Hz, CH_3-5'); TLC ($CHCl_3$-CH_3OH, 4:1) R_f 0.3. Anal. ($C_{47}H_{46}N_2O_{11}$·0.8$CHCl_3$) C, H, Cl, N.

5-Iminodoxorubicin Hydrochloride (4). A solution of 1.073 g (1.32 mmol) of 8 in 70 mL of 80% AcOH was stirred at 23 °C in the dark for 5 h, frozen, and lyophilized. The residue was dissolved in 100 mL of $CHCl_3$–CH_3OH (1:1), the solution was stirred, and 14.3 mL (1.32 mmol) of 0.092 M methanolic hydrogen chloride was added dropwise, followed by 200 mL of Et_2O. The resulting precipitate was collected and washed with $CHCl_3$ (3 × 25 mL) and with Et_3O (3 × 50 mL) to afford 0.642 g (82%) of hydrochloride 4: IR 2.90 (OH, NH), 5.78 (C=O), 6.30 μm (H-bonded quinone); 100-MHz NMR (Me_2SO-d_6) δ 15.80 (s, 1, OH-11), 13.48 (br s, 1, OH-6), 9.55 (br s, 1, NH), 8.00 (d, 1, J = 8 Hz, H-1), 7.93 (br s, 3, NH_3^+), 7.78 (t, 1, J = 8 Hz, H-2), 7.56 (d, 1, J = 8 Hz, H-3), 5.46 (br s, 1, H-1'), 4.94 (br s, 1, H-7), 4.59 (s, 2, H-14), 4.19 (m, 1, H-5'), 4.09 (s, 3, OCH_3), 3.59 (br s, 1, H-4'), 3.38 (m, 1, H-3'), 2.84 (br s, 2, H-10), 2.07 (br s, 2, H-8), 1.72 (m, 2, H-2'), 1.15 (d, 3, J = 6.5 Hz, CH_3-5'); UV-vis λ_{max} (CH_3OH) 221 nm (ε 30 200), 233 sh (25 500), 252 (31 600), 305 (7200), 335 sh (4500), 360 sh (4050), 520 sh (8900), 551 (16 700), 592 (19 600); MS [as the $(Me_3Si)_6$ derivative], m/e 959 (M - CH_3), 944 (M - $2CH_3$); TLC ($CHCl_3$–CH_3OH–2 N AcOH, 40:10:1) R_f 0.11; HPLC 4.6 × 250 mm Altex Ultrasphere Octyl 5 μm, 0.01 M H_3PO_4–CH_3OH (30:70), 1.0 mL/min, UV at 254 nm, retention time 3.9 (5.3%, 5-iminoadriamycinone), 7.9 (0.3%), 15.6 (0.3%), and 25.7 min (94.1%, 5-iminodoxorubicin). Anal. ($C_{27}H_{30}N_2O_{10}$·HCl·H_2O) C, H, Cl, N.

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Synthesis and Antitumor Activity of Cysteinyl-3,4-dihydroxyphenylalanines and Related Compounds

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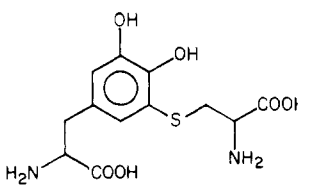
The natural catecholic amino acid 5-*S*-cysteinyl-3,4-dihydroxyphenylalanine (1) was selectively toxic to a variety of human tumor cell lines in culture and exhibited antitumor activity against L1210 leukemia and B-16 melanoma in mice at doses which were not toxic to the host. Structural analogues of 5-*S*-cysteinyl-3,4-dihydroxyphenylalanine, including several new compounds, were synthesized and tested for growth inhibition of cultured cells of human neuroblastoma YT-nu and Chinese hamster fibroblast Don-6. Some were also examined for antitumor activity against L1210 and B-16 in vivo. 4-*S*-Cysteinylcatechols and 2- and 4-*S*-cysteinylphenols, which cannot be prepared by conventional methods, were synthesized by the reaction of catechols and phenols with cystine in boiling aqueous HBr. 5-*S*-Cysteinyl- and 2-*S*-Cysteinyl-3,4-dihydroxyphenylalanine (1 and 2), L-3,4-dihydroxyphenylalanine (L-Dopa), and 2- and 4-*S*-cysteinylphenol (14 and 15) were toxic to the YT-nu cell line only, while 4-*S*-cysteinylcatechol (6), 3-*S*-cysteinyl-5-methylcatechol (8), 5-*S*-cysteaminyldopamine (9), and 4-methylcatechol were strongly toxic to both cell lines. Compounds 1 (1000 mg/kg), 6 (500 mg/kg), and 8 (400 mg/kg) increased the life span of L1210-bearing mice by 50, 50, and 43%, respectively, and compounds 1 and 8 were marginally effective against B-16 melanoma as well. Compound 9 was too toxic to show any activity. There was a good correlation between the cytotoxicity and the in vivo activity.

