NON-SPECIFIC TRITIATION OF SOME CARCINOGENIC AROMATIC AMINES

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SUMMARY

2-Aminofluorene, 4-amino-3-methylbiphenyl, 4-aminobiphenyl and 4-amino-4'-fluorobiphenyl were tritiated by acid catalyzed exchange of the corresponding nitro compounds followed by catalytic reduction. The exchange reactions were carried out by heating the nitro compounds in [^{3}H]-trifluoroacetic acid with a catalytic amount of trifluoromethanesulphonic acid (TFMS). No loss of tritium could be detected during the conversion of the tritiated nitro compounds into the corresponding amines by catalytic hydrogenation. Incorporation into the ortho position is very low ($< 4\%$). During the metabolic activation and binding of the tritiated N-acetyl-2-aminofluorene to rat liver DNA in vivo, no tritium exchange occurred.

Key Words : Tritiation, Exchange, Carcinogenic Aromatic Amines

INTRODUCTION

Investigations on the mechanism of action of carcinogenic aromatic compounds have led to the synthesis of several [^{3}H]- and [^{14}C]-labelled derivatives of these compounds. In the case of N-acetyl-2-aminofluorene the [2'-^{3}H]-, the [1'-^{14}C]- and the [9-^{14}C]-labelled compounds have been widely used for studying the interaction of metabolites of this carcinogen with macromolecules in the cell in vivo and in vitro (1, 2). The

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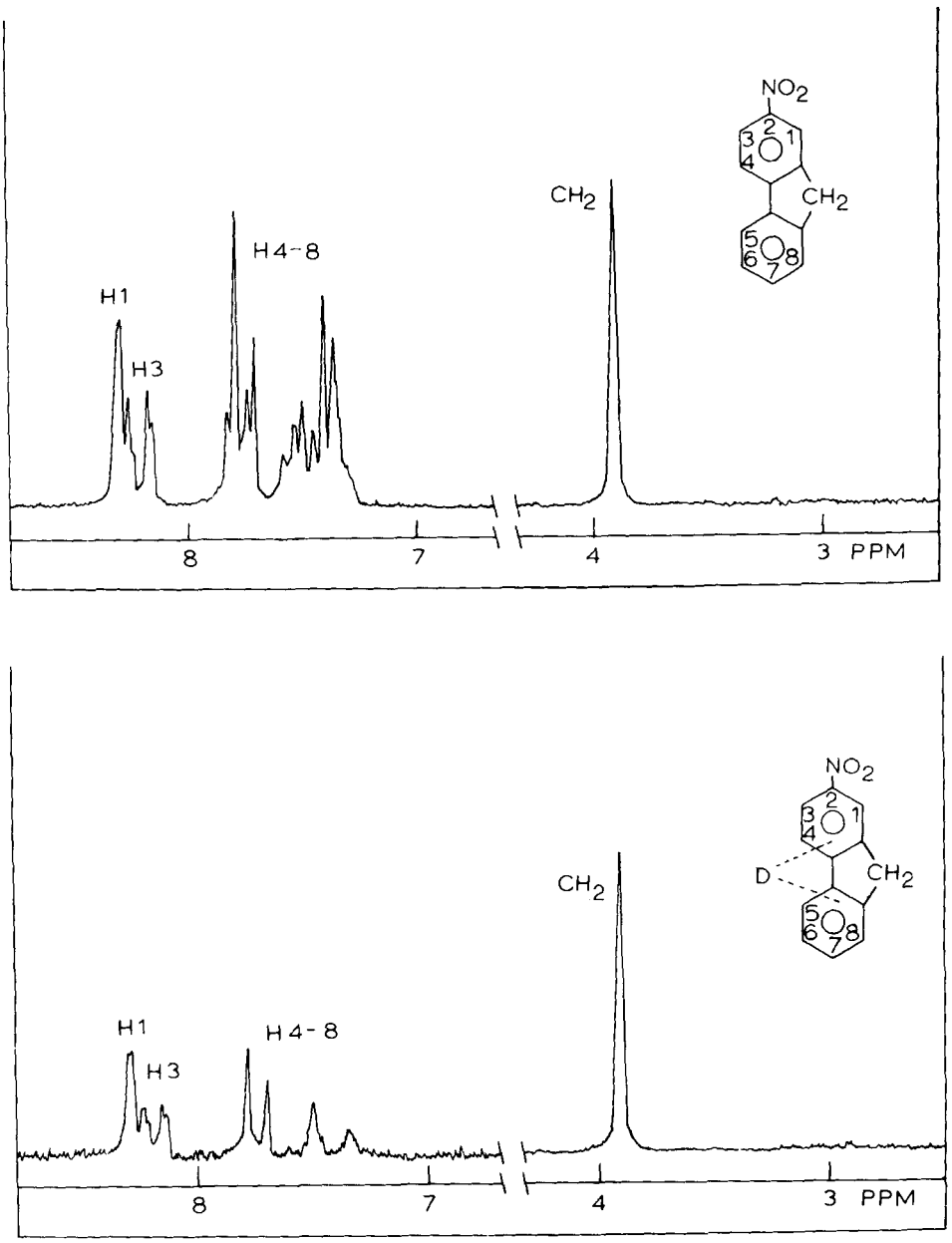
(2'-^{3}H]- and the [1'-^{14}C]-labelled compounds are unsuitable to show the presence of deacetylated bound residues and with the [9-^{14}C]-derivatives only a limited specific activity can be obtained. High specific activities can be obtained by the procedure of Gutmann et al. who describe the synthesis of different isomers of [9-^{3}H]-labelled aminofluorene (4). Because this method fails in the case of biphenyl amines, we developed a simple general method for labelling the nitro compounds in the aromatic moiety.

