Et_2O (3:2 v/v): total yield 53%. The later crops each had mp 77–78°. A sample recrystallized twice from C_6H_6–cyclohexane had mp 79–80°. *Anal.* ($C_{15}H_{24}N_2O_7$) C, H, N.

Diethyl Acetamido[4-(2-oxo-3-oxazolidinyl)butyl]malonate (2b). Alkylation of the Na derivative of 1 with 3-(4-chlorobutyl)-2-oxazolidinone[13] was carried out on a 0.614-mol scale as described for the preparation of **2a**. The Norit treatment and filtration (silica gel mat) of an EtOAc solution of the crude residue (from removal of the DMAC) were performed twice. Following the removal of EtOAc, the orange oil was redissolved in EtOAc, and addition of ligroine (bp 30–60°) caused partial separation of crude **2b** (85.2 g), mp 71–77°, which was recrystallized from C_6H_6–cyclohexane to give pure **2b** (69.6 g), mp 80–81°. The residue from evaporation of the EtOAc–ligroine filtrate was chromatographed on a silica gel column, and elution with EtOAc led to additional crops of crude product, which were recrystallized from C_6H_6–cyclohexane to give pure **2b** (29.2 g): mp 81–83°; total yield 45%. An analytical sample (from C_6H_6–cyclohexane) had mp 81–82°. *Anal.* ($C_{16}H_{26}N_2O_7$) C, H, N.

Diethyl Acetamido[3-(2-bromoethylamino)propyl]malonate Hydrobromide (3a). A solution of **2a** (25.0 g) in freshly prepared 30% dry HBr in AcOH solution (125 ml) was stirred at 25–30° for 43 hr. Addition of Et_2O (500 ml) precipitated **3a** as a viscous gum, which was washed with three 500-ml portions of Et_2O by decantation. The remaining gum was extracted with portions of boiling EtOAc (4 l. total), and evaporation of the clarified EtOAc solution gave a crystalline residue. Recrystallization from MeCN–Et_2O gave pure **3a**, mp 111–113°, in 63% yield (22.6 g). *Anal.* ($C_{14}H_{25}BrN_2O_5 \cdot HBr$) C, H, N.

Diethyl Acetamido[4-(2-bromoethylamino)butyl]malonate Hydrobromide (3b). A solution of **2b** (99.1 g) in 30% dry HBr in AcOH solution (500 ml) was stirred at 25–30° for 72 hr and then added in a thin stream to stirred Et_2O (2.5 l.). The clear supernatant was removed by decantation from the gummy precipitate, which was stirred with three more 2.5-l. portions of Et_2O. The still-gummy residue was dissolved in boiling EtOAc (900 ml), and a small crop (5.1 g) of **3b** separated from the cooled solution. The filtrate was evaporated, and the gummy residue was dissolved in EtOAc–EtOH solution (9:1, 750 ml). Evaporation of this solution gave a solid residue, which was combined with the small first crop and recrystallized from MeCN–Et_2O to give pure **3b**, mp 147–149°, in 65% yield (85.4 g). *Anal.* ($C_{15}H_{27}BrN_2O_5 \cdot HBr$) C, H, Br, N.

***S*-2-[4-Acetamido-4,4-bis(ethoxycarbonyl)butylamino]ethyl Hydrogen Thiosulfate (5a).** A mixture of 20.00 mmol each of $Tl_2S_2O_3$ (10.418 g) and **3a** (9.244 g) in H_2O (35 ml) was stirred at 25–30° for 64 hr, filtered from TlBr, and evaporated to dryness (bath at 25–30°, final pressure <1 mm). The residual syrup was stirred with EtOH (250 ml); the solution was clarified by filtration and evaporated to dryness. The glassy residue was again dissolved in EtOH (300 ml), and the Norit-treated and filtered (Celite) solution was evaporated as above to give **5a** as an amorphous, deliquescent, solidified foam, which was pulverized under Et_2O, collected under N_2, and dried *in vacuo* (25–30°, P_2O_5): yield 77% (6.40 g); ir (KBr) 3400 (NH), 2980, 2830 (aliphatic CH), 1730 (ester C=O), 1660 (amide I), 1500 (amide II), 1190, 1015, 620 cm^{-1} (SSO_3^-); pmr (DMSO-d_6–TMS) δ 1.15, 1.90, 0.8–2.2 (t, s, m, 13, CH_3CH_2, $COCH_3$, $CCH_2CH_2CH_2$), 2.6–3.5 (m, 6, $CH_2NCH_2CH_2S$), 4.12 (q, 4, CH_3CH_2), 8.2 (s, 1, CONH), 8.0–8.6 (2, br s, NH_2^+). *Anal.* ($C_{14}H_{26}N_2O_8S_2$) C, H, N, S. A 30.3-mmol run gave **5a** in 87% yield (12.5 g); ir spectrum identical with that of analytical sample.

