

4-Chloroacetamido-1-naphthyl Acetate (III).—To a suspension of 4.7 g of 4-amino-1-naphthol hydrochloride in 25 ml of glacial acetic acid containing 15 g of sodium acetate, 3.5 ml of chloroacetyl chloride was added dropwise. The resulting reaction mixture was stirred for 3 hr and diluted with 200 ml of water. The purplish precipitate was collected, washed well with water, air-dried, and then recrystallized from 50% ethanol to give 3.3 g (78%) of crude III, mp 154–156° dec. Recrystallization from 95% ethanol and then ethyl acetate afforded pure III as pink purplish fluffy needles: μ 161–162; λ^{CHCl_3} 2.96, 5.67, 5.92, and 6.2 μ .

Anal. Calcd for $\text{C}_{14}\text{H}_{12}\text{ClNO}_3$: C, 60.6; H, 4.4; N, 5.1. Found: C, 60.9; H, 4.5; N, 5.3.

A 0.2-g sample of III was suspended in 10 ml of 2 *N* NaOH. After 1 hr, a green solution resulted. After standing for 1 additional hr, this solution was neutralized with glacial acetic acid and the precipitate was collected and recrystallized twice from 5% ethanol to give light pink needles, mp 194–196°. A mixture melting point with II as prepared above, showed no depression.

4-Iodoacetamide-1-naphthol (I).—A 0.9-g sample of II dissolved in 25 ml of acetone was added to 0.3 g of NaI. A homogenous solution resulted at first, which turned turbid in a few seconds and NaCl precipitated on standing at room temperature overnight. The NaCl was separated and the filtrate was diluted with 75 ml of cold water. The pink precipitate was collected and recrystallized from 100 ml of hot ethyl acetate, 0.7 g (57%), mp 198° dec; it was again recrystallized from aqueous methanol, mp 199° dec. A mixture melting point with II was found to be 186–187.5° dec.

Anal. Calcd for $\text{C}_{12}\text{H}_{10}\text{INO}_2$: C, 44.1; H, 3.1; N, 4.4. Found: C, 44.2; H, 3.1; N, 4.2.

4-Iodoacetamido-1-naphthyl acetate (IV) was prepared in a similar procedure in 87% yield, mp 186–187°, as pink leaflets, after recrystallization from aqueous acetone.

Anal. Calcd for $\text{C}_{14}\text{H}_{12}\text{INO}_3$: C, 45.6; H, 3.3; N, 3.8. Found: C, 45.3; H, 3.2; N, 3.9.

Histochemical Procedure.—The procedure used is similar to that published earlier⁷ for another chromogenic reagent. Tissues were fixed for 24 hr in 1% trichloroacetic acid in 80% ethanol, dehydrated, embedded in paraffin, cut at 6 μ , and rehydrated. The sections on glass slides were then incubated for 1 hr at 50° in the following medium, prepared by adding 4-iodoacetamido-1-naphthol (25 mg in 15 ml of ethanol) to 35 ml of 0.1 *M* phosphate buffer at pH 7.0. The sections were then washed with 30% ethanol and water and then treated for 3 min at room temperature with fast blue BBN (1 mg/ml of 0.1 *M* phosphate buffer at pH 7.4). They were then washed with water, dehydrated, cleared with xylene, and mounted in Permount. The similarity of the staining reaction for sulfhydryl groups in Figure 1 may be compared with the earlier method.⁷

(7) R. J. Barnett and A. M. Seligman, *Science*, **116**, 323 (1952).

cis-4-Aminomethylcyclohexanecarboxylic Acid

WALTER E. MEYER

Organic Chemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

Received February 11, 1966

Both stereoisomers of 4-aminomethylcyclohexanecarboxylic acid (AMCHA) were isolated as early as 1900.¹ Although the *trans* isomer has been described as effecting marked inhibition of the fibrinolytic enzyme system, detailed evidence for assigning this configuration to the more active isomer was lacking.² These configurations have now been confirmed by fusion, whereby only the less active β form produced a lactam. The structure of this lactam was established by spectral evidence (see Experimental Section). After this work had been completed, Shimizu,

(1) (a) A. Einhorn and C. Ladisch, *Ann. Chem.*, **310**, 194 (1900). (b) The two isomers were designated α and β . The α form was reported to have a softening point at 270° and the β form, probably an isomeric mixture, was described as decomposing between 220–229°.