The catecholic amino acid 5-*S*-cysteinyl-3,4-dihydroxyphenylalanine (5-*S*-cysteinyl-Dopa, 1) is the chief building stone of pheomelanins, yellow to reddish-brown melanins.[1] High levels of this amino acid have been found in the urine of patients with melanoma metastases.[2] Recently, Wick et al. showed that L-3,4-dihydroxyphenylalanine (L-Dopa) is toxic to melanoma cells in vitro[3] and that its analogues exhibit antitumor activity in several experimental tumor systems.[4-6] Later, we found that 1 is much more toxic to a variety of human tumor cell lines in culture than is L-Dopa and possesses antitumor activity against murine L1210 leukemia and B-16 melanoma with no untoward effects on the host.[7] It was suggested that the mechanism of action of these catechols may involve oxidation to an *o*-benzoquinone with subsequent sulfhydryl scavenging and inhibition of enzymes essential for DNA synthesis.[6,7]



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In an attempt to obtain antitumor agents that are more effective than 1 and to elucidate the mechanism of action of the catechols, we have synthesized structural analogues of 5-*S*-cysteinyl-Dopa (1) and tested these compounds (1–16, Chart I) for growth inhibition of cultured cells of human neuroblastoma YT-nu and Chinese hamster fibroblast Don-6. Some of the compounds (1, 6, 8, 9, and 15) were also examined for their effects on the life span of mice bearing L1210 leukemia or B-16 melanoma.

Chemistry. Physical constants and spectral data for the compounds prepared for this work are summarized in Table I. Among them, compounds 6, 9, and 14–16 had not been previously described and compounds 1–4 were obtained in the crystalline form for the first time.

The simplest method for the attachment of a thioether group to the C-3 position of a catechol involves the oxi-

(1) G. Prota, *J. Invest. Dermatol.*, **75**, 122 (1980).
(2) G. Agrup, P. Agrup, T. Andersson, L. Hafström, C. Hansson, S. Jacobsson, P.-E. Jönsson, H. Rorsman, A.-M. Rosengren, and E. Rosengren, *Acta Dermatovener. (Stockholm)*, **59**, 381 (1979).
(3) M. M. Wick, L. Byers, and E. Frei III, *Science*, **197**, 468 (1977).
(4) M. M. Wick, *Nature (London)*, **269**, 513 (1977).
(5) M. M. Wick, *Science*, **199**, 775 (1978).
(6) M. M. Wick, *Cancer Treat. Rep.*, **69**, 991 (1979).
(7) K. Fujita, S. Ito, S. Inoue, Y. Yamamoto, J. Takeuchi, M. Shamoto, and T. Nagatsu, *Cancer Res.*, **40**, 2543 (1980).

0022-2623/81/1824-0673$01.25/0

Table I. Synthesis and Properties of Cysteinyl-3,4-dihydroxyphenylalanines and Related Compounds

compd	method[a]	mp, °C (dec)	emp formula	anal.	UV[b] λ_{max}, nm (ϵ)	UV[b] λ_{min}, nm (ϵ)
1[c]	B[c]	209	$C_{12}H_{16}N_2O_6S \cdot H_2O$	C, H, N, S	293 (2930), 253 (3630)	273 (1340), 247 (3440)
2[d]	B[c]	231	$C_{12}H_{16}N_2O_6S \cdot 2.5H_2O$	C, H, N, S	294 (3380), 255 (2450)	272 (1210), 249 (2330)
3[e]	C[e]	255	$C_{12}H_{16}N_2O_6S \cdot 0.25H_2O$	C, H, N, S	292 (3110), 253 (5840)	274 (1870), 242 (5080)
4[f]	A[g]	233	$C_{15}H_{21}N_3O_8S_2 \cdot 2H_2O$	C, H, N, S	303 (3420), 273 (9550)	297 (3340), 251 (3710)
5[f]	A[f]	175[f]	$C_9H_{11}NO_4S \cdot HCl \cdot H_2O$	f	288 (2610), 252 (3530)[f]	271 (1380), 241 (2690)
6	C	208	$C_9H_{11}NO_4S$	C, H, N, S	288 (3210), 253 (5900)	272 (2140), 237 (4500)
7[f]	A[f]	262[f]	$C_{12}H_{16}N_2O_6S_2 \cdot 2H_2O$	f	298 sh (3200), 270 (10 800)[f]	246 (3710)
8[h]	B[h]	196[h]	$C_{10}H_{13}NO_4S$	C, H, N	293 (2880), 252 (3490)[h]	272 (1100), 244 (3170)
9	B	204	$C_{10}H_{16}N_2O_6S \cdot 2HCl$	C, H, N, S; Cl[i]	291 (2850), 251 (3580)	271 (1240), 244 (3310)
13[e]	C[e]	192	$C_{12}H_{16}N_2O_5S \cdot H_2O$	C, H, N, S	289 (3140), 251 (2880)[j]	269 (1270), 242 (2550)[j]
14	C	175	$C_9H_{11}NO_3S$	C, H, N, S	283 (3050), 250 (2940)[k]	266 (1490), 236 (2100)[k]
15	C	201	$C_9H_{11}NO_3S$	C, H, N, S	251 (7150)[l]	237 (5520)[l]
16[m]	m	280	$C_9H_{14}N_4O_4S \cdot 0.5H_2O$	C, H, N, S	253 (7610), 219 (5270)	233 (4660)

