Synthesis and Biological Activity of Cocaine Analogues. 2. 6*H*-[2]Benzopyrano[4,3-*c*]pyridin-6-ones

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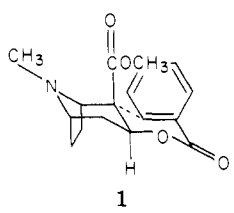
1,2,3,4-Tetrahydro-2-methyl-6*H*-[2]benzopyrano[4,3-*c*]pyridin-6-one (**20**) and *cis*- and *trans*-1,2,3,4,4a,10b-hexahydro-2-methyl-6*H*-[2]benzopyrano[4,3-*c*]pyridin-6-one (**3a** and **3b**) were synthesized. The design of **3b** was based on the proposal that the active conformation of cocaine is one in which the phenyl and amino groups are arranged in a manner that will superimpose upon a β-phenethylamine in a trans-staggered conformation. The compounds were compared with cocaine and tropacocaine for their ability to inhibit uptake of [^{3}H]norepinephrine by rat brain synaptosomal preparations. The test compounds (IC_{50} = 3.2 × 10^{-4} M, **20**; 6.5 × 10^{-4} M, **3a**; and 3.2 × 10^{-4} M, **3b**; respectively) were considerably weaker than cocaine (IC_{50} = 5.8 × 10^{-7} M) and tropacocaine (IC_{50} = 5.6 × 10^{-6} M). Compound **3b** showed selectivity at 1 × 10^{-5} M for inhibiting the uptake of norepinephrine (36%). It inhibited dopamine (3%) and serotonin (0%) uptake to a much lesser extent, if at all, at this concentration.

Structural modification of cocaine has been undertaken to obtain compounds that interact at the same biological sites as cocaine but with an altered or reduced spectrum of activity.[1] Such an approach might ultimately lead to a new antidepressant or a compound with the ability to reverse or block cocaine activity at the receptor level. It may also provide a compound which is more selective than cocaine as an inhibitor of biogenic amine uptake. Fluoxetine, a selective inhibitor of serotonin reuptake,[2] and nisoxetine, a selective inhibitor of norepinephrine reuptake,[3] have generated clinical interest.

In this paper, we describe the synthesis and biogenic amine uptake inhibition activity of compounds based upon what has been proposed as an active conformation of cocaine, **1**.[4] In this conformation, the phenyl ring and



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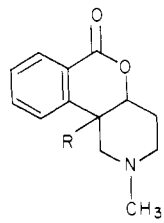
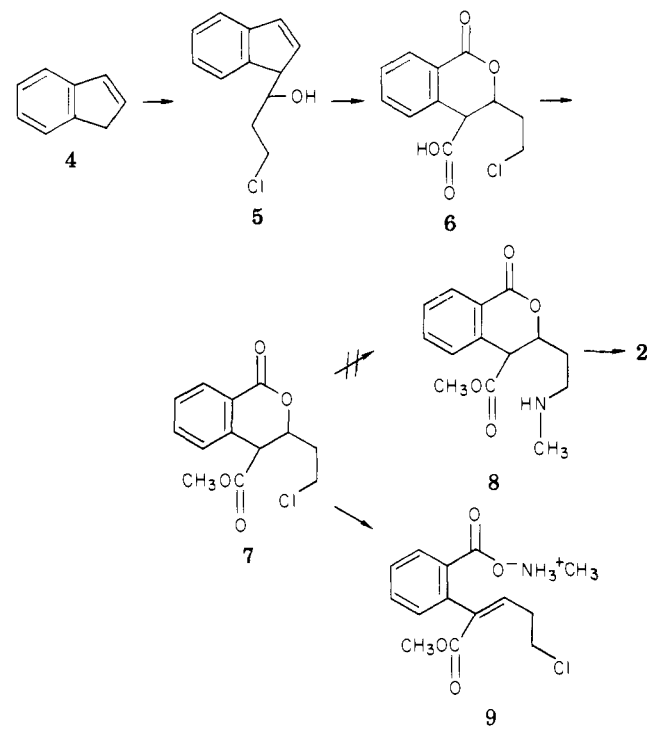


2, R = $COOCH_3$
3, R = H

nitrogen are arranged in a manner that will superimpose upon a trans-staggered phenethylamine. Removal of the ethylene bridge on cocaine and formation of a bond between the phenyl and piperidine rings provided the novel 6*H*-[2]benzopyrano[4,3-*c*]pyridin-6-one system represented by **2** and **3**. Compound **3** is a rigid analogue of tropacocaine (cocaine without the carbomethoxy group). Tropacocaine has been shown to possess weak cocaine-like activity.[5] The synthesis and activity of **3a** (cis), **3b** (trans), and unsaturated analogue **20** are described.

