

$\alpha$ -Fluoro- and  $\alpha$ -Hydroxypyridylalanines<sup>1</sup>

P. TIMOTHY SULLIVAN, CYNTHIA B. SULLIVAN, AND S. J. NORTON\*

Department of Chemistry, North Texas State University, Denton, Texas 76203

Received August 7, 1970

The four isomeric  $\alpha$ -hydroxypyridylalanines and three of the four isomeric  $\alpha$ -fluoropyridylalanines have been synthesized. All of the synthetic amino acids have been studied for growth inhibition properties in *Escherichia coli* 9723, *Leuconostoc dextranicum* 8086, and *Lactobacillus arabinosus* 17-5. The  $\alpha$ -hydroxypyridylalanines possess very little inhibitor activity, whereas certain of the  $\alpha$ -fluoropyridylalanines were found to be competitive antagonists of phenylalanine.

The replacement of H by F is often advantageous in the constructing of compounds displaying antimetabolic properties.<sup>2,3</sup> Because of previous syntheses and demonstrations of biological activity of various F-substituted amino acids<sup>4,5</sup> and pyridine ring substituted amino acids,<sup>6,7</sup> the synthesis and biological study of certain fluoropyridylalanines was undertaken. The compounds synthesized were the  $\alpha$ -fluoropyridylalanines, with the alanine substituent positioned on the ring in such a manner that pseudo ortho, meta, and para isomers resulted. In addition, the 4 isomeric  $\alpha$ -hydroxypyridylalanines<sup>8</sup> were synthesized in anticipation that certain of them would be effective tyrosine antagonists;  $\beta$ -(5-hydroxy-2-pyridyl)-DL-alanine was previously found to be a potent tyrosine antagonist in certain microorganisms.<sup>9</sup> All of the amino acid analogs were studied for growth inhibition properties in *Escherichia coli* 9723, *Leuconostoc dextranicum* 8086, and *Lactobacillus arabinosus* 17-5.

**Chemistry.**—The  $\alpha$ -fluoro- and  $\alpha$ -hydroxypyridylalanines were synthesized through the usual malonic ester condensation synthesis. Allylic bromination with NBS of the appropriate fluoropicoline gave the desired bromomethyl derivative which was isolated as the HBr salt. Condensation of the bromomethyl intermediates (1-4) with ethyl sodioacetamidomalonate followed by acidic hydrolysis yielded the  $\alpha$ -hydroxypyridylalanines (12-15). For the synthesis of the fluoropyridylalanines (16-18), the bromomethyl intermediates were condensed with ethyl sodioacetamidocynoacetate, followed by mild alkaline hydrolysis. Under reflux conditions alkaline hydrolysis resulted in displacement of the F substituent as evidenced by uv

spectra, an observation in contrast with the reported stability of an  $\alpha$ -F on the pyridine ring.<sup>10</sup> Repeated attempts to prepare  $\beta$ -(2-fluoro-4-pyridyl)-DL-alanine were unsuccessful.

**Biological Studies.**—A summary of the biological activities in several microorganisms of the fluoro- and hydroxypyridylalanines is presented in Table I. The

TABLE I  
SUMMARY OF MICROBIAL GROWTH INHIBITIONS BY  
FLUORO- AND HYDROXYPYRIDYLALANINES

| Compd | —Minimal concentration, $\mu$ g/ml, for complete—<br>growth inhibition |                                            |                                           |
|-------|------------------------------------------------------------------------|--------------------------------------------|-------------------------------------------|
|       | <i>E. coli</i> 9723 <sup>a</sup>                                       | <i>L. dextranicum</i><br>8086 <sup>a</sup> | <i>L. arabinosus</i><br>17-5 <sup>a</sup> |
| 12    | b                                                                      | b                                          | >600 <sup>c</sup>                         |
| 13    | b                                                                      | b                                          | 600                                       |
| 14    | b                                                                      | b                                          | 600                                       |
| 15    | b                                                                      | b                                          | 60 <sup>d</sup>                           |
| 16    | 200                                                                    | >600 <sup>c</sup>                          | 0.6                                       |
| 17    | >600 <sup>c</sup>                                                      | 20                                         | 0.6                                       |
| 18    | 6                                                                      | >600 <sup>c</sup>                          | 2                                         |

<sup>a</sup> Growth media are described in Experimental Section. <sup>b</sup> No observable growth inhibition at the max level tested (600  $\mu$ g/ml). <sup>c</sup> 40-80 % inhibition at 600  $\mu$ g/ml. <sup>d</sup> About 90% inhibition at 20  $\mu$ g/ml.

