Synthesis of D- and L-Thalidomide and Related Studies

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The optical isomers of thalidomide were synthesized by a route based on D- and L-isoglutamine. The thalidomide isomers were obtained in high yields by treating the optical isomers of N^2-phthaloylisoglutamine at 0° with thionyl chloride in dimethylformamide. L-Thalidomide was also obtained by using N,N'-carbonyldiimidazole to close the glutarimide ring. Application of these two methods, together with the active (cyanomethyl) ester method, to both N^2-phthaloyl-L-isoglutamine and N^2-phthaloyl-L-glutamine showed that L-thalidomide is formed more readily and with less risk of racemization from the isoglutamine derivative. DL-Thalidomide was also prepared under much milder conditions than those previously used.

THE DESIRABLE SEDATIVE, the undesirable neurotoxic, and the tragic teratogenic properties of thalidomide (IX) are well known. Summaries of these properties have been published (*e.g.*, 1–3), and numerous other publications of biological and biochemical studies have appeared. The synthesis of thalidomide consists, in essence, of the formation of two imide rings. In most of the recorded methods of synthesis, the final step was the formation of the glutarimide ring at temperatures of 150–250° (4–15). The glutarimide ring has also been formed before the introduction of the phthalimido group (16, 17). Prior to the development of *N*-ethoxycarbonylphthalimide for the phthaloylation of amino acids (18), the preparation of phthaloylglutamic acid derivatives was usually accompanied by some racemization (19, 20), although procedures have been devised to obviate or minimize racemization (*e.g.*, 19, 21). However, even when optically active glutamic acid or glutamine was employed for thalidomide synthesis, racemization apparently occurred, either during the phthaloylation with phthalic anhydride, or at the high temperatures used for glutarimide-ring formation. Syntheses of L-thalidomide (22) and of both D- and L-thalidomide (23) have been reported in preliminary communications. Several biological studies (24–29) that have included D- and L-thalidomide have been published without revealing the method of preparation of the optical isomers. In this report a full account of the studies resulting in a synthesis of D- and L-thalidomide is presented (23).

These investigations began with attempts to form the glutarimide ring from N^2-phthaloyl-L-glutamine (L-I) by using peptide-forming reagents. The preliminary investigations produced evidence of the formation of some thalidomide under relatively mild conditions and, also, of partial conversion of the amide group to a nitrile group by dehydrating agents. Dehydration of glutamine and asparagine derivatives to nitriles by certain peptide-forming reagents has been reported (30–35). The active-ester method was investigated by utilizing the cyanomethyl ester of L-I, since it is conveniently prepared. In the strongly basic medium of one equivalent of sodium methoxide in dimethyl sulfoxide (DMSO), the L-I cyanomethyl ester (L-II, $[\alpha]_D^{25}$ −46.6° in dimethylformamide [DMF]) was rapidly cyclized at room temperature to thalidomide in a yield of 70%. Cyclization in good yield also occurred, at a slower rate, with less than one equivalent of methoxide. The conditions employed were much milder than those previously reported; even so, DL-thalidomide (DL-IX) was the product of both experiments.

The preliminary evidence of difficulties in basing a synthesis of L-thalidomide on L-I was suggestive, rather than conclusive, because the application of peptide-forming reactions was not thoroughly investigated. During the course of these studies, Liberek presented evidence that phthaloyl derivatives (36) and cyanomethyl esters (37) of amino acids are especially susceptible to base-catalyzed racemization. Since our evidence and evidence from the literature suggested that dehydrating-type reagents might produce considerable nitrile from glutamine derivatives (30–35) and that base-catalyzed cyclizations might cause racemization, these studies were subsequently based on isoglutamine for the following reasons. Activation of the carboxyl group of glutamine derivatives increases the acidity of the C—H bond of the adjacent asymmetric carbon atom and, thereby, enhances the tendency to racemize. In isoglutamine

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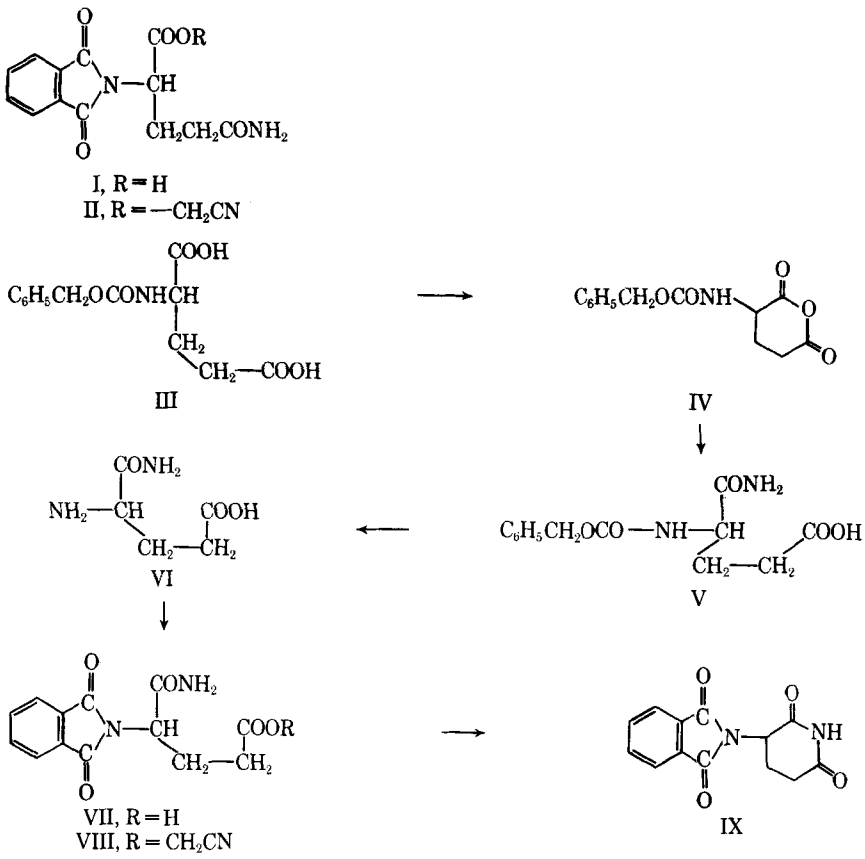

