

residue was stirred with ether and filtered to give a solid (1.8 g), mp = 212–4 °C dec. This solid (0.98 g, 5 mmol) was converted to its acyl sulfonamide by the procedure described for general preparation of acyl sulfonamides 14a–j to afford **6** (210 mg), mp = 176–8 °C. Anal. ( $C_{14}H_{12}N_2O_5S$ ) C, H, N.

**5-( $\alpha$ -Carboxyphenethyl)pyridine-2-carboxylic Acid (5).** Lithium diisopropylamide (5 mmol) was prepared from a solution of redistilled diisopropylamine (0.7 mL) in dry tetrahydrofuran (50 mL) under argon at –20 °C by adding a solution of *n*-butyllithium (1.6 M in hexane, 3.15 mL). After 10 min at –20 °C the mixture was cooled to –70 °C and diester **3a** (1.0 g, 5 mmol) in tetrahydrofuran (20 mL) added. After 20 min at –70 °C a solution of benzyl bromide (0.75 mL, 11 mmol) in tetrahydrofuran (20 mL) was added. After 1 h at –70 °C the mixture was allowed to warm to ambient temperature and neutralized with glacial acetic acid. The mixture was evaporated to dryness and partitioned between water and ethyl acetate. The organic extracts were washed with brine, dried, and evaporated to an oil. This oil was chromatographed on a Merck silica gel column (Art. 10401) eluting with dichloromethane/ethyl acetate (9:1) to give a colorless oil (800 mg). This oil was dissolved in methanol (10 mL) and 1 N sodium hydroxide (7.5 mL) was added. The mixture was refluxed for 2 h and evaporated to a small volume, and water (6 mL) added. The aqueous phase was extracted with ether, and then the extracts were discarded. The aqueous phase was acidified to pH 1–2 with 2 N hydrochloric acid and extracted twice with ethyl acetate. The combined organic phases were dried and evaporated to a solid. After recrystallization from acetonitrile, **5** was obtained (300 mg), mp = 177–8 °C. Anal. ( $C_{15}H_{13}NO_4$ ) C, H, N.

**Ethyl 2-(Ethoxycarbonyl)pyridine-5-propionate (9).** To a solution of triethyl phosphonoacetate (3.8 g, 17 mmol) in dry tetrahydrofuran (40 mL) cooled to –60 °C, under argon, was added at this temperature a 1.6 M solution of *n*-butyllithium in hexane (11.3 mL, 17 mmol). After the addition aldehyde **7b** (3 g, 17 mmol) in dry tetrahydrofuran (80 mL) was added and the mixture kept at –60 °C for 30 min. After warming to room temperature the mixture was evaporated to dryness and the residue partitioned between ether and water. The organic extracts were combined, washed with water, dried, and evaporated to give **8b** (4.1 g) mp = 70–3 °C. A mixture of tellurium metal (1.3 g, 10 mmol), sodium borohydride (0.9 g, 24 mmol), and ethanol was heated to reflux for 30 min to give a purple solution. This was cooled to –20 °C and a deoxygenated solution of glacial acetic acid (1.2 mL) in ethanol (3 mL) was added. The black suspension was stirred at –20 °C for 5 min and a solution of the diethyl ester **8b** in ethanol (25 mL) and dichloromethane (25 mL) added. After warming to room temperature the mixture was filtered through Celite and the filtrate evaporated to dryness. The residue was partitioned

between water and ethyl acetate. The combined organic layers were dried and evaporated to dryness. The residue was chromatographed on a Merck silica gel column (Art. 10102) eluting with ethyl acetate/hexane (1:1) to give **9** as an oil (0.7 g). Anal. ( $C_{13}H_{17}NO_4$ ) C, H, N.

**2-Carboxypyridine-5-propionic Acid (11).** Compound **9** (251 mg, 1 mmol) was dissolved in methanol (10 mL) and 1 N sodium hydroxide (4 mL, 4 mmol) was added. The mixture was stirred at room temperature for 3 h and evaporated to dryness, and the residue dissolved in the minimum volume of water. The pH of the solution was adjusted to 4 with dilute hydrochloric acid and then extracted twice with ethyl acetate. The combined extracts were dried and evaporated to a white solid. This solid was boiled with absolute ethanol and filtered, and the filtrate evaporated to yield **11** (60 mg), mp >250 °C. Anal. ( $C_9H_9NO_4Na_2$ ) C, H, N.

