Synthesis of 2-[3′-(Trifluoromethyl)anilino]-5-hydroxynicotinic Acid

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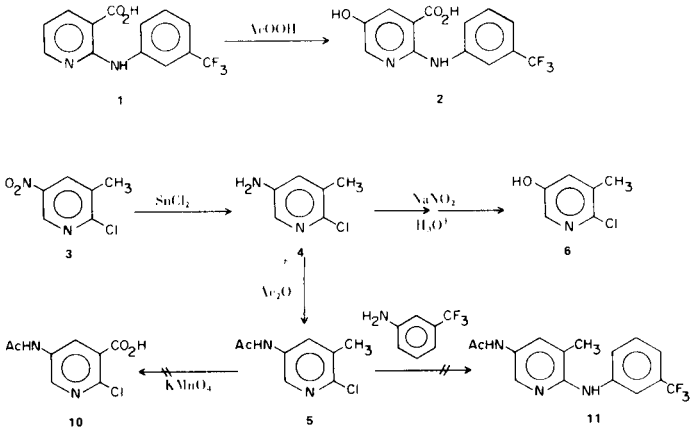
2-[3′-(Trifluoromethyl)anilino]-5-hydroxynicotinic acid (**2**) was synthesized by two routes: a) by direct hydroxylation of 2-[3′-(trifluoromethyl)anilino]nicotinic acid (**1**); and b) by the following sequence starting from 2-chloro-3-methyl-5-nitropyridine (**3**) *via* 5-amino-2-chloro-3-methylpyridine (**4**), 2-chloro-5-hydroxy-3-methylpyridine (**6**), 5-acetoxy-2-chloro-3-methylpyridine (**7**), 5-acetoxy-2-chloronicotinic acid (**8**), and 2-chloro-5-hydroxynicotinic acid (**9**). The correlation of **2** with one of the metabolites of **1** has been accomplished, and the identities of both compounds have been proven.

During metabolic studies of 2-[3′-(trifluoromethyl-^{14}C)-anilino]nicotinic acid (niflumic acid) (**1**) in humans and dogs, a mixture of glucuronic acid and sulfate conjugates of niflumic acid, as well as two of its metabolites, was isolated (1). Based on the results from low- and high-resolution mass spectrometry and 100-MHz proton magnetic resonance spectrometry, structures were assigned (2).

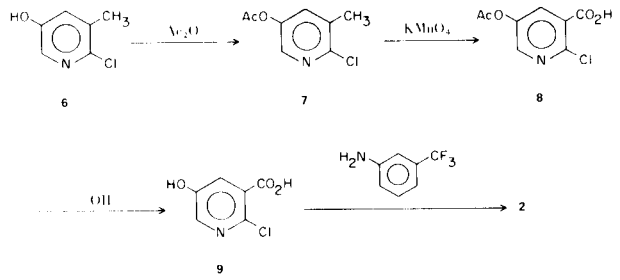
We wish to report the synthesis of 2-[3′-(trifluoromethyl)anilino]-5-hydroxynicotinic acid (**2**) and the correlation of this compound with one of the metabolites (M2b), the structure of which was believed to be identical with the acid **2**, (1). Essentially, two chemical approaches employed for the synthesis of the hydroxy acid **2** were considered.

Niflumic acid **1**, described earlier (3) and readily available, served as a starting material for one of the two approaches. The reaction considered was the direct hydroxylation of the acid **1** by peracetic acid. It has been reported (4) that the preparation of heteroaromatic *N*-oxides is sometimes accompanied by side reactions, particularly when peracetic acid is used as an oxidizing agent. β-Hydroxyheterocycles, along with α-oxo heterocycles, *N*-hydroxy-α-oxo heterocycles, and their *N*-oxides are among the byproducts. Moreover, further reactions may result if a molecule contains an additional susceptible group. Presence of an amino group, for example, may give rise to the formation of an hydroxyamino derivative. In the present case, however, one of the above-mentioned byproducts of the *N*-oxide formations, *i.e.* β-hydroxyheterocyclic derivative **2**, represented the desired product. A possible mechanism for the formation of 5-hydroxy acid **2** by direct hydroxylation of acid **1**, utilizing peracetic acid, may involve electrophilic attack of the peracetic acid at the 5-position of the pyridine ring (4). The



