250 ± 0.5°. The results obtained are summarized in Figures 1–6 and 8 and Table III.

Thermal Anomerization of α-D-Xylopyranose.—Samples (3 mg) of the sugar were placed in small aluminum pans. The pans were sealed and heated isothermally for 12.5 min, at several temperatures ranging from 127 to 200°, in a Fisher-Johns melting point apparatus. The heated samples were silylated and analyzed for the α- and β-D-xylopyranose forms by glc.[30] Each experiment was repeated four times to determine the reproducibility of the data. The results obtained are given in Table I.

Condensation of D-Xylose.—Samples of free sugar (3 mg) were heated in the tga instrument at 200° to several levels of weight loss. The heated samples were analyzed by glc and tlc methods before and after hydrolysis with 1 *N* HCl at 100° following the method described by Laver and associates.[31] The glc results are given in Table II.

A heated sample of D-xylose (weight loss 9.5%) had C, 42.67; H, 6.36 [$C_5(H_2O)_{4.5}$ requires C, 42.55; H, 6.38].

Cleavage of Phenyl β-D-Xylopyranosides.—Samples of phenyl β-D-xylopyranoside were heated at 250° and analyzed as in the previous experiment. This gave the amounts of glycoside remaining intact and the lost aglycone. The loss of the carbohydrate moiety was calculated by difference from the total weight loss recorded by tga. The results are shown in Table IV.

For the $ZnCl_2$-treated phenyl β-D-xylopyranosides, the samples were heated under flowing nitrogen in a small test tube, at temperatures just above their first endotherm and analyzed for the carbohydrates as before and for the free phenols by direct glc. The data obtained are given in Table V.

Esr Spectroscopy.—Samples of the glycosides (one part) were mixed with ground glass (nine parts) and ground together thoroughly to ensure uniform mixing. The ground samples (4–7 mg) were accurately weighted into a 2-mm capillary tube. The tube was placed into the cavity of a Varian E-3 esr spectrometer equipped with a specially designed variable temperature accessory, which was previously heated to 270 ± 0.5°. Temperature was controlled by means of a Research Incorporated Thermac series 6000 temperature controller employing a copper–constantan thermocouple feedback.

The intensity of the esr signal was plotted against time. The relative rates were measured at the inflection points of the resulting sigmoid curves, which correspond to the maximum rate of increase in signal amplitude. The resulting data are presented in Figure 7 and Table VI.

Registry No.—α-D-Xylopyranose, 6763-34-4; methyl β-D-xylopyranoside, 612-05-5; *p*-methoxyphenyl β-D-xylopyranoside, 13299-09-7; phenyl β-D-xylopyranoside, 4756-31-4; *p*-phenylphenyl β-D-xylopyranoside, 13299-14-4; *p*-chlorophenyl β-D-xylopyranoside, 3325-47-1; *p*-nitrophenyl β-D-xylopyranoside, 2001-96-9.

Acknowledgment.—The authors thank the National Science Foundation for supporting this work through Grant No. GP-15556.

(30) R. Bentley and N. Botlock, *Anal. Biochem.*, **20**, 312 (1967).

(31) M. L. Laver, D. F. Root, F. Shafizadeh, and J. C. Lowe, *Tappi*, **50**, 618 (1967).

Potential Folic Acid Antagonists. VI. The Syntheses of 1- and 3-Deazamethotrexate[1,2]

ROBERT D. ELLIOTT, CARROLL TEMPLE, JR.,* JERRY L. FRYE, AND JOHN A. MONTGOMERY

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Received February 12, 1971

The reaction of ethyl 6-amino-4-chloro-5-nitro-2-pyridinecarbamate (**4a**) and ethyl 4-amino-6-chloro-5-nitro-2-pyridinecarbamate (**4b**), respectively, with the oxime of methyl *p*-[(3-aminoacetonyl)methylamino]benzoate gave the corresponding 4- and 6-(acetonylamino)pyridine oximes **6a** and **6b**. Reductive cyclization of these compounds with Raney nickel gave the 1,2-dihydropyrido[3,4-*b*]pyrazine (**9a**) and 3,4-dihydropyrido[2,3-*b*]-pyrazine (**9b**) ring systems. Oxidation of **9a** and **9b** with $KMnO_4$ in acetone and DMAC gave the heteroaromatic compounds **10a** and **10b**, which were hydrolyzed with ethanolic KOH to give *p*-[[(5,7-diaminopyrido-[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoic acid (**13a**) and *p*-[[(6,8-diaminopyrido[2,3-*b*]pyrazin-2-yl)-methyl]methylamino]benzoic acid (**13b**). The amino groups of **13a** and **13b** were acetylated with Ac_2O to give **14a** and **14b**, which were coupled with diethyl L-glutamate to give **11a** and **11b**. Treatment of **11a** and **11b** with aqueous NaOH hydrolyzed both the ester and acetyl groups to give 1-deazamethotrexate {*N*-[*p*-[[(5,7-diaminopyrido[3,4-*b*]pyrazin-3-yl)methyl]methyloamino]benzoyl]-L-glutamic acid} (**12a**) and 3-deazamethotrexate {*N*-[*p*-[[(6,8-diaminopyrido[2,3-*b*]pyrazin-2-yl)methyl]methylamino]benzoyl]-L-glutamic acid} (**12b**).

Previously, we reported unambiguous methods for the preparation of 6-substituted 2,4-diamino-1- and -3-deazapteridines[3] and, recently, the synthesis of 2,4-diaminopteridines by a method involving the construction of the pyrazine ring containing the *p*-(methyleneamino)benzoyl moiety of folic acid and its antagonists, aminopterin and methotrexate (**12**, X = Y = N).[4] We now wish to report the preparation of both 1- and 3-deazamethotrexate by these methods.

Initially, studies were directed toward the preparation of 1-deazaaminopterin. Ethyl *p*-[(3-aminoacetonyl)amino]benzoate oxime[4] was alkylated with **4a**[5] to give the nitropyridine **1**, which was hydrolyzed in HCl to give the corresponding ketone **2**. The nitro group of **2** was hydrogenated in the presence of Raney nickel and the resulting 5-aminopyridine cyclized *in situ* to give the dihydro-1-deazapteridine **3**. Careful oxidation of **3** with a dilute solution of $KMnO_4$ in acetone and DMAC gave the heteroaromatic 1-deazapteridine **5**. However, hydrolysis of the ester and urethane groups of **5** with KOH in refluxing EtOH under N_2 resulted in extensive decomposition, and no further work was carried out on this compound.

The instability of **5** was attributed to the lability of the CH_2–NH bond under basic conditions.[4,6] The

(1) This work was supported by funds from the C. F. Kettering Foundation, and Chemiotherapy, National Cancer Institute, National Institutes of Health, Contract NIH-71-2021.

