THE ESTIMATION OF *N*-BENZYL-*N'N''*-DIMETHYLGUANIDINE (BW 467C60) IN URINE AND SOME OBSERVATIONS ON ITS REACTION WITH HYPOBROMITE

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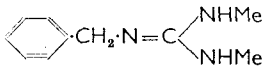
N-Benzyl-*N'N''*-dimethylguanidine, a new anti-adrenergic drug, was estimated in urine by treatment with alkaline hypobromite, extraction of the bromo derivative with carbon tetrachloride and spectrophotometric estimation of the iodine liberated from acidified potassium iodide. Some peculiarities of the bromo derivative are described.

N-BENZYL-*N'N''*-DIMETHYLGUANIDINE (BW 467C60; I) is a new anti-adrenergic drug with properties resembling both bretylium and guanethidine (Boura and Green, 1962). Being a trisubstituted guanidine, the methods used for estimation of monosubstituted guanidines were not



(I)

applicable to its assay, but during attempts to modify the Sakaguchi test it was found that addition of alkaline hypobromite to an aqueous solution of the drug gave a product that was readily extracted by chloroform or carbon tetrachloride. This product behaved like an *N*-bromo compound and liberated iodine from acidified potassium iodide. The iodine liberated was a measure of the amount of drug in the original solution but only one instead of the two atoms of iodine expected was liberated per molecule of drug. This result, and the possibility that the bromo derivative might serve to isolate and purify the drug excreted in urine, led to further examination of its chemistry. This proved to be unexpectedly complicated.

The *N*-bromo compound decomposed in carbon tetrachloride solution at room temperature to give appreciable amounts of benzyl bromide, methylamine and carbon dioxide. This decomposition, reminiscent of the Hofmann degradation of acid amides, did not occur at 0° and the product could be isolated as a yellow solid which was stable when stored at this temperature. It was decomposed by acidified potassium bromide, but not apparently by hydrobromic acid, in a complicated manner. In this reaction rather more than two moles of hydrogen bromide were absorbed per mole of bromo compound with elimination of a small proportion of methylamine and the evolution of about one-third mole of carbon dioxide. Little bromine was produced. The main reaction product had properties suggesting a quaternary ammonium salt of the

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empirical formula $C_{14}H_{23}Br_2N_4O_2$. It lacked any readily observable effect in rats at 30 mg./kg. intraperitoneally and since the determination of its structure was likely to be prolonged the investigation was discontinued.

EXPERIMENTAL

BW 467C60 iodide and sulphate were supplied by Dr. E. Walton.

Chromatograms were developed in s-butanol : acetic acid : water (11 : 5 : 3). The drug could be visualised on paper by Dragendorff's reagent (sensitivity about 30 μg.).

Assay of BW 467C60 in urine. Bromine (0·5 ml.) was dissolved in freshly prepared 2N-sodium hydroxide (50 ml.) as required. Potassium iodide (0·5 g.) was dissolved in 0·02N-sodium hydroxide (25 ml.).

The sample of urine (15 to 30 ml.) was adjusted to pH 7 and passed down a column (10 × 1 cm.) of Zeo-Karb 226 (H^+) and the column washed with water to remove interfering substances, mainly urea and creatinine. Bases were eluted with 0·1N hydrochloric acid (17 ml.), the eluate neutralised by 2N-sodium hydroxide (freshly made) and the volume adjusted to 20 ml. Aliquots (6 ml.) were taken for assay. To each was added carbon tetrachloride (5 ml.) followed by alkaline hypobromite (0·2 ml.). The mixture was shaken for a few min., lightly centrifuged and 4 ml. of the solvent layer pipetted into a tube containing potassium iodide solution (0·1 ml.). 2N-Sulphuric acid (0·3 ml.) was added, the mixture shaken, lightly centrifuged, and iodine in the carbon tetrachloride estimated at 510 mμ.

Carbon dioxide inhibited the liberation of iodine. The bromo derivative could be extracted by chloroform but it was unstable in this solvent. Warming with chloroform and alkali led to the odour of carbylamines.

