An Unusual Decarboxylative Maillard Reaction between L-DOPA and D-Glucose under Biomimetic Conditions: Factors Governing Competition with Pictet−Spengler Condensation

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Received January 22, 2001

In 0.1 M phosphate buffer at pH 7.4 and 37 °C, the tyrosine metabolite L-3,4-dihydroxyphenylalanine (L-DOPA) reacts smoothly with D-glucose to afford, besides diastereoisomeric tetrahydroisoquinolines **1** and **2** by Pictet−Spengler condensation, a main product shown to be the unexpected decarboxylated Amadori compound *N*-(1-deoxy-D-fructos-1-yl)-dopamine (**3**). Under similar conditions, dopamine gave only tetrahydroisoquinoline products **4** and **5**, whereas L-tyrosine gave exclusively the typical Amadori compound **6**. Fe^{3+} and Cu^{2+} ions, which accumulate in relatively high levels in parkinsonian substantia nigra, both inhibited the formation of **3**. Cu^{2+} ions also inhibited the formation of **1** and **2** to a similar degree, whereas Fe^{3+} ions increased the yields of **1** and **2**. Apparently, the formation of **3** would not be compatible with a simple decarboxylation of the initial Schiff base adduct, but would rather involve the decarboxylative decomposition of a putative oxazolidine-5-one intermediate assisted by the catechol ring. These results report the first decarboxylative Maillard reaction between an amino acid and a carbohydrate under biomimetic conditions and highlight the critical role of transition metal ions in the competition with Pictet−Spengler condensation.

Introduction

L-3,4-Dihydroxyphenylalanine (L-DOPA), the primary product of tyrosine metabolism in neural crest-derived cells and the main percursor of catecholamine neurotransmitters, has gained widespread credit over the past decades as being the mainstay for the pharmacological treatment of Parkinson's disease,[1] a disabling neurodegenerative disorder characterized by selective loss of pigmented dopaminergic neurons of the substantia nigra. However, despite having initially beneficial effects, in the long term this drug causes severe, adverse (untoward) effects, including involuntary movements, on−off fluctuation of effectiveness, and dyskinesias at peak doses, possibly as a result of the hastened degeneration of nigrostriatal neurons.[2] Although several hypotheses have been put forward, the chemical mechanisms underlying these side effects have remained elusive; consequently, progress in the development of an effective therapeutic regimen based on L-DOPA has been considerably slowed.

A possible contributory mechanism in L-DOPA-induced side effects involves Pictet−Spengler condensation with aldehyde metabolites leading to tetrahydroisoquinoline derivatives and oxidation products thereof. Tetrahydroisoquinolines are generally regarded as neurotoxic compounds and are putatively involved in a variety of pathological conditions of the central nervous system, including alcoholism,[3] phenylketonuria,[4] and neurodegenerative disorders such as Parkinson's disease.[5] In this framework, a plausible class of target compounds for L-DOPA is provided by carbohydrate-related metabolites, which play a very prominent role in neuron energetics and functioning. Glucose, the brain's source of energy, and other hexoses are transported across the blood brain barrier and may react with L-DOPA, usually administered at relatively high doses, and other catecholamines to give tetrahydroisoquinolines and other potentially neurotoxic species that could exacerbate neuronal damage in the long term. Along this line of thought, we recently demonstrated that L-DOPA[6] as well as the catecholamine neurotransmitter dopamine[7] reacts under physiologically relevant conditions with D-glyceraldehyde, the simplest member of the carbohydrate family, to give stereoisomeric tetrahydroisoquinolines. Transition metal cations of wide occurrence in biological systems, including the parkinsonian substantia nigra,[8] were found to cause dychotomous effects on the reaction course: whereas Fe^{3+} accelerated tetrahydroisoquinoline formation, Cu^{2+} caused an opposite and rate-decreasing effect. Overall, the results were consistent with a mechanistic picture governed by Schiff base formation as the key event that is

