CHEMOTHERAPY OF BACTERIAL INFECTIONS

Part II. Synthesis of Some Sulphanilamide Derivatives and the Relation of Chemical Constitution to Chemotherapeutic Action

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DOMAGK's discovery of the remarkable therapeutic properties of 'Prontosil' in *hemolytic streptococcal* infections has brought about a renaissance in the Chemotherapy of Bacterial Infections. Extensive researches and clinical trials have proved sulphanilamide and 2-N[1]-sulphanilamidopyridine* (M & B 693, sulfapyridine or dagenan) to possess irrefutable therapeutic properties in many bacterial infections such as those due to the *streptococci, pneumococci, gonococci, meningococci* and a few others. There are further many indications of the possibility of these types of compounds exerting good therapeutic effects in other types of infections though, as yet, no compound has been discovered showing such brilliant results as those obtained with the two drugs in the above-mentioned infections. These drugs do exhibit certain—at times even serious—toxic manifestations which require to be eliminated to make them absolutely harmless in their extensive applications. The synthetic work undertaken is to investigate these possibilities and to throw some light on the relation of chemical constitution to chemotherapeutic action in this class of compounds.

Many derivatives of sulphanilamide have been synthesised according to the usual method.[1] Of these compounds, the following have been tested independently by many workers abroad: 4-N[1]-sulphanilamidobenzenesulphonic acid[3,4]; 4-N[1]-sulphanilamidoaniline,[5,6,7] N-sulphanilylglycine[6 8,9] and the isomeric N[1]-sulphanilamidobenzoic acids.[3] Of the remaining compounds, seventeen were kindly tested by Dr. G. A. H. Buttle in experimental *streptococcal* and *pneumococcal* infections in mice, and the results have been reported and discussed elsewhere.[2]

Subsequently, some more compounds were tested here. Though the results will be reported elsewhere in detail,[35b] some of the interesting observations are recorded here. The compound, 2-N[1]-sulphanilamidothiazol


(I, R = H) which has since been reported by Fosbinder and Walter[35] and 2 : 8-sulphanilamidoacridine (II) possess striking protective action in experimental *streptococcal* and *pneumococcal* infections in mice. Till now, very pronounced *anti-pneumococcal* propeity was the sole monopoly of 2-N^1-sulphanilamidopyridine but these results give hope that other compounds might be discovered to beat its record. A number of sulphanilamide derivatives of thiazol of formula (I) and acridine are being prepared and tested. The dye (III), an interesting analogue of prontosil, was found to be unfit for use due to its highly staining properties. The corresponding azodye (IV) is far inferior to sulphanilamidopyridine in *pneumococcal* infections. It has been reported that 6-N^1-sulphanilamidoquinoline[2] and 8-N^1-sulphanilamidoquinoline[12] are extremely toxic and possess no appreciable therapeutic properties.
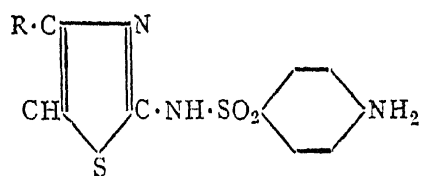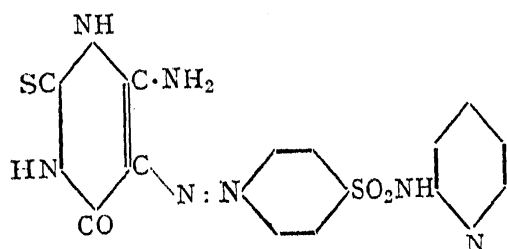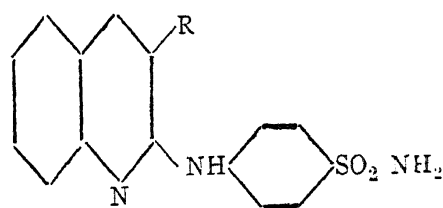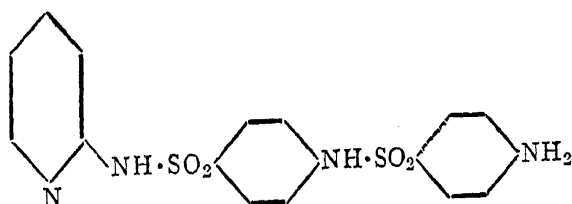

