BACTERIAL CHEMOTHERAPY

V. Synthesis of Phenolic Azo-dyes derived from the Sulphonamides

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The effective chemotherapy of many bacterial infections with the sulphonamide group of drugs which has been possible in recent times owes its origin to the introduction of an azo-dye, prontosil in clinical practice by Domagk. While very different explanations for the ways in which the various azo-dyes in therapeutic use exert their antiseptic action have been advanced, opinion concerning the mode of action of the azo-dyes derived from the sulphonamides, of which prontosil is one, is at the present time almost unanimous. Although by no means fully proved, it is generally believed that the therapeutic properties of the sulphonamido azo-dyes are primarily the therapeutic properties of the sulphanilamide itself, which has resulted from reductive cleavage of the azo-linkage in vivo and this explanation, to a great extent, the increasing disuse of the prontosil types in the treatment of acute bacterial infections.

It should be remembered that in addition to the sulphonamides resulting from these azo-dyes, amino phenols or aromatic polyamines are also at the same time liberated by the in vivo cleavage at the azo double bonds. It is, therefore, of interest to enquire whether or not the latter contribute in any manner to the observed final physiological effect. Previous knowledge of biological oxidations and the detoxication mechanisms of amino phenols, aromatic polyamines and the anti-haemorrhagic compounds would suggest the possibility of production in vivo of quinonoid bodies from the "second ring" not bearing the sulphonamidino group. This assumes great interest because many natural and synthetic quinones have recently come to be recognised as powerful antibacterial agents and many quinones, in addition to their occurrence in some bacteria, are known to constitute essential growth factors for many types of bacteria. Furthermore, the observed influence of other compounds on the detoxication of the sulphonamides themselves and the phenomenon of synergism of action of the sulphonamides...
and azochloramide, urea or some purines, which is finding increasing application in actual practice, would appear to have a bearing on enquiries directed towards the mode of action of the sulphonamido azo-dyes. Such enquiries would appear to be somewhat justified in view of past literature on the sulphonamido Schiff’s bases; the Schiff’s bases derived from the sulphonamides are hydrolysed, after oral administration, into the component parts; the possibility of the “aldehyde half” of the molecule or its derivative elaborated in vivo exerting a modifying influence on the chemotherapeutic efficacy of the active half has already been indicated in an earlier paper. That the second ring in azo-dyes may well be of significance was indicated by Gley and Girard’s finding that whereas molecular equivalents of prontosil and sulphanilamide were of equal therapeutic value in experimental streptococcal infections, the carboxy derivative of prontosil, which could liberate no more sulphanilamide than prontosil, was twice as effective, therapeutically, as prontosil. The possibility envisaged by Gley and Girard may be construed to receive support from the activities reported for the azo-dyes derived from sulphanilamide and resorcinol, 3:5-diamino-benzoic acid, p-hydroxy phenyl glycine and histidine, pyrrole and indole and some purines; prontosil and neoprontosil may also probably be included in this group.

A scrutiny of the existing literature on the sulphonamido azo-dyes brings to light many curious generalities. For instance, while quite a considerable number of dyes derived from sulphanilamide itself is known, a comparatively smaller number of their heterocyclic analogues has been disclosed. Again, as against the basic dyes, very few phenolic dyes have, so far, been revealed. Furthermore, only a small proportion of either the basic or acidic dyes have been studied in detail in respect of their activity and their detoxication mechanisms. That a study of the sulphonamido azo-dyes need not be considered entirely fruitless is supported by the use of other dyes still in medicine and the notable success achieved recently by Congo red in even the sulphonamide-resistant bacterial infections.

