

SYNTHESIS OF CARBON-14 AND TRITIUM LABELED N,N'-(2-CHLORO-5-CYANO-1,3-PHENYLENE)DIOXAMIC ACID DI-THAM SALT

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SUMMARY

Acetylation of tritium and carbon-14 labeled 4-chloro-3,5-diaminobenzonitrile (Ia and Ib) with ethyl oxalyl chloride afforded diethyl N,N'-(2-chloro-5-cyano-1,3-phenylene)dioxamate (IIa and IIb). The diester II was hydrolyzed to give N,N'-(2-chloro-5-cyano-1,3-phenylene)dioxamic acid, which was isolated as the *tris*-hydroxymethylaminomethane (THAM) salt III.

Key Words: Carbon-14, Tritium, N,N'-(2-Chloro-5-cyano-1,3-phenylene)dioxamic Acid, Synthesis, Stability

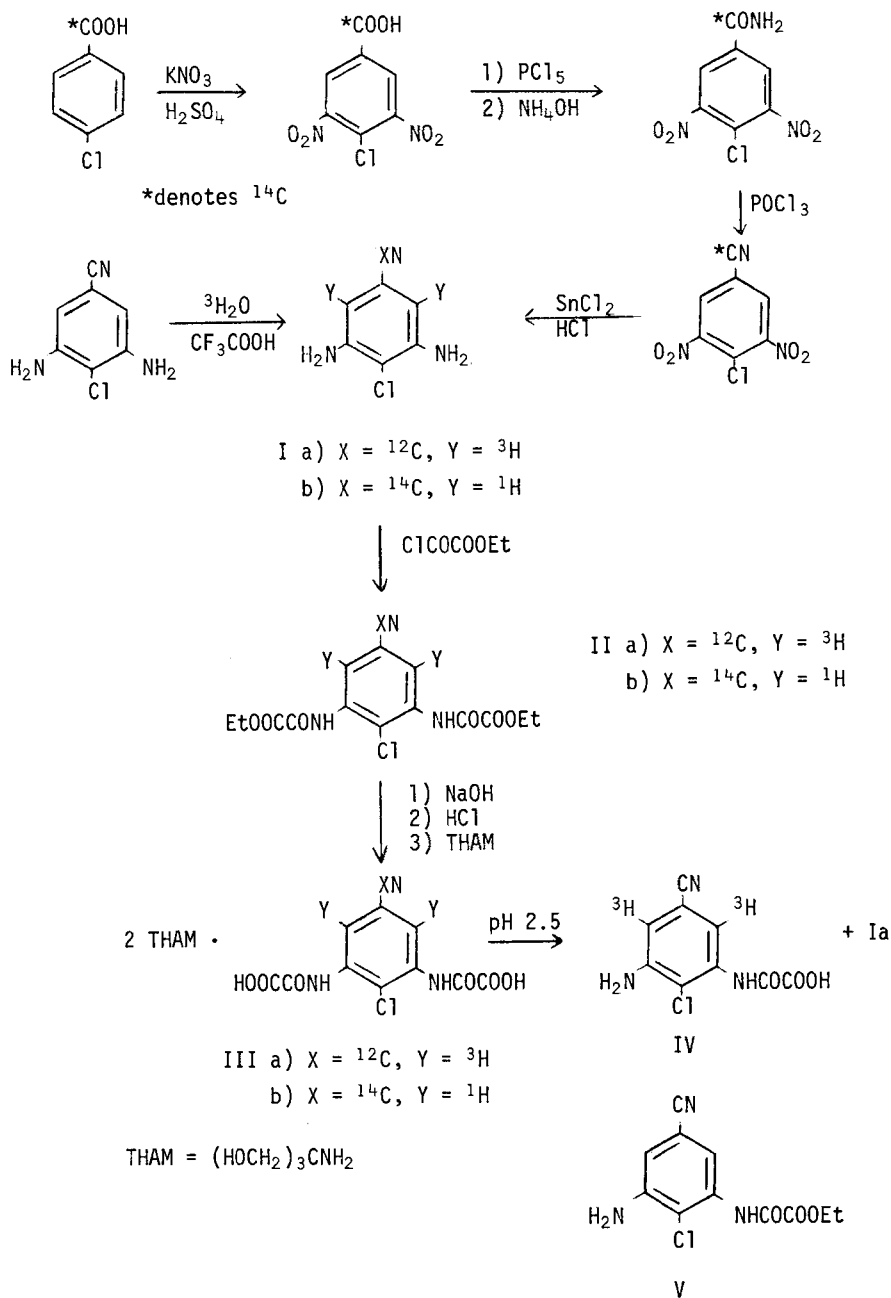
INTRODUCTION

N,N'-(2-chloro-5-cyano-1,3-phenylene)dioxamic acid di-THAM salt (III) is a potential new anti-asthma agent. Radioactive forms of the compound were needed for conducting studies on its absorption, excretion and metabolism in