(I)
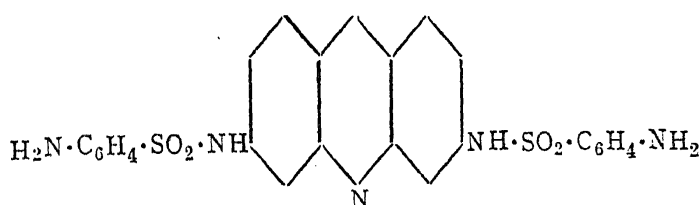
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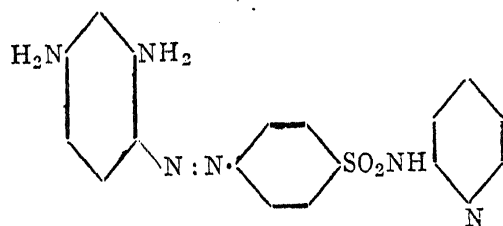
(III)

(IV)

(V)

(VI)

Recently, Gray[13] has reported N (2-quinolyl) sulphanilamide (V, R = H) to be inactive. We have found the corresponding carboxylic acid (V, R = CO₂H) to be comparatively of low toxicity and to possess some *antistreptococcal* and very little *antipneumococcal* properties. The three isomeric N^1-sulphanilamido-nitrobenzenes appear to be superior to sulphanilamide in *streptococcal* infections though far more toxic. The interesting pyridine derivative, 2-(4'-sulphanilamidobenzenesulphonamido) pyridine (VI) has been synthesised but it was disappointing to find that it possessed very little protective effect in both the infections.

Chemical Constitution and Chemotherapeutic Action

In the " race " to find out new effective therapeutic compounds in this sulphanilamide group, the therapeutic properties of nearly 500 derivatives have till now been reported, while about 150 compounds have also been described in the chemical and patent literature without any reference to their individual chemotherapeutic properties. From the nebulous data available, the elucidation of the relationship between the chemical constitution and chemotherapeutic action in this group proves to be a complex problem due to many reasons. Analogies from the investigations with the therapeutic and pharmacological effects of certain groupings in other class of compounds— *e.g.*, the arsenicals and the antimalarials—do not hold good in the present case. Though, at present, we are not able to enunciate definite rules without exceptions even within small limits of structural changes, opportunity is however taken, for future guidance, to give in broad outlines the effects produced by the structural changes.

1. The Fundamental Nucleus

Though the seat of chemotherapeutic activity (*antistreptococcal*) appears to be the grouping (VII),[14,2] compounds known to be highly active all possess the radical (VIII). The activity is almost lost if the relative positions of these groupings are changed or additional substituents introduced into the benzene ring;[29] as an exception, the compound (IX) is reported to be active.[15] The grouping (VII) is not indispensable for activity because, compounds with no sulphur atoms as the diphenyloxide or diphenylmethane derivatives,[8] nitrobenzoic acid, some arsenic derivatives,[16] and some nonbenzenoid compounds as dipyridylsulphide,[16] are all known to possess definite, though small, activity.

$$-N-\left\langle\bigcirc\right\rangle-S-R \qquad -N-\left\langle\bigcirc\right\rangle-SO_2-R \qquad HO\left\langle\bigcirc\right\rangle-SO_2NH_2$$
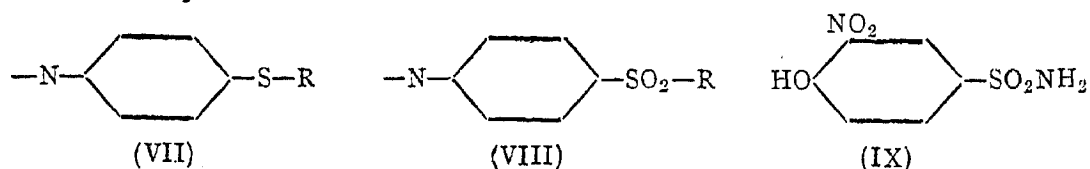
(VII)   (VIII)   (IX)

