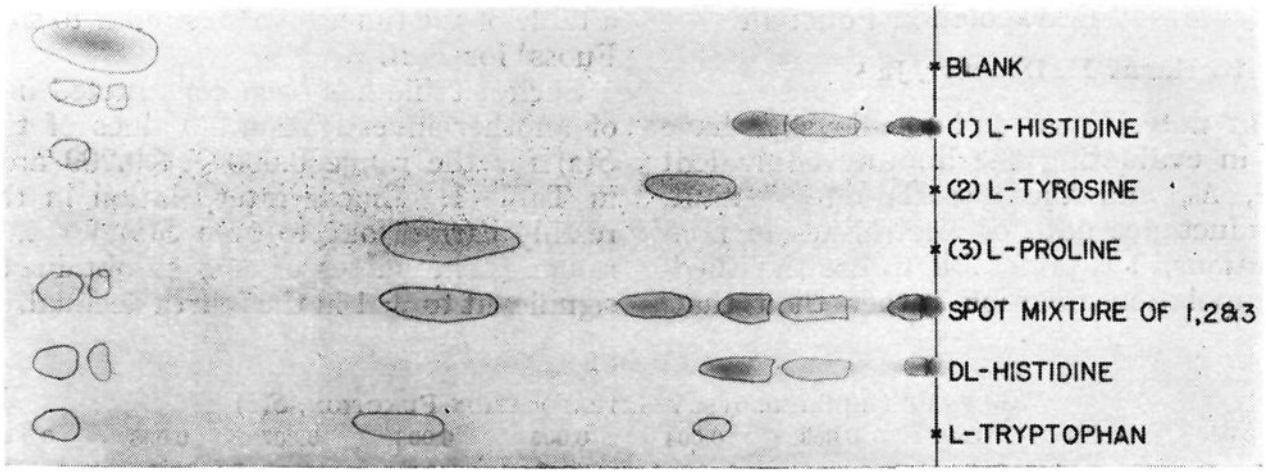

Fig. 1.—Chromatogram of the azo dyes of L-histidine, L-tyrosine, L-proline, DL-histidine and L-tryptophan; irrigated with *s*-butanol–H_2O for 40 hr. at 13°.

amino-acids overlap or lie sufficiently close to histidine that the identification of histidine is not always possible. The identification of tyrosine with this method was difficult because phenylalanine and tryptophan gave color reactions similar to tyrosine and lay near enough to tyrosine on a one-dimensional chromatogram that the identity of the spots was questionable.

The present method affords a more positive identification of histidine and tyrosine because the azo dyes from histidine could be resolved into a distinct pattern of three different colored spots while the azo dye from tyrosine gave a colored spot with an R_f value greater than any of the spots of histidine.

It has been found that a satisfactory chromatographic separation of histidine and tyrosine can be made by modifying the method described by Hossfeld for the partition chromatography of simple phenols.[3] Histidine and tyrosine were coupled with diazotized *p*-nitroaniline[4] in the presence of 4% sodium carbonate. The resulting azo dyes were spotted on Whatman No. 1 filter paper and developed by descending irrigation with secondary butanol (1 vol.)–water (1 vol.). After drying at room temperature, the chromatogram was sprayed with 4% sodium carbonate to intensify the color of the spots (Fig. 1). The azo dyes from L-histidine gave a pattern of three-colored spots: purple, yellow-orange and orange-red in the order of their increasing R_f values. A brown spot remained at the origin. L-Tyrosine gave a purple spot having an R_f value greater than that for the orange-red spot of L-histidine.

If an excess of diazotized *p*-nitroaniline was used in the coupling procedure, a red spot appeared near the solvent front, usually followed by a small yellow spot. The ultraviolet absorption curve of an absolute methanol extract of these colored areas was similar to the absorption curve obtained for the red spot from the blank (Fig. 1).

The possibility of separating the isomeric forms of a racemic mixture of histidine was investigated (Fig. 1); however, the pattern of the azo dyes from DL-histidine was similar to that from L-histidine.

As a control measure, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, proline, hydroxyproline, valine, leucine, phenylalanine, tryptophan, arginine, lysine, methionine, cystine, histamine and glucosamine were treated with diazotized *p*-nitroaniline under the above conditions and the resulting colored products chromatographed. Proline, hydroxyproline, tryptophan and arginine yielded derivatives which when chromatographed gave yellow spots. The spot due to the azo dye of hydroxyproline followed that of proline and the two dyes when spotted together on a filter paper strip were easily separated. The derivatives of histamine gave two spots: one was purple having approximately the same R_f value as the yellow-orange spot of histidine, the other was orange-red corresponding in position to an area between proline and tyrosine. The spots resulting from the arginine, tryptophan, proline, hydroxyproline and histamine derivatives did not interfere with the identification of histidine and tyrosine.

