BIOORGANIC CHEMISTRY **24,** 169–177 (1996)
ARTICLE NO. 0015

Synthesis and Use of Deuterated 3,4-Dihydroxyphenylglycolaldehyde as an Internal Standard for Determination of Dopegal in Brain Tissue by Gas Chromatography–Mass Spectrometry

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Received January 11, 1996

Trideuterated 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL-d_3) was synthesized from tetradeuterated catechol. After derivatization with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide, the identity of DOPEGAL-d_3 was confirmed by comparison with unlabeled DOPEGAL by gas chromatography–mass spectrometry (GC–MS); they have the same pattern of mass fragmentation. The deuterated DOPEGAL was used as an internal standard for the determination of DOPEGAL in human locus ceruleus by GC–MS in the selected-ion-monitoring mode.

INTRODUCTION

3-4-Dihydroxyphenylglycolaldehyde (DOPEGAL) is formed by oxidative deamination of noradrenaline (NA) by monamine oxidase (MAO-A) (*1*, *2*). In tissues this aldehyde is either enzymatically reduced to 3,4-dihydroxyphenylglycol (DOPEG) or oxidized to 3,4-dihydroxymandelic acid (*1*, *3*). Although estimates of this compound in tissues have been made (*3–6*), it has not been previously measured in human brain using gas chromatography–mass spectrometry (GC–MS). This has been due to lack of deuterated DOPEGAL internal standard.

We have reported both biological (*6*) and chemical (*7*) synthesis of DOPEGAL. This DOPEGAL standard enabled the development of a method using high-performance liquid chromatography with electrochemical detection (HPLC–EC) to measure tissue levels of DOPEGAL (*6*). However, GC–MS is a more reliable quantitative method of measuring extremely low levels of bioactive compounds in tissue. Capillary gas chromatography with selected-ion-monitoring (SIM) mass spectrometry affords a combination of great selectivity and sensitivity. The high accuracy can be obtained with the stable isotope dilution method. Using deuterated analogues as the corresponding internal standard, SIM provides an ideal basis for specific quantitative analysis by GC–MS.

We present here the synthesis and use of deuterated DOPEGAL (**1**) as an

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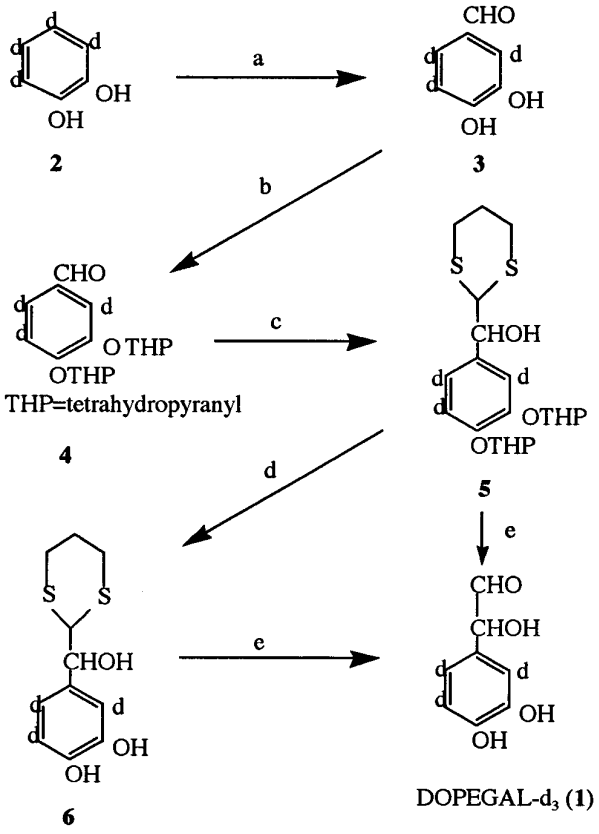

SCHEME 1. (a) *N*-Methylformanilide, $POCl_3$; (b) dihydropyran, pyridinum *p*-toluenesulfonate; (c) 2-lithio-1,3-dithiane; (d) pyridinum *p*-toluenesulfonate; and (e) $HgCl_2$, HgO, acetonitrile.

internal standard to determine the concentration of DOPEGAL in tissue samples by GC–MS–SIM. We apply this method to the measurement of DOPEGAL in human brain tissue from the locus ceruleus (LC).

