated products which vary greatly in consistency; they may be waxy solids, limpid fluids possessed of a sheen, clear gels or worm-like growths, and are in almost all cases strongly birefringent. The high degree of hydration seems essential for their production, for, if allowed to form from 50% or higher strengths of alcohol, they usually separate in the solid crystalline state, a totally distinct phase amenable to filtration and processes of washing. It is only in this manner in many cases that it is possible to obtain the products pure and free from inorganic salts. As a rule, the products are readily salted out from aqueous solutions by sodium chloride in a caseous form which lends itself to filtration. It has not been possible to decide whether this caseous phase is a new phase, in the same sense that the curds obtained in the soap-boiling process are a new phase giving an *X*-ray pattern, unobtainable from the anisotropic fluid phase of soaps, or is merely a stage in the dehydration of the anisotropic fluids. Such qualitative evidence as we possess suggests that under the dehydrating action of sodium chloride or alcohol it is possible to pass gradually from a limpid anisotropic fluid in equilibrium with its saturated isotropic solution to the caseous form in equilibrium with a concentrated solution of sodium chloride or alcohol. The intermediate anisotropic fluid appears to possess a certain degree of rigidity, but always flows under pressure of a cover-slip. In many cases, the caseous form passes with lapse of

time into the crystalline solid phase, probably with some change in the degree of hydration. It follows from what has been said about the hydration of the anisotropic fluid phase that, if the pure solid crystalline form be redissolved in the minimum volume of boiling water, then, on cooling, the anisotropic fluid phase may separate and, owing to its high degree of hydration, may combine with the whole of the water present, giving a single anisotropic fluid phase. Such we believe we have observed in the case of the monosodium salt of *m*-aminobenzoyl-2-naphthylamine-5 : 7-disulphonic acid (see Experimental). In other cases, a biphasic fluid may result from equilibrium of the anisotropic fluid with its isotropic saturated aqueous solution. The stability of such solutions varies. The specific instance quoted above lends itself particularly to demonstration purposes, as the viscous anisotropic fluid may persist at room temperature for months and then pass into a sparingly soluble, microscopically fine crystalline solid in equilibrium with a limpid saturated solution. To the naked eye both conditions of the solution possess a sheen and are indistinguishable. In the case of the tetrasodium salt of the *s*-carbamide of *m'*-aminobenzoyl-*m*-aminobenzoyl-2-naphthylamine-6 : 8-disulphonic acid, it has been possible to observe, by means of the polarising microscope, the slow crystallisation of the solid phase at the expense of the surrounding anisotropic fluid. In other cases, the anisotropic fluid phase appears to be permanently stable, as samples have been kept for 2 years.

The converse phenomenon, formation of the anisotropic fluid phase at the expense of the crystalline solid phase, has been observed in the case of the disodium salt of *m'*-nitrobenzoyl-*m*-aminobenzoyl-2-naphthylamine-6 : 8 -disulphonic acid and in the case of the free acid, *m'*-nitrobenzoyl-*m*-aminobenzoyl-2-naphthylamine-4 : 8-disulphonic acid (see Experimental).

In appearance the anisotropic fluids, whether in presence of and in equilibrium with their saturated isotropic aqueous solutions or not, usually possess a sheen, and examined under the highest power of the microscope are never particulate. Under the polarising microscope they present alternations of dark and light patches and under pressure of a cover-slip show streaming of the light patches. The densities of the anisotropic fluid phase and the isotropic fluid phase in equilibrium are usually very close, as there is no evidence of settling, but on prolonged centrifuging a partial separation may be effected, the supernatant phase being clear and isotropic and the lower portion now exhibiting an orientation of the sheen in spiral form. The production of a sheen is by no means invariable, and cases are described in the experimental portion of clear anisotropic gels and of clear anisotropic viscous solutions.

The mimicry of crystalline form by liquid crystals has often been observed (Lehmann, Vörlander) and a good instance of such in aqueous media is presented by the sodium hydrogen salt of *m'*-aminobenzoyl-*m*-aminobenzoyl-2-naphthylamine-4 : 8-disulphonic acid.

The derivatives of 1-naphthylamine-4 : 6 : 8-trisulphonic acid, on the other hand, show none of these phenomena and as a rule are extremely soluble in water and salted out with difficulty. The following table shows the incidence of this property :

Salted out.	Not salted out.
RNH_2	$(RNH)_2CO$
NO_2·Bz·NHR	NH_2·Bz·NHR
NO_2·Bz·NH·Bz·NHR	$(RNH·Bz·NH)_2CO$
NH_2·Bz·NH·Bz·NHR (diNa salt)	NH_2·Bz·NH·Bz·NHR (triNa salt)
	$(RNH·Bz·NH·Bz·NH)_2CO$

It is of interest that the substance Bayer 205 (I), which only differs from the last member here tabulated by two methyl groups, is extremely soluble in water and is also not salted out. It occurs commercially as a white powder devoid of crystalline structure, but according to our own observations it may readily be crystallised by solution in a fraction of its weight of water, addition of several volumes of pure methyl alcohol, followed by gradual addition of acetone. It separates after some delay in clusters of needles.

It is a pleasure to recognise our indebtedness to the following : to Miss Durham and Miss Marchal of this Department for carrying out the many biological experiments; to Dr. A. Schedler of the Clayton Aniline Co. for gifts of pure wool, silk, and cotton; to Dr. J. T. Conroy of the United Alkali Co. for a free and liberal supply of carbonyl chloride, and to the Pharmazeutische Abteilung of the I.G. Farbenindustrie Aktiengesellschaft for a gift of 1-naphthylamine-4 : 6 : 8-trisulphonic acid.

Experimental.

m-*Nitrobenzoyl*-1-*naphthylamine*-3 : 6-*disulphonic Acid.*—1-Naphthylamine-3 : 6-disulphonic acid (Freund's acid, 12·1 g.) was dissolved in 40 c.c. of *N*-sodium hydroxide (1 mol.) and 20 c.c. of water and treated with 14·85 g. (2 mols.) of *m*-nitrobenzoyl chloride, all at once, and the mixture was shaken for ½ hour with addition of a few drops of ether. Three successive portions (each 40 c.c.) of *N*-sodium hydroxide were then added at half-hourly intervals with subsequent shaking. The solution was made distinctly acid to Congo-paper, the *m*-nitrobenzoic acid removed by ether, the aqueous liquor neutralised to litmus and concentrated under reduced pressure at 50°, and two crops of the *disodium* salt collected (yield, 75%). This salt is readily soluble in water (1 in 26) and is precipitated in microscopic, triangular leaflets on addition of sodium chloride.

For analysis it was crystallised from 50% alcohol (Found : Loss at 140°, 16·3. $C_{17}H_{10}O_9N_2S_2Na_2,5\frac{1}{2}H_2O$ requires H_2O, 16·6%. Found in dried salt : Na, 9·1. $C_{17}H_{10}O_9N_2S_2Na_2$ requires Na, 9·3%). An aqueous solution of the salt is neutral to litmus and gives crystalline precipitates on addition of calcium, magnesium, and barium chlorides. The salt will also crystallise unchanged from solutions which are acid to Congo-paper.

m-*Aminobenzoyl*-1-*naphthylamine*-3 : 6-*disulphonic Acid*.—To a solution of 25 g. of the crude sodium salt (above described) in 195 c.c. of 2*N*-sodium hydroxide at 0°, ferrous chloride (70 g.; 7 mols.) in 100 c.c. of water was added slowly with stirring, followed by 195 c.c. of 2*N*-sodium hydroxide to make the solution definitely alkaline. After filtration the ferric hydroxide sludge was extracted twice with 250 c.c. of 0·2*N*-sodium hydroxide each time and the solution was neutralised and concentrated at 50°; the sparingly soluble azoxy-compound (1·7 g.) separated first, followed by the *disodium* salt (13·5 g.) of the required amino-acid. This crystallises from water, in which it is very readily soluble, in fine needles (Found : Loss at 160°, 16·6. $C_{17}H_{12}O_7N_2S_2Na_2,5H_2O$ requires H_2O, 16·2%. Found in anhydrous salt : Na, 9·5. $C_{17}H_{12}O_7N_2S_2Na_2$ requires Na, 9·9%). It gives a moderately easily soluble *diazo*-compound free from chloride but containing sodium, as a primrose-yellow precipitate of needles. This couples with glyoxaline derivatives in aqueous sodium carbonate, the solutions having a cherry-red colour. The disodium salt in concentrated solution gives a *calcium* salt, microscopic prisms, and a *barium* salt, sheaves of flat prisms, on addition of saturated calcium or barium chloride. The *silver* salt separates similarly as a microcrystalline powder.

