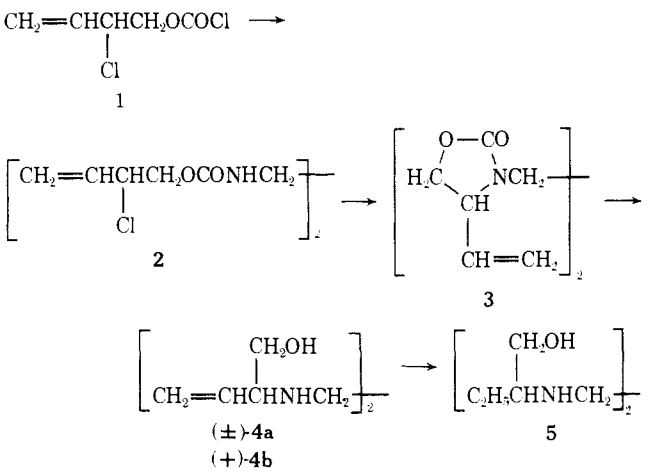
lenediimino)di-3-buten-1-ol (**4a**) which was resolved by fractional crystallization of its neutral (−)-malate. The less soluble salt yielded the (+) base **4b** which was eventually reduced to a tetrahydro derivative **5**, identical with ethambutol (Scheme I).

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).

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

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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

Table I. Isomer Distribution in the Chlorination and Bromination of L-Phenylalanine[a]

Cl_2/phe	Solvent	Temp, °C	Product phe	ortho	meta	para	2,5	3,4	2,3	Remarks
1.00	1 *N* HCl	0								Only dec products and phe
1.00	1 *N* HCl	20								Only dec products and phe
1.00	CH_3CN	20	40.6	32.5	3.3	23.7	0.0	1.0	0.0	After 1 week
0.50	96% H_2SO_4	0	51.0	27.9	4.4	17.6	0.0	0.0	0.0	Cl_2 bubbled directly into H_2SO_4
0.80	6 *N* HCl	0	22.4	44.3	3.1	28.0	0.7	1.5	0.0	Complete in 4 hr
0.80	6 *N* HCl	−12	23.2	43.5	3.0	23.2	0.0	7.1	0.0	Complete in 24 hr
1.00	6 *N* HCl	20	3.7	53.5	3.3	32.8	4.4	2.2	Trace	Complete in 0.5 hr
1.04	6 *N* HCl	0	5.8	48.0	2.9	30.5	3.2	9.5	Trace	Complete in 4 hr
1.95	6 *N* HCl	20	0.0	5.0	0.0	10.8	37.9	8.3	9.8	Only 72% of amino acid accounted for
1.00 Br_2	1 *N* HBr	60	27.3	24.7	5.0	41.3	1.5	0.0	0.0	Duration, 5 hr
7.60 Br_2	None	20	20.7	32.2	3.5	36.8	4.5	2.2	0.2	Reference 8

[a]Determined with the Beckman Model 120C amino acid analyzer, pH 5.26, 0.35 *N* citrate buffer.

Table II. Retention Times for Substituted Phenylalanines on Beckman Model 120C Amino Acid Analyzer, pH 5.26, 0.35 *N* Citrate Buffer

