CHEMOTHERAPY OF BACTERIAL INFECTIONS

Part VII. Synthesis of Sulphanilamide Derivatives of the Pyrimidine Group

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In a previous publication, one of us reported that the guanidine radical in sulphanilylguanidine does not undergo condensation with the β-ketonic esters, β-diketones and α:β-unsaturated ketones as readily as guanidine or amidines. Further studies of these reactions showed that, in some cases, such a condensation could be effected in the presence of sodium ethoxide and this paper presents the results of these investigations.

Sulphanilylguanidine* condensed with ethyl acetoacetate in the presence of sodium ethoxide to yield a compound now identified to be 2-sulphanilamido-4-methylpyrimidone† (vide infra). This compound could also be prepared by condensing acetsulphanilylguanidine with ethyl acetoacetate and hydrolysing the corresponding acetsulphanilamido derivative obtained with about 4 N hydrochloric acid. Similarly, α-methyl, ethyl, n-butyl, iso-amyl and n-hexyl derivatives of ethyl acetoacetate condensed with sulphanilylguanidine yielding the corresponding 2-sulphanilamido-4-methyl-5-alkylpyrimidones in varying yields. These compounds as a class were soluble in alkali, very stable and exhibited the properties of the pyrimidones. In these condensations, only one product could be isolated in each case. Under these conditions, sulphanilylguanidine failed to condense with ethyl malonate and ethyl cyanoacetate.

For sulphanilylguanidine, two structures (I) and (II) are possible and it is very difficult from purely chemical methods to decide unequivocally

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* Just before the foot-note in the paper of White et al., establishing the identity of sulphanilylguanidine with the product obtained by Buttle et al. by the condensation of sulphanilamide with dicyandiamide, came to our notice, we also had come to the same conclusion not only by comparing the two compounds as such, but also the products obtained from them on condensation with ethyl acetoacetate.

† All the compounds described as pyrimidones in this paper can have the tautomeric 6-hydroxypyrimidine structure as well.
between the two. Basing on the facts that the compound does not dissolve in alkali and does not condense with the \( \beta \)-ketonic esters as readily as the amidines, the structure (II) was suggested by one of us as more probable; but the limitations of this argument are obvious. For the compound obtained by condensing sulphanilylguanidine with ethyl acetoacetate, which hereafter will for brevity be referred to by our serial number 76, four structures (III), (IV), (V) and (VI) (where \( R = H \)) are possible on the basis of the two formulæ (I) and (II) for sulphanilylguanidine. The formulæ (III) and (IV) can be considered to be tautomeric but there is no information as to the influence of the sulphanilyl group on the stabilising effect of the tautomeric forms. Since the compound 76 is soluble in alkali, the formula (VI) is to be rejected because a compound of this structure should be insoluble in alkali. A definite decision between the other possible formulæ (III), (IV) and (V) appeared to be possible by specific hydrolysis of the compound 76 at the imino or amino group attached to the carbon atom 2 of the pyrimidone ring for, by such a process, the compounds of formulæ (III) or (IV) should yield 4-methyluracil while that of formula (V) should furnish 3-sulphanilyl-4-methyluracil. But such a hydrolysis could not be effected. On boiling with about 30\% sodium hydroxide for 6 hours, the compound 76 was recovered unchanged. While a little of it was decomposed by boiling with 4 N hydrochloric acid for about 2 hours, it underwent almost complete hydrolysis yielding sulphanilic acid and 2-amino-4-methyl-pyrimidine on refluxing with concentrated hydrochloric acid for about 6 hours. The isolation of 4-methyl-2-aminopyrimidine definitely proves that the compound 76 contains the 4-methylpyrimidine radical. The hydrolysis of the sulphanilamido derivatives with concentrated hydrochloric acid in many instances is known to cause fission at the sulphur-nitrogen linkage yielding the corresponding amine and sulphanilic acid\(^6,7\); but a compound of formula (V)
could be expected to be hydrolysed even with 4-6 N hydrochloric acid. For instance, it has been observed that N-acetsulphanilylimidazole on boiling with 15% hydrochloric acid for half an hour yields imidazole and sulphanilic acid. So, we are of opinion that the results of the hydrolysis of the compound 76 suggests the rejection of the formula (V) for it. As a supplementary evidence it may be added that the pyrimidone derivative (76 Ac.) obtained by condensing acetsulphanilylguanidine with ethyl acetoacetate (which on hydrolysis yielded the compound 76) is insoluble in dilute hydrochloric acid indicating the absence of the free imino grouping.