[a] Method A, enzymatically generated quinone plus cysteine; method B, chemically generated quinone plus cysteine or cysteamine; method C, catechol or phenol plus cystine in aqueous HBr. [b] In 0.1 M HCl, unless otherwise stated. [c] Reference 16. [d] E. Fattorusso, L. Minale, S. DeStefano, G. Cimino, and R. A. Nicolaus, *Gazz. Chim. Ital.*, **99**, 969 (1969). [e] Reference 9. [f] Reference 12. [g] Reference 8. [h] G. Prota, G. Scherillo, and R. A. Nicolaus, *Gazz. Chim. Ital.*, **97**, 1451 (1967), mp 192–194 °C dec. [i] Cl: calcd, 23.54; found, 23.11. [j] In 0.1 M NaOH: λ_{max} 311 nm (ϵ 5600); λ_{min} 279 nm (ϵ 1140). [k] In 0.1 M NaOH: λ_{max} 305 nm (ϵ 5590); λ_{min} 274 nm (ϵ 1280). [l] In 0.1 M NaOH: λ_{max} 260 nm (ϵ 15 670); λ_{min} 228 nm (ϵ 5120). [m] To be published elsewhere.

dation of the catechol with mushroom tyrosinase in the presence of a thiol (method A). For example, enzymatic oxidation of a mixture of L-Dopa and L-cysteine yielded 5- and 2-*S*-cysteinyl-Dopa (**1** and **2**, respectively) in the ratio of 5:1.[8] The reaction proceeds via formation of Dopaquinone, followed by 1,6-addition of the sulfhydryl group of cysteine. 2,5-*S*,*S*-Dicysteinyl-Dopa (**4**), the product of two consecutive 1,6-addition reactions of cysteine, was also produced in a low yield.[8] Small amounts (10–100 mg) of compounds **1**, **2**, **4**, **5**, **7**, and **9** were easily prepared by this method using L-Dopa, dopamine, or pyrocatechol as a catechol and L-cysteine or cysteamine as a thiol. However, there is a disadvantage in this method: the preparation of the adducts in gram quantitites requires large amounts of the enzyme tyrosinase. Therefore, some of the compounds examined for in vivo antitumor activity were prepared by the reaction of thiols with *o*-benzoquinones, formed chemically by oxidation of the corresponding catechols with silver oxide (method B). Thus, the oxidation of *N*-acetyl-L-Dopa ethyl ester, followed by the addition of L-cysteine and acid hydrolysis, gave a mixture of **1** and **2**, which was separated by chromatography on Dowex 50W using 2 M HCl as eluent.[8] This method provided us with gram quantities of **1** as a crystalline product. Similarly, 5-*S*-cysteaminyldopamine (**9**) was obtained from *N*-acetyldopamine and cysteamine. The structure of compound **9** was verified by UV, NMR, and elemental analyses.

Since the addition of thiols to *o*-benzoquinones takes place almost exclusively at the C-3 position, an alternative approach was required for the introduction of a thioether group at the C-4 position. One of us (S. Ito) has previously reported that the phenolic amino acids tyrosine and Dopa reacted with cystine in boiling aqueous HBr to give 3-*S*-cysteinyltyrosine (**13**) and 5-, 2-, and 6-*S*-cysteinyl-Dopa (**1**–**3**), respectively (method C).[9] The reaction proceeds by electrophilic substitution of the cysteine sulfenyl cation, $HOOCCH(NH_2)CH_2S^+$, formed under the strongly acidic conditions,[10] on the phenol and catechol rings. We reexamined the reaction and applied it for the synthesis of 4-*S*-cysteinylcatechol (**6**) and 2- and 4-*S*-cysteinylphenol (**14** and **15**). Reaction of L-Dopa with L-cystine in boiling aqueous 47% HBr for 2–6 h gave crystalline 6-*S*-cysteinyl-Dopa (**3**) in 6–7% yields, together with lesser amounts of 5- and 2-S isomers (**1** and **2**).[11] The 6-S isomer (**3**) had previously been isolated as a product of the enzymatic oxidation in less than 1% yield.[8] Similarly, pyrocatechol reacted with L-cystine in hot aqueous HBr to afford a 1:10 mixture of 3- and 4-*S*-cysteinylcatechol (**5** and **6**), from which **6** was isolated as a crystalline product in 35% yield. The 3-S isomer (**5**) had previously been prepared by the enzymatic method.[12] 3-*S*-Cysteinyltyrosine (**13**) was obtained in 12% yield from L-tyrosine and L-cystine by heating for 6 h.[13] Reaction of phenol and L-cystine for 2 h gave a 2:5 mixture of 2- and 4-*S*-cysteinylphenol (**14** and **15**), from which **14** and **15** were isolated by fractional crystallization in 2.3 and 37% yields, respectively. These newly synthesized compounds **6**, **14**, and **15**, as well as **3** and **13**, were characterized by UV, NMR, and elemental analyses. Thus, the cystine–HBr method proved suitable for the preparation of 4-*S*-cysteinylcatechols and 2- and 4-*S*-cysteinylphenols which appear difficult to prepare by

(8) S. Ito and G. Prota, *Experientia*, **33**, 1118 (1977).

