162. *The Preparation and Properties of Certain Poly-sulphanilamide Compounds.*

By Frederick G. Mann and James Watson.

A number of compounds having two, three, or four sulphanilamide groups in the molecule have been prepared and investigated. One compound proved to have slight antibacterial action; otherwise they were therapeutically inactive.

THE vast amount of chemical work on derivatives of sulphanilamide which has followed the discovery of the therapeutic value of the parent compound has been directed mainly in two channels, (*a*) the effect of introducing and interchanging various groups in the sulphanilamide molecule, and (*b*) that of linking the sulphanilamide group to various heterocyclic systems (for a summary of results up to 1940, see Northey, *Chem. Rev.*, 1940, **27**, 85). We have now investigated a third aspect, *viz.*, the effect of introducing several sulphanilamide groups into one molecule, whereby the therapeutic action might be affected both by the number of groups and by their relative spatial position within the molecule. The latter factor is one which has hitherto received comparatively little attention.

It was recognised that any increase in therapeutic action due to the multiplicity of the sulphanilamide groups might be largely counterbalanced by the low solubility of compounds of this type; therefore only poly-sulphanilamido-derivatives of simple aliphatic compounds were prepared, in order to obtain comparatively low molecular weights and hence, it was hoped, reasonably high solubilities. Furthermore, the sulphanilamide units were always linked through the amide groups, in order to leave the *p*-amino-groups unsubstituted, an essential condition for high bactericidal action in the sulphanilamide series.

The condensation of tetrakisaminomethylmethane, $C(CH_2{\cdot}NH_2)_4$ (Litherland and Mann, J., 1938, 1588), in alkaline solution with *p*-acetamidobenzenesulphonyl chloride could only be effected by working at 45—50° and at great dilution, and by adding sodium hydroxide and the chloride successively in very small quantities; a smooth condensation to give *tetra*-(p-*acetamidobenzenesulphonamidomethyl*)*methane* (I, R = Ac) in high yield then resulted. Hydrolysis with hydrochloric acid gave the required *tetra*-p-*aminobenzene* derivative (I, R = H). Clearly, in this compound the points of attachment of the four sulphanilamido-residues are tetrahedrally arranged around the central carbon atom.

(I.) $C(CH_2{\cdot}NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHR)_4$ $N(CH_2{\cdot}CH_2{\cdot}NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHR)_3$ (II.)

Similar treatment of ββ′β″-triaminotriethylamine, $N(CH_2{\cdot}CH_2{\cdot}NH_2)_3$ (Mann and Pope, *Proc. Roy. Soc.*, 1925, *A*, **109**, 444), gave *tri*-(β-p-*acetamidobenzenesulphonamidoethyl*)*amine* (II, R = Ac) which on acid hydrolysis gave the required *tri*-p-*aminobenzenesulphonamido*-derivative (II, R = H). Although in this molecule the normal oscillations of the three valencies of the central nitrogen atom are possibly affected by the heavy groups to which they are attached, the molecule can be considered as being virtually planar, with the sulphonamidoethyl groups arranged at 120° to one another.

All similar attempts to prepare the corresponding sulphonamido-derivative of γγ′γ″-triaminotripropylamine failed: this amine differs from the ethyl homologue in having a markedly basic tertiary amine group capable of forming stable salts (Mann and Pope, J., 1926, 482, 489) and it was hoped that this property would cause a valuable increase in the water-solubility of the final product.

αα′-Diamino*iso*propyl alcohol (Mann, J., 1927, 2913), when similarly condensed with the sulphonyl chloride and then subjected to acid hydrolysis, gave αα′-*di*-(p-*aminobenzenesulphonamido*)iso*propyl alcohol* (III, R = H): this compound, which was more soluble in water than any other member of the series, has the two sulphonamidomethyl groups held at the tetrahedral angle as in (I). A fourth compound, structurally allied to (III), was obtained from αβγ-triaminopropane, which furnished αβγ-*tri*-(p-*aminobenzenesulphonamido*)*propane* (IV, R = H).

(III.) $CH(OH)(CH_2{\cdot}NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHR)_2$ $NHR{\cdot}C_6H_4{\cdot}SO_2{\cdot}NH{\cdot}CH(CH_2{\cdot}NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHR)_2$ (IV.)

