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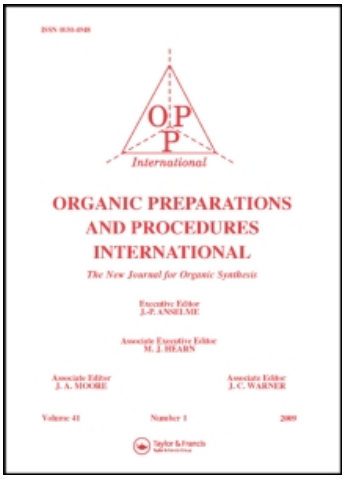

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CONVENIENT SYNTHESES OF SELECTED ^{14}C- AND ^{3}H-LABELED AROMATIC HYDROXYLAMINES

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CONVENIENT SYNTHESES OF SELECTED ^{14}C- AND ^{3}H-LABELED AROMATIC HYDROXYLAMINES

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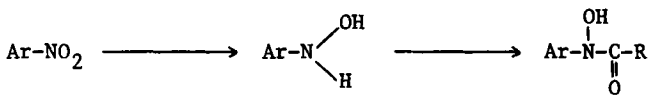
Recent studies regarding the role of arylhydroxamic acid acyl transferase in the metabolic activation of aromatic amines[1-3] prompted us to undertake the synthesis of several radiolabeled hydroxamic acid derivatives and related intermediates. In general, the hydroxamic acids were conveniently prepared by acylation of the corresponding N-hydroxy compounds which had, in turn, been prepared by reduction of the appropriate nitro substituted precursors. The compounds prepared to date are listed in the Table.



Earlier reports of the preparation of N-hydroxy-2-aminofluorene[4] and other aromatic hydroxylamines[5-7] utilized the method of Willstatter and Kubli[8] in which an ethanolic solution of an aromatic nitro compound was sequentially saturated with ammonia and hydrogen sulfide. We have improved this basic procedure by using commercially available aqueous ammonium sulfide and a co-solvent (e.g., DMF), which eliminates effort, odor, and the hazard of using hydrogen sulfide gas. The extent of reduction is controlled

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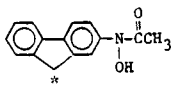
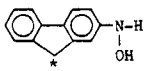
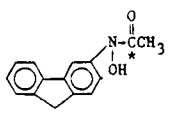
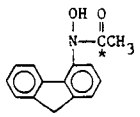
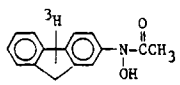
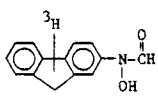
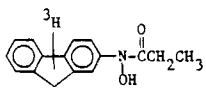
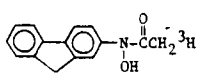
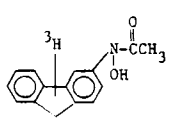
TABLE. ^{14}C- AND ^{3}H-LABELED AROMATIC HYDROXYLAMINES

Compound No.	Structure (* = ^{14}C)	Recrystallization Solvent	TLC System[k]	TLC Rf	Specific Activity (mCi/mmol)	% Yield
I [a]		THF:Hexanes (1:4) -20°; or EtOH:EtOAc:Cyclo-hexane (1:4:4)	A	0.75	11	22
II [b]		-	B	0.26	8.3	31
III [c]		THF:Hexanes (1:10) 0°	B	0.30	13.6	58
IV [c]		THF:Hexanes (1:15) 0°	B	0.25	10.4	16
V [a]		THF:Hexanes (1:4) -20°	D	0.30	137	33
VI		THF:Hexanes (1:1) -20°; or EtOH:EtOAc (1:9)	A	0.47	45	26
VII [d]		EtOH:H_2O (1:1)	A	0.65	80	43
VIII [e]		THF:Hexanes (1:4) -20°	B	0.50	60	36
IX [c]		EtOH:H_2O (1:1)	D	0.60	10	22

(continued)

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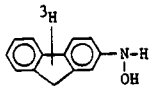
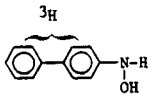
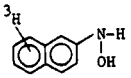
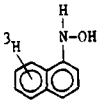
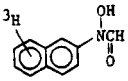
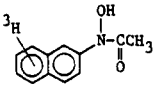
SELECTED ^{14}C- AND ^{3}H-LABELLED AROMATIC HYDROXYLAMINES

X [b]		-	B	0.25	85	23
XI [f]		Chloroform:Hexanes (1:3)	B	0.45	66	37
XII [g]		THF:Hexanes (3:1)	C	0.24	610	30
XIII [h]		Ether:Hexanes (1:4) -20°	C	0.32	392	41
XIV [i]		THF:Hexanes (1:3)	D	0.37	52	60
XV [j]		Benzene:Hexanes (1:4)	D	0.37	51	58

