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Reaction of Dopamine with D-Glyceraldehyde under Biomimetic Conditions: Stereoselective Formation of Tetrahydroisoquinolines and Rate-Accelerating Effects of Transition Metal Ions

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Abstract—In 0.05 M phosphate buffer, pH 7.4, and at 37°C, dopamine underwent a smooth Pictet–Spengler condensation with D-glyceraldehyde and D,L-glyceraldehyde-3-phosphate to afford diastereoisomeric tetrahydroisoquinolines. In the case of D-glyceraldehyde **1a/1b** were formed in ca. 2:1 ratio. Treatment with carbonyldiimidazole converted **1a** and **1b** into the corresponding oxazinoisoquinolinones **2a** and **2b** which were separated and stereochemically characterised by NMR analysis. Transition metal ions commonly occurring in biological systems (e.g. Cu^{2+} and Fe^{3+}) markedly accelerated the formation of **1a**–**1b** without affecting the product ratio. Mechanistic evidence suggested the reversible generation of Schiff base intermediates, detected by ^1H NMR, which undergo stereoselective cyclisation according to the Felkin–Anh model. Metal-chelation at the catechol group facilitates the rate-determining nucleophilic attack to the imine moiety by enhancing the electron density at the site of cyclisation. These results highlight an apparently overlooked effect of transition metal ions on the Pictet–Spengler reaction under biomimetic conditions and provide a chemical basis to postulate a role of carbohydrate metabolites as modulatory agents of dopaminergic neurotransmission via conversion to potentially bioactive tetrahydroisoquinoline derivatives.

Introduction

The reaction of β-arylethylamines with aldehydes and activated carbonyl compounds, first reported by Pictet and Spengler in 1911, provides a most straightforward access to tetrahydroisoquinolines and related ring systems forming the core structural moiety of alkaloids and other important classes of biologically active products.[1–3] Because of the cogent biological implications, studies of this reaction have been driven equally by the purely synthetic option and the investigative value of biomimetic processes. In the latter context, interest has recently been heightened by the increasing implication of tetrahydroisoquinolines in certain physiological and pathological alterations of catecholamine metabolism. Paradigmatic in this regard are the production of salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) as putative determinant of the pharmacological and behavioural effects produced by either acute or chronic ingestion of alcohol;[4] the identification of the potent β-adrenergic agonist tetrahydropapaveroline as the product of the action of monoamine oxidase on dopamine in patients with Parkinson's disease;[5] and the identification of 1-carboxy-1-phenylmethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, a dopamine-β-hydroxylase inhibitor, in patients with phenylketonuria.[6]

Lesser attention has so far been paid to the possible interaction of dopamine and other catecholamine neurotransmitters with aldehydes relating to carbohydrate metabolism.[7] Interest in this issue is warranted by the high concentrations of glucose in the brain (cerebral utilization = 77 mg/min) and body fluids (0.65–0.95 mg/mL in whole blood and more than 0.4 mg/mL in cerebrospinal fluid) and the crucial role of carbohydrate metabolism, as interwoven with lipid and amino acid metabolism, in neurone energetics and functioning.[8] Critical enzymes and intermediates of the glycolytic pathway, such as D-glyceraldehyde-3-phosphate, pyruvate, lactate, are present in relatively high levels in brain tissues (e.g. 0.03 μmol/g of D-glyceraldehyde-3-phosphate) and in body fluids, where significant catecholamine levels are also present (unconjugate and conjugate dopamine concentrations are 48 and 594

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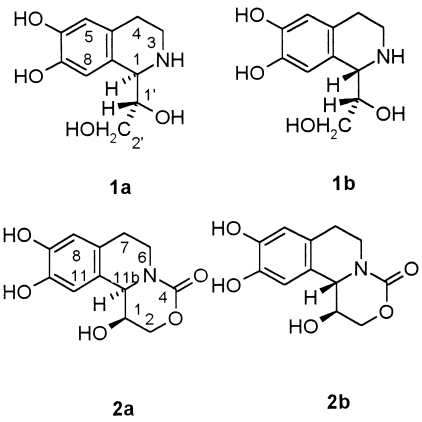
pg/mL in cerebrospinal fluid, and 86 and 2926 pg/mL in plasma, respectively) and may fluctuate to reach much higher values.[9] Conditions, therefore, exist to envisage efficient covalent interactions between carbohydrate metabolites and catecholamines, both intra- and extracellularly, possibly through the Pictet–Spengler route, to afford a range of potentially neurotoxic and/or neuromodulatory agents. Experimental evidence supporting viability of these reactions under physiologically relevant conditions is however lacking. In this paper we report the reaction of dopamine with D-glyceraldehyde, a convenient model of glycolytic intermediate and the lowest member of the carbohydrate family, affording in a stereoselective fashion tetrahydroisoquinoline products at physiological pH and temperature, and provide evidence for hitherto overloaded rate-accelerating effects of certain transition metal cations commonly found in biological systems.

