ACTA CHEMICA SCANDINAVICA 26 (1972) 1045–1048

Studies on Flavins

The Preparation of Heavy Atom Derivatives

LAJOS MARON

Institute of Inorganic and Physical Chemistry, University of Stockholm, S-104 05 Stockholm 50, Sweden

The preparation of the following bromine substituted compounds, required for our X-ray crystallographic investigations, are described: 4-amino-3-bromo-5-nitro-*o*-xylene, 3-bromo-4-methylamino-5-nitro-*o*-xylene, 4,5-diamino-3-bromo-*o*-xylene, 5-amino-3-bromo-4-methylamino-*o*-xylene, 9-bromo-7,8-dimethylalloxazine, 9-bromo-3,7,8-trimethylalloxazine, 9-bromo-7,8,10-trimethylisoalloxazine, 9-bromo-3,7,8,10-tetramethylisoalloxazine, 9-bromo-1,3,7,8-tetramethylalloxazine, 5-acetyl-9-bromo-5,10-dihydro-7,8-dimethylalloxazine, 5-acetyl-9-bromo-5,10-dihydro-3,7,8-trimethylalloxazine, 5-acetyl-9-bromo-5,10-dihydro-7,8,10-trimethylalloxazine, 5-acetyl-9-bromo-5,10-dihydro-3,7,8,10-tetramethylalloxazine, 5-acetyl-9-bromo-5,10-dihydro-1,3,7,8-tetramethylalloxazine, 5-acetyl-9-bromo-5,10-dihydro-1,3,7,8,10-pentamethylalloxazine, 9-bromo-5,10-dihydro-1,3,7,8,10-pentamethylalloxazine and riboflavine silver-chelate perchlorate.

The vital role of the flavo-proteins in biological redox processes has inspired extensive research on the chemistry of the constituent molecules of this group of enzymes. The research in this field has been reviewed in several recent articles.[1–5]

The preparation of heavy atom derivatives of flavins is a part of our research project concerning structural studies of flavin compounds in different states of oxidation. The numbering of the atoms forming the alloxazine ring system of the flavins is shown in Fig. 1.

The bromination of 4-amino-5-nitro-*o*-xylene and 4-methylamino-5-nitro-*o*-xylene, respectively, in glacial acetic acid-sodium acetate solution afforded I and II. The reduction of I and II with stannous chloride in concentrated hydro-

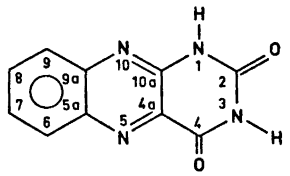

Fig. 1. The numbering of atoms adopted for the alloxazine molecule.

chloric acid gave 4,5-diamino-3-bromo-*o*-xylene (III) and 5-amino-3-bromo-4-methylamino-*o*-xylene (IV).

To yield 9-bromo-7,8-dimethylalloxazine (V), 9-bromo-3,7,8-trimethylalloxazine (VI), 9-bromo-7,8,10-trimethylisoalloxazine (VII) and 9-bromo-3,7,8,10-tetramethylisoalloxazine (VIII), III and IV were condensed with alloxan [6] and 3-methylalloxan,[7] respectively.

The methylation of V with dimethyl sulphate in dimethylformamide solution [8] gave 9-bromo-1,3,7,8-tetramethylalloxazine (IX).

The reductive acetylation of V, VI, VII, VIII, and IX in acetic acid-acetic anhydride solution with zinc powder [8] afforded 5-acetyl-9-bromo-5,10-dihydro-7,8-dimethylalloxazine (X), 5-acetyl-9-bromo-5,10-dihydro-3,7,8-trimethylalloxazine (XI), 5-acetyl-9-bromo-7,8,10-trimethylalloxazine (XII), 5-acetyl-9-bromo-5,10-dihydro-3,7,8,10-tetramethylalloxazine (XIII) and 5-acetyl-9-bromo-5,10-dihydro-1,3,7,8-tetramethylalloxazine (XIV).

The methylation of XIV with dimethyl sulphate in dimethylformamide solution [8] gave 5-acetyl-9-bromo-5,10-dihydro-1,3,7,8,10-pentamethylalloxazine (XV).

The mild acid hydrolysis [8] of XV afforded 9-bromo-5,10-dihydro-1,3,7,8,10-pentamethylalloxazine (XVI).

To yield riboflavine silver-chelate perchlorate (XVII), riboflavine perchlorate was treated with silver nitrate in slightly acidic acetone solution. The crystal and molecular structure of VIII,[9] XIV,[10] XV,[11] and XVI [12] has been determined by X-ray diffraction methods.