The *sodium hydrogen* salt is much less soluble than the disodium salt and rapidly separated in flattened prisms when the latter (2·9 g.) in 5 c.c. of water was treated with 1 c.c. of concentrated hydrochloric acid (yield, 2·6 g.) (Found : Loss at 160°, 15·6. $C_{17}H_{13}O_7N_2S_2Na,4\frac{1}{2}H_2O$ requires H_2O, 15·4%. Found in anhydrous salt : Na, 5·1. $C_{17}H_{13}O_7N_2S_2Na$ requires Na, 5·2%).

The *azoxy-compound* is readily soluble in 8 parts of hot water and crystallises in yellow, silky, hair-like needles (Found : Loss at 95°, 18·5. $C_{34}H_{20}O_{15}N_4S_4Na_4,12H_2O$ requires H_2O, 18·6%. Found in anhydrous salt : Na, 9·2. $C_{34}H_{20}O_{15}N_4S_4Na_4$ requires Na, 9·7%).

m′-*Nitrobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-3 : 6-*disulphonic Acid*.—The disodium salt of the preceding acid (10·7 g., 87% anhydrous salt) dissolved in 50 c.c. of water was nitrobenzoylated with two equivalents of acid chloride as described in the first stage of the synthesis. The final solution was made distinctly acid to

Congo-paper and the gelatinous precipitate was collected and dissolved in water. The two solutions were then thoroughly extracted with ether, combined, neutralised, and concentrated at 50°, and the successive crops of gelatinous nitro-acid collected. These were dissolved in boiling water and treated with 10 volumes of alcohol and the solution was kept until the gelatinous nitro-compound which first separated had changed into a crystalline mass of soft, white needles (yield, 11 g.) (Found in product from 90% alcohol: Loss at 160°, 11·0. $C_{24}H_{15}O_{10}N_3S_2Na_2,4H_2O$ requires H_2O, 10·5%. Found in anhydrous salt: Na, 7·6. $C_{24}H_{15}O_{10}N_3S_2Na_2$ requires Na, 7·5%). This *nitro-amide* is readily soluble in water with a neutral reaction and couples only faintly with Pauly's reagent in sodium carbonate solution. It is fairly readily soluble in boiling methyl alcohol, but does not crystallise well from it.

m′-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-3 : 6-*disulphonic Acid.*—The preceding nitro-acid (10·2 g.) was reduced with ferrous chloride exactly as described at a previous stage. The alkaline filtrates and extracts of the ferric hydroxide were made neutral to Congo-paper; the thick, gelatinous precipitate which first separated soon became wholly crystalline (yield, 10·1 g.). The mother-liquors on concentration only deposited a further 0·25 g. This *amino-acid* is a *monosodium* salt and is soluble in 3 volumes of boiling water, separating as a thick felt of small, silky needles on cooling (Found: Loss at 160°, 24·0. $C_{24}H_{18}O_8N_3S_2Na,10H_2O$ requires H_2O, 24·2%. Found in anhydrous salt: Na, 3·9. $C_{24}H_{18}O_8N_3S_2Na$ requires Na, 4·1%). An aqueous solution of the salt colours Congo-paper faintly blue. The salt forms a sparingly soluble *diazo*-compound, crystallising in microscopic needles, which couples with alkaline β-naphthol with the usual red colour. The diazo-compound is free from chloridion but contains sodium.

s-*Carbamide of* 1-*Naphthylamine*-3 : 6-*disulphonic Acid.*—Freund's acid (5 g.) in 250 c.c. of water containing 35 g. (20 mols.) of anhydrous sodium carbonate was submitted to a slow stream of carbonyl chloride until the reaction was acid to Congo-paper. The main bulk of the carbamide had separated (5·1 g.). When dissolved in 33 c.c. of boiling water and neutralised to litmus paper, it separated on cooling in long, silky needles with a faint pink colour. It gave no reaction for amino-groups and was free from chloride (Found: Loss at 160°, 25·6. $C_{21}H_{12}O_{13}N_2S_4Na_4,14H_2O$ requires H_2O, 25·9%. Found in anhydrous salt: Na, 12·6. $C_{21}H_{12}O_{13}N_2S_4Na_4$ requires Na, 12·8%). This *carbamide* is soluble in cold water to the extent of 8%, but is very readily salted out by sodium chloride.

s-*Carbamide of* m-*Aminobenzoyl*-1-*naphthylamine*-3 : 6-*disulphonic Acid.*—Five g. of the *m*-aminobenzoyl amide were phosgenated as

described in the foregoing section. The solid which had separated was collected, dissolved in 50 c.c. of hot water, and inoculated with the needle form of the carbamide. The required carbamide then separated in balls of long, silky needles (yield, 3·75 g.). This *carbamide* separates from concentrated aqueous solutions in gelatinous, rounded masses which are optically anisotropic, showing black crosses under the polarising microscope. On keeping in more dilute solution, they become transformed into the needle form. It is also readily salted out in gelatinous, anisotropic masses (Found: Na, 7·2; loss at 160°, 21·7. $C_{35}H_{22}O_{15}N_4S_4Na_4,14\frac{1}{2}H_2O$ requires Na, 7·5; H_2O, 21·4%).

s-*Carbamide of* m′-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-3 : 6-*disulphonic Acid.*—The phosgenation was carried out in the same way as the preceding. Complete separation of the partly precipitated carbamide was effected by addition of sodium chloride. The gelatinous solid from 6 g. of amino-compound was dissolved in 30 c.c. of hot water, neutralised, and treated with 7 volumes of alcohol. On keeping, the carbamide separated in finely divided and partly anisotropic particles free from chloride. The product was again crystallised and gave 3·6 g. of carbamide (Found: Loss at 160°, 17·0; Na, 6·2. $C_{49}H_{32}O_{17}N_6S_4Na_4,14H_2O$ requires H_2O, 17·4; Na, 6·3%). This *carbamide*, so prepared, is very soluble in water and separates from concentrated solutions in voluminous, anisotropic, waxy masses. It is precipitated by strong mineral acid as a fine, amorphous precipitate, but is only salted out with difficulty and yields a sparingly soluble, amorphous *barium* salt.

m-*Nitrobenzoyl*-2-*naphthylamine*-5 : 7-*disulphonic Acid.*—Amino-J-acid (10·2 g. = 9·7 g. of anhydrous salt) in 100 c.c. of water was nitrobenzoylated as described above. After acidification, extraction with ether, and neutralisation the solution was concentrated at 50° and the successive crops were collected without washing, until sodium chloride began to separate. The crude solid was dissolved in 50 c.c. of hot water and treated with 50 c.c. of alcohol; it then gradually separated in clusters of small needles (yield, 15·2 g.) (Found: Loss at 160°, 16·9; Na, 7·4. $C_{17}H_{10}O_9N_2S_2Na_2,5\frac{1}{2}H_2O$ requires H_2O, 16·6; Na, 7·7%). This *nitro-amide* is extremely readily soluble in water, and very concentrated aqueous syrupy solutions deposit fibre- or worm-like anisotropic growths at first, but on keeping, fine needles.

