Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIV, No. 4

METALLATION/REDUCTION AS A NEW APPROACH TO TRITIUM LABELING. THE SYNTHESIS OF [^{3}H]IBOGAINE.[1]

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SUMMARY

A new method is presented for the tritiation of aryl compounds with tritium gas which is conducted on the underivatized substrate. Combined directed ortho metallation and facile reduction of the carbon-potassium bond affords incorporation of tritium at high specific activity under mild conditions. The metallation/reduction method is demonstrated for the preparation of [^{3}H]ibogaine.

Key Words: Directed ortho metallation, carbon-potassium bond, reduction, ibogaine, tritium NMR, tritiation, tritium gas

INTRODUCTION

A novel and potentially general approach to the incorporation of tritium into aryl compounds has been developed. Standard methods[2] for the preparation of tritiated compounds typically employ catalytic tritiation of an olefin or halide derivative of the target compound. Both methods require the prior synthesis of the derivative which can involve a majority of the overall effort. Furthermore, functional groups in the target molecule that may suffer unwanted reduction under catalytic tritiation conditions limit the application of the method. In this paper we detail a new approach for the tritiation of aryl compounds that requires neither prior derivatization of the compound to be labeled nor the use of a catalyst and results in a product of high specific activity. The method melds directed ortho metallation with the facile reduction of the carbon-metal bond.

The first of the two reaction steps (A, Figure 1) that provide the basis of the new tritiation procedure is directed ortho metallation[3-5] by metal-hydrogen exchange. Aryl

CCC 0362–4803/94/040367–09

Received 12 October, 1993
Revised 29 October, 1993

compounds with a wide variety of heteroatom substituents including sulfonamide, amide, carbamate, ether, and amine moieties are readily metallated in the ortho position by treatment with n-butyllithium. Similar treatment with n-butylpotassium affords the corresponding arylpotassium compound with greater facility. The metallation step is directed by coordination of the alkyllithium with the heteroatom of the DMG to give the ortho lithiated aryl.[6]

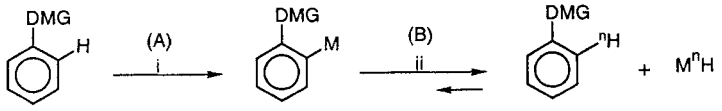

Figure 1. Metallation/Reduction of Aryl Compounds

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The second reaction step (B) utilizes the remarkably facile reduction of the carbon-metal bond by hydrogen to afford a C-^{n}H bond and the corresponding metal hydride.[7,8] These reactions proceed at -25 °C to 35 °C in the absence of a hydrogenation catalyst for organopotassium[9] and organolithium[8] compounds. The process of reduction of the arylmetallic by hydrogen can be viewed as an acid - base reaction with hydrogen as the acid. For the equilibrium to be shifted to product, the resulting Ar-H must be a weaker acid than hydrogen (pKa approx. 37).[7] Furthermore, the nature of the metal influences the ease and rate of the reduction. The potassium-carbon bond reduces significantly faster than the corresponding lithium-carbon bond.[8] By employing the above sequence of reactions, a one flask approach to the tritiation of a broad class of underivatized aryl compounds can be envisioned.

This new metallation/reduction approach is demonstrated for the synthesis of [^{3}H]-ibogaine. Ibogaine[10] is an indole alkaloid that has received recent attention as a potential anti-addiction treatment for cocaine abuse.[11] A tritiated analog was needed for metabolism, distribution, and binding studies, and the usual derivatives could not be conveniently prepared for use in the standard tritiation methods. For the application of the approach to ibogaine, further issues regarding the chemistry of the metallation of indoles need also be recognized. Specifically, indoles with an unsubstituted nitrogen

metallate only on the nitrogen when n-butyllithium is the base.[12] However, with n-butylpotassium, prepared in situ from n-butyllithium and potassium t-butoxide, indoles can be dimetallated.[13,14] This suggested that ibogaine could be employed as a substrate for tritiation without derivatization or protection by the use of n-butylpotassium as shown in Figure 2.

