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Synthesis of a Biochemically Important Aldehyde, 3,4-Dihydroxyphenylacetaldehyde

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3,4-Dihydroxyphenylacetaldehyde (DOPAL) is an important precursor of the major brain metabolites of dopamine, 3,4-dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid. A new method for the synthesis of DOPAL from piperonal is described. We report for the first time the physical and chemical characteristics of DOPAL. Its importance for research in Parkinson's disease and Alzheimer's disease is discussed.

INTRODUCTION

3,4-Dihydroxyphenylacetaldehyde (DOPAL) is formed by oxidative deamination of dopamine (DA) by monoamine oxidase (MAO) (*1*, *2*). In tissues this aldehyde is either enzymatically oxidized to 3,4-dihydroxyphenylacetic acid or reduced to 3,4-dihydroxyphenylethanol (DOPET) (*2*, *3*). The synthesis of DOPAL is important for several reasons (see Fig. 1). First, a standard will allow an accurate measurement in human and animal tissues. Second, the human brain, in a number of disorders such as Parkinson's disease and Alzheimer's disease, is studied primarily using postmortem tissue. Deficits in catecholamines, including DA, are thought to play a role in these disorders (*4*, *5*). DOPAL is the precursor of other major brain metabolites of DA, including 3,4-dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid or homovanillic acid (*6*). Carlsson and Winblad (*7*) have suggested that in postmortem brain the sum of a catecholamine plus its major metabolites is a better index of the level of the catecholamine in brain at the time of death than the catecholamine alone. This is supported by the findings of Warsh *et al.* (*8*) that catecholamines are metabolized in the brain after death. The measurement of DOPAL will therefore contribute to a more accurate estimation of premortem levels of DA in postmortem human brain. In addition, because the enzymes involved in metabolism of DOPAL (aldehyde reductase, aldehyde dehydrogenase, and catechol-*O*-methyltransferase) either are in very low amounts (*9*) or are extraneuronal (*10–12*), DOPAL may be an index of the intraneuronal metabolism of newly formed DA (*13*).

Finally, DOPAL is the initial MAO-B metabolite of dopamine. Blashko (*1*) has suggested that aldehyde metabolites generated by the action of MAO on amines

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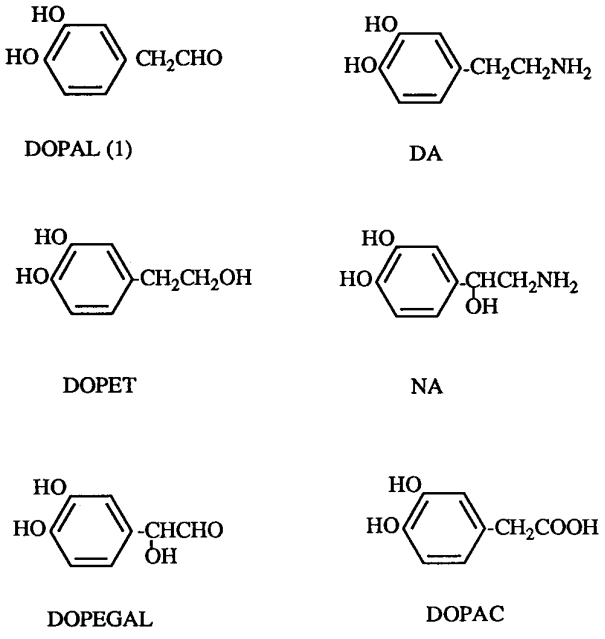
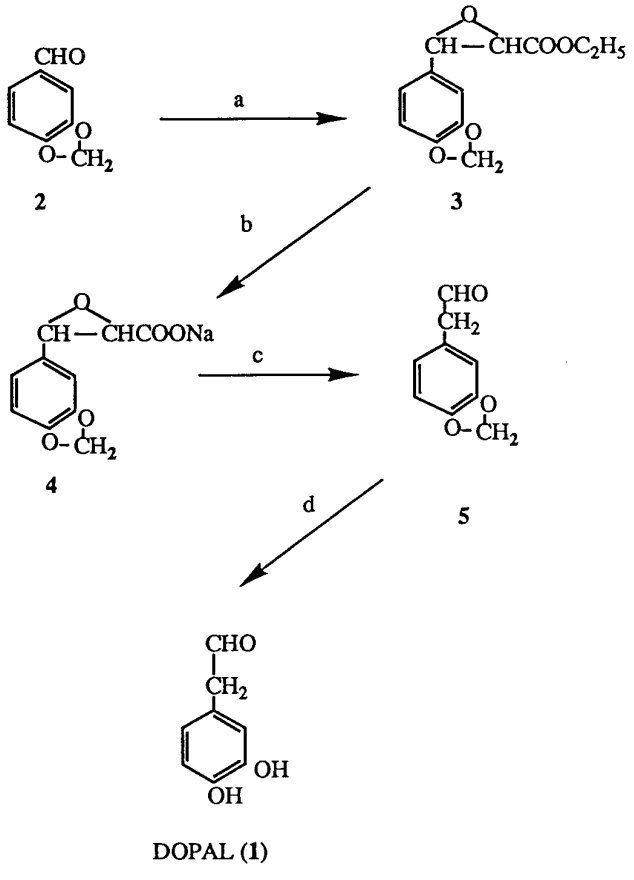