Scheme I

(VI) derivatives, however, the carboxyl group to be activated for ring-closure is separated from the asymmetric carbon atom by two methylene groups. In addition, there is evidence from peptide chemistry (*e.g.*, 38–40) of rather facile imide formation from α-glutamyl peptides.[1]

The synthetic route to D-and L-thalidomide is outlined in Scheme I, and details, including some refinements of procedures reported in the literature for intermediates (III–VI), are described in *Experimental.* The method of Nefkens *et al.* (18), which introduces the phthaloyl group without racemization, was used to prepare the phthaloyl derivatives (I, VII) of L-glutamine (already mentioned), L-isoglutamine, and D-isoglutamine. After work on the synthesis of these compounds had been completed, Schröder and Klieger (41) reported the synthesis of L-I and L-VII by this method, although the optical rotation of L-VII was not recorded. The formation of the glutarimide ring was effected readily by thionyl chloride in DMF at 0° or, over a longer period of time, by *N,N'*-carbonyldiimidazole (42). The thionyl chloride-DMF method afforded L- and D-thalidomide (L-IX and D-IX), with optical rotations comparable to those of subsequently purified specimens, in yields of 75–85%. The optical rotations[2] of L- and D-thalidomide recrystallized from DMF–water were −64.6° and +64°, respectively, in dry DMF at 25°. The optical isomers and commercial DL-thalidomide moved identically on thin-layer chromatograms and gave identical nuclear magnetic resonance spectra in DMSO-d_6. The infrared spectra, determined by the potassium bromide disk method, of D- and L-thalidomide were identical, as expected, and were also identical with a published spectrum (43) of DL-thalidomide, except for minor differences ascribable to the fact that the spectra were determined in the solid state.

Tipson (20) had found that the phthalimide ring of phthaloylglutamic acid is readily opened by dilute aqueous base, and Williams (45) reported that DL-thalidomide undergoes hydrolysis in the neutral pH range to at least 12 compounds.[3] These findings led us to design our

[1] Battersby and Reynolds (40) reported the failure of a γ-glutamyl (glutamine-type) peptide to cyclize under conditions that gave a low yield of a glutarimide from an α-glutamyl (isoglutamine-type) peptide.

[2] Values reported in the literature for L-thalidomide are $[\alpha]_D^{20}$ −62° in dioxane (22) and $[\alpha]_D^{20}$ −45.7° in DMF (26) and for D-thalidomide, $[\alpha]_D^{20}$ +53.2° in DMF (26). A very recent publication (44) on some biological properties of L- and D-thalidomide gave $[\alpha]_D^{20}$ −58° and +60°, respectively, in DMF.

[3] A complete description of the studies of Williams and coworkers on hydrolysis has subsequently been published (26). Other reports of facile hydrolysis have also been published (*e.g.*, 46, 47).

preparative and isolation procedures with the aim of preventing, or minimizing insofar as possible, hydrolysis of the imide rings, as well as racemization. In addition, after the thalidomide isomers had been obtained, we attempted to determine the stability of L-thalidomide and its immediate precursor (L-VII) in DMF and aqueous DMF. Both compounds were in DMF solution (containing trace amounts of water) during the preparation of L-thalidomide and during the determination of their optical rotations, and L-thalidomide was exposed to aqueous DMF during isolation and recrystallization. Optical rotation data indicated that L-thalidomide suffered little or no change in dry DMF or in 80% DMF during observation periods of 18 and 43 hr., respectively, and that L-VII was stable in both of these solutions for at least 24 hr. However, a specimen of L-thalidomide that had not been recrystallized (after isolation from a thionyl chloride–DMF reaction) exhibited a decrease in specific rotation in both dry DMF and in 80% DMF; the decrease was more rapid in the latter solution. Subsequently, at least one recrystallization from DMF–water was performed on the day of the preparation of L- or D-IX. As a further precaution, the preparation of L-VII at a pH lower than that of the customary Nefkens phthaloylation was performed, but there appeared to be no advantage in this modification.

In the course of our studies, comparison of L-glutamine and L-isoglutamine derivatives as precursors of thalidomide was made. L-I and L-VII were treated with thionyl chloride in DMF in experiments identical in procedure and isolation methods. One pair of reactions was allowed to proceed for 0.5 hr.; another pair, for 2 hr. L-VII gave L-thalidomide in yields of 75–80%; L-I gave a low yield (9–10% calculated as thalidomide) of crude material that contained sulfur, had an optical rotation greater in magnitude than that of L-thalidomide, and produced 2–3 spots (one of which was thalidomide) on thin-layer chromatograms. From the filtrate of the 2-hr. reaction of L-I, a yield of 11% of crude thalidomide was obtained. Although L-I gave a small amount of thalidomide of unknown optical purity, L-VII obviously gave vastly superior results in this procedure.

