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Reactions of D-glucose with phenolic amino acids: further insights into the competition between Maillard and Pictet–Spengler condensation pathways

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Abstract—The reactions of 5-*S*-cysteinyldopa (**1**), L-α-methyldopa (**2**) and DL-*m*-tyrosine (**3**) with D-glucose were investigated at 90 °C in phosphate buffer at pH ranging from 5.0 to 9.0. Whereas **1** gave mainly the double Maillard condensation product *N*,*N*′-bis(1″-deoxy-D-fructos-1″-yl)-5-*S*-cysteinyldopa, as an inseparable mixture of β-D-fructopyranosyl and α,β-D-fructofuranosyl derivatives, **2** and **3** gave both Maillard and Pictet–Spengler products, although to different extents and with different regio- and stereochemistry. A peculiar pattern of reactivity was displayed by **2** which gave, besides the Maillard product and the expected 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline C-1 diastereoisomeric pairs, the unprecedented 7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline derivative via the *ortho* cyclization pathway. Pictet–Spengler cyclization of **2** and **3** proceeded with Felkin–Anh-type asymmetric induction, favouring the 1*R* isomer throughout the pH range 5.0–9.0. These results, which highlight the first example of carbohydrate-derived 7,8-dihydroxytetrahydroisoquinoline, provide new insights into the factors governing competition between Maillard and Pictet–Spengler condensation pathways.

Keywords: Phenolic amino acid; D-Glucose; Pictet–Spengler reaction; Maillard reaction

1. Introduction

Reactions of carbohydrates with 3,4-dihydroxyphenylethylamine compounds, comprising catecholic amino acids such as L-DOPA and catecholamines, for example, dopamine, occur at neutral pH through the initial formation of Schiff base intermediates which may partition between two competitive pathways, the Pictet–Spengler phenolic condensation[1,2] and the Maillard reaction.[3] The former involves an intramolecular cyclization of the aldimine, which may proceed with *para* and *ortho* regiochemistry to give 6,7- and 7,8-dihydroxytetrahydroisoquinolines, respectively, as diastereoisomeric pairs at C-1. The *ortho* cyclization route is generally thwarted, probably because of steric factors, and the *para* isomer is usually the major, if not the sole, product. The Maillard reaction, on the other hand, is a complex process leading to the formation of *N*-(1-deoxy-D-fructos-1-yl)amine derivatives via the so-called Amadori rearrangement (Scheme 1).

Despite the importance of these reactions in the field of carbohydrate research, organic synthesis and biomedical chemistry, for example, in relation to non-enzymatic glycation processes in diabetes, aging and neurodegenerative disorders,[4–7] knowledge of the factors governing the competition between the Maillard and Pictet–Spengler pathways has remained poor. Previous studies have shown that L-DOPA reacts with D-glucose to give diastereoisomeric 6,7-dihydroxytetrahydroisoquinolines (1*R*:1*S* ratio of ca. 5:3), according to the Felkin–Anh model of asymmetric induction, and an unusual decarboxylated Amadori compound, whereas dopamine gives

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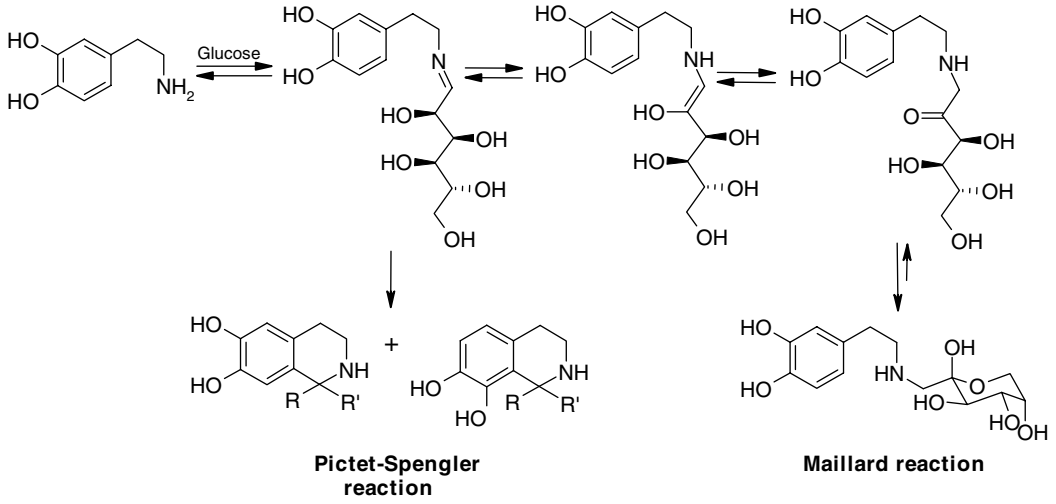
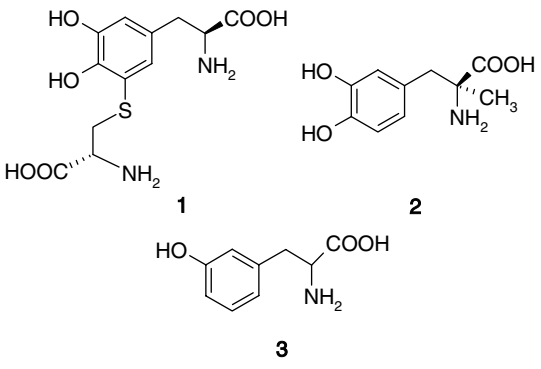


Scheme 1. Mechanistic pathways of the Pictet–Spengler and Maillard reactions.