FIGURE 1

may be toxic to cells in which they are formed. Markey *et al.* (*14*) have shown that the exogenous toxin 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine, which causes a form of Parkinson's disease, becomes toxic to neurons only after its oxidation to the pyridinium form by MAO-B. We have previously synthesized 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL), the MAO-A metabolite of noradrenaline (NA) and adrenaline (*15*). DOPEGAL is a major NA metabolite in animal and postmortem human tissue (*16*, *17*). DOPEGAL, but not other noradrenaline metabolites, is toxic to cultured PC-12 cells (*18*) and triggers apoptotic cell death (*19*), the form of neuronal death found in Alzheimer's disease (*20*) and Parkinson's disease (*21*).

Attempts have been made to synthesize pure DOPAL (*22–25*). However, none of the methods provide detailed procedures and physical or chemical characterization of DOPAL other than its molecular weight by GC–MS. Such a method is important because, in order to study a potential role for DOPAL in neuron death in Alzheimer's disease and Parkinson's disease, it is necessary to develop a reliable method for synthesis and purification of large amounts of chemically pure DOPAL. The chemically pure compound is required for *in vitro* and *in vivo* tests of DOPAL toxicity as well as for quantification of DOPAL in brain neurons affected in Parkinson's disease and Alzheimer's disease (*21*, *26–28*).

In this paper we describe a new synthetic method for DOPAL and its physical characterization. The successful synthetic route uses commercially available piperonal as outlined in Scheme 1. Conversion of **2** to the ethyl glycidate (**3**) was accomplished by reaction with ethyl chloroacetate in the presence of sodium ethoxide.



SCHEME 1. (a) Ethyl chloroacetate, $NaOC_2H_5$; (b) NaOH, ethanol; (c) acetic acid, benzene; and (d) $BCl_3/S(CH_3)_2$, 1,2-dichloroethane.

Hydrolysis of **3** with sodium hydroxide gave the sodium salt (**4**) which upon treatment with acetic acid in benzene generated the aldehyde group to afford **5.** Final deprotection of the methylenedioxy group with boron trichloride–methyl sulfide complex gave crude **1,** which was purified by chromatography over silica gel.

EXPERIMENTAL

Melting points were determined on a Fisher–Johns melting point apparatus and uncorrected. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Gemini 300 spectrometer with Me_4Si as an internal standard. Field strength of various proton resonances is expressed as s, d, t, q, the center of which is given. IR spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR spectrometer.

Ultraviolet spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. GC–mass spectrometry was obtained using an HP 5890 Series II gas chromatograph coupled with a HP 5971 mass selective detector. The gas chromatograph was equipped with Alltech Econo Cap SE-45 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Molecular masses were determined via a VG70-250 SEQ hybrid-tandem spectrometer. Column chromatography was performed on silica gel 60 (70–230 mesh ASTM) and TLC analysis on silica gel 60 F254 precoated plates (layer thickness 0.2 mm).