Results and Discussion

In 0.05 M phosphate buffer, pH 7.4, and at 37°C dopamine (0.5 mM) reacted smoothly with equimolar amounts of D,L-glyceraldehyde-3-phosphate under an oxygen-depleted atmosphere to give the corresponding tetrahydroisoquinolines as main detectable products (HPLC and spectral evidences). Because of some difficulties in the isolation and characterisation of these products, in subsequent experiments D-glyceraldehyde was preferably used as a more convenient substrate. Under the above conditions, reaction with dopamine afforded an intimate mixture of two main products which, though chromatographically distinguishable at nanomole to micromole levels, escaped all attempts at separation at the higher concentrations required for preparative purposes. Eventually, the products were isolated as a mixture by preparative HPLC and were identified as the stereoisomeric tetrahydroisoquinolines **1a** and **1b** by extensive NMR analysis (HOHAHA,[10] homonuclear and heteronuclear COSY).

Both HPLC and ^{1}H NMR spectra indicated formation of the two stereoisomers in an approximate ratio of 2:1. Since the two isomeric products differed exclusively in the configuration of the stereogenic centre at C-1, their relative stereochemistry was investigated by conversion into cyclic derivatives and straightforward inspection of coupling constants between H-11b and H-1. Thus, treatment of the isolated mixture with carbonyldiimidazole afforded two main products which were identified as (1*S*,11b*R*)-1,9,10-trihydroxy-1,6,7,11b-tetrahydro-2*H*, 4*H*-[1,3]oxazino[4,3-*a*]isoquinolin-4-one (**2a**) and (1*S*,11b*S*)-1,9,10-trihydroxy-1,6,7,11b-tetrahydro-2*H*,4*H*-[1,3]oxazino[4,3-*a*]isoquinolin-4-one (**2b**).

In the ^{1}H NMR spectrum of the most abundant isomer, the proton at C-11b appeared as a broad singlet at δ 4.78, whereas in the spectrum of the minor isomer the same proton gave a doublet at δ 4.43 ($J = 5.4$ Hz). These results were suggestive of different dihedral angles between the H11b-C11b-C1 and the C11b-C1-H1 planes, as expected for **2a** and **2b**.



Inspection of Dreiding models and molecular mechanics considerations (MM2 force field[11]) consistently indicated a dihedral angle of about 60° for minimised structure **2a** and an angle of about 180° for minimised structure **2b**. Based on Karplus correlation data, predicting the smaller coupling constant for the 60°C dihedral angle, the more abundant isomer was assigned structure **2a** and, by implication, the parent tetrahydroisoquinoline (main isomer) was accordingly formulated as **1a**. This structural relationship was secured by acid hydrolysis of **2a**, which afforded exclusively the most abundant tetrahydroisoquinoline, i.e. **1a**. In 0.05 M phosphate buffer and at 37°C, the reaction of dopamine with glyceraldehyde was first order in both substrates and exhibited pH-dependent kinetics (Fig. 1).

Periodical monitoring by ^{1}H NMR (400 MHz) of the reaction of 330 mM dopamine with 495 mM D-glyceraldehyde in phosphate buffered D_2O, pD 7.8, and 37°C (Fig. 2) revealed after 30 min the presence in the low field region of two small, yet distinct signals at δ 8.43 and 8.47, which persisted throughout the whole course of the reaction (Fig. 2B), suggesting the generation of isomeric Schiff bases.

