SYNTHESIS OF DL-TYROSINE-2'-*t* AND TYRAMINE-2'-*t*

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SUMMARY

Lithiation of 4-benzyloxy-2-bromotoluene and subsequent quenching of the lithiated product affords 4-benzyloxytoluene-2-t which is oxidized directly to 4-benzyloxybenzaldehyde-2-t with ceric ammonium nitrate. Transformation of the aldehyde 6 by standard procedures affords DL-tyrosine-2'-t and tyramine-2'-t.

INTRODUCTION

In connection with studies relating to the utilization of tyrosine and tyramine in the biosynthesis of alkaloids of the mesembrine family[1] a synthesis of the title compounds was undertaken. There are many metabolic pathways both in animal and plant metabolism, in which one or the other of these two compounds are involved and since a number of these involve reactions at the 2',6'-position, it would appear that the title compounds may be generally useful.[2]

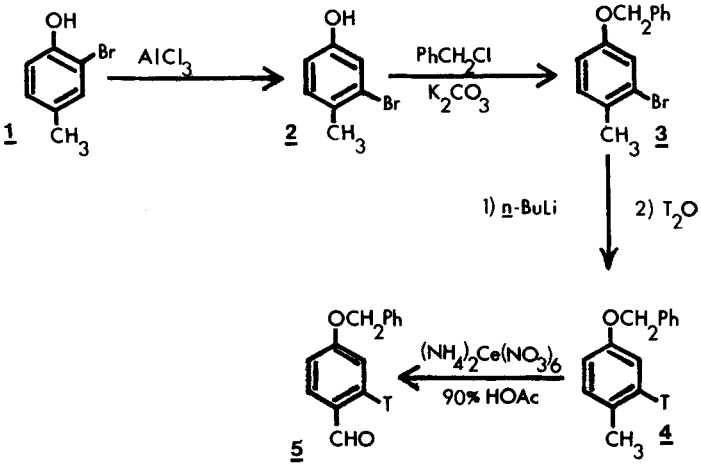
DISCUSSION

Lithiation of aryl bromides and subsequent quenching of the aryl lithium compound provides a potentially convenient and in-

expensive method for the introduction of tritium at a selected site of the aromatic ring. The application of this method is complicated when the aryl bromide also contains an oxygen function since lithiation of the ring ortho to the oxygen occurs at a rate which is often competitive with halogen-metal interchange.[3] In the reaction sequence to be described, unwanted tritium introduced at the equivalent 3',5'-positions of tyramine or tyrosine is easily removed by acid catalyzed exchange during the final step of the synthesis.

The conversion of 2-bromo-4-methylphenol (1) to the 3-bromo-isomer 2 (Scheme 1) was effected by heating with aluminum chloride at 120-127°C for one hour.[4] After protecting the phenolic hydroxyl of 2 as the benzyl ether, the product 3 was lithiated with one equivalent of n-butyl lithium in ether at 0°C. The course of the lithiation reaction could be followed by gas chromatography (see experimental) and when complete, the reaction was quenched by the addition of tritiated water.[5] Oxidation of the resulting *t*-labelled 4-benzyloxytoluene to the corresponding aldehyde 5

