BACTERIAL CHEMOTHERAPY

IV. Synthesis of N^4, N^1-Diacyl Sulphanilamides*

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Received January 3, 1944

(Communicated by Lt.-Col. S. S. Sokhey, M.A., M.D., I.M.S., F.A.SC.)

THAT sulphanilamide acts by blocking the enzyme concerned in the utilisation of *para*-aminobenzoic acid (PAB), an "essential metabolite" in the bacterial cells of the susceptible organisms, by virtue of its structural resemblance to PAB, was first postulated by Fildes and Woods.[2,3] This concept has been extended,[4] in recent times, to cover the other well-known sulphonamides also: the varying potencies of the latter have been explained by the additional effect of the heterocyclic halves of their molecules on other metabolic processes peculiar to or more important to the particular bacterium under consideration.[5,6] In addition the hypothesis of Fildes and Woods receives its strongest support from the work of McIlwain and his collaborators, who by a study of the nutritional requirements of bacteria both in the presence and absence of antibacterial agents, were able to lay down successfully rules regarding structural specifications required for drug action in possible chemotherapeuticals.[7,8,9]

Despite the proved adequacy of the Fildes-Woods theory of the mechanism of action of the sulphonamides, yet another theory, namely, the Acidic Dissociation Theory has been recently formulated by Schmelkes[10] and Fox and Rose.[11] According to these authors, considering the present theory as only a slight elaboration of the original concept of Fildes and Woods and the non-specificity of sulphonamide action observed by other workers,[12] the sulphonamides act by virtue of the similarities of their ions $R \cdot S\langle^{O}_{O} -NH-$ to the $R \cdot C\langle^{O}_{O-}$ of PAB. They also state that the anions of the sulphonamide *acids*, rather than the cations or the undissociated acids, exert the bactericidal action. In support, they adduce evidence to show that the minimal amount of each drug (ionised drug) needed for bacteriostasis is approximately the same, irrespective of the test organisms used.

* A preliminary note on a few compounds reported in this communication appeared in *Current Science*.[1]

Although the acidic dissociation theory appears to open up new aspects of chemotherapeutical possibilities, exceptions can be taken to the theory in its present form. For instance, the hypothesis is founded on the classical conception of acids and consequently lays emphasis on acidic dissociation of the sulphonamides as an essential prerequisite for the antibacterial activity of the latter. On this basis it is difficult to explain the activity of the sulphonamides in which both the hydrogen atoms at the $-SO_2.NH_2$ have been substituted and which in all probability are unlikely to give rise *in vivo* to ionisable derivatives: examples of this class of compounds are the sulphanilyl derivatives of dimethyl amine,[13,14] diethylamine,[13] diethanolamine[15,13] and, of the α-pyridyl, α-thiazolyl and α-thiazolinyl methylamines.[17] In contrast to the Fildes-Woods theory which is based on the structural specificity of the sulphonamide types, the acidic dissociation theory maintains that the heterocyclic halves of the sulphonamides contribute only to a greater capacity of the molecules for acidic dissociation. However, conception of the sulphonamides as acids in the more comprehensive, modern sense, postulated by Brönsted[18] and Lowry,[19] helps to remove the above and other apparent anomalies in the acidic dissociation theory which, it will be remembered, arise from the interpretation of acids in the classical restricted sense by the sponsors of the theory.

The acidic dissociation theory suggests the possibility that if sulphonamides could be rendered more "acidic" by suitable means more potent chemotherapeuticals than those hitherto available could be obtained. It also raises the question whether by transformation of the sulphanilamide molecule itself compounds of the same acidity and the same degree of activity as the most powerful sulphonamides now known cannot be prepared. It appeared that these objects could be achieved by the incorporation of acyl radicals at the sulphonamide part of the molecule instead of the more difficult task of introducing heterocyclic ring systems at the N^1-position of sulphanilamide. A search of the literature reveals that many compounds of the N^1-acyl sulphanilamide type have already been disclosed.[16,28] Although they have not been investigated at length with the exception of "albucid", many of the straight chain derivatives and the N^1-isocyclicacyl sulphanilamides are stated to be, weight for weight, as good as sulphanilamide in experimental streptococcal infections. On an equimolecular basis, these derivatives—some contain less than fifty per cent. of sulphanilamide—were definitely superior to sulphanilamide. These results are parallel to those obtained with some the higher straight chain N^4-acyl sulphonamides,[20] which include a few therapeutically valuable members.[21] The case of albucid is remarkable: recent studies[22] have revealed it to possess not only a low order of toxicity

but also effectiveness, at least in some infections, in even sulphathiazole-resistant cases. Interesting data of a far-reaching nature may therefore be expected to accrue by a detailed study of members of the N^1-acyl sulphanilamide group and by attempting correlation of the degree of dissociation of the N^1-acyl sulphanilamides with their activity in experimental infections.