Integration of relevant signals allowed to estimate a total Schiff base concentration of about 1%. Apart from the signals ascribed to the intermediate Schiff base, analysis of the resonance patterns revealed the generation of **1a** and **1b** as the main detectable products since the onset of the reaction. Eventually, when all of the dopamine had disappeared, the spectrum was virtually superimposable to that of **1a**–**1b** isolated by preparative HPLC, with the sole exception of the signals due to excess glyceraldehyde. These latter clearly indicated that under the reaction conditions glyceraldehyde was present mostly in the hydrated form,[12] with an equilibrium concentration of the aldehydic monomer form of less than 9%.

Overall, these data were consistent with a mechanism involving the rapidly reversible formation of the Schiff base which then suffered irreversible cyclisation in the rate determining step. Within this scheme, the stereoselective formation of **1a** would be dictated by the asymmetric centre in the glyceraldehyde-derived moiety, and the transition state ensuing from cyclisation of the Schiff base conformer in Figure 3 would reflect the Felkin–Anh model for asymmetric induction,[13] in the assumption

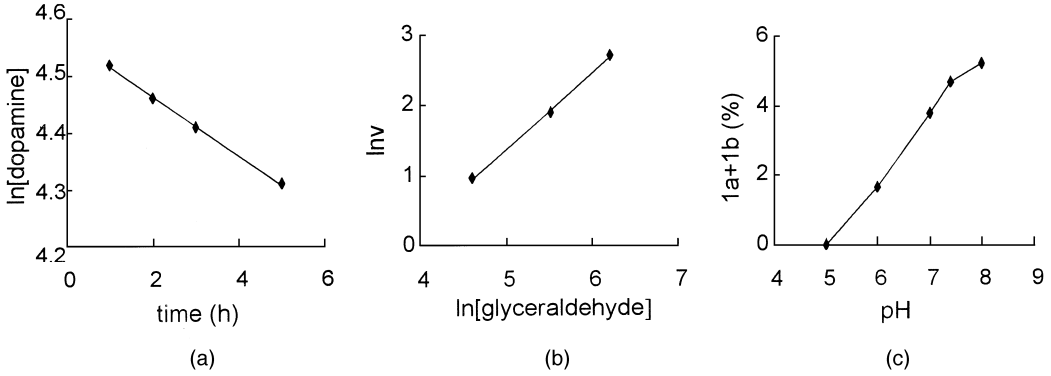


Figure 1. First-order dependence of the reaction kinetics on dopamine (a) and glyceraldehyde (b) concentrations, and pH-dependence of product yield (c). Data are averages of at least three determinations. S.D. did not exceed ±5% of mean values. (a) Dopamine = 50 μM; glyceraldehyde = 500 μM; $r^2 = 0.998$. (b) Dopamine = 500 μM; glyceraldehyde = 100–500 μM; initial rates (v) were determined at 30 min; slope = 1.094. (c) Dopamine = 1 mM; glyceraldehyde = 1 mM; product yield determined at 6 h.