Spectrophotometric assay of iodine in carbon tetrachloride was possible down to 10 μg./ml. (optical density about 0·03 for a 1 cm. light path). This is equivalent to about 300 μg. of BW 467C60 iodide in the urine sample treated as described.

By comparison of the amount of iodine liberated by a known amount of BW 467C60 with a standard solution of iodine in carbon tetrachloride it was found that 1 mole drug corresponded to 1 atom iodine (Found : 1·04) instead of the 2 atoms expected. This result was confirmed by iodometric titration (Found : 0·92 moles sodium thiosulphate per mole BW 467C60 sulphate).

Recovery of 1 mg. quantities of drug averaged 80 $\pm$ 4 per cent in 4 trials using aqueous solutions. Recovery of 8 mg. added to normal urine was 85, 90 per cent in 2 trials. Normal urine gave negligible blank values.

Excretion of BW 467C60 by patients. Urine was collected from a subject who had received 40 mg. of the sulphate orally. Table I shows the progress of excretion during 12 hr.

Table II shows the daily excretion of the drug by a patient who received 45 mg. of the sulphate daily (40 mg. on the first day). To check the assay, 50 μg. of drug was added to 25 ml. of the day 6 sample. Recovery of the additional drug was 52 μg.

Experiments with Brominated BW 467C60

At room temperature. BW 467C60 sulphate (1 g.) with a trace of α-benzyl-^{14}C-labelled drug in water (5 ml.) was shaken with hypobromite (70 ml. ≡ 0·7 ml. bromine) and carbon tetrachloride (10 ml.). The product was extracted with further portions of solvent. The extract was yellow and soon deposited a white solid while acquiring a pungent odour and lachrymatory property due to benzyl bromide. The aqueous layer, which contained 34 per cent of the added ^{14}C, smelled of amines, and when distilled gave bases equivalent to 518 ml. N acid per mole drug. On cooling, the solution in the distilling flask deposited white plates m.p., 82–84°, which were strongly radioactive. The distilled base was identified as methylamine by conversion to the picrate, m.p. 206–211° (no depression with authentic material). Solids in the carbon tetrachloride were re-dissolved by a little ethanol. 2N-Sulphuric acid (10 ml.) was added, followed by gradual addition of potassium bromide (4 g. in water, 40 ml.). Gas containing carbon dioxide (baryta) was evolved at each addition of bromide and the mixture assumed a deep orange colour but there was no precipitate. The aqueous phase contained 60 per cent of the added ^{14}C, leaving a trace in the solvent. The aqueous layer when basified yielded 0·14 g. of a basic oil to ether. Using manometric methods it was shown that 1 mole drug eliminated 1 mole carbon dioxide.

TABLE I

EXCRETION OF BW 467C60 IN URINE AFTER A SINGLE ORAL DOSE (40 MG.) OF THE SULPHATE

Collection interval	80 min.	167 min.	173 min.	85 min.	215 min.	Aggregate (12 hr.)
Urine vol. (ml.)	192	97	165	210	580	—
Mg. drug	0·32	0·66	1·52	1·29	3·54	7·33
μg./min.	4·0	4·0	8·8	15·2	16·5	—

TABLE II

EXCRETION OF BW 467C60 IN URINE DURING PROLONGED ORAL DOSAGE
40 mg. of the sulphate was given in the first day, and 45 mg. daily subsequently

