Synthesis of 2,6-Dioxo-3-phthalimidopiperidine-3,4,4,5,5-d_5 and 2,5-Dioxo-3-phthalimidopyrrolidine-3,4,4-d_3 from L-Deuterio-Glutamic Acid and L-Deuterio-Aspartic Acid

By HAROLD J. RHODES, SEN MAW FANG, and MARTIN I. BLAKE

The isolation and characterization of L-deuterio-glutamic acid and L-deuterio-aspartic acid from the ionic fraction of the hydrolysate of the algae *Scenedesmus obliquus* are described. These deuteriated amino acids were employed for the synthesis of 2,6-dioxo-3-phthalimidopiperidine-3,4,4,5,5-d_5 (VIII) and 2,5-dioxo-3-phthalimidopyrrolidine-3,4,4-d_3 (VII) which are analogs of thalidomide. The following sequence of reactions was utilized: phthaloylation with *N*-carbethoxyphthalimide, heating with acetic anhydride, and heating with urea.

THE MASS CULTURING of a variety of algae (1) in 99.6% deuterium oxide has made available a source of supply of fully deuteriated sugars and amino acids. The isolation and characterization of deuterio-mannose[1] and deuterio-glucose from the nonionic fraction and L-deuterio-aspartic acid, deuterio-glycine, L-deuterio-alanine, and L-deuterio-glutamic acid from the ionic fraction of the algae hydrolysate have been reported (2). The deuteriated sugars have been employed as carbon sources in the cultivation of heterotrophic organisms (3–5). Li *et al.* (6) studied the deuterium isotope effects on the rate of mutarotation of fully deuteriated glucose and mannose. The deuterium isotope effects in the nonenzymatic transamination of L-deuterio-alanine (7) and L-deuterio-glutamic acid (8) have been noted.

In the present study L-deuterio-glutamic acid and L-deuterio-aspartic acid were isolated from the ionic fraction of the hydrolysate of the algae *Scenedesmus obliquus*. They then were employed as starting compounds in the synthesis of 2,6-dioxo- 3 - phthalimidopiperidine - 3,4,4,5,5 - d_5 and 2,5 - dioxo - 3 - phthalimidopyrrolidine - 3,-4,4-d_3 according to Scheme I. Inasmuch as these compounds are the partially deuteriated forms of thalidomide and *N*-phthalylaspartimide (9, 10), respectively, they should prove of value in the study of the effect of deuteriation on the pharmacodynamic and pharmacokinetic properties of medicinal agents.

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[1] The prefix "deuterio-" as used in this paper indicates that nonexchangeable hydrogen atoms have been replaced with deuterium atoms.

EXPERIMENTAL[2]

Isolation and Characterization of L-Deuterio-Glutamic Acid and L-Deuterio-Aspartic Acid.—The ionic fraction[3] from the hydrolysate of residual cell wall of *S. obliquus* grown in deuterium oxide (2) served as the source material for L-deuterio-glutamic acid and L-deuterio-aspartic acid. Isolation of these amino acids was effected through a two-step procedure utilizing the following ion-exchange resins: Dowex 50-X8 (chromatographic grade, 200–400 mesh) for concentration and desalting, and Dowex 1-X8 (chromatographic grade, 200–400 mesh) for elution analysis (11).

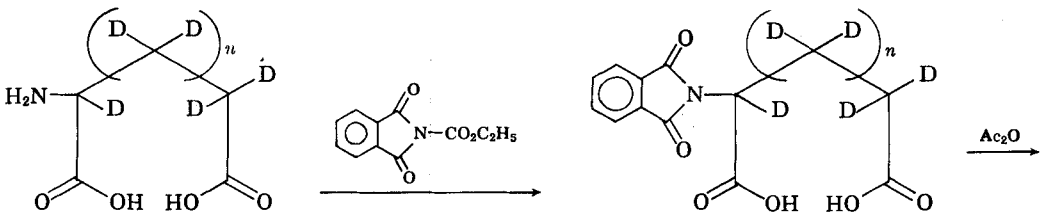
Dowex 50-X8 (80 Gm.) in the acid form was suspended in a sufficient quantity of distilled water to allow removal of fines by decantation and then packed as a slurry into a chromatographic column (1.8 × 40.0 cm.). The packed column then was washed with distilled water (500 ml.) until the effluent was neutral and free of residue.