m-*Aminobenzoyl*-2-*naphthylamine*-5 : 7-*disulphonic Acid.*—The nitro-amide was reduced at room temperature with ferrous chloride and alkali as described above. The combined alkaline filtrates and extracts of the ferric hydroxide on neutralisation to Congo-paper gave a copious, white, microcrystalline precipitate of the amino-

compound. The yield was practically quantitative. The solid was dissolved in the minimum volume of boiling water (about 30 volumes). When cold, the product consisted of an opaque, viscous, anisotropic fluid with elastic properties and possessed of a crystalline sheen. There was no separation into two phases on prolonged centrifuging. The liquid crystalline condition was unaltered by addition of dilute acids, but either alcohol or sodium chloride precipitated anisotropic gelatinous masses. On keeping for various periods, 5 days to 3 months, the viscous fluid passed spontaneously into a more stable condition with separation of microscopic needles in almost quantitative amount (Found : Loss at 160°, 14·4; Na, 4·6. $C_{17}H_{13}O_7N_2S_2Na,4H_2O$ requires H_2O, 14·0; Na, 4·4%). When diazotised in dilute acid solution, it yields a gelatinous *diazo-compound*. The *disodium* salt is extremely soluble in water, the solution soon becoming brown.

m′-*Nitrobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-5 : 7-*disulphonic Acid*.—The foregoing amino-compound (10·3 g.) in 170 c.c. of water was nitrobenzoylated as described for previous members. The final solution was neutral and contained the required nitro-amide in suspension as a finely-divided, crystalline solid. This was collected and the mother-liquors after removal of nitrobenzoic acid and concentration gave only a small quantity of nitro-amide. The combined solids crystallised from 150 c.c. of boiling water, with final addition of 150 c.c. of alcohol, in small, silky needles (yield, 14·0 g.) (Found: Loss at 160°, 21·1; Na, 5·6. $C_{24}H_{15}O_{10}N_3S_2Na_2,9H_2O$ requires H_2O, 20·9; Na, 5·9%). This *amide* is readily soluble in warm water and separates from the solution, on cooling, as a white, anisotropic, gelatinous mass. From very dilute solutions it separates in microscopic needles. Its aqueous solution is neutral to litmus, and sodium chloride produces an anisotropic, gelatinous precipitate.

m′-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-5:7-*disulphonic Acid*.—The foregoing nitro-compound (7·7 g.) in 250 c.c. of water was reduced at 30—35° with ferrous chloride and alkali. The faintly alkaline mixture was stirred for 2 hours; all traces of the white, gelatinous ferrous salt had then disappeared. The filtrate and alkaline extracts of the ferric hydroxide on neutralisation to Congo-paper gave a gelatinous, anisotropic precipitate of the amino-compound. The mother-liquors gave a further small crop of 0·65 g. The product was crystallised from 125 c.c. of boiling water with addition of 125 c.c. of alcohol and gave 5·35 g. of soft balls of microscopic, silky needles of the *sodium hydrogen* salt (Found : Loss at 160°, 15·3; Na, 3·8. $C_{24}H_{18}O_8N_3S_2Na,5\frac{1}{2}H_2O$ requires H_2O, 15·0; Na, 3·5%). This sodium salt is readily soluble in boiling water, and on cooling, the solution yields a white, turbid, anisotropic

fluid with a crystalline sheen. Clear aqueous solutions give gelatinous precipitates at high dilutions with 3*N*-hydrochloric acid. It diazotises and then couples in the usual way with β-naphthol. The *disodium* salt is readily soluble in cold water and crystallises in soft, woolly needles (Found: Loss at 160°, 17·1; Na, 6·3. $C_{24}H_{17}O_8N_3S_2Na_2,6\frac{1}{2}H_2O$ requires H_2O, 16·7; Na, 6·5%).

s-*Carbamide of* 2-*Naphthylamine*-5 : 7-*disulphonic Acid*.—When this naphthylamine was phosgenated in 50 volumes of water with sodium carbonate (20 mols.), there was no separation of solid, but on saturation with sodium chloride the carbamide was precipitated in short needles. It was collected and re-phosgenated under the same conditions and again precipitated. It was dissolved in hot water, neutralised, and crystallised by addition of 3 volumes of alcohol. It was recrystallised and gave 5·0 g. from 10·0 g. of the naphthylamine (Found: Loss at 160°, 14·4; Na, 11·0. $C_{21}H_{12}O_{13}N_2S_4Na_4,7H_2O$ requires H_2O, 14·9; Na, 10·9%). This *carbamide* retains chloride with persistency. It crystallises well in clusters of long needles and is very soluble in water.

s-*Carbamide of* m-*Aminobenzoyl*-2-*naphthylamine*-5 : 7-*disulphonic Acid*.—The amino-compound (10 g.) was phosgenated twice as described in the preceding paragraph. The partial separation of leaflets was completed by saturation with sodium chloride. The product after neutralisation was twice crystallised from hot water by addition of 3 volumes of alcohol (yield, 9·9 g.) (Found: Loss at 160°, 22·1; Na, 7·3. $C_{35}H_{22}O_{15}N_4S_4Na_4,15H_2O$ requires H_2O, 22·0; Na, 7·5%). This *carbamide* is very soluble in water, crystallising therefrom in pointed leaflets, and is precipitated by sodium chloride in microscopic rods or narrow leaflets. Addition of concentrated hydrochloric acid to a warm solution precipitates leaflets, possibly of a free sulphonic acid. The *barium* salt is sparingly soluble and crystallises in leaflets.

s-*Carbamide of* m′-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-5 : 7-*disulphonic Acid*.—Five g. of the amino-compound in 500 c.c. of water were phosgenated in the usual way. The resultant acid product consisted of an opalescent jelly similar to raw white of egg. It was optically anisotropic and devoid of visible particles. When neutralised, it became definitely biphasic to the eye with separation of the sodium salt. Separation was completed by addition of sodium chloride. The product was collected, dissolved in 120 c.c. of boiling water, and treated with 120 c.c. of alcohol. If the alcohol be omitted, the solution sets to a clear anisotropic gel. The product from 50% alcohol was amorphous (yield 3·6 g.) (Found: Loss at 160°, 10·3; Na, 6·5. $C_{49}H_{32}O_{17}N_6S_4Na_4,8H_2O$ requires H_2O, 10·7; Na, 6·9%). This *carbamide* is readily precipit-

ated, possibly as the free sulphonic acid, by concentrated hydrochloric acid as a crystalline liquid, but from more dilute solutions, at first as a clear anisotropic gel but later in discrete crystalline growths. If the solution be heated and allowed to cool, it deposits circular tufts of leaflets. The *calcium* and *barium* salts are both sparingly soluble.

m-*Nitrobenzoyl*-2-*naphthylamine*-4 : 8-*disulphonic Acid*.—C-Acid (12·0 g = 9·7 g. of anhydrous monosodium salt) in 210 c.c. of water was nitrobenzoylated in the usual way. The solid which had separated was collected, dissolved in the minimum volume of water (900 c.c.), acidified, and extracted with ether. On neutralisation to litmus, the nitro-amide (12·8 g.) separated in a crystalline condition. The mother-liquors and the original mother-liquors of the benzoylation gave a further 2·5 g. on concentration. The solid crystallised from 9 volumes of boiling water in clusters of soft, pale yellow needles (Found: Loss at 160°, 15·2; Na, 7·6. $C_{17}H_{10}O_9N_2S_2Na_2,5H_2O$ requires H_2O, 15·4; Na, 7·8%). The most striking property of the salt is the ease with which it is salted out from aqueous solutions. Such solutions are neutral to litmus and give no precipitate with hydrochloric acid.

