^{14}C- AND ^{2}H-LABELLED 7-METHYLBENZ[c]ACRIDINE

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SUMMARY

7-Methylbenz[c]acridine-7-^{14}C, specific activity 12.2 mCi/mmole, and deuterated 7-methylbenz[c]acridine were prepared from acetic anhydride-1-^{14}C and acetic anhydride-2-$^{2}H_6$ respectively. The deuterated compound was not specifically labelled at the 7-methyl group because isotopic exchange of the hydrogen atoms occurred during synthesis.

KEY WORDS. 7-Methylbenz[c]acridine-7-^{14}C, deuterated 7-methylbenz-[c]acridine, polycyclic heteroaromatic compounds, air pollutants.

Recent studies of the metabolism and of the metabolically activated DNA-binding properties of polycyclic aromatic hydrocarbons (such as benzo[a]pyrene) and their derivatives have shed considerable light on the possible mechanisms of chemical carcinogenesis mediated by these compounds[1]. Polycyclic heteroaromatic compounds such as dibenz[a,h]acridine and alkylbenz[c]acridines are weakly carcinogenic compounds found as air pollutants[2,3]. The metabolism and DNA-binding ability of such compounds are of interest, and to facilitate such studies 7-methylbenz[c]acridine-^{14}C and deuterated 7-methylbenz[c]acridine were synthesized.

The synthesis of ^{14}C-labelled 7-methylbenz[c]acridine (4) using the Bernthsen reaction of N-phenyl-1-naphthylamine (1) with acetic acid and zinc chloride has previously been reported[4]. In developing the method of choice for the highest radiochemical yield we investigated variation of the molar proportions of reagents (Table 1) and other experimental conditions (see end of paper).

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When acetic anhydride (in a 2:1 molar ratio relative to N-phenyl--1-naphthylamine (1)) replaced acetic acid used originally, and N-acetyl-N-phenyl-1-naphthylamine (2) was allowed to form at room temperature and then converted to 7-methylbenz[c]acridine without prior isolation, the yield obtained (69%) exceeded those encountered in large scale preparations[5]. The yield fell to 52% when N-phenyl--1-naphthylamine (1) was heated with acetic anhydride immediately after mixing. The best yield based on acetic anhydride was obtained with an equimolar ratio of acetic anhydride to amine (1). Under these conditions 7-methylbenz[c]acridine-7-^{14}C was obtained in 43% yield from acetic anhydride-2-^{14}C, and was of greater than 98% radiochemical purity.

TABLE 1

Effect of experimental conditions on the yield of 7-methylbenz[c]-acridine in sealed tube experiments.

Molar ratio of acetic anhydride to amine (1)	Catalyst	Time of heating in h[a]	Yield(%) based on acetic anhydride	Yield(%) based on amine(1)
2:1	$ZnCl_2$	6	21	42
2:1	$ZnCl_2$	24[b]	26	52
2:1	$ZnCl_2$	24	35	69
2:1	$ZnCl_2$	72	34	67
1:1	$ZnCl_2$	24	44	44
2:1	Polyphosphoric acid	24	14	27
1:1	P_2O_5	24[c]	11	21

[a] All reaction mixtures except those specified otherwise were stood at room temperature for 48 h before being heated to 240°.

[b] No prior standing at room temperature.

[c] Reaction temperature, 180°.

The reaction was repeated under identical conditions with a mole equivalent of acetic anhydride-2-$^{2}H_{6}$. The product obtained showed a cluster of peaks in the chemical ionisation mass spectrum (CI-MS) of m/e from 244 to 252 (Figure 1) indicating that deuterium scrambling occurred during synthesis. Such exchange was not surprising since a mole of water and of acetic acid are produced during the reaction. In the presence of excess Lewis acids deuterium-protium exchange may be expected for both the aromatic and methyl hydrogen atoms[6]. For example, extensive scrambling of the label occurred even at low temperatures during acetylation of a deuterated paracyclophane in the presence of aluminium chloride[7].

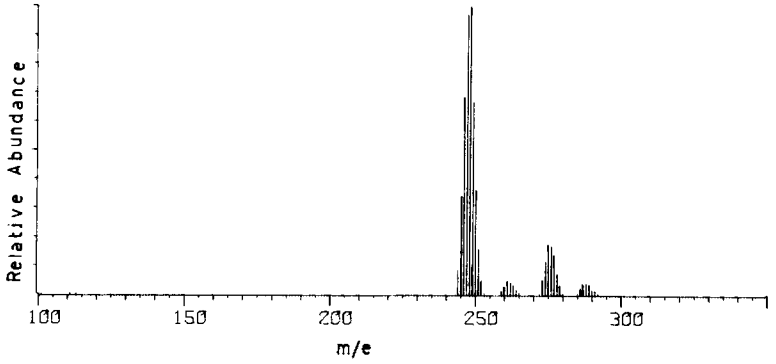


Figure 1. Chemical ionisation mass spectrum of deuterated 7-methylbenz[c]acridine.

