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Pictet–Spengler Condensation of the Antiparkinsonian Drug L-DOPA with D-Glyceraldehyde. Opposite Kinetic Effects of Fe^{3+} and Cu^{2+} Ions and Possible Implications for the Origin of Therapeutic Side Effects

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Abstract—In 0.05 M phosphate buffer, pH 7.4, and at 37 °C, L-DOPA, a widely used antiparkinsonian drug, reacted smoothly with D-glyceraldehyde to afford diastereoisomeric (1*R*,1′*S*,3*S*)-3-carboxy-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**1**) and (1*S*,1′*S*,3*S*)-3-carboxy-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**2**) in an approx. 3:2 ratio. The prevalent formation of **1** over **2** reflects stereoselective cyclisation of a transient Schiff base in accord with the Felkin–Anh model. Fe^{3+} ions, present at relatively high levels in parkinsonian brains, markedly accelerated formation of **1** and **2**, whereas Cu^{2+} decreased the reaction rate, due apparently to different sites of chelate formation between L-DOPA and the metal ions. Both metal ions markedly decreased the stereoselectivity of the reaction. Product **1** exhibited chelating properties toward metal ions comparable or stronger than those of L-DOPA. These results throw new light on the effects of transition metal ions on the Pictet–Spengler reaction and suggest a possible role of tetrahydroisoquinoline products from L-DOPA and carbohydrate metabolites in the severe side effects of the drug.

Introduction

In spite of increasing efforts toward innovative pharmacological treatments of Parkinson's disease, current therapeutic regimens still make extensive recourse to the classical dopamine-related drug L-DOPA (levodopa).[1–3] First introduced in the clinical practice in the 1960s, L-DOPA is now established as the mainstay of symptomatic treatment of the disease, often leading to more than 50% amelioration of clinical manifestations.[4] However, in the long term, its efficay declines and chronic administration at high oral doses eventually causes drug-induced involuntary movements, on–off fluctuation of effectiveness and dyskinesias at peak doses, possibly as a result of hastened degeneration of nigrostriatal neurons.[5] These problems and the lack of a detailed knowledge of the molecular mechanisms underlying the antiparkinsonian effects warrant continuing interest in the biochemical transformations of L-DOPA in the brain.

One attractive explanation for the origin of DOPA-induced dyskinesias is the conversion of the drug into potent neurotoxic metabolites counteracting the positive effects derived from partial replacement of deficient dopamine. In this general context, tetrahydroisoquinoline products derived from Pictet–Spengler condensations with aldehydes would deserve particular interest because of their prominent position among the catecholamine metabolites under suspicion of inducing neuronal degeneration. Moreover, the susceptibility of L-DOPA to suffer condensation with aldehydes is well documented on a chemical basis[6,7] and is supported, in vivo, by the increased excretion of salsolinol and tetrahydropapaveroline in the urine of patients with Parkinson's disease during oral treatment with L-DOPA.[8]

Our entry into this issue was driven by the hypothesis that aldehydes derived from carbohydrate metabolism may provide suitable targets for L-DOPA in view of their elevated levels and almost ubiquitous distribution in the brain. An attractive candidate and useful model compound, in this regard, is D-glyceraldehyde, the lowest member of the carbohydrate family, a representative glycolytic intermediate and a potential glycating agent

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Table 1. NMR Data for Products **1** and **2** (in D_2O)

	1		**2**	
Carbon	δ_C	δ_H (mult., *J* Hz)	δ_C	δ_H (mult., *J* Hz)
1	58.2	4.86–4.88 (m)	58.0	4.91 (d, 2.9)
3	54.2	4.86–4.88 (m)	53.5	4.31 (m)
4	29.0	3.17 (dd, 17.5, 7.3), 3.87 (dd, 17.5, 5.7)	28.6	3.16 (dd, 16.7, 11.7), 3.24 (dd, 16.7, 5.1)
4a	125.3		124.8	
5	117.9	6.87 (s)	117.7	6.92 (s)
6	144.9		145.7	
7	144.7		144.6	
8	116.2	6.76 (s)	116.1	6.84 (s)
8a	120.3		121.8	
1′	73.2	4.34 (m)	72.7	4.48 (m)
2′	64.0	3.59 (dd, 11.5, 5.9), 3.75 (dd, 11.5, 4.4)	64.1	3.86 (dd, 12.0, 4.3), 3.91 (dd, 12.0, 3.7)
COOH	173.0		172.7	

that may contribute to neurone damage.[9] Several processes occurring in the parkinsonian substantia nigra under oxidative stress conditions would account for the generation of locally high levels of D-glyceraldehyde and/or of D-glyceraldehyde-3-phosphate. These include the iron-promoted oxidative degradation of ascorbate,[10] and the nitric oxide-mediated inhibition of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase.[11–13]

In the present paper, we report a detailed investigation of the reaction of L-DOPA with D-glyceraldehyde as a model system for testing the reactivity of the drug with carbohydrate-derived aldehydes. Since iron and other transition metal ions are known to accumulate at locally high levels in the substantia nigra in Parkinson's disease, compared to matched control subjects,[14,15] particular attention has been focused on their effects on the kinetic and stereochemical course of the reaction.

