CHEMOTHERAPEUTIC STUDIES IN BACTERIOSTASIS

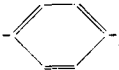
PART I. SYNTHETIC COMPOUNDS CONTAINING THE SKELETON OF *p*-TOLUIDINE

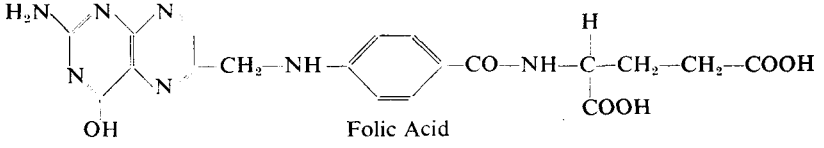
BY W. H. LINNELL AND S. V. VORA

From the Pharmaceutical Chemistry Research Laboratories of the School of Pharmacy, University of London

Received July 24, 1951

THE subject of metabolite antagonism has created widespread interest amongst chemists and biologists during recent years. Not only has it provided a clue to the activity of many antibacterial agents, but it has also opened up a new approach to the design of better chemotherapeutic agents.

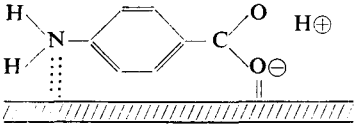
The stimulus for many of the investigations along this line came from the discovery of the striking relationship between *p*-aminobenzoic acid and the sulphonamides. All the active sulphonamides have in common the basic structure of *p*-aminobenzoic acid (H_2N-  -) and are completely antagonised by a relatively small amount of this acid. Woods[1] first suggested that the activity of sulphonamides might be explained on the basis of their interference with the utilisation of *p*-aminobenzoic acid in the metabolic system. A large amount of subsequent work has supported this view. The recent discovery of the constitution of pteroic acid and pteroyl glutamic acid (folic acid) and their relation to sulphonamide action[2] have confirmed and added considerable weight to Woods's[1] ideas.

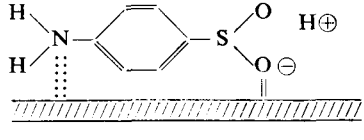


Folic Acid

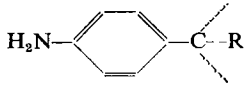
It is now generally believed that sulphonamides exert their bacteriostatic effect by displacing *p*-aminobenzoic acid from the enzyme surface on which the pteroyl glutamic acid molecule is built up.

The study of several sulphur-free compounds related to *p*-aminobenzoic acid[3,4,5,6] indicate that a structure analogous to this acid is more important for bacteriostatic activity reversible by *p*-aminobenzoic acid than resemblance to sulphanilamide. This probably means that the active centres of the enzymes involved are designed to accommodate any molecule in which an aromatic amino group is separated from an electron attracting group ($R\rightarrow\ddot{O}:$) by a suitable system of conjugated double bonds, such as a benzene ring.





Any compound having this basic configuration and which forms an enzyme-drug complex with a low degree of dissociation may act as a potential bacteriostatic agent. A survey of the literature shows that several compounds of this type have been prepared, all of which exhibit bacteriostatic activity. One aspect of this problem, however, still remains untouched—that of preparing compounds, in which the skeleton of *p*-aminobenzoic acid is combined with another molecule which itself exhibited toxic action against micro-organisms. Accordingly investigations of compounds of the following type were undertaken.

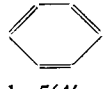


where R is a fragment toxic to bacteria.

The investigations of these compounds and their properties have been conducted with two basic objects in mind:—(a) to develop another line of evidence in support of the concept that the resemblance of sulphonamides to *p*-aminobenzoic acid is the basis of their activity and (b) to observe whether the incorporation of the skeleton of a metabolite into a toxic molecule serves as a means of bringing these compounds to a specific site of action.

