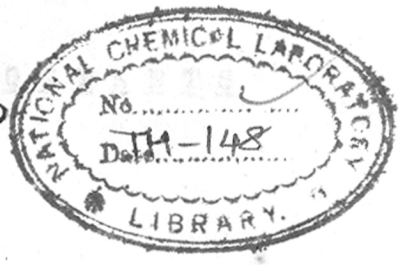


# SURFACE-ACTIVE CHEMOTHERAPEUTICALS

A Thesis

submitted by

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# C O N T E N T S

# Part I.

## AMMONIUM SALTS OF SULPHANILAMIDES AND WETTING-AGENTS CONTAINING A SODIUM SULPHONATE GROUP

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# INTRODUCTION.

The bactericidal and lytic action of surface active agents such as soaps and certain naturally occurring detergents (e.g. bile salts) have been recognized for some years.[1] Surface active agents which when added to water give it higher degree of wetting against a contiguous phase are referred to as "Wetting agents".[2] Domagk[3] in 1935 first established the bactericidal property of detergents like Zephiran and recommended its use for disinfection of skin diseases. Baker et al.[4] made a critical study of a variety of synthetic wetting agents and observed that (1) all cationic detergents studied were effective inhibitors of bacterial metabolism in high dilutions (1:30,000). Few of the anionic detergents showed equal efficiency as the catonoid compounds. (2) Gram-positive and Gram-negative organisms were equally sensitive to cationic detergents. The anionic detergents on the other hand selectively inhibited the metabolism of Gram-positive organisms. (3) Inhibitory action of both types of detergents was markedly influenced by pH. Cationic detergents exhibited maximum activity in the alkaline range while anionic detergents were effective in the acid solution. (4) In the homologous series of straight chain alkyl sulphates ($C_8$ - $C_{18}$) inhibition was maximum for $C_{12}$ - $C_{16}$ compounds. Gershenfeld and his collaborators[5] studied the antibacterial aspects of wetting agents and their influence upon the efficacy of

antiseptic materials and found that many of them possessed marked activity. The mechanism of this synergistic action is still obscure[5]. In a recent paper, Gershenfeld and Sagin[6] have studied the synergistic action of detergents such as Aerosol O.T., Tergitol 4, and Triton K12 on the *in vitro* activity of sulphaguanidine and succinyl sulphathiazole. They report a definite synergistic action and suggest the possible use of non-toxic detergents in sulphonamide therapy especially as intestinal antiseptics. A review of literature on the various attempts to impart surface activity to established chemotherapeutics by the introduction of surface active groups in the molecule of the drug are recorded in Part IV of the thesis, and a special reference has been made to their probable utility in combating bacteria such as the tubercle and lepromata which are known to possess a fatty cuticle. In the field of sulphonamides Crossley *et al*.[7] prepared $N^1$-acyl and $N^1$-$N^4$-diacylsulphanilamides using long chain fatty acids with a view of their probable activity against the lypoid-covered tubercle bacteria, and they found that $N^1$-dodecylsulphanilamide exhibited such action. Rajagopalan[8] similarly prepared a series of $N^4$-acyl and $N^1$+$N^4$-diacylsulphanilamides from different fatty acids with the same object, but the physiological activity of these derivatives are not reported.

## PRESENT WORK.

The work carried out in this laboratory[9] on the structural characteristics of organic compounds which lead to surface activity in relation to their use as textile auxiliary agents suggested an extension of these investigations in the field of chemotherapy. In this part the attempts to prepare surface-active sulphanilamides are described.

The arylamine salts of naphthalene sulphonic acids are useful in the characterisation of both the amines and the sulphonic acids.[10] Sulphanilamide salts were prepared in order to synthesise surface active compounds from wetting agents containing sodium sulphonate groups and different sulphonamides. By virtue of their stability to mineral acid, the salts should pass through the stomach, and under the alkaline condition in intestines liberate the drug component. Such an action would probably make these salts active intestinal bacteriostatic agents. The surface active component should also lead to advantages due to the spreading, penetration, and emulsification properties associated with it.

Sulphanilamide salts of benzenesulphonic acid (m.p. 213-217°)(decomp.), phenol-p-sulphonic acid (m.p.216-220°) (decomp.), sulphosalicylic acid (m.p. 214-220)[11,12], camphor sulphonic acid[13] and salicylic and acetylsalicylic acid[14], phenylglycollic and adipic acid[15] have been mentioned, but

they do not appear to have been isolated in pure form and characterised. It will be noticed that the decomposition points of the first three salts in the above list are very close to each other. In order to confirm these results, the salt (IX) of sulphanilamide with phenol-p-sulphonic acid was prepared and it was found that the pure substance had m.p. 241-42° (decomp.) as against 216-20° (decomp.) recorded in the literature.[16] A mixture of the authentic salt and sulphanilamide hydrochloride gave the m.p. 216-20° (decomp), showing that the compound described earlier was probably a mixture. The bacteriostatic activity of some of the salts has been stated to be better than sulphonilamides, although the salts contained only 40-50% of the sulpha component.[11]

As difficulty was encountered in isolating salts from surface-active agents (Igepon T, Aerosol OT, etc.) in analytically pure form, a preliminary study of salts prepared from naphthalene-β-sulphonic acid and a series of sulphanilamides was undertaken. The salts were prepared by adding a boiling aqueous solution of the sodium salt of the sulphonic acid (1.0 mole) to the hot solution of the sulphanilamide (1.0 mole) in water containing hydrochloric acid (about 1.1 mole), and boiling the contents for about two minutes. The salts generally separated quickly on cooling, although in some cases it was necessary to

leave the reaction mixture overnight or even longer or to concentrate it to smaller bulk.

The salts of sulphanilamides and sulphathiazole with naphthalene-2-sulphonic acid (I and II respectively) were easily prepared, but salt formation in the case of sulphanilamides with basic $N^1$-substituents such as -- sulphapyridine, sulphapyrimidine, and sulphaguanidine proved to be difficult in the early experiments in which an equivalent quantity of hydrochloric acid was used. In order to find out whether the basic $N^1$-substituent was in any way responsible for the failure of the amino group to form the ammonium salt, or its inability to separate from the aqueous solution, a series of salts of naphthalene-2-sulphonic acid with 2-aminothiazole (X), 2-aminopyrimidine (XI) and 2-amino-4:6-dimethylpyrimidine (XII) were prepared. In the preparation of these salts it was found that addition of excess of hydrochloric acid was necessary for salt formation. The salt from 2-aminopyridine could not be isolated under any of the conditions which have so far been tried.

Using excess of hydrochloric acid (up to 4.0 mole) salts from sulphapyridine (III), sulphadiazine (IV), sulphamerazine (V), and sulphaguanidine (VI) were readily prepared. Salts from $N^1$-acetylsulphanilamide (VII) and from p-aminobenzoic acid (VIII) were also prepared using

an excess of hydrochloric acid. Crystallisation of most of these salts was effected from dilute hydrochloric acid.

Most of the salts, and particularly those derived from sulphanilamides with basic $N^1$-substituents, gave stable aqueous solutions only at a pH below 5. Many of the salts separated from the reaction mixture directly in pure crystalline form. Salt formation was in some cases sensitive to changes in experimental conditions such as dilution, time of reaction, and acidity of the reaction mixture, and it was important to carefully adhere to specified conditions.

The purity of the salts such as (I) and (II), which gave neutral solutions in water, could be determined by titration against 0.1 N sodium hydroxide solution. Using Alkali Blue 6B or phenolphthalein as indicators the alkali absorbed corresponded to both the salt and sulphonamido groups ; when Methyl Red or Methyl Orange was used the titre corresponded exclusively to the salt linkage. The purity of a series of sulphanilamides was also determined by titration against 0.1 N sodium hydroxide.

The preparation of salts from wetting agents containing a sodium sulphonate group proved to be much more difficult. The observation of Forster *et al.*[10] regarding the formation of arylamine salts of aromatic sulphonic acids, has been applied by Kertess[17] as well as by Kling and Puschell[18] to fatty alcohol sulphates and sul-

phonates. The latter workers used benzidine hydrochloride for salt formation and claimed that the method could be employed for estimation of fatty alcohol sulphates and sulphonates. Shirolkar and Venkataraman[19], however, could not confirm the above observations and found that such arylamine salt formation, while serving as an excellent method for characterising arylamines and sulphonic acids, cannot be employed for the estimation of even simple benzene and naphthalene sulphonic acids. A precipitate is obtained on mixing aqueous solutions of benzidine hydrochloride and the sodium salt of oleyl-N-methyltaurine (Igepon T), but it has not been possible to prepare an ammonium salt in crystalline form, and the present experiments have confirmed that the procedure is not suitable for the estimation of surface-active sulphates or sulphonates.

Judging from the ease with which aromatic sulphonic acids such as phenol-p-sulphonic and naphthalene-β-sulphonic acid gave salts with the different sulphanilamides, one of the first wetting agents which was used for salt formation with sulphanilamide was Nekal BX (di-isopropylnaphthalene-sodium sulphonate). While most of the alkali salt merely decomposed to the free alkyl naphthalene sulphonic acid under the acid conditions of the reaction, a small quantity of the desired ammonium salt (XIII) (m.p. 220-222°) could be isolated and characterised.

Sodium di-octylsulphosuccinate (Aorosol O.T.) gave a light brown hygroscopic salt (XIV) with sulphanilamide. It could not be crystallised, but the analysis and the properties of the substance gave definite indication of ammonium salt formation.

Lauroyl-p-toluidide-2-sodium sulphonate,[20,21] which is a powerful wetting agent, gave a hygroscopic amorphous light brown salt (XV) with sulphanilamide possessing very good wetting power, and this compound was obtainable in analytically pure condition.

With the object of determining the relative ease with which p-aminobenzoic acid (an essential growth factor for the bacteria-enzyme system) and sulphanilamide combine with organic anions such as the sulphonic radical, the salt of naphthalene-2-sulphonic acid was treated with p-aminobenzoic acid under various conditions. While p-aminobenzoic acid replaces sulphanilamide appreciably at elevated temperature (about 100°) to give the salt of the former with the sulphonic acid, replacement could be noticed to a small extent even at room temperature (26-29°), when an aqueous solution of p-aminobenzoic acid and sulphanilamide-sulphonic acid salt was kept for 10 days.

## DISCUSSION OF BACTERIOSTATIC ACTIVITY OF THE AMMONIUM SALTS.

The *in vitro* tests indicated that the salts are more

active than the parent sulphanilamide in spite of the lower contents (40-50%) of the active sulpha components.

Sulphanilamides or naphthalene-β-sodium sulphonate when tested separately in amounts present in the salts were inactive. The increased potency of the salts could not be due to increased solubility as mentioned by Smyth and Carpenter,[11] as the salts had equal or less solubility as compared to the sulpha components.

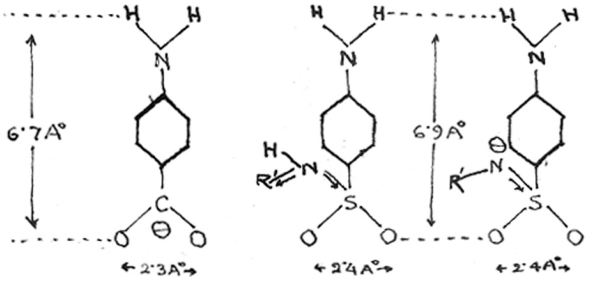
In order to explain the increased activity of the salts, the different hypotheses which have been suggested regarding the mode of action of the sulphanilamides were considered. While for a detailed account reference must be made to Henry's excellent review, it is of interest to summarise these hypotheses from the present point of view.

Woods[22] demonstrated that p-aminobenzoic acid prevents the bacteriostatic action of sulphanilamide and sulphapyridine. Woods and Fildes[23] suggested that p-aminobenzoic acid is an essential metabolite associated with one or more of the enzymatic processes involved in bacterial growth. They pointed out the close structural relationship between the sulphanilamides and this acid, and suggested that the former may act by blocking the enzyme system or systems with which p-aminobenzoic acid is involved and on which many bacteria depend for normal growth and development. The essential nature of p-aminobenzoic acid[24-25].

and the competitive inhibition produced by sulphanilamide derivatives with respect to p-aminobenzoic acid[26,27] has been confirmed recently.

If the bacteriostatic action of the sulphanilamide type of compounds is due to a competition with the required p-aminobenzoic acid for an essential enzyme system, then the more closely the competitor compound resembles this acid, the greater should be its blocking or bacteriostatic effect. The characteristic groups in p-aminobenzoic acid are the carboxyl group and the amino group. Apart from "geometric configuration" probably the most important property of the amino and the carboxyl groups is their positive and negative character. Since the sulphanilamide derivatives also contain an acidic sulphanilamido group and a basic p-amino group, Bell and Roblin[28] compared the acidity or the basicity of these groups with the strength of the carboxyl and amino groups in p-aminobenzoic acid by determination of the acid and base dissociation constants. The basic dissociation constants of p-aminobenzoic acid and a number of $N^1$-substituted sulphanilamide derivatives varied between very small limits, but their acid dissociation constants varied between a wide range and could be related with their chemotherapeutic activity. Since p-aminobenzoic acid and sulphanilamides have the $N^4$-amino group in common, and since the carboxyl group in the former is completely

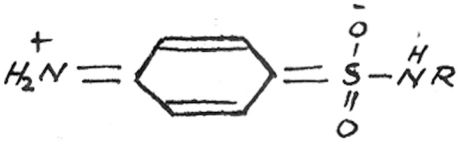
ionized at pH 7 it follows that for sulphanilamide to be closely similar to p-aminobenzoic acid and hence have maximum bacteriostatic activity, the $-SO_2-$ group must be comparable in acidity to the carboxyl ion in p-aminobenzoic acid. The similarity in the geometric configuration of sulphanilamides and of p-aminobenzoate ion is as indicated:




The activity will depend on the ionisation of the $-SO_2-NHR$ which in turn will depend on the electron attracting power of R. In brief the theory may be stated that the more the negative character of $N^1$-substituted sulphanilamide, the greater is its bacteriostatic power. Since acid constants are related to both structure of the $N^1$-substituents and the activity of the derivatives, an indirect co-relation is established between structure and chemotherapeutic activity. Knowing the electron attracting power of the $N^1$-substituent it is possible to predict for the first time the bacteriostatic power of any new sulphanilamide derivative. Bell and Roblin[28] conclude that the maximum bacteriostatic activity of the sulphanilamides has been reached in sulphathiazole and sulphadiazine, and further researches should be directed

only in modifying pharmacological properties of the sulpha drugs. The acidity of sulphaguanidine and the inactivity of sulphanilylurea and sulphanilyl-$N^1$-methylpyridine cannot be explained by the above theory.

Kumler and his collaborators[29,30] on the basis of dipole moment determinations showed that the resonance form (1) makes about the same contribution in sulphanilamides



(1)

as the analogous resonating form with a separation of charge makes in the methyl ester of p-aminobenzoic acid, and that the same was true for p-aminobenzoic acid. They suggested that the fundamental factor for activity is the contribution of the 'resonating form' (1) characterised by the coplaner amino group and a separation of charge. The increase in relative charge of $SO_2$ group would increase the contribution of the resonating form (1). Thus Bell and Roblins' (loc. cit) observation that the bacteriostatic activity is associated with the negative character of the $-SO_2-$ group is compatible with Kumler's hypothesis. Kumler explained the activity of the compounds like sulphaguanidine and the inactivity of sulphanilylurea which appears to be exceptions to Bell and Roblin's theory.

Bordwell and Klotz[31] have controverted Kumler and Daniel's hypothesis on the fundamental basis of the theory of absorption of light in ground and excited states. They pointed out that the explanation put forward by Kumler and Daniel to account for the inactivity of sulphanilylurea could as well be applied to the highly active sulphanilamides like sulphadiazine.

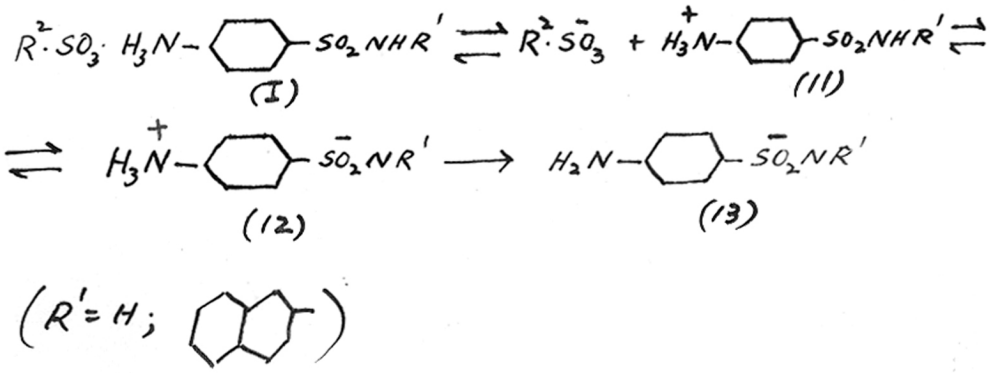
Kumler and Daniels' hypothesis has been further challenged by Bell, Roblin, and Bone[32] on the basis of absorption spectra in molecular (1 N NaCl) and ionic (1 N NaOH) form. They further contradict the hypothesis on the ground that there is no corelation between the basic constants and the bacteriostatic activity.

Klotz[33,34] has suggested that inhibition of bacterial growth could be explained quantitatively on the assumption that the action was due to a reversible complex formation between the basic 'form' of the drug and the neutral form of the protein, and that the law of mass action was applicable. Deviations between the observed and predicted bacteriostatic activities were explained by Klotz on the grounds of combination with extraneous proteins in addition to combination with growth enzymes.

On the basis of the above discussion regarding the activity of the sulphanilamides the important fact emerges, that in sulphanilamide and p-aminobenzoic acid both the

acidic and basic parts of the molecule are involved in the bacteriostatic activity. They would appear to enter into a complex formation with basic and acidic active centres of the bacterial enzyme, either separately or at the same time, since the latter is amphoteric in character and contains both the positively and negatively charged groups between the extreme limits of pH 2-13 like many other proteins.[35]

A sulphanilamide should then have maximum activity if it possesses positively and negatively charged -- groups (Zwitterion structure), which the parent sulphanilamide itself has to a very small extent. The potentiation of the sulphanilamide by the ammonium salt formation could, therefore, be due to the fact that by ionisation, the sulpha components would acquire the more active zwitterion form (12).

