Synthesis and Biological Evaluation of Fragmented Derivatives of Tetrahydroisoquinolines. 2. Trimetoquinol Studies

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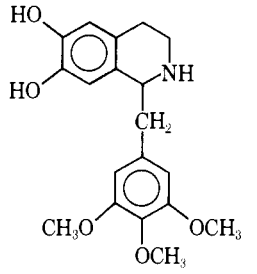
The synthesis of *N*-(3′,4′,5′-trimethoxyphenylethyl)-3,4-dihydroxyphenylethylamine (**2**) and 1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**1**) is presented. Comparative pharmacological effects of the optical isomers of 1 and compound **2** are reported in guinea pig atria, rat adipose tissue, guinea pig trachea, and guinea pig aortic strip preparations. In the β-adrenoreceptor preparations, (−)-1 was shown to be more potent than (+)-1 or **2**. Racemic 1 and **2** were shown to have equal α-antagonist properties in the inhibition of norepinephrine-induced contractions of guinea pig aorta.

In a study of derivatives of tetrahydroisoquinolines, Yamato[1] found trimetoquinol [1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**1**)] to be among the most potent bronchodilating agents known. Clinical studies have indicated trimetoquinol to be a useful agent in treating asthma.[2] The bronchodilatory activity of **1** is related to its β-adrenergic activity.[3] We are presently investigating the relationship between adrenergic activity and structure alteration of **1**. In order to study the importance of the intact tetrahydroisoquinoline nucleus of **1** for adrenergic activity, and as an aid in the development of selective adrenergic drugs, we have prepared a fragmented derivative of **1** in which the bond between C_1 and the catechol ring system is broken, as shown with **2**.



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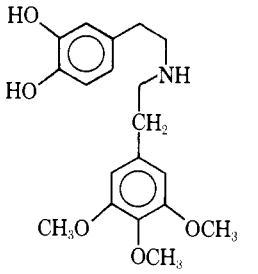

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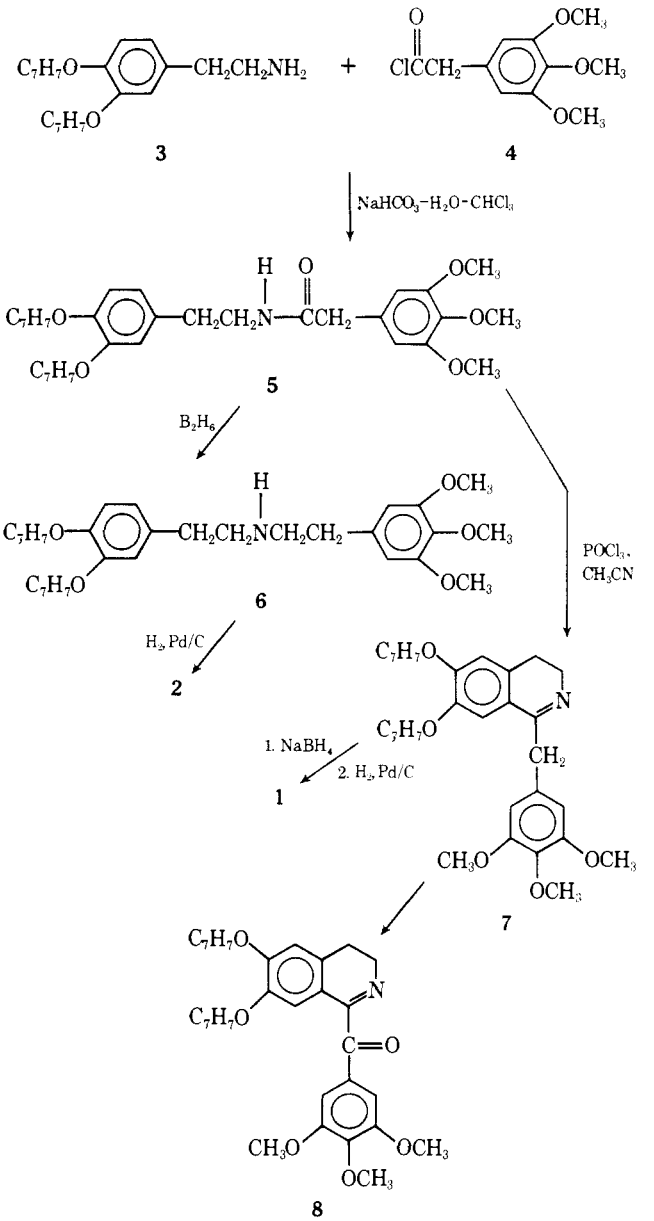
It has been suggested that β-adrenergic receptors should be subclassified on the basis of agonist[4] and antagonist[5] activity. According to this subdivision β-receptors are divided into β-1 (heart muscle, lipolysis) and β-2 (bronchial muscle).[6] Farmer et al.[7] have reported that trimetoquinol is a selective β_2 type receptor stimulant. However, a recent study by Buckner and Abel[8] does not support this high degree of selectivity reported for **1**. Our results are more in agreement with the latter authors.

