2-Hydroxymethyl-3-methylquinoxaline 1:4-Dioxide: a Metabolite of 2:3-Dimethylquinoxaline 1:4-Dioxide Active against Gram-negative Bacteria

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The antibacterial activity of certain quinoxaline 1:4-dioxides has been described by McIlwain (1943), Wiedling (1945), Frisk (1946), and Iland (1948), and previous papers from this Laboratory described their action on *Entamoeba histolytica* (Jones, Landquist & Stewart, 1953), and on viruses of the lymphogranuloma-psittacosis group (Hurst *et al.* 1953). We have found that quinoxaline 1:4-dioxides show activity against Gram-negative bacteria, including *Salmonella dublin* and *Salm. typhi*, either *in vitro* or in infections in mice (Table 1). Similar observations have recently been published by Coulthard & Hale (1955).

2:3-Dimethylquinoxaline 1:4-dioxide (7218) proved to be one of the most effective agents against *Salm. dublin* infections in mice, being more active than aureomycin, but it had little activity *in vitro*. Similar results were obtained with *Escherichia coli* and with *Clostridium welchii*, which was the most sensitive. It appeared, therefore, that an active metabolite was formed *in vivo*, and it was found that the blood and urine of sheep dosed with 7218 became active against *Salm. dublin* and *Cl. welchii* within an hour of dosing. Isolation of the metabolite from sheep's urine was attempted, the urine being treated with lead acetate until no further

precipitation of lead salts occurred, then concentrated, and subjected to various extraction procedures. The concentration of the biologically active fraction was followed at first by assays against *Cl. welchii* and later by examination of the ultraviolet absorption spectra for bands characteristic of quinoxaline di-*N*-oxides (Fig. 1). Undue exposure of the solutions to light was avoided. The active metabolite was finally separated from unchanged 7218 by partition chromatography and was obtained as crystalline solid with an elementary composition corresponding to $C_{10}H_{10}O_3N_2$. On the basis of the absence of obvious phenolic characteristics, a comparison of its absorption spectrum with those of numerous quinoxaline derivatives, and its non-identity with the di-*N*-oxide of 5- or 6-hydroxy-2:3-dimethylquinoxaline, it was concluded that the metabolite is 2-hydroxymethyl-3-methylquinoxaline 1:4-dioxide, and this was confirmed by synthesis of this compound (12811).

Fig. 1. Ultraviolet absorption spectra of 2:3-dimethylquinoxaline 1:4-dioxide (full line) and metabolite (broken line) in water + 0.5% methanol.

2-Methylquinoxaline-3-aldehyde (Seyhan, 1951) was made by an improved method and was reduced to 2-hydroxymethyl-3-methylquinoxaline. This carbinol was also obtained, in very poor yield, by the reduction of ethyl 2-methylquinoxaline-3-carboxylate with lithium aluminium hydride; reduction with lithium borohydride gave rather better results. The carbinol could not be oxidized directly to the di-*N*-oxide by performic acid, but acetylation, followed by oxidation with peracetic acid, gave 2-acetoxymethyl-3-methylquinoxaline 1:4-dioxide. This was hydrolysed to 2-hydroxymethyl-3-methylquinoxaline 1:4-dioxide (12811), which was identical with the metabolite isolated from sheep's urine.

Table 1. *Therapeutic activity of some quinoxaline 1:4-dioxides against Salm. dublin infections in mice*

Doses were given twice daily for five days.

Code no.	Substituents	Dose (mg.)	Average survival time (days)
8173	None	5	5.2
8333	6-Chloro	5	7.4
7218	2:3-Dimethyl	5	14.2
		10	12.4
9947	6:7-Dimethyl	5	8.0
10389	6-Chloro-7-methyl	10	6.6
Controls	—	—	1.4

Both synthetic 12811 and the natural metabolite showed the expected antibacterial and amoebicidal properties, being slightly less active than 7218 in experiments *in vivo*. Spectroscopic determination of blood levels of 7218 and 12811 in mice dosed with 7218 showed that formation of the metabolite was rapid and extensive.

The metabolic change undergone by 7218 is rather unexpected. Substitution in the quinoxaline nucleus is known to be difficult, but it is surprising that 7218 was hydroxylated in the side chain without undergoing other changes, such as reduction of the *N*-oxide group. Further metabolic changes certainly occurred, as the quinoxaline derivatives excreted in sheep's urine contained only a minor proportion of 12811.

EXPERIMENTAL

Therapeutic test against Salm. dublin. Mice were dosed orally twice daily for 5 days and were injected intraperitoneally, 2 hr. after the first dose of the compound, with 0.25 ml. of a 24 hr. blood-broth culture of *Salm. dublin* (Lovell). They were observed for 11 days and survivors were assumed to live for 15 days. The majority of the controls died within 24 hr. (Table 1).

