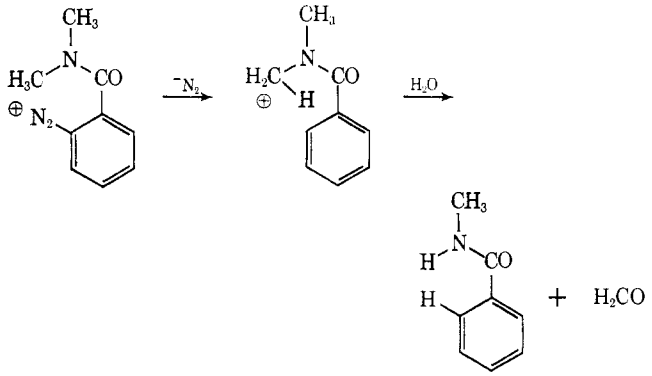

3-Deoxypyridoxine and its phosphate have been investigated in a number of biological systems as inhibitors of B_6 kinases and amino acid decarboxylases, notably glutamic decarboxylase.[6-10] We have noted antitumor activity; 3-deoxypyridoxine has an effect upon Murphy lymphosarcoma. At 4 mg/rat, the compound caused significant arrest of growth of lymphosarcoma implants in rats maintained on a pyridoxine-deficient diet, while the effect produced by the diet alone was not significant.[11]

Experimental Section

2-Methyl-3-chloro-4-methoxymethyl-5-hydroxymethylpyridine Hydrochloride from Pyridoxine Hydrochloride Mother Liquors.—A crude residue (600 g) from pyridoxine hydrochloride mother liquors was dissolved in water, neutralized (NaOH) to pH 7, and extracted with benzene. After washing well with water the benzene solution was treated with Norit, filtered, and concentrated to dryness. The residue was taken up in ethanol, and treated with dry HCl. The crystalline solid which separated was recrystallized from ethanol to constant mp 180–182°; the yield was 34 g; ultraviolet spectrum, $\lambda_{max}^{0.1\ N\ HCl}$ 292 mμ ($A_{1cm}^{1\%}$ 290), $\lambda_{max}^{pH\ 7\ borate}$ 277 mμ ($A_{1cm}^{1\%}$ 178). $FeCl_3$ and Gibbs tests for 3-hydroxyl were negative.

Anal. Calcd for $C_9H_{12}Cl_2NO_2$: C, 45.39; H, 5.51; Cl, 29.81; N, 5.88. Found: C, 45.11; H, 5.62; Cl, 29.86; N, 5.93.

3-Deoxypyridoxine Hydrochloride from Pyridoxine Hydrochloride Mother Liquors.—A portion of the aqueous layer from the above isolation was made strongly alkaline with 48% NaOH and extracted continuously with ether overnight in a liquid-liquid extractor. The dried ether solution was treated with HCl to yield 28 g of mixed hydrochlorides which gave a positive $FeCl_3$ test for the 3-hydroxy group of pyridoxine. This contaminant was removed by passing an aqueous solution of the hydrochlorides over a column containing an excess of IRA-400 resin on the ^-OH cycle. A second passage over fresh IRA-400 was necessary to remove the last trace of pyridoxine. The eluate, which now gave a negative $FeCl_3$ test, was made acidic with HCl, evaporated to dryness, and recrystallized from boiling ethanol; yield 13.3 g; mp 206–208° (lit.[4,5] mp 202–203°); depressed the melting point of pyridoxine hydrochloride; ultraviolet spectrum, $\lambda_{max}^{0.1\ N\ HCl}$ 264 mμ ($A_{1cm}^{1\%}$ 333); $\lambda_{max}^{pH\ 7\ borate}$ 265 mμ ($A_{1cm}^{1\%}$ 194); Gibbs test negative. The nmr spectrum[12] showed two ring protons at τ 1.48 and 2.55.

Anal. Calcd for $C_8H_{12}ClNO_2$: C, 50.66; H, 6.38; N, 7.38. Found: C, 50.90; H, 6.53; N, 7.36.