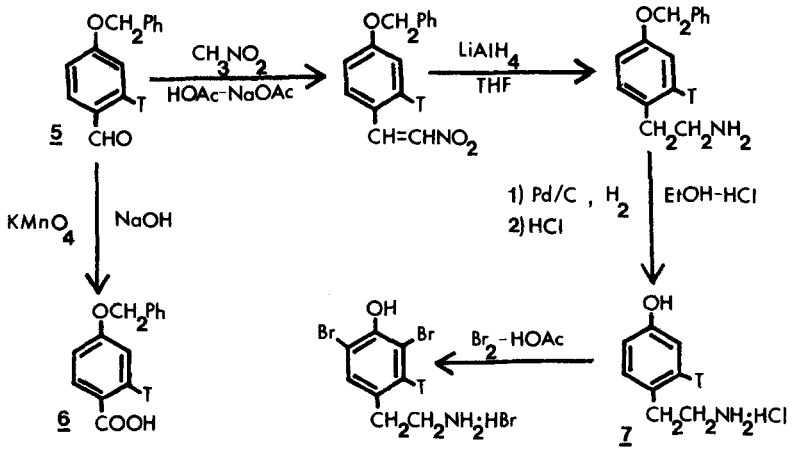
SCHEME 1



was accomplished most satisfactorily with ceric ammonium nitrate.[6] The aldehyde was purified by silica gel chromatography and oxidation to the corresponding acid 6 occurred with no loss of specific activity and demonstrates the absence of tritium in the aldehyde group of 5.

The conversion of 5 to tyramine is summarized in Scheme 2 and follows the previously described procedures.[7,8] Removal of the benzyl group by hydrogenolysis gave radiolabeled tyramine with an 18% loss of tritium.[9] A further 26% loss of tritium from the 3',5'-positions occurred when the tyramine was heated with 9N HCl at 100° in a sealed tube for 24 hr. The absence of any residual tritium at the 3',5'-positions of the labelled tyramine (7) after the acid treatment was demonstrated by its conversion to 3',5'-dibromotyramine hydrobromide which had the same specific activity as the starting material.

SCHEME 2

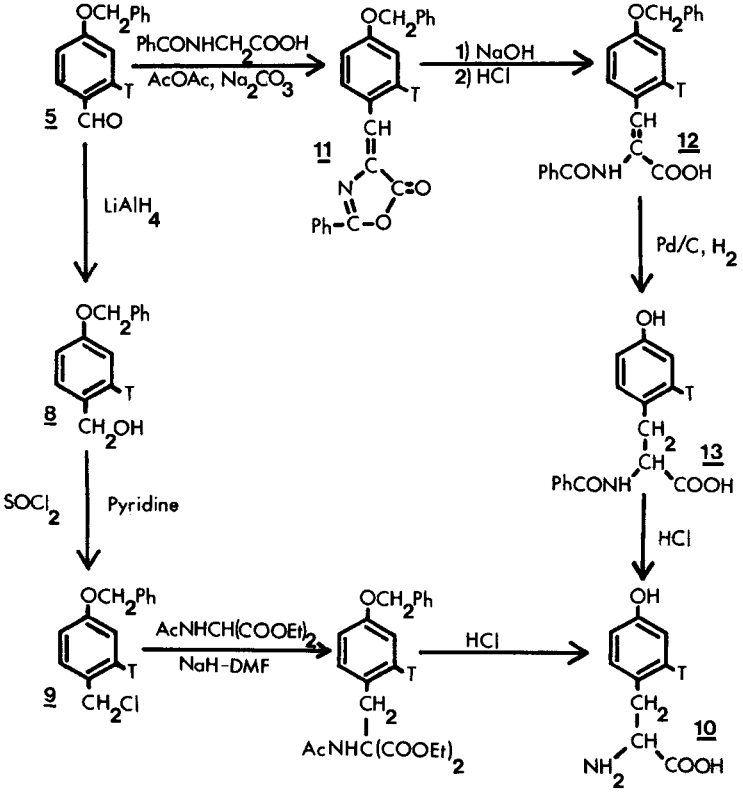


The two methods which were used for the conversion of the labelled aldehyde 5 to DL-tyrosine-2'-*t* are depicted in Scheme 3. In the first method reduction of the aldehyde to the alcohol 8 with

lithium aluminum hydride was followed by conversion of the latter to 4-benzyloxybenzyl chloride (9) with thionyl chloride. Consendation of 9 with diethyl acetamidomalonate and subsequent hydrolysis of the product in refluxing 6N hydrochloric acid gave DL-tyrosine-2'-*t* (10). Bromination of 10 to 3',5'-dibromotyrosine occurred without any loss of specific activity and indicated that the unwanted label at the 3',5'-positions was completely removed under the conditions of the acid hydrolysis.

