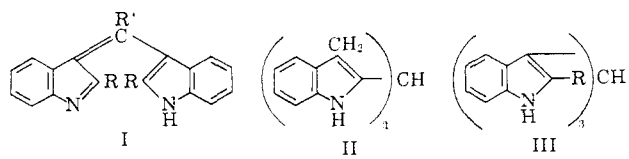
TABLE I
ETHYLENE DERIVATIVES
YCH=CHZ

| No. | Y | Z | Method | M.p.,[a] °C. | Yield, % | Formula | Ultraviolet absorption $\lambda_{max}$, mμ (log ε) in methanol | in acetic acid | Calcd., % C | Calcd., % H | Found,[b] % C | Found,[b] % H | $ED_{50}$,[c] γ/ml. | T/C (mg./kg.)[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3-Indolyl | 4-Quinolyl | A | 241–243[e,f] | 30 | $C_{19}H_{13}N_3$ | 380 (4.3) 495 | 480 (4.5) | 84.42 | 5.22 | 84.30 | 5.27 | 3 | |
| 2 | 3-Indolyl | 2-Quinolyl | A | 212–213[g,f] | 49 | $C_{19}H_{13}N_3$ | 375 (4.4) | 465 (4.6) | 84.42 | 5.22 | 84.38 | 5.19 | 4 | 1 (1600) |
| 3 | 3-Indolyl | 1-Isoquinolyl | A | 239–241[e,f] | 45 | $C_{19}H_{13}N_3$ | 385 (3.6) | 465 (4.0) | 84.42 | 5.22 | 84.30 | 5.45 | 10 | 1 (1600) |
| 4 | N-Methyl-3-indolyl | 4-Quinolyl | B | 172–173[h,i] | 4 | $C_{20}H_{15}N_3$ | 380 (3.9) 495 (3.8) | 485 (4.5) | 84.20 | 6.00 | 83.91 | 5.63 | 2 | |
| 5 | 3-Pyridyl | 4-Quinolyl | A | 109–110[j,k] | 2 | $C_{16}H_{12}N_3$ | 325 (4.3) | 325 (4.2) | 82.72 | 5.21 | 82.64 | 5.66 | 72 | |
| 6 | 3-Pyridyl | 2-Quinolyl | A | 96[f,l] | 55 | $C_{16}H_{12}N_3$ | 325 (4.4) | 350 (4.4) | 82.72 | 5.21 | 82.54 | 5.11 | 3 | |
| 7 | 2-Thiophenyl | 4-Quinolyl | B[m] | 86[f,n] | 24 | $C_{15}H_{11}NS$ | 340 (4.0) | 317 (3.9) 405 (3.8) | 75.95 | 4.64 | 76.08 | 4.77 | 6 | 1 (100)[o] |
| 8 | 6-(N-Methyl-1,2,3,4-tetrahydroquinolyl) | 4-Quinolyl | B[p] | 137[n] | 25 | $C_{21}H_{20}N_2$ | 415 (4.2) | 545 (4.5) | 84.00 | 6.66 | 84.50 | 6.80 | 1 | |

[a] Corrected for thermometer stem exposure; determined with Thiele tube. [b] Average of two determination by Weiler and Strauss, Oxford, England. [c] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory and Southern Research Institute. [d] We are grateful to Professor Alexander Haddow, Mr. J. E. Everett, and Mr. B. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single i.p. injection in Arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor bearing animals were sacrified approximately 8 days later and the average weights of tumors in treated and untreated hosts are reported as the ratio *T/C*. [e] Recrystallized from ethanol. [f] Yellow crystals. [g] Recrystallized from methanol. [h] Recrystallized from isooctane and from ethanol. [i] Tan crystals. [j] Recrystallized from isopropyl ether. [k] White crystals. [l] Recrystallized from isohexane. [m] Reacted 0.5 hr. at 130°. [n] Recrystallized from isooctane. [o] Killed 1 of 3 rats at 200 mg./kg. [p] Reaction temperature 155–165°; time, 2 hr.

