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Research Papers

The diuretic activity of clorexolone and some related phthalimides and 1-oxoisoindolines

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N-Substituted 4-chloro-5-sulphamoylphthalimides showed diuretic activity in the rat. Reduction of the carbonyl group in the position *para* to the 5-sulphamoyl group in the phthalimides produced even more active compounds. The relationship between potency and the nature of the *N*-substituent was examined in 32 phthalimides and 10 reduced derivatives. Maximum potency was found in the *N*-cycloalkyl and *N*-cycloalkylmethyl compounds, one of which, 5-chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline (clorexolone), was 300 times as active as chlorothiazide in the rat. This compound was examined in more detail in both the rat and the dog.

THE discovery of clinically useful diuretic activity amongst sulphamoylbenzothiadiazines (I; Novello & Sprague, 1957) has been followed by the synthesis of a great number of derivatives and analogues, many of which proved to be potent compounds.

We decided to investigate some new major structural modifications of the heterocyclic ring of the benzothiadiazines (Cornish, Lee & Wragg, 1963; Lee & Wragg, 1963), leaving intact the *o*-chlorobenzenesulphonamide part of the molecule apparently essential to diuretic activity. It was already known that this approach could result in new structures with diuretic activity, for example, the saccharin derivative (II; Merck & Co. Inc., 1960), and the 1,2,3,4-tetrahydro-1-oxoquinazoline (III; quinethazone; Cohen, Klarberg & Vaughan, 1959). On the other hand, the approach could lead to inactive compounds, for example, the disulphimide (IV; Logemann, Giraldi & Galimberti, 1959).

The present work concerns the 4-chloro-5-sulphamoylphthalimide (V) and 5-chloro-1-oxo-6-sulphamoylisoindoline (VI) analogues of chlorothiazide (I; R=H). Of 32 *N*-substituted derivatives of the type (V) prepared (Table 1), most had diuretic activity (Lee & Wragg, 1960). Chemical reduction of a number of these phthalimides gave the corresponding 1-oxoisoindolines (VI) which proved to be even more active diuretics (Table 2; Lee & Wragg, 1961). One of these (VI; R=cyclohexyl; compound No. 39; clorexolone) was considered of sufficient interest to justify clinical trials (Simpson, 1964).

A 5-chloro-1-oxo-6-sulphamoylisoindoline structure (VI) was assigned to the products obtained by chemical reduction of the phthalimides (V) on the basis of the following evidence:

(*a*) Theoretically, the reaction could also yield the isomeric 6-chloro-1-oxo-5-sulphamoylisoindolines (VII), but in practice only a single reduction product is formed.

(*b*) In the most interesting case, the single reduction product, clorexolone, was assigned the structure (VI; R=cyclohexyl) because the same compound was obtained by the alternative route 1 (Fig. 1) from 5-chloro-2-cyclohexyl-1-oxoisoindoline (VIII; R=H).

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(*c*) To minimise the possibility of ambiguity, the 6-chloro-1-oxo-5-sulphamoylisoindoline isomer (VII; R=cyclohexyl) of clorexolone (VI; R=cyclohexyl) has also been synthesised by the similar route 2 (Fig. 1), starting from 6-chloro-2-cyclohexyl-1-oxoisoindoline (IX; R=H). The product (VII; R=cyclohexyl) and the starting material (IX; R=H) were both shown to be different from their isomers clorexolone (VI; R=cyclohexyl) and 5-chloro-2-cyclohexyl-1-oxoisoindoline (VIII; R=H) respectively.

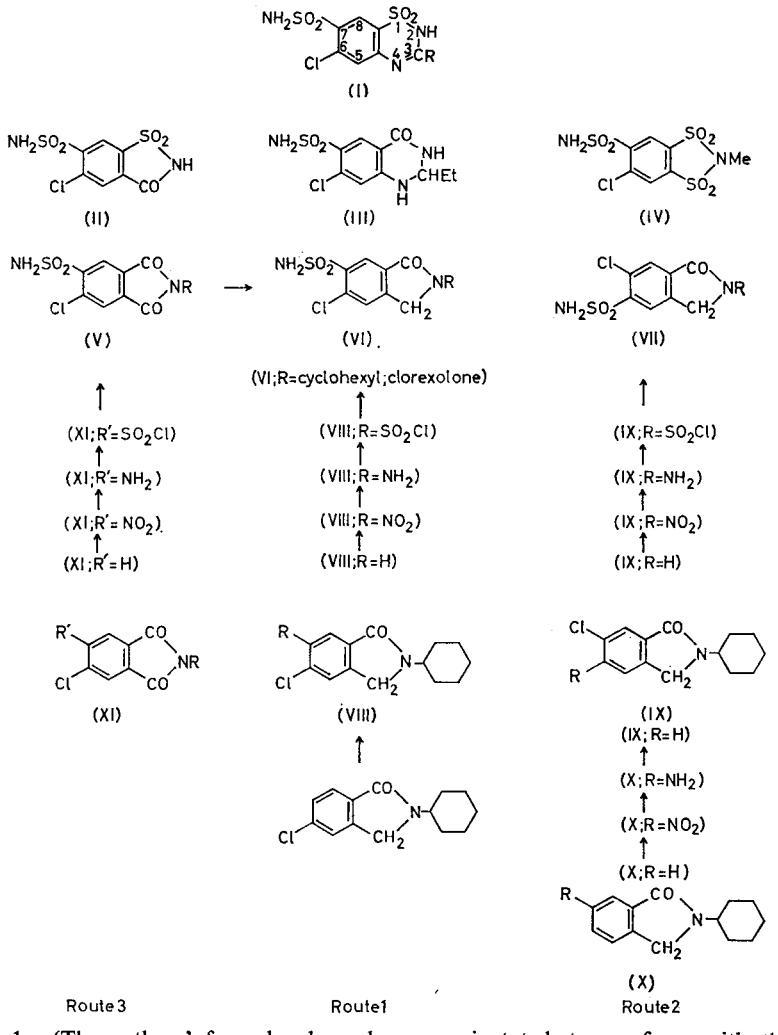


FIG. 1. (The authors' formulae have been reorientated to conform with this Journal's practice).

(*d*) The structure assigned to the starting material (IX; R=H) depended on its preparation from 2-cyclohexyl-6-nitro-1-oxoisoindoline (X; R=NO_2),

prepared in turn from 2-cyclohexyl-1-oxoisoindoline (X; R=H) under nitration conditions which, when applied to 2-methyl-1-oxoisoindoline, had been shown to give the 6-nitro-derivative (Borsche, Diacont & Hanau, 1934).

Since this work was completed certain 4-substituted 5-sulphamoylphthalimides have been described as diuretics in a patent (Novello, 1962) in which an entirely different synthetic route to that described above was used.

