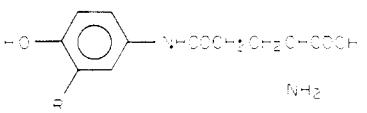
Structural Analogues of L-Glutamic Acid γ-(4-Hydroxyanilide) and γ-(3,4-Dihydroxyanilide) as Potential Agents against Melanoma

A. Rosowsky,* M. M. Wick, and S.-H. Kim

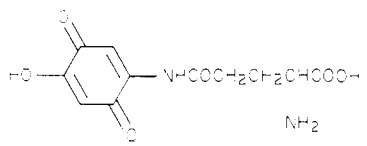
The Sidney Farber Cancer Institute and Departments of Pharmacology and Dermatology, Harvard Medical School, Boston, Massachusetts 02115. *Received March 12, 1979*

Nine heretofore unknown mono- and dihydroxyanilide analogues of the cytotoxic mushroom metabolites L-glutamic acid γ-(4-hydroxyanilide) (1) and L-glutamic acid γ-(3,4-dihydroxyanilide) (3, agaridoxin) were synthesized and tested as inhibitors of the growth of B16 mouse melanoma cells in culture. The naturally occurring anilides 1 and 3 had ID_{50} values of 0.10 and 0.27 mM, respectively. The analogue of 1 in which the γ-L-glutamyl moiety was replaced by β-L-aspartyl showed only a threefold decrease in activity, whereas attachment of the phenolic OH group to the meta instead of the para position resulted in a tenfold decrease. Other structural modifications, such as O-methylation or deletion of the carboxyl or amino group in the side chain, led to compounds of still lower activity (ID_{50} >1.0 mM). The only analogue in the series with more activity than either 1 or 3 against B16 cells was L-glutamic acid γ-(2,5-dihydroxyanilide) (14), which had an ID_{50} value of 0.051 mM. These data suggest that the γ-L-glutamyl side chain in 1 or 3 plays a significant role in the biological action of these compounds, though some flexibility appears to exist insofar as the positioning of OH groups on the aromatic ring is concerned.

L-Glutamic acid γ-(4-hydroxyanilide) (1) is a natural



1, R = H
3, R = OH



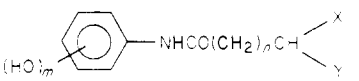
2

growth-regulatory substance which occurs in the gills of the common mushroom *Agaricus bisporus* and is an excellent substrate for the enzyme tyrosinase.[1] In the presence of this enzyme, the colorless phenol is converted rapidly to a red product with an absorbance maximum at 490 nm. Although it was originally proposed that this is an ortho quinone,[2,3] more recent data favor alternative structures such as **2**.[4] Metabolic conversion of 1 to the 490-nm quinone was postulated to occur via the transient intermediate L-glutamic acid γ-(3,4-dihydroxyanilide) (**3**),[1,3] and this compound was, in fact, isolated subsequently from the closely related mushroom species *Agaricus campestris* and named agaridoxin.[5] The catechol structure of **3** was confirmed by chemical synthesis.[5]

The natural role of the putative quinone metabolite in the life cycle of the mushroom is thought to be the induction and maintenance of spore dormancy, i.e., a "cryptobiotic state" in which energy utilization and macromolecular synthesis virtually cease.[6] The quinone is a potent thiol reagent and can apparently inhibit energy production in rat liver mitochondria by irreversibly alkylating cysteine residues in succinic dehydrogenase, α-ketoglutarate dehydrogenase, pyruvate dehydrogenase, xanthine dehydrogenase, dihydroorotate dehydrogenase, and other SH-containing enzymes.[7] It has also been found to inhibit RNA polymerase in *Escherichia coli*[8] and DNA polymerase from L1210 mouse leukemia and four other murine tumor lines in vitro.[9] Inhibition of DNA polymerase from L1210 cells was more profound than with either *p*-chloromercuribenzoate or *N*-ethylmaleimide and was less readily reversed by dithioerythritol.[9] Preincubation of the enzyme with the 490-nm quinone prior to the addition of deoxynucleotide precursors markedly decreased DNA synthesis, and it was suggested that DNA polymerase may have specific binding sites with a high affinity for this particular quinone.