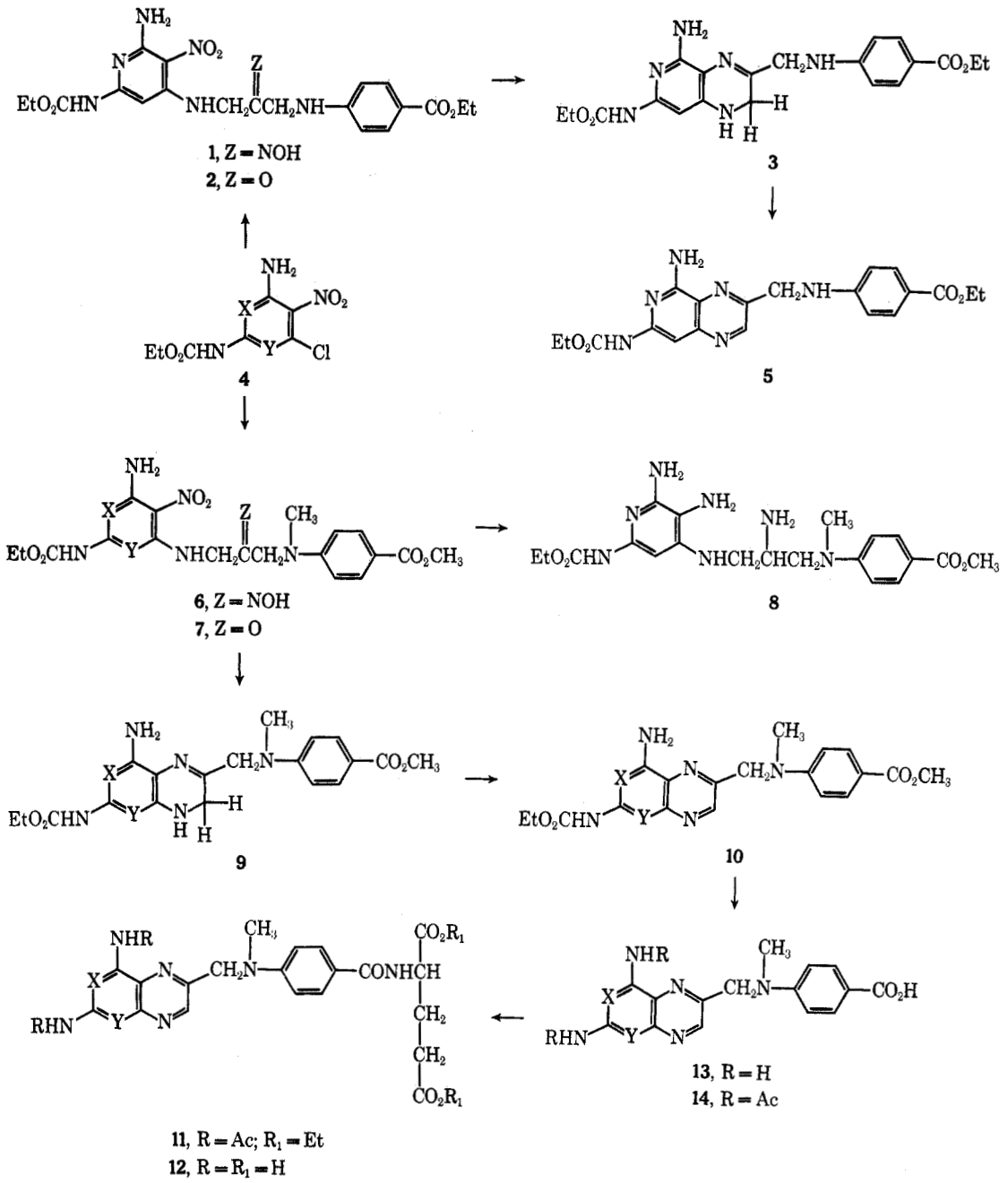
(2) A part of this work was presented at the Conference on Folate Antagonists as Chemotherapeutic Agents, New York Academy of Sciences, January 12, 1971.

(3) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **33**, 533, 2393 (1968).

(4) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *ibid.*, **35**, 1676 (1970).

(5) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *ibid.*, **31**, 1890 (1966).

(6) D. R. Seeger, D. B. Cosulich, J. M. Smith, Jr., and M. E. Hultquist, *J. Amer. Chem. Soc.*, **71**, 1753 (1949).

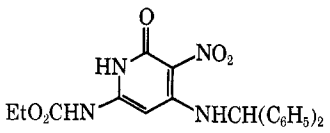


a (1-deaza series), X = N; Y = CH
b (3-deaza series), X = CH; Y = N

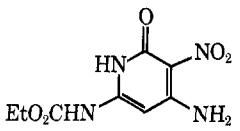
stability of the $-CH_2NCH_3$ moiety in a related pteridine (**13**, X = Y = N)[4] directed our efforts to the preparation of the methotrexate analogs **12a** and **12b**. Alkylation of a mixture of the syn and anti oximes of methyl *p*-[(3-aminoacetonyl)methylamino]benzoate[4] with **4a** gave the nitropyridine **6a**. Hydrolysis of the oxime function of **6a** with hydrochloric acid on a small scale gave a 76% yield of the ketone **7a**. Reduction of the nitro group of **7a** with Raney nickel and hydrogen and cyclization *in situ* of the intermediate aminopyridine gave the dihydro-1-deazapteridine **9a**. However, the hydrolysis of the oxime **6a** on a large scale gave only a 27% yield of **7a**. The low yield was attributed to cleavage of the acetonyl side chain, probably *via* a Beckmann rearrangement. This surmise was supported by the identification of ethyl 4,6-diamino-5-nitro-2-pyridinecarbamate[5] and methyl *p*-(methylamino)benzoate in the reaction residue. Another route to **9a** involved the hydrogenation of the oxime **6a**. Reduction of the oxime function before the nitro group of **6a** would result in the formation of **8**, whereas, reduction of the nitro group before the oxime function might result in the formation of **9a** by a transamination type of reaction. Both **8** (10%) and **9a** (64%) were isolated as analytically pure samples from this hydrogenation reaction. Oxidation of a solution of **9a** in DMAC with $KMnO_4$ in acetone gave **10a**, which was treated with ethanolic KOH to hydrolyze both the ester and urethane groups to give **13a**. The condensation of **13a** with diethyl L-glutamate in the presence of *N*,*N*′-dicyclohexylcarbodiimide to give the diethyl ester of **12a** was unsuccessful. However, acylation of the amino groups of **13a** with Ac_2O gave **14a**, which was coupled with diethyl L-glutamate to give **11a**. Hydrol-

ysis of the blocking groups of the latter with aqueous sodium hydroxide gave the desired 1-deazamethotrexate **12a**.

For the synthesis of 3-deazamethotrexate (**12b**), an intermediate isomeric with **4a** was prepared from **15**.[5] Treatment of **15** with HBr in HOAc removed the diphenylmethyl group to give **16**, which was treated with $POCl_3$ to give **4b**. The alkylation of a



15



16

mixture of the syn and anti oximes described above with **4b** gave **6b**. Treatment of **6b** with hydrochloric acid to give **7b** resulted in cleavage of the side chain to give ethyl 4,6-diamino-5-nitro-2-pyridinecarbamate. The hydrogenation of **6b** with Raney nickel, however, gave a good yield of **9b**. The latter was converted to 3-deazamethotrexate (**12b**) by the same sequence of reactions that was described above for the preparation of **12a**.