Conversion of the aldehyde 5 to DL-tyrosine-2'-*t* was also accomplished by a standard azlactone synthesis by the reactions shown in Scheme 3. In this sequence the final step involving the removal of the N-benzoyl group by refluxing in 6N hydrochloric acid also served to remove, completely, residual tritium at the 3',5'-positions.

SCHEME 3



The advantages of the procedures described is that they constitute a safe and inexpensive method for the synthesis of the title compounds at levels of specific activity which should permit their use as tracers in many biological systems.

EXPERIMENTAL

The reactions reported were first carried out with inactive materials and the products were characterized by their melting point or boiling point, spectral and chromatographic properties and were analyzed by combustion analysis or high resolution mass spectral methods. Gas chromatographic analyses were performed on an F and M Model 402 chromatograph using a 6 ft x 0.25 in glass columns packed with 3% SE-30 or 3% OV-17 on Aeropak 30 (100-200 mesh). Radiolabelled samples were purified to constant activity and were analyzed by thin layer chromatography on precoated silica gel plates followed by subsequent scanning with a Varian-Berthold Radioscanner Model 6000-10. Liquid scintillation counting was carried out on a Beckman LS-100 system using the previously described scintillation mixture.[1] $CDCl_3$ was used throughout as a solvent for nmr spectral measurements.

3-Bromo-4-methylphenol

2-Bromo-4-methylphenol (45 g, 0.24 mole) was mixed with aluminum chloride (65 g, 0.49 mole) and heated at 120-127°C for one hour. The reaction was quenched with ice water and extracted with benzene. The organic layer was extracted with 5% NaOH, followed by acidification of the aqueous layer with concentrated HCl and extraction with benzene. After drying and removal of the solvent, the oil was fractionally distilled and the fraction boiling at 102-112°C (6.5 Torr) contained 10.5 g (16%) of 90% 3-bromo-4-methylphenol and 10% p-cresol by gc: ir 3220 cm^{-1} (OH); nmr (60 Mz) δ 7.13 - 6.55 (m, 3H, ArH), 6.00 (s, 1H, OH, D_2O replaceable), 2.26 (s, 3H, CH_3).

4-Benzyloxy-2-bromotoluene

A mixture of 3-bromo-4-methylphenol (6.69 g, 35.6 mmole), absolute ethanol (75 ml), and anhydrous K_2CO_3 (9.5 g) was brought to reflux with stirring before benzyl chloride (4.5 g, 36.8 mmole) was added dropwise. After the reaction had refluxed for 8 hours, the mixture was cooled, filtered, and the solvent removed in vacuo. A chloroform solution of the product was extracted with 5% NaOH and once with water, then dried and filtered. After removal of the chloroform, the residual oil was fractionally distilled to yield 5.52 g (55.8%) of a colorless oil which solidified upon cooling: bp 172-177°C (6.5 Torr); 99% pure by gc: ir 1602 cm^{-1}, 1505 cm^{-1}; nmr (60 Mz) δ 7.33 (s, 5H, ArH), 7.20-6.65 (m, 3H, ArH), 4.97 (s, 2H, $ArCH_2O$), 2.25 (s, 3H, CH_3).

Anal. Calcd for $C_{14}H_{13}OBr$: m/e 277.0140. Found: m/e 277.0142.

4-Benzyloxytoluene-2-*t*

An anhydrous ethyl ether solution (80 ml) of 4-benzyloxy-2-bromotoluene (2.78 g, 10 mmole) was stirred at 0°C, while adding fresh n-butyllithium (5 ml, 2.4 mole/liter, 12 mmole) in hexane, dropwise. After 15 minutes the reaction had gone to 95% completion by gc and was immediately quenched with tritiated water (0.1 ml. 500 mCi). After 15 minutes of stirring, additional water was added and the organic layer was separated, dried and filtered. After removal of the solvents, the colorless oil, 1.95 g (98%), was converted to the benzaldehyde (6) without further purification: nmr (60 Mz) δ 7.35 (s, 5H, ArH), 7.10 (d, 2H, J = 9 Hz, H_A), 6.85 (d, 2H, J = 9 Hz, H_B), 4.99 (s, 2H, $ArCH_2O$), 2.25 (s, 3H, $ArCH_3$).