Results and Discussion

Reaction of L-DOPA (500 μM) with D-glyceraldehyde (500 μM) in 0.05 M phosphate buffer, pH 7.4 and at 37 °C, with rigorous exclusion of oxygen led to the gradual formation of two main products in an approximate ratio of 3:2 which could be obtained in pure form by preparative HPLC. Mass spectrometric and NMR analysis clearly indicated that the products were the expected (1*R*,1′*S*,3*S*)-3-carboxy-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**1**) and (1*S*,1′*S*,3*S*)-3-carboxy-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**2**) differing exclusively in the configuration of the stereogenic C-1 carbon. Detailed inspection of the cross peaks in the ^{1}H-^{1}H COSY, ^{1}H–^{13}C HETCOR and ^{1}H–^{13}C HMBC spectra permitted complete assignment of proton and carbon resonances for the products (Table 1).

To determine the relative stereochemistry of the products, the ^{1}H NMR and the ^{13}C NMR spectra were analysed with the assistance of computer aided molecular modelling (MM+ force field), based also on the extensive arguments in the literature regarding the compression effect in ^{13}C NMR for 1,4-gauche interactions in trans 1,3-disubstituted tetrahydroisoquinolines[16] and β-carbolines.[17] For the energy-minimised stereostructure of the isomer **1**, in which the carboxyl group and the dihydroxyethyl chain are in a *cis* relationship and occupy pseudo-equatorial positions, no ^{13}C γ-effect would be anticipated for the carbon signals for C-1 and C-3 (Fig. 1).

By contrast, one energy-minimised conformation of the isomer **2** bearing the COOH group on a pseudoequatorial position would experience a ^{13}C γ-effect, shifting

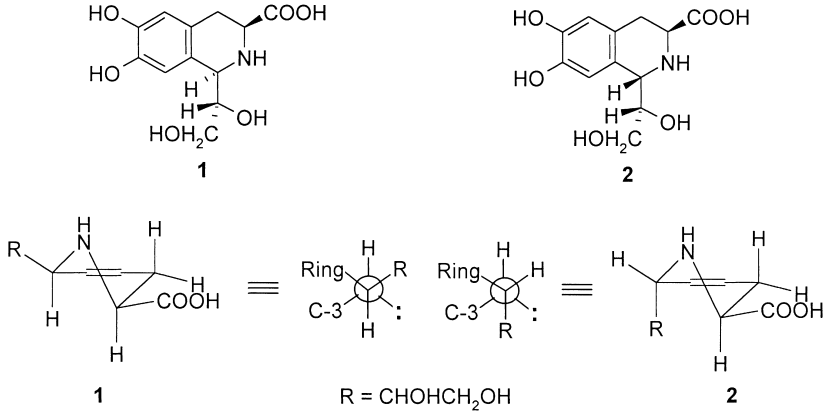


Figure 1. Structures of tetrahydroisoquinolines **1** and **2** formed by reaction of L-DOPA with D-glyceraldehyde, and schematic representation highlighting the γ-gauche effect in **2**.

the C-1 and C-3 carbon signals at higher fields.[18] On this basis, structure **2** was assigned to the minor isomer, in which the resonances for the C-1 and C-3 carbons appeared 0.2 and 0.7 ppm upfield compared to those of the main product. NOe experiments designed to substantiate this assignment did not provide conclusive evidence. However, the relatively upfield resonance of the H-1 proton in the major stereoisomer (δ 4.87 versus 4.91 for the minor isomer) lent further support to the above stereochemical assignment, in view of the similar shift observed in the case of 3-carboxysalsolinol stereoisomers[19] and of the dopamine–glyceraldehyde adducts.[20]

Interestingly, in the main product (**1**) the H-3 proton appeared about 0.5 ppm downfield (δ 4.87) compared to the H-3 proton in **2** (δ 4.31) and to the H-3 proton in L-DOPA (δ 4.29). A possible explanation came from inspection of the energy-minimised stereostructures of **1** and **2**. In **1**, the carboxyl group occupies invariably a pseudoequatorial position, forcing the H-3 proton in a pseudoaxial position oriented toward the periphery of the deshielding region of the aromatic ring. In at least one stable conformation of tetrahydroisoquinoline **2**, on the other hand, the carboxyl group occupies the pseudoaxial position, so that the adjacent H-3 proton on the pseudoequatorial position is kept away from the aromatic ring (Fig. 2).