The specific rotation of **12b** was determined in 1 *N* NaOH. Although a solution of **12a** in this medium was opaque to sodium D light, the rotation of a solution of the blocked derivative **11a** in EtOH was observed. To estimate the extent of racemization, if any, of **12a** and **12b** during the base hydrolysis, the rotation of a solution of *N*-(*p*-aminobenzoyl)-L-glutamic acid in EtOH and 1 *N* NaOH was determined before and after heating at reflux temperature of 1 hr. There was no observed decrease in optical activity within the limits of experimental error.

The apparent pK_a values (determined spectrophotometrically) of **12a** and **12b** indicate that **12a** (pK_a 4.5) was less basic than methotrexate (pK_a 5.5) and that **12b** (pK_a 7.4) was more basic.

Experimental Section

Melting points were determined on a Kofler Heizbank or, when indicated, on a Mel-Temp or Mettler FP1 apparatus. The ultraviolet absorption spectra were determined in aqueous solution[7] with Cary Model 14 and 17 spectrophotometers, whereas the infrared absorption spectra were determined in pressed KBr disks with a Perkin–Elmer Model 521 spectrophotometer. The pmr spectra were determined in a 5–7% w/v solution in DMSO-d_6 or CF_3CO_2D, when indicated, with a Varian A-60A spectrometer at a probe temperature of about 37° with tetramethylsilane as an internal reference. The relative peak areas are given to the nearest whole number, and chemical shifts quoted in the case of multiples are measured from the approximate center.

Ethyl 6-Amino-4-[[3-(*p*-ethoxycarbonylanilino)acetonyl]amino]-5-nitro-2-pyridinecarbamate Oxime (1).—A mixture of ethyl *p*-[(3-aminoacetonyl)amino]benzoate oxime (1.63 g, 6.49 mmol),[4] **4a** (1.69 g, 6.49 mmol),[5] Et_3N (656 mg, 6.49 mmol), and EtOH (30 ml) was heated at 65° under N_2 for 18 hr. The mixture was cooled to 20°, and the yellow product was collected by filtration, washed with cold EtOH, and dried *in vacuo* over P_2O_5: yield 1.83 g (59%); mp 203° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 278 (15.9), 308 (20.4), 343 (13.1); pH 7, 261 (16.8), 308 (21.9), 352 (13.5); 0.1 *N* NaOH, 264 (16.0), 313 (23.9), 359 (14.2); ν_{max}, cm^{-1}, 1740, 1700 (C=O); 1590, 1553 (C=C, C=N).

Anal. Calcd for $C_{20}H_{25}N_7O_7$: C, 50.52; H, 5.30; N, 20.62. Found: C, 50.55; H, 5.40; N, 20.69.

Ethyl 6-Amino-4-[[3-(*p*-ethoxycarbonylanilino)acetonyl]amino]-5-nitro-2-pyridinecarbamate (2).—A suspension of finely powdered **1** (1.60 g, 3.36 mmol) in 1.0 *N* HCl (65 ml, 65 mmol) and EtOH (10 ml) was stirred at 50° for 6.5 hr. The mixture at 0° was made slightly basic with NH_4OH, and the product was collected by filtration, washed with H_2O, and dried *in vacuo* over P_2O_5: yield 1.48 g (96%); mp ~199° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 221 (30.3), 276 (18.6), 303 (22.0), 342 (14.8); pH 7, 227 (20.4), 311 (17.7), 352 (14.7); 0.1 *N* NaOH, 258 (14.9), 305 (broad) (14.3), 360 (shoulder) (27.9), 386 (30.2); ν_{max}, cm^{-1}, 1737, 1685 (C=O); 1597, 1550 (C=C, C=N).

Anal. Calcd for $C_{20}H_{24}N_6O_7$: C, 52.17; H, 5.25; N, 18.25. Found: C, 51.94; H, 5.22; N, 18.07.

Ethyl 5-Amino-1,2-dihydro-3-[(*p*-ethoxycarbonylanilino)methyl]pyrido[3,4-*b*]pyrazine-7-carbamate Ethanolate (3).—A suspension of **2** (1.17 g, 2.54 mmol) in EtOH (50 ml) was hydrogenated in the presence of Raney Ni (4.4 g, weighed wet with EtOH) at room temperature and atmospheric pressure for 24 hr. The resulting mixture was diluted with EtOH (30 ml), heated to dissolve the product, filtered under N_2, and refrigerated. The yellow crystalline product was collected by filtration, washed with cold EtOH, and dried *in vacuo* over P_2O_5: yield 690 mg (66%); mp ~168° (darkens from 158°, Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 254 (34.4), 302 (20.1); pH 7, 223 (17.3), 250 (14.4), 324 (17.2); ν_{max}, cm^{-1}, 1728, 1670 (C=O); 1597, 1518 (C=C, C=N).

Anal. Calcd for $C_{20}H_{24}N_6O_4 \cdot C_2H_6O$: C, 57.63; H, 6.60; N, 18.33. Found: C, 57.76; H, 6.35; N, 18.56.

Ethyl 4-Amino-6-chloro-5-nitro-2-pyridinecarbamate (4b).[5]—A mixture of **16** (16.6 g) and $POCl_2$ (560 ml) was heated with stirring at 80° for 2 hr. The resulting solution was concentrated *in vacuo* to a small volume and added with stirring to crushed ice. The solid (17.4 g) that deposited was collected by filtration and recrystallized from 1:1 EtOH–H_2O, yield 15.1 g (84%), mp 185–186°.[8]

Ethyl 5-Amino-3-[(*p*-ethoxycarbonylanilino)methyl]pyrido[3,4-*b*]pyrazine-7-carbamate (5).—A mixture of **3** (600 mg, 1.31 mmol), $MgSO_4$ (600 mg), and DMAC (14 ml) was treated dropwise with an 0.27% solution of $KMnO_4$ in acetone (56.3 ml, 0.961 mmol). The resulting solution was charcoaled, filtered, concentrated on a water bath *in vacuo* to ~2 ml, diluted with H_2O (25 ml), and refrigerated. The orange precipitate was collected by filtration, washed with H_2O, recrystallized from EtOH (~10 ml), and dried *in vacuo* over P_2O_5: yield 122 mg (23%); mp ~201° dec (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 232 (19.6), 255 (sh) (17.2), 310 (32.2); ν_{max}, cm^{-1}, 1728, 1680 (C=O); 1593, 1520 (C=C, C=N).

Anal. Calcd for $C_{20}H_{22}N_6O_4$: C, 58.53; H, 5.40; N, 20.48. Found: C, 58.32; H, 5.37; N, 20.33.