4-Benzyloxybenzaldehyde-2-*t*

The 4-benzyloxytoluene-2-*t* (665 mg, 3.36 mmole) was dissolved in 90% acetic acid (18 ml) and rapidly stirred while ceric ammonium nitrate 0.36 g, 13.43 mmole), in 90% acetic acid, was added dropwise at room temperature. After 22 hours of stirring, the reaction was

quenched with water and extracted with ethyl ether. The ethereal solution was extracted with saturated sodium carbonate until the washings were basic. After removal of the solvent, the orange oil was purified with a silica gel column using 5% ethyl ether in hexane as eluant. The resulting light yellow oil was recrystallized from methanol-water to yield 208 mg (29%) of platelets: mp 70.5-72.5 C (lit[10] 72°C); ir 1680 cm^{-1} (C=O); nmr (60 Mz) δ 9.93 (s, 1H, CHO), 7.90 (d, 2H, J = 4 Hz, H_A), 7.40 (s, 5H, ArH), 7.10 (d, 2H, J = 4 Hz, H_B), 5.17 (s, 2H, $ArCH_2O$). Specific Activity = 11.8 mCi/mmole.

4-Benzyloxybenzoic-2-*t* Acid

A mixture of 4-benzyloxybenzaldehyde-2-*t* (250 mg, 1.17 mmole, Specific Activity = 85 μCi/mmole) and water (10 ml) was brought to reflux with stirring, then potassium permanganate (360 mg, 2.34 mmole) in water (20 ml) was added dropwise over a period of ten minutes. After reacting for 30 minutes, additional potassium permanganate (360 mg, 2.34 mmole) in water (20 ml) was added dropwise and the reaction gently refluxed for 2 hours. After cooling and basification with 10% KOH, the manganese dioxide was filtered and the clear solution was acidified. The copious white precipitate was filtered and recrystallized from benzene-hexane. mp 191-192°C; ir 2875 cm^{-1} (OH), 1680 cm^{-1} (C=O); nmr (60 Mz) δ 8.11 (d, 2H, J = 9 Hz, H_A), 7.53 (m, 5H, ArH), (d, 2H, J = 9 Hz, H_B), 5.33 (s, 2H, $ArCH_2O$); Specific Activity = 84 μCi/mmole.

Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.69; H, 5.26. Found: C, 73.36; H, 5.20.

4-Benzyloxy-β-nitrostyrene-2-*t*

A mixture of 4-benzyloxybenzaldehyde-2-*t* (213 mg, 1 mmole), ammonium acetate (77 mg, 1 mmole), nitromethane (152 mg, 2.5 mmole), and acetic acid (1 ml) was gently refluxed for 2 hours. After removing the acetic acid in vacuo, the yellow-green precipitate was crystallized from 95% ethanol to yield 140 mg (55%) as yellow-green plates: mp 118-

119°C (lit[11] 120°C); ir (no carbonyl); nmr (60 Mz) δ 7.83 (d, 1H, J = 13 Hz, $ArCH_A$) 7.33 (d, 1H, J = 13 Hz, = CH_BNO_2), 7.33 (d, 2H, J = 9 Hz, ArH_A), 7.30 (s, 5H, ArH), 6.87 (d, 2H, J = 9 Hz, ArH_B), 5.03 (s, 2H, $ArCH_2O$); Specific Activity = 11.8 mCi/mmole.