Due to the variance in the effects of the isomers of catecholamines in α-adrenergic tissues,[9] we have investigated the isomers of **1** and the fragmented derivative **2** in an α-adrenergic system, guinea pig aorta. This paper also describes an alternate synthesis of **1** to that reported by Yamato et al.[1]

Chemistry. *N*-(3′,4′,5′-Trimethoxyphenethyl)dopamine (**2**) was prepared by allowing dibenzyloxyphenethylamine (**3**) to react with 3′,4′,5′-trimethoxyphenylacetyl chloride according to the procedure of Cava and Buck[10] to give the desired amide **5**.[11] Attempts were made at reducing **5** to give amine **6** using $LiAlH_4$; however, it was found that B_2H_6 was highly superior for this reduction. Removal of the benzyl-protecting groups via hydrogenolysis gave the desired catecholamine **2** (Scheme I).

We have also prepared trimetoquinol (**1**) by an alternate route to that previously reported by Yamato,[1] utilizing the amide **5** as a starting point. The amide was allowed to undergo a Bischler–Napieralski reaction using $POCl_3$ in acetonitrile to give 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dibenzyloxy-3,4-dihydroisoquinoline (**7**). If air was not rigorously excluded from the reaction and work-up, small amounts of the oxidized material **8** could also be isolated along with **7**. Recrystallization of **7** in benzene in the presence of air also gave a quantitative yield of **8**. This type of oxidation has previously been reported with 1-benzyldihydroisoquinoline.[12,13] The dihydroisoquinoline **7** was reduced using $NaBH_4$ followed by hydrogenolysis, using 10% Pd/C as a catalyst, to give trimetoquinol (**1**).

Scheme I



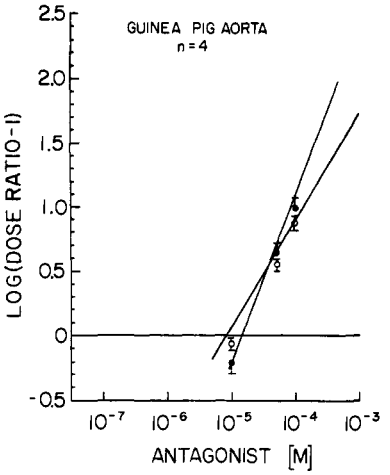


Figure 1. Plot of log (dose ratio − 1) vs. log molar antagonist concentration of racemic trimetoquinol (**1**) and *N*-(3′,4′,5′-trimethoxyphenethyl)dopamine (**2**). Key: (●-●), (+)-trimetoquinol; (O-O) *N*-(3′,4′,5′-trimethoxyphenethyl)dopamine (**2**). Values plotted represented the mean ± SE of $n = 4$ on guinea pig aorta. Calculated pA_2 values for 1 and **2** were 4.8 and 5.0, respectively. Slope values determined for 1 and **2** were 1.20 and 0.85, respectively.