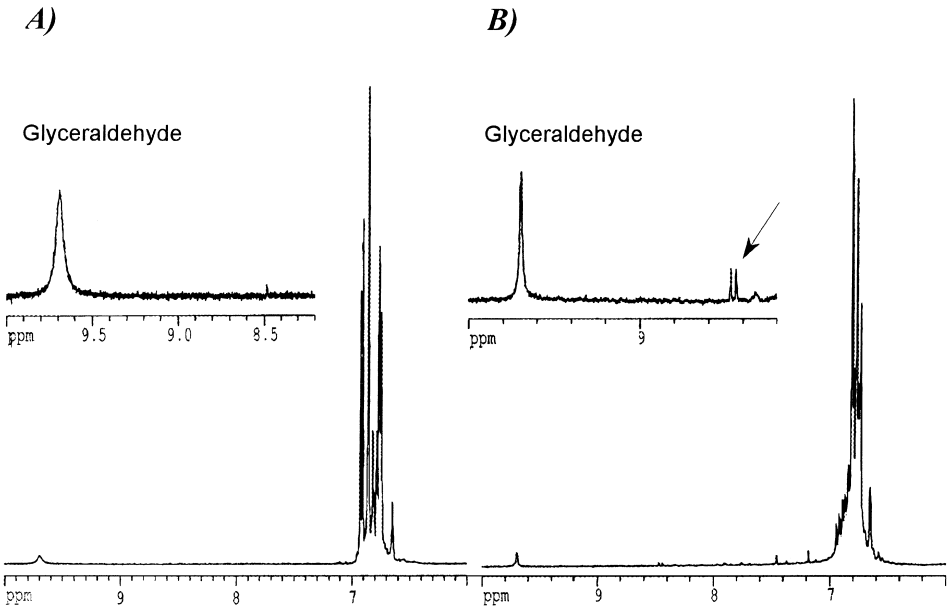


Figure 2. ^{1}H NMR spectrum (400 MHz, low field region) of the reaction mixture of dopamine (330 mM) with glyceraldehyde (495 mM) in phosphate buffered D_2O. A, $t = 0$; B, $t = 30$ min. Insets show expanded region of the spectrum comprising Schiff base protons, indicated by the arrow.

that the hydroxyl group at C-1′ provides the lowest energy σ^*_{C-X} orbital.[14] In such an arrangement, which maximises orbital interactions with the imine π system, the entering nucleophile would preferably attack from the side opposite the OH group, so to experience the smallest steric hindrance in the cyclisation step.

Molecular mechanics calculations indicated similar steric energies ($\Delta E = 0.3$ kcal/mol) for minimised structures of **1a** and **1b**, implying that stereoelectronic effects, rather than, or in addition to, merely steric contributions, should be important in determining relative energies of incipient transition states and π-facial differentiation.

Tetrahydroisoquinolines **1a**–**b** were previously prepared for synthetic purposes by reacting dopamine with glyceraldehyde in refluxing methanol for 2 days,[15] and under such conditions reported stereoselectivity was much greater (estimated isomer ratio 9:1). The effects of the medium on the **1a**:**1b** product ratio were thus investigated by conducting the reaction in different solvents (e.g. methanol and DMF) and at different temperatures, with both substrates at 10 mM concentration. In all cases, the kinetics were much slower in the organic solvents, being virtually negligible at 37°C and reaching values of 792 μM/h (DMF) and 208 μM/h (methanol) at 60°C, versus values of 490 μM/h at 37°C and 2 mM/h at 60°C in aqueous phosphate buffer, pH 7.4. Notably, stereoselective control was much higher in organic solvents, approximating a **1a**:**1b** product ratio of 4:1 both in methanol and DMF at 60°C, whereas in aqueous phosphate buffer the **1a**:**1b** product ratio remained constant at ca. 2:1 in the temperature range between 25 and 60°C. In all cases, the isomer formation ratio remained constant throughout the reaction, ruling out the possible equilibration of **1a** and **1b**.

While the faster reaction kinetics in aqueous medium could be ascribed to the enhanced nucleophilicity of the partially deprotonated catechol ring in the transient Schiff base,[16,17] the partial loss of stereochemical control could not be attributed tout-court to the usual

decrease in selectivity accompanying faster reaction rates, since no differences in product ratio were observed with increasing temperature. Specific solvation effects might possibly be considered, affecting critical orbital interactions invoked in the Felkin–Anh model, for example, by accentuating the energy gap between the polarisable imine π-orbital and the σ^*-orbital, whereby their mutual interaction would be weakened. This point is currently under investigation in our laboratories.

Notably, when the reaction of dopamine with glyceraldehyde was carried out in the presence of some metal ions of common occurrence in biological systems, a marked acceleration was observed which depended on both the nature and concentration of the metal (Fig. 4). Of the various ions tested, Cu^{2+} proved the most effective followed by Fe^{3+}, whereas Zn^{2+}, Al^{3+}, Mn^{2+} at physiologically relevant concentrations were scarcely or not effective.

The reaction rates were first order in Cu^{2+} and Fe^{3+}, and a maximum accelerating effect was apparent at a metal-to-dopamine ratio of 1:2, no significant increase being observed at higher ratios. No variation was observed in the ratio of formation of **1a**:**1b**. These data suggested that the accelerating effects derive from formation of a complex between the metal ion and the intermediate Schiff base, lowering the energy of the transition state in the rate-determining cyclisation step. Two possible target functionalities for metal ions were considered, namely the aromatic *o*-dihydroxy group, which would expectedly exhibit larger electron donating effects following chelation, and the imine group flanked by the hydroxyl, which might be susceptible of activation by the metal ion acting as a Lewis acid.

