SYNTHESIS OF SELECTED ^{14}C-LABELED CARCINOGENIC AROMATIC AMINES

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SUMMARY

4,4'-(Methylene-^{14}C)bis[N,N-dimethylbenzenamine] (2), 2,4-^{14}C,6-trimethylbenzenamine hydrochloride (7), and 4-chloro-2-(methyl-^{14}C)benzenamine hydrochloride (18) were synthesized for an investigation of the metabolism of carcinogenic compounds. Evaluations of synthetic routes led to the following preferred methods: the $LiAlH_4$-$AlCl_3$ reduction of carbonyl-labeled Michler's ketone for 2, the Hofmann-Martius rearrangement of the salt formed from 2,6-dimethylbenzenamine and iodomethane-^{14}C for 7, and the nitration of (methyl-^{14}C)benzene followed by reduction with stannous chloride in hydrochloric acid for 18. The free bases of 7 and 18 were isolated from complex product mixtures by preparative thin-layer chromatography, but the free base of 7 was not completely separated from a tetramethylbenzenamine.

Key Words: Reduction, 4,4'-(Methylene-^{14}C)bis[N,N-dimethylbenzenamine], Rearrangement, 2,4-^{14}C,6-Trimethylbenzenamine, Nitration, 4-Chloro-2-(methyl-^{14}C)benzenamine

INTRODUCTION

The synthesis of the title compounds with specific activities of ~3 mCi/mmol was undertaken in order to provide ^{14}C-labeled aromatic amines for an investigation of the metabolism of carcinogenic compounds. In unlabeled form, these amines have industrial uses and have shown varying degrees of carcinogenicity in experimental animals (1-3). Moreover, 4-chloro-2-methylbenzenamine[1] is a metabolite of a widely used acaricide (4). These are but a few of

[1]Parent names used for key compounds in this paper derive from Chemical Abstracts: benzenamine (aniline), methylbenzene (toluene), diphenylmethanone (benzophenone).

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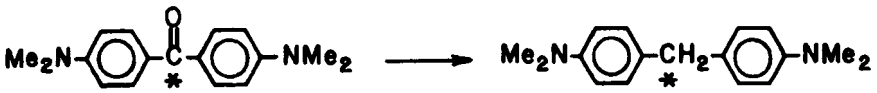
the suspected carcinogens among aromatic amines, three of which (benzidine, 2-naphthylamine, 4-biphenylamine) are known to induce urinary bladder cancer in man (5).

4,4'-(METHYLENE-^{14}C)BIS[N,N-DIMETHYLBENZENAMINE]

Bis[4-(dimethylamino)phenyl]methanone-^{14}C (1), wanted per se for a separate investigation and available commercially, was chosen as the starting material for the preparation of title compound 2. The reduction of unlabeled 1 (Michler's ketone) with aluminum chloride and lithium aluminum hydride (6),



1 2

which form aluminum hydride, and with sodium bis(2-methoxyethoxy)aluminum hydride (7) were compared on a scale adaptable to the preparation of 2. For these experiments, purple commercial Michler's ketone was freed of gentian violet, an ionic by-product of the usual preparation, by successive recrystallizations from ethanol, decahydronaphthalene, and ethanol again.

The forcing conditions necessary for the reduction with sodium bis(2-methoxyethoxy)aluminum hydride gave rise to complex reaction mixtures, which could have resulted in part from alkylations of the product via fragmentation of the reducing agent as reported for the hydrogenolysis of benzophenone (8). Furthermore, the product by necessity was isolated by preparative thin-layer chromatography (TLC) and, after recrystallization, still contained several impurities. On the other hand, the reduction with aluminum chloride-lithium aluminum hydride gave a good yield of product that did not require further purification by chromatography or recrystallization; no colored by-products were observed, and very little contamination was detected by TLC.