Resolution of the azo dyes of L-histidine, L-tyrosine and L-proline was effected from superimposed spots of a mixture of the dyes at one location on the paper (Fig. 1).

Experimental

Preparation of the Azo Dyes.—Diazotized *p*-nitroaniline was prepared according to the method of Smith and Irwin[4] and coupled to the amino-acids in a 4% sodium carbonate solution using 0.01 molar quantities of the reactants. The resulting reaction mixtures were spotted directly by means of a small nichrome wire loop at the appropriate position on the paper strip (22 × 56 cm. Whatman No. 1 filter paper).

Development of the chromatograms was accomplished using the method described by Hossfeld[3] except that the chromatograms were sprayed with 4% sodium carbonate after development instead of before. Trailing of the spots was decreased by developing the chromatograms at 13°, controlling the temperature (±0.5°).

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RECEIVED MARCH 1, 1951

(3) R. L. Hossfeld, THIS JOURNAL, **73**, 852 (1951).
(4) L. I. Smith and W. B. Irwin, *ibid.*, **63**, 1036 (1941).

The Preparation of *trans*-4-Chlorocyclohexanol

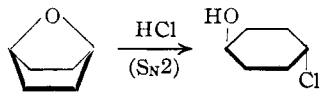
BY EDWARD A. FEHNEL, SETHA GOODYEAR AND JOAN BERKOWITZ

The action of concentrated hydrochloric acid on 1,4-cyclohexanediol is reported to yield an oil which has been characterized as a mixture of *cis*- and *trans*-4-chlorocyclohexanol.[1,2] The separation

(1) L. N. Owen and P. A. Robins, *J. Chem. Soc.*, 320 (1949).
(2) L. Palfray and B. Rothstein, *Compt. rend.*, **189**, 701 (1929).

of these isomers has not been accomplished, but the phenylurethans of both forms have been described (m.p. 133–134°[1] and 99°[2]), the higher-melting derivative being regarded as the *trans*-compound. Attempts to prepare a stereochemically pure form of 4-chlorocyclohexanol by treatment of the pure *cis* or *trans* forms of the diol with hydrochloric acid have also led to mixtures from which neither isomer has been isolated in a pure condition.[2]

In view of the inversion which is generally observed to accompany the nucleophilic displacement reactions of epoxides,[3] it would be expected that the action of hydrogen chloride on the *cis*-ether, 1,4-epoxycyclohexane, should lead exclusively to *trans*-4-chlorocyclohexanol. When the addition of hydrogen chloride to this epoxide was carried out under conditions similar to those described for the preparation of tetramethylene chlorohydrin from tetrahydrofuran,[4] a waxy crystalline solid was obtained which melted at 82–83° after several recrystallizations from cyclohexane. This product gives a phenylurethan which melts at 132–133° and which is presumably identical with the phenylurethan of *trans*-4-chlorocyclohexanol described by Owen and Robins.[1] The α-naphthylurethan was also prepared to further characterize the compound.



Experimental[5]

1,4-Epoxycyclohexane.[6]—A mixture of 200 g. of hydroquinone and 200 ml. of methanol was hydrogenated over 20 g. of Raney nickel at 150° and 120 atmospheres until no more hydrogen was absorbed. Two hundred grams of freshly roasted activated alumina was added to the crude mixture of *cis*- and *trans*-1,4-cyclohexanediol which remained after evaporation of the methanol, and the mixture was heated at 240° under a 30-cm. Vigreux column for 5 hours. The distillate (b.p. 120–133°) thus obtained consisted of two layers which were separated and the lower aqueous layer was extracted several times with ether. The combined oil layer and ether extracts were dried over anhydrous magnesium sulfate and distilled through a 60-cm. Fenske column to give as the main fraction 87.0 g. (49% over-all yield) of the epoxide, b.p. 117–118° (uncor.).