The fifth compound, differing in general type from the above derivatives, was obtained from *NN′*-di-(β-aminoethyl)ethylenediamine, $C_2H_4(NH{\cdot}C_2H_4{\cdot}NH_2)_2$. When this base in pyridine solution was treated with the sulphonyl chloride, NN′-*di*-(p-*acetamidobenzenesulphonyl*)-NN′-*di*-(β-p-*acetamidobenzenesulphonamidoethyl*)-*ethylenediamine* (V, R = Ac) was obtained. This compound on hydrolysis gave the *tetra*-(p-*aminobenzenesulphonamido*)-derivative (V, R = H).

(V.) $[{\cdot}CH_2{\cdot}N(SO_2{\cdot}C_6H_4{\cdot}NHR){\cdot}CH_2{\cdot}CH_2{\cdot}NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHR]_2$

In spite of the many polar groups in the above *p*-amino-compounds, they all had very low solubilities in water, (III, R = H) being the most soluble. It is significant that Dr. G. Brownlee, of the Wellcome Physiological Research Laboratories, who has investigated these compounds, finds that only the compound (III) has perceptible antibacterial action, and this is too slight to be of value in enteric infections; none of the compounds is apparently absorbed from the gut of the mouse.

Since certain sulphanilamide compounds are known to have an effective action upon monkey malaria, it is always possible that new compounds of this type might prove of value in the treatment of human malaria. Dr. Ann Bishop, of the Molteno Institute of Parasitology, has therefore kindly tested the action of the above polysulphanilamido-compounds upon infections of *Plasmodium relictum* (*a*) transmitted to canaries by intramuscular inoculation of infected blood, and (*b*) induced in canaries by sporozoites. Dr. F. Hawking and Miss I. M. Tonkin, of the National Institute for Medical Research, have also tested the action of compounds (I, III, IV, and V, R = H) on 10-day old chicks which had been infected with *Plasmodium gallinaceum* by intravenous blood injection. None of these compounds, however, showed any antimalarial activity in these tests.

In view of Dr. Brownlee's results with mice, however, it is possible that these compounds are also absorbed only to a negligible extent from the gut of the birds used, and that this factor may have wholly or partly

invalidated the above antimalarial tests, in all of which the compounds were administered orally. Dr. Bishop has therefore further examined compound (III), the most promising of this group, by giving direct intramuscular injections of it to a canary previously infected with *P. relictum* by an intramuscular inoculation of infected blood. Again, no antimalarial action could be detected. In view of these results, it is highly probable that these compounds would also be without effect on human malaria.

Experimental.

All the sulphonamido-compounds described below were dried for 2 hours at either 80°/15 mm. or 130°/15 mm. (depending on their m. p.'s) before analysis and final m. p. determination : all were colourless when pure. The acid used throughout for hydrolysing the acetamido-compounds was concentrated hydrochloric acid diluted with an equal volume of water.

Tetra-(p-*acetamidobenzenesulphonamidomethyl*)*methane* (I, R = Ac).—10% Aqueous sodium hydroxide solution (12·2 c.c., 4 mols.) was added to a suspension of tetrakisaminomethylmethane dihydrogen sulphate (2·5 g.) in water (200 c.c.), and the clear solution of the free base was then warmed to 45—50°. Finely powdered *p*-acetamidobenzenesulphonyl chloride (0·4 g.) and 10% aqueous sodium hydroxide (0·7 c.c.) were then added (the alkali being just sufficient to neutralise the hydrogen chloride liberated by the condensation), and the mixture vigorously shaken for 5 mins. and then reheated to 45—50° for a further 10 mins. Eighteen further similar additions of the chloride and the alkali were then made, followed by the shaking and heating at 45—50° as before, the total addition of the chloride (7·6 g.) being thus 4·3 mols. Excess (25 c.c.) of 10% sodium hydroxide was then added, and the mixture mechanically shaken overnight. The *tetra-acetamido*-compound (I, R = Ac) (3·15 g., 45% of theoretical) which had separated was collected, washed with water and was then pure. A second, less pure fraction was obtained by acidifying the filtrate with hydrochloric acid; the precipitate thus produced was collected, washed with water, dissolved in dilute sodium hydroxide solution, reprecipitated with a slight excess of hydrochloric acid, and finally treated with sodium carbonate solution. This second fraction (1·5 g., 22% of theoretical) was recrystallised from a mixture of glycol monomethyl ether and water. The compound (I, R = Ac) is insoluble in all the usual cold solvents except glycol monomethyl ether : it has m. p. 304—306° (Found : C, 47·9; H, 4·9; N, 11·9. $C_{37}H_{44}O_{12}N_8S_4$ requires C, 48·2; H, 4·8; N, 12·2%).