The ionic fraction from *S. obliquus*, obtained as a thick syrup (50 ml.), subsequently was loaded on the column and washed with distilled water for 4 days at a flow rate of 60 ml./hr. The amino acids then were eluted from the column with 5% NH_4OH. The effluent was collected in 50-ml. portions at a flow rate of 50 ml./hr. on an automatic fraction collector. Fractions 4 through 6 yielded a ninhydrin-positive light brown residue when evaporated to dryness at room temperature.

Dowex 1-X8 (2 Kg.) in the chloride form was freed of fines in the manner described previously and packed as a slurry into a chromatographic column (5.5 × 107.0 cm.). The resin then was washed with 2 *N* sodium acetate (12 L.) at a flow

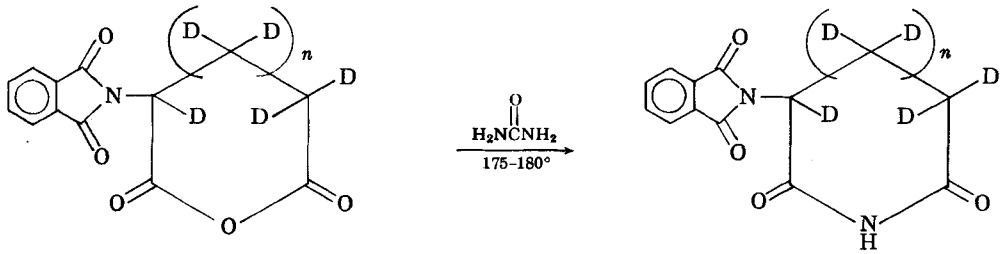
[2] All melting points were determined in a modified Hershberg apparatus and are corrected. Infrared spectra were obtained in Nujol mull with either a Perkin-Elmer 137B spectrophotometer or a Beckman model IR4 spectrophotometer. NMR spectra of the samples in either DCl–D_2O or pyridine solution were measured at 60 Mc/sec. with a Varian A-60 instrument, employing tetramethylsilane as the internal standard. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill.

[3] The authors thank J. J. Katz and H. L. Crespi, Chemistry Division, Argonne National Laboratory, Argonne, Ill., for supplying the ionic fraction used in this study.



I, n = 0 (L-Deuterio-aspartic acid)
II, n = 1 (L-Deuterio-glutamic acid)

III, n = 0 (N-Phthalyl-L-aspartic acid-2,3,3-d_3)
IV, n = 1 (N-Phthalyl-L-glutamic acid-2,3,3,4,4-d_5)



V, n = 0 (N-Phthalyl-L-aspartic anhydride-2,3,3-d_3)
VI, n = 1 (N-Phthalyl-L-glutamic anhydride-2,3,3,4,4-d_5)

VII, n = 0 (2,5-Dioxo-3-phthalimidopyrrolidine-3,4,4-d_3)
VIII, n = 1 (2,6-Dioxo-3-phthalimidopiperidine-3,4,4,5,5-d_5)

Scheme I

rate of 90 ml./hr., and finally with distilled water (4 L.) until the effluent was neutral and free of residue.

Fractions 4 through 6 were dissolved in 0.5 N acetic acid (100 ml.) and transferred to the Dowex 1-X8 column. Fractionation of the mixture was then effected by elution with 0.5 N acetic acid. The effluent was collected in 200-ml. portions on an automatic fraction collector at a flow rate of 50 ml./hr. On evaporation to dryness at room temperature, fractions 19 through 25 and 51 through 60 yielded L-deuterio-glutamic acid and L-deuterio-aspartic acid, respectively, as white crystalline residues. Preliminary identification of the deuterio-amino acids was based on comparison of their R_f values with those of the corresponding protio-amino acids, obtained by descending paper chromatography on Whatman No. 1 paper with the monophasic solvent system, 1-butanol–acetic acid–water (12:3:5) (12). After development for 18 hr. in a Chromatocab, the papers (18.25 × 22.5 in.) were dried at room temperature before being sprayed with freshly prepared ninhydrin reagent (1.0 Gm. of ninhydrin, 10 ml. of pyridine, 500 ml. of acetone). Color development was allowed to take place at room temperature. The R_f values for the protio-amino acids, L-glutamic acid and L-aspartic acid, were 0.28 and 0.23, respectively. Lower R_f values were obtained with the deuterio- amino acids: 0.26 for L-deuterio-glutamic acid and 0.21 for L-deuterio-aspartic acid. Samples from the combined fractions, 19 through 25 and 51 through 60, when passed through a Spinco 120 amino acid analyzer, showed only one peak; in each case, the peak obtained corresponded with that obtained with the protio-amino acids.