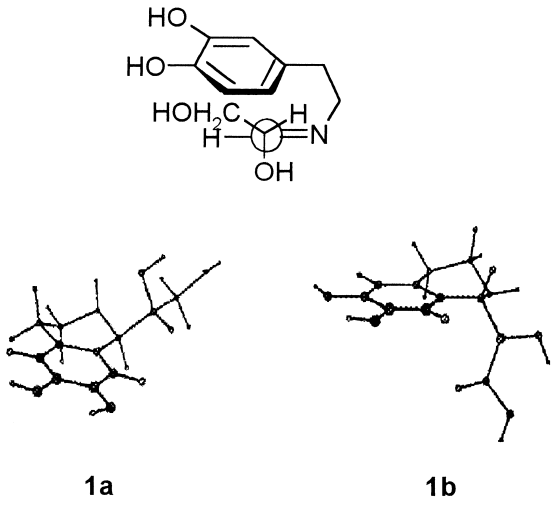


Figure 3. Suggested conformation of the Schiff base for the intramolecular cyclisation according to the Felkin–Anh model and minimised structures (MM2) for diastereoisomeric tetrahydroisoquinolines **1a** and **1b**.

That the former option was mechanistically more relevant was argued on the basis of the established ability of Cu^{2+} and Fe^{3+} ions to form strong chelates with catechol compounds,[18,19] and was corroborated by separate experiments showing that metal ions have no detectable influence on the reaction of glyceraldehyde with the *O,O*-dimethylether of dopamine, in which metal chelation at the catechol functionality was clearly precluded. Furthermore, a comparable rate hastening effect of metal ions was observed in the Pictet–Spengler condensation of dopamine with other aldehydes, e.g. formaldehyde and acetaldehyde (not shown), indicating a general mechanism of activation independent of the nature of the aldehyde but requiring a free catechol functionality. ^{1}H NMR analysis showed no significant variation in the chemical shift and integrated area of the intermediate imine signals at δ around 8.4 obtained by reaction of dopamine (330 mM) with D-glyceraldehyde (495 mM) in the presence of 33 mM Cu^{2+}.

On this basis, a plausible mechanistic scheme can be proposed (Fig. 5) in which the catecholamine is envisaged to form a 2:1 chelate complex with Cu^{2+} and other active metal ions.[18] Condensation of the chelate with glyceraldehyde would then give rise to a Schiff base–metal complex, which would suffer cyclisation at a faster rate because of the enhanced electron donating capacity of the metal-chelated *o*-dihydroxy functionality. In this frame, metal ions would affect the kinetics of the rate determining step but not the stereochemical control imparted by the asymmetric hydroxyl group,

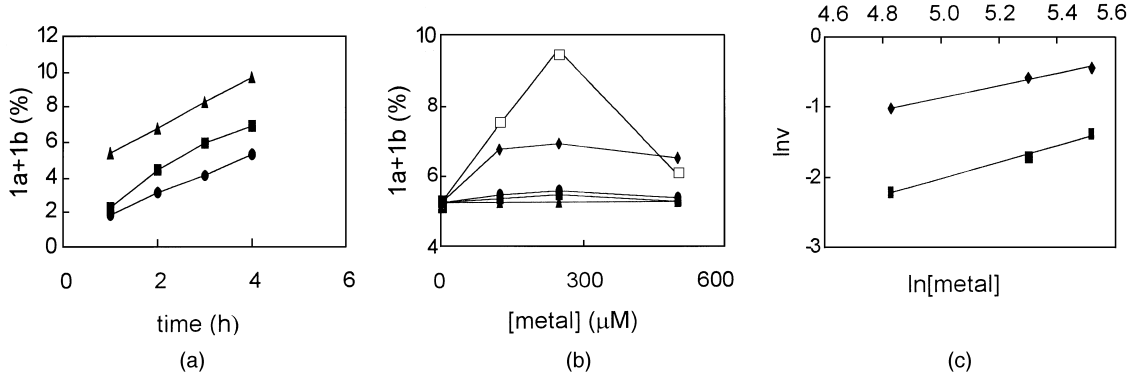


Figure 4. Effects of transition metal ions on the kinetics of the reaction of dopamine with glyceraldehyde. Data are averages of at least three determinations. S.D. did not exceed ±5% of mean values. (a) Dopamine = 500 μM; glyceraldehyde = 500 μM; metal = 250 μM; (▲), Cu (II); (■), Fe (III); (●), control. (b) Dopamine = 500 μM; glyceraldehyde = 500 μM; (□), Cu (II); (◆), Fe (III); (■), Zn (II), (●), Mn (II); (▲), control. (c) First order dependence of the reaction kinetics on metal ion concentration; dopamine = 500 mM; glyceraldehyde = 500 μM; metal = 125-500 μM; initial rates (v) were determined at 30 min; (◆), Fe (III), slope 0.87, and (■), Cu (II), slope 1.15.