m-*Aminobenzoyl*-2-*naphthylamine*-4 : 8-*disulphonic Acid*.—The preceding nitro-amide (equivalent to 9·9 g. of anhydrous salt) was dissolved in 275 c.c. of water and reduced with ferrous chloride and alkali at room temperature. The alkaline extracts of the ferric hydroxide on neutralisation to Congo-paper deposited the main bulk of the required amino-acid, and the mother-liquors on concentration gave a further small quantity (yield, practically quantitative). It crystallised from the minimum volume of boiling water as a voluminous mass of soft, silky needles (Found: Loss at 160°, 18·6; Na, 4·3. $C_{17}H_{13}O_7N_2S_2Na,5\frac{1}{2}H_2O$ requires H_2O, 18·2; Na, 4·2%). This *sodium hydrogen* salt is sparingly soluble in cold water, yielding a solution neutral to litmus. In dilute hydrochloric acid, it gives a sparingly soluble *diazosulphonate*, which separates in anisotropic, filamentous growths. The *disodium* salt was obtained by dissolving the hydrogen salt in a little water with the aid of sufficient sodium hydrogen carbonate to make the reaction faintly alkaline to litmus. It crystallised in fine needles, but it also occurs in plates. It is soluble in 10 volumes of water at room temperature (Found: Loss at 160°, 22·8. $C_{17}H_{12}O_7N_2S_2Na_2,7\frac{1}{2}H_2O$ requires H_2O, 22·5%. Found in anhydrous salt: Na, 9·7. $C_{17}H_{12}O_7N_2S_2Na_2$ requires Na, 9·9%).

m′-*Nitrobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-4 : 8-*disulphonic Acid*.—The foregoing acid (10·2 g.) in 320 c.c. of water was nitrobenzoylated in the usual way. The final solution was clear, very viscous, and anisotropic. It was diluted with 400 c.c. of water,

acidified to Congo-paper, and extracted with ether. It was then neutralised to litmus, which restored the anisotropic gel condition. If the solution was more dilute, a gel was not formed, but anisotropic worm- or thread-like growths separated in quantity. These showed the phenomenon of black crosses under the polarising microscope, especially at the ends of growth. In either case, the solution was heated in the boiling water-bath and saturated with sodium chloride with stirring. When cold, the precipitated gelatinous solid was collected in quantitative yield, without washing. 5 G. of the air-dried material were dissolved in 75 c.c. of hot water and treated with 20 c.c. of concentrated hydrochloric acid. The solution rapidly deposited a thick felt of needles. These were filtered off, dissolved in 25 c.c. of hot water, and heated with 10 c.c. of concentrated hydrochloric acid. Fine, long needles rapidly separated, which were filtered off as dry as possible, rubbed on porous plate, and dried in a vacuum over sulphuric acid and sodium hydroxide. In this way, 2·35 g. of almost pure, anhydrous *disulphonic acid* were obtained free from chloride (Found: Na, 0·4%. 0·249 G. was equivalent to 8·2 c.c. of *N*/10-sodium hydroxide, agreeing with the value required for a mixture of 95% of disulphonic acid and 5% of disodium salt). A repetition of the procedure would probably remove the last traces of sodium. The disulphonic acid, treated with a little water, swells up, forming a clear, viscous, anisotropic fluid in equilibrium with the surrounding aqueous solution. More dilute solutions are clear and set to an anisotropic gel on addition of silver nitrate. If the crude crystalline disulphonic acid obtained after one treatment of the crude disodium salt with hydrochloric acid be dissolved in a small volume of hot water and treated with an equal volume of alcohol, the neutral *disodium* salt separates in soft balls of extremely minute needles (Found: Loss at 160°, 12·7. $C_{24}H_{15}O_{10}N_3S_2Na_2,5H_2O$ requires H_2O, 12·8%. Found in anhydrous salt: Na, 7·4. $C_{24}H_{15}O_{10}N_3S_2Na_2$ requires Na, 7·5%). The mother-liquors, however, are strongly acid to Congo-paper. This salt may be obtained directly from the original crude disodium salt by dissolving it in hot water and adding alcohol, but the process through the disulphonic acid is preferable. The disodium salt is moderately easily soluble in cold water and on addition of a little sodium chloride the solution sets to a clear anisotropic gel, a state also obtained by dissolving the disodium salt in warm water and allowing the solution to cool. This gel remains unchanged in a sealed vessel for many months. The *barium* and *calcium* salts are very sparingly soluble, the former crystallising in hairs. The *magnesium* salt crystallises in clusters of needles moderately easily soluble in water.

m′-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-4:8-*disulphonic Acid*.—The foregoing disodium salt (7·6 g.) in 250 c.c. of water was reduced with ferrous chloride and alkali at 25°. The filtrate and alkaline extracts of the ferric hydroxide were made faintly acid to Congo-paper; the amino-compound then separated as a voluminous, anisotropic, gelatinous product which, on filtration, was left as a white, soapy solid. It was dissolved in 350 c.c. of boiling water and treated with an equal volume of alcohol; it then separated slowly in gelatinous, oat-shaped, anisotropic crystals (yield, 6·15 g.) (Found: Loss at 160°, 17·1; Na, 3·3. $C_{24}H_{18}O_8N_3S_2Na,6\frac{1}{2}H_2O$ requires H_2O, 17·2; Na, 3·4%). This *sodium hydrogen* salt is sparingly soluble in hot water, and a boiling saturated aqueous solution when allowed to cool gives two phases, a clear supernatant liquor and anisotropic aggregates simulating ill-formed, crystalline growths. On filtration on hardened paper under pressure, both phases passed through, yielding a macroscopically homogeneous filtrate with a crystalline sheen. Examined microscopically, it showed complete absence of particles, the whole fluid being anisotropic under polarised light. On prolonged centrifuging there is a separation of a clear aqueous upper layer and a more uniform orientation of the sheen in spiral form in the lower layer.

s-*Carbamide of* 2-*Naphthylamine*-4:8-*disulphonic Acid*.—This carbamide was prepared by a double phosgenation of the parent amine (5·0 g.), separation being completed by addition of sodium chloride. After neutralisation it was twice crystallised from water and separated in clusters of microscopic needles (yield, 3·5 g.) (Found: Loss at 160°, 11·3; Na, 11·1. $C_{21}H_{12}O_{13}N_2S_4Na_4,5H_2O$ requires H_2O, 11·1; Na, 11·3%). This *carbamide* is soluble in water at room temperature to the extent of 5%. It is not very readily salted out and is not precipitated by concentrated hydrochloric acid.

s-*Carbamide of* m-*Aminobenzoyl*-2-*naphthylamine*-4:8-*disulphonic Acid*.—For the preparation of this carbamide, 80 volumes of water were required and a double phosgenation was necessary. At the end of the phosgenation the crude carbamide separated in the liquid crystalline state, in the form of stringy, anisotropic masses. After saturation with sodium chloride, the product could be filtered off on hardened paper and after neutralisation with alkali it was dissolved in 9 volumes of hot water and treated with an equal volume of alcohol. The carbamide separated on keeping in clusters of small needles. The yield was 10·5 g. from 10·0 g. of initial material (Found: Loss at 160°, 18·4; Na, 7·5. $C_{35}H_{22}O_{15}N_4S_4Na_4,12H_2O$ requires H_2O, 18·4; Na, 7·8%). This *carbamide* is readily soluble in water, is fairly readily salted out, and is precipitated by excess

of concentrated hydrochloric acid in the liquid crystalline state; if, however, the solution be warmed and allowed to cool slowly, the carbamide separates in discrete crystalline growths. The *calcium* and *barium* salts are sparingly soluble, the latter notably so.

s-*Carbamide of* m'-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-4 : 8-*disulphonic Acid.*—The parent amine (5 g.) was phosgenated in the usual way, and precipitation of the crude product completed by addition of sodium chloride. It was dissolved in 30 c.c. of boiling water, neutralised, and treated with 70 c.c. of alcohol. On keeping, the carbamide separated without definite recognisable structure, but the product was anisotropic (yield, 4·7 g.) (Found : Loss at 160°, 18·2; Na, 5·7, 6·1. $C_{49}H_{32}O_{17}N_6S_4Na_4,14\frac{1}{2}H_2O$ requires H_2O, 17·9; Na, 6·3%). This *carbamide* is soluble in water and crystallises from concentrated solutions in microscopic, circular, crystalline aggregates. It is readily precipitated, possibly as the free tetrasulphonic acid, by addition of concentrated hydrochloric acid in a similar crystalline form. The *magnesium*, *barium*, and *calcium* salts are sparingly soluble.