reaction conditions employed here were basically identical with those used for the formation of *N*-oxides. Niflumic acid **1** was allowed to react with peracetic acid. The reaction, when carried out at 0°, proceeded extremely slowly. An improvement was achieved at 90°. Based on the considerations mentioned above, a rather complicated reaction mixture was anticipated. In fact, it was shown by tlc that the resulting reaction mixture contained at least sixteen different components. Nevertheless, by a combination of preparative tlc and sublimation technique, it was possible to isolate, in 8.4% yield, a compound that was hydroxylated in the 5-position of the pyridine ring. This compound was shown to be identical in all respects (ir spectrum, m.p., mixture melting point and Rf) with 2-[3′-(trifluoromethyl)anilino]-5-hydroxynicotinic acid (**2**) synthesized by the second route.

A coupling reaction between 3-trifluoromethylaniline and either 2-halogen-5-hydroxynicotinic acid or some suitable precursor of it was considered to be the approach



in the second pathway. For this purpose, 2-chloro-3-methyl-5-nitropyridine (**3**), the synthesis of which has already been reported by Hawkins and Roe (5), was utilized as the starting material. Reduction of the 5-nitro group was effected by use of stannous chloride. The resulting 5-amino-2-chloro-3-methylpyridine (**4**) was obtained in 99% yield. The amino group in **4** was then protected by acetylation with acetic anhydride at ambient temperature. 5-Acetamido-2-chloro-3-methylpyridine (**5**) was obtained in 99% yield. The analytical data were consistent with the proposed structure. The ir spectrum displayed bands at 3295, 3245, 3170 cm^{-1} (NH stretching), 3095 (pyridine ring CH stretch vibration), 1605 and 1415 cm^{-1} (ring stretching vibrations), 1700 cm^{-1} (amide carbonyl stretch absorption). The nmr spectrum showed two low-field one-proton signals at τ 1.95 and 1.56, split as doublets, with the magnitude of the splitting 3 Hz, which was in accord with the presence of two pyridine protons located at C-4 and C-6, respectively, and with the expected value for meta coupling $J_{4,6} = 3$ Hz. The chemical shifts of the six remaining protons were located high-field as two sharp singlets at τ 7.78 (Py-CH_3) and 7.58 (CH_3CO) (6,7). Potassium permanganate oxidation of the 3-methyl group in derivative **5** was attempted at both room and elevated temperatures. However, the expected acid **10** was not isolated, and starting material **5** was recovered unchanged (82%). Similarly unsuccessful was the coupling reaction between acetamido derivative **5** and 3-trifluoromethylaniline, which was carried out at 175-180°. The recovery of unchanged starting material **5** was 75% and no trifluoromethyl derivative **11** was isolated. Failure of these two attempted reactions initiated use of the other route in which 5-hydroxy derivative **6**, or preferably, its acetoxy derivative **7**, was used instead of derivative **5**. Replacement of the 5-amino group in **4** by a hydroxy group was achieved *via* diazotization. The intermediate diazonium salt was not isolated, but was decomposed *in situ*, at 90-95°. This procedure produced 2-chloro-5-hydroxy-3-methylpyridine (**6**) in 79% yield. A broad absorption band in the ir spectrum of this compound, located between 2950-2550 cm^{-1} (OH), and the absence of a peak at approximately 1670 cm^{-1} (CO), are in agreement with the structure of 5-hydroxy derivative **6**, in which the hydroxy-oxo tautomerism does not occur (8,9). The nmr spectrum of **6** showed a downfield shift of the two heteroaromatic proton signals by approximately 0.2 ppm in comparison with those in amino derivative **4**. This shift is apparently due to the different increments by which amino and hydroxy groups contribute to the chemical shift of the protons involved (10). For the next step, namely the oxidation of the 3-methyl group to the corresponding carboxyl derivative, it was better to protect the free 5-hydroxy group in **6**. Acetic anhydride, at 