

CHEMOTHERAPY OF TUBERCULOSIS

Part IV. Synthesis of Phthalyl and *ortho*-Toluoyl Derivatives of the Sulphonamides and Sulphones as Possible Mycobacterial Antagonists

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Received October 15, 1949

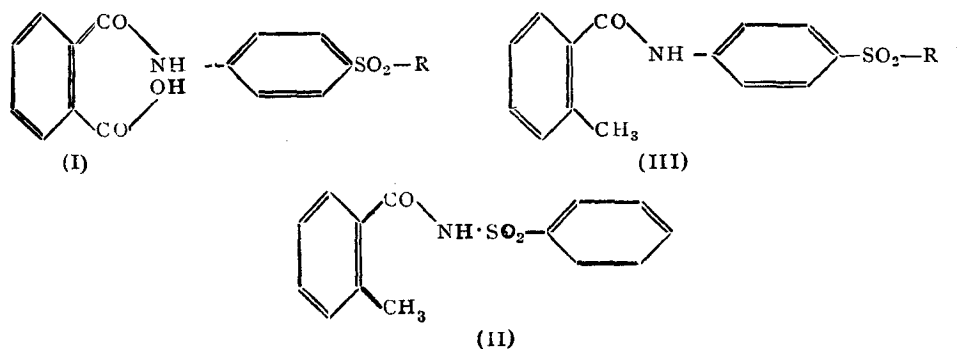
(Communicated by Major-General Sir S. S. Sokhey, Kt., F.A.Sc.)

THE Fildes-Woods¹ theory of the mechanism of action of sulphanilamides and other antibacterial agents connotes the possibility that chemotherapeutic agents could be obtained from among compounds that simulate the essential growth factors or "essential metabolites" of the bacteria from the stereochemical or structural points of view.² The expectation is that these compounds would displace the "essential metabolites" in the enzyme reactions concerned but themselves cannot be utilised for the growth or proliferation of the bacteria. With this as the working hypothesis, attempts were made to synthesise and study compounds as possible chemotherapeutic agents for tuberculosis.

We do not at present have enough knowledge about the exact nutritional requirements of *M. tuberculosis*³ or about the vital enzyme reactions concerned. In view of the facts that a 1:4-naphthoquinone derivative (phthiocol) has been obtained as a cellular constituent of *M. tuberculosis*⁴ and that the closely related acid fast organism, *Johne's bacillus*, requires a 1:4-naphthoquinone derivative as an essential growth factor,⁵ it appears likely that some 1:4-naphthoquinone derivative would be an essential metabolite of *M. tuberculosis*. So, derivatives of 1:4-naphthoquinone or compounds resembling 1:4-naphthoquinone in spatial configuration appear to be worthy of trial as possible chemotherapeutic agents against the tubercle bacilli. Lloyd and Middlebrook⁶ tested fifteen naphthoquinones against tubercle bacilli *in vitro*; of these 3-sulphanilyl-1:4-naphthoquinone appeared to be promising in view of its effectiveness and low toxicity. Gronwell and Zatterberg⁷ have studied the tuberculo static effect of compounds obtained by coupling 2-methyl-naphthoquinone with sulphanilamide, sulphapyridine and sulphathiazole but the details of the work of these authors are not available to us to form an opinion.

In view of the bacteriostatic effect of sulphanilamides and sulphones and the naphthoquinones against the tubercle bacilli *in vitro*, attempts were made, in the first instance, to prepare and study simple derivatives of sulphanilamides and diamino-diphenylsulphones which would show a formal resemblance to the naphthoquinones also.

Accordingly, derivatives of phthalic and *ortho*-toluic acids of the types (I), (II) and (III) were synthesised. The two acids were chosen partly because of the disputed claim that the biological property (antihemorrhagic) of the vitamin K is possibly due to the end product of metabolism which is phthalic acid⁸ and if this were so, *ortho*-toluic acid itself can serve as an inhibitor.