(9) S. Ito and G. Prota, *J. Chem. Soc., Chem. Commun.*, 251 (1977). In the title of this paper, cystine was erroneously printed as cysteine.

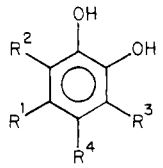
(10) R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **80**, 1966 (1958).

(11) Products before crystallization may be composed of diastereomers as the result of racemization at the α-carbon atoms of cystine. However, as judged by the $[\alpha]_D$ values of crystals of **1** and **2**, crystallization appears to give rise to practically pure isomers having the L,L configuration.

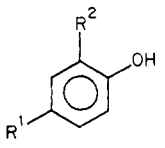
(12) S. Ito and J. A. C. Nicol, *Biochem. J.*, **161**, 499 (1977).

(13) The stereochemical purity of this preparation could not be determined because of the lack of a suitable method.

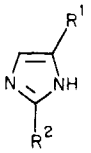
Chart I



		R^1	R^2	R^3	R^4
1	5-*S*-cysteinyl-Dopa	Ala[a]	H	Cys[b]	H
2	2-*S*-cysteinyl-Dopa	Ala	Cys	H	H
3	6-*S*-cysteinyl-Dopa	Ala	H	H	Cys
4	2,5-*S*,*S*-dicysteinyl-Dopa	Ala	Cys	Cys	H
5	3-*S*-cysteinyl-catechol	H	Cys	H	H
6	4-*S*-cysteinyl-catechol	Cys	H	H	H
7	3,6-*S*,*S*-dicysteinyl-catechol	H	Cys	Cys	H
8	3-*S*-cysteinyl-5-methyl-catechol	Me	H	Cys	H
9	5-*S*-cysteaminyldopamine	$CH_2CH_2NH_2$	H	$SCH_2CH_2NH_2$	H
10	L-Dopa	Ala	H	H	H
11	dopamine	$CH_2CH_2NH_2$	H	H	H
12	4-methyl-catechol	Me	H	H	H



13-15



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		R^1	R^2
13	3-*S*-cysteinyltyrosine	Ala[a]	Cys[b]
14	2-*S*-cysteinylphenol	H	Cys
15	4-*S*-cysteinylphenol	Cys	H
16	2-*S*-cysteinylhistidine	Ala	Cys

[a] Ala = $CH_2CH(NH_2)COOH$. [b] Cys = $SCH_2CH(NH_2)$-COOH.

conventional methods. It should be mentioned that the prolonged heating in aqueous HBr resulted in some racemization at the α-carbon atoms of amino acids, especially of cystine. Under the experimental conditions, the optical purity of recovered L-cystine decreased to 54–56% after heating for 6 h in aqueous HBr. However, the racemization may be avoided to a large extent by shortening the reaction time, although this may decrease the yields of products.

Biological Activity. Cysteinyl-3,4-dihydroxyphenylalanines (**1**–**4**) and related compounds (**5**–**16**) were tested for growth inhibition of cultured cells of human neuroblastoma YT-nu and Chinese hamster fibroblast Don-6. These cell lines are of neoplastic origin and of nonneoplastic origin, respectively. The results are summarized in Table II. Compounds **1**, **2**, **10**, **14**, and **15** exhibited moderate to strong toxicity against YT-nu but relatively weak toxicity against Don-6. Replacement of the alanyl moiety of **1** by a methyl group (compound **8**) greatly increased cytotoxicity and decreased selectivity. Similar phenomenon was also observed with L-Dopa (**10**) and 4-methylcatechol (**12**). Removal of the 2-carboxyl group in **1** led to a great increase in cytotoxicity with complete loss of selectivity, as in compound **9**. Similarly, removal of the carboxyl group in L-Dopa (**10**) to give dopamine (**11**) increased cytotoxicity and decreased selectivity. From these results it appears that, if substituents on the catechol rings are similar in electronegativity, more hydrophilic compounds exhibit lower, but more selective, cytotoxicities. This may be attributed to the less efficient uptake into the cells.