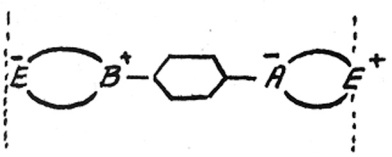


Klotz and Gruen[36] have shown that zwitterion forma-

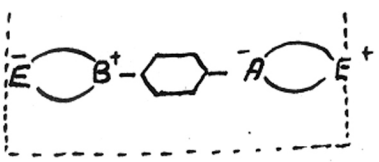
tion in sulphanilamide is negligible, but p-aminobenzoic acid is present as zwitterions to the extent of 3% at pH 7. Bell and Roblin (loc.cit) obtained indirect evidence to suggest that the ionised carboxyl group in p-aminobenzoic acid increased the basicity of the amino groups threefold.

Complexes formed by the combination of ionic forms of the sulpha drugs or p-aminobenzoic acid with the -- amphoteric bacterial enzyme may be represented as follows, where $A^-$ and $B^+$ represent the charged acidic and basic ends of sulphanilamides or p-aminobenzoic acid respectively, and $E^+$ and $E^-$ represent the charged active centres in the enzyme:

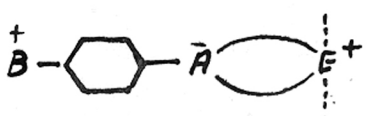
COMPLEXES WITH ZWITTERIONS.



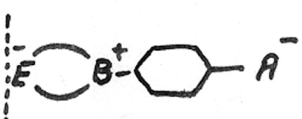
(14) Intramolecular combination.



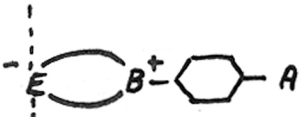
(15) Intermolecular combination.



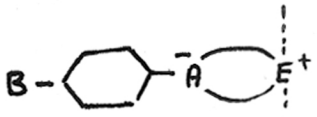
(16) Positively charged complexes.



(17) Negatively charged complexes.




(18) Complexes under strongly acidic conditions.




(19) Complexes under strongly alkaline conditions.

The mechanism underlying the action of antagonists structurally related to metabolites has been recently -- reviewed by Roblin.[37] According to him "the metabolite and antagonists are assumed to compete for an active centre or receptor in which there are polar groups oriented in such a manner as to exert a strong, specific attractive force on the metabolite..... The relative attractive forces between the metabolite and the active centres compared with the attraction of the antagonist to the active centres, will determine the efficiency of the antagonist. This efficiency is reflected in the molar ratio between antagonist and the metabolite. With few exceptions these ratios are greater than unity, because the firmness of the binding of the metabolite to the receptor is usually greater than the binding between the antagonist and the receptor."[37]

In the light of this general consideration of antagonist-metabolite relationships the formation of complexes (14-19) by sulpha drugs (antagonists) in competition with complex-formation by p-aminobenzoic acid (essential -- metabolite) will depend on the relative strength of the

electrical charges on the p-aminobenzoic acid and the sulpha drugs, as also on the charges at the points of attachment in the enzyme.

When the ammonium salt (I) ionises ultimately to (12) the zwitterionic character of (12) will be further stabilised by complementary inductive effects of the -- positively charged ammonium and negatively charged sulphonamides groups. The increased activity of the ammonium salts could be explained on the basis of increased attraction between their zwitterionic forms, e.g. (12) and active centres in the enzyme as compared to the parent sulphanilamides; the increased attraction being due to the increased electrical charges on the amino and the sulphonamido groups in the form (12). Under optimum -- conditions one might even expect these attractive forces to compare in strength with the attractive forces between the essential metabolite, p-aminobenzoic acid, and the active centres in the enzyme system. The sulphonic anion might make a small contribution to the bacteriostasis by removing part of the essential metabolite, p-aminobenzoic acid, by salt formation as also by complex formation with the -- positively charged parts of the enzyme molecule. As mentioned earlier it has been found that the replacement of sulphanilamide from its salts with naphthalene-β-sulphonic acid takes place to a small extent even at room

temperature (26-29°) when an aqueous solution of p-amino-benzoic acid and the sulphanilamide salt (I) is left for 10 days. Inactivation of sulpha drugs *in vivo* during metabolism by $N^4$-acetylation might perhaps be prevented or minimised by the use of the sulphanilamide salt -- instead of the parent sulpha components.

The variation in the activity of the different $N^1$-substituted sulphanilamides could be explained on the basis of the difference in the contribution of the zwitterionic form due to the variation in the acidity of the sulphonamide groups, depending on $N^1$-substituent (c.f. Bell and Roblin, *loc.cit*). The greater the acidity of the sulphonamido group, the greater will be its inductive effect on the positive charge on the $N^4$-ammonium group. It is in fact conceivable that with high activity of the sulphonamido group, its ionisation might precede the formation of (12). The effect of changes in pH on the activity of the sulphanilamides could also be explained similarly.

Compounds derived by $N^1$-substitution of sulphanilamides include most of the therapeutically important sulpha drugs, substitution in other positions invariably leading to inactive products. The sulphanilamide salts now prepared are more active than the parent sulpha drug in spite of the modification of the $N^4$-amino group. The

physiological properties of the sulphanilamides such as absorption-excretion characteristics, toxicity, easy accessibility to the site of infection, etc., could also be modified by varying the nature of the sulphonic anion in the salts.

# E X P E R I M E N T A L.

## Part I.

The sulphanilamides used were of pure grade for research purposes obtained from the Calco (Chemical Division of the American Cyanamide Co.).

Technical naphthalene-β-sodium sulphonate was recrystallised from hot water after clarifying (norit) and used. Phenol-p-sulphonic acid and p-aminobenzoic acid (m.p. 185°C) used in the present work were Theodore Schuchardt's and B. D. H. products respectively.

Nekal Bx (commercial product purified by extraction with dry benzene) and Lauryl-p-toluidide-2-sodium sulphonate used in the present work were of analytical purity. Aerosol OT was available commercially in chemically pure form and was directly used.

Sulphanilamide salt of naphthalene-β-sulphonic acid (I)

A hot aqueous solution naphthalene-2-sodium sulphonate [4.6 g. in water (100 c.c.)] was added to a hot solution of sulphanilamide (3.44 g.) in water (20 c.c.) and concentrated hydrochloric acid (2.5 c.c.) After boiling for about 2 minutes and decolourising (norit) the solution was cooled gradually to room temperature when lustrous white flakes of the salt separated, m.p. 262-263° (5.0 g.) raised to 263-264°C on further recrystallisation from hot water (Found: N, 7.3; S, 17.2. $C_{16}H_{16}O_5N_2S_2$ requires N, 7.4; S, 16.8%)

Sulphathiazole salt of naphthalene-2-sulphonic acid (II)

The salt was prepared from naphthalene-2-sodium sulphonate (4.6 g.), sulphathiazole (5.1 g.), concentrated hydrochloric acid (2,5 c.c.) and water (250 c.c.in total) following the general method employed for (I). The salt separated from the reaction mixture as white needles (8.0 g.), and on recrystallisation gave m.p. 214-215°C (Found: N, 9.2; S, 20.5. $C_{19}H_{17}O_5N_3S_3$ requires N, 9.1 ; S, 20.7%).

2-Aminothiazole salt of naphthalene-2-sulphonic acid (X)

A solution of naphthalene-2-sodium sulphonate (0.92 g.) in water (5.0 c.c.) was added to a hot solution of 2-aminothiazole (0.4 g.) in 1.5 N-hydrochloric acid (5.27 c.c., 2 mols), and the mixture was boiled for 2 minutes. On cooling in ice light brown crystalline powder separated (0.68 g.), softens above 140°, m.p. 160-170°C. On crystallisation from water after clarifying (norit) the salt gave colourless hexagonal tablets, m.p. 160-163°C, raised to 162-164°C by further recrystallisation. It was dried at 125° under reduced pressure for one hour and analysed (Found: N, 8.9. $C_{13}H_{12}O_3N_2S_2$ requires N, 9.1%).

2-Aminopyrimidine salt of naphthalene-2-sulphonic acid (XI):

The salt was prepared from 2-aminopyrimidine (0.376 g.) using the same quantities of the other reactants as above. The milky reaction mixture was cooled gradually to room temperature and finally in the refrigerator overnight. It was decolourised (norit), concentrated (ca 4 c.c.) and cooled when colourless short needles (0.625 g.) of the salt separated, m.p. 192-196°C. After three crystallisations from hot water the salt melted at 190-192°; mixed with 2-aminopyrimidine hydrochloride (m.p. 196°C) the salt gave m.p. 160-170°. (Found: N, 13.2; S, 10.1. $C_{14}H_{13}O_3N_3S$ requires N, 13.9; S, 10.5%).

2-Amino-4:6-dimethylpyrimidine salt of naphthalene-2-sulphonic acid (XII)

A boiling solution of naphthalene-2-sodium sulphonate (4.6 g.) in water (30 c.c.) was poured into a solution of 2-amino-4:6-dimethylpyrimidine (2.46 g.) in dilute hydrochloric acid (5.0 c.c. of concentrated

acid, 2 mols., diluted with 10 c.c. water). The solution was boiled for 2 minutes, clarified (norit), concentrated and cooled to room temperature when white needles of the salt separated, m.p. 107-110°C (8.5 g.) raised to 155-156°C after drying in vacuum at 100°C. After two recrystallisations from water the anhydrous product melted at 159-160°C (Found: N, 11.7; S, 9.4. $C_{16}H_{17}O_3N_3S$ requires N, 12.6; S, 9.6%)

Sulphapyridine salt of naphthalene-2-sulphonic acid (III)

A solution of naphthalene-2-sodium sulphonate (2.3 g.) in water (10.0 c.c.) was added to a boiling solution of sulphapyridine (2.48 g.) in water (20 c.c.) acidified with concentrated hydrochloric acid (2 c.c., ca 2 mols.). The mixture was boiled for 2 minutes, clarified (norit) and gradually cooled in ice when an oil settled down. The supernatent liquid was decanted from the oil and both cooled in a freezing mixture. The oil solidified to a cream coloured solid which was separated quickly pressed on a filter paper and crystallised from alcohol after decolourising (norit). On cooling cream coloured needles separated, soften at

above 40°C, m.p. 60-75°C (1.5 g.), raised to 70-75°C by three recrystallisations from alcohol. The supernatent liquid gave a white crystalline product on cooling (1.3 g.) which gave lustrous colourless needles of the salt after crystallisation from alcohol, m.p. 68-75°C. It was further purified by three more recrystallisations from alcohol, washed with ether and dried for 3 hours at room temperature under reduced pressure (5 m.m.), m.p.70-75°. (Found: N, 8.34. $C_{21}H_{19}O_5N_3S_2, C_2H_6O$ requires N, 8.35%). A portion after drying at 100°C under reduced pressure gave a cream coloured powder, m.p. 152-153° (Found: N, 9.6. $C_{21}H_{19}O_5N_3S_2$ requires N, 9.2%)

Sulphadiazine salt of naphthalene-2-sulphonic acid (IV)

A solution of naphthalene-2-sodium sulphonate (2.3 g.) in water (15 c.c.) was added to a hot clear solution of sulphadiazine (2.5 g.) in boiling dilute hydrochloric acid (40 c.c. water containing 40 c.c. 4 mols. of concentrated acid). The mixture was boiled for about 2 minutes, decolourised (norit) and cooled gradually to about 30-35°C, when pale lemon yellow needles separated, m.p. 188-189°C (3, 72.g). The mother liquor on cooling in ice gave lustrous cream coloured short needles of the salt (IV), m. p. 188°C (0.55 g.). It was dried at 120-130°C/~~per~~ 150 m. m. for one hour, melting point being unaltered.

after drying (Found: N, 11.9; S, 13.6. $C_{20}H_{18}O_5N_4S_2$ requires N, 12.2; S, 14.0%)

The first crop of the salt (150 mg.) when boiled with water (2 c.c.) gave the insoluble sulphadiazine due to dissociation which did not go in solution on dilution with water (25 c.c.). On the addition of concentrated hydrochloric acid (0.15 c.c.) a clear solution was again obtained which gave colourless needles of sulphadiazine on cooling in a freezing mixture. The mother liquor after separation of sulphadiazine was acidified with concentrated hydrochloric acid (2 drops) and concentrated to about 3 c.c. and cooled (0.5°C). The solution was seeded with the salt (IV) (m.p. 188°C) when short needles of the salt separated, m.p. 186-188°C. It is obvious that the salt (IV) readily dissociates in water and gives stable aqueous solutions only under acidic conditions.

Sulphamerazine salt of naphthalene-2-sulphonic acid (V):

The salt was prepared as above starting from naphthalene-2-sodium sulphonate (4.6 g.), sulphamerazine (5.28 g.) in boiling water (50 c.c.) containing concentrated hydrochloric acid (5 c.c., 2.5 mol.).

The clarified (norit) reaction mixture on cooling to room temperature gave cream coloured needles of the salt, m.p. 117-120°C (9.5 g.), raised to 118-120°C on further crystallisation from water acidified with a few drops of concentrated hydrochloric acid (Found: S, 13.0. $C_{21}H_{20}O_4N_4S_2$ requires S, 13.5%). Further crystallisation leads to decomposition of the salt to give sulphamerazine.

Sulphaguanidine salt of naphthalene-2-sulphonic acid (VI):

A solution of naphthalene-2-sodium sulphonate (2.3 g.) in water (15 c.c.) was added to a hot solution of sulphaguanidine (2.14 g.) in water (20 c.c.) containing concentrated hydrochloric acid (1 c.c.). The solution was boiled for 2 minutes and left overnight at room temperature. Colourless flat needles of the salt separated, softens at 186-190° m.p. 224-226°C (3.4 g.). Melting point of sulphaguanidine used, 186-190°C. Mixed m.p. of the salt and sulphaguanidine, 160-175°C. The mother liquor after concentration (ca 15 c.c.) gave a second crop of the salt, m.p. 175-185°C (0.32 g.)

The first crop on recrystallisation from hot acidulated water after clarifying (norit) gave lustrous

long flat needles which were dried at 120-130°C/~~per~~ 150 m.m. for one hour, softens at 186°, m.p. 225-226°C (Found: N, 13.1; S, 15.8. $C_{17}H_{18}O_5N_4S_2$ requires N, 13.3; S, 15.2%)

N'-Acetylsulphanilamide salt of naphthalene-2-sulphonic acid (VII):

A solution of naphthalene-2-sodium sulphonate (2.3 g. in 10 c.c. water) was added to a hot solution of the amine (2.14 g.) in dilute hydrochloric acid (20 c.c. water acidified with 1 c.c. of concentrated acid). The mixture was boiled for 2 minutes, clarified (norit) and cooled gradually to room temperature. Lustrous flakes of the salt separated, m.p.250-255°C (partial decomposition) (1.3 g.). The filtrate after cooling in ice gave a second crop of the salt (0.35 g.) and a third crop was collected from the mother liquor by concentration to about 15 c.c. (1.0 g.). The first crystallised product on further recrystallisation from hot acidulated (0.1 c.c. of 3% hydrochloric acid) water (10 c.c.) gave m.p. 253-254°C (partial decomposition). (Found: N, 7.0; S, 15.5. $C_{18}H_{18}O_6N_2S_2$ requires N, 6.3; S, 15.1%).

p-Aminobenzoic acid salt with naphthalene-2-sulphonic acid (VIII):

The salt was prepared in the usual manner from naphthalene-2-sodium sulphonate (1.15 g.), p-aminobenzoic acid (0.7 g.), concentrated hydrochloric acid (0.5 c.c.) and water (15 c.c.). The reaction mixture was boiled for 2 minutes and on cooling gave lustrous flakes of the salt, softened at 252°, m.p. above 260°C (decomposition) (1.31 g.), unaltered by further recrystallisations from water. (Found: N, 3.9. -- $C_{17}H_{18}O_6NS$ requires N, 4.1%)

Sulphanilamide salt of phenol-p-sulphonic acid (IX):

Sulphonic acid (8.7 g.) was dissolved in dilute sodium hydroxide (50 c.c. containing 2.1 g. of the alkali) and added to a hot solution of sulphanilamide (8.6 g.) in dilute hydrochloric acid (75 c.c. containing 7.0 c.c. of concentrated acid). The mixture was boiled, decolourised (norit) and cooled to room temperature. White needles of the salt separated, m.p. 240-241°C (11.0 g.) raised to 241-242° on further recrystallisation from acidulated water (Found: N, 8.3. $C_{12}H_{14}O_6N_2S_2$ requires N, 8.1%).

Sulphanilamide salt of Nekal BX (XIII) :

A solution of Nekal BX (9.42 g.) in water (50 c.c.) was added to a hot solution of sulphanilamide (5.16 g.) in dilute hydrochloric acid (3.65 c.c. of concentrated acid diluted with 50 c.c. water). The solution was boiled for 2 minutes, clarified (norit) and cooled to room temperature and finally in ice, when heavy oil separated. The clear supernatent liquid was decanted, concentrated and cooled in the refrigerator for a week when white flakes of the salt separated, m.p. 195-200°C (3.0 g.). On recrystallisation from acidulated water the salt melted at 220-222°C (Found: N, 5.9. $C_{22}H_{31}O_6N_2S_2$ requires N, 6.0%). The heavy oil from the crude reaction mixture was identified as Nekal BX sulphonic acid (Diisopropylnaphthalene sulphonic acid) (Found: S, 11.0. $C_{16}H_{33}O_3S$ requires S, 10.9%).

Sulphanilamide salt of Aerosol OT (XIV) :

An aqueous solution of Aerosol OT (13.2 g. in 150 c.c.) was added to a boiling solution of sulphanilamide (5.15 g.) in dilute hydrochloric acid

(60 c.c., 1.5 N Hcl.) when a turbid solution was obtained. The solution remained turbid on further boiling and on cooling to room temperature an oil separated. The reaction mixture was evaporated to dryness on a water-bath and soxhlet extracted with absolute alcohol when an inorganic residue was left behind. The alcoholic extract gave a cream coloured viscous solid after distillation of the solvent, analysis of which indicates that salt formation has taken place.