2-(4-Benzyloxyphenyl-2-*t*)ethylamine

The 4-benzyloxy-β-nitrostyrene-2-*t* (140 mg, 0.543 mmole) was dissolved in dry tetrahydrofuran (25 ml) and added dropwise to a stirring solution of lithium aluminum hydride (125 mg, 3.29 mmole) in dry tetrahydrofuran (5 ml) at room temperature. The reaction was refluxed for 3 hours, then cooled to 0°C and quenched with 10% NH_4Cl solution. After filtering the reaction mixture, the aluminum salts were washed with hot ethyl acetate and the combined organic phase was dried and the solvents removed under reduced pressure. The amine was crystallized from absolute ethanol-ethyl ether to give 67.8 mg (47%) of a dark brown solid: mp 202-204°C (lit[2]-204°C); nmr (60 Mz) δ 7.33 (s, 5H, ArH), 7.10 (d, 2H, J = 4 Hz, H_A), 7.03 (d, 2H, J = 4 Hz, H_B), 5.00 (s, 2H, $ArCH_2O$), 3.6-2.6 (m, 4H, CH_2CH). Specific Activity = 11.8 mCi/mmole.

Tyramine-2'-*t*

The 2-(4-benzyloxyphenyl)ethylamine-2-*t* hydrochloride (67.8 mg, 0.256 mmole) was dissolved in 95% ethanol (3 ml) containing 10% Pd/C (40 mg) and hydrogenolyzed at 1 atm hydrogen at 25°C overnight. The catalyst was removed by filtration and the solvent removed in vacuo to afford a light brown solid, 44 mg (100%). Two crystallizations from ethanol-ethyl ether gave 30 mg of a tan solid. mp 268-269°C. A radioscan of the TLC showed only one detectable spot, corresponding to the R_f of authentic tyramine. Specific Activity = 9.70 mCi/mmole.

Back Exchange of Tyramine-2'-*t*

The tyramine was dissolved in 1 ml of 9N HCl and heated in a sealed tube under nitrogen at 100°C for 24 hours. The tube was opened, diluted with methanol, and the solvents removed to give a quantative yield

of material. The specific activity of the back exchange tyramine (7.18 mCi/mmole) was comparable to the 3',5'-dibromotyramine-2'-*t* (7.15 mCi/mmole) obtained by the bromination of tyramine-2'-*t* (7) with bromine in acetic acid.

4-Benzyloxybenzyl-2-*t* Alcohol

A solution of 4-benzyloxybenzaldehyde-2-*t* (4.00 g, 18.8 mmole) in dry ethyl ether (50 ml) was added dropwise to a refluxing suspension of lithium aluminum hydride (720 mg, 18.8 mmole) in dry ethyl ether (20 ml). After the addition, the solution was refluxed for 30 minutes, then quenched with 10% NH_4Cl solution. The solution was filtered and the aluminum salts washed with ether. The combined ethereal solutions were dried, filtered, and the ether removed in vacuo. The solid was recrystallized from ligroin to yield 3.4 g (85%) as white flakes: mp 87-88°C (lit[12] 87-88°C). Specific Activity = 17.6 μCi/mmole.

4-Benzyloxybenzyl-2-*t* Chloride

The benzyloxybenzyl-2-*t* alcohol (350 g, 16.35 mmole) was converted to the benzyl chloride (9) by the procedure of Barton.[2] Yield = 3.22 g (85%). Specific Activity = 15.4 μCi/mmole.

Diethyl (4-Benzyloxybenzyl-2-*t*)acetamidomalonate

A mixture of diethyl acetamidomalonate (1.19 g, 5.5 mmole), sodium hydride (236.5 mg, 56% in mineral oil, 5.5 mmole) and DMF (5 ml) was stirred at 50°C under nitrogen for 30 minutes, before adding the 4-benzyloxybenzyl-2-*t* chloride (1.16 g, 5 mmole) in DMF (5 ml). After the addition, the mixture was stirred at 50°C for 3 hours, then quenched with water and extracted with ethyl ether. After drying, filtering, and removing the ether, the residue was chromatographed on alumina (Act III) with benzene. The resulting solid was crystallized from benzene-petroleum ether to yield 1.10 g (53%) of a white crystalline solid: mp 109-111°C; ir 3300 cm^{-1} (NH), 1725 cm^{-1} (carboxyl C=O), 1760 cm^{-1} (amide C=O); nmr (60 Mz) δ 7.40 (s, 5H, ArH), 6.93

(s, 4H, ArH), 5.00 (s, 2H, $ArCH_2O$), 4.25 (q, 4H, OCH_2), 3.63 (s, 2H, $ArCH_2C$), 1.97 (s, 3H, $NHCOCH_3$), 1.25 (t, 6H, CH_3); Specific Activity = 14.7 μCi/mmole.