test animals and man. We prepared both tritium and carbon-14 labeled III because each labeled version would have some advantages and drawbacks. Tritium labeled III could be obtained with high specific activities, but the chemical stability of this label would be relatively low, as will be discussed later. On the other hand, although only lower specific activities would be achievable with carbon-14 labeled III, the compound should retain the radioisotope label, provided that it is not located in the side-chains.

DISCUSSION

The synthetic route leading to tritium labeled IIIa is outlined in Scheme 1. The hydrogen atoms at the 2- and 6-positions in 4-chloro-3,5-diaminobenzonitrile readily undergo acid catalyzed exchange with tritium, since they are

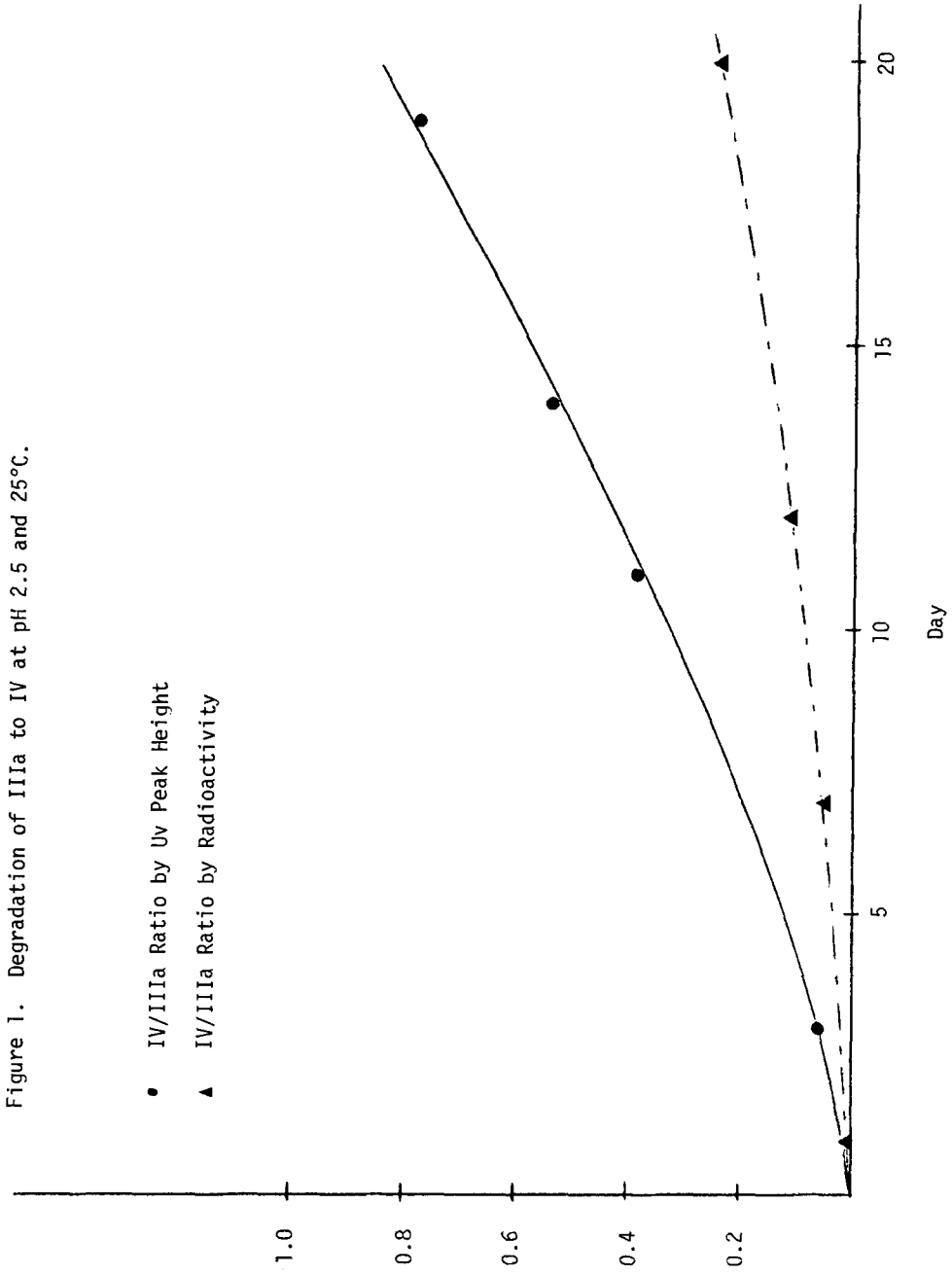
Scheme 1. Synthesis of Carbon-14 and Tritium Labeled N,N'-(2-Chloro-5-cyano-1,3-phenylene)dioxamic Acid Di-THAM Salt