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g E. Boyland, D. Manson and R. Nery, J. Chem. Soc., 606 (1962).
h R. Willstatter and H. Kubli, Ber., 41, 1936 (1908).
i E. Boyland and D. Manson, Biochem. J., 99, 189 (1966).
j E. Boyland and D. Manson, Biochem. J., 101, 84 (1966).
k E. Merck silica gel plates spotted and developed in the absence of UV light. Solvent systems: A, ethyl acetate; B, benzene:methanol (9:1); C, chloroform:methanol (25:1); D, hexanes:acetone (1:1).

by reaction temperature, concentration of reactants, and time. That is, at low temperatures (5-15°) the hydroxylamine is formed almost exclusively, although somewhat slowly, while at room temperature or above the amine is generally the major product.[9] Further, the rate of reduction may be increased somewhat by increasing the amount of aqueous ammonium sulfide although doing so may cause precipitation of the nitro precursor; addition of DMF will redissolve the starting material, but extraction of the hydroxylamine from dilute solution becomes more difficult. Typically, with a 40:1 molar excess of ammonium sulfide only ∿ 10% of the aryl nitro compound is reduced in 3-6 hr at 10°, indicating that long reaction times (∿ 24 hr) as well as low temperature (0-10°) are required for a good yield of hydroxylamine.

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A specific improvement has also been realized with regard to the preparation of the 9-^{14}C labeled fluorene derivatives I and II. The pathway of Heidelberger and Rieke[10] was utilized to prepare fluorene-9-^{14}C. However, due to the difficulty in effectively controlling the small-scale nitration of fluorene-9-^{14}C using the original procedure (HNO_3/HOAc) we investigated the use of sodium nitrate in trifluoroacetic acid as the nitrating agent.[11] As expected, the reaction proceeded smoothly at room temperature, resulting in a consistent 90-95% yield of 2-nitrofluorene-9-^{14}C as compared to the 71% and 79% yields reported by Heidelberger and Rieke[10] and Ray and Geiser,[12] respectively.

EXPERIMENTAL

Tritiated nitro compounds were prepared from their unlabeled analogs by catalytic exchange (New England Nuclear or Amersham Corporation) or were prepared in-house.[13] Other starting materials were obtained or prepared as described in the literature (see references in the Table). Ammonium sulfide

(Fisher "light," 24% in water) was purchased from Fisher Scientific Company. The identity of labeled compounds was determined by comparison of UV spectra and chromatographic behavior relative to authentic, fully characterized, unlabeled standards. With the exception of the unstable hydroxylamines, chemical and radiochemical purities of all compounds were ≥ 97% as determined by TLC radiochromatogram scanning (Packard Model 7201 radiochromatogram scanner) and autoradiography. Radioactivity was determined with a Packard Model 3003 liquid scintillation counter using Aquafluor® or Liquifluor® (New England Nuclear) as the counting medium. The IR spectra were determined with a Beckman Acculab I and the UV spectra were recorded with a Varian Superscan III spectrophotometer.

The compounds listed in the Table were prepared using procedures which varied little with respect to aromatic moiety, and therefore detailed descriptions are provided below for four representative compounds. The examples given thus represent generally applicable procedures. In addition, a detailed experimental description is also provided for the nitration of fluorene-9-^{14}C since this method represents a simple, general nitration method for polynuclear aromatic hydrocarbons. All reactions and subsequent workup procedures were carried out under yellow light and a blanket of argon gas.

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N-Hydroxy-N-acetyl-3-aminofluorene-(acetyl-1-^{14}C) (III).- To 250 mg (1.175 mmol) of 3-nitrofluorene in 20 ml of DMF was added 13 ml of 24% ammonium sulfide solution. After stirring for 24 hr at 5-10° the reaction mixture was chilled to 0° and treated with 30 ml of water, after which it was extracted with ether (4 x 30 ml). The ethereal hydroxylamine solution was maintained at 0° under a stream of argon, thereby reducing the volume to ∿ 50 ml. The resulting solution was washed with water (2 x 5 ml), dried (anhydrous Na_2SO_4) and immediately added to 75 µl (∿ 15 mCi) of acetyl chloride-1-^{14}C and 100 µl of triethylamine which had been vacuum transferred into a 100 ml round bottom flask. After stirring 1.5 hr at 0° the reaction mixture containing III was washed with water (2 x 5 ml), dried (anhydrous Na_2SO_4) and evaporated to dryness (in vacuo). The residue was dissolved in 10 ml of ethyl acetate and extracted into 1 N potassium hydroxide (3 x 10 ml). The aqueous solution was washed with ethyl acetate (10 ml) and acidified with 2 N hydrochloric acid. The hydroxamic acid was

then extracted into ethyl acetate (3 x 30 ml), the combined extracts dried (anhydrous Na_2SO_4) and evaporated to dryness (in vacuo). Crystallization of the residues from THF:hexanes (1:10) at 0° gave 164 mg (9.3 mCi, 13.6 mCi/mmol, 58% yield) of III with a radiochemical and chemical purity of ≥ 98%.