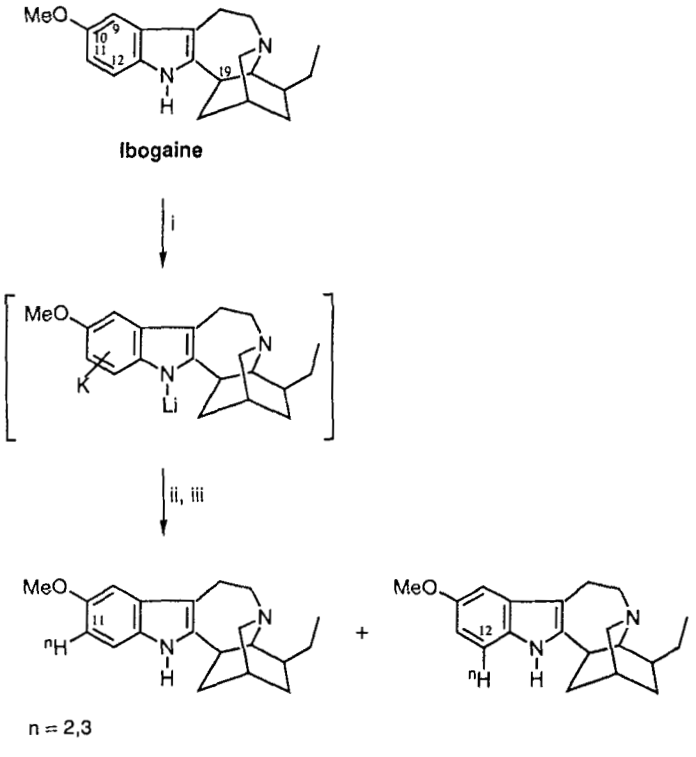

Figure 2. Metallation/Reduction of Ibogaine

RESULTS AND DISCUSSION

A pilot reaction to probe the conditions for the metallation of ibogaine was conducted in ether at -30 °C with four molar equivalents of n-buLi/t-buOK (LiCKOR). Maximum carbon metallation of 65-70% was reached within 1 h at -30 °C as determined by trapping with trimethylstannyl chloride and monitoring by HPLC (Figure 3).

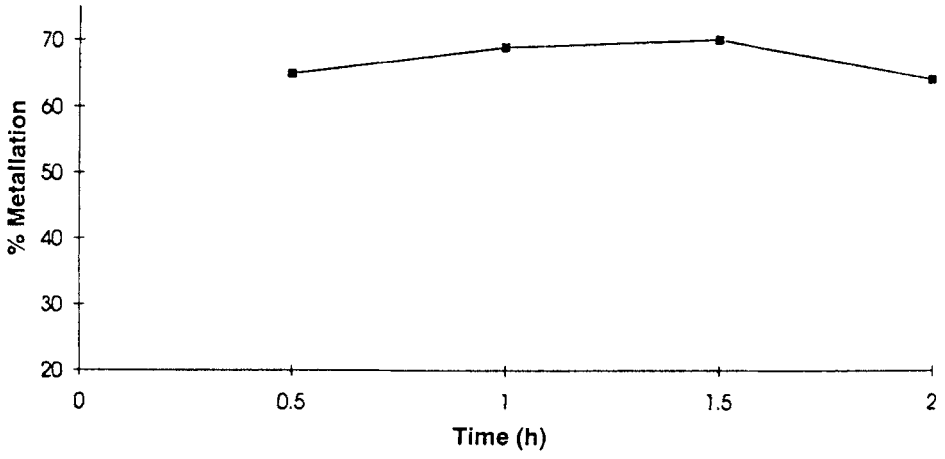

Figure 3. Percent Metallation vs Time at -30 °C

The percent of stannylated to unstannylated ibogaine agreed to within 2-3% of the level of d_1 incorporation of a parallel aliquot quenched into MeOD and analyzed by mass spectrometry. Hence, this was taken to be indicative of the extent of metallation. If the metallation was allowed to reach ambient temperature, incorporation levels were erratic and often dropped to zero. An early tritiation, however, involved a 15 min ambient temperature treatment and gave good incorporation levels. Not withstanding, our experience indicates that the ambient temperature metallation was unnecessary and occasionally counter productive.

The rate and extent of reduction in the presence of a deuterium atmosphere was monitored as the extent of deuterium incorporation (mass spectrometry) versus time (Figure 4). The results indicate that d_1 incorporation increased at a rate of 20%/h at -30 °C (toward a theoretical maximum of 65% of metallation) and that up to approximately -10 °C the rate increased slightly then dropped off as 0 °C was approached. Since competing quenching of the metallated ibogaine by proton abstraction from the ether solvent becomes significant above -30 °C, this drop-off in incorporation could be due, in part, to reaction with solvent at higher temperatures.

Taken together these results suggest that maximum tritium incorporation would be best obtained by maintaining the temperature for the reduction at -30 °C for 3-4 h. In the case of ibogaine, this implies a maximum specific activity by this approach of approximately 20 Ci/mmol.