m-*Nitrobenzoyl*-2-*naphthylamine*-6 : 8-*disulphonic Acid.*—G-Acid (9·8 g. of anhydrous sodium hydrogen salt) in 120 c.c. of water was nitrobenzoylated in the usual way. The solution was diluted with 425 c.c. of water to dissolve the solid which had separated; it was then acidified and extracted with ether. After neutralisation the solution was concentrated at 50° and the successive crops of gelatinous solid were collected without washing. The combined crops were made up to 150 c.c. with boiling water, and 150 c.c. of alcohol added. On keeping, the nitro-amide separated in soft, woolly needles (yield, 14·55 g.) (Found : Loss at 160°, 19·9; Na, 7·7. $C_{17}H_{10}O_9N_2S_2Na_2,7H_2O$ requires H_2O, 20·3; Na, 7·4%). This *amide* is readily soluble in hot water and separates on cooling as an anisotropic, gelatinous phase, possibly a semi-rigid liquid crystalline phase, as it flowed when submitted to pressure under a coverslip. Addition of concentrated hydrochloric acid gave an anisotropic gelatinous precipitate, as also did the addition of sodium chloride, which even in small quantities has a salting-out effect.

m-*Aminobenzoyl*-2-*naphthylamine*-6 : 8-*disulphonic Acid.*—The foregoing nitro-compound (12·4 g.) in 250 c.c. of water was reduced with ferrous chloride and alkali at 25°. On acidification, the filtrate and alkaline extracts of the ferric hydroxide gave a crystalline precipitate, and the mother-liquors on concentration a further small crop. These were dissolved in 50 c.c. of boiling water and treated with 20 c.c. of alcohol. The amide separated on cooling as a compact mass of needles (yield, 8·8 g.) (Found : Loss at 160°, 18·2. $C_{17}H_{13}O_7N_2S_2Na,5\frac{1}{2}H_2O$ requires H_2O, 18·2%. Found in anhydrous

solid: Na, 5·1. $C_{17}H_{13}O_7N_2S_2Na$ requires Na, 5·2%). This *amide* is readily soluble in hot water. A hot concentrated solution, when cold, becomes transformed into a viscous liquid crystalline mass with a sheen. This remains unchanged when kept in a stoppered tube for months. The amide forms a sparingly soluble *diazo-compound*, crystallising in needles with a primrose-yellow colour.

m′-*Nitrobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-6 : 8-*disulphonic Acid*.—The preceding amino-compound (16·3 g.) in 370 c.c. of water was nitrobenzoylated in the usual way. The very viscous solution which resulted was acidified and mixed with an equal volume of alcohol. On keeping at 0°, the precipitate crystallised (yield, 19·5 g.). It separated from hot 65% alcohol in minute, soft needles (Found: Loss at 160°, 14·7. $C_{24}H_{15}O_{10}N_3S_2Na_2,6H_2O$ requires H_2O, 14·9%. Found in anhydrous solid: Na, 7·2. $C_{24}H_{15}O_{10}N_3S_2Na_2$ requires Na, 7·5%). This *amide* is soluble in 14 parts of boiling water and separates in anisotropic gelatinous globules. If the amide be left in contact with 18 volumes of water, the original white powder disappears and the whole of the fluid becomes a white elastic mass with a sheen. Under the polarising microscope it retained the anisotropic sheen and flowed under uneven pressure of the cover-slip. A hot solution treated with concentrated hydrochloric acid deposits a microcrystalline precipitate, possibly of the free di-sulphonic acid. The amide is very readily salted out and gives sparingly soluble precipitates with calcium, magnesium, and barium chlorides.

m′-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-6:8-*disulphonic Acid*.—The preceding nitro-amide (17·4 g.) was dissolved in 600 c.c. of water at 30° and treated with 84 c.c. of 2*N*-sodium hydroxide. The solution set to a clear anisotropic gel, which gradually dis-integrated on addition of 33·6 g. of ferrous chloride in 50 c.c. of water. Finally, a further 84 c.c. of 2*N*-sodium hydroxide were added, the final reaction being made faintly alkaline to litmus. After being stirred for 1 hour, the solution was warmed to 50° to ensure complete solution and reduction of the nitro-compound. The filtrate and alkaline extracts of the ferric hydroxide on acidification deposited the amino-compound in soft, matted, microscopic needles. Precipitation was completed by addition of sodium chloride. The collected solid was dissolved in the minimum volume (700 c.c.) of boiling water and treated with 350 c.c. of alcohol; on cooling, the amino-amide crystallised in clusters of soft, white needles (yield, 14·7 g.) (Found: Loss at 160°, 14·5; Na, 3·5. $C_{14}H_{18}O_8N_3S_2Na,5\frac{1}{2}H_2O$ requires H_2O, 15·0; Na, 3·5%). This *amino*-compound is soluble in boiling water; the solution, on cooling, develops a white sheen, due to separation of the liquid crystalline phase. If a small amount of

sodium chloride be added to a clear dilute solution, this develops the liquid crystalline sheen, but only temporarily, the amide separating eventually in the solid crystalline condition. Hydrochloric acid also precipitates a liquid crystalline phase, and in very dilute hydrochloric acid a clear solution gives a liquid crystalline *diazosulphonate* on addition of sodium nitrite. The *disodium* salt is readily obtained as well-formed needles by dissolving the sodium hydrogen salt in warm sodium hydrogen carbonate solution and allowing it to cool. The *magnesium*, *calcium*, and *barium* salts are sparingly soluble in water.

s-*Carbamide of* 2-*Naphthylamine*-6 : 8-*disulphonic Acid*.—G-Acid (5 g.) was submitted to a thrice-repeated phosgenation under the usual conditions, sodium carbonate (20 mols.) being used each time. Separation of the solid was completed by addition of sodium chloride. After neutralisation it crystallised from boiling water, in which it was very soluble, in fine, colourless needles (Found : Loss at 160°, 19·9. $C_{21}H_{12}O_{13}N_2S_4Na_4,10H_2O$ requires H_2O, 20·0%. Found in the dried salt : Na, 12·6. $C_{21}H_{12}O_{13}N_2S_4Na_4$ requires Na, 12·8%). This *carbamide* has a sweet taste. It is readily soluble in water and is very readily salted out, in needles. It gives a sparingly soluble *barium* salt and with concentrated hydrochloric acid a precipitate of fine needles, probably the free tetrasulphonic acid.

s-*Carbamide of* m-*Aminobenzoyl*-2-*naphthylamine*-6 : 8-*disulphonic Acid*.—The parent amino-compound (5 g.) was submitted to a treble phosgenation. Separation of the solid, which consisted of very fine, small needles, was completed by saturation with sodium chloride. The product was dissolved in 50 c.c. of hot water and neutralised. It separated in round, weakly anisotropic nodules, which coalesced on attempted washing. It was redissolved in 25 c.c. of water, and 25 c.c. of alcohol added ; it then slowly separated in clusters of needles (yield 3·5 g.) (Found in substance crystallised from water : Loss at 160°, 19·8. $C_{35}H_{22}O_{15}N_4S_4Na_4,13H_2O$ requires H_2O, 19·6%. Found in anhydrous salt : Na, 10·0. $C_{35}H_{22}O_{15}N_4S_4Na_4$ requires Na, 9·6%). This *carbamide* is very readily salted out. It gives sparingly soluble salts with magnesium, calcium, and barium chlorides. With concentrated hydrochloric acid it gives a precipitate of needles, probably the free tetrasulphonic acid.