EXPERIMENTAL

All melting points are uncorrected. Evaporations were carried out under reduced pressure at a bath temperature below 40°.

Chromatography. Paper: Whatman No. 1. Thin layer chromatography. Absorbent: Kieselgel G nach Stahl, Merck. Solvent: butanol-formic acid-water 77:10:13. The spots on the chromatograms were located with UV-light.

4-Amino-3-bromo-5-nitro-o-xylene (I). 4-Amino-5-nitro-*o*-xylene (16.6 g) and sodium acetate (8.5 g) were dissolved in acetic acid (100 ml). Bromine (16 g) dissolved in acetic acid (50 ml) was added with stirring during 2 h at room temperature. A few crystals of iodine were used as catalyst. The stirring was continued for 3 h, the acetic acid evaporated *in vacuo* and the residue treated with water. Recrystallization from acetic acid gave crystals (18.0 g), m.p. 128–130°. (Found: C 39.1; H 3.73; N 11.3. Calc. for $C_8H_9BrN_2O_2$: C 39.2; H 3.73; N 11.4.)

3-Bromo-4-methylamino-5-nitro-o-xylene (II). 4-Methylamino-5-nitro-*o*-xylene (18.0 g) sodium acetate (8.5 g) and a few crystals of iodine were dissolved in acetic acid (100 ml) and treated with bromine (16 g) dissolved in acetic acid (50 ml) with stirring for 2 h at room temperature. The stirring was continued for 3 h, the acetic acid evaporated *in vacuo* and the residue treated with water. Recrystallization from ethanol gave crystals (19 g), m.p. 85–87°. (Found: C 41.8; H 3.85; N 10.8. Calc. for $C_9H_{11}BrN_2O_2$: C 41.9; H 3.91; N 10.9.)

4,5-Diamino-3-bromo-o-xylenedihydrochloride (III). 4-Amino-3-bromo-5-nitro-*o*-xylene (24 g) was dissolved in 37 % hydrochloric acid (250 ml) and treated with $SnCl_2.2H_2O$ (110 g) with stirring. The solution was heated to boiling for 15 min and cooled to room temperature. The mixture was neutralized with sodium hydroxide (300 g) dissolved in water (400 ml) and extracted with ethyl ether; the ether extract was dried with sodium sulphate and filtrated. The diamine was precipitated with hydrogen chloride as the dihydrochloride. Recrystallization from ethanol-water 1:1 containing some hydrochloric acid gave crystals (21 g), m.p. 125–127°. (Found: C 33.3; H 4.05; N 9.7. Calc. for $C_8H_{13}BrCl_2N_2$: C 33.4; H 4.20; N 9.7.)

5-Amino-3-bromo-4-methylamino-o-xylene dihydrochloride (IV). 3-Bromo-4-methylamino-5-nitro-*o*-xylene (25.8 g) was dissolved in 37 % hydrochloric acid (250 ml) and treated with $SnCl_2.2H_2O$ (110 g) with stirring. The solution was heated to boiling for 15 min and cooled to room temperature. The mixture was neutralized with sodium hydroxide (300 g) dissolved in water (400 ml) and extracted with ethyl ether. The ether extract was dried with sodium sulphate and filtered. The diamine was precipitated with hydrogen chloride as the dihydrochloride. Recrystallization from ethanol-water containing some hydrochloric acid gave crystals (30 g), m.p. 120 – 122°. (Found: C 35.8; H 4.70; N 9.3. Calc. for $C_9H_{15}BrCl_2N_2$: C 35.9; H 4.69; N 9.3.)

9-Bromo-7,8-dimethylalloxazine (V). 4,5-Diamino-3-bromo-*o*-xylene dihydrochloride (5.8 g) was dissolved in acetic acid (150 ml) containing sodium acetate (3.3 g) with stirring at 70°. Alloxan monohydrate (4.8 g) and boric acid (1.9 g) were dissolved in acetic acid (50 ml) at 70°. The two solutions were brought together and stirred overnight. The precipitated alloxazine was filtered off and washed with water and acetic acid. Recrystallization from acetic acid gave crystals (8 g), m.p. 325 – 327°. (Found: C 44.8; H 2.75; N 17.4. Calc. for $C_{12}H_9BrN_4O_2$: C 44.9; H 2.82; N 17.5.)