112°, was used as an acetylating agent. Thus 5-acetoxy-2-chloro-3-methylpyridine (**7**) was obtained in 94% yield. The ir spectrum of this compound showed strong absorption for carbonyl at 1770 cm^{-1}. The nmr spectrum demonstrated two methyl groups (CH_3CO- and CH_3-Py) by the presence of two three-proton singlets at τ 7.65 and 7.73, respectively. The potassium permanganate oxidation of **7** was incomplete, even with an excess of oxidizing agent and use of different reaction media and temperatures. One of the best conditions for this reaction seemed to be the use of an aqueous acetic acid solution of **7** at a temperature between 30-50°. Under these conditions, hydrolytic replacement of the 2-chloro atom occurred only to a small extent. However, as revealed by tlc, deacetylation always accompanied this reaction, so that a mixture of acetoxy acid **8** and hydroxy acid **9** was formed. To facilitate isolation of **8**, it was found convenient to reacetylate the crude reaction mixture. The yield of 5-acetoxy-2-chloronicotinic acid (**8**) was 12%; 64% of unchanged starting material **7** was recovered. Satisfactory analytical data were not achieved until the analytical sample had been dried *in vacuo* of 0.1 mm. at the elevated temperature of 80° because of the tendency of **8** to bind solvent molecules. (Precautions should be taken, since the compound sublimed at approximately 140°). The ir spectrum of acetoxy acid **8** displayed a broad band between 2950-2350 cm^{-1} (the pyridine carboxylic acid OH stretch absorption), and strong peaks at 1780 and 1735 cm^{-1} (aliphatic and heteroaromatic carbonyls, respectively), which are consistent with the assigned structure. Supporting evidence was also found in the nmr spectrum. This acid was deacetylated by sodium hydroxide in a methanol-water solution at room temperature for six hours. In spite of **8** having a reactive α-halogen, no byproduct caused by nucleophilic replacement, which could accompany the deacetylation, has been found. 2-Chloro-5-hydroxynicotinic acid (**9**) was obtained as the sole product in 96% yield. The ir spectrum showed the loss of the acetate carbonyl peak at 1780 cm^{-1}, and the position of the nicotinic acid carbonyl band was shifted to the lower frequency of 1695 cm^{-1}. The two heteroaromatic protons at C-4 and C-6 gave rise to two doublets centered at τ 2.33 and 1.97, respectively, which had a common line separation of 3 Hz.

The final reaction of the sequence was the nucleophilic substitution of the halogen in 2-chloro-5-hydroxynicotinic acid (**9**) by 3-trifluoromethylaniline. The best results were achieved when the reaction was carried out at 175° in the presence of sodium iodide and under nitrogen atmosphere. The optimal reaction time was less than 4 hours. The 3-trifluoromethylaniline was used in large excess, thereby serving as a solvent as well. The product, 2-[3′-(trifluoromethyl)anilino]-5-hydroxynicotinic acid (**2**), was isolated in 68% yield. The ir spectrum of acid **2** displayed a strong broad band between 3000-2550 cm^{-1} (OH stretch absorption). The τ-values for the two heteroaromatic protons in this compound were very much the same as in its precursor (acid **9**). The signals that appeared as doublets at τ 2.17 and 1.96, with a common value of J = 3 Hz, were assigned to the protons at C-4 and C-6 of the pyridine ring, respectively. The four aromatic ring protons had chemical shifts of τ 1.85, 2.28, 2.61, and 2.81. The singlet signal at τ 1.85 showed signs of fine splitting, evidence that this proton is weakly coupled to others, *e.g.* in meta position. This signal could, therefore, have arisen from the proton on C-2′. The next proton to be discussed had a signal centered at 2.61. It appeared, as a first approximation, as a triplet with J = 7.5 Hz, which is in agreement with the diortho-coupled proton at C-5′. The chemical shifts of the two remaining benzenoid protons had τ-values of 2.28 and 2.81, and showed 8- and 7.5 Hz splittings, respectively; however, the assignment remained uncertain.

