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The synthesis of the new 1-ethyl-5,6-dimethyl-7-(4-methyl-1-piperazinyl)-1,4-dihydro-4-oxo-1*H*-pyrrolo[3,4-*b*]pyridine-3-carboxylic acid (**12**) was carried out using ethyl 4-amino-2-methylpyrrole-3-carboxylate as the starting material. Compound **12** was devoid of antibacterial activity *in vitro*.

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In the preceding communication [2], the synthesis of a series of 4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylic acids was described. The other two classes of fused pyrrolopyridines, namely 4,7-dihydro-7-oxopyrrolo[3,2-*b*]pyridine-6-carboxylic acids and 1,4-dihydro-4-oxopyrrolo[3,4-*b*]pyridine-3-carboxylic acids have been described by Sowell *et al.* [3].

We now wish to report the synthesis of a member of the pyrrolo[3,4-*b*]pyridines which bears the piperazine ring in the 7-position. This was done on account of the greater potency and broader spectrum of activity obtained when this substituent was introduced on the nalidixic acid molecule. This compound (**12** in the Scheme of Synthesis), however, was devoid of antibacterial activity *in vitro* at concentrations less than 128 $\mu\text{g/ml}$. This result is surprising because the analogue compounds bearing a phenyl group in position 7 and different substituents on the pyrrole nitrogen were found to exhibit a broad spectrum of activity [3].

Chemistry.

Our synthetic route differs from that reported by Sowell *et al.* in that the pyridine ring was not constructed through the usual Gould-Jacobs reaction [4]. The starting β -aminopyrrole **2** was obtained in 74% yield by cyclization of the enamine **1** according to the procedure previously described [5]. The enamine **1** was prepared in 80% yield by a modification of the original procedure [6]. Contrary to what was observed in the case of 2-aminopyrroles, the amino group of **2** had to be protected with the carbobenzyloxy (Cbz) group both to selectively methylate the pyrrole nitrogen to give **4** and to confer stability to the molecule in the subsequent steps.

The bromination of the 5 position with bromine in carbon tetrachloride using diazabicyclooctane (Dabco) as an acid acceptor gave **5** which could not be obtained in a pure form notwithstanding the different solvents used for the recrystallization. For this reason compound **5** was not isolated and made to react with an excess of 1-methylpiperazine in ethanol solution to give **6** in 63% yield. The sodium salt of the 4-carbobenzyloxyamino group was then alkylated with ethyl iodide in DMF to afford **7**. In the nmr-spectrum, compound **7** appeared as a mixture of two isomers in the ratio 3:1, probably due to the hindered rotation around the Cbz-nitrogen bond. Deprotection of the

amino group was effected with palladium on carbon and formic acid [7] and the resulting 4-ethylamino group was made to react with an excess of ethyl acrylate using acetic acid as a catalyst [8]. The overall yield from **6** to **9** was 22%. The Dieckmann cyclization of **9** to yield **10** was obtained with sodium bis(trimethylsilyl)amide in ether. Other bases were not tried on account of the good results obtained with pyrrolo[2,3-*b*]pyridines [2]. The sequence halogenation-dehydrohalogenation in order to obtain **11** was not expedient in this case due to various by-products which formed by treating **10** with an ethanolic solution of iodine and potassium acetate. Better results were obtained by the dehydrogenation of **10** in ethanol:cyclohexene (1:10) using palladium on carbon as the catalyst [9,10]. The yield was low (30%) but compound **11** could be readily obtained in a pure form. The same yield was obtained when the dehydrogenation was run in toluene with the same amount of catalyst. However, five hours of reflux were necessary and the recovery of the product required a chromatographic purification.