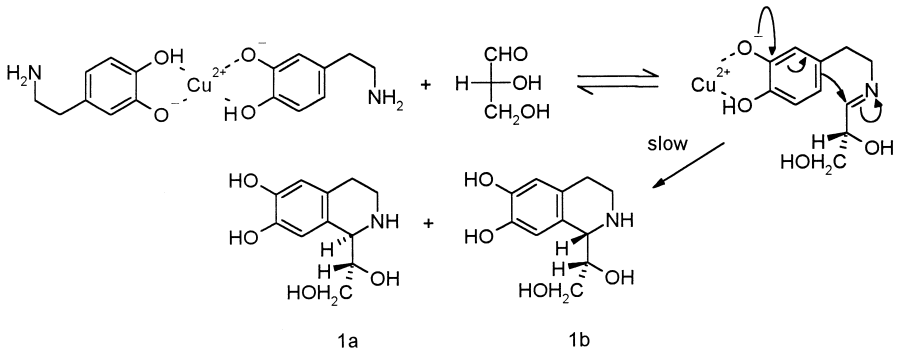


Figure 5. Proposed mechanism for the Pictet–Spengler condensation of dopamine with D-glyceraldehyde highlighting the effect of metal ions.

consistent with chelate formation occurring distal to the interacting sites in the cyclisation reaction.

Conclusions

The results of the present study provide a chemical background to postulate novel mechanisms of modulation of catecholaminergic neurotransmission by carbohydrate-related metabolites, disclosing specific rate-accelerating effects of transition metal ions on the Pictet–Spengler condensation under physiologically-relevant conditions. In addition to the possible intervention of enzymatic activities, which have recently been implicated in the generation of endogenous tetrahydroisoquinolines,[20] the marked rate hastening effects of transition metal ions widespread in brain tissues would extend the expected relevance of this reaction to pathological situations associated with abnormal accumulation of metal ions (e.g. in Parkinson's disease) in which iron levels in the substantia nigra pars compacta increase by ca. 35%, in progressive supranuclear palsy, in which iron levels in the substantia nigra pars compacta increase by ca. 70%, and in Huntington's disease, in which iron levels increase by 56% in caudate nucleus and copper levels increase by 64% in putamen and 68% in the substantia nigra pars compacta.[21] Additional instances in which formation of endogenous tetrahydroisoquinolines related to **1** seems plausible include conditions of altered glucose metabolism associated with inhibition of glyceraldehyde-3-phosphate dehydrogenase. This would apply especially to oxidative stress and other circumstances in which nitric oxide production is increased, resulting in a covalent binding of NAD^+ to the enzyme.[22–25] The occurrence of tetrahydroisoquinolines by reactions of catecholamines with intermediary products of carbohydrate metabolism and the biological activity of these compounds are currently under investigation in our laboratories.

Experimental

Optical rotations were measured using a Perkin–Elmer 141 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 Avance spectrometer. Tetramethylsilane (TMS) was used as the internal standard, with the exception of samples in D_2O solutions, for which sodium 3-(trimethylsilyl)-2,2,3,3[d_4]propionate (TSP) was used. Assignments with identical superscripts may be interchanged. EI-MS spectra were recorded using the electron ionization (EI) at 70 eV. TLC was performed on 0.25 or 0.5 mm silica gel F_{254} plates. Analytical and preparative reverse phase HPLC was carried out on C18 columns (4.6 × 250 mm and 22 × 250 mm), with flow rates of 1 and 10 mL/min, respectively. Dopamine was from Aldrich, D-(+)-glyceraldehyde from Fluka and 1,1′-carbonyldiimidazole from Merck. Metal ion solutions were prepared from $FeCl_3$, $CuSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}H_2O$, $Al_2(SO_4)_3{\cdot}16H_2O$. *O,O*-Dimethyldopamine was prepared as previously described.[26]