In another pair of identical experiments, reactions of L-I and of L-VII with *N,N'*-carbonyldiimidazole gave a 41% yield of L-thalidomide ($[\alpha]_D^{25}$ −58°) from L-VII and a 30% yield of DL-thalidomide from L-I.

Cyclization of the cyanomethyl ester (L-VIII) of phthaloyl-L-isoglutamine with sodium methoxide in DMSO in experiments identical with those employed for the L-glutamine derivative (L-II) likewise gave DL-thalidomide. However, during the preparation of L-VIII in the presence of triethylamine, optically active thalidomide, as well as L-VIII, was obtained. This observation alone did not necessarily demonstrate a greater propensity of L-VIII to cyclize, since the reaction time required to prepare L-VIII was greater than that used to prepare L-II. However, it did show that L-VIII would cyclize under less basic conditions than those of the sodium methoxide–DMSO reagent. The product isolated after treating L-VIII with triethylamine in DMSO was partially racemized L-thalidomide ($[\alpha]_D^{25}$ −45°), whereas L-II in an identical experiment was racemized but failed to cyclize. Thus, isoglutamine derivatives were cyclized much more readily by the three methods investigated and were much less susceptible to racemization than the corresponding glutamine derivatives.

EXPERIMENTAL[4]

Infrared spectra were recorded with a Perkin-Elmer model 521 spectrophotometer using samples in potassium bromide disks. Melting points were determined on a Kofler Heizbank melting point apparatus unless otherwise noted. TLC was performed by the ascending method on layers of Silica Gel H[5] of standard 0.25 mm. thickness supported on glass plates. Crude and purified specimens of the thalidomides were applied to the adsorbent in DMF, although D- and L-thalidomide could also be applied in ethyl acetate. Generally, 20- and 80-mcg. portions of each sample were spotted, and commercial DL-thalidomide and the starting material were used as reference compounds. At 80 mcg. the thalidomide spots were usually convex. Spots were detected with an ultraviolet lamp, which emits principally at 254 mμ, after the developed chromatograms had been sprayed with Ultraphor WT.[6] A spray of basic potassium permanganate was sometimes also used to detect nonabsorbing impurities. Unless otherwise indicated, either 95:5 (v/v) chloroform–methanol or 85:15 (v/v) benzene–methanol was used to develop the chromatograms. In these solvent systems L- and D-VII remain at the origin, but they migrate in 1:1 chloroform–methanol.

N^2-Benzyloxycarbonyl-L-isoglutamine (L-V)—*N*-Benzyloxycarbonyl-L-glutamic acid anhydride (L-IV) was prepared by the method of Bergmann and Zervas (48) and recrystallized from chloroform–ether (49): yields, 80–83%; m.p. 94–95°; $[\alpha]_D^{25}$ −43 to −45.0° (c = 10.0 in glacial acetic acid).

[4] The authors are indebted to Drs. W. J. Barrett, W. C Coburn, Jr., and P. D. Sternglanz of the Analytical and Physical Chemistry Division of this Institute for spectral determinations and elemental analyses; to Mrs. Frances Dean for optical rotations; and to Misses Kathleen Hewson and Imogene Baswell for thin-layer chromatographic determinations.

[5] Brinkmann Instruments Inc., Westbury, N. Y.

[6] An optical whitening agent by BASF Colors and Chemicals, Inc., Charlotte, N. C.

Lit. values $[\alpha]_D$, m.p.: −44.1°, 94° (48); −43°, 91–92° (49); −45.1° (50). Although the melting point and optical rotation of a batch of commercial *N*-benzyloxycarbonyl-L-glutamic acid (L-III) were in agreement with values reported in the literature, partially racemized anhydride was obtained unless the commercial material was purified as follows. A solution of 98 Gm. of commercial *N*-benzyloxycarbonyl-L-glutamic acid in 600 ml. of ethyl acetate was washed twice with 100-ml. portions and 4 times with 50-ml. portions of water, dried with magnesium sulfate, and diluted with 2.5 L. of petroleum ether. The white precipitate was washed twice on a filter with 500-ml. portions of petroleum ether: yield, 83.5 Gm.; m.p. 120–122°; $[\alpha]_D^{25}$ −7.8° (c = 3.00 in glacial acetic acid).

The procedure of Bergmann and Zervas (48) for the preparation of L-V was modified. Anhydrous ammonia was introduced during approximately 30 min. into a stirred solution (protected from atmospheric moisture with a tube of calcium sulfate) of 84 Gm. of the anhydride (L-IV) in 1 L. of freshly distilled chloroform, 500 ml. of anhydrous ether, and 150 ml. of dry, purified dioxane at 0°. The mixture was stirred at 0° for an additional 75 min., the solvents were removed *in vacuo* at 30–40°, the residue was dissolved in 700 ml. of water, and the aqueous solution was acidified to pH 3.4 at 0°. The precipitated product was washed four times with 300-ml. portions of water and recrystallized from 1.8 L. of water: yield, 54.6 Gm. (61%); m.p. 174–175°; $[\alpha]_D^{25}$ −7.1° (c = 2.00 in methanol). Lit. m.p. 175° (48), 174.5–175° (51); $[\alpha]_D^{19}$ −6.0° (methanol) (51).