It was proven unequivocally that the structure of the metabolite M2b was identical with 2-[3′-(trifluoromethyl)anilino]-5-hydroxynicotinic acid (**2**). A mixture melting point was undepressed, Rf (chloroform-methanol 2:1 and 3:2) values were identical, ir (potassium bromide), nmr (perdeuteriomethanol) and mass spectra were superimposable. Since the metabolite M2b was ^{14}C-labeled (in the trifluoromethyl group), an additional proof of identity with **2** with obtained by isotope dilution technique.

EXPERIMENTAL

The ir spectra were measured with a Perkin-Elmer 257 grating infrared spectrophotometer, the nmr spectra were obtained at ambient temperature on Perkin-Elmer R 12B and Varian Associates XL-100 spectrometers. Tetramethylsilane was used as an internal standard, perdeuteriomethanol was used as the solvent, and the radiofrequency was internally locked to deuterium in the solvent. The mass spectra were obtained on an Associated Electrical Industries model MS 902 double-focusing mass spectrometer. Melting points were determined with a Fisher-Johns melting point apparatus and were not corrected. Thin-layer chromatography plates used were Quanta/Gram precoated glass plates, both silica gel Q1F (analytical) and PQ1F (preparative), with fluorescent indicators. All chemicals used were reagent grade unless otherwise specified. Petroleum ether refers to the fraction, b.p. 20-40°. Unless stated otherwise, analytical samples were dried at room temperature in vacuum of 0.1 mm. for 20 hours. Radioactivity was determined in a model 3375 Packard Tri-Carb liquid-scintillation spectrometer, using the automatic external standard channel ratios technique for quench corrections. Bray's liquid scintillator was used for counting the methanol solutions (11).

Hydroxylation of 2-[3′-(Trifluoromethyl)anilino]nicotinic Acid (**1**).

Preparation of 2-[3′-(Trifluoromethyl)anilino]-5-hydroxynicotinic Acid (**2**).

A solution of acid **1** (564 mg., 2.0 mmoles) in 30% hydrogen peroxide (1.0 ml.) and glacial acetic acid (25 ml.) was kept at 0 ± 5° for 66 hours. Tlc, utilizing solvent system chloroform-methanol (5:1), was used for monitoring the course of the reaction. After this period of time, the reaction mixture still contained starting material **1** as the major component. The solution was heated at 90 ± 5°, with magnetic stirring, for 7 hours. Evaporation of the solution *in vacuo* and repeated codistillation of the syrup with water (4 x 5 ml.) led to a brown amorphous residue (595 mg.). Tlc (chloroform-methanol [5:1]) revealed a mixture of compounds (16 spots uv-detection). The major constituent was starting material (R_f 0.6). This mixture was subjected repeatedly to preparative tlc (chloroform-methanol [5:1], and ethyl acetate-ethanol [2:1]). The band of R_f 0.4 (chloroform-methanol [5:1]) was extracted with boiling methanol. Methanol extracts were concentrated *in vacuo*, dissolved in a mixture of methanol-water (1:1), and treated with Dowex 50W (H^+). Solid material obtained after evaporation of the solvents was subjected to sublimation *in vacuo* (0.05 mm.) at 200-210° (bath temperature) and yielded 49 mg. of **2** (8.4%), m.p. 247-248° (recrystallization from methanol-water [3:2]). A mixed melting point with **2** prepared by the second route was not depressed, ir spectra were superimposable, and R_f values in chloroform-methanol (5:1), ethyl acetate-ethanol (2:1), and chloroform-methanol (2:1) were identical.