2. The Function of the (N⁴)-Nitrogen in (VII & VIII)

(*a*) The compounds, wherein this nitrogen atom functions as a nitro, nitroso or hydroxylamino and not a hydrazo-group, are all highly active, especially when they are benzenesulphonamide derivatives.[17]

(*b*) When the nitrogen atom is functioning as an azo-linkage, compounds of varied activity are produced ;[15,21,22,40e] but the activity of these dyes appear to be due to the amino compounds formed from them by reduction *in vivo*.[18,40]

(*c*) When (N⁴)-nitrogen functions as an amino-group, compounds of great therapeutic advantage are obtained. Substitutions at this amino-group in a variety of ways have not resulted in the production of compounds of far greater activity[18] ; there are some suggestions[18,36,39,40e] that the activity of these also may be due to the free amine liberated *in vivo*.

Substitution of the alkyl or (substituted alkyl group with amino, hydroxyl or carboxyl, and not the sulphonic [6, 19] radicals (or aralkyl groups at the N⁴-amino group decreases or destroys the activity.[22,23, 29,30] Acylation of the amino-group decreases or destroys the activity, [20,22,23] although valeryl, caproyl[20] and 2-pyridone 5-carboxyl groups[21] have been stated not to do so. Conversion of the amino-group into a urea[22] or a thiourea[2] derivative destroys the activity, while the guanidine derivative is reported[8] to be quite active. Conversion into Schiff's bases produces compounds of about the same activity statistically, but of far less toxicity.[21,2] Conversion into other types of heterocyclic derivatives results in the loss of activity.[23]

3. The Function of the Radical R in (VII & VIII)

(*a*) The compounds, wherein the radical R is a hydrogen atom, hydroxyl group or chlorine atom in (VII) or (VIII), are only of theoretical interest to indicate that some distinct, but little, activity is obtainable even in the absence of sulphonamide group. Thus, *p*-acetaminothiophenol[24] (and not the nitro or amino derivative[8]), *p*-acetaminobenzenesulphinic acid[41] (and not the sulphochloride[25]), dinitro and diamino diphenylidisulphides[8,26] all possess some distant activity.

(*b*) (i) Very highly active compounds are produced when R is a benzene ring with a nitro, amino or acetamino groups in the *para*-positions, especially in (VIII). 4 : 4′-Diaminodiphenylsulphone tops the list of compounds possessing *antistreptococcal* activity in experimental infections in mice, being about 100 times as active as sulphanilamide.[8,14,27,28] The introduction of other groupings into the benzene rings destroys the activity. The corresponding diphenylsulphoxides are less and the diphenylsulphides much less active.[8]

(ii) When R is an aliphatic chain in (VIII), the activity of the compounds is very little or nil[14] excepting when it is an amyl or β-ethanol radical, in which case the compounds obtained are a little less active than sulphanilamide.[8,9]

(c) *Sulphonamide Derivatives* (R = NHR' in VIII).—Derivatives of this ' type offer a great deal of variations and possibilities. The two compounds now extensively in use and those that show promise all belong to this group.