6,7-dihydroxytetrahydroisoquinolines with a 1*R*:1*S* ratio of ca. 4:1 as expected, but no detectable 7,8-dihydroxytetrahydroisoquinolines or the Amadori compound were observed.[8] L-Tyrosine, on the other hand, gives exclusively the typical Amadori product,[8] due to the lack of the OH group at C-3 of the aromatic ring, which is an essential requisite for Pictet–Spengler-type phenolic cyclization.[9] Apart from these examples, the reactivity patterns of phenolic amino acids with D-glucose have remained virtually unexplored. The attractive mechanistic issues raised by this chemistry, and the opportunities offered for the preparation of new potentially bioactive 6,7-dihydroxytetrahydroisoquinoline derivatives,[8,10–13] prompted us to extend this reaction to other phenolic and catecholic amino acids, in an attempt to rationalize the influence of substituents on the competitive Maillard and Pictet–Spengler processes, as well as on the stereo- and regiochemical outcome of the cyclization pathway.

2. Results and discussion

The substrates investigated in the present study include 5-*S*-cysteinyldopa (**1**), L-α-methyldopa (**2**) and DL-*m*-tyrosine (**3**). These substrates were selected on the basis of their investigative value, commercial availability or biological and pharmacological interest. For example, **1** is a natural melanogen with antioxidant properties involved in the biosynthesis of pheomelanins, the characteristic pigments of red hair and feathers,[14–17] and **2** is a centrally acting drug with antihypertensive properties.[18] The reactions were typically carried out in phosphate buffer at pH 7.4 and at 90 °C.



2.1. Reaction of 5-*S*-cysteinyldopa (1) with D-glucose

When reacted with D-glucose in 0.1 M phosphate buffer, pH 7.4, **1** gave a rather complex mixture of products after 4 h. A major component of the mixture could eventually be obtained by repeated ion-exchange column chromatography on a Dowex 50W-X4(H^+) resin and was sub-

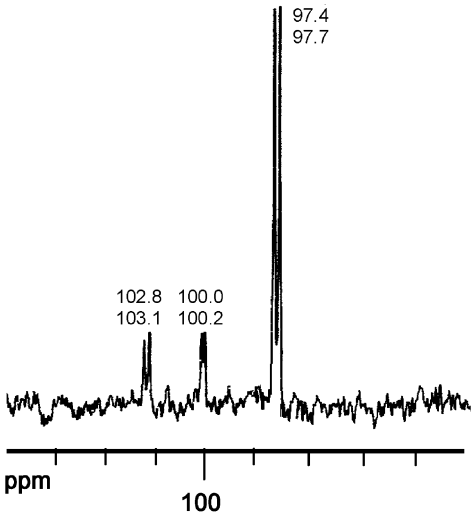
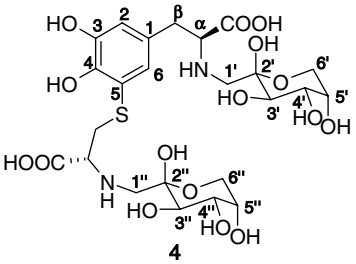


Figure 1. Aromatic carbons region of ^{13}C NMR spectrum (D_2O) of compound **4**.

jected to extensive spectroscopic characterization. The ^{1}H NMR spectrum displayed, besides two singlets at δ 6.89 and 7.00, a double pattern of signals between δ 3.0 and 4.5 reminiscent of those of a fructosamine moiety. In line with this suggestion, in the ^{13}C NMR spectrum the product displayed six signals in the anomeric carbon region appearing as three tight pairs (Fig. 1) one of which (δ 97.4/97.7) was relatively more intense and was suggestive of β-fructopyranosyl structures, whereas the others at δ 100.0/100.2 and 102.8/103.1 denoted β- and α-fructofuranosyl structures, respectively.[19,20] This suggested that the compound existed as a mixture of isomers differing in the fructosamine unit.

On the basis of 1D/2D NMR data summarized in Table 1, the product was identified as *N*,*N*′-bis(1″-deoxy-D-fructos-1″-yl)-5-*S*-cysteinyldopa (**4**), that is, the double Maillard condensation product. The structure was supported by (ESI$^+$)MS showing pseudo-molecular ion peaks $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ at *m*/*z* 641, 663 and 679, respectively, and a peak for a $[M-H_2O+H]^+$ ion at *m*/*z* 623.[19]



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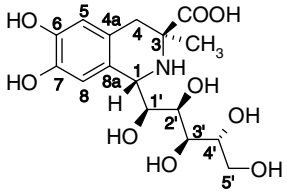
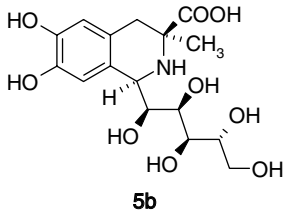
Unfortunately, attempts to isolate minor reaction products met with failure because of their small amounts and difficulties encountered with separation by ion-exchange chromatography and/or preparative HPLC. The lack of significant amounts of Pictet–Spengler products from **1** is presumably due to steric interactions between the bulky cysteinyl residue and the polyol side chain hindering intramolecular phenolic cyclization.