A mixture of this acetamido-compound (6·3 g.) and dilute hydrochloric acid (250 c.c.), when refluxed for 2 hours, gave a clear pale brown solution. Cooling, followed by addition of excess sodium carbonate, precipitated the crude pale pink *compound* (I, R = H) (5 g., 97%). This product was most satisfactorily purified by warming with water (1 l.), adding 10% sodium hydroxide until a clear solution was obtained, and boiling this with charcoal. The solution was then cooled, filtered, and hydrochloric acid added until the precipitated amino-compound redissolved; the boiling with charcoal was now repeated in the acid solution, which was then again cooled, filtered, and the pure amino-compound (3·4 g.) precipitated by sodium carbonate solution. It is soluble at room temperature only in acetone, and was recrystallised from aqueous acetone; m. p. 243·5—244° (Found : C, 46·5; H, 4·9; N, 14·8. $C_{29}H_{36}O_8N_8S_4$ requires C, 46·2; N, 4·8; N, 14·9%).

Tri-(β-p-*acetamidobenzenesulphonamidoethyl*)*amine* (II, R = Ac).—The same method was employed as for the previous acetyl derivative. A solution of ββ′β″-triaminotriethylamine trihydrochloride (2·1 g.) in water (140 c.c.) was decomposed by addition of 10% sodium hydroxide solution (9·8 c.c.), and warmed to 45—50°. Twenty-two successive additions, each of *p*-acetamidobenzenesulphonyl chloride (0·3 g.) and 10% alkali (0·5 c.c.) were then made with vigorous intermediate shaking, and finally 4 further additions of alkali (0·5 c.c.). After 1 hour's final shaking, excess sodium hydroxide was added, and carbon dioxide bubbled through the filtered solution to precipitate the *triacetamido*-compound (5·3 g., 87%). The product, recrystallised from aqueous alcohol (charcoal), softened at 115° and melted at 198·5—200·5° (Found : C, 48·6; H, 6·0; N, 13·3. $C_{30}H_{39}O_9N_7S_3$ requires C, 48·8; H, 5·3; N, 13·3%).

For hydrolysis, a mixture of the triacetamido-compound (4·5 g.) and dilute hydrochloric acid (90 c.c.) was refluxed for 1 hour, a clear solution being readily obtained. This was cooled, diluted with water (150 c.c.), and the crude *tri-p-aminobenzene* derivative (II, R = H) precipitated as a red viscous product by sodium carbonate. It was purified by boiling in hydrochloric acid solution (charcoal), reprecipitation with sodium carbonate, and finally by dissolution in sodium hydroxide solution, followed by precipitation with carbon dioxide. The pure derivative (3·2 g., 86%) separated as a fine powder, which was recrystallised from aqueous acetone; m. p. 178·5—180° (decomp.) (Found : C, 47·5; H, 5·5; N, 16·5. $C_{24}H_{33}O_6N_7S_3$ requires C, 47·15; H, 5·4; N, 16·0%).

All attempts to condense *p*-acetamidobenzenesulphonyl chloride with an aqueous solution of γγ′γ″-triaminotripropylamine failed. Attempted interaction of these compounds in pyridine solution, in boiling ethereal solution in the presence of potassium carbonate, and finally in an acetone solution vigorously agitated with 33% aqueous sodium hydroxide solution, also failed.