**2-Carboxypyridine-5-propenoic Acid (10).** A solution of *tert*-butyl dimethyl phosphonoacetate (1.12 g, 50 mmol) in dry tetrahydrofuran (10 mL) was cooled under argon to –60 °C and a 1.6 M solution of *n*-butyllithium in hexane (3.2 mL) added. After the addition, a solution of aldehyde **7a** (0.83 g, 50 mmol) in dry tetrahydrofuran (20 mL) was added. The mixture was allowed to warm to ambient temperature for 1 h and evaporated to dryness, and the residue partitioned between ether and water. The combined organic extracts were dried and evaporated to yield **8a** (1 g), mp 120–3 °C. **8a** (0.4 g) in trifluoroacetic acid (15 mL) was stirred at room temperature for 30 min and then evaporated to dryness. The residue was dissolved in saturated sodium bicarbonate solution and extracted with ether, and the aqueous phase acidified to pH 4 with 2 M hydrochloric acid. The aqueous phase was extracted twice with ethyl acetate. The combined organic extracts were dried and evaporated to a solid (0.25 g), mp = 220–3 °C. This solid was stirred with 1 N sodium hydroxide solution (3.4 mL) for 30 min at room temperature, diluted with an equal volume of water, and extracted with ether. The aqueous phase was acidified to pH 4 with 2 M hydrochloric acid and the solid filtered. Recrystallization from aqueous dimethylformamide gave **10** (89 mg), mp = 271–3 °C dec. Anal. ( $C_9H_7NO_4$ ) C, H, N.

**2-[(Methylsulfonyl)carbamoyl]pyridine (17).** This compound was prepared by the method described for the general preparation of acyl sulfonamides, but using pyridine-2-carboxylic acid (2.46 g, 20 mmol) as starting material gave **17** (2.3 g, 58%), mp = 105–8 °C. Anal. ( $C_7H_8N_2O_3S$ ) C, H, N.

**Acknowledgment.** We thank Dr. T. J. Franklin, M. Hitchin, and C. J. Cunliffe for providing the biological data and Dr. L. Furlong for detailed guidance of its statistical evaluation. We are also grateful to Dr. N. J. Hales and Dr. H. Tucker for many useful discussions.

## Novel Inhibitors of Prolyl 4-Hydroxylase. 2. 5-Amide Substituted Pyridine-2-carboxylic Acids

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A series of 5-[(arylcabonyl)amino]- and 5-(arylcabamoyl)pyridine-2-carboxylic acids has been prepared and tested for activity as inhibitors of the enzyme prolyl 4-hydroxylase (EC 1.14.11.2). All the analogues prepared were inhibitors of the enzyme *in vitro*, the best compounds being equipotent with the known inhibitor pyridine-2,5-dicarboxylic acid (**9**). Like **9** these amidic analogues were not active in a cultured embryonic chick tendon cell model, considered to be a predictor of *in vivo* activity. The activity of the amides is not consistent with the model described for the mode of action of **9** with the enzyme and aspects of this are discussed.

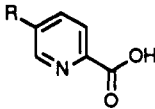
### Introduction

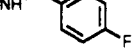
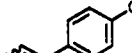
The reasons for our interest in inhibitors of prolyl 4-hydroxylase (EC 1.14.11.2), which is critically important in the biosynthesis of collagen, have been discussed in an earlier work.<sup>1</sup> A model of the mode of action of prolyl

hydroxylase has been described<sup>2</sup> and has been used by these authors to predict that pyridine-2,5-dicarboxylic acid

(1) Dowell, R. I.; Hadley, E. M. Novel Inhibitors of Prolyl 4-Hydroxylase. *J. Med. Chem.*, preceding paper in this issue.