***S*-2-[5-Acetamido-5,5-bis(ethoxycarbonyl)pentylamino]ethyl Hydrogen Thiosulfate (5b).** Treatment of **3b** (28.57 g, 60.00 mmol) with an equimolar amount of $Tl_2S_2O_3$ (31.25 g) in H_2O (100 ml) and subsequent work-up like that described for **5a** gave deliquescent **5b** as an amorphous glass in 96% yield (24.7 g): ir (KBr) 3380 (NH), 2980, 2870 (aliphatic CH), 1740 (ester C=O), 1665 (amide I), 1515 (amide II), 1190, 1020, 625 cm^{-1} (SSO_3^-); pmr (D_2O–DSS) δ 1.23, 2.05, 0.9–2.4 (t, s, m, 15, CH_3CH_2, $COCH_3$, $CCH_2CH_2CH_2CH_2$), 3.1 (br t, 2, $CH_2CH_2CH_2N$), 3.2–3.7 (m, 4, NCH_2CH_2S), 4.27 (q, 4, CH_3CH_2). *Anal.* ($C_{15}H_{28}N_2O_8S_2$) C, H, N, S.

N^5-(2-Mercaptoethyl)ornithine Dihydrochloride (6a). A solution of **5a** (10.983 g, 26.50 mmol) in 6 *N* HCl (200 ml) was refluxed under N_2 for 4.5 hr, cooled to ~80°, treated with a solution of $Ba(OAc)_2$ (6.769 g, 26.50 mmol) in H_2O (50 ml), kept at ~80° for 30 min, cooled in an ice bath, and stored in a refrigerator overnight. The mixture was filtered from $BaSO_4$, and the filtrate was evaporated to near dryness. The remaining syrup was dissolved in H_2O (50 ml), and the solution was treated with Norit, filtered (Celite), and evaporated to ~20 ml. The colorless solution was freeze-dried to give deliquescent **6a** as a frothy glass, which was dried further *in vacuo* (25–30°, P_2O_5), broken up under Et_2O, collected under N_2, and redried as before: yield 97% (6.80 g); ir (KBr) 3300–2100 (NH_3^+), 1960 (amino acid hydrochloride), 1740 (C=O), 1580 (NH_3^+ deformation), 1200 cm^{-1} (CO_2H deformation);† pmr (DMSO-d_6–TMS) δ 1.5–2.3 (br m, 4, $CCH_2CH_2CH_2$), 2.5–3.6 (br m, 6, $CH_2NCH_2CH_2SH$), 3.9 (br m, 1, $CHNH$), 9 (v br s, ~7, NH_3^+, NH_2^+, OH, SH; not observed separately because of exchanging). *Anal.* ($C_7H_{16}N_2O_2S \cdot 2HCl \cdot 0.6H_2O$) C, H, Cl, N, S; SH (by iodometric titration).

N^6-(2-Mercaptoethyl)lysine Dihydrochloride (6b). Hydrolysis of **5b** (13.1 g) and subsequent treatment of the solution with $Ba(OAc)_2$ was carried out as described for the conversion of **5a** to **6a**. The filtered solution was evaporated under reduced pressure to dryness with filtrations at half-volume (to remove a small amount of crystalline $BaCl_2$) and at ~50 ml (to remove a trace of other insoluble material). The clear glassy residue was kept *in vacuo* over P_2O_5 for 66 hr, pulverized under Et_2O, collected under N_2, and dried *in vacuo* (25–30°, P_2O_5) to give deliquescent **6b** in 70% yield (6.1 g): ir (KBr) 3300–2100 (NH_3^+), 1980 (amino acid hydrochloride), 1735 (C=O), 1580 (NH_3^+ deformation), 1200 cm^{-1} (CO_2H deformation);† pmr (D_2O–DSS) δ 1.2–2.3 (m, 6, $CCH_2CH_2CH_2CH_2$), 2.7–3.6 (m, 6, $CH_2CH_2NCH_2CH_2S$), 4.10 (t, 1, $NCHCH_2$). *Anal.* ($C_8H_{18}N_2O_2S \cdot 2HCl \cdot 0.3H_2O$) C, H, Cl, N; SH: calcd, 11.62; found, 11.18 (by iodometric titration).

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† *Cf.* ref 14 for the interpretation of amino acid spectra.