Chemistry. A direct five-step route to **2** which failed in the fourth step is illustrated in Scheme I. The magnesium iodide salt of indene (**4**) was prepared by reaction of **4** with ethylmagnesium iodide. This was allowed to react with β-chloropropionaldehyde to give **5**. Ozonolysis, followed by oxidative workup, gave the diacid, which lactonized in situ to give **6**. Esterification of **6** was achieved with diazomethane. At this point, the goal was to convert **7** to the amine **8** and cyclize to the piperidine ring via a Mannich reaction. However, rather than displace the halide, methylamine abstracted the benzylic proton of **7**

Scheme I



and the benzoate ester eliminated to give **9**. Attempts to aminate **5** or **6** to circumvent this problem were unsuccessful and the route had to be abandoned.

The synthesis of **3** is illustrated in Scheme II. This route was based on the synthesis of 1-methyl-3-phenyl-4-piperidone from ethyl α-phenylacrylate by Patchett and Giarrusso.[8] Homophthalic acid (**10**) was prepared by oxidation of **4** according to an established procedure.[9] Esterification of **10** was carried out according to the procedure of Sheehan and O'Neill.[10] Carboxylation with lithium diisopropylamide and carbon dioxide according to a general procedure for esters[11] provides **12**. Formation of Mannich base **13** with formaldehyde and diethylamine, followed by in situ HOAc/NaOAc decarboxylation and elimination, gave **14**. The conversion of **11** to **14** basically followed a procedure by Carlson et al.[12] for synthesis of α-methylene lactones from lactones.

Conjugate addition of ethyl β-(methylamino)propionate to **14** gave **15**. Dieckmann condensation was carried out

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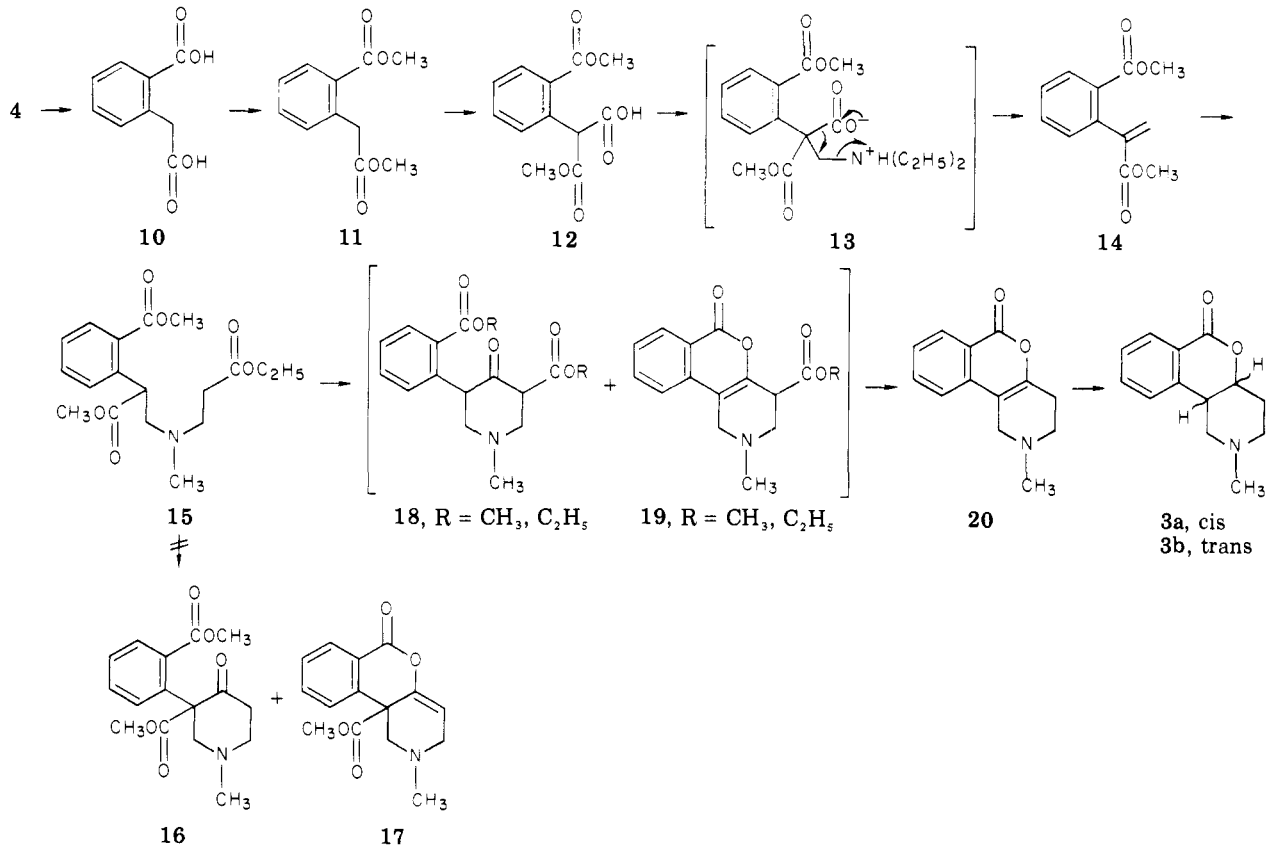
Scheme II