The synthesis of deuterated DOPEGAL (DOPEGAL-d_3) (**1**) was performed as illustrated in Scheme 1. The deuterated catechol-d_4 (**2**) used as the starting material was prepared from catechol and deuterated water according to the procedure described by Clemo and Swan (*9*). The deuterated 3,4-dihydroxybenzaldehyde-d_3 (**3**) was prepared from **2** by utilizing *N*-methylformanilide–$POCl_3$. The synthetic route from deuterated **3** to the desired compound (**1**) was based on that of unlabeled DOPEGAL which we published (*7*). However, we optimized and modified all of the reaction conditions and some of the reaction agents were changed, such as using pyridinum *p*-toluenesulfonate instead of *p*-toluenesulfonic acid, and butyl lithium instead of lithium diisopropylamide. The last two reactions, removal of the protective group, tetrahydropyranyl, and unmasking of the desired carbonyl function, could be combined into one step by using mercury chloride and mercuric

oxide. Therefore, the purity of products was much better and they were easy to purify.

EXPERIMENTAL

Chemicals and Materials

DOPEGAL was prepared according to our previous paper (*7*). All other chemicals were purchased either from Aldrich (Milwaukee, WI) or from Sigma (St. Louis, MO). The brain tissue was obtained from the Saint Louis University Alzheimer Brain Bank. Melting points were determined on a Fisher–Johns melting point apparatus and uncorrected. IR spectra were recorded on a Perkin–Elmer 1320 spectrophotometer. Molecular masses were determined via a VG 70-250 SEQ hybrid-tandem spectrometer. All HPLC analyses were done using a Waters 6000A multisolvent delivery system with an Applied Biosystems Model 783 programmable detector. Column chromatography was performed on silica gel 60 (70–230 mesh) and TLC analysis on silica gel 60 F254 precoated plates (layer thickness 0.2 mm). Compounds for which only mass spectral data are given have purities >98% as measured by TLC or HPLC.

Synthesis of DOPEGAL-d_3 (**1**)

*3,4-Dihydroxybenzaldehyde-d*3 (**3**). A solution of catechol-d_4 (**2**) (11.4 g, 0.1 mol) in a mixture of *N*-methylformanilide (12.4 ml, 0.11 mol) and benzene (60 ml) was treated with phosphorus oxychloride (10 ml, 0.11 mol) at 10–15°C. The reaction mixture was stirred at room temperature overnight and then heated to 65–70°C for 2 h. The supernatant was poured out, and the residue was mixed with ice water, neutralized with 40% NaOH, and extracted with ethyl acetate. The extract was washed with saline, dried over anhydrous Na_2SO_4, and concentrated. The crude compound was purified by chromatography over silica gel using 2% CH_3OH in CH_2Cl_2 as eluting solvent. All the fractions corresponding to product were combined and cooled to 0°C. The separated solid was collected; yield 4.2 g (30%); mp 151–153°C. MS calcd for di-TMS derivative ($C_{13}H_{19}D_3O_3Si_2$) of 3,4-dihydroxybenzaldehyde-d_3: 285.00. Found: 285 (M^+).

3,4-Ditetrahydropyranoxybenzaldehyde-d_3 (**4**). Pyridinum *p*-toluenesulfonate (500 mg) was added to a solution of 3,4-dihydroxybenzaldehyde-d_3 (7.05 g, 0.05 mol) and dihydro-2*H*-pyran (11 ml, 0.12 mol) in CH_2Cl_2 (20 ml). The reaction mixture was stirred for 5 h at room temperature, then washed with 10% $NaHCO_3$ and saline and dried over anhydrous Na_2SO_4. Removal of the solvent gave the crude compound which was purified by chromatography on silica gel column eluting with 30% CH_2Cl_2 in hexane. The desired fractions were pooled and concentrated to afford 13 g (84%): mp 72–74°C. MS $(FAB)^+$ calcd for ($C_{17}H_{19}D_3O_5$): 309.00. Found: 310 (MH^+).