TABLE II
INDOLE DERIVATIVES



I II III

| No. | Compd. type | R | R' | $ED_{50}$[a] | *T/C*[b] | @ mg./kg. |
|---|---|---|---|---|---|---|
| 1 | I | H | H[c] | 3 | 0.7 | 80[d] |
| 2 | I | $CH_3$ | H[e] | 2 | 1.1 | 80[f] |
| 3 | I | $CH_3$ | $CH_3$[g] | 55 | 1.0 | 320[h] |
| 4 | II | ... | ...[i] | 33 | 0.7 | 1600 |
| 5 | III | H | H | 100 | 1.2 | 1600 |
| 6 | III | $CH_3$ | $CH_3$[j] | 30 | 1.0 | 1600 |

[a] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory and Southern Research Institute. [b] See footnote *d*, Table I. [c] W. König, *J. prakt. Chem.*, **84,** 194 (1911). [d] Killed 3/3 at 160 mg./kg. [e] M. Scholtz, *Ber.*, **46,** 2138 (1913). [f] Killed 2/3 at 160 mg./kg. [g] A. K. Kiang and F. G. Mann, *J. Chem. Soc.*, 594 (1953). [h] Killed 2/2 at 625 mg./kg. [i] H. V. Dobeneck and H. Pritzel, *Z. physiol. Chem.*, **299,** 214 (1955). [j] M. Passerini, *Gazz. chim. ital.*, **68,** 480 (1938).

### Experimental

The methods of synthesis of compounds in Table I are illustrated below.

**Method A.**—A mixture of 10.0 g. of indole-3-carboxaldehyde and 12 g. of 2-methylquinoline was heated 21 hr. in a closed container at 125–135°. The hot mixture was then triturated with 150 ml. of isopropyl alcohol. After recrystallization from methanol and from ethanol the yellow crystals melted at 210–211°; yield 8.9 g.

**Method B.**—A mixture of 7.0 g. of N-methylindole-3-carboxaldehyde and 12.5 g. of lepidine hydrochloride was heated at 160–170° until it solidified (2 hr.). The solid was dissolved in methanol and the solution was neutralized by addition of 8 *N* NaOH, then diluted with water and cooled. The solid was recrystallized once from octane and three times from methanol to yield 2.9 g. of tan crystals, m.p. 172–173°.

Method A tended to give a cleaner product, but only method B was successful with N-methylindole-3-carboxaldehyde, and neither method was satisfactory for N-acetylindole-3-carboxaldehyde.

**3,3',3''-Triindolylmethane.**—A solution of 0.10 mole of indole-3-carboxaldehyde, 0.20 mole of indole, and 1 drop of concentrated HCl in 200 ml. of methanol was boiled under reflux for 0.6 hr. The solid product was recrystallized from benzene to give white crystals, m.p. 254–256° cor., yield 80%.

*Anal.*[5] Calcd. for $C_{25}H_{19}N_3$: C, 83.07; H, 5.30. Found: C, 82.98; H, 5.24.

(5) Analysis by Galbraith Laboratories.

## α- and γ-Glutamyl Derivatives of Aminobenzoic Acids

RODNEY F. LLOYD,[1a] CHARLES G. SKINNER, WILLIAM SHIVE,

*Clayton Foundation Biochemical Institute, and The Department of Chemistry, The University of Texas, Austin, Texas*

AND R. JOHN STEDMAN

*Smith Kline and French Laboratories, Philadelphia, Pennsylvania*

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A previous report assigned the γ-glutamyl structure to the *o*-, *m*-, and *p*-(L-glutamylamino)benzoic acids prepared by condensation of N-carbobenzoxy-L-glutamic anhydride with the methyl esters of the appropriate aminobenzoic acids and subsequent removal of the protecting groups.[1b] The γ-amide I rather than the α-amide II structure was favored because with ninhydrin the materials gave a strong purple color, which was taken to be characteristic of α-amino acids having a free carboxyl group, and because, with phenylisocyanate, derivatives which appeared to be hydrates of hydantoins were obtained. A reinvestigation of the

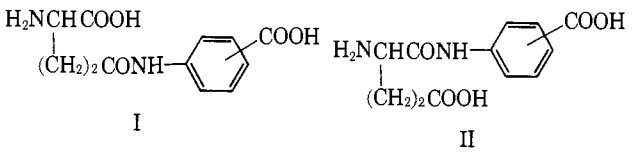
(1) (a) Predoctoral Fellow (1F1 GM-19,443-01), National Institute of General Medical Sciences. (b) D. L. Ross, C. G. Skinner, and W. Shive, *J. Med. Chem.*, **6,** 208 (1963).