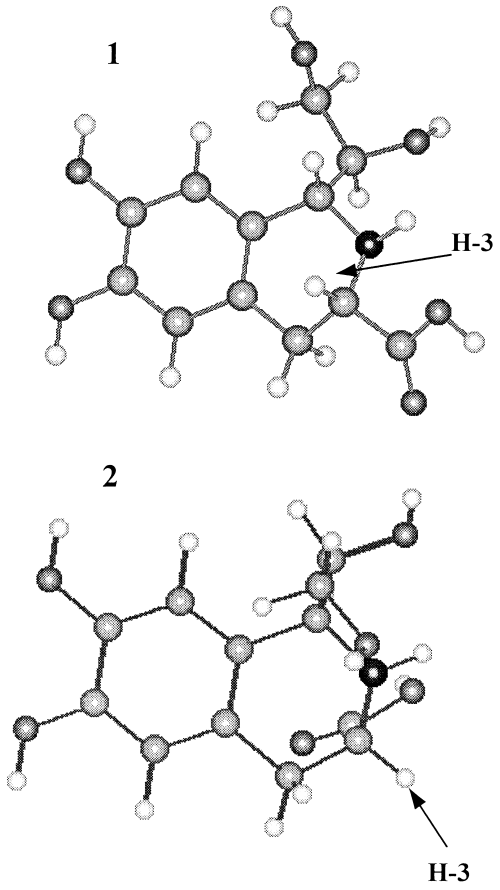


Figure 2. Minimised stereostructures (MM+) for diastereoisomeric tetrahydroisoquinoline **1** (1) and **2** (2) highlighting the different orientations of the H-3 proton.

Formation of **1** and **2** was first order with respect to both L-DOPA and D-glyceraldehyde concentrations and exhibited a pH-dependent kinetic profile, the rate increasing with increasing pH (data not shown). This behaviour is in accord with a typical Pictet–Spengler mechanism proceeding through the intermediacy of a transient Schiff base. The latter would suffer stereoselective cyclisation in compliance with the Felkin–Anh model for asymmetric induction as indicated by the preferential formation of the 1*R* stereoisomer.[21,22] It may be noted that the reaction of L-DOPA with D-glyceraldehyde proceeds with lower stereoselectivity compared to the similar reaction of dopamine.[20] This evidently reflects the influence of the carboxyl group of L-DOPA which would counteract, for example by intramolecular hydrogen bonds with the OH groups, the effects of the asymmetric centre in the aldimine moiety of the Schiff base intermediate.

Notably, when the reaction of L-DOPA with D-glyceraldehyde was carried out in the presence of Cu^{2+} and Fe^{3+} ions under rigorously anaerobic conditions, opposite effects were observed on the kinetic course of the reaction: while Fe^{3+} considerably accelerated product formation, Cu^{2+} exerted a significant rate-decreasing effect (Fig. 3). Both metals acted in a concentration-dependent way, no further increase or decrease of product formation rates being generally observed at metal-to-substrate ratios greater than 1:2. Other metal ions did not affect the reaction course to measurable degrees (not shown).

The opposite effects of Cu^{2+} and Fe^{3+} on the kinetics of the Pictet–Spengler reaction of L-DOPA with D-glyceraldehyde can be ascribed to the different nature of the chelate complexes that these metals can form with L-DOPA and, hence, with the transient Schiff base at pH 7.4. In particular, Fe^{3+} would bind mainly to the catechol moiety of the drug (purple complex, λ_{max} 560 nm),[23] favouring partial deprotonation of the phenoxyl groups to give a metal-bound catecholate group. The binding of transition metal cations to catecholic compounds is well documented[24] and is generally accompanied by a marked bathochromic shift of the chromophore, similar to that observed in alkaline media. Following metal chelation, the aromatic ring of L-DOPA would become more susceptible to electrophilic attack by the imine double bond of the transient Schiff base intermediate, the apparent rate-limiting step in tetrahydroisoquinoline formation. An alternative explanation would involve activation of the electrophilic component by Fe^{3+} acting as a Lewis acid. Though plausible, however, this view would be in contrast with the marked pH-dependence of the Pictet–Spengler condensations of catecholamines, which proceed fastly in alkaline media, where phenolic ionisation becomes significant, and at considerably slower rates at acidic pH, under conditions where at least partial protonation of the Schiff base would be expected to accelerate the reaction kinetics. To settle this mechanistic issue and to distinguish between the two possible activation mechanisms, in another series of experiments we investigated the effects of Fe^{3+} on the reaction of 4-methyl-

catechol, a model compound for L-DOPA, with formaldehyde, leading to 4-hydroxymethyl-5-methylcatechol (**3**) (Scheme 1).