2-(3,4-Ditetrahydropyranoxy-α-hydroxybenzyl)-1,3-dithiane-d_3 (**5**). A solution of 1,3-dithiane (1.19 g, 9.9 m*M*) in 40 ml of THF was treated with 3.96 ml of 2.5 *M* butyl lithium at −60°C under nitrogen. The reaction mixture was stirred at −40°C

for 2 h and then cooled to −70°C. A solution of **4** (3.06 g, 9.9 mM) in THF (10 ml) was added to the above solution of 2-litho-1,3-dithiane at −70°C. After that, the reaction mixture was stirred at −60°C for 2.5 h and then allowed to come to room temperature overnight. Removal of the THF at room temperature gave a residue which was treated with ice water and extracted with CH_2Cl_2. The extract was washed with water, dried over anhydrous Na_2SO_4, and concentrated. The crude compound was purified by chromatography over silica gel using 30% hexane in CH_2Cl_2 as eluting solvent. All the fractions corresponding to the product were pooled and concentrated: yield 2.5 g (58.8%); IR (Nujol) 3450 (OH) cm^{-1}. MS (FAB^+) calcd for ($C_{21}H_{27}D_3S_2O_5$): 429.00. Found: 430 (MH^+).

2-(3,4-Dihydroxy-α-hydroxybenzyl)-1,3-dithiane-d_3 (**6**). Pyridinium *p*-toluenesulfonate (140 mg) was added to a solution of **5** (2.4 g, 5.6 mM) in 20 ml of 70% CH_3OH. The reaction mixture was stirred at room temperature for 5 h and extracted with ethyl acetate. The extract was washed with 10% $NaHCO_3$ and water and dried over anhydrous Na_2SO_4. Removal of solvent gave the crude compound which was recrystallized with alcohol to afford 1.29 g (88%); mp 99–100°C. MS (FAB^-) calcd for ($C_{11}H_8D_3S_2O_3$): 261.00. Found: 260 $(M-H)^+$.

DOPEGAL-d_3. Method A: To a solution of **5** (690 mg, 1.62 mM) in 70 ml of 80% acetronitrile was added mercury chloride (1.4 g) and mercuric oxide (1.5 g). The reaction mixture was refluxed for 3 h and filtered. The acetonitrile solution was concentrated and extracted with ethyl acetate. Removal of the solvent gave the crude product which was purified by chromatography over silica gel eluting with 1% CH_3OH in CH_2Cl_2. The desired fractions were pooled and concentrated to afford 174 mg (62.8%); mp 105–107°C; NMR (DMSO-d_6) δ 9.50 (d, 1H, CH). MS (FAB+) calcd for ($C_8H_5D_3O_4$): 171.00. Found: 172 (MH+).

Method B: Mercury chloride (906 mg) and mercuric oxide (718 mg) were added to a solution of **6** (321 mg, 0.75 mM) in 35 ml of 80% acetonitrile. The reaction mixture was refluxed for 3 h and extracted with ethyl acetate. The extract was washed with saline, dried over anhydrous Na_2SO_4, and concentrated. The crude compound was purified by chromatography over silica gel eluting with 1% CH_3OH in CH_2Cl_2. The desired fractions were pooled and concentrated to afford 82 mg (64%); mp 105–107°C [Lit. 105–107°C (*7*)]. MS (FAB^+) calcd for ($C_8H_5D_3O_4$): 171.00. Found: 172 (MH^+).

Derivatization of DOPEGAL and Calibration Graphs

For direct analysis of standards, the pure compounds were dissolved in acetonitrile and derivatized under nitrogen with *N*-methy-*N*-trimethylsilyltrifluoroacetamide (MSTFA) at 70°C for 1 h. A standard curve was generated by adding various amounts of unlabeled DOPEGAL to a fixed concentration of deuterated DOPEGAL. After derivatization, 1 μl of the mixture of DOPEGAL and deuterated DOPEGAL was injected into the GC–MS. Calculations were made on the basis of the DOPEGAL/DOPEGAL-d_3 peak-area ratios using the ions at *m/z* 355 and 358, respectively. The regression equation is $y = 1.057\chi + 0.521$ and the correlation coefficient (r^2) is 0.9846.