TABLE I

γ-L-GLUTAMYLAMINOBENZOIC ACIDS AND DERIVATIVES

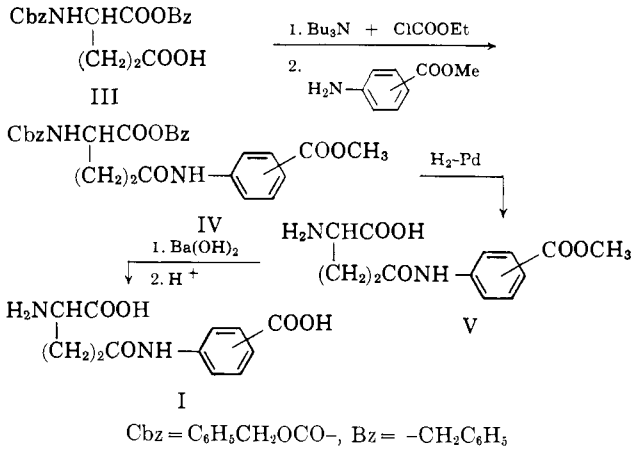
| Compd. | M.p., °C. | Yield, % | Formula | Calcd., % C | Calcd., % H | Calcd., % N | Found, % C | Found, % H | Found, % N |
|---|---|---|---|---|---|---|---|---|---|
| (N-Carbobenzoxy-α-benzyl-γ-L-glutamyl)aminobenzoic Acid Methyl Esters (IV) | | | | | | | | | |
| *o*- | 156–159 | *a* | $C_{28}H_{28}N_2O_7$ | 66.66 | 5.59 | 5.55 | 66.91 | 5.94 | 5.63 |
| *m*- | 87–91 | 84 | $C_{28}H_{28}N_2O_7$ | 66.66 | 5.59 | 5.55 | 66.83 | 6.03 | 5.51 |
| *p*- | 150–154 | 36 | $C_{28}H_{28}N_2O_7$ | 66.66 | 5.59 | 5.55 | 66.85 | 5.73 | 5.54 |
| γ-L-Glutamylaminobenzoic Acid Methyl Esters (V) | | | | | | | | | |
| *o*- | 178.5–179 | 14[b] | $C_{13}H_{16}N_2O_5$ | 55.70 | 5.75 | 10.00 | 55.95 | 6.00 | 9.86 |
| *m*- | 190–190.5 | 46 | $C_{13}H_{16}N_2O_5$ | 55.70 | 5.75 | 10.00 | 55.60 | 6.08 | 10.00 |
| *p*- | 206–207 | 44 | $C_{13}H_{16}N_2O_5$ | 55.70 | 5.75 | 10.00 | 55.51 | 5.90 | 10.11 |
| γ-L-Glutamylaminobenzoic Acids (I) | | | | | | | | | |
| *o*- | 178–178.5 | 58 | $C_{12}H_{14}N_2O_5$ | 54.13 | 5.30 | 10.52 | 54.15 | 5.63 | 10.49 |
| *m*- | 198.5–199 | 64 | $C_{12}H_{14}N_2O_5$ | 54.13 | 5.30 | 10.52 | 54.22 | 5.52 | 10.57 |
| *p*- | 258–259 | 83 | $C_{12}H_{14}N_2O_5$ | 54.13 | 5.30 | 10.52 | 54.43 | 5.58 | 10.69 |

[a] Difficult to isolate, so hydrogenolyzed directly to *o*-V. [b] Represents over-all yield through two steps.




structures was suggested by one of the present authors (R. J. S.) because in a similar case of the opening of the carbobenzoxyglutamic anhydride by a weakly basic amine, the α- rather than the γ-amide was the predominant product.[2] Furthermore, when the crude mixture from the reaction of the anhydride with methyl *o*-aminobenzoate was subjected to electrophoresis at pH 4,[3] two new anionic materials, presumably the α- and γ-amides, were found, the slower moving component being the more plentiful. This component was readily isolated and had the properties of the reported *o*-[(N-carbobenzoxy-L-glutamyl)amino]benzoic acid methyl ester.[1] Its electrophoretic mobility indicated that it was the isomer with the less strongly acidic carboxyl group,[4] and this argued that it was the α-isomer.[5] The structural assignments of all the reported compounds were therefore re-examined, and the unambiguous synthesis of the desired γ-amides was undertaken.