2-Chloro-3-methyl-5-nitropyridine (**3**).

This compound was prepared according to the procedure of Hawkins and Roe (5) starting from 2-amino-3-methylpyridine. Melting point 47°, ir (potassium bromide) ν: 3070 (w), 1595 (m), 1515 (s), 1355 (s) cm^{-1}; nmr (perdeuteriomethanol) τ: 7.47 (s, 3H, CH_3), 1.48 (d, 1H, J3Hz, 4-H), 1.00 (d, 1H, J3Hz, 6-H). The elemental analysis was in accord with the calculated percentages.

5-Amino-2-chloro-3-methylpyridine (**4**).

To a solution of nitro derivative **3** (1.73 g., 10 mmoles) in concentrated hydrochloric acid (17 ml.) was added a solution of stannous chloride (8.54 g., 45 mmoles) in concentrated hydrochloric acid (10 ml.) at room temperature during 20 minutes. The reaction mixture was heated at 85° with stirring for 1 hour. The solution was cooled to 10°, diluted with water (60 ml.), evaporated *in vacuo* to a small volume, and dissolved in water (30 ml.), then the *p*H was brought to 7 by addition of potassium bicarbonate. The white slurry was exhaustively extracted with ether, and the combined extracts were dried over magnesium sulfate. Removal of ether left 1.41 g. (99% yield) of white crystals of **4**, m.p. 93-94°, which sublimed as low as 50° as 20 mm. This compound was recrystallized from carbon tetrachloride and a mixture of ether-hexane (4:1) to give an analytical sample of **4**, m.p. 93.5-94.5°; ir (potassium bromide) ν: 3320 (s), 3190 (s), 3080 (shoulder), 1660 (m), 1605 (m), 1580 (m), 1470 (s), 1415 (s), 1385 (m) cm^{-1}; nmr (perdeuteriomethanol) τ: 7.78 (s,

3H, CH_3), 3.01 (d, 1H, J3Hz, 4-H), 2.41 (d, 1H, J3Hz, 6-H).

Anal. Calcd. for $C_6H_7ClN_2$: C, 50.54; H, 4.95; Cl, 24.87; N, 19.65. Found: C, 50.65; H, 5.18; Cl, 24.70; N, 19.63.

5-Acetamido-2-chloro-3-methylpyridine (**5**).

A solution of amino derivative **4** (2.35 g., 16.5 mmoles) in acetic anhydride (30 ml.) was allowed to stand at room temperature for 17 hours. Evaporation under diminished pressure, followed by drying of the residue, gave 3.02 g. (99% yield) of **5**, m.p. 172-173°. Recrystallization from the mixture of acetone-hexane (1:1) afforded pure **5**, m.p. 173.5-174°; ir (potassium bromide) ν: 3295 (m), 3245 (s), 3170 (m), 3095 (m), 1700 (s), 1605 (s), 1465 (m), 1415 (s), 1395 (s) cm^{-1}; nmr (perdeuteriomethanol) τ: 7.78 (s, 3H, Py-CH_3), 7.58 (s, 3H, CH_3CO), 1.95 (d, 1H, J3Hz, 4-H), 1.56 (d, 1H, J3Hz, 6-H).

Anal. Calcd. for $C_8H_9ClN_2O$: C, 52.04; H, 4.92; Cl, 19.21; N, 15.17. Found: C, 51.92; H, 4.76; Cl, 19.32; N, 15.13.

Oxidation of 5-Acetamido-2-chloro-3-methylpyridine (**5**).