Synthesis of *dl*-3-(Hydroxymethyl)tyrosine

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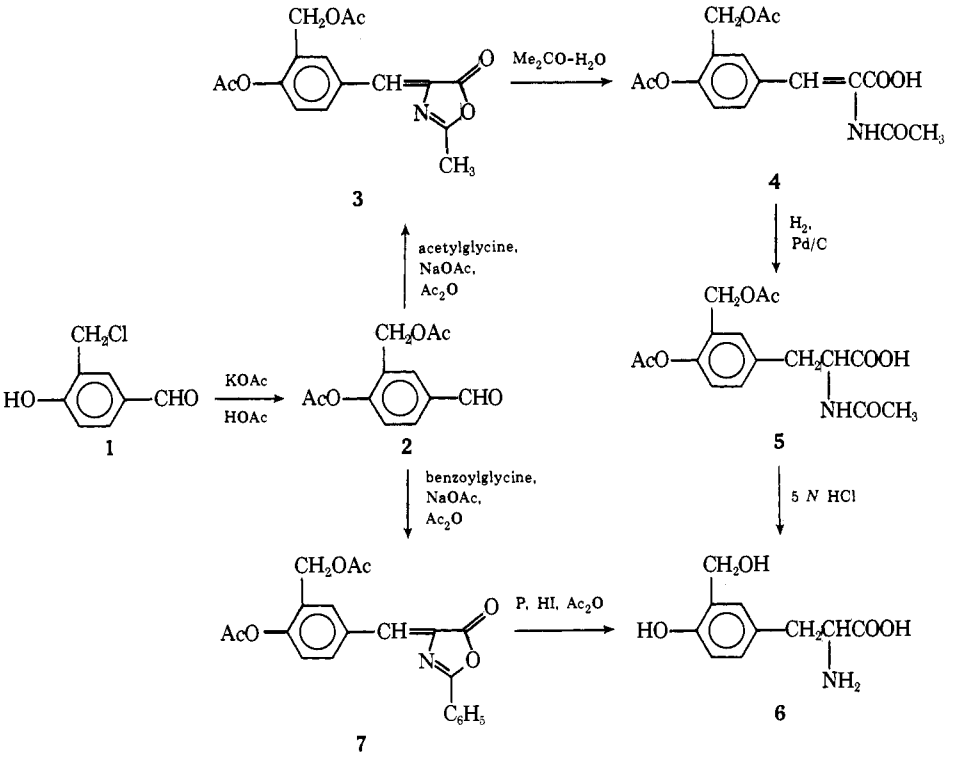
Dopa has been widely used in the treatment of Parkinson's disease. The efficacy of *l*-Dopa has been attributed to the penetration of *l*-Dopa and its metabolic conversion into dopamine in the extrapyramidal brain centers.[1,2]

Scheme I



Since dopamine cannot penetrate into the brain tissue, potentiation of *l*-Dopa therapy has been achieved by peripheral decarboxylase inhibitors preventing formation of dopamine before *l*-Dopa penetration can occur.[3]

Inactivation of dopamine in brain tissue occurs through metabolic breakdown by monoamine oxidase (MAO) or catechol *O*-methyltransferase (COMT) enzymes. As a result, potentiation of the central action of *l*-Dopa has been obtained by MAO and COMT inhibitors.[4,5]

Recent reports[6] indicate that replacement of the meta phenolic group of the catechol by related functions stable to COMT does not destroy β-adrenergic-stimulating activity displayed by selected catecholamines. Therefore, it occurred to us that 3-(hydroxymethyl)tyrosine, where a hydroxymethyl function stable to COMT replaces the meta phenolic group of Dopa, may possess greater and longer lasting anti-Parkinsonian activity than *l*-Dopa.†

Synthesis. The synthetic scheme used for the preparation of *dl*-3-(hydroxymethyl)tyrosine (6) is outlined in Scheme I. Acetolysis of the chloromethyl aldehyde 1 to the acetoxymethylaldehyde 2 was followed by azlactone formation[8] to produce compound 3. Hydrolysis[9] of the azlactone 3 to the unsaturated carboxylic acid 4 was followed by hydrogenation to *dl*-3-(hydroxymethyl)tyrosine triacetate (5). Finally, hydrolysis of the triacetate 5 produced *dl*-3-(hydroxymethyl)tyrosine (6).

In another approach compound 7 obtained by azlactone formation from the aldehyde 2 could be converted by reductive cleavage[10] into *dl*-3-(hydroxymethyl)tyrosine (6).