with sodium hydride. Theoretically, the condensation could have given **16** and **17**, as well as **18** and **19**. Reduction of **17** would have provided **2**; however, only **18** and **19** were detected as reaction products. The failure of **16** or **17** to form may be due to their relative instability compared to **18** and **19**. Under the reaction conditions, any of **16** that might have initially formed could have been converted back to starting material and ultimately to **18** or **19**. Hydrolysis and decarboxylation to **20** were carried out in refluxing 10% hydrochloric acid. Catalytic reduction with platinum oxide gave **3a** and **3b** in a ratio of 13:1.

Biological Results and Discussion. Compounds **3a** and **3b**, as well as their unsaturated precursor **20**, were compared to cocaine and tropacocaine for their ability to inhibit the reuptake of [^{3}H]norepinephrine by rat-brain synaptosomal preparations. The results are shown in Figure 1.

The compounds may be separated into three groups on the basis of their potency. Cocaine was the most potent compound with an IC_{50} of approximately 5.75×10^{-7} M. The IC_{50} of tropacocaine had an intermediate value of approximately 5.6×10^{-6} M. The three other compounds formed a group of weak inhibitors. Compounds **3b** and **20** were equipotent with IC_{50} values of approximately 3.2×10^{-4} M. Compound **3a** was somewhat weaker, with an IC_{50} of about 6.5×10^{-4} M.

Compounds **3a** and **3b** were also evaluated in a separate study[13] using a similar test. Both compounds were evaluated at concentrations of 1×10^{-5} M. Compound **3b** inhibited reuptake of norepinephrine by 36%, while **3a** showed no inhibition at all. At 1×10^{-5} M (Figure 1), **3b** inhibited reuptake by about 11%. Compound **3a** (Figure 1) was not tested at this concentration, but if the line obtained for **3a** is extrapolated to 1×10^{-5} M a value of 0% is not unreasonable. Some variations between the values shown in Figure 1 and those obtained by Ferris and Maxwell[13] may be expected, since slightly different procedures were used. Also, as indicated by the bars in Figure 1, variation occurred to some extent for each point on the curve.

The design of **3** was based on what has been proposed to be the active conformation of cocaine. The trans-fused ring system **3b** is superimposable on the proposed active conformation represented by **1**, whereas the cis-fused system **3a** is not. Therefore, it was expected that **3b** would be more potent than **3a**. Although the results in Figure 1 show that the IC_{50} of **3b** is slightly less than half that of **3a**, the low potency of both compounds and small difference in activity is not adequate to conclude that **3b** is an accurate representation of the active conformation of cocaine. Therefore, these results do not lend any support to the active conformation of cocaine proposed by Maxwell et al.[4] The unsaturated compound **20** was found to be about equipotent with **3b**. Assuming that both compounds act at a cocaine site, this suggests that the enol double bond has no observable effect on potency.

In an examination of uptake inhibitors, Koe[14] proposed that the active conformation of most of these compounds is represented by an extended phenylbutylamine. Accordingly, he proposed that the active conformation of cocaine was that in which the benzoate ester was extended, rather than folded back as in **1**. Koe's proposal did not rule out the possibility that uptake inhibitors can act in a phenethylamine-type orientation. He postulated alternate binding sites for aromatic rings to permit the uptake site to accommodate either a phenethylamine or a phenylbutylamine.