Biological Results. The (+) and (−) isomers of trimetoquinol (**1**) along with **2** were evaluated for their ability to interact with α- and β-adrenergic tissues. All compounds studied were agonists in the β-adrenergic receptor systems, lipolysis, guinea pig atria, and guinea pig trachea. In each case the (−) isomer of **1** was considerably more active than either the (+) isomer of **1** or compound **2**. Isomeric activity differences calculated for the trimetoquinol isomer [−log ED_{50} (−)-**1** minus −log ED_{50} (+)-**1**] were found: 1.77 (lipolysis), 1.30 (guinea pig atria), and 2.00 (guinea pig trachea) log units. Compound **2** shows comparable activity to (+)-**1** in lipolysis and trachea but is considerably less active as a stimulant of the chronotropic response in guinea pig atria. In guinea pig atria **2** is approximately 100 times less active than (+)-**1**.

The interactions of racemic trimetoquinol **1** and **2** in guinea pig aortic strips are presented in Figure 1. None of the compounds tested (+)-, (−)-, and (±)-**1** or -**2** possessed any stimulatory actions in this α-adrenergic receptor system. These compounds were found to be competitive inhibitors of norepinephrine-induced contractions of aortic strips. Little differences could be detected in the blocking properties of racemic **1** and **2** (Figure 1). Although not presented, no stereoselective action was observed with the isomers of **1**. The pA_2 values obtained for (+)- and (−)-**1** were 4.7 and 4.8, respectively.

Discussion

It has been shown that some tetrahydroisoquinoline derivatives, which may be considered cyclized phenethylamines, are more or as active as isoproterenol on tracheal relaxation,[1,3] in mobilization of free fatty acids from adipose tissue,[14-16] and in guinea pig atria.[8] The stereochemistry of the substitution at the 1 position of tetrahydroisoquinolines appears to be quite important for β-adrenergic stimulant activity as illustrated in Figure 2. Farmer et al.[7] and Brittain et al.[17] reported that trimetoquinol was a selective β_2-receptor stimulant. Our results along with those of Buckner and Abel[8] do not support this claim. At the present time we are not able to explain the discrepancy observed for the interaction of **1** in guinea pig heart preparations. While the fragmented derivative **2** is considerably weaker than (−)-**1** in all β systems studied and (+)-**1** in guinea pig atria, it has similar activity to (+)-**1** in lipolysis and guinea pig trachea. It appears that stimulation of guinea pig heart muscle is more dependent upon a tetrahydroisoquinoline nucleus than the other two β-adrenergic systems employed. Farmer et al.[7] reported that racemic **1** was a partial agonist in guinea pig trachea. In our study we found both isomers of **1** to be partial agonists while **2** produced near maximal effect. In addition, (+)-**1** and **2** were found to be partial agonists in lipolysis.

It appears from this work that an intact tetrahydroisoquinoline nucleus and proper stereochemistry are important for highly potent β-adrenergic activity, while neither appears important for moderate α-antagonist properties (Figure 1).

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover melting point apparatus. Spectral data were obtained using a Perkin-Elmer 237 infrared spectrophotometer and a Varian A-60A nuclear magnetic resonance spectrometer. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Analytical results for C, H, and N were with ±0.4% of the theoretical values.