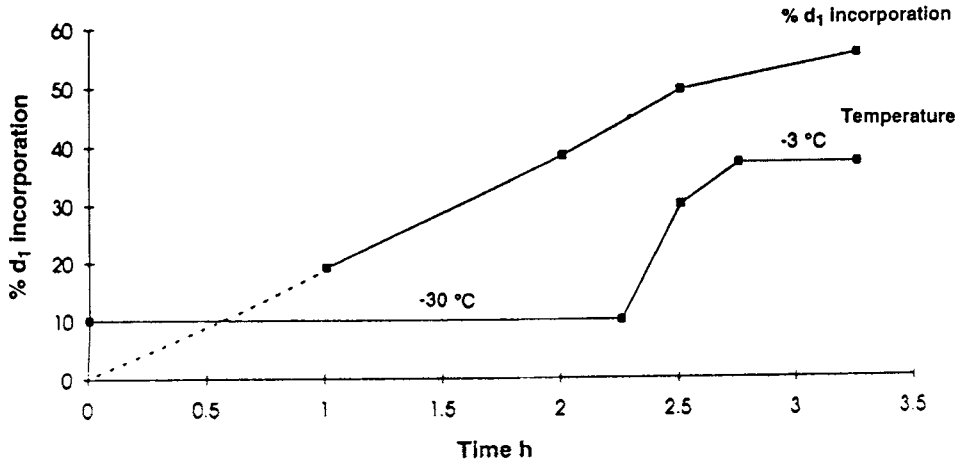

Figure 4. Reduction of Metallated Ibogaine versus Time

Tritiated ibogaine was obtained by similar treatment of ibogaine with two equivalents[15] each of n-butyllithium and potassium t-butoxide, followed by tritium gas. Predominantly a single component was obtained (TLC-radioscan, 85%; HPLC, 82%) which was purified by HPLC. Potential metallation/reduction sites on ibogaine are C-9 and C-11 ortho to the methoxyl[12] and C-12 ortho[16] and C-19 beta[13] to the indole nitrogen. Tritium NMR of the product was especially revealing as it unambiguously demonstrated the sites of labeling as the C-11 and C-12 aromatic positions.[17] From the absence of tritium-tritium coupling it was also clear that the product was a 1:1 mixture of mono-C-11 and mono-C-12 tritiated ibogaine and not 11,12-ditritiated ibogaine. The presence of two labeled sites ensures the maintenance of label in the vast majority of metabolites while the specific activity of 13.8 Ci/mmol enables a variety of pharmacological studies to be conducted.

The C-11 and C-12 incorporation demonstrates the directing effect of the methoxyl and indole nitrogen groups respectively. The lack of incorporation at C-19 is in contrast to the lateral metallation observed for 2-alkyl indoles.[13] This can reasonably be attributed to the poor acidity of the benzylic methine proton relative to a benzylic methyl or methylene[18] and the fact that the resulting carbanion at C-19 would also be conformationally restricted from overlap stabilization with the aryl system.[13,16] This made lateral metallation of the C-19 benzylic position less favorable than ortho metallation of the C-12 position.

A direct method for the incorporation of tritium into aromatic compounds has been demonstrated for the preparation of high specific activity [^{3}H]ibogaine. It is expected that this new approach of metallation/reduction will have applicability to the tritiation of a broad class of aryl compound since the method employs chemistry which demonstrates a wide scope.

EXPERIMENTAL

^{1}H- and ^{3}H-NMR spectra were obtained on a Bruker AMX 500 MHz spectrometer. Mass spectra were obtained on a model 5989A Hewlett Packard mass spectrometer with a direct insertion probe. The HPLC was a Waters system with a model 484 tunable UV detector. Ibogaine hydrochloride was obtained from Sigma.

Metallation of Ibogaine

Ibogaine (104 mg, 0.34 mmol), potassium t-butoxide (95%, 158 mg, 1.34 mmol) and a few crystals of 1,10-phenanthroline indicator[19] were dissolved in anhydrous ether (6 mL) under dry argon in a flame dried flask. The mixture was cooled to -78 °C with stirring and treated with 1.4 M n-butyllithium in hexane until a persistent red end point was observed (0.3 mL, 0.42 mmol). A further addition of n-butlyllithium (0.72 mL, 1.0 mmol) was made. The solution was warmed to -30 °C and stirred for 2 h. Aliquots (0.4 mL) were removed at half hour intervals after first cooling to -78 °C and added to trimethylstannyl chloride (9 eq.) in 0.5 mL anhydrous ether at ambient temperature. The samples were partitioned between ether (2x) and water and the combined ether layers were dried (Na_2SO_4). The residue upon evaporation of the volatiles was dissolved in methanol and analyzed by HPLC (Waters C-18 RCM Radial-Pak, 8 mm x 10 cm, 10 μ, 90% MeOH - 10% [MeOH/H_2O/Et_2NH/85% H_3PO_4 (400:400:0.86:0.34; pH 6), 280 nm, 2 mL/min]) to determine the extent of metallation; R_t ibogaine 5.5 min, stannylibogaines 9.5 min; % Sn-ibogaines (0.5, 1, 1.5, 2 h) = 65, 69, 70, 64%.