F analogs are active to some extent in all of the microorganisms tested. On the other hand, the OH analogs are completely inactive in *E. coli* 9723 and *L. dextranicum* 8086, but do exhibit some inhibitory properties in *L. arabinosus* 17-5.

It is surprising that  $\beta$ -(6-hydroxy-3-pyridyl)-DL-alanine (14) exhibits little if any inhibitory activity in the organisms studied, since  $\beta$ -(5-hydroxy-2-pyridyl)-DL-alanine is a potent tyrosine antagonist in *E. coli* 9723 and *L. dextranicum* 8086.<sup>9</sup> A plausible explanation for this finding may be that the  $\alpha$ -OH of 14 exists predominantly as the pyridone tautomer at the physiological pH of the growth medium. The  $\beta$ -OH of  $\beta$ -(5-hydroxy-2-pyridyl)-DL-alanine, however, exists as the enol tautomer and thus more closely resembles tyrosine structurally.

Each of the fluoropyridylalanines is quite toxic to the growth of *L. arabinosus* 17-5. The data in Table I indicate that in certain experiments  $\beta$ -(2-fluoro-3-pyridyl)-DL-alanine (16) and  $\beta$ -(6-fluoro-3-pyridyl)-DL-alanine (17) completely inhibited the growth of this organism at concentrations as low as 0.6  $\mu$ g/ml. In other more detailed experiments (Table II) attempts were made to reverse the inhibitions of 16 and 17 by phenylalanine. Virtually complete growth inhibition occurs at

(1) This work was supported by grants from the Robert A. Welch Foundation of Texas (B-133) and from a Faculty Research Grant of North Texas State University (2024).

(2) C. Heidelberger, N. K. Chadhuri, P. Dannenberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Plevin, and J. Scheiner, *Nature (London)*, **179**, 663 (1957).

(3) F. M. Singer, J. P. Januszka, and A. Borman, *Proc. Soc. Exp. Biol. Med.*, **102**, 370 (1959).

(4) H. K. Mitchell and C. Niemann, *J. Amer. Chem. Soc.*, **69**, 1232 (1947).

(5) W. F. Coulson, E. Wardlee, and J. B. Jepson, *Biochim. Biophys. Acta*, **167**, 99 (1968).

(6) E. Lansford and W. Shive, *Arch. Biochem. Biophys.*, **38**, 347 (1952).

(7) S. J. Norton and P. Timothy Sullivan, *J. Heterocycl. Chem.*, **7**, 699 (1970).

(8) (a) It is well established that the tautomeric equilibrium favors the pyridone over the pyridinol structure in neutral soln.<sup>9b</sup> However, for simplicity and for comparative purposes with the  $\alpha$ -fluoro- $\alpha$ -pyridylalanines, the name,  $\alpha$ -hydroxy- $\alpha$ -pyridylalanine, will be employed throughout the discussion rather than the more correct name,  $\beta$ -(1,2-dihydro-2-oxo- $\alpha$ -pyridyl)-DL-alanine; (b) "The Chemistry of Heterocyclic Compounds," Part III, "Pyridine and its Derivatives," Erwin Klingsberg, Ed., Wiley, New York, N. Y., pp 619-631, and ref cited therein.

(9) S. J. Norton, C. G. Skinner, and W. Shive, *J. Org. Chem.*, **26**, 1495 (1961).

(10) H. L. Bradlow and C. A. Vanderwerf, *ibid.*, **14**, 509 (1949).