Experimental

SYNTHETIC METHODS

Preparation of the N-*substituted phthalimides* (V). Each of the compounds examined (Table 1) was prepared from 4-chloro-5-sulphamoylphthalimide (V; R=H) by reaction with the appropriate primary amine (Method A), or halogeno-compound in the presence of a suitable condensing agent (Method B).

The primary intermediate (V; R=H) was prepared from 4-chlorophthalimide (XI; R=R′=H) by the reaction sequence shown as route 3 (Fig. 1).

4-*Chloro*-5-*nitrophthalimide* (XI; R=H, R′=NO_2). 4-Chlorophthalimide (20 g; Levy & Stephen, 1931) was added to a mixture of 20% oleum (200 ml) and fuming nitric acid (24 ml). The solution was heated at 80° for 30 min, cooled, and poured on to ice (2 kg) to give, after recrystallisation from ethanol, 4-chloro-5-nitrophthalimide, m.p. 198–200°. Found: C, 42·4; H, 2·1; N, 12·3; $C_8H_3ClN_2O_4$ requires C, 42·4; H, 1·3; N, 12·4%.

Similarly prepared was 4-chloro-*N*-methyl-5-nitrophthalimide (XI; R=Me, R′=NO_2) m.p. 173–175°, from chloroform. Found: N, 11·6; Cl, 15·5; $C_9H_5ClN_2O_4$ requires N, 12·05; Cl, 14·8%.

5-*Amino*-4-*chlorophthalimide* (XI; R=H, R′=NH_2). 4-Chloro-5-nitrophthalimide (8 g) was added to a solution of stannous chloride (28 g) in concentrated hydrochloric acid (150 ml). The mixture was heated at 60° for 30 min. When cold, the precipitated solid was filtered off, washed well with water, and recrystallised from dimethylformamide to give 5-amino-4-chlorophthalimide, m.p. 314–315°. Found: C, 48·9; H, 3·05; N, 14·5; $C_8H_5ClN_2O_2$ requires C, 48·9; H, 2·55; N, 14·25%.

Similarly prepared was 5-amino-4-chloro-*N*-methylphthalimide (XI; R=Me, R′=NH_2), m.p. 209–211°, from ethanol. Found: N, 14·0; Cl, 17·0; $C_9H_7ClN_2O_2$ requires N, 13·3; Cl, 16·8%.

4-*Chlorophthalimide*-5-*sulphonyl chloride* (XI; R=H, R′=SO_2Cl). Preparation of this intermediate by direct chlorsulphonation of 4-chlorophthalimide failed even at 150°. The following procedure was therefore adopted:

5-Amino-4-chlorophthalimide (20 g) was diazotised at 0–5° with sodium nitrite (9 g) in concentrated hydrochloric acid (200 ml). The diazonium solution was added to a solution of sulphur dioxide (40 ml) in glacial

acetic acid (110 g) containing cuprous chloride (1 g). The reaction product was filtered off, washed with water and dried. Recrystallisation from benzene gave 4-chlorophthalimide-5-sulphonyl chloride, m.p. 169–171°. Found: C, 34·7; H, 2·5; N, 5·2; $C_8H_3Cl_2NO_4S$ requires C, 34·3; H, 1·1; N, 5·0%.

Similarly prepared was 4-chloro-*N*-methylphthalimide-5-sulphonyl chloride (XI; R=Me, R′=SO_2Cl), m.p. 161–163° Found: N, 4·9; S, 11·3; $C_9H_5Cl_2NO_4S$ requires N, 4·7; S, 10·9%.

4-*Chloro*-5-*sulphamoylphthalimide* (V; R=H). 4-Chlorophthalimide-5-sulphonyl chloride (20 g) was added to liquid ammonia (200 ml). The solution was evaporated to dryness and the solid product triturated with water. The solid was filtered off and heated with concentrated hydrochloric acid (100 ml) on a steam-bath for 30 min. The product was filtered off and washed well with water. Recrystallisation from methanol gave 4-chloro-5-sulphamoylphthalimide, m.p. 292–294°. Found: C, 36·8; H, 2·45; N, 10·6; $C_8H_5ClN_2O_4S$ requires C, 36·9; H, 1·9; N, 10·75%.

Similarly prepared was 4-chloro-*N*-methyl-5-sulphamoylphthalimide (V; R=Me), m.p. 238–240°. Found: Cl, 12·6; S, 11·5; $C_9H_7ClN_2O_4S$ requires Cl, 12·9; S, 11·65%.

Typical example of the use of preparative Method A. 4-Chloro-5-sulphamoylphthalimide (10 g) was refluxed in amyl alcohol (100 ml) with cyclohexylamine (3·8 g) for 4 hr. The reaction mixture was cooled. The crystalline solid was filtered off and recrystallised from methanol to give 4-chloro-*N*-cyclohexyl-5-sulphamoylphthalimide (V; R=cyclohexyl), m.p. 216–218°. Found: N, 8·3; Cl, 10·6; $C_{14}H_{15}ClN_2O_4S$ requires N, 8·2; Cl, 10·4%.

The success of this route was dependent, amongst other factors, on the volatility and the degree of steric hindrance of the primary amine used. For instance, t-butylamine failed to react under these conditions. Amine in excess of one mole with more stringent reaction conditions led to side reactions; this reaction with undiluted benzylamine in excess at reflux resulted in the formation of 3,*NN*-tribenzyl-4-sulphamoylphthaldiamide.

Typical example of the use of preparative Method B. 4-Chloro-5-sulphamoylphthalimide (10 g) was dissolved in dry dimethylformamide (40 ml). To this solution was added sodium hydride (50% oil dispersion; 1·81 g). The mixture was heated to 70° and methyl iodide (5·9 g) in dry dimethylformamide (10 ml) was added. The solution was stirred at 70° for 1 hr, cooled, and poured on to water. The precipitated solid was filtered off and recrystallised from methanol to give 4-chloro-*N*-methyl-5-sulphamoylphthalimide (V; R=Me), m.p. 238–240°. Found: C, 39·5; H, 2·9; N, 10·1; $C_9H_7ClN_2O_4S$ requires C, 39·35; H, 2·55; N, 10·2%.

This route could feasibly have given the isomeric derivative with the methyl group on the sulphonamide nitrogen atom. This possibility was excluded by the preparation of an authentic sample from 4-chloro-*N*-methylphthalimide (XI; R=Me; R′=H) *via* route 3 (Fig. 1), a synthesis already detailed above. Mixed melting-points and infrared

spectra showed the two samples of 4-chloro-*N*-methyl-5-sulphamoylphthalimide (V; R=Me) to be identical.