The author's interest in the N^4-, N^1-diacyl sulphanilamides was stimulated by the earlier attempt[23] to render the sulphonamides mycobactericidal by the further introduction of fatty acid groupings in their molecules. The present communication deals with the synthesis of a series of N^4, N^1-diacyl derivatives of sulphanilamide which are listed in the table.

Serial No.	Name	M.P./°C.	Nitrogen percentage	
			Found	Required
42	N^4-*n*, Caproyl, N^1-acetyl sulphanilamide ..	166–69 dec.	8·8	9·0
43	N^4-*n*, Caproyl, N^1-*n*, butyryl sulphanilamide ..	164–68	8·2	8·2
44	N^4, N^1-Di (*n*, caproyl) sulphanilamide ..	164–72	7·9	7·6
45	N^4-*n*, Caproyl, N^1-*n*, heptoyl sulphanilamide ..	148–52	7·7	7·3
46	N^4-*n*, Caproyl, N^1-palmityl sulphanilamide ..	123–26	5·6	5·5
47	N^4-*n*, Caproyl, N^1-stearyl sulphanilamide ..	127–30	5·0	5·2
48	N^4, N^1-Di (*n*, butyryl-) sulphanilamide ..	217–20	8·7	9·0
49	N^4, N^1-Di (*n*, heptoyl-) sulphanilamide ..	131–34	7·6	7·1
50	N^4-*n*, Caproyl, N^1-benzoylsulphanilamide ..	180–83	8·0	7·5
51	N^4-*n*, Caproyl, N^1-cyclohexoylsulphanilamide ..	185–87	7·3	7·3
52	N^4-*n*, Capryol, N^1-cinnamoyl sulphanilamide ..	228–31	7·1	7·0
53	N^4-*n*, Caproyl, N^1-(*a*, naphthoyl-) sulphanilamide	154–57	6·8	6·5
54	N^4-*n*, Caproyl, N^1-(*m*, nitrobenzoyl-) sulphanilamide	173–78	10·1	10·4
55	N^4-*n*, Caproyl, N^1-(*p*, nitrobenzoyl-) sulphanilamide	222–30	10·0	10·4
56	N^4, N^1-Dibenzoyl sulphanilamide	239–40	..	..
57	N^4, N^1-Dicyclohexoyl sulphanilamide	248–50	7·0	7·1
58	N^4, N^1-Dicinnamoyl sulphanilamide	216–18	6·8	6·5
59	N^4, N^1-Di (*p*-nitrobenzoyl-) sulphanilamide ..	251 dec.	11·7	11·9
60	N^4, N^1-Difuroyl sulphanilamide	255 dec.	7·8	7·7
72	N^4, N^1-Di (*p*-nitrobenzoyl-) sulphapyridine ..	232–34 dec.	13·0	12·9

From the standpoint of both the acidic dissociation and the Fildes-Woods theories, the presence of the grouping $H_2N \cdot C_6H_4 \cdot SO_2 \cdot NH$ in the sulphonamide molecules is considered to be of fundamental physiological significance.[3,5,11,24,25] A stable structural alteration in this unit has been observed to be accompanied by a destruction of therapeutic activity and is comprehensible in the light of the finding that similar permanent alterations in the molecule of PAB depress or nullify[3,2] the growth-promoting action of PAB.[37] The synthesis of N^4, N^1-diacyl sulphanilamides may, perhaps, be considered as unproductive of interesting results on the ground that the significant, free amino group is not present in their molecules.