3-Deoxypyridoxine Hydrochloride from 4-Methoxypyridoxine Hydrochloride Mother Liquors.—A solution of 4-methoxypyridoxine hydrochloride after the diazotization was concentrated, neutralized to pH 7, and thoroughly extracted with butanol. The dried butanol solution was treated with an excess of dry HCl and the hydrochlorides were allowed to crystallize. A first crop melted at 175–178° and yielded pure 4-methoxypyridoxine hydrochloride upon recrystallization from ethanol. A second crop, amounting to one third of the whole, melted below 170°. This was acetylated with an excess of boiling Ac_2O, which was removed by concentration, and the crude acetylated hydrochloride was filtered off and washed with ethanol; mp 132–135°. Extraction with hot ethyl acetate, in which it was sparingly soluble, raised the melting point to 165–167°. The lower melting point is in agreement with the one which was previously reported.[6] This diacetate proved to be identical by melting point and mixture melting point with the diacetate prepared by acetylating the 3-deoxypyridoxine hydrochloride described above. *Anal.* Calcd for $C_{12}H_{16}ClNO_4$: C, 52.65; H, 6.06; N, 5.17. Found: C, 52.95; H, 6.06; N, 5.17.

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(11) We are obliged to Dr. R. N. Arison for these test results.

(12) W. Korytnyk and R. P. Singh, *J. Am. Chem. Soc.*, **85**, 2813 (1963).

Cyclohexyl Derivatives of Dopacetamide and Dopamine[1]

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The enzymatic hydroxylations[2-4] of phenylalanine to tyrosine and of tyrosine to dopa have been determined to be important steps in norepinephrine biosynthesis, and several investigators[2,5] have suggested that the inhibition of such aromatic hydroxylation steps may aid in developing useful cardiovascular and psychopharmacological agents.

Efforts toward such an approach have been initiated by Carlsson, Corrodi, and co-workers who reported the synthesis[6-8] of some derivatives of dihydroxyphenylacetamides and their inhibitory activities[8] on the enzymatic hydroxylation of phenylalanine to tyrosine, of tryptophan to 5-hydroxytrytophan, and of tyrosine to dopa, without inhibiting catechol O-methyltransferase.

In order to expand on the structure–activity relationship in this series we undertook the synthesis of α-cyclohexyl-3,4-dihydroxyphenylacetamide (**4a**, α-cyclohexyldopacetamide) and β-cyclohexyl-β-(3,4-dihydroxyphenyl)ethylamine (**5**, β-cyclohexyldopamine) and studied general synthetic pathways to compounds of these types.[9-12]

The synthetic sequence for the preparation of α-cyclohexyl-3,4-dihydroxyphenylacetamide (**4a**) and β-

(1) Presented in part before the Division of Medicinal Chemistry at the 151st National Meeting of the American Chemical Society, Pittsburgh, Pa., March, 1966.

(2) S. Udenfriend, *Pharmacol. Rev.*, **18**, 43 (1966).

(3) S. Udenfriend, P. Zaltzman-Nirenberg, and T. Nagatzu, *Biochem. Pharmacol.*, **14**, 837 (1965).

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(8) A. Carlsson and H. Corrodi, *ibid.*, **47**, 1340 (1964).

(9) K. I. Melville, H. E. Shister, and S. Huq, *Can. Med. Assoc. J.*, **90**, 761 (1964).

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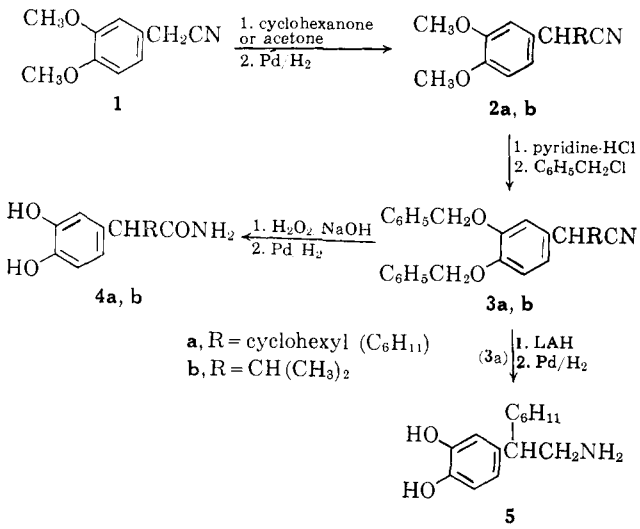
(11) S. Casadio, G. Pala, T. Bruzzese, E. Crescenzi, E. Marazzi-Uberti, and G. Coppi, *ibid.*, **8**, 594 (1965).