Sulphanilamide salt of Lauryl-p-toluidide-2-sodium sulphonate (XV):

A solution of the wetting agent (5.9 g.) in water (70 c.c.) was added to a hot solution of sulphanilamide (2.5 g.) in dilute hydrochloric acid (50 c.c. acidified with 1.65 c.c. of concentrated acid), and the mixture was boiled for 2 minutes. On cooling gradually, the mixture set to a thick jelly, and it was therefore evaporated to dryness on water bath. The residue was extracted with absolute alcohol in a soxhlet when sodium chloride (0.54 g.) obtained during salt formation was left behind. The alcoholic extract gave the salt in the form of a light brown hygroscopic powder after removal of the solvent (Found: N, 8.1; S, 11.2. $C_{25}H_{39}O_6N_3S_2$ requires N, 7.7 ; S, 11.8%).

Substitution of sulphanilamide from its salt (I) by p-aminobenzoic acid to give (VIII) :

Method A.

A solution of salt (I) (0.38 g.) and p-aminobenzoic acid (0.137 g.) in water (20 c.c.) was boiled under reflux for one hour and the solution cooled gradually to 0-5° when lustrous flakes separated, m.p. 240-245°C (decomposition). The product was recrystallised from water when it gave m.p. 260-280°C (decomposition) (0.045 g.), underpressed when mixed with (VIII).

Sulphanilamide is, therefore, displaced from its salt by p-aminobenzoic acid.

The filtrate after separation of the crude reaction product, m.p. 240-245 (decomposition) was concentrated to about 8 c.c. and cooled when colourless flakes separated, m.p. 200-240°C (decomposition).

Method B.

The above experiment was repeated, using identical quantities of the (I) and p-aminobenzoic acid but this time an aqueous solution of two (60 c.c. water) was left for 10 days at room temperature (26-29°C). The solution was evaporated in an open dish at room

temperature and the light brown residue was vigorously shaken with water (16 c.c.) for 30 minutes and filtered and the mother liquor was stored. The residue was washed thrice with water (3 c.c.) and dried, m.p. 200-280°C (decomposition) (0.105 g.). On recrystallisation from boiling water (5 c.c.) after clarifying (norit) lustrous small flakes were obtained, m.p. 259-263°C; unaltered when mixed with(I)

The mother liquor was clarified (norit) and concentrated to about 4 c.c. and cooled gradually to 0-5°C when pale brown needles separated, m.p. 175-185°C (decomp.). The product was recrystallised from boiling water (ca 2 c.c.) when pale brown flakes separated, m.p. 260-285°C (decomp.), undepressed when mixed with (VIII).

The experiment shows substitution by p-aminobenzoic acid takes place even at room temperature.

Method C.

A mixture of identical quantities of the salt (I) and p-aminobenzoic acid as above dissolved in dilute 1% sodium hydroxide (16 c.c., 4.0 mol.) in cold, and the solution acidified with concentrated hydrochloric

acid (0.5 c.c., 5.0 mols.) under stirring at room temperature. Small rods separated, m.p. 260-267° (0.175 g.) which were identical with (I). The filtrate after separation of the above product was cooled in crushed ice, when lustrous colourless flakes separated, m.p. 260-280° (decomp.) (0.075 g.), undepressed when mixed with (VIII).

In the first step the salt (I) is hydrolysed by alkali to the free sulphanilamide and in the next step of acidification both the latter and p-aminobenzoic acid are available for salt formation with the sulphonic acid part. The experiment indicates the competitive activity of p-aminobenzoic acid.

Titration of sulphanilamides and sulphanilamides salts of naphthalene-2-sulphonic acid :

Apart from sulphanilamide and sulphaguanidine which could not be titrated the other sulpha drugs such as sulphathiazole, sulphapyridine, sulphadiazine, sulphamerazine and N'-acetylsulphanilamide could be accurately titrated against 0.1 N sodium hydroxide using phenolphthalein or Alkali Blue 6B as indicators. Titrations using Alkali Blue as indicator were

carried out in alcoholic solution and sharp N-points (Blue - Red) were obtained. The sulpha drugs were not acidic to either Methyl Red or to Methyl Orange.

In the titrations of the salts it was found that the titre using Alkali Blue 6B or phenolphthalein corresponded to both the salt and sulphonamide groups while when Methyl Red or Methyl Orange was used the titre corresponded exclusively to the salt linkage.

The results of the alkali titrations are recorded in Table I.

TABLE I.

| Compounds | Weight taken in gms. | Indicator | c.c. of 0.1N NaOH | |
|---|---|---|---|---|
| | | | Titre observed | Titre required |
| I | 0.2 | Alkali Blue 6B | 5.3 | 5.2 |
| I | 0.2 | Methyl Red | 5.37 | 5.2 |
| Sulpha-thiazole | 0.2 | Alkali Blue 6B | 8.15 | 8.0 |
| II | 0.2 | Alkali Blue 6B | 8.64 | 8.64 |
| II | 0.2 | Methyl Red | 4.2 | 4.3 |
| VI | 0.2 | Phenolphtha-lein | 4.5 | 4.7 |

The sulphanilamides and their salts were tested for in vitro bacteriostatic activity against **E.coli.** and Staphylococcus aureus, using the following media: (1) 1% Casein Hydrolysate, (2) Muir's Synthetic medium containing 1% Casein Hydrolysate, (3) Beef Extract (0.4%) (Lab-Lemco, Oxo-Ltd., London), peptone (1%), and sodium chloride (0.5%).

The tests were carried out at pH 7.6 and 37°C and growth of the bacteria was followed by the appearance of turbidity. The concentration of the

drug in milligrams per cent required to prevent the growth after 72 hours was determined. When Muir's synthetic medium alone was used no growth was observed with Staphylococcus aureus. Satisfactory results were, however, obtained when Muir's medium containing 1% Casein Hydrolysate was used as in (2). *In vitro* tests of some of the salts using the media (1), (2) and (3) are summarised in Tables II, III and IV respectively.

## Table II

| | Sulpha Drug | Per cent of sulpha drug in Aryl Salt. | Minimum effective concentration in mgm % | | | |
|---|---|---|---|---|---|---|
| | | | Staphylococcus aureus. | | Escherichia coli. | |
| | | | Salt | Sulpha | Salt | Sulpha |
| I | Sulphanilamide | 45 | 500 | 500 | 500 | 500 |
| II | Sulphathiazole | 55.1 | 8 | 12 | 8 | 10 |
| VI | Sulphaguanidine | 50.7 | 250-300 | 250 | 250 | 300 |
| VII | N-Acetylsulphanilamide[1] | 50.7 | 150 | 200 | 150 | 225 |
| IV | Sulphadiazine | 54.3 | 8 | 20 | 10 | 22 |
| III | Sulphapyridine | 54.4 | 12 | 22 | 14 | 22 |
| V | Sulphamerazine | 56.1 | 20 | 40 | 30 | 30 |

Table III.

| Sulpha Drug | Per cent of sulpha drug in Aryl salt | Minimum effective concentration in mgm % | | | |
|---|---|---|---|---|---|
| | | Staphylococcus aureus | | Escherichia coli | |
| | | Salt | Sulpha | Salt | Sulpha |
| Sulphanilamide | 45 | 400 | 600 | 500 | 500 |
| Sulphathiazole | 55.1 | 8 | 10 | 8 | 12 |
| Sulphaguanidine | 50.7 | 250 | 250-300 | 250 | 250-300 |
| N-Acetyl sulphanilamide | 50.7 | 150 | 200 | 175 | 225 |
| Sulphadiazine | 54.3 | 12 | 20 | 10 | 22 |
| Sulphapyridine | 54.4 | 14 | 22 | 12 | 22 |
| Sulphamerazine | 56.1 | 20 | 40 | 30 | 30 |

Table IV.

| | Sulpha Drug | Per Cent of sulpha drug in aryl salt | Minimum effective concentration in mgm.% | | | |
|---|---|---|---|---|---|---|
| | | | Staphylococcus aureus | | Escherichia coli | |
| | | | Salt | Sulpha | Salt | Sulpha |
| I | Sulphanilamide | 45 | 1050 | 1050 | 500 | 800 |
| II | Sulphathiazole | 55.1 | 300 | 400 | 400 | 500 * |
| VI | Sulphaguanidine | 50.7 | 400 | 500 * | 500 | 500 * |
| VII | $N^1$-Acetyl sulphanilamide | 50.7 | 400 | 500 * | 500 | 500 * |

* Limiting concentration could not be determined due to insolubility of the compounds beyond 500 mg.

Bacteriostatic activities of sulphanilamides using the inhibitor-free media 1, 2 now determined were comparable to results obtained by other workers (Bell and Roblin, loc. cit.) J.A.C.S., 1942, 64, 2905) and the small difference between the above values and the values obtained by others is undoubtedly due to difference in the media employed. The salts proved to be more active than their sulpha drug components inspite of the fact that they contained only 40 - 50% of the sulpha components.

When beef extract-peptone medium was employed considerable quantities of the sulpha drugs and their salts were required when inhibitor free media such as 1 and 2 were employed. In beef extract-peptone media most of the salts had nearly equal bacteriostatic activity, inspite of the divergent activity of the sulpha components.

Tests could not be carried out for (XIV) due to insufficient quantity of the analytical specimen. Tests on other surface-active substances, viz. XIV and XV are in progress. In vivo tests will be shortly undertaken. *

A 500 mg.% solution of a mixture of sulphanilamide (275 mg.) and Naphthalene-2-sulphonate (225 mg.) was prepared in Muir's medium containing 1% casein hydrolysate.

Three tubes containing 500 mg.%, 400 mg.% and 200 mg.% of the above solution were inoculated with E.coli. and

* Potentiation of sulphanilamide and sulphathiazole was observed by the synergic action with naphthalene-2-sodium sulphonate.

incubated 37°C. No growth was observed in any of the tubes, which indicates that there is a definite synergistic action, for separately both the sulphanilamides and sodium salt give growth with these concentrations. Similarly, synergistic action was observed with a mixture of sulphathiazole and naphthalene-2-sulphonate, using 10 mg.%, 8 mg.% and 4 mg.% solutions. Here also no growth was observed in all the tubes.

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## Part II.

# SOME SULPHANILAMIDES AND OTHER DERIVATIVES OF *p*-CYMENE.

# INTRODUCTION

## SOME SULPHANILAMIDE AND OTHER DERIVATIVES OF p-CYMENE.

After Tréfouël et al.[1] showed that the active part in Prontosil was p-aminobenzene-sulphonamide, a vigorous search for improved sulphanilamides was undertaken by various workers and has led to a tremendous amount of research and some three thousand odd derivatives of sulphanilamides have been synthesised.[2] There are 8 hydrogen atoms in sulphanilamide and the effect of replacing one or more hydrogen atoms have been exhaustively studied. The following briefly summarises the findings:

$N^1$-Derivatives : While the introduction of alkyl groups like methyl or ethyl group caused little change in the activity, longer alkyl chains lowered the activity.[2] None of the isocyclic derivatives were active. Heterocyclic derivatives include the most powerful and widely used sulpha drugs, viz., sulphapyridine,[3] which is effective against pneumococcal, streptococcal and coli infections ; sulphathiazole[4] and sulphadiazine[5], which are less toxic and possess the highest activity amongst the known sulpha drugs. Amongst the acyl derivatives only three are

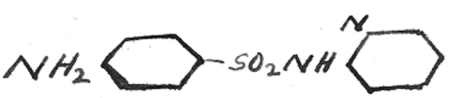
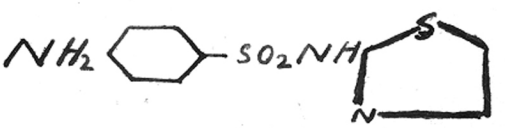
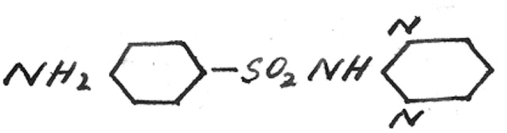
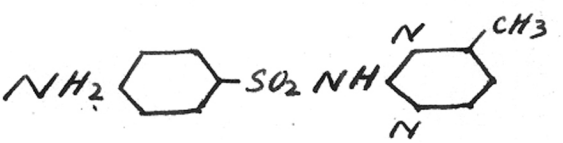
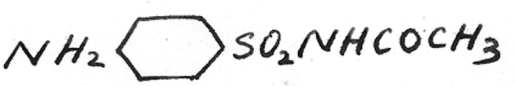
of practical importance. Sulfacetamide[6] is used for eye and urinary infections. Irgafen[7] is said to be as potent as sulphapyridine. Sulphaguanidine,[8] which is an N[1]-acylsulphanilamide theoretically derived from carbonic acid is very useful in intestinal diseases.

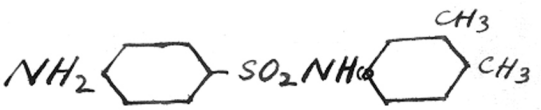
N[4]-derivatives: "Unless the substituting group in the N[4]-position is hydrolysed, reduced, or otherwise removed in vivo, it appears that the derivative will have little, if any, activity. That such processes do occur has been amply demonstrated by finding of a diazotisable amine in the blood after feeding 4-nitro-, hydroxylamino-, azo-, N[4]-acyl-, anil and reduced anil, aldehyde-bisulphite, and aldehyde-sulfoxalate sulphanilamides, and by the isolation of sulphanilamide from the urine of amimals so treated. It has not been proved that the activities of these compounds are entirely the result of cleavage with liberation of sulphanilamide, but there is much which points to such a mechanism. It is quite possible that the -- superior properties claimed for certain derivatives of this type are a result of slow cleavage with -- prolonged maintenance of therapeutic blood levels of sulphanilamide, or whatever active form may be derived in vivo from sulphanilamide."[2] Important examples

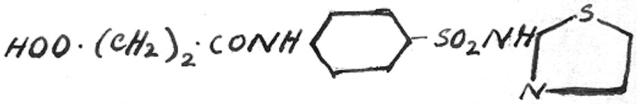
in this series are Prontosil,[9] Prontosil soluble, and Septazine ($N^1$-Benzylsulphanilamide).

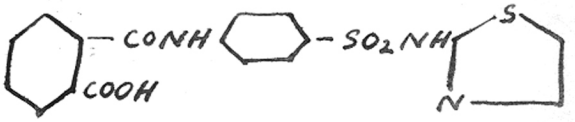
Important $N^1$:$N^4$-derivatives are : Succinyl sulphathiazole,[10] and Phthaloyl sulphathiazole[11], both of which are powerful intestinal antiseptics. A number of therapeutically active sulpha drugs are listed in Table I.

Table I

| Trade or Proprietary name | Formula |
|---|---|
| Sulphapyridine |  |
| Sulphathiazole |  |
| Sulphadiazine |  |
| Sulphamerazine |  |
| Sulphacetanide |  |

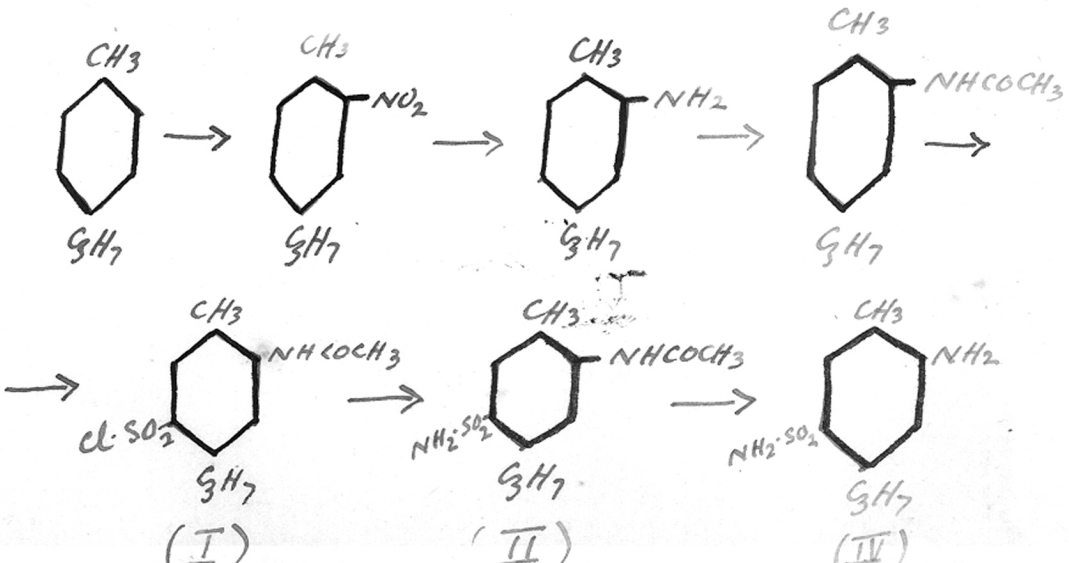
"Irgafen" 