s-*Carbamide of* m′-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-6 : 8-*disulphonic Acid*.—The parent amino-compound (5 g.) was submitted to a double phosgenation. Separation of the carbamide was completed by addition of sodium chloride. It was then dissolved in 150 c.c. of boiling water with the aid of sufficient alkali to adjust the reaction to neutrality. To the hot solution 150 c.c.

of alcohol were added and on keeping the carbamide separated. It was again crystallised from 100 c.c. of water and 325 c.c. of alcohol (yield, 3·8 g.) (Found: Loss at 160°, 19·9. $C_{49}H_{32}O_{17}N_6S_4Na_4,16\frac{1}{2}H_2O$ requires H_2O, 19·9%. Found in anhydrous salt: Na, 7·6. $C_{49}H_{32}O_{17}N_6S_4Na_4$ requires Na, 7·7%). This *carbamide* separates from water–alcohol mixtures in weakly anisotropic, gelatinous particles. It is soluble in water; a hot concentrated solution on cooling deposits circular anisotropic clusters, but the surrounding aqueous medium also is anisotropic and flows under pressure of a cover-slip. Crystallisation of the solid phase proceeds at the expense of the liquid crystalline phase. Addition of dilute hydrochloric acid to a solution of the carbamide gives a clear anisotropic gel. The *magnesium*, *calcium*, and *barium* salts are sparingly soluble.

1-m-*Nitrobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—H-Acid (14·7 g.) was suspended in 70 c.c. of water with addition of 5·5 g. of hydrated sodium acetate (1 mol.) and nitrobenzoylated as described for earlier members. After acidification to Congo-paper, the heavy crop of crystals was collected, dried, and extracted with ether, leaving 18·2 g. of crude amide. The mother-liquors gave a further 2·6 g. on concentration. These combined crops were dissolved in boiling water and treated with a quarter of its volume of alcohol. On keeping, the pure amide separated in compact clusters of needles (Found: Loss at 120°, 19·3. $C_{17}H_{10}O_{10}N_2S_2Na_2,7H_2O$ requires H_2O, 19·7%. Found in anhydrous salt: Na, 8·9. $C_{17}H_{10}O_{10}N_2S_2Na_2$ requires Na, 9·0%). This *amide* is readily soluble in warm water and is not precipitated by concentrated hydrochloric acid. It is only salted out with difficulty and separates in a crystalline state. The *barium* salt, tufts of needles, is readily soluble in warm water but much less soluble in cold.

1-m-*Aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—The foregoing nitro-amide (12·8 g.) was reduced with ferrous chloride and alkali at 0° in the usual way. The filtrate and alkaline extracts of the ferric hydroxide on neutralisation gave a copious crystalline precipitate, which was collected, dissolved in 200 c.c. of boiling water, and treated with 100 c.c. of alcohol. After an interval, the pure amino-compound separated in delicate needles (yield, 9·3 g.) (Found: Loss at 160°, 17·4. $C_{17}H_{13}O_8N_2S_2Na,5H_2O$ requires H_2O, 16·4%. Found in dried salt: Na, 4·7. $C_{17}H_{13}O_8N_2S_2Na$ requires Na, 5·0%). This *sodium hydrogen* salt is not very soluble in warm water and it separates in anisotropic gelatinous particles. A dilute aqueous solution gives an immediate anisotropic precipitate on addition of dilute hydrochloric acid, probably of the disulphonic acid, which separates in microscopic leaflets if the solution be warmed

and allowed to cool. The *calcium*, *magnesium*, and *barium* salts are sparingly soluble, gelatinous precipitates. The *disodium* salt is very soluble in water and crystallises in needles.

1-m′-*Nitrobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—The foregoing amino-compound (10·7 g.) was nitrobenzoylated as described at the previous stage. The resulting solution consisted of a viscous syrup containing anisotropic stringy or worm-like masses in suspension. It was diluted with 2000 c.c. of water, acidified, and extracted with ether. The solution was neutralised and then set to a clear anisotropic gel. It liquefied on rise of temperature and was concentrated at 50° to one-half its bulk and acidified with 200 c.c. of concentrated hydrochloric acid. This caused the precipitation of anisotropic liquid threads from which the mother-liquor could be decanted. When these were dissolved in hot water (140 c.c.) and treated with 280 c.c. of alcohol, the required salt separated in a filterable condition (yield, 11·8 g.) (Found: Loss at 160°, 17·4. $C_{24}H_{15}O_{11}N_3S_2Na_2,7\frac{1}{2}H_2O$ requires H_2O, 17·6%. Found in dried salt: Na, 7·3. $C_{24}H_{15}O_{11}N_3S_2Na_2$ requires Na, 7·3%). This *disodium* salt is soluble in boiling water and, on cooling, separates in anisotropic, worm-like growths. A dilute aqueous solution treated with a little sodium chloride sets to a clear anisotropic gel. Under the most favourable conditions for crystallisation—a little water and excess of alcohol—it separates in anisotropic, gelatinous aggregates devoid of recognisable structure.

1-m′-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—The preceding nitro-compound (9·0 g.) was reduced at 0° with ferrous chloride and alkali. The filtrate and alkaline extracts of the ferric hydroxide were neutralised to Congo-paper and on being kept for 24 hours deposited almost the whole of the amino-compound as an amorphous precipitate. This was dissolved in 100 c.c. of boiling water and treated with 50 c.c. of alcohol; it then separated slowly in the amorphous condition (Found: Loss at 160°, 16·9. $C_{24}H_{18}O_9N_3S_2Na,6\frac{1}{2}H_2O$ requires H_2O, 16·8%. Found in anhydrous salt: Na, 4·3. $C_{24}H_{18}O_9N_3S_2Na$ requires Na, 4·0%). This *sodium hydrogen* salt is not very soluble in boiling water. It separates on cooling in microscopic spicules or leaflets. Its dilute aqueous solution is precipitated by concentrated hydrochloric acid as a gelatinous, anisotropic product, which dissolves on warming and then crystallises in fine needles. It gives an orange colour on diazotisation.

ON-*Carbonyl*-1 : 8-*aminonaphthol*-3 : 6-*disulphonic Acid*.—H-Acid (5 g.) was dissolved in 100 c.c. of water containing 10 c.c. of 2*N*-sodium hydroxide and 20 g. of anhydrous sodium carbonate. The solution was phosgenated till acid to Congo-paper. The solid was

collected and crystallised from hot water (40 c.c.) (yield, 4 g.) (Found : Loss at 150°, 16·1. $C_{11}H_5O_8NS_2Na_2,4H_2O$ requires H_2O, 15·6%. Found in dried salt : Na, 11·8. $C_{11}H_5O_8NS_2Na_2$ requires Na, 11·8%). This *disodium* salt is soluble in 10 parts of cold water to a neutral solution. It crystallises in needles which darken on exposure to the air. It is readily salted out by sodium chloride and gives a very sparingly soluble *barium* salt. Unlike all the other derivatives of H-acid described in this paper, which give an intense magenta-red colour with Pauly's reagent in sodium carbonate solution, this salt gives no coloration.

s-*Carbamide of* 1-m-*Aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—Five g. of the aminobenzoylamide were submitted to a double phosgenation as described in previous instances. The precipitated solid was collected, dissolved in 15 c.c. of boiling water, neutralised, and treated with 7 c.c. of alcohol. The carbamide separated in clusters of gelatinous leaflets (yield, 3·0 g.) (Found : Loss at 160°, 19·8. $C_{35}H_{22}O_{17}N_4S_4Na_4,13\frac{1}{2}H_2O$ requires H_2O, 19·7%. Found in anhydrous salt : Na, 9·2. $C_{35}H_{22}O_{17}N_4S_4Na_4$ requires Na, 9·3%). This *carbamide* is soluble in boiling water and crystallises, on cooling, in the same way as from dilute alcohol. A solution in dilute nitric acid gives an orange-red solution on boiling. With magnesium, calcium, and barium chlorides it yields sparingly soluble, gelatinous precipitates.

s-*Carbamide of* 1-m′-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—Five g. of the amino-compound were phosgenated in the usual way. The solution was saturated with sodium chloride, and the solid collected. It was dissolved in 260 c.c. of boiling water, neutralised, and treated with 200 c.c. of alcohol. The carbamide separated in gelatinous particles which were isotropic (yield, 3·3 g.) (Found : Loss at 160°, 20·6; Na, 6·0. $C_{49}H_{32}O_{19}N_6S_4Na_4,17\frac{1}{2}H_2O$ requires H_2O, 20·4; Na, 6·0%). This *carbamide* is not very soluble in water. It is readily salted out and gives sparingly soluble salts with magnesium, barium, and calcium chlorides.