Chemical Procedure. 2-(3,4-Dibenzyoxyphenyl)ethylamine (**3**) was prepared according to the procedure of Cava and Buck.[10]

***N*-(3,4-Dibenzyloxyphenylethyl)-3′,4′,5′-trimethoxyphenylacetamide (5).** To a solution of 10 g (0.044 mol) of 3,4,5-trimethoxyphenylacetic acid in 100 ml of absolute ether and 50 ml of dry THF was added 4 ml (0.044 mol) of thionyl chloride and 2 drops of pyridine. After sitting at room temperature for 24 hr with occasional shaking, the mixture was filtered and solvent removed in vacuo to give the acid chloride which was dissolved in 100 ml of chloroform. This solution was added dropwise, with mechanical stirring, to a mixture of 13.9 g (0.042 mol) of amine **3** in 100 ml of chloroform and 8.2 g of sodium carbonate in 100 ml of water. After stirring for an additional 1 hr, the chloroform layer was separated and washed successively with 10% aqueous hydrochloric acid, satu-

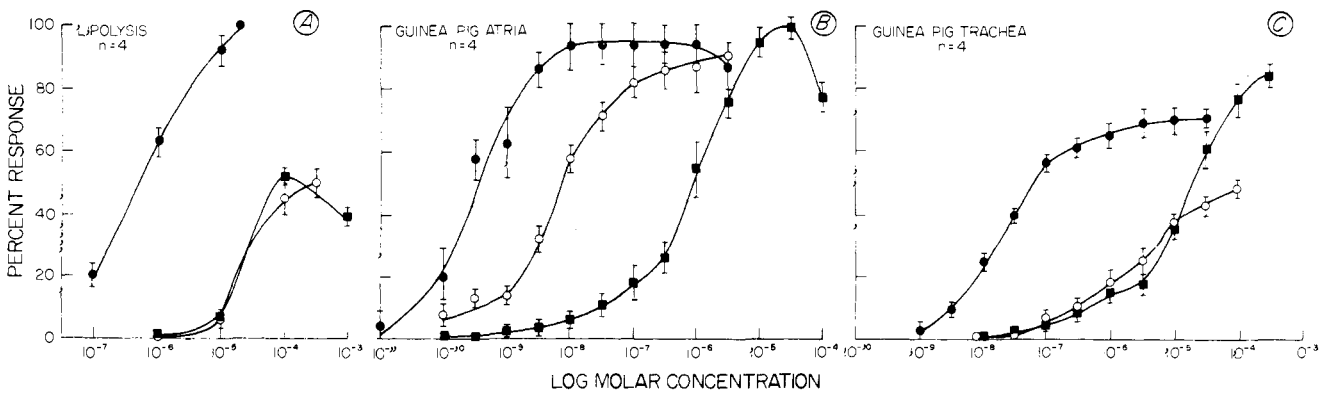


Figure 2. Comparative effects of the stereoisomers of trimetoquinol (**1**) and *rac*-*N*-(3′,4′,5′-trimethoxyphenethyl)dopamine (**2**) on lipolysis, guinea pig atria, and guinea pig trachea. Key: (●-●), (−)-trimetoquinol; (O-O), (+)-trimetoquinol; (■-■), *N*-(3′,4′,5′-trimethoxyphenethyl)dopamine. Values plotted represent the mean ± SE of $n = 4$.

rated aqueous $NaHCO_3$, and water. After drying ($MgSO_4$) the filtrate was evaporated to give a light brown oil which crystallized on trituration in benzene and ether (1:50). Recrystallization of the solid in hot benzene–ether solvent gave a white solid: 11.8 g (56%); mp 108–109° (lit.[11] 109–112°).

***N*-(3′,4′,5′-Trimethoxyphenylethyl)-3,4-dibenzyloxyphenylethylamine (6).** The amide **5** (5 g, 9.4 mmol) in 50 ml of dry THF was added slowly to 15 ml (0.016 mol) of 1 *M* borane in THF while stirring under nitrogen in an ice bath. The mixture was refluxed for 3 hr, then cooled again in an ice bath, and treated with ether saturated with HCl gas until effervescence ceased. The solvent was removed in vacuo to give a residue which was dissolved in 150 ml of $CHCl_3$ and washed with 10% NaOH and water. After drying ($MgSO_4$) the filtrate was evaporated to give an oil, 4.2 g (80%). The HCl salt of **6** was prepared: mp 151.5–153.5°.