In this model reaction, the aldehyde, as electrophilic counterpart of the catechol, would be unlikely to form strong complexes with the metal ions. The results shown in Figure 4 indicated again a marked accelerating effect of Fe^{3+} on the kinetic course of the reaction, which would hardly be ascribed to activation of the electrophile and would merely denote activation of the catechol system. Notably, Cu^{2+} proved more effective than Fe^{3+} in accelerating the reaction of 4-methylcatechol with formaldehyde.

Unlike Fe^{3+}, Cu^{2+} can interact with L-DOPA in different, pH-dependent modes, binding chiefly to the amino acid functionality in moderately acidic media and to the catechol moiety at alkaline pH.[25] At physiological pH, therefore, substantial Cu^{2+} chelation at the aminoacidic functionality of L-DOPA may be anticipated, which may hinder Schiff base formation slowing down the overall rate of the reaction. Since Cu^{2+} is also complexed by glycols, it is possible that interaction with D-glyceraldehyde or the D-glyceraldehyde-derived moiety in the Schiff base plays a role in the rate decreasing effects of the metal. To address in more detail this and other issues relating to the roles of metal ions in Pictet–Spengler condensations, we investigated the effects of Fe^{3+} and Cu^{2+} on the reaction of L-DOPA with the simplest aldehyde, formaldehyde, to give 3-carboxy-6,7-dihydroxytetrahydroisoquinoline (**4**). The results, reported in Figure 5, confirmed both the accelerating properties of Fe^{3+} and the rate decreasing effect of Cu^{2+} on the kinetics of tetrahydroisoquinoline formation.

This result would rule out any significant involvement of the glycolic substituent in the L-DOPA-D-glyceraldehyde reaction in the presence of metal ions. Since Cu^{2+} and Fe^{3+} both accelerated the analogous Pictet–Spengler condensation of dopamine with D-glyceraldehyde,[20] as well as the reaction of 4-methylcatechol with formaldehyde (Fig. 4) it can be concluded that the changing effect of Cu^{2+} in the case of L-DOPA depends critically on the presence of the carboxyl group in the latter which prevents in part the metal from binding to the catechol moiety.

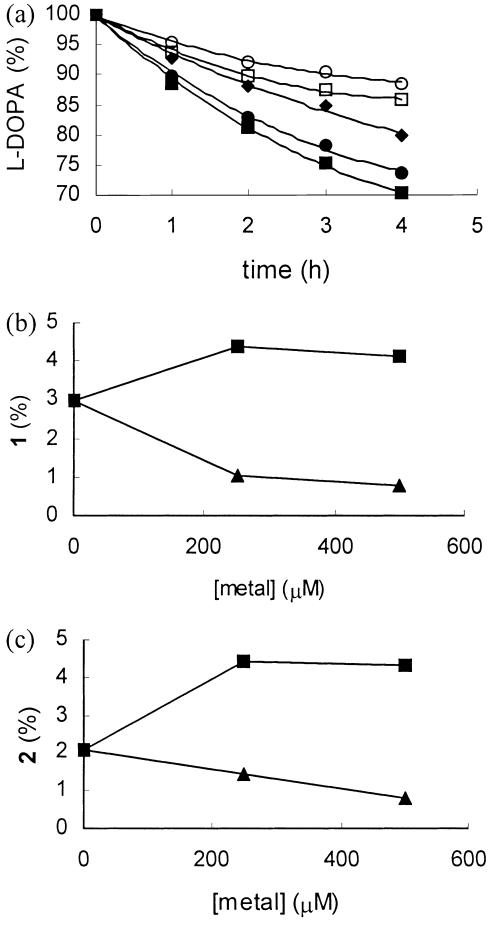
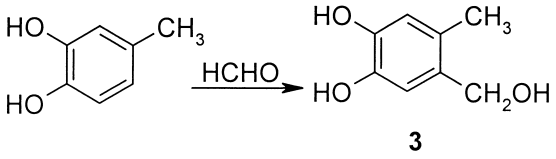


Figure 3. Effects of transition metal ions on the kinetics of the reaction of L-DOPA with D-glyceraldehyde (a) and tetrahydroisoquinoline formation (b and c). Data are averages of at least three determinations; sd did not exceed ±5% of means values: (a) L-DOPA = 500 μM; glyceraldehyde = 500 μM; ◇, control; □, Cu (II) 250 μM; ○, Cu (II) 500 μM; ■, Fe (III) 250 μM; ●, Fe (III) 500 μM. (b) and (c) L-DOPA = 500 μM; glyceraldehyde = 500 μM; ■, Fe (III); △, Cu (II).



Scheme 1.