Other workers have attempted to modify the structure of cocaine in an attempt to obtain a stimulant or antidepressant. 3β-Phenyltropan-2-ols[15] and 3β-phenyltropane-2-carboxylate esters[16] have shown cocaine-like

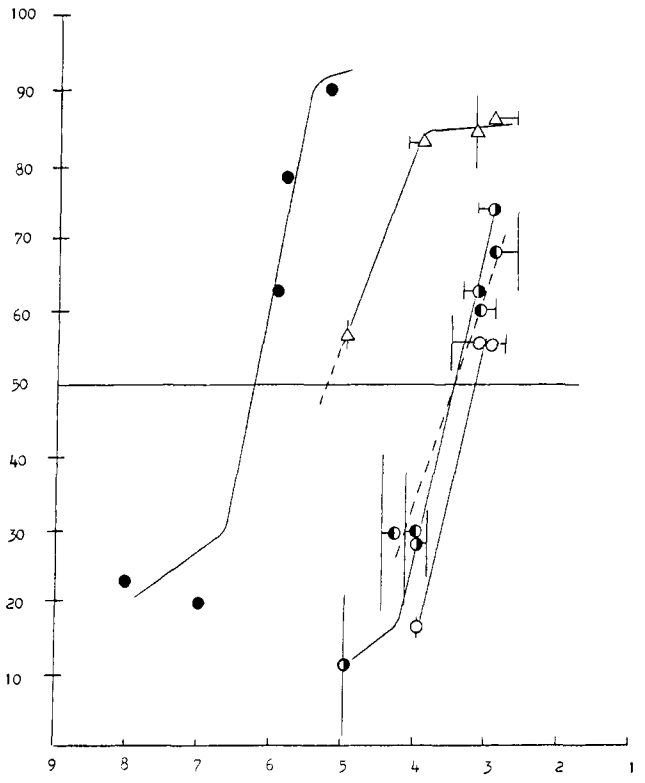


Figure 1. Relative potencies of cocaine (●), tropacocaine (△), **20** (◐), **3b** (◑), and **3a** (○) as inhibitors of norepinephrine uptake by rat-brain synaptosomal preparations: abscissa, -log [drug]; ordinate, percent inhibition. The points on the curve represent the average percent inhibition of two determinations (except for cocaine, which had six determinations) at each concentration. The bars extend out to the two values that were used to determine each point.

Table I. Inhibition of Uptake of Biogenic Amines by **3b**[a]

biogenic amine	% inhibn at 10^{-5} M
norepinephrine	36
serotonin	0
dopamine	3

[a] Data obtained by Ferris and Maxwell[13] according to the procedure in ref 19.

activity in various tests. In both of these systems, removal of the ethylene bridge from the tropane ring resulted in loss of activity. If this represents a general rule at the cocaine receptor, lack of an ethylene bridge might contribute to the low activity of **3a**, **3b**, and **20**.

The data in Table I suggest that at 1×10^{-5} M **3b** exhibits selectivity in inhibition of biogenic amine uptake. At this concentration, **3b** inhibited norepinephrine uptake by 36% while having virtually no effect on serotonin or dopamine uptake. If appropriate substitutions on **3b** or perhaps **20** could increase potency while retaining selectivity, a useful compound might result.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover unimelt apparatus and are uncorrected. Elemental analyses were performed by Microlytics, Inc., South Deerfield, Mass., and are within ±0.4% of the theoretical values. All compounds gave IR and proton NMR spectra compatible with their assigned structures. NMR spectra were obtained on a Hitachi Perkin-Elmer R-24 high resolution NMR spectrometer, using Me_4Si as an internal standard with $CDCl_3$ or external with D_2O. IR spectra were obtained on a Beckman Acculab 3. Mass spectra were obtained with an A.E.I. Scientific Inc. mass spectrometer MS902. TLC were run on Baker-flex silica gel 1B-F sheets, 7.52 × 2.5 cm. Column chromatography was run on silica gel 60, particle size 0.063–0.200 μm (70–230 mesh ASTM; EM Reagents). References to removing or evaporating solvent under reduced pressure refer to evaporations on a rotary flash evaporator. Centrifugations at 10000*g* were carried out on a Sorvall superspeed RC2-B automatic refrigerated centrifuge. Centrifugation at 23000*g* was carried out on a Beckman L2-65B ultracentrifuge.