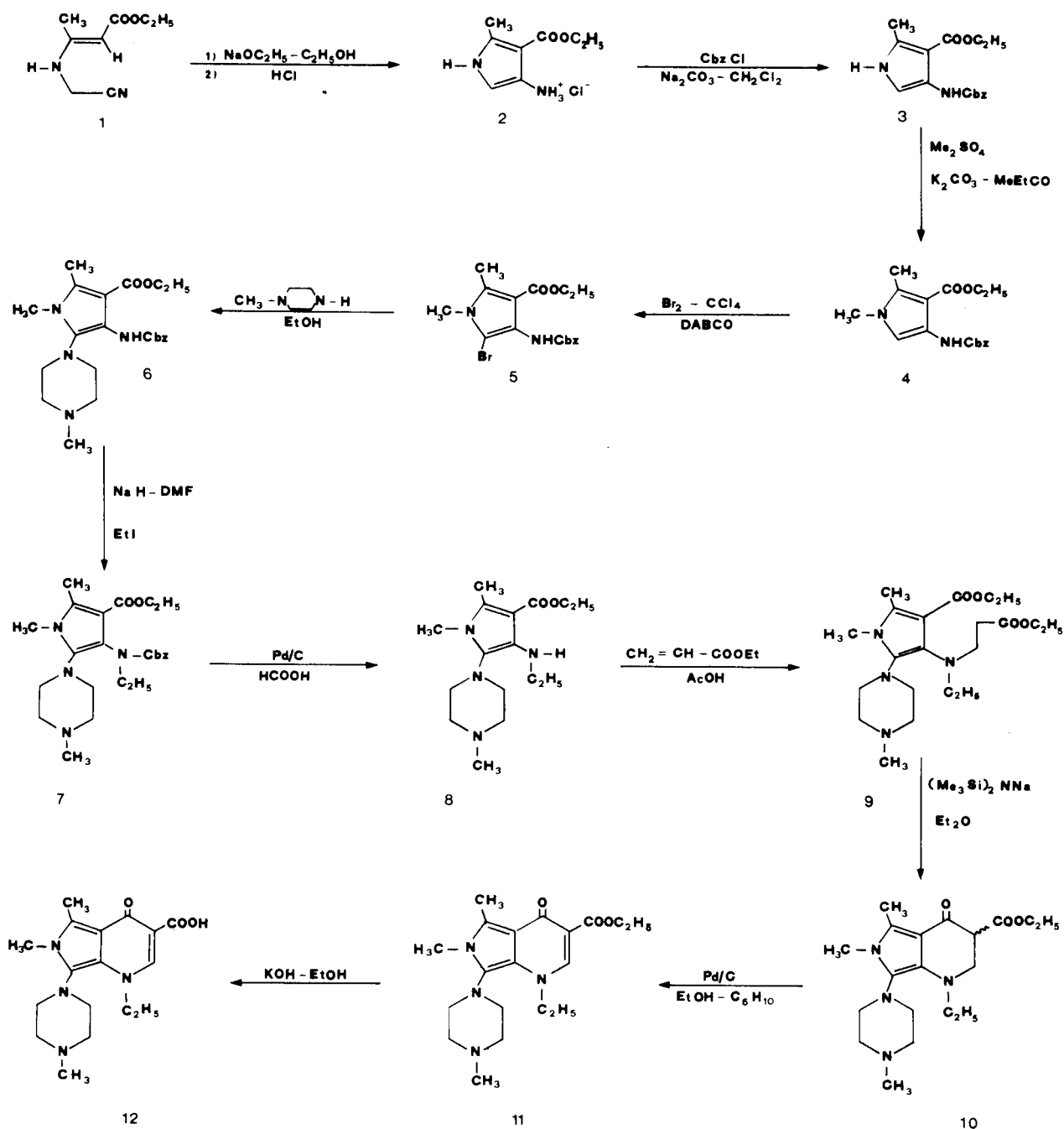
Finally, the hydrolytic cleavage of the ester function with potassium hydroxide in ethanol gave **12** in 42% yield.

It is interesting to note that in compounds **11** and **12**, due to the substitution pattern and the rigid ring system, ring inversion and rotation around the piperazine-pyrrole bond, is probably prevented. The axial and equatorial protons in the piperazine part are clearly separated. Due to the fixed geometry, the equatorial protons appear as doublets ${}^2J = 12$ Hz and their axial counterparts as triplets ${}^2J = {}^3J = 12$ Hz. Due to the substitution, the resonance lines at lower field are assigned to the protons adjacent to the pyrrole moiety and at higher field (δ 2.80, 2.29) to the protons adjacent to N-CH₃-piperazine.

EXPERIMENTAL

Melting points were determined on a Büchi SMP-510 capillary apparatus and are uncorrected. The ir spectra were obtained with a Perkin-Elmer 297 spectrophotometer, ν are given in cm^{-1} . The ¹H-nmr spectra were recorded on a Bruker WH-270 MHz spectrometer, chemical shifts are given in ppm (δ) relative to tetramethylsilane and J in Hz. The elemental analyses were performed by the Analytical Department of the Gruppo Lepetit. The tlc were performed on Merck silica gel plates, type 60 F-254. The purifications on silica gel columns were all obtained by the flash chromatography procedure [11].