Ethyl-3-(3,4-Methylenedioxyphenyl) Glycidate (**3**)

To 65 ml of absolute alcohol was added 3.08 g (0.134 gram atom) of sodium at room temperature. The reaction mixture was heated under reflux until the solid sodium disappeared and then cooled to 0–5°C. A mixture of piperonal (**2**) (17.8 g, 0.119 M) and redistilled ethyl chloroacetate (15.93 g, 0.13 M) was added to the above sodium ethoxide solution at −5°C during 1 h. The reaction mixture was stirred at 0°C for 1 h then allowed to warm to room temperature overnight and poured into a mixture of ice (100 g) and acetic acid (2 ml). The mixture was extracted with dichloromethane (50 ml × 4) and the extracts were washed with water and dried over anhydrous sodium sulfate. Removal of the solvent gave a crude product which was purified by chromatography on a silica gel column eluting with 50% dichloromethane in hexane. The desired fractions were identified with TLC, pooled, and evaporated to afford an oil, 18.8 g (67%) of 3; NMR ($CDCl_3$), δ 6.84 (dd, 1H, aromatic), 6.78 (d, 1H aromatic), 6.70 (d, 1H aromatic), 5.97 (s, 2H, CH_2), 4.28 (q, 2H, CH_2), 4.02 (d, 1H, J = 2 Hz, CH), 3.46 (d, 1H, J = 2 Hz, CH), 1.30 (t, 3H, CH_3); IR (nujol) 1740 (CO), 931 (OCH_2O), 1235 (–O–), cm^{-1}; MS (GC–MS), calcd for $C_{12}H_{12}O_5$: 236.00. Found: 236 (M^+).

Sodium 3-(3,4-methylenedioxyphenyl) Glycidate (**4**)

To a solution of **3** (11.48 g, 0.049 M) in 35 ml of ethanol was added 25 ml of 4 N NaOH and 10 ml of water. The reaction mixture was stirred at room temperature overnight. The solid which separated was collected, washed with ethanol, and dried over P_2O_5 in a desiccator: yield 9.1 g (81%) of **4,** mp >300°C. The dried salt was used without further purification.

3,4-Methylenedioxyphenylacetaldehyde (**5**)

To a suspension of **4** (8.87 g, 0.038 M) in 100 ml of benzene was added 10 ml of acetic acid. The suspension was heated under reflux for 4 h. The benzene solution was washed with saturated sodium bicarbonate solution then water and dried over anhydrous sodium sulfate. Removal of the benzene gave a crude compound which was purified by chromatography on a silica gel column with 30% of dichloromethane in hexane as the eluting solvent. The desired fractions were identified with TLC, pooled, and concentrated: yield 3.6 g (57%) of **5,** mp 60–62°C; NMR ($CDCl_3$), δ 9.70 (t, 1H, CHO), 7.22 (dd, 1H, aromatic), 7.19 (d, 1H aromatic), 6.85 (d, 1H, aromatic), 6.10 (s, 2H, CH_2), 3.59 (d, 2H, CH_2); IR (nujol), 1720 (CO), 927 (–OCH_2O–) cm^{-1}; MS (GC–MS), calcd for ($C_9H_8O_3$): 164.00. Found: 164 (M^+).

3,4-Dihydroxyphenylacetaldehyde (**1**)

To a solution of **5** (560 mg, 3.42 mM) in 15 ml of 1,2-dichloroethane was added 3.5 ml of 2 M boron trichloride–methyl sulfide complex solution in dichloromethane. The reaction mixture was heated under reflux for 24 h and then cooled to room temperature. Ten milliliters of water was added and the mixture was stirred for 1 h at room temperature. The organic layer was separated and the water layer was extracted with 1,2-dichloroethane (15 ml × 3). The combined extracts were washed with water and dried over anhydrous sodium sulfate. Removal of the solvent gave the crude product which was purified by chromatography on a silica gel using 2% methanol in dichloromethane as the eluting solvent. The desired fractions were identified with TLC, pooled, and concentrated to afford 75 mg (14%) of **1:** NMR (DMSO-d_6) δ 9.67 (t, 1H, CHO), 7.27 (dd, 1H, aromatic), 7.20 (d, 1H, aromatic), 6.90 (d, 1H, aromatic), 3.50 (d, 2H, CH_2); mp 110–112°C; IR (nujol), 3425 (OH), 1690 (CHO) cm^{-1}; UV (methanol) λ max 228 nm (ε = 9173), 262 nm (ε = 8181); MS (CI), calcd for $C_8H_8O_3$: 152.00. Found: 153 (MH^+). *Anal.* Calcd for $C_8H_8O_3H_2O$: C, 56.45%; H, 5.92%. Found: C 56.18%; H, 6.18%.

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