146. DERIVATIVES OF SULPHANILAMIDE AND CHOLIC ACID

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It was suggested to the author by Prof. J. H. Dible that use might be made of sulphanilamide derivatives which would be concentrated in the bile. It seemed possible that compounds between cholic acid and sulphanilamide might be so excreted, and the following substances have therefore been prepared: N-(p-sulphonamido) phenyl cholamide (I), and (probably) α -cholyl β -p-aminobenzene-sulphonyl hydrazine (II).

The compound (I) was isolated, apparently as a hydrate, after direct condensation of sulphanilamide with triformyl cholyl chloride, as prepared by Cortese & Baumann [1935].

For the preparation of the hydrazine derivative (II), theoretically equivalent amounts of pure cholyl hydrazine ('cholic acid hydrazide', III, Bondi & Müller [1906]; Caldwell [1938]) and pure *p*-acetamidobenzenesulphonyl chloride were mixed in pyridine solution. Reaction took place at once, but the product could not be isolated in crystalline form and free from solvent. Hydrolysis gave (II) as a white, possibly crystalline, powder which, by titration, diazo-colour measurement and elementary analysis appeared to be pure. It could not, however, be recrystallized from solvents and had no definite melting point; such behaviour is, of course, not uncommon among cholic acid derivatives. For further characterization, the compound (II) or the original condensation product was boiled with alkali. There was thus obtained an acid, decomposing at 160°, which was also the product of alkaline hydrolysis of cholyl hydrazine (III). At *ca.* 80°, this acid seemed to lose solvent and was then indistinguishable from cholic acid. On oxidation with chromium trioxide, it was readily changed into dehydrocholic acid, which was also isolated by direct oxidation of (II) or of the original condensation product.

Finally, for comparison with (II), there were synthesised α -benzoyl β -p-acetamidobenzenesulphonyl hydrazine (IV, $R_1 = \text{CO} \cdot \text{CH}_3$), and α -benzoyl β -p-aminobenzenesulphonyl hydrazine (IV, $R_1 = \text{H}$). The method used was the same as for the preparation of (II), and both products were crystalline and easily purified. Comparison by titration of the amino-compound (IV, $R_1 = \text{H}$) with (II) gave the expected result.

Condensation of *p*-acetamidobenzenesulphonyl chloride with the 'hydrazides' (such as III) appears to offer a new and simple method for the preparation of derivatives of sulphanilamide and non-ketonic carboxylic acids. The products here described seem to be stable; they are sufficiently acidic to dissolve in ammonia and sodium carbonate solutions.

Preliminary tests by Prof. Dible indicate that the substance (II) has considerable bacteriostatic activity *in vitro*.

EXPERIMENTAL

All melting points are uncorrected. Elementary analyses and Rast molecular weight determinations were by Dr G. Weiler.

N-(*p*-Sulphonamido-)phenyl cholamide (I). Triformyl cholic acid [cf. Cortese & Baumann, 1935] was prepared by warming 20 g. of cholic acid with 40 ml. of 98/100 % formic acid at 40° until solution occurred. After 16 hr. at room temperature, the mixture was diluted with water and stirred until the product solidified. The collected and washed solid was dissolved in alcohol, warmed to ca. 60° and diluted, with mixing, until crystals formed, when gradual cooling gave the formyl compound as needles of m.p. 204–206°. Yield, 12–14 g.

The above compound (2 g.), dissolved in redistilled thionyl chloride (2 ml.), was kept at 0° for 1 hr. Dilution with light petroleum gave a gum, which was washed and rubbed with fresh petroleum until it formed a white powder. This was dried in air and used at once. 0.5 g. of formyl derivative was thus converted into the chloride, and the product was heated with 0.5 g. of sulphanilamide in 3 ml. of pyridine for 1 hr. at 100°. The cooled mixture was diluted with HCl solution, and the precipitate was washed and then warmed for 15 min. with 25 ml. of 5 *N* NaOH. After re-acidification with HCl, the gelatinous product was collected, washed and dissolved in alcohol. Considerable dilution with water gave small white needles (0.2 g.) of m.p. 238–245°. Recrystallization gave substance (I) of m.p. 244–246°, decomp. (Found: C, 61.8; H, 8.3; N, 5.0; S, 5.0 %. $\text{C}_{30}\text{H}_{46}\text{O}_6\text{N}_2\text{S} \cdot \text{H}_2\text{O}$ requires C, 62.1; H, 8.3; N, 4.8; S, 5.5 %.)