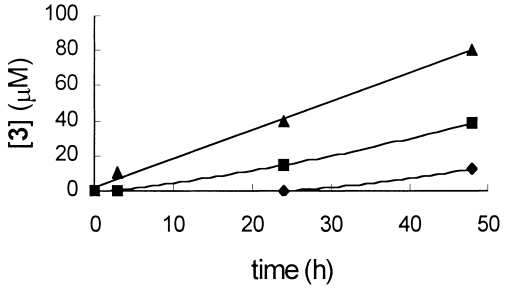


Figure 4. Effects of transition metal ions on the kinetics of the reaction of 4-methylcatechol with formaldehyde. Data are averages of at least three determinations; sd did not exceed ±5% of means values: 4-methylcatechol = 0.16 M; formaldehyde = 1.6 M; ◆, control; ■, Fe(III) 0.16 M; ▲, Cu(II) 0.16 M.

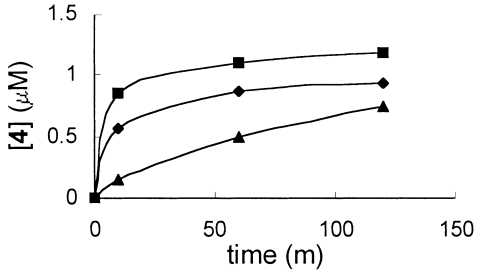
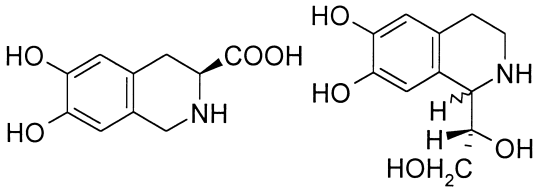


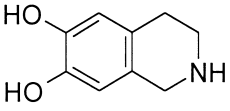
Figure 5. Effects of transition metal ions on the kinetics of the reaction of L-DOPA with formaldehyde. Data are averages of at least three determinations; sd did not exceed ±5% of means values: L-DOPA = 500 μM; formaldehyde = 500 μM; ◆, control; ■, Fe(III) 250 μM; ▲, Cu(II) 250 μM.

In addition to the kinetic effects, Fe^{3+} and Cu^{2+} also influenced to some extent the stereochemical course of Schiff base cyclisation, virtually suppressing the modest stereoselectivity of the reaction (Fig. 2). This effect may be ascribed again to the formation of a chelate complex between the aminoacidic functionality and metal ions, partially overwhelming the stereoinducing properties of the glyceraldehyde moiety. This conclusion would be supported by the observation that Cu^{2+} and Fe^{3+} did not affect the stereochemical course of the reaction of dopamine with D-glyceraldehyde.[20]

Finally, in another set of experiments we explored the metal-chelating properties of **1** in comparison with those of **4**, of (1′*S*)-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**5**, the analogue from dopamine and D-glyceraldehyde, mixture of stereoisomers),[20] of the parent 6,7-dihydroxytetrahydroisoquinoline (**6**) as well as of L-DOPA and dopamine. The aims of the experiments were to discriminate the relative contributions of the various potential metal binding sites.



4 **5**



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The data reported in Table 2 indicate that in the presence of Fe^{3+} the tetrahydroisoquinoline **1** forms a purplish complex absorbing at 520 nm, that is shifted ipsochromically with respect to the analogous complexes from **4** and L-DOPA. The maximum chromophore intensity was observed at a metal/**1** ratio of ca. 2. A quite similar behaviour was displayed by the tetrahydroisoquinoline **5**. In the presence of Cu^{2+}, on the other hand, less pronounced chromophoric changes could be detected, resulting with all substrates in the appearance of a shoulder at around 300 nm. The complexes of **4** with Fe^{3+} and Cu^{2+} were closely similar to those of the corresponding complexes with L-DOPA (Table 2), and no significant difference was observed between the Fe^{3+} and Cu^{2+} complexes of **1** and those of the stereoisomer **2** (not shown).