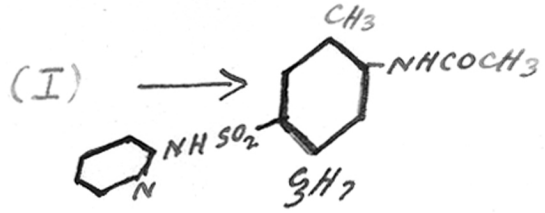
Succinylsulphathiazole 

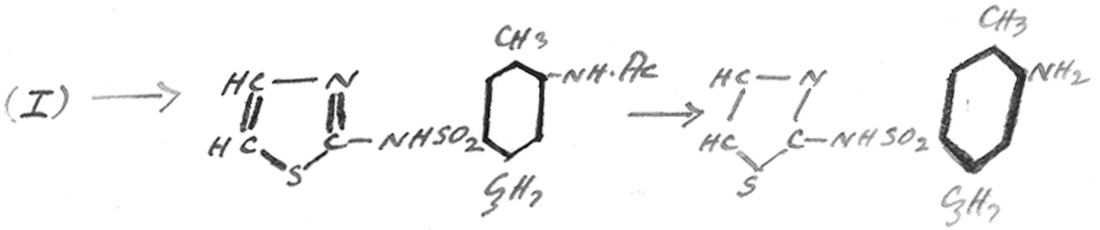
Phthaloylsulphathiazole 

Nuclear substitution : Substitution in the hydrogen atoms in the benzene ring of sulphanilamide has not been investigated in detail firstly because the substituted derivatives are difficult to synthesise, and secondly because the simpler derivatives were found to be generally inactive. It was found that introducton of halogen[12], amino,[13] sulphonamide,[14] methyl,[15] or carboxyl group[16] in the ring completely destroyed the activity. Definite conclusions that any substitution in the ring will destroy activity should not be drawn, however, since 3:5-dimethyl-sul-phanilamide[17] is said to have some activity. In some cases, anthelmintic activity of the nuclear substituted derivatives has been reported. As a further study of

the effect of nuclear substitution on the bacteriostatic activity of sulphanilamides, two C-alkyl derivatives of sulphanilamide and sulphathiazole have now been synthesised, starting from the terpene-hydrocarbon p-cymene. p-Cymene occurs in many essential oils (oil of carraway and eucalyptus oil), and the p-cymene skeleton has been recognized in various naturally occurring terpenes and camphors. It will also be remembered that thymol (hydroxy-p-cymene) is a useful antiseptic and anthelmintic. In addition to examining bacteriostatic activity, anthelmintic activity of the newly prepared neuclear substituted sulphanilamide was also determined. 5-Methyl-2-isopropyl sulphanilamide (IV) and 5-methyl-2-isopropyl sulphathiazole (VII) were prepared according to the following scheme :







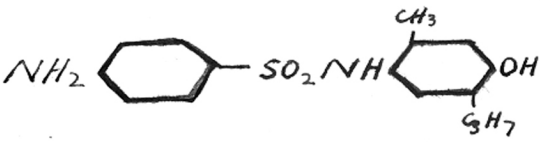
p-Cymene was nitrated[19] and the pure 2-nitro-cymene (B.P. 127-132°/12 mm.) was reduced according to Pekka[20] by iron and dilute hydrochloric acid. The crude reduction product was boiled and steam-distilled. Examination of the distillate indicated the presence of p-toluidine, but vacuum distillation gave pure cymidine (B.P. 110°C/ 10 mm.). 2-Cymidine was acetylated with glacial acetic acid, and the acetyl derivative (m.p. 70°C) was then treated with cold chlorosulphonic acid as in the standard preparation of sulphanilamide, to give 4-acetamido-5-methyl-2-isopropylbenzene sulphenyl chloride (I), m.p. 176-78°C (decomp.). On treatment with ammonia, (I) gave the sulphonamide (II), which crystallised from aqueous alcohol in plates, m.p. 225-26°C. Hydrolysis of the acetyl group was carried out with concentrated hydrochloric acid to give 5-methyl-2-isopropyl-sulphanilamide (IV), which crystallised

from hot water in plates, m.p. 160-61°C. The new sulphanilamide (IV) was characterised by preparing the $N^1$-$N^4$-diacetyl derivative (III), the $N^4$-benzoyl derivative (V), and the azo dye (VI), obtained by diazotising (IV), and coupling it with β-naphthol. The dye (VI) was also examined for its anthelmintic activity.

5-Methyl-2-isopropyl-sulphathiazole (VIII) was prepared in a similar manner, (I) being condensed with 2-aminothiazole in pyridine, and the acetyl derivative (VII) hydrolysed with dilute sodium hydroxide to give (VIII), m.p. 246-47°C. Similarly by condensing (I) with 2-aminopyridine, 5-methyl-2-isopropyl-4-acetamido sulphapyridine (IX) was obtained which crystallised from alcohol in plates, m.p. 190-91°C. This acetanilide derivative could not be hydrolysed by acid or alkali to the desired amine. Dr. Mahal has kindly examined (IV) for bacteriostatic activity and found it to be inactive. It had also no anthelmintic action.

p-Amino-N-(3'-hydroxy-5'-methyl-2'-isopropyl-phenyl)benzene sulphonamide (XI) was prepared by condensing acetylsulphanilyl chloride with 6-amino-thymol in distilled pyridine and hydrolysing the

acetyl derivative with sodium hydroxide. It has been prepared earlier,[21] and has been reported to be inactive.




It was, however, again prepared with a view to examine its anthelmintic action, which was found to be poor.

With a view to study the effect of nuclear substitution on the antiseptic property of salicylanilide, a well known textile antiseptic used for the prevention of mildew growth in cotton goods, the anilide (XII) was prepared by condensing cymidine with salicylic acid. The anilide (XIII) of 5-chlorosalicylic acid was also prepared. The antiseptic property of (XII) and (XIII) was compared against -- standard fungicides like β-naphthol, salicylic acid, phenol, and salicylanilide, and both the new compounds were found to be inferior in their activity.

The present experiments confirm the view that in general nuclear substitution of sulphanilamides destroys the bacteriostatic activity of the parent drugs. The nuclear substituted sulphanilamides prepared had also no anthelmintic action. Fungicidal potency was likewise reduced by nuclear substitution

as shown by the results of tests on a number of nuclear substituted salicylanilides.

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# EXPERIMENTAL.

5-Methyl-2-isopropyl-4-acetamidobenzene sulphonyl chloride (I) :

Acetyl-p-cymidine (5.5 g. ; 1.0 mole.) was added gradually to chlorosulphonic acid (15 c.c. ; 6.0 moles.) under good stirring keeping the temperature below 20°C by external cooling. The mixture was allowed to come to room temperature and finally heated on water-bath for 1 hour at 60-70°C. After cooling to room temperature the mixture was poured over chipped ice (60 gms.). A cream coloured sticky product separated which solidified on standing. The solid (6.0 g.) was washed free from acid with ice-cold water (I). Crystallised in rods from dry toluene, m.p. 176-178°C (Found: N, 5.2 ; Cl, 12.0. $C_{12}H_{16}NO_3SCl$ requires N, 4.8 ; Cl, 12.2%).

5-Methyl-2-isopropyl-4-acetamidobenzene sulphonamide (II) :

Crude product (5.0 g.) from above was thoroughly pasted with 25% ammonia (50 c.c.) for half an hour and then warmed on water bath (70°C) for one hour. The mixture was cooled in ice bath and then acidified with dilute sulphuric acid (1:1) till acidic to Congo Red. It was further cooled in ice bath for half an hour when (II) separated in crystalline form

(2.5 g.) ; recrystallised from aqueous alcohol in plates, m.p. 225-26°C. (Found: N, 10.3 ; S, 11.5. $C_{12}H_{18}N_2O_3S$ requires N, 10.4 ; S, 11.8%).

4-N'-N-Diacetyl-5-methyl-2-isopropylbenzene sulphonamide (III) :

Compound (II) (1.0 g.) was heated for an hour under reflux with acetic anhydride (1.2 g,) in toluene to which a drop of pyridine was added. After cooling, white crystalline solid separated (1.0 g.); diacetyl derivative (III) gave plates from alcohol, m.p. 260-61°C (Decomp.) (Found: N, 9.1 ; S, 10.5. $C_{14}H_{20}N_2SO_4$ requires N, 8.9 ; S, 10.3 %).

5-Methyl-2-isopropylbenzene sulphonamide (IV):

Compound (II) (1.0 g.) was heated under reflux with concentrated hydrochloric acid, sp.gr. 1.16 (40 c.c.) for 6 hours. The reaction mixture was cooled and diluted with water (25 c.c.), decolourised and finally made weaky alkaline with sodium carbonate. Crystallised from hot water in needles, m.p. 165-66°C (0.6 g.). (Found: N, 12.3 ; S, 14.0. $C_{10}H_{16}N_2SO_2$ requires N, 12.3 ; S, 14.2 %).

$N^4$-Benzoyl derivative (V) from compound IV :

Compound (IV) (1.0 g.) was dissolved in 2.5 N Sodium hydroxide (4.0 c.c.) and benzoyl chloride (0.6 c.c.) was added with constant shaking. White sticky paste separated, which solidified after scratching with a glass rod. Crystallised from pyridine in needles, m.p. 214-16°C (Found: N, 8.5 ; S, 9.8. $C_{17}H_{21}N_3SO_3$ requires N, 8.4 ; S, 9.6 %).

Azo dye from Compound (IV) and β-naphthol (VI) :

Compound (IV) (1.1 g.) was diazotised with concentrated hydrochloric acid (2.5 c.c.), water (10 c.c.) and sodium nitrite (0.5 g.). The clear diazotised solution was coupled with β-naphthol (0.72 g.) dissolved in 10% sodium hydorxide (10 c.c.). The dye (VI) (1.6 g.) was precipitated by dilute hydrochloric acid and recrystallised from glacial acetic acid in needles, m.p. 275-76°C. (Found: N, 10.7; S, 8.6. $C_{20}H_{21}N_3SO_3$ requires N, 10 ; S, 8.3 %).

5-Methyl-2-isopropyl-4-acetamido sulphathiazole (VII):

2-Aminothiazole (3.9 g.), dissolved in dry pyridine (10 c.c.) was added with good shaking to sulphonyl chloride (I) (4.4 g.). The mixture was

warmed on water-bath for one hour, cooled and poured over a mixture of chipped ice and concentrated hydrochloric acid, when crystals of (VII) separated (4.0 g.) ; recrystallised from aqueous alcohol in plates, m.p. 250-51°C) (Found: N, 11.7 ; S, 18.5. $C_{15}H_{19}N_3S_2O_3$ requires N, 11.8 ; S, 18.1 %).

5-Methyl-2-isopropyl sulphathiazole (VIII) :

The above acetyl derivative (0.7 g.) was hydrolysed by boiling with 10% sodium hydroxide (10 c.c.) for 2 hours. After cooling and neutralisation with dilute hydrochloric acid (1:1) the amino compound (VIII) separated ; recrystallised from aqueous alcohol in plates, m.p. 246-47°C. (Found: N, 13.2 ; S, 20.8. $C_{13}H_{17}N_3S_2O_2$ requires N, 13.5 ; S, 20.5 %).

5-Methyl-2-isopropyl-4-acetamido-sulphapyridine (IX):

2-Aminopyridine (1.5 g.; 1.0 mole.) was dissolved in dry pyridine (6.0 c.c.) and the sulphonyl chloride (I) (4.5 g.) was slowly added with -- shaking. The mixture was maintained at room temperature by cooling in water bath. When all the sulphonyl chloride was added the mixture was left overnight. The mixture was heated at 60°C for 20 minutes, cooled

and poured over chipped ice and hydrochloric acid, when cream coloured product separated (IX) ; crystallised from aqueous alcohol in needles, m.p. 273-74°C. (Found: N, 12.1 ; S, 9.6. $C_{17}H_{21}N_3SO_3$ requires N, 12.1 % ; S, 9.25 %). It has not been found possible to hydrolyse (IX) to the corresponding alkyl sulphapyridine.

N$^4$-Acetylsulphanilyl-6-aminothymol (X) :

6-Aminothymol (4.5 g. ; 1.0 mole.), prepared according to Kobbe and Doumani,[19] was dissolved in sodium dry pyridine (15 c.c.) and N$^4$-acetylsulphanilyl-chloride (6.0 g. ; 1.0 mole.) was added slowly with shaking. The mixture, which became warm on addition of the sulphonyl chloride was maintained at room temperature by cooling in water bath. When all the sulphonyl chloride was added the mixture was left overnight. After heating at 60°C for twenty minutes the mixture was cooled and poured over a mixture of chipped ice and concentrated hydrochloric acid, when the acetyl derivative (X) separated (6.5 g. ; 90%); crystallised from aqueous alcohol in needles, m.p. 228-30°C. (Found: N, 7.2 ; S, 8.7. $C_{28}H_{32}N_2SO_4$ requires N, 7.7 ; S, 8.5 %).

Sulphanilyl-6-aminothymol (XI) :

$N^4$-Acetylsulphanilyl-6-aminothymol (3.0 g.) was dissolved in 10% sodium hydroxide (50 c.c.) and the solution was refluxed for one hour when the -- coloured changed from bluish green to dark brown. The solution was cooled, filtered and the filtrate acidified with dilute hydrochloric acid (1:1) when a cream-coloured powder was obtained (2.1 g.). Compound (XI) crystallised from aqueous alcohol in golden yellow long needles, m.p. 220-22°C (Found: N, 8.5 ; S, 10.3. $C_{16}H_{20}N_2SO_3$ requires N, 8.75 ; S, 10.4 %).

Salicyl-p-cymidide (XII) :

p-Cymidine (3.0 g. ; 1.0 mole.) and salicylic acid (8.2 g. ; 3.0 mole.) were suspended in dry toluene (150 c.c.) and heated on water bath at 60-70°C for half an hour. Phosphorous trichloride (70 c.c.; 3 moles.) was then gradually added in half an hour. The mixture was further heated on water bath for half an hour at 60-70°C and finally heated under reflux at 120-30°C in an oil bath for 10 hours. After cooling the supernatant liquid was decanted from phosphoric acid which settled down as a sticky yellow residue. Toluene was removed by distillation and the product

obtained was boiled repeatedly with water to remove traces of salicylic acid. The sticky yellow residue solidified after cooling, and after crystallisation from alcohol gave salicyl-p-cymidide (XII) as needles, m.p. 144-45°C. (Found: N, 5.1 ; $C_{17}H_{19}NO_2$ requires N, 5.2 %).

5-Chlorosalicyl-p-cymidide (XIII) :

Preparation of 5-chlorosalicylic acid : Salicylic acid (21.0 g.) was dissolved in glacial acetic acid (210° c.c.) and cooled to 0°C in ice-bath and chlorine gas (10.5 g.) was slowly passed into the cold solution (60-70 bubbles per minute). The resulting solution was cooled overnight in refrigerator when 5-chlorosalicylic acid separated in needles, m.p. 170-71°C.

p-Cymidine (1.5 g.; 1.0 mole.) and 5-chlorosalicylic acid (5.0 g.; 3.0 mole.)were suspended in toluene (150 c.c.) and heated under reflux on water-bath at 60-70°C. Phosphorous trichloride (4.0 c.c.) was added in drops in course of half an hour. The whole mixture was finally heated for 10 hours at 120-123°C in an oil bath and then cooled. Phosphoric acid formed was separated, toluene removed by distillation,

and the sticky powder obtained was repeatedly washed with hot water and finally crystallised, when 5-chloro salicyl-p-cymidide (XIII) separated as needles from dilute alcohol, m.p. 149-50°C (Found: N, 4.8 ; Cl, 11.25. $C_{17}H_{18}NO_2Cl$ requires N, 4.6 ; Cl, 11.04%).

Compounds (XII) and (XIII) were examined for their antiseptic power by the test tube method as follows:

5 c.c. of various concentrations of antiseptics were added to 5 c.c. of a paste containing 2% farina and 4% agar. The contents of the tube were mixed, the tube plugged and sterilized in an autoclave at 15 lbs. pressure for twenty minutes. After sterilizing, the tubes were allowed to cool in a slanting position. The tubes were then incubated with a culture of Aspergillus niger van Tiegh and observations were made after 4 days. The inhibition concentration was taken as that which just prevented the growth of the organism when observed for 4 days. The results are recorded in the following table, along with figures of other antiseptics for comparison.

| Compounds | Inhibition concentration % |
|---|---|
| 1. Salicylanilide | 0.003 |
| 2. β-naphthol | 0.005 |
| 3. Salicylic acid | 0.04 |
| 4. Phenol | 0.06 |
| 5. XII | More than 0.2 |
| 6. XIII | 0.12 |

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## Part III.

# AZO DYES FROM SULPHANILAMIDES.

# INTRODUCTION.

## AZO DYES FROM SULPHANILAMIDES

Historical:

Although Gelmo[1] first prepared p-aminobenzene-sulphonamide (Sulphanilamide) in 1908, a quarter of a century had to pass before its chemotherapeutic properties were discovered. In 1909[2], Hoerlein and his co-workers of I.G.Farbenindustrie, synthesised azo dyestuffs containing sulphonamide and substituted groups and found them superior in colour-fastness to the corresponding azo dyes not containing sulphonamide groups. Certain azo dyes apparently formed a stable combination with the protein constituting the wool and silk fibres, and so it was suggested that the azo dyes might react with the bacterial protoplasm. In 1913, Eisenberg[2] observed the bactericidal action *in vitro* of chrysoidine and suggested its use in chemotherapy. Heidelberger and Jacobs[3] observed that p-aminobenzenesulphonamide would be liberated by the break down in the tissues of p-sulphonamidobenzene-azo-chrysoidine. Unfortunately, further investigations were not continued and to Domagk[4] in 1935 goes the credit for discovering the chemotherapeutic value of Prontosil, a dye which was

first prepared by Klarer and Mietzsch[5] in 1932. -- Prontosil is the hydrochloride of 4'-sulphonamido-2:4-diamino-azo benzene, and was found highly effective clinically in haemolytic streptococcal infections. Tréfouël et al.[6], while studying the antibacterial action of Prontosil, demonstrated that the azo linkage (-N=N-) was not necessary for bactericidal potency, Prontosil being split in the tissue at this linkage to yield sulphanilamide and they stressed the importance of the sulphonamido group ($-SO_2NH_2$). The azo dyes were thus antibacterial by virtue of yielding sulphanilamide, and Bliss and Long[7] soon confirmed these findings by reducing Prontosil soluble with cysteine hydrochloride and showed the resulting sulphanilamide to be active against haemolytic streptococci. Henceforward, the interest shifted from azo dyes to sulphanilamide itself.

It seems probable that lack of absorption or resistance to cleavage will account for most of the inactive dyes. Apart from the sulphonamides resulting from the cleavage at the azo linkage, aminophenols or aromatic polyamines are also liberated, and it is to be expected that they will also exercise desirable or undesirable physiological action. It is possible

that the amino components may exert synergistic --- action on the liberated sulphanilamide or may in themselves be independently active. If this component is a powerful surface-active agent or an anthelmintic or possesses good "substantivity", it is possible that the activity of the sulphanilamide component would be enhanced. With these considerations in view, azo dyes were prepared by coupling diazotised sulphanilamide derivatives with (a) 4-n-hexylresorcinol, (b) thymol and (c) arylamides of 2-hydroxy-3-naphthoic acid.