Conclusive evidence of the α-amide structure of the three reported glutamylaminobenzoic acids was provided by a quantitative ninhydrin assay of the free α-amino acid groupings present.[6] In spite of the misleading color reaction, there was no significant liberation of carbon dioxide; it follows that the α-carboxyl group of the glutamic acid cannot be free and the γ-glutamyl structures (I) must be excluded. The derivatives obtained with phenylisocyanate are therefore uncyclized hydantoic acid amides rather than hydrated hydantoins as had been supposed; their apparent loss of water on high-temperature drying is not attributable to hydration. For the synthesis of the authentic γ-L-glutamylaminobenzoic acids (I), several different routes were explored. The only satisfactory procedure found was to condense N-carbobenzoxy-L-glutamic acid α-benzyl ester[7] (III) with the appropriate methyl aminobenzoate by the mixed anhydride technique[8] to yield the protected intermediates (IV). Hydrogenolysis removed the benzyl ester and carbobenzoxy groups to give the methyl γ-glutamylaminobenzoates (V), which were hydrolyzed with alkali to afford the desired products (I). The results of the preparative work are summarized in Table I. A paper chromatographic comparison




Cbz = $C_6H_5CH_2OCO-$, Bz = $-CH_2C_6H_5$

of the newly prepared authentic γ-glutamylaminobenzoic acids with the previously reported materials showed them to be distinct and apparently homogenous com-

TABLE II

GLUTAMYL DERIVATIVES OF *o*-, *m*-, AND *p*-AMINOBENZOIC ACIDS

| Glutamyl derivative | M.p., °C. | $R_f$ on paper chromatogram[a] Solvent A | Solvent B | Quant. ninhydrin, %[b] |
|---|---|---|---|---|
| *o*-Aminobenzoic Acid | | | | |
| α-[c] | 258–259 dec. | 0.56 | 0.23 | Neg.[d] |
| γ- | 178–178.5 | 0.44 | 0.25 | 97 |
| *m*-Aminobenzoic Acid | | | | |
| α-[c] | 162–163 | 0.55 | 0.13 | Neg.[d] |
| γ- | 198.5–199 | 0.41 | 0.12 | 108 |
| *p*-Aminobenzoic Acid | | | | |
| α-[c] | 297–300 dec. | 0.59 | 0.12 | Neg.[d] |
| γ- | 258–259 | 0.42 | 0.10 | 104 |

[a] Solvent solutions: A, *n*-butyl alcohol–acetic acid–water (4:1:1); B, isoamyl alcohol–pyridine–water (7:7:6). [b] Based on the evolution of carbon dioxide.[6] [c] Previously reported as γ-isomer.[1] [d] Values obtained were less than 5% of theory for an α-aminocarboxylic acid.

(2) R. J. Stedman, unpublished studies of the reaction of carbobenzoxyglutamic anhydride with 6-aminopenicillanic acid.

(3) Paper electrophoresis was carried out in a pH 4 acetate buffer to which half a volume of methanol had been added. The products showed brilliant blue fluorescence under ultraviolet light.

(4) In deducing the relative acidities of the two isomers from their electrophoretic mobilities, we are assuming that factors such as adsorption on the paper, etc., are the same in both cases.

(5) W. J. Le Quesne and G. T. Young, *J. Chem. Soc.*, 1954 (1950).

(6) D. D. Van Slyke, D. A. MacFadyen, and P. Hamilton, *J. Biol. Chem.*, **141**, 671 (1941).

(7) M. Sachs and E. Brand, *J. Am. Chem. Soc.*, **75**, 4610 (1953).

(8) (a) M. Sachs and E. Brand, *ibid.*, **75**, 4608 (1953); (b) M. L. Kornguth, A. Neidle, and H. Waelsch, *Biochemistry*, **2**, 740 (1963).

pounds. The chromatographic properties of the *ortho*, *meta*, and *para* isomeric pairs, together with their melting points and ninhydrin assays, are presented in Table II.