Preparation of the N-*substituted* 1-*oxoisoindolines* (VI). Each compound (Table 2) was prepared by the tin and hydrochloric acid reduction of the corresponding phthalimide (V).

Typical reduction, yielding 5-*chloro*-2-*cyclohexyl*-1-*oxo*-6-*sulphamoylisoindoline* (VI; R=cyclohexyl; compound No. 39; clorexolone). 4-Chloro-*N*-cyclohexyl-5-sulphamoylphthalimide (V; R=cyclohexyl) (1000 g) was dissolved in a mixture of dimethylformamide (5·65 litres) and methanol (5·65 litres). Granulated tin (835 g) was added, followed by concentrated hydrochloric acid (3·45 litres). Some heat was applied from a steam-bath to the stirred reaction mixture, whereupon an exothermic reaction was initiated. The reaction mixture was gently refluxed for 3 hr with stirring and the solution was decanted from the tin residue and concentrated *in vacuo* on a steam-bath until crystallisation started. Concentrated hydrochloric acid (2·5 litres) was added with stirring and the suspension cooled and filtered. The solid was washed with hydrochloric acid and finally water. The damp solid was dissolved in 2N sodium hydroxide, filtered, diluted with water and poured into rapidly stirred 2N hydrochloric acid (3 litres). After stirring for 1 hr the solid was filtered off and washed thoroughly with water. The product was dried at 80° to give a white solid which was recrystallised from a 50/50 mixture of dimethylformamide and methanol to give 5-chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline (548 g, 55%) as a white solid, m.p. 266–268°. Found: N, 8·4; Cl, 10·7; $C_{14}H_{17}ClN_2O_3S$ requires N, 8·5; Cl, 10·8%.

Alternative preparation of 5-*chloro*-2-*cyclohexyl*-1-*oxo*-6-*sulphamoylisoindoline* (VI; R=cyclohexyl) *via* route 1 (Fig. 1). 4-Chlorophthalimide (263 g) was reacted in amyl alcohol (2·6 litres) with cyclohexylamine (143·5 g, 1 mole) at reflux temperature for 16 hr. The cooled solution was filtered and the solid was recrystallised from ethanol to give 4-chloro-*N*-cyclohexylphthalimide (250 g, 66%) as a solid, m.p. 134–136°. Found: N, 5·5; Cl, 13·5; $C_{14}H_{14}ClNO_2$ requires N, 5·3; Cl, 13·5%.

4-Chloro-*N*-cyclohexylphthalimide (250 g) was dissolved in glacial acetic acid (2·5 litres). Concentrated hydrochloric acid (555 ml) and tin (278 g) were added and the suspension was heated on a steam-bath for 16 hr. The cooled solution was filtered and concentrated to dryness *in vacuo* to give a white solid. This solid was dissolved in water and the precipitated oil extracted with chloroform. The chloroform solution was dried and concentrated *in vacuo* to give a solid which, after recrystallisation from acetone, yielded 5-chloro-2-cyclohexyl-1-oxoisoindoline (VIII; R=H; 103 g, 43%), m.p. 140–142°. Found: N, 5·6; Cl, 14·5; $C_{14}H_{16}ClNO$ requires N, 5·5; Cl, 14·2%.

5-Chloro-2-cyclohexyl-1-oxoisoindoline (102·9 g) was dissolved in concentrated sulphuric acid (665 ml) and potassium nitrate (723 g) in concentrated sulphuric acid (166 ml) was added at 0°. The reaction mixture was allowed to warm to room temperature and stirred at 25° for

12 hr. The reaction mixture was poured on to ice to give a cream solid which, after recrystallisation from benzene, gave 5-chloro-2-cyclohexyl-6-nitro-1-oxoisoindoline (VIII; R=NO_2; 46·7 g, 44%) as a white solid, m.p. 164–168°. Found: N, 9·3; Cl, 12·2; $C_{14}H_{15}ClN_2O_3$ requires N, 9·55; Cl, 12·0%.

5-Chloro-2-cyclohexyl-6-nitro-1-oxoisoindoline (93·9 g) was reduced in concentrated hydrochloric acid (1970 ml) with stannous chloride (376 g), whereupon the reaction temperature rose to 70°. The resulting solution was cooled in ice and filtered. The product was washed well with water, filtered and dried to give 6-amino-5-chloro-2-cyclohexyl-1-oxoisoindoline (VIII; R=NH_2; 74·1 g, 87·6%) which, after recrystallisation from benzene, had m.p. 216–218°. Found: N, 10·7; Cl, 13·7; $C_{14}H_{17}ClN_2O$ requires N, 10·6; Cl, 13·4%.

6-Amino-5-chloro-2-cyclohexyl-1-oxoisoindoline (42·5 g) was dissolved in concentrated hydrochloric acid (425 ml) and the solution diazotised at 0–5° by the addition of sodium nitrite (21·25 g) in water (125 ml). The resulting diazonium salt solution was added to a solution of liquid sulphur dioxide (93 ml) in glacial acetic acid (243 ml) containing cuprous chloride (2·25 g). A yellow solid was precipitated; this was filtered off, washed, dried and recrystallised from benzene to give 5-chloro-2-cyclohexyl-1-oxoisoindoline-6-sulphonyl chloride (VIII; R=SO_2Cl; 45 g, 80%) as a cream coloured solid, m.p. 171–174°. Found: N, 4·1; Cl, 19·6; $C_{14}H_{15}Cl_2NO_3S$ requires N, 4·0; Cl, 20·4%.

This sulphonyl chloride (23·7 g) was reacted with liquid ammonia (237 ml). Crystallisation of the product from methanol gave 5-chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline (VI; R=cyclohexyl; 14·2 g, 53%), m.p. 259–261°, identical (mixed m.p.; infrared spectrum) with the sample prepared above by chemical reduction of the corresponding phthalimide (V; R=cyclohexyl). Found: N, 8·2; Cl, 11·0; $C_{14}H_{17}ClN_2O_3S$ requires N, 8·5; Cl, 10·8%.

Preparation of 6-chloro-2-cyclohexyl-1-oxo-5-sulphamoylisoindoline (VII; R=cyclohexyl) *via* route 2 (Fig. 1). *N*-Cyclohexylphthalimide (9 g) was dissolved in glacial acetic acid (100 ml). To this solution was added tin (10 g) followed by concentrated hydrochloric acid (50 ml). The solution was heated on a steam-bath until all the tin had dissolved. The cooled solution was filtered and poured into water (250 ml) and the oil was extracted into ether. The extract was dried and concentrated. The yellow residue was dissolved in acetone and then water was added. The crude solid thus formed was recrystallised from acetone to give 2-cyclohexyl-1-oxoisoindoline (X; R=H; 4·3 g, 51%), m.p. 92–97°.