(i) When R' is an aliphatic chain without or with the groupings amino, hydroxyl, carboxyl, sulphonic, phenyl, etc., the activity of the compounds is much decreased or lost excepting when it is a methyl or ethyl radical.[2,9,8,21,22,29,30]

(ii) When R' is an acetyl radical the activity is decreased.[18,31,32] Compounds superior to sulphanilamide are claimed to be produced when R' is a p-aminobenzenesulphonyl radical.[33,8]

(iii) When R' is an aromatic ring, a great variety of interesting compounds are produced by substitution in the second benzene ring. The parent compound, p-aminobenzenesulphonanilide itself is active.[21] Substituents as nitro, amino and dimethyl amino-groups increase the activity.[3,6] The compounds with the carboxyl and sulphonic acid groupings in the *ortho*-positions are quite active,[3,2] though the acrylic acid grouping produces an inactive compound.[2] A sulphonamide or substituted sulphonamide group in the *para* produces active compounds with practical advantages.[8,11,33] The aminobenzenesulphonamide grouping in the *meta* or *para* and not in the *ortho*-position, produces active compounds, the activity of which being further increased by an additional sulphonic acid grouping in the second benzene ring.[3]

When R' is a reduced benzene ring the activity is lost.[21,30]

(iv) When R_6 is a heterocyclic ring, very interesting compounds are produced and the entire possibilities in this direction have not yet been fully explored. Though the data available is scanty, from the results so far obtained, the suggestion appears justifiable that the nature of this heterocyclic ring plays a remarkable rôle in intensifying the therapeutic properties of the parent sulphanilamide, especially in those infections, in which it is little or not effective.

A morpholine[30] or a piperidine[21] ring destroys the activity. When R' is a pyridine ring, compounds remarkably active in the *pneumococcal* infection are obtained.[34,35] The corresponding quinoline derivatives are very toxic and appear to be of no use.[2,12,13] Acridine derivatives show some promise.

Thiazole derivatives[35] also appear to show as good results as the pyridine compounds in many experimental infections in mice. The other ring systems have not yet been tried.

4. The Specificity of Antibacterial Action

Sulphanilamide possesses a striking polyvalent action, being very effective in *β-hemolytic streptococcal, meningococcal* and *gonococcal* infections ; less in the *pneumacoccal* and far less in the *straphylococcal* infections. Substitution at the amino part of the molecule greatly suppresses all the properties excepting its action in *streptococcal* infections.[5,37,38,39] On the other hand, particular types of substituents in the amide part of the molecule (R'), *e.g.,* a pyridine or a thiazol ring, enhance the activity in *pneumococcal* and *staphylococcal* infections. Some compounds also show some viruscidal properties.[42] The diaminodiphenylsulphone and its derivatives, though far more active than sulphanilamide and 2-sulphanilamidopyridine in *streptococcal* infections in mice, is far inferior to 2-sulphanilamidopyridine in *pneumococcal* infections. Levaditi *et alia*[43] have reported the diphenylsulphoxide derivatives to possess very specific activity in *gonococcal* infections " just as the arsenicals ". Adams *et alia* [23,30] have provided us with a large number of instances of sulphanilamide derivatives which are practically inactive in *streptococcal* infections but highly active in *meningococcal* infections in mice.

These and many other instances appear to indicate the possible existence of some sort of specificity of the different compounds towards particular bacterial infections. This implies that, we may, by skilful manipulations of the molecular structure of sulphanilamide, be able to produce derivatives, each of which being effective in a particular infection. This is quite reasonable to anticipate in view of the fact that the bacterial infections vary so widely in their ætiology that the nature of the compounds required to combat them should possess the requisite variety of properties (varying from persistent moderate action over a long period to intense quick action for a short time) which can be provided by suitable changes in the molecular structure. The whole problem thus consists not merely in synthesising different types of compounds but also in testing them in many types of infections.

Experimental

The yields in all experiments, unless otherwise stated, are all understood to be good varying from 75 to 90 per cent.

4'-Hydroxy 3'-methoxybenzlyidene 4-aminobenzenesulphonamide.—*p*-Aminobenzenesulphonamide (3·4 g.) was dissolved in alcohol (30 c.c.), vanillin (3·0 g.) added and the mixture heated under reflux for 30 min. It is then

diluted with water and the alcohol distilled off whereby the anil crystallised out. It is then recrystallised from dilute alcohol, m.p. 198°–99°. [Found: N, 9·0 ; S, 10·1 ; $C_{24}H_{14}N_2O_4S$ requires N, 9·1 ; S, 10·4%.]