Further heating of the pyridine mixture lowered the yield of final product, which, in any case, was not always obtained in the amount mentioned above. Compound (I) could be separated from more acidic material by saturation of an alkaline solution with CO_2 , when (I) was precipitated. The compound gave a red colour in the Liebermann-Burchard test; it was easily soluble in alcohol (from which it only crystallized after great dilution of a cold solution with water), in acetone, acetic acid and solutions of KOH, NaOH etc. Alkaline solutions very readily formed gels on cooling, even at great dilution, e.g. 50 mg./100 ml.

α -Cholyl β -p-aminobenzenesulphonyl hydrazine (II). A suspension of cholic acid (20 g.) in ethyl alcohol (150 ml.) was saturated with diazomethane. After evaporation of solvent, the solid residue was heated under reflux for 20 hr. with 5 ml. of pure hydrazine hydrate and enough alcohol to dissolve the mixture. The solid contents of the flask were then washed out with hot alcohol, and after considerable dilution with water there slowly separated long needles (16 g.) of cholyl hydrazine (III), of m.p. 193–195° [compare Bondi & Müller, 1906]. An identical product was obtained by the same procedure from triformyl cholic acid.

Cholyl hydrazine (10 g.) in pyridine (50 ml.) was mixed with a solution of *p*-acetamidobenzenesulphonyl chloride (5.5 g., m.p. 150–151°) in pyridine (50 ml.). The temperature rose at once to 40°. After 15 min. the solution was poured, with stirring, into excess of dilute HCl. A slightly yellow solid separated in almost quantitative yield. After collection, washing and drying at room temperature *in vacuo*, and at 50–60°, this product was purified by solution in acetone, filtration from a small amount of yellow insoluble matter and precipitation with light petroleum. The solid which formed appeared crystalline; it softened at *ca.* 160° and decomposed with frothing above 180°.

This substance gave a red colour in the Liebermann-Burchard test and a negative diazo-test; it dissolved readily in alcohol, acetone, acetic acid and solutions of NaOH, KOH, NH₃ and Na₂CO₃, from which it was precipitated with CO₂.

The whole of the wet crude product from a preparation as above was dissolved in 2*N* NaOH (150 ml.) and refluxed for 45 min. The cooled solution was diluted to *ca.* 500 ml. and saturated with CO₂. The white solid precipitate was collected, washed and dried *in vacuo* and then at 75°. Yield, 7 g. of compound (II).

The bicarbonate mother liquors were acidified with HCl, and the precipitated acid was collected, washed and purified by solution in dilute NaHCO₃. Acidification of the filtered NaHCO₃ solution gave 1.5 g. (dried) of acid which, from dilute alcohol, formed long white needles that frothed at 160°. On drying at 70–80°, these appeared to lose solvent and then had m.p. 194–196°, not depressed by cholic acid (m.p. 196–197°). The same acid was isolated when compound (II) (0.2 g.) was boiled for 1.5 hr. with 2*N* NaOH (2 ml.), or when cholyl hydrazine (1 g.) was refluxed for 1 hr. with 2*N* NaOH (10 ml.). The acid was oxidized in the usual way to dehydrocholic acid, m.p. 232–234°, decomp. Compound (II) was purified by solution in acetone and precipitation with light petroleum, followed by solution in dilute NaOH and precipitation with CO₂. After drying, it melted at *ca.* 150° and decomposed with frothing above 200°. (Found, on a sample dried to constant weight at 80° *in vacuo*: C, 62.5; H, 8.4; N, 7.2; S, 5.4%; mol. wt. (by titration and comparison with (IV), $R_1 = H$) = 573,545. C₃₀H₄₇O₆N₃S; calcd. C, 62.4; H, 8.1; N, 7.3; S, 5.5%; mol. wt. = 577.)