***N*-(3′,4′,5′-Trimethoxyphenylethyl)-3,4-dihydroxyphenylethylamine (2).** A solution of 3.0 g (5.3 mmol) of **6** (HCl salt) in 100 ml of absolute MeOH was hydrogenated with 300 mg of 10% Pd/C on a Parr shaker apparatus, at 40 psi. After 6 hr when hydrogen uptake had ceased, the mixture was filtered and the solvent was evaporated in vacuo. Recrystallization of the residue from MeOH–Et_2O gave 1.8 g of **2** (89%), mp 191–192.5°.

1-(3′,4′,5′-Trimethoxybenzyl)-6,7-dibenzyloxy-3,4-dihydroisoquinoline (7); A mixture of 10 g (18.5 mmol) of amide **5** in 120 ml of dry acetonitrile and 3.6 ml (39 mmol) of $POCl_3$ was refluxed in an oil bath under nitrogen for 2 hr. On cooling to room temperature, the excess solvent and $POCl_3$ were evaporated in vacuo and the residue, dissolved in 200 ml of $CHCl_3$, was washed with 10% aqueous $NaHCO_3$ and water. After drying ($MgSO_4$), the filtrate was evaporated in vacuo to give an oil, 6.8 g (70%). The HCl salt was prepared and recrystallized from ethyl acetate: mp 186–188° (lit.[11] 185°).

1-(3′,4′,5′-Trimethoxybenzyl)-6,7-dibenzyloxy-3,4-dihydroisoquinoline (8). A solution of 2.0 g (3.8 mmol) of the free base **7** in 20 ml of benzene was refluxed using a steam bath for 15 min. On cooling, 40 ml of hexane was added and allowed to sit until crystals formed. Recrystallization of the solid from benzene–hexane gave **8**: 2.1 g (88%); mp 149–150.5°.

1-(3′,4′,5′-Trimethoxybenzyl)-6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline (9). To a solution of the free base of **7** (4.0 g, 7.6 mmol) in 70 ml of MeOH was added 4.5 g (119 mmol) of $NaBH_4$ during 15 min. After stirring for an additional 1.5 hr at room temperature, the solvent was distilled off to give a white residue which was suspended in water and basified with 10% aqueous NaOH. The mixture was extracted with ether, washed with water, and dried ($MgSO_4$). Removal of the solvent in vacuo gave a clear oil of **9**, 3.4 g (86%). The hydrochloride recrystallized from Et_2O–MeOH as white microcrystals: mp 199–202° (lit.[11] mp 202–206°).

1-(3′,4′,5′-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1). A solution of 1.5 g (2.68 mmol) of the hydrochloride salt of **9** in 50 ml of absolute methanol was hydrogenated with 250 mg of 10% Pd/C on a Parr apparatus at 40 psi. When hydrogen uptake had ceased after 5 hr, the mixture was filtered and the solvent removed in vacuo. Recrystallization of the glassy residue from MeOH–Et_2O gave 0.7 g of **1** (67%), mp 125–129° (lit.[11] mp 125–126°).

Biological Testing. Isolated Tracheal Strip Preparation. Guinea pigs of either sex weighing 300–500 g were killed by a sharp blow on the head. The trachea of each animal was isolated and cleaned free of fatty tissue. From each guinea pig two spiral tracheal strips were prepared and mounted in a 12-ml jacketed muscle chamber containing a physiological solution maintained at 37° through which a mixture of 95% O_2–5% CO_2 was bubbled. Drug-induced effects were recorded on a Grass polygraph (Model 7C) via a force displacement transducer. Strips were allowed to equilibrate for 1–1.5 hr before each experiment under a tension of 1 g. Carbachol (3×10^{-7} *M*) was used to increase the tone of each preparation and cumulative dose–response curves were obtained for each drug. Individual plots of tracheal relaxation, expressed as a percent of the maximum relaxation obtained with 10^{-5} *M* isoproterenol added at the end of each experiment vs. log molar concentration of each drug, were prepared and the ED_{50} values determined individually. In all biological experiments the ED_{50} values represent the concentration of each agonist required to produce a response equal to one-half of its maximal response in the appropriate system.