2-Cyclohexyl-1-oxoisoindoline (5 g) was dissolved in concentrated sulphuric acid (33 ml). A solution of potassium nitrate (3·66 g) in concentrated sulphuric acid (8·3 ml) was added to the stirred solution at 0°. The solution was stirred at 0° for 6 hr and then poured on to ice/water (200 ml). The precipitated crude solid was recrystallised from ethanol to give 2-cyclohexyl-6-nitro-1-oxoisoindoline (X; R=NO_2; 4·5 g, 85%) as a white solid, m.p. 125–128°. Found: C, 64·8; H, 6·5; N, 11·2; $C_{14}H_{16}N_2O_3$ requires C, 64·6; H, 6·15; N, 10·75%.

2-Cyclohexyl-6-nitro-1-oxoisoindoline (132 g) was added to a solution of stannous chloride (454 g) in concentrated hydrochloric acid (2770 ml). The reaction mixture was warmed to 50° on a steam-bath. The solution was filtered hot and the filtrate cooled. The white solid which crystallised out was dissolved in the minimum quantity of water and was treated with a large excess of sodium hydroxide (2N). The white solid precipitated was washed with water and dried *in vacuo*. The dried solid (80 g, 70%) was 6-amino-2-cyclohexyl-1-oxoisoindoline (X; R=NH_2).

The crude amino-compound (80 g) was added to concentrated hydrochloric acid (809 ml). To this suspension was added a solution of sodium nitrite (41 g) in water (100 ml) at 0–5°. The solution of diazonium salt was poured into a solution of cuprous chloride (110 g) in concentrated hydrochloric acid (715 ml). The precipitated solid was recrystallised from acetone to give 6-chloro-2-cyclohexyl-1-oxoisoindoline (IX; R=H; 70 g, 80%), m.p. 134–137°. Found: N, 5·8; Cl, 14·3; $C_{14}H_{16}ClNO$ requires N, 5·6; Cl, 14·25%). This product was different (mixed m.p., infrared spectrum) from a sample of the isomer 5-chloro-2-cyclohexyl-1-oxoisoindoline (VIII; R=H) prepared, as described above, *via* route 1 (Fig. 1).

6-Chloro-2-cyclohexyl-1-oxoisoindoline (69 g) was added at below 10° to concentrated sulphuric acid (460 ml). To this solution was added at 0° during 30 min a solution of potassium nitrate (50 g) in concentrated sulphuric acid (115 ml). The solution was stirred at 0° for a further 5 hr and then poured on to ice/water. The precipitated solid was recrystallised from benzene to give 6-chloro-2-cyclohexyl-5-nitro-1-oxoisoindoline (IX; R=NO_2), 10·4 g, m.p. 158–162°.

6-Chloro-2-cyclohexyl-5-nitro-1-oxoisoindoline (10·4 g) was dissolved in a solution of stannous chloride (40·8 g) in concentrated hydrochloric acid (214 ml). The solution was heated at 85° for 1 hr, cooled in an ice-bath and then filtered. The solid was recrystallised from methanol to give 5-amino-6-chloro-2-cyclohexyl-1-oxoisoindoline (IX; R=NH_2; 9·0 g), m.p. 188–190°. Found: N, 10·5; Cl, 13·5; $C_{14}H_{17}ClN_2O$ requires N, 10·4; Cl, 13·45%.

5-Amino-6-chloro-2-cyclohexyl-1-oxoisoindoline (7·7 g) was diazotised in concentrated hydrochloric acid (77 ml) at 0–5° with sodium nitrite (3·85 g) in water (22 ml). The diazonium solution was added to a solution of sulphur dioxide (16·7 ml) and cuprous chloride (0·4 g) in glacial acetic acid (44 ml). The 6-chloro-2-cyclohexyl-1-oxoisoindoline-5-sulphonyl chloride (IX; R=SO_2Cl; 8·8 g, 87%), m.p. 167–169°, was used without further purification.

The crude 6-chloro-2-cyclohexyl-1-oxoisoindoline-5-sulphonyl chloride (8·8 g) was added to liquid ammonia (88 ml). After the excess liquid ammonia had evaporated the crude solid was washed with water and recrystallised from methanol to give 6-chloro-2-cyclohexyl-1-oxo-5-sulphamoylisoindoline (VII; R=cyclohexyl; compound No. 36; 5·0 g, 86%), m.p. 234–236°. Found: N, 8·4; Cl, 10·85; $C_{14}H_{17}ClN_2O_3S$ requires N, 8·5; Cl, 10·8%.

This product was different (mixed m.p., infrared spectrum) from the sample of the isomer 5-chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline (VI; R=cyclohexyl) prepared, as described above, *via* route 1 (Fig. 1).

PREPARATION OF THE COMPOUNDS NOS. 33–35

5-Chloro-2-cyclohexyl-3-hydroxy-1-oxo-6-sulphamoylisoindoline (compound No. 33). 4-Chloro-*N*-cyclohexyl-5-sulphamoylphthalimide (1·0 g) was dissolved in a mixture of methanol (50 ml) and 5N sulphuric acid (11 ml). The solution was electrolysed at room temperature for 15 min at a current density of 0·03 A cm^{-2} using a lead cathode and a carbon anode contained in a Visking sausage skin. The solvent employed above was also used as anolyte. The potential of the working cathode was −0·7 V referred to a saturated calomel electrode. After about 8 min a precipitate appeared in the catholyte and precipitation was complete after 15 min. The catholyte was filtered and the filtrates from 14 such reductions were bulked, rendered neutral with solid sodium carbonate, filtered and concentrated. The residue was recrystallised from methanol to give 5-chloro-2-cyclohexyl-3-hydroxy-1-oxo-6-sulphamoylisoindoline (2·8 g, 20%), m.p. 198–200° (decomp.). Found: C, 50·4; H, 5·6; Cl, 10·1; N, 8·2; S, 8·4; $C_{14}H_{17}ClN_2O_4S$ requires C, 48·8; H, 4·9; Cl, 10·3; N, 8·1; S, 9·3%.

That the hydroxyl group was in position 3 in the foregoing product was established by its reduction to 5-chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline (VI; R=cyclohexyl), as follows.

5-Chloro-2-cyclohexyl-3-hydroxy-1-oxo-6-sulphamoylisoindoline (10 g) was dissolved in a mixture of dimethylformamide (56 ml) and methanol (56 ml). Concentrated hydrochloric acid (34 ml) and tin (8·5 g) were added. The suspension was stirred and heated on a steam-bath for 18 hr, after which the hot reaction mixture was filtered and the filtrate concentrated. The residue was treated with concentrated hydrochloric acid (100 ml) and the residue filtered off. This residue was dissolved in sodium hydroxide (2N, 50 ml) and then treated with hydrochloric acid (2N, 200 ml). The precipitated solid was filtered off, dried and recrystallised from dimethylformamide/methanol to give 5-chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline (VI; R=cyclohexyl; 4·7 g, 50%), m.p. 259–261°, which was identical (mixed m.p. and infrared spectrum) with an authentic sample.