Table I



compd	R	mp, °C	formula <sup>a</sup>	IC <sub>50</sub> , μM	
				enzyme	cell
5		194-6	C <sub>13</sub> H <sub>11</sub> FN <sub>2</sub> O <sub>2</sub>	31	80
6	OCH <sub>2</sub> Ph	144-6 <sup>b</sup>	C <sub>13</sub> H <sub>11</sub> NO <sub>3</sub>	39	
7		222	C <sub>14</sub> H <sub>10</sub> ClNO <sub>2</sub> <sup>c</sup>	150	
8	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> <sup>d</sup>			149	
9	CO <sub>2</sub> H <sup>d</sup>			5.5	NA

<sup>a</sup>All compounds were analyzed for C, H, N. <sup>b</sup>Literature mp 143-4 °C (ref 12). <sup>c</sup>Anal. C: calcd, 64.74; found, 64.2. <sup>d</sup>Aldrich Chemical Co. Ltd.

(9) would be an effective inhibitor of the enzyme. Recent work from these laboratories has shown that even more potent inhibitors than pyridine-2,5-dicarboxylic acid are obtained when the 5-carboxylic acid group is replaced by acyl sulfonamides,<sup>1</sup> which are well-known carboxylic acid group mimics. These findings are consistent with the mode of action model referred to above and confirm the importance of an acidic function at the 5-position of the pyridine-2-carboxylic acid. Moreover, the activity of the acyl sulfonamides extends the model by demonstrating that there is a greater degree of steric freedom around the 5-position of pyridine-2-carboxylic acids than was originally anticipated.

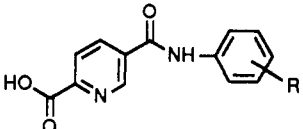
The report that 5-[(4-fluorophenyl)methyl]amino]pyridine-2-carboxylic acid (5) is an inhibitor of prolyl hydroxylase<sup>3</sup> was particularly interesting since, if it were to bind to the enzyme in the same way as pyridine-2,5-dicarboxylic acid, there would be a basic benzylamino group interacting with the proposed acid binding site. Regardless of the mode of binding, the inhibitory activity of 5 opens up the possibility that other nonacidic functional groups can be tolerated in the 5-position of pyridine 2-carboxylic acids. In this publication we describe our work aimed at elucidating the nature of the 5-substituent in inhibitors of prolyl hydroxylase based upon pyridine 2-carboxylic acids.

### Chemistry

The *N*-phenylpyridine-5-carboxamides 1 were prepared by reaction of acid chloride 2 with the corresponding aniline in pyridine solution (Scheme I). Acid chloride 2 was prepared from the known half-ester<sup>4</sup> 3 using thionyl chloride. The 5-(aminocarbonyl)pyridine derivatives 4 were obtained by reaction of 5-aminopyridine-2-carboxylic acid methyl ester with the appropriate benzoyl halide in pyridine solution followed by base hydrolysis of the methyl ester. The 5-aminopyridine-2-carboxylic acid methyl ester used as starting material was prepared by trifluoroacetic acid hydrolysis of the known *tert*-butyl carbamate.<sup>5</sup>

- (2) Hanauske-Abel, H. M.; Guenzler, V. A. Stereochemical Concept for the Catalytic Mechanism of Prolylhydroxylase, Applicability to Classification and Design of Inhibitors. *J. Theor. Biol.* 1982, 94, 421-455.
- (3) Finch, N. Treating hypertension with substituted 5-aminopyridinecarboxylic acids. U.S. Patent 4,273,779, 1981.
- (4) Isagawa, K.; Kawai, M.; Fushizaki, Y. Syntheses of Isochinchomeric Acid Monoesters. *Nippon Kagaku Zasshi* 1967, 88, 553-556.