2.2. Reaction of L-α-methyldopa (2) with D-glucose

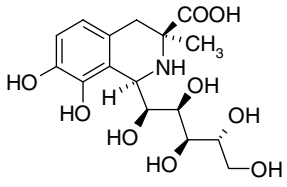
Reaction of **2** with D-glucose was carried out as above to give four major products. These could be isolated by ion-exchange chromatography followed by preparative HPLC and were identified as (1*S*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**5a**), (1*R*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**5b**), (1*R*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-3-methyl-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**6**) and *N*-(1′-deoxy-β-D-fructopyranos-1′-yl)-L-α-methyldopa (**7**).



5a



5b



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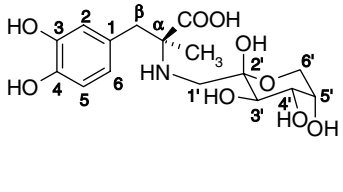

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Structural assignments for compounds **5a**,**b** and **6** were based on the (ESI$^+$)MS spectra, showing pseudo-molecular ion peaks $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ at *m*/*z* 374, 396 and 412, respectively, and on extensive 1D/2D NMR analysis (Table 2).

In particular, the configuration *S* at C-1 of compound **5a** and *R* at C-1 of compound **5b** was inferred on the basis of the ROESY spectra displaying for the latter a diagnostic cross peak indicating a contact between the H-1 proton at δ 4.64 and the methyl protons at δ 1.38. This reflected a spatial proximity of such protons as

Table 1. Spectroscopic data of compound **4** (D_2O)

Carbon	δ_C	δ_H (mult., *J* Hz)	Carbon	δ_C	δ_H (mult., *J* Hz)
1	129.5	—	2′	97.4	—
2	119.9	6.89 (s)	2″	97.7	—
3	147.9	—	3′	72.3	3.71 (d, 9.6)
4	147.3	—	3″	72.3	3.77 (d, 9.6)
5	119.9	—	4′	71.3	3.85 (dd, 9.6, 3.2)
6	128.0	7.00 (s)	4″	71.3	3.91 (dd, 9.6, 3.6)
α	63.6	4.10 (m)	5′	70.9	3.98 (m)
β	36.5	3.16 (m)	5″	70.9	4.02 (m)
CH_2S	36.1	3.30–3.42 (m)	6′	66.2	3.72 (d, 12) 3.96 (d, 12)
CH	61.3	3.87 (m)	6″	66.4	3.75 (d, 12) 4.02 (d, 12)
1′	54.5	3.30–3.42 (m)	α-COOH	172.5	—
1″	54.5	3.30–3.42 (m)	COOH	171.4	—

Table 2. Spectroscopic data of compounds **5a**,**b**, **6** and **7** (D_2O)

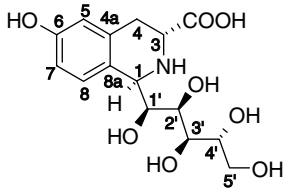
Carbon	5a		5b		6		Carbon	7	
	δ_C	δ_H (mult., J Hz)	δ_C	δ_H (mult., J Hz)	δ_C	δ_H (mult., J Hz)		δ_C	δ_H (mult., J Hz)
1	61.5	5.04 (s)	58.0	4.64 (d, 3.6)	55.7	4.95 (d, 4.0)	1	127.4	—
3	64.1	—	63.3	—	63.9	—	2	117.7	6.74 (d, 2.4)
4	37.1	2.95 (d, 16.4)	36.1	2.89 (d, 16.0)	36.2	2.91 (d, 16.0)	3	145.3	—
		3.16 (d, 16.4)		3.12 (d, 16.0)		3.05 (d, 16.0)			
4a	126.3	—	126.4	—	125.4	—	4	145.0	—
5	117.9	6.76 (s)	118.2	6.82 (s)	117.6	6.77 (d, 8.0)	5	119.0	6.67 (d, 8.0)
6	145.7	—	146.0	—	122.7	6.93 (d, 8.0)	6	123.8	6.86 (dd, 8.0, 2.4)
7	145.3	—	144.9	—	144.7	—	α	43.8	—
8	115.0	6.68 (s)	115.5	6.90 (s)	143.1	—	β	30.6	2.99 (d, 14.0)
									3.10 (d, 14.0)
8a	121.3	—	122.1	—	118.3	—	1′	51.7	3.26 (s)
1′	73.7	4.36 (s)	71.2	4.35 (s)	74.0	4.28 (s)	2′	96.3	—
2′	71.0	3.73 (s)	73.4	4.03 (s)	71.9	3.95 (s)	3′	70.2	3.73 (d, 9.6)
3′	74.4	3.62 (m)	72.4	3.7–3.8 (m)	72.2	3.7–3.8 (m)	4′	72.1	3.83 (dd, 9.6, 2.8)
4′	72.4	3.62 (m)	73.3	3.7–3.8 (m)	72.5	3.7–3.8 (m)	5′	70.7	3.97 (s)
5′	63.8	3.62 (m)	63.8	3.7–3.8 (m)	63.7	3.7–3.8 (m)	6′	65.1	3.71 (d, 13.2)
		3.55 (m)		3.63 (dd, 11.2, 4.8)		3.5–3.6 (m)			3.98 (d, 13.2)
COOH	176.8	—	178.4	—	172.4	—	COOH	175.6	—
CH_3	25.4	1.65 (s)	21.4	1.38 (s)	20.5	1.28 (s)	CH_3	19.9	1.49 (s)

evidenced by the geometry-minimized (MM+) structure of **5b** (not shown).