A separate study also quenched aliquots into excess MeOD at ambient temperature. These were partitioned between ether and water to remove exchangeable deuterium from the ibogaine molecule. Mass spectrometry determined the d_1 incorporation levels to be within 2-3% of the stannylation levels.

Reduction of Metallated Ibogaine

The above reaction mixture was cooled to -130 °C and the atmosphere was exchanged with deuterium (dried through a -130 °C trap). The temperature was raised

to -30 °C, and the mixture was stirred vigorously for 2.25 h. Over the next 0.5 h the temperature was allowed to rise to -3 °C and maintained at that temperature for another 0.5 h. Aliquots (0.4 mL) were removed at 1, 2, and 2.5 h and they and the reaction were quenched with EtOH and worked up as above. The quenches from the $^2H_2$-hydrogenation were analyzed by MS to determine deuterium incorporation: 19, 38, 50 and 55% d_1, respectively.

[11-^{3}H] and [12-^{3}H]Ibogaine

A flame dried 1 mL tritiation flask with a septum sealed side arm was charged with a stir bar and potassium t-butoxide (9.6 mg, 0.086 mmol) under a dry argon atmosphere and evacuated and backfilled with dry argon on a tritiation manifold. Anhydrous ether (0.3 mL) was added via syringe and the mixture was cooled to -30 °C and treated with n-butyllithium in hexane (45 µL, 0.086 mmol) with stirring. After 15 min, ibogaine (13.4 mg, 0.043 mmol) in 0.3 mL anhydrous ether was added, the cooling bath was removed and the reaction stirred as it warmed to room temperature. After 15 min at room temperature, the reaction was frozen in a liquid nitrogen bath, evacuated and tritium (5 Ci, 0.086 mmol) was introduced from a flask that was cooled to -78 °C (to retain any water that was potentially present). The reaction flask was warmed to -20 °C, stirred for 2 h and then 2 h further at room temperature. The reaction was quenched by injection of 0.1 mL of degassed EtOH and stirred overnight. The EtOH was removed in vacuo and the residue was exchanged twice more with 2 mL EtOH each to obtain 300 mCi of product which was dissolved in 10 mL of absolute ethanol. Partitioning a 3 mCi aliquot between pH 10 phosphate buffer and EtOAc afforded 99% of the recovered radioactivity in the organic phase, demonstrating no exchangeable tritium in the product. TLC-radioscan (SiO_2, 10 % Et_3N/Et_2O, UV) showed a major (85%) radioactive peak at the Rf of ibogaine. HPLC [Waters C-18 Radial-Pak, 8 mm x 10 cm, 10 µ, 90% MeOH/H_2O/H_3PO_4/Et_2NH (400:400:0.34:0.86; pH 6) -10% MeOH, 280 nm, 2 mL/min] showed a major peak (82%) at the R_t of ibogaine (5.6 min). The product was concentrated to 0.5 mL and chromatographed on the above HPLC system in five portions employing a 5 - 100% MeOH/buffer gradient (Waters #7, 2 mL/min, 12 min). The pure ibogaine fractions (HPLC) were combined in ethanol, concentrated in vacuo to remove volatiles, diluted with HPLC mobile phase, reconcentrated, and the aqueous residue was adjusted to pH 10 with $NaHCO_3$. The aqueous solution was loaded on a

Waters C-18 Sep-Pak, washed with 10 mL of water and then 10 mL of MeOH. All the radioactivity (59 mCi) eluted in the organic phase which was then diluted to 100 mL with absolute EtOH. The specific activity was 13.8 Ci/mmol as determined by counting and UV quantitation. TLC-radioscan and HPLC as above demonstrated 99% radiochemical and chemical purity. Tritium NMR ($CDCl_3$, proton decoupled) δ 6.76 (s, 1H, 11-H), 6.92 (s, 0.1 H, 9-H), 7.13 (s, 1 H, 12-H); (proton coupled) δ 6.76 (d, 1 H, J = 7.4 Hz, 11-H), 6.92 (s, 0.1H, 9-H), 7.13 (d, 1H, J = 8.8 Hz, 12-H); compare with the aryl region proton spectrum of unlabeled ibogaine: δ 6.76 (dd, 1 H, J = 8.6, 2.4 Hz, 11-H), 6.93 (d, 1 H, J = 2.4 Hz, 9-H), 7.14 (d, 1 H, J = 8.7 Hz, 12-H).

ACKNOWLEDGMENT

This work was supported by the National Institute on Drug Abuse on Contract No. 271-91-7311. The tritium NMR spectra were obtained by Kevan Gaetano.

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