In a series of comparative experiments in which 5 mg. of drugs were given orally to mice twice daily for 5 days, only 9 of 85 mice treated with aureomycin were alive 9 days after infection, but 54 out of 100 treated with 7218 were alive.

Bacteriostatic tests. Serial threefold dilutions of drugs were incorporated with Lemco-agar medium, inoculated with a diluted (1:1 000) 24 hr. blood-broth culture of *Salm. dublin* and incubated for 18 hr. at 37°. Tests against *Cl. welchii* were performed similarly, but glucose was added to the medium to a final concentration of 1%, and after inoculation with a 24 hr. Robertson's meat-broth culture of *Cl. welchii* the tubes were incubated in a McIntosh and Fildes jar with hydrogen. Consistent results were obtained when inoculation was confined to a single streak with a standard loopful of the culture. The dilutions of 7218 and its metabolite which just prevented growth are shown in Table 2.

Isolation of the metabolite. Two sheep were dosed orally with 7218 (50 mg./kg.) twice daily for 2 days and the urine was collected for 2–3 days, stored at 0–5° over chloroform, and protected from the light. After each dosing schedule, the animals were rested for a few days.

Urine (3 l.) containing the metabolite was treated with a small excess of powdered lead acetate and filtered from

Table 2. *Bacteriostatic action of 7218 and its metabolite*

Substance	Minimum inhibitory concentration	
	<i>Salm. dublin</i>	<i>Cl. welchii</i>
7218	1:1 000	1:100 000
Metabolite	1:9 000	1:2 700 000
12811	1:9 000	—

lead salts. The pH of the filtrate was adjusted to about 7.5 by addition of concentrated ammonium carbonate solution to remove excess of lead, and the liquid was filtered and concentrated under reduced pressure to one-tenth of the original volume. To avoid the exposure to light inseparable from conventional extraction techniques, the concentrated urine was adsorbed on silica gel (100 g. of silica gel to 60 ml. of concentrate) and the resulting powder was eluted with chloroform until a sample of the eluate showed only a faint reaction for quinoxaline derivatives [2–3 ml. was evaporated to dryness, heated for 30 sec. with acetic acid (2–3 ml.) and zinc dust, cooled and treated with acetylacetone (1 drop); a pink colour showed the presence of *o*-phenylenediamine formed by scission of the quinoxaline derivatives]. The combined chloroform extracts were concentrated to 30–50 ml. under reduced pressure, benzene (200 ml.) was added and the solution was again concentrated. This was repeated until the chloroform had been removed, and the benzene solution (30–50 ml.) was filtered from tarry material and put on a column (5 cm. diameter) of silica gel (100 g.) moistened with water (60 ml.), with A.R. benzene as the solvent phase. Elution with benzene removed first 7218 and unidentified metabolites, and then the active metabolite (approx. 30 mg.) which crystallized from benzene in faintly brown crystals, m.p. 185° corr. (Found: C, 57.9; H, 4.65; N, 13.9; C₁₀H₁₀O₃N₂ requires C, 58.25; H, 4.85; N, 13.6%.) (Table 3.)

Synthesis of the metabolite

2-Methylquinoxaline-3-aldehyde. 2:3-Dimethylquinoxaline (20 g.), freshly sublimed selenium dioxide (15 g.) and ethyl acetate (250 ml.) were stirred and boiled under reflux for 4 hr. After filtration from selenium, the solvent was distilled off and the residue steam-distilled. The aqueous distillate was saturated with salt and extracted with ether. The extract was evaporated, leaving the crude aldehyde (9.8 g., m.p. 132–137°) which was crystallized from light petroleum (b.p. 80–100°), giving 7.7 g., m.p. 140°. The use of other solvents, unsublimed selenium dioxide or other proportions of reagents gave inferior yields.

2-Hydroxymethyl-3-methylquinoxaline. This was synthesized by two methods. In method (a), 2-methylquinoxaline-3-aldehyde (34.2 g.), formalin (68 ml.) and potassium

Table 3. *Chromatographic separation of solvent extract of urine from sheep dosed with 7218*

Fraction	Vol. (ml.)	Colour	Bacteriostatic activity against <i>Cl. welchii</i>	Absorption spectrum of quinoxaline oxide type	Acetylacetone reaction
1	200	Brown	—	—	—
2*	200	Yellow	+	+	+
3	400	Almost colourless	—	—	—
4	1000	Yellow	+	+	+

* Unchanged 7218

hydroxide (46 g.) in water (200 ml.) were shaken together for 3 hr. The dark red-brown solution formed at first became colourless and a cream solid separated. This was collected and dried (21.6 g., m.p. 115–118°), and crystallized from light petroleum (b.p. 80–100°), giving white crystals of the *carbinol* (19.2 g.), m.p. 116–118°. (Found: N, 16.2; $C_{10}H_{10}ON_2$ requires N, 16.1%). The aqueous mother liquor was extracted with ether, yielding impure *carbinol* (10 g.), m.p. 95–114°, which could not easily be purified by re-crystallization.