## (A) AZO DYES FROM 4-n-HEXYLRESORCINOL.

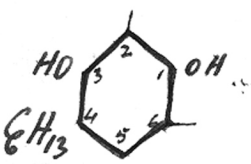
A great many acyl and alkyl resorcinols have been studied for their germicidal properties, and 4-n-hexylresorcinol in particular has been found to be highly active (with a Phenol coefficient 50) and to have low toxicity in comparison with resorcinol derivatives carrying shorter or longer paraffin groups. Azo dyes from 4-n-hexylresorcinol and diazotised sulphanilamides have been prepared. By virtue of the $C_6$-alkyl chain the compounds might have surface-activity, the possible advantages of which have been indicated in Part I. The azo dye (I) from diazotised sulphanila-

mide coupled with n-hexylresorcinol has already been described by Tréfouël et al.[6], but has been prepared again for studying its properties and for comparison with the azo dyes from other sulphanilamides.

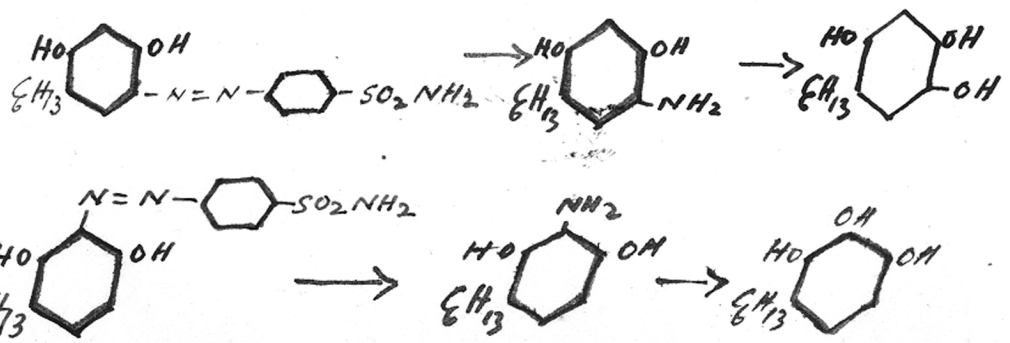
Resorcinol couples so readily that even with one mole of diazobenzene chloride, about 10% of a disazo dye, is formed.[8] Using two moles of the diazo salt, two disazo dyes are obtainable, depending on the pH of the reaction mixture. If the coupling is carried out at pH 5-8, the product is 2:4-benzeneazoresorcinol. Under strongly alkaline conditions the product is the 4:6-disazo compound ; in the present case this symmetrical dye cannot be obtained, since the 4-position of resorcinol is already occupied by the hexyl group. With regard to the monoazo dye, it has been assumed by analogy that the product is 5-p-sulphonamidobenzeneazo-2:6-dihydroxyhexyl benzene (I), and attempt has been made to separate the very small amount of the 3-isomer which may have been formed.

When the diazo coupling was effected in alkaline solution (sodium hydroxide or sodium carbonate), a mixture of the mono and disazo dyes was formed even with one mole of the diazonium salt. The mono and disazo dyes were separated by fractional crystallisation,

the mono azo being more soluble than the diazo dyes. The yield of the mono azo dye was also poor. When the coupling was carried out in pyridine-ammonia solution, the nearly pure disazo dye was formed, while in acetic acid medium, the mono azo dye was the predominant product (60-70 per cent.). Some of the dyes could not be crystallised and had to be purified by precipitation.



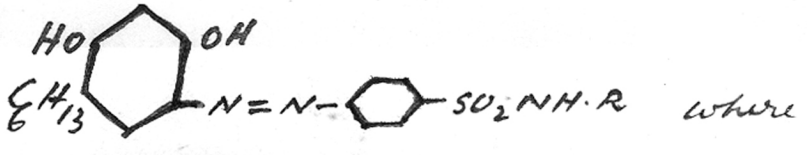

4-n-Hexylresorcinol has two coupling positions (2- and 6-), and attempts were made to determine the orientation of the azo group in the mono azo dyes. The method was to obtain an aminohexylresorcinol, which could then be converted to a hexyl-trihydroxy benzene and characterisation of the latter as a pyrogallol or hydroxy hydroquinone derivative depending on the orientation of the azo group in (I).



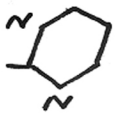
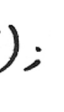
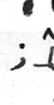
The hydroxy compounds could then be identified by suitable colour reactions. The dye was reduced with sodium hydrosulphite in sodium hydroxide solution, when the deep wine colour was discharged and a dark-coloured nitrogenous product separated, which had a high melting point and could not be crystallised. The mother liquor after concentration gave a white crystalline product, which was identified as sulphanilamide. Reduction with zinc dust, acetic acid, and alcohol gave similar results. On account of the instability of the amino-resorcinol derivative, the next attempt was to proceed through the dimethyl ether of the azo dye (I). The dimethyl ether ( X ) was therefore prepared, but it was not possible to convert it ultimately into the desired amino-resorcinol.

Petyunin[9] prepared mono azo dyes from 4-chlororesorcinol by coupling diazotised aniline, p-nitroaniline, sulphanilic acid, and parachloroaniline, and assumed that the coupling took place solely in the 6-position. The following mono azo and disazo dyes have been prepared and characterised by analysis.

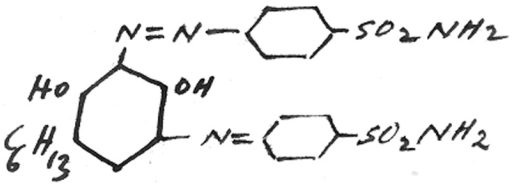
Monoazo dyes:-

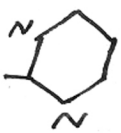



where

R = H (I);  (III);  (IV);  (VI);  (VIII)

Disazo dyes:



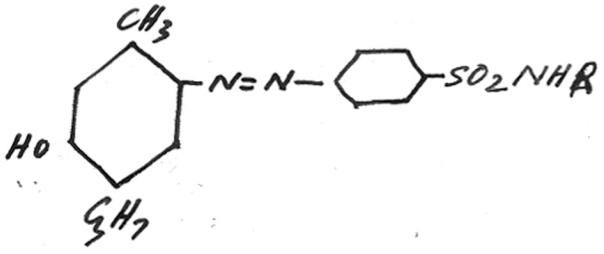
where R = H (II);  (V);  (VII);  (IX)

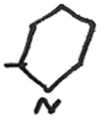
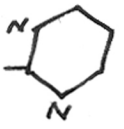
The azo dyes were tested in vitro for bacteriostatic activity. The tests were carried out at pH 8 and 37°C. with Staphylococcus aureus, Escherichia coli, and E.typhosus. All the azo dyes proved to be inactive. The wetting power of the dyes, as determined by the Herbig Number technique was very poor (Herbig Number 12-14). (Herbig No. of water 18·4)

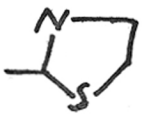
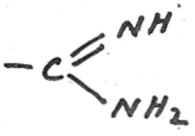
## (B) AZO DYES FROM THYMOL.

Thymol is a powerful antiseptic and anthelmintic. As part of the attempts to combine the bacteriostatic action of sulphanilamides and the physiological activity of other components possessing anthelmintic and anti-

septic properties, the azo dyes (XI - XV) have been prepared by coupling diazotised sulphanilamides with thymol:



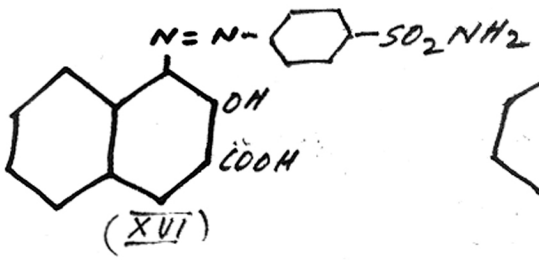
where R = H (XI);  (XII);  (XIII);

 (XIV);  (XV)

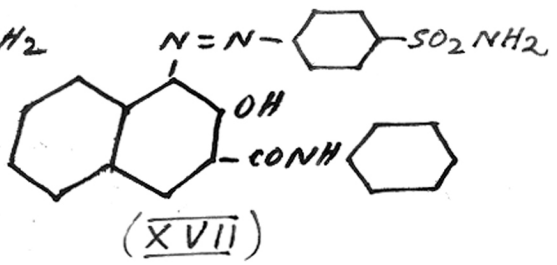
The azo dye (XI) has already been prepared[10] but has been prepared again for an examination of its properties and for comparison with the azo dyes from other sulphanilamides. The couplings were carried out under usual alkaline conditions and the alkali soluble dye was precipitated with dilute hydrochloric acid. The dyes were readily crystallisable, but some of them did not possess a sharp melting point. The dyes were inactive in vitro and had no anthelmintic activity.

## (C) AZO DYES FROM NAPHTHOLS.

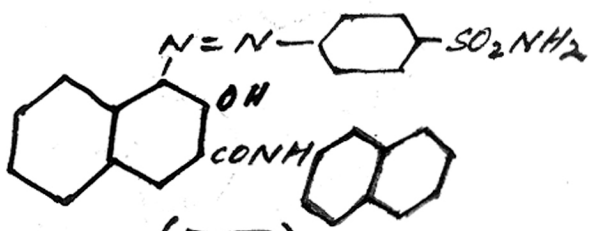
With a view to study the relationship between affinity for or substantivity to textile fibres and bacteriostatic activity, azo dyes were prepared by diazotising sulphanilamide and coupling it in alkaline medium with the following "naphthols" in increasing order of substantivity :- 2÷3-hydroxy naphthoic acid, Naphthol AS, Naphtol AS-SW, and Naphtol AS-S.



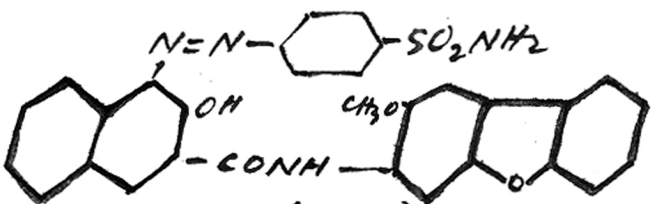

(XVI)



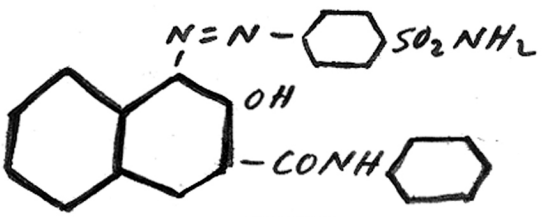

(XVII)




(XVIII)




(XIX)




XX

These azo dyes, except (XVI), were insoluble in aqueous alkali, acids and alcohol but crystallised readily from pyridine or nitrobenzene. An alkali soluble azo dye (XX) from Naphtol AS was then prepared by coupling it with diazotised N'-Acetylsulphanilamide.

The azo dyes (XVI) and (XX) were found to be inactive *in vitro* and also had no anthelmintic action.

The above azo dyes prepared have proved inactive on the basis of *in vitro* tests against Escherichia coli, Staph.aureus and E.typhosus at pH 8 and 37°C. It is possible, as in the case of Prontosil, that these dyes may show activity *in vivo*, due to the liberated sulphanilamide and the antiseptic, anthelmintic and substantive components. *In vivo* tests will, therefore be shortly undertaken.

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# EXPERIMENTAL.

## (A) AZO DYES FROM 4-n-HEXYLRESORCINOL AND SULPHA DRUGS.

4-n-Hexylresorcinol having two coupling positions, monoazo dyes were difficult to obtain exclusively. When the coupling was done in alkaline medium (sodium carbonate ar sodium hydroxide), a mixture of mono and disazo dyes were formed, which were separated by fractional crystallisation. The yield of monoazo dyes was poor (25-30%). When the coupling was done in pyridine (B.P. 114-116°C), almost pure disazo dyes were obtained, while in acetic acid medium about 60-70% of monoazo dyes were formed along with the disazo dyes. The sulphanilamides were diazotised and coupled at 5-10°.

4-Hexyl-6-(4'-sulphonamide)phenylazoresorcinol (I):
and
4-Hexyl-2:6-bis(4'-sulphonamide)phenylazoresorcinol (II):

Sulphanilamide (1.8 g. ; 1.0 mole) was diazotised with concentrated hydrochloric acid (8 c.c.), sodium nitrite (0.5 g.) and water (10 c.c.). The clear diazotised solution was coupled with a solution of hexylresorcinol (1.94 g.) in 5% sodium carbonate (200 c.c.) and sodium acetate (4.0 g.) with vigorous stirring. After three hours the solution was acidified with dilute hydrochloric acid (1:1) and the brick-red dye filtered and

washed free from acid (3.6 g.). The crude dye softened at 165° and melted at 189-90°C. Mono and disazo dyes were separated from the crude dyes by fractional crystallisation from alcohol. The disazo dye was sparingly soluble as compared to the mono azo dye, which was more soluble. The monoazo dye (I) crystallised from alcohol in needles, m.p. 204-205°C (1.0 g. ; 25%) (Found: N, 11.5; S, 9.0. $C_{18}H_{23}O_4N_3S$ requires N, 11.2 ; S, 8.75%).

Disazo dye (II) crystallised from alcohol in needles, m.p. 253-55°C (2.0 g.) (Found: N, 10.2 ; S, 11.7. $C_{24}H_{28}N_6O_6S_2$ requires N, 10.0 ; S, 11,4%).

Monoazo dye from sulphapyridine (III) :

Sulphapyridine (2.5 g.; 1.0 mole) was diazotised with concentrated hydrochloric acid (0.6 c.c.), sodium nitrite (1.0 g.), and water (20 c.c.). The diazotised solution was coupled with hexylresorcinol (1.94 g.; 1.0 mole) dissolved in acetic acid (30 c.c.) and sodium acetate (12.0 g.). After vigorous stirring for 3 hours at 10-12°C, deep orange dye separated. It was filtered, washed with ice-cold water (4.5 g.) and the mono and disazo dyes were separated by fractional crystallisation from alcohol. The monoazo dye was difficult to crystallise from usual organic solvents. It was, therefore, purified by dissolving in sodium hydroxide and

precipitating with dilute hydrochloric acid, m.p. 193-96°C (3.4 g. ; 75%) (Found: N, 12.1 ; S, 7.5. $C_{23}H_{28}N_4O_4S$ requires N, 12.1 ; S, 7.0%).

Monoazo dye from sulphadiazine (IV) :

Sulphadiazine (0.5 g.; 1.0 mole) was diazotised with concentrated hydrochloric acid (0.6 c.c.), sodium nitrite (0.36 g.), and water (15 c.c.). The clear diazotised solution was coupled with hexylresorcinol (0.4 g.; 1.0 mole) dissolved in acetic acid (20 c.c.) containing sodium acetate (3 g.) under vigorous stirring for three hours, when brick red dye separated. The monoazo dye (IV) was separated by fractional crystallisation from alcohol (0.95 g. ; i.e. 75%) ; recrystallised from glacial acetic acid in needles, m.p. 235-38°C (Found: N, 15.5 ; S, 14.5. $C_{22}H_{25}N_5SO_4$ requires N, 15.3 ; S, 14.0%).

Disazo dye from sulphadiazine (V) :

Sulphadiazine (0.5 g.) was diazotised as above and added to hexylresorcinol (0.2 g.) dissolved in pyridine (10 c.c.) and 5 c.c. alcohol. The disazo dye which separated was filtered and washed with dilute hydrochloric acid to remove traces of pyridine, and finally washed free from acid. It was crystallised from aqueous acetic acid in needles, m.p. 284°C (0.9 g.; 85%) (Found: N, 19.8; S, 9.2. $C_{32}H_{32}N_{10}S_2O_6$ requires N, 19.5 ; S, 8.9%).

Monoazo (VI) and disazo (VII) dyes from sulphaguanidine:

Sulphaguanidine (0.84 g. ; 1.0 mole) was diazotised with concentrated hydrochloric acid (2.0 c.c.), sodium nitrite (0.72 g.), water (20 c.c.). The clear diazotised solution was coupled with hexylresorcinol (0.8 g.) dissolved in acetic acid (40 c.c.) containing sodium acetate (4.0 g.). After vigorous stirring for 3 hours a red dye separated which was filtered and washed with ice-cold water (1.5 g.). The dye was soluble in common organic solvents. Mono and disazo dyes formed were separated by fractional crystallisation from alcohol. The monoazo dye was purified by precipitation from an alkaline solution, m.p. 192-93°C (0.8 g. ; 65%)(Found: N, 16.5 ; S, 8.0. $C_{18}H_{25}N_5SO_4$ requires N, 17.0 ; S, 7.6%).

Disazo dye crystallised from acetic acid in -- needles, m.p. 360°C (0.4 g. ; 25%) (Found: N, 21.3 ; S, 10.2. $C_{26}H_{32}N_{10}S_2O_6$ requires N, 21.7 ; S, 9.9%).

Monoazo dye (VIII) and disazo dye (IX) from sulphathiazole:

Sulphathiazole (2.5 g. ; 1.0 mole) was diazotised with concentrated hydrochloric acid (4.0 c.c.), sodium nitrite (1.8 g.), and water (40 c.c.). The clear diazotised solution was coupled with hexylresorcinol (1.9 g.; 1.0 mole) dissolved in acetic acid (80 c.c.) containing sodium acetate (12.0 g.) with vigorous stirring. The

azo dye which separated was filtered and washed free from acid (3.5 g.). The mono and disazo dyes were separated in the usual way. Both the mono and disazo dyes were difficult to crystallise from ordinary organic solvents in which they were soluble. Purified by dissolving in a sodium hydroxide and precipitating with dilute hydrochloric acid.

Monoazo dye; m.p. 136-40°C (2.4 g.; 70%)(Found: N, 11.4 ; S, 14.1. $C_{21}H_{24}N_4O_4S_2$ requires N, 12.1 ; S, 13.8%).

Disazo dye: m.p. 240-42°C (1.2 g.); amorphous powder from aqueous alcohol (Found: N, 16.2 ; S, 9.9. $C_{30}H_{28}N_8S_4O_6$ requires N, 16.7 ; S, 9.5%).