both ortho and para to the two amino functions. Treatment of the diamine with tritiated water in the presence of trifluoroacetic acid afforded tritium labeled Ia. Acylation of Ia with ethyl oxalyl chloride gave the dioxamic ester IIa, which was hydrolyzed to the corresponding dioxamic acid, and subsequently converted to the di-THAM salt IIIa.

Although the tritium-protium exchange occurs rapidly in Ia, because of the presence of the amino groups, introduction of the acyl side chains stabilizes the labels by delocalization of the electron pairs on the nitrogens. The stabilizing effects of the N-acyl groups, however, are negated by the fact that the side chains are easily cleaved in acidic media such as those found in the stomach juice of some test animals. In aqueous solution at pH 2.5 and at room temperature, IIIa was found to lose the side chains to first give 3'-amino-2'-chloro-5'-cyanooxanilic acid (IV), and eventually Ia. The loss of even one oxalyl side chain would be sufficient to render labile both of the tritium labels because of their ortho and para relationship to the resulting amino group. These occurrences were observed by means of reverse phase high pressure liquid chromatography (hplc) which separated compounds IV and IIIa with detection by ultraviolet light (uv, 254 nm) absorption. An aqueous solution of IIIa maintained at pH 2.5 and room temperature was analyzed at selected time intervals by hplc and the recovered fractions corresponding in retention time to compounds IV and IIIa were measured for radioactivity. The ratios of uv peak heights and the ratios of radioactivities attributable to IIIa and IV were plotted against time (Figure 1). The increase in IV/IIIa radioactivity ratio (broken line) lagged behind the increase in IV/IIIa uv peak height ratio (solid line)*. The loss of an oxalyl side chain exposed the tritium labels to loss by back-exchange in the acidic medium, thus decreasing the specific activity in IV, and hence the apparent slower rate of formation of IV as measured by radioactivity in comparison to uv measurements.

*The bias in the uv peak height ratios, because of different uv responses of IIIa and IV, should reinforce our conclusion, since the ϵ values at 254 nm are 23,150 for IIIa and 9,250 for IV.