1-(1-Hydroxy-3-chloropropyl)-1*H*-indene (5). EtMgI was prepared from 116.7 g of EtI (0.748 mol) dissolved in 120 mL of anhydrous Et_2O and 17.8 g (0.74 mol) of magnesium turnings. The vigorous reaction was slowed by the rapid addition of 160 mL of anhydrous Et_2O. After the addition of EtI was complete, the reaction mixture was warmed on a hot water bath for an additional 1 h, then cooled to room temperature, and a solution of 86 g of indene (0.74 mol) in 220 mL of toluene was added rapidly. The condenser was removed and the reaction heated at 100 °C with stirring for 10 h. The brown reaction mixture was then cooled in an ice bath, causing indenemagnesium iodide to precipitate. As much brown supernatant as possible was decanted, and the precipitated salt was dissolved in anhydrous Et_2O. The solvent was removed under reduced pressure, leaving a pea-green paste which was dissolved in 350 mL of anhydrous Et_2O. An ethereal solution of β-chloropropionaldehyde[17] prepared earlier was added. The reaction temperature was kept at 15 °C or below by cooling in ice. The flask was packed in ice and stirred overnight. The reaction mixture was then poured into 350 mL of ice–water saturated with NH_4Cl and extracted with Et_2O (12 × 300 mL). The combined Et_2O extracts were washed with saturated NH_4Cl solution (2 × 400 mL) and saturated NaCl solution (2 × 400 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give 116 g of a red-brown oil. After seeding and cooling in a freezer, 43.3 g of dark-brown crystals was obtained. The seed was obtained by column chromatography purification of an earlier reaction [silica gel, petroleum ether–Et_2O (7:3), which eluted some impurity, followed by petroleum ether–Et_2O (1:1), which eluted the product which readily solidified]. The product was recrystallized from hexane four times to give 26.8 g (0.128 mol, 17.4%) of light-yellow product, mp 73.5–76.5 °C. After several days in a desiccator, the product turned light brown. Therefore, it was sublimed to give 16.2 g of stable, white, analytically pure product, mp 75–77 °C. Anal. ($C_{12}H_{13}C_{1O}$) C, H.

***trans*-3,4-Dihydro-1-oxo-3-(2-chloroethyl)-1*H*-2-benzopyran-4-carboxylic Acid (6).** A solution of sublimed **5** (16.2 g, 77.6 mmol) and 385 mL of anhydrous EtOH was cooled with stirring in an ice bath. Ozone was bubbled into the reaction via a Welsbach T-23 ozonator for 3 h and 10 min. The EtOH was removed under reduced pressure to give 23.9 g of a sticky foamy oil, which was dissolved in 108 mL of glacial HOAc and 163 mL of 15% H_2O_2. The mixture was warmed, with stirring, for 17 h in an oil bath set at 55 °C. The solvent was removed under reduced pressure, and the residue was dissolved in 200 mL of Et_2O and carefully extracted (violent reaction) with a 10% solution of $NaHSO_3$ (7 × 25 mL) to destroy the excess H_2O_2. The ether phase was then extracted with 10% Na_2CO_3 (4 × 25 mL). The combined base extracts were washed with Et_2O (2 × 30 mL) and then made acidic with 3 N HCl. The cloudy aqueous phase was extracted with Et_2O (4 × 50 mL). The combined Et_2O extracts were dried (saturated NaCl solution extract; then Na_2SO_4) and filtered, and the solvent was removed under reduced pressure to give 11 g of yellow oil. The product was crystallized from a small amount of $CHCl_3$ with a few drops of hexane. Two crops gave 5.2 g. The residue from the mother liquors was purified again by the base–acid extractions to give another 0.64 g of product, for a total of 5.84 g (23.3 mmol, 30%). An analytical sample, mp 134–135.5 °C, was obtained by recrystallization from chloroform: NMR ($CDCl_3$) decoupling at δ 2.0 shows J_{CH-CH} = 6 Hz. On this basis, the trans configuration was assigned. Anal. ($C_{12}H_{11}ClO_4$) C, H.

Methyl *trans*-3,4-Dihydro-1-oxo-3-(2-chloroethyl)-1*H*-2-benzopyran-4-carboxylate (7). An ethereal solution of diazomethane prepared from 8.56 g (40 mmol) of Diazald® (*p*-tolysulfonylmethylnitrosamide, Aldrich) was added to a solution of **6** (650 mg, 2.55 mmol) dissolved in 50 mL of Et_2O. The reaction mixture was allowed to sit in a hood overnight. The faint yellow reaction mixture was then filtered, and the solvent was removed under reduced pressure to give a yellow oil. The product was crystallized from acetone–hexane to give 300 mg (1.116 mmol,

44%), mp 97–99 °C. An analytical sample, mp 99.5–101 °C, was obtained by recrystallization from acetone–hexane. Anal. ($C_{13}H_{13}ClO_4$) C, H.

Methylammonium *o*-[1-(Methoxycarbonyl)-4-chlorobut-1-enyl]benzoate (9). CH_3NH_2 gas was bubbled into a solution of 222 mg (0.826 mmol) of **7** in 14 mL of anhydrous Et_2O for 15 min. The mixture was cooled and filtered to give 194 mg (0.647 mmol, 78%) of analytically pure product: mp 121–122 °C. Anal. ($C_{14}H_{18}ClNO_4$) C, H, N.