2-m-*Nitrobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—2R-Acid (24·2 g. of commercial disodium salt) was dissolved in 150 c.c. of water and nitrobenzoylated in the usual way. The highly coloured but clear solution was acidified to Congo-paper and on being kept for 12 hours at 0° gave 37 g. of crystalline solid, which, on ether extraction, gave 27·0 g. of amide. The original aqueous liquors after extraction with ether and concentration gave a further 4·2 g. of amide. The combined crops crystallised from 100 c.c. of water in small prisms (yield, 25·2 g.) (Found : Loss at 160°, 13·6. $C_{17}H_{10}O_{10}N_2S_2Na_2,4\frac{1}{2}H_2O$ requires H_2O, 13·7%. Found in anhydr-

ous salt: Na, 8·7. $C_{17}H_{10}O_{10}N_2S_2Na_2$ requires Na, 9·0%). This *disodium* salt is readily soluble in hot water but sparingly soluble in cold. It forms a *barium* salt, sparingly soluble in water, which crystallises in microscopic clusters of needles. This amide is readily salted out; it gives an eosin-like colour in sodium carbonate solution with Pauly's reagent. When boiled with dilute nitric acid it gives an orange-red solution.

2-m-*Aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—The above-described nitro-compound (20·8 g.) was reduced at 0° with ferrous chloride and alkali in the usual way. The filtrate and alkaline extracts of the ferric hydroxide on neutralisation to Congo-paper deposited 16·8 g. of small needles. The mother-liquor on concentration gave a further 2·1 g. The product was recrystallised from 10 volumes of boiling water (Found: Loss at 160°, 13·6. $C_{17}H_{13}O_8N_2S_2Na,4H_2O$ requires H_2O, 13·5%. Found in anhydrous salt: Na, 4·7. $C_{17}H_{13}O_8N_2S_2Na$ requires Na, 5·0%). This *amide* has a faint acid reaction to Congo-paper. It forms a *diazosulphonate*, fine needles, and it gives a sparingly soluble *barium* salt, granular crystals. It is very readily salted out.

2-m′-*Nitrobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—The preceding amino-compound (10·6 g.) in 100 c.c. of water was nitrobenzoylated by the usual process. The final product was a clear anisotropic gel. Addition of a little concentrated hydrochloric acid gave a limpid fluid, but excess gave a clear anisotropic gel. This was stirred with sodium chloride; it then coagulated and could be filtered. It was dissolved in 150 c.c. of boiling water and treated with 400 c.c. of alcohol. On keeping, the nitro-compound separated in the amorphous state, but gradually transformed into clusters of microscopic needles (yield, 11·5 g.) (Found in product from 50% alcohol: Loss at 160°, 17·1. $C_{24}H_{15}O_{11}N_3S_2Na_2,7H_2O$ requires H_2O, 16·7%. Found in dried salt: Na, 7·2. $C_{24}H_{15}O_{11}N_3S_2Na_2$ requires Na, 7·3%). This *nitro-compound* is best obtained white and in needles by stirring it with just insufficient boiling water to dissolve it and adding alcohol gradually to complete its solution and then several volumes of alcohol. The *calcium* and *barium* salts are sparingly soluble.

2-m′-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—The foregoing nitro-compound (12·0 g.) was dissolved in 222 c.c. of 0·5*N*-sodium hydroxide and reduced with ferrous chloride and alkali at 15° in the usual way. The filtrate and alkaline extracts of the ferric hydroxide were acidified and separation of the anisotropic solid was completed by saturation with sodium chloride. The gelatinous solid obtained on filtration was dissolved in 100 c.c. of boiling water and treated with 300 c.c. of

alcohol. On keeping at 0° for some days, a gelatinous, anisotropic mass filled the fluid. This was collected by filtration and washed free from chloride by 90% alcohol. The mother-liquors were concentrated to a small volume and re-treated in a similar way. The total yield of solid, dried in a vacuum, was 9·0 g. (Found : Loss at 160°, 20·6. $C_{24}H_{18}O_9N_3S_2Na,8\frac{1}{2}H_2O$ requires H_2O, 20·9%. Found in dried salt : Na, 4·3. $C_{24}H_{18}O_9N_3S_2Na$ requires Na, 4·0%). This *amino-compound* is readily soluble in hot water and separates on cooling in gelatinous, anisotropic needles. It is very readily salted out and if such a solution be warmed until clear and be allowed to cool, it sets to a clear anisotropic gel. A dilute solution in hydrochloric acid gives a clear anisotropic, gelatinous solution on addition of sodium nitrite and the product couples with alkaline β-naphthol. Hot dilute nitric acid gives a red coloration, and an alkaline solution couples with diazotised sulphanilic acid with production of an eosin-like coloration.

s-*Carbamide of* 2 : 8-*Aminonaphthol*-3 : 6-*disulphonic Acid*.—2R-Acid (5·0 g.) in 250 c.c. of water containing 26 g. of anhydrous sodium carbonate was phosgenated until the reaction was acid to Congo-paper. The solution was neutralised and saturated with sodium chloride and on being kept for several days deposited the carbamide mixed with some unchanged amine. It was redissolved, submitted to a second phosgenation, and when the conditions were adjusted for its isolation it slowly separated. It crystallised from 2 volumes of boiling water in rods (yield, 45%) (Found : Loss at 160°, 19·2. $C_{21}H_{12}O_{15}N_2S_4Na_4,10H_2O$ requires H_2O, 19·3%. Found in dried salt : Na, 12·3. $C_{21}H_{12}O_{15}N_2S_4Na_4$ requires Na, 12·2%). This *carbamide* is readily soluble in water and is not readily salted out. It gives no precipitate with concentrated hydrochloric acid, whereas 2R-acid gives a precipitate of needles. Unlike 2R-acid, it gives a microcrystalline *barium* salt on addition of concentrated barium chloride solution.

s-*Carbamide of* 2-m-*Aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid*.—The parent amine (5·8 g.) was submitted to a double phosgenation in the usual way. The precipitated solid was dissolved in 17·5 c.c. of boiling water with neutralisation and precipitated by addition of an equal volume of alcohol (yield of material dried in a vacuum, 2·8 g.) (Found : Loss at 160°, 13·6. $C_{35}H_{22}O_{17}N_4S_4Na_4,8\frac{1}{2}H_2O$ requires H_2O, 13·4%. Found in dried solid : Na, 9·2. $C_{35}H_{22}O_{17}N_4S_4Na_4$ requires Na, 9·3%). This *carbamide* is readily soluble in water and crystallises from very concentrated solutions as a microcrystalline powder, but better by addition of sodium chloride. The *calcium* and *barium* salts are sparingly soluble.

s-*Carbamide of* 2-m′-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3 : 6-*disulphonic Acid.*—The parent amine (5 g.) in 100 volumes of water containing 20 g. of sodium carbonate was submitted to a double phosgenation at 30°. The precipitated solid was neutralised, dissolved in 120 c.c. of boiling water, and treated with 120 c.c. of absolute alcohol. The yield of material dried in a vacuum over sulphuric acid was 3·0 g. (Found: Loss at 160°, 11·9. $C_{49}H_{32}O_{19}N_6S_4Na_4,9H_2O$ requires H_2O, 11·7%. Found in dried solid: Na, 7·6. $C_{49}H_{32}O_{19}N_6S_4Na_4$ requires Na, 7·5%). This *carbamide* is easily soluble in warm water and is readily salted out as a weakly anisotropic solid. Concentrated hydrochloric acid precipitates a weakly anisotropic, finely divided substance, possibly the free tetrasulphonic acid. The *magnesium*, *calcium*, and *barium* salts are sparingly soluble, gelatinous substances.