(2) S. Okamoto, S. Sato, Y. Takada, and U. Okamoto, *Keio J. Med.*, **13**, 177 (1964).

et al., reported configurational assignments based on a stereospecific synthesis of each isomer from methyl *cis*- and *trans*-4-carboxamidocyclohexanecarboxylate and the conversion of the separate AMCHA isomers to their respective known *cis* and *trans* forms of 1,4-cyclohexanedicarboxylic acid.³

Experimental Section⁴

***cis*- and *trans*-4-Aminomethylcyclohexanecarboxylic Acids.**⁵—Each isomer was purified by partition chromatography with a 1-butanol–ethyl acetate–acetic acid–water (100:50:5:50) system and the purity was confirmed by thin layer chromatography. The fibrinolytically more active isomer was designated α : mp⁶ 295–300°; infrared absorption (KBr) at 1528, 1381, and 1325 cm^{-1} , and the less active isomer, β : mp⁶ 252°; infrared absorption (KBr) at 1640, 1563, 1515, 1403, and 1308 cm^{-1} .

4-Aminomethylcyclohexanecarboxylic Acid Lactam (I).—A 30-mg sample of the β isomer was fused over an open flame in a test tube that had been equipped with a cold finger condenser. The reaction mixture was brought to room temperature after effervescing had ceased and the residue had begun to darken. This residue and a distillate were combined and triturated two times with 1 ml of ethyl ether. The combined extract was dried (MgSO_4), filtered, and evaporated giving 5.3 mg (17.7 wt % recovery) of the crystalline lactam (I). A 720-mg sample of accumulated lactam from five 800-mg runs was purified by recrystallizing twice from 20 ml of hexane followed by a sublimation at 100° (2.5×10^{-2} mm); mp⁷ 104°; infrared absorption (KBr) at 1661 (“amide I”), 1421, 1325, and 1205 cm^{-1} , but lacking the “amide II” band of small and medium ring lactams;⁸ nmr peaks (CDCl_3) at 432 (1 H broad, CONH), 199 (2 H triplet, $>\text{CHCH}_2\text{NH}-$), and between 100–160 cps (10 H multiplet); after an active hydrogen exchange, at 196 cps (2 H doublet, $J = 8$ cps, $>\text{CHCH}_2\text{N}<$), and between 100–160 cps (10 H multiplet).

Anal. Calcd for $\text{C}_6\text{H}_{13}\text{NO}$: C, 67.03; H, 9.41; N, 10.07. Found: C, 67.27; H, 9.13; N, 10.41.

Acknowledgment.—The author wishes to thank Mr. L. M. Brancone and staff for microanalyses and differential thermal analyses, Mr. W. Fulmor and staff for infrared and nmr spectroscopic determinations, Mr. C. Pidacks and staff for chromatographic purifications, and Dr. P. Bell and staff for inhibition assays. In addition, thanks is expressed to Dr. J. S. Webb for pertinent suggestions and discussions.

(3) M. Shimizu, T. Naito, A. Okano, and T. Aoyagi, *Chem. Pharm. Bull.* (Tokyo), **13**, 1012 (1965).

(4) The corrected melting point was determined on a Kofler micro hot stage melting point apparatus. The nmr spectra were obtained on a Varian A-60 spectrometer using tetramethylsilane as an internal standard. Infrared spectra were obtained on a Perkin-Elmer Model 21 infrared spectrometer. Inhibitory activities were determined by measuring the prolongation of clot-lysis times as described by F. B. Ablondi, J. J. Hagan, M. Philips, and E. C. DeRenzo, *Arch. Biochem. Biophys.*, **82**, 153 (1959).

(5) The extent of inhibition of fibrinolytic activity, infrared spectra, and melting points of our pure isomers are in complete agreement with the data reported in a recent patent [Daiichi Seiyaku, Dutch Patent 6,414,942 (1965)]. For reasons unknown to us the infrared maxima for these isomers reported by Shimizu, *et al.*,³ do not agree with our findings or with the maxima described in the above Daiichi patent.

(6) As an endotherm, determined on a Du Pont Model 900 differential thermal analyzer. Nonreproducible melting point values were obtained using ordinary methods, probably because of thermal polymerization.

(7) The melting point was recorded at the point where, under crossed Nichol prisms, birefringence was lost. The crystal form, however, was only slowly lost thereafter over a wide temperature range.

(8) U. Schiedt, *Angew. Chem.*, **66**, 609 (1954).

Some Solvatochromic Chelating Agents

A. MUELLER AND J. P. PHILLIPS

Chemistry Department, University of Louisville, Louisville, Kentucky 40208

Received February 23, 1966

Reactions of 5-formyl-8-quinolinol¹ with *N*-methylated heterocycles containing an active methyl group readily produce com-

(1) G. R. Clemo and R. Howe, *J. Chem. Soc.*, 3552 (1955).