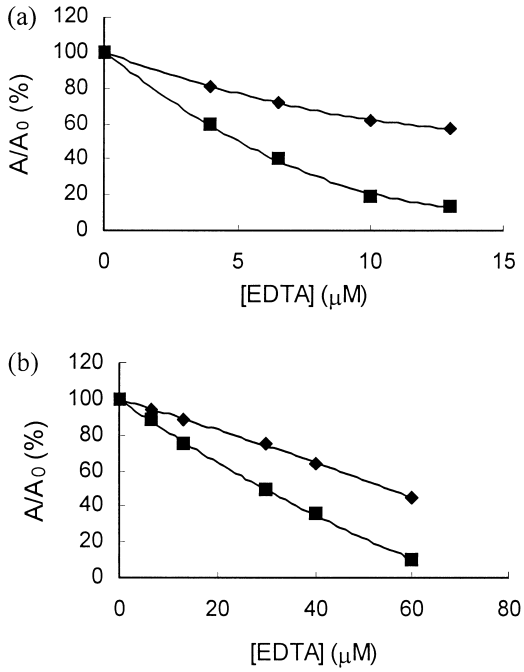

Figure 6. Effect of EDTA on the Fe^{3+} and Cu^{2+} chelates of **1** and L-DOPA. A_0 = absorbance in the absence of EDTA, A = absorbance in the presence of EDTA. **1** and L-DOPA were 0.25 mM, Fe^{3+} and Cu^{2+} were 0.125 mM: (a) ◆, **1**-Fe^{3+} chelate, $\lambda = 550$ nm; ■, L-DOPA-Fe^{3+} chelate, $\lambda = 520$ nm; (b) ◆, **1**-Cu^{2+} chelate, $\lambda = 295$ nm; ■, L-DOPA-Cu^{2+} chelate, $\lambda = 303$ nm.

Interestingly, whereas the complexes of **1** and L-DOPA with Fe^{3+} proved of comparable stability, as evidenced spectrophotometrically by comparing the effects of increasing concentrations of EDTA, the Cu^{2+}/**1** complex proved to be more stable than the Cu^{2+}/L-DOPA complex (Fig. 6).

Overall, these data showed that the tetrahydroisoquinoline **1** exhibits significant metal coordinating

Table 2. Spectrophotometric properties of Fe^{3+} and Cu^{2+} chelates of **1**, **4**, **5**, **6**, L-DOPA and dopamine

	λ_{max} (nm) (absorbance)					
Ligand/metal[a]	**1**	**4**	L-DOPA	**5**	**6**	Dopamine
Fe^{3+}						
0.5	520 (0.15)	540 (0.27)	550 (0.13)	522 (0.40)	543 (0.17)	548 (0.21)
1	520 (0.12)	540 (0.19)	550 (0.10)	522 (0.33)	543 (0.14)	548 (0.10)
2	520 (0.07)	540 (0.15)	550 (0.05)	522 (0.22)	543 (0.09)	548 (0.08)
4	520 (0.04)	540 (0.14)	550 (0.03)	522 (0.15)	543 (0.09)	548 (0.06)
Cu^{2+}						
0.5	303 (1.10)	295 (1.72)	295 (1.52)	300 (2.00)	300 (0.96)	293 (1.12)
1	303 (0.87)	295 (1.45)	295 (1.26)	300 (1.72)	300 (0.87)	293 (0.87)
2	303 (0.76)	295 (1.24)	295 (1.09)	300 (1.29)	300 (0.66)	293 (0.72)
4	303 (0.58)	295 (1.20)	295 (0.62)	300 (0.86)	300 (0.50)	293 (0.46)

[a]Ligand concentration was maintained at 0.25 mM and metal varied as indicated.

properties involving the catechol functionality. The different absorption maxima of the Fe^{3+}-chelates of **1** and **4** as well as of **5** and **6** would indicate that the dihydroxyethyl chain at C-1 affects to some degree the coordination chemistry of the 6,7-dihydroxytetrahydroisoquinoline system, probably by participating to some extent in chelate formation. This is not entirely unexpected considering that the vicinal diol functionality may act as a bidentate ligand. On the other hand, the apparent similarity between the spectrophotometric properties of the Fe^{3+}-chelates of **1** and **5**, or of **4** and **6**, would rule out more than a marginal influence of the carboxyl group at C-3 on the binding of Fe^{3+} to the catechol moiety.

Conclusions

Under physiologically relevant conditions the antiparkinsonian drug L-DOPA was found to react smoothly with D-glyceraldehyde to give diastereoisomeric tetrahydroisoquinoline products with moderate Felkin–Anh-type stereoselectivity. Major outcomes of this study include the demonstration of the divergent and specific kinetic effects of Fe^{3+} and Cu^{2+} ions on the reaction of L-DOPA with biologically relevant aldehydes, which have no precedent in the literature on Pictet–Spengler chemistry, and a detailed analysis of the metal binding properties of the resulting tetrahydroisoquinolines. In the degenerating substantia nigra in Parkinson's disease the increased iron load may exceed the storage capacity of ferritin, and could be present as weak, low molecular weight chelate complexes, that may release the metal when exposed to relatively high levels of L-DOPA,[26] an effective chelating agent. This could favour interaction of the drug with aldehydes and carbohydrate metabolites leading to tetrahydroisoquinoline products, which may chelate iron as well and may evoke a neurotoxic response akin to that caused, for example, by norsalsolinol and tetrahydropapaveroline,[27] thus contributing to the long term side effects of L-DOPA. Whether and to what extent the proposed reaction pathways are operative and compete with other possible routes to tetrahydroisoquinolines, including enzymatic ones, remains the focus for future work.