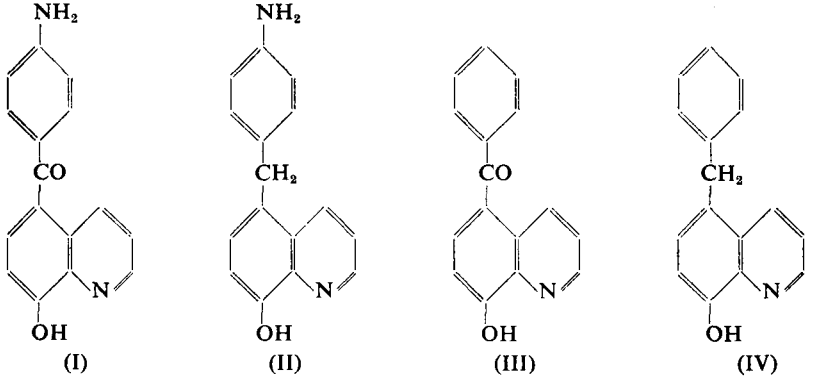
The following classes of antibacterial substances were chosen for study in the present investigations:—(i) 8-hydroxyquinoline; (ii) *p*-chlorophenol; (iii) long chain aliphatic tertiary amines and quaternary ammonium compounds; (iv) 2:8-diaminoacridine.

The syntheses of the derivatives of 8-hydroxyquinoline are described in this paper. 8-Hydroxyquinoline (oxine) has been used as a bactericide and fungicide for many years and several modifications of this drug have been tested[7]. Albert and his colleagues[8,9] have recently published some interesting reports on the mode of action of 8-hydroxyquinoline. These authors have suggested that oxine, by virtue of its chelating properties, deprives the bacteria of necessary trace metals, leading to their death. A considerable number of oxine derivatives containing the same chelating arrangement have been prepared. Not only do they possess similar or greater antibacterial power but the derivatives formed by the introduction of the alkyl group in the 5 position are less toxic. McMaster and Burner[10], who synthesised 5-benzyl-8-hydroxyquinoline, reported it to be as active as oxine and considerably more so than phenol. They also showed it to be much less toxic than either of the latter two compounds. Recently Rosenmund and Karst[11] have reported the preparation of certain 5-acyl derivatives of oxine; one of these, 5-benzoyl-8-hydroxyquinoline showed promising results against both Gram-positive and Gram-negative organisms.

The above survey of the activity of oxine derivatives led to the suggestion that the introduction of the *p*-aminobenzoic acid structure (skeleton of *p*-toludine H_2N—  —C) in position 5 of the oxine molecule would give compounds 5(4′-aminobenzoyl)-8-hydroxyquinoline (I) and 5(4′-aminobenzyl)-8-hydroxyquinoline (II), which might exhibit

more pronounced chemotherapeutic properties than those of oxine, since the molecule embodies the metabolite structure in addition to the general activity associated with the oxine group.

The activity, if any, of the above compounds would be due, either to the whole molecule as such or to the oxine portion. In order to ascertain which of these two possibilities applied, it was decided to synthesise related compounds without the amino group. The molecular weight being virtually the same, any additional activity could be ascribed to the metabolite skeleton. It is also important to observe whether the activity of these compounds is affected in any way by *p*-aminobenzoic acid. Any change in activity or competitive antagonism should provide information for the suggested mode of action of these compounds. The following 4 compounds were synthesised and tested under identical conditions.