9-Bromo-3,7,8-trimethylalloxazine (VI). 4,5-Diamino-3-bromo-*o*-xylene dihydrochloride (5.8 g) was dissolved in acetic acid (150 ml) containing sodium acetate (3.3 g) with stirring at 70°. 3-Methyl-alloxan monohydrate (5 g) and boric acid (1.9 g) were dissolved in acetic acid (50 ml) at 70°. The two solutions were brought together and stirred overnight. The precipitated alloxazine was filtered off and washed with water and acetic acid. Recrystallization from acetic acid gave crystals (8.2 g), m.p. 335 – 340°. (Found: C 46.5; H 3.25; N 16.7. Calc. for $C_{13}H_{11}BrN_4O_2$: C 46.6; H 3.31; N 16.6.)

9-Bromo-7,8,10-trimethylisoalloxazine (VII). 5-Amino-3-bromo-4-methylamino-*o*-xylene dihydrochloride (4.1 g) was dissolved in water (100 ml) with stirring at 90° and alloxan monohydrate (3 g) was added in one portion. The stirring was continued for 20 min and the precipitated alloxazine was filtered off, washed with water and dried. Recrystallization from acetic acid-water 2:1 gave crystals (3.6 g), m.p. 270 – 272°. (Found: C 46.6; H 3.23; N 16.7. Calc. for $C_{13}H_{11}BrN_4O_2$: C 46.6; H 3.31; N 16.7.)

9-Bromo-3,7,8,10-tetramethylisoalloxazine (VIII). 5-Amino-3-bromo-4-methylamino-*o*-xylene dihydrochloride (4 g) was dissolved in water (100 ml) with stirring at 90° and 3-methylalloxan monohydrate (3.5 g) was added in one portion. The stirring was continued for 20 min and the precipitated alloxazine was filtered off, washed with water and dried. Recrystallization from acetic acid-water 2:1 gave crystals (3.5 g), m.p. 246 – 248°. (Found: C 48.3; H 3.61; N 16.0. Calc. for $C_{14}H_{13}BrN_4O_2$: C 48.2; H 3.75; N 16.0.)

9-Bromo-1,3,7,8-tetramethylalloxazine (IX). 9-Bromo-7,8-dimethylalloxazine (3.2 g) and potassium carbonate (4 g) in dimethylformamide (35 ml) were heated to 90° with stirring. Into the reaction mixture dimethyl sulphate-dimethylformamide 1:1 (9.5 ml) was added dropwise during 1 h. The stirring was continued for 1 h and potassium carbonate (2.4 g) was added again. After 3 h of stirring the dimethylformamide was evaporated *in vacuo* and the residue washed with 2 M ammonium hydroxide and water. Recrystallization from the acetic acid gave crystals (3 g), m.p. 240 – 245°. (Found: C 48.0; H 3.70; N 16.0. Calc. for $C_{14}H_{13}BrN_4O_2$: C 48.1; H 3.75; N 16.0.)

5-Acetyl-9-bromo-5,10-dihydro-7,8-dimethylalloxazine (X). 9-Bromo-7,8-dimethylalloxazine (4 g) was dissolved in acetic acid-acetic anhydride 1:1 (80 ml) and heated to boiling with stirring. Zinc powder (4 g) was added during 1 h. The refluxing was continued for 30 min, the mixture filtered hot and evaporated *in vacuo*. The residue was washed with water, dissolved in 1 M sodium hydroxide and precipitated with acetic acid. Recrystallization from acetic acid gave crystals (2.5 g), m.p. 280 – 282°. (Found: C 46.0; H 3.51; N 15.2. Calc. for $C_{14}H_{13}BrN_4O_3$: C 46.0; H 3.59; N 15.3.)

5-Acetyl-9-bromo-5,10-dihydro-3,7,8-trimethylalloxazine (XI). 9-Bromo-3,7,8-trimethylalloxazine (5 g) was dissolved in acetic acid-acetic anhydride 1:1 (100 ml) and heated to boiling with stirring. Zinc powder (5 g) was added during 1 h. The refluxing was continued for 30 min, the mixture filtered hot and evaporated *in vacuo*. The residue was washed with water, then dissolved in 1 M sodium hydroxide and precipitated with acetic acid. Recrystallization from acetic acid gave crystals (3 g), m.p. 290 – 295°. (Found: C 47.4; H 4.02; N 14.7. Calc. for $C_{15}H_{15}BrN_4O_3$: C 47.5; H 3.99; N 14.8.)

