SYNTHESIS OF CARBON-13 LABELLED 6-SUBSTITUTED BENZO(a)PYRENES

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SUMMARY

Sodium formate-^{13}C, prepared by hydrolysis of isopropyl formate, was allowed to react with N-methylaniline hydrochloride to prepare the formylating agent N-methylformanilide-1-^{13}C. Formylation of benzo(a)pyrene with N-methylformanilide-1-^{13}C gave the 6-^{13}CHO derivative which was reduced to 6-$^{13}CH_3$ and 6-$^{13}CH_2OH$ benzo(a)pyrenes. 6-$^{13}CH_2Cl$ benzo(a)pyrene was prepared from the 6-$^{13}CH_2OH$ derivative.

Key Words: 6-substituted benzo(a)pyrenes, N-methylformanilide-1-^{13}C

INTRODUCTION

The carcinogenic activities of benzo(a)pyrene and related polycyclic aromatic hydrocarbons are well known although mechanisms whereby these chemical carcinogens induce cell transformation are not yet understood. As part of a program designed to synthesize ^{13}C-enriched benzo(a)pyrenes and their derivatives in order to use ^{13}C-NMR as a probe for the determination of structures of the interaction products of chemical carcinogens and biomolecules, we report here the synthesis of benzo(a)pyrene derivatives with ^{13}C-enriched substituents at position 6. Included among these are the carcinogenic 6-formyl and 6-hydroxymethyl derivatives (1) which may be formed metabolically from benzo(a)pyrene (2,3). The following scheme summarizes the reactions used in the preparation of these various carbon-13 labelled 6-substituted benzo(a)pyrenes.

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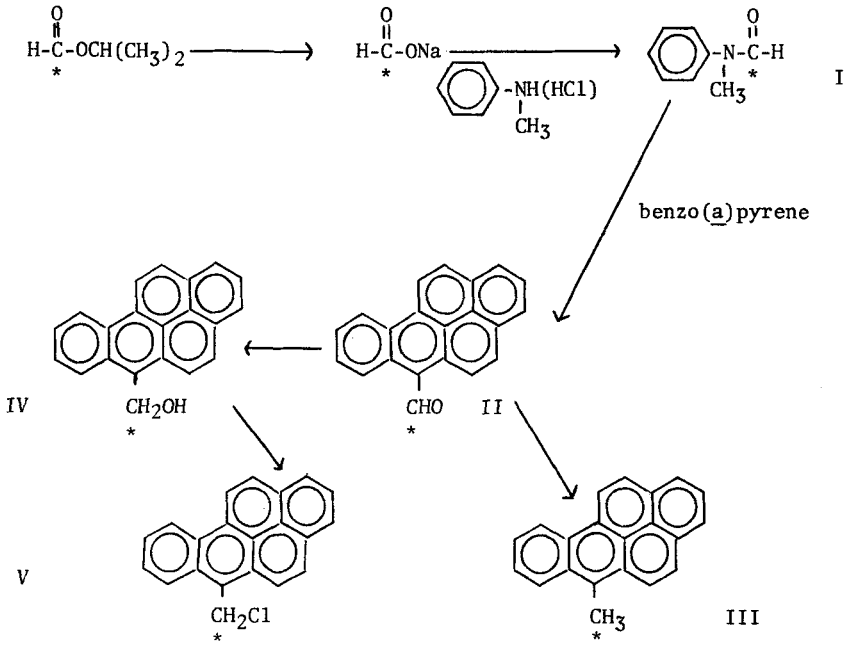
SCHEME 1