To a solution of 5-acetamido-2-chloro-3-methylpyridine (**5**) (185 mg., 1.0 mmole) in glacial acetic acid (12 ml.), an aqueous solution (6 ml.) of potassium permanganate (348 mg., 2.2 mmoles) was added gradually during a 1 hour period at room temperature. The magnetically stirred solution was allowed to stand at ambient temperature for 3 hours and was then heated at 108° for 30 minutes. The reaction mixture was cooled, evaporated *in vacuo*, and codistilled with water (3 x 20 ml.), then the residue was extracted with ether (4 x 15 ml.). Ether extracts afforded 152 mg. (82% of recovery) of unchanged starting material **5** (m.p. 170-172°, mixed melting point with authentic **5** was undepressed).

Coupling Reaction Between 5-Acetamido-2-chloro-3-methylpyridine (**5**) and 3-Trifluoromethylaniline.

A solution of 5-acetamido-2-chloro-3-methylpyridine (**5**) (185 mg., 1.0 mmole) in 3-trifluoromethylaniline (1.0 ml.) was stirred for 17 hours at 175-180°. Precipitation with acetone-ether-hexane afforded 138 mg. (75% recovery) of unchanged starting material **5** (m.p. 171-173°, confirmed by mixed melting point).

2-Chloro-5-hydroxy-3-methylpyridine (**6**).

A solution of amino derivative **4** (428 mg., 3.0 mmoles) in 1*N* sulfuric acid (20 ml.) was cooled to 3° and an aqueous solution (4 ml.) of sodium nitrite (276 mg., 4.0 mmoles) was added slowly with stirring, the temperature being kept below 3°. This solution was stirred for 20 minutes after the addition of sodium nitrite had been completed, and was then heated at 90-95° for another 20 minutes. The reaction mixture was cooled, neutralized with potassium bicarbonate, saturated with sodium sulfate, and extracted exhaustively with ether. The combined extracts were decolorized with charcoal, dried over magnesium sulfate, filtered, and evaporated to dryness to give 342 mg. (79% yield) of **6**, m.p. 123-128°. Recrystallization in a mixture of benzene-hexane (2:1) led to white needles of **6**, m.p. 131.5-132°; ir (potassium bromide) ν: 3060 (w), 2950-2550 (s, broad), 1610 (w), 1590 (s), 1460 (s), 1420 (s), 1385 (m) cm^{-1}; nmr (perdeuteriomethanol) τ: 7.69 (s, 3H, CH_3), 2.83 (d, 1H, J3Hz, 4-H), 2.25 (d, 1H, J3Hz, 6-H).

Anal. Calcd. for C_6H_6ClNO: C, 50.19; H, 4.21; Cl, 24.70; N, 9.76. Found: C, 49.99; H, 4.08; Cl, 24.52; N, 9.47.

5-Acetoxy-2-chloro-3-methylpyridine (**7**).

Hydroxy derivative **6** (7.18 g., 50 mmoles) in acetic anhydride (85 ml.) was stirred and heated for 18 hours at 112°. The yellowish reaction liquid was evaporated at 40° *in vacuo* to a thick syrup. Crystals appeared when the syrup was triturated with hexane. The product was successively recrystallized from carbon tetrachloride-hexane-pentane (3:5:1), carbon tetrachloride-petroleum ether (1:3), and carbon tetrachloride, to give 8.71 g. (94% yield) of white crystals, m.p. 38-39°; ir (potassium bromide) ν: 3070 (w), 1770 (s), 1605 (w), 1575 (m), 1465 (m), 1405 (s), 1375 (m) cm^{-1}; nmr (perdeuteriomethanol) τ: 7.73 (s, 3H, Py-CH_3), 7.65 (s, 3H, CH_3CO), 2.47 (d, 1H, J3Hz, 4-H), 1.97 (d, 1H, J3Hz, 6-H).

Anal. Calcd. for $C_8H_8ClNO_2$: C, 51.77; H, 4.34; Cl, 19.10; N, 7.55. Found: C, 51.55; H, 4.21; Cl, 19.16; N, 7.29.