TABLE II  
REVERSAL OF FLUOROPYRIDYLALANINE TOXICITIES IN *L. arabinosus* 17-5 BY DL-PHENYLALANINE<sup>a,b</sup>

| Analog,<br>μg/ml                              | Supplement, DL-phenylalanine, μg/ml |      |      |      |
|-----------------------------------------------|-------------------------------------|------|------|------|
|                                               | None                                | 0.20 | 0.60 | 2.00 |
| <b>β-(6-Fluoro-3-pyridyl)-DL-alanine (17)</b> |                                     |      |      |      |
| 0                                             | 0                                   | 0    | 0    | 0    |
| 0.2                                           | 0                                   |      |      |      |
| 0.6                                           | 83                                  | 43   |      |      |
| 2                                             | 96                                  | 88   | 68   |      |
| 6                                             | 100                                 | 96   | 75   | 35   |
| 20                                            |                                     | 100  | 88   | 51   |
| 60                                            |                                     | 100  | 99   | 58   |
| 200                                           |                                     |      | 100  | 89   |
| 600                                           |                                     |      |      | 100  |
| <b>β-(2-Fluoro-3-pyridyl)-DL-alanine (16)</b> |                                     |      |      |      |
| 0                                             | 0                                   | 0    | 0    | 0    |
| 0.2                                           | 16                                  |      |      |      |
| 0.6                                           | 94                                  | 31   |      |      |
| 2                                             | 100                                 | 79   | 58   |      |
| 6                                             | 100                                 | 87   | 74   | 49   |
| 20                                            |                                     | 96   | 77   | 59   |
| 60                                            |                                     | 100  | 90   | 58   |
| 200                                           |                                     |      | 100  | 77   |
| 600                                           |                                     |      |      | 100  |

<sup>a</sup> Incubated 36 hr at 30°. <sup>b</sup> Growth media was supplemented with 0.04 μg/ml of phenylalanine.

2 μg/ml for both **16** and **17**. Phenylalanine reverses the inhibition of both analogs in a competitive fashion over a tenfold range of increasing phenylalanine concentrations. The inhibition index (ratio of inhibitor to substrate necessary for complete inhibition of growth) was found to be between 100 and 300 for both analogs. *p*-Fluorophenylalanine has been reported to inhibit growth of *L. arabinosus* at 16 μg/ml and to be reversed competitively by phenylalanine with an inhibition index of approximately 10.<sup>11</sup>

It is apparent that the fluoropyridylalanines, **16** and **17**, are more inhibitory to *L. arabinosus* at lower concentrations than is *p*-fluorophenylalanine, while the inhibition indices of **16** and **17** are higher than that exhibited by *p*-fluorophenylalanine inhibition.<sup>11</sup> This paradox may possibly be due to the actual utilization of the latter antagonist in protein biosynthesis, which is known to occur in this microorganism.<sup>12</sup> This could necessitate a larger relative concentration of phenylalanine to reverse growth inhibition caused by both the blocking of phenylalanine utilization as well as the formation of nonfunctional protein.<sup>13</sup> Further studies to determine whether **16** and **17** are utilized by *L. arabinosus* could be informative in this regard.

The variations in the toxicities of **17** and **18** [β-(6-fluoro-2-pyridyl)-DL-alanine] in *E. coli* and *L. dextranicum* were not anticipated (Table I). Compound **17** competitively antagonizes phenylalanine in *L. dextranicum*, with an inhibition index of 300, over a 30-fold

range of increasing phenylalanine concentrations (Table III). There seems to be no apparent explanation for

TABLE III  
REVERSAL OF β-(6-FLUORO-3-PYRIDYL)-DL-ALANINE TOXICITY IN *Leuconostoc dextranicum* 8086 BY DL-PHENYLALANINE<sup>a,b</sup>

| β-(6-Fluoro-3-pyridyl)-DL-alanine (17), μg/ml | Supplement, DL-phenylalanine, μg/ml |      |      |      |      |
|-----------------------------------------------|-------------------------------------|------|------|------|------|
|                                               | None                                | 0.20 | 0.60 | 2.00 | 6.00 |
| 0                                             | 0                                   | 0    | 0    | 0    | 0    |
| 2                                             | 9                                   | 9    |      |      |      |
| 6                                             | 37                                  | 22   | 16   | 0    |      |
| 20                                            | 100                                 | 81   | 34   | 0    | 11   |
| 60                                            | 100                                 | 100  | 80   | 23   | 4    |
| 200                                           |                                     |      | 100  | 36   | 16   |
| 600                                           |                                     |      |      | 85   | 65   |
| 2000                                          |                                     |      |      |      | 97   |