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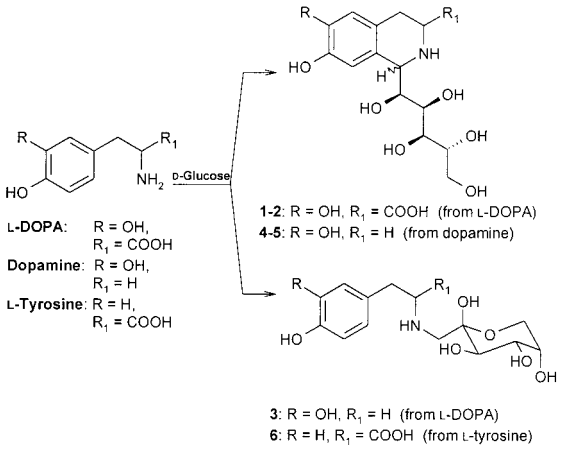
10.1021/jo010078d CCC: $20.00
Published on Web 06/23/2001

Scheme 1. Main Products Obtained by Reaction of L-DOPA and L-Tyrosine with D-Glucose



followed by a finely modulated ring-closure reaction in accordance with the Felkin−Anh model of asymmetric induction.[9]

As an extension of that study, we have now investigated the reaction of L-DOPA and dopamine with D-glucose under physiologically relevant conditions to specifically elucidate the nature of competing pathways, the possible role of transition metal cations, and the chemical viability of nonenzymatic glycation processes in relation to the motor problems attending long-term L-DOPA therapy.

Results and Discussion

Reaction of L-DOPA with D-Glucose. In 0.1 M phosphate buffer at pH 7.4 and 37 °C under an argon atmosphere, L-DOPA (50 mM) reacted smoothly with D-glucose (1.5 M) to give eventually three main products, two of which were readily identified as (1*R*,1′*S*, 3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**1**) and (1*S*,1′*S*, 3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**2**) (25% and 15% yields, respectively), differing exclusively in the configuration of the stereogenic center at C-1. Detailed inspection of the NMR spectra permitted the complete assignment of all proton and carbon resonances for the products. The configuration at C-1 was assigned on the basis of the following arguments: (a) In the ^{13}C NMR spectra of 1,3-disubstituted tetrahydroisoquinolines[10] and β-carbolines,[11] both C-1 and C-3 carbons are shifted upfield in the *trans* isomer due to a compression effect for 1,4-gauche interactions[12] that are precluded in the *cis* isomer. (b) The H-1 proton is usually upfield in the *cis* isomer.[6,7,13] The preferential formation of the 1*R* isomer (the *cis* form) is predicted by the Felkin−Anh model of asymmetric induction[9] and is consistent with previous observations in the case of the reaction of L-DOPA with D-glyceraldehyde.[6]

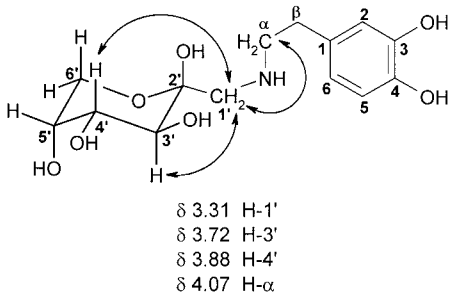


Figure 1. ROESY correlations for compound **3**.

Besides **1** and **2**, HPLC analysis of the reaction mixture displayed another major condensation product (about 30% yield) that was subjected to extensive spectral analysis and was shown to be the Amadori compound *N*-(1-deoxy-D-fructos-1-yl)-dopamine (**3**) (Scheme 1). Formation of **3** is remarkable since it represents, to the best of our knowledge, the first example of a decarboxylative Amadori rearrangement involving D-glucose and an amino acid. Indeed, the Amadori reaction, the initial step in Maillard type condensations of amino acids[14] and proteins[15] with carbohydrates, involves two sequential tautomerization steps in converting an aldimine (the Schiff base) into a ketoamine, which proceeds normally without concomitant decarboxylation.

Diagnostic features in support of the proposed decarboxylated structure were the lack of the carboxyl carbon resonance and the presence of four CH_2 carbons versus only three aliphatic CH signals. The noticeable finding was that the product appeared to be in a single form, as β-fructopyranose, at variance with the mixtures of forms in equilibrium that are commonly reported for Amadori compounds derived from D-glucose and amino acids.[16] This fact confirms the suggested participation of the free carboxy group in ring-opening and ring-closing reactions of Amadori compounds.