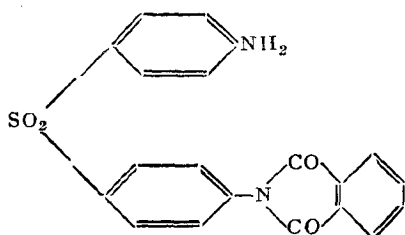


We were encouraged in this to find that the compound N¹-*o*-toluoyl-sulphanilamide has been described in a patent with the claim that it shows high antibacterial activity and low toxicity.⁹ Another compound, 4-amino-4'-toluamidodiphenyl-sulphone has also been mentioned in a patent.¹⁰ The twenty-six compounds synthesised in the course of this study are given in the table.

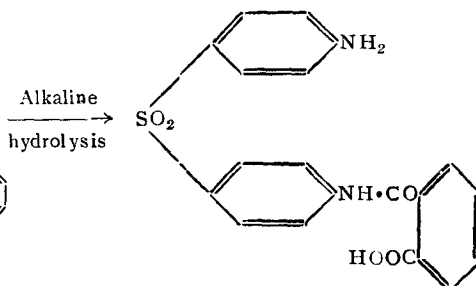
Of the phthalyl derivatives here reported, those of sulphadiazine and sulphamerazine appear to have been synthesised¹¹ before by workers at the Sharp and Dohme, Inc. and they have been examined for their potentialities as intestinal antiseptics.^{11,12} Similarly, 4-amino-4'-phthalamido-diphenyl sulphone (No. 84), which results by the interaction of one or two molecular proportions of phthalic anhydride with 4:4'-diamino-diphenyl-sulphone in alcohol medium, has been reported by Gray and Platt.¹³ These authors found that by the action of phthalic anhydride on 4:4'-diaminodiphenylsulphone in pyridine medium, they obtained only the mono- and *bis*-imides (IV and V) which by hydrolysis with aqueous alkali gave rise to the same monophthalamido derivative (VI). Mild hydrolysis of (V) however yielded them the corresponding *bis*-amidé (VII).