For the reasons stated above, it was therefore, considered worthwhile to synthesise a series of azo-dyes derived on the one hand, from sulphonamides which have already earned a definite place in chemotherapy and on the other, from mono- and poly-hydric phenolic compounds. In the hope that useful information likely to lead to a better understanding of the evolution of future chemotherapeutics could be gained, it was further planned to investigate first their usefulness in experimental bacterial infections and later, the mechanism of action of the active members. The phenolic dyes which were prepared in this connection are represented in the table.
<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Name</th>
<th>Nitrogen percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>2:4-Dihydroxy, (p-sulphamyl phenyl azo-) benzene</td>
<td>Found: 13.8</td>
</tr>
<tr>
<td>100</td>
<td>2:4-Dihydroxy, 1:5-bis (p-sulphamyl phenyl azo-) benzene</td>
<td>Required: 14.3</td>
</tr>
<tr>
<td>101</td>
<td>4-Hydroxy, (p-sulphamyl phenyl azo-) naphthalene</td>
<td>Found: 17.4</td>
</tr>
<tr>
<td>102</td>
<td>3-Hydroxy, 4 (p-sulphamyl phenyl azo-) phenanthrene</td>
<td>Required: 17.7</td>
</tr>
<tr>
<td>103</td>
<td>2-Methyl, 4-hydroxy, 5-isopropyl, (p-sulphamyl phenyl azo-) benzene</td>
<td>Found: 12.3</td>
</tr>
<tr>
<td>104</td>
<td>2:3:4-Trihydroxy, (p-sulphamyl phenyl azo-) benzene</td>
<td>Required: 12.8</td>
</tr>
<tr>
<td>105</td>
<td>2:4-Dihydroxy, (p-sulphoguanidyl phenyl azo-) benzene</td>
<td>Found: 11.3</td>
</tr>
<tr>
<td>106</td>
<td>2:4-Dihydroxy, (p-N(^2), 2'-pyridyl sulphamyl phenyl azo-) benzene</td>
<td>Required: 11.1</td>
</tr>
<tr>
<td>107</td>
<td>2:4-Dihydroxy, (p-N(^2), 2'-thiazolyl phenyl azo-) benzene</td>
<td>Found: 14.8</td>
</tr>
<tr>
<td>108</td>
<td>2:4-Dihydroxy, (p-N(^2), 2'-thiazolinyl sulphamyl phenyl azo-) benzene</td>
<td>Required: 15.1</td>
</tr>
<tr>
<td>109</td>
<td>2:4-Dihydroxy, 5 (p-N(^2), 2'-thiazolyl sulphamyl phenyl azo-) quinoline</td>
<td>Found: 14.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Required: 14.8</td>
</tr>
</tbody>
</table>
Two compounds listed in the table have previously been reported but in insufficient detail and were therefore included with the object of a comparative and detailed study in animal experiments: the compounds are No. 99 and No. 103. Four of the dyes (Nos. 106-109) have, so far, been subjected by Dr. B. B. Dikshit to preliminary tests in experimental *Pasteurella pestis* infections in mice. Although the results obtained were somewhat interesting, none of them exhibited an activity greater than that of sulpha-thiazole, the control used in comparative bio-assays of the efficacies of new sulphanilamide derivatives in this Institute.

**Experimental**

The dyes, Nos. 99-104, 106-109 were all prepared by the following general procedure which is illustrated by the preparation of the compound, No. 99. A solution of sulphanilamide (17.2 g., 1 mol.) in sodium hydroxide (4 g. in 100 c.c. of water), cooled to 15°, was treated with a solution of sodium nitrite (7.4 g. in 20 c.c. of water; 1.08 mol.) and the resulting solution poured with stirring on to a mixture of concentrated hydrochloric acid (21.2 c.c. of D, 1.18; 2.5 mols.) and crushed ice (120 g.). The diazonium salt mixture, after standing 15-20 minutes, was then added with good stirring to a solution of resorcinol (11 g., 1 mol.) in sodium hydroxide (20 g. in 80 c.c. of water; 5 mols.) which had previously been cooled to about 0-5° C. by addition of ice (80 g.). The resulting solution was allowed to stand at ordinary temperature for one half hour, filtered and acidified with hydrochloric acid when the dye separated out as a voluminous precipitate.

For the compound, No. 105, a slightly different process was adopted. This consisted in the direct diazotisation of a dilute hydrochloric acid solution of sulphaguanidine itself by means of sodium nitrite followed by coupling with resorcinol in alkaline medium on the same lines as has been worked out for the other azo-dyes.

The attempts to crystallise the dyes from the usual organic solvents or mixtures of them did not meet with success. Resort was therefore had, for purposes of purification, to repeated fractional precipitation of the dyes from their aqueous alkaline solutions (Norite) by acetic acid, the first and final fractions being rejected. The dyes after washing with water and drying in an air-oven, were then obtained as powders without any definite melting points or crystalline natures but possessing one or other grades of redness.

The yields of the pure dye-stuffs should, in all the instances, be considered good since they average from 75-90 per cent. of the theoretical.
The author wishes to express his gratefulness to Col. S. S. Sokhey for his kind interest in this investigation and to the Lady Tata Memorial Trust for the award of a Research Scholarship.

Summary

The synthesis of a series of phenolic azo-dyes derived from the sulphonamides with the object of exploring their therapeutic potentialities and of gaining an insight into their mode of action, has been effected and reported.

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