The fructopyranose structure **3** was inferred from the chemical shift of the anomeric carbon, resonating at a δ of 97.0 (fructofuranose anomeric carbons usually appear at a δ of ca. 103).[17] The β configuration of the anomeric carbon was deduced from a ROESY experiment indicating a close spatial proximity between the CH_2NH protons of fructose and the axial protons on C-3′ and C-4′ (Figure 1). Finally, the values of the coupling constants between H-3′ and H-4′ protons and between H-4′ and H-5′ protons indicated a $^2C_5$ conformation of the pyranose ring.[14a]

Kinetic analysis indicated that the product ratios do not change during the course of the reaction and remain unaffected at higher temperatures, e.g., 80 °C. The reaction showed pH-dependent kinetics, increasing in alkaline medium (Figure 2), without significant changes in product distribution.

Reactions of Dopamine and L-Tyrosine with D-Glucose. From the complex of these results, it appears that L-DOPA can react with D-glucose via two main competing pathways, viz., a Pictet−Spengler condensation and a Maillard process leading in the initial steps to a decarboxylated Amadori compound. Because of the

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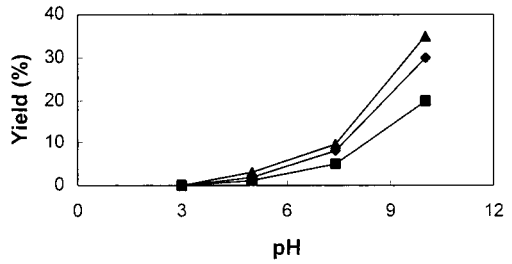

Figure 2. pH dependence of product yields of the reaction of L-DOPA with D-glucose. Data are averages of at least three determinations. Standard deviation did not exceed ±5% of mean values. L-DOPA = 10 mM; D-glucose = 100 mM. Product yields determined at 3 days: (◆) 1, (■) 2, (▲) 3.

cogent biological implications, a more detailed understanding of the structural and experimental factors governing this competition was desirable. This was pursued through a detailed analysis of the reactivity of two biologically relevant compounds related to L-DOPA, namely, the catecholamine dopamine, lacking the carboxyl group, and the amino acid L-tyrosine, lacking the hydroxyl group on the 3-position of the aromatic ring,[18] which is critical to activate the 6-position toward Pictet–Spengler type cyclizations.

Under conditions comparable to those of the L-DOPA–D-glucose reaction, dopamine reacted at a rate similar to that of L-DOPA to give the diastereoisomeric tetrahydroisoquinolines **4** and **5** in an approximate ratio of 4:1 (Scheme 1). These products were isolated by preparative HPLC and identified by comparing the ^{1}H NMR spectra with that reported in the literature.[19]

Notably, no detectable formation of products from Maillard reactions was observed.

A quite different behavior was displayed by L-tyrosine under the same experimental conditions. This amino acid reacted with D-glucose more slowly than did L-DOPA (initial rate ratio of $v_{DOPA}/v_{Tyr} = 8.5$) to give a single major product that proved to be the typical Amadori compound **6**, in which the carboxyl group of the amino acid was retained (Scheme 1).[20] The structure was assigned on the basis of ^{1}H NMR and ^{13}C NMR. No trace of Pictet–Spengler products could be detected in the L-tyrosine–D-glucose reaction.

Effects of Transition Metal Cations on the Reaction of L-DOPA with D-Glucose. In further experiments, the possible effect of transition metal cations of biological interest (e.g., Fe^{3+} and Cu^{2+}) on the reactions of L-DOPA with D-glucose was investigated. Fe^{3+} ions markedly accelerated tetrahydroisoquinoline formation at the expenses of the Maillard process, which was significantly inhibited. Conversely, Cu^{2+} ions decreased the kinetics of both processes without affecting their relative extents. Other metal cations, e.g., Zn^{2+} and Mn^{2+}, did not affect the kinetic and chemical course of the reaction. Both Fe^{3+} and Cu^{2+} ions also accelerated tetrahydroisoquinoline formation from dopamine and D-glucose; this observation supported the general character of the metal-dependent effects and ruled out any role of the polyol side chain of glucose in the metal-assisted Pictet–Spengler reaction.

