was heated at 85° under N_2 for 3 min and filtered through Celite under N_2. The filtrate was diluted with H_2O (200 ml) and refrigerated. The resulting orange crystalline precipitate was collected by filtration, washed successively with cold H_2O–DMAC (2:1) and H_2O, and dried at 65° *in vacuo* (P_2O_5): yield 5.19 g (60%); mp ~161° dec (Mettler FP1 apparatus); λ_{max} nm ($\epsilon \times 10^{-3}$), pH 7, 244 (29.2), 312 (30.5). *Anal.* ($C_{20}H_{23}N_5O_5$) C, H, N.

Ethyl 3-[(*p*-Methoxycarbonyl-*N*-methylanilino)methyl]-5(6*H*)-oxopyrido[3,4-*b*]pyrazine-7-carbamate (5). A stirred suspension of finely powdered **4** (100 mg, 0.242 mmol) in H_2O (10 ml), EtOH (1 ml), and 1 *N* NaOH (0.32 ml, 0.32 mmol) was treated dropwise with a 0.27% solution of $KMnO_4$ (9.44 ml, 0.161 mmol) in H_2O. After filtration, the filtrate was neutralized with 1 *N* HCl. The resulting yellow precipitate was collected by centrifugation, washed with H_2O, and dried at 65° *in vacuo* (P_2O_5): yield 81 mg (81%); mp ~225° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 7, 253 (17.4), 313 (39.9), 375 (5.25). *Anal.* ($C_{20}H_{21}N_5O_5$) C, H, N.

***p*-[[(7-Amino-5(6*H*)-oxopyrido[3,4-*b*]pyrazin-3-yl)methyl]-methylamino]benzoic Acid (6).** A stirred solution of **5** (2.23 g, 5.43 mmol) and KOH (11.2 g) in EtOH (200 ml) was refluxed under N_2 for 16 hr and evaporated to dryness *in vacuo*. A solution of the residue in H_2O (100 ml) was filtered and acidified with 6 *N* HCl to pH 3.5. The precipitate of **6**, containing partially hydrolyzed **5**, was retreated with KOH in EtOH as described above to give pure **6** as an orange precipitate. This solid was collected by filtration, washed with water, and dried at 100° *in vacuo* (P_2O_5): yield 1.61 g (91%); melting point indefinite, turned black at ~220°; λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 7, 252 (17.1), 316 (28.7), 417 (3.57); 0.1 *N* NaOH, 273 (30.0), 433 (4.32); ν_{max}, 1673, 1660, 1650 (C=O and NH_2), 1604, 1564, 1512 cm^{-1} (C=C, C=N); pmr δ 3.21 (3, NCH_3), 4.77 (2, NCH_2), 5.59 (1, 8-CH), 6.22 (2, NH_2), 6.82, 7.76 (m, 4, C_6H_4), 8.42 (1, 2-CH), ~10 (br, CO_2H, NH). *Anal.* ($C_{16}H_{15}N_5O_3$) C, H, N.

***p*-[[(7-Acetamino-5(6*H*)-oxopyrido[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoic Acid Hydrochloride (2:1) (7).** A solution of **6** (1.27 g, 3.91 mmol) in Ac_2O (127 ml) was refluxed under N_2 for 1 hr and evaporated to dryness *in vacuo*. The residual gum was stirred with H_2O (50 ml) for 2 days until a homogeneous powder formed. The resulting suspension was treated with concentrated NH_4OH (3 ml) to give a solution, which was immediately filtered and acidified to pH 3 with 6 *N* HCl. The brown precipitate was collected by centrifugation, washed with H_2O (pH 3), and dried *in vacuo* (P_2O_5): yield 1.39 g (92%); melting point indefinite; λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 7, 256 (19.0), 300 (25.8). *Anal.* ($C_{18}H_{17}N_5O_4 \cdot 0.5HCl$) C, H, N.

Diethyl *N*-[*p*-[[(7-Acetamido-5(6*H*)-oxopyrido[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoyl]-L-glutamate Hydrate (5:4) (8). A suspension of **7** (1.01 g, 2.62 mmol) and *N,N'*-dicyclohexylcarbodiimide (540 mg, 2.62 mmol) in anhydrous pyridine (50 ml) was treated with diethyl-L-glutamiate hydrochloride (628 mg, 2.62 mmol), stirred at 25° for 44 hr, filtered to remove *N,N'*-dicyclohexylurea, and evaporated to dryness *in vacuo*. A solution of the residue in $CHCl_3$ (20 ml) was filtered and washed successively with 0.3 *N* HCl (2 × 20 ml), H_2O (10 ml), saturated $NaHCO_3$ solution (10 ml), and H_2O (2 × 10 ml). The resulting solution was dried over $MgSO_4$ and evaporated *in vacuo* to give a brown foam (1.05 g). A solution of the foam in $CHCl_3$ (5 ml) was adsorbed on a short column (28 mm diameter) containing 30 g of silica gel[‡] equilibrated with $CHCl_3$. The column was developed with $CHCl_3$ (50 ml) followed by 98:2 $CHCl_3$–MeOH (50 ml) and 96:4 $CHCl_3$–MeOH. The major yellow fraction was evaporated *in vacuo* to give 524 mg (35%) of yellow product: melting point indefinite with softening at ~97°; λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 7, 254 (16.2), 311 (32.7), 374 (br, 4.85). *Anal.* ($C_{27}H_{32}N_6O_7 \cdot 0.8H_2O$) C, H, N.