The other tangible difference between the three formulæ for the compound 76 is that in (III) there are two hydrogen atoms replaceable by sodium while in (IV) and (V) there is only one. When titrated with N/20 sodium hydroxide using phenolphthalein as the indicator, the compound 76 took about one molecular equivalent of alkali; but 2-sulphanilamido-4-methylpyrimidine and also 2-amino-4-methylpyrimidone under the same conditions took far less than one molecular equivalent of sodium hydroxide though both of these substances dissolved freely in alkali. Next, the compound of formula (III) should on methylation yield a dimethyl derivative insoluble in alkali and/or a monomethyl derivative soluble in alkali, while the compounds of formulæ (IV) and (V) should yield only a monomethyl derivative insoluble in alkali. The methylation of the compound 76 with dimethyl sulphate in alkaline medium was therefore tried. Under a particular set of conditions, a compound insoluble in alkali was obtained which analysed correctly for a dimethyl derivative and this, from all the evidences taken together, can be represented as 2-sulphanilylmethylamido-1:4-dimethylpyrimidone (VII). In addition, a product, which so far could not be obtained as a sharp melting one, was also isolated from the methylation products; this was soluble in alkali and appeared to be a mixture of the two possible isomeric monomethyl derivatives (VIII) and (IX) of the compound 76. These results therefore support the structure
(III, \( R = H \)) for the compound 76 and also (III, \( R = \text{alkyl} \)) for the others (compounds 101 to 104) obtained by condensing sulphanilyl guanidine with the \( \alpha \)-alkyl acetoacetates.*

Attempts are being made to convert the \( N^4 \)-acetyl derivative of the compounds 76 and 99 into the known 2-sulphanilamido-pyrimidines through the intermediate chloro-compound according to the standard procedure. On treating this \( N^4 \)-acetyl derivative (76 Ac.) with phosphorus oxychloride according to the usual procedure, a chloro-compound not melting below 280° was obtained; this was soluble in alkali (indicating the presence of the sulphonamide grouping) and insoluble in all usual organic solvents. The analytical figures agreed well for the chloro-compound of structure (X) but its properties were quite unusual. Treatment of this with zinc dust and water did not lead to any definite results. Attempts to condense 2-amino-4-methyl-6-chloropyrimidine with acetsulphanilylchloride to prepare the chloro-compound of formula (X) were unsuccessful.

To obtain a compound of the structure (III, \( R = \text{H} \)) by the converse way, acetsulphanilylchloride was condensed with 2-amino-4-methylpyrimidone. While in the presence of pyridine only dark products were obtained, the condensation proceeded in good yields in alkaline (sodium bicarbonate) medium. The product obtained was soluble in dilute hydrochloric acid and insoluble in alkali. On boiling it with 2.5 N sodium hydroxide or 4 N hydrochloric acid for a few minutes, the starting material, 2-amino-4-methylpyrimidone was obtained in good yields. Thus, we assign the structure of the sulphonate (XI) to this compound in preference to the alternative structure of \( 1 \)-acetsulphanilyl-2-imino-4-methylthiopyrimidine (acetyl derivative of VI). In support of the sulphonate structure,

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\begin{align*}
\text{Ac-NH} & \quad \text{SC-NH-C} \\
\text{N} & \quad \text{C-Me} \\
\text{H} & \quad \text{CH} \\
\text{N} & \quad \text{C-Cl} \\
\text{H}_{2}N-C & \quad \text{CH} \\
\text{N} & \quad \text{C-O-SO}_{2} \text{NHAc} \\
\end{align*}
\]

\[\text{(X)}\]

\[
\begin{align*}
\text{N} & \quad \text{C-Me} \\
\text{H}_{2}N-C & \quad \text{CH} \\
\text{Me-N} & \quad \text{CO} \\
\text{(XII)} \\
\end{align*}
\]

\[\text{XI}\]