5-Chloro-2-cyclohexyl-6-methylsulphamoyl-1-oxoisoindoline, (compound No. 34). 4-Chloro-*N*-cyclohexyl-5-methylsulphamoylphthalimide (10·0 g) was reduced with tin (8·05 g) and a mixture of hydrochloric (33 ml) and acetic (100 ml) acids to give after filtration, concentration and dilution with water, a solid. This solid was recrystallised from methanol to give 5-chloro-2-cyclohexyl-6-methylsulphamoyl-1-oxoisoindoline as a white solid (1·75 g, 20%), m.p. 233–234°. Found: N, 8·15; S, 9·5; $C_{15}H_{19}ClN_2O_3S$ requires N, 8·2; S, 9·35%.

6-Acetylsulphamoyl-5-chloro-2-cyclohexyl-1-oxoisoindoline (compound No. 35). 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline (10 g)

was dissolved in acetic anhydride (100 ml). The solution was heated at reflux for 2 hr. The excess acetic anhydride was removed *in vacuo* and the sticky residue triturated to a cream coloured solid with water. Recrystallisation from 50% aqueous acetic acid gave 6-acetylsulphamoyl-5-chloro-2-cyclohexyl-1-oxoisoindoline as a white solid (8·7 g, 80%), m.p. 145° (decomp.). Found: Cl, 9·6; N, 7·5; $C_{15}H_{19}ClN_2O_4S$ requires Cl, 9·6; N, 7·55%.

Pharmacological methods

DIURETIC ACTIVITY

The method was similar to that of Lipschitz, Hadidian & Kerpscar (1943).

Eight groups of four male albino rats were starved overnight and were then given by stomach tube, isotonic saline at 37°, 25 ml/kg, in which the test compound was dispersed. The doses used were chosen after a preliminary experiment so as to produce an approximately threefold increase in urine excretion. Four randomly chosen groups received the standard compound, chlorothiazide, and the remaining groups received the test compound. Samples of urine were collected for 5 hr. Na^+ and K^+ in the urine were determined by flame photometry, and Cl^- was estimated with a potentiometric microtitration apparatus. Control experiments were made 2 days before and four days after the test experiment, the object being first to establish, and then to check, the base-line urinary excretion pattern for each group of 4 rats.

Quantitative dose-response relationships. To compare accurately the dose-response relationships, two of the *N*-substituted phthalimides and two reference compounds, chlorothiazide and hydrochlorothiazide, were assayed at 3 dose levels (in the ratio 1 : 2 : 4) selected to fall on the maximum slope portion of the dose-response curves. At weekly intervals, all 32 rats received a single dose level of one of these compounds on a random basis. Otherwise, the experimental conditions were as described above. The hydrochlorothiazide was obtained from powdered 25 mg tablets (Ciba Laboratories Ltd.).

ACUTE ORAL TOXICITY IN MICE

Three groups of 5 albino mice, each weighing 16–18 g, were used. The mice were starved overnight and then given, by stomach tube, 1, 2 and 3 g/kg, respectively, of the compound under test suspended in 0·6% tragacanth solution. Observations were made for 2 weeks after dosing.

SUPPLEMENTARY METHODS USED TO STUDY 5-CHLORO-2-CYCLOHEXYL-1-OXO-6-SULPHAMOYLISOINDOLINE (VI; R=cyclohexyl; compound No. 39; clorexolone)

A cross-over test (cf. British Pharmacopoeia, 1963, p. 1093) was performed in the rat with clorexolone and chlorothiazide.

The two compounds were also compared in the dog using a cross-over test. Perineostomised female dogs were starved overnight and given

distilled water, 40 ml/kg, by stomach tube. One hr later the bladder was emptied by catheterisation. Compounds were given orally by capsule, and the bladder emptied hourly for 5 hr. Control values were obtained the day before an experiment. Na^+, K^+ and Cl^- were estimated in the urine. When the pH was measured the urines were collected under liquid paraffin. Each dog received individual doses in a randomly selected order at weekly intervals.

In creatinine clearance estimations in dogs, 200 mg/kg creatinine hydrochloride was injected subcutaneously as a 12·5% solution. Supplementary creatinine and water were administered to achieve constant conditions. Two control clearance determinations preceded administration of clorexolone, and, thereafter, clearance determinations were made and compared with similar observations in the same animals when they had not received a diuretic. Creatinine in the urine and plasma was estimated by the alkaline picrate method of Bosnes & Taussky (1945).

The carbonic anhydrase inhibition *in vitro* was studied by the method of Philpot & Philpot (1936). The carbonic anhydrase extract was prepared from sheep or pig erythrocytes using an ethanol : chloroform mixture and was stored at −70° (Booth & Roughton, 1946).

In some experiments the urine was made acid or alkaline by the oral administration of ammonium chloride or sodium bicarbonate respectively. Rats received 0·5 g/kg/day ammonium chloride, or 1 g/kg/day sodium bicarbonate for 7 days (Farah, Bender, Kruse & Cafruny, 1959). Dogs received 100 m-equiv./dog/day of the salts for 7 days (Baer, Russo & Beyer, 1959).

Acute oral and intravenous LD50 values were determined in mice and rats.

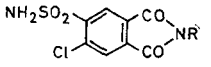
Results

The diuretic potency of the *N*-substituted 4-chloro-5-sulphamoylphthalimides (V) is summarised in Table 1. Highest activity was found when the *N*-substituent in (V) was a cyclohexyl, a methyl-substituted cyclohexyl, cycloheptyl or cyclo-octyl ring. Such compounds (Nos. 11 to 15) were about six times as potent as chlorothiazide. Reduction in size of the *N*-substituent to a cyclopentyl ring (No. 10) diminished activity, whilst the larger cyclododecyl ring eliminated activity (No. 16).

The pattern of excretion of water, Na^+, K^+ and Cl^- was similar to that produced by equipotent doses of any of these compounds or of chlorothiazide. More detailed comparisons of these excretions showed that compounds Nos. 10 and 11 (V; R=cyclopentyl and cyclohexyl respectively) produced dose-response curves which were parallel to those obtained for chlorothiazide and hydrochlorothiazide (e.g. see Fig. 2). Compounds Nos. 10 and 11 were 3·3 and 6·0 times as active as chlorothiazide, and 0·14 and 0·30 times as active as hydrochlorothiazide with respect to sodium excretion.