Biochemical and Pharmacological Activities. The antitremorine effects were determined in male albino mice of the Charles River strain weighing 20–30 g each. Two doses of the test compounds were administered intraperitoneally to groups of ten mice 0.5 hr before intraperitoneal injection of tremorine (20 mg/kg). The fraction showing lethality and prevention of tremors, as well as other effects on the CNS, is shown in Table I.

Compound 6 is not a substrate for Dopa decarboxylase as determined by the method of Sourkes[11] using Worthington (lot no. 25A) *l*-Dopa decarboxylase enzyme. Compound 6 was also tested for tyrosine hydroxylase inhibitory activity.

In vivo tyrosine hydroxylase inhibitors are known to produce sedation and can, therefore, be screened by observation. The injection of 1 mmol/kg of 6 into the tail vein of mice produced no sedation, no ptosis, and no piloerection. No significant effects could be observed when 3 mmol/kg was injected; these results, therefore, suggest that 6 produces no alteration of amine function through tyrosine hydroxylase inhibition.

In vitro testing of tyrosine hydroxylase[12] was made with bovine tyrosine hydroxylase and screened in triplicate at 10^{-3}, 10^{-4}, and $10^{-5}M$, using the method of Nagatsu.[13] After the appropriate quench and blank corrections were applied, the results shown in Table II were obtained.

As a conclusion, 6 does not act *in vivo* or *in vitro* as an inhibitor of tyrosine hydroxylase. The lack of gross effects at 3 mmol/kg in mice further suggests no significant interference with overall catecholamine biosynthesis or storage.

These results indicate that compound 6 is not likely to be useful in the treatment of Parkinson's disease. The same conclusion was reached by Atkinson, *et al.*,[7] for *l*-3-(hydroxymethyl)tyrosine.

Experimental Section

Microanalyses were within ±0.3% of the theoretical values as performed by Galbraith Laboratories, Knoxville, Tenn. Melting points were obtained on a Fisher-Johns hot stage and are corrected. Ir spectra were recorded on a Perkin-Elmer 337 grating ir spectrophotometer. Nmr spectra were run on Varian A-60A and HA-100 spectrometers in $(CD_3)_2SO$ with Me_4Si as internal reference. Uv spectra were recorded on a Bausch & Lomb spectronic 505 spectrophotometer. Mass spectra were determined on a Hitachi RMU-6D double-focusing spectrometer at 70 eV. Type QIF

† After our original manuscript was submitted for publication, the preparation of *l*-3-(hydroxymethyl)tyrosine was reported by an independent synthesis.[7]

Table I

Compd	Dosage, mg/kg ip	Fraction showing prevention of tremors	Fraction showing lethality	Other effects
3	500	0/10	10/10	CNS depression
	1000	0/10	10/10	Writhing movements
7	500	9/10	3/10	
	1000	10/10	3/10	
6	500	0/10	3/10	
	1000	3/10	6/10	
dl-Dopa	500	0/10	5/10	
	1000	9/10	6/10	
l-Dopa	250	3/10	1/10	
	500	10/10	5/10	

Table II

	M	% control[a]	% inhibn[a]
l-Monoiodotyrosine	10^{-5}	42	58
6	10^{-5}	95	5
6	10^{-4}	97	3
6	10^{-3}	102	

[a]Means of triplicate.

silica gel plates from Quantum Industries were used for tlc development. Ir, nmr, uv, mass spectra, and tlc were all appropriate.

4-Hydroxy-3-hydroxymethylbenzaldehyde Diacetate (2). A mixture of 1[14] (1.7 g, 0.01 mol), KOAc (2 g, 0.2 mol), and AcOH (15 cc) was stirred at 60° for 5 hr and then at 25° for 16 hr. The solvent was evaporated to yield a solid, to which 100 g of ice-H_2O was added. The product was extracted into Et_2O, and the solvent was evaporated to yield crystals. Recrystallization from Et_2O provided **2** (1.1 g, 57% yield), mp 99–100°. Compound **2** gave a positive 2,4-DNP and negative $FeCl_3$ test. *Anal.* ($C_{12}H_{12}O_5$) C, H.

4-[4′-Hydroxy-3′-(hydroxymethyl)benzylidene]-2-methyl-2-oxazolin-5-one Diacetate (3). A mixture of **2** (23.6 g, 0.1 mol), acetylglycine (11.7 g, 0.1 mol), NaOAc (8.23 g, 0.1 mol), and Ac_2O (80 ml) was stirred at 110° for 1 hr and then poured into 500 g of ice-H_2O. The obtained solid was filtered and crystallized from EtOAc-Et_2O to give **3** (26 g, 81%), mp 116–118°. *Anal.* ($C_{16}H_{15}NO_6$) C, H, N.