* Note added in proof: After this paper was sent for publication, Sprague, Kissinger and Lincoln (J. Amer. Chem. Soc., 1941, 63, 3028) have described the preparation of a compound, 2-sulphanilamido-4-methyl-6-hydroxyprymidine, m.p. 253-5-4°, by condensing acetsulphanilylchloride with 2-amino-4-methyl-6-ethoxypyrimidine and then hydrolysing the product obtained. The melting point of this agrees with that of our compound 76 and we are establishing the identity of the two by mixed melting points.
it has been found that 6-hydroxy-2-amino-pyridine and also some aminophenols yield only sulphonate derivatives with acetsulphanilylchloride. In some cases of the aminophenols, it has been observed that when sodium bicarbonate was used as the condensing agent the sulphonates are formed but with sodium acetate the sulphanilamido derivatives are obtained. However, the condensation of 2-amino-4-methylpyrimidone with acetsulphanilylchloride did not proceed well in the presence of sodium acetate, the products obtained being acetsulphanilic acid and the starting amine. The sulphonate derivative (XI) could not successfully be condensed with another molecule of acetsulphanilylchloride. 2-Amino-4-methylpyrimidone was very readily methylated in alkaline medium with dimethylsulphate and the product obtained (XII) is probably identical with one of the compounds isolated by Majima by condensing methylguanidine with ethyl acetooacetate. This methyl derivative also could not be condensed with acetsulphanilylchloride to obtain a product of formula (IX). It is of interest that while the 2-amino group in the pyrimidine derivatives is very reactive, the same grouping in the pyrimidone is comparatively inert.

Ethyl formylacetate condensed with sulphanilylguanidine in the presence of sodium ethoxide and the compound obtained can now, by analogy, be represented as 2-sulphanilamidopyrimidone (XIII). Formylacetone (hydroxymethylenacetone) was subjected to a similar condensation anticipating 2-sulphanilamido-4-methylpyrimidine; but the product obtained in bad yield, even during crystallisation from water, decomposed yielding the starting sulphanilylguanidine. Hydroxymethylene methylbutylketone did not yield any appreciable amount of the condensation product with sulphanilylguanidine. These results are not unexpected since such a condensation even with guanidine proceeds only in bad yields. Other conditions are being investigated.

Mesityloxide condensed with sulphanilylguanidine to yield, in different experiments but not simultaneously, two products, with m.p. 190–93° (compound 77a) and 135° (compound 77) respectively, and the conditions under which these are produced could not be defined exactly. These two compounds were insoluble in alkali and were of the same composition. It appears that they are dimorphic and this phenomena is being observed to an increasing extent in the compounds of the sulphanilamide group. Acetsulphanilylguanidine, on the other hand, condensed with mesityloxide in the presence of sodium ethoxide to yield a mixture of two products: (1) a compound, with m.p. 238–40°, insoluble in alkali and dilute hydrochloric acid, which on hydrolysis yielded the above described amino compound, melting at 190–93° (77a) and (2) a compound with m.p. 217–18°, soluble in
alkali and insoluble in dilute hydrochloric acid, which on hydrolysis yielded another amino compound having m.p. 230° (77b) and soluble in alkali. These two amino compounds (77a) and (77b) have been provisionally represented by the two possible structures (XIV) and (XV) respectively. The identity of the latter with the one obtained by condensing 2-amino-4 : 6-trimethyl dihydropyrimidine with acetsulphanilylchloride and subsequent hydrolysis¹ is being established.

Further work on the structures of these compounds is in progress. The results of condensing sulphanilylguanidine and acetsulphanilylguanidine with β-diketones will be presented in a separate communication.