Reactions of dopamine with D-glyceraldehyde. Appropriate amounts of dopamine hydrochloride and D-(+)-glyceraldehyde were incubated in 0.05 M phosphate buffer, pH 7.4, thermostatted at 37°C in a rubber capped tube under argon. Addition of excess $NaClO_4$ to control ionic strength did not affect reaction rates to any significant extent. When necessary, aliquots of stock aqueous solutions of metal ions were added. For product analysis, aliquots of the reaction mixtures were withdrawn with a syringe, acidified to pH 3 and injected into the HPLC. All kinetic experiments were run at least in triplicate. For dopamine and total tetrahydroisoquinoline quantitation, 3 mM octane-1-sulphonic acid in 0.2 M H_3PO_4, pH 2.5-acetonitrile 92:8 v/v was used as the eluant. To estimate relative concentrations of isomeric tetrahydroisoquinolines 0.3% HCOOH was preferably used as the eluant, although dopamine analysis was often unreproducible due to partial co-elution with one of the diastereoisomers.

Isolation of (1*R*, 1′*S*)-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1a) and (1*S*, 1′*S*)-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1b). Dopamine hydrochloride (1 g) and D-(+)-glyceraldehyde (955 mg) were dissolved in 0.1 M phosphate buffer, pH 8, (530 mL) previously purged with argon and heated at 60°C. The reaction was periodically monitored by HPLC, and when most of the dopamine had disappeared (after about 3 h), the mixture was acidified to pH 4 with 2 M HCl and evaporated under reduced pressure. The residue was taken up in water and passed through a Dowex X-8 H^+ cation exchange column. After extensive washings with water, the

products were eluted with 1 M HCl along with residual dopamine. Preparative HPLC, eluant 0.3% HCOOH eventually afforded pure **1a**:**1b** as a glassy solid (700 mg): UV (0.5 M HCl) λ_{max} nm (log ε): 284 (3.72); $[\alpha]_D^{23}$ + 10.9° (*c* 1.6, methanol); ^{1}H NMR, (D_2O): δ 2.90 (m, 2H, H-4_{1b}), 2.96 (m, 2H, H-4_{1a}), 3.21 (m, 1H, H-3_{1b}), 3.39 (m, 1H, H-3_{1a}), 3.60 (m, 1H, H-3_{1a}), 3.69 (m, 1H, H-3_{1b}), 3.69 (m, 2H, H-$2'_{1b}$), 3.80 (m, 2H, H-$2'_{1a}$), 4.13 (q, *J* = 4.7 Hz, 1H, H-$1'_{1a}$), 4.35 (q, *J* = 4.2 Hz, 1H, H-$1'_{1b}$), 4.58 (d, *J* = 5.3 Hz, 1H, H-1_1a), 4.75 (d, *J* = 4.4 Hz, 1H, H-$1'_{1b}$), 6.74[c] (s, 1H, H-5), 6.79[d] (s, 1H, H-5), 6.81[c] (s, 1H, H-8), 6.81[d] (s, 1H, H-8); ^{13}C NMR, (D_2O): δ 26.4 (t, C-4_{1a}), 27.1 (t, C-4_{1b}), 41.5 (t, C-3_{1a}), 43.3 (t, C-3_{1b}), 58.6 (d, C-1_{1a}), 60.4 (d, C-1_{1b}), 64.7 (t, C-$2'_{1b}$), 65.0 (t, C-$2'_{1a}$), 73.1 (d, C-$1'_{1b}$), 73.3 (d, C-$1'_{1a}$), 116.4[c] (d, C-5_{1b}), 116.9[d] (d, C-5_{1a}), 118.6[d] (d, C-8_{1a}), 118.6[c] (d, C-8_{1b}), 122.4[e] (s, C-9_{1b}), 123.2[f] (s, C-9_{1a}), 127.7[f] (s, C-10_{1a}), 128.0[e] (s, C-10_{1b}), 145.7[g] (s, 1C×2, C-7_{1a1b}), 146.9[g] (s, 1C×2, C-6_{1a1b}); EI-MS (*m*/*z*): 225 (M^+, 5%), 194 (12), 164 (100). Anal. calcd for $C_{11}H_{15}NO_4$: C, 58.64; H, 6.72; N, 6.22%. Found: C, 58.59; H, 6.70; N, 6.21%.