Analytical samples were prepared by crystallizing the corresponding combined residues from water and drying the first crops in an Abderhalden drying pistol over P_2O_5 under reduced pressure at 100° for 5 hr.

Pertinent physical properties and analysis results are summarized in Table I.

Suitable semimicro synthetic procedures were developed using L-protio-glutamic and L-protio-aspartic acids. Characterization of the protio-intermediates and final products was based on a comparison of melting points and infrared spectra with those cited in the literature (13, 14). In the case of 2,6-dioxo-3-phthalimidopiperidine,[4] a mixed melting point determination with an authentic sample showed no depression.

TABLE I.—PHYSICAL PROPERTIES AND ANALYSIS RESULTS FOR DEUTERIATED AMINO ACIDS

	L-Deuterio-Aspartic Acid $C_4H_4D_3NO_4$	L-Deuterio-Glutamic Acid $C_5H_4D_5NO_4$
M.p.	270° dec.	208–209° dec.
C, %		
Calcd.	35.30	39.46
Found	35.79	39.70
H + D, %		
Calcd.	7.40	9.27
Found	7.49	8.93
N, %		
Calcd.	10.29	9.20
Found	10.12	9.00
I.R. spectra	3105 (NH)	3100 (NH)
$\nu_{max.}^{Nujol}$ cm.$^{-1}$	2640, 1585, 1250 (COO^-)	2630, 1585, 1260 (COO^-)
NMR spectra	Absorption peaks for CH and CH_2 absent	Absorption peaks for CH and CH_2 absent

[4] An authentic sample and its infrared spectrum were obtained through the courtesy of Dr. Henry M. Fales, Laboratory of Chemistry of Natural Products, National Heart Institute, U. S. Public Health Service, Bethesda, Md.

N-Phthalyl-L-glutamic Acid-2,3,3,4,4-d_5 (IV).—This compound was prepared by the method of Nefkens (13) with the following modifications. L-Deuterio-glutamic acid (500 mg., 3.29 mmoles) was first dissolved in aqueous 1.0 *M* sodium carbonate (5 ml., 5 mmoles). *N*-Carbethoxyphthalimide (793 mg., 3.61 mmoles) then was added portion-wise to the stirred mixture at room temperature. After the addition was completed, the reaction mixture was stirred for a period of 1.5 hr. at room temperature, then acidified with aqueous hydrochloric acid (36%) to a pH of 0, and finally cooled to 0°. The precipitated phase, after filtration and recrystallization from water and subsequent drying, yielded 713 mg. (61.2%) of *N*-phthalyl-L-glutamic acid-2,3,3,4,4-d_5, m.p. 160–162°. I.R. $\nu_{max.}^{Nujol}$ cm.$^{-1}$: 1710, 753 (phthalimido), 2536, 2510, 1280 (COOH).

Anal.—Calcd. for $C_{13}H_6D_5NO_6$: C, 55.30; H + D, 5.71; N, 4.96. Found: C, 55.85; H + D, 5.86; N, 4.73.

N-Phthalyl-L-glutamic Anhydride-2,3,3,4,4-d_5 (VI).—The anhydride was prepared by dissolving *N*-phthalyl-L-deuterio-glutamic acid (500 mg., 1.77 mmoles) in warm acetic anhydride (75 ml.), concentrating the solution under reduced pressure to a thin syrup, and adding anhydrous ether (20 ml.) (15). Trituration of the syrup under ether resulted in the formation of a white crystalline phase. After filtration and washing with anhydrous ether, the cake was recrystallized from acetic anhydride and anhydrous ether. The product, *N*-phthalyl-L-glutamic anhydride-2,3,3,4,4-d_5, crystallized out in the form of colorless prisms, m.p. 202–204°. The yield was 250 mg. (53.4%). I.R. $\nu_{max.}^{Nujol}$ cm.$^{-1}$: 1719, 775 (phthalimido), 1810, 1763 (anhydride).