5-Acetoxy-2-chloronicotinic Acid (**8**).

The picoline derivative **7** (1.86 g., 10 mmoles) was dissolved in acetic acid (35 ml.) and water (75 ml.). The solution was mixed with potassium permanganate (4.74 g., 30 mmoles) in one portion with vigorous stirring. Highest yields resulted when the reaction mixture was allowed to react for 16 hours at 30° and was then heated at 45-50° for 5 hours. Manganese dioxide was removed by filtration and washed with 90° water (3 x 30 ml.). The filtrate was extracted four times with carbon tetrachloride, and the combined extracts were dried over magnesium sulfate. Evaporation afforded the unreacted starting material **7** (1.19 g., 64% recovery). The extracted water layer was evaporated *in vacuo* to dryness and codistilled several times with a mixture of benzene-toluene (1:1). Tlc of the residue (chloroform-methanol [3:2]) revealed that the product was a mixture of acetyl and desacetyl derivatives **8** and **9**, respectively. The residue was then heated under reflux with acetic anhydride (15 ml.) for four hours. Excess acetic anhydride was removed *in vacuo* and the resulting material was dissolved in water (4 ml.), then the *p*H was brought to 1 by the addition of concentrated hydrochloric acid. This solution was extracted with ether (25 ml. plus 4 x 15 ml.), and the combined ether extracts were washed with water (6 x 1.5 ml.) and dried over magnesium sulfate. Removal of solvent yielded 251 mg. (12%) of the acid **8**, m.p. 143-147°. Tlc (chloroform-methanol [5:1], chloroform-methanol [2:1], and chloroform-methanol [3:2]) showed acetyl derivative **8** as a major product; hydroxy acid **9** was present in traces only. Successive recrystallizations from chloroform, chloroform-hexane (9:1), acetone-carbon tetrachloride-hexane (4:6:5), and dioxane-hexane (2:1) led to white needles of **8**, m.p. 162.5-163.5°. For a satisfactory elemental analysis, the analytical sample had to be dried at 80° in a vacuum of 0.1 mm. for 20 hours. The compound sublimes at approximately 140°; ir (potassium bromide) ν: 3080 (w), 2950-2350 (s, broad), 1780 (s), 1735 (s), 1580 (s), 1425 (s), 1375 (m) cm^{-1}; nmr (perdeuteriomethanol) τ: 7.71 (s, 3H, CH_3), 1.92 (d, 1H, J3Hz, 4-H), 1.66 (d, 1H, J3Hz, 6-H).

Anal. Calcd. for $C_8H_6ClNO_4$: C, 44.57; H, 2.81; Cl, 16.45; N, 6.50; N.E. 215.6. Found: C, 44.84; H, 2.89; Cl, 16.55; N, 6.61; N.E. (sodium hydroxide), 215.

2-Chloro-5-hydroxynicotinic Acid (**9**).

A solution of acetoxy acid **8** (151 mg., 0.70 mmole) in methanol (10 ml.) was mixed at room temperature with 1*N* sodium hydroxide (1.4 ml.), and the clear reaction mixture was set aside at ambient temperature. The reaction was followed by tlc (chloroform-methanol [3:2]) until the starting material had disappeared (6 hours). The solution was demineralized with Dowex 50W (H+) and the eluate was evaporated *in vacuo* to dryness to give 116 mg. (96% yield) of the product, m.p. 234-236°. Tlc (chloroform-methanol [3:2]) showed one major spot (R_f 0.4). The analytical sample of hydroxy acid **9** was obtained by recrystallization from dioxane, m.p. 237.5-238°. Before elemental analysis, the sample was dried at 80° in vacuum (0.1

mm.) for 18 hours; ir (potassium bromide) ν: 3220 (s, broad), 3080 (w), 2950-2250 (s, broad), 1695 (s), 1605 (m), 1590 (s), 1435 (s) cm^{-1}; nmr (perdeuteriomethanol) τ: 2.33 (d, 1H, J3Hz, 4-H), 1.97 (d, 1H, J3Hz, 6-H).