Comparative studies of the interaction of different aromatic amides to DNA of various tissues in vivo in our laboratory prompted us to the synthesis of tritiated 4-aminobiphenyl, 4-amino-4'-fluorobiphenyl, 4-amino-3-methylbiphenyl and 2-aminofluorene. As the ortho positions of carcinogenic aromatic amines and amides are biologically active and play a role in the binding of these compounds to macromolecules in the cell (3), incorporation of tritium at the ortho position has to be avoided. Homogeneous acid catalyzed exchange of the corresponding nitro compounds appeared to be the most favourable technique for this purpose. In order to obtain some information about the reactivity with regard to different acids and the selectivity of the exchange, preliminary experiments were carried out with deuterated acids. Although with D_2SO_4 at 50° exchange in the aromatic ring occurred, the yields were poor as a result of decomposition and sulphonation. In addition, the poor solubility in D_2SO_4 limited its use as a medium for efficient exchange. Trifluoroacetic acid (5) appeared to be a better solvent but hydrogen exchange was only achieved if a catalytic amount of sulphuric acid was added. In this case, however, sulphonation was also difficult to avoid and better results were obtained when using trifluoromethanesulphonic acid instead of sulphuric acid.



NMR spectrum of 2-nitrofluorene and [^{2}H(G)]-2-nitrofluorene. The ratio of the integrals of the signals of H1 + H3 : H4-8 : CH_2 = 2 : 5 : 2 in the spectrum of 2-nitrofluorene (above) changed to a value of 2 : 2,1 : 2 in the spectrum of the deuterated compound (below).