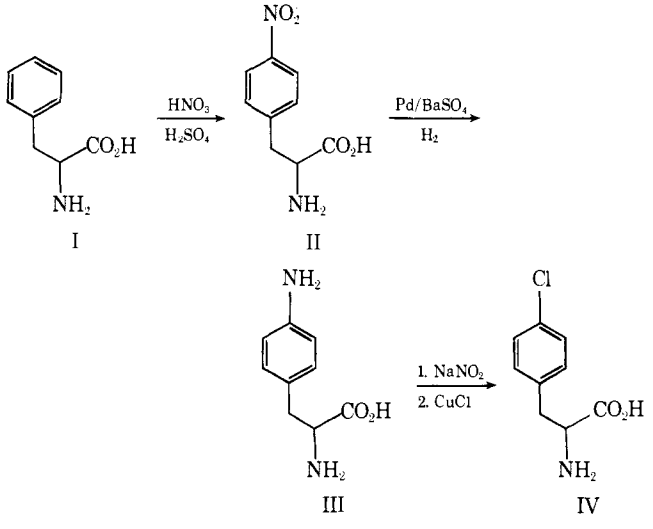
Substituted phenylalanine	Retention time, min	Substituted phenylalanine	Retention time, min
Phenylalanine	55	3,5-Dichloro	192
o-Chloro	83	*o*-Nitro	63
m-Chloro	98	*m*-Nitro	75
p-Chloro	120	*p*-Nitro	96
2,3-Dichloro	177	*o*-Bromo	103
2,4-Dichloro	187	*m*-Bromo	126
2,5-Dichloro	129	*p*-Bromo	156
2,6-Dichloro	109	*p*-Amino	68
3,4-Dichloro	248		

Table III. Isomer Distribution in the Nitration of L-Phenylalanine

Expt	Nitration conditions[a] Time of addn, min	Concn of HNO_3, %	Temp, °C	Ortho	Meta	Para
1	5	100	0	23	26	51
2	20	100	0	20	22	58
3	20	70	0	21	22	57
4	100	100	0	19	21	60
5	50	100	−25 to −12	16	21	63

[a]Vigorously mechanically stirred; 100% HNO_3, *d* 1.51, was prepared by distillation of fuming HNO_3; isomer ratios were determined by automatic amino acid analysis.

an adequate separation for the anticipated isomers could be found. This was realized by nitration where the pure para isomer could be easily obtained. Reduction, selective diazotization, and displacement by chloride then gave pure *p*-chlorophenyl-L-alanine (IV).



The usual method for preparing optically active *p*-nitrophenyl-L-alanine (II) follows the original procedure by Erlenmeyer and Lipp[9] as improved by Bergel and Stock.[10] Contrary to the 80–90% yields of II reported by these authors, we found a mixture of approximately 20% ortho, 25% meta, and 55% *p*-nitrophenyl-L-alanine. Variation in the reaction conditions brought about only minor changes in the isomer ratio (Table III). Pure (99.9+%) II (as the hydrate) can be obtained in 40% yield by recrystallization several times from water.† Using the pure *p*-nitro compound II obtained above, reduction to *p*-aminophenyl-L-alanine (III) was accomplished easily and quantitatively.[11] By the same procedure, *o*-nitrophenylalanine will form 1-hydroxy-3-aminodihydrocarbostyril.[12]

The preparation of *p*-chlorophenyl-L-alanine (IV) then proceeded *via* selective diazotization of aminophenylalanine.[13] Reaction of this diazonium phenylalanine with CuCl gave an 85% yield of pure IV. The optical purity of the chloro compound was determined by hydrogenolysis of IV to give L-phenylalanine, which had an optical purity of 99.5%.

By the procedure outlined above it is thus possible to obtain pure *p*-nitrophenyl-L-alanine (II) and from it optically pure *p*-chlorophenyl-L-alanine (IV) without resolution. This procedure should also be applicable to the preparation of other para-substituted derivatives of L-phenylalanine.

Experimental Section

Nmr's were obtained with a Varian T-60 spectrometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. A Carl Zeiss automatic polarimeter was used to determine optical rotations. Amino acid analyses were performed in the Analytical Laboratory, University of California, Berkeley, using a Beckman Model 120C automatic amino acid analyzer.

Preparation of *dl* Chlorinated Phenylalanines. The prepara-

†A large number of commercial samples of *p*-nitro, *p*-amino-, and *p*-chlorophenylalanine were examined for amino acid content and isomer and optical purity. Although there was considerable variation, in no case did we find a sample of complete purity. Since these materials are being used in biological experiments, caution is clearly indicated.

tion of *o*-, *m*-, and *p*-chlorophenylalanine and the dichlorinated phenylalanines was accomplished by treating the appropriate α-chlorotoluene with sodium acetamidomalonate in absolute ethanol; the properties of the resulting substituted benzyl acetamidomalonates were in accord with those reported.[7] The ring-substituted phenylalanines were prepared by acid hydrolysis with boiling 6 *N* HCl; their physical properties agreed with the reported values and amino acid analysis gave only a single peak (Table II). Previous references to 2,3-, 2,5-, 2,6-, and 3,5-dichlorophenylalanine were not found.