N^2-Benzyloxycarbonyl-D-isoglutamine (D-V)—*N*-Benzyloxycarbonyl-D-glutamic acid (D-III: m.p. 122°; $[\alpha]_D^{25}$ +7.7°, c = 10.0 in glacial acetic acid) was prepared by the method of Klieger and Gibian (52) and purified by the procedure used for the L-isomer. It was converted *via* *N*-benzyloxycarbonyl-D-glutamic anhydride (52) (D-IV: 84% yield; m.p. 92–93°; $[\alpha]_D^{25}$ +42.6°, c = 10.0 in glacial acetic acid) to D-V (56% yield; m.p. 174°; $[\alpha]_D^{25}$ +6.1°, c = 1.96 in methanol) by the procedures used in the preparation of the L-isomer. Klieger and Gibian report specific rotations of +7.6° and +43.1° for D-III and D-IV, respectively.

D-Isoglutamine (D-VI)—A solution of 8.0 Gm. of D-V, 680 ml. of 50% aqueous methanol, and 2 ml. of acetic acid was prepared and was treated with hydrogen in the presence of 5% palladium-charcoal catalyst (2 Gm.) at atmospheric pressure with stirring for 2 hr. (Slight warming may be required to prepare the 50% methanol solution of D-V, and rewarming to 35–40° during the early part of the hydrogenation may be necessary if some precipitation occurs.) The system was flushed with fresh hydrogen after 15- and 75-min. intervals. The filtrate from the catalyst was evaporated to dryness *in vacuo* at 25–30°, and the residue was dissolved in 25 ml. of water and reprecipitated with 250 ml. of acetone. The white precipitate was collected by filtration from the chilled mixture, washed with acetone, and dried *in vacuo* over phosphorus pentoxide at room temperature: yield, 3.78 Gm. (90%); m.p. 188°; $[\alpha]_D^{22}$ −20.6° (c = 5.39 in water). Recrystallization of 2.1 Gm. of crude product from water (20 ml.) and acetone (120 ml.) gave 1.95 Gm. (93% recovery); m.p. 188°; $[\alpha]_D^{22}$ −21.3° (c = 5.41 in water).

Anal.—Calcd. for $C_5H_{10}N_2O_3$: C, 41.09; H, 6.90; N, 19.17. Found: C, 41.17; H, 6.94; N, 19.03.

L-Isoglutamine (L-VI) was prepared from L-V by the procedure used for D-VI: yield, 89%; m.p. 187° (Heizbank), 178–179° (capillary); $[\alpha]_D^{22}$ +20.6°(c = 5.4 in water). Lit. m.p. 186–186.5° (51); $[\alpha]_D^{22}$ + 21.1° (48), + 21.2° (51).

N^2-Phthaloyl-L-isoglutamine (L-VII)—To a cold (0–5°) solution consisting of 6.9 Gm. (47 mmoles) of L-isoglutamine (L-VI), 7.0 Gm. (56.5 mmoles) of sodium carbonate monohydrate, and 100 ml. of water was added 14.6 Gm. (66.6 mmoles) of *N*-ethoxycarbonylphthalimide (18), and the resulting suspension was stirred vigorously for 10 min. at 0–5°. The initial pH was approximately 9. The ice bath was removed, and the mixture was stirred for 50 min. as it warmed to room temperature. The unreacted *N*-ethoxycarbonylphthalimide was removed by filtration, the filtrate was acidified at 0° to pH 4 with concentrated hydrochloric acid, and the solution was allowed to stand (for about 10 min.) until a white precipitate began to form. The mixture was then acidified further to pH 2, stirred at 0–5° for 1 hr., and filtered. The precipitate was washed with three 20-ml. portions of water at 0–5° and dried *in vacuo* over phosphorus pentoxide at room temperature. A solution of the product (m.p. 150–151°) in 125 ml. of hot ethanol was diluted with 100 ml. of petroleum ether (b.p. 30–60°), cooled slowly, and stored at 0–5° for 2 hr. The recrystallized product was collected by filtration, washed with petroleum ether, and dried as before: yield, 8.3 Gm. (63%); m.p. 151–152° and 158–160°; $[\alpha]_D^{25}$ −27±0.5°(c = 2 in DMF).

Anal.—Calcd. for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14. Found: C, 56.46; H, 4.42; N, 10.11.

In view of the facile formation of thalidomide from L-VII by methods described below, the product of this reaction was examined by TLC (95:5 chloroform–methanol). At the high spotting level of 500 mcg. a trace amount of thalidomide was detected in the crude product, but not in the recrystallized sample. Schröder and Klieger (41) reported a melting point of 122–124° and did not give $[\alpha]_D$. We have sometimes observed melting of apparently pure samples in this temperature range or lower. For example, one specimen melted at 110–120°, resolidified, and remelted at 150°; the analytical data (C, 56.41; H, 4.40; N, 10.24) and rotation (−27.2°) were satisfactory. Most specimens would melt at 150–152° (Heizbank or capillary) and, when induced to resolidify, would remelt at 158–161°.

The procedure described above was similar to that used by Nefkens, Tesser, and Nivard (18) to prepare phthaloyl-L-glutamic acid. The studies of Williams (45) on the stability of DL-thalidomide and those of Tipson (20) on phthaloyl-L-glutamic acid suggested that reopening of the phthalimide ring might occur in some degree in the basic solution. Therefore, a similar experiment was performed in a less basic solution in which the initial, millimolar proportions of L-isoglutamine, *N*-ethoxycarbonylphthalimide, and sodium carbonate monohydrate were 10:13.8:1.58 in 25 ml. of water. The pH of the mixture at the beginning was 7.4, and solid sodium carbonate monohydrate (6.22 mmoles) was added in portions

so as to maintain the pH at 7.3–7.7. The reaction time after the removal of the mixture from the ice bath was 70 min. This modified procedure did not give improved results; the product, isolated as before, was obtained in 56% yield; m.p 150°; $[\alpha]_D^{25}$ −27.2°(c = 2 in DMF).