After the administration of compounds Nos. 1 and 2 (V; R=H and Me respectively) the urine was a bright yellow colour; tests for bile

TABLE 1. 4-CHLORO-5-SULPHAMOYL-*N*-SUBSTITUTED-PHTHALIMIDES (V): CHEMICAL CHARACTERISTICS AND DIURETIC ACTIVITY IN THE RAT

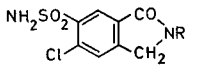


(V)

No.	R	Preparative method; m.p.; crystallisation solvent	Analytical figures			Diuretic activity (chlorothiazide = 1)
				N %	Cl %	
1	H	, 293–295°, methanol	Found	10·6		0·25
			$C_8H_5ClN_2O_4S$ req.	10·75		
2	Methyl	B, 242–244°, methanol	Found	10·3		0·5
			$C_9H_7ClN_2O_4S$ req.	10·2		
3	Allyl	A, 173–174°, ethanol	Found	9·2	12·0	0·25
			$C_{11}H_9ClN_2O_4S$ req.	9·3	11·8	
4	Prop-2-ynyl	B, 188–191° ethanol	Found	9·3	11·8	1
			$C_{11}H_7ClN_2O_4S$ req.	9·4	11·9	
5	Butyl	A, 178–181°, amyl alcohol	Found	8·4	11·2	1
			$C_{12}H_{13}ClN_2O_4S$ req.	8·8	11·3	
6	Dodecyl	A, 169–171°, ethanol	Found	6·65	8·0	0·5
			$C_{20}H_{29}ClN_2O_4S$ req.	6·5	8·3	
7	Isobutyl	A, 177–178°, methanol	Found	8·85	11·4	1
			$C_{12}H_{13}ClN_2O_4S$ req.	8·85	11·2	
8	2-Bromoethyl	(1), 191–193°, benzene	Found	7·85		1
			$C_{10}H_8BrClN_2O_4S$ req.	7·6		
9	2-Hydroxyethyl	A, 193–195°, ethanol	Found	9·4	11·8	1
			$C_{10}H_9ClN_2O_5S$ req.	9·2	11·7	
10	Cyclopentyl	A, 182–185°, ethanol	Found	8·4	10·9	3
			$C_{13}H_{13}ClN_2O_4S$ req.	8·5	10·8	
11	Cyclohexyl	A, 216–218°, methanol	Found	8·1	10·6	6
			$C_{14}H_{15}ClN_2O_4S$ req.	8·2	10·4	
12	4-Methylcyclohexyl	A, 206–209°, benzene	Found	7·6	10·0	6
			$C_{15}H_{17}ClN_2O_4S$ req.	7·9	9·9	
13	3-Methylcyclohexyl	A, 170–172°, benzene	Found	7·7	9·5	6
			$C_{15}H_{17}ClN_2O_4S$ req.	7·9	9·9	
14	Cycloheptyl	A, 206–208°, ethanol	Found	7·8	10·05	6
			$C_{15}H_{17}ClN_2O_4S$ req.	7·9	9·9	
15	Cyclo-octyl	A, 185–187°, ethanol	Found	7·6	9·6	6
			$C_{16}H_{19}ClN_2O_4S$ req.	7·55	9·6	
16	Cyclododecyl	A, 228–230°, glacial acetic acid	Found	6·3	8·3	0
			$C_{20}H_{27}ClN_2O_4S$ req.	6·5	8·3	
17	Bicyclohexyl-4-yl	A, 197–200°, benzene	Found	6·9	8·45	0
			$C_{20}H_{25}ClN_2O_4S$ req.	6·6	8·4	
18	3-Phenylnorborn-2-yl	A, 217–218°, glacial acetic acid	Found	6·4	8·1	0
			$C_{21}H_{19}ClN_2O_4S$ req.	6·5	8·2	
19	Decahydronaphth-2-yl	A, 249–251°, n-butanol	Found	6·9	8·9	0
			$C_{16}H_{21}ClN_2O_4S$ req.	7·1	9·0	
20	1,2,3,4-Tetrahydronaphth-2-yl	A, 210–213°, amyl alcohol	Found	6·5	8·6	0·25
			$C_{20}H_{15}ClN_2O_4S$ req.	6·8	8·9	
21	Benzyl	A, 213–215°, amyl alcohol	Found	7·6	10·2	1
			$C_{15}H_{11}ClN_2O_4S$ req.	8·0	10·1	
22	Phenethyl	A, 255–257°, amyl alcohol	Found	7·3	9·65	1
			$C_{16}H_{13}ClN_2O_4S$ req.	7·7	9·7	
23	*p*-Methylbenzyl	A, 215–218°, ethanol	Found	7·7	9·5	1
			$C_{16}H_{13}ClN_2O_4S$ req.	7·7	9·7	
24	*p*-Isopropylbenzyl	A, 190–191°, ethanol	Found	6·9	9·1	0·25
			$C_{18}H_{17}ClN_2O_4S$ req.	7·1	9·05	
25	*p*-Chlorobenzyl	A, 219–221°, methanol	Found	7·5	18·2	1
			$C_{15}H_{10}Cl_2N_2O_4S$ req.	7·3	18·5	
26	*m*-Trifluoromethylbenzyl	B, 200–202°, isopropanol	Found	(C = 46·3)	(H = 2·2)	<1
			$C_{16}H_{10}ClF_3N_2O_4S$ req.	(C = 45·9)	(H = 2·4)	
27	*p*-Nitrobenzyl	B, 242–244°, glacial acetic acid	Found	10·4		0·5
			$C_{15}H_{10}ClN_3O_6S$ req.	10·6		
28	Cyclohexylmethyl	B, 161–164°, isopropanol	Found	7·7	9·8	1
			$C_{15}H_{17}ClN_2O_4S$ req.	7·85	9·95	
29	Cyclopentylmethyl	B, 174°, methanol	Found	8·2	10·4	1
			$C_{14}H_{15}ClN_2O_4S$ req.	8·2	10·4	
30	Norborn-2-ylmethyl	A, 182°–184°, ethanol	Found	7·6	9·6	1
			$C_{16}H_{17}ClN_2O_4S$ req.	7·8	9·5	
31	Tetrahydrothiopyran-4-yl	A, 243–246°, n-butanol	Found	7·4		1
			$C_{13}H_{13}ClN_2O_4S_2$ req.	7·75		
32	Tetrahydropyran-2-ylmethyl	A, 221–223°, amyl alcohol	Found	8·0	9·8	1
			$C_{14}H_{15}ClN_2O_5S$ req.	7·8	9·9	

(1) by the action of PBr_3 on compound No. 9.