The content and distribution of deuterium in the deuterated 7-methylbenz[c]acridine (Table 2) was determined by ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and by CI mass spectrometry. The CI-MS of the deuterated sample showed the presence of an average of 3.7 deuterium atoms per molecule which agreed with the corresponding figure of 3.9 from ^{1}H NMR (Table 2). Assuming complete scrambling of hydrogen, the theoretical content of deuterium is 4.02 atoms per molecule (based on stated isotope content of acetic anhydride-$^{2}H_{6}$ of 98%).

TABLE 2

^{1}H Chemical shifts of, and deuterium incorporation in deuterated 7-methylbenz[c]acridine.

Position	δ[a]	Incorporation of ^{2}H (as atoms of ^{2}H)	
		From ^{1}H NMR[b]	From CI-MS[c]
CH_3	2.82	0.6	
C-1	9.48	<0.2	
C-2,C-3,C-4	7.55-7.8		
C-5	7.50	<0.27	
C-6	7.76	~0.3	
C-8	8.05		
C-9	7.71	0.31	
C-10	7.45	0.24	
C-11	8.29		
Total	-	3.9	3.7

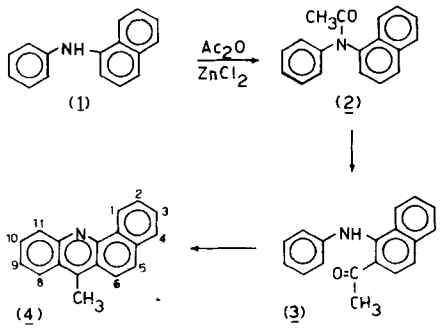
[a] Aromatic ^{1}H signals are assigned by low-power ^{1}H-^{1}H decoupling, nuclear Overhauser enhancement measurements, and comparison with related compounds (unpublished results).

[b] The deuterium content at a particular aromatic position is estimated from the line-shape and integrated intensity of the ^{1}H NMR signal of an adjacent ortho proton; that at the methyl group from the integrated intensities of the CH_3, CH_2D and CHD_2 components of the methyl ^{1}H signal (the CD_3 component being negligible). The integrated intensity of the methyl signal, extrapolated to the case where deuterium incorporation is zero, serves as integration standard in determining the content of deuterium in the whole molecule.

[c] The average deuterium content was determined from the relative peak heights at m/e values from 243 to 252. Correction was made for the contributions due to ^{13}C and ^{15}N, and the proportion of m/e 243 to 244 in the spectrum of unlabelled compound run under identical GC-MS conditions. The actual abundances were d_0 1.6%, d_1 6.9%, d_2 16.0%, d_3 22.1%, d_4 24.0%, d_5 16.5%, d_6 8.0%, d_7 3.6%, and d_8 1.2%.

Some evidence for the mechanism of the Bernthsen reaction as postulated by Lacassagne et al.[3], and shown in Scheme 1 for 7-methylbenz[c]acridine, was obtained. The N-acetate (2) formed at room temperature was isolated in fair yield in one experiment.

SCHEME I



Both intermediate (2) and the C-acetyl compound (3) were converted in low yield to 7-methylbenz[c]acridine (4) on heating with zinc chloride. These yield were not optimised. The rearrangement of compound (2) to compound (3) has not been demonstrated under our conditions. The C-acetyl compound (3) was prepared from 2-acetyl--1-naphthol by the Knoevenagel synthesis (see Experimental).

EXPERIMENTAL

Radioactivity was determined in a Packard model 3255 liquid scintillation spectrometer using a counting medium containing PPO (1.5 g), dimethyl POPOP (50 mg) and ethanol (100 ml) per L in toluene. Radiochemical purity was determined in a Nuclear Chicago Actigraph III radiochromatogram scanner. Methane CI-MS were run on a Finnigan 3200E gas chromatograph mass spectrometer and associated Finnigan 6110 data system. Compounds were injected onto 30 cm of 3% OV1 at 150° and the temperature was raised to 260° at 10° per min. ^{1}H NMR spectra were measured at 100 MHz in chloroform-^{2}H containing tetramethylsilane as internal standard.