EXPERIMENTAL

N-methylformanilide-1-^{13}C (I) - Isopropyl formate (90.93% ^{13}C in the formyl carbon) as an 82% by weight solution in 2-propanol was obtained from the Los Alamos Scientific Laboratory (Group H-11). This solution (38.3 g, 0.35 mol isopropyl formate) was added to 100 ml of 40% aqueous ethanol containing 0.35 mol NaOH, and the resulting solution was refluxed for 2 hr, after which the solvents were removed on a rotary evaporator. The resulting sodium formate was used directly. N-Methylaniline hydrochloride (65 g, 0.45 mol) was added to the 0.35 mol sodium formate (^{13}C) and the mixture heated at 150° for 1 hr, after which the water formed was removed by distillation. After cooling, 5% HCl (50 ml) was added and the resulting aqueous solution was extracted 4 times with 50 ml portions of diethyl ether. After drying over $MgSO_4$, the ether was removed over a steam bath, and the ^{13}C labelled N-methylformanilide (I) was distilled at 8 Torr. The fraction bp 114-121° (36.4 g, 76%) was collected. (lit(4) bp 114-121°/

8 Torr).

6-Benzo(a)pyrenecarboxaldehyde-formyl-^{13}C (II) - Benzo(a)pyrene (4 g, 15.9 mmol), N-methylformanilide-1-^{13}C (4.5 g, 33 mmol), and phosphorus oxychloride (4.5 g, 29 mmol) were warmed over a steam bath for 2.5 hr in a flask fitted with a reflux condenser. The system was flushed with dry nitrogen before heating the mixture. After cooling, the solution was poured into 200 ml of 10% aqueous sodium acetate, and the crude product was filtered and recrystallized twice from chloroform giving 3.25 g of product, mp 202-203°. The residue from the mother liquors was chromatographed on alumina with benzene, giving an additional 0.7 g, mp 202.5-203.5°; (lit. (5) mp 202.5-203.5°); total yield 88%.

6-Methyl-^{13}C-benzo(a)pyrene (III) - 6-Benzo(a)pyrenecarboxaldehyde-formyl-^{13}C (0.7 g, 2.5 mmol), hydrazine hydrate (0.66 ml, 0.013 mol), diethylene glycol (40 ml) and KOH (0.44 g, 7.9 mmol) were heated with stirring for 30 min at 100° and then for 2 hr at 140-150°. After cooling the mixture, water was added to precipitate the product which was filtered and dried, and chromatographed on alumina with benzene. After concentration of the eluant, ethanol was added to crystallize the product, giving 0.42 g (60%) of product, mp 214-215°. Sublimation and a second benzene/ethanol crystallization gave material mp 215 -216° (lit. (6) mp 216.2-216.7°).

6-Benzo(a)pyrenemethanol-^{13}C (IV) - 6-Benzo(a)pyrenecarboxaldehyde-formyl-^{13}C (3.25 g, 11.6 mmol), aluminum isopropoxide (16.25 g, 79 mmol) and 2-propanol (325 ml) were distilled slowly with a Hahn condenser until no more acetone was collected. The remaining solution (150 ml) was poured into cold 5% HCl (600 ml). The crude product was filtered and was recrystallized 3 times from benzene, giving 3.1 g (95%) of product, mp 228-229° (d); in a sealed tube, mp 230-231° without decomposition, followed by solidification and remelting at 290° with decomposition. (lit. (1) mp 232-233°).

6-Chloromethyl-^{13}C-benzo(a)pyrene (V) - 6-Benzo(a)pyrenemethanol-

^{13}C (0.20 g, 0.71 mmol), $SOCl_2$ (0.20 g, 1.7 mmol) and benzene (7 ml) were refluxed for 30 min with stirring, after which time the solvent was removed. The crude product was recrystallized twice from benzene giving 0.17 g (80%) of product, mp 218-219° (lit. mp (7) 215-218° (d).)

Table I. ^{13}C Chemical Shifts of 6-Substituted-Benzo(a)pyrenes

Compound	Chemical Shift (ppm)
II	194.8
III	14.3
IV	55.8
V	39.5

All spectra taken in DMSO-d_6; Chemical shifts are ppm downfield from TMS; spectra were obtained with a Varian XL-100 CW/FT (Nicolet TT 100 FT System) on FT mode at 25.2 MHz, solution concentrations 6 mg/ml, using 1000 pulses FT-CMR.

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