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The Synthesis of NN-Di-(2-chloroethyl)[$3,5-{}^{3}H_{2}$]aniline of High Specific Activity

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The synthesis of *NN*-di-(2-chloroethyl)aniline specifically tritiated in the positions *meta* to the nitrogen mustard group by catalytic halogen-tritium exchange with the 3,5-di-iodo-derivative is described.

ISOTOPICALLY labelled NN-di-(2-chloroethyl)aniline has been prepared by Warwick and his collaborators on various occasions. ¹⁴C-Labelling, both in the aromatic ring and in the aliphatic side chain,¹ has been used to give products of specific activity $0.5-0.63 \ \mu c/mg$. $(109-136 \ \mu c/mmole)$. More recently, tritium labelling has been used and a generally ring labelled ³H-product of specific activity 353 mc/mmole has been described.² In view of recent work showing that metabolism of the para-position may be implicated in the antitumour action of this compound,³ a requirement arose for a molecule of high specific activity labelled at a site other than the 4-hydrogen. It occurred to us that it might be possible to prepare NN-di-(2-chloroethyl)[3,5-³H₂]aniline of high specific activity by the catalytic halogen-tritium exchange method from NN-di-(2-chloroethyl)-3,5-diiodoaniline. The intermediate required was prepared from 3,5-di-iodoaniline by hydroxyethylation with ethylene oxide in aqueous acetic acid and chlorination of the resulting diol with phosphoryl chloride. This compound was smoothly hydrogenolysed over palladiumcharcoal containing a small percentage of platinum oxide. The purity of the product was significantly improved by conducting this reaction in the dark. From the specific activity of the product obtained after dilution of the partially purified product with nonradioactive material it seems that the specific activity of the initial tritiation product was in excess of 48 c/mmole.

Solutions of the radioactive product [0.3% in light petroleum (b.p. $30-40^{\circ}$)] appeared fairly stable when stored at -20° . Solid samples, even when stored in the dark, became discoloured, overnight at the highest specific activity and during several weeks at levels of 11 mc/mmole. Details are recorded in the Experimental section.

EXPERIMENTAL

Melting points were determined with a Kofler hot-stage apparatus. Measurements of radioactivity were made with a Packard Tricarb model **3375** liquid scintillation counter, with naphthalene-PPO or dimethyl POPOP in ethanol, dioxan, and toluene as phosphor.

NN-Di-(2-hydroxyethyl)-3,5-di-iodoaniline.— 3,5-Di-iodoaniline ⁴ (4 g.) was dissolved in glacial acetic acid (90 ml.); water (10 ml.) was added and the solution was cooled (5°) before the addition of ethylene oxide (8 ml.). The flask

was stoppered and set aside overnight at room temperature. Next day a trace of solid was filtered off, the filtrate was poured into water, and the solution was neutralised with sodium hydrogen carbonate. The oil which separated was extracted with ethyl acetate, the extract was dried (Na₂SO₄), and the solvent was evaporated off *in vacuo*. The residual pale yellow oil was dissolved in hot ethanol (charcoal), the solution was filtered, and water was added. When the solution was cool, large plates of NN-*di*-(2-*hydroxyethyl*)-3,5-*di*-*iodoaniline* separated (49%), m.p. 128–129° (Found: C, 28·1; H, 3·1; I, 60·4; N, 3·3. C₁₀H₁₃I₂NO₂ requires C, 27·7; H, 3·0; I, 58·6; N, 3·2%).