(12) G. Pala, S. Casadio, T. Bruzzese, E. Crescenzi, and E. Marazzi-Uberti, *ibid.*, **8**, 698 (1965).

SCHEME I



cyclohexyl-β-(3,4-dihydroxyphenyl)ethylamine (**5**) is outlined in Scheme I.

α-Isopropyl-3,4-dihydroxyphenylacetamide[7] (**4b**) was prepared in good yield using the same synthetic method, and the reaction sequence for the preparation of the isopropyl analog is also outlined in Scheme I. The complete synthetic procedures are presented in the Experimental Section.

Biological Studies.[13]—Evaluation of the effect of α-cyclohexyldopacetamide (**4a**) on brain norepinephrine levels was carried out using intraperitoneal administrations of 200 mg/kg (single doses) in DMSO to two rats (see Table I). The rats were sacrificed after 5.5 hr;

TABLE I
DETERMINATION OF BRAIN NOREPINEPHRINE

Rat	Brain norepinephrine concn, μg/g: Control (DMSO)	4a (200 mg/kg ip)
1	0.53	0.50
2	0.56	0.57

the brains were extracted according to the procedure of Anton and Sayre[14] and analyzed for norepinephrine according to the method of Shore and Olin.[15] No significant differences in norepinephrine levels could be detected between drug treated and control animals. Solvent controls showed no effect on norepinephrine levels.

Experimental Section[16]

Cyclohexylidene-3,4-dimethoxyphenylacetonitrile and Isopropylidene-3,4-dimethoxyphenylacetonitrile.—To a solution of 6.45 g (0.28 g-atom) of sodium in 350 ml of absolute alcohol was added 50 g (0.28 mole) of 3,4-dimethoxyphenylacetonitrile (**1**) and 13.9 g (0.14 mole) of cyclohexanone. The reaction mixture was allowed to reflux for 1 hr, cooled, and added to 1 l. of ice–water. The mixture was extracted with ether, the extracts were dried, and the solvent was distilled *in vacuo* to yield a viscous liquid which was crystallized from dilute ethanol to give 23.3 g (64%) of the product, mp 79–82°. A 5.0-g sample was recrystallized from isopropyl alcohol to yield 4.4 g of white crystalline solid, mp 83.5–85.5°, $\lambda_{max}^{CCl_4}$ 4.53 μ (conjugated CN), λ_{max}^{EtOH} 274 mμ (ε 6850) and 223 (19,780).

Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.65; H, 7.44; N, 5.45. Found: C, 74.14; H, 7.56; N, 5.49.

Isopropylidene-3,4-dimethoxyphenylacetonitrile was prepared from **1** (0.57 mole) and acetone (0.62 mole) as described above, bp 120–127° (0.04 mm), mp 97–98°, in 54% yield.

Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.86; H, 6.96; N, 6.45. Found: C, 71.92; H, 7.18; N, 6.81.

α-Cyclohexyl-3,4-dimethoxyphenylacetonitrile (2a) and α-Isopropyl-3,4-dimethoxyphenylacetonitrile (2b).—To a solution of 10.0 g (0.04 mole) of cyclohexylidene-3,4-dimethoxyphenylacetonitrile in 250 ml of absolute ethanol was added 1.0 g of 10% Pd–C, and the mixture was shaken with hydrogen (4.13 kg/cm²) until the theoretical amount of hydrogen had been taken up in 10 min. The catalyst was removed by filtration and the filtrate was reduced *in vacuo* to yield a clear syrup which was crystallized from ethanol to yield 7.4 g (70.4%) of a white crystalline solid, mp 84–86°, λ_{max}^{Nujol} 4.48 μ, λ_{max}^{EtOH} 280 mμ (ε 3080).