SCHEME 1



$\text{Cbz} = \text{C}_6\text{H}_5\text{CH}_2\text{OCO}$

Ethyl 3-cyanomethylamino-2-butenoate (1).

To a solution of aminoacetonitrile hydrochloride (45.9 g, 0.496 mole) in methanol (260 ml) was added a solution of triethylamine (69.13 ml, 0.496 mole) in the same solvent (60 ml) under a nitrogen atmosphere and 10 minutes later a solution of ethyl acetoacetate (49.71 g, 0.382 mole) in

toluene (620 ml) containing a catalytic amount (0.2 g) of *p*-toluenesulfonic acid.

The reaction mixture was heated at reflux and the azeotropic mixture was distilled with a Dean-Stark trap during 4 hours. Toluene (300 ml) was added at about 50° followed by a 2% aqueous solution of sodium bicar-

bonate (170 ml). The organic phase was separated dried and concentrated to about 50 ml to give 51.7 g (80%) of crude **1** mp 98-100°; mp lit [6], 103-104° after recrystallization from 1-propanol; ir (nujol): 3300 (NH), 1640, 1600, 1580 (CO-C=C-N).

Anal. Calcd. for $C_8H_{12}N_2O_4$: C, 57.13; H, 7.19; N, 16.66. Found: C, 56.92; H, 7.48; N, 16.53.

Ethyl 4-Amino-2-methylpyrrole-3-carboxylate Hydrochloride (**2**).

To a solution of sodium (3.63 g, 0.158 g-atom) in absolute ethanol (400 ml) at 0° and under argon was added in portions **1** (23.8 g, 0.141 mole) and the stirring was maintained for 2 hours until all the solid dissolved. After staying at 5° for 3 days, the solution was acidified with hydrogen chloride and diluted with ethyl ether (800 ml). The precipitate was collected by filtration to give 29.5 g of a mixture of **2** and sodium chloride which was used as such in the next step. The amount of **2**, as determined by titration with 0.1 *N* sodium hydroxide was 72.2% and the yield was 74%. An analytical sample was obtained by dissolving **2** in absolute ethanol and diluting with ethyl ether, mp 211-214° dec; ir (nujol); 3310 (NH), 1660 (COOEt); ¹H-nmr (deuteriochloroform): δ 1.30 (t, J = 6, 3H, CH₃-CH₂), 2.41 (s, CH₃), 4.35 (q, 2H, CH₂-CH₃), 7.05 (s, H-C5), 10.26 (br s, NH₃⁺), 12.26 (br s, NH).

Anal. Calcd. for $C_8H_{12}N_2O_4 \cdot HCl$: C, 46.95; H, 6.40; N, 13.69. Found: C, 47.08; H, 6.42; N, 13.86.

Ethyl 4-Benzoyloxycarbonylamino-2-methylpyrrole-3-carboxylate (**3**).

To a dispersion of **2** (2.84 g of mixture with sodium chloride, 10 mmoles) in methylene chloride (20 ml) were added, from two dropping funnels, a solution of benzyl chloroformate (3.94 ml, 25 mmoles) in methylene chloride (12 ml) and a solution of sodium carbonate (2.65 g, 25 mmoles) in water (12 ml). The reaction mixture was stirred at room temperature for 30 minutes the organic phase was separated, washed with 10% aqueous ammonium acetate, dried and evaporated. The residue was chromatographed on a silica gel column by eluting with 20% ethyl acetate in methylene chloride to give 2.33 g (77%) of **3**, mp 126-128° after recrystallization from ethyl ether-hexane; ir (nujol); 3360, 3250 (NH), 1720 (O-CO-N), 1650 (COOEt); ¹H-nmr (deuteriochloroform): δ 1.38 (t, J = 6, 3H, CH₂-CH₃), 2.47 (s, CH₃), 4.38 (q, 2H, CH₂-CH₃), 5.38 (s, CH₂ Cbz), 7.15 (s, H-C5), 7.44-7.66 (m, 5 H-aromat), 8.20 (br s, NH-pyrrole), 8.89 (br s, NH-CO).

Anal. Calcd. for $C_{17}H_{18}N_2O_4$: C, 63.56; H, 6.00; N, 9.26. Found: C, 63.40; H, 5.97; N, 9.30.

Ethyl 4-Benzoyloxycarbonylamino-1,2-dimethylpyrrole-3-carboxylate (**4**).