In conclusion, all the compounds described in the previous paper[1b] should be corrected to read as α-glutamyl derivatives rather than as the corresponding γ-isomers. The condensation products with phenylisocyanate are (N-phenylcarbamyl)-α-L-glutamylaminobenzoic acids. The authentic γ-L-glutamylaminobenzoic acids have now been prepared by the mixed anhydride coupling of N-carbobenzoxy-L-glutamic acid α-benzyl ester with the appropriate methyl aminobenzoate followed by removal of the protecting groups.

### Experimental[9]

Since all the γ-glutamylaminobenzoic acid derivatives herein described were prepared by similar procedures, complete experimental data are given only for the *para* isomer, and the data on the remaining analogs are presented in Table I. Comparative data for the α- and γ-glutamyl derivatives are presented in Table II.

**p-[(N-Carbobenzoxy-α-benzyl-γ-L-glutamyl)amino]benzoic Acid Methyl Ester (*p*-IV).**—A 12.6-g. sample of N-carbobenzoxy-L-glutamic acid α-benzyl ester[7] and 8 ml. of tri-*n*-butylamine were dissolved in 80 ml. of dimethylformamide–tetrahydrofuran (1:1). the mixture was cooled in an ice bath, and 3.3 ml. of ethyl chloroformate was added dropwise to form the mixed anhydride.[8] After stirring for about 30 min. in the cold, 5.13 g. of methyl *p*-aminobenzoate in 45 ml. of the dimethylformamide–tetrahydrofuran solvent mixture was added dropwise, and the reaction mixture was allowed to come to room temperature. Stirring was continued overnight. The solvent was evaporated *in vacuo*, and the residue was dissolved in 100 ml. of ethyl acetate and extracted with three 50-ml. portions of 2 *N* HCl. The ethyl acetate phase was then evaporated *in vacuo* to a pale yellow oil which was crystallized from ethanol-water to yield 11.4 g. of solid. Recrystallization of this material from toluene produced 6.3 g. of product, m.p. 150–154°. The *meta* isomer crystallized with difficulty, and crystallization of the *ortho* isomer was sufficiently difficult that it was isolated only for elemental analysis. The reaction mixture of the *ortho* derivative was used directly without purification in the subsequent hydrogenolysis step.

**p-(γ-L-Glutamylamino)benzoic Acid Methyl Ester (*p*-V).**—A 3.55-g. sample of *p*-IV was dissolved in 90 ml. of 90% ethanol and hydrogenolyzed at atmospheric pressure and room temperature in the presence of 0.25 g. of palladium black catalyst for about 5 hr. The reaction product precipitated on the catalyst, which was collected and extracted with 300 ml. of boiling water. Upon cooling, the aqueous filtrate yielded 0.87 g. of product, which was recrystallized from water-ethanol, m.p. 206–207°.

**p-(γ-L-Glutamylamino)benzoic Acid (*p*-I).**—A 0.5-g. sample of *p*-V was dissolved in 175 ml. of saturated $Ba(OH)_2$ solution, and after about 15 min. the resulting crystalline precipitate was filtered, washed with water, and dried to yield 0.64 g. of crude barium salt. This was suspended in 50 ml. of water, adjusted to pH 2.5 with 0.6 *M* $H_2SO_4$ to precipitate the barium ions as the insoluble sulfate, taken to pH 8 with 5 *N* NaOH to solubilize the reaction product, and finally filtered. The filtrate was then adjusted to pH 5 with 0.6 *M* $H_2SO_4$ to precipitate 0.4 g. of product, m.p. 227–228° dec. After two recrystallizations from water, which had been adjusted to pH 3 by the addition of dilute $H_2SO_4$, a white solid was obtained, m.p. 258–259°. This material was chromatographically homogeneous in two different solvent systems and gave the anticipated yield of $CO_2$ by the quantitative ninhydrin technique.[6] The alkaline hydrolysis of each of these methyl esters was extremely rapid, and the crude barium salt was isolated only during the hydrolysis of *p*-V; for the *meta* and *ortho* isomers, the reaction mixture was processed directly without any intermediate separation of the barium salt.