Anal. Calcd for $C_{16}H_{21}NO_2$: C, 74.09; H, 8.16; N, 5.40. Found: C, 73.67; H, 8.07; N, 5.41.

α-Isopropyl-3,4-dimethoxyphenylacetonitrile (**2b**) was prepared from isopropylidene-3,4-dimethoxyphenylacetonitrile as described above, mp 45.5–49°, in 84% yield.

Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.18; H, 7.83; N, 6.39. Found: C, 71.13; H, 7.73; N, 6.53.

α-Cyclohexyl-3,4-dihydroxyphenylacetonitrile and α-Isopropyl-3,4-dihydroxyphenylacetonitrile.—A mixture of 5.0 g (0.019 mole) of **2a** and 13.9 g (0.12 mole) of pyridine hydrochloride was heated in an oil bath for 3.5 hr at 200–220°. The clear solution was poured into 200 ml of ice–water, and the resulting solid was collected and crystallized from 50% 2-propanol to yield 3.7 g (84%) of a white powder, mp 172–173.5°, λ_{max}^{Nujol} 2.91 and 3.01 μ, λ_{max}^{EtOH} 282 mμ (ε 3550).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.62; H, 7.60; N, 6.22.

α-Isopropyl-3,4-dihydroxyphenylacetonitrile was prepared from **2b** as described above, mp 134.5–136°, in 78% yield.

Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.86. Found: C, 69.05; H, 6.85.

α-Cyclohexyl-3,4-dibenzyloxyphenylacetonitrile (3a) and α-Isopropyl-3,4-dibenzyloxyphenylacetonitrile (3b).—A mixture of 45.8 g (0.2 mole) of α-cyclohexyl-3,4-dihydroxyphenylacetonitrile, 56.6 ml (62.3 g, 0.5 mole) of benzyl chloride, and 77 g (0.56 mole) of K_2CO_3 in 1 l. of ethanol was refluxed for 4 hr and filtered while warm and cooled. The crystalline solid was collected and recrystallized from 700 ml of methanol to yield 70 g (86%) of a white solid, mp 90–92°, λ_{max}^{Nujol} 4.49 μ.

Anal. Calcd for $C_{28}H_{29}NO_2$: C, 81.74; H, 7.08; N, 3.40. Found: C, 81.41; H, 7.33; N, 3.72.

α-Isopropyl-3,4-dibenzyloxyphenylacetonitrile (**3b**) was prepared from α-isopropyl-3,4-dihydroxyphenylacetonitrile as described above, mp 76–78°, in 84% yield.

Anal. Calcd for $C_{25}H_{25}NO_2$: C, 80.82; H, 6.78; N, 3.77. Found: C, 80.94; H, 6.89; N, 3.50.

α-Cyclohexyl-3,4-dibenzyloxyphenylacetamide and α-Isopropyl-3,4-dibenzyloxyphenylacetamide.—To a solution of 0.85 g (0.02 mole) of NaOH in 2 ml of water was added a slurry of 4.1 g (0.01 mole) of **3a** in 40 ml of methanol. The mixture was heated to 50° and 8 ml of 30% H_2O_2 was added in 5 min. The temperature was maintained at 50° for 10 hr and at room temperature for 7 hr. The mixture was diluted with 400 ml of water and cooled, and the solids were collected, washed with water, and recrystallized once from methanol and twice from ethanol to yield 1.4 g (32.5%) of a light white solid, mp 197–198°, λ_{max}^{Nujol} 6.12 μ.

Anal. Calcd for $C_{28}H_{31}NO_3$: C, 78.29; H, 7.28; N, 3.26. Found: C, 78.50; H, 7.17; N, 3.42.

α-Isopropyl-3,4-dibenzyloxyphenylacetamide was prepared from **3b** as described above, mp 131–134°, in 53% yield.

Anal. Calcd for $C_{25}H_{27}NO_3$: C, 77.11; H, 6.98; N, 3.60. Found: C, 77.11; H, 7.08; N, 3.62.

