A Novel Octahydropyridobenzothiazepine Metabolite in Human Urine: Biomimetic Formation from the Melanogen 5-*S*-Cysteinyldopa and Formaldehyde via a Peculiar Sulfur-Controlled Double Pictet−Spengler Condensation

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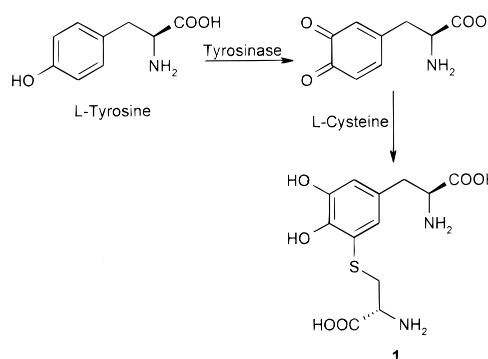
Received December 28, 1999

HPLC evidence is reported demonstrating the occurrence in some human urine samples of a novel catecholic metabolite, (3*R*,7*S*)-3,7-dicarboxy-10,11-dihydroxy-2,3,4,5,6,7,8,9-octahydropyrido[4,3-*g*][1,4]benzothiazepine (**2**). The compound was shown to arise by a double Pictet−Spengler condensation of the urinary melanogen 5-*S*-cysteinyldopa (**1**) with formaldehyde, in which regioselective formation of the six-membered ring ortho to the activating hydroxyl group lends assistance to the subsequent closure of the seven-membered 1,4-thiazepine moiety. Under physiologically relevant conditions, i.e., in 0.1 M phosphate buffer pH 7.4 and at 37 °C, the 7,8-tetrahydroisoquinoline **5** was the sole detectable intermediate in the formation of **2**. *N*-Acetylcysteinyldopa (**4**) reacted likewise with formaldehyde to give the 7,8-dihydroxytetrahydroisoquinoline **6**. The anomalous regiochemistry underlying formation of **5** and **6** was rationalized with the aid of AM1/PM3 calculations on the model alkylthiocatechol **10**, predicting a higher HOMO-controlled reactivity on the position ortho rather than para to the activating hydroxyl group. The potential of the reported chemistry as a convenient synthetic access to the 2,3,4,5-tetrahydro[1,4]benzothiazepine ring system is suggested by the efficient conversion of the cysteinylcatechol **3** to **8** in the presence of formaldehyde.

Introduction

Since its first isolation from human urine in 1973,[1] 5-*S*-cysteinyldopa (3-[(*R*)-2-amino-2-carboxyethylthio]-5-[(*S*)-2-amino-2-carboxyethyl]-1,2-dihydroxybenzene, **1**) has spurred considerable interest because of its central role in the biosynthesis of pheomelanins,[2] the characteristic pigments responsible for the UV susceptibility trait in red-haired, fair-complexioned individuals.[3] Biogenetically, **1** is generated within functionally active cutaneous melanocytes via nucleophilic addition of L-cysteine to dopaquinone produced by tyrosinase-catalyzed oxidation of L-tyrosine (Scheme 1)[4] and is continuously excreted into body fluids at levels of 3.7 ± 1.4 nmol/L in urine and 1.2 ± 0.3 nmol/L in plasma.[5] The documented correlation between the blood and urine levels of **1** and the functional status of melanocytes in patients with malignant melanoma has warranted exploitation of **1** as a specific metabolic marker of tumor progression and for the early detection of occult metastases.[4]

Scheme 1. Biosynthesis of 1 by Reaction of l-Cysteine with Dopaquinone



Additional incentives for studies of **1** have derived more recently from the demonstration of its powerful inhibitory effects on OH radical-induced damaging processes[6] and lipid peroxidation[7] promoted by iron-dependent Fenton-type systems. However, despite the increasing academic and practical interest of **1**, its general reaction behavior and metabolic fate under nonoxidative conditions have remained virtually unexplored, and no metabolite of **1** has so far been isolated from biological sources.