Since tritium labeled IIIa appeared only suitable for use in studies where acidic media would not be encountered, carbon-14 labeled IIIb was prepared as outlined in Scheme 1. 4-Chlorobenzoic acid- α - ^{14}C was nitrated according to known procedure (1,2) to give 4-chloro-3,5-dinitrobenzoic acid- α - ^{14}C in 82.2% yield. The acid was converted to the corresponding acid chloride by treatment with phosphorous pentachloride, and subsequently to the carbon-14 labeled 4-chloro-3,5-dinitrobenzamide (3) in 95.7% yield with ammonium hydroxide. The high yield of the amide was achieved by minimizing displacement of the 4-chlorine by ammonia, by carrying out the reaction at low temperatures and in a two-phase water-benzene mixture. Dehydration of the amide with phosphorous oxychloride produced 4-chloro-3,5-dinitrobenzotrile- α - ^{14}C in 91.6% yield. The nitro groups were reduced with stannous chloride in hydrochloric acid to give 4-chloro-3,5-diaminobenzotrile- α - ^{14}C (Ib) in 97% yield. Consistently high yields were obtained in the reduction when it was carried out below room temperature. The conversions from Ib to the dioxamic ester IIB and then to the dioxamic acid THAM salt IIIb paralleled those leading from the tritium labeled Ia to IIIa *via* IIA. The overall yield of IIIb from 4-chlorobenzoic acid- α - ^{14}C was 40%.

EXPERIMENTAL

Radioactivity determinations were carried out in Ditol with a Packard Tri-Carb Model 2425 liquid scintillation spectrometer using the external standard method. Hplc analyses were carried out with a Chromatronix Model 3510 liquid chromatograph equipped with a Model 230 UV (254 nm) detector. The 1 m x 2.1 mm I.D., 316 stainless steel column was packed with Corasil/C-18 (Waters Associates). The mobile phase was a mixture of 30 ml of 85% H_3PO_4 and 100 ml of MeOH q.s. to 1 μ with H_2O . Tlc analyses were carried out on 1" x 4" glass plates coated with a 250 μm thick layer of silica gel GF (Analtech). Developed zones were visualized under ultraviolet (uv) light (254 nm). Radioactive zones were detected with a Vanguard Model 880 Autoscaner equipped with Model 885 Glass Plate Scanner. Uv spectra were obtained with a Cary Model 15

spectrometer. Melting points were obtained with a Thomas-Hoover Unimelt and were uncorrected.

4-Chloro-3,5-diaminobenzonitrile-2,6-³H₂ (Ia)

A solution of 100 mg (0.6 mmole) of 2-chloro-5-cyano-1,3-phenylenediamine in 0.4 ml of CF₃COOH was stirred overnight at room temperature with 30 Ci of tritiated water.* The mixture was concentrated *in vacuo*. The residue was stirred with 2 ml of 0.1N NaOH for 20 min. Labile tritium was removed *in vacuo* with MeOH. The resulting crude Ia was recrystallized from a mixture of 2 ml of MeOH and 17.5 ml of H₂O to give 61 mg of Ia, sp. act. 14.6 mCi/mg or 2.45 Ci/mM, radiochemically pure by tlc (5% v/v MeOH in CH₂Cl₂, Rf 0.51).

4-Chloro-3,5-dinitrobenzoic Acid- α -¹⁴C

To a solution of 478 mg (3.05 mmoles, 278.6 μ Ci/mg or 43.6 mCi/mM) of 4-chlorobenzoic acid- α -¹⁴C ** in 5.2 ml of conc H₂SO₄ at 73°C was added 1.55 g (15.3 mmoles) of KNO₃. The reaction temperature was increased to 125°C and the mixture was stirred under N₂ for 3.25 hrs and poured onto 25 g of ice. The mixture was stirred for 10 min, filtered, and the crystals washed with 4 x 10 ml of H₂O and dried, 619 mg, 82.2% yield, mp 159-160°C; sp. act. 176 μ Ci/mg or 43.5 mCi/mM; radiochemically pure by tlc (upper phase of a mixture of 9 parts by volume of EtOAc, 2 parts of HOAc, 5 parts of isooctane, and 10 parts of H₂O, Rf 0.50).