α-Cyclohexyl-3,4-dihydroxyphenylacetamide (4a) and α-Isopropyl-3,4-dihydroxyphenylacetamide (4b).—To a solution of 11.5 g (0.027 mole) of α-cyclohexyl-3,4-dibenzyloxyphenylacetamide in 250 ml of absolute alcohol was added 0.5 g of 10%

(13) Biological studies were conducted by Professor A. Horita, Department of Pharmacology, School of Medicine, University of Washington, Seattle, Wash. The authors gratefully acknowledge Professor Horita for his permission to include his results.

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(15) P. A. Shore and J. S. Olin, *ibid.*, **122**, 295 (1958).

(16) Melting points were taken with a Thomas–Hoover capillary apparatus and are corrected. The infrared spectra were determined with a Beckman spectrophotometer, Model IR8, and the ultraviolet spectra were determined with a Beckman spectrophotometer, Model DK2A.

Pd–C. The mixture was shaken with hydrogen (1.5 kg/cm²) until the theoretical amount had been taken up in 1 hr. The catalyst was removed by filtration and the filtrate was reduced *in vacuo* to yield a light red glass which was dissolved in 300 ml of ether and filtered to remove the insoluble material. The filtrate was treated with activated carbon and concentrated to yield 4.4 g (65.6%) of a white glass, mp 77° dec, λ_{max}^{Nujol} 2.98 μ.

Anal. Calcd for $C_{14}H_{19}NO_3$: N, 5.62. Found: N, 5.22.

α-Isopropyl-3,4-dihydroxyphenylacetamide (**4b**) was prepared from α-isopropyl-3,4-dibenzyloxyphenylacetamide as described above, mp 59° dec, in 93.8% yield.

Anal. Calcd for $C_{11}H_{15}NO_3$: N, 6.70. Found: N, 6.41.

β-(Cyclohexyl)-β-(3,4-dibenzyloxyphenyl)ethylamine Hydrochloride.—To a dispersion of 1.94 g (0.051 mole) of $LiAlH_4$ in 50 ml of ether was added 7 g (0.017 mole) of **3a** in 200 ml of ether in 15 min. The mixture was stirred at room temperature for 2 hr and refluxed for 8 hr. It was decomposed with 10 ml of water and the solids were removed by filtration. The filtrate was dried and adjusted to acidity by the addition of ethereal HCl. The precipitated solid was collected and recrystallized from methanol to yield 6.3 g (82.1%) of a light-textured white solid, mp 208–210°, λ_{max}^{EtOH} 281 mμ (ε 2810).

Anal. Calcd for $C_{18}H_{34}ClNO_2$: C, 74.40; H, 7.58; N, 3.10. Found: C, 74.40; H, 7.72; N, 2.98.

β-(Cyclohexyl)-β-(3,4-dihydroxyphenyl)ethylamine Hydrochloride (5).—To a solution of 3.05 g (0.0068 mole) of β-(cyclohexyl)-β-(3,4-dibenzyloxyphenyl)ethylamine hydrochloride in 200 ml of ethanol was added 0.5 g of 10% Pd–C. The mixture was shaken with hydrogen (2.81 kg/cm²) until the theoretical amount had been taken up in 5 hr. The catalyst was removed by filtration, and the filtrate was concentrated to yield a glassy solid which was dried thoroughly *in vacuo* at room temperature to yield 1.83 g (100%) of a gray-white powder, mp 71°, ν_{max}^{Nujol} 2.98 μ, λ_{max}^{EtOH} 283 mμ (ε 3330).

Anal. Calcd for $C_{14}H_{22}ClNO_2$: C, 61.86; H, 8.16; Cl, 13.05; N, 5.16. Found: C, 61.68; H, 8.07; Cl, 13.02; N, 4.85.

Acknowledgment.—The authors wish to thank Mr. E. Kluchesky, Mr. D. Dusterhoft, and the staff of our Analytical Department for many of the analyses and ultraviolet and infrared spectra.