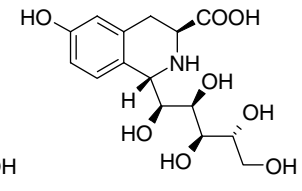
Additional arguments supporting stereochemical assignments at C-1 for **5a**,**b** came from comparative scrutiny of the carbon resonances, revealing an upfield shift of the C-1 and C-3 carbon signals ascribable to a γ-gauche effect typical of the 1*R* diastereoisomers in the case of **5b**.[21–23] The pattern of substitution of tetrahydroisoquinoline **6** at C-7 and C-8 was readily apparent from a couple of doublets at δ 6.77 and 6.93, denoting *ortho*-coupled aromatic protons ($J = 8.0$ Hz), whereas the *R* configuration at C-1 was deduced from the cross peak in the ROESY spectrum correlating the H-1 resonance at δ 4.95 with the methyl protons signal at δ 1.28. To the best of our knowledge, the *ortho* cyclization pathway is unprecedented in the panorama of Pictet–Spengler phenolic condensations with carbohydrates. The β-fructopyranosyl moiety in **7** was inferred from the chemical shift of the anomeric carbon at δ 96.3 (Table 2). Moreover, the coupling constant between the H-3′ and the H-4′ proton signals ($J = 9.6$ Hz) suggested a *trans* diaxial relationship and, hence, a $^2C_5$ conformation of the β-pyranose ring.[19] Structural assignments for compound **7** was also confirmed by the (ESI$^+$)MS spectrum, showing pseudo-molecular ion peaks $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ at *m*/*z* 374, 396 and 412, respectively, and a peak for a $[M-H_2O+H]^+$ ion at *m*/*z* 356.

2.3. Reaction of DL-*m*-tyrosine with D-glucose

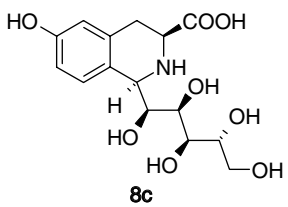
DL-*m*-Tyrosine (**3**) reacted with D-glucose in 0.1 M phosphate buffer, pH 7.4, at 90 °C to give after 3 h four main products, which could be isolated by ion-exchange chromatography followed by preparative HPLC. By this method, two products could be obtained in pure form whereas the other two resisted all attempts at separation and were characterized as an intimate mixture. Spectral analysis of these latter (Table 3) indicated two isomers in a ratio of ca. 3:1, which were formulated as (1*R*,1′*S*,3*R*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (**8a**), the major isomer, and (1*S*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (**8b**). Of the other two products, one displayed spectral features similar to those of **8a** and **8b**, and was identified as (1*R*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (**8c**) (Table 3). The 1*S*,3*R* isomer was not formed in appreciable amounts.



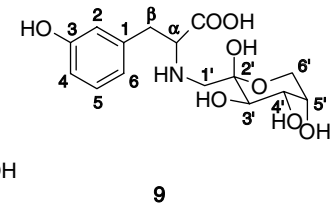
8a



8b



8c



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All products displayed the expected pseudomolecular ion peaks in the (ESI$^+$)MS spectra at *m*/*z* 344 ($[M+H]^+$), 366 ($[M+Na]^+$) and 382 ($[M+K]^+$). The configuration at C-1 for compounds **8a**–**c** was deduced from extensive 2D NMR analysis and by comparison of the

Table 3. Spectroscopic data of compounds **8a–c** and **9** (D_2O)