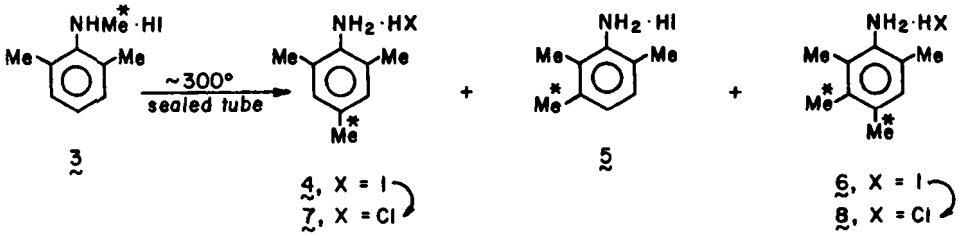
These results translated well to the preparation of 2, although the final product was recrystallized.

2,4-^{14}C,6-TRIMETHYLBENZENAMINE HYDROCHLORIDE

The usual synthesis of unlabeled 2,4,6-trimethylbenzenamine (mesidine) from acetone (9-11) gives a low yield (13-15%) in the first of three steps, a major disadvantage if ^{14}C-labeled acetone were used. The simplest alternative appeared to be the Hofmann-Martius rearrangement of a salt formed from an appropriately substituted benzenamine and iodomethane-^{14}C. Two versions of this approach were investigated with unlabeled starting materials; these were based on reported sealed-tube reactions of iodomethane with N,N-dimethylbenzenamine at 300° (12) and with 2,4-dimethylbenzenamine at 260° (13), but 2,6-dimethylbenzenamine, in which the dominant para position (14) is unoccupied, was used in place of the 2,4-isomer. The crude products isolated in typical experiments were analyzed by gas-liquid chromatography (GLC) in conjunction with mass spectrometry (MS). The most favorable results were obtained when 2,6-dimethylbenzenamine and iodomethane were heated at ~300° for ~4.5 hours [17% unchanged 2,6-dimethylbenzenamine, 53% mesidine, 8% 2,3,6-trimethylbenzenamine, and 16.5% 2,3,4,6-tetramethylbenzenamine]. (2,4,5-Trimethylbenzenamine was named as a product of Hofmann-Martius rearrangements of N-methylbenzenamine hydrohalides with no mention of mesidine (15), the first such study to identify products by GLC.) Preliminary experiments also prescribed the conditions for, and thus the degree of, salt (N,2,6-trimethylbenzenamine hydroiodide) formation prior to heating. On two occasions the sealed tube broke during heating when the mixture was allowed to stand overnight because the salt that formed set to a rigid crystalline mass. Thereafter, the period after sealing and before heating was limited to one hour at room temperature or four hours at ice temperature.

These studies indicated a satisfactory one-step method, but the problem of separating mesidine from a complex mixture remained. Good separation of major components was observed by TLC on an analytical scale with a solvent system suggested by a reported separation of aromatic amines (16), but problems of overloading, detection and overlapping of bands, discoloration, and the adherence of acetic acid were encountered on a preparative scale. Rechromatographing the band containing mesidine (in all cases difficult to define) and conversion to the stable hydrochloride became the standard procedure. On the chromatogram, mesidine was concentrated in a faintly blue, narrow band flanked by impurities, all merging into a uniform band when seen under ultraviolet light. Nevertheless, preparative TLC produced mesidine of 95% purity. The major impurity (2.6%) was identified by GLC-MS as a tetramethylbenzenamine, which was rationalized as the 2,3,4,6-isomer because of the probability that it resulted from methylation of the major product. In TLC, mesidine and its persistent impurity, which migrated in front of mesidine, gave blue spots with ninhydrin; 2,4,5-trimethylbenzenamine gave a red spot.

The above experiments defined the major products (4-6) to be expected from a thermal rearrangement of the ^{14}C-labeled salt 3 under optimized conditions. The observed limitations of preparative TLC predicted contamination of the target product 7 with the doubly labeled by-product 8.