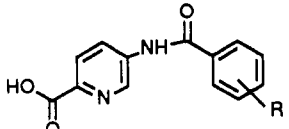
Table II



compd	R	mp, °C	formula <sup>a</sup>	IC <sub>50</sub> , μM	
				enzyme	cell
10	H	>300	C <sub>13</sub> H <sub>9</sub> N <sub>2</sub> NaO <sub>3</sub>	26.5	
11	2-Cl	194-5	C <sub>13</sub> H <sub>8</sub> ClN <sub>2</sub> O <sub>3</sub>	16.3	NA
12	3-Cl	220-3	C <sub>13</sub> H <sub>8</sub> ClN <sub>2</sub> O <sub>3</sub>	32.2	
13	4-Cl	>300	C <sub>13</sub> H <sub>8</sub> ClN <sub>2</sub> NaO <sub>3</sub>	17.4	
14	2-F	182-3	C <sub>13</sub> H <sub>8</sub> FN <sub>2</sub> O <sub>3</sub> ·0.75H <sub>2</sub> O	11	
15	4-F	194-5	C <sub>13</sub> H <sub>8</sub> FN <sub>2</sub> O <sub>3</sub>	16.1	
16	4-Me	224-5	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> ·0.75AcOH	8.3	NA
17	2-EtO	204-5	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	32.9	
18	4-EtO	221-3	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	5.0	
19	4-Me <sub>2</sub> N	215-7	C <sub>15</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub> ·0.7H <sub>2</sub> O	4.1	NA
20	2-MeO	184-5	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>4</sub> ·0.3H <sub>2</sub> O	14.8	NA
21	4-MeO	>300	C <sub>14</sub> H <sub>11</sub> N <sub>2</sub> NaO <sub>4</sub> ·0.25H <sub>2</sub> O	21.1	
22	2,4-(MeO) <sub>2</sub>	183	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub>	16.6	NA
23	3,4,5-(MeO) <sub>3</sub>	210-11	C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>6</sub> ·0.25AcOH	26.5	
24	4-NO <sub>2</sub>	238-40	C <sub>13</sub> H <sub>8</sub> N <sub>3</sub> O <sub>5</sub> ·AcOH <sup>b</sup>	44	

<sup>a</sup>All compounds were analyzed for C, H, N. <sup>b</sup>Anal. C: calcd, 51.87; found, 51.4.

Table III



compd	R	mp, °C	formula <sup>a</sup>	IC <sub>50</sub> , μM	
				enzyme	cell
25	H	227-8	C <sub>13</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> ·AcOH	25.6	
26	4-Cl	146-8	C <sub>13</sub> H <sub>9</sub> ClN <sub>2</sub> O <sub>3</sub> ·1.5H <sub>2</sub> O	22.5	
27	4-Me	236-7	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> ·AcOH	16.5	NA
28	4-EtO	232-5	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub> ·0.25H <sub>2</sub> O	11.2	NA
29	4-F	210-2	C <sub>13</sub> H <sub>9</sub> FN <sub>2</sub> O <sub>3</sub> ·AcOH <sup>b</sup>	51.2	NA
30	4-Me <sub>2</sub> N	234-5	C <sub>15</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub> ·H <sub>2</sub> O	11.3	NA
31	2-F	315-320	C <sub>13</sub> H <sub>8</sub> FN <sub>2</sub> NaO <sub>3</sub> ·0.8H <sub>2</sub> O	33.8	NA

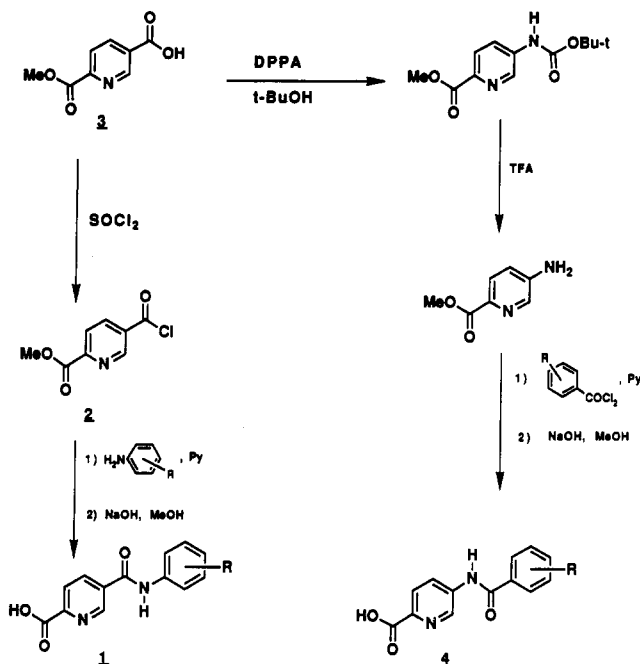
<sup>a</sup>All compounds were analyzed for C, H, N. <sup>b</sup>Anal. C: calcd, 56.2; found, 56.7.