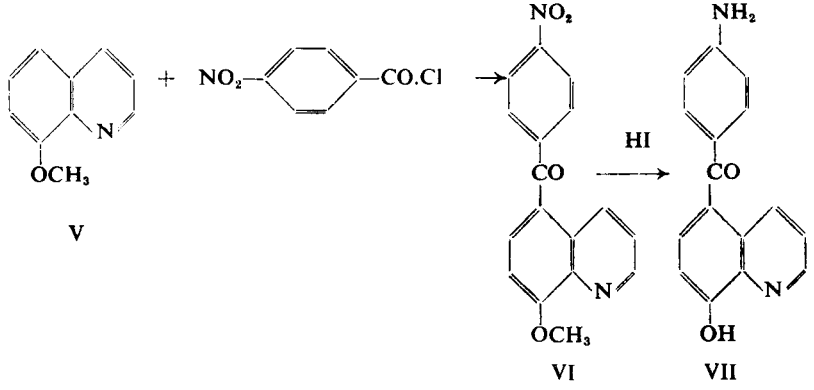


5-Benzoyl-8-hydroxyquinoline (III) was obtained in 50 per cent. yield by condensing benzoyl chloride with 8-hydroxyquinoline using anhydrous aluminium chloride as the condensing agent and nitrobenzene as solvent, according to the conditions of Matsumura[12]. The product twice crystallised from methanol melted at 112°C. (recorded m.pt. 118°C.). Our product also differed from that of Matsumura, particularly in the melting-points of its derivatives. The identity of the product was, therefore, established by micro-analysis and by the Beckmann rearrangement of its oxime to 5-benzoylamino-8-hydroxyquinoline.

Condensation of 8-hydroxyquinoline with *p*-nitrobenzoyl chloride in nitrobenzene under similar conditions to those used for the compound III did not succeed. However, when the OH group was protected by methylation, and the nitrobenzene replaced by carbon disulphide the reaction went smoothly, giving the required 5(4′-nitrobenzoyl)-8-methoxyquinoline (VI) in 6 per cent yield. The product crystallised from chlorobenzene to pale yellow cubes, m.pt. 200°C., and formed a well-defined picrate, hydrochloride and semicarbazone.

Demethylation of compound VI was accomplished by heating it with constant boiling hydriodic acid. This reagent was in fact doubly successful, as reduction of the nitro group was accomplished in addition to

the desired demethylation, giving compound VII in 1 stage in 85 per cent. yield. 5(4′-aminobenzoyl)-8-hydroxyquinoline crystallised from chlorobenzene to give a white crystalline product of m.pt. 255°C. The



V VI VII

picrolonate was obtained as a yellow crystalline solid and was shown by analysis to have the formula $B_1(C_{16}H_{12}N_2O_2)_1$.

The constitution of compound VII was readily confirmed by its diazotisation and deamination with ethanol to 5-benzoyl-8-hydroxyquinoline. The compound and its picrate both showed identical melting-points and mixed melting-points with the authentic sample.

The reduction of the ketones (I and III) presented no difficulty. Both were easily reduced by the Haung-Minlon modification of the Wolfe-Kischner process[15]. Equimolecular quantities of the ketone and hydrazine hydrate (90 per cent.) were refluxed in ethylene glycol in the presence of alkali (sodium hydroxide), the water formed during the reaction was distilled off and the hydrazone produced was decomposed by refluxing the mixture for a further 2 hours. The reduction products can be isolated from the glycol solution by dilution and saturation with carbon dioxide.

The bacteriological results will be communicated later.

Experimental

5-Benzoyl-8-hydroxyquinoline. This was obtained according to the conditions of Matsumura[12]. Benzoyl-8-hydroxyquinoline crystallised from dilute methanol in colourless silky needles, m.pt. 112°C. (recorded m.pt. 118°C.). Yield 50 per cent. It was easily soluble in the usual organic solvents and in dilute mineral acids, moderately soluble in hot water, but soluble with difficulty in dilute sodium hydroxide solution. It gave an intense green colour with ferric chloride and a red colour with diazotised sulphanilic acid. Found: C, 77·8; H, 4·10; N, 5·50; $C_{16}H_{11}NO_2$ requires C, 77·1; H, 4·41; N, 5·62 per cent.

The following derivatives of this compound were isolated.

Monopicrate: Deep yellow needles from dilute ethanol, m.pt. 123°C. (recorded 143°C.).

Hydrochloride ($C_{16}H_{11}NO_2$,HCl):—Pale yellow silky needles from dilute ethanol; hydrolysed in water, m.p. 242° to 244°C. (recorded 252° to 260°C.).

Acid sulphate $C_{16}H_{11}N_1O_2$,H_2SO_4:—Pale yellow needles from ethanol; hydrolysed in water, m.p. 208° to 209°C. (recorded 219° to 220°C.).