Ethyl 6-Amino-4-[[3-(*p*-methoxycarbonyl-*N*-methylanilino)-acetonyl]amino]-5-nitro-2-pyridinecarbamate Oxime (6a).—A mixture of methyl *p*-[(3-aminoacetonyl)methylamino]benzoate oxime (2.14 g, 8.51 mmol),[4] **4a** (2.22 g, 8.51 mmol),[5] Et_3N (862 mg, 8.51 mmol), and MeOH (30 ml) was refluxed for 16 hr under N_2 and cooled to 25°. The yellow crystalline product was collected by filtration, washed with cold MeOH, and dried *in vacuo* over P_2O_5: yield 3.26 g (81%); mp 197° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 233 (24.3), 277 (10.2), 317 (22.4); pH 7, 259 (13.3), 318 (25.2), 353 (sh) (11.5); 0.1 *N* NaOH, 261 (12.2), 323 (26.6), 360 (sh) (11.9); ν_{max}, cm^{-1}, 1735, 1710, 1690, 1680 (C=O); 1600 (NH_2); 1550, 1515 (C=C, C=N).

Anal. Calcd for $C_{20}H_{25}N_7O_7$: C, 50.52; H, 5.30; N, 20.62. Found: C, 50.56; H, 5.29; N, 20.64.

Ethyl 4-Amino-6-[[3-(*p*-methoxycarbonyl-*N*-methylanilino)-acetonyl]amino]-5-nitro-2-pyridinecarbamate Oxime (6b).—A mixture of methyl *p*-[(3-aminoacetonyl)methylamino]benzoate oxime (60.5 g, 241 mmol),[4] **4b** (62.7 g, 241 mmol), Et_3N (24.4 g, 241 mmol), and MeOH (800 ml) was refluxed under N_2 for 18 hr and cooled to 20°. The orange product was collected, washed with MeOH (200 ml), and dried *in vacuo* over P_2O_5: yield 91.6 g (80%); mp 183° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 223 (31.0), 272 (12.1), 315 (23.5); pH 7, 257 (13.8), 318 (27.0); 0.1

(7) Each solution contains 10% dissolving solvent and 90% appropriate aqueous solvent: (a) EtOH; (b) DMSO–MeOH (4:46); (c) 0.01 *N* NaOH.

(8) Previously, the melting point of **4b** was incorrectly reported as 284–286°; see ref 5.

N NaOH, 260 (sh) (13.4), 324 (29.0); ν_{max}, cm^{-1}, 1720, 1670 (C=O); 1601 (NH_2); 1555, 1535, 1523 (C=C, C=N).

Anal. Calcd for $C_{20}H_{25}N_7O_7$: C, 50.52; H, 5.30; N, 20.62. Found: C, 50.34; H, 5.46; N, 20.48.

Hydrolysis of **6b** in a refluxing solution of 1 *N* HCl and CH_3OH gave, as the major product, ethyl 4,6-diamino-5-nitro-2-pyridinecarbamate,[5] identified by tlc.

Ethyl 6-Amino-4-[[3-(*p*-methoxycarbonyl-*N*-methylanilino)-acetonyl]amino]-5-nitro-2-pyridinecarbamate (7a).—A mixture of **6a** (476 mg, 1.00 mmol), 1 *N* HCl (20 ml), and MeOH (2 ml) was stirred at 50° for 16 hr. The resulting mixture was made basic with NH_4OH, and the precipitate was collected by filtration, washed with cold H_2O, and dried *in vacuo* over P_2O_5. A solution of the crude product in DMSO (15 ml) at 80° was diluted with H_2O (4 ml), and the crystalline product was collected by filtration, washed with DMSO–H_2O (4:1), then H_2O, and dried *in vacuo* over P_2O_5: yield 350 mg (76%); mp ~234° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7b] 0.1 *N* HCl, 276 (14.0), 313 (25.7); ν_{max}, cm^{-1}, 1750, 1728, 1693 (C=O); 1610 (NH_2); 1561, 1528, 1495 (C=C, C=N).

Anal. Calcd for $C_{20}H_{24}N_6O_7$: C, 52.17; H, 5.25; N, 18.25. Found: C, 51.98; H, 5.30; N, 18.56.

Hydrolysis of **6a** on a 21-mmol scale by the above procedure gave a 27% yield of the ketone **7a**. Addition of H_2O to the mother liquor gave several products, one of which was identified as ethyl 4,6-diamino-5-nitro-2-pyridinecarbamate[5] by its tlc, and another after sublimation as methyl *p*-methylaminobenzoate by its tlc, melting point, and pmr spectrum.

Ethyl 5-Amino-1,2-dihydro-3-[(*p*-methoxycarbonyl-*N*-methylanilino)methyl]pyrido[3,4-*b*]pyrazine-7-carbamate (9a) and Ethyl 5,6-Diamino-4-[[2-amino-3-(*p*-methoxycarbonyl-*N*-methylanilino)propyl]amino]-2-pyridinecarbamate (8). A.—A suspension of **7a** (1.45 g, 3.15 mmol) in EtOH (300 ml) was hydrogenated in the presence of Raney Ni (3.4 g, weighed wet with EtOH) at room temperature and atmospheric pressure. After 72 hr, additional catalyst (3 g) was added to the mixture, which was hydrogenated at 50° for 30 min, then at 25° for 48 hr to give a total H_2 ptake of ~410 ml. The solvent containing suspended product was decanted from the catalyst, which was extracted with additional hot EtOH (three 100-ml portions). The EtOH suspension was refluxed under N_2 for a short time, filtered, and concentrated by boiling to ~180 ml. The brown crystalline **9a** was collected by filtration, washed with EtOH, and dried *in vacuo* over P_2O_5: yield 780 mg (60%); mp ~170° (Mettler FP1); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 254 (36.2), 312 (25.5); pH 7, 313 (29.0); ν_{max}, cm^{-1}, 1708, 1690 (C=O); 1603 (NH_2); 1520 (C=C, C=N); pmr δ 1.18 (t, 3, CCH_3), 3.05 (3, NCH_3), 3.73 (OCH_3), ~4 (m, OCH_2, 2-CH_2, NCH_2), 5.13 (2, NH_2), 6.37 (2, 8-CH, NH), 6.78, 7.76 (m, 4, C_6H_4), 8.94 (1, NH).

Anal. Calcd for $C_{20}H_{24}N_6O_4$: C, 58.24; H, 5.87; N, 20.38. Found: C, 58.43; H, 5.66; N, 20.22.

B.—A suspension of **6a** (10.0 g, 21.0 mmol) in EtOH (1250 ml) was hydrogenated at 45° for 72 hr in the presence of Raney Ni (20.0 g, weighed wet with EtOH). The reaction mixture, which had taken up 2.22 l. (91.0 mmol) of H_2, was evaporated to dryness *in vacuo* and the residue triturated with EtOH (100 ml). The mixture was filtered under N_2 and the residue washed with additional EtOH (100 ml). The catalyst-containing product was stirred with DMAC (160 ml) at 90° and filtered through Celite under N_2. The Celite was rinsed with DMAC (20 ml). The hot filtrate was diluted with H_2O (180 ml) and allowed to cool. The crystalline **9a** was collected by filtration, washed with cold DMAC–H_2O (1:1), then H_2O, and dried *in vacuo* over P_2O_5. The product was then triturated with $CHCl_3$ (40 ml) and redried *in vacuo* over P_2O_5, yield 5.50 g (64%), mp ~172° dec (Mettler FP1).