The compounds described are being tested in experimental streptococcal, pneumococcal and P. pestis infections in mice and the results will be described in detail in due course. The compounds 76, 77a and 99 (XIII) were found to be devoid of therapeutic activity.

**Experimental**

2-Sulphanilamidopyrimidone (XIII) : Compound 99.—A mixture of ethylformate (14.8 g.) and ethylacetate (17.6 g.) was added gradually to powdered sodium (4.6 g.) suspended in dry ether (200 c.c.). The reaction mixture under ice cooling was stirred for about 3 hours and then allowed to stand overnight at the room temperature. The solid cake was separated from ether and mixed with sulphanilylguanidine (15.3 g.) and a solution of sodium ethoxide prepared by dissolving sodium (4.6 g.) in absolute ethyl alcohol (150 c.c.). The whole was heated under reflux for 2–3 hours, the alcohol distilled off, the residue shaken with water and filtered. The insoluble portion was the unreacted sulphanilylguanidine and the condensation product separated from the alkaline filtrate on acidification (yield, 50–60% of theory). On crystallisation from water, it separated in yellowish needles; m.p. 268–69°. (Found: N, 21.3; C₁₀H₁₀N₄O₃S requires N, 21.1%).

2-Sulphanilamido-4-methyl-5-alkylpyrimidone (III).—The compounds of this group were prepared by the following general procedure:
To a solution of sodium ethoxide prepared by dissolving sodium (about 1.5 molecular proportions) in absolute ethyl alcohol (about 10 times the quantity of sodium) was added successively about 1 molecular proportion each of sulphanilylguanidine and then ethyl acetoacetate or its α-alkyl derivative. The mixture was refluxed for 3–8 hours. The solid usually went into solution and then a crystalline product began to separate. Most of the alcohol was distilled off and on triturating the residue with water, the unchanged sulphanilylguanidine separated and was filtered off. The condensation product was isolated by just acidifying the alkaline filtrate with acetic acid. All the compounds crystallised well from dilute acetic acid or water. They were all soluble in alkali and dilute hydrochloric acid. The solubility in water was very moderate and as a class, the melting points were not very sharp and depended upon the rate of heating.

The condensation of acetsulphanilylguanidine with ethyl acetoacetate or its alkyl derivative was carried out in the same way as described above. The acetsulphanilamido derivatives obtained were insoluble in dilute hydrochloric acid and soluble in dilute alkali. They were hydrolysed to the sulphanilamido derivatives by boiling with about 8–10 times the amount of 4 N hydrochloric acid till the solid went completely into solution (20–30 minutes) and then neutralising the clear filtered solution. By this process, the compounds obtained were identical with those prepared by the foregoing process.

The pyrimidone derivatives thus prepared are listed in the following table:

| Serial No | R = | Yield % | M.P. °C. | Molecular Formula | % Nitrogen 
|-----------|-----|---------|---------|------------------|-------------
|           |     |         |         |                  | Found       | Required |
| 76 Ac.    |     |         |         |                  |             |          |
| 76        | Hydrogen | 75  | 253–54  | C₁₁H₁₄N₄O₃S  | 20.1 | 20.0  |
| 104       | Methyl-   | 40  | 238–39  | C₁₂H₁₄N₄O₃S  | 18.9 | 19.0  |
| 100       | Ethyl-    | 70  | 208–09  | C₁₂H₁₄N₄O₃S  | 18.2 | 18.2  |
| 101       | n-Butyl-  | 30  | 121–22  | C₁₂H₁₄N₄O₃S  | 16.2 | 16.7  |
| 102       | iso-Amyl- | 20  | 190–93  | C₁₂H₁₄N₄O₃S  | 15.6 | 16.0  |
| 103       | n-Hexyl-  | 60  | 108–10  | C₁₇H₂₄N₄O₃S  | 15.2 | 15.4  |

2-Acetsulphanilamido-4-methyl-5-(R)-pyrimidone (Compound 76).—On boiling the product with 10 times the amount of about 4 N hydrochloric acid for 2 hours, a good deal of the starting material was
recovered unchanged. On boiling it with concentrated hydrochloric acid (37%) for 1/2 hour there was some hydrolysis. The following is a typical experiment wherein the hydrolysed products were isolated in good yields.