Phthalic and *O*-Toluic Acid Derivatives of Sulphonamides and Sulphones

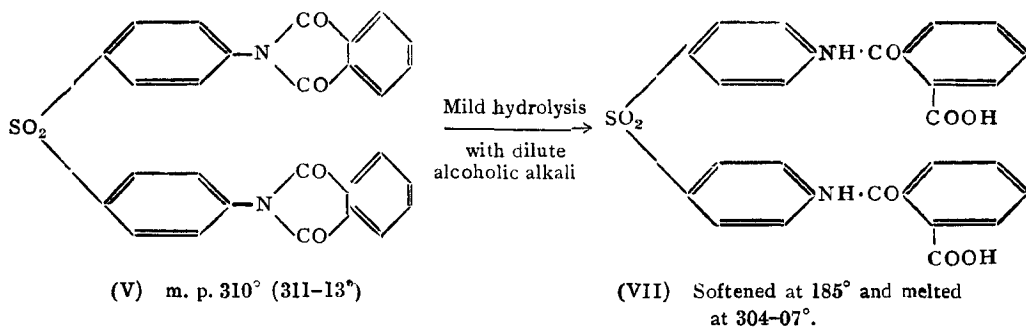
| Serial No. | Name of the Compound | Molecular Formula | M.P./°C. | Nitrogen percentage | |
|------------|---|---|---|---------------------|----------|
| | | | | Found | Required |
| 79 | Phthalyl sulphadiazine | C ₁₈ H ₁₄ O ₅ N ₄ S | 280 (dec.) | 14.1 | 14.4 |
| 80 | Phthalyl sulphamerazine | C ₁₈ H ₁₆ O ₅ N ₄ S | 273-76 | 13.6 | 13.6 |
| 81 | Phthalyl sulphamethazine | C ₂₀ H ₁₈ O ₅ N ₄ S | 156-60 (resolidifying and melting at 212-13) | 13.2 | 13.1 |
| 82 | 4-Nitro-4'-phthalamidodiphenyl sulphone | C ₂₀ H ₁₄ O ₇ N ₂ S | 173-75 | 13.2 | 13.1 |
| 83 | 4-Acetamido-4'-phthalamidodiphenyl sulphone | C ₂₂ H ₁₈ O ₆ N ₂ S | 228-29 (dec.) | 6.7 | 6.4 |
| 84 | 4-Amino-4'-phthalamidodiphenyl sulphone | C ₂₀ H ₁₆ O ₅ N ₂ S | 251-54 | 6.7 | 7.1 |
| 85 | N ⁴ - <i>o</i> -Toluoyl sulphanilamide | C ₁₄ H ₁₄ O ₃ N ₂ S | 240-41 | 9.7 | 9.7 |
| 86 | N ⁴ - <i>o</i> -Toluoyl sulphathiazole | C ₁₇ H ₁₆ O ₃ N ₃ S ₂ | 245-46 | 11.2 | 11.3 |
| 87 | N ⁴ - <i>o</i> -Toluoyl sulphathiazoline | C ₁₇ H ₁₇ O ₃ N ₃ S ₂ | 220-21 | 11.2 | 11.1 |
| 88 | N ⁴ - <i>o</i> -Toluoyl sulphapyridine | C ₁₈ H ₁₈ O ₃ N ₃ S | 210-11 | 11.2 | 11.4 |
| 89 | N ⁴ - <i>o</i> -Toluoyl sulphadiazine | C ₁₈ H ₁₆ O ₃ N ₄ S | 257-58 | 15.3 | 15.2 |
| 90 | N ⁴ - <i>o</i> -Toluoyl-5-bromo-sulphadiazine | C ₁₈ H ₁₅ O ₃ N ₄ SB _r | 248-49 (dec.) | 12.6 | 12.5 |
| 91 | N ⁴ - <i>o</i> -Toluoyl sulphamerazine | C ₁₉ H ₁₇ O ₃ N ₄ S | 242-43 | 14.5 | 14.7 |
| 92 | N ⁴ - <i>o</i> -Toluoyl-5-bromo-sulphamerazine | C ₁₉ H ₁₆ O ₃ N ₄ SB _r | 252-54 (dec.) | 12.2 | 12.1 |
| 93 | 4-Nitro-4'- <i>o</i> -toluamidodiphenyl sulphone | C ₂₀ H ₁₆ O ₅ N ₂ S | 189-90 | 7.2 | 7.1 |
| 94 | 4-Amino-4'- <i>o</i> -toluamidodiphenyl sulphone | C ₂₀ H ₁₈ O ₃ N ₂ S | 230-40 | 7.4 | 7.7 |
| 95 | 4:4'-Di- <i>o</i> -toluamidodiphenyl sulphone | C ₂₈ H ₂₄ O ₄ N ₂ S | 244-45 | 5.6 | 5.8 |
| 96 | N ⁴ -Formyl-N ¹ - <i>o</i> -toluoyl sulphanilamide | C ₁₅ H ₁₄ O ₄ N ₂ S | 195-200 (Softg. at 187) | 8.3 | 8.8 |
| 97 | N ⁴ -Acetyl-N ¹ - <i>o</i> -toluoyl sulphanilamide | C ₁₆ H ₁₆ O ₄ N ₂ S | 225-27 | 8.5 | 8.4 |
| 98 | N ⁴ - <i>n</i> -Butyryl-N ¹ - <i>o</i> -toluoyl sulphanilamide | C ₁₈ H ₂₀ O ₄ N ₂ S | 226-29 | 7.8 | 7.8 |
| 99 | N ⁴ - <i>n</i> -Caproyl-N ¹ - <i>o</i> -toluoyl sulphanilamide | C ₂₀ H ₂₄ O ₄ N ₂ S | 175-76 | 7.5 | 7.3 |
| 100 | N ⁴ - <i>n</i> -Heptoyl-N ¹ - <i>o</i> -toluoyl sulphanilamide | C ₂₁ H ₂₆ O ₄ N ₂ S | 176-80 (Softg. at 170) | 6.9 | 7.0 |
| 101 | N ⁴ - <i>cyclo</i> -Hexoyl-N ¹ - <i>o</i> -toluoyl sulphanilamide | C ₂₁ H ₂₂ O ₄ N ₂ S | 216-20 | 6.9 | 7.0 |
| 102 | N ⁴ -Cinnamoyl-N ¹ - <i>o</i> -toluoyl sulphanilamide | C ₂₃ H ₂₀ O ₄ N ₂ S | 239-41 | 6.8 | 6.7 |
| 103 | N ⁴ , N ¹ -Di- <i>o</i> -toluoyl sulphanilamide | C ₂₂ H ₂₀ O ₄ N ₂ S | 229-31 | 6.9 | 6.9 |



(IV) m.p. 256-58 °C.