TABLE 2. 2-SUBSTITUTED 1-OXOISOINDOLINES (VI): CHEMICAL CHARACTERISTICS AND DIURETIC ACTIVITY IN THE RAT



(VI)

No.	R	M.p.; crystallisation solvent	Analytical figures	N%	Cl%	Diuretic activity (chlorothiazide = 1)
37	Isobutyl	242–243°, methanol	Found	8·9	11·9	75–100
			$C_{12}H_{15}ClN_2O_3S$ req.	9·3	11·75	
38	Cyclopentyl	281–285°, methanol	Found	8·8	11·2	100
			$C_{13}H_{15}ClN_2O_3S$ req.	8·9	11·3	
39	Cyclohexyl	266–268°, dimethylformamide/methanol	Found	8·4	10·6	300
			$C_{14}H_{17}ClN_2O_3S$ req.	8·5	10·8	
40	4-Methylcyclohexyl	265–270°, methanol	Found	8·0	10·3	100
			$C_{15}H_{19}ClN_2O_3S$ req.	8·2	10·4	
41	3-Methylcyclohexyl	245–250°, methanol	Found	8·1	10·0	100
			$C_{15}H_{19}ClN_2O_3S$ req.	8·2	10·4	
42	3,4-Dimethylcyclohexyl	274–276°, methanol	Found	7·9	10·1	200
			$C_{16}H_{21}ClN_2O_3S$ req.	7·9	10·0	
43	Cycloheptyl	261–262°, methanol	Found	8·1	10·4	50–100
			$C_{15}H_{19}ClN_2O_3S$ req.	8·2	10·6	
44	Cyclo-octyl	252–253°, methanol	Found	7·9	10·0	100
			$C_{16}H_{21}ClN_2O_3S$ req.	7·9	10·0	
45	Norborn-2-yl	274–275°, methanol	Found	8·0	9·0	100
			$C_{15}H_{17}ClN_2O_3S$ req.	8·2	9·4	
46	Cyclohexylmethyl	220–222°, methanol	Found	8·0	10·1	200
			$C_{15}H_{19}ClN_2O_3S$ req.	8·2	10·4	

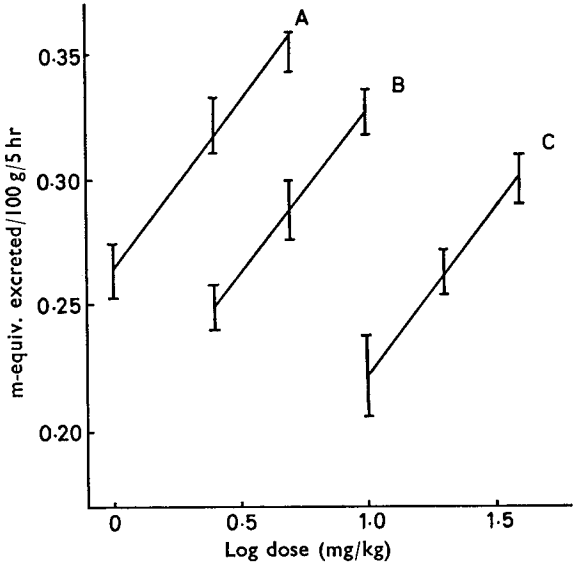


FIG. 2. Sodium ion excretion after hydrochlorothiazide (A), Compound No. 11 (B) and chlorothiazide (C). Vertical lines represent standard errors of the group mean value.

pigments were negative. Apart from this, the observation was not studied further.

With the exception of compounds Nos. 3 and 8, the *N*-substituted phthalimides (V) were not lethal to mice in oral doses of 3 g/kg. No toxic symptoms were noted. Compounds Nos. 3 (V; R=allyl) and 8 (V; R=2-bromoethyl) had LD50 values between 1–2 g/kg and 2–3 g/kg respectively.

Reduction of one of the carbonyl groups of the phthalimide (V; R=cyclohexyl) to –CH(OH)– giving the corresponding 3-hydroxy-1-oxoisoindoline (compound No. 33) produced a 10-fold increase in activity, whilst complete reduction of this carbonyl group to $-CH_2-$ to form the corresponding 1-oxoisoindoline (Table 2, compound No. 39; clorexolone), increased activity 50 fold. Table 2 illustrates the activity of 10 such 1-oxoisoindolines. Methylation or acetylation of the sulphonamido- group of 5-chloro-2-cyclohexyl-1-oxo-6-sulphamoylisoindoline produced at least a 10-fold decrease in diuretic activity (compound Nos. 34 and 35 respectively).

Reduction of the other carbonyl group of the phthalimide (V; R=cyclohexyl) to $-CH_2-$, giving the isomeric 6-chloro-5-sulphamoylisoindoline (VII; R = cyclohexyl; compound No. 36), abolished diuretic activity. The foregoing oxoisoindolines were not lethal to mice at 3 g/kg.

The most active compound in the above tests, clorexolone, was chosen for further study. In a cross-over test using 32 rats, it was 450 times as potent as chlorothiazide with respect to water excretion (fiducial limits

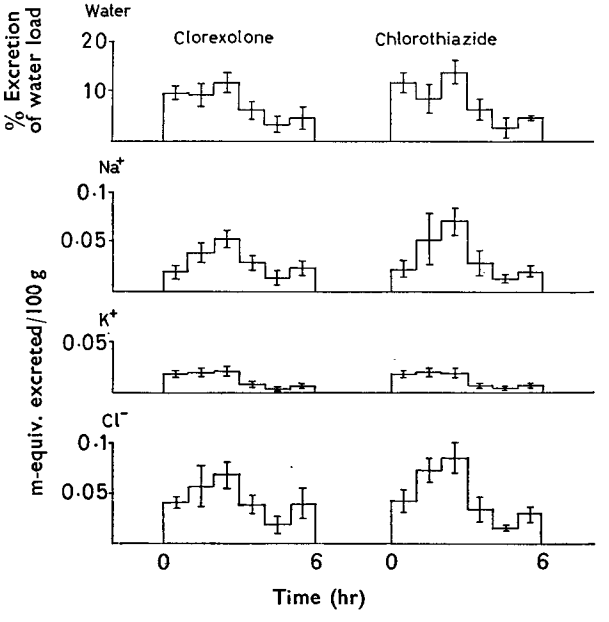


FIG. 3. Electrolyte and water excretion in rats after approximately equipotent doses of clorexolone, 0·025 mg/kg orally, and chlorothiazide, 10 mg/kg orally.

of error, P=0·05, 57–176%), and 320 times as potent with respect to sodium excretion (fiducial limits of error, P=0·05, 64–155%). The effect of approximately equipotent doses of the two compounds on hourly excretions of water, Na^+, K^+ and Cl^- are shown in Fig. 3. Both compounds produced their maximum effects in the third hr after an oral dose.