### Results

5-[(4-Fluorophenyl)methyl]amino]pyridine-2-carboxylic acid (5) is reported to be an inhibitor of prolyl hydroxylase.<sup>3</sup> We have confirmed this in our test system and find it to be some 5-fold less potent than pyridine-2,5-dicarboxylic acid (9). We have taken 5 as our starting point for an investigation into the effect of the 5-substituent in pyridine-2-carboxylic acids on inhibition of prolyl hydroxylase. We prepared the related oxygen analogue 6 and found that it was equipotent with 5 (Table I), which indicates that it is not necessarily the basicity of the heteroatom that is important for inhibitory activity. A carbon analogue (7) which lacks the heteroatom but would be expected to pick up any lipophilic aromatic ring interaction with the enzyme was much less potent. Fusaric acid (8), an antihypertensive agent which is reported to inhibit the

- (5) Marsham, P. R.; Hughes, L. R.; Jackman, A. L.; Hayter, A. J.; Oldfield, J.; Wardleworth, J. M.; Bishop, J. A. M.; O'Connor, B. M.; Calvert, A. H. Quinazoline Antifolate Thymidylate Synthase Inhibitors: Heterocyclic Benzoyl Ring Modifications. *J. Med. Chem.* 1991, 34, 1594-1605.

Scheme I



enzyme dopamine- $\beta$ -hydroxylase,<sup>6</sup> was also a poor inhibitor of prollyl hydroxylase.

It would appear, therefore, that in this small set of compounds the introduction of the heteroatom in the 5-side chain has improved the inhibitory potency. One of the possible roles for the heteroatom in improving potency could be through participation in hydrogen-bond formation with a complementary site on the enzyme. We tested this hypothesis by preparing analogues containing groups in the 5-position which were capable of taking part in such interactions and chose the amide group as our starting point. It is clear from the data presented in Tables II and III that the introduction of the amide moiety has given compounds with potencies which are at least comparable to that found for 5 and in some cases, e.g., 16, 18, and 19, comparable with pyridine-2,5-dicarboxylic acid (9) itself.

A more detailed consideration of the test data in Table II shows that there is a relatively narrow range of potencies between the most and least potent analogues, 19 and 24. The position of the substituent in the anilide ring has little effect on potency although the lower potency found for 17 may be a result of unfavorable steric interactions. There is a trend toward greater potency with electron-donating substituents, cf. 18 with 13 and 24; however the effect of the electron-donating substituents does not appear to be additive, cf. 21 with 22 and 23.

Similar trends in potency are found for the isomeric substituted (benzoylamino)pyridines listed in Table III, in which the amide groups can be considered to have been "reversed".

Although pyridine-2,5-dicarboxylic acid is a potent inhibitor of prollyl hydroxylase, it is unable to inhibit the enzyme in cultured embryonic chick tendon cells at the testing dose of 20  $\mu$ g/mL,<sup>7</sup> a test which is considered to be a predictor of activity in vivo. Pyridine-2,5-dicarboxylic

acid (9) is reported to be an inhibitor of prollyl hydroxylase in cultured human skin fibroblasts at a dose approximately 1000 times its  $K_i$  value in vitro.<sup>8</sup> The reason for this inactivity is attributed to the inability of the highly polar pyridine diacid to penetrate the cell membrane and endoplasmic reticulum adequately and gain access to the intracellular enzyme. In this same test system 5 did inhibit hydroxylation in the cells but with an IC<sub>50</sub> of 80  $\mu$ M. It was hoped that the less polar (compared to 5) amides would cross the cell membrane and inhibit the intracellular enzyme, thus giving inhibitors with activity in vivo. However no in vivo activity was found with any of the amides investigated.