Table II. Growth Inhibition of YT-nu and Don-6 Cell Lines by Cysteinyl-3,4-dihydroxyphenylalanines and Related Compounds

compd	concn, mM	growth inhibn[a] YT-nu	Don-6
1	1.0	59 ± 6	11 ± 7
2	1.0	35 ± 3	0
3	1.0	61 ± 0	41 ± 1
4	1.0	56 ± 4	44 ± 4
5	1.0	20 ± 5	32 ± 2
6	1.0	76 ± 5	81 ± 2
	0.1	71 ± 1	69 ± 1
7	1.0	40 ± 8	30 ± 0
8	1.0	84 ± 4	82 ± 4
	0.1	44 ± 4	25 ± 1
9	1.0	65 ± 2	83 ± 1
	0.1	48 ± 1	73 ± 1
10	1.0	24 ± 2	6 ± 1
11	1.0	40 ± 2	30 ± 3
12	1.0	93 ± 1	76 ± 1
	0.1	48 ± 2	33 ± 2
13	1.0	7 ± 2	5 ± 0
14	1.0	51 ± 1	5 ± 1
15	1.0	36 ± 0	5 ± 4
16	1.0	7 ± 2	0

[a] Percentage of growth inhibition according to the formula [(number of control cells − number of treated cells)/number of control cells] × 100 by comparison with parallel control cultures that were manipulated similarly except that they did not have a drug added. Each value represents the mean plus or minus SE of three determinations.

4-*S*-Cysteinylcatechol (**6**), the S-homologue of L-Dopa, was highly toxic to both cell lines. This may be related to the fact that **6** is quite susceptible to autoxidation, forming a puple pigment. Graham et al. have shown that the rate of autoxidation of catechols correlates with their cytotoxicity.[14] The *S*-cysteinyl moiety itself is not a prerequisite for cytotoxicity, as seen in compounds **13** and **16**. The addition of a second cysteinyl moiety to **1** or **2** and to **5** (compounds **4** and **7**) did not improve the activity.

The antitumor activities of compounds **1**, **6**, **8**, **9**, and **15** were then examined in L1210 leukemia bearing mice. These compounds were selected because they were readily available in gram quantities and because they exhibited either selective toxicity to YT-nu cells or nonselective, but higher, toxicity to both cell lines. Compounds **1**, **6**, and **8** significantly prolonged the life span (Table III). 4-*S*-Cysteinylcatechol (**6**) at 500 mg/kg was toxic to the mice, as judged by their weight loss, whereas 5-*S*-cysteinyl-Dopa (**1**) did not exhibit any toxicity at 1000 mg/kg.[7] Postmortem examinations of mice treated with compound **6** disclosed extensive dark-green coloration of the peritoneal cavity. 5-*S*-Cysteaminyldopamine (**9**) was too toxic to show any activity. Phenol **15** could not be administered at doses higher than 250 mg/kg because of its limited water solubility. Compounds exhibiting activity against L1210 leukemia were then examined for the effects on the life span of B-16 melanoma bearing mice. High doses of **1** and **8** increased the life span, although marginally, but compound **6** was toxic at doses below those required for antitumor

(14) D. G. Graham, S. M. Tiffany, W. R. Bell, Jr., and W. F. Gutknecht, *Mol. Pharmacol.*, **14**, 644 (1978).

Table III. Antitumor Activity of Cysteinyl-3,4-dihydroxyphenylalanine and Related Compounds against L1210 Leukemia and B-16 Melanoma

tumor	compd[a]	dose, (mg/kg)/ day	wt change,[b] g	% T/C[c]
L1210	1	500	+1.7	137[d,e]
		1000	+1.7	150[d,e]
	6	100	+1.4	113[f]
		300	+0.5	138[e]
		500	−1.0	150[e]
	8	400	+1.6	143[f]
		800	+0.5	143
	9	100	+1.1	100
		150	−2.1	88
	15	250	+3.0	100
B-16	1	500	+2.6	108[d]
		1000	+2.1	127[d,g]
	6	100	+1.8	109
		300	−1.3	114
		500	−1.5	95
	8	400	+0.7	100
		800	−0.4	126[f]

[a] Compounds were injected ip for 7 days to L1210-bearing mice and 12 days to B-16-bearing mice. [b] Average weight gain or loss between days 1 and 5. [c] % T/C = (median survival time of treated animals/that of control animals) × 100. [d] Cited from ref 7 for comparison. [e] $p < 0.001$. [f] $p < 0.01$. [g] $p < 0.05$.

activity. Compound 8 was somewhat toxic at 800 mg/kg.

It should be noted that the results of the in vitro and in vivo assays correlate well with each other. 5-*S*-Cysteinyl-Dopa (1), which showed selective toxicity in vitro, was also selectively toxic to tumor cells in vivo, and the corresponding decarboxy analogue, 5-*S*-cysteaminyldopamine (9), which was strongly cytotoxic, was also highly toxic in vivo. The observed in vivo activity of 8 in both L1210 and B-16 systems may be attributable to the low but significant selectivity in cytotoxicity.

Two mechanisms have been proposed for cytotoxicity of catechols:[14] (1) production of cytotoxic hydrogen peroxide, superoxide, and hydroxyl radicals as the result of autoxidation and (2) production of the corresponding *o*-benzoquinones, which kill cells through inhibition of sulfhydryl enzymes. Strong toxicity of compounds 6, 9, and others to both YT-nu and Don-6 cell lines may be a reflection of one or both of these mechanisms. However, selective toxicity of 1 and others to YT-nu cells cannot be explained by these mechanisms alone; therefore, there must be other factors for the selectivity.

