# SYNTHESIS OF N-PHENYL-2-[1,4,5,8-$^{14}$C]NAPHTHYLAMINE, N-PHENYL-2-[8-$^{13}$C] NAPHTHYLAMINE, AND N-[U-$^{14}$C]PHENYL-2-NAPHTHYLAMINE

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## SUMMARY

N-Phenyl-2-naphthylamine has been synthesized (1) with $^{14}$C in positions 1, 4, 5 and 8, (2) with $^{13}$C in position 8 (small amounts of [1-$^{13}$C] naphthalene and 2-[8-$^{13}$C]naphthylamine are formed as by-products), and (3) with the N-phenyl nucleus uniformly labelled with $^{14}$C.

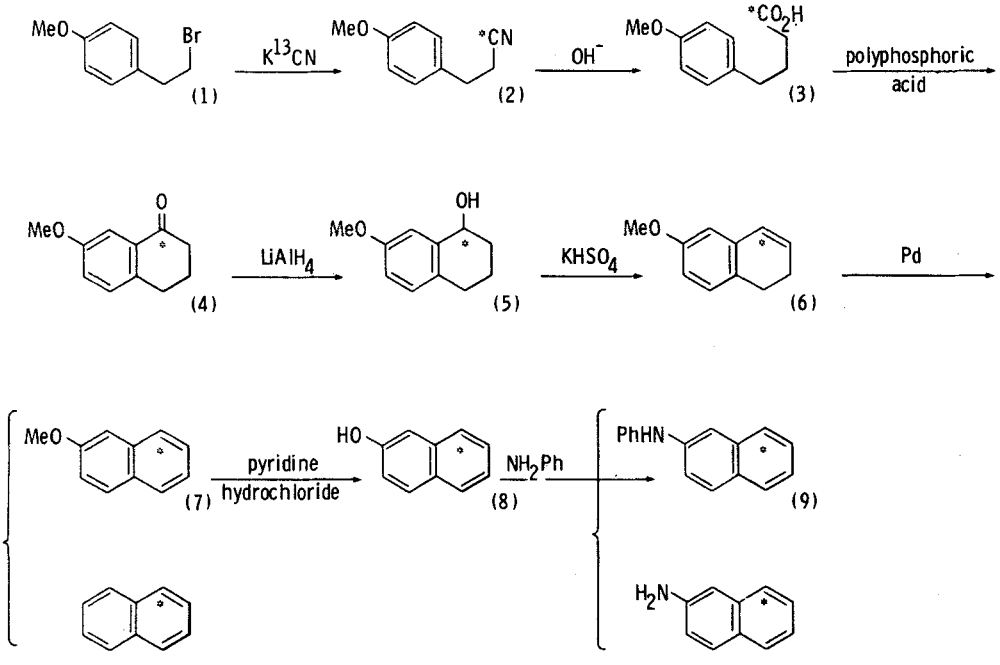
Forms of N-phenyl-2-naphthylamine that were labelled in the naphthalene nucleus with $^{14}$C and $^{13}$C were required for an investigation in animals of the possibility that this important rubber antioxidant may undergo oxidative N-dephenylation. The $^{14}$C-labelled form is necessary for monitoring N-phenyl-2-naphthylamine metabolites, and $^{13}$C-labelled material facilitates identifications involving mass spectrometry. In the event of 2-naphthylamine being formed, the nature of the leaving-group would have to be determined, and in that connexion, N-[U-$^{14}$C]phenyl-2-naphthylamine is also required.

While N-phenyl-2-[1,4,5,8-$^{14}$C]naphthylamine and N-[U-$^{14}$C]phenyl-2-naphthylamine are obtained by unit synthesis, the preparation of