On preincubation with tyrosinase, solutions of compound 1 have been shown to become toxic to bacteria and cultured leukemic cells.[7] Without prior tyrosinase activation, the phenol itself reportedly causes some inhibition of growth of B16 melanoma in the mouse, this selective antitumor effect being ascribed to the presence of much higher tyrosinase levels in melanoma cells than in normal tissues of the host.[10,11] Interestingly, catechol **3** is said to have no experimental antitumor activity, although details are lacking.[5]

In order to help elucidate the mode of action of L-glutamic acid γ-(4-hydroxyanilide) and its putative catechol metabolite agaridoxin, we have performed a comparative study of the properties of a series of structural analogues. This paper describes the chemical synthesis of these compounds and summarizes the structure–activity correlations that have been established in bioassays against B16 melanoma cells in culture. The structural variations that have been made in order to define the structure–activity requirements of the series are expressed in the general structure i.



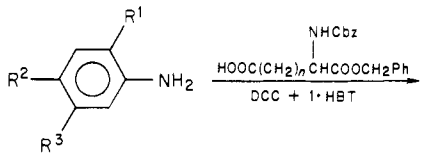
i

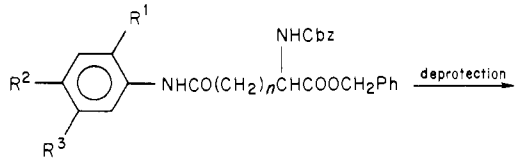
Chemistry. The syntheses of monohydroxyanilides and dihydroxyanilides accomplished during this work are outlined in Schemes I–IV.

p-(Benzyloxy)aniline and *N*-(carbobenzyloxy)-L-glutamic acid α-benzyl ester were condensed in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (1-HBT),[12] and the resultant γ-glutamylanilide 4 was deprotected by catalytic hydrogenolysis to obtain compound **1**. Similar reactions of *p*-anisidine, *m*-(benzyloxy)aniline, 3,4-(methylenedioxy)aniline, and 3,4- and 2,5-dimethoxyaniline yielded the γ-glutamylanilides **5–9**, respectively. Condensation of *p*-(benzyloxy)aniline with *N*-(carbobenzyloxy)-L-aspartic acid α-benzyl ester afforded the protected γ-aspartylanilide **10**, in which the side chain is shortened by one carbon. Hydrogenolysis of compounds **5–7** and **10** gave the expected products **11–13** and **15**. Overall yields of these anilides were 40–60%, except for compound **12** which was obtained in 80% overall yield. For the preparation of the dihydroxyanilides **3** and **14**, compounds **8** and **9** were treated with boron tribromide in dichloromethane at room temperature overnight.[13]

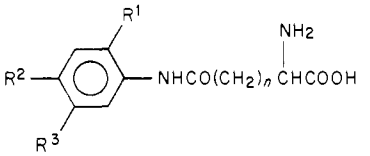
0022-2623/79/1822-1034$01.00/0

Scheme I. Synthesis of L-Glutamic Acid γ-(4-Hydroxyanilide) and Related Compounds



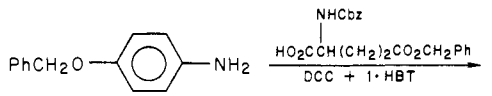


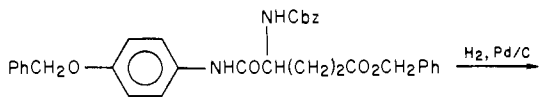
	n	R¹	R²	R³
4	2	H	$PhCH_2O$	H
5	2	H	MeO	H
6	2	H	H	$PhCH_2O$
7	2	H	$-OCH_2O-$	
8	2	H	MeO	MeO
9	2	MeO	H	MeO
10	1	H	$PhCH_2O$	H



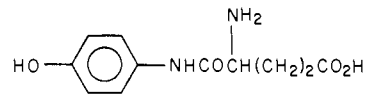
	n	R¹	R²	R³
1	2	H	HO	H
3	2	H	HO	HO
11	2	H	MeO	H
12	2	H	H	HO
13	2	H	$-OCH_2O-$	
14	2	HO	H	HO
15	1	H	HO	H

Scheme II. Synthesis of L-Glutamic Acid α-(4-Hydroxyanilide)





16



17

Overall yields of the dihydroxy derivatives were 20–30%. Use of boron tribromide was very advantageous because it permitted removal of N- and C-protecting groups and bis-O-demethylation *in a single step.*

As shown in Scheme II, condensation of *p*-(benzyloxy)aniline with *N*-(carbobenzyloxy)-L-glutamic acid γ-benzyl ester and hydrogenolysis of the resultant α-glutamyl anilide **16** gave a 49% overall yield of **17**, which is a positional isomer of **1**.