4-Chloro-3,5-dinitrobenzamide- α -¹⁴C

To a solution of 608 mg (2.47 mmoles) of 4-chloro-3,5-dinitrobenzoic acid- α -¹⁴C in benzene (5 ml) was added 565 mg (2.71 mmoles) of PCl₅, and the mixture was stirred under N₂ at 52°C overnight. After removal of benzene and

*The tritiation portion of this preparation was carried out at New England Nuclear Corporation, Boston, Mass. according to procedures suggested by Dr. C.M. Hall of The Upjohn Company.

**Supplied by New England Nuclear Corp., Boston, Mass.

POCl_3 formed in the reaction at 52°C and 25 mm Hg, the solid residue was dissolved in benzene (5 ml), the solution cooled in an ice bath, and 3 ml of conc NH_4OH was added dropwise with vigorous stirring. The mixture was stirred at 0°C for 20 min and filtered. The resulting crystals were washed with 10 ml each of benzene and cold water, and dried, 580 mg, 95.7% yield, mp $180\text{--}183^\circ\text{C}$; sp. act. $177 \mu\text{Ci}/\text{mg}$ or $43.4 \text{ mCi}/\text{mM}$; radiochemically pure by tlc (1:1 v/v Me_2CO -hexane, Rf 0.40).

4-Chloro-3,5-dinitrobenzonitrile- α - ^{14}C

A mixture of 573 mg (2.34 mmoles) of 4-chloro-3,5-dinitrobenzamide- α - ^{14}C and 5 ml of POCl_3 was stirred under N_2 at 56°C for 12.5 hrs. The excess POCl_3 was removed at 56°C and 25 mm Hg and the residue was chromatographed on a column of 100 g (2.2 x 52 cm) of silica gel 60 (Mallinckrodt, 70-230 mesh) eluted with CH_2Cl_2 . After a forerun of 325 ml (column holdup volume was 190 ml), 250 ml of the eluate was collected, which, after removal of CH_2Cl_2 , produced 486 mg (91.6% yield) of product, mp $141\text{--}142.5^\circ\text{C}$; sp. act. $194 \mu\text{Ci}/\text{mg}$ or $44.1 \text{ mCi}/\text{mM}$; radiochemically pure by tlc (1:1 v/v Me_2CO -hexane, Rf 0.65). This material was of suitable purity for use in the next step without recrystallization.

4-Chloro-3,5-diaminobenzonitrile- α - ^{14}C (Ib)

To a stirred solution at 0°C of 3.389 g (15.02 mmoles) of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 8.2 ml of conc HCl was added in portions 478 mg (2.10 mmoles) of 4-chloro-3,5-dinitrobenzonitrile- α - ^{14}C . The mixture containing solids was stirred while the ice bath was allowed to melt over a 1.5 hr period. The mixture was again cooled to 0°C and basified to pH 12-14 by dropwise addition of 50% NaOH. The mixture was partitioned between cold H_2O and EtOAc, and the aqueous phase was extracted with 4 x 40 ml of EtOAc. The combined EtOAc extracts were washed with 2 x 50 ml of H_2O , 100 ml of saturated NaCl solution, and dried over MgSO_4 . Removal of solvent under vacuum afforded 341 mg (96.9% yield) of Ib, mp $169\text{--}171^\circ\text{C}$; sp. act. $260 \mu\text{Ci}/\text{mg}$ or $43.6 \text{ mCi}/\text{mM}$; radiochemically pure by tlc (5% v/v MeOH in CH_2Cl_2 , Rf 0.51).

Carbon-14 Labeled (Iib) and Tritium Labeled (IIa) Diethyl N,N'-(2-Chloro-5-cyano-1,3-phenylene)dioxamate