The combined EtOH extract and wash from the above reaction was evaporated to dryness *in vacuo*. A solution of the residue (2.10 g) in $CHCl_3$ (5 ml) was refrigerated for 96 hr. The crystalline **8** was collected by filtration, washed with cold $CHCl_3$, and dried *in vacuo* over P_2O_5: yield 940 mg (10%); melting point indefinite; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 235 (40.0), 306 (28.5); pH 7, 232 (36.8), 308 (26.8); ν_{max} cm^{-1}, 1720, 1680 (C=O); 1600 (NH_2); 1520 (C=C, C=N).

Anal. Calcd for $C_{20}H_{29}N_7O_4$: C, 55.67; H, 6.77; N, 22.72. Found: C, 55.54; H, 6.73; N, 22.31.

Ethyl 8-Amino-3,4-dihydto-2-[(*p*-methoxycarbonyl-*N*-methylanilino)methyl]pyrido[2,3-*b*]pyrazine-6-carbamate (9b).—A suspension of **6b** (13.3 g, 28.0 mmol) in EtOH (1600 ml) was hydrogenated at 45° for 3 days in the presence of Raney Ni (15 g, weighed wet with EtOH). The reaction mixture, which had taken up 2800 ml (115 mmol) of H_2, was evaporated to dryness *in vacuo*, and the residue was triturated with EtOH (100 ml). The mixture was filtered under N_2 and the residue washed with additional EtOH (200 ml). The catalyst-containing product was stirred with DMAc (200 ml) at 100° for 15 min and filtered through Celite under N_2. The Celite was rinsed with DMAC (10 ml) and the filtrate diluted with H_2O (150 ml) and allowed to cool to 25°. The crystalline product was collected by filtration, washed with cold DMAC–H_2O(1:1), then H_2O, and dried at 100° *in vacuo* over P_2O_5: yield 7.4 g (64%); mp ~250° dec (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[8a] 0.1 *N* HCl, 232 (28.7), 313 (26.9); ν_{max}, cm^{-1}, 1719, 1683 (C=O); 1640, 1597, 1555, 1516 (NH_2, C=C, C=N); pmr, δ 1.18 (t, 3, CCH_3), 3.05 (3, NCH_3), 3.75 (3, OCH_3), ~4 (m, 6, OCH_2, 3-CH_2, NCH_2), 5.48 (2, 8-NH_2), 5.82 (1, NH), 6.49 (1, 7-CH), 6.80, 7.77 (m, 4, C_6H_4), 8.85 (1, NH).

Anal. Calcd for $C_{20}H_{24}N_6O_4$: C, 58.24; H, 5.87; N, 20.38. Found: C, 58.39; H, 5.94; N, 20.54.

Ethyl 5-Amino-3-[(*p*-methoxycarbonyl-*N*-methylanilino)methyl]pyrido[3,4-*b*]pyrazine-7-carbamate (10a).—A solution of **9a** (500 mg, 1.21 mmol) in DMAC (12 ml) was treated (~2 drops per second) with a 0.27% solution of $KMnO_4$ in acetone (57 ml, 0.974 mmol), stirred with $MgSO_4$ (500 mg) for 30 sec, and filtered under N_2. The precipitate was washed well with acetone, and the combined filtrate and wash were evaporated at 25° *in vacuo* to leave a solution of the product in DMAC. This solution was treated dropwise with H_2O (18 ml) and stirred for 5 min to give a yellow product which was collected by filtration, washed with DMAC–H_2O (2:3), then H_2O, and dried *in vacuo* over P_2O_5: yield 422 mg (85%); mp 217°; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 133 (20.7), 258 (sh) (15.7), 312 (37.0); ν_{max}, cm^{-1}, 1745, 1695 (C=O); 1608 (NH_2); 1520 (C=C, C=N); pmr δ 1.26 (t, 3, CCH_3), 3.24 (3, NCH_3), 3.75 (3, OCH_3), 4.15 (q, OCH_2), 4.90 (2, NCH_2), ~6.9 (NH_2), 6.87, 7.75 (m, C_6H_4), 7.43 (1, 8-CH), 8.78 (1, 2-CH), 9.62 (1, NH).

Anal. Calcd for $C_{20}H_{22}N_6O_4$: C, 58.53; H, 5.40; N, 20.48. Found: C, 58.72; H, 5.45; N, 20.24.

Ethyl 8-Amino-2-[(*p*-methoxycarbonyl-*N*-methylanilino)methyl]pyrido[2,3-*b*]pyrazine-6-carbamate (10b).—A suspension of **9b** (2.25 g, 5.46 mmol) in DMAC (70 ml) was stirred at 45° for 10 min, treated dropwise (rapidly) with a 0.27% solution of $KMnO_4$ in acetone (184 ml, 3.15 mmol), stirred with $MgSO_4$ (2.25 g) for 30 sec, and filtered rapidly. The MnO_2 residue containing product was washed well with acetone and dried under N_2. This residue was extracted at 95° with DMAC (25 ml), filtered through Celite, and diluted with H_2O (10 ml). The crystalline product was collected by filtration, washed with DMAC–H_2O (2:1), then H_2O, and dried at 100° *in vacuo* over P_2O_5, yield 334 mg. Product also crystallized from the reaction filtrate, which was collected by filtration, washed with acetone, and dried at 100° *in vacuo* over P_2O_5, yield 771 mg. The mother liquor was concentrated at 60° *in vacuo* to ~60 ml and diluted with H_2O (25 ml). The crystalline product was collected, washed with DMAC–H_2O (2:1), then H_2O, and dried at 100° *in vacuo* over P_2O_5: yield 586 mg; the ultraviolet spectra and thin layer chromatograms of the three crops were found to be identical; total yield 1.69 g (76%), mp >360° (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 224 (38.4), 260 (sh) (13.2), 315 (38.1); ν_{max}, cm^{-1}, 1737, 1690 (C=O); 1610 (NH_2); 1575, 1527 (C=C, C=N); pmr (CF_3CO_2D) δ 1.45 (t, 3, CCH_3), 3.75 (3, NCH_3), 4.07 (3, OCH_3), 4.48 (q, 2, OCH_2), 5.50 (2, NCH_2), 6.73 (1, 7-CH), 7.87, 8.29 (m, 4, C_6H_4), 9.00 (1, 3-CH).

Anal. Calcd for $C_{20}H_{22}N_6O_4$: C, 58.53; H, 5.40; N, 20.48. Found: C, 58.46; H, 5.47; N, 20.32.