Carbon	8a		8b		8c		Carbon	9	
	δ_C	δ_H (mult., J Hz)	δ_C	δ_H (mult., J Hz)	δ_C	δ_H (mult., J Hz)		δ_C	δ_H (mult., J Hz)
1	58.3	4.85 (d, 5.2)	58.5	5.02 (d, 5.2)	60.2	4.75 (s)	1	137.5	—
3	55.4	4.6–4.8 (m)	55.2	4.6–4.8 (m)	57.1	3.92 (m)	2	117.5	6.8–6.9 (m)
4	30.2	3.1–3.3 (m)	30.3	3.1–3.3 (m)	30.7	3.08 (d, 16.0)	3	157.3	—
		3.4–3.5 (m)		3.4–3.5 (m)		3.25 (d, 16.0)			
4a	135.4	—	134.7	—	136.7	—	4	116.2	6.8–6.9 (m)
5	116.6	6.81 (s)	116.8	6.8–6.9 (m)	116.7	6.84 (s)	5	131.9	7.27 (t, 8.0)
6	157.0	—	156.5	—	157.0	—	6	122.8	6.8–6.9 (m)
7	116.2	6.87 (d, 8.4)	116.1	6.8–6.9 (m)	116.3	6.87 (d, 8.0)	α	53.8	4.12 (m)
8	130.0	7.23 (d, 8.4)	130.0	7.15 (d, 8.4)	129.4	7.29 (d, 8.0)	β	36.6	3.23 (d, 5.6)
8a	122.0	—	121.4	—	122.6	—	1′	54.2	3.31 (s)
1′	74.1	4.27 (m)	70.9	4.40 (m)	72.9	4.41 (s)	2′	96.5	—
2′	70.9	4.11 (s)	72.6	3.5–3.9 (m)	71.6	4.05 (s)	3′	70.2	3.71 (d, 9.6)
3′	74.1	3.5–3.9 (m)	72.9	3.5–3.9 (m)	73.7	3.7–3.8 (m)	4′	71.1	3.84 (dd, 9.6, 2.8)
4′	72.3	3.5–3.9 (m)	74.1	3.5–3.9 (m)	72.3	3.7–3.8 (m)	5′	70.6	3.9–4.1 (m)
5′	63.7	3.5–3.9 (m)	63.8	3.5–3.9 (m)	63.8	3.7–3.8 (m)	6′	65.2	3.71 (d, 13.2)
						3.64 (m)			3.9–4.1 (m)
COOH	176.3	—	176.4	—	174.9	—	COOH	172.8	—

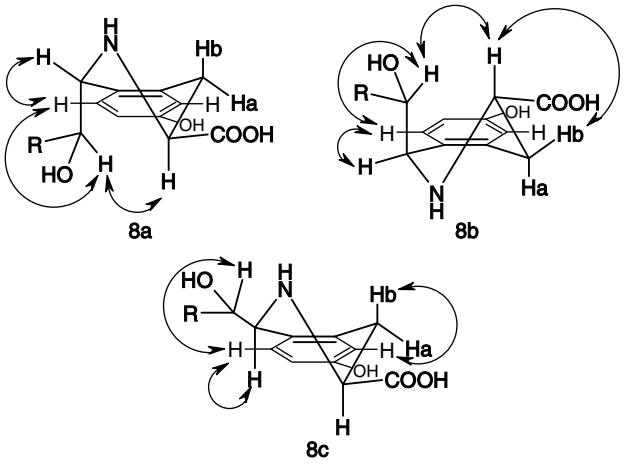

Figure 2. Salient NOE contacts observed in the ROESY spectra of compounds **8a–c**.

H-1 and H-2′ proton shifts with those of tetrahydroisoquinolines **5a**,**b** and of other diastereoisomeric 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines reported in the literature. Diagnostic of the *trans* relationship between the carboxyl group and the polyalcoholic side chain in **8a** and **8b** were also distinct cross peaks in the ROESY spectra denoting spatial proximity between the H-1′ and the H-3 protons. A summary of NOE contacts for products **8a–c** is given in Figure 2.

The spectral features of the remaining product isolated from **3** were those expected for the Amadori compound *N*-(1′-deoxy-β-D-fructopyranos-1′-yl)-*m*-tyrosine (**9**) (Table 3), including the pseudo-molecular ion peaks in the (ESI$^+$)MS spectra at m/z 344 $[M+H]^+$, 366 $[M+Na]^+$ and 382 $[M+K]^+$, and a peak for a $[M-H_2O+H]^+$ ion at m/z 326. The sugar ring structure was inferred from a carbon resonance at δ 96.5 suggestive of a β-pyranosyl system, whilst the conformation was argued to be $^2C_5$ on the basis of a coupling constant $J = 9.6$ Hz between the H-3′ and H-4′ proton signals, denoting a *trans* diaxial relationship.

2.4. Mechanistic issues

The effects of pH and of some transition metal cations of biological relevance, for example, Fe^{3+} and Cu^{2+},[10,12] on product yield and distribution for the reactions of **2** and **3** with D-glucose are summarized in Table 4.

The data showed that Maillard and Pictet–Spengler reactions proceed to different extents depending on the substrate, the presence of metal cations and the pH of the medium. One major determinant of the reactivity of glucose with amino acids is the protonation of the amine group, since the initial condensation step involves

Table 4. Yields of the products obtained by the reactions of **2** and **3** with D-glucose under different conditions

Compound	Yields (%)[a]				
	Reaction conditions[b]				
	pH 5.0	pH 7.4	pH 9.0	pH 7.4+Cu^{2+}	pH 7.4 + Fe^{3+}
2	16	—	2	34	—
5a	7	14	17	12	16
5b	31	37	34	23	35
6	14	25	24	16	28
7	28	22	13	13	19
3	62	21	—	23	38
8a	3	13	20	2	4
8b	1	4	10	1	1
8c	7	16	28	3	8
9	25	31	19	5	27

[a] Product yield were determined by HPLC.
[b] All reactions were carried out in 0.1 M phosphate buffer as detailed in the Experimental section.