<sup>a</sup> Incubated 30 hr at 30°. <sup>b</sup> Growth media described in Experimental Section.

the complete lack of toxicity of **17** in *E. coli*. *p*-Fluorophenylalanine, isosteric to **17**, was reported by Bergmann<sup>14</sup> to inhibit completely the growth of *E. coli* (ATCC 9637) at an inhibitor concentration of 60 μg/ml. On the other hand, **18** (F meta to the alanine side chain) is fairly toxic to *E. coli* 9723 as shown in Table IV. An

TABLE IV  
REVERSAL OF β-(6-FLUORO-2-PYRIDYL)-DL-ALANINE TOXICITY IN *E. coli* 9723 BY DL-PHENYLALANINE<sup>a,b</sup>

| β-(6-Fluoro-2-pyridyl)-DL-alanine (18), μg/ml | Supplement, DL-phenylalanine, μg/ml |      |      |      |      |
|-----------------------------------------------|-------------------------------------|------|------|------|------|
|                                               | None                                | 0.02 | 0.06 | 0.20 | 0.60 |
| 0                                             | 0                                   | 0    | 0    | 0    | 0    |
| 0.6                                           | 0                                   | 0    |      |      |      |
| 2                                             | 25                                  | 17   | 18   | 12   |      |
| 6                                             | 98                                  | 49   | 39   |      | 13   |
| 20                                            | 100                                 | 71   | 47   | 35   | 41   |
| 60                                            |                                     | 95   | 71   | 47   | 58   |
| 200                                           |                                     |      | 93   | 73   | 65   |
| 600                                           |                                     |      |      | 88   | 75   |
| 2000                                          |                                     |      |      |      | 94   |

<sup>a</sup> Incubated 15 hr at 37°. <sup>b</sup> Growth media described in Experimental Section.

inhibition index of 3000 was demonstrated for **18**, and tyrosine and tryptophan also reversed the inhibition to some extent.

## Experimental Section

**General Procedures.**—A Thomas-Hoover capillary melting point apparatus was employed for all melting point determinations, and the melting points reported are uncorrected. UV spectra were determined with a Beckman DBG recording spectrophotometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the calcd values unless otherwise specified. The aminopicolines were obtained from Aldrich Chemical Co., Inc. and J. T. Baker Laboratory Chemicals.

For the microbiological assays employing *E. coli* 9723 as test organism a previously reported inorganic salts-glucose medium<sup>15</sup> was employed, and the experimental details have been reported

(11) D. E. Atkinson, S. Melvin, and S. W. Fox, *Arch. Biochem. Biophys.*, **31**, 205 (1951).

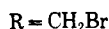
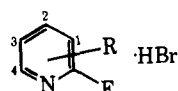
(12) J. E. Johnson and S. W. Fox, *Biochim. Biophys. Acta*, **28**, 318 (1958).

(13) L. Fowden, S. Neale, and H. Tristram, *Nature (London)*, **199**, 35 (1963).

(14) E. D. Bergmann, S. Sieber, and B. E. Volcani, *Biochem. J.*, **54**, 1 (1953).

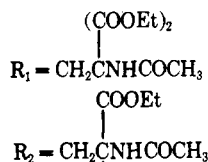
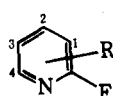
(15) E. H. Anderson, *Proc. Nat. Acad. Sci. U. S.*, **32**, 120 (1946).

TABLE V



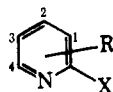
| No. | R<br>(position) | Mp, °C dec | Yield, % | Formula <sup>a</sup>                                          |
|-----|-----------------|------------|----------|---------------------------------------------------------------|
| 1   | 1               | 105–120    | 52       | C <sub>6</sub> H <sub>5</sub> Br <sub>2</sub> FN              |
| 2   | 2               | 93–105     | 75       | C <sub>6</sub> H <sub>5</sub> Br <sub>2</sub> FN <sup>b</sup> |
| 3   | 3               | 114–120    | 77       | C <sub>6</sub> H <sub>5</sub> Br <sub>2</sub> FN              |
| 4   | 4               | 108–112    | 52       | c                                                             |

<sup>a</sup> All compds indicated by the mol formula were analyzed for C, H, N. <sup>b</sup> Anal. (C<sub>6</sub>H<sub>5</sub>Br<sub>2</sub>FN) H, N; C: calcd, 26.60; found, 27.04. <sup>c</sup> Not analyzed.