4-(4'-Hydroxy 3'-methoxybenzylamino) benzenesulphonamide.—To the above Schiff's base dissolved in glacial acetic acid was added zinc dust and the whole warmed on the steam-bath till the yellow solution was decolourised. The reduction product, isolated in the usual way, crystallised from dilute alcohol in shining leaflets, m.p. 167°. [Found: N, 8·7 ; S, 10·0 ; $C_{14}H_{16}N_2O_4S$ requires N, 9·1 ; S, 10·4%.]

The Schiff's base from *p*-nitrobenzaldehyde and sulphanilamide did not undergo such a smooth reduction with zinc dust and acetic acid.

a-N¹-Acetsulphanilamidodihydrocinnamic acid : (N-acetsulphanilyl-β-phenyl-a-alanine.—To phenylalanine (6·2 g.) dissolved in 2·5 N sodium hydroxide (30 c.c.) was added with good stirring *p*-acetaminobenzenesulphochloride (8·8 g.). Everything quickly went into solution and after a short time set to a crystalline mass. It was dissolved in the minimum amount of water, filtered and acidified with hydrochloric acid. The oil which separated rapidly became granular. It was filtered and on recrystallisation from dilute alcohol was obtained in shining needles, m.p. 205–6°. [Found: N, 7·2 ; S, 9·1 ; $C_{17}H_{18}N_2O_5S$ requires N, 7·7 ; S, 8·8%.]

a-N¹ Sulphanilamidodihydrocinnamic acid hydrochloride (N-sulphanilyl-β-phenyl a-alanine).—The above acetyl compound (5·6 g.) was heated on the steam-bath with 5·5 N hydrochloric acid (40 c.c.) for 1 hour. The clear solution was diluted with ethyl alcohol whereby the hydrochloride of the base crystallised out in thick prismatic needles. Recrystallised from alcohol containing a little hydrochloric acid it has m.p. 196–7° (decomp.). [Found: N, 7·3 ; Cl, 9·6 ; $C_{15}H_{17}N_2O_4SCl$ requires N, 7·9 ; Cl, 10·0%.]

-(N¹ Sulphanilamido) ethylene sulphonic acid (N-sulphanilyltaurine).—To *dl*-taurine (5·8 g.) dissolved in 4 N sodium hydroxide (15 c.c.) was added *p*-acetaminobenzenesulphochloride (11·5 g.) with good shaking. When everything had gone into solution, the clear filtrate was acidified with con. hydrochloric acid. The product obtained was filtered and hydrolysed by boiling with 5·5 N hydrochloric acid for 45 minutes. The free base obtained crystallised from water in beautiful elongated plates. [Found: N, 9·8 ; S, 22·3 ; $C_8H_{12}N_2O_5S_2$ requires N, 10·0 ; S, 22·9%.]

I-N¹-Sulphanilamidonaphthalene-3 : 6 disulphonic acid.—To I-amino-naphthalene 3 : 6-disulphonic acid (6·0 g.) dissolved in 4 N sodium-hydroxide (15 c.c.) was added finely powdered *p*-acetaminobenzenesulphochloride (4·6 g.) and well shaken till everything went into solution. It was filtered,

acidified and the product, which separated, was boiled with 5·5 N hydrochloric acid for 1½ hours. The amine hydrochloride separated on cooling in fine needles and was recrystallised from dilute alcohol. (Found : N, 5·4 ; Cl, 7·2 ; $C_{16}H_{15}N_2O_8 S_3Cl$ requires N, 5·7 ; Cl, 7·2 %.)

2-N¹-Sulphanilamidonaphthalene 5 : 7-disulphonic acid was prepared as above. The free base crystallised from dilute alcohol in fine needles. The aqueous solution showed a blue fluorescence. [Found : N, 5·7 ; $C_{16}H_{14}N_2O_8S_8$ requires N, 5·1 %.]