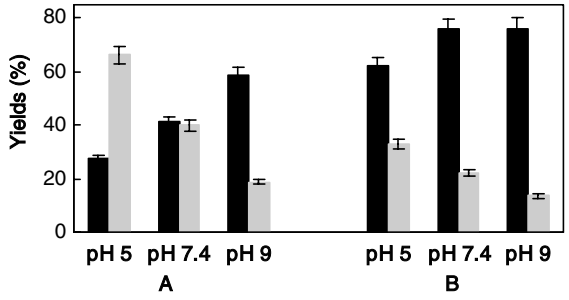

Figure 3. Effect of pH on the reaction of D-glucose with **3** (A) or **2** (B). Yields of Pictet–Spengler (black bars) and Maillard (grey bars) products. For Pictet–Spengler products, the overall yield of diastereoisomeric tetrahydroisoquinolines were considered. Analytical and reaction conditions as detailed in the Experimental Section.

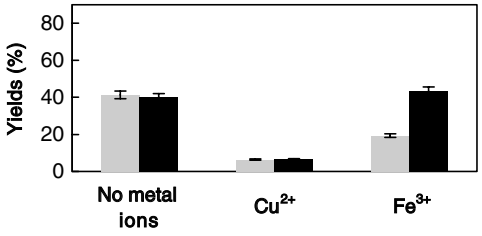

Figure 4. Effect of transition metal ions on the reaction of D-glucose with **3**. Yields of Pictet–Spengler (grey bars) and Maillard (black bars) products. For Pictet–Spengler products the overall yield of diastereoisomeric tetrahydroisoquinolines were considered. Analytical and reaction conditions as detailed in the Experimental Section.

the unprotonated form, and this explains why substrate consumption decreased at pH 5.0. An interesting issue concerns the unexpected formation of **6** from **2**. 7,8-Dihydroxy-1,2,3,4-tetrahydroisoquinoline derivatives have already been described by reactions of **2** with phenylpyruvic acid,[24] and of 3-hydroxyphenylethylamine derivatives with formaldehyde and acetaldehyde,[11,25,26] but no *ortho* cyclization product has ever been obtained from L-DOPA or dopamine with bulky carbonyl compounds. It is possible that the α-methyl group in **2** affects the relative stability of transition states along the *para* versus *ortho* cyclization pathways whereby the energy gap governing kinetic control is decreased and the otherwise unfavoured route becomes more accessible. Whether this is due to simple steric effects or more complex factors is difficult to assess at the present level of investigation. Scrutiny of Table 4 revealed that Pictet–Spengler products increase with increasing pH. This trend is readily apparent from Figure 3, which reports overall yields of Pictet–Spengler versus Maillard products for **2** and **3** at the various pH values examined, and can be ascribed to the increased ionization of the C-3 OH group at alkaline pH enhancing reactivity of the aromatic ring towards the imine function and side-tracking the latter in part from the Amadori rearrangement. Furthermore, Pictet–Spengler products prevail over Maillard products throughout the pH range 5–9 in the case of **2**, but only at alkaline pH in the case of **3**, as a result of the higher nucleophilic reactivity of the catechol ring compared to that of the monophenol ring.

Metal ions exerted an overall inhibitory effect on the condensation of **3** with D-glucose; in particular, Cu^{2+} inhibited both Maillard and Pictet–Spengler reactions, as a reflection of chelate formation with the amino acidic group at neutral pH interfering with Schiff base formation (Fig. 4).[27] Fe^{3+}, however, selectively inhibited the Pictet–Spengler reaction, probably because of the formation of chelate complexes with the Schiff base that pose conformational constraints to intramolecular cyclization (Fig. 4). Both Cu^{2+} and Fe^{3+} proved quite ineffective on the reaction of **2** with D-glucose, due probably to prevalent chelation at the catechol moiety distal from the aldimine functionality.[28–30]

With regard to the stereochemistry of Pictet–Spengler condensations, the Felkin–Anh model for asymmetric induction predicts the prevalence of the 1*R* tetrahydroisoquinolines, assuming that the CH–OH group provides the lowest energy σ^*_{C-X} orbital and the C–O bond is perpendicular to the imine group. Such a conformation, which maximizes the orbital interactions with the imine π system, entails that the entering aromatic ring attacks preferably from the side opposite to the OH group, so as to experience the smallest steric hindrance during the cyclization step.[31,32]

The 1*R*:1*S* ratio values for the (3*S*)-tetrahydroisoquinolines from **2** and **3** (Fig. 5) indicate that the Pictet–Spengler reaction occurs under Felkin–Anh control, and that the stereoselectivity is higher in the case of **3**, decreasing with increasing pH for both compounds. Both effects would be consistent with the expected loss of stereocontrol due to the increasing rates of Pictet–Spengler reaction observed in the case of **2** and **3** at higher pH.