Methiodide $C_{16}H_{11}N_1O_2CH_3I$:—Garnet coloured prisms from hot water, m.p. 187°C.

2 : 4 Dinitrophenylhydrazone ($C_{22}H_{16}N_5O_5$):—Saffron coloured prisms m.p. 175°C.

Oxime:—Colourless prisms from dilute ethanol, easily soluble in the usual organic solvents, m.p. 133° to 134°C. (recorded 147° to 148°C.).

Beckmann rearrangement of the oxime. A mixture of oxime (0·3 g.) glacial acetic acid (2·4 g.) and acetic anhydride (0·6 g.) was saturated with hydrogen chloride gas in the cold and then maintained at 100°C. for 3 hours in a sealed tube. On cooling, the contents were made alkaline with sodium carbonate and shaken with chloroform. On concentrating the solvent, colourless crystals were obtained (0·24 g.). It formed yellowish white plates from chloroform, m.p. 237°C. The recorded m.p. of 5-benzoylamino-8-hydroxyquinoline is 237° to 238°C.[13]

8-Methoxyquinoline. This was prepared by the modification of the Bedall Fischer process[14]. 8-Hydroxyquinoline (100 g.) and methyl iodide (100 g.) were added to a solution of potassium hydroxide (40 g.) in methanol (400 ml.) and the mixture was refluxed for 6 hours. After distilling off the methanol, the residue was treated with dilute sodium hydroxide, extracted with benzene, dried over anhydrous sodium sulphate and distilled. 8-Methoxyquinoline distilled at 164°C./14 mm., m.pt. 38° to 40°C. It was purified by redistillation and crystallisation from light petroleum (b.pt. 100° to 120°C.), m.pt. 45° to 46°C. Yield 68 g. *Picrate* m.pt. 160°C.; *Chloroplatinate* m.pt. 206°C. (decomp.).

5-(4′-Nitrobenzoyl)-8-methoxyquinoline. *p*-Nitrobenzoyl chloride (11·5 g.) and 8-methoxyquinoline (10 g.) were dissolved in dry carbon disulphide (100 ml.) and powdered aluminium chloride (20 g.) was added in one lot. A vigorous reaction set in; the yellow precipitate which first formed went into solution and a red semi-solid mass separated. The mixture was heated on a water-bath at 60° to 70°C. and mechanically stirred for 6 hours. After the removal of the solvent, the residue was decomposed with ice and hydrochloric acid, cooled and filtered.

The separated white solid mass was boiled with 5 per cent. aqueous sodium carbonate to remove *p*-nitrobenzoic acid, crystallised twice from dilute hydrochloric acid (one decolorising with charcoal) and treated with sodium acetate which liberated the base 5-(4′-nitrobenzoyl)-8-methoxyquinoline as a pale greenish yellow solid. It was purified by crystallisation from ethanol and recrystallisation from chlorobenzene. Yield 1·24 (6 per cent.). m.pt. 201° to 202°C. It gave a red colour with dilute sodium hydroxide, but no green colour with ferric chloride. Found:

C, 66·09; H, 3·67; N, 9·18; $C_{17}H_{12}N_2O_4$ requires C, 66·23; H, 3·89; N, 9·20 per cent. *Picrate*, m.pt. 208° to 209°C. (decomp.). Found: C, 52·50; H, 2·28; N, 13·25; $C_{23}H_{15}N_5O_{11}$ requires C, 52·59; H, 3·02; N, 13·04 per cent. *Hydrochloride* m.pt. 133° to 136°C. *Semicarbazone* m.pt. 192° to 193°C.

5-(4'-Aminobenzoyl)-8-hydroxyquinoline. 5-(4'-Nitrobenzoyl)-8-methoxyquinoline (1 g.) was heated with constant boiling hydriodic acid (15 ml.) at 120° to 130°C. for 5 hours, the solution being stirred continuously. The cooled solution was diluted with water, decolorised with sulphur dioxide and made alkaline with solid sodium carbonate, when 5-(4'-aminobenzoyl)-8-hydroxyquinoline came down as a pale green precipitate. It was purified by boiling with a little ethanol and recrystallising from chlorobenzene. Yield 1·6 g. (86 per cent.). It formed colourless silky needles m.pt. 255°C. insoluble in benzene, light petroleum, sparingly soluble in ethanol, chlorobenzene, and gave a deep green colour with ferric chloride. Found: C, 73·18; H, 5·33; N, 10·74; $C_{16}H_{12}N_2O_2$ requires C, 72·72; H, 5·34; N, 10·6 per cent.