αα′-*Di-*(p-*acetamidobenzenesulphonamido*)iso*propyl alcohol* (III, R = Ac).—0·3N-Sodium hydroxide solution was added to a solution of αα′-diamino*iso*propyl alcohol dihydrochloride (1 g.) until the solution was just alkaline to phenolphthalein. The powdered sulphonyl chloride (4·6 g., 3·2 mols.) was added, the mixture warmed to 45—50°, 0·3N-alkali (25 c.c.) added, and the mixture shaken for 15 mins. Successive additions (25 c.c.) of the alkali and shakings at 45—50° were continued until a clear solution was obtained. Cooling, followed by acidification with acetic acid, precipitated the crude *compound* (III, R = Ac) (2·8 g., 95%). This compound is insoluble in most hot liquids, but dissolves in hot nitrobenzene, acetic acid, and the glycol monoalkyl ethers. It was purified by successive extraction with hot water, alcohol, and acetone, followed by crystallisation from aqueous glycol monomethyl ether; m. p. 232·5—233·5° (Found : C, 47·6; H, 5·2; N, 11·4. $C_{19}H_{24}O_7N_4S_2$ requires C, 47·1; H, 5·0; N, 11·6%).

When a mixture of this compound (2·4 g.) and dilute hydrochloric acid (40 c.c.) was refluxed for ¾ hour, and the clear solution then cooled in ice and treated with sodium carbonate, the *di-*(p-*aminobenzenesulphonamido*)-compound (III, R = H) was precipitated, m. p. 177—179°, after recrystallisation from hot water (Found : C, 45·4; H, 5·4; N, 14·0. $C_{15}H_{20}O_5N_4S_2$ requires C, 45·0; H, 5·0; N, 14·0%).

αβγ-*Tri-*(p-*acetamidobenzenesulphonamido*)*propane* (IV, R = Ac).—αβγ-Triaminopropane trihydrochloride monohydrate was prepared by Mann and Pope's method (*Proc. Roy. Soc.*, 1925, *A*, **107**, 80). The highest yield of its tri-*p*-acetamido-derivative was obtained when 21 additions, each of the sulphonyl chloride (0·4 g.) and of 0·5% aqueous sodium hydroxide (13·7 c.c.), were made successively to a solution of the above monohydrate (2·5 g.) in 1% sodium hydroxide (138 c.c.), the product being meanwhile shaken vigorously and kept at 45—50°. Finally, excess of 10% sodium hydroxide solution (*ca.* 50 c.c.) was added, and the shaking continued. A portion of the *tri-*p-*acetamido*-compound (IV, R = Ac) which had not dissolved in the alkali was now collected, and a second crop obtained by treating the filtrate with a small excess of hydrochloric acid and then with much sodium carbonate to redissolve any sulphanilic acid; the two crops when united (5·9 g., 75%) and recrystallised from aqueous acetic acid gave the pure acetamido-compound, m. p. 218·5—220·5° (Found : C, 47·5; H, 5·0; N, 12·4. $C_{27}H_{32}O_9N_6S_3$ requires C, 47·6; H, 4·7; N, 12·35%). The use

of more concentrated alkali in the above preparation, or its replacement by pyridine, caused a marked reduction in the yield of the final product.

For hydrolysis, a mixture of the triacetamido-compound (12 g.) and dilute hydrochloric acid (200 c.c.) was refluxed for 1·5 hours, and the clear brown solution was cooled and treated, first with 30% sodium hydroxide solution until a faint precipitate was obtained, and finally with sodium carbonate solution until precipitation was complete. The *tri*-(p-*aminobenzenesulphonamido*)*propane* (IV, R = H) thus obtained separated at first as a red, and later as a colourless, solid. Purification was best effected by adding the total product to hot water (2 l.), adding 10% sodium hydroxide until complete dissolution was obtained, and then boiling with charcoal for 5 mins. The solution was filtered, treated with hydrochloric acid until a clear solution was again obtained, and the charcoal treatment repeated. Filtration gave a colourless solution, which was cooled and treated cautiously with sodium carbonate solution; the compound (IV, R = H) separated as fine crystals (6·0 g., 62%), which were collected, washed with water, and recrystallised from aqueous acetone; m. p. 234·5—236° (decomp.) with softening at 220° (Found: C, 45·3; H, 4·6; N, 15·3. $C_{21}H_{26}O_6N_6S_3$ requires C, 45·5; H, 4·7; N, 15·2%). This compound is freely soluble in acetone, but almost insoluble in the other common solvents.