Metal ions had no significant effect on the stereochemical course of the reaction of **2**, however, it is worth noting the increase in Felkin–Anh stereoselectivity induced

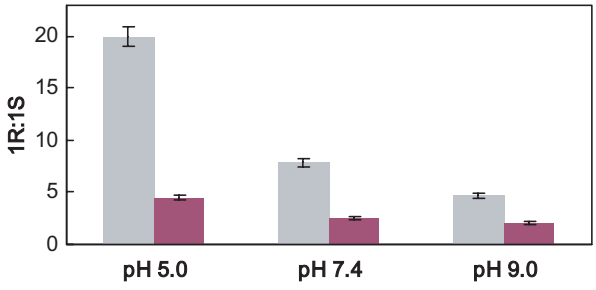

Figure 5. Effect of pH on the 1*R*:1*S* ratio for the tetrahydroisoquinoline derivatives obtained from the reaction of D-glucose with **2** (grey bars) or **3** (black bars). Analytical and reaction conditions as detailed in the Experimental Section.

by Fe^{3+} in the case of **3** (Table 4). This effect would seem in contrast with the commonly observed metal-induced inversion of stereoselectivity described in the Cram-chelated model, but it is conceivable that the carboxyl group could compete favourably with the OH on C-1′ forming a complex chelate with the imine functionality that does not alter the conformational arrangement of the Felkin–Anh model.

Data reported in Table 4 revealed that the regiochemistry of the Pictet–Spengler reaction of **2** with D-glucose was not affected by the pH or metal cations, with the *para* regioisomer **5b** always prevailing over the *ortho* isomer **6**. The same behaviour was observed in the case of **3**, for which the formation of the *ortho* isomer was invariably below detection limits.

3. Experimental

3.1. General methods

(ESI$^+$)MS spectra were recorded with a Waters ZQ quadrupole mass spectrometer. HRESI mass spectra were obtained with a Finnegan MAT 90 instrument. NMR spectra were recorded in D_2O with a Bruker DRX-400 MHz instrument. ^1H and ^{13}C NMR spectra were recorded at 400.1 and 100.6 MHz, respectively, using *tert*-butyl alcohol (δ_H 1.23, δ_C 31.0) as the internal standard. ^1H–^1H COSY, ^1H–^{13}C HMQC, ^1H–^{13}C HMBC and ROESY NMR experiments were run at 400.1 MHz using standard pulse programs from the Bruker library. Analytical and preparative HPLC were carried out on a Gilson apparatus equipped with a UV detector set at 280 nm using a Sphereclone ODS (5 μm, 4.6×250 mm) or Econosil (10 μm, 22×250 mm) column, respectively. For analytical runs, the following eluants were used at a flow rate of 1 mL/min: 0.1 M HCOOH/acetonitrile 98:2 (v/v) (eluant A) and 5×10^{-3} M octane-1-sulfonic acid in 0.1 M H_3PO_4 pH 2.5/acetonitrile 95:5 (v/v) (eluant B). In preparative runs, elution conditions were 0.1 M HCOOH/acetonitrile 95:5 (v/v) (eluant C), at a flow rate of 10 mL/min. Ion-exchange chromatography was performed using a DOWEX 50W-X4 (H^+) resin.

3.2. Materials

β-(3,4-Dihydroxyphenyl)-α-methyl-L-alanine (L-α-methyldopa) sesquihydrate and D-glucose were from Aldrich. DL-*m*-Tyrosine and $CuSO_4 \times 5H_2O$ were purchased from Fluka. $FeNH_4(SO_4)_2 \times 12H_2O$ was from Carlo Erba. Organic solvents were HPLC quality; 0.1 M phosphate buffers (pH 5.0, 7.4 and 9.0) were treated with Chelex-100 resin before use to remove transition metal contaminants. 5-*S*-Cysteinyldopa was prepared by a literature method.[33]

3.3. Reaction of phenolic amino acids with D-glucose: general procedure

A solution of the appropriate phenolic amino acid (0.042 M) in 0.1 M phosphate buffer, pH 7.4 previously purged with argon, was treated under vigorous stirring with D-glucose (15 mol equiv) and kept at 90 °C. After 3 h, the mixture was acidified to pH 2 with 3 M HCl and analyzed by HPLC using eluant A. Similar experiments were performed with the addition of $FeNH_4(SO_4)_2 \times 12H_2O$ (1 mol equiv) or $CuSO_4 \times 5H_2O$ (1 mol equiv), or using 0.1 M phosphate buffer at pH 5.0 or 9.0 as reaction medium.

3.4. *N*,*N*′-Bis(1″-deoxy-D-fructos-1″-yl)-5-*S*-cysteinyldopa (4)

5-*S*-Cysteinyldopa (100 mg, 0.32 mmol) was dissolved in 0.1 M phosphate buffer, pH 7.4 (4 mL) previously purged with argon, treated under vigorous stirring with D-glucose (600 mg, 3.3 mmol), and kept at 90 °C. After 3 h, the reaction mixture was acidified to pH 2 with 3 M HCl and subjected to ion-exchange chromatography (DOWEX 50W-X4 (H^+), 2×60 cm column) using water (250 mL), 0.1 M HCl (250 mL), 0.5 M HCl (500 mL) and 1 M HCl (500 mL) as eluants. The fraction eluted with 1 M HCl was re-subjected to ion-exchange chromatography (DOWEX 50W-X4 (H^+), 2×60 cm column) using water (100 mL), 0.1 M HCl (100 mL), 0.5 M HCl (100 mL) and 1 M HCl (250 mL) as eluants. The fraction eluted with 1 M HCl was collected and evaporated to dryness under reduced pressure to afford **4** (57 mg, 28% yield, $t_R = 10$ min, eluant B). Purity of compound **4** was at least 98% as determined by HPLC analysis. (ESI$^+$)MS: *m*/*z* 623 $[M-H_2O+H]^+$, 641 $[M+H]^+$, 663 $[M+Na]^+$, 679 $[M+K]^+$. Accurate mass: $[M+H]^+$ calcd for $C_{24}H_{37}N_2O_{16}S$, 641.1864; found, 641.1861. For ^1H and ^{13}C NMR data of compound **4**, see Table 1.