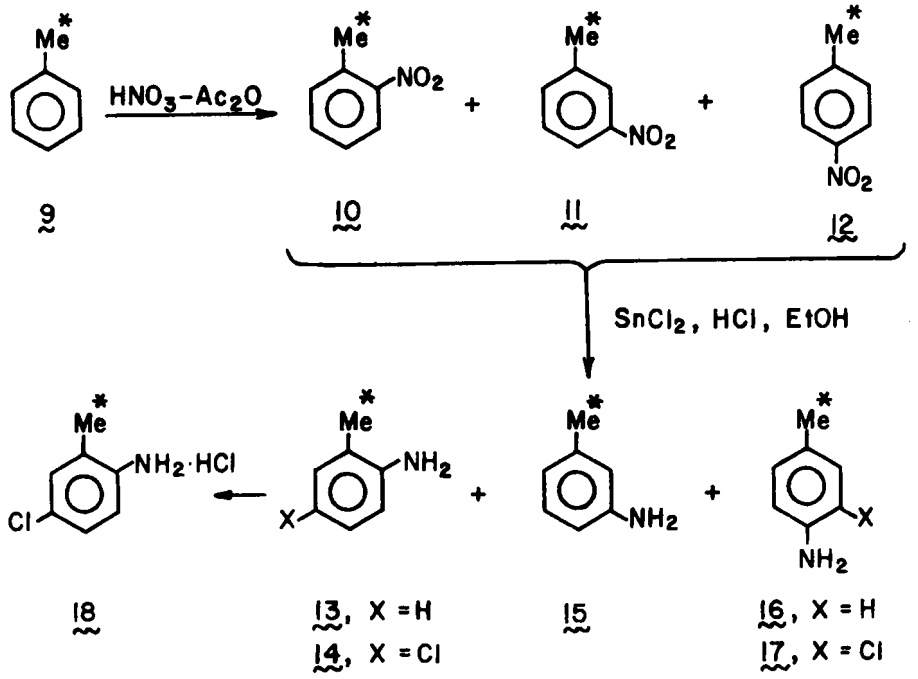
4-CHLORO-2-(METHYL-^{14}C)BENZENAMINE HYDROCHLORIDE

What appeared to be the most direct route to the title compound 18, (methyl-^{14}C)benzene (9) being available, was suggested by the reported chlorination accompanying the reduction of 1-methyl-2-nitrobenzene with stannous chloride in hydrochloric acid (17). Any known alternatives (18-21) to this step, which would follow the nitration of 9, would lengthen the scheme considerably. The reported results of recent mononitrations of methylbenzene with a variety of agents indicate that an isomeric mixture containing ~60% of the 2-isomer would be a reasonable expectation (22-27). For this work, the method chosen on the basis of simplicity and adaptability to a small scale was that described for the nitration of cyclopropylbenzene (25, 28).

The proposed synthesis appeared initially to hinge on the development of preparative chromatographic separations of the products of each step. An intensive search, however, failed to uncover an entirely satisfactory solvent system for the TLC separation of isomeric methylnitrobenzenes. On the other hand, the TLC behavior of model benzenamine derivatives indicated the feasibility of a preparative separation of 4-chloro-2-methylbenzenamine with the solvent system already used for the separation of mesidine from product mixtures.

The development of procedures applicable to the preparation of 18 involved successful demonstrations of the nitration and reduction steps with chromatographic separation reserved for the reduction products. The proposed nitration gave an 81% yield of mononitro isomers (63.5% 2-, 0.6% 3-, and 35.9% 4- as determined by GLC), and an unoptimized reduction of 1-methyl-2-nitrobenzene in refluxing 96% ethanol and 25% HCl with stannous chloride gave a product that could be separated by preparative TLC from its major contaminant, 2-methylbenzenamine, and converted to the hydrochloride in acceptable yield. The reduction of a typical mixture of methylnitrobenzenes gave a product that was

shown by TLC to contain four major components (4-methyl-, 2-methyl-, 4-chloro-2-methyl-, and probably 2-chloro-4-methylbenzenamine) and one minor component (probably 3-methylbenzenamine). As a final demonstration, unlabeled methylbenzene was nitrated, the resulting product reduced, and 4-chloro-2-methylbenzenamine isolated by preparative TLC and converted to the hydrochloride, which as the free base still contained ~24% 2-methylbenzenamine. Conversion of the hydrochloride to the free base and rechromatographing gave a 99% pure product. The low overall yield (15%) was not unattractive when alternative methods were considered. These observations were predictive of the isomeric mixtures depicted below; the yield and purity of 18 slightly exceeded the predictions.