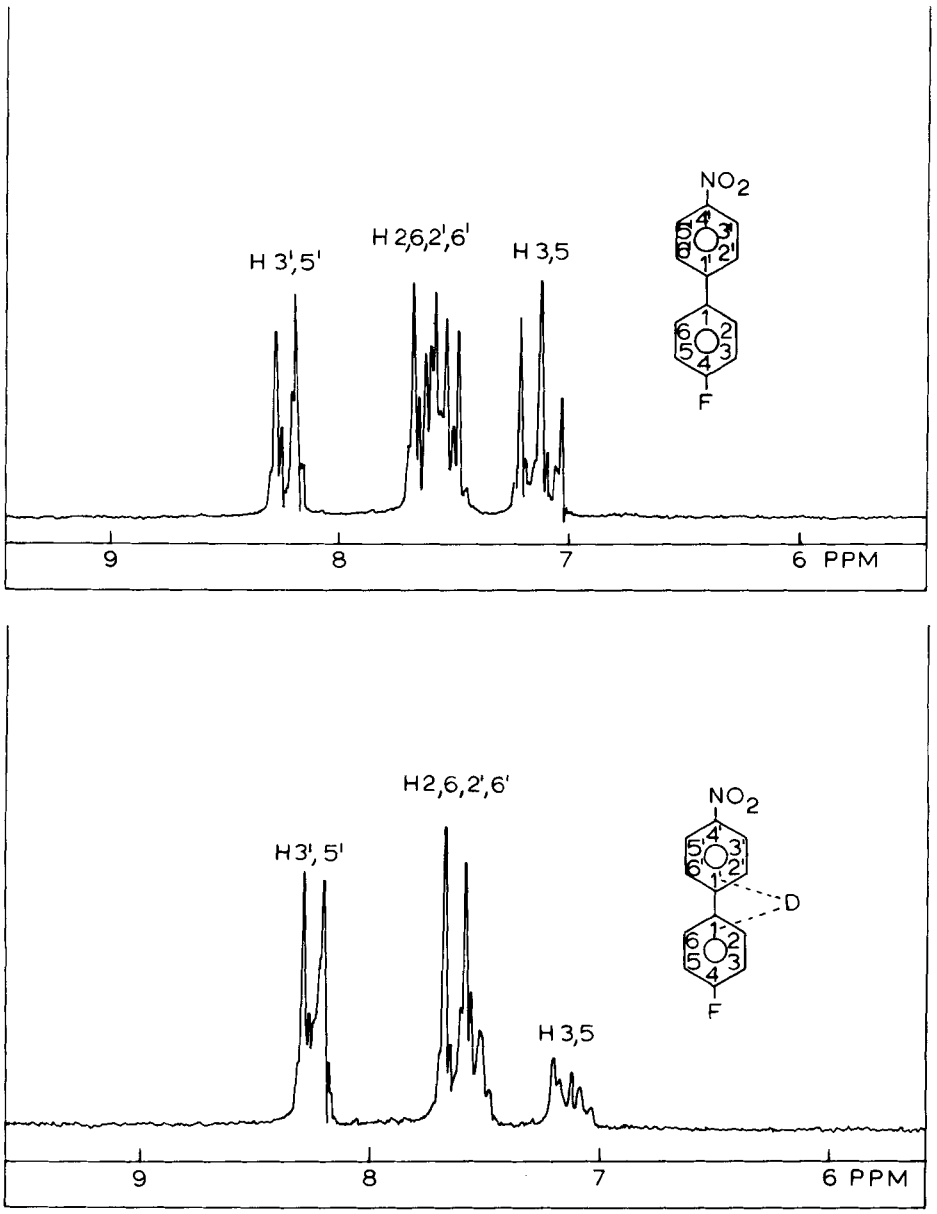


Figure 2

NMR spectrum of 4-fluoro-4'-nitrobiphenyl and [^{2}H(G)]-4-fluoro-4'-nitrobiphenyl. 45% of the deuterium is incorporated at the positions 3 and 5. Partly collapsing of the doublet structure of the signals H 3'5' after exchange shows that incorporation occurs at the positions 2' and 6' to some extend.