N^2-Phthaloyl-D-isoglutamine (D-VII) was prepared by the first procedure described above: yield, 64%; m.p. 151°; $[\alpha]_D^{25}$ + 26.5 ± 0.5° (c = 2.0 in DMF). Found: C, 56.79; H, 4.09; N, 10.06. The infrared spectrum was identical with that of the L-isomer.

L-Thalidomide (L-N-(2,6-Dioxo-3-piperidyl)phthalimide) (L-IX)—A solution of 2.00 Gm. (7.25 mmoles) of phthaloyl-L-isoglutamine (L-VII) in 50 ml. of dry DMF was cooled to −5 to 0° in an ice-salt bath. To the cold solution, which was under a current of nitrogen and was protected from moisture with a tube of calcium sulfate, 0.65 ml. (9 mmoles) of thionyl chloride was added dropwise with vigorous stirring during 4 min.[7] The solution was then stirred at −5 to 0° for 1 hr. and poured slowly (4 min.) into a mechanically stirred mixture of 1.78 Gm. (18.1 mmoles) of potassium acetate, 150 ml. of ice-cold water, and 75 Gm. of crushed ice. The white precipitate was collected after 30 min. of stirring, washed with three 10-ml. portions of cold water, and dried *in vacuo* over phosphorus pentoxide at room temperature: yield, 1.63 Gm. (87%); $[\alpha]_D^{25}$ −65.5°(c = 2.00 in dry DMF). Values of $[\alpha]_D^{25}$ (c = 2 in dry DMF) of the products of several experiments in which the reaction times were 0.5–2 hr. were 64 ± 1.5°; melting temperatures were similar to those of purified specimens.

Specimens of L-thalidomide were purified by reprecipitation from 1:4 (v/v) DMF–water[8] as follows. A solution of a specimen of L-thalidomide (55–65 mg./ml.) in dry DMF was filtered and chilled to −5 to 0°. Cold water (4 volumes) was introduced dropwise with vigorous mechanical stirring at a rate (20–40 min.) that kept the temperature at 0 ± 4°. The mixture was stirred at 0° for an additional 0.5 hr.; and the precipitate was collected on a filter, washed with cold water, and dried *in vacuo* over phosphorus pentoxide at room temperature. The recovery after each reprecipitation was 90–94%. The product obtained from the reaction described above was recrystallized three times from DMF–water, the first recrystallization being performed on the same day that the reaction was performed. The optical rotation was essentially unchanged after the first reprecipitation; $[\alpha]_D^{25}$ −64.6 ± 0.5° (c = 2.00 in DMF).

Anal.—Calcd. for $C_{13}H_{10}N_2O_4$: C, 60.46; H, 3.91; N, 10.85. Found: C, 60.63; H, 3.73; N, 10.94.

D-, L-, and DL-Thalidomide (applied in DMF) moved identically and near the front on thin-layer plates of silica gel developed in 95:5 chloroform–methanol or in 85:15 benzene–methanol. DMSO-d_6 solutions (prepared without heating) of D-, L-, and DL-thalidomide gave identical NMR spectra. The melting point observed for L-thalidomide depended on the techniques used in the determination, the variability being due evidently to thermal racemization. Pure specimens melted at 244–245° when sprinkled along the gradiently heated bar of a Kofler Heizbank apparatus. Specimens that melted in this range would resolidify and remelt at a higher temperature, usually near the melting point of DL-thalidomide. Melting in the region of 240–250° could be observed in a capillary tube, provided that the specimen was inserted near its melting point.

Reference infrared spectra recorded for specimens obtained by recrystallizing commercial DL-thalidomide from dioxane, from 1:4 DMF–water by the procedure described for the optical isomers, or from glacial acetic acid displayed minor differences. The spectra of an untreated commercial sample, the specimen from dioxane, and several specimens prepared by the cyanomethyl ester method (below) were all identical with a subsequently published spectrum (43). The spectra of L- and D-thalidomide were identical with the published spectrum of DL-thalidomide except that a broad, weak band near 860–850 cm.$^{-1}$ in the spectrum of DL-IX was replaced by a weak, poorly resolved doublet near 850 and 830 cm.$^{-1}$ in the spectra of the optical isomers. The spectra of specimens of DL-IX (either commercial or prepared from L-II or L-VIII) that had been recrystallized from DMF–water (by the procedure described for the optical isomers) differed somewhat more from the spectra of the optical isomers, although the spectra were very similar. The principal differences have already been listed (23); the difference in the 860–800 cm.$^{-1}$ region was more pronounced since the band near 850 cm.$^{-1}$ was considerably weaker in the spectrum of DL-IX recrystallized in this way. Differences between the spectra of D- or L-IX and DL-IX are undoubtedly due to the fact that all spectra were determined in the solid state.