Chlorination of L-Phenylalanine. The halogenations summarized in Table I were carried out as follows. A magnetically stirred solution of 1.65 g (10 mmol) of L-phenylalanine in 200 ml of 6 *N* HCl was maintained at room temperature while 0.71 g (10 mmol) of chlorine in 125 ml of the same solvent was added over a 10-min period. The reaction was maintained at the stated temperature until the disappearance of the yellow color (15 min) and then stirred for an additional 15 min. Solvent was then removed *in vacuo* and the crude product was subjected to amino acid analysis (Table I). Tlc on Eastman cellulose sheets No. 6065 (methyl ethyl ketone–pyridine–water–HOAc, 70:15:15:2) gave $R_{f(phe)}$ 0.36, $R_{f(o)}$ 0.44, $R_{f(m,\ p,\ and\ 2,3)}$ 0.57, $R_{f(2,5\ and\ 3,4}$ 0.64.

Separation of Chlorinated L-Phenylalanines. The separation of 2.0 g (10 mmol) of the isomeric mixture prepared above was attempted using Bio Rad AG 50W cation exchange resin (100–1000 mequiv/mequiv of amino acid) with cross linking from 2 to 8%, a solvent variance from 2 *N* HCl to 6 *N* HCl, and the same solvents containing an organic phase (methanol, 2–10%). All attempted variations failed to give an adequate separation or yield of pure IV. The fractions were analyzed by tlc and amino acid analysis. Attempted fractional crystallization also failed.

***p*-Nitrophenyl-L-alanine Monohydrate (II).** The Bergel and Stock[10] procedure was followed (Table III) except that the crude product obtained was recrystallized from water (3 ml/g) four times to give a 40% yield of pure nitro-L-phenylalanine monohydrate (II): mp 240–242° dec (lit.[10] mp 238–241°); $[\alpha]^{26}D$ +7.9 ± 0.2°. (*c* 1.77, 1.0 *N* HCl) (lit.[10] $[\alpha]^{26}D$ +9.8 ± 0.2°); nmr [D_2O–DCl, 3-(trimethylsilyl)propanesulfonic acid as internal reference] δ 3.48 (d, *J* = 7.0 Hz, 2 H, $-CH_2-$), 4.45 (t, *J* = 7.0 Hz, 1 H, $-CHCO_2H$), 7.55 (d, *J* = 7.0 Hz, 2 H, aromatic protons, ortho to $-CH_2-$), 8.08 (d, *J* = 7.0 Hz, 2 H, aromatic protons ortho to $-NO_2$). *Anal.* ($C_9H_{10}N_2O_4$, H_2O removed by heating in high vacuum) C, H.

***p*-Aminophenyl-L-alanine Monohydrate (III).** The method of Bergmann[11] was used and gave a quantitative yield of pure amino-L-phenylalanine by filtering off the catalyst: mp 242–247° dec (lit.[11] mp 235–242°); $[\alpha]^{25}D$ −42.0 ± 0.5° (*c* 1.5, H_2O) (lit.[14] $[\alpha]^{25}D$ −42°); amino acid analysis gave a single peak (Table II); nmr (D_2O) δ 3.25 (d, *J* = 7.0 Hz, 2 H, $-CH_2-$), 4.00 (t, *J* = 7.0 Hz, 1 H, $-CHCO_2H$), 7.30 (s, 4 H, aromatic).