Mechanistic Issues. L-DOPA, dopamine, and L-tyrosine displayed quite different reactivities with D-glucose that could not be rationalized in terms of the currently accepted mechanism. In particular, the following points seem worthy of attention:

L-DOPA behaves differently from all of the other amino acids investigated so far in that it suffers an exclusively decarboxylative Maillard condensation with D-glucose.

L-Tyrosine does not give rise to Pictet–Spengler products, whereas dopamine does not produce Maillard adducts. These findings imply that the OH group on the 3-position of the aromatic ring is essential for cyclization to tetrahydroisoquinoline products, whereas the carboxyl group is critical for Maillard reaction.

The most plausible explanation would invoke a critical effect of the OH group on the 3-position of L-DOPA, which would not only activate the 6-position toward Schiff base cyclization to give tetrahydroisoquinolines but somehow also induce decarboxylation and assist the critical Amadori rearrangement that drives Maillard condensation. Formation of tetrahydroisoquinoline products is clearly indicative of a Pictet–Spengler reaction involving a transient Schiff base, which might well be an intermediate in the Maillard process, as is commonly reported.[14b,15] The Schiff base per se is not prone to decarboxylation and, hence, is unlikely to be the ultimate precursor of the Maillard product. An attractive hypothesis is that decarboxylation occurs at the expense of an oxazolidine-5-one intermediate formed by intramolecular cyclization of the Schiff base. This intermediate would thus afford an azomethine ylide that would rapidly establish tautomerization and protonation equilibria to yield the decarboxylated Amadori product (Scheme 2).

Evolution of the putative azomethine ylide must be accompanied by a concomitant proton shift to yield the enol intermediate in the Amadori rearrangement. This azomethine ylide route is clearly precluded from dopamine in which the Schiff base would be more prone to cyclization to Pictet–Spengler products than to the Maillard process.

Whereas reversible formation of the putative oxazolidine-5-one can be envisaged as a common route to all Schiff bases,[21] its facile decomposition, even at room temperature, is unique to the L-DOPA reaction, and seems to be the critical step affected by the catechol ring. The mechanism by which the catechol ring would facilitate decarboxylation from the putative oxazolidine-5-one system is intriguing. Theoretical analysis by semiempirical methods (AM1, PM3) identifies the 6- and 2-positions as the most reactive in terms of HOMO coefficients and total charge density, respectively.[22] It is therefore tempting to speculate that the electron rich catechol ring can be engaged in intramolecular interactions with the oxazolidine-5-one system and lower, in some way, the barrier for decarboxylation by stabilizing the developing positive charges, e.g., on the acyl carbon during fission of the C–C bond or on the nitrogen center via interaction with the NH proton. Alternately, perhaps the role of the OH group on the 3-position of L-DOPA is to enable the

(18) For the sake of simplicity, we adopted the common system for nomenclature of catecholamines, which assigns positions 3 and 4 to the aromatic carbons bearing the hydroxyl groups.

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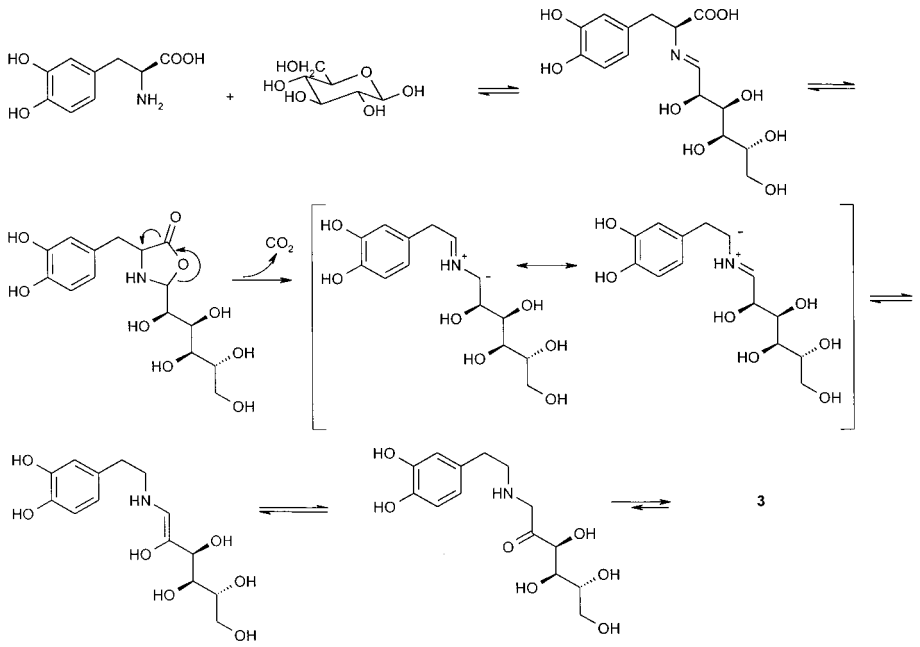
Scheme 2. Proposed Mechanism for the Formation of 3 by Reaction of L-DOPA with D-Glucose