To a stirred solution of 337 mg (2.01 mmoles) of Ib and 864 mg (8.54 mmoles) of Et₃N in 5 ml of dry DMF was added 1.165 g (0.95 ml, 8.54 mmoles) of ethyl oxalyl chloride. The mixture containing precipitates was stirred under N₂ at 85°C for 2 hrs and 30 ml of H₂O was added dropwise. The resulting mixture was filtered and the collected crystals were washed with H₂O and dried. The crude product was dissolved in 10 ml of hot Me₂CO, and 30 ml of hot hexane was slowly added. The mixture was kept at room temperature for 15 hrs and the crystals were filtered, washed with hexane and dried, 630 mg. This material was further purified by chromatography on a 40 g column of silica gel eluted with 5% v/v EtOH in CH₂Cl₂. The residue from the first 250 ml of eluate was recrystallized from a mixture of 10 ml of Me₂CO and 30 ml of hexane to give 607 mg (82.0% yield) of Iib, mp 180-180.5°C; sp. act. 120 μCi/mg or 44.1 mCi/mM; radiochemically pure by tlc (5% v/v MeOH in CH₂Cl₂, Rf 0.71).

Similarly, from 59 mg of Ia and 23 mg of non-radioactive 2-chloro-5-cyano-1,3-phenylenediamine, there was obtained 77 mg of IIa, sp. act. 4.79 mCi/mg or 1.76 Ci/mM, radiochemically pure by tlc (5% v/v MeOH in CH₂Cl₂, Rf 0.71).

Carbon-14 Labeled (IIIb) and Tritium Labeled (IIIa) N,N'-(2-Chloro-5-cyano-1,3-phenylene)dioxamic Acid Di-THAM Salt

A mixture of 276 mg (0.75 mmole) of Iib, 6 ml of H₂O and 9 ml of 0.2 N NaOH was stirred at room temperature for 30 min. Traces of fluffy precipitates were removed by filtration and the filter funnel was washed with 2.5 ml of H₂O. The combined filtrate and washing were added dropwise with stirring in 5 min to 5 ml of 0.5 N HCl at 0°C. The mixture containing copious amounts of fine precipitates was stirred at 0°C for 5 more min and kept in the refrigerator for 20 min. The mixture was filtered and the cake was washed with 10 ml of ice water in portions, briefly air dried, and finally dried *in vacuo* (25°C, 50 μm Hg) overnight (20 hrs), 212 mg (91% yield). To a filtered, warm solution of 156 mg (0.50 mmole) of the crude diacid in 8 ml of MeOH was added 12.5 ml (1.00 mmole)

of 0.08 M solution of THAM in MeOH. Copious amounts of precipitates appeared during 3 hrs of stirring. The crystals were filtered, washed with 3 ml of MeOH, followed by 5 ml of 1:1 MeOH-Et₂O and 6 ml of Et₂O, briefly air-dried, and finally dried *in vacuo* (25°C, 50 μm Hg), 182 mg (77.4% yield) of IIIb, sp. act.* 80.1 μCi/mg or 44.3 mCi/mM; $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 204 nm (ϵ 19,200), 243 (29,400), $\lambda_{\text{sh}}^{\text{H}_2\text{O}}$ 305 nm (ϵ 2,750), 317 (1,400); single radioactive component by tlc (7:2:1 *i*-PrOH-NH₄OH-H₂O, R_f 0.82; 5% v/v MeOH in CH₂Cl₂, R_f 0); 99.4% radiochemically pure by hplc.**

Similarly, 61 mg of the tritium labeled diester IIa was hydrolyzed to the diacid and converted to the di-THAM salt IIIa, 40 mg; sp. act.* 3.21 mCi/mg or 1.77 Ci/mM; $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 242 nm (ϵ 29,400), $\lambda_{\text{sh}}^{\text{H}_2\text{O}}$ 305 nm (ϵ 2,600), 316 nm (ϵ 1,200); radiochemically pure by tlc (10% v/v MeOH in CH₂Cl₂, R_f 0.02; 7:2:1 v/v *i*-PrOH-NH₄OH-H₂O, R_f 0.82), 99.1% radiochemically pure by hplc.**

Ethyl 3'-Amino-2'-chloro-5'-cyanooxanilate (V)