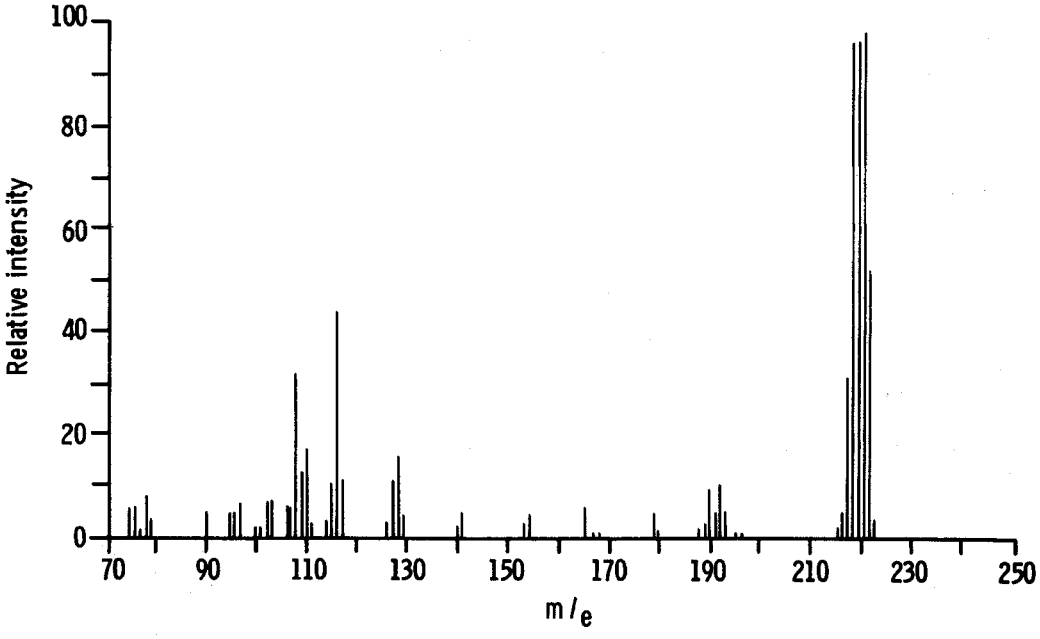
N-phenyl-2-[8-$^{13}$C]naphthylamine (9) *via* 7-methoxy-1-[1-$^{13}$C]tetralone (4) (Campbell *et al.*, 1942) is more difficult, since dehydrogenation of the tetralin prepared by reduction of 7-methoxy-1-tetralone gives an equimolar mixture of 2-methoxynaphthalene and naphthalene, and since step-wise synthesis of 2-[8-$^{14}$C]naphthylamine from 3-(*p*-anisyl) propyl bromide (1) according to Catch *et al.*, (1953) affords only a low yield of the carcinogen.

We considered that the analysis of all of the crude reaction mixtures by gas chromatography and that the isolation of the major component at each stage (*v.* Reaction Scheme) would lead to improved synthesis of 2-[8-$^{13}$C]naphthol (8) from (1) by a reaction sequence which differed in several processes from that of Catch *et al.* (1953) for 2- 8-$^{14}$C naphthol. A better yield of pure (1) was obtained by reaction of 3-(*p*-anisyl) prop-2-en-1-ol with aqueous 48% HBr, not with Horeau's (1948) $PBr_3$ reagent, and nearly an 80% yield of pure (3) *via* nucleophilic replacement of bromide with $^{13}CN^-$. Step-wise synthesis of 2-methoxy-[8-$^{13}$C]naphthalene (7) from (4) *via* (5) and (6) (*v.* Reaction Scheme) gave a high yield of product,

Reaction Scheme



contaminated only with 3% of [1-$^{13}$C]naphthalene and 3% of starting-material. Nucelophilic replacement of the hydroxyl group of (8) yielded N-phenyl-2-[8-$^{13}$C] naphthylamine (9) with a 5.5 atom % enrichment of the $^{13}$C isotope, compared with the theoretical 6.4 atom % enrichment from $^{13}$C isotope in the K$^{13}$CN source. Hence, the overall yield of (9) from (1) exceeded 30%, and this takes into account a low yield (54%) of (4) resulting from polyphosphoric acid cyclization, whereas had this stage been replaced by HF cyclization (Campbell et al., 1942) with its attendant high yield of (4), a 50.5% overall yield of (9) might have been attained.




The mass spectrum of N-phenyl-2-[8-$^{13}$C]naphthylamine (Fig. 1) shows strong peaks at 221, 220, 219 and 218 due to $^{13}$C, $^{12}$C peaks for the (M+1) and (M-1) mass ions; peaks at 128 and 127 correspond to the naphthyl fragments and those at 116 and 115 to mass fragments that have lost a C(=NH)Ph group.

Animal experiments with the $^{14}$C and $^{13}$C-labelled forms of N-phenyl-2-naphthylamine will be reported elsewhere.