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10.1021/jo991969c CCC: $19.00 © 2000 American Chemical Society
Published on Web 06/17/2000

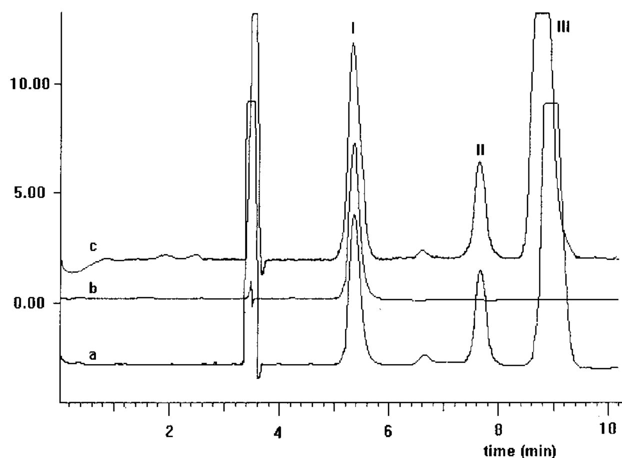

Figure 1. HPLC-ED traces of (a) catecholamine-containing fraction from human urine; (b) main 1-HCHO adduct; (c) a plus b. Peaks: I = 1-HCHO adduct; II = L-dopa; III = dopamine. Elution system: eluant a; retention time of 1 = 20 min.

We report herein the first identification of a urinary metabolite of **1** featuring the hitherto unknown octahydropyridobenzothiazepine skeleton. The apparent origin of this heterocyclic ring system from an intriguing double condensation of **1** with formaldehyde prompted us to examine in detail the reaction of 5-alkylthiocatecholamines[8] with formaldehyde under biomimetic Pictet−Spengler[9] conditions.

Results and Discussion

Urine Analysis. Because of our continuing interest in biologically active tetrahydroisoquinolines,[10] our search for novel metabolites of **1** was initially directed toward possible reaction products with biologically relevant aldehydes. Adsorption of lipid-free human urine on acid-washed alumina followed by selective elution with formic acid, under conditions reported for isolation of tetrahydroisoquinoline metabolites,[11] yielded a fraction exhibiting on HPLC-ED (high-pressure liquid chromatography with electrochemical detection) a well-defined pattern of electrochemically detectable compounds. Comparative analysis of similar urine fractions from 5 healthy individuals revealed the presence in 2 samples, besides L-dopa, dopamine and **1**, of an electrochemically detectable peak coeluting with a major peak produced by reaction of **1** with formaldehyde under physiologically relevant conditions, i.e., in 0.1 M phosphate buffer, pH 7.4 and at 37 °C (Figure 1).

The identity of the urinary metabolite with the formaldehyde−**1** adduct was further corroborated by comparison of their electrochemical behaviors. Plots of relevant peak areas versus the working electrode potential gave curves that were totally superimposable in the range 0.1−0.4 V, denoting the same redox potential, and

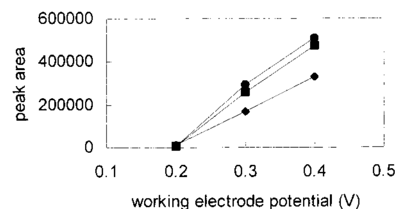

Figure 2. HPLC-ED response of the urinary metabolite and synthetic 1-HCHO adduct with respect to L-dopa as reference: ●, urinary metabolite; ■, synthetic 1-HCHO adduct; ◆, L-dopa.

different from that of L-dopa taken as a reference catecholamine (Figure 2).

Moreover, virtually identical electrochemical responses were obtained with two sequential working electrodes set at +0.4 and −0.2 V indicating reversibility of the oxidation reaction and identical lifetimes of the oxidized counterparts.

Attempts to isolate the compound from the eluted fraction were defeated despite considerable efforts, due both to the exceedingly low concentrations, far below the UV detection limits, and the presence of strongly UV-absorbing, electrochemically silent impurities. Mass spectrometric analysis of the relevant peak proved also of little value. Accordingly, insight into the structure of the compound was sought through investigation of the more accessible synthetic adduct and the chemistry underlying its formation.