Analogues of **1** in which the amino or carboxyl group of the glutamyl side chain is replaced by hydrogen were also prepared (Scheme III). Condensation of *p*-(benzyloxy)-aniline with *N*-(carbobenzyloxy)-4-aminobutyric acid via the mixed anhydride method gave anilide **18**, and hydrogenolysis of **18** led to the decarboxy analogue **19** in 30% overall yield. On the other hand, when *p*-(benzyloxy)-aniline was condensed with glutaric acid monomethyl ester via DCC coupling and the resultant anilide **20** was hydrogenolyzed (to **21**) and saponified, the deamino analogue **22** was obtained in 83% overall yield.

A bifunctional derivative was also synthesized as shown in Scheme IV. Starting from glutaric anhydride, condensation with *p*-(benzyloxy)aniline yielded the monoanilide **23**, which was coupled with a second molecule of *p*-(benzyloxy)aniline via the mixed anhydride route. Deprotection of the resultant dianilide **24** afforded the bis(4-hydroxyanilide) **25** in 35% overall yield. It may be noted that in the synthetic schemes depicted in Schemes III and IV the use of a mixed anhydride reaction was necessitated by the unexpected failure of *N*-(carbobenzyloxy)-4-aminobutyric acid and of compound **23** to undergo DCC-mediated coupling with *p*-(benzyloxy)-aniline.

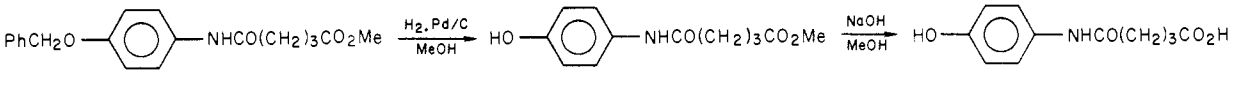
Physical constants for the new compounds prepared during the course of this work are presented in Tables I and II.

Table I. Protected Amino Acid Hydroxyanilides

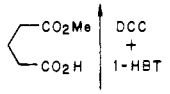
no.	meth[a]	% yield	mp, °C	emp form.	anal.
4	A	57	158–160	$C_{33}H_{32}N_2O_6$	C, H, N
5	A	55	140–142	$C_{27}H_{28}N_2O_6$	C, H, N
6	A	80	123–125	$C_{33}H_{32}N_2O_6$	C, H, N
7	A	51	170–173	$C_{29}H_{26}N_2O_7$	C, H, N
8	A	59	155–158	$C_{28}H_{30}N_2O_7$	C, H, N
9	A	51	113–116	$C_{28}H_{30}N_2O_7$	C, H, N
10	A	87	173–175	$C_{32}H_{30}N_2O_6$	C, H, N
16	A	52	151–153	$C_{33}H_{32}N_2O_6$	C, H, N
18	B	55	158–161	$C_{15}H_{26}N_2O_4$	C, H, N
20	A	83	120–123	$C_{19}H_{21}NO_4$	C, H, N
21	C	~100	93–95	$C_{12}H_{15}NO_4$	C, H, N
23	D	84	165–168	$C_{18}H_{19}NO_3 \cdot 0.75H_2O$	C, H, N
24	B	44	240–243	$C_{31}H_{30}N_2O_4 \cdot 0.1H_2O$	C, H, N

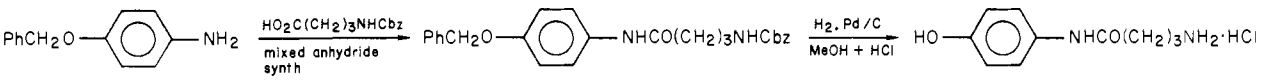
[a] Method A: DCC + 1-HBT coupling; method B: mixed anhydride synthesis (*i*-BuOCOCl/Et_3N); method C: H_2, 10% Pd/C in MeOH–HCl; method D: glutaric anhydride/AcOH.