Anal. Calcd. for $C_6H_4ClNO_3$: C, 41.52; H, 2.32; Cl, 20.43; N, 8.07; N.E., 173.6. Found: C, 41.66; H, 2.60; Cl, 20.52; N, 8.29; N.E. (sodium hydroxide), 172.

2-[3′-(Trifluoromethyl)anilino]-5-hydroxynicotinic Acid (**2**).

A fine suspension of sodium iodide (75 mg., 0.5 mmole) in a solution of hydroxy acid **9** (208 mg., 1.2 mmoles) in 3-trifluoromethylaniline (98% purity) (10 ml.) was heated at 175 ± 5° (bath temperature) in a closed flask under nitrogen atmosphere for 4 hours with vigorous stirring. The reaction mixture was cooled, diluted with carbon tetrachloride (10 ml.), and extracted repeatedly with concentrated ammonium hydroxide. Alkaline extracts were combined and extracted with carbon tetrachloride (4 x 1.5 ml.). These extracts were discarded, and the ammonium hydroxide layer was evaporated *in vacuo* to dryness. The residue was washed several times with boiling acetone, the acetone liquors were discarded, and the solid residue was subjected to vacuum sublimation (0.05 mm.) at bath temperature 195 ± 5°. The yield was 244 mg. (68%) of pale yellow sublimate, m.p. 239-241°, showing on tlc (chloroform-methanol [5:1]) one major spot of desired product (R_f 0.4) and one minor spot (R_f 0.7) corresponding to 3-trifluoromethylaniline. Recrystallizations from methanol-water (3:2) gave yellowish platelets of an analytically pure acid **2**, m.p. 247-248°, R_f 0.59 (chloroform-methanol [3:2]); ir (potassium bromide) ν: 3100 (w), 3000-2550 (s, broad), 1670 (m), 1620 (m), 1600 (m), 1565 (s), 1500 (m), 1420 (m) cm^{-1}; nmr (perdeuteriomethanol) τ: 2.81 (d, 1H, J7.5Hz, 6′ or 4′-H), 2.61 (t, 1H, J7.5Hz, 5′-H), 2.28 (d, 1H, J8Hz, 4′ or 6′-H), 2.17 (d, 1H, J3Hz, 4-H), 1.96 (d, 1H, J3Hz, 6-H), 1.85 (s, 1H, 2′-H).

Anal. Calcd. for $C_{13}H_9F_3N_2O_3$: C, 52.36; H, 3.04; F, 19.11; N, 9.39; N.E., 298.2. Found: C, 52.65; H, 3.08; F, 19.38; N, 9.49; N.E. (sodium hydroxide), 293.

Cocrystallization of the Metabolite M2b (^{14}C) with 2-[3′-(Trifluoromethyl)anilino]-5-hydroxynicotinic Acid (**2**).

A methanolic solution of the crude metabolite M2b (^{14}C) containing 82,000 disintegrations per minute was combined with a solution of synthetic hydroxy acid **2** (12.9 mg., m.p. 247-248°) in methanol (1.0 ml.), filtered and evaporated to dryness in a nitrogen stream. The residue was dissolved in hot methanol (180 μl.), water was added (100 μl.), and the solution was allowed to crystallize. The first crystallization provided 12.0 mg. of material, m.p. 240-243°, having specific activity 3873 dpm/mg. The second recrystallization, from ethanol (100 μl.) - water (100 μl.), led to 8.8 mg. of crystals, m.p. 242-244°, having 4038 dpm/mg. The third recrystallization, from isopropyl alcohol (100 μl.) - water (200 μl.), yielded 7.9 mg. of crystals, m.p. 246-248°, having activity 3782 dpm/mg. The apparent differences in specific activity seen upon successive recrystallizations are considered to be within the limits of error of these determinations.

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