Reaction of 1 and Related Alkylthiocatechol Derivatives with Formaldehyde. Synthesis of the formaldehyde−**1** adduct was readily achieved by reacting **1** with aqueous formaldehyde in 0.1 M phosphate buffer, pH 7.4, in the absence of oxygen, to prevent substrate oxidation. Under all the conditions investigated, the reaction resulted in the smooth formation of the desired product in good to excellent yield, depending on substrate concentration and temperature. As formed, the product partially separated from the mixture as a white solid slightly soluble in most organic solvents and freely soluble in DMSO. 1D and 2D NMR analysis coupled with FAB/MS data ($(M + H)^+ = 341$ *m/z*) suggested a 2:1 formaldehyde−**1** adduct. The lack of aromatic proton resonances and a telltale couple of AB quartets in the range 4.2−4.7 ppm, suggesting diastereotopic protons on two separate methylene groups, argued moreover in favor of a doubly cyclized derivative of **1**. On these bases, the compound was unambiguously formulated as (3*R*,7*S*)-3,7-dicarboxy-10,11-dihydroxy-2,3,4,5,6,7,8,9-octahydropyrido-[4,3-*g*][1,4]benzothiazepine (**2**) characterized by a hitherto unknown heterocyclic skeleton. The energy-minimized stereostructure of **2** revealed a twisted conformation for the tetrahydropyridine moiety, in accordance with literature data,[12] and a boatlike conformation for the 1,4-thiazepine ring (Figure 3).

With the authentic sample available, excretion values of **2** of 1.2 ± 0.2 nmol/L and 0.9 ± 0.1 nmol/L in two different urine samples could be determined by comparison with external calibration curves.

Systematic inspection of the mixtures obtained by reaction of **1** with various amounts of formaldehyde revealed the presence in the early stages of a single intermediate which, on further reaction with formalde-

(8) For the sake of simplicity, we adopt 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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Table 1. NMR Data for Compounds 5 and 6 (in D_2O/DCl)

	5				6			
carbon	δ_C	δ_H (mult, J Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC	δ_C	δ_H (mult, J Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
1	44.0	4.41 (d, 16.0)[a]	4.74		43.3	4.18 (d, 16.0)	4.67	
		4.74 (d, 16.0)[b]	4.41			4.67 (d, 16.0)	4.18	
3	54.5	4.39 (dd, 11.0, 5.6)	3.16, 3.32	3.16, 3.32, 4.74	54.5	4.06 (m)	3.01−3.10, 3.19	3.01−3.10
4	28.3	3.16 (dd, 17.0, 11.0)[a]	3.32, 4.39	4.39, 6.85	28.7	3.01−3.10 (m)	3.19, 4.06	
		3.32 (dd, 17.0, 5.6)[b]	3.16, 4.39			3.19 (dd, 12.8, 8.0)	3.01−3.10, 4.06	
4a	123.3			4.39, 6.85	124.3			6.76
5	117.9	6.85 (s)			116.8	6.76 (s)		
6	115.8			3.33, 3.47	116.5			3.07, 3.39
7	146.9			6.85	145.5			6.76
8	144.8				144.0			
8a	124.1			4.41, 4.74, 6.85	122.8			4.18, 4.67, 6.76
3-COOH	171.4			4.39	177.3			3.01−3.10
CH_2S	34.5	3.33 (dd, 14.8, 4.8)	3.47, 4.27	4.27	34.5	3.07 (dd, 14.0, 7.2)	3.39, 4.36	4.36
		3.47 (dd, 14.8, 5.8)	3.33, 4.27			3.39 (dd, 14.0, 3.6)	3.07, 4.36	
CH	53.0	4.27 (t, 5.8, 4.8)	3.33, 3.47	3.33, 3.47	55.6	4.36 (dd, 7.2, 3.6)	3.07, 3.39	
COOH	170.4			4.27	174.1			4.36
CONH					174.7			3.39, 4.36
CH_3					21.9	1.84 (s)		

[a] Pseudoaxial orientation. [b] Pseudoequatorial orientation.