***trans*-4-Chlorocyclohexanol.**—The epoxide (45.7 g.) was heated to the boiling point in a three-necked flask fitted with a reflux condenser, a thermometer dipping into the liquid, and a capillary tube through which a slow stream of gaseous hydrogen chloride was admitted near the bottom of the flask. The temperature rose gradually to *ca.* 150° over a period of 2 hours, after which the reaction was discontinued. On cooling, the contents of the flask crystallized to a greasy solid, which was taken up in hot benzene and recrystallized by the addition of petroleum ether; yield 42.8 g. (68%) of waxy crystals melting at 68–72°. After several recrystallizations from cyclohexane, the pure product was obtained in the form of colorless leaflets, m.p. 82–83°; b.p. 105° at 14 mm., 99° at 10 mm.

Anal. Calcd. for $C_6H_{11}ClO$: C, 53.54; H, 8.24. Found: C, 53.27; H, 8.14.

The **phenylurethan**, prepared in the usual way by heating the alcohol with phenyl isocyanate, melted at 132–133° after recrystallization from cyclohexane (lit.,[1] 133–134°).

The **α-naphthylurethan** was prepared by heating the alcohol with an equal weight of α-naphthyl isocyanate for 5 minutes and recrystallizing the solid product several times from cyclohexane. The pure derivative was obtained in the form of colorless needles melting at 157–158°.

Anal. Calcd. for $C_{17}H_{18}ClNO_2$: C, 67.18; H, 5.97. Found: C, 67.45; H, 5.85.

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RECEIVED JUNE 20, 1951

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(4) D. Starr and R. M. Hixon, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N Y., 1943, p. 571.

(5) Microanalyses were performed by the Clark Microanalytical Laboratory, Urbana, Illinois.

(6) *Cf.* R. C. Olberg, H. Pines and V. N. Ipatieff, THIS JOURNAL, **66**, 1096 (1944).

Preparation of an Intermediate for Synthesis of Lysine: ε-Bromocaproic Acid[1]

BY SIDNEY W. FOX, ERNEST H. POLAK, MILON W. BULLOCK AND YUTAKA KOBAYASHI

Successful conversion of cyclopentanone to δ-bromopentanoic acid[2] through an oxidation step with hydrogen peroxide has led to interest in the corresponding conversion of cyclohexanone to ε-bromocaproic acid in this Laboratory and in that of Heine and Jones.[3] In the present project, the ultimate goal has been the synthesis of lysine.

Experiences with this oxidation confirm the easy explosions recorded by Heine and Jones. The explosion problem has been overcome here by destroying the peroxides in the acidified aqueous solution of the lactone by addition of sufficient sodium sulfite to obtain a negative starch–iodide test, or by destroying the diethyl peroxides in the ether extracts by drying the ether solution over a mixture of sodium sulfate and sodium sulfite. As presented in the Experimental, the yields of bromoacid have been superior to those earlier recorded.

Attempts to isolate the lactone by distillation have been unfruitful. The product obtained was a mixture which distilled over a large range, and left a large residue. Apparently the lactone is polymeric and not readily purified by distillation. Oxidation of cyclohexanone with Caro's acid also has been shown to yield a product that cannot be purified by distillation,[4,5] although dehydration of ε-hydroxycaproic acid obtained in the production of hexamethylene glycol has given a distillable lactone.[6]

The ε-bromocaproic acid, directly prepared from the crude oxidation product, was converted to the α,ε-dibromocaproic acid by classical α-bromination as described by Merchant, Wickert and Marvel.[7] Although satisfactory aminations of α,ε-dibromo acid have not been carried out in these laboratories, conditions for this conversion have been reported.[8,9]

Experimental

ε-Bromocaproic Acid.—To 440 ml. of 20% sodium hydroxide solution in a three-liter flask equipped with an effi-

(1) Journal Paper No. J-1941 of the Iowa Agricultural Experiment Station, Ames, Project 1110, Preparation of Chemicals for Agricultural Utility. This work was also supported by the Industrial Science Research Institute of Iowa State College.

(2) M. Fling, F. N. Minard and S. W. Fox, THIS JOURNAL, **69**, 2466 (1947).

(3) H. W. Heine and H. Jones, *ibid.*, **73**, 1361 (1951).

(4) R. Robinson and L. H. Smith, *J. Chem. Soc.*, 371 (1937).

(5) R. P. Linstead and H. N. Rydon, *ibid.*, 1995 (1934).

(6) F. J. Van Natta, J. W. Hill and W. H. Carothers, THIS JOURNAL, **56**, 455 (1934).

(7) R. Merchant, J. N. Wickert and C. S. Marvel, *ibid.*, **49**, 1828 (1927).

(8) D. C. Sayles and E. F. Degering, *ibid.*, **71**, 3161 (1949).

(9) E. F. Degering and L. G. Boatright, *ibid.*, **72**, 5137 (1950).