Attempts were made to locate the orientation of the azo group in the monoazo dyes derived from 4-n-hexylresorcinol which has two coupling positions. With this view attempts were made to prepare aminohexylresorcinol from the azo dye (I). The dye was reduced as follows : The dye (0.1 g.) was dissolved in 5N sodium hydroxide (2 c.c.) and heated to boiling. Sodium hydrosulphite was added in small portions. The deep red colour gradually discharged and black precipitate separated. The nitrogenous product had no melting point. It gave a bluish violet solution in sodium hydroxide. The mother liquor

after separation of the above product gave sulphanilamide as a white crystalline solid after cooling, m.p. 164-66°C.

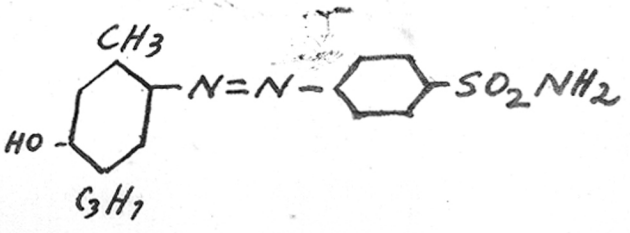
Aminohexylresorcinol being unstable, the azo dye (I) was methylated and subsequently reduced.

4-Hexyl-6(4'-sulphonamide)phenylazoresorcinol dimethyl ether (X) :

The dye (1.0 g.) was dissolved in acetone (15 c.c.) and anhydrous potassium carbonate (2.0 g.) was added, followed by addition of dimethylsulphate (0.5 c.c.). The mixture was refluxed for 12 hours, and the reaction product was poured over water, excess acetone removed, and the red precipitate filtered. The dimethoxy derivative (X) crystallised from alcohol in needles, m.p. 108°C (Found: N, 9.7. $C_{20}H_{27}N_3O_4S$ requires N, 10.9% and $C_{20}H_{27}N_3O_4S, C_2H_5OH$ requires N, 9.8%). This compound being insoluble in aqueous alkali it was reduced with zinc dust and acetic acid in alcohol. A sticky dark product separated which could not be identified.

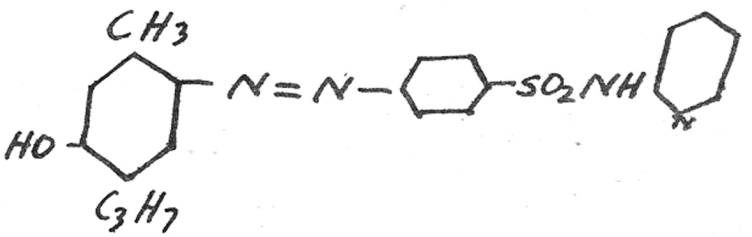
## (B) AZO DYES FROM THYMOL AND SULPHA DRUGS

p-(4-Hydroxy-5-isopropyl-2-methyl)-phenylazobenzene sulphonamide (XI) :



Sulphanilamide (3.4 g. ; 1.0 mole) was diazotised with concentrated hydrochloric acid (7.0 c.c.), sodium nitrite (1.7 g.) and water (20 c.c.) at 5.10°C. The clear diazotised solution was coupled with thymol (3.0 g.) dissolved in sodium hydroxide (9 c.c. of 30% solution), alcohol (5 c.c.) and water (50 c.c.). Coupling was carried out with vigorous stirring for 4 hours. The yellow dye (4.0 g.) obtained by acidification with dilute hydrochloric acid crystallised from acetic acid in plates, m.p. 205°C (Found; N, 12.6 ; S, 10.2. $C_{16}H_{19}N_3SO_3$ requires N, 12.7 ; S, 9.9%).

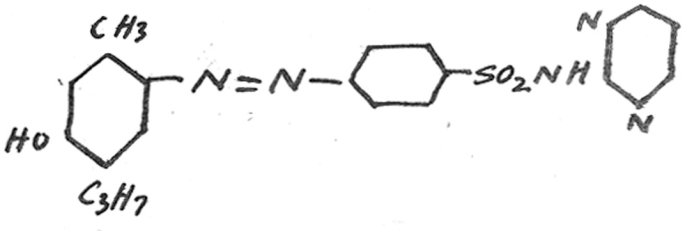
p-(4-hydroxy-5-isopropyl-2-methyl)phenylazobenzene sulphanilyl-2'-aminopyridine (XII)




Sulphapyridine (5.0 g.) was diazotised with concentrated hydrochloric acid (10 c.c.), sodium nitrite (3.5 g.), and water (40 c.c.). The diazotised solution was added gradually to a solution of thymol (3.0 g.) in sodium hydroxide (10 c.c. of 50%) and water (80 c.c.), and stirred at 5-10°. The azo dye was precipitated with dilute hydrochloric acid and the yellow precipitate (8.0 g.)

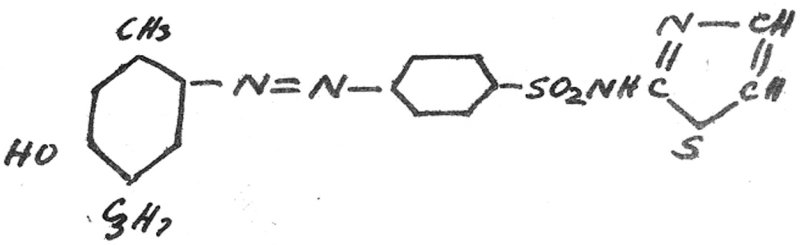
obtained crystallised from aqueous acetic acid in needles, m.p. 222-25°C (Found: N, 13.2; S, 8.0. $C_{21}H_{21}N_4SO_3$ requires N, 13.2 ; S, 7.7%).

p-(4-hydroxy-5-isopropyl-2-methyl)phenylazobenzene sulphanilyl-2'-aminopyridine (XIII) :



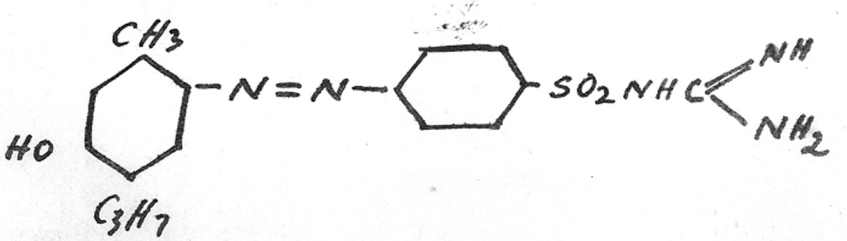

Sulphadiazine (2.55 g.) was diazotised with concentrated hydrochloric acid (3.0 c.c.), sodium nitrite (1.8 g.) and water (100 c.c.). The diazotised solution was coupled with thymol (1.5 g.) in sodium hydroxide (5 c.c. of 50%) and water (40 c.c.) for 4 hours. The dye was precipitated with dilute hydrochloric acid and yellow coloured product obtained was filtered and washed free from acid (3.8 g.). The dye crystallised from aqueous acetic acid in needles, m.p. 132-33°C) (Found: N, 16.2 ; S, 8.0. $C_{20}H_{21}N_5SO_3$ requires N, 17.0 ; S,7.6%).

p-(4-hydroxy-5-isopropyl-2-methyl)-phenylazobenzene-sulphanilyl-2'-aminothiazole (XIV)



Sulphathiazole (2.75 g. ; 1.0 mole) was diazotised with concentrated hydrochloric acid (5.0 c.c.), sodium nitrite (1.8 g.) and water (20 c.c.). The diazotised solution was coupled with thymol (1.5 g.) dissolved in sodium hydroxide (5 c.c. of 50%) and water (20 c.c.) as above. The dye was precipitated with dilute hydrochloric acid and yellowish-brown precipitate was filtered, washed free from acid (3.5 g.), and crystallised from aqueous acetone in bunches of needles, m.p. 170-72°C (Found: N, 14.2 ; S, 15.8. $C_{19}H_{20}N_4S_2O_3$ requires N, 13.5 ; S, 15.3 %).

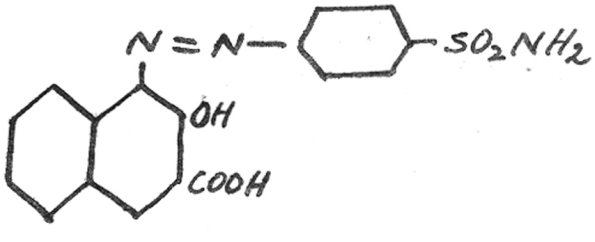
p-(4-Hydroxy-5-isopropyl-2-methyl)-phenylazobenzene-sulphanilylguanidine (XV) :



Sulphaguanidine (4.2 g.) was diazotised with concentrated hydrochloric acid (10.0 c.c.), sodium nitrite (3.6 g.) and water (25 c.c.). The clear diazotised solution was coupled with ice-cold solution of thymol (1.5 g.) dissolved in sodium hydroxide (5 c.c. of 50%) and water (20 c.c.). The dye was precipitated with dilute hydrochloric acid and the yellow solid obtained was filtered and washed free from acid (6.0 g.). The dye crystallised from aqueous acetic acid in needles, m.p. 260-63°C(Found: N, 18.8 ; S, 8.5. $C_{17}H_{21}N_5SO_3$ requires N, 19.1 ; S,8.0%).

## (C) AZO DYES FROM SULPHANILAMIDE AND "NAPHTHOLS".

Monoazo dye from 2-hydroxy-3-naphthoic acid (XVI) :

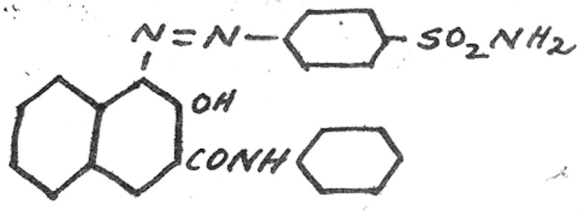



Sulphanilamide (5.25 g. ; 1.0 mole) was diazotised with concentrated hydrochloric acid (6.5 c.c.), sodium nitrite (2.0 g.) and water (25 c.c.). The clear diazotised solution was poured into a solution of 2-hydroxy-3-naphthoic acid (5.75 g ; 1.0 mole), alcohol (10 c.c.), sodium hydroxide (10 c.c. of 50%) and water (90 c.c.) and

the coupling was carried out under vigorous stirring for 4 hours in ice-bath. The azo dye was precipitated with dilute hydrochloric acid (8.0 g.).

The azo dye was sparingly soluble in acetic acid, alcohol, chlorobenzene, and soluble in pyridine and nitrobenzene. It crystallised from distilled nitrobenzene in needles, m.p. 310-11°C (Found: N, 11.0 ; S, 9.55. $C_{16}H_{13}N_3SO_4$ requires N, 11.2 ; S, 9.0%).

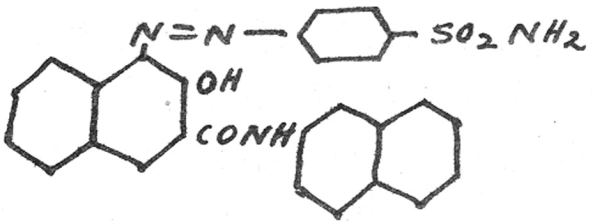
Monoazo dye from Naphthol AS (XVII):




Sulphanilamide (3.4 g.; 1.0 mole) was diazotised as above and the clear diazotised solution was coupled with a solution of Naphthol AS (5.2 g. ; 1.0 mole) dissolved in alcohol (100 c.c.), sodium hydroxide (10 c.c.) of 50%) and water (100 c.c.) as in the previous experiment. The dye separated as a brick-red precipitate (8.0 g.).

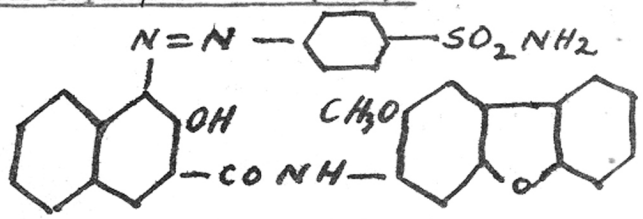
The azo dye was sparingly soluble in alkali, acids, alcohol, acetic acid, and chlorobenzene. It crystallised from aqueous pyridine in needles, m.p. 310-11°C (Found: N, 10.5 ; S, 7.8. $C_{23}H_{18}N_4SO_4$ requires N, 9.7; S, 7.2%).

Azo dye from Naphthol AS-SW (XVIII) :




Sulphanilamide (3.44 g.) was diazotised as in the above experiment, and the diazotised solution coupled with Naphthol AS-SW (6.25 g.) dissolved in sodium hydroxide (10 c.c. of 50%), alcohol (5 c.c.) and water (100 c.c.). The azo dye (9.5 g.) which was sparingly soluble in alkali, acids, alcohol and acetic acid, was crystallised from pyridine in needles, m.p. 305-07°C (Found: N, 11.0 ; S, 6.9. $C_{27}H_{20}N_4SO_4$ requires N, 11.2 ; S, 6.5%).

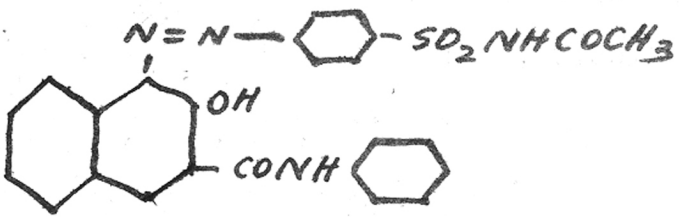
Azo dye from Naphthol AS-S (XIX) :




Sulphanilamide (1.72 g. ; 1.0 mol.) was diazotised as above and coupled with Naphthol AS-S (5.5 g.) dissolved in sodium hydroxide (10 c.c. of 10%) and water (100 c.c.) in the usual manner. A brick-red dye was obtained (6.0 g.). The dye was sparingly soluble in alkali, acids,

alcohol, chlorobenzene and acetone; it was, however, soluble in pyridine and nitrobenzene. It crystallised from nitrobenzene in long needles, m.p. 358-60°C (Found: N, 10.1 ; S, 6.1. $C_{20}H_{23}N_4SO_5$ requires N, 10.1 ; S,5.8%).

Azo dye from N'-Acetylsulphanilamide and Naphthol AS (XX):




N'-Acetylsulphanilamide (4.3 g.; 1.0 mole) was diazotised with concentrated hydrochloric acid (6.0 c.c.), sodium nitrite (1.5 g.) and water (30 c.c.). The clear diazotised solution was coupled with an ice-cold solution of Naphthol AS (5.2 g.; 1.0 mole) dissolved in sodium hydroxide (10 c.c. of 50%), alcohol (5 c.c.) and water (100 c.c.), in the usual manner. The alkali soluble dye was precipitated with dilute hydrochloric acid (8.5 g.) and crystallised from pyridine in needles, m.p.270-71°C (Found: N, 11.1 ; S, 6.8. $C_{25}H_{20}N_4SO_4$ requires N, 11.5; S, 6.5%).

# R E F E R E N C E S.


1. Gelmo, J. pr. Chem., 1908, 77, 369-283.

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## Part IV.

# FATTY ACID DERIVATIVES OF MAGENTA P.S. AND PARAROSANILINE.

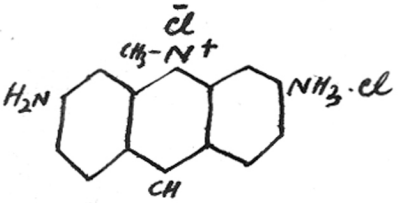
# INTRODUCTION.

It was known as early as 1891 by the work of Koch and Uffelman that certain dyes had the power to prevent the growth of bacteria. The credit for laying the foundation of chemotherapy is, however, largely due to -- Ehrlich (1912) who observed that some dyes could stain certain bacteria, and conceived the idea that these compounds would destroy the parasite which they stain, without injury to the hosts. In the same year Churchman[1] found that gentian violet was more toxic to the Gram-positive organisms, and Browning and Gilmour showed that specific affinity for certain bacteria was shown by fuchsin, brilliant green, gentian violet, methylene blue, and congo red. It was found that most of the coal-tar dyes were in varying amount toxic to the tissues. The toxicity of these dyes has been studied by Hueker and Lee[2], who examined a large number of medicinal dyes by injecting them into the udders of cows, and then comparing the physical condition of the udder and the appearance and chemical composition of the milk. They were able by these tests to compare the penetrative as well as the bacteriostatic action of these dyes. It may be of interest to give a brief survey of the important medicinal dyes.

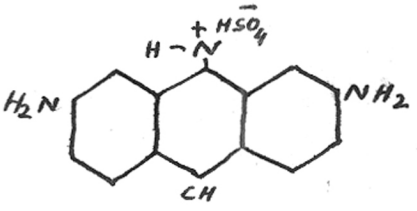
## (A) ACRIDINE DYES.

The therapeutic property of acridine dyes was first

observed by Ehrlich, who noticed that they were effective



Acriflavin.

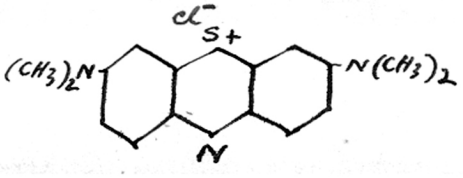


against some trypanosomes, and the available data indicate that they have valuable bacteriostatic properties. It has been found that they are more effective in broth than in serum of the same pH; alkalis like sodium carbonate increase the efficiency of these dyes. Acriflavin is used as a urinary antiseptic. Mitchell and Buttle[3] have suggested the application of dry powdered Proflavin to wounds instead of aqueous solution.

## (B) AZO DYES.

The medicinal azo dyes are mostly dyes derived from β-naphthol. Examples are scarlet red, scarlet red sulphonate and dimazon. Pyridium (3-phenylazo-2:6-diaminopyridine monohydrochloride) is used as a genito urinary antiseptic. Prontosil is another example of this class of dyes.

## (C) THIAZINE DYES.



Methylene blue was one of the first of the dyes to be used medicinally, finding employment as a urinary antiseptic (Einhoven, 1891). Ehrlich observed that methylene blue rapidly stained the plasmodia of malaria and suggested its use in the chemotherapy of this disease. In 1933, Ryrie[4] observed the selective action of methylene blue and employed intravenous injections of the drug in man, but found no improvement. The French workers Montel et al.[5] on the other hand claimed remarkable success with this dye. There is again conflicting opinion regarding the effect of this dye on Myco. tuberculosis. It has been found to penetrate the tubercle bacilli and exert a definite bacteriostatic action, but there is yet no proof of its bactericidal and therapeutic efficacy.