Isomeric Mannich Bases Derived from Ethyl 5-Hydroxy-2-methylindole-3-carboxylate

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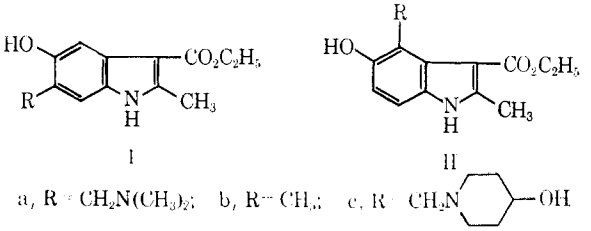
The condensation of ethyl 5-hydroxy-2-methylindole-3-carboxylate (I, R = H) with dimethylamine and formaldehyde is reported to yield a Mannich base in which the dimethylaminomethyl group has been assigned to the 6 position (Ia).[1] The discovery that this indole derivative exhibits hypoglycemic activity in rats led us to seek direct evidence for the position of the dimethylaminomethyl group, as the most likely alternative structure IIa cannot be excluded.



I

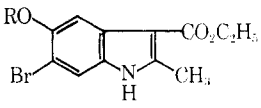
II

a, R = $CH_2N(CH_3)_2$; b, R = CH_3; c, R = CH_2N⟨⟩—OH

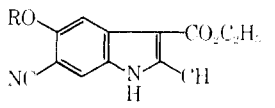
The dimethylaminomethyl side chain of the Mannich base was degraded to a methyl group by Raney nickel in refluxing alcohol to give an indole which must be the 4-methyl derivative IIb since it was different from the known 6-methyl isomer Ib.[2,3] Thus the Mannich base must be the product of substitution at the 4 and not the 6 position, a conclusion which is supported by the nmr spectra.[4]

The structure of the Mannich base does not resemble that of either major class of useful synthetic insulin substitutes, the sulfonylureas or the biguanides. Consequently a number of analogs were prepared by varying the amine component in the Mannich condensation in the hope of finding an improved insulin substitute. One member of this group, the 4-hydroxypiperidinomethyl derivative IIc, appeared to be as active in rats as the dimethylaminomethyl derivative and somewhat less toxic.

The original plan for synthesis of the corresponding 6-substituted derivatives, Ia and Ic, involved blocking the 4 position with a bromine atom followed by a Mannich reaction and removal of the blocking atom by a reduction process. Surprisingly, bromination of I (R = H), its O-acetate or O-benzyl ether in acetic acid led, in high yield, to the 6-bromo derivatives IIIa–c. The nmr spectra provided decisive evidence for these structures as the aromatic proton resonances appeared as two unsplit peaks. The reason for the contrasting courses of the bromination and Mannich reactions is not apparent. The 6-bromo derivatives were nevertheless useful since the bromine atom could be replaced by a nitrile group which in turn could be transformed to the desired dimethylaminomethyl function.



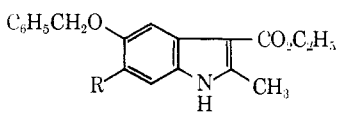
III



IV

a, R = H; b, R = $COCH_3$; c, R = $CH_2C_6H_5$

The benzyl ether IIIc was converted in high yield to the nitrile IVc by use of cuprous cyanide in a Rosenmund–von Braun reaction.[5,6] Hydrogenation catalyzed by platinum in acetic acid then yielded the 6-aminomethyl derivative Va. Methylation by the Eschweiler–Clarke procedure gave Vb which furnished the 6-dimethylaminomethyl-5-hydroxyindole Ia upon hydrogenation over palladium in alcohol.[7] This new



V

a, R = CH_2NH_2
b, R = $CH_2N(CH_3)_2$

(1) E. A. Steck, U. S. Patent 2,852,527 (June 1, 1956); *Chem. Abstr.*, **53**, 8163 (1959).

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(3) G. R. Allen, Jr., C. Pidacks, and M. J. Weiss, *J. Am. Chem. Soc.*, **88**, 2536 (1966).

(4) This finding was cracked independently by S. A. Monti, O. Johnson, and H. White, *Tetrahedron Letters*, 4459 (1966).

(5) O. T. Mowry, *Chem. Rev.*, **42**, 207 (1948).

(6) L. Friedman and H. Shechter, *J. Org. Chem.*, **26**, 2522 (1961).

(7) Mr. A. E. Sorin of our Development Laboratory reports that Ia may be prepared in good yield by hydrogenating an ethanol solution of the nitrile IVc and a large excess of dimethylamine in the presence of 10% Pd–C