Trimethylene-1-(4-aldoximinopyridinium)-1′-(4-cyanopyridinium) Dibromide (V).—A solution of 5.76 g (0.018 mole) of 1,3′-bromopropyl-4-aldoximinopyridinium bromide and 11.1 g (0.107 mole) of 4-cyanopyridine in 100 ml of N,N-dimethylformamide was maintained at 65° for 48 hr. The yellow crystals were collected, washed with 2-propanol and ethyl ether; yield 4.06 g (53%) of product, mp 228–229° dec. Recrystallization from methanol–ether yielded yellow-green needles, mp 225–226° dec. Compounds VI–VIII were made with appropriate modification of the general method described above. The results are listed in Table II.

Trimethylene-1-(4-aldoximinopyridinium)-1′-(4-pyridone) Picrate (IX).—A solution of 428 mg (0.001 mole) of trimethylene-1-(4-aldoximinopyridinium)-1′-(4-cyanopyridinium) dibromide in 10 ml of water was mixed with 5 ml of 1.0 *N* NaOH, maintained at 0–5° for 10 min and then adjusted to pH 9.9. The product was isolated from the reaction mixture by column chromatography using a Dowex 50-X12 (Na^+ form) with 0.005 *M* sodium carbonate, pH 9.9, as the eluent, and desalted with charcoal (Barnebey-Cheney RC-2) utilizing ethanol–0.1 *N* HCl (1:1, v/v) as the eluent. The solvent was removed *in vacuo*. The extremely hygroscopic oil was crystallized as the picrate salt from absolute ethanol. Recrystallization from absolute ethanol yielded yellow needles (35 mg, 60%), mp 184–186° dec.

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Preparation of 4-Iodoacetamido-1-naphthol as a Histochemical Reagent for Sulfhydryl Groups[1]

K. C. TSOU

Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

AND ARNOLD M. SELIGMAN

Departments of Surgery, Sinai Hospital of Baltimore, and The Johns Hopkins University School of Medicine, Baltimore, Maryland

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Since the inhibitory action of iodoacetic acid on sulfhydryl groups of protein depends on a stoichiometric reaction, $ICH_2COOH + RSH \rightarrow RSCH_2COOH + HI$, one may anticipate 4-iodoacetamido-1-naphthol (I) to react similarly with sulfhydryl groups in protein to form a thioether which could then couple with diazotized 4-amino-2,5-diethoxybenzanilide (fast blue BBN) to form a blue dye[2] and thereby demonstrate the location of SH groups in *situ*. The dye derived from the parent compound can be easily washed out by organic solvents and does not interfere with the result. The preparation of 4-chloroacetamido-1-naphthol (II) has been reported.[3] A reinvestigation of this sample, softening at 175–180°, mp 199.5–201.5°, as reported by the early workers shows that it is a mixture of II and 4-chloroacetamido-1-naphthyl acetate (III) from infrared spectra. Modified preparations of both II and III are given in this note. The corresponding iodoacetyl derivatives I and IV react with sulfhydryl groups in protein. Owing to the alkali sensitivity of the O-acetyl linkage in IV, it still slowly couples with fast blue BBN at pH 7.5 and is therefore equally satisfactory for our study under appropriate conditions. A preliminary account of histochemical data has been given[4] and its usefulness in demonstrating the bound SH groups in plant tissues has also been reported.[5]