2-(Methoxycarbonyl)-2-[2-(methoxycarbonyl)phenyl]acetic Acid (12). A solution of diisopropylamine (25.3 g, 253 mmol) in 250 mL of dry THF under an N_2 atmosphere was cooled to between −10 and −15 °C in a dry ice–acetone bath, and 162 mL of 1.6 N *n*-butyllithium was added. After stirring the solution for 15 min, 45 g (216 mmol) of **11** dissolved in 170 mL of dry THF was added over a 30-min period at −30 to −15 °C. After the addition was complete, the N_2 inlet was removed and CO_2 was vigorously bubbled through the reaction mixture for 20 min. The mixture was maintained at 2 °C or less and slowly acidified with 10% HCl. The reaction mixture was then extracted with $CHCl_3$ (1 × 375 mL, 3 × 100 mL). The combined $CHCl_3$ extracts were extracted with 10% Na_2CO_3 (2 × 200 mL, 4 × 100 mL). The combined aqueous extracts were cooled below 2 °C and were made acidic by the careful addition of, initially, 6 N HCl and then 10% HCl as the pH approached neutrality. The product was extracted into $CHCl_3$ (5 × 200 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4), and the solvent removed under reduced pressure to give 38 g (150 mmol, 69%) of **12** as a light-yellow solid. Recrystallization from Et_2O gave 30.8 g (122 mmol) of white crystals, the yield now 56.5%. An analytical sample, mp 117–118 °C, was obtained by a second recrystallization from Et_2O. Anal. ($C_{12}H_{12}O_6$) C, H.

Methyl 2-[2-(Methoxycarbonyl)phenyl]acrylate (14). A solution of 100 mL of diethylamine and 190 mL of 35–40% formaldehyde was cooled in an ice bath and 51.5 g (204 mmol) of **12** was added. The reaction mixture was stirred at room temperature for 15 min and then heated on a steam bath for 30 min. The reaction was removed from the steam bath, and 19.1 g of NaOAc and 190 mL of glacial HOAc were added carefully. The reaction was heated for an additional 15 min and cooled, and the contents of the flask were extracted with Et_2O (1 × 800 mL, 1 × 200 mL). The combined Et_2O extracts were washed with 10% HCl (3 × 200 mL), H_2O (2 × 200 mL), saturated $NaHCO_3$ solution (2 × 200 mL), and saturated NaCl solution (2 × 200 mL). The Et_2O was then dried (Na_2SO_4), filtered, and evaporated to give 40.8 g (185 mmol, 91%) of **17** as a colorless oil. An analytical sample was obtained by distillation: bp 90–93 °C (0.06 mm). Anal. ($C_{12}H_{12}O_4$) C, H.

Methyl α-[[*N*-[2-(Ethoxycarbonyl)ethyl]-*N*-methylamino]methyl]-*o*-(methoxycarbonyl)phenylacetate (15). Ethyl β-(methylamino)propionate[18] (13.8 g, 105 mmol) and 21 g (95.4 mmol) of **14** were stirred overnight at room temperature. The reaction mixture was then dissolved in 150 mL of cold 2 N HCl and extracted with 100 mL of Et_2O. The Et_2O was back extracted with 70 mL of 2 N HCl. The combined acid extracts were made basic with 20% NaOH, and the product was extracted with Et_2O (6 × 100 mL). The ether solution was dried (Na_2SO_4) and evaporated to give **18** as an oil. An attempt was made to distill the product (160–168 °C, 0.15 mm); however, examination of the distillate showed it was not **15** but probably the retro-Michael product **14**. Since the product apparently decomposed upon distillation, it was purified by filtering through silica gel, eluting with benzene–ether (1:1). This separated the product from any remaining ethyl β-(methylamino)propionate, which remained in the silica gel. The product was an oil, 19.3 g (55 mmol, 55%). No sample was submitted for elemental analysis. The NMR spectrum was consistent for the structure of **15**. An attempt was made to make a hydrochloride salt, but it proved to be gummy and hygroscopic: NMR ($CDCl_3$) δ 7.2–8.0 (4 H, m, aromatic), 4.9 (1 H, m, benzylic proton), 4.1 (2 H, m, $-CH_2OCO-$), 3.9 (3 H, s, phenyl $COOCH_3$), 3.65 (3 H, s, C-$COOCH_3$), 2.3 (3 H, s, N-CH_3), 1.2 (3 H, t, CH_3CH_2OCO-).