It may be speculated that compounds exhibiting selective cytotoxicity cannot enter the cells rapidly and therefore act mostly outside of their cell membrane, while highly toxic compounds enter the cells rapidly and act therein. In fact, studies in progress in our laboratory have shown that the toxicity of 5-*S*-cysteinyl-Dopa (1) to YT-nu cells is completely depressed by the addition of catalase to the medium, indicating hydrogen peroxide to be the mediator of the cytotoxicity. The mechanism by which phenols 14 and 15 are selectively toxic to YT-nu cells is not clear at present. However, it may be suggested that these phenols would be converted to the corresponding catechols within the cells which may contain tyrosine hydroxylase.[15]

In conclusion, catechols which are strongly toxic to both YT-nu and Don-6 cells are also toxic in vivo, and catechols which are selectively toxic to YT-nu cells possess antitumor activity with fewer toxic effects. It also appears that more hydrophilic catechols exhibit higher selectivity.

Experimental Section

Chemistry. All melting points were determined in capillary tubes and are uncorrected. Ultraviolet spectra were recorded on a Beckman Model UV 5260 spectrophotometer, and optical rotations were measured in 1 M HCl with a JASCO DIP-4 automatic polarimeter at 25 °C. NMR spectra were taken in 2 M DCl–D_2O with a JEOL JNM-PMX 60 spectrometer or a NEVA NV-21 90-MHz spectrometer, using as internal reference the methyl signal of 2-methyl-2-propanol which appears at δ 1.28. Amino acid analyses were carried out on a JEOL JLC-6AH amino acid analyzer. Microanalyses were performed by the Microanalytical Laboratory, Faculty of Science, Osaka University, and by the Analytical Laboratory, Faculty of Pharmacy, Meijo University. Column chromatography utilized Dowex 50W-X2 resin (200–400 mesh) which had been equilibrated with diluted HCl. Solvents were removed with a rotary evaporator.

5- (1) and 2-*S*-Cysteinyl-3,4-dihydroxyphenylalanine (2). Method B. The previously reported method of Prota et al.[16] was slightly modified. To a solution of 4.00 g (15 mmol) of *N*-acetyl-L-Dopa ethyl ester[16] in 200 mL of methanol containing 2 mL of 98% formic acid, cooled in an ice bath, were added 12 g of anhydrous sodium sulfate and 12 g of silver oxide. The mixture was vigorously stirred for 3 min and filtered through a layer of sodium sulfate, and the filtered mass was washed with methanol. The combined, wine-red filtrate was added dropwise over 10 min to a stirred solution of 3.51 g (20 mmol) of L-cysteine hydrochloride monohydrate in 60 mL of water and 60 mL of methanol at room temperature. The resultant pale-yellow suspension was evaporated to dryness, and the residue was dissolved in 80 mL of 6 M HCl and refluxed for 1 h. The hydrolysate was evaporated to dryness, and the residue was taken up in 2 M HCl and applied on a column (3.6 × 30 cm) of Dowex 50W (equilibrated with 2 M HCl). The column was eluted with 2 M HCl, and fractions of 20 mL were collected and analyzed by UV. Fractions 121–151 and 155–200 contained **2** and **1**, respectively, which were evaporated to give pale-yellow, crystalline HCl salts of the amino acids. They were crystallized from aqueous 1% sodium metabisulfate adjusted to pH 6 with solid sodium acetate to afford ~3.5 g (70%) of **1** and ~0.4 g (7%) of **2** as colorless crystals: **1**, $[\alpha]_D$ +62° (c 0.5); **2**, $[\alpha]_D$ +59° (c 0.5). These crystalline preparations of 1 and 2 were homogeneous as determined by amino acid analyses.

5-*S*-Cysteaminyldopamine (9). Method B. Since the reaction conditions have not been optimized, the yield of **9** may be much improved. To a solution of 9.48 g (50 mmol) of dopamine hydrochloride in 10 mL of water was added in portions 40 mL of acetic anhydride over a period of 3 h. The pH of the reaction mixture was kept at 3.0 ± 0.1 by the occasional addition of solid sodium acetate. TLC analysis revealed the presence of unchanged dopamine, *N*-acetyldopamine, *O*-acetyldopamine, and *N,O*-diacetyldopamine. The resultant solution was extracted with ethyl acetate (3 × 300 mL), washed with water (2 × 100 mL), and evaporated to leave a mixture of *N*-acetyldopamine and *N,O*-diacetyldopamine; dopamine and *O*-acetyldopamine remained in the aqueous layer. The oily mixture was dissolved in 400 mL of methanol containing 4 mL of 98% formic acid and then cooled in an ice bath. To the solution were added 24 g of anhydrous sodium sulfate and 24 g of silver oxide. The mixture was vigorously stirred for 3 min and filtered through a layer of sodium sulfate, and the filtered mass was washed with methanol. The combined, wine-red filtrate was added dropwise over 10 min to a stirred solution of 5.68 g (50 mmol) of cysteamine hydrochloride in 100 mL of water and 100 mL of methanol. The resultant pale-yellow suspension was evaporated to dryness and the residue was dissolved in 100 mL of 6 M HCl and refluxed for 2 h. The hydrolysate was evaporated and the residue was chromatographed on a column (3.6 × 25 cm) of Dowex 50W (equilibrated with 3 M HCl). The column was eluted with 3 M HCl, and fractions of 20 mL were collected and analyzed by UV. Fractions 46–70 and 128–205 contained dopamine (3.3 g as the HCl salt, 35%)

(15) S. Imashuku, H. Takeda, T. Sawada, T. Nakamura, and E. H. LaBrosse, *Cancer*, **36**, 450 (1975).