Picrolonate. m.pt. 236°C. (decomp.). Found: C, 58·87; H, 3·92; N, 15·87; $C_{26}H_{20}N_6O_7$ requires C, 59·09; H, 3·8; N, 15·9 per cent. *Monopicrate*, m.pt. 180°C. (decomp.).

Constitution of 5-(4'-aminobenzoyl)-8-hydroxyquinoline. Compound VII (0·5 g.) was dissolved in hydrochloric acid (4 ml.) and diazotised with sodium nitrite at 0°C. Ethanol (5 ml.) was then added and the solution heated on a water-bath for 30 minutes. After removal of the ethanol the solution was made alkaline with sodium carbonate and extracted with light petroleum (b.pt. 100° to 120°C.). Evaporation of the solvent gave 5-benzoyl-8-hydroxyquinoline (0·3 g.) m.pt. 112° to 113°C. The mixed m.pt. with the authentic sample showed no depression.

5-(4'-Aminobenzyl)-8-hydroxyquinoline. The ketone VII (3 g.) was added to ethylene glycol (35 ml.) containing hydrazine hydrate 90 per cent. (3 ml.) and sodium hydroxide (2·5 g.). The whole was refluxed over a naked flame for 1 hour. The condenser was then removed and the thermometer fixed so that the bulb was immersed in the liquid; the refluxing was continued thus until the thermometer recorded 195°C.; when the condenser was replaced and refluxing continued for a further 3 hours. After cooling, the contents of the flask were diluted with water (100 ml.) and saturated with carbon dioxide, when 5-(4'-aminobenzyl)-8-hydroxyquinoline came down as a pale yellow precipitate. It was recrystallised from dilute ethanol, m.pt. 175°C. Yield 2·4 g. Found: C, 76·4; H, 5·46; N, 10·98; $C_{16}H_{14}N_2O_1$ requires C, 76·8; H, 5·60; N, 11·20 per cent.

5-Benzyl-8-hydroxyquinoline. This was obtained from 5-benzoyl-8-hydroxyquinoline in the same way as described above. 5-Benzoyl-8-hydroxyquinoline crystallised from light petroleum (100° to 120°C.) in colourless needles, m.pt. 114°C.

REFERENCES

1. Woods, *Brit. J. Expt. Path.*, 1940, **21,** 74.
2. Ivanovics, *J. Path. Bact.*, 1941, **51,** 91.
3. Kuhn, Moller and Wendt, *Ber. dtsch. chem. Ges.*, 1943, **76,** 405.
4. Kuhn, Moller, Wendt and Beinert, *ibid.*, 1942, **75,** 711.
5. Peters, *J. Pharmacol.*, 1943, **79,** 32.
6. Wyss, Rubin and Strandskov, *Proc. Soc. Expt. Biol. Med.*, 1943, **52,** 155.
7. Altpeter, *Pharm. Zbl.*, 1929, **70,** 805.
8. Albert, *Pharm. J.*, 1947, **158,** 275.
9. Albert and Magrath, *Biochem. J.*, 1947, 534.
10. McMaster and Burner, *J. Amer. chem. Soc.*, 1935, **57,** 1697.
11. Rosenmund and Karst, *Arch. Pharm.*, 1941, 154.
12. Matsumura, *J. Amer. chem. Soc.*, 1930, **52,** 4433.
13. Kostenski, *Ber. dtsch. chem. Ges.*, 1891, **24,** 152.
14. Bedall and Fischer, *ibid.*, 1881, **14,** 2570.
15. Haung-Minlon, *J. Amer. chem. Soc.*, 1946, **68,** 2487.