TABLE VI  
INTERMEDIATE ETHYLACETAMIDOMALONATES AND  
ETHYLACETAMIDOCYANOACETATES

| No. | R              | Position | Mp, °C  | Recrystn <sup>b</sup><br>solvent | Yield<br>purifd,<br>% | Formula <sup>a</sup>                                           |
|-----|----------------|----------|---------|----------------------------------|-----------------------|----------------------------------------------------------------|
| 5   | R <sub>1</sub> | 1        | 119–120 | W                                | 41                    | C <sub>15</sub> H <sub>19</sub> FN <sub>2</sub> O <sub>5</sub> |
| 6   | R <sub>2</sub> | 1        | c       |                                  |                       |                                                                |
| 7   | R <sub>1</sub> | 2        | 123–124 | W                                | 14                    | C <sub>15</sub> H <sub>19</sub> FN <sub>2</sub> O <sub>5</sub> |
| 8   | R <sub>1</sub> | 3        | 127–128 | W                                | 40                    | C <sub>15</sub> H <sub>19</sub> FN <sub>2</sub> O <sub>5</sub> |
| 9   | R <sub>2</sub> | 3        | 134–136 | E-A                              | 38                    | C <sub>15</sub> H <sub>19</sub> FN <sub>2</sub> O <sub>5</sub> |
| 10  | R <sub>1</sub> | 4        | 108–110 | E                                | 42                    | C <sub>15</sub> H <sub>19</sub> FN <sub>2</sub> O <sub>5</sub> |
| 11  | R <sub>2</sub> | 4        | 92–93   | W                                | 24                    | C <sub>15</sub> H <sub>19</sub> FN <sub>2</sub> O <sub>5</sub> |

<sup>a</sup> See footnote a, Table V. <sup>b</sup> A = Me<sub>2</sub>CO, E = Et<sub>2</sub>O, W = H<sub>2</sub>O. <sup>c</sup> Oil, not purified further or analyzed; but hydrolyzed directly to give 16.

TABLE VII  
FLUORO- AND HYDROXY-SUBSTITUTED PYRIDYLALANINES

| No. | X               | R<br>(position) | Mp, °C dec | Uv (λ <sub>max</sub> ) | Recrystn<br>solvent | Yield<br>purifd, % | Formula <sup>a</sup>                                                                           |
|-----|-----------------|-----------------|------------|------------------------|---------------------|--------------------|------------------------------------------------------------------------------------------------|
| 12  | OH <sup>b</sup> | 1               | 227–229    | 300, 229               | W-A                 | 46                 | C <sub>8</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> ·H <sub>2</sub> O                 |
| 13  | OH              | 2               | 283–284    | 297, 228               | W-A                 | 59                 | C <sub>8</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> ·H <sub>2</sub> O                 |
| 14  | OH              | 3               | 215–216    | 302, 232               | W                   | 76                 | C <sub>8</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> ·H <sub>2</sub> O                 |
| 15  | OH              | 4               | 257–258    | 303, 229               | W                   | 60                 | C <sub>8</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> ·0.5H <sub>2</sub> O <sup>d</sup> |
| 16  | F               | 1               | 198–200    | 262                    | W-A                 | c                  | C <sub>8</sub> H <sub>9</sub> FN <sub>2</sub> O <sub>2</sub>                                   |
| 17  | F               | 3               | 252–253    | 265                    | W-A                 | 49                 | C <sub>8</sub> H <sub>9</sub> FN <sub>2</sub> O <sub>2</sub>                                   |
| 18  | F               | 4               | 220–221    | 264                    | W-A                 | 40                 | C <sub>8</sub> H <sub>9</sub> FN <sub>2</sub> O <sub>2</sub>                                   |