***N*-[*p*-[[(7-Amino-5,6-dihydro-5-oxopyrido[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoyl]-L-glutamic Acid Hydrate (9).** A stirred solution of **8** (500 mg, 0.883 mmol) in EtOH (20 ml) and 1 *N* NaOH (8.50 ml, 8.50 mmol) was refluxed for 1 hr under N_2 and evaporated to dryness *in vacuo*. A solution of the residue in H_2O (12 ml) was filtered and acidified with 1 *N* HCl to pH 3. The red precipitate was collected, washed with H_2O at pH 3, and dried *in vacuo* (P_2O_5) at 100°: yield 340 mg (82%); melting point indefinite, darkens above 188°; λ_{max}, nm ($\epsilon \times 10^{-3}$), 0.1 *N* HCl, 262 (16.2), 329 (30.2), 495 (br, 4.81); pH 7, 225 (18.4), 255 (sh, 16.8), 318 (36.0), 413 (br, 3.73); 0.1 *N* NaOH, 272 (28.1), 309 (28.3), 432 (br, 3.90); ν_{max} 1710 (sh), 1640 (C=O and NH_2), 1598, 1565, 1500 cm^{-1} (C=C, C=N); pmr δ 1.8–2.5 (m, 4, CH_2CH_2), 3.19 (3, NCH_3), 4.36 (m, 1, NCH), 4.74 (2, NCH_2), 5.56 (8-CH), 6.18 (2, NH_2), 6.81, 7.75 (m, 4, C_6H_5), 8.18 (d, 1, NHCO), 8.40 (1, 2-CH), ~11 (br, CO_2H, NH). *Anal.* ($C_{21}H_{22}N_6O_6 \cdot H_2O$) C, H, N.

***N*-[*p*-[[(7-Amino-1,2,5,6-tetrahydro-5-oxopyrido[3,4-*b*]pyrazin-3-yl)methyl]methylamino]benzoyl]-L-glutamic Acid (10).** A solution of **9** (24 mg, 0.06 mmol) in 1 *N* NaOH (8 ml) was hydrogenated for 80 min at 25° in the presence of 5% palladium on charcoal (25 mg) to absorb 1.2 ml (0.05 mmol) of hydrogen. An aliquot portion (1 ml) of the filtered solution was treated with 1 *M* 2-mercaptoethanol (2.5 ml) and 1 *N* HCl (0.9 ml) for ultraviolet spectral determination: λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 7, 309 (~24). The remaining solution of **10** was treated with ascorbic acid (70 mg) and tested in the KB cell culture screen.

Acknowledgment. This investigation was supported by Contract NIH-71-2021 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health. The authors are indebted to the members of the Sections of Southern Research Institute directed by Dr. W. R. Laster, Jr., for L1210 leukemia testing, to Dr. R. F. Pittillo for bacteriological testing, to Dr. L. J. Wilkoff for cytotoxicity testing, to Miss Suzanne Straight for enzymological testing, and to Dr. W. C. Coburn, Jr., for microanalytical and spectral determinations.

References

(1) J. A. Montgomery, R. D. Elliott, S. L. Straight, and C. Temple, Jr., *Ann. N. Y. Acad. Sci.*, **186**, 227 (1971).
(2) R. D. Elliott, C. Temple, Jr., J. L. Frye, and J. A. Montgomery, *J. Org. Chem.*, **36**, 2818 (1971).
(3) B. S. Gorton, J. M. Ravel, and W. Shive, *J. Biol. Chem.*, **231**, 331 (1958).
(4) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **35**, 1676 (1970).
(5) W. R. Boon and T. Leigh, *J. Chem. Soc.*, 1497 (1951).
(6) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **31**, 1890 (1966).
(7) B. R. Baker, B. T. Ho, and T. Neilson, *J. Heterocycl. Chem.*, **1**, 88 (1964).

‡SilicAR TLC-7 (fines removed), Mallinckrodt Chemical Works.