The substance gave a red colour in the Liebermann-Burchard test; it dissolved readily in alcohol, acetone, acetic acid, and also in NaOH, KOH, Na₂CO₃ and NH₃ solutions, from which it could be precipitated with CO₂. It was almost insoluble in water, chloroform, benzene, ether and ethyl acetate, and in dilute acids.

A solution of (II) in 30% alcohol gave quantitatively the amount of colour, calculated on a molecular weight basis, as sulphanilamide similarly treated by the diazotization method of Bratton & Marshall [1939]. Results on both compounds by this method were not exactly reproducible.

For oxidation, a solution of 0.2 g. of (II) in 2 ml. of acetic acid was cooled in water and treated gradually with 1.5 ml. of a solution of CrO₃ (2 g. dissolved in the minimal amount of water and brought to 10 ml. with acetic acid). Reaction with effervescence and formation of a precipitate occurred immediately. A little water was added to dissolve the precipitate and the mixture was kept for 15 min. at room temperature. It was then poured into excess of water and left overnight. The precipitate was collected, washed and stirred with dilute NaHCO₃. The filtered solution was acidified with HCl, and the precipitate collected, washed and recrystallized from dilute alcohol, from which it formed long needles of m.p. 229–232°, decomp., not depressed by dehydrocholic acid. The same acid was similarly obtained from the original condensation product.

α -Benzoyl β -p-acetamidobenzenesulphonyl hydrazine (IV, $R_1 = \text{CO} \cdot \text{CH}_3$). Benzoyl hydrazine (1 g.) and p-acetamidobenzenesulphonyl chloride (1.7 g.) were mixed in pyridine (10 ml.). The temperature rose to 47°. After 15 min., the mixture was diluted with HCl and water and the precipitated solid collected, washed and recrystallized from dilute alcohol, from which it formed white needles of m.p. 219–220°, decomp. Yield, 1.8 g. (Found: C, 53.9; H, 4.5; S, 10.0%; mol. wt. (Rast) 315. $\text{C}_{15}\text{H}_{15}\text{O}_4\text{N}_3\text{S}$ requires C, 54.1; H, 4.5; S, 9.6%; mol. wt. 333.)

α -Benzoyl β -p-aminobenzenesulphonyl hydrazine (IV, $R_1 = \text{H}$). The above substance (IV, $R_1 = \text{CO} \cdot \text{CH}_3$) (1 g.) was refluxed with 2N NaOH (10 ml.) for 20 min. The mixture frothed, became cloudy and evolved an almond odour. After dilution, it was saturated with CO_2 , when crystals were deposited. These were collected, washed and recrystallized from dilute alcohol, from which (IV, $R_1 = \text{H}$) separated as long white needles of m.p. 190–192°, decomp. Yield, 0.4 g. (Found: C, 54.2; H, 4.5; N, 14.4; S, 11.4%; mol. wt. (Rast) 316. $\text{C}_{13}\text{H}_{13}\text{O}_3\text{N}_3\text{S}$ requires C, 53.6; H, 4.5; N, 14.4; S, 11.0%; mol. wt. 291.)

After treatment with HCl, the bicarbonate liquors from the above hydrolysis yielded a small amount of benzoic acid. With a solution of 2:4-dinitrophenylhydrazine in HCl, they gave the 2:4-dinitrophenylhydrazone of benzaldehyde.

Compound IV ($R_1 = \text{H}$) gave a positive diazo-reaction; it dissolved in dilute acids and alkalis.

SUMMARY

For therapeutic trial, there have been prepared two compounds of cholic acid and sulphanilamide. One of these substances has a free $-\text{SO}_2 \cdot \text{NH}_2$ group, the other a free $-\text{NH}_2$ group. The latter derivative was made by reaction between cholic acid 'hydrazide' and p-acetamidobenzenesulphonyl chloride. It is suggested that this simple method can be used generally for preparation of compounds of sulphanilamide and non-ketonic carboxylic acids. For comparison, and as a further example, the corresponding benzoic acid derivative was synthesized.

REFERENCES

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