Scheme III. Synthesis of Deamino and Decarboxy Analogues of L-Glutamic Acid γ-(4-Hydroxyanilide)



20 **21** **22**





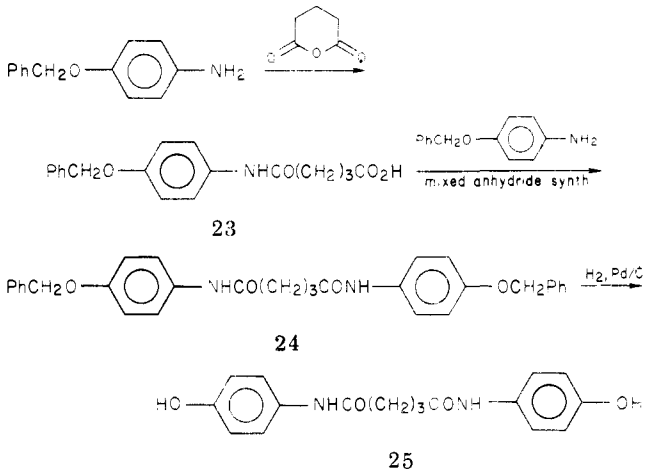
18 **19**

Table II. Amino Acid Hydroxyanilides

no.	meth[a]	% yield	mp, °C (dec)	emp form.	anal.
1	C	~100	220[b]		
3	E	52	205[c]	$C_{11}H_{14}N_2O_4 \cdot 0.5H_2O$	C, H, N
11	C	85	210–212	$C_{12}H_{16}N_2O_4$	C, H, N
12	C	~100	180–183	$C_{11}H_{14}N_2O_4 \cdot 0.75H_2O$	C, H, N
13	C	81	195–197	$C_{12}H_{14}N_2O_5 \cdot 0.75H_2O$	C, H, N
14	E	42	180	$C_{11}H_{14}N_2O_5 \cdot 0.33H_2O$	C, H, N
15	C	56	250–252	$C_{10}H_{12}N_2O_4$	C, H, N
17	C	94	288	$C_{11}H_{14}N_2O_4 \cdot 0.33H_2O$	C, H, N
19·HCl	C	54	160–167	$C_{10}H_{14}N_2O_2 \cdot HCl \cdot 0.33H_2O$	C, H, N
22	F	~100	197–199	$C_{11}H_{13}NO_4 \cdot 0.5H_2O$	C, H, N
25	C	96	232–235	$C_{17}H_{18}N_2O_4 \cdot 0.5H_2O$	C, H, N

[a] Method C: H_2, 10% Pd/C in MeOH-HCl; method E: BBr_3 in CH_2Cl_2; method F: NaOH saponification. [b] Lit.[3] mp 226 °C. [c] Lit.[5] mp 220–221 °C.

Scheme IV. Synthesis of Glutaric Acid Bis(*p*-hydroxyanilide)