## EXPERIMENTAL

Melting points (m.p.) were determined on a Kofler microscope hot-stage. $^{14}C$ activities were measured by liquid scintillation methods using an Intertechnique SL30 spectrometer. Merck t.l.c. plates (250μ,$GF_{254}$) were used to monitor purity; preparative plates were coated with Merck Kieselgel HR 0.5 mm thick. Radiochemical purity was monitored using Kodak X-ray film. Gas chromatography (g.l.c.) was carried out on a Pye Unicam Series 104 instrument equipped with flame ionisation detection and using 5 ft columns (inside diameter $\frac{1}{8}$ in of Johns Manville Chromosorb W (AWDMCS), (1) 60-80 mesh size, that had been coated with 5% of PEGS and run at 170°, (2) 80-100 mesh size, coated with 5% of DEGA and run at 175°, or (3) 60-80 mesh size, coated with 5% of OV-1 and run at 150°. The flow rate for $N_2$ through all three columns was 60 ml $min^{-1}$. G.L.C. was used to estimate the proportions of product and by-products in the crude reaction mixtures, and to compare retention times of $^{13}C$-labelled products with those of reference compounds. Mass spectra were made with an A.E.I. high resolution M.S.9. instrument, using the solid-sample insertion probe.

3-(p-Anisyl)propyl bromide was prepared by conventional methods. - Thus, Döbner-Knoevenagel condensation of anisaldehyde and malonic acid in the presence of piperidine afforded a 92% yield of p-methoxycinnamic acid, which crystallized from ethanol in needles, m.p. 172° (lit., 170°). Reductive hydrogenation in the presence of 2% palladized charcoal yielded 80% of 3-(p-anisyl) propionic acid (phloretic acid methyl ether), which crystallized from aqueous ethanol in needles, m.p. 104-105° (lit., 104-105°) (Found: C, 66.4; H, 6.9.$C_{10}H_{12}O_3$ requires C,66.7;H,6.7%), and $LiAlH_4$ reduction in boiling ether gave a 91% yield of pure (by g.l.c.) 3-(p-anisyl) propan-1-ol, b.p. 122°/2.0 mm, with an 8.5 min retention time on column (1). 3-(p-Anisyl)propan-1-ol (44g) was gradually added with stirring to a solution containing constant-boiling aq. 48% HBr (52 ml) and $H_2SO_4$ (14 ml, d 1.84), and the mixture was refluxed for 5 h. Ether

extraction of the chilled reaction mixture gave an oil which was distilled to give pure 3-(p-anisyl) propyl bromide (42g; 73% yield), b.p. 124-127°/2mm (cf. Catch et al., 1953; Horeau, 1948) (Found: C, 52.2, H, 5.7; Br, 35.0 $C_{10}H_{13}OBr$ requires C,52.4;H,5.7;Br, 34.9).

3-(4-Anisyl)propyl[$^{13}$C]nitrile

1.0g of $K^{13}CN$ (with an 88 atom % enrichment of the $^{13}C$ isotope) (B O C Limited, Prochem, London SW19 3UF) was added to a solution of 3-(p-anisyl)propyl bromide (5.24g) in dimethyl sulphoxide (130 ml), and the reaction mixture was stirred for 3 h at 90-95°, when the resulting solution was poured into water (1.5 l). n-Hexane extraction gave an oil (2.8g), which by g.l.c. was 95% pure, and which had a retention time of 15.4 min on column (1).

4-(4-Anisyl)butan[$^{13}$C]oic acid

3-(4-Anisyl)propyl[$^{13}$C]nitrile (2.79g) was added to a solution of NaOH (12.5g) plus KOH (12.5g) in a mixture of ethane-1,2-diol(185 ml) and water (125 ml), and the reaction mixture was stirred for 6 h at 115° Ether extracts of the chilled solution were rejected and those of the acidified aqueous phase gave the acid, 2.46 g (79.2% yield), sufficiently pure for the next stage.

7-Methoxy-1-[1-$^{13}$C]tetralone

(cf. Campbell and Todd, 1942; Haworth and Sheldrick, 1934) 4-(4-Anisyl)butan[$^{13}$C]oic acid (2.43 g) was added to polyphosphoric acid (75 g), and the reaction mixture was stirred for 2 h at 120°. The chilled mixture was poured on to ice and the ether extracts were washed successively with 2N-NaOH and water. Evaporation of the dry solvent ($MgSO_4$) left 1.2 g (54%) of pure (by g.l.c.) 7-methoxy-1-[1-$^{13}$C]tetralone, which had a 10.7 min retention time on column (1).

7-Methoxy-1,2,3,4-tetrahydro-[1-$^{13}$C]naphth-1-ol

An ether solution (75 ml) of 7-methoxy-1-[1-$^{13}$C]tetralone (1.2 g) was

added dropwise to a stirred solution of $LiAlH_4$ (100 mg) in boiling ether. After one hour's refluxing, excess reagent was destroyed by ethyl acetate (3 ml). Ether extraction of the acidified (2N-HCl) reaction mixture gave an oil (1.21 g), which was found to be pure by g.l.c., and which had a 14.6 min retention time on column (2).