The deuterium incorporation could be followed with the aid of the NMR spectra (Figs.1 and 2). From the ratio between the integrals of the signals of the ortho protons and the methylene protons (Fig. 1) it could be concluded that in the case of 2-nitrofluorene exchange at the ortho position occurred to a minor extend only. Similar results were found for 3-methyl-4-nitrobiphenyl. The total number of incorporated deuterium was calculated by the isotope ratio of the molecular ion peak in the mass spectra. From these spectra it was concluded that between 0 and 6 deuteriums were incorporated. The number of incorporated deuteriums was also determined by measuring the integrals of the aromatic protons with regard to the ortho protons in the NMR spectra, assuming that only negligible exchange had taken place at the ortho positions. That this statement was correct, was confirmed by the agreement of the data obtained from the mass spectra and the NMR spectra (Table I). For the 4-nitrobiphenyl and the 2-nitrofluorene the exchange at the ortho position was determined more precisely by catalytic hydrogenation of the tritiated nitro compounds and conversion of the obtained amines into the ortho dibromo derivatives with bromine in acetic acid (6). The specific activities of the nitro compounds and the brominated amines were equal within 2%.

As was to be expected the ease of exchange was as follows: 2-nitrofluorene > 3-methyl-4-nitrobiphenyl > 4-nitrobiphenyl > 4-fluoro-4'-nitrobiphenyl (see Table I). The reactions were carried out in boiling trifluoroacetic acid in order to obtain sufficient solubility. Too high concentrations of TFMS gave rise to decomposition and low yields, while too low TFMS concentrations gave poor exchange. With the concentrations noted in Table I incorporation of 2 or 3 deuteriums was achieved. Using [^{3}H]-trifluoroacetic acid, the specific activities of the labelled nitro compounds did not correspond to the number of in-

Table I. Results of the exchange reactions in deuterium trifluoro acetate.

Compound	Vol. % TFMS [a]	Concentration nitro compound in g/ml [b]	Reaction time in hours	Number of inc. deuteriums [a] Mass spectrum	NMR spectrum	Yield in %
2-Nitrofluorene	1.3	0.07	3.5	3.1 ± 0.1	2.9 ± 0.3	80
3-Methyl-4-nitro-biphenyl	2.0	0.10	7	3.0 ± 0.1	2.8 ± 0.3	89
4-Nitrobiphenyl	4.8	0.10	24	3.4 ± 0.1	3.0 ± 0.3	90
4-Fluoro-4'-nitro-biphenyl	17	0.08	24	2.8 ± 0.1	2.7 ± 0.3	81

a) Unlabelled TFMS was added, so that in fact the number of exchanged protons is somewhat higher.

b) At this concentration the nitro compound was completely dissolved at 60°.

corporated deuteriums in Table I (see experimental section). This is probably due to the high sensitivity of the exchange reaction to water; with [^{2}H]-trifluoroacetic acid no exchange occurred, when 1% water was added. (Although isotope effects may also play a role.) Starting from tritiated water (90 mC/mmole) specific activities of the aromatic nitro compounds of about 100 mC/mmole were obtained. Conversion of the nitro compounds into the corresponding amines was carried out by catalytic reduction with Pd/C. No change in specific activities was detected within 2%.

In order to check metabolic displacement of the aromatic hydrogens in vivo, N-acetyl-2-amino-[^{3}H]-fluorene (96 mC/mmole) was mixed with a quantity of [9-^{14}C]-N-acetyl-2-amino-fluorene (20 mC/mmole). The doubly labelled material (^{3}H/^{14}C = 3.75 ± 0.06) was injected intraperitoneally into male rats of the strain R-Amsterdam as described (3). After 20 hr the rat liver DNA was isolated. In this DNA a ^{3}H/^{14}C-ratio of 3.55 ± 0.06 was found.

EXPERIMENTAL

Materials.

D_2O (99.75% D), trifluoroacetic acid, trifluoromethanesulphonic acid and 2-nitrofluorene were obtained from Merck, West Germany. 4-Nitrobiphenyl was obtained from Fluka A.G., Switzerland and 4-fluoro-4'-nitrobiphenyl from Koch Light Lab., England. 3-Methyl-4-nitrobiphenyl was synthesized by the reaction of N-nitroso-3-methyl-4-nitroacetanilide with benzene as described (7). The nitro compounds were purified by chromatography on a column of silicagel (50 x 3 cm) using benzene as eluent. The purity was checked by T.L.C., G.L.C. and melting point.