Biological Activity. All the anilides described above were tested as inhibitors of B16 murine melanoma cells in culture. This is a moderately pigmented tumor which is known to contain tyrosinase[14] and should therefore be capable of biotransforming L-glutamic γ-(4-hydroxyanilide) (1) and other phenolic compounds to catechols and quinones. As shown in Table III, compound 1 had an ID_{50} value against this cell line of 0.1 mM, which is close to the data reported for L-Dopa against mouse, as well as human melanoma cells in culture.[15] In compound 17, replacement of the γ-L-glutamyl moiety by α-L-glutamyl led to a greater than tenfold decrease in activity. A similar loss of activity accompanied removal of the α-carboxy and α-amino group in the side chain (compounds 19 and 22, respectively). Replacement of γ-L-glutamyl by β-L-aspartyl (compound 15) led to a threefold increase in the ID_{50} value, to 0.31 mM. Placement of the phenolic OH group on the meta position, as in compound 12, likewise led to some decrease in activity (ID_{50} = 1.0 mM) but not as great a loss as other structural modifications. O-Methylation, as in 11, resulted in a greater than tenfold loss of activity, indicating that catabolic *O*-methyl cleavage probably does not occur in these cells. Interestingly, catechol 3, the putative metabolite of 1, is less toxic to B16 cells than the parent phenol. Since tyrosinase oxidation of 3 to the 490-nm quinone has been shown to occur very rapidly under cell-free conditions,[4,11,16] it is possible that the threefold reduction in cytotoxicity of the catechol which we observe is due to less efficient uptake across the cell membrane. Blocking of the diol as a methylenedioxy derivative (compound 13), which would in effect represent a prodrug, did not restore activity. Thus, despite evidence in the literature that methylenedioxyphenyl compounds are potentially metabolizable to catechols by mammalian cells,[17,18] our data indicate that the capacity of B16 cells to effect this type of biotransformation is limited. Of the 11 compounds presented in Table III, only the hydroquinone analogue 14 was more active than the parent phenol 1. Although the increase in cytotoxicity of 14 was only twofold, this finding is of some interest, since it is obvious from the chemical structure of 14 that this compound can potentially give rise to the same 490-nm quinone metabolite 2 as the naturally occurring catechol 3.

Table III. Cytotoxic Effect of Monohydroxyanilide and Dihydroxyanilide Derivatives on B16 Mouse Melanoma Cells in Culture

no.	ID_{50}, mM
1	0.10
3	0.27
11	>1.0
12	1.0
13	>1.0
14	0.051
15	0.31
17	>1.0
19	>1.0
22	>1.0
25	>1.0

[a] Moderately pigmented B16 melanoma cells were grown in monolayer culture in McCoy's 5A medium with 10% fetal calf serum. Cells were in exponential growth for the assay. Drug was added 24 h after plating, and incubation was continued for another 48 h. Four dose levels were tested (in triplicate) between 0.1 and 1.0 mM, except with compound 14 where doses between 0.01 and 0.1 mM were used. Standard deviations for all the ID_{50} values listed were within ±10% of the mean.

In summary, in bioassays against B16 melanoma cells in culture the natural product L-glutamic acid γ-(4-hydroxyanilide) exhibits cytotoxicity comparable to that of other types of phenolic compounds that are thought to undergo bioactivation in the presence of the enzyme tyrosinase.[14,15,19] The activity of this compound shows considerable side-chain specificity, suggesting that the γ-L-glutamyl moiety (1) may facilitate uptake into cells, (2) may promote bioactivation to a cytotoxic metabolite (e.g., quinone 2). or (3), if it is still present in the active metabolite, may provide increased affinity to the ultimate intracellular target site. It also appears from our data that relocation of the OH group from the para to the meta position and the addition of a second OH group are structural modifications that do not violate the minimal structure-activity requirements of the system. Further biochemical and pharmacological studies with these compounds are in progress and will be reported at a later date.

Experimental Section

Melting points (uncorrected) were determined in Pyrex capillary tubes by means of a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) at a heating rate of approximately 2 °C/min. Infrared spectra were recorded on a Perkin-Elmer Model 137B double-beam recording spectrophotometer, ultraviolet spectra on Cary Models 11 and 15 spectrophotometers, and NMR spectra on a Varian T60A instrument. TLC analyses were performed on Eastman 13181 silica gel or Eastman 13254 cellulose sheets with fluorescent indicator. Spots were visualized at 254 nm in a viewing chamber or under laboratory light after spraying with ninhydrin or 3% alcoholic $FeCl_3$. Dry-column chromatography was performed on Woelm activated grade III/30 mm silica gel (ICN Pharmaceuticals, Inc., Cleveland, Ohio). Protected amino acids were purchased from Bachem, Inc., Torrance, Calif.; boron tribromide was obtained from the Ventron Corp., Danvers, Mass.; 1-hydroxybenzotriazole (1-HBT), *N,N'*-dicyclohexylcarbodiimide (DCC), and other chemicals came from Aldrich Chemical Co., Milwaukee, Wis.