EXPERIMENTAL

4,4'-(Methylene-^{14}C)bis[N,N-dimethylbenzenamine] (2)

Bis[4-(dimethylamino)phenyl]methanone-^{14}C (1, 9 mCi, 6 mCi/mmol; New England Nuclear Corp.) was reduced in two runs of approximately equal scale. Dry, powdered $AlCl_3$ (344 mg, 2.58 mmol) was added to magnetically stirred anhydrous ether (10 ml) in a 2-neck 25-ml flask, the vertical neck stoppered and the slanted neck fitted with a reflux condenser and Drierite tube. After bubbling ceased, $LiAlH_4$ (49 mg, 1.3 mmol) was added in portions, the vigorous reaction subsiding after a few additions. 1 (198 mg, 0.740 mmol) was added during 1 hour through a powder funnel so that it fell directly into the stirred mixture; the additions were spaced so that the transitory orange color that formed disappeared between additions. The mixture was warmed until persistent brown spots disappeared, chilled in an ice bath, treated successively and dropwise with 5 ml each of 1:1 ethyl acetate-ether (v/v) and aqueous 20% NaOH, and stirred until the lumps became finely divided. Diluted with water (5 ml) and transferred to a separatory funnel, the mixture was allowed to separate into layers. The aqueous layer was extracted with ether (15 ml) and the combined ether layers washed with water (3 x 20 ml), dried ($MgSO_4$), filtered, and evaporated to dryness[2]: yield 173 mg (92%). In the second reduction of 1 (214 mg, 0.797 mmol), emulsions prompted double extractions with ether. The product, exceeding the theoretical yield, was combined with the first in hot ethanol. The hot solution was filtered and concentrated to ~3 ml. The platelets that formed were collected after refrigeration and dried *in vacuo*: total yield 276 mg (71%), excluding a brownish residue (63 mg) recovered from the filtrate.

The above product was combined in ether with 276 mg of unlabeled 2 (ICN Pharmaceuticals, Inc.) that had been thrice-recrystallized from ethanol [mp 87-

[2]Evaporations were usually done with a rotary evaporator and water aspirator; drying to constant weight was done *in vacuo* (oil pump) over P_2O_5 and NaOH.

89°; lit. mp 90.5° (6)]; evaporation and drying left 542 mg: specific activity determined by liquid scintillation counting 2.8 mCi/mmol. The radiochemical purity determined by TLC [Analtech silica gel GF, 3:1 cyclohexane-ethyl acetate (v/v), 0.3-cm zonal scraping] was 98.3%. The chemical purity determined by GLC [2 m x 2 mm (i.d.) glass column packed with 3% OV-17 on 80-100 mesh Gas-Chrom Q; port 175°; column temperature programmed from 150° to 240° at 4°/min, holding at 240°] was 99.3%, whereas that of the unlabeled diluent was 98.9%.

2,4-^{14}C,6-Trimethylbenzenamine Hydrochloride (7)

Iodomethane-^{14}C (639 mg, 4.50 mmol; 18.45 mCi, 4.1 mCi/mmol; California Bionuclear Corp.) was transferred from an opened ampoule to a constricted combustion tube [Corning, 200 mm x 10 mm (o.d.), 8 mm (i.d.)] containing freshly distilled 2,6-dimethylbenzenamine (0.555 ml, 4.49 mmol), both ampoule and tube being kept cold in dry ice-acetone baths; the transfer was made with a Hamilton jacketed syringe kept cold by air swept over dry ice. The tube, cooled with dry ice-acetone, was sealed, allowed to stand at room temperature for an hour, and heated to 296° during ~2 hours and kept at 295-298° for ~4.5 hours in an electrically heated, nitrogen-purged, 2-liter stainless steel pressure vessel containing some xylene for counterbalance of pressure. The tube was opened at dry-ice-acetone temperature and its contents repeatedly extracted with 1 N HCl (~60 ml total). The extract was distilled as such (~8 ml collected and discarded) and after strong basification with 50% NaOH (~50 ml collected). The basic distillate was extracted with ether (3 x 15 ml); the extract was dried ($MgSO_4$) and concentrated to an oil (25% 2,6-dimethylbenzenamine, 49% mesidine, 6.4% 2,3,6-trimethylbenzenamine, and 12% 2,3,4,6-tetramethylbenzenamine), which was dissolved in a little chloroform and chromatographed on two 20 x 20-cm Analtech 2-mm silica

gel GF plates with 15:2:3 benzene-ethyl acetate-acetic acid (v/v/v). The major band was cut from each in a glove bag under nitrogen, combined, and eluted with ether. Evaporation left a yellow oil, which darkened during overnight refrigeration and was rechromatographed as above. The eluted oil was treated with ethanolic hydrogen chloride solution and evaporated to dryness with several additions of ethanol. This process was repeated and the crystalline residue dried to constant weight: yield 241 mg (31%).