Preparation of NN′-*Di*-(*β*-*aminoethyl*)*ethylenediamine*.—Ethylene dibromide (20 c.c.) and ethylenediamine hydrate (54 c.c., 3 mols.) were mixed in a flask fitted with an efficient reflux condenser. After a few minutes, a vigorous reaction ensued with much heat evolution; when this reaction subsided, the mixture was heated at 130—140° for 4 hours. The product was cooled, treated with much powdered potassium hydroxide, and the dried liquid residue distilled first at atmospheric pressure to remove unchanged ethylenediamine and finally at reduced pressure to obtain the above pure tetramine, b. p. 150—152°/16 mm., 158—160°/20 mm.; a high-boiling by-product remained in the distillation flask.

A solution of the above amine (0·62 g.) in dry pyridine (5 c.c.) was added in 1-c.c. portions to a similar solution of the sulphonyl chloride (4·2 g., 4·25 mols.) in pyridine (5 c.c.), the latter being kept vigorously agitated meanwhile. The mixture became warm, developed a dark red colour, and deposited a small quantity of solid. It was then shaken for 2 hours, poured into water (150 c.c.), and the red precipitate of crude *tetra-acetamido*-compound (V, R = Ac) (2·2 g., 55%) collected, washed with water, and recrystallised from aqueous pyridine (charcoal); m. p. 290·5—291·5° (Found: C, 48·0; H, 5·3; N, 11·7. $C_{38}H_{46}O_{12}N_8S_4$ requires C, 48·7; H, 5·0; N, 12·0%). It is freely soluble only in hot pyridine, nitrobenzene, and the lower glycol monoalkyl ethers.

After a mixture of this acetamido-compound (5 g.) and dilute hydrochloric acid (200 c.c.) had been refluxed for 2 hours, some solid still remained undissolved, but a clear solution was obtained by subsequent dilution with hot water (600 c.c.). This solution, when cooled and treated with excess of sodium carbonate, gave the *tetra*-p-*aminobenzenesulphonamido*-compound (V, R = H) (3·4 g., 83%). It was purified by recrystallisation from aqueous glycol monomethyl ether (charcoal), a second crop being obtained by cautious addition of water to the filtrate; m. p. 208—209° (Found: C, 47·4; H, 5·4; N, 14·9. $C_{30}H_{38}O_8N_8S_4$ requires C, 47·0; H, 5·0; N, 14·6%). The compound is freely soluble only in hot nitrobenzene and glycol monomethyl ether.

Dr. Brownlee reports as follows on the compounds (I)—(V), where in all cases R = H:

"In Vitro *Tests*.—*Method A*: where a variable inoculum, diluted serially to the simple powers of 10, is used and the drug concentration is kept constant. The range of organisms included *Streptococcus*, *Staphylococcus*, *B. coli*, *B. typhosus*, *Shiga*, *Flexner* and *Sonne*. Saturated solutions of compounds (I)—(V) in broth have been tested and found inactive except (III), which was effective against all organisms tested except *Staphylococcus*.

Method B: where the inoculum is kept constant and the drug concentration is diluted serially to the simple powers of 10. Only the compound (III) was tested: this compound at a dilution of 1 in 2000 in broth inhibited the growth of *Streptococcus*, *B. typhosus*, and *B. dysenteriæ Shiga*.

Therapeutic Tests.—Compounds (I)—(V) in doses of 1 mg. and 10 mg. were tested orally for activity against *Streptococci* and *Staphylococci* in groups of six mice. No protection was shown by any compound.

Tests for absorption from the gut. Compounds (I)—(V) were fed to mice in doses of 25 mg. per 100 g. of mouse, and the animals bled at hourly intervals for 8 hours. Estimations were made on the blood to determine the drug concentrations, but no drug could be detected in the blood stream. It is concluded that the drugs are not absorbed."

The help received from Dr. G. Brownlee, Dr. A. Bishop, Dr. F. Hawking, and Miss I. M. Tonkin is gratefully acknowledged.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, October 5th*, 1943.]