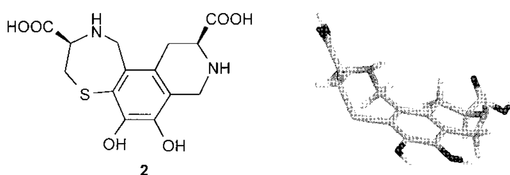
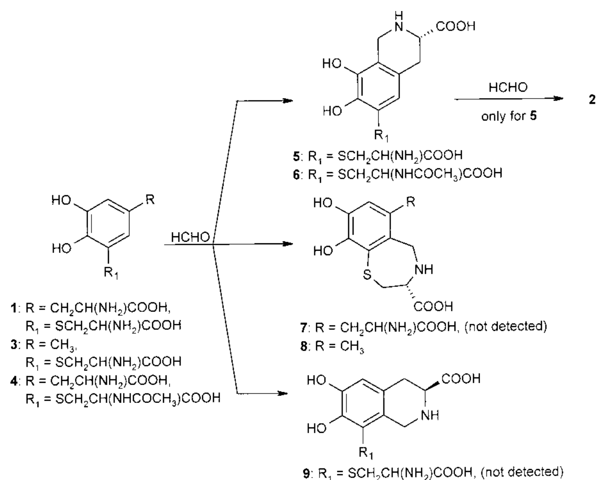


Figure 3. Structure and energy-minimized stereostructure of **2** (MM+).

Scheme 2. Reactions of 5-Alkylthiocatechols with Formaldehyde



hyde, was converted into **2**. Straightforward analysis of the NMR spectra allowed formulation of the compound as (3*S*)-6-[(*R*)-2-amino-2-carboxyethylthio]-3-carboxy-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**5**) (Scheme 2).

The presence of a tetrahydroisoquinoline ring was deduced from careful analysis of the ^1H and ^{13}C NMR data. Especially insightful was a well-discernible correlation in the $^1H-^{13}C$ HMBC spectrum (Table 1) between the carbon signal at δ 54.5, ascribed to the aliphatic CH carbon of the former dopa moiety, and the doublet at δ 4.74, due to one of the methylene protons on the $NHCH_2$ grouping. Such a correlation was taken moreover to indicate for this proton a pseudoequatorial orientation, warranting a relatively high $^3J_{CH}$ coupling.[13] The geminal H-4 protons at δ 3.16 and 3.32 could be proposed as having a pseudoaxial and a pseudoequatorial orientation, respectively, from analysis of coupling constants based on the vicinal Karplus correlation graph.

Notably, no evidence for the presence of the 1,4-benzothiazepine **7** or of the 6,7-dihydroxytetrahydroisoquinoline **9** in the reaction mixture of **1** with formaldehyde was obtained.

To check whether formation of the 1,4-benzothiazepine ring was peculiar to the reaction of **1** with aldehydes or reflected a general behavior of 3-(2-aminoethylthio)-1,2-dihydroxybenzene derivatives, the model compound **3** was reacted with formaldehyde at pH 7.4 and at 60 °C and was found to give the expected 1,4-benzothiazepine **8** in good yields (Scheme 2).

Mechanism of Formation of 2: Regiochemical Issues. Formation of structure **2** is remarkable in that it involves two Pictet−Spengler-type condensations in which a seven-membered 1,4-thiazepine moiety is formed at a site where the more facile closure of the six-membered ring would be anticipated. Indeed, condensations of dopamine, L-dopa, and their congeners with formaldehyde and other aldehydes normally proceed with preferential formation of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines over their 7,8-dihydroxy isomers,[14] due to the pronounced directing effect of the activating OH group to the para rather than ortho position. In the case of **2**, the isolation of **5** as the sole precursor suggested a fast, regiochemically anomalous formation of the six-membered ring preceding the second cyclization step.