Isolated Right Atrial Preparation. Guinea pigs of either sex were killed by a sharp blow on the head. The atrium was dissected from extraneous tissue and placed in a 12-ml jacketed muscle bath. The atrium was allowed to equilibrate for a 1-hr period in a physiological solution maintained at 37° through which a mixture of 95% O_2–5% CO_2 was bubbled. The increase in atrial rate was recorded on a Grass polygraph (Model 7C) via a force displacement transducer.

In each experiment, the atrium was exposed to a test dose of a drug and the atrial rate recorded during a 3-min period. Individual recordings were made at 1- and 3-min intervals. Cumulative dose–response curves were obtained for each analog. The data were plotted on a log scale and the chronotropic responses expressed in terms of the maximum response obtained in the presence of 10^{-5} *M* isoproterenol added at the end of each experiment. ED_{50} values were determined from individual plots.

Aortic Strip Preparation. Male guinea pigs were killed by a sharp blow on the head and strips from the thoracic aortic were mounted in a 10-ml jacketed tissue bath containing physiological solution maintained at 37.5°. Width and length of strips varied from 2 to 4 mm and 20 to 40 mm, respectively. A mixture of O_2–CO_2 (95% O_2–5% CO_2) was bubbled through the solution during the experiments. Drug-induced responses were recorded via isotonic lever. Two spiral strips were prepared from each animal. After a 1-hr equilibration, the tissue was exposed to varying concentrations of antagonists for 15 min and cumulative dose–response curves were constructed with (−)-norepinephrine. The pA_2 values for antagonists were obtained according to the method of Arunlakshana and Schild.[18]

Adipose Tissue Preparation. Epididymal fat pads from four to six male Harlan-Wistar rats (200–250 g) per experiment were transferred to Krebs Ringer bicarbonate buffer, pH 7.4, and minced with scissors to yield adipose tissue fragments. Various concentrations (10^{-9}–3×10^{-4} *M*) of each agonist and 300 mg of adipose tissue were incubated at 37° for 60 min in 2.5 ml of bicarbonate buffer which contained 4% bovine serum albumin.[14] After 60 min, the reaction was terminated by the addition of 1 ml of trichloroacetic acid (10%). The rate of lipolysis was determined from the glycerol formed, by oxidation and assay of the resulting formaldehyde by the method of Nash.[19] In each experiment, a maximal rate of glycerol released was obtained with 2×10^{-5} *M* (−)-norepinephrine. This maximal release rate of glycerol was used to calculate the percent response of adipose tissue to varying concentrations of each agonist to obtain dose–response curves to permit calculation of negative log ED_{50} values. Both isomers of each compound were tested on a given day from a given batch of fat pads.

Drugs. All drugs were prepared in normal saline containing 0.05% sodium metabisulfite.

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Porphyria-Inducing Activity of a Series of Pyridine and Dihydropyridine Compounds. Investigation in a Cell Culture System

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A series of analogs of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) was prepared and tested for porphyria-inducing activity in chick embryo liver cells. One of the analogs tested, viz. 3,5-di-*tert*-butoxycarbonyl-1,4-dihydro-2,6-dimethylpyridine, was found to be highly active despite the absence of a 4-alkyl substituent. It was concluded that *tert*-butoxycarbonyl groups are resistant to enzymic hydrolysis and that compounds containing such groups are resistant to inactivation by chick embryo liver cells. Several analogs of DDC were found with considerably higher activity. These should be useful in inducing high levels of δ-aminolevulinic acid synthetase prior to undertaking the isolation of the enzyme.