2-Methoxy-5,6-dihydro-[8-$^{13}$C]naphthalene

7-Methoxy-1,2,3,4-tetrahydro-[1-$^{13}$C]naphth-1-ol (1.2 g) was stirred with freshly-prepared, fused $KHSO_4$ powder (900 mg) at 180° for 1h. The ether extracts of an aqueous solution (50 ml) of the cooled reaction mixture were dried ($MgSO_4$). Evaporation of the solvent left an oil, which was shown by g.l.c. to be a pure product with a 2.8 min retention time on column 3.

2-Methoxy-[8-$^{13}$C]naphthalene

2-Methoxy-5,6-dihydro-[8-$^{13}$C]naphthalene (1.0 g) was stirred with 1 g of 10% palladized charcoal at 220°/550 mm for 1 h. Ether extraction of the cooled product afforded crystals (713 mg; 72% yield), which were shown by g.l.c. on column (3) to consist of 3% of [1-$^{13}$C]naphthalene with a retention time of 1.2 min, 3% of unchanged 2-methoxy-5,6-dihydro-[8-$^{13}$C]naphthalene with a retention time of 2.8 min, and 94% of 2-methoxy-[8-$^{13}$C]naphthalene with a retention time of 3.2 min.

2-[8-$^{13}$C]Naphthol

Crude 2-methoxy-[8-$^{13}$C]naphthalene (710 mg) was stirred with pyridine hydrochloride (3 g) at 210° for 6 h, when the cooled melt was dissolved in N-HCl. Ether extraction afforded 2-[8-$^{13}$C]naphthol (669 mg), which had a retention time of 4.0 min on g.l.c. column (3), and which was further purified (613 mg; 95% yield) by extraction into alkali.

N-Phenyl-2-[8-$^{13}$C]naphthylamine

A mixture of 2-[8-$^{13}$C]naphthol (613 mg), aniline (790 mg) and

p-toluenesulphonic acid (10 mg) were heated under $N_2$ for 1 h at 190°, and then for 6 h at a temperature slowly rising from 190° to 240°; the reaction vessel facilitated the escape of water vapour that was produced during the reaction. A solution of the cooled melt in acetone-n-hexane (1:1, v/v) mixture was applied to the head of a 100 mesh $SiO_2$-gel column (10 mm diam. x 8 cm long), which was eluted with acetone-n-hexane (1;9 v/v) mixture until drops of eluate, when injected into a gas chromatograph using column (3), no longer contained N-phenyl-2-naphthylamine. Dropwise addition of water to the warm glacial acetic acid (3 ml) solution of the evaporate (925 mg) produced fawn needles, which were dissolved in ethyl acetate, and washed with N-NaOH to remove any unchanged 2-[8-$^{13}$C]napthol. The residual solvent phase was washed free from alkali, and dried ($MgSO_4$). Crystallization of the evaporate from a small volume of ethyl acetate afforded 606 mg of pure (by g.l.c.)N-phenyl-2-[8-$^{13}$C]naphthylamine, with an 8.7 min retention time on column 3 operating at 200°, and with a 5.5 atom % enrichment of the $^{13}$C isotope. For this purification, t.l.c. was used with toluene as developing solvent. In that system, the $R_F$ value of N-phenyl-2-naphthylamine was 0.53, of 2-naphthol was 0.10 and of 2-naphthylamine was 0.17. Whilst the crude reaction mixture contained traces of 2-[8-$^{13}$C]naphthylamine, this substance was entirely absent from the purified product.

Similarly, N-phenyl-2-[1,4,5,8-$^{14}$C]naphthylamine (95.1% chemical yield, 95.1% radiochemical yield; specific activity 1.44 mCi/mM) and N-[U-$^{14}$C]phenyl-2-naphthylamine (36.1% chemical yield, 25.8% radiochemical yield; specific activity 64.2μCi/mM) were synthesized by the final reaction process in the Reaction Scheme for N-phenyl-2-[8-$^{13}$C]naphthylamine, but using respectively 2-[1,4,5,8-$^{14}$C]naphthol (sp. activity, 1.59 mCi/m mole) and [U-$^{14}$C]aniline hydrogen sulphate (sp. activity, 248μCi/m mole) to introduce the $^{14}$C label. These reagents were prepared by dilution, with the correspondingly unlabelled chemicals, of radiochemical (obtained from the Radiochemical Centre, Amersham,

Buckinghamshire, England). Since predictable specific activities for the two preparations were respectively 1.59 and 0.248 mCi/m mole, the radiochemical yield that was obtained for the second synthesis was disappointingly low. Autoradiography of materials that were run on thin-layer plates showed them to be radiochemically pure.

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