In this connection, it is worth noting that while formation of **2** in 0.1 M phosphate buffer, pH 7.4, proceeded smoothly at 37 °C (65% yield), the model compound **3** failed to give detectable amounts of the 1,4-benzothiazepine **8** by reaction with formaldehyde at the same temperature, substantial conversion to the product being observed only under more forcing conditions, e.g., 60 °C. Such a different reactivity suggests that the initial formation of the six-membered ring in **5** sets the stage

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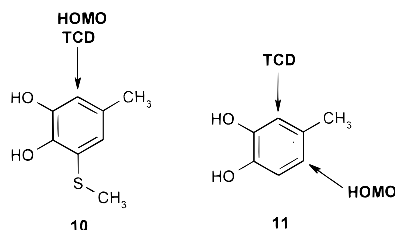

Figure 4. Predicted sites of attack of electrophilic agents on the model structures **10** and **11** based on AM1/PM3 calculations of the HOMO coefficients and total charge densities (TCD).

Table 2. Total Charge Densities (TCD) and HOMO Coefficients for Carbons 3 and 5 in Model Structures 10 and 11

		AM1		PM3	
compd	carbon[a]	HOMO coefficient	TCD	HOMO coefficient	TCD
10	3	0.298	−0.182	0.278	−0.155
	5	0.193	−0.151	0.122	−0.124
11	3	0.041	−0.172	0.037	−0.155
	5	0.347	−0.126	0.347	−0.100

[a] For the sake of simplicity, we adopt for compound **11** (3-methylthio-5-methylcatechol) the same numbering system used for 4-methylcatechol. Accordingly, positions 3 and 5 denote the same position ortho and para to the orienting OH group, respectively.

for the subsequent seven-membered ring-forming step in **2**, possibly by increasing the electron density on the catechol system. Interestingly, *N*-acetylcysteinyldopa (3-[(*R*)-2-acetylamino-2-carboxyethylthio]-5-[(*S*)-2-amino-2-carboxyethyl]-1,2-dihydroxybenzene, **4**), to which the benzothiazepine route was clearly precluded, reacted with formaldehyde to give only the 7,8-dihydroxytetrahydroisoquinoline **6** (Table 1), confirming the anomalous regioselectivity of the Schiff base cyclization in the presence of the sulfur-containing substituent (Scheme 2).

To address this regiochemical issue more in depth, the properties of structure **10**, a computationally more convenient model of **1** and **4**, were briefly explored by AM1/PM3 calculations in comparison with 4-methylcatechol (**11**) (Figure 4). For both structures, total charge distributions (Table 2) identified the positions ortho to the activating OH group as the most reactive toward electrophilic agents. By contrast, HOMO coefficients correctly predicted the para position as the preferred site of attack of electrophiles on **11**, and the ortho position in the case of **10**. Overall, these data would be consistent with a mechanism involving HOMO-controlled cyclization of Schiff base intermediates derived from **1** and **4** in which the sulfur substituent apparently dictates the regiochemical outcome.

Concluding Remarks

In the present paper, we have described a novel octahydropyridobenzothiazepine metabolite in human urine. Details concerning the occurrence of **2** in biological fluids and its significance will be reported separately in due course. From the chemical viewpoint, the results of this study have disclosed novel facets of the time-honored Pictet−Spengler chemistry of catecholamines and have highlighted the critical role of sulfur substituents, subverting the normal regiochemistry of cyclization of Schiff bases leading to 7,8-dihydroxytetrahydroisoquinolines. The potential of the reaction of 5-*S*-cysteinylcatechols with aldehydes as a convenient synthetic entry to the 2,3,4,5-tetrahydro[1,4]benzothiazepine system is also adumbrated.[15] Finally, the structural elucidation of **2** holds interest in relation to the reported use of formaldehyde for detection of **1** in pigmented tissues by the classic Falck histochemical test.[16]

Experimental Section

General Methods. Melting points are uncorrected. ^{1}H NMR spectra were obtained at 400 MHz with *tert*-butyl alcohol (δ 1.23) as internal standard. ^{13}C NMR spectra were run at 100 MHz. ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR, and ^{1}H−^{13}C HMBC NMR experiments were obtained 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). The flow rate was maintained at 1 mL/min for analytical runs and at 10 mL/min for preparative chromatography.