2-N¹-Sulphanilamido 8-naphthol 3 : 6-disulphonic acid.—To 2-amino 8-naphthol 3 : 6-disulphonic acid (10 g.) dissolved in 4 N sodium hydroxide (25 c.c.) was added *p*-acetaminobenzenesulphochloride (7 g.) and shaken till everything went into solution. The clear solution was acidified and the product which separated was heated on the steam-bath with 5·5 N hydrochloric acid for 1½ hours. The product that separated on cooloing was filtered, dissolved in dilute sodium hydroxide, and the clear solution acidified with acetic acid, whereby the acid gradually crystallised out. It was filtered, washed with alcohol and dried. (Found : N, 5·8 ; S, 20·9 ; $C_{16}H_{14}N_2O_9S_3$ requires N, 5·8 ; S, 20·2 %.)

1-N¹ Sulphnilamido 8-hydroxynaphthalene 3 : 6-disulphonic acid was preprared as described in the previous experiment. (Found : N, 5·7 ; S, 21·0 ; $C_{16}H_{14}N_2O_9S_3$ requires N, 5·9 ; S, 20·2 %.)

6-N¹-Sulphanilamidoquinoline.[45]—6-Aminoquinoline (5 g.) and *p*-aceta, amino benzenesulphochloride (8 g.) in pyridine (25 c.c.) was heated to boiling, cooled and diluted with water. The crystalline condensation product was filtered and boiled with 5·5 N hydrochloric acid for 30 minutes. The clear solution was just made alkaline with ammonia whereby the free amine separated. It recrystallised from dilute alcohol in rectangular plates 201°. (Found : N, 13·7 ; S, 10·6 ; $C_{15}H_{13}N_3O_2S$ requires N, 14·0 ; S, 10·7 %.)

p-(Phenylthiocarbamido) benzenesulphonamide.—A mixture of *p*-aminobenzenesulphonamide (17·2 g.), phenylisothiocyanate (13·5 g.) and alcohol (250 c.c.) was heated under reflux for 30 minutes. The crystalline solid that separated was filtered and recrystallised from alcohol, m.p. 189°. [Found : N, 13·9 ; $C_{13}H_{13}N_3O_2S_2$ requires N, 13·7 %.]

4 : 4'-Di (w-allylthiocarbamido) diphenylsulphone.—4 : 4'-Diamino-diphenylsulphone (3 g.) in ethyl alcohol (20 c.c.) was boiled with allyl isothiocyanate (2 g.) for 4 hours. The solution was then diluted with water, the crystalline product that separated was filtered, washed with dilute hydrochloric acid and crystallised from alcohol, m.p. 183°. [Found : N, 12·5 ; S, 21·4 ; $C_{22}H_{22}N_4O_2S_3$ requires N, 12·4 ; S, 21·5 %.]

4-N¹-Sulphanilamidoaniline.—This compound has been described previously[1] and given the m.p. 155°. Just subsequent to this, Webster and Powers[44] obtained it by the reduction of the corresponding nitro-compound and gave the m.p. 155–56°. On repeating our own experiments on a larger scale, we could obtain a product of m.p. 137–8°, the mixed melting point with the previous specimen being undepressed. Recently Bauer[6] has obtained this compound by the reduction of the corresponding nitro-compound with sodium hydrosulphite and records the melting point 138°. This interesting discrepancy is being investigated.

2-N¹-Sulphanilamidonitrobenzene.—The acetyl derivative of this compound has been described by Webster and Powers[44] who use dimethylaniline as the condensing agent. We find that the reaction proceeds smoothly with pyridine.