Experimental

Optical rotations were measured using a JASCO P-1010 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 Avance spectrometer using *tert*-butyl alcohol (δ 1.23) as internal standard. Mass spectra were recorded using the fast atom bombardment (FAB–MS) technique with glycerol as the matrix and the electron ionisation (EI–MS) technique. Analytical and preparative reverse phase HPLC were carried out on C18 columns (4.6×250 and 22×250 mm), with flow rates of 1 and 10 mL/min, respectively. L-DOPA was from Aldrich, D-(+)-glyceraldehyde was from Fluka and 4-methylcatechol was from Sigma. Metal ion solutions were prepared from $FeCl_3$ and $CuSO_4{\cdot}7H_2O$.

Reactions of L-DOPA with D-glyceraldehyde

Appropriate amounts of L-DOPA 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 L-DOPA and tetrahydroisoquinoline quantitation, 0.1 M HCOOH/acetonitrile 97:3 v/v was used as the eluant.

Reactions of 4-methylcatechol with formaldehyde

Appropriate amounts of 4-methylcatechol and formaldehyde were incubated in 0.05 M phosphate buffer, pH 8, thermostatted at 50 °C in a rubber-capped tube under argon. 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, reduced with sodium borohydride, acidified to pH 3 and injected into the HPLC. All kinetic experiments were run at least in triplicate. For **3** quantitation, 0.5 M HCOOH/methanol 90:10 v/v was used as the eluant.

Isolation of (1*R*, 1′*S*, 3*S*)-3-carboxy-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1) and (1*S*, 1′*S*, 3*S*)-3-carboxy-1-(1′,2′-dihydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (2). L-DOPA (1 g) and D-(+)-glyceraldehyde (1.37 g) were dissolved in 0.1 M phosphate buffer, pH 8, (508 mL) previously purged with argon and heated at 60 °C. The reaction was periodically monitored by HPLC, and when most of the L-DOPA had disappeared (after about 3 h), the mixture was acidified to pH 3 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 0.5 M HCl along with residual L-DOPA. Preparative HPLC, eluant 0.1 M HCOOH/acetonitrile 95:5 v/v afforded ca. 600 mg of pure **1** and ca. 400 mg of pure **2**.

1: UV (0.5 M HCl) λ_{max} nm (log ε): 280 (3.47); $[\alpha]_D^{15}$ +26.3° (*c* 0.04, 0.5 M HCl); H^1 NMR (D_2O) and ^{13}C NMR (D_2O): see Table 1; MS (FAB) *m*/*z*: 270; HRMS calcd for $C_{12}H_{16}NO_6$ ($M^+ + 1$) 270.0978, found 270.0973.

2: UV (0.5 M HCl) λ_{max} nm (log ε): 280 (3.44); $[\alpha]_D^{15}$ −49.5° (*c* 0.01, 0.5 M HCl); H^1 NMR (D_2O) and ^{13}C NMR (D_2O): see Table 1; MS (FAB) *m*/*z*: 270; HRMS calcd for $C_{12}H_{16}NO_6$ ($M^+ + 1$) 270.0978, found 270.0971.

Isolation of 4-hydroxymethyl-5-methylcatechol (3). 4-Methylcatechol (200 mg) and $FeCl_3$ (520 mg) were dissolved in 0.1 M phosphate buffer, pH 8, (200 mL) previously purged with argon and heated at 50 °C. After 10 min, formaldehyde (600 μL) was added to the mixture. After 24 h TLC analysis ($CHCl_3$/MeOH 9:1)

showed one main product at R_f 0.4 besides traces of the starting material. The reaction was stopped by addition of $NaBH_4$, the mixture was acidified to pH 3 with HCl and extracted twice with ethyl acetate. The organic layer was essiccated with anhydrous sodium sulphate and evaporated under reduced pressure. The residue was then chromatographed on TLC preparative plates ($CHCl_3$/MeOH 9:1) to give **3** (50 mg) as yellow oil.

3: UV (MeOH) λ_{max} nm (log ε): 285 (3.57); ^{1}H NMR (CD_3OD): δ 2.07 (s, 3H, CH_3), 3.60 (s, 2H, CH_2OH), 6.34 (s, 1H, H-3), 6.59 (s, 1H, H-6); ^{13}C NMR (CD_3OD): δ 19.7 (q, CH_3), 37.2 (t, CH_2OH), 118.6 (d, C-3), 119.2 (d, C-6), 129.5 (s, C-5), 132.5 (s, C-4), 144.9 (s, 2C, C-1, C-2); EI–MS (*m*/*z*): 154 (M^+). Anal. calcd for $C_8H_{10}O_3$: C, 62.31; H, 6.54%. Found: C, 62.19; H, 6.47%.