A mixture of **3** (2.33 g, 7.7 mmoles), anhydrous potassium carbonate (1.93 g, 14 mmoles) and dimethyl sulfate (1.54 ml, 16 mmoles) in methyl-ethylketone (35 ml) was heated at reflux under vigorous stirring for 7 hours. The inorganic salts were removed by filtration, the solvent was evaporated and the residue crystallized from ethyl ether-hexane to give 2.15 g (88%) of **4**, mp 112-113°; ir (nujol): 3350 (NH), 1720 (O-CO-N), 1660 (COOEt); ¹H-nmr (deuteriochloroform): δ 1.43 (t, J = 6, 3H, CH₂-CH₃), 2.49 (s, CH₃), 3.36 (s, N-CH₃), 4.39 (q, 2H, CH₂-CH₃), 5.28 (s, CH₂ Cbz), 7.19 (s, H-C5), 7.47-7.63 (m, 5 H-aromat), 8.93 (br s, NH).

Anal. Calcd. for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.85. Found: C, 64.62; H, 6.36; N, 8.92.

Ethyl 4-Benzoyloxycarbonylamino-1,2-dimethyl-5-(4-methylpiperazinyl)pyrrole-3-carboxylate (**6**).

To a solution of **4** (19.2 g, 0.06 mole) and 1,4-diazabicyclo[2.2.2]octane (7.5 g, 0.066 mole) in carbon tetrachloride (1500 ml) was added -10° and under stirring a 0.4 *M* solution of bromine in the same solvent (163 ml, 0.065 mole) during 3 hours. The disappearance of **4** from the reaction mixture was checked on tlc (20% ethyl acetate in benzene). The reaction mixture was filtered and an excess of 1-methylpiperazine (50 ml) was added to the filtrate. Carbon tetrachloride was evaporated, the residue was dissolved in ethanol (160 ml) and the solution was heated at reflux under argon for 5 days. The solvent and the excess of methylpiperazine were distilled under reduced pressure, the residue was taken up with ethyl acetate and saturated sodium chloride solution. The organic phase was

dried, evaporated and the residue was chromatographed on a silica gel column by eluting with 5% methanol in chloroform. The unreacted compound **5** was first recovered (1.43 g) followed by 15.78 g (63%) of **6**.

Compound **5** is sensitive to light and correct analytical data could not be obtained, mp 78° with dec after recrystallization from ethyl ether; ir (nujol): 3280 (NH), 1705 (O-CO-N), 1685 (COOEt); ¹H-nmr (deuteriochloroform): δ 1.63 (t, J = 6.3, 3H, CH₂-CH₃), 2.61 (s, CH₃), 3.67 (s, N-CH₃), 4.34 (q, 2H, CH₂-CH₃), 5.32 (s, CH₂ Cbz), 6.68 (br s, NH), 7.47-7.63 (m, 5 H-aromat).

An analytical sample of **6** was obtained by recrystallization from ethyl ether-hexane, mp 105-107°; ir (nujol): 3180 (NH), 1720 (O-CO-N), 1680 (COOEt); ¹H-nmr (deuteriochloroform): δ 1.19 (t, J = 6, 3H, CH₂-CH₃), 2.36 (s, N-CH₃-pip), 2.49 and 3.11 (br m, 8H, CH₂-pip), 2.49 (s, CH₃), 3.40 (s, N-CH₃ pyrrole), 4.20 (q, 2H, CH₂-CH₃), 5.21 (s, CH₂ Cbz), 5.20 (br s, NH), 4.38-7.53 (m, 5 H-aromat).

Anal. Calcd. for $C_{22}H_{30}N_4O_4$: C, 63.74; H, 7.29; N, 13.51. Found: C, 63.87; H, 7.29; N, 13.61.

Ethyl *N*-[3-Carboxy-1,2-dimethyl-5-(4'-methyl)piperazinyl-4-pyrryl]-*N'*-ethyl-3-aminopropionate (**9**).