The overproduction of porphyrins in liver cells induced by a variety of drugs results from an enhanced synthesis of the first enzyme in the porphyrin biosynthetic pathway, viz. δ-aminolevulinic acid (ALA) synthetase.[1-4] As a result of recent study it was concluded that for a chemical to induce porphyria it must remain in the liver for a period of at least several hours in order to induce and maintain high levels of ALA synthetase.[5] Consequently it is clear that a porphyria-inducing drug must possess chemical features that prevent it from being rapidly metabolized and inactivated by the liver. Previous studies on the relationship between chemical structure and porphyria-inducing activity of a series of compounds related to 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC, **1a**) and the related pyridine **2a** led to the suggestion that the underlying critical feature for activity was an ester group which is sterically hindered from hydrolysis.[6-9] Recently Parker and Weinstock[10] have studied the metabolism of 1,4-dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylic acid diethyl ester in the dog. Principle urinary metabolites were isolated and identified and showed that the above compound is aromatized and hydrolyzed during biotransformation. The two *o*-methyl substituents adjacent to each ethoxycarbonyl group of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine and related compounds were thought to protect the ethoxycarbonyl group from hydrolysis to yield the inactive free diacid. This explained the inactivity of 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethylpyridine (**1b**). If this interpretation was correct, it appeared possible that active analogs of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine might be obtained in which the ester group could be protected from enzymic hydrolysis by other means than two *o*-alkyl substituents. It was thought that replacement of the ethyl ester substituent by more bulky groups such as *tert*-butyl ester or benzyl ester groups might yield active analogs in compounds in which the 4-methyl substituent of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine was replaced by hydrogen. To test this idea the following four analogs of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine were synthesized: 3,5-di-*tert*-butoxycarbonyl-1,4-dihydro-2,6-dimethyl (**1h**) and the corresponding pyridine **2h**, 3,5-dibenzyloxycarbonyl-1,4-dihydro-2,6-dimethyl (**1f**) and the corresponding pyridine **2f** and their activity compared with that of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) and 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine (OX-DDC, **2a**). For comparative purposes the analogs containing 4-methyl substituents were synthesized (**1g**, **1i**, **2g**, **2i**).

For further progress in studies of the mechanism of ALA synthetase induction by drugs, it would be desirable to isolate this enzyme in pure form. This would enable an antibody to be prepared to this enzyme and would facilitate direct measurement of ALA synthetase. Before attempting the isolation of this enzyme it would be desirable to induce high levels of this enzyme. For this reason a study of DDC analogs was undertaken to find a drug of high activity. The following drugs were synthesized and their porphyrin-inducing activity measured: 3,5-dimethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (**1e**) and the corresponding pyridine **2e**.

In previous studies the porphyria-inducing activity of DDC analogs was assessed by a qualitative procedure which involved the observation of the fluorescence intensity of chick embryo liver cells 20 hr after incubation with a drug. In the present study the amount of porphyrin in chick embryo liver cells and growth medium was measured quantitatively.

Experimental Section

All melting points are uncorrected. The structures of all compounds are supported by their ir, uv, and NMR spectra. Spectra were recorded on Perkin-Elmer Model 173E, Unicam SP800 and a Varian A-60 spectrometer (Me_4Si). All compounds were analyzed for C, H, and N and the results were within ±0.4% of the theoretical value. Benzyl acetoacetate was obtained as a gift from Dr. S. F. MacDonald, N.R.C., Ottawa. *tert*-Butyl acetoacetate and methyl acetoacetate were purchased from Aldrich Chemical Co.

Culture of Chick Embryo Cells on Petri Dishes. The procedure of Granick[4] was used with the following modifications. The enzyme solution for preparing the cell suspension, from four chick embryo livers, consisted of 5 ml of 2.5% trypsin in saline (Microbiological Associates) and 5 ml of magnesium and calcium free Earle's solution. The suspension was centrifuged at low speed and the supernatant discarded. The sediment was resuspended in culture medium (Eagle basal medium, Microbiological Associates,