Another run of identical scale in which the reaction temperature was less carefully controlled and rose to ~305° gave a product mixture (21% 2,6-dimethylbenzenamine, 35% mesidine, 12% 2,3,6-trimethylbenzenamine, and 16.5% 2,3,4,6-tetramethylbenzenamine) that yielded only 94 mg (12%) of 7. The labeled samples were combined with an unlabeled sample (109 mg) in methanol, the solution being treated with Norit and filtered through Celite. Evaporation of the filtrate to dryness left 430 mg, specific activity 15.5 μCi/mg or 2.7 mCi/mmol (uncorrected for purity). For determinations of chemical purity by GLC and radiochemical purity by TLC, the base from ~1 mg of 7 was freed in 0.4 ml of 19:1 methanol-50% NaOH (v/v). Thin-layer chromatograms of up to 4 μl of this solution were sectioned (0.3-cm zonal scraping) and counted; the radioactivity was distributed between a major peak (~92%) and a slightly overlapping, slower-traveling minor peak (~8%). The chemical purity determined by GLC was 95.6%. The identity of the major impurity as doubly labeled 8 accounted for the difference between chemical and radiochemical purity.

4-Chloro-2-(methyl-^{14}C)benzenamine Hydrochloride (18)

Fuming nitric acid (0.90 ml; Baker's Analyzed, sp gr 1.5) was added from a Drierite-protected dropping funnel to magnetically stirred, cold (-60°, dry ice-acetone bath) acetic anhydride contained in a thermometer-equipped 2-neck 15-ml flask at such a rate that the reaction temperature did not exceed -40°.

The nitric acid remaining in the funnel was rinsed down with acetic anhydride (3 x 0.1 ml). Then (methyl-^{14}C)benzene [20.3 mCi, 3.0 mCi/mmol; 6.8 mmol (0.72 ml); California Bionuclear Corp.], which had been transferred from a cold ampoule to a pressure-equalizing dropping funnel by syringe and rinsing with acetic anhydride (0.5 ml), was added dropwise during 30 minutes to the stirred nitrating mixture kept at -55° to -60° and rinsed down with acetic anhydride (3 x 0.1 ml). The partially frozen mixture was allowed to thaw at -35° to -40°, stirred at this temperature for 30 minutes until freezing began again, and at -25° to -20° for 15 minutes. The cold mixture was added to hot water (50 ml), stirred 1 hour, and extracted with dichloromethane (20 ml, 3 x 10 ml). The combined extracts were washed successively with water (20 ml), saturated $NaHCO_3$ solution (3 x 10 ml), and water (3 x 10 ml); dried ($MgSO_4$); filtered; and evaporated to an oil (862 mg).

A stirred solution of the oil in 96% ethanol (7.3 ml) and 25% HCl (7.3 ml) was heated to reflux and treated dropwise during 45 minutes with a solution of $SnCl_2 \cdot 2\ H_2O$ (4.9 g) in 25% HCl (7.3 ml). Refluxing was continued for 15 minutes. The solution was diluted with water (15 ml) and distilled until ~20 ml of distillate had been collected. The remaining solution was diluted with water (15 ml), made strongly alkaline by dropwise addition of 50% NaOH, and distilled until ~50 ml of distillate had been collected. The second distillate was extracted with dichloromethane (5 x 10 ml), an emulsion forming each time. The combined extracts were dried ($MgSO_4$), filtered, and evaporated to a pink oil (669 mg), which was stored overnight under N_2 in a freezer.

The oil, dissolved in a little dichloromethane, was chromatographed on two preparative plates like 7. The major bands were cut, extracted with ether and rechromatographed on two plates. The eluted oil was treated with an ethanolic solution of hydrogen chloride and evaporated to dryness with three

additions each of ethanol-dry HCl and methanol. The residual hydrochloride was dried to constant weight: yield 220 mg (18% from methylbenzene).