5-Acetyl-9-bromo-5,10-dihydro-7,8,10-tetramethylalloxazine (XII). 9-Bromo-7,8,10-trimethylisoalloxazine (4 g) was dissolved in acetic acid-acetic anhydride 1:1 (80 ml) and heated to boiling with stirring. Zinc powder (4 g) was added during 1 h. The refluxing

was continued for 30 min, the mixture filtered hot and evaporated *in vacuo*. The residue was washed with water, then dissolved in 1 M sodium hydroxide and precipitated with acetic acid. Recrystallization from acetic acid gave crystals (2.5 g), m.p. 350°. (Found: C 47.3; H 3.90; N 14.7. Calc. for $C_{15}H_{15}BrN_4O_3$: C 47.5; H 3.99; N 14.8.)

5-Acetyl-9-bromo-5,10-dihydro-3,7,8,10-tetramethylalloxazine (XIII). 9-Bromo-3,7,8, 10-tetramethylisoalloxazine (2 g) was dissolved in acetic acid-acetic anhydride 1:1 (40 ml) and heated to boiling with stirring. Zinc powder (2 g) was added during 1 h. The refluxing was continued for 30 min, the mixture filtered hot and evaporated *in vacuo*. The residue was washed with water, then dissolved in 1 M sodium hydroxide and precipitated with acetic acid. M.p. 330°. (Found: C 48.9; H 4.38; N 14.1. Calc. for $C_{16}H_{17}BrN_4O_3$: C 48.9; H 4.36; N 14.2.)

5-Acetyl-9-bromo-5,10-dihydro-1,3,7,8-tetramethylalloxazine (XIV). 9-Bromo-1,3,7,8-tetramethylalloxazine (2 g) was dissolved in acetic acid-acetic anhydride 1:1 (40 ml) and heated to boiling with stirring. Zinc powder (2 g) was added during 1 h. The refluxing was continued for 30 min, the mixture filtered hot and evaporated *in vacuo*. The residue was washed with water, then dissolved in 1 M sodium hydroxide and precipitated with acetic acid. Recrystallization from acetone gave crystals (1 g), m.p. 228 – 230°. (Found: C 49.0; H 4.46; N 14.1. Calc. for $C_{16}H_{17}BrN_4O_3$: C 48.9; H 4.36; N 14.2.)

5-Acetyl-9-bromo-5,10-dihydro-1,3,7,8,10-pentamethylalloxazine (XV). 5-Acetyl-9-bromo-5,10-dihydro-1,3,7,8-tetramethylalloxazine (2 g) and potassium carbonate (4 g) in dimethylformamide (30 ml) were heated to 60°C with stirring. Dimethylsulphate-dimethylformamide 1:1 (4.7 ml) was added dropwise to the reaction mixture during 1 h and the stirring was continued for 3 h. The dimethylformamide was evaporated *in vacuo* and the residue was treated with water, the pH adjusted to 5 with acetic acid and the precipitate filtered. Recrystallization from ethanol gave crystals (0.7 g), m.p. 235 – 236°C. (Found: C 50.2; H 4.95; N 13.8. Calc. for $C_{17}H_{19}BrN_4O_3$: C 50.1; H 4.70; N 13.8.)

9-Bromo-5,10-dihydro-1,3,7,8,10-pentamethylalloxazine (XVI). 5-Acetyl-9-bromo-5, 10-dihydro-1,3,7,8,10-pentamethylalloxazine (1 g) was heated in ethanol-2 M hydrochloric acid 1:1 (40 ml) to 80°C for 2 h. The precipitate was filtered off and dried. Recrystallization from ethanol gave crystals (0.4 g), m.p. 238 – 240°C. (Found: C 49.5; H 4.41; N 15.3. Calc. for $C_{15}H_{17}BrN_4O_2$: C 49.3; H 4.69; N 15.3.)

Riboflavine silver-chelate perchlorate (XVII). Riboflavine (0.3764 g) was dissolved in acetone (20 ml) containing perchloric acid (0.2100 g) at 40°C. Silver nitrate (0.1699 g) was dissolved in acetone (30 ml) at 40°C. The two solutions were brought together and slowly crystallized to give crystals (0.600 g), m.p. 285 – 290°C with decomposition. (Found: C 35.0; H 3.61; N 9.4. Calc. for: $C_{17}H_{21}AgClN_4O_{10}$: C 34.9; H 3.62; N 9.5.)

Acknowledgements. The present work has received financial support from the *Tri-Centennial Fund of the Bank of Sweden* and from the *Swedish Natural Science Research Council*. The author is indebted to Professor Peder Kierkegaard for his active interest throughout this work.

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Received June 3, 1971.