N-Hydroxy-N-formyl-2-aminofluorene-(ring-^{3}H) (VI).- To a chilled solution (5-10°) of 2-nitrofluorene-(ring-^{3}H) (528 mg, 2.5 mmol, ∿ 100 mCi) in 50 ml of DMF was added 25 ml of 24% ammonium sulfide solution. The resulting suspension was stirred vigorously overnight, warming gradually to 23° and forming a dark solution. Ice water (∿ 50 ml) was added and the resulting suspension was extracted with chilled (5-10°) ether until no further tritium could be extracted as determined by liquid scintillation counting of 100 µl samples of the extracts. The resulting ether solution was washed with water (2 x 5 ml), dried (anhydrous K_2CO_3) and evaporated to dryness (in vacuo). The light yellow solid (hydroxylamine) was dissolved in 60 ml of ether and chilled to 0°. Acetic-formic anhydride was prepared according to Fieser[14] and 300 µl of this mixed anhydride was added to the ether solution containing the hydroxylamine and after stirring 1 hr, the mixture was filtered, yielding 205 mg of ∿ 95% pure VI. Crystallization from THF:hexanes (1:1) at -20° afforded two crops of VI totaling 145 mg (29 mCi, 45 mCi/mmol, 26% yield) with a chemical and radiochemical purity ≥ 97%.

N-Hydroxy-N-propionyl-2-aminofluorene-(ring-^{3}H) (VII).- To a chilled solution (5-10°) of 2-nitrofluorene-(ring-^{3}H) (528 mg, 2.5 mmol, ∿ 200 mCi) in 50 ml of DMF was added 25 ml of 24% ammonium sulfide solution. The resulting suspension was allowed to stir overnight while gradually warming to 20°. Water (∿ 50 ml) was added and the brown solution extracted with ether until no further tritium could be detected in the extracts. The resulting ether

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solution was washed with water (2 x 5 ml), dried (anhydrous K_2CO_3) and evaporated (in vacuo) yielding the light yellow hydroxylamine. The hydroxylamine was dissolved in 50 ml of ethyl acetate to which 300 µl of triethylamine had been added, and the resulting solution chilled to 0°. Propionyl chloride (0.2 ml) was then added and the reaction mixture was stirred for 1.5 hr. The resulting mix was washed with water (2 x 10 ml) and the product extracted into 1 N potassium hydroxide. After washing the alkaline solution with ether (2 x 10 ml), it was acidified with 2 N hydrochloric acid. The hydroxamic acid was then extracted into ether (3 x 30 ml), the ether solution dried (anhydrous K_2CO_3) and evaporated (in vacuo) yielding an off-white solid. Crystallization of the solid from ethanol:water (1:1) afforded 272 mg (86 mCi, 80 mCi/mmol, 43% yield) of VII with a chemical and radiochemical purity ≥ 98%.

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N-Hydroxy-1-aminonaphthalene-(ring-^{3}H) (XIII).- To a stirred solution of 137 mg (0.78 mmol, ∿ 335 mCi) of 1-nitronaphthalene-(ring-^{3}H) in 6 ml of DMF at 0° was added (dropwise) 5 ml of 24% ammonium sulfide solution. After stirring for 5 hr at 5-10° the TLC (silica gel) chloroform:methanol (25:1) showed the reaction to be nearly complete. Upon cooling to 0°, 5 ml of water was added and the solution was extracted with ether (6 x 10 ml). The combined ethereal extracts were kept at ∿ 0° under a stream of argon while being washed with water (3 x 5 ml) and dried (anhydrous Na_2SO_4). Following evaporation of the solvent (in vacuo) at 0° to ∿ 5 ml, the solution was again washed with water (1.5 ml) to remove traces of DMF and was dried (anhydrous Na_2SO_4). After addition of ∿ 16 ml of hexanes, the solution was chilled to -20°, yielding 51 mg (128 mCi, 392 mCi/mmol, 41% yield) of crystalline XIII with a chemical and radiochemical purity ≥ 90%.

2-Nitrofluorene-9-^{14}C.- Fluorene-9-^{14}C, 1.3 g (9.15 mmol, 300 mCi, 39 mCi/mmol) was dissolved in 31 ml of trifluoroacetic acid and 876 mg of sodium nitrate was added to the stirred solution in small portions over a period of 2 hr. After 4 hr at room temperature the reaction was quenched by addition of 100 ml of water and 130 ml of 3 N sodium hydroxide. The resulting slurry was extracted with ether (3 x 200 ml) and the combined ethereal extracts washed sequentially with 100 ml of water, 100 ml of 3 N sodium hydroxide, and 100 ml of water. After drying (anhydrous Na_2SO_4) the ether was evaporated (in vacuo) and the crude product crystallized from acetic acid, yielding 1.75 g (275 mCi, 39 mCi/mmol, 91% yield) of 2-nitrofluorene-9-^{14}C with a radiochemical purity $\geq$ 97%.

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