(1*S*,11b*R*)-1,9,10-Trihydroxy-1,6,7,11b-tetrahydro-2*H*,4*H*-[1,3]oxazino[4,3-*a*]-isoquinolin-4-one (2a) and (1*S*,11b*S*)-1,9,10-trihydroxy-1,6,7,11b-tetrahydro-2*H*,4*H*-[1,3]oxazino[4,3-*a*]isoquinolin-4-one (2b). A mixture of **1a** and **1b** (1 g) and carbonyldiimidazole (800 mg) was dissolved in anhydrous DMF (10 mL) and heated at 60°C.[27] After 4 h TLC analysis ($CHCl_3$:MeOH 9:1) showed two main products at R_f 0.2 and 0.3. The solvent was then evaporated under reduced pressure and the residue was repeatedly chromatographed on preparative TLC plates ($CHCl_3$:MeOH, 9:1) to give eventually 200 mg of pure **2a** and 75 mg of **2b** as pale yellow oils. Impurities of imidazole may contaminate the products and can be removed by repeated TLC purification.

2a: UV (MeOH) λ_{max} nm (log ε): 221 (4.21, sh), 286 (3.93), 293 (3.84, sh); $[\alpha]_D^{23}$ + 5.8° (*c* 0.5, methanol); ^{1}H NMR (CD_3OD): δ2.49 (dt, *J* = 15.4, 2.6 Hz, 1H, H-7_{eq}), 2.80 (ddd, *J* = 15.4, 4.4 Hz, 1H, H-7_{ax}), 2.95 (ddd, *J* = 12.1, 2.8 Hz, 1H, H-6_{ax}), 4.26 (dd, *J* = 11.4, 2.4 Hz, 1H, H-2), 4.35 (brs, 1H, H-1), 4.44 (m, 1H, H-6_{eq}), 4.45 (dd, *J* = 11.4, 1.2 Hz, 1H, H-2), 4.78 (brs, 1H, H-11b), 6.56[c] (s, 1H, H-11), 6.69[c] (s, 1H, H-8); ^{13}C NMR (CD_3OD): δ 29.2 (t, C-7), 43.4 (t, C-6), 60.0 (d, C-11b), 65.9 (d, C-1), 72.0 (t, C-2), 113.7[c] (d, C-11), 116.5[c] (d, C-8), 124.8 (s, C-11a), 129.1 (s, C-7a), 145.8 (s, 1C×2, C-9, C-10), 155.7 (s, 1C, C-4); EI-MS (*m*/*z*): 251 (M^+, 75%), 208 (100), 164 (81). Anal. calcd for $C_{12}H_{13}NO_5$: C, 57.35; H, 5.22; N, 5.58%. Found: C, 57.29; H, 5.10; N, 5.49%.

2b:. UV (MeOH) λ_{max} nm (log ε): 221 (4.38, sh), 286 (4.15), 293 (4.07, sh); $[\alpha]_D^{23}$ −19.4° (*c* 0.6, methanol); ^{1}H NMR (CD_3OD): 2.51 (dt, *J* = 15.4, 2.6 Hz, 1H, H-7_{eq}), 2.85 (ddd, *J* = 15.4, 4.4 Hz, 1H, H-7_{ax}), 3.00 (ddd, *J* = 12.1, 2.8 Hz, 1H, H-6_{ax}), 4.00–4.13 (m, 1H×3, H-1, 2×H-2), 4.24 (m, 1H, H-6_{eq}), 4.43 (d, *J* = 5.4 Hz, 1H, H-11b), 6.56[c] (s, 1H, H-11), 7.10[c] (s, 1H, H-8); ^{13}C NMR (DEPT), (CD_3OD): δ 31.0 (C-7), 45.2 (C-6), 61.8 (C-11b), 68.3 (C-1), 69.4 (C-2), 113.6[c] (C-11), 116.6[c] (C-8); EI-MS (*m*/*z*): 251 (M^+, 22%), 208 (100), 164 (48). Anal. calcd for $C_{12}H_{13}NO_5$: C, 57.35; H, 5.22; N, 5.58%. Found: C, 57.26; H, 5.17; N, 5.51%.

Acknowledgements

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