Days	1	2	3	4	5	6	7
mg.	8·9	2·9	3·6	4·9	2·4	3·2	1·9

With ice cooling. The above experiment was repeated using 2 g. of drug and ice cooling throughout. The carbon tetrachloride acquired no odour of benzyl bromide and deposited no solid material. The aqueous layer now retained only 6 per cent of the added ^{14}C and gave no amine when distilled. Addition of sulphuric acid and then potassium bromide solution caused separation of an oil in the aqueous phase. Gas was not evolved until the addition of bromide was almost complete. The oil passed into the solvent layer which became deep orange in colour and soon deposited a sticky red mass (2·35 g.) which contained 90 per cent of the added ^{14}C. The aqueous layer was neutral, contained 3 per cent of the

added ^{14}C, and after basifying, gave bases on distillation equivalent to 260 ml. of N acid per mole drug. The red mass was drained on porous tile to give 1·8 g. of a yellow solid which was washed with acetone and crystallised from aqueous acetone in white prisms, m.p. 198–199° (Found: C, 38·6; H, 5·3; N, 12·9; Br, 36·6; loss at 100°, 2·2. Calc. for $C_{14}H_{23}Br_2N_4O_2$: C, 38·4; H, 5·3; N, 12·8; Br, 36·6 per cent). It was easily soluble in water, slightly soluble in ethanol but not in other common solvents. The bromine was wholly ionic in aqueous solution. There was a weak ultra-violet absorption peak at 253 mμ. With aqueous potassium iodide, it gave a white precipitate, m.p. 205°, containing 45·0 per cent iodide ion, and it gave a brown stain with Dragendorff's reagent on paper.

Dry distillation at about 200° gave benzyl bromide (44 per cent) identified by conversion to benzyltrimethylammonium bromide, m.p. 219–220° (Found: Br, 34·3. Calc. for $C_{10}H_{16}BrN$, 34·8 per cent).

Distillation of the bromide in 5N sodium hydroxide led to evolution of methylamine during 30 min., equivalent to 1·66 mole per mole $C_{14}H_{23}Br_2N_4O_2$. The aqueous residue deposited a white precipitate which crystallised from aqueous ethanol, m.p. 146–150°.

In further experiments using standard methods it was found that 1 mole drug consumed 1 mole hypobromite during bromination. During electrometric titration with 2N sulphuric acid and potassium bromide solution, added in small portions alternately at 0°, there was disappearance of 2·4 g. hydrogen ion and consumption of 2·6 atom bromine per mole drug. 2·0 atom of the total bromine used per mole drug was not ionised. During the reaction only 0·006 atom bromine per mole drug was swept out by a current of nitrogen. 0·33 mole of carbon dioxide was recovered.

Discussion

The urinary elimination of BW 467C60 by two subjects after oral dosage was similar to that found in cats (Boura, Duncombe, Robson and McCoubrey, 1962). About one-fifth of the oral dose was excreted by this route during 24 hr. The fall in urinary excretion during prolonged dosage suggested that the drug might be metabolised after a period of adaptation but there was no evidence for any metabolites in cats (Boura and others, 1962). The mono-*N*-demethylated product gave about 44 per cent of the colour intensity due to BW 467C60 in the assay as described, while *N*-benzylguanidine could not be detected. Both these possible metabolites, unlike BW 467C60, could be detected on paper chromatograms by conventional spray reagents for guanidines but they could not be found by spraying chromatograms of the urines examined.

The reactions of brominated BW 467C60 proved to be an interesting chemical problem. The product decomposed at room temperature, at least partially, in a manner similar to the Hofmann degradation of *N*-bromoamides. A search for an anticipated hydrazine was not successful though a small amount of an unidentified basic oil was isolated. There are two possible *N*-bromo derivatives of BW 467C60 but it is difficult to conceive how either could absorb 2 hydrogen ions in company with 2 bromine atoms. The empirical formula of the main reaction product

isolated after treatment with acid potassium bromide at 0° (a C_{14} compound or possibly some other multiple of C_7) must arise by condensation of 2 or more molecules of BW 467C60 (C_{14}) but to obtain a C_{14} compound containing benzyl and 2 methylamine residues from 2 molecules of BW 467C60 can only be achieved by cleavage of a benzyl group (reaction with the solvent was not excluded). Since the product had no detectable biological activity in the conscious rat at a moderate dose level, investigation of the purely chemical problem was discontinued, being outside the scope of chemical pharmacology. The action of alkaline hypobromite on guanidines has not been described since 1926 when Cordier found differences in the degree of elimination of nitrogen between individual guanidines.

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