Metal complexes

Formation of Fe^{3+} and Cu^{2+} chelates of tetrahydroisoquinolines **1**, **4**, **5** and **6**, dopamine and L-DOPA was studied spectrophotometrically at different metal to ligand ratios. In a typical experiment, the metal was added at the stated concentration to a solution of 0.25 mM catechol in 0.1 M phosphate buffer (pH 7.4) which had been fluxed with argon for at least 20 min. The UV spectrum was recorded after 3 min equilibration. In competition experiments, varying amounts of EDTA were added to a solution of 0.25 mM **1** or L-DOPA and 0.125 mM $FeCl_3$ or $CuSO_4$ in phosphate buffer (pH 7.4) under an argon atmosphere.

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References

1. Napolitano, A.; Pezzella, A.; Misuraca, G.; Prota, G. *Exp. Opin. Ther. Patents* **1998**, *8*, 1249.
2. Dunnett, S. B.; Björklund, A. *Nature* **1999**, *399*, A32.
3. Yahr, M. D. *Neurology* **1993**, *60*, 11.
4. Cedarbaum, J. M.; Scheilfer, L. S. In *Pharmacological Basis of Therapeutics*, 18th ed.; Godman L. S., Gilmann A. (Eds.); Macmillan: New York, 1990; Vol. 29, p 537.
5. Fahn, S. In *The Scientific Basis for the Treatment of Parkinson's Disease*. Olanow C. W., Lieberman A. N., (Eds.), Parthenon: Carnforth, UK, 1992; Vol. 89, p 111.
6. Brossi, A.; Focella, A.; Teitel, S. *Helv. Chim. Acta* **1972**, *55*, 15.
7. Daxenbichler, M. E.; Kleiman, R.; Weisleder, D.; VanEtten, C. H.; Carlson, K. D. *Tetrahedron Lett.* **1972**, *18*, 1801.
8. Sandler, M.; Bonham Carter, S.; Hunter, K. R.; Stern, G. M. *Nature*, **1973**, 439.
9. Finch, C. E.; Cohen, D. M. *Exp. Neurol.* **1997**, *143*, 82.
10. Mlakar, A.; Batna, A.; Dudda, A.; Spiteller, G. *Free Rad. Res.* **1996**, *25*, 525.
11. Zhang, J.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9382.
12. McDonald, L. J.; Moss, J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6238.
13. Marin, P.; Maus, M.; Bockaert, J.; Glowinsky, J.; Premont, J. *Biochem. J.* **1995**, *309*, 891.
14. Dexter, D. T.; Carayon, A.; Javoy-Agid, F.; Agid, Y.; Wells, F. R.; Daniel, S. E.; Lees, A. J.; Jenner, P.; Marsden, C. D. *Brain* **1991**, *114*, 1953.
15. Riederer, P.; Sofic, E.; Rausch, W. D.; Schmidt, B.; Reynolds, G. P.; Jellinger, K.; Youdim, M. B. H. *J. Neurochem.* **1989**, *52*, 515.
16. Ibañez, A. F.; Yaculiano, G. B.; Moltrasio Iglesias, G. Y. *J. Heterocyclic Chem.* **1989**, *26*, 907.
17. Ungemach, F.; Soerens, D.; Weber, R.; DiPierro, M.; Campos, O.; Mokry, P.; Cook, J. M.; Silverton, J. V. *J. Am. Chem. Soc.* **1980**, *102*, 6976.
18. Whitesell, J. K.; Minton, M. A. In *Stereochemical Analysis of Alicyclic Compounds by ^{13}C NMR Spectroscopy*; Chapman and Hall: London/New York, 1987.
19. Brossi, A.; Focella, A.; Teitel, S. *Helv. Chim. Acta* **1972**, *55*, 15.
20. Manini, P.; d'Ischia, M.; Lanzetta, R.; Parrilli, M.; Prota, G. *Bioorg. Med. Chem.* **1999**, *7*, 2525.
21. Eliel, E. L., Wilen, S. H., Mander, L. N. In *Stereochemistry of Organic Compounds*; John Wiley and Sons: New York, 1995, p 115.
22. Collins, P. M., Ferrier, R. J. In *Monosaccharides, their Chemistry and their Roles in Natural Products*; John Wiley and Sons: New York, 1995, p 115.
23. Gerard, C.; Chehhal, H.; Hugel, R. P. *Polyhedron* **1994**, *13*, 591.
24. Walaas, E.; Walaas, O.; Haavaldsen, S. *Arch. Biochem. Biophys.* **1963**, *100*, 97.
25. Kwik, W. L.; Purdy, E.; Stiefel, E. I. *J. Am. Chem. Soc.* **1974**, *96*, 1638.
26. Monteiro, H. P.; Winterbourn, C. C. *Biochem. Pharmacol.* **1989**, *38*, 4177.
27. Naoi, M.; Maruyama, W.; Niwa, T.; Nagatsu, T. *J. Neural Transm.* **1994**, *41*, 197.