2 g. of the compound 76 was boiled with 20 c.c. hydrochloric acid (37%) for about 6 hours. The solution was evaporated to dryness whereby a mass of crystalline product separated. This was triturated with 25 c.c. of water whereby a portion of it went into solution. The portion remaining undissolved was crystallised from water and was identified to be sulphanilic acid. The solution was neutralised and the product obtained was crystallised from water and identified to be 2-amino-4-methylpyrimidone.

In none of the many experiments carried out could sulphanilamide be isolated.

Action of alkali on 2-sulphanilamido-4-methylpyrimidone.—On boiling the compound with ten times the amount of sodium hydroxide (30%) no change was perceptible and the starting substance was recovered unchanged on diluting the solution and acidifying it with acetic acid.

Methylation of 2-sulphanilamido-4-methylpyrimidone : Isolation of 2-sulphanilylmethylamido-1 : 4-dimethylpyrimidone (VII).—The following is one of the typical experiments wherein the methylated product was obtained with considerable ease. To 3 g. of the compound 76 dissolved in sodium hydroxide (10 c.c. of 2.5 N), acetone (25 c.c.) was added followed by dimethyl sulphate (4 c.c.). The mixture was refluxed for about 1 hour and allowed to stand for a day. The solvent was evaporated off, the residue triturated with a little alkali to make it distinctly alkaline and filtered. The solid was washed well with sodium hydroxide solution (about 5%) then with water and repeatedly crystallised from water, m.p. 160-65° (with previous shrinking). (Found: N, 18.3; C_{13}H_{18}N_{4}O_{3}S requires N, 18.2%). This is undoubtedly the dimethyl derivative (VII) of the compound 76. The alkaline mother liquor after the separation of this product was acidified with acetic acid whereby a gummy product separated which so far could not be obtained as a well-defined crystalline product. This product is being purified. It is definitely different from the starting compound 76 and is probably a mixture of the two monomethyl derivatives of formulæ (VIII) and (IX).

Action of phosphorousxychloride on 2-acetsulphanilamido-4-methylpyrimidone.—This acetyl compound (2 g.) was gently boiled with phosphorus oxychloride (6 c.c.) for about 2 hours, cooled and poured into ice. A solid product separated. It was filtered, dissolved in dilute sodium hydroxide solution and the filtered clear solution acidified whereby the chloro compound separated. It was insoluble in almost all organic solvents and
as such could not be recrystallised. It did not melt below 280°. [Found: N, 16·8; C_{13}H_{13}N_{4}O_{2}SCl (formula X) requires N, 16·5%]

On boiling with zinc dust and water, the product obtained also did not melt below 280° and was not free from chlorine.

2-Amino-1:4-dimethylpyrimidine (XI).—2-Amino-4-methylpyrimidone (5·0 g.) dissolved in sodium hydroxide (2·5 N, 25 c.c.) was treated with dimethylsulphate (6 c.c.) with good shaking. In a very short time a white crystalline precipitate separated. It was allowed to stand for about an hour, filtered and washed first with dilute sodium hydroxide and then with water. On crystallisation from water it separated in beautiful long needles; m.p. above 280°. (Found: N, 30·2; C_{6}H_{5}N_{3}O requires N, 30·2%.)

[2′-Amino-4′-methyl-(6) pyrimidonyl] 4-acetaminobenzenesulphonate (XI): To a solution of 2-amino-4-methylpyrimidone (6·0 g.) in sodium hydroxide (2·5 N, 50 c.c.) was added sodium bicarbonate (13·0 g.) and then acetsulphanilylchloride (12 g.) with sufficient acetone to keep it in solution. The solution was stirred for about 3 hrs.; a white crystalline precipitate gradually separated. This was separated off and then another crop was obtained from the mother liquors on concentration. (Total yield, 8·2 g.) It separated from water in colourless needles and had m.p. 193-94°. (Found: N, 16·7; C_{13}H_{14}N_{4}O_{2} requires N, 17·4%). This compound was insoluble in alkali but soluble in dilute hydrochloric acid. On boiling with 4 N hydrochloric acid or 2·5 N sodium hydroxide for about a few minutes, it furnished in very good yields the starting 2-amino-4-methylpyrimidone.