Dieckmann Condensation of 15 with NaH. Isolation of Ethyl 1,2,3,4-Tetrahydro-2-methyl-6-oxo-6*H*-[2]benzopyrano[4,3-*c*]pyridine-4-carboxylate (19). Benzene was distilled from a solution of 13.2 g (37.6 mmol) of **15** in 250 mL of anhydrous benzene until the volume was reduced to 200 mL to remove any traces of water. NaH–mineral oil dispersion (50% sodium hydride, 3.94 g of dispersion, 1.97 g of NaH, 82.4 mmol) was added to a dry flask, the mineral oil was removed by rinsing with hexane, and then 80 mL of anhydrous benzene was added. The solution of **15** was then poured into the reaction flask. The mixture was refluxed, and gradually began to darken. After 6.75 h, the reaction mixture was cooled in an ice bath and poured into 100 mL of ice-cold 2 N HCl. After shaking the mixture, the aqueous phase was separated, and the benzene was extracted with more 2 N HCl (4 × 25 mL). The combined acid washes were extracted with Et_2O (2 × 50 mL) and then neutralized with 10% NaOH. As soon as the aqueous solution remained cloudy, it was extracted with 100 mL of Et_2O. The process was repeated twice, and then the aqueous phase was extracted four times with 100 mL of Et_2O. The combined extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give 7.9 g of a light-yellow amorphous solid. This material was then used directly in the next reaction without further purification.

In an earlier run of the same reaction, the product was characterized. TLC of the crude product (silica gel, benzene–Et_2O, 1:1) showed four spots between R_f 0.23 and 0.48. There was also a small amount of material with a higher R_f and some at the base line. Crude product (1.6 g) was passed through a silica gel column (36 g), eluting with benzene–Et_2O, 1:1. Only the compound with the lowest R_f among the group of four with close R_f values was isolated in pure form. Spectral evidence and elemental analysis were consistent with the proposed structure **19** (R = CH_3). An analytical sample, mp 122–123.5 °C, was obtained by four recrystallizations from methanol. The fact that this structure had a methyl ester at the 4 position indicated that ester exchange occurred. Mechanistic considerations and NMR spectra of the crude products suggested structures **18** and **19** (R = C_2H_5) for the other compounds in the group of four with R_f values between 0.23 and 0.48. None of the spectra were consistent for structures **16** or **17**: NMR ($CDCl_3$) δ 7.25–8.45 (4 H, m, aromatic), 3.75 (3 H, s, methyl ester), 3.65 (2 H, br s, N-$CH_2C{=}C$), 2.65–3.5 (3 H, m, N-CH_2CH), 2.5 (3 H, s, N-CH_3); IR (KBr) broad band centering on 1720 cm^{-1}, ester and enol lactone carbonyl; MS calcd for M^+, 273.0996; found, 273.1001. Anal. ($C_{15}H_{15}NO_4$) C, H, N.

1,2,3,4-Tetrahydro-2-methyl-6*H*-[2]benzopyrano[4,3-*c*]pyridin-6-one (20). The crude product from the previous reaction (7.9 g) was dissolved in 120 mL of 10% HCl and refluxed for 3.5 h. The hot reaction mixture was filtered, and the filtrate was cooled to give 4.56 g (18.1 mmol) of product as the HCl salt, an overall 48% yield based on **18**. Recrystallization from water gave an analytical sample, which decomposed at 285–287 °C. Anal. ($C_{13}H_{14}ClNO_2$) C, H, N.

The free base was prepared by dissolving 4.46 g (17.7 mmol) of the HCl salt in 20 mL of warm H_2O and cautiously adding 50 mL of saturated Na_2CO_3 solution. After cooling and filtration of the mixture, 3.6 g (16.7 mmol, 94%) of free base, mp 111.5–113 °C, was obtained. An analytical sample, mp 112–113 °C, was obtained by recrystallization from hexane. Anal. ($C_{13}H_{13}NO_2$) C, H, N.