It may, therefore, be well, at this stage, to state the theoretical considerations for undertaking the synthesis of N^4, N^1-diacyl sulphanilamides. In the synthesis of this type of compounds, in addition to the possibility of their proving mycobactericidal, a primary consideration was the relative ease with which they could be prepared as compared to the strictly N^1-acyl derivatives of sulphanilamide. Considering the smoothness with which some of the N^4-acyclic acyl sulphanilamides are cleaved to sulphanilamide[24,27] and the facility with which the N^4-acyl group is split off from N^4, N^1-diacyl sulphanilamides,[28] the N^4-acyclic acyl, N^1-substituted sulphanilamides (Nos. 42–55) will, in all probability, give rise *in vivo* to the respective N^1-acyl sulphanilamides. It was, therefore, of particular interest to ascertain whether or not the observed therapeutic effect of this class of compounds may not after all be that of the N^1-acyl sulphanilamides themselves, modified or otherwise by the presence of the fatty acids simultaneously liberated. Such an enquiry was expected to lead to a study of the detoxication mechanisms involved in the passage of the active members through the animal body both in a state of health and disease. Again, the synthesis of the N^4, N^1-isocyclic acyl sulphanilamides (Nos. 56–72) needs explanation. It is true that the lack of activity of the N^4-isocyclic or heterocyclic acyl sulphonamides, hitherto reported, is generally understood to bedue to the inability of experimental animals to hydrolyse aroyl or heteroöyl amide linkages.[16] Nevertheless, there are a few instances of this kind of N^4-substituted sulphanilamides, for example, N^4-furoyl sulphapyridine[29] and the N^4-nicotinyl,[16,29,30] N^4-quinolinyl,[31] and N^4-(5, pyrrolidone-2, carbonyl-[32]) and the N^1, 4′-(*p*-anisylidene-) or the N^1, 4′-(*p*-dimethylaminobenzylidene-) aminophenyl, N^4- acetyl sulphanilamides,[16] whose activity is consequently surprising and has not yet been plausibly explained. In the contemplation of the synthesis of the N^4, N^1-diisocyclic acyl sulphanilamides, it was encouraging to find that a further substitution of the acyl group at the sulphonamide portion, even though exemplified so far in the isolated case of N^4, N^1-dibenzoyl sulphanilamide,[33] is sufficient to render active an otherwise inert N^4-acyl sulphanilamide.[34] Unfortunately, an explanation for the activity of dibenzoyl sulphanilamide founded on experimental data has not yet been offered by the original investigators. Such an explanation would no doubt have a important bearing on the Fildes-Woods theory of sulphonamide action which lays emphasis on the role of a free or potential amino group in conditioning the physiological action of the sulphonamides. A study of the compounds, Nos. 56–72 was, for obvious reasons, expected to answer ultimately the question of whether or not the activity of N^4, N^1-dibenzoyl sulphanilamide is the consequence of an inherent characteristic of this type

of compounds. The case of the *p*-nitrobenzoyl derivatives (Nos. 55, 59, 72) deserves mention. Judging from analogy,[35] they are to be expected to be reduced *in vivo* to the corresponding amino bodies; these latter, besides probably fulfilling the requisites for high anionic dissociation, would then represent compounds bearing a structural resemblance to the bacterial "essential metabolite", PAB different from the type obtaining in other sulphonamides.

Whether any or all of the expectations of this study will be realised can be settled only by actual biological trials. It is however reasonable to hope that even if no highly antibacterial agents are encountered in the group of compounds now synthesised, the present study would be atleast instrumental in shedding additional and useful light on the existing theories* of the mechanism of action of the sulphonamides.

Experimental

For the N^4-*n*, caproyl, N^1-substituted derivatives (Nos. 42, 43, 45, 46, 47, 50–55) of sulphanilamide. N^4-*n*, caproyl sulphanilamide — reported[34] to be as antistreptococcal as sulphanilamide itself, but much less toxic— constituted the starting material: the condensations with the desired acid chlorides were carried out in pyridine medium. The remaining N^4, N^1-diacyl sulphanilamides (Nos. 44, 48, 49, 56–60) resulted directly by the operation of slightly more than 2 mols. of the appropriate acid chlorides on sulphanilamide itself in pyridine solution.

The condensation products, obtained by dilution of the reaction mixtures with excess of water, were severally purified usually through precipitation from their dilute sodium hydroxide solutions (charcoal) by acidification. They were mostly recrystallised from alcohol, the exceptions being Nos. 53, 56, 57 and 60 which were crystallised from acetone and No. 59 which was obtained from nitrobenzene. All these compounds (Nos. 42–60) were obtained as colourless needles.

The disubstituted pyridine derivative (No. 72) was prepared by similar action of 2 mols. of *p*-nitrobenzoyl chloride on sulphapyridine in the presence of pyridine. It separated from acetone as colourless plates.

The yields of the compounds reported in this paper were, in all instances, good.

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* While this MS. was under preparation, there has appeared an interesting paper by Bell and Roblin,[36] admirably correlating ionic dissociation with activities of the sulphonamide types.

The melting points of many of the compounds listed are not at all sharp in spite of their many recrystallisations and homogeneous crystalline structure: this appears to be an inherent characteristic, as has previously been observed[28] in the case of the N^1-acyl sulphanilamide derivatives.

I desire to take this opportunity of expressing my indebtedness to Col. S. S. Sokhey but for whose kind interest and encouragement this investigation would not have been possible. I am also thankful to the Lady Tata Memorial Trust for the award of a Scholarship.

Summary

With the object of exploring the group of N^4, N^1-diacyl sulphanilamides for practicable antibacterial agents and in view of the possibility of shedding further light on the existing theories of the mode of sulphonamide action by a study of the N^4, N^1-diacyl sulphanilamide type of compounds, a number of N^4, N^1-diacyl sulphanilamides, twenty in all, have so far been synthesised.

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