In rats, clorexolone (0·07 mg/kg), and chlorothiazide (20 mg/kg) produced a threefold increase in the total excretion of Na^+; they increased the concentration of Na^+ from a control value of 0·99 m-equiv./litre to 1·24 and 1·29 m-equiv./litre respectively.

In dogs, a comparison of clorexolone and chlorothiazide by a cross-over test is shown in Table 3. Although the doses chosen from a pre-

TABLE 3. COMPARISON OF THE DIURETIC ACTIVITIES OF CLOREXOLONE AND CHLOROTHIAZIDE IN DOGS

(The same 5 dogs, 9–19 kg, were used to obtain each result)

Compound	mg/kg orally	% Excretion water load	m-equiv. excreted/kg/5 hr		
			Na^+	K^+	Cl^-
None		67 ± 3*	0·43 ± 0·10	0·19 ± 0·03	0·37 ± 0·05
Clorexolone	0·1	81 ± 4	1·20 ± 0·16	0·45 ± 0·13	1·95 ± 0·20
,, ...	0·3	91 ± 4	1·54 ± 0·29	0·54 ± 0·17	2·41 ± 0·30
Chlorothiazide	1·0	79 ± 3	0·90 ± 0·20	0·43 ± 0·10	1·38 ± 0·20
,, ...	3·0	86 ± 5	1·11 ± 0·08	0·43 ± 0·09	1·57 ± 0·09

* Standard error of the mean.

liminary experiment were not equipotent, it can be shown graphically that clorexolone was about 50 times as active as chlorothiazide with respect to Na^+ excretion. The lowest dose of clorexolone (0·1 mg/kg) produced a threefold increase in Na^+ excretion, whereas at 1 mg/kg, chlorothiazide produced only a twofold increase in Na^+ excretion.

Both 0·1 and 0·3 mg/kg of clorexolone decreased the pH of the urine by 0·9 to 1·8 units. Clorexolone did not affect the clearance of exogenous creatinine in the conscious dog.

Carbonic anhydrase inhibition was studied in the presence of three doses of clorexolone and chlorothiazide, and in controls, with three observations at each concentration. The results may be summarised by stating that about 50% inhibition of carbonic anhydrase action *in vitro* was obtained by adding 1 ml of $2·2 \times 10^{-5}$M clorexolone, or 6×10^{-5}M chlorothiazide to the reaction mixture; clorexolone was therefore about three times as active as chlorothiazide.

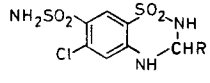
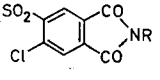
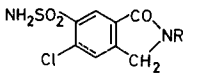
Clorexolone was diuretic in acidotic animals (8 rats, 1 dog; urine pH 0·5–0·9 units < controls) and in alkalotic animals (8 rats, 1 dog; urine pH 0·3–0·4 units > controls). The rats were given 0·07 mg/kg and the dogs 0·1 mg/kg of clorexolone. These treatments increased the excretion of water in the acidotic animals from 30 to 84% in the rats, and 49 to 85% in the dog, with corresponding increases in Na^+ excretion of 0·5 to 2·3 and 0·75 to 1·41 m-equiv./kg/5 hr. These treatments increased the excretion of water in alkalotic animals from 46 to 110% in the rats and 62 to 82% in the dogs with corresponding increases in Na^+ excretion of 1·0 to 3·1 and 0·6 to 1·49 m-equiv./kg/5 hr.

Acute oral toxicity tests showed that clorexolone, 6 g/kg, was not lethal to mice and 10 g/kg was not lethal to rats. No toxic symptoms were observed in either species. The acute intravenous LD50 in mice was 230 mg/kg (fiducial limits of error at P=0·05 were 83–120%). All deaths occurred immediately after injection; the mice convulsed and died of respiratory arrest. The acute intravenous LD50 in rats was 120 mg/kg (limits of error at P = 0·05 were 91–110%). Hypopnoea and convulsions were observed 30 to 60 min after injection and the rats died of respiratory arrest.

Discussion

Diuretic activity was as widespread amongst *N*-substituted 4-chloro-5-sulphamoylphthalimides (Table 1), as it was already known to be amongst 2-substituted and, particularly, 3-substituted derivatives of hydrochlorothiazide (Schlittler, de Stevens & Werner, 1962). Moreover there was a considerable degree of similarity in the pattern of structure-activity relationship amongst the *N*-substituted phthalimides (V) compared with the 3-substituted derivatives of hydrochlorothiazide (Table 4). There

TABLE 4. SUBSTITUENTS R ARRANGED IN ORDER OF THE DIURETIC ACTIVITY THEY CONFER IN THE 3 DIFFERENT SERIES

3-substituted derivatives of hydrochlorothiazide*	*N*-substituted derivatives of the phthalimides (V)	2-substituted 1-oxo-isoindolines (VI)	
			Order in which values of R confer increased activity
R=	R=	R=	
hydrogen methyl cyclopentyl butyl benzyl, phenethyl, isobutyl, *cyclohexyl*, cycloheptyl, cyclo-octyl cyclohexylmethyl cyclopentylmethyl	hydrogen methyl butyl, isobutyl benzyl, phenethyl cyclohexylmethyl, cyclopentylmethyl cyclopentyl *cyclohexyl*, cycloheptyl, cyclo-octyl, 3-methylcyclohexyl, 4-methylcyclohexyl	isobutyl cyclopentyl, cycloheptyl, cyclo-octyl 3-methylcyclohexyl, 4-methylcyclohexyl cyclohexylmethyl *cyclohexyl*	↓

* From Schlittler, de Stevens & Werner (1962).

was, however, one notable point of difference; in the former series (V), a cycloalkyl substituted on the nitrogen atom conferred greater activity than a cycloalkylalkyl substituent, while in the latter series this relationship was reversed.

Reduction of the phthalimides (V) to the 1-oxoisoindolines (VI) gave compounds with greatly enhanced activities (Table 2), paralleling the increase in activity seen on reduction of the C=N bond in the 3,4-position in chlorothiazide (I; R=H).

The comparisons in Table 4 again show a parallel structure-activity relationship resulting from varying the substituent R in position 2 in the

1-oxoisoindolines (VI); in this instance a cyclohexyl group conferred the most activity.

The fact that the *N*-substituted 4-chloro-5-sulphamoylphthalimides (V) and the corresponding 1-oxoisoindolines (VI) produce similar excretion of Na^+, K^+ and Cl^- to chlorothiazide and to hydrochlorothiazide, suggests that they all act on the same tubular mechanism for the reabsorption of Na^+.

In vivo inhibition of carbonic anhydrase would appear to contribute little to the activity of the phthalimides (V) or the 1-oxoisoindolines (VI) in effective diuretic doses.

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