Diethyl *N*-[*p*-[[(5,7-Diacetamidopyrido[3,4-*b*]pyrazin-3-yl)-methyl]methylamino]benzoyl]-L-glutamate Hydrate (11a).—A solution of **14a** monohydrate (2.00 g, 4.70 mmol) in anhydrous pyridine (15 ml) was treated with *N*,*N*′-dicyclohexylcarbodiimide (969 mg, 4.70 mmol). After stirring for 5 min, this mixture was treated with diethyl L-glutamate hydrochloride (1.13 g, 4.70 mmol), and the whole was stirred for 3 days. The precipitate of *N*,*N*′-dicyclohexylurea was removed by filtration under N_2 and rinsed with pyridine (10 ml). The combined filtrate and wash was evaporated to dryness at 40° on a spin evaporator, and the residue was triturated with Et_2O (20 ml) and dried *in vacuo* over P_2O_5. A solution of the crude product in $CHCl_3$ (50 ml) was gently extracted with 0.3 *N* NH_4OH (four 50-ml portions). The interface was retained with the organic layer after each extraction. The organic layer was evaporated to dryness *in vacuo*. A filtered

solution of the residue in $CHCl_3$ (100 ml) was adsorbed on a short column (6.5 cm diameter, containing 25 g of SilicAR TLC-7 equilibrated with $CHCl_3$). The column was eluted with $CHCl_3$ (1200 ml) followed by 99:1 $CHCl_3$–CH_3OH, and the fractions were analyzed by tlc (97:3 $CHCl_3$–CH_3OH). A small forerun was discarded, and the main fraction was collected and evaporated to dryness. The yellow residue was triturated with Et_2O and dried at 65° *in vacuo* over P_2O_5: yield 1.28 g (45%); mp ~91° (Mettler FP1); $[\alpha]^{25}D$ −15.6 ± 2.1° (*c* 1.0, EtOH); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 252 (32.2), 306 (32.6), 383 (5.39); pH 7, 261 (40.0), 305 (29.4), 374 (5.64); ν_{max}, cm^{-1}, 1725, 1690 (C=O); 1600, 1500 (NH, C=C, C=N).

Anal. Calcd for $C_{29}H_{35}N_7O_7 \cdot H_2O$: C, 56.95; H, 6.10; N, 16.03. Found: C, 56.86; H, 5.77; N, 15.79.

The NH_4OH extracts from the above reaction were combined, filtered, and acidified to pH 3 with 6 *N* HCl. The brown precipitate of recovered **8a** was separated by centrifugation, washed with H_2O, and dried *in vacuo* over P_2O_5, yield 420 mg (21%).

Diethyl *N*-[*p*-[[(6,8-Diacetamidopyrido[2,3-*b*]pyrazin-2-yl)methyl]methylamino]benzoyl]-L-glutamate (11b).—A mixture of **14b** (5.34 g, 12.6 mmol), diethyl L-glutamate hydrochloride (3.03 g, 12.6 mmol), *N*,*N*′-dicyclohexylcarbodiimide (2.60 g, 12.6 mmol), and anhydrous pyridine (76 ml) was stirred for 72 hr. The precipitate of *N*,*N*′-dicyclohexylurea was removed by filtration under N_2 and washed with pyridine until free of colored products. The combined filtrate and wash was evaporated to dryness at 25° under high vacuum. A solution of the crude product in $CHCl_3$ (380 ml) was extracted gently with 0.3 *N* NH_4OH (five 125-ml portions). The interface was retained with the organic layer after exch extraction. The organic layer was evaporated to dryness *in vacuo*. A filtered solution of the residue in $CHCl_3$ (300 ml) was adsorbed on a short column (9 cm d, containing 80 g of SilicAR TLC-7 equilibrated with $CHCl_3$). The column was eluted with $CHCl_3$ (100 ml) followed by 99:1 $CHCl_3$–CH_3OH, and the fractions analyzed by tlc (95:5 $CHCl_3$–CH_3OH). A small forerun was discarded and the main fraction collected and evaporated to dryness. The yellow product was triturated with Et_2O and dried at 100° *in vacuo* over P_2O_5: yield 3.6 g (48%); mp ~239° dec (Mettler FP1); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7b] 0.1 *N* HCl, 310 (31.2), 332 (sh) (26.9), 347 (sh) (21.6); pH 7, 251 (29.6), 308 (29.5); 0.1 *N* NaOH, 305 (26.7); ν_{max}, cm^{-1}, 1728, 1714, 1694 (C=O), 1620, 1604, 1572, 1548, 1516 (NH, C=C, C=N).

Anal. Calcd for $C_{29}H_{35}N_7O_7$: C, 58.68; H, 5.94; N, 16.52. Found: C, 58.87; H, 6.19; N, 16.55.

***N*-[*p*-[[(5,7-Diaminopyrido[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoyl]-L-glutamic Acid Hydrochloride Hydrate (10:1:16) (12a).**—A solution of **11a** (133 mg, 0.217 mmol) in EtOH (5 ml) and 1 *N* NaOH (2.17 ml, 2.17 mmol) was refluxed under N_2 for 1 hr and evaporated to dryness *in vacuo*. A solution of the residue in H_2O (5 ml) was filtered and carefully acidified with 1 *N* HCl to pH 3. The orange product was collected by centrifugation at 4°, washed with cold H_2O at pH 3, and dried at 65° *in vacuo* over P_2O_5: yield 101 mg (96%); melting point indefinite, turned black at ~170° (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7c] 0.1 *N* HCl, 245 (22.8), 321 (29.8), 427 (br) 3.81); pH 7 and 0.1 *N* NaOH, 268 (28.3), 306 (28.0), 427 (br) (3.81); ν_{max}, cm^{-1}, 1700 (sh) (CO), 1650 (sh) (NH_2); 1595, 1495 (C=C, C=N); pmr δ 1.8–2.6 (m, CH_2CH_2), 3.23 (3, NCH_3), 4.4 (m, NCH), 4.85 (2, NCH_2), 5.99 (8-CH), 6.8 (NH_2), 6.95, 7.87 (m, C_6H_4), 8.24 (d, 1, NH), 8.62 (1, 2-CH), 3–7 (NH_2, HCl, OH).

Anal. Calcd for $C_{21}H_{23}N_7O_5 \cdot 0.1HCl \cdot 1.6H_2O$: C, 51.91; H, 5.46; Cl, 0.73; N, 20.18. Found: C, 51.80; H, 5.19; Cl, 0.71; N, 20.03.

***N*-[*p*-[[(6,8-Diaminopyrido[2,3-*b*]pyrazin-2-yl)methyl]methylamino]benzoyl]-L-glutamic Acid Hydrochloride (10:1) Monohydrate (12b).**—A solution of **11b** (594 mg, 1.00 mmol) in EtOH (23 ml) and 1 *N* NaOH (10.0 ml, 10.0 mmol) was refluxed under N_2 for 1 hr and evaporated to dryness *in vacuo*. A solution of the residue in H_2O (15 ml) was filtered and carefully acidified with 1 *N* HCl to pH 5. The yellow product was collected by centrifugateion at 4°, washed with cold H_2O at pH 5, and dried at 100° *in vacuo* over P_2O_5: yield 428 mg (90%); mp ~228° dec (darkens above 200°, Mel-Temp); $[\alpha]^{25}D$ 28.0 ± 1.9° [*c* 1.0, 1 *N* NaOH]; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7c] 0.1 *N* HCl, 223 (49.1), 313 (br) (21.4), 330 (br) (20.9); pH 7, 221 (48.9), 264 (14.6), 306 (27.2), 345 (sh) (15.6); 0.1 *N* NaOH, 219 (44.1), 262 (21.2), 305 (25.8), 355 (br) (12.4); ν_{max}, cm^{-1}, 1648 (C=O); 1597, 1540, 1496 (NH_2, C=C, C=N); pmr δ 1.8–2.6 (m, CH_2CH_2), 3.18 (3, NCH_3), 4.33 (m, 1, NCH), 4.82 (2, NCH_2), 6.07 (1, 7-CH), 6.4–7.6 (NH_2, HCl, OH), 6.81, 7.73 (m, C_6H_4), 8.03 (d, 1, NH), 8.52 (1, 3-CH).