formation of a ketotautomer that can assist as a general acid in protonating the azomethine ylide as it is formed.[23]

To gain some insight into the mechanism of the Amadori reaction, L-DOPA was incubated with D-glucose under the same conditions as described above using, however, phosphate-buffered D_2O as the medium. The reaction proceeded at a significantly slower rate (initial rate ratio of $v_H/v_D = 3.1$) to give a similar product mixture from which a compound that was chromatographically indistinguishable from **3** was isolated and subjected to spectral analysis. The ^{1}H NMR spectrum (Figure 3A) revealed complete deuteration of the methylene group of the sugar moiety linking the secondary amino functionality and replacement of only one of the CH_2 protons flanking the nitrogen on the opposite side. This result, which is supported by the ^{13}C NMR spectrum (Figure 3B), in which the carbon signals for the relevant methylene groups are evidently affected by deuteration, is not in contrast with the postulated pathway. In particular, monodeuteration on the former carboxyl-bearing carbon is compatible with conventional decarboxylation mechanisms, including that from the oxazolidine-5-one. Complete deuteration of the alternate $NHCH_2$ group is likely to be indicative of rapid tautomeric equilibria involving the ketoamine functionality in the Amadori compound.

In this frame, the kinetic isotope effect observed in the presence of deuterium is likely to be due to a decrease in the rate of Schiff base formation since both of the competing Pictet−Spengler and Amadori processes are affected in accordance with the proposed scheme.

Experimental Section

General Methods. ^{1}H NMR spectra were obtained at 400 and 200 MHz with *tert*-butyl alcohol (δ 1.23) as the internal standard. ^{13}C NMR spectra were run at 50 MHz. ^{1}H−^{1}H COSY and ROESY experiments were performed at 400 MHz. Mass spectra were recorded using the fast atom bombardment (FAB) technique with glycerol as the matrix. Analytical and preparative HPLC were carried out on C18 columns (4.6 × 250 mm and 22 × 250 mm, respectively) with UV detection at 280 nm for L-DOPA and dopamine reactions and at 260 nm for L-tyrosine reactions. The flow rate was maintained at 1 mL/min for analytical runs and at 15 mL/min for preparative chromatography.

L-DOPA, dopamine, and D-glucose were purchased from Aldrich, while L-tyrosine was purchased from Sigma. Metal ion solutions were prepared from $Fe(NH_4)(SO_4)_2$ and $CuSO_4 \cdot 7H_2O$.

Reactions of L-DOPA and Dopamine with D-Glucose. Appropriate amounts of L-DOPA or dopamine and D-glucose were incubated in 0.1 M phosphate buffer, pH 7.4, kept at 37 °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, acidified to pH 3, and injected into the HPLC system. All kinetic experiments were run at least in triplicate. The eluant used for L-DOPA−glucose reactions was 5 mM octane-1-sulfonic acid in 0.1 M H_3PO_4 (pH 3)/acetonitrile 97:3 v/v (eluant a), while the eluant used for the dopamine−glucose reactions was 5 mM octane-1-sulfonic acid in 0.1 M H_3PO_4 (pH 3)/acetonitrile 95:5 v/v (eluant b).