This material was combined in methanol containing a few drops of ethanol-dry HCl with a second labeled sample (99 mg) prepared similarly. The methanol solution was evaporated with two additions of methanol, dried to constant weight (315 mg), and stored dry in a freezer. One solution (1.18 mg diluted to 1.00 ml with 19:1 methanol-50% NaOH) was used for determinations of purity and specific activity. The chemical purity of the free base determined by GLC [glass column (2 m x 2 mm i.d.) packed with 3% Carbowax 20M on 100-120 mesh Gas-Chrom Q, port 230°, column programmed from 70° to 200° at 2°/min] was 96.9% with three impurities (1.3, 1.2, and 0.6%), all with short retention times and all present in the methanol used in the product workup. No radiochemical impurities were seen by strip-scanning thin-layer chromatograms of 1-μl and 10-μl aliquots. The specific activity of the hydrochloride was 17.5 μCi/mg or, corrected for chemical purity, 3.04 mCi/mmol, which agreed with the supplier's value for (methyl-^{14}C)benzene.

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1. Unpublished results, Division of Cancer Cause and Prevention, National Cancer Institute.
2. Morris H. P. and Wagner B. P. - Acta Unio Int. Cancrum 20: 1364 (1964).
3. Homburger F., Friedell G. H., Weisburger E. K., and Weisburger J. H.- Toxicol. Appl. Pharmacol. 22: 280 (1972).
4. Knowles C. O. - J. Agr. Food Chem. 18: 1038 (1970).
5. Clayson D. B. and Garner R. C. -Chemical Carcinogens (ACS Monograph 173). 366-461, Ed. C. E. Searle, American Chemical Society, Washington (1976).
6. Brown B. R. and White A. M. S. - J. Chem. Soc. 3755 (1957).
7. Cerny M. and Malek J. - Collect. Czech. Chem. Commun. 35: 1216 (1970).
8. Cerny M. and Malek J. - Tetrahedron Lett. 691 (1972).
9. Adams R. and Hufferd R. W. - Organic Syntheses, Collective Vol. I, 341 (1941).
10. Powell G. and Johnson F. R. - Organic Syntheses, Collective Vol. II, 449 (1943).
11. Conroy H. and Firestone R. A. - J. Am. Chem. Soc. 78: 2290 (1956).
12. Ingold C. K. and Piggott H. A. - J. Chem. Soc. 125: 168 (1924).
13. Porter C. W. and Thurber F. H. - J. Am. Chem. Soc. 43: 1194 (1921).
14. Hughes E. D. and Ingold C. K. - Quart. Rev. (London) 6: 34 (1952).
15. Ogata Y., Tabuchi H., and Yoshida K. - Tetrahedron 20: 2717 (1964).
16. Bassl A., Heckemann H.-J., and Baumann E. - J. Prakt. Chem. 36 [4]: 265 (1967).
17. Blanksma J. J. - Rec. Trav. Chim Pays-Bas 25: 365 (1906).
18. Wynne W. P. - J. Chem. Soc. 61: 1042 (1892).
19. Schuloff R., Pollak R., and Riesz E. - Ber. 62: 1846 (1929).
20. Reverdin F. and Crepieux P. - Ber. 33: 2497 (1900).

21. Nishihara A., Kato H., Jimbo Y., Tomoda Y., and Omi J. - German Patent 2,337,621 (1974); through Chem. Abstr. 80: 145730z (1974).

22. Ingold C. K., Lapworth A., Rothstein E., and Ward D. - J. Chem. Soc. 1959 (1931).

23. Jones W. W. and Russell M. - J. Chem. Soc. 921 (1947).

24. Brown H. C. and Bonner W. H. - J. Am. Chem. Soc. 76: 605 (1954).

25. Ketcham R., Cavestri R., and Jambotkar D. - J. Org. Chem. 28: 2139 (1963).

26. Knowles J. R., Norman R. O. C., and Radda G. K. - J. Chem. Soc. 4885 (1960).

27. Coon C. L., Blucher W. G., and Hill M. E. - J. Org. Chem. 38: 4243 (1973).

28. Levina R. Y., Shabarov Y. S., and Potapov V. K. - J. Gen. Chem. USSR 29: 3196 (1959).