D-Thalidomide (D-IX) was prepared by the thionyl chloride–DMF procedure described for the L-isomer: yield of crude product, 85%; m.p. (Kofler Heizbank), 243°; $[\alpha]_D^{25}$ + 63.2° (c = 2.00 in DMF). The D-isomer was reprecipitated from DMF–water by the procedure described for the L-isomer: m.p. 244–245° (variable as for the L-isomer); $[\alpha]_D^{25}$ + 64 ± 0.5° (c = 2.00 in dry DMF). The infrared spectrum was identical with that of L-IX.

Anal.—Found: C, 60.52; H, 3.68; N, 10.76.

Optical Rotatory Stability of L-Thalidomide and L-VII—Since it was considered possible that trace amounts of water in DMF solutions of thalidomide and L-VII might effect some hydrolysis, observations of the specific rotation over a period of time were first made with solutions prepared from DMF containing small amounts of water. The amount of water was determined by vapor-phase chromatography. A solution of a recrystallized specimen of L-thalidomide in DMF, containing less than 0.1% water, was allowed to stand for 18 hr. The initial (−63.4°, determined within 20 min. of the addition of DMF) and the final (−63.2°) rotations (c = 2.0) were in agreement within experimental error. Addition of sufficient water to form an 80% (v/v) DMF solution caused the rotation to drop to −61.4°. This change was presumably due to the change of solvents (DMF to 80% DMF) since values of $[\alpha]_D^{25}$ were in the range of 60.9 ± 0.5° during the next 43 hr., at which time the experiment was dis-

[7] Another experiment was identical except that the thionyl chloride was first dissolved in a small portion of DMF, and this solution was then added similarly. Both the yield (20%) and the optical rotation (−60°) were lower.

[8] A decrease in optical rotation was observed after a crude specimen of L-thalidomide had been recrystallized from acetic acid. Dioxane gave a low recovery when used to recrystallize DL-thalidomide.

continued. These data indicate little, if any, degradation. In contrast, a specimen of L-thalidomide obtained directly from a thionyl chloride–DMF procedure showed a decrease in $[\alpha]_D^{25}$ (c = 2) in dry DMF (less than 0.01% water, distilled from calcium hydride and dried further over molecular sieves) from an initial value of −63.5° (determined 5–10 min. after the addition of DMF) to the following values (hr. after initial determination in parentheses): −60.1°(7), −52.8°(24), −47.6°(96), −46.7°(103), −46.4°(119). Values of $[\alpha]_D^{25}$ (c = 1.6) for a solution of the same specimen of L-thalidomide in 80% DMF decreased from an initial value of −61.7° as follows (hr. in parentheses): −58.8°(1), −56.8°(2), −54.7°(3), −52.6(4), −51.3°(6), −43.3°(24), −42.4°(29.5), −39.5°(94), −39.4(118). The infrared spectra of the residues recovered (by evaporation of the solvents *in vacuo*) from the last two solutions after approximately 5 days were intermediate between that of L-thalidomide and that of DL-thalidomide recrystallized from DMF–water.

The specific rotations of a specimen of N^2-phthaloyl-L-isoglutamine (L-VII) in dry DMF (less than 0.01% water) and in 80% (v/v) DMF were within the range of −26.5 ± 0.2° (c = 2.0) and 26.8 ± 0.2° (c = 1.6), respectively, during a 24-hr. observation period.

N^2-Phthaloyl-L-glutamine Cyanomethyl Ester (L-II)—Chloroacetonitrile (1.63 ml.) was added to a mixture (protected from atmospheric moisture with a tube of calcium sulfate) consisting of 3.0 Gm. of phthaloyl-L-glutamine (L-I) (18, 39), 1.6 ml. of anhydrous triethylamine, and 10 ml. of purified, anhydrous dioxane. The reaction mixture was kept at 60°, with stirring, for 1 hr. The solid phase dissolved, and a new solid later precipitated. The reaction mixture was chilled in an ice bath; and the precipitate was separated by filtration, washed with several portions of water, and dried *in vacuo* over phosphorus pentoxide at room temperature: yield, 1.5 Gm. (44%); m.p. 189–190°; $[\alpha]_D^{25}$ −46.4° (c = 2.01 in DMF). A specimen was recrystallized twice from dioxane: m.p. 189–190°; $[\alpha]_D^{25}$ −46.6° (c = 1.98 in DMF).

Anal.—Calcd. for $C_{15}H_{13}N_3O_5$: C, 57.14; H, 4.16; N, 13.33. Found: C, 57.38; H, 4.00; N, 13.12.

N^2-Phthaloyl-L-isoglutamine Cyanomethyl Ester (L-VIII) and Thalidomide from Phthaloyl-L-isoglutamine (L-VII)—A solution of 4.0 Gm. of L-VII 3.2 ml. of triethylamine, 3.2 ml. of chloroacetonitrile, and 20 ml. of purified, anhydrous dioxane was stirred at 60–65° for 4 hr. Examination of aliquots from an earlier experiment by TLC had shown that a considerable quantity of L-VII remained after 1.25 hr. The mixture, containing a precipitate, was poured into 150 ml. of ether; the new mixture was stirred at 0° for 30 min.; the ether was decanted; the gummy residue was treated again with ether (50 ml.), which was also decanted; and the flocculent residue was stirred at 0° with 150 ml. of water. After the solid had been separated by filtration, washed with water, and dried *in vacuo* at room temperature over phosphorus pentoxide, it weighed 3.2 Gm.; m.p. 163° to a cloudy liquid; $[\alpha]_D^{25}$ −35° (c = 2.0 in DMF). TLC (85:15 benzene–methanol) indicated that this fraction was a mixture of L-VIII and thalidomide; the latter presumably was formed from L-VIII.