(16) G. Prota, G. Scherillo, and R. A. Nicolaus, *Gazz. Chim. Ital.*, **98**, 495 (1968).

and cysteaminyldopamines, respectively. Rechromatography of fractions 128–205 on the same column and crystallization from 6 M HCl–acetone afforded 1.13 g (7.5%) of **9** as colorless crystals: ^{1}H NMR δ 2.96 (2 H, br d, J = 6.5 Hz, CH_2), 3.20 (4 H, s, CH_2CH_2), 3.23 (2 H, br d, J = 6.5 Hz, CH_2), 6.87 (1 H, d, J = 2 Hz, ArH), 6.96 (1 H, d, J = 2 Hz, ArH).

2,5-*S*,*S*-Dicysteinyl-3,4-dihydroxyphenylalanine (4). The HCl salt (56 mg) of **4**, prepared by the enzymatic method,[8] was converted to the free amino acid by neutralization of a 1% sodium metabisulfate solution with sodium acetate: colorless crystals; yield 26 mg; $[\alpha]_D$ +152° (c 0.2).

6-*S*-Cysteinyl-3,4-dihydroxyphenylalanine (3). Method C. A mixture of 1.97 g (10 mmol) of L-Dopa and 2.40 g (10 mmol) of L-cystine in 100 mL of 47% HBr was refluxed for 6 h. The resultant yellow solution was evaporated to dryness, and the residue was chromatographed on a column (3.6 × 23 cm) of Dowex 50W (equilibrated with 2 M HCl). The column was eluted with 2 M HCl, and fractions of 20 mL were collected and analyzed by UV. Evaporation of fractions 24–42 and 45–62 left cystine and Dopa as the HCl salts, respectively, which were converted to the free amino acids: cystine, yield 1.90 g (79.2%); $[\alpha]_D$ −117° (c 0.5); Dopa, yield 1.36 g (69.0%); $[\alpha]_D$ −10.1° (c 1.0). Authentic L-cystine and L-Dopa had $[\alpha]_D$ values of −210° (c 0.5) and −10.3° (c 1.0), respectively. Evaporation of fractions 66–98, 102–138, and 168–212 afforded 385 (9.5% as estimated by UV), 116, and 153 mg of the HCl salts of **3**, **2**, and **1**, respectively. Neutralization of a solution of **3** in 1% sodium metabisulfate with sodium acetate gave 196 mg (6.0%) of free **3** as colorless prisms: $[\alpha]_D$ +85° (c 0.5); ^{1}H NMR δ ~3.5 (2 H, m, AB part of an ABX system, CH_2), 3.51 (2 H, d, J = 7–8 Hz, CH_2), 4.38 (1 H, t, J = 5.5 Hz, CH), 4.38 (1 H, br t, J = 7–8 Hz, CH), 6.94 (1 H, s, C_2 H), 7.20 (1 H, s, C_5 H). Crystalline 5- and 2-*S*-cysteinyl-Dopa (**1** and **2**) were also obtained in yields of 47 (1.4%) and 66 mg (1.8%), respectively. They had $[\alpha]_D$ values of +59° (c 0.1)[11] and +57° (c 0.1).[11] Reaction of L-Dopa with L-cystine for 2 h yielded 418 (10.4% as estimated by UV), 84, and 91 mg of the HCl salts of **3**, **2**, and **1**, respectively. Thus, the overall yield of cysteinyl-Dopa isomers is higher at 6 h than at 2 h. However, for the preparation of the 6-S isomer (**3**), the 2-h reaction is preferred.

4-*S*-Cysteinylcatechol (6). Method C. A mixture of 22.0 g (200 mmol) of pyrocatechol and 12.0 g (50 mmol) of L-cystine in 500 mL of 47% HBr was refluxed for 1 h. The yellow solution was evaporated to dryness, and the residue was dissolved in 2 M HCl (50 mL) and extracted with ethyl acetate (3 × 100 mL) to remove unreacted pyrocatechol and other byproducts. The aqueous layer was evaporated and the residue was chromatographed as described above. Fractions 56–120 contained 7.24 g of a mixture of the HCl salts of **5** and **6** (ca. 1:10 by UV analysis), which was crystallized from 1% sodium metabisulfate adjusted to pH 6 with sodium acetate to give 4.04 g (35.2%, based on cystine) of **6** as colorless to pale-violet prisms: $[\alpha]_D$ +80° (c 0.5); ^{1}H NMR (90 MHz) δ 3.48 (2 H, d, J = 5.5 Hz, CH_2), 4.26 (1 H, t, J = 5.5 Hz, CH), 6.92 (1 H, dd, J = 8.5 and 0.7 Hz, C_6 H), 6.96 (1 H, dd, J = 8.5 and 2.0 Hz, C_5 H), 7.11 (1 H, dd, J = 2.0 and 0.7 Hz, C_3 H). Amino acid analysis of the sample revealed no contamination by the isomer **5**.