(1*R*,1′*S*,3*S*)-1-(D-*gluco*-Pentitol-1′-yl)-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1), (1*S*,1′*S*,3*S*)-1-(D-*gluco*-Pentitol-1′-yl)-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (2), and *N*-(1-Deoxy-D-fructos-1-yl)-dopamine (3). A solution of L-DOPA (100 mg, 0.5 mmol) in 0.1 M phosphate buffer, pH 7.4 (10 mL), previously purged with argon, was treated with D-glucose (1.4 g, 15 mmol) at 80 °C. After 4 h, HPLC analysis (eluant a) revealed three main products along with small amounts of the starting material. The mixture was acidified to pH 2 with 3 M HCl and subjected to ion-exchange chromatography (Dowex 50W-X4 (H^+), 2 × 60 cm) using water (250 mL), 0.1 HCl (250 mL), 0.5 M HCl (500 mL), and 1 M HCl (500 mL) as the eluants. Fractions eluted with 0.5 and 1 M HCl were collected, evaporated to dryness under reduced pressure, and subjected to preparative HPLC (eluant, 0.1 M HCOOH/acetonitrile 97:3 v/v) to afford **1** (25%), **2** (15%), and **3** (30%).

1: UV λ_{max} (log ϵ) (H_2O) 286 (2.79) nm; $[\alpha]^{21}_D$ +6.76 (*c* 0.5, 0.5 M HCl); ^{1}H NMR (D_2O) δ 3.03−3.21 (2H, m), 3.67−3.82 (1H × 4, m), 4.10−4.14 (1H × 2, m), 4.40 (1H, s), 4.75 (1H, s), 6.83 (1H, s), 6.91 (1H, s); ^{13}C NMR (D_2O) δ 28.7 (t), 55.9 (d), 60.5 (d), 63.4 (t), 71.0 (d), 72.0 (d), 72.5 (d), 73.4 (d), 114.6 (d), 117.1 (d), 121.7 (s), 126.0 (s), 144.3 (s), 145.0 (s), 173.4 (s); MS

(23) We acknowledge a suggestion by one of the referees.