On condensing 2-amino-4-methylpyrimidone with two molecules of acetsulphanilylchloride the same condensation product was isolated. When sodium acetate was used as the condensing agent in the place of sodium bicarbonate, only the starting amine and acetsulphanilic acid could be isolated from the reaction mixture.

Condensation of acetsulphanilylguanidine with mesityloxide: Isolation of 2-sulphanilylimido-4: 4: 6-trimethyl-2: 3: 4: 5-tetrahydropyrimidine (XIV) 2-sulphanilamido-4: 4: 6-trimethyl-4: 5-dihydropyrimidine (XV).—To a solution of sodium (2·5 g.) in absolute ethyl alcohol (200 c.c.) was added finely powdered acetsulphanilylguanidine (25·6 g.) followed by mesityloxide (12·0 g.). The mixture was boiled under reflux for 3-5 hours. The red solution gradually lost its tint. After the refluxing was over, most of the solvent was distilled off and the residue triturated with water. A gummy product separated which on scratching solidified. This was filtered off (product A) and the alkaline filtrate acidified whereby another product (B) was obtained. The former formed the main bulk of the condensation product.
The product (A) was crystallised from dilute acetic acid or water; m.p. 241-42°. (Found: N, 17·0; C_{13}H_{20}N_{4}O_{3}S requires N, 16·7%) This compound is insoluble in dilute hydrochloric acid and dilute alkali and is provisionally fixed to the N^4-acetyl derivative of (XIV). On hydrolysis with 4 N hydrochloric acid as usual, a compound crystallising in needles from water was obtained; m.p. 190-93°. (Found: N, 18·8; C_{13}H_{18}N_{4}O_{2}S requires N, 19·0%) This compound (77a) is soluble in dilute hydrochloric acid and insoluble in dilute sodium hydroxide and is provisionally represented as (XIV).

The product B, which was formed only in small quantities, crystallised from water acidified with acetic acid; m.p. 217-18°. This is soluble in dilute sodium hydroxide and insoluble in dilute hydrochloric acid. This is provisionally represented as the N^4-acetyl derivative of the compound of formula (XV). On hydrolysis with 4 N hydrochloric acid as usual, a product m.p. 228-30° was obtained. (Found: N, 19·6; C_{13}H_{15}N_{4}O_{2}S requires N, 19·0%) This compound (77b) is soluble in dilute hydrochloric acid and sodium hydroxide and is provisionally represented as (XV).

Condensation of sulphanilylguanidine with mesityl oxide.—This was carried out as described in the above case and 2 products were isolated in different experiments; but the exact conditions under which they are produced could not be defined. One of them (Compound 77) crystallised from alcohol in fine plates, m.p. 130-35°. (Found: N, 18·5; C_{13}H_{18}N_{4}O_{2}S requires N, 19·0%). Another product (77a) obtained was identical with the one described above, m.p. 190-93°. Both these compounds are insoluble in alkali. The melting points of these vary from sample to sample in a small range.

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**Summary**

Sulphanilylguanidine condensed with ethyl acetoacetate and its α-alkyl derivatives to yield 2-sulphanilamido-4-methylpyrimidone and 2-sulphanilamido-4-methyl-5-alkylpyrimidones respectively. Experiments have been recorded which support these structures and a number of intermediate compounds are described. Ethyl formylacetate condensed with sulphanilylguanidine to furnish 2-sulphanilamidopyrimidone. Hydroxymethyleneacetone and hydroxymethylene ethylbutyrlketone failed to condense with sulphanilylguanidine to yield the corresponding pyrimidines in appreciable yields. Mesityl oxide, on the other hand, has yielded two products which are represented provisionally as the two possible pyrimidine derivatives (XIV) and (XV).
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