A mixture of *ortho*-nitraniline (14 g.), *p*-acetaminobenzenesulphochloride (24 g.), acetone (80 c.c.) and pyidine (16 c.c.) was refluxed for 1 hour, allowed to stand for 2 hours and diluted with water whereby an oil separated which slowly solidified. The solid was washed with dilute hydrochloric acid and boiled with alcoholic hydrochloric acid for $1\frac{1}{2}$ hours. The solution was diluted after distilling off the alcohol and carefully neutralised with sodium-bicarbonate, whereby the free amine was thrown down. It crystallised from dilute alcohol in golden yellow needles, m.p. 167°. [Found : N, 14·0 ; $C_{12}H_{11}N_3O_4S$ requires N, 14·3 %.]

N⁴-(3-Carboxy 2-quinolyl) sulphanilamide ; [2-(4'-sulphanilamidobenzene amino) *quinoline 3-carboxylic acid*].—An intimate mixture of finely powdered sulphanilamide (1·5 g.) and 2-chloroquinoline 3-caboxylic acid (1·7 g.) was heated in a bath at 165–70° for 30 minutes. The mixture softened to a yellow mass and became brittle. It was cooled, dissolved in dilute sodium hydroxide, filtered and precipitated with acetic acid. It separated from di-acetic acid as a crystalline powder, m.p. not below 280°. [Found : N, 14·2 ; $C_{16}H_{13}N_3O_4S$ requires N, 14·7 %.]

Though cholesteryl chloride has been condensed with aniline, xylidene, etc., all attempts to react it with sulphanilamide ended in failure. So also, chloracetylcholesterol[46] failed to react with sulphanilamide. When cholesterylanilide was treated with chlorosulphonic acid with the hope of obtaining the benzene substituted sulphochloride, a more deep-seated reaction appeared to take place. Other methods to obtain the desired compounds are being tried.

2 : 8-Di (N¹-sulphanilamido) acridine (II).—To 2 : 8-diaminoacridine (6 g.) suspended in pyridine (10 c.c.), acetone (25 c.c.) and water (10 c.c.) was

added gradually with good stirring *p*-acetaminobenzenesulphochloride (15 g.). The whole of it went gradually into solution with the liberation of heat and the temperature was kept below 45° C. by cooling. The mixture was kept shaking for 2 hours. It was then diluted with water (100 c.c.) and sodium-hydroxide solution added to make the solution just alkaline. After about an hour, it was filtered (about 2·5 g. of the diaminoacridine was recovered) and the filtrate acidified with 50% acetic acid whereby 2 : 8-di-N¹-acetsulphanil-amidoacridine was thrown down. It was filtered, washed with water, dissolved once again in dilute sodium hydroxide solution and the clear solution treated with a saturated solution of ammonium chloride till there was no further increase in the precipitate. This was filtered and boiled with 10% sodium hydroxide for 1½ hours. On cooling, the sodium salt of the free amine separated. It was dissolved in water and acidified with acetic acid. From the hot alkaline solution itself, by the addition of a saturated solution of ammonium sulphate the base can be obtained. It was obtained from dilute acetic acid as a red powder. (Yield about 3·5–4 g.). [Found : N, 13·0 ; $C_{25}H_{21}O_4N_5S_2$ requires N, 13·5 %.] It is insoluble in all common organic solvents excepting acetic acid. It is soluble to the extent of 1 in 12,000 in water and the aq. solution is yellow. The sodium salt which can be obtained as a crystalline mass, is gradually hydrolysed by water.

In the above experiment even when one molecule of the sulphochloride was used, only the disubstituted product was obtained.

2 (4'-N¹Sulphanilamidobenzenesulphonamido) pyridine (VI).—To 2-sulpha-nilamidopyridine (2·1 g.) in 50% acetone (20 c.c.) and pyridine (1 c.c.) was added gradually *p*-acetaminobenzenesulphochloride (2 g.) with grinding in a mortar. It is kept for 1 hour with frequent shaking, diluted with water and alkali was added whereby everything went into solution. On acidifying with acetic acid, the condensation product separated as an oil and soon crystallised. It was filtered and boiled with 5 N hydrochloric acid for ¾ hour. The clear solution was carefully neutralised with sodium bicarbonate, when the free amine separated. It crystallised from alcohol in rosettes of shining needles, m.p. 236–38°. [Found : N, 13·3 ; $C_{17}H_{16}N_4S_2O_4$ requires N, 13·9%.]