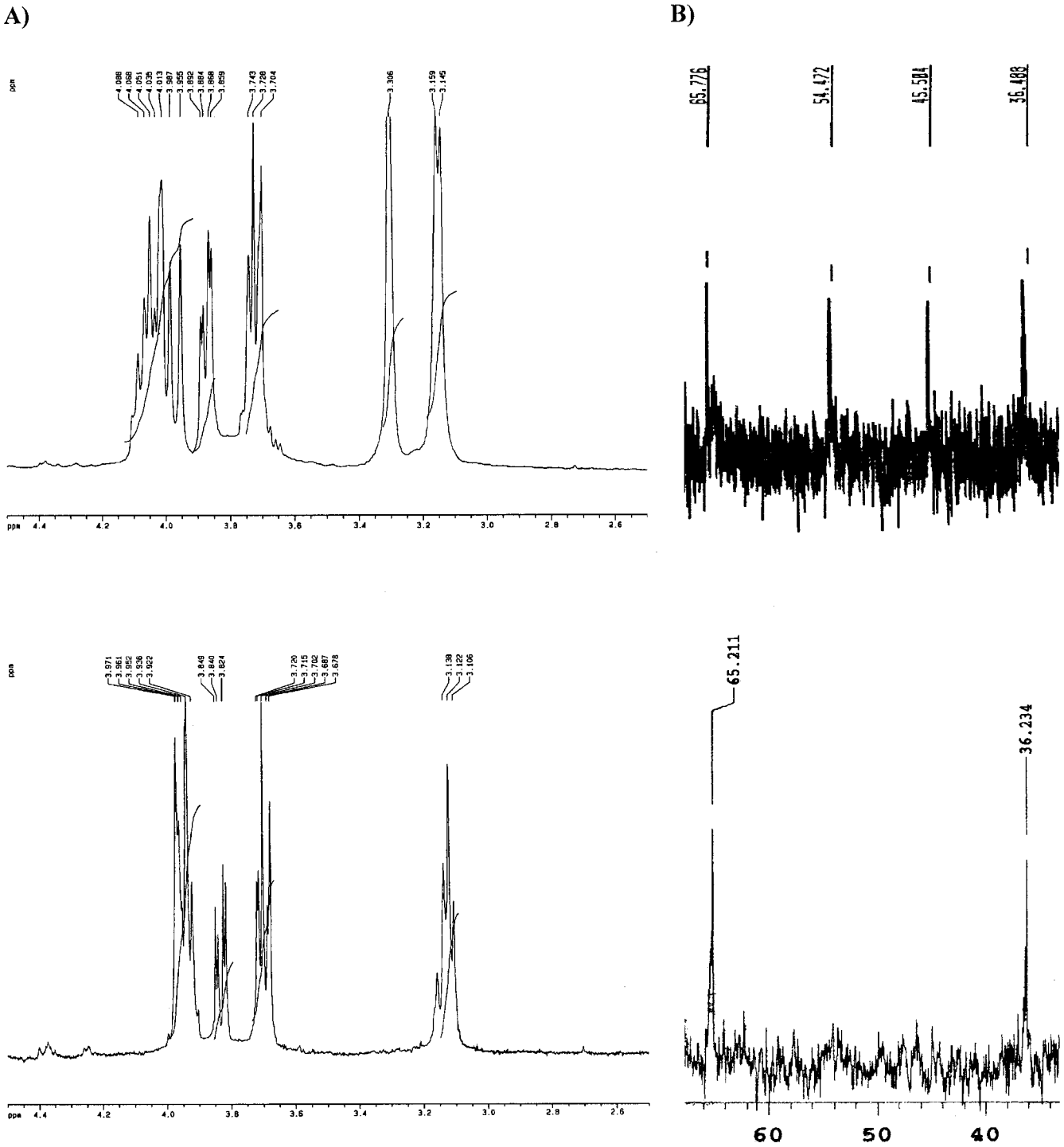


Figure 3. (A) ^{1}H NMR spectrum (400 MHz, high-field region) of **3** obtained in phosphate-buffered H_2O (upper trace) and in phosphate-buffered D_2O (lower trace). (B) ^{13}C NMR spectrum (50 MHz, high-field region) of **3** obtained in phosphate-buffered H_2O (upper trace) and in phosphate-buffered D_2O (lower trace).

(FAB) *m*/*z* 360; HRMS ($M^+ + 1$) calcd for $C_{15}H_{22}NO_9$ 360.1295, found 360.1297. Anal. Calcd for $C_{15}H_{21}NO_9$: C, 50.12; H, 5.89; N, 3.90. Found: C, 50.10; H, 5.85; N, 3.88.

2: UV λ_{max} (log ϵ) (H_2O) 286 (2.76) nm; $[\alpha]^{21}_D$ −4.33 (*c* 0.7, 0.5 M HCl); ^{1}H NMR (D_2O) δ 3.17 (1H, dd, J = 17.0, 4.5 Hz), 3.31 (1H, dd, J = 17.0, 5.2 Hz), 3.57−3.80 (1H × 5, m), 4.39 (1H, m), 4.67 (1H, t, J = 5.2 Hz), 4.99 (1H, d, J = 4.6 Hz), 6.77 (1H, s), 6.85 (1H, s); ^{13}C NMR (D_2O) δ 28.9 (t), 54.6 (d), 58.7 (d), 63.6 (t), 70.3 (d), 72.0 (d), 72.9 (d), 73.2 (d), 115.0 (d), 117.7 (d), 120.8 (s), 124.6 (s), 144.8 (s), 145.5 (s), 173.1 (s); MS (FAB) *m*/*z* 360; HRMS ($M^+ + 1$) calcd for $C_{15}H_{22}NO_9$ 360.1295, found 360.1296. Anal. Calcd for $C_{15}H_{21}NO_9$: C, 50.12; H, 5.89; N, 3.90. Found: C, 50.11; H, 5.87; N, 3.87.

3: UV λ_{max} (log ϵ) (H_2O) 281 (2.64) nm; $[\alpha]^{21}_D$ −2.77 (*c* 0.9, 0.5 M HCl); ^{1}H NMR (D_2O) δ 3.15 (2H, m), 3.31 (2H, s), 3.72 (1H, d, J = 9.6 Hz), 3.73 (1H, d, J = 12 Hz), 3.88 (1H, dd, J = 9.6, 3.4 Hz), 3.97 (1H, d, J = 12 Hz), 4.01 (1H, d, J = 3.4 Hz), 4.07 (2H, m), 6.73 (1H, dd, J = 8, 2.5 Hz), 6.85 (1H, d, J = 2.5 Hz), 6.92 (1H, d, J = 8 Hz); ^{13}C NMR (D_2O) δ 36.5 (t), 45.5 (t), 54.5 (t), 65.8 (t), 70.7 (d), 71.0 (d), 71.9 (d), 97.0 (s), 118.5 (d), 119.0 (d), 123.7 (d), 128.6 (s), 145.2 (s), 145.9 (s); MS (FAB) *m*/*z* 316; HRMS ($M^+ + 1$) calcd for $C_{14}H_{22}NO_7$ 316.1396, found 316.1394. Anal. Calcd for $C_{14}H_{21}NO_7$: C, 53.31; H, 6.72; N, 4.44. Found: C, 53.29; H, 6.71; N, 4.42.