## (D) TRIPHENYLMETHANE DYES (ROSANILINE DYES)

This group includes malachite green, brilliant green, gentian violet, crystal violet, fuchsin and pararosaniline.

Malachite green is used as a wound antiseptic in about 1:1000 solutions and for the treatment of ulcers. Brilliant green, the tetraethyl analogue of malachite green, is also used for antiseptic washes and dressings.

The rosaniline dyes are bactericidal against Gram-positive organisms in dilutions of 1:1000,000. They are

particularly effective against staphylococci, B.diphtheriae, and B.pyocyaneus. The acid-fast bacteria , though they take the stain, are little affected. The Gram-negative bacteria are very resistant to the rosaniline dyes.

FACTORS GOVERNING THE ACTION OF MEDICINAL DYES.

(a) Electrostatic character : The selective action of most of these medicinal dyes against bacteria is closely related to the iso-electric point of the constituent cell. McCalla[6] explains that staining of bacteria is an exchange reaction, the staining replacing similar charged ions already adsorbed by the cells. For Gram-positive cells, the iso-electric point centres around pH 2-3, while for Gram-negative cells it centres around pH 5.0. Accordingly, antiseptic dyes are divided into two groups. The electro-positive dyes, such as basic dyes, have a special affinity for the Gram-positive organisms, while the electro-negative dyes, such as acid dyes, are more active against Gram-negative organisms. The former is more active in basic medium, while the latter is more active in acid medium.

(b) Hydrogen ion concentration : Hydrogen ion concentration has pronounced effect on the action of dyes. Thus Browning, Gulbransen and Kennaway (1920) reported that the effect of diamino-acridine methyl chloride is enhanced

more than hundred-fold by changing the pH from 4 to pH 11. Stearn and Stearn[7] (1926) have shown the effect of pH on the toxicity of the dyes. They found that with acid dyes, the growth was most inhibited in the most acid medium while in case of basic dyes, the reverse was noted.

(c) Effect of temperature : Cameron (1930) observed that most coal-tar dyes are more bacteriostatic at lower temperatures than at higher temperatures.

## BACTERIOSTATIC PROPERTY AND SURFACE-ACTIVITY.

In the case of acid fast bacteria such as those of tuberculosis and leprosy, it is very probable that if substances which are known to exercise favourable action are modified in their chemical constitution by the introduction of structural features which lead to surface-activity, solubility in fats and fat-solvents, ability to lower the interfacial tension between aqueous systems and oils, and the general properties of colloidal electrolytes, they would be preferentially absorbed by the waxy or lipoid cell-walls of bacteria, and would penetrate them with a resultant increase in the cumulative effect. An affinity or substantivity for the cuticle of the parasite has been considered by Ehrlich to be a requisite for -- compound with chemotherapeutic activity ; a parallel in

a different field is the substantivity of wetting agents and detergents in textile processing which may be regarded as a desirable property.[8] The advantages of Naphthols with fatty attributes have been explained by Ramachandran,[9] and dyes with long chain alkyl and aryl radicals have been the subject of many patents (c.f. Siedel and Engelfried.[10] ; I.C.I. and du Pont patents on dyes typified by the Carbolan Colours of I.C.I.). Bergmann and Haskelberg[11] have drawn attention to the disinfectant potency of quarternary ammonium compounds like Cetab and Roccal containing long chain alkyl groups. The same authors also studied the influence of lipophilic acyl radicals on the toxicological and chemotherapeutical property of Atoxyl (Sodium-N-acetylarsanilate). In view of the probability that some combination of chaulmoogric acid with sulphanilamide might penetrate the tubercle of, and be effective against tuberculosis, and on account of the inaccessibility of chaulmoogric acid, Crossley et al.[12] prepared $N^1$-acyl-sulphanilamides and $N^1$:$N^4$-diacylsulphanilamides using other long chain fatty acids. The sodium salts of the former, as would be anticipated, were freely water-soluble and had the characteristics of soaps ; the acyl derivatives were themselves soluble in fats and fat solvents. Pharmacological tests indicated that $N^1$-dodecyl-sulphanilamide was effective against infections of β-haemolytic streptococci

and arrested the spread of tuberculosis in cavies.

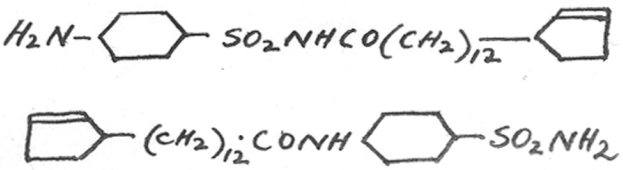
The conflicting claims put forward as regards the efficacy of methylene blue in combating leprosy have already been mentioned. Certain other dyes have been also used to some extent against leprosy. Leger[13] found Trypaflavin of value, but Schwerz[14] failed to obtain any improvement with this dye. Montel et al.[15] found that phenolsulphothalein localised in the leprous nodules and produced some improvement. Grimes et al.[16] claim that gentian violet can be used with advantage. The evidence as regards the ability of certain dyes to stain and therefore destroy the lepromata selectively is still of a controversial nature, and further research in this field is desirable.. In vitro leprocidal activity of some non-chaulmoogryl compounds has been studied by Emerson and Salle.[17] Triphenylmethane dyes represented a group of highly active compounds, but they suggested a comprehensive pharmacloogical evaluation of these compounds before they could be clinically utilised. Fischer[18] studied the effect of triphenylmethane leuco bases on bacteriostasis produced by dyes and observed that malachite green, gentian violet and their carbinol bases have bacteriostatic effect of the same order, but that their leuco bases have no appreciable bacteriostatic effect ;

in fact, when applied simultaneously, they inhibit the bacteriostatic action of dyes and carbinol bases. Fuchsin, however, behaved differently. Its leuco base was bacteriostatic, and it did not inhibit the action of the dyes or of their carbinol bases.

It has already been stated that Gram-negative bacteria are very resistant to rosaniline dyes. The acid fast bacteria could be stained, but are little affected. Acid fast bacteria like those of tuberculosis or leprosy are known to be enveloped by a waxy coating and the possibility that surface-activity might render a drug more effective against such organisms has also been mentioned. It is well known that sulphanilamides are feebly active against myco-bacterial diseases, and this is probably due to the outer "waxy" layer of the organism which prevents the access of the drug into the interior of the organism. Efforts have been made by many workers to make drugs lipophilic so that they could penetrate the myco-bacteria. Haskelberg et al[19] have prepared lipophilic derivatives of therapeutically active dyes like 4-benzeneazo-1-naphthylamine or its 2-isomer by acylation with fatty acids. These modified dyestuffs do not appear to have been tested against myco-bacterial diseases, although they were expected to give promising results.

As already mentioned Crossley et al.[12] have --

prepared lipophilic derivatives from sulphanilamide by introducing fatty acyl groups in the $N^4$-position and observed that only $N^4$-Dodecanoylsulphanilamide was found to be active against experimental tuberculosis in guinea pigs. Steenbach and Duca[20], Muschenheim[21], and Arnold[22] on the other hand have not confirmed this observation. Rajagopalan[23] also synthesised a series of $N^4$-acylsulphanilamides from several fatty acids, but has not reported their physiological activity. Wagner-Jauregg (quoted by Arnold)[24] used chaulmoogric acid to obtain lipophilic derivatives of sulphanilamide, but they have not been --

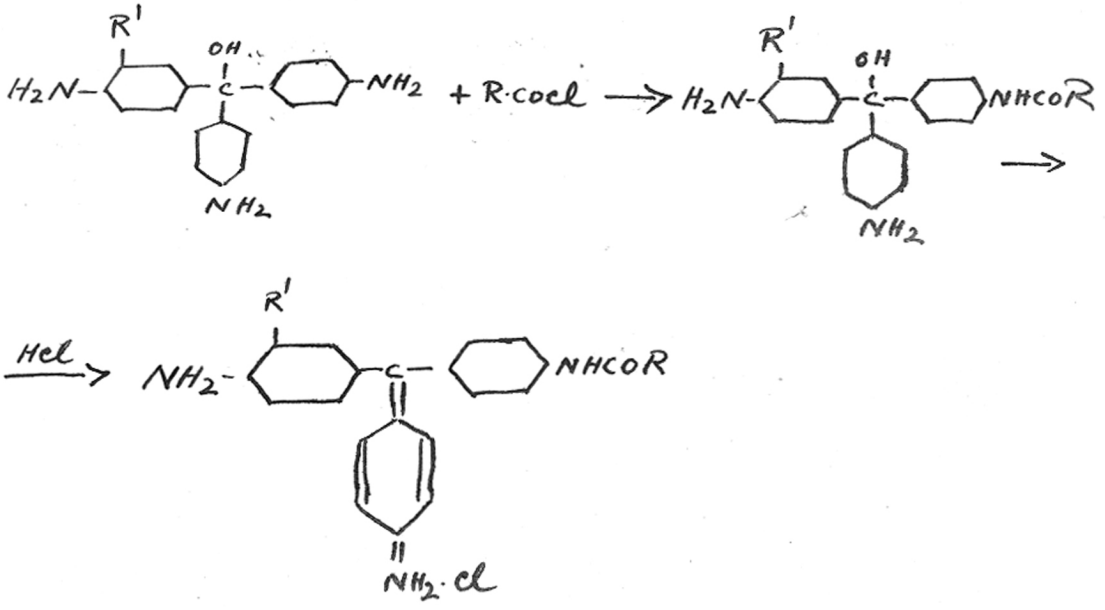


tested so far against myco-bacterial diseases. Lloyd and Middlebrook[25] (1944) prepared $N^1$-$N^4$-dichaulmoogryl derivative of p-p'-diaminodiphenyl-sulphone and examined it for its *in vitro* activity against tubercle bacilli with negative results.

## PRESENT WORK

With a view to prepare lipophilic derivatives of triphenylmethane dyes and make them active against acid-fast bacteria, fatty acid derivatives of Magenta and

Pararosaniline have been prepared by acylation with a series of fatty acids. The benzoyl derivative of pararosaniline was prepared for comparison. Starting from the carbinol base derived from the dyes, one amino group was acylated by condensing with one mole of acid chloride in presence of solvent naphtha. The carbinol base of the monoacyl derivative was then converted to the dye by treatment with the calculated amount of hydrochloric acid.




where $R' = CH_3$ and $R = n\cdot C_3H_7$ (I); $n\cdot C_5H_{11}$ (III); $n\cdot C_{11}H_{23}$ (V).

where $R' = H$ and $R = n\text{-}C_3H_7$ (VIII); $n\text{-}C_5H_{11}$ (IX); $CH_3\cdot(CH_2)_3\cdot\underset{C_2H_5}{CH}$ (XI);

$n = CH_3\cdot(CH_2)_7\text{-}CH=CH\cdot(CH_2)_7\text{-}CO-$ (XV); $C_6H_5$ (XVII).

$n\cdot C_{11}H_{23}$ (XIII)

The fatty acids used were butyric acid, caproic acid, 2-ethylhexoic acid, oleic acid and lauric acid.

Careful examination in this laboratory of the properties of a series of soaps has demonstrated the outstanding value of the dodecyl member of the fatty acid series. Ruggli and Braun[26] associate the amido group with substantivity. Very frequent occurrence of the amido group in wetting agents has been mentioned by Shirolkar and Venkataraman.[27] In an earlier work Uppal and Venkataraman[28] have drawn conclusions regarding the relationship between chemical constitution and wetting power. They condensed a series of saturated fatty acids (caproic to stearic) with sulphanilic acid to study the effect of the length of the alkyl chain and found that solubility in water decreased with increase in C-atoms, but the Herbig Number rose continually up to $C_{12}$ (lauric acid derivative) and became practically constant when the alkyl chain was further increased. Shirolkar and Venkataraman[27] also studied the effect of the C-alkyl chain in lauryl-m-sodium sulphonate and observed that the wetting power and protective colloidal action increased with increase in the length of the fatty acid chain, being optimum with the lauric acid.

The fatty acid derivatives of magenta and pararosaniline were soluble in water, the solubility decreasing

with the increase in the length of the alkyl chain. All the fatty acid derivatives have been found to be insoluble in fats like cocoanut oil and fat-solvents like light-petroleum (60-80°) and solvent naphtha.

In vitro bacteriostatic tests were carried out on the acyl derivatives, using broth medium of the following composition: Peptone (1%), Beef-extract (0.4%) and sodium chloride (0.5%). pH was controlled at 7.6 (Phenol red) and incubation temperature kept at 37°C. Growth was observed every 24 hours. It was found that introduction of long acyl chains decreased the activity. The parent dyes inhibited the growth of Staphylococcus aureus in dilutions of 1:1000,000, whereas the acyl derivatives inhibited the growth at 1:100,000. Against E.coli and E. typhosus, the acyl derivatives had no activity.

It has been reported[29] that triphenylmethane dyes have marked anthelminthic action. Hence the acyl derivatives now prepared were also examined for their anthelminthic activity and compared with the parent dyes, magenta and pararosaniline. It was found that the acyl derivatives were superior in anthelminthic action to the parent dyes. Here also the anthelminthic action increased with the increase in the number of carbon atoms of the fatty acid chain (Tables I and II). The anthelmintic activity of the acyl derivatives compared favourably with the values

obtained for some standard anthelminthics like carbon tetrachloride, thymol, β-naphthol and santonin (Table III). The anthelminthic action was determined in terms of the time required to kill 6 earth worms and that required to kill half the number (Tables I, II and III). Anthelminthic activity of all the acyl derivatives prepared was more than santonin. In addition to being more active than santonin, the lauryl derivatives (V) and (XIII) were also more active than carbon tetrachloride and thymol.

The acyl derivatives dyed wool and tannin mordanted cotton, but gave shades which were inferior in light fastness as compared to the parent dyes.

To see whether the introduction of long acyl chains imparted wetting power to the parent dyes, the acyl derivatives were tested by the Herbig Number technique but showed no improvement over the parent dyes. The Herbig Number was 12-14 (Herbig Number for water 18.4). The wetting power of the acyl derivatives of Magenta was also compared in terms of their ability to lower interfacial tension, employing the Gardner and Holdt's surface tension meter. By this method the "drop No." (the number of drops formed by a definite volume of liquid) was obtained which gave the measure of the --

interfacial tension as the latter is inversely proportional to the "drop No.". Judged by the drop-number technique, the acyl derivatives had better wetting power than the parent dye, Magenta. The wetting power increased with the increase in the alkyl chain, the lauryl derivative (V) being the most efficient of the three.

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# EXPERIMENTAL.

## FATTY ACID DERIVATIVES OF MAGENTA P.S.

Butyroyl derivative of Magenta P.S. (I) :

Magenta base (6.1 g. ; 1.0 mole) was acylated with freshly prepared butyroyl chloride (2.1 g. ; 1.0 mole) in solvent naphtha (50 c.c.). The mixture was heated for 6 hours in an oil bath at 150-160°C. The mixture was cooled and the product obtained was filtered and washed free from unreacted butyric acid. Yield (7.0 g.). The dye (I) crystallised from alcohol in needles. High melting point. (Found: N, 10.5 ; Cl, 8.9. $C_{24}H_{24}N_3OCl$ requires N, 10.37 ; Cl, 8.67%).

The corresponding carbinol base (II) obtained by treating the aqueous solution of the dye with dilute sodium hydroxide ; the base was difficult to crystallise. (Found: N, 11.3. $C_{24}H_{27}O_3N_3$ requires N, 11.2%).

Caproyl derivative of Magenta P.S. (III) :

Magenta base (6.1 g. ; 1.0 mol.) was acylated with freshly prepared caproyl chloride (2.7 g. ; 1.1 mole) as in the previous experiment. The product obtained after cooling the reaction mixture was thoroughly agitated with ether to remove traces of caproic acid. The green lustrous powder was crystallised from alcohol, when (III) separated in needles (7.0 g.) (Found: N, 9.8 ; Cl, 7.9. $C_{26}H_{30}N_3OCl$ requires N, 9.4 ; Cl, 8.1%).

The corresponding carbinol base (IV) was precipitated from the aqueous solution of the dye, with dilute sodium hydroxide. The high melting base was difficult to crystallise, and was therefore, purified by reprecipitation with sodium hydroxide (Found: N, 10.3. $C_{26}H_{32}N_2O_2$ requires N, 10.0%).

Lauroyl derivative of Magenta P.S. (V) :

Magenta base (6.1 g.) was suspended in solvent naphtha (50 c.c.) and freshly prepared lauroyl chloride (4.5 g. ; 1.1 mole) was slowly added with shaking. The mixture was heated for 6 hours in an oil bath at 150-160°C The mixture was cooled, filtered and the residue obtained was agitated with light petroleum (60-80) to remove traces of lauric acid, filtered and dried. Yield (5.0 g.) (I). The green shining powder was crystallised from alcohol when (I) separated in prisms, m.p. above 360°C. (Found: N, 8.65 ; Cl, 7.43. $C_{32}H_{42}N_3O.Cl$ requires N, 8.84; Cl, 7.8%).

The dye was dissolved in water and the corresponding carbinol base (VI) precipitated by dilute sodium hydroxide (10%). The high melting base was difficult to crystallise, and was, therefore, purified by precipitation from its solution with sodium hydroxide (Found: N, 8.5. $C_{32}H_{43}N_3O_2$ requires N, 8.3%).

## FATTY ACID DERIVATIVES OF PARAROSANILINE.

Butyroyl derivative of Pararosaniline (VIII) :

Pararosaniline base (6.05 g. ; 1.0 mole) was acylated with freshly prepared butyroyl chloride (2.1 g.; 1.1 mole) as in (I). After the green dye was filtered and washed free from traces of butyric acid with ether (7.5 g.), it was dissolved in water and precipitated with dilute sodium hydroxide. The carbinol base (VII) was difficult to crystallise, and was, therefore, purified by reprecipitation. (Found: N, 10.8. $C_{23}H_{25}N_3O_2$ requires N, 11.2%).