5-*S*-Cysteinyldopa (3-[(*R*)-2-amino-2-carboxyethylthio]-5-[(*S*)-2-amino-2-carboxyethyl]-1,2-dihydroxybenzene, **1**) and 3-[(*R*)-2-amino-2-carboxyethylthio]-1,2-dihydroxy-5-methylbenzene (**3**) were prepared by literature methods.[17,18] Formaldehyde (37%), L-dopa, and cerium ammonium nitrate were purchased from Aldrich. *N*-Acetyl-L-cysteine was purchased from Fluka.

Molecular mechanics (MM+) and AM1/PM3 calculations were carried out with Hyperchem 5.0 package produced by Hypercube Inc. (Waterloo, Ontario, Canada), 1997.

Collection and Extraction of Human Urine. Samples of human urine were from healthy individuals of either sex and were immediately stored after treatment with 0.5 mg/mL of $NaHSO_3$ at −20 °C until use. Urine (500 mL) was treated with 10 μL of a 10 μg/mL solution of α-methyldopa in 0.1 M HCl as internal standard, acidified with 0.1 M HCl (500 mL), and extracted twice with ethyl acetate to remove lipids. The aqueous phase was then treated with $NaHSO_3$ (400 mg) and ethylenediaminetetraacetic acid (EDTA) (6 g), buffered to pH 8.4 with 1 M trishydroxymethylaminomethane (Tris) (150 mL), and adsorbed on acid-treated alumina[19] (30 g) that had been prewashed with 0.1 M Tris buffer (250 mL). The suspension was stirred for 10 min and then centrifuged. The aqueous phase was removed, and the alumina was washed twice with distilled water (500 mL) and centrifuged again. The alumina was eventually eluted by shaking for 10 min with 0.5 M HCOOH (300 mL) and centrifugation. The acidic solution was evaporated under reduced pressure, dissolved in 0.1 M HCl (500 μL), and injected into the HPLC.

Analysis of Urinary Metabolites. HPLC-ED determinations were performed with a Gilson instrument equipped with a model 305 pump. A 5100A ESA Coulochem detector equipped with dual fully porous graphite electrodes was used directly after the column for electrochemical detection with sensitivity set at 200 nA. The first electrode was set at −0.1 V and the second was set at +0.4 V for urine analysis. For studies of the electrochemical properties, the first electrode was set at a variable positive potential and the second at −0.2 V. Analyses were carried out on a Sphereclone ODS reversed-phase column using two different eluants: (a) 0.1 M HCOOH−acetonitrile

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96:4 v/v; (b) 5 mM octane-1-sulfonic acid in 0.1 M H_3PO_4, pH 2.5 acetonitrile 96:4 v/v.

(3*R*,7*S*)-3,7-Dicarboxy-10,11-dihydroxy-2,3,4,5,6,7,8,9-octahydropyrido[4,3-*g*][1,4]benzothiazepine (2). A solution of **1** (100 mg, 0.32 mmol) in 0.1 M phosphate buffer, pH 7.4 (15 mL), previously purged with argon, was treated with formaldehyde (250 μL, 3.2 mmol) and refluxed for 2 h. The white solid that separated was collected by centrifugation, washed with water, and desiccated under vacuum to give pure **2** (50 mg). The surnatant was acidified to pH 2 with 3 M HCl and subjected to preparative HPLC (eluant 0.1 M HCOOH−acetonitrile 94:6 v/v) to afford another 30 mg of **2** (overall yield 75%).