3-*S*-Cysteinyltyrosine (13). Method C. A mixture of 3.62 g (20 mmol) of L-tyrosine and 4.81 g (20 mmol) of L-cystine in 200 mL of 47% HBr was refluxed for 6 h. The pale-yellow solution was evaporated to dryness and the residue was chromatographed on a column (3.6 × 21 cm) of Dowex 50W (equilibrated with 3 M HCl). Fractions 10–27 and 37–66 afforded 3.86 g (80.2%) of cystine, $[\alpha]_D$ −113° (c 0.5), and 2.83 g (78.2%) of tyrosine, $[\alpha]_D$ −8.8° (c 1.0). Authentic L-tyrosine had $[\alpha]_D$ −10.0° (c 1.0). Evaporation of fractions 91–158 gave 1.37 g (16.3% by UV) of the HCl salt of **13** as a pale-yellow oil. Free amino acid **13** was crystallized by adding ethanol to an aqueous solution of the HCl salt adjusted to pH 6 with pyridine: yield 766 mg (12.0%); $[\alpha]_D$ +49° (c 0.5);[13] ^{1}H NMR (90 MHz) δ ~3.24 (2 H, m, AB part of an ABX system, CH_2), ~3.51 (2 H, m, AB part of an ABX system, CH_2), 4.31 (1 H, t, J = 5.5 Hz, CH), 4.40 (1 H, m, CH), 7.01 (1 H, d, J = 8.3 Hz, C_5 H), 7.25 (1 H, dd, J = 8.3 and 2.0 Hz, C_6 H), 7.47 (1 H, d, J = 2.0 Hz, C_2 H).

2- (14) and 4-*S*-Cysteinylphenol (15). Method C. A mixture of 18.8 g (10 mmol) of phenol and 12.0 g (50 mmol) of L-cystine in 500 mL of 47% HBr was refluxed for 2 h. The orange solution was worked up as described for **6**. Fractions 71–170 contained 7.08 g of a mixture of the HCl salts of **14** and **15** (ca. 2:5 by UV analysis), which by crystallization from water–sodium acetate gave 5.07 g (47.6%, based on cystine) of a crystalline mixture of free amino acids. Fractional crystallization from water afforded 0.40 g (2.3%) of **14** as colorless needles and 3.94 g (37.0%) of **15** as colorless plates. **14**: $[\alpha]_D$ +83° (c 0.5); ^{1}H NMR δ 3.47 (2 H, d, J = 5.5 Hz, CH_2), 4.26 (1 H, t, J = 5.5 Hz, CH), 6.8–7.7 (4 H, m, ArH). **15**: $[\alpha]_D$ +93° (c 0.5); ^{1}H NMR δ 3.48 (2 H, d, J = 5.5 Hz, CH_2), 4.28 (1 H, t, J = 5.5 Hz, CH), 6.95 (2 H, d, J = 8.5 Hz, C_2 and C_6 H), 7.47 (2 H, d, J = 8.5 Hz, C_3 and C_5 H). These isomeric cysteinylphenols were practically (purity, ~99%) free from the isomer as determined by amino acid analyses.

Biology. In Vitro. The cell line of human neuroblastoma YT-nu was a gift from Professor T. Suzuki, Department of Pathology, School of Medicine, Niigata University, and Chinese hamster fibroblast Don-6, was a gift from the Aichi Cancer Center Research Institute (Nagoya). Single cell suspensions in McCoy's 5A medium, supplemented with 15% fetal calf serum, 100 units/mL of penicillin, and 100 μg/mL streptomycin, were inoculated into 60-mm Falcon petri dishes, and the cells were allowed to attach for 24 h prior to exposure to a drug. After the cells were washed, 1 mL of a Hanks' balanced salt solution containing 1 or 0.1 mM of a drug was added, and cultures were incubated at 37 °C for 1 h. The exposed cells were then grown in the McCoy's 5A medium for 48 h. Cells were harvested by trypsinization with 0.25% trypsin–EDTA and counted in a Model Z Coulter counter.

In Vivo. L1210 leukemia and B-16 melanoma have been maintained by following National Cancer Institute protocols.[17] On day 0, young C57 BL/6 × DBA/2 F_1 male mice (10 mice per group) weighing about 20 g were given ip inoculations of either 1×10^5 leukemia cells or 5×10^6 melanoma cells. Starting on day 1, drugs were given as a 0.9% NaCl solution ip once a day for 7 days to L1210 leukemia bearing mice and for 12 days to B-16 leukemia bearing mice.

(17) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbot, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).