To a solution of **6** (11.91 g, 0.029 mole) in anhydrous DMF (144 ml) was added in portions at room temperature 55% sodium hydride in mineral oil (1.5 g, 0.034 mole) and stirring was maintained for 1 hour under argon. A solution of ethyl iodide (2.88 ml, 0.034 mole) in DMF (10 ml) was then dropped into the reaction mixture kept at about 0° and stirring was continued for 15 minutes. The dispersion was poured into 10% aqueous ammonium acetate (1400 ml) and extracted with ethyl acetate. The organic phase was dried and evaporated to give a residue (11.94 g) containing **7** and various by-products. This residue was dissolved in 98% formic acid (50 ml) and added to a dispersion of 10% palladium on carbon (10 g) in 98% formic acid. After stirring under argon at room temperature for 2 hours, the reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was taken up with ethyl acetate and 5% aqueous sodium bicarbonate, the organic layer was dried and evaporated to give 7.26 g of impure **8**, which was immediately dissolved in an excess of ethyl acrylate (106 ml) under argon. To this solution was dropped glacial acetic acid (47 ml) during 20 minutes. The stirring was maintained for 90 minutes at 50-60° then the excess of ethyl acrylate and acetic acid were removed under reduced pressure. The residue was taken up with ethyl acetate and 5% aqueous sodium bicarbonate, the organic layer was dried and evaporated to give 8.65 g of impure **9**. Purification was accomplished by chromatography on a silica gel column eluted with 5% methanol in chloroform to afford 2.61 g (22%) of **9** as an oil.

The analytical sample of **7** had mp 113-115° (hexane); ir (nujol): 1700 (O-CO-N), 1680 (COOEt); ¹H-nmr (deuteriochloroform): major component δ 1.14 and 1.20 (2t, J = 6, 6H, CH₂-CH₃), 2.31 (s, CH₃-pip), 2.43, 2.93 and 3.07 (each m, 8H, CH₂-pip), 2.51 (s, CH₃), 3.38 (s, N-CH₃), 4.13-4.43 (m, 4H, CH₂-CH₃), 5.09-5.17 (CH₂-Cbz), ²J = 12 AB system, 7.35-7.50 (m, 5H aromat); for the minor component only the values of resonance lines different from major compound are given; 2.33 (s, NCH₃-pip), 2.43 (s, CH₃), 3.60-3.87 (CH₂-CH₃), 5.23 (CH₂-Cbz).

Anal. Calcd. for $C_{24}H_{34}N_4O_4$: C, 65.13; H, 7.74; N, 12.66. Found: C, 65.30; H, 7.80; N, 12.79.

The analytical sample of **8** had mp 78-80° (pentane); ir (nujol): 3320 (NH), 1665 (COOEt); ¹H-nmr (deuteriochloroform): δ 1.18 (t, J = 6, 3H, NCH₂-CH₃), 1.33 (t, J = 6, 3H, CO₂CH₂CH₃), 2.38 (s, NCH₃-pip), 2.44 (s, CH₃), 2.53 and 3.13 (br m, 8H, CH₂-pip), 3.31 (q, 2H, NCH₂-CH₃), 3.40 (s, N-CH₃), 4.33 (q, 2H, CH₂-CH₃).

Anal. Calcd. for $C_{16}H_{28}N_4O_4$: C, 62.31; H, 9.15; N, 18.16. Found: C, 62.04; H, 9.12; N, 17.77.

The analytical sample of **9** hydrochloride had mp 154-156° dec (ethanol-ethyl ether); ir: the base (oil) 1750 (propionate), 1685 (ester pyrrole); ¹H-nmr (deuteriochloroform): δ (base) 1.07 (t, J = 6.2, 3H, NCH₂-CH₃), 1.22 and 1.36 (t, J = 6, 6H, CO₂CH₂CH₃), 2.36 (s, NCH₃-pip), 2.44 (s, CH₃), 2.50 (t, J = 6.3, 2H, NCH₂CH₂CO₂Et), 3.04 (q, 2H, NCH₂-CH₃), 3.18 (br m, 8H, N-CH₂-pip), 3.32 (t, J = 6.3, 2H, N-CH₂-CH₂CO₂Et), 3.38

(s, N-CH₃), 4.13 and 4.31 (q, 4H, CH₂-CH₃ ester).

Anal. Calcd. for C₂₁H₃₆N₄O₄.HCl: C, 56.68; H, 8.38; N, 12.59. Found: C, 56.42; H, 8.18; N, 12.59.

Ethyl 1-Ethyl-1,2,3,4-tetrahydro-5,6-dimethyl-7-(4-methyl-1-piperazinyl)-4-oxo-6H-pyrrolo[3,4-b]pyridine-3-carboxylate (**10**).