2: mp >400 °C dec; UV λ_{max} (log ϵ) (0.5 M HCl) 255 (3.70), 295 (3.59) nm; $[\alpha]^{20}_D$ = −10.1 (*c* 3.96, 0.5 M HCl); ^{1}H NMR (D_2O/DCl) δ 3.13 (1H, m), 3.23 (1H, dd, *J* = 17.0, 11.6 Hz), 3.40 (1H, m), 3.54 (1H, dd, *J* = 17.0, 4.8 Hz), 4.25 (1H, d, *J* = 16.4 Hz), 4.42 (1H, dd, *J* = 11.6, 4.8 Hz), 4.55 (1H, d, *J* = 16.4 Hz), 4.56 (1H, d, *J* = 14.4 Hz), 4.65 (1H, m), 4.67 (1H, d, *J* = 14.4 Hz); ^{13}C NMR (D_2O/DCl) δ 27.1 (t), 33.9 (t), 42.0 (t), 46.3 (t), 55.2 (d), 62.0 (d), 118.0 (s), 124.6 (s), 125.4 (s), 126.6 (s), 143.5 (s), 143.9 (s), 170.3 (s), 171.7 (s); MS (FAB) *m*/*z* 341; HRMS calcd for $C_{14}H_{17}N_2O_6S$ (M^+ + 1) 341.0807, found 341.0801. Anal. Calcd for $C_{14}H_{16}N_2O_6S$: C, 49.41; H, 4.74; N, 8.23; S, 9.42. Found: C, 49.50; H, 4.78; N, 8.26; S, 9.47.

(3*S*)-6-[(*R*)-2-Amino-2-carboxyethylthio]-3-carboxy-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline (5). A solution of **1** (100 mg, 0.32 mmol) in 0.1 M phosphate buffer, pH 7.4, (15 mL), previously purged with argon, was treated with formaldehyde (250 μL, 3.2 mmol) at 37 °C. After 6 h, HPLC analysis (eluant a) revealed two main products in a 4:1 ratio along with little amounts of the starting material. The mixture was acidified to pH 2 with 3 M HCl and subjected to preparative HPLC (eluant 0.1 M HCOOH−acetonitrile 98:2 v/v) to afford **5** (17 mg, 16%) along with **2** (75 mg, 70%).

5: yellowish oil; UV λ_{max} (log ϵ) (0.5 M HCl) 256 (3.52), 294 (3.61) nm; $[\alpha]^{20}_D$ = −19.4 (*c* 2.54, 0.5 M HCl); ^{1}H NMR (D_2O/DCl) see Table 1; ^{13}C NMR (D_2O/DCl) see Table 1; MS (FAB) *m*/*z* 329; HRMS calcd for $C_{13}H_{17}N_2O_6S$ (M^+ + 1) 329.0807, found 329.0800. Anal. Calcd for $C_{13}H_{16}N_2O_6S$: C, 47.56; H, 4.91; N, 8.53; S, 9.76. Found: C, 47.50; H, 4.87; N, 8.50; S, 9.71.

(3*R*)-3-Carboxy-8,9-dihydroxy-6-methyl-2,3,4,5-tetrahydro[1,4]benzothiazepine (8). A solution of **3** (100 mg, 0.39 mmol) in 0.1 M phosphate buffer, pH 7.4 (15 mL), previously purged with argon, was treated with formaldehyde (290 μL, 3.9 mmol) and refluxed for 2 h. The pale brown solid that separated was collected by centrifugation, washed with water, and desiccated under vacuum to give pure **8** (45 mg). The surnatant was acidified to pH 2 with 3 M HCl and subjected to preparative HPLC (eluant 0.1 M HCOOH−acetonitrile 95:5 v/v) to afford another 25 mg of **8** (overall yield 70%).