(1*R*,1′*S*)-1-(D-*gluco*-Pentitol-1′-yl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (4) and (1*S*,1′*S*)-1-(D-*gluco*-Pen-

titol-1′-yl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (5). A solution of dopamine (100 mg, 0.5 mmol) in 0.1 M phosphate buffer, pH 7.4 (10 mL), previously purged with argon, was treated with D-glucose (1.4 g, 15 mmol) at 80 °C. After 4 h, HPLC analysis (eluant b) revealed two main products along with small amounts of the starting material. The mixture was acidified to pH 2 with 3 M HCl and subjected to ion-exchange chromatography (Dowex 50W-X4 (H^+), 2 × 60 cm) using water (250 mL), 0.1 M HCl (250 mL), 0.5 M HCl (250 mL), 1 M HCl (500 mL), and 2 M HCl (500 mL) as the eluants. Fractions eluted with 1 and 2 M HCl were collected, evaporated to dryness under reduced pressure, and subjected to preparative HPLC (eluant, 0.1 M HCOOH/acetonitrile 95:5 v/v) to afford **4** (60%) and **5** (15%).

NMR and other spectral data were in accordance with literature.[19]

***N*-(1-Deoxy-D-fructos-1-yl)-L-tyrosine (6).** A solution of L-tyrosine (100 mg, 0.6 mmol) in 0.1 M phosphate buffer, pH 7.4 (6 mL), was treated with D-glucose (1.4 g, 15 mmol) at 100 °C. After 24 h, HPLC analysis (eluant, 0.1 M HCOOH/acetonitrile 98:2 v/v) revealed one main product along with large amounts of the starting material. The mixture was acidified to pH 2 with 3 M HCl and subjected to ion-exchange chromatography (Dowex 50W-X4 (H^+), 2 × 60 cm) using water (250 mL), 0.1 M HCl (250 mL), 0.5 M HCl (500 mL), and 1 M HCl (500 mL) as the eluants. Fractions eluted with 0.5 and 1 M HCl were collected, evaporated to dryness under reduced pressure, and subjected to preparative HPLC (eluant, 0.1 M HCOOH/acetonitrile 97:3 v/v) to afford **6** (15%).

6: ^{1}H NMR (D_2O) δ 3.31 (2H, m), 3.43 (2H, s), 3.75 (1H, d, J = 10 Hz), 3.76 (1H, d, J = 12 Hz), 3.92 (1H, dd, J = 10, 3.4 Hz), 4.03 (1H, d, J = 3.4 Hz), 4.04 (1H, d, J = 12 Hz), 4.34 (1H, m), 6.92 (1H × 2, dd, J = 8.4, 2.7 Hz), 7.23 (1H × 2, dd, J = 8.4, 3 Hz); ^{13}C NMR (DEPT) (D_2O) δ 35.2 (t), 46.4 (d), 53.5 (t), 65.3 (t), 70.1 (d), 70.2 (d), 71.0 (d), 117.4 (d), 132.3 (d); MS (FAB) m/z 344; HRMS ($M^+ + 1$) calcd for $C_{15}H_{22}NO_8$ 344.1345, found 344.1341. Anal. Calcd for $C_{15}H_{21}NO_8$: C, 52.31; H, 6.44; N, 4.07. Found: C, 52.29; H, 6.41; N, 4.02.

Acknowledgment. This work was supported by grants from MURST (PRIN 1997) and CNR (Rome). We thank Miss Silvana Corsani for technical assistance, the Centro Interdipartimentale di Metodologie Chimico-Fisiche (CIMCF, University of Naples) for NMR spectra, and the Servizio di Spettrometria di Massa del CNR e dell'Università (Naples) for mass spectra.

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, and ROESY spectra of compounds **1**, **2**, **3**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010078D