Evaporating the solvent from the ether decantates and stirring the residue with cold water gave 0.4 Gm. of solid (m.p. 238–244°, $[\alpha]_D^{25}$ −56° in DMF) that was shown by TLC (1 spot) and infrared spectroscopy to be thalidomide.

Recrystallization of the first fraction (3.2 Gm.) from dioxane–petroleum ether gave a first crop of 1.9 Gm. (m.p. 170°, $[\alpha]_D^{25}$ −26.8°) and a second crop, by further dilution with petroleum ether, of 701 mg. of impure thalidomide (m.p. 230–250°, $[\alpha]_D^{25}$ −42.6°), which was identified by TLC. Recrystallization of the first crop (1.9 Gm.) from dioxane–petroleum ether gave 1.5 Gm. of L-VIII: m.p. 173–174°; $[\alpha]_D^{25}$ −25.3° (c = 2.00 in dry DMF); TLC (85:15 benzene–methanol), 1 spot.

Anal.—Calcd. for $C_{15}H_{13}N_3O_5$: C, 57.14; H, 4.16; N, 13.33. Found: C, 57.22; H, 4.31; N, 13.25.

DL-Thalidomide from the Cyanomethyl Esters (L-II and L-VIII)—A solution of 368 mg. (1.17 mmoles) of phthaloyl-L-glutamine cyanomethyl ester (L-II) in 5 ml. of dry dimethyl sulfoxide was added dropwise during 10 min. to a stirred mixture of 63 mg. (1.17 mmoles) of sodium methoxide and 10 ml. of dry dimethyl sulfoxide at 20°. The dimethyl sulfoxide had been distilled from calcium hydride, and the reaction mixture was protected from atmospheric moisture by a stream of nitrogen and a calcium sulfate drying tube. After the addition had been completed, 0.1 ml. of glacial acetic acid was added to neutralize the base and the solution was concentrated *in vacuo* at room temperature to a paste. Addition of 20 ml. of water produced a solid that was collected by filtration, washed with water, and dried *in vacuo* at room temperature: yield, 212 mg. (70%); m.p. 269–273° (cap.); $[\alpha]_D^{25}$ 0.0°(c = 2.06 in dioxane).

In another experiment the amount of sodium methoxide was reduced to 13% on a molar basis, and the appearance of thalidomide and the disappearance of the cyanomethyl ester (L-II) were monitored by TLC (9:1 chloroform–methanol). The disappearance of L-II was essentially complete within 3 hr.; and thalidomide, isolated by the procedure described above, was obtained in 80% yield. It also was racemic.

Treatment of phthaloyl-L-isoglutamine cyanomethyl ester (L-VIII) with sodium methoxide in dimethyl sulfoxide likewise caused racemization. DL-Thalidomide was isolated in 78% yield from an experiment, analogous to the first experiment described above, in which L-VIII was added to an equimolar amount of sodium methoxide during 10 min. From an experiment in which L-VIII was treated with 16 mole percent of sodium methoxide for 2.5 hr. by the second procedure described for L-II, DL-thalidomide was also obtained in 78% yield.[9] Isolation of thalidomide from both of these experiments was effected by pouring the reaction solutions into a mixture of crushed ice and water. All samples of DL-thalidomide obtained from L-II and L-VIII were identified by their melting points and infrared spectra (compared with commercial material), by TLC, and by the absence of optical rotation in DMF or dioxane.

[9] The reaction time employed in this experiment was similar to that used for L-II. Since L-VIII cyclizes more readily, a much shorter reaction time might be adequate and might afford optically active thalidomide.

Comparison of L-I and L-VII as Precursors of L-Thalidomide—*Thionyl Chloride–DMF Method*—In identical experiments carried out simultaneously, phthaloyl-L-glutamine (L-I) and phthaloyl-L-isoglutamine (L-VII) were treated with thionyl chloride in DMF. The procedures were identical with that described above for the preparation of L-thalidomide except that the reactions were allowed to proceed for 2 hr. From L-VII, L-thalidomide was obtained in 76% yield; $[\alpha]_D^{25}$ −64.5° (c = 2.00 in DMF). From L-I, only a small quantity of a pink solid was obtained (by exactly the same isolation procedure) $[\alpha]_D^{25}$ −83° (c = 1.0 in DMF). This material contained sulfur (5.9%), and TLC revealed the presence of 2–3 components, one of which was thalidomide. The amount (150 mg. from 1.600 Gm. of L-I) corresponded to a yield of 10%, calculated as thalidomide. A second crop amounted to 12 mg., and finally a third portion of 173 mg. (11.5%) of material shown by its infrared spectrum and by TLC (2 spots) to be impure thalidomide was isolated from the filtrate residue.

Another pair of identical experiments in which the reaction time was 0.5 hr. gave similar results in that L-VII gave L-thalidomide (80% yield, $[\alpha]_D^{25}$ −64.2°, c = 2.00 in DMF), whereas L-I afforded only a small amount (9% calculated as thalidomide) of material that was similar [judged by its infrared spectrum, optical rotation (−88°), and thin-layer chromatogram] to the crude product of the 2-hr. reaction.