7-Methylbenz[c]acridine-7-^{14}C. N-Phenyl-1-naphthylamine (1) (24.4 mg), fused zinc chloride (75 mg) and acetic anhydride-1-^{14}C (11.4 mg, 27 mCi/mmole) were sealed under nitrogen in a glass tube and allowed to stand at room temperature for 48 h. After being

heated at 230-240° for 24 h the then tarry reaction mixture was refluxed with 4M sulphuric acid (30 ml) and toluene (10 ml). The product, isolated by treatment of the aqueous phase with excess 5M sodium hydroxide solution and extraction with toluene, was purified by thin-layer chromatography (TLC) using 2% acetone in cyclohexane for development. The 7-methylbenz[c]acridine-7-^{14}C obtained (11.5 mg, specific activity 12.2 mCi/mmole) was radiochemically 98% pure by TLC. During trial experiments with unlabelled acetic anhydride a yellow crystalline compound separated from the sulphuric acid phase. Recrystallisation from ethanol-chloroform gave the sulphate salt of 7-methylbenz[c]acridine as a hydrate, mp 252-254°; m/e (CI) 244 (MH^+); m/e (electron impact) 243 (M^+), and 242 (base peak) (Found: C, 58.8; H, 4.9; N, 3.7; S, 8.8%. Calculated for $C_{18}H_{15}NO_4S.1\frac{1}{2}H_2O$: C, 58.7; H, 4.9; N, 3.8; S, 8.7%).

Deuterated 7-methylbenz[c]acridine. N-Phenyl-1-naphthylamine (1) (500 mg), acetic anhydride-2-$^2H_6$ (0.25 ml, 98% $(CD_3CO)_2O$) and zinc chloride (1.5 g) were sealed under nitrogen in a glass tube and reacted as above. The product was isolated as above and purified by column chromatography on neutral alumina. Elution with light petroleum and recrystallisation from the same solvent afforded a product (48%), mp 129-132°, having CI-MS shown in Figure 1.

7-Methylbenz[c]acridine from "Bernthsen intermediates". (i) From N-acetyl-N-phenyl-1-naphthylamine (2): N-Acetyl-N-phenyl-1-naphthylamine (0.30 g) and fused zinc chloride (0.75 g) were heated under nitrogen at 230-240° for 24 h. The tarry reaction mixture was worked up as above using TLC separation to give 7-methylbenz[c]-acridine (4) in 32% yield. (ii) From N-phenyl-2-acetyl-1-naphthylamine (3): N-Phenyl-2-acetyl-1-naphthylamine (0.10 g) and fused zinc chloride (0.30 g) were reacted and worked up as in (i) to afford 7-methylbenz[c]acridine (4) in 8% yield.

N-Acetyl-N-phenyl-1-naphthylamine (2). (i) N-Phenyl-1-naphthylamine (1) (1.01 g), acetic anhydride (4.7 g) and freshly fused

zinc chloride (3.0 g) were allowed to stand at room temperature for 48 h. The reaction mxiture was poured into water and the product isolated with ether. Recrystallisation from ethanol afforded N-acetyl-N-phenyl-2-naphthylamine (2) (53%), mp 124-6° (lit.[8] mp 115°); δ2.00(Ac), 7.1-8.2 ppm (arom.H); m/e (CI) 262 (MH^+).

(ii) To N-phenyl-1-naphthylamine (1) (20 g) in acetic anhydride (43 g) was added 0.2 ml of concentrated sulphuric acid, and the mixture was worked up to give the N-acetyl derivative (2) (17 g), mp 125-6°.

N-Phenyl-2-acetyl-1-naphthylamine (3). 2-Acetyl-1-naphthol (7.2 g), aniline (3.6 g) and iodine (1.2 g) were heated under reflux in a Dean-Stark apparatus. The lost aniline was returned to the reaction flask during the reaction, and after 19 h the reaction mixture was cooled and diluted with chloroform. After extraction with aqueous sodium hydroxide the chloroform was distilled from the organic phase. The residue was distilled under reduced pressure affording N-phenyl-2-acetyl-1-naphthylamine (3) (4.1 g), bp 120-135°/0.3 mm, which was recrystallised from ethanol giving needles, mp 129-131°; δ2.37 (Ac), 6.9-7.8 (10 arom.H), 8.50 ppm (H-3); m/e (CI) 262 (MH^+) (Found: C, 82.4; H, 6.02; N, 5.38%. Calculated for $C_{18}H_{15}NO$: C, 82.8; H, 5.75; N, 5.36%).

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