Anal.—Calcd. for $C_{13}H_4D_5NO_5$: C, 59.06; H + D, 5.42; N, 5.30. Found: C, 58.76; H + D, 5.08; N, 5.15.

2,6 - Dioxo - 3 - phthalimidopiperidine - 3,4,4,5,5-d_5 (VIII).—A mixture of *N*-phthalyl-L-glutamic anhydride-2,3,3,4,4-d_5 (200 mg., 0.76 mmole) and urea (100 mg., 1.72 mmoles) was heated in an oil bath at a temperature of 175–180° for a period of 20 min. After cooling, the melt was crystallized from 95% ethyl alcohol. Further purification of this material by recrystallization from dioxane yielded 40 mg. (20.0%) of 2,6-dioxo-3-phthalimidopiperidine-3,4,4,5,5-d_5, m.p. 269–270°. I.R. $\nu_{max.}^{Nujol}$ cm.$^{-1}$: 1709, 760 (phthalimido), 3305, 3111 (CONH—). NMR spectra: Absorption peaks for CH_2 and aliphatic CH were absent.

Anal.—Calcd. for $C_{13}H_5D_5N_2O_4$: C, 59.29; H + D, 5.74; N, 10.66. Found: C, 58.77; H + D, 5.15; N, 10.70.

N-Phthalyl-L-aspartic Acid-2,3,3-d_3 (III).—A mixture of L-deuterio-aspartic acid (620 mg., 4.56 mmoles), *N*-carbethoxyphthalimide (1100 mg., 5.02 mmoles), and aqueous 1.0 *M* sodium carbonate (7.0 ml., 7.0 mmoles) was reacted and worked-up in the same manner as indicated for the preparation of compound IV. *N*-Phthalyl-L-aspartic acid-2,3,3-d_3 was obtained in a yield of 610 mg. (50.3%), m.p., 217–218° dec. I.R. $\nu_{max.}^{Nujol}$ cm.$^{-1}$: 1719, 760 (phthalimido), 2535, 2510, 1280 (COOH).

Anal.—Calcd. for $C_{12}H_6D_3NO_6$: C, 54.15; H + D, 4.54; N, 5.26. Found: C, 54.08; H + D, 4.57; N, 5.74.

N-Phthalyl-L-aspartic Anhydride-2,3,3-d_3 (V).—Except for the following modification, the procedure cited for the preparation of compound VI was followed. *N*-Phthalyl-L-deuterio-aspartic acid (480 mg., 1.80 mmoles) was dissolved in warm acetic anhydride (40 ml.) and then concentrated under reduced pressure to a syrup. The procedure was repeated twice with 40- and 50-ml. portions of acetic anhydride. The product, *N*-phthalyl-L-aspartic anhydride-2,3,3-d_3, crystallized in the form of small colorless needles from a mixture of acetic anhydride and anhydrous ether and melted at 216–217° dec. The total yield was 320 mg. (71.6%). I.R. $\nu_{max.}^{Nujol}$ cm.$^{-1}$: 1719, 745 (phthalimido), 1865, 1782 (anhydride).

Anal.—Calcd. for $C_{12}H_4D_3NO_5$: C, 58.07; H + D, 4.06; N, 5.64. Found: C, 57.54; H + D, 3.81; N, 5.60.

2,5 - Dioxo - 3 - phthalimidopyrrolidine - 3,4,4 - d_3 (VII).—A mixture of *N*-phthalyl-L-aspartic anhydride-2,3,3-d_3 (287 mg., 1.09 mmoles) and urea (135 mg., 2.25 mmoles) was reacted in the same manner as described in the preparation of compound VIII. 2,5-Dioxo-3-phthalimidopyrrolidine-3,4,4-d_3 was isolated as colorless prisms after two crystallizations from absolute ethanol in a yield of 60 mg. (21%), m.p., 206–207° dec. (10). I.R. $\nu_{max.}^{Nujol}$ cm.$^{-1}$: 1715, 755 (phthalimido), 3227, 3118 (—CONH—). NMR spectrum: absorption peaks for CH_2 and aliphatic CH were absent.

Anal.—Calcd. for $C_{12}H_5D_3N_2O_4$: C, 58.30