Method (b). A solution of ethyl 3-methylquinoxaline-2-carboxylate (22 g.) in dry ether (120 ml.) was added during 1.5 hr. to lithium borohydride (1.2 g.), stirring in dry ether. When addition was completed, the mixture was heated under reflux for 30 min., cooled and treated with water (50 ml.). After separation, the aqueous phase was extracted twice with ether and the combined ethereal solutions were dried (Na_2SO_4) and evaporated in the cold. The residue, which soon darkened, was scratched, cooled and filtered, the solid (1.85 g.) being washed with benzene containing a little cyclohexane. Crystallization from benzene-cyclohexane gave the *carbinol*, m.p. 116–118°.

2-Acetoxymethyl-3-methylquinoxaline. 2-Hydroxymethyl-3-methylquinoxaline (19.2 g.), pure, dry pyridine (42 ml.) and acetic anhydride (21 ml., A.R. quality) were mixed, the temperature rising to 45°. After 18 hr., the mixture was poured into water (500 ml.), left for 1 hr., made faintly acid (pH 5–6) with conc. HCl, and extracted with ether (3 × 300 ml.). The extract was washed successively with 0.5N-HCl (2 × 100 ml.), saturated $NaHCO_3$ solution (2 × 200 ml.), and water (100 ml.), dried (Na_2SO_4) and evaporated. The oily residue (21 g.) crystallized on cooling (m.p. 55–56°). 2-Acetoxymethyl-3-methylquinoxaline gave colourless crystals from light petroleum (b.p. 40–60°), m.p. 57–58° (Found: C, 67.1; H, 5.8; N, 12.9; $C_{12}H_{12}O_3N_2$ requires C, 66.67; H, 5.55; N, 12.96%).

2-Acetoxymethyl-3-methylquinoxaline 1:4-dioxide. 2-Acetoxymethyl-3-methylquinoxaline (15.9 g.) and 1.25N peracetic acid (240 ml.) were heated at 50° overnight, concentrated under reduced pressure, neutralized (to pH 7.0) by addition of ice and sodium hydroxide solution, and extracted with chloroform. Evaporation of the dried (Na_2SO_4) extract gave a brown syrup (16 g.) containing much unoxidized material. Partition chromatography (benzene-water-silica gel) was unpromising, and retreatment of the crude product with peracetic acid did not effect much improvement. The crude product (13.5 g.) dissolved in 50% aqueous methanol (1 l.) was stirred overnight with an acid-washed sulphonated phenolic resin (Amberlite

IR-105 H) (50 g.), left for 2 days (protected from light) and filtered. The resin was re-extracted with 50% aqueous methanol (3 × 1 l.) and the combined filtrates were evaporated under reduced pressure. The residual black tar was extracted with ethylene dichloride-light petroleum (b.p. 100–120°), affording yellow-brown needles of the *di-N-oxide* (1.6 g.), m.p. 165–167°. (Found: C, 58.5; H, 5.1; N, 13.0; $C_{12}H_{12}O_4N_2$ requires C, 58.07; H, 4.84; N, 11.3%). Re-extraction of the tar gave 0.6 g., m.p. approx. 160°.

2-Hydroxymethyl-3-methylquinoxaline 1:4-dioxide. The acetoxy compound (2.5 g.) and 2N-HCl (100 ml.) were boiled under reflux for 15 min., treated with carbon, and filtered. The cooled solution was neutralized (pH 7.0) with dil. NaOH and extracted with chloroform (3 × 120 ml.). Evaporation of the dried (Na_2SO_4) extract gave a crystalline residue (1.7 g., m.p. 171–180°) which crystallized from benzene, yielding 2-hydroxymethyl-3-methylquinoxaline 1:4-dioxide (0.9 g.), m.p. and mixed m.p. with the metabolite of 7218, 182–184°. (Found: C, 58.0; H, 4.8%; $C_{10}H_{10}O_3N_2$ requires C, 58.25; H, 4.85%.)

SUMMARY

1. 2:3-Dimethylquinoxaline 1:4-dioxide has little activity against *Salmonella dublin* and *Clostridium welchii* *in vitro*, but is converted *in vivo* into a highly active metabolite.

2. The metabolite has been isolated and identified as 2-hydroxymethyl-3-methylquinoxaline 1:4-dioxide.

3. The synthesis of the metabolite is described.

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