The base was converted into the hydrochlorine by pasting it with required quantity of concentrated hydrochloric acid and evaporating to dryness on a water bath. The dye (VIII) crystallised from dilute alcohol in -- needles (Found: N, 10.7 ; Cl, 9.25. $C_{23}H_{24}N_3O.Cl$ requires N, 10.6 ; Cl, 9.0%).

Caproyl derivative of Pararosaniline dye (IX) :

Pararosaniline carbinol base (6.0 g. ; 1.0 mole) was acylated with freshly prepared caproyl chloride (2.7 g. ; 1.1 mole) as in (I) and the reaction product was filtered and washed free from traces of caproic acid. The greenish crystalline powder was soluble in alcohol and water, and after recrystallisation from hot water (IX) gave needles (Found: N, 10.1 ; Cl, 8.45. $C_{25}H_{28}N_3$

O.Cl requires N, 10.0 ; Cl, 8.41 %).

The corresponding carbinol base (X) which was precipitated from the aqueous solution with dilute sodium hydroxide was difficult to crystallise (Found: N, 10.7. $C_{25}H_{29}N_3O_2$ requires N, 10.4 %).

2-Ethylhexoic acid derivative of Pararosaniline dye (XI):

Pararosaniline carbinol base (6.0 g. ; 1.0 mole) was acylated with freshly prepared 2-ethylhexoyl chloride (3.3 g. ; 1.1 mole), and the product obtained after cooling was filtered and washed with ether to remove traces of the decomposed 2-ethylhexoic acid. Yield (8.0 g.). The dye was dissolved in water and the carbinol base precipitated with dilute sodium hydroxide. The base was reconverted into the dye by pasting it with required quantity of concentrated hydrochloric acid and taking the mixture to dryness on water bath. Dark green lustrous powder of the dye (XI) was recrystallised from alcohol in needles (Found: N, 9.5; Cl, 8.0. $C_{27}H_{33}N_3O.Cl$ requires N, 9.35 ; Cl, 7.8%).

The carbinol base (XII) was obtained by treating the aqueous solution of the pure dye with dilute sodium hydroxide (10%). The base (XII) was difficult to crystallise, and was purified by precipitation as before. (Found: N, 10.1. $C_{27}H_{33}N_3O_2$ requires N, 9.8%).

Lauroyl derivative of Pararosaniline dye (XIII) :

Pararosaniline base (6.0 g.; 1.0 mole) was acylated with freshly prepared lauroyl chloride (4.5 g.; 1.1 mole) as in (I). The dye obtained after cooling was agitated with light petroleum (60-80) to remove traces of lauric acid, filtered and dried (110°C). The sticky product obtained solidified after pasting with benzene and evaporating on water bath. The dye (XIII) was difficult to crystallise. It gave an amorphous shining powder from dilute alcohol (Found: N, 8.8 ; Cl, 8.1. $C_{31}H_{40}N_3O.Cl$ requires N, 8.4 ; Cl, 7.9%).

The carbinol base (XIV) was obtained by treating the purified dye solution with dilute sodium hydroxide (10%). The base was difficult to crystallise (Found: N, 9.4. $C_{31}H_{41}N_3O_2$ requires N, 8.8%).

Oleyl derivative of Pararosaniline (XV) :

Pararosaniline carbinol base (2.0 g. ; 1.0 mole) was acylated with freshly prepared oleyl chloride (2.1 g.; 1 mol.). The reaction mixture after cooling gave a green coloured dye (XV). It was agitated with ether, filtered and the green shining powder (2.5 g.) crystallised from hot water in needles (Found: N, 6.55 ; Cl, 5.6. $C_{37}H_{51}N_3OCl$ requires N, 6.35 ; Cl, 5.35).

The carbinol base (XVI) was obtained by treating

the dye solution with dilute sodium hydroxide (Found: N, 7.85. $C_{37}H_{52}N_3O_2$ requires N, 7.35%).

Benzoyl derivative of Pararosaniline (XVII) :

Pararosaniline base (3.05 g.; 1.0 mole) was dissolved in toluene (30 c.c.) and acylated with benzoyl chloride (1.5 g.) by heating under reflux for 6 hours at 120-30°C. The dye which was obtained on cooling (3.8 g.) was dissolved in water and the carbinol base was precipitated with dilute sodium hydroxide. The base was filtered, washed free from alkali and was re-converted into dye by treatment with hydrochloric acid in the usual way. The dye (XVII) crystallised from alcohol in needles (Found: N, 9.8 ; Cl, 8.1. $C_{28}H_{23}N_3OCl$ requires N, 9.8 ; Cl, 8.25%).

The carbinol base (XVIII) was obtained by treatment of the pure dye solution with dilute sodium hydroxide (10%). (Found: N, 10.7. $C_{26}H_{24}N_3O_2$ requires N,10.2%).

The acyl derivatives prepared above and the parent dyes were examined for their anthelminthic action as follows:

The dye was dissolved in minimum quantity of N/10 sodium hydroxide and then diluted to 250 c.c. Six worms were placed in the solution. After the worms became

motionless, they were transferred to fresh tap water to make sure that they were really dead and showed no signs of recovery. Time required to kill half the number of worms and that required to kill all the worms was also noted. The results are recorded in Tables I and II.

From the two tables, it is clear that acyl derivatives of magenta and pararosaniline are superior in their anthelmintic action as compared to the parent dyes, the activity increasing with the increase in carbon atoms of the fatty acid chain. The acyl derivatives could be well compared with standard anthelminthic like carbon tetrachloride, thymol, and β-naphthol, and Santonin, the values of which are recorded in Table III.

[ P.T.O. for Tables I, II and III ]

## Table I

### Anthelmintic action of Magenta P.S. and its acyl derivatives.

| Substance. | % composition | Time in minutes required to kill 6 worms. | Time required to kill 3 worms | Minimum time taken when one of the two worms was found dead after 24 hours when placed in tap water. | Minimum time taken when both the worms removed were found dead after 24 hrs. |
|---|---|---|---|---|---|
| Magenta P.S. | 0.1 | 80 | 70 mts. | 65 mts. | 65 mts. |
| | 0.05 | 110 | 90 " | 80 " | 80 " |
| | 0.025 | 135 | 125 " | 110 " | 120 " |
| | 0.01 | 160 | 145 " | 140 " | 140 " |
| Butyryl derivative | 0.1 | 70 | 60 " | 50 " | 55 " |
| | 0.05 | 95 | 85 " | 70 " | 80 " |
| | 0.025 | 110 | 100 " | 90 " | 90 " |
| | 0.01 | 145 | 135 " | 125 " | 130 " |

Table I (Contd.)

| Substance. | % composition. | Time in minutes required to kill 6 worms | Time required to kill 3 worms. | Minimum time taken when one of the two worms was found dead after 24 hours when placed in tap water. | Minimum time taken when both the worms removed were found dead after 24 hrs. |
|---|---|---|---|---|---|
| Caproyl derivative | 0.1 | 55 | 45 mts. | 40 mts. | 45 mts. |
| | 0.05 | 75 | 60 " | 55 " | 55 " |
| | 0.025 | 95 | 85 " | 75 " | 80 " |
| | 0.01 | 130 | 110 " | 100 " | 105 |
| Lauryl derivative | 0.1 | 45 | 35 " | 30 " | 30 " |
| | 0.05 | 65 | 55 " | 45 " | 50 " |
| | 0.025 | 80 | 70 " | 60 " | 65 " |
| | 0.01 | 115 | 95 " | 85 " | 90 " |

## Table II

Anthelmintic action of Pararosaniline ~~hydrochloride~~ and its acyl derivatives.

| Substance | % Composition. | Time required to kill 6 worms | Time required to kill 3 worms | Minimum time when one of the two worms removed was found dead after 24 hrs. in tap water. | Minimum time when both the worms removed were found dead after 24 hrs. |
|---|---|---|---|---|---|
| Pararosaniline. ~~Hydrochloride~~ | 0.1 | 85 mts. | 72 mts. | 60 mts. | 65 mts. |
| | 0.05 | 115 " | 90 " | 80 " | 80 " |
| | 0.025 | 140 " | 130 " | 110 " | 125 " |
| | 0.01 | 170 " | 145 " | 145 " | 140 " |
| Butyryl derivative | 0.1 | 75 " | 60 " | 50 " | 55 " |
| | 0.05 | 100 " | 95 " | 75 " | 80 " |
| | 0.025 | 110 " | 100 " | 80 " | 90 " |
| | 0.01 | 150 " | 140 " | 135 " | 127 " |

Table II (Contd.)

| Substance. | % composition | Time required to kill 6 worms | Time required to kill 3 worms | Minimum time when one of the two worms removed was found dead after 24 hrs. in tap water. | Minimum time taken when -- both the worms removed were found dead after 24 hrs. |
|---|---|---|---|---|---|
| Caproyl derivative | 0.1 | 60 mts. | 52 mts. | 43 mts. | 40 mts. |
| | 0.05 | 73 " | 70 " | 56 " | 50 " |
| | 0.025 | 96 " | 84 " | 78 " | 82 " |
| | 0.01 | 142 " | 130 " | 110 " | 105 " |
| Lauryl derivative | 0.1 | 40 " | 30 " | 25 " | 30 " |
| | 0.05 | 65 " | 55 " | 55 " | 45 " |
| | 0.025 | 85 " | 70 " | 62 " | 60 " |
| | 0.01 | 120 " | 95 " | 80 " | 90 " |

Table III

| Substance | % solution. | Time taken to kill 6 worms. | Time taken to kill 3 worms |
|---|---|---|---|
| Carbon tetrachloride | 0.1 | 30 mts. | 25 mts. |
| | 0.05 | 50 " | 40 " |
| | 0.025 | 65 " | 50 " |
| | 0.01 | 75 " | 65 " |
| Thymol | 0.1 | 50 " | 40 " |
| | 0.05 | 60 " | 50 " |
| | 0.025 | 70 " | 60 " |
| | 0.01 | 80 " | 70 " |
| β-Naphthol | 0.1 | 20 " | 15 " |
| | 0.05 | 30 " | 20 " |
| | 0.025 | 38 " | 30 " |
| | 0.01 | 48 " | 30 " |
| Santonin | 0.1 | 130 " | 115 " |
| | 0.05 | 165 " | 145 " |
| | 0.025 | 190 " | 170 " |
| | 0.01 | over 4 hrs. | over 4 hours. |

It was expected that introduction of the long chain acyl group may impart surface activity to Magenta PS and pararosaniline. The following determinations were thus made to determine the comparative surface-activity of the dyes and their acyl derivatives.

Determination of Wetting Power : Herbig Number :

The Herbig number tests were carried out in the modified Herbig apparatus (Forster, Uppal and Venkataraman, J.Soc. Dyers Col., 1938, 54, 465). This apparatus is a

modification of the Evan's apparatus (ibid., 1935, 5, 233). The Herbig machine consists of a screwed spindle which is attached to the main frame of the machine through an axle in such a way that it can be rotated in a vertical position, when centrifuging the yarn. On the top of the -- spindle a hook of steel is attached by means of nuts. The spindle rotate by means of a mechanical friction clutch, one component of which is coupled to a single phase A.C. 1/8 H.P. motor with a reversing switch.

To maintain a constant number of revolutions of the spindle during centrifuging, there is a nut which moves along the length of the spindle either up or down. At one end of the nut there is a projection which, when the nut is moving up , pushes a knob of the switch which is attached to the main frame at the top. This breaks the circuit of the electric motor and the spindle stops. When the spindle is in this position, a hank is attached to the hook of the spindle and the main switch of the electric motor is switched off, as the switch at the top cuts off the electric current only when the knob is pressed. The lower component of the friction clutch is then detatched by means of a handle attached to it and is kept in this position by means of an iron strip. This iron strip is attached to another strip of iron (fitted to the main frame by means of a pin so that it can move in the vertical

phase only). The lower end of the strip holds the clutch in position, which the top end is kept pulled by means of a spring attached to the main frame. After the clutch is detached, the spindle is brought to a horizontal position by means of a lever, so that the hank (attached to the hook) can dip in the solution to be tested. After the hank has been dipped for the requisite length of time, it is brought out of the solution, the weight attached to the hank removed, and the motor started. This time the motor should rotate in a direction which will bring the nut moving along the spindle down. The spindle is then pressed into the vertical position which pushes in iron strip holding the lower component of the friction clutch, and the spindle starts rotating. The spindle is automatically stopped by means of the strip attached to the moving nut which disengages the lower component of the clutch by pushing it down. The main switch is then switched off.

In carrying out the test as described by Evans (loc cit.)approximately 0.5 g. of hanks of 20's grey yarn, free from size and conditioned at room temperature were used. A 10 g. weight was employed to keep the yarn weighted in the solution which was contained in a test tube, care being taken to ensure complete immersion of the whole length of the yarn. In all cases, the period of

immersion was 30 seconds. Five hanks were used for each test, and three such readings were taken in each case as these were generally found to lead to a representative average result. The "Herbig Number" is the weight of liquor absorbed by 100 g. of the textile material.

The Herbig Number tests were carried in the modified Herbig apparatus with 0.25, 0.15 and 0.10% solutions of the dyes at 30°C. The results are recorded in Table IV.

Table IV.

Herbig Numbers at 30°C

| Compound | % concentration of solutions. | | |
|---|---|---|---|
| | 0.25 | 0.15 | 0.10 |
| Magenta | 11.5 | 12.0 | 11.0 |
| I | 12.1 | 11.5 | 11.2 |
| III | 14.0 | 12.8 | 12.1 |
| V | 12.8 | 13.3 | 14.2 |
| Pararosaniline | 10.6 | 11.0 | 11.2 |
| VIII | 11.7 | 12.4 | 11.9 |
| IX | 10.9 | 10.3 | 11.3 |
| XI | 12.9 | 12.0 | 11.4 |
| XIII | 10.8 | 11.5 | 12.7 |
| XV | 10.3 | 11.7 | 11.6 |
| XVII | 10.1 | 9.5 | 9.7 |

On the basis of the above values it appears that introduction of long fatty acid chains in Magenta and Pararosaniline did not impart wetting power to these dyes.

The comparative surface-activities of the above dyes and their acyl derivatives were also determined by interfacial tension measurements of aqueous solutions of the compounds against kerosene. A 5 c.c. Gardner and Holdt surface tension was used for the purpose as follows:

The apparatus was filled with dye solution to the mark and fixed exactly vertical with the lower end dipping in 80 c.c. of dry kerosene in a 100 c.c. beaker. The rate at which drops issued from the tip was regulated, so as not to allow too rapid a formation of drops. The rate of drops was kept constant at 120-130 seconds for 100 drops. The temperature, the depth of immersion of the capillary tip was kept constant and the beaker was always filled to the same height and the instrument rested on the bottom of the beaker. The number of drops formed in allowing 5 c.c. of the dye solution to flow out of the kerosene was noted. The number of drops formed by a definite volume of liquid being inversely proportional to the interfacial tension, the greater the number of drops, the less the interfacial tension and hence greater the wetting power of the compound. The results of the tests for derivatives from Magenta PS are given in Table V.

Table V.

| Compound | % concentration. | Drop No. | Drop rate in seconds |
|---|---|---|---|
| Magenta P.S. | 0.05 | 75 | 130 |
| | 0.1 | 95 | 128 |
| | 0.25 | 183 | 130 |
| I | 0.05 | 97 | 125 |
| | 0.1 | 160 | 126 |
| | 0.25 | 220 | 128 |
| III | 0.05 | 100 | 125 |
| | 0.1 | 163 | 126 |
| | 0.25 | 198 | 128 |
| V | 0.05 | 120 | 130 |
| | 0.1 | 258 | 127 |
| | 0.25 | 320 | 125 |

The above values show that acylation of long fatty acids increased the surface-activity of Magenta P.S., the surface-activity increasing with the increase in the length of the fatty acid chain.

Bacteriostatic Tests :

Magenta P.S., Pararosaniline and their acyl derivatives were tested in vitro for bacteriostatic activity as

the culture media prepared from Beef-extract (0.4%), Peptone (1%) and sodium chloride (0.5%). The pH of the solution was adjusted to 7.6 (Phenol-Red) and after sterilizing, the solutions were inoculated with Staphylococcus aureus, Escherichia coli and E.Typhosus. The minimum effective concentration which prevented the growth upto 72 hours was determined. The results are recorded in Tables VI and VII.

The results show that as against expected improvement in bacteriostatic activity, the acyl derivatives were actually less active than the parent dyes.

[ P.T.O. for Tables VI and VII ]

## Table VII

Bacteriostatic activity of pararosaniline hydrochloride and its acyl derivatives.

Staphylococeus Aureus, 18 hr., 37°C, pH. 7.6

| | Pararosaniline hydrochloride | | | VIII | | | IX | | | XI | | | XIII | | | XV | | | XVII | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hrs. | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 16 | - | - | - | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + |
| 24 | - | - | - | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + |
| 48 | - | - | - | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + |
| 72 | - | - | - | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + |

E. Coli and E. Typhosus 18 hr. 37°C pH.7.6

| | Pararosaniline hydrochloride | | | | VIII | | | | IX | | | | XI | | | | XIII | | | | XI | | | | XVII | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hrs. | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 16 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 24 | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 48 | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 72 | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

Dilutions : Same as in Magenta P.S. T.

## Table VI

Bacteriostatic activity of Magenta PS and its acyl derivatives.

Staphlococeus Auseus, 18 hr. culture, pH 7.6 37°C.

| Compound | Magenta | | | I | | | III | | | V | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hrs. | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 16 | - | - | - | - | - | + | - | - | + | - | - | + |
| 24 | - | - | - | - | - | + | - | - | + | - | - | + |
| 48 | - | - | - | - | - | + | - | - | + | - | - | + |
| 72 | - | - | - | - | - | + | - | - | + | - | - | + |

where 1 = 1:10,000
2 = 1:100,000
3 = 1:1000,000

Escherichia Coli and E.Typhosus 18 hr. culture, pH. 7.6, 32°C

| Compound | Magenta | | | | I | | | | III | | | | V | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hrs. | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 16 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 24 | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 48 | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 72 | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

where 1 = 1 : 500
2 = 1 : 1000
3 = 1 : 2000
4 = 1 : 5000

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