To a solution of 1.676 g (10 mmoles) of 2-chloro-5-cyano-1,3-phenylenediamine and 2.024 g (20 mmoles) of Et₃N in 25 ml of Me₂CO was added dropwise with stirring during 30 min 1.365 g (10 mmoles) of ethyl oxalyl chloride in 20 ml of CH₂Cl₂. The mixture was kept at room temperature overnight and concentrated at reduced pressure. The residue was washed with 50 ml of hexane. The solids, a mixture of unreacted starting diamine (I), the desired monoacylation product (V), and the diacylated product (II), were dissolved in 15 ml of CH₂Cl₂ and chromatographed on a column of 180 g of silica gel eluted with 2.5 μ of 10% v/v EtOAc in CHCl₃, which was collected in 12 ml fractions at 4 min/fraction. The combined residue from fractions 67-110 (2.038 g) was chromatographed on a column of 180 g of silica gel eluted with 10% v/v EtOH in CH₂Cl₂, which was collected

*Compounds IIIa and IIIb in MeOH solution tend to plate out from Diotol onto counting vial walls and lead to erratic counting results. This problem was alleviated by counting aqueous solutions of IIIa and IIIb in Diotol.

**At a mobile phase flow rate of 1.4 ml/min, the effluent was collected in 0.7 ml fractions, which were counted in 15 ml portions of Diotol, each containing 1 ml of MeOH to help solubilize the effluent. Retention time was 2.3 min for I, 9.5 min for IV, and 17 min for III.

in 12 ml fractions at 3.5 min/fraction. The combined residue from fractions 23-29 (1.875 g), which was essentially free of (II) but still contained (I) and (V), was further chromatographed twice on 180 g columns of silica gel eluted with 5% v/v Me₂CO in benzene at 15 ml/2.5 min to afford 965 mg of crude (V). The crude was dissolved in 20 ml of hot Me₂CO and the solvent was replaced, by boiling, with a final volume of 15 ml of benzene. The solution on cooling afforded 864 mg (32.3% yield) of V, mp 151-152.5°C; single component by tlc (5% v/v Me₂CO in benzene, R_f 0.21); $\lambda_{\max}^{\text{EtOH}}$ 211 nm (ϵ 18,900), 229 (22,150), 248 (20,250) and 334 (4,100); infrared (ir) ν_{\max} (Nujol) 2,240 cm⁻¹ (C=N), 1,730 cm⁻¹ (ester C=O), 1,705 cm⁻¹ (amide C=O).

Anal. Calc'd. for C₁₁H₁₀ClN₃O₃ (M.W. 267.67); C, 49.36; H, 3.77; Cl, 13.25; N, 15.70. Found: C, 49.32; H, 3.67; Cl, 13.58; N, 15.77.

3'-Amino-2'-chloro-5'-cyanooxanilic Acid (non-radioactive form of IV)

To a solution of 804 mg (3 mmoles) of V in 30 ml of Me₂CO was added with stirring 3.3 ml of 1N KOH. The solidified mixture was diluted with more Me₂CO, filtered, and the cake washed with Me₂CO and dried. The solids were suspended in 50 ml of 95% EtOH and stirred for 2 hrs with 6.5 g of Amberlyst 15 resin (RSO₃H form, 50-100 mesh) which had been thoroughly washed with hot H₂O, EtOH, and Me₂CO. The clear supernate was filtered, further stirred with 3.5 g of fresh resin for 15 min and again filtered. The filtrate was concentrated at 30°C and 35 mm Hg to give 487 mg of crude product. Recrystallization from a mixture of 4 ml of Me₂CO and 10 ml of hexane afforded 435 mg (61% yield) of product, single component by hplc analysis; *mp 203-204°C (dec); $\lambda_{\max}^{\text{EtOH}}$ 247 nm (ϵ 28,950), 332 (4,200); ir ν_{\max} (Nujol) 2,240 cm⁻¹ (C=N), 1,755 cm⁻¹ (acid C=O), 1,710 cm⁻¹ (amide C=O).

Anal. Calc'd. for C₉H₆ClN₃O₃ (M.W. 239.62): C, 45.11; H, 2.52; Cl, 14.80; N, 17.54. Found: C, 45.05; H, 2.78; Cl, 14.83; N, 17.50.

*Retention time 9.5 min with mobile phase flow rate of 1.4 ml/min.

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