Tritiated water (90 mC/mmole) and biphenyl-[2-^{14}C]carboxylic acid (20 mC/mmole) were purchased from The Radiochemical Centre Ltd., Amersham, England. NMR spectra were recorded on a Varian H.A. 100 in deuterochloroform with TMS as standard. Mass spectra were taken on a Varian M.S. 9 at 160°, 70 eV. Isotope determinations of the molecular ion peaks were calculated as the average values of 5 scans and corrected for the natural isotope distribution.

Methods.

Trifluoroacetic anhydride was prepared by adding 45 ml trifluoroacetic acid slowly to 60 g P_2O_5 at 0°, followed by distillation through a vigreux (15 cm). The fraction distilling at 39-41° was collected. Trifluoroacetic acid [^{3}H] and -[^{2}H] were prepared by adding 38 ml trifluoroacetic anhydride to 4.5 ml tritiated water (90 mC/mmole) and 5 ml D_2O respectively at -10°. The acid was distilled through a vigreux (15 cm) and the fraction boiling at 70-71.5° was collected. From the NMR spectrum it was concluded that the obtained CF_3COOD did not contain more than 4% CF_3COOH.

Exchange reactions were carried out by distilling the labelled trifluoroacetic acid on 1 g of the nitro compounds (for reaction times and quantities see Table I) and adding a catalytic amount of trifluoromethanesulphonic acid. The reaction mixture was stirred and refluxed in a nitrogen atmosphere at 80°. At the end of the reaction 90% of the trifluoroacetic acid was quickly distilled off and could be used for another exchange reaction. To the residue 50 ml water were added and the precipitate was filtered and washed with water. The dried product was dissolved in 10 ml chloroform/benzene (1:1) and the solution was brought onto a column of silicagel and eluted with benzene. The products

were crystallized from ethanol; specific activities: [^{3}H(G)]-2-nitrofluorene, 98 mC/mmole; [^{3}H(G)]-4-fluoro-4'-nitrobiphenyl, 124 mC/mmole; [^{3}H(G)]-3-methyl-4-nitrobiphenyl, 104 mC/mmole; [^{3}H(G)]-4-nitrobiphenyl, 84 mC/mmole. Radioactive purity was checked by scanning a thinlayer chromatogram (silicagel, chloroform/ethanol 19:1) with a Berthold scanner.

[^{3}H(G)]-N-acetyl-2-aminofluorene was prepared by hydrogenation of 78 mg [^{3}H(G)]-2-nitrofluorene (98 mC/mmole) in 7 ml benzene with 40 mg Pd/C 5% at 1 at. After 2 hr the calculated amount of hydrogen was consumed and the reaction mixture was filtered. To the filtrate 0.5 ml acetic anhydride was added and after 2 hr at room temperature the solvent was evaporated and the residue crystallized from ethanol/water. The yield was 54 mg [^{3}H(G)]-N-acetyl-2-aminofluorene (96 mC/mmole). Its purity was checked by scanning a thinlayer chromatogram (silicagel, benzene) with a Berthold scanner and the I.R. spectrum.

[^{3}H(G)]-3,5-dibromo-4-aminobiphenyl and [^{3}H(G)]-1,3-dibromo-2-aminofluorene were synthesized by catalytic reduction of the nitro compounds as described above and conversion of the corresponding amines into the dibromo compounds as described in the reference cited (6). [^{3}H(G)]-4-aminobiphenyl (84 mC/mmole), [^{3}H(G)]-4-amino-3-methylbiphenyl (105 mC/mmole), [^{3}H(G)]-2-aminofluorene (98 mC/mmole) and 4-amino-4'-fluorobiphenyl (121 mC/mmole) were obtained by catalytic reduction as described above. The amines were crystallized from ethanol/water.

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