4-[4′-Hydroxy-3′-(hydroxymethyl)benzylidene]-2-phenyl-2-oxazolin-5-one Diacetate (7). Compound **7** was prepared from **2** in the same way as described for the synthesis of compound **3**, except the reaction time at 110° was extended for 6 hr. Obtained was **7** (28.1 g, 74%), mp 156–157°. *Anal.* ($C_{21}H_{17}NO_6$) C, H, N.

2-Acetamido(3-acetoxymethyl-4-acetoxy)cinnamic Acid (4). Compound **3** (12.2 g, 0.04 mol) in 75% aqueous Me_2CO (400 ml) was heated at reflux until a clear solution was obtained (approximately 6 hr). The solvent was evaporated to give a solid. Crystallization from THF-Et_2O gave **4** (10.4 g, 78%): mp 183–185°; tlc (Me_2CO-MeOH, 1:1) R_f 0.78. *Anal.* ($C_{16}H_{17}NO_7$) C, H, N.

***dl*-3-(Hydroxymethyl)tyrosine Triacetate (5).** A solution of **4** (3.4 g, 0.01 mol) in 75% aqueous MeOH (80 ml) was hydrogenated at 25° over 10% Pd/C (350 mg) at an initial H_2 pressure of 50 lb/in.^{2}. When hydrogenation was complete the catalyst was filtered, the solvent was evaporated, and the residue was crystallized from Me_2CO to give **5** (3.04 g, 91%): mp 157–158°; tlc (C_6H_6-EtOAc-MeOH, 7:3:3) R_f 0.50. *Anal.* ($C_{16}H_{19}NO_7$) C, H, N.

***dl*-3-(Hydroxymethyl)tyrosine (6). Procedure A.** A mixture of **5** (3.4 g, 0.01 mol) and 5 *N* HCl (100 ml) was stirred at reflux until a clear solution was obtained (approximately 5 hr). The solution was concentrated (to 20 ml) under reduced pressure, treated with activated charcoal, filtered, and kept at 0° for 2 days. The precipitated crystals were filtered and washed with EtOH and then with Me_2CO to yield the HCl salt of **6** (1.71 g, 71%), mp >300°. *Anal.* ($C_{10}H_{14}NO_4Cl$) C, H, N, Cl.

The HCl salt was dissolved in H_2O (30 ml), dilute NH_4OH was added to pH 5.5, and the solution was concentrated. The precipitated crystals were filtered and washed with H_2O to give **6** (1.34 g, 64%): mp 290–294° dec; tlc (96% EtOH-34% NH_4OH, 7:3) R_f 0.85; tlc (*n*-BuOH-H_2O-HOAc-pyridine, 15:5:3:2) R_f 0.62. Compound **6** gave a positive ninhydrin test: λ max (0.01 *N* HCl) 226 mμ (log ε 3.81) and 275 (3.58). Corresponding data for *dl*-Dopa: λ max (0.01 *N* HCl) 224 mμ (log ε 3.79) and 279 (3.40). *Anal.* ($C_{10}H_{13}NO_4$) C, H, N.

Procedure B. To a cooled mixture of **7** (7.6 g, 0.02 mol) and purified red P (4.65 g, 0.15 mol) in Ac_2O (60 ml) was added dropwise with stirring 55% HI (50 g, 0.2 mol). The mixture was refluxed for 1 hr and then cooled. The excess P was removed by filtration; the solvent was evaporated under reduced pressure to give a solid. The solid was partitioned between H_2O-Et_2O (400 ml, 1:1) and after separation the aqueous solution was adjusted to pH **6** with dilute NH_4OH. The solution was concentrated and the precipitated crystals were filtered and washed with H_2O, MeOH, and then Me_2CO to give **6** described in procedure A.

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Amino–Imino Tautomerism in the Antibiotic Formycin A as Studied by CNDO/2 Molecular Orbital Theory

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In a recent communication Krugh[1] reported that carbon-13 nmr studies of the nucleoside antibiotic formycin A (**1**) show that this molecule is involved in prototropic tautomeric equilibria. These results for formycin A, which is a cytotoxic adenosine analog, are particularly interesting since adenosine does not give any evidence of tautomerization in its ^{13}C spectra and is known to exist in the amine form.[2,3] In the present communication we show that CNDO/2 molecular orbital calculations indicate that, relative to adenine, there are significant changes in the stability of the amino and imino forms of formycin A and that this suggests a possible mechanism for the biological activity of this drug.

Using the Pople and Segal[4] version of CNDO/2, we have computed the total ground-state energy of formycin