(VI) Frothed at 176° and on continued heating solidified to give (IV),



The melting points together with other particulars reported by these authors have been given in the above flowsheet. We were able to obtain in our experiments only the monophthalamido diphenylsulphone, melting at 251-54° with decomposition and dissolving in cold dilute sodium bicarbonate with effervescence.

The other phthalamido derivatives reported herein were similarly prepared by the action of phthalic anhydride on the appropriate amino compound usually in alcohol medium. These were also soluble in cold dilute sodium bicarbonate with liberation of carbon dioxide.

Among the N⁴-acylated sulphanilamides chosen for condensation with *o*-toluyl chloride, figure N⁴-*cyclo*-hexoyl sulphanilamide and N⁴-cinnamoyl sulphanilamide in view of bacteriostatic effect claimed against mycobacteria for *cyclo*-hexyl grouping¹⁴ and the cinnamyl radical.¹⁵

From knowledge available on the phthalyl derivatives of the sulphanilamides^{11,16} the compounds now prepared and tested would not appear to be adequately absorbed after oral administration. Some of them might perhaps constitute excellent intestinal antiseptics, but the low concentrations in which they would be met with in the blood stream following their oral administration would militate against their possible usefulness as anti-mycobacterial agents. They would necessarily have to be given parenterally in order to ascertain their antimycobacterial potentialities.

EXPERIMENTAL

Phthalyl derivatives (Nos. 79-84)—were prepared by refluxing an alcoholic solution of calculated amounts of phthalic anhydride and the appropriate amino compound. The crude phthalamido derivatives obtained by dilution of the reaction mixtures with excess of water were purified by repeated solution in dilute sodium hydroxide (charcoal) and reprecipitation with excess of dilute hydrochloric acid. The yields of the purified materials

were above 90 per cent. of the theoretical. The compounds thus purified were then crystallised.

*N*⁴-*o*-toluoyl derivatives of the sulphonamides (Nos. 85-89, 91) and the sulphones (Nos. 93, 95)—resulted by the interaction of the requisite sulphonamide or sulphone with slightly more than the calculated quantity of freshly distilled *o*-toluoyl chloride in presence of pyridine.

The *N*⁴-acyl-*N*¹-*o*-toluoyl sulphanilamides (Nos. 96-103)—were obtained by the action of a slight excess of *o*-toluoyl chloride on the suitable *N*⁴-acyl-sulphanilamide in pyridine.

*N*⁴-*N*¹-*Di-o*-toluoyl sulphanilamide (No. 104)—was prepared by the action of slightly more than two molecular equivalents of *o*-toluoyl chloride on sulphanilamide in pyridine medium.

All the *o*-toluoyl derivatives readily separated out on dilution of the reaction mixtures with water. Occasionally, partial neutralisation of pyridine contained in the diluted reaction mixtures with dilute hydrochloric acid effected the separation of the bulk of the condensation products. Unlike the *N*⁴-toluoyl derivatives of the sulphonamides and the toluamidodiphenyl-sulphones, the *N*⁴, *N*¹-disubstituted sulphonamides (Nos. 96-104) were contaminated with traces of the starting materials. The *N*⁴, *N*¹-disubstituted sulphanilamides were found to be more acidic than the *N*⁴-acyl-sulphanilamides. This characteristic difference was utilised to purify the *N*⁴-acyl-*N*¹-toluoyl sulphanilamides. By merely neutralising a dilute alkaline solution of the reaction products with dilute hydrochloric acid (litmus), only the *N*⁴-acyl sulphanilamides were precipitated out; by acidification of the filtrate with excess of dilute hydrochloric acid (Congo red) the desired *N*⁴-acyl-*N*¹-*o*-toluoyl sulphanilamides were obtained in a high state of purity.