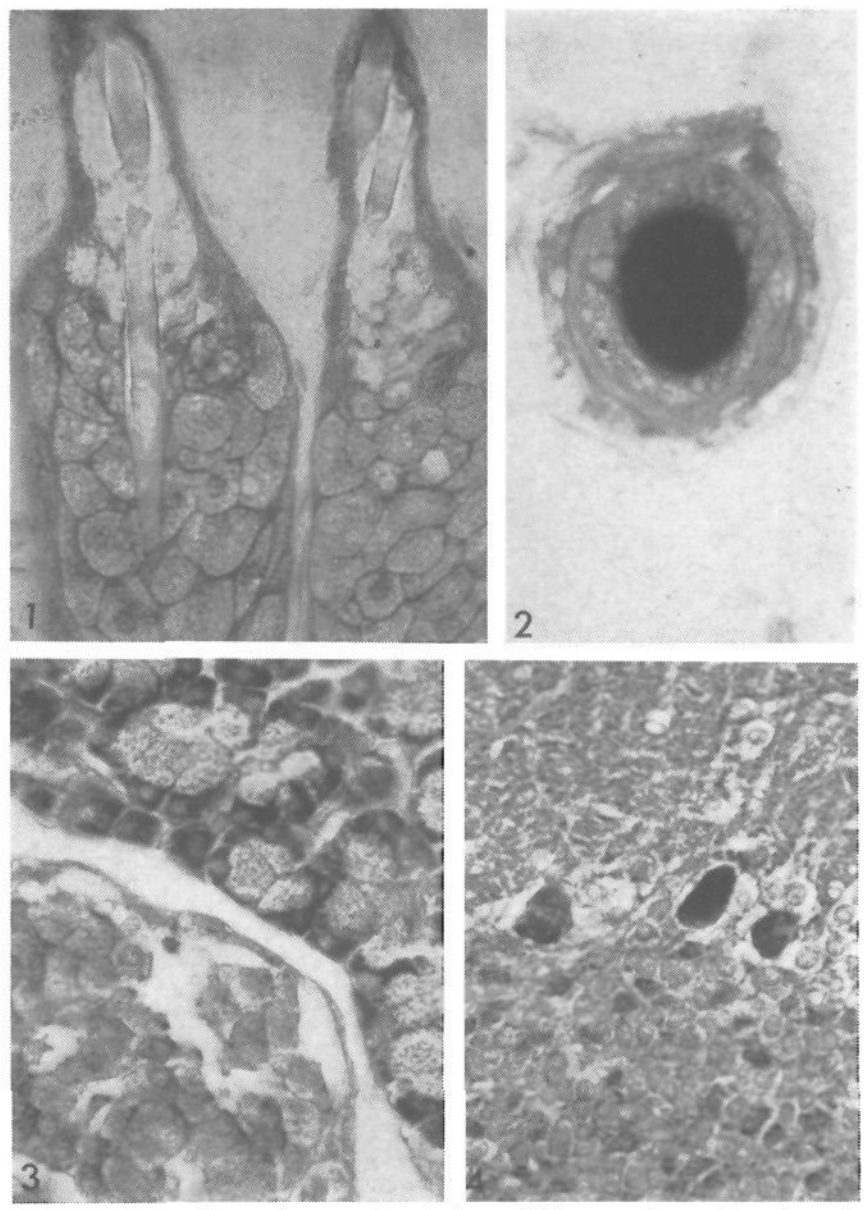

Figure 1.—Rat tissues fixed in trichloracetic acid–ethanol, embedded in paraffin, cut 6 μ thick, and stained for protein-bound sulfhydryl groups with 4-iodoacetamido-1-naphthol and fast blue BBN. 1: The epithelial cells of the sebaceous glands stain prominently. 2: At a deeper level in the dermis, in the region of horny transformation, the hair shaft is stained. 3: The basal and perinuclear region of the pancreatic acinar cells (top) is prominently stained as compared to the very faint stain of cells of the islets of Langerhans (bottom). 4: Purkinje cells of the cerebellum are more prominently stained than cells of the molecular layer (top) and granular layer (bottom).

Experimental Section[6]

4-Chloroacetamido-1-naphthol (II).—4-Amino-1-naphthol hydrochloride (4.9 g) was dissolved in a mixture of 50 ml of glacial acetic acid and 15 ml of dry dioxane, by warming slightly on a steam cone. Then 3.0 g of sodium acetate and chloroacetyl chloride were added in a rapid stream to this solution and the reaction mixture was stirred for 0.5 hr. The excess reagents were hydrolyzed by pouring into 350 ml of ice water with good stirring. The dark purple precipitate was collected, air dried, and recrystallized from ethyl acetate to yield 2.0 g (34%) of shiny purplish small needles, mp 195° dec. Further recrystallization from 95% alcohol and from aqueous methanol gave a sample of mp 196° dec.

Anal. Calcd for $C_{12}H_{12}ClNO_2$: C, 61.2; H, 4.3; N, 5.9. Found: C, 61.1; H, 4.2; N, 5.8.

It is only slightly soluble in $CHCl_3$ and ligroin, gives a positive Beilstein test, and couples readily with diazonium salt in a $NaHCO_3$ suspension.

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(1) This investigation was supported by U. S. Public Health Service Research Grant CA-07339 and CA-02478.

(2) Tyrosine in protein does not form a blue dye with this diazonium salt.

(3) W. A. Jacobs, M. Heidelberger, and I. P. Rolf, *J. Am. Chem. Soc.*, **41**, 458 (1919).

(4) R. J. Barrnett, K. C. Tsou, and A. M. Seligman, *J. Histochem. Cytochem.*, **3**, 406 (1955).

(5) L. W. Roberts, *Science*, **124**, 628 (1956).

(6) All melting points are corrected; analyses by Dr. C. K. Fitz, Needham Heights, Needham, Mass. Acknowledgment for histochemical technical assistance is due Mrs. Hannah L. Wasserkrug.

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: mp 161–162; λ^{CHCl_3} 2.96, 5.67, 5.92, and 6.2 μ.

Anal. Calcd for $C_{14}H_{12}ClNO_3$: 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 $C_{12}H_{10}INO_2$: 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 $C_{14}H_{12}INO_3$: 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 earlier[7] for another chromogenic reagent. Tissues were fixed for 24 hr in 1% trichloracetic 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. Barrnett 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

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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, *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]

(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).

Experimental Section[4]

***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: mp[6] 295–300°; infrared absorption (KBr) at 1528, 1381, and 1325 cm^{-1}, and the less active isomer, β: mp[6] 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); mp[7] 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, $>CHCH_2NH-$), and between 100–160 cps (10 H multiplet); after an active hydrogen exchange, at 196 cps (2 H doublet, J = 8 cps, $>CHCH_2N<$), and between 100–160 cps (10 H multiplet).

Anal. Calcd for $C_8H_{13}NO$: C, 67.03; H, 9.41; N, 10.07. Found: C, 67.27; H, 9.13; N, 10.41.

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(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 inihibition 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

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Reactions of 5-formyl-8-quinolinol[1] with N-methylated heterocycles containing an active methyl group readily produce com-

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