## Discussion

The central feature of the Guenzler and Hanauske-Abel model, which successfully predicted the ability of pyridine-2,5-dicarboxylic acid to inhibit prollyl hydroxylase, was that 2-ketoglutarate interacted with the enzyme by formation of a bidentate ligand with the enzyme-bound Fe(II) atom using the keto group and its adjacent carboxylate and the 5-carboxylate interacted with an anion binding site. Pyridine-2,5-dicarboxylic acid is considered to interact with the enzyme in the same manner with the pyridine N atom and 2-carboxylate forming the bidentate ligand and the 5-carboxylate interacting with the anionic binding site. The report that the 5-carboxylate can be replaced by acyl sulfonamides is consistent with the model and shows, in addition, that there is a large degree of steric freedom about the 5-position of the pyridine-2-carboxylates. The inhibitory potency of 5 is difficult to reconcile with this model since, if it were to bind in the same mode, it would require a basic group to interact at the anionic binding site. This suggests that 5 binds to the enzyme in a different mode to pyridine-2,5-dicarboxylic acid and, as a corollary to this, an acidic function in the 5-position of the pyridine-2-carboxylic acids is not an absolute requirement for inhibitory activity. A comparison of the potencies of 5 and 6 with those of 7 and 8 highlighted the role of the heteroatom, and given the disparity in basicity between the heteroatoms, the beneficial influence on potency was attributed to the ability of the heteroatom to participate in hydrogen-bond formation. The amide group can behave as a hydrogen-bond donor or acceptor, and it is not clear from the data which of these effects dominates; however, the potency of the oxygen analogue 6 would tend to favor a hydrogen-bond acceptor as being important.

## Summary

The introduction of amide substituents into the 5-position of pyridine-2-carboxylic acid has given a series of inhibitors which, in the best cases, are of comparable potency to pyridine-2,5-dicarboxylic acid. These compounds have provided us with less polar inhibitors of prollyl hydroxylase which are believed to bind to the enzyme in a different manner than pyridine-2,5-dicarboxylic acid and have opened up new opportunities for the design of novel inhibitors of prollyl hydroxylase.

## Experimental Section

The following general procedures were followed unless stated otherwise. Melting points were determined using an Electrothermal melting point apparatus and are uncorrected. <sup>1</sup>H NMR spectra were recorded on a Bruker AC250, Bruker AM200, Varian EM390, or JEOL FX90Q instrument using dilute solutions in [2H<sub>6</sub>]DMSO or [2H]CHCl<sub>3</sub> with tetramethylsilane as internal standard. NMR spectra were run on all isolated intermediates and on final products and were consistent with the structural

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- (7) Tschank, G.; Raghunath, M.; Guenzler, V.; Hanauske-Abel, H. M. Pyridinecarboxylates, the first mechanism-derived inhibitors for prollyl 4-hydroxylase, selectively suppress cellular hydroxyprolyl biosynthesis. *Biochem. J.* 1987, 248, 625-633.

(8) Franklin, T. J. Personal communication.

assignments. Column chromatography was carried out under slight positive pressure ("flash chromatography") on silica gel (Merck type 9385). Extracts in water-immiscible solvents were dried over anhydrous magnesium sulfate and the organic solvents were evaporated under reduced pressure using a Büchi rotary evaporator.

**5-[[4-(4-Ethoxyphenyl)amino]carbonyl]pyridine-2-carboxylic Acid (18).** 4-Ethoxybenzenamine (5.4 mL, 41.7 mmol) was added dropwise (rapidly) to a stirred suspension of 5-(chlorocarbonyl)-2-(methoxycarbonyl)pyridine (8.31 g, 41.7 mmol) in pyridine (30 mL). There was an initial exothermic reaction and the mixture was stirred at ambient temperature for 20 h. The reaction mixture was evaporated to dryness and the residue obtained was triturated with cold methanol and filtered. The solid product was crystallized from methanol (400 mL) to give 5-[[4-(4-ethoxyphenyl)amino]carbonyl]-2-(methoxycarbonyl)pyridine, (8.67 g, 69%), mp 197–200 °C. Anal. ( $C_{16}H_{16}N_2O_4$ ) C, H, N.