The yields of the recrystallised *o*-toluoyl derivatives ranged from 70-90 per cent. of theory, and were implicitly dependent on the degree of dryness of the starting materials.

4-Amino-4'-*o*-toluamidodiphenylsulphone (No. 94)—resulted by the action of *o*-toluoyl chloride on excess of 4:4'-diaminodiphenyl sulphone suspended in water. The fraction which was soluble in dilute hydrochloric acid and dilute ammonium hydroxide was subjected to repeated fractional crystallisation from dioxane and alcohol. The more soluble fractions constituted the required amino toluamidodiphenylsulphone which was obtained in yields of 25-40 per cent.

5-Bromoderivatives of *N*⁴-toluoylsulphadiazine (No. 90) and *N*⁴-toluoyl sulphamerazine (No. 92)—were obtained by bromination of *N*⁴-toluoyl

sulphadiazine (No. 89) and N⁴-toluoyl sulphamerazine (No. 91) respectively by adoption of the method of Price, *et al.*¹⁷ for the preparation of 2-(N⁴-acetyl-sulphanilamido)-5-bromo-4-methyl-6-methoxymethyl pyrimidine. However in contrast to the experience of Price and his associates,¹⁷ the bromo compounds were invariably contaminated with varying quantities of the starting materials (Nos. 89 and 91). The brominated derivatives were only very feebly acidic compared to the parent compounds and could easily be obtained by washing the reaction mixtures with dilute ammonia which dissolved the distinctly acidic unreacted substances (Nos. 89 and 91). The yields of the recrystallised bromoderivatives of toluoyl sulphadiazine and toluoyl sulphamerazine varied from 40-55 per cent.

We express our grateful thanks to Major-General Sir Sahib Singh Sokhey, Director, Haffkine Institute, Bombay, and Dr. K. Ganapathi, for their kind interest in these investigations. We are also thankful to Mr. M. H. Shah, B.Sc., for carrying out all the analysis.

SUMMARY

In order to fit the sulphonamides and sulphones out better for use in the chemotherapy of tuberculosis and leprosy, synthesis has been effected of a series of *o*-phthalic and *o*-toluic acid derivatives of well-known sulphonamides and disubstituted diphenyl sulphones as possible mycobacterial antagonists.

Phthalic and *o*-toluic acid derivatives were chosen for synthesis in view of the recent theory advanced by Shemiakin that the antihæmorrhagic activity of substances of a vitamin K nature, intimately connected with the metabolism of mycobacteria, is due to phthalic acid which arises from the antihæmorrhagic substances. The study of the compounds designed as possible mycobacterial antagonists is expected to shed additional light on the mode of action of antihæmorrhagic compounds and the relation between antihæmorrhagic action and antimycobacterial efficacy.

The twenty-six compounds synthesised include *o*-phthalic acid derivatives of sulphanilamidopyrimidine, sulphanilamido-4-methyl, and sulphanilamido-4:6-dimethyl pyrimidines and of 4-nitro-, 4-acetamido-diphenyl-sulphones and 4:4'-diaminodiphenyl sulphone. They also include N⁴-*o*-toluoyl derivatives of sulphanilamide, sulphathiozole, sulphathiazoline, sulphapyridine, sulphadiazine, sulphamerazine, 5-bromo-sulphadiazine, and 5-bromosulphamerazine. Other toluic acid derivatives prepared are N⁴, N¹-di-*o*-toluoyl sulphanilamide, 4:4'-*o*-ditoluoylamido-diphenyl sulphone and 4-nitro- and 4-amino-4'-*o*-toluoylamido-diphenyl sulphones. In addition,

N¹-*o*-toluoyl derivatives have been obtained from N⁴-formyl-, N⁴-acetyl-, N⁴-*n*-butyryl-, N⁴-*n*-valeryl-, N⁴-*n*-caproyl-, N⁴-*p*-heptoyl-, N⁴-cyclohexoyl and N⁴-cinnamoyl-sulphanilamides.

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