To a solution of sodium bis(trimethylsilyl)amide (7.38 g, 0.04 mole) in anhydrous ethyl ether (360 ml) under argon was added a solution of **9** (2.5 g, 6.1 mmoles) in anhydrous ethyl ether during 75 minutes. Quenching of the reaction was effected by addition of 5% aqueous sodium phosphate monobasic (360 ml). The aqueous phase was brought to pH 7 with 10% sodium hydroxide, saturated with sodium chloride, and extracted with methylene chloride after separation of the ether. The organic phases were collected dried and evaporated.

The hexamethyldisilazane was distilled at 50° (0.3 mm Hg) and the residue was chromatographed on a silica gel column by eluting with 5% methanol in chloroform to give 1.19 g (54%) of **10** as an oil. This compound gave rise to many unidentified by-products when kept at about 5° for a week; ir (film): 1730 (COOEt), 1650 (CO), 1590, 1520 (C=C); ¹H-nmr (deuteriochloroform): δ (two epimers) 1.07 (t, J = 6, 3H, NCH₂CH₃), 1.26 (t, J = 6, 3H, CO₂CH₂CH₃), 2.36 (s, N-CH₃-pip); 2.44 (s, CH₃), 2.48 and 3.11 (m, 8H, CH₂-pip), 3.25-3.60 resonance lines of NCH₂CH₃ with overlapping of H-C3 and 2H-C2, 3.36 (s, NCH₃), 4.29 AB part of an ABX₃ system 2H, CO₂CH₂-CH₃).

The hydrochloride monohydrate of **10** had mp 118° dec (ethanol-ethyl ether).

Anal. Calcd. for C₁₉H₃₀N₄O₃.HCl.H₂O: C, 54.73; H, 7.98; N, 13.44. Found: C, 54.24; H, 7.76; N, 13.40.

Ethyl 1-Ethyl-5,6-dimethyl-7-(4-methyl-1-piperazinyl)-1,4-dihydro-4-oxo-6H-pyrrolo[3,4-b]pyridine-3-carboxylate (**11**).

To a solution of **10** (0.5 g, 1.38 mmoles) in ethyl alcohol (2 ml) and cyclohexene (20 ml) was added 10% palladium on carbon (1 g) and the dispersion was heated at reflux under vigorous stirring for 2½ hours. The mixture was filtered, the solvents were evaporated and the residue was triturated with ethyl ether to give 150 mg (30%) of **11**. The analytical sample was obtained by recrystallization from ethyl acetate, mp 206-208°; ir (nujol): 1660 (COOEt), 1620 (CO), 1600, 1565 (C=C); ¹H-nmr (deuteriochloroform): δ 1.42 (2t, J = 6, 6H, CH₂-CH₃), 2.29 and 3.53, 2.80 and 3.07 (m, 8H, CH₂-pip), 2.42 (s, NCH₃-pip), 2.71 (s, CH₃), 3.51 (s, NCH₃), 4.36 and 4.41 (q, 4H, CH₂CH₃), 8.22 (s, H-C2).

Anal. Calcd. for C₁₅H₂₈N₄O₃: C, 63.31; H, 7.83; N, 15.54. Found: C, 63.26; H, 7.87; N, 15.50.

1-Ethyl-5,6-dimethyl-7-(4-methyl-1-piperazinyl)-1,4-dihydro-4-oxo-1H-pyrrolo[3,4-b]pyridine-3-carboxylic Acid (**12**).

To a solution of **11** (360 mg, 1 mmole) in ethanol (5 ml) was added 2 ml of a 1 N solution of potassium hydroxide in the same solvent. After 4 hours of reflux, the solvent was evaporated and 2 ml of a 1 N aqueous solution of hydrochloric acid was added to the residue. Compound **12** was isolated by extraction with boiling ethyl acetate which was then concentrated to a small volume and filtered to afford 140 mg (42%) of **12**, mp 249° dec; ir (nujol): 1685 (COOH), 1620 (CO), 1600, 1565 (C=C); ¹H-nmr (deuteriochloroform): δ 1.46 (t, J = 6, 3H, CO₂CH₂-CH₃), 2.30 and 3.53, 2.84 and 3.07 (m, 8H, CH₂-pip), 2.40 (s, NCH₃-pip), 2.70 (s, CH₃), 3.67 (s, N-CH₃), 4.42 (q, 2H, CH₂CH₃), 8.28 (s, H-C2), 15.45 (s, COOH).

Anal. Calcd. for C₁₇H₂₄N₄O₃: C, 61.42; H, 7.27; N, 16.85. Found: C, 60.98; H, 7.28; N, 16.51.

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