(9) All melting points are corrected, and were determined by the capillary technique using a well-stirred liquid bath. The paper chromatograms were prepared by the ascending procedure using the indicated solvent systems on Whatman No. 1 paper, and were subsequently developed with ninhydrin reagent.

## Synthesis of N,N-Bis(2-haloethyl) Aliphatic Amides

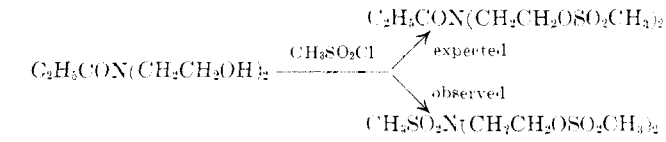
CARLOS H. GAOZZA

*Catedra de Química Orgánica I, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina*

*Received April 25, 1964*

With the purpose of contributing to the series of the N,N-bis(2-chloroethyl) aliphatic amides[1] with potential cytotoxic activity, four amides were prepared by acylation of bis(2-chloroethyl)amine with acid chlorides in pyridine–chloroform solution (Table I).

In order to obtain the iodo analogs by means of the corresponding methanesulfonates (mesylates),[2] N,N-bis-(2-hydroxyethyl)propionamide was obtained by ester aminolysis with diethanolamine, and this compound was mesylated with methanesulfonyl chloride in pyridine–chloroform solution at 3–5°. Methanesulfonyl chloride, besides esterifying the hydroxyl groups, displaced the acyl by the sulfonyl group. The same results




were obtained at different temperature and with or without a solvent. The N,N-bis(2-hydroxyethyl)-acetamide behaved like the propionamide.

The N,N-bis(2-mesylethyl)methanesulfonamide was also synthesized from diethanolamine. The diiodo derivative was obtained from the mesylate by displacement with sodium iodide. The structure of the compounds was confirmed by their infrared spectra.

$$CH_3SO_2N(CH_2CH_2OSO_2CH_3)_2 \xrightarrow{NaI} CH_3SO_2N(CH_2CH_2I)_2$$

**Biological Results.**—The drugs were tested in rats with transplanted Sarcoma 180 and Ehrlich ascitic carcinoma. The results are shown Table II.

### Experimental

**N,N-Bis(2-chloroethyl)amides.**—The acid chloride (0.04 mole) in dry chloroform was added slowly to a stirred solution of bis-(2-chloroethyl)amine prepared from the hydrochloride (0.045 mole) and pyridine (0.05 mole) in chloroform. Stirring was continued for 1 hr. and the mixture then was poured into water. Distillation of the solvent from the dried solution yielded thick oils which solidified by cooling in the case of caproamide (see Table I).

**N,N-Bis(2-hydroxyethyl)propionamide.**—Equimolar quantities of ethyl propionate and diethanolamine were mixed and refluxed for 10 hr. Excess solvent was removed by vacuum distillation and the product distilled at less than 1 mm.; yield 60%; colorless oil, b.p. 134–135° (0.35 mm.).

*Anal.* Calcd. for $C_7H_{15}NO_3$: C, 52.1; H, 9.31; N, 8.70. Found: C, 51.8; H, 9.42; N, 8.92.

**Attempt to Obtain N,N-Bis(2-mesylethyl)propionamide.**—Methanesulfonyl chloride (0.18 mole) in chloroform (10 ml.)

(1) (a) D. H. Peacock, *J. Chem. Soc.*, 1303 (1934); (b) A. F. Childs, *ibid.*, 2174 (1948); (c) E. R. H. Jones, *ibid.*, 547 (1949); (d) F. J. Buckle, *ibid.*, 912 (1949); (e) H. Brintzinger, *Chem. Ber.*, **82**, 389 (1949); (f) G. Drefahl, *ibid.*, **87**, 1628 (1954); (g) I. Ailso, *J. Pharm. Soc. Japan*, **75**, 418 (1955); (h) W. C. J. Ross, *J. Chem. Soc.*, 3616 (1959); (i) Y. Kuwanda, *Chem. Pharm. Bull.* (Tokyo), **8**, 77 (1960).

(2) F. Kagan, *J. Am. Chem. Soc.*, **81**, 3026 (1959).