3.5. (1*S*,1′*S*,3*S*)-1-(D-*gluco*-Pentitol-1′-yl)-3-carboxy-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (5a), (1*R*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (5b), (1*R*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-3-methyl-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6), *N*-(1′-deoxy-β-D-fructopyranos-1′-yl)-L-α-methyldopa (7)

L-α-Methyldopa (100 mg, 0.42 mmol) was dissolved in 0.1 M phosphate buffer, pH 7.4 (10 mL) previously purged with argon, treated under vigorous stirring with D-glucose (1.2 g, 6.7 mmol), and kept at 90 °C. After 3 h, the reaction mixture was acidified to pH 2 with 3 M HCl and subjected to ion-exchange chromatography (DOWEX 50W-X4 (H^+), 2×60 cm column) using water (250 mL), 0.1 M HCl (250 mL), 0.5 M HCl (500 mL) and 1 M HCl (500 mL) as eluants. The fraction eluted with

1 M HCl was collected, evaporated to dryness under reduced pressure, and subjected to preparative HPLC (eluant C) to afford **5a** (15 mg, 10% yield, $t_R = 5$ min, eluant A), **5b** (47 mg, 30% yield, $t_R = 9$ min, eluant A), **6** (32 mg, 20% yield, $t_R = 11$ min, eluant A), **7** (25 mg, 16% yield, $t_R = 19$ min, eluant A). Purity of compounds **5a**,**b**, **6** and **7** was at least 98% as determined by HPLC analysis. (ESI$^+$)MS: **5a**, *m*/*z* 374 $[M+H]^+$, 396 $[M+Na]^+$, 412 $[M+K]^+$; **5b**, *m*/*z* 374 $[M+H]^+$, 396 $[M+Na]^+$, 412 $[M+K]^+$; **6**, *m*/*z* 374 $[M+H]^+$, 396 $[M+Na]^+$, 412 $[M+K]^+$; **7**, *m*/*z* 356 $[M-H_2O+H]^+$, 374 $[M+H]^+$, 396 $[M+Na]^+$, 412 $[M+K]^+$. Accurate mass: **5a** $[M+H]^+$ calcd for $C_{16}H_{24}NO_9$, 374.1451; found, 374.1449; **5b** $[M+H]^+$ calcd for $C_{16}H_{24}NO_9$, 374.1451; found, 374.1453; **6** $[M+H]^+$ calcd for $C_{16}H_{24}NO_9$, 374.1451; found, 374.1448; **7** $[M+H]^+$ calcd for $C_{16}H_{24}NO_9$, 374.1451; found, 374.1450. For ^{1}H and ^{13}C NMR data of compounds **5a,b**, **6** and **7**, see Table 2.

3.6. (1*R*,1′*S*,3*R*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (8a), (1*S*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (8b), (1*R*,1′*S*,3*S*)-1-(D-*gluco*-pentitol-1′-yl)-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (8c), *N*-(1′-deoxy-β-D-fructopyranos-1′-yl)-*m*-tyrosine (9)

DL-*m*-Tyrosine (100 mg, 0.56 mmol) was dissolved in 0.1 M phosphate buffer, pH 7.4 (6 mL) previously purged with argon, treated under vigorous stirring with D-glucose (1.4 g, 7.8 mmol), and kept at 90 °C. After 3 h, the reaction mixture was acidified to pH 2 with 3 M HCl and subjected to ion-exchange chromatography (DOWEX 50W-X4 (H^+), 2 × 60 cm column) using water (250 mL), 0.1 M HCl (250 mL), 0.5 M HCl (500 mL) and 1 M HCl (500 mL) as eluants. The fraction eluted with 1 M HCl was collected, evaporated to dryness under reduced pressure, and subjected to preparative HPLC (eluant C) to afford fractions containing **8a** plus **8b** (24 mg, 13% yield, $t_R = 4.5$ min, eluant A), **8c** (19 mg, 10% yield, $t_R = 8$ min, eluant A) and **9** (46 mg, 25% yield, $t_R = 20$ min, eluant A). Purity of compounds **8c** and **9** was at least 98% as determined by HPLC analysis. (ESI$^+$)MS: **8a** plus **8b**, *m*/*z* 344 $[M+H]^+$, 366 $[M+Na]^+$, 382 $[M+K]^+$; **8c**, *m*/*z* 344 $[M+H]^+$, 366 $[M+Na]^+$, 382 $[M+K]^+$; **9**, *m*/*z* 326 $[M-H_2O+H]^+$, 344 $[M+H]^+$, 366 $[M+Na]^+$, 382 $[M+K]^+$. Accurate mass: **8c** $[M+H]^+$ calcd for $C_{15}H_{22}NO_8$, 344.1345; found, 344.1342. For ^{1}H and ^{13}C NMR data of compounds **8a**–**c** and **9**, see Table 3.

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