2-N¹-Sulphanilamidothiazol(I, R = H).—This compound, also reported by Fosbinder and Walter,[35] can best be prepared by the following method which, after many trials, we have adopted to synthesise in large quantities for clinical trials.

To 2-aminothiazol (45 g.) in acetone (50 c.c.) and pyridine (20 c.c.) is added gradually with good stirring *p*-acetaminobenzenesulphochloride (about 100 g.) freshly prepared from acetanilide (75 g.) and chlorosulphonic

acid (160 c.c.) and dried on porous plate. The whole of it goes into solution with warming up. After all is added, it is allowed to stand with frequent shaking for two hours and then diluted with water. The product that separated is filtered after some time, dissolved in sodium hydroxide solution and the clear filtered solution acidified with acetic acid when 2-acetamino-benzenesulphonamidothiazol is thrown down. It is filtered and dried. (Yield about 35 g.). In the above experiment, in the place of pyridine, sodium carbonate can be used but this offers no advantage.

The hydrolysis can be carried out in almost quantitative yields by boiling 20 g. of the above acetyl derivative with 150 c.c. of about $3 \cdot 5$ N hydrochloric acid till everything goes into solution (20 min.) and carefully neutralising with ammonia. If excess of ammonium hydroxide is added, the dissolved base can be liberated by acetic acid. The product obtained as such melts at 190–93° and by one more crystallisation at 197–98°. The solubility of this compound is about twice as that of 2-sulphanilamidopyridine. The amino-group of the thiazol derivative is diazotisable and so for estimation the same method as that used for sulphanilamide or 2-sulphanilamidopyridine can be used.

4-Amino 5-(4'-sulphanilamidophenylazo) uracil.—To 4-aminouracil ($8 \cdot 0$ g.) dissolved in 2 N sodium hydroxide (150 c.c.), was added under cooling a solution of diazotised sulphanilamide (5 g.) after removing the excess of nitrous acid with urea. The red solution was kept stirred for 15 min. and then acidified whereby the red dye separated. It was then obtained from pyridine as a red powder. [Found : N, $26 \cdot 7$; $C_{11}H_{11}O_4N_6S$ requires N, $27 \cdot 1\%$.]

4-Amino 5-(4'-sulphonamidophenylazo) thiouracil.—This was prepared as above by diazotising sulphanilamide and coupling with 4-aminothiouracil and obtained as a red infusible powder. [Found : N, $26 \cdot 3$; $C_{10}H_{10}O_3N_6S_2$ requires N, $25 \cdot 8\%$.]

Similarly, 2-N^1-sulphanilamidopyridine diazotised and coupled with 4-aminothiouracil and *meta*phenylenediamine yielded the corresponding dyes (IV and III) respectively.

The nitrogen in most of these compounds was estimated by Kjeldahl's method and sulphur by oxidising the substance and estimating as barium sulphate.

Summary

The synthesis and the chemotherapeutic effects in experimental *streptococcal* and *pneumococcal* infections in mice of new sulphanilamide derivatives are described. 2-N¹sulphanilamidothiazol appears to possess a very striking effect in these infections.

The realtionship of chemical constitution to chemotherapeutic action in this group of compounds has been outlined basing on a study of the therapeutic properties of about 500 compounds till now reported. While the substitution at the amino radical of sulphanilamide does not produce compounds of increased activity but only suppress the polyvalent action of the original compound, the substitution at the amide part of the molecule, especially by a heterocyclic ring, produces compounds of increased activity, particularly in infections in which sulphanilamide is very little effective. The highly active diaminodiphenylsulphone derivatives have to be properly modified to be used in practical therapy. There does appear to be a sort of specificity of the compounds towards different types of infections so that the new compounds produced should be tested in different types of infections also.

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