8: mp >400 °C dec; UV λ_{max} (log ϵ) (0.5 M HCl) 253 (3.62), 296 (3.68) nm; ^{1}H NMR (D_2O/DCl) δ 2.26 (3H, s), 3.11 (1H, m), 3.38 (1H, m), 4.47 (1H, d, *J* = 14.5 Hz), 4.58 (1H, dd, *J* = 6.8, 2.5 Hz), 4.63 (1H, d, *J* = 14.5 Hz), 6.75 (1H, s); ^{13}C NMR (D_2O/DCl) δ 20.2 (q), 34.1 (t), 47.3 (t), 62.0 (d), 119.3 (d), 125.0 (s), 126.8 (s), 133.1 (s), 143.5 (s), 145.9 (s), 170.5 (s); MS (FAB) *m*/*z* 256; HRMS calcd for $C_{11}H_{14}NO_4S$ (M^+ + 1) 256.0644, found 256.0648. Anal. Calcd for $C_{11}H_{13}NO_4S$: C, 51.55; H, 5.51; N, 5.47; S, 12.51. Found: C, 51.57; H, 5.55; N, 5.50; S, 12.57.

3-[(*R*)-2-Acetylamino-2-carboxyethylthio]-5-[(*S*)-2-amino-2-carboxyethyl]-1,2-dihydroxybenzene (4). A solution of L-dopa (500 mg, 2.5 mmol) in 2 M sulfuric acid (12.5 mL) was treated with cerium ammonium nitrate (2.78 g, 5.1 mmol) in 2 M sulfuric acid (25 mL) under stirring. The resultant yellow-orange mixture was rapidly added to a solution of *N*-acetyl-L-cysteine (1.63 g, 10 mmol) in 2 M sulfuric acid (12.5 mL). After 10 min the reaction mixture was subjected to ion-exchange chromatography (Dowex 50W-X4 (H^+), 2 × 60 cm), using water (400 mL), 0.1 M HCl (200 mL), and 0.5 M HCl (1 L) as the eluants. Fractions eluted with 0.5 M HCl (λ_{max} 255 and 286 nm) were collected and evaporated to dryness under reduced pressure to afford pure **4** (600 mg, 61% yield) as a glassy oil, homogeneous on HPLC (eluant: 0.1 M HCOOH−acetonitrile 98:2 v/v): UV λ_{max} (log ϵ) (0.5 M HCl) 255 (3.49), 286 (3.40) nm; ^{1}H NMR (D_2O) δ 1.89 (3H, s), 3.03 (1H, ddd, *J* = 14.4, 7.6 Hz), 3.13−3.23 (1H, m), 3.18 (1H, dd, *J* = 14.4, 8.4 Hz), 3.39 (1H, dd, *J* = 14.4, 4.4 Hz), 4.17 (1H, dd, *J* = 7.6, 5.2 Hz), 4,40 (1H, dd, *J* = 8.4, 4.4 Hz), 6.77 (1H, d, *J* = 2.0 Hz), 6.86 (1H, d, *J* = 2.0 Hz); MS (FAB) *m*/*z* 359; HRMS calcd for $C_{14}H_{19}N_2O_7S$ (M^+ + 1) 359.0913, found 359.0919.

6-[(*R*)-2-Acetylamino-2-carboxyethylthio]-3(*S*)-carboxy-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6). A solution of **4** (100 mg, 0.23 mmol) in 0.1 M phosphate buffer, pH 7.4, (15 mL) previously purged with argon, was treated with formaldehyde (173 μL, 2.3 mmol) and heated to 60 °C for 3 h. The mixture was acidified to pH 2 with 3 M HCl and subjected to preparative HPLC (eluant 0.1 M HCOOH−acetonitrile 95:5 v/v) to afford **6** (75 mg, 60%).

6: UV λ_{max} (log ϵ) (0.5 M HCl) 259 (3.00), 297 (2.98); $[\alpha]^{20}_D$ = −13.6 (*c* 2.5, 0.5 M HCl); ^{1}H NMR (D_2O) see Table 1; ^{13}C NMR (D_2O) see Table 1; MS (FAB) *m*/*z* 371; HRMS calcd for $C_{15}H_{19}N_2O_7S$ (M^+ + 1) 371.0913, found 371.0907. Anal. Calcd for $C_{15}H_{18}N_2O_7S$: C, 48.64; H, 4.90; N, 7.56; S, 8.66. Found: C, 48.61; H, 4.87; N, 7.52; S, 8.62.

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.

JO991969C