***N*-(Carbobenzyloxy)-L-glutamic Acid γ-[3-(Benzyloxy)anilide] α-Benzyl Ester (6). Method A.** To a solution of 3-(benzyloxy)aniline (0.4 g, 0.002 mol), *N*-(carbobenzyloxy)-L-glutamic acid α-benzyl ester (0.74 g, 0.002 mol), and 1-HBT (0.65 g, 0.0048 mol) in dry tetrahydrofuran (10 mL) at 0 °C was added a cold solution of DCC (0.46 g, 0.0022 mol) in the same solvent (2 mL). The mixture was kept at 0 °C for 1 h and at room temperature overnight. A small amount of glacial acetic acid was then added, the mixture was stirred for 15 min and filtered, and the filter cake was washed with tetrahydrofuran (20 mL). Evaporation of the combined filtrate and wash solution under reduced pressure left a semisolid residue which was taken up in ethyl acetate (100 mL). A small amount of undissolved solid was filtered off, and the ethyl acetate solution was washed successively with 10% citric acid, water, saturated sodium bicarbonate, and finally water. Vacuum evaporation gave a crude product, which was purified by dry-column chromatography using 9:1 chloroform–ethyl acetate as the eluent. Evaporation of appropriate TLC-homogeneous fractions, trituration with petroleum ether (bp 30–60 °C), and drying in vacuo yielded 0.88 g (80%) of **6** as a colorless solid.

***N*-(Carbobenzyloxy)-4-aminobutyric Acid *p*-(Benzyloxy)anilide (18). Method B.** Isobutyl chloroformate (1.3 mL) was added to a mixture of *N*-(carbobenzyloxy)-4-aminobutyric acid (2.4 g, 0.01 mol) in dry tetrahydrofuran (10 mL) containing triethylamine (1.5 mL) and kept at −15 °C by means of a dry ice–methanol bath. After another 15 min at −15 °C, a solution of *p*-(benzyloxy)aniline (2 g, 0.01 mol) in dry tetrahydrofuran (3 mL) precooled to −15 °C was added, and the reaction mixture was stirred for 1 min at this temperature and then overnight at room temperature. Vacuum evaporation, trituration of the residue with water, suction filtration, washing, air-drying, and recrystallization from ethyl acetate gave compound **18** as a colorless solid (2.3 g, 55%).

L-Glutamic Acid α-(4-Hydroxyanilide) (17). Method C. A suspension of compound **16** (0.49 g) in methanol (40 mL) containing 2 mL of 1 N HCl was placed in a 500-mL Parr hydrogenation bottle, a slurry of 10% Pd/C (120 mg) in water (2 mL) was added, and the mixture was shaken at room temperature overnight under 1 atm of hydrogen. After filtration through a Celite pad and washing of the pad with methanol (10 mL) and water (10 mL), the combined filtrate and wash solutions were evaporated to a small volume under reduced pressure. A small amount of insoluble material was filtered off, and the remaining solution was adjusted to pH 5 with dilute ammonia and then lyophilized. Recrystallization of the light-brown residue from a small volume of water with the aid of decolorizing charcoal gave 0.19 g (94%) of **17** as a white powder.

Glutaric Acid 4-(Benzyloxy)anilide (23). Method D. *p*-(Benzyloxy)aniline (0.4 g, 0.002 mol) was added to a solution of glutaric anhydride (0.46 g, 0.004 mol) in glacial acetic acid (4 mL), and the mixture was stirred at room temperature overnight. Evaporation of the acetic acid under reduced pressure (bath temperature below 35 °C) and addition of water (10 mL) gave a white precipitate, which was collected, washed with cold water, and dried: yield 0.5 g (84%).