Ethambutol. Synthesis of an Unsaturated Analog

Luigi Bernardi,* Maurizio Foglio, and Aldemio Temperilli

Istituto Ricerche Farmitalia, Milano, Italy.
Received October 23, 1973

We have recently[1] reported a new synthesis of ethambutol (**5**) based on the key intermediate **1**, easily obtainable in high yield by the reaction of phosgene on 3,4-epoxy-1-butene. The same intermediate has now been employed for the synthesis of (+)-2,2'-(ethylenediimino)di-3-buten-1-ol (**4b**), an unsaturated analog of ethambutol. Although the biological activity of this compound has been found inferior to that of the parent compound, its potential use in the preparation of tritium-labeled ethambutol warrants in our opinion the report of the details of its synthesis.†

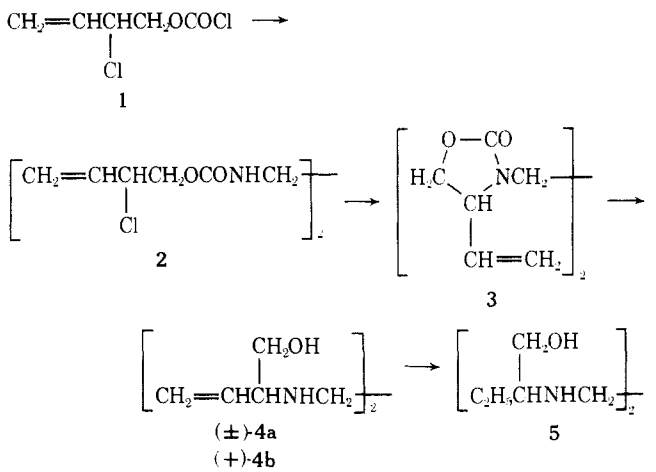
2-Chloro-3-buten-1-yl chloroformate (**1**) was condensed in chloroform solution with ethylenediamine to give the symmetric bisurethane **2** that, by treatment with KOH, yielded a 50:50 mixture of meso and DL forms of 3,3'-ethylene-di(4-vinyl-2-oxazolidone) (**3**). Separation of the two forms was achieved by fractional crystallization from cyclohexylamine, in which the meso form is less soluble, and from pyridine, in which on the contrary the DL form is less soluble.

Alkaline hydrolysis of **3** (DL form) gave (±)-2,2'-(ethy-

†We are indebted to Dr. A. Sanfilippo of these laboratories for the biological data.

lenediimino)di-3-buten-1-ol (**4a**) which was resolved by fractional crystallization of its neutral (−)-malate. The less soluble salt yielded the (+) base **4b** which was eventually reduced to a tetrahydro derivative **5**, identical with ethambutol (Scheme I).

Scheme I



The ED_{50} of **4b** was four times that of ethambutol (200 and 50 mg/kg/day, respectively, administered by sc route for 15 days) against a uniformly fatal (21-25 days) *Mycobacterium tuberculosis* H37Rv intravenous infection in mice based on survival of the mice.[2] The concentration of **4b** required to give 100% inhibition on *Mycobacterium tuberculosis* H37Rv in Dubos albumin medium (Difco) after 14 days at 37° was four times that of ethambutol (25 and 6.25 μg/ml, respectively).

Experimental Section

Di-2-chloro-3-buten-1-yl Ethylenedicarbamate (2). To a solution of 1 (200 g, 1.19 mol) in $CHCl_3$ (2000 ml), 74 g (1.22 mol) of ethylenediamine was added under stirring at 0°. After 2 hr the suspension was filtered and the filtrate concentrated *in vacuo*; the residue was taken up in a small amount of ethyl ether and filtered to give 164 g (85%) of **2**, mp 92-94°. *Anal.* ($C_{12}H_{18}Cl_2N_2O_4$) C, H.

3,3′-Ethylenedi(4-vinyl-2-oxazolidone) (3). To a solution of **2** (200 g, 0.61 mol) in EtOH (800 ml), 96 g of 85% KOH in EtOH (600 ml) was added at 40°. The solution was refluxed for 5 min, then concentrated and extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated *in vacuo*; the residue was taken up in a small amount of EtOAc and filtered to give 110 g (71%) of a 50:50 mixture of DL- and *meso*-3,3′-ethylenedi(4-vinyl-2-oxazolidone), mp 94-96°. The mixture was dissolved in cyclohexylamine (1100 ml); the solution was left at room temperature for 4 hr and then filtered to give 61 g of a solid A. The mother liquors were evaporated to dryness and the residue was crystallized from 50 ml of pyridine. After standing 2 hr at room temperature, the solution was filtered; 21.5 g of DL-3,3′-ethylenedi(4-vinyl-2-oxazolidone) (96% pure by gc‡), mp 114-116° was collected. *Anal.* ($C_{12}H_{16}N_2O_4$) C, H.