<sup>a</sup> See footnote a, Table V. <sup>b</sup> See ref 8. <sup>c</sup> See footnote c, Table VI. <sup>d</sup> Anal. (C<sub>8</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>·0.5H<sub>2</sub>O) H, N; C: calcd, 50.25 found, 50.84.

elsewhere.<sup>16</sup> For the assays in which *L. dextranicum* 8086 and *L. arabinosus* 17-5 served as the test organism, a previously described amino acid medium was used,<sup>17</sup> except that the phenylalanine and tyrosine were omitted from the basal medium, unless otherwise noted, and the tryptophan and aspartic acid concns were increased threefold. For the assay of *L. dextranicum*, this medium was supplemented with 0.05 μg/ml of pantothenic acid. The amino acid analogs were dissolved in sterile H<sub>2</sub>O and added aseptically to sterile assay tubes in all cases. The amt of growth was determined photometrically at 600 mμ after the appropriate

incubation period. The optical density readings were then converted into per cent inhibitions.

The following reaction procedures are given for specific compds; those indicated by reference to the particular table were prepared in like manner.

**α-Fluoropicolines.**—The appropriate aminopicoline was diazotized as previously reported<sup>18,19</sup> utilizing HBF<sub>4</sub> and NaNO<sub>2</sub>. The boiling points agreed in all cases with those reported above.

**2-Fluoro-3-bromomethylpyridine·HBr** (Table V, 1–4).—To 11.1 g of (0.10 mole) of 2-fluoro-3-methylpyridine in 300 ml of MgSO<sub>4</sub>-dried CCl<sub>4</sub> was added 17.8 g (0.10 mole) of NBS and 1 g of benzoyl peroxide as catalyst. After heating under reflux for several hours, the succinimide was removed by filtration, and the filtrate was coned *in vacuo* to about 50 ml. The CCl<sub>4</sub> soln was then washed with an equal vol of each of the following: 4% NaOH, H<sub>2</sub>O, and 2% HBr. To the CCl<sub>4</sub> soln Et<sub>2</sub>O was added to make a total vol of 150 ml and the soln was dried (MgSO<sub>4</sub>). The dried soln was satd with anhyd HBr at 0°. The pptd salt was rapidly filtered by suction, washed several times with anhyd Et<sub>2</sub>O, and stored in a desiccator over P<sub>2</sub>O<sub>5</sub>. The product was extremely hygroscopic and a powerful lachrymator. Attempts to recryst the product resulted in appreciable decompn. However, the product was sufficiently pure (physical constants and analyses, Table V) for further synthetic work.

**Ethyl 2-Acetamido-2-(2-fluoro-3-pyridylmethyl)malonate** (Table VI, 5, 7, 8, 10).—To a soln of 6.51 g (0.03 mole) of ethyl acetamidomalonate in 180 ml of Mg-dried EtOH contg 1.38 g (0.06 g-atom) of Na was added 8.13 g (0.03 mole) of 2-fluoro-3-bromomethylpyridine·HBr. The reaction mixt was heated under reflux until the pH of an aliquot dissolved in distd H<sub>2</sub>O had decreased to approximately pH 5–6. The reaction mixt was taken to dryness *in vacuo*, and the product extd (Et<sub>2</sub>O). It was then crystd from Et<sub>2</sub>O–pet ether and recrystd from H<sub>2</sub>O. The condensation leading to 7 and 10 was carried out in the same vol (as above) of 1:1 C<sub>6</sub>H<sub>6</sub>–EtOH. For 7 a molar excess of ethyl acetamidomalonate and Na was used and the halide was added portionwise over 1 hr (Table VI).

**Ethyl 2-Acetamido-2-(2-fluoro-3-pyridylmethyl)cyanoacetate** (Table VI, 6, 9, 11).—The same reaction procedure was followed as for the corresponding malonate intermediate, 5, except that ethyl acetamidocyanoacetate was employed as condensing reagent. A cryst product could not be obtained for 6 so the oil was used directly in the Ba(OH)<sub>2</sub> hydrolysis. A 1:1 C<sub>6</sub>H<sub>6</sub>–EtOH

solvent was used in the preparation of 11. Physical constants and analyses are given in Table VI.