L-Glutamic Acid γ-(2,5-Dihydroxyanilide) (14). Method E. To a solution of compound **9** (0.25 g, 0.00049 mol) in dry dichloromethane (5 mL) cooled to −78 °C (dry ice–acetone) was added dropwise under nitrogen a solution of boron tribromide (1.3 g) in the same solvent (5 mL), likewise precooled to −78 °C. The reaction mixture was allowed to come to room temperature overnight, with careful moisture exclusion throughout. After a total of 45 h, the suspension was cooled and treated carefully with degassed methanol (3 mL). Vacuum evaporation yielded a light-brown residue, which was triturated with deionized water and extracted with ether (5 × 10 mL). The filtered aqueous layer was lyophilized and the residue dissolved in a small volume of deionized water. This solution was applied to a column of Dowex-50WX8 (H^+) resin which had previously been equilibrated with 0.4 M ammonium formate (pH 4.0). Elution with the same buffer and lyophilization of pooled TLC-homogeneous fractions gave a yellowish-brown powder (0.065 g). Trituration of this crude product with alcohol and ether yielded 0.052 g (42%) of compound **14** as a pale tan powder. Aqueous solutions of this compound cannot be stored at room temperature without extensive darkening and decomposition.

Glutaric Acid 4-Hydroxyanilide (22). Method F. To a solution of compound **21** (0.31 g) in methanol (5 mL) was added 3 mL of 1 N sodium hydroxide, and the mixture was stirred at room temperature under a nitrogen atmosphere for 24 h. After acidification with 1 N HCl and evaporation of the methanol, the suspension of precipitated product was kept at 0–5 °C overnight. The solid was then filtered, washed with cold water, and dried: yield 0.27 g (ca. 100%).

Acknowledgment. This work was supported in part by Grants CA 23151 and CA 06516 from the National Cancer Institute, DHEW.

References and Notes

(1) R. F. Weaver, K. V. Rajagopalan, P. Handler, P. Jeffs, W. L. Byrne, and D. Rosenthal, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 1050 (1970).
(2) R. F. Weaver, K. V. Rajagopalan, P. Handler, D. Rosenthal, and P. W. Jeffs, *J. Biol. Chem.*, **246**, 2010 (1971).
(3) R. F. Weaver, K. V. Rajagopalan, P. Handler, and W. L. Byrne, *J. Biol. Chem.*, **247**, 2015 (1971).
(4) S. W. Tiffany, D. G. Graham, F. S. Vogel, M. W. Cass, and P. W. Jeffs, *Cancer Res.*, **38**, 3230 (1978).
(5) A. Szent-Gyorgi, R. H. Chung, M. J. Boyajian, M. Tishler, B. H. Arison, E. F. Schoenwaldt, and J. J. Wittick, *J. Org. Chem.*, **41**, 1603 (1976).
(6) F. S. Vogel and R. F. Weaver, *Exp. Cell Res.*, **75**, 95 (1972).
(7) R. F. Weaver, K. V. Rajagopalan, and P. Handler, *Arch. Biochem. Biophys.*, **149**, 541 (1972).
(8) F. S. Vogel, S. J. McGarry, L. A. K. Kemper, and D. G. Graham, *Am. J. Pathol.*, **76**, 165 (1974).
(9) F. S. Vogel, L. A. K. Kemper, S. J. McGarry, and D. G. Graham, *Am. J. Pathol.*, **78**, 33 (1975).
(10) D. G. Graham, R. W. Tye, and F. S. Vogel, *Cancer Res.*, **37**, 436 (1977).
(11) F. S. Vogel, L. A. K. Kemper, P. W. Jeffs, M. W. Cass, and D. G. Graham, *Cancer Res.*, **37**, 1133 (1977).
(12) W König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(13) A. M. Felix, *J. Org. Chem.*, **39**, 1429 (1974).
(14) H. Sugano, I. Sugano, K. Jimbow, and T. B. Fitzpatrick, *Cancer Res.*, **35**, 3126 (1975).
(15) M. M. Wick, L. Byers, and E. Frei III, *Science*, **197**, 468 (1977).
(16) We have observed that on incubation with mushroom tyrosinase all the mono- and dihydroxyanilides described in this paper are converted into red products, though at different rates. Further biochemical and pharmacologic studies with these compounds will be reported separately.
(17) J. Casida, J. Engel, E. Essac, F. Kamienski, and S. Kuwatsuka, *Science*, **153**, 1130 (1966).
(18) G. M. Marquardt, V. DiStefano, and L. L. Ling, *Biochem. Pharmacol.*, **27**, 1503 (1978).
(19) P. A. Riley, B. Sawyer, and M. A. Wolff, *J. Invest. Dermatol.*, **64**, 86 (1975).