NN-Di-(2-chloroethyl)-3,5-di-iodoaniline.—A solution of the above hydroxy-compound (4 g.) in benzene (40 ml.) was treated with redistilled phosphoryl chloride (8 ml.) and then boiled under reflux for 1 hr. Evaporation of the solvent was followed by addition and removal of several further portions of benzene. The dark oily residue was taken up in benzene and put on a column of alumina (Spence type H; 30×3 cm.). Elution with benzene followed by evaporation of the solvent left a yellow solid, which gave white needles (30%) of NN-di-(2-chloroethyl)-3,5-di-iodoaniline, m.p. 97— 98° (from ethanol-ether) (Found: C, 25.6; H, 2.5; Cl, 15.3; I, 53.8; N, 3.1. C₁₀H₁₁Cl₁I₂N requires C, 25.6; H, 2.4; Cl, 15.1; I, 54.0; N, 3.0%).

Reductive Deiodination of NN-Di-(2-chloroethyl)-3,5-diiodoaniline.—A solution of the di-iodo compound (470 mg.) in dry dioxan (5 ml.) containing triethylamine (0·3 ml.), 5% palladium-charcoal (150 mg.), and Adams platinum oxide (5 mg.) was stirred in the dark in an atmosphere of hydrogen overnight at room temperature. When uptake had ceased (49 ml., 2 equiv.) the catalyst was filtered off (Hyflo) and washed with ethyl acetate, and the combined filtrate was washed with dilute hydrochloric acid, then with water, and dried (Na₂SO₄). Removal of the solvent left an oil which solidified when chilled in ice. The solid gave prisms of di-2-chloroethylaniline, m.p. 43—44° (lit.⁵ 45°) [from light petroleum (b.p. 30—40°)], $R_{\rm F}$ (silica gelbenzene) and i.r. spectrum identical with those of an authentic specimen.

NN-Di- $(2\text{-}chloroethyl)[3,5-^{3}H_{2}]$ aniline.—Tritiation was carried out at the Radiochemical Centre by the reductive deiodination procedure described above, with use of 100 c tritium gas $(98\% {}^{3}H_{2}/H_{2})$. The product, after one recrystallisation from light petroleum ether, was recrystallised from the same solvent after the addition of non-radioactive di-(2-chloroethyl)aniline (1 g.). This gave batch A of product (958 mg.); the mother liquor was treated with non-radioactive di-(2-chloroethyl)aniline (2 g.) and a second batch (B; $1\cdot856 \text{ g.})$ was obtained. This procedure was repeated twice more to obtain in all four batches of product of specific activities 5070, 750, 88.2, and 11.4

- 4 C. Willgerodt and E. Arnold, Ber., 1901, 34, 3343.
- ⁵ R. Robinson and J. S. Watt, J. Chem. Soc., 1934, 1536.

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² G. P. Warwick, Biochem. Pharmacol., in preparation.

³ M. E. Whisson and T. A. Connors, Nature, 1965, 206, 689.

1339

mc/mmole. Batches C and D weighed 1.579 and 2.355 g. respectively. From the radioactivity measured and the weight of the initial crude product it appears that the tritiation product had a specific activity of at least 48 c/mmole.

Stability of Tritiated Di-(2-chloroethyl)aniline.—After storage in the solid form in the refrigerator for 4 weeks, the various batches of product were again assayed for radiochemical purity. Samples were chromatographed on silica gel thin layers with benzene as developing solvent. Portions were scraped off at 1 cm. intervals directly into the phosphor and assayed by scintillation counting. The percentages of the radioactivity appearing on the plate in regions distinct from the peak corresponding to the authentic material were 56, 8.5, 9.2, and 7.3 in batches A, B, C, and D respectively. Batch A was black and the remainder were pink-purple of varying degrees of darkness. Even on the day after preparation crystals of A had turned pink. In an attempt to stabilise it the compound was dissolved in light petroleum (b.p. $30-40^{\circ}$) (3 mg./ml.) and stored at -20° . After 9 weeks under these conditions, the solution was assayed for radiochemical purity as above and gave a value of >97%. However during this period the specific activity had dropped to 3.83 c/mmole as a result of deposition of radiolysis products from the solution. Radiochemically pure material could be obtained from any of the above fractions by passage through a column of silica gel in benzene solution.

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