Aqueous NaOH (25 mL, 2 N) was added to a suspension of this ester (10.3 g, 34.4 mmol) in methanol, the mixture was stirred at laboratory temperature for 20 h and filtered to give an off-white solid. This solid was triturated with hot aqueous acetic acid, cooled, and filtered. The resulting yellow solid was crystallized from aqueous DMF to give 18 (6.9 g, 70%), mp 222–3 °C. Anal. ( $C_{15}H_{14}N_2O_4$ ) C, H, N.

This procedure was used to prepare the other analogues listed in Table II.

**5-[[4-(4-Methylphenyl)carbonyl]amino]pyridine-2-carboxylic Acid (27).** A mixture of 5-amino-2-(methoxycarbonyl)pyridine (0.8 g, 5.3 mmol) and 4-methylbenzoyl chloride (0.82 g, 5.3 mmol) in pyridine (10 mL) was stirred at laboratory temperature for 20 h. The reaction mixture was poured into water (100 mL) and the precipitate was collected, washed with water, and dried to yield 1.3 g (90%). This material was used for the next step without further purification.

The ester (0.6 g, 2.2 mmol) was stirred with aqueous NaOH (1.1 mL, 2.2 mmol) in ethanol (10 mL) for 2 h. The reaction mixture was diluted with water (20 mL) and filtered. The solid obtained was crystallized from glacial acetic acid to give 27 as its acetate salt (0.433 g, 61%), mp 236–7 °C. Anal. ( $C_{14}H_{12}N_2O_3 \cdot AcOH$ ) C, H, N.

This procedure was used to prepare the other analogues listed in Table III.

**5-[2-(4-Chlorophenyl)ethenyl]pyridine-2-carboxylic Acid (7).** A solution of *n*-butyllithium in hexane (1.25 mL of 1.6 M solution) was added to a stirred suspension of (4-chlorobenzyl)-triphenylphosphonium chloride (0.92 g, 2.17 mmol) in tetrahydrofuran (THF) (15 mL) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 15 min and a solution of 5-formyl-2-(methoxycarbonyl)pyridine in THF (15 mL) was added.

The reaction mixture was stirred at 0 °C for 1 h and then evaporated to a small volume and partitioned between EtOAc and water. The EtOAc extract was dried and the residue obtained was subjected to chromatography (eluant EtOAc). 5-[2-(4-Chlorophenyl)ethenyl]-2-(methoxycarbonyl)pyridine was obtained as a 3:2 mixture of *E* and *Z* isomers, yield 150 mg (30%), which was used without further purification.

A mixture of the methyl ester (described above) (150 mg) and aqueous NaOH (2 mL, 1 N) in methanol (10 mL) was stirred for 1 h and then evaporated to dryness. The solid obtained was crystallized from glacial acetic acid to give 7, as an indeterminate mixture of *E* and *Z* isomers, mp 222 °C. Anal. ( $C_{14}H_{10}ClNO_2$ ) C, H, N.

**Biological Testing.** The enzyme prolyl 4-hydroxylase was isolated from the leg tissue of 17-day-old chick embryos and purified by the method of Kedersha and Berg<sup>9</sup> and appeared homogeneous when examined by SDS/polyacrylamide gel electrophoresis.<sup>10</sup> The inhibitory potency of the compounds against prolyl hydroxylase was determined using the indirect assay described by Cunliffe et al.<sup>10</sup> in which the conversion of the labeled cofactor 2-ketoglutarate into labeled succinate is measured. All points were determined in duplicate and  $IC_{50}$  values were obtained by interpolation on six-point dose-response curves. A compound is significantly different in potency to pyridine-2,5-dicarboxylic acid (9) if its  $IC_{50}$  value differs by a factor of more than 2. The inhibitory potency of the compounds against prolyl hydroxylase in cultured embryonic chick tendon cells was carried out following the procedure described by Franklin and Hitchen.<sup>11</sup> The percent inhibition caused by 20 µg/mL of the test compound is measured.

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