m-*Nitrobenzoyl*-1-*naphthylamine*-4 : 6 : 8-*trisulphonic Acid.*—The parent naphthylaminetrisulphonic acid (9·0 g. of disodium salt) was nitrobenzoylated in the usual way. After removal of nitrobenzoic acid the neutralised liquors on concentration gave the required amide, which was recrystallised from a small volume of water (yield, 13·55 g.) (Found: Loss at 160°, 11·7. $C_{17}H_9O_{12}N_2S_3Na_3,4\frac{1}{2}H_2O$ requires H_2O, 11·9%. Found in dried substance: Na, 11·4. $C_{17}H_9O_{12}N_2S_3Na_3$ requires Na, 11·5%). This *amide* is very readily soluble in water and is only salted out with difficulty. It is best crystallised from diluted alcohol and then separates in clusters of needles. It forms a readily soluble *calcium* salt and a somewhat less soluble *barium* salt, crystallising in woolly needles.

m-*Aminobenzoyl*-1-*naphthylamine*-4 : 6 : 8-*trisulphonic Acid.*—13·3 G. of the above nitro-amide were reduced with ferrous chloride and alkali. The neutralised filtrates, freed from iron, were concentrated under reduced pressure at 80° and as much sodium chloride removed as possible by evaporation and filtration and precipitation with alcohol. The amino-compound was then precipitated as an oil by excess of alcohol and gradually solidified. The yield of crude material containing some sodium chloride was 11·1 g. It was not obtained sufficiently pure for analysis. It is extremely soluble in water and is not salted out. It is also soluble in hot methyl alcohol. It diazotises and then couples with β-naphthol. From ethyl-alcoholic solutions containing a little water it crystallises in needles or prisms.

m′-*Nitrobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-4 : 6 : 8-*trisulphonic Acid.*—8 G. of the above amino-compound were nitrobenzoylated in the usual way. The neutralised liquor on concentration deposited a felted mass of needles, which were collected

without washing, dissolved in 10 c.c. of hot water, and treated with 50 c.c. of alcohol. When cool, the required nitro-amide had separated as a felt of very fine needles (yield, 7·4 g.). For analysis, it was recrystallised from 90% alcohol (Found: Loss at 160°, 16·7. $C_{24}H_{14}O_{13}N_3S_3Na_3,8H_2O$ requires H_2O, 16·7%. Found in dried substance: Na, 9·4. $C_{24}H_{14}O_{13}N_3S_3Na_3$ requires Na, 9·6%). This *nitro-amide* is very easily soluble in water, but is readily salted out. On addition of calcium or barium chloride the *calcium* or *barium* salt is precipitated as a gelatinous, anisotropic product, but on keeping the barium salt crystallises in tufts of woolly needles.

m′-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-4 : 6 : 8-*trisulphonic Acid*.—The preceding nitro-compound (7·5 g.) was reduced at 0° with ferrous chloride in the usual way. The solution, neutralised to Congo-paper and free from iron, was concentrated to about 60 c.c.; it then set to a paste of fine, short needles. These were collected without washing, dissolved in 20 c.c. of hot water, and treated with 25 c.c. of absolute alcohol. The product separated as an anisotropic, gelatinous mass filling the solution. It was filtered off and well washed with 80% alcohol, but could not thus be freed from chloride. The yield of material dried in a vacuum was 4·5 g. This product is a *disodium* salt and can be salted out from aqueous solutions, but not very readily. For analysis it was dissolved in a small volume of hot water, neutralised to litmus, and treated with several volumes of alcohol. On keeping, the solution deposited balls of woolly needles of the *trisodium* salt (Found in material dried in a vacuum: Loss at 160°, 8·7. $C_{24}H_{16}O_{11}N_3S_3Na_3,3\frac{1}{2}H_2O$ requires H_2O, 8·4%. Found in anhydrous solid: Na, 9·6. $C_{24}H_{16}O_{11}N_3S_3Na_3$ requires Na, 10·0%). The trisodium salt is not salted out and is extremely readily soluble in water. If the reaction of the original liquors from the reduction be neutral to litmus instead of neutral or acid to Congo, no amino-compound separates until almost all the sodium chloride has been removed and the solution concentrated to a syrup.

s-*Carbamide of* 1-*Naphthylamine*-4 : 6 : 8-*trisulphonic Acid*.—The parent amine (10 g.) was phosgenated at 30° in a solution containing 100 g. of anhydrous sodium carbonate in 250 c.c. of water. The solution was neutralised and concentrated repeatedly under reduced pressure with intermediate removal of as much sodium chloride as possible. The residual filtrate was evaporated almost to dryness in a vacuum, treated with a few c.c. of warm water, and decanted from undissolved sodium chloride. On cooling, the carbamide separated in long, delicate needles, which were recrystallised from a little water. In this way, 4·1 g. of the carbamide were obtained, still containing some sodium chloride which could not be removed

from the limited quantities at our disposal. This *carbamide* is extremely readily soluble in water, is not salted out, and gives no precipitate on addition of concentrated hydrochloric acid or alkaline-earth chlorides.

s-*Carbamide of* m-*Aminobenzoyl*-1-*naphthylamine*-4 : 6 : 8-*trisulphonic Acid.*—The unisolated amino-compound prepared from 10 g. of the corresponding nitro-compound was made up to 500 c.c. with water and treated with 40 g. of anhydrous sodium carbonate, and carbonyl chloride passed in until the solution was acid. The neutralised solution was concentrated and as much sodium chloride removed as possible, first by direct crystallisation, then by addition of ethyl alcohol, and finally by addition of methyl alcohol. The liquor was then concentrated to a small volume and several volumes of methyl alcohol were added so long as there was precipitation of sodium chloride; after a few days, below 0°, the required carbamide separated in soft needles (yield, 6·8 g.). For analysis, it was recrystallised under the same conditions from 66% methyl alcohol at — 5° (Found in material dried in a vacuum : Loss at 160°, 6·1. $C_{35}H_{20}O_{21}N_4S_6Na_6,4H_2O$ requires H_2O, 5·8%. Found in dried solid : Na, 11·4. $C_{35}H_{20}O_{21}N_4S_6Na_6$ requires Na, 11·9%). This *carbamide* is hygroscopic and extremely readily soluble in water or in 98% methyl alcohol and is not salted out.

s-*Carbamide of* m'-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-4 : 6 : 8-*trisulphonic Acid.*—The base prepared from the nitro-compound (13·8 g. anhydrous) without isolation was phosgenated in 500 c.c. of water in presence of 50 g. of anhydrous sodium carbonate. The carbamide was isolated in the same way as the preceding member. The yield of crystalline solid containing a little chloride was 9·65 g. For analysis, it was dissolved in 10 c.c. of hot water and treated with 35 c.c. of 98% methyl alcohol; it then slowly crystallised, below 0°, in clusters of white needles. It was washed with 80% methyl alcohol, cooled to — 5° (yield, 8·1 g.). As it was hygroscopic, it was dried in a vacuum (Found : Loss at 160°, 9·3. $C_{49}H_{30}O_{23}N_6S_6Na_6,8H_2O$ requires H_2O, 9·3%. Found in dried solid : Na, 9·5. $C_{49}H_{30}O_{23}N_6S_6Na_6$ requires Na, 9·8%). This *carbamide* is very soluble in water or methyl alcohol and is not salted out. With saturated barium chloride solution, it gives after some delay a *barium* salt in anisotropic particles. It gives no precipitate with lead acetate, but is precipitated by basic lead acetate.

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