Anal. Calcd for $C_{21}H_{23}N_7O_5 \cdot H_2O \cdot 0.1HCl$: C, 53.10; H, 5.32; Cl, 0.74; N, 20.64. Found: C, 53.15; H, 5.00; Cl, 0.73; N, 20.55.

***p*-[[(5,7-Diaminopyrido[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoic Acid (13a).**—A solution of **10a** (296 mg, 0.721 mmol) and KOH (1.48 g, 26.4 mmol) in EtOH (30 ml) was refluxed under N_2 for 17 hr and evaporated to dryness at 60° *in vacuo*. A solution of the residue in H_2O (15 ml) was filtered and carefully acidified to pH 4 with 6 *N* HCl. The red solid was collected by filtration, washed with H_2O, and dried *in vacuo* over P_2O_5: yield 220 mg (94%); melting point indefinite, turned black above 245° (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7c] 0.1 *N* HCl, 242 (21.4), 322 (34.3), 433 (br) (3.44); pH 7, 270 (30.7), 300 (sh) (23.4), 432 (br) (3.82); 0.1 *N* NaOH, 270 (31.4), 300 (sh) (23.4), 432 (br) (3.82); ν_{max}, cm^{-1}, 1673 (CO), 1610 (sh), 1596, 1525 (NH_2, C=C, C=N); pmr δ 3.18 (3, NCH_3), 4.75 (2, NCH_2), 5.9 (3, NH_2, 8-CH), 6.75 (br) (NH), 6.74, 7.72 (m, C_6H_4), 8.47 (1, 2-CH).

Anal. Calcd for $C_{16}H_{16}N_6O_2$: C, 59.25; H, 4.97; N, 25.91. Found: C, 59.19; H, 5.00; N, 25.83.

***p*-[[(6,8-Diaminopyrido[2,3-*b*]pyrazin-2-yl)methyl]methylamino]benzoic Acid Monohydrochloride (13b).**—A solution of **10b** (5.90 g, 14.4 mmol) and KOH pellets (29.5 g) in EtOH (590 ml) was refluxed under N_2 for 16 hr and cooled in an ice bath. The yellow precipitate was collected by filtration, washed with cold EtOH, and dried *in vacuo* over P_2O_5. A solution of the precipitate in boiling H_2O (100 ml) was filtered, acidified to pH 1 with 6 *N* HCl, and stirred for 30 min. The yellow product was collected, washed with H_2O at pH 1, and dried at 100° *in vacuo* over P_2O_5, yield 4.1 g (80%), mp 265° dec (Mel-Temp).

Anal. Calcd for $C_{16}H_{16}N_6O_2 \cdot HCl \cdot H_2O$: C, 50.73; H, 4.79; Cl, 9.36; N, 22.19. Found: C, 51.05; H, 5.04; Cl, 9.51; N, 22.13.

Further drying at 100° gave the anhydrous compound, mp 266° dec, from which spectral data were obtained: λ_{max}, nm ($\epsilon \times 10^{-3}$),[7c] 0.1 *N* HCl, 223 (45.2), 313 (26.6); pH 7, 221 (41.2), 265 (sh) (16.1), 295 (21.4), 340 (14.1); 0.1 *N* NaOH, 219 (36.5), 263 (21.9), 293 (21.2), 355 (11.4); ν_{max}, cm^{-1}, 1685 (C=O); 1655 (NH_2); 1595, 1553, 1520 (C=C, C=N); pmr δ 3.23 (NCH_3), 4.90 (s, NCH_2), 6.19 (1, 7-CH), 6.84, 7.74 (m, C_6H_4), 7.6–8.4 (4, NH_2), 8.67 (1, 3-CH), ~12 (br) (2, OH, HCl).

Anal. Calcd for $C_{16}H_{16}N_6O_2 \cdot HCl$: C, 53.26; H, 4.75; Cl, 9.83; N, 23.29. Found: C, 53.12; H, 5.24; Cl, 9.66; N, 22.93.

The EtOH filtrate from the reaction mixture was evaporated to a yellow oil which was dissolved in boiling H_2O (200 ml), acidified to pH 1 with 6 *N* HCl, and cooled in an ice bath to give additional product, yield 0.50 g (10%), mp 261° dec.

***p*-[[(5,7-Diacetamidopyrido[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoic Acid Monohydrate (14a).**—A mixture of **13a** (4.31 g, 13.3 mmol) and Ac_2O (400 ml) was refluxed under N_2 for 4 hr and evaporated to dryness under high vacuum at 40–45°. The gummy residue was stirred with H_2O (130 ml) until it solidified to a homogeneous powder (~4 hr). The resulting mixture was treated with concentrated NH_4OH (7.25 ml) and stirred for 10 min to give a solution which was filtered and acidified with 1 *N* HCl to pH 4. The brown precipitate was collected by filtration, washed with H_2O, and dried *in vacuo* at 78° over P_2O_5: yield 5.10 g (90%); mp ~370° (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] 0.1 *N* HCl, 251 (29.4), 309 (31.5); pH 7, 261 (38.2), 290 (25.1); ν_{max}, cm^{-1}, 1690 (C=O); 1600, 1520 (NH, C=C, C=N).

Anal. Calcd for $C_{20}H_{20}N_6O_4 \cdot H_2O$: C, 56.33; H, 5.20; N, 19.71. Found: C, 56.50; H, 5.02; N, 19.87.

***p*-[[(6,8-Diacetamidopyrido[2,3-*b*]pyrazin-2-yl)methyl]methylamino]benzoic Acid Hydrochloride Hydrate (10:1:6) ((14b).**—A solution of **13b** (872 mg, 2.42 mmol) in Ac_2O (74 ml) was refluxed under N_2 for 2 hr and evaporated to dryness at 45°. The residue was stirred with H_2O (22 ml) until it solidified to a homogeneous powder (~1 hr). The resulting mixture was treated with concentrated NH_4OH (1.20 ml) and stirred for several minutes to give a solution which was filtered and acidified with 1 *N* HCl to pH 3–4. The yellow product was collected by filtration, washed with H_2O, and dried *in vacuo* at 78° over P_2O_5: yield 946 mg (92%); mp 275–283° dec (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$),[7c] 0.1 *N* HCl, 227 (30.5), 312 (33.4), 332 (sh) (28.3), 346 (sh) (22.1); pH 7, 223 (28.9), 251 (34.3), 295 (22.7), 338 (15.1); 0.1 *N* NaOH, 222 (29.8), 285 (23.1), 345 (11.4); ν_{max}, cm^{-1}, 1705 (C=O); 1600, 1550, 1520, 1508 (NH, C=C, C=N).