N,N′-Carbonyldiimidazole Method—To a solution of 1.265 Gm. of L-VII in 40 ml. of dry DMF (distilled from calcium hydride) was added 0.810 Gm. (91% pure by gas chromatographic determination of liberated carbon dioxide) of *N,N′*-carbonyldiimidazole (40). The reaction solution (protected from moisture by calcium sulfate) was stirred in an atmosphere of nitrogen for 96 hr. at room temperature. Glacial acetic acid (0.34 ml.) was added to the solution, the mixture was concentrated *in vacuo* at room temperature to a viscous oil, and the mixture resulting from the addition of 20 ml. of water was stirred at 0° for 30 min. A white precipitate was removed by filtration, washed with water, and dried *in vacuo* at room temperature: yield, 491 mg. (41%); $[\alpha]_D^{25}$ −57.9° (c = 1.99, DMF). Two reprecipitations from DMF–water (1:4) effected a modest increase in the specific rotation; $[\alpha]_D^{25}$ −59° (c = 2 in DMF).

An identical experiment with phthaloyl-L-glutamine (L-I) afforded DL-thalidomide in 30% yield. The infrared spectrum was identical with that of a commercial specimen. Recrystallization of the DL-thalidomide obtained from L-I from DMF–water (1:4) gave a specimen, m.p. 274–276° (cap.) with an infrared spectrum identical with that of the commercial specimen after it had been recrystallized in the same way; m.p. 275–276° (cap.).

Cyanomethyl Ester Method—As shown above, the cyanomethyl esters of both phthaloyl-L-glutamine and phthaloyl-L-isoglutamine were converted to DL-thalidomide by sodium methoxide in DMSO. In view of the fact that optically active thalidomide was isolated during the preparation of L-VIII, a weaker base was tried. A solution (under a nitrogen atmosphere and protected from moisture with a tube of calcium sulfate) of 315 mg. (1 mmole) of L-VIII, 16.5 ml. of dry DMSO, and 3.5 ml. of a solution containing 0.04 ml. of triethylamine per ml. of DMSO (1 mmole of triethylamine) was stirred at 34–37° for 72 hr. The formation of thalidomide was monitored by TLC (85:15 benzene–methanol). The solution was neutralized with glacial acetic acid and poured into a mixture of 40 Gm. of water and 40 Gm. of crushed ice. After 30 min. of stirring, the product was collected on a filter, washed with water, and dried *in vacuo* at room temperature: yield, 189 mg. (73%, not corrected for the removal of aliquots for TLC); $[\alpha]_D^{25}$ −45.3° (c = 2.02, DMF); TLC showed only thalidomide. The infrared spectrum was identical with that of L-thalidomide except that the doublet at 850 and 830 cm.$^{-1}$ in the L-thalidomide spectrum was replaced by a band at 850 cm.$^{-1}$ with a shoulder at 830 cm.$^{-1}$.

An identical experiment with the glutamine derivative (L-II) caused racemization without appreciable cyclization. Phthaloyl-DL-glutamine cyanomethyl ester was isolated by pouring the reaction solution into 40 ml. of a mixture of water and crushed ice: yield, 263 mg. (84%); m.p. 176–177°; $[\alpha]_D^{25}$ 0.0° (c = 2.02, DMF). This material and the starting material (L-isomer) traveled together on thin-layer plates (9:1 chloroform–methanol) and gave very similar infrared spectra.

Anal.—Calcd. for $C_{15}H_{13}N_3O_5$: C, 57.14; H, 4.16; N, 13.33. Found: C, 56.93; H, 3.95; N, 13.22.

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Keyphrases

Thalidomide, D and L—synthesis
TLC separation
Optical rotation—identity
IR spectrophotometry—structure

Study of Absorption, Translocation, and Residue Properties of 2,3,5-Triiodobenzoic Acid in Field-Grown Soybeans

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The synthesis of carboxyl-^{14}C-2,3,5-triiodobenzoic acid is described. The translocation, biological half-life, and residues remaining in the plant after application to field-grown soybeans are discussed.

INCREASED KNOWLEDGE of plant metabolism has led to a search for chemicals which stimulate or inhibit the growth of plants. One such chemical is 2,3,5-triiodobenzoic acid, commonly referred to as TIBA. TIBA has been shown by Galston (1) to decrease apical dominance and to affect the flowering of soybean plants, causing an increased number of flowers. Anderson (2) reported that the result of several combined effects is an increased yield of soybeans per acre, making TIBA a useful agricultural chemical. However, since soybean products are used in animal feeds and for human consumption, the residue and metabolism properties of TIBA must be determined prior to general usage.

Wheeler and Johns (3) reported the first synthesis of TIBA by reacting anthranilic acid with iodine monochloride. The product was diazotized, and allowed to react with potassium iodide yielding TIBA. Olivier and Combe (4) were able to obtain 95% of the theoretical yield of TIBA by making minor changes in the method of Wheeler and Johns (3). Munakata and Nakai (5), and Ice, Breckinridge, and Christian (6) described the synthesis of 2(^{131}I),3,5-triiodobenzoic acid. They followed closely the procedure of Olivier and Combe (4) substituting sodium iodide-131 for potassium iodide. Jarboe (7) synthesized 2,3(^{131}I),5(^{131}I)-triiodobenzoic acid by substituting iodine-131 monochloride for iodine monochloride.

EXPERIMENTAL

Synthesis—To facilitate tracing the TIBA molecule through the entire growth period of the soybean plant from the flowering to the bean stage, radioactive TIBA was synthesized containing carbon-14 in the carboxyl position. One hundred millicuries (2.584 Gm.) of anthranilic acid[1] ($C_6H_4{}^{14}CO_2H$-2-NH_2), was suspended in 60 ml. of

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[1] Volk Radiochemical Co., Inc., New York, N. Y.