**β-(1,2-Dihydro-2-oxo-3-pyridyl)-DL-alanine** (Table VII, 12–15).—Compound 5 (3.5 g, 0.011 mole) was hydrolyzed in the presence of 50 ml of refluxing 6 N HCl for 8 hr. The soln was coned to dryness *in vacuo* and the residue was dissolved in 100 ml of H<sub>2</sub>O. The warm aq soln was neutralized (Amberlite IR-45), and the resulting filtrate was decolorized with Darco G-60 and coned to a smaller volume. Me<sub>2</sub>CO was added to the turbidity point and the amino acid crystd out in the cold (Table VII).

(16) F. W. Dunn, J. M. Ravel, and W. Shive, *J. Biol. Chem.*, **219**, 809 (1956).

(17) J. M. Ravel, L. Woods, B. Felsing, and W. Shive, *ibid.*, **206**, 391 (1954).

(18) J. T. Miner, G. E. Hawkins, C. A. Vanderwerf, A. Roe, *J. Amer. Chem. Soc.*, **71**, 1125 (1949).

(19) A. Roe, P. H. Cheek, and G. F. Hawkins, *ibid.*, **71**, 4152 (1949).

$\beta$ -(2-Fluoro-3-pyridyl)-DL-alanine (Table VII, 16-18).—The cyanoacetate intermediate, **6**, was added to a warm slurry of 15% Ba(OH)<sub>2</sub> in H<sub>2</sub>O, and the reaction mixt was heated at 70° with stirring for 3 days. Periodically, during the course of the reaction, aliquots were removed from the reaction mixture and the uv spectra determined to insure that hydrolysis of the F substituent was not occurring. At the completion of the reaction, insolubles were removed by filtration and chunks of Dry Ice were

added to the filtrate until the pH had fallen to approximately 7. After removing the pptd BaCO<sub>3</sub>, the pH was carefully lowered in the cold to pH 4.5 by addn of 10% H<sub>2</sub>SO<sub>4</sub>. The BaSO<sub>4</sub> was filtered off and the filtrate was concd to dryness *in vacuo* keeping the amino acid soln at 35° or less. The product was dissolved in a minimal amt of H<sub>2</sub>O and Me<sub>2</sub>CO added to near the cloud point. After standing in the refrigerator for several hours, the amino acid crystallized (Table VII).

## Quaternary Furyl-, Thienyl-, and Pyrrolylpyridinium Salts. Oral Hypoglycemic Agents

GRETCHEN E. WIEGAND,\* VICTOR J. BAUER, S. R. SAFIR,

*Organic Chemical Research Section*

D. A. BLICKENS, AND S. J. RIGGI

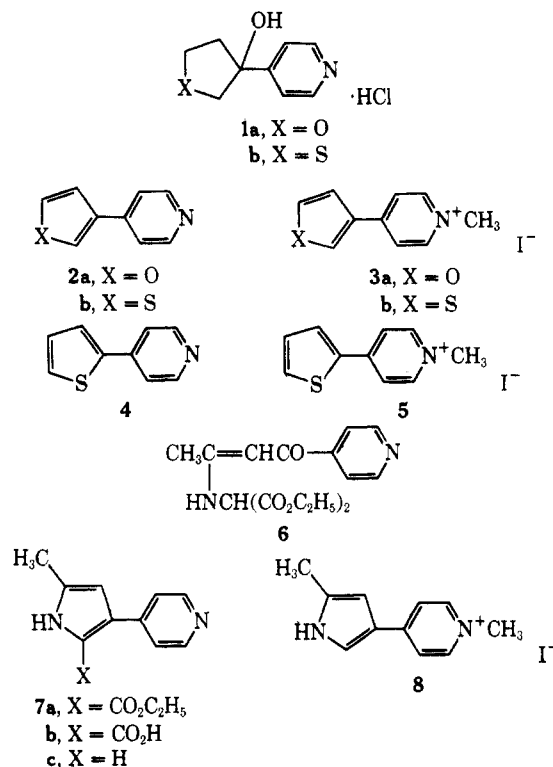
*Department of Metabolic Chemotherapy, Lederle Laboratories, A Division of American Cyanamid Company,  
Pearl River, New York 10965*

*Received August 14, 1970*

1-Methyl-4-(3-furyl)pyridinium iodide, 1-methyl-4-[2(and 3)-thienyl]pyridinium iodide, and 1-methyl-4-(5-methyl-3-pyrrolyl)pyridinium iodide have been synthesized as representatives of these classes of quaternary pyridinium salts. Blood glucose concentration of normal mice was decreased following oral administration of these compounds.