Anal. Calcd for $C_{20}H_{20}H_6O_4 \cdot 0.1HCl \cdot 0.6H_2O$: C, 56.81; H, 5.08; Cl, 0.84; N, 19.87. Found: C, 56.79; H, 4.82; Cl, 0.98; N, 19.81.

Ethyl 4-Amino-5-nitro-6(1*H*)-oxy-2-pyridinecarbamate (16).—A solution of **15**[5] (1.00 g, 2.45 mmol) in 10% HBr in HOAc (15 ml) containing phenol (50 mg) was stirred at room temperature for 24 hr. The solid (0.55 g) that deposited was collected by filtration; the filtrate was evaporated to dryness, and the residue was washed with ether to give an additional amount of product (0.20 g). The combined crops were recrystallized from 4:3 ethanol–water to give white crystals: yield 0.43 g (73%); mp 293–294° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] pH 7, 261 (6.53), 341 (13.8).

Anal. Calcd for $C_8H_{10}N_4O_5$: C, 39.67; H, 4.16; N, 23.13. Found: C, 39.45; H, 3.98; N, 23.26.

In a large-scale run (148 g), the crude product was washed with boiling ethanol to give material suitable for use in the next step, yield 81.5 g (93%), mp 285–290° dec.

Registry No.—**1**, 30768-44-6; **2**, 30768-45-7; **3**, 30768-46-8; **4b**, 6502-04-1; **5**, 30768-47-9; **6a**, 30826-43-8; **6b**, 30768-48-0; **7a**, 30826-44-9; **8**, 30768-49-1; **9a**, 30768-50-4; **9b**, 30768-51-5; **10a**, 30826-45-0; **10b**, 30768-52-6; **11a**, 30826-46-1; **11b**, 30826-47-2 **12a**, 30768-53-7; **12b**, 30826-48-3; **13a**, 30768-54-8; **13b**, 30826-49-4; **14a**, 30826-50-7; **14b**, 30826-51-8; **16**, 30768-55-9.

Acknowledgments.—The authors are indebted to Mr. R. B. Duncan for his work on the preparation of **4b** and to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectral determinations reported.

Microbiological Oxygenation of *cis*-5-Acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct[*b*]indole with *Calonectria decora*

THOMAS L. LEMKE, ROY A. JOHNSON,*[1] HERBERT C. MURRAY, DAVID J. DUCHAMP, CONSTANCE G. CHIDESTER, JACKSON B. HESTER, JR., AND RICHARD V. HEINZELMAN

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

Received December 11, 1970

Oxygenation of *cis*-5-acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct[*b*]indole (**3**) with *Calonectria decora* (CBS) gave *cis*-5-acetyl-5,5a,6,7,8,10,11,11a-octahydro-9*H*-cyclooct[*b*]indol-9-one (**4**) as the major product and *cis*-5-acetyl-5,5a,6,7,9,10,11,11a-octahydro-8*H*-cyclooct[*b*]indol-8-one (**5**) as the minor product. Dehydrogenation of **4** and **5**, followed by deacetylation, gave isomeric hexahydrocyclooct[*b*]indolones **8** and **9**, respectively. The positions of the ketones in these compounds were shown to be at C-8 and C-9 by nmr analysis. Final structure assignments, including the cis-ring junction in the octahydro compounds, were based on an X-ray crystallographic structure determination of the *p*-bromophenylhydrazone (**10**) of major product **4**.

In connection with other work in these laboratories, we have examined the microbiological oxygenation of *cis*-5-acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct-[*b*]indole (**3**). The substrate **3** was prepared by zinc–hydrochloric acid reduction[2] of 6,7,8,9,10,11-hexahydro-5*H*-cyclooct[*b*]indole (**1**),[3] followed by acetylation of the resulting product **2**. At the outset, the stereochemistry of the saturated ring juncture of **2** was unknown but was suspected to be cis on the basis of the nmr signals for the C-5a (δ 3.95, triplet, $W_{1/2}$ = 21 cps) and C-11a (δ 3.28, triplet, $W_{1/2}$ = 21 cps) protons. The $W_{1/2}$ expected for these protons if a trans ring juncture were present is 12–15 cps while a $W_{1/2}$ of 12–21 cps is expected for cis protons.

Oxygenation of **3** with the fungus *Calonectria decora* gave two ketones, **4** (38% yield) and **5** (7% yield), as the only isolable products. The presence of optical activity in the minor product **5** was demonstrated by a CD spectrum (see Experimental Section).[4] Product **4**, however, was devoid of optical activity by the same criterion. Nmr analysis at this point showed that oxygenation had not occurred at the C-6 or C-11 positions in either product, since the signals for the C-5a and C-11a protons remained as broad multiplets. Presence of a ketone at C-6 (or C-11) would eliminate two protons α to the bridgehead and thus simplify the nmr signal for the bridgehead proton at C-5a (or C-11a).

Dehydrogenation of **4** and **5** over palladium on carbon gave the *N*-acetylindoles **6** and **7**, respectively. It now was possible, on the basis of the nmr spectra of these two compounds, to eliminate positions C-7 or C-10 as sites of oxygenation in either compound. As above, a ketone at either position would again simplify the signal of the geminal protons (at C-6 or C-11) between the indole ring and the carbonyl group. However, such signals were not apparent in the spectra of **6** or **7** (see Experimental Section). This conclusion is confirmed by the nmr spectra of the indoles **8** and **9** obtained from deacetylation of **6** and **7**, respectively. Ultraviolet spectra (Experimental Section) of compounds **6–9** were consistent with the *N*-acetylindole (**6** and **7**) and indole **8** and **9** structures of these compounds.[5] The positions of the ketones in these compounds were thus narrowed to the C-8 and C-9 carbons.

An X-ray crystallographic analysis was sought in order to make a final decision as to the position of the ketones in the products and, at the same time, to clear up the uncertainty with regard to the stereochemistry of the ring juncture in the octahydro compounds. To this end, the *p*-bromophenylhydrazone (**10**) of the major ketonic oxygenation product was prepared.

The crystal structure of **10** was determined by the heavy-atom method. X-Ray results were used to pre-

(1) To whom correspondence should be addressed.

(2) N. K. Kochetkov, N. F. Kucherova, and I. G. Zhukova, *Zh. Obshch. Khim.*, **31**, 924 (1961).

(3) B. Witkop, J. B. Patrick, and M. Rosenblum, *J. Amer. Chem. Soc.*, **73**, 2641 (1951).

(4) We are indebted to Dr. W. C. Krueger for determination of this spectrum.

(5) *Cf.* A. I. Scott, "Interpretation of the Ultraviolet Spectra of Organic Molecules," Macmillan, New York, N. Y., 1964, p 172.