***p*-Chlorophenyl-L-alanine (IV).** A solution of 7.23 g (40 mmol) of *p*-amino-L-phenylalanine in 24 ml of 4.0 *N* HCl was mechanically stirred at 0° while 2.00 g (40 mmol) of $NaNO_2$ in 6 ml of water was added over a 20-min period. After stirring for 5 min longer, the resulting solution was added to 5.35 g (54 mmol) of CuCl in 24 ml of concentrated HCl at 0° over 20 min. The reaction was quite frothy (a 500-ml flask is adequate for the reaction) with the bubbling continuing as the reaction was heated to 60° for 30 min with vigorous stirring. The resulting mixture was dissolved in 350 ml of water and H_2S was bubbled through the solution until the filtrate was clear. This clear aqueous solution was evaporated *in vacuo*, the residue was dissolved in H_2O (500 ml) and neutralized (pH 6.5) with 3 *N* NaOH, and the solution was again evaporated to dryness. Chromatography of the residue on 120 g of silica with CH_3OH–17% NH_3–$CHCl_3$ (10:1.5:13.5) as eluent gave 7.50 g (86%) of pure IV: mp 241–243° dec (lit.[7] 236–242°); $[\alpha]^{26}D$ −3.9 ± 0.3° (*c* 2.0, 1 *N* HCl) (lit.[6] −3.5); $[\alpha]^{26}D$ −27.8 ± 0.2° (*c* 0.4, H_2O); nmr (D_2O–DCl) δ 3.35 (d, *J* = 6.5 Hz, 2 H, $-CH_2-$), 4.40 (t, *J* = 6.5 Hz, 1 H, $-CHCO_2H$), 7.32 (d, *J* = 8.0 Hz, 2 H, aromatic protons ortho to $-CH_2-$), 7.36 (d, *J* = 8.0 Hz, aromatic protons ortho to −Cl). *Anal.* ($C_9H_{10}ClNO_2$) C, H, N.

Optical Purity of IV. Hydrogenation of 0.603 g (3.02 mmol) of IV in 100 ml of water in the presence of 0.20 g of 10% Pd/C was carried out with hydrogen at 40 psi for 2 hr. After filtering off the catalyst and washing it thoroughly with H_2O, the resulting solution was adjusted to pH 5.50 with 1.0 *N* NaOH and lyphilized. The rotation of the residue (0.678 g; theory, 0.675 g; 100.5%) was $[\alpha]^{26}D$ −33.90 ± 0.05° (*c* 2.0, H_2O), allowing for the presence of NaCl; L-phenylalanine has $[\alpha]^{26}D$ −34.13 ± 0.03°. A series of control experiments using L-phenylalanine and L-phenylalanine hydrochloride was performed to ensure that there was no change of optical activity in the L-phenylalanine during the hydrogenation, neutralization, or lyphilization. The amino acid analysis showed only the phenylalanine peak and the total absence of IV in the hydrogenated material.

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Potential Bioreductive Alkylating Agents. 3. Synthesis and Antineoplastic Activity of Acetoxymethyl and Corresponding Ethyl Carbamate Derivatives of Benzoquinones

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A number of benzo- and naphthoquinone derivatives with one or two side chains capable of alkylation after reduction were found (a) to possess inhibitory activity against the growth of transplantable tumors of mice and (b) to cause inhibition of nucleic acid biosynthesis and of the activities of coenzyme Q mediated enzyme systems.[1-3] *In vivo* conversion to an active form is hypothesized to involve reduction, presumably by a pyridine nucleotide-requiring quinone reductase,[4,5] to a dihydroquinone which spontaneously decomposes to an *o*-quinonemethide. This extremely reactive intermediate has the capacity to alkylate cellular components. Although no evidence is currently available to support the existence of *o*-quinonemethide *in vivo*, chemical evidence has been obtained to substantiate the formation of quinonemethide in the reductive amination of 2,3-dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone by aniline and morpholine.[6] The theoretical potential of compounds of this type against solid neoplasms has been discussed.[2]

The proposed action mechanism for bioreductive alkylating agents suggests that the biochemical activation of compounds of this type would be significantly influenced by their oxidation–reduction potentials. Compounds with relatively high (positive) redox potentials should be more susceptible to reduction than derivatives with more negative ones, and thereby the former types of agents might possess greater antineoplastic activity. Since substitution of the quinone ring with electron-donating groups, such as methyl or methoxy, should change the redox potentials of