***cis*- and *trans*-1,2,3,4,4a,10b-Hexahydro-2-methyl-6*H*-[2]benzopyrano[4,3-*c*]pyridin-6-one (3a and 3b).** PtO_2 (150 mg), **23** (2 g, 9.29 mmol), and 50 mL of glacial HOAc were shaken under a hydrogen atmosphere that ranged from 58 to 20 psi for 21 h. TLC of the product (free base on silica gel, eluted with $CHCl_3$ with 5% MeOH) showed that there was some starting material left. An additional 0.5 g (2.32 mmol) of **23** and 150 mg of PtO_2 catalyst were added to the reaction mixture and shaken under 60 psi of hydrogen. After 18.5 h, the pressure had decreased to 48 psi and the reaction was stopped. The reaction mixture was filtered and the catalyst rinsed with a small amount of glacial HOAc. The HOAc was subsequently evaporated under reduced pressure, providing a residual oil which was dissolved in H_2O and made basic with 10% Na_2CO_3. The product was extracted into $CHCl_3$ (3 × 25 mL). The combined $CHCl_3$ extracts were washed with saturated NaCl solution (2 × 20 mL), dried (Na_2SO_4), filtered, and evaporated to give 2.3 g of oil which according to TLC showed no starting material: R_f ($CHCl_3$, 5% MeOH) **23**, 0.52; **3b**, 0.47; **3a**, 0.34. The product was purified by passing through a 65-g silica gel column. The fractions containing pure **3b** were combined and gave 56 mg (0.258 mmol, 2.2%). The fractions containing pure

3a were combined to give 456 mg. The fractions containing a mixture were combined and concentrated. The resulting oil was passed through a second column to give an additional 272 mg of **3a** for a total of 728 mg (3.35 mmol, 29%). Analytical samples of **3b**, mp 101–101.5 °C, and **3a**, mp 83–84 °C, were prepared by recrystallizations from hexane. Anal. ($C_{13}H_{15}NO_3$) C, H, N. The hydrochloride salts of **3a**, 279–280 °C dec, and **3b**, mp 268–270 °C, were also prepared and recrystallized from water. Anal. ($C_{13}H_{16}ClNO_2$) C, H, N.

Biological Test Methods. Eight 60-day-old Charles River C.D. albino male rats weighing between 300 and 400 g were sacrificed, and their brains were immediately dissected on ice. The midbrains, containing the hypothalamus, hippocampus, and striatum were separated and homogenized in 0.32 M sucrose (10% w/v) using eight rapid up and down strokes with a size B Thomas Teflon pestle tissue homogenizer spinning at approximately 800 rpm. The homogenate was centrifuged at 3000*g* for 10 min, and the supernatant was separated and centrifuged at 10000*g* for 10 min. The pellet was resuspended in 0.32 M sucrose and layered on sucrose gradients consisting of 1.2 and 0.8 M sucrose. The gradients were centrifuged at 23000*g* for 70 min. The white synaptosomal layer, between the 0.8 and 1.2 M sucrose, was pipetted off and diluted with an equal volume of distilled water. The resulting suspension was diluted with an equal volume of 0.32 M sucrose and centrifuged at 10000*g* for 20 min, and the resulting pellet was resuspended in Kreb's Ringer phosphate buffer containing 1.6 mg of pargyline hydrochloride and 237 mg of glucose per 100 mL.

The incubation tubes contained 20 μL of test drug (except three control tubes), 20 μL of 4.5% NaCl solution, 50 μL of 10^{-5} M norepinephrine, 10 μCi of a 1 μCi/10 μL solution of [^{3}H]norepinephrine (except two control tubes), and 500 μL of synaptosomal suspension. The tubes were incubated at 30 °C for 5 min. Immediately after incubation, 10 μL of the [^{3}H]norepinephrine was added to the set of two control tubes. Approximately 3 mL of the pargyline–glucose buffer solution was added to each tube as it was removed from the incubation bath to dilute the suspension and, therefore, to quench uptake of biogenic amine by the synaptosomes. The tubes were kept in ice and after 2 min were centrifuged at 10000*g* for 15 min. The supernatant was discarded and the sides of the tubes were carefully cleaned with Q-tips® without touching the pellet. Two glass beads and 200 μL of 50% EtOH were added to each tube. The tubes were then vortexed for 30 s, and 100 μL of the final suspension was added to 10 mL of Bray's solution in a scintillation vial. The radioactivity was counted on a Packard Tri-Carb liquid scintillation spectrometer Model 3380. The set of two control tubes represented the amount of [^{3}H]norepinephrine taken up by the synaptosomes after the incubation period was over. This value (cpm) was subtracted from the average cpm obtained in each of the other sets of vials. Percent inhibition was then calculated for each sample as (control cpm − sample cpm)/control cpm. The cpm were not converted to dpm, since counting efficiency varied only ±0.5% (35–36%) between samples.

Uptake studies on **3a** and **3b** were also conducted by Dr. R. M. Ferris and Dr. R. A. Maxwell[13] at Burroughs Wellcome Co. in Research Triangle Park, North Carolina. The procedure was similar to the one just described but contained some variations.[19]

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References and Notes

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