An extensive series of quaternary 4-azolylpyridinium salts including pyrazolyl,<sup>1</sup> isoxazolyl,<sup>2-5</sup> 1,2,4-oxadiazolyl,<sup>6</sup> thiazolyl,<sup>7</sup> and oxazolyl<sup>8</sup> derivatives has been found to display hypoglycemic activity in laboratory animals. In marked contrast, the quaternary 1,2,4-triazolyl-, 1,3,4-thiadiazolyl-, imidazolyl-, and tetrazolylpyridinium salts were ineffective in lowering blood sugar levels, and a 1,3,4-oxadiazolylpyridinium salt induced a slight non-dose-related hypoglycemia.<sup>9</sup> To further delineate the structural requirements for hypoglycemic activity following oral administration of quaternary pyridinium salts, we have synthesized representative furyl, thienyl, and pyrrolyl analogs and measured their effects on the blood glucose concentration of mice.

For the synthesis of 1-methyl-4-(3-furyl)pyridinium iodide (**3a**), the general method of Wynberg, *et al.*,<sup>10</sup> for the preparation of arylthiophenes was employed. Addition of 4-pyridyllithium<sup>11</sup> to tetrahydrofuran-3-one yielded the carbinol **1a**, which was dehydrated-dehydrogenated to 4-(3-furyl)pyridine (**2a**). Quaternization of **2a** gave the desired salt **3a**.



Reaction of the known 4-(3-thienyl)pyridine (**2b**)<sup>12</sup> and 4-(2-thienyl)pyridine (**4**)<sup>12,13</sup> with MeI gave the representative quaternary thienylpyridinium salts **3b** and **5**, respectively.

The pyrrolylpyridinium analog **8** was synthesized by a general method of Umio, *et al.*,<sup>14</sup> for the preparation of

(1) V. J. Bauer, H. P. Dalalian, W. J. Fanshawe, S. R. Safir, E. C. Tocus, and C. R. Boshart, *J. Med. Chem.*, **11**, 981 (1968).

(2) V. J. Bauer, W. J. Fanshawe, H. P. Dalalian, and S. R. Safir, *ibid.*, **11**, 984 (1968).

(3) S. J. Rigg, D. A. Blickens, and C. R. Boshart, *Diabetes*, **17**, 646 (1968).

(4) D. A. Blickens and S. J. Rigg, *Toxicol. Appl. Pharmacol.*, **14**, 393 (1969).

(5) D. A. Blickens and S. J. Rigg, *Diabetes*, **18**, 612 (1969).

(6) W. J. Fanshawe, V. J. Bauer, S. R. Safir, D. A. Blickens, and S. J. Rigg, *J. Med. Chem.*, **12**, 381 (1969).

(7) G. E. Wiegand, V. J. Bauer, S. R. Safir, D. A. Blickens, and S. J. Rigg, *ibid.*, **12**, 891 (1969).

(8) G. E. Wiegand, V. J. Bauer, S. R. Safir, D. A. Blickens, and S. J. Rigg, *ibid.*, **12**, 943 (1969).

(9) V. J. Bauer, G. E. Wiegand, W. J. Fanshawe, and S. R. Safir, *ibid.*, **12**, 944 (1969).

(10) H. Wynberg, A. Logothetis, and D. Verploeg, *J. Amer. Chem. Soc.*, **79**, 1972 (1957).

(11) A. Murray, III and W. H. Langham, *ibid.*, **74**, 6289 (1952).

(12) H. Wynberg, T. J. van Bergen, and R. M. Kellogg, *J. Org. Chem.*, **34**, 3175 (1969).

(13) K. Kahmann, H. Sigel, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 1754 (1964).

(14) S. Umio, K. Kariyone, K. Tanaka, T. Kishimoto, and H. Noguchi, U. S. Patent 3,367,944, Feb 6, 1968; *Chem. Abstr.*, **65**, 13661a (1966).