Solid A was repeatedly crystallized from morpholine to give 15 g of *meso*-3,3′-ethylenedi(4-vinyl-2-oxazolidone), mp 96-98°. *Anal.* ($C_{12}H_{16}N_2O_4$) C, H.

(±)-2,2′-(Ethylenediimino)di-3-buten-1-ol (4a). A solution of **3** (20 g, 0.08 mol) in EtOH (75 ml) containing 25 g of NaOH was refluxed for 3 hr, then neutralized with HCl, and evaporated to dryness. The residue was taken up in MeOH (100 ml), MeONa was added until basic to thymolphthalein, and again the suspension was evaporated to dryness. The residue was taken up in boiling tetrahydrofuran and filtered. The solution was concentrated to a volume of 30 ml; on cooling 12 g (75%) of **4a**, mp 106-108° (dihydrochloride, mp 186-188°), separated. *Anal.* ($C_{10}H_{20}N_2O_2$) C, H.

‡The separation was monitored by gc on glass columns (2 m), packed with Apiezon L or SE-30 (10%) on Chromosorb, at 170°. In these conditions the DL form shows the lowest retention time.

(+)-2,2′-(Ethylenediimino)di-3-buten-1-ol (4b). To a warm solution of (−)-malic acid (8.04 g, 0.06 mol) in EtOH (240 ml), **4a** (12 g, 0.06 mol) was added; the solid that separated on cooling was crystallized once from EtOH (50 ml). The neutral salt (7.4 g) was collected: mp 137-139°; $[\alpha]^{20}D$ +20° (*c* 5, H_2O). *Anal.* ($C_{10}H_{20}N_2O_2 \cdot C_4H_6O_5$) C, H. The salt was dissolved in H_2O and the solution was passed through an ion-exchange column containing Amberlite-IRA 400 (OH^- cycle). After evaporation of the solvent to dryness, **4b** (4.3 g, 70%), mp 117-119°, $[\alpha]^{20}D$ +5.5° (*c* 5, H_2O), was collected. The dihydrochloride had mp 178-180°, $[\alpha]^{20}D$ +32.6° (*c* 5, H_2O). *Anal.* ($C_{10}H_{20}N_2O_2 \cdot 2HCl$) C, H, Cl.

(+)-2,2′-(Ethylenediimino)di-1-butanol (Ethambutol) (5). A solution of **4b**·2HCl (2 g, 0.073 mol) in 90% MeOH (30 ml) was hydrogenated in the presence of 10% Pd/C (0.05 g). The solvent was evaporated and the residue crystallized from EtOH to give 1.9 g (94%) of a solid identical with ethambutol (melting point, ir, nmr, and optical rotation).

References

(1) L. Bernardi, M. Foglio, P. Giardino, and A. Temperilli, *Chim. Ind. (Milan)*, in press.
(2) D. McKenzie, B. S. Malone, S. Kushner, J. J. Oleson, and J. SubbaRow, *J. Lab. Clin. Med.*, **33**, 1249 (1948).

Synthesis of Pure *p*-Chlorophenyl-L-alanine from L-Phenylalanine

Richard A. Houghten and Henry Rapoport*

Department of Chemistry and Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720.
Received November 15, 1973

It has long been known that *p*-chlorophenylalanine (IV) inhibits serotonin (5-hydroxytryptamine) formation in the tissues of laboratory animals.[1] This fact has lead to a large number of publications on the effects of this inhibition, a few of which include altered sleep patterns in cats,[2] variations in learning in rats,[3] changes in social behavior in rats,[4] and alteration of normal sexual patterns in rats, cats, and rabbits.[5] The resolution of this physiologically important compound did not appear until 1971,[6] and the method used gave poor yields of the optical isomers of IV. We sought a simple, direct method of preparation using L-phenylalanine as the optically active starting material, thus avoiding a resolution step, and we now present the details of such a method.

A simple means for obtaining IV, which appeared quite attractive, was the direct chlorination of L-phenylalanine (I) with molecular chlorine in acidic medium, the acid functioning as an amine protecting group. The difficulties encounted with this method were the production of other isomers (ortho, meta, and dichlorinated) and the complete separation of these isomers from the desired product (IV). The isomeric composition varied little with the solvent or the ratio of chlorine to phenylalanine employed (Table I). The components of the isomer mixture were identified through amino acid analysis by comparison with authentic samples prepared by the method of Burchalter and Stevens[7] (Table II). All attempts to separate pure IV in practical yield from the mixture, using cation exchange resins with aqueous acid as eluent, failed. A recent procedure,[8] which has been used to separate isomeric bromophenylalanines on Sephadex, was considered and rejected because the separation and yields were poor for the bromophenylalanines, and the differences between isomeric chlorophenylalanines are smaller than for the corresponding bromophenylalanines.

Although the preparation of IV by direct chlorination of phenylalanine was abandoned as impractical, the use of L-phenylalanine as starting material was still attractive if