Anal. Calcd for $C_{23}H_{27}NO_6$: C, 66.83; H, 6.54; N, 3.39. Found: C, 66.96; H, 6.43; N, 3.19.

Tyrosine-2'-*t*

The radioactive malonate (14) (120 mg, 0.372 mmole) was refluxed with 6N HCl (5 ml) for 3 hours. After reflux, the solution was reduced to half its original volume and basified with concentrated ammonium hydroxide to a pH of 6-7. The white precipitate was filtered and the solid recrystallized from water-ethanol to yield 62 mg (92%) of a fluffy white solid: mp 300-302°C; Specific Activity = 10.0 μCi/mmole.

4-(4-Benzyloxybenzylidine-2-*t*)-2-phenyl-2-oxazoline-5-one

The oxazolin-5-one (11) was prepared from 4-benzyloxybenzaldehyde-2-*t* (320 mg, 1.5 mmole) by the procedure of Battersby.[13] Yield = 192 mg (36%).

α-Benzoylamino-4-benzyloxycinnamic-2-*t* Acid (12)

The oxazolin-5-one (11) (190 mg. 0.54 mmole) was dissolved by warming in methanol (4 ml) and the resulting solution was treated with 5% sodium hydroxide (4 ml). After standing overnight at room temperature, the mixture's volume was reduced to 4 ml, heated to reflux and treated with 5% HCl to give a volume of 10 ml. On cooling the cinnamic acid formed fine crystals which were filtered and recrystallized from methanol. mp 232°C (dec) yield = 185 mg (92%); uv max 320 nm (ε 16,400), 210 (ε 28,100); ir 3230 cm^{-1}, 3000 cm^{-1}, 1700 cm^{-1}, 1650 cm^{-1}, 1600 cm^{-1}; nmr (60 Mz) δ 9.3 (s, 1H, CONH), 8.1 (d, 2H, ArH), 7.7 (s, 1H, COOH), 7.6-7.4 (m, 5H, phenyl), 6.95 (d, 2H, ArH), 5.1 (s, 2H, $ArCH_2O$).

Anal. Calcd for $C_{23}H_{19}NO_4$: C, 73.98; H, 5.13. Found: C, 73.59 H, 5.03.

N-Benzoyltyrosine-2'-*t*

The cinnamic acid (12) (185 mg, 0.49 mmole) was dissolved in methanol (10 ml) and acetic acid (2 ml) containing 10% Pd/C (10 mg) and concentrated HCl (0.1 ml). After stirring under one atm. of hydrogen at 25°C for one hour, the catalyst was filtered and the solvents removed under reduced pressure. Recrystallization from water gave 110 mg (78%) of material: mp 192-195°C; Specific Activity = 1.72 mCi/mmole.

Tyrosine-2'-*t*

The N-benzoyltyrosine-2'-*t* (110 mg, 0.39 mmole) was refluxed in 6N HCl for one hour, then cooled and filtered through celite. The filtrate was evaporated to dryness and then dissolved in a minimum amount of 10% NaOH. The solution was heated to boiling before cautiously acidifying the mixture with acetic acid. On cooling, 62 mg (85%) of white crystals were formed. Specific Activity = 1.72 mCi/mmole. The identity of the tyrosine synthesized by both methods was established by thin layer chromatography in butanol-acetic acid-water, 3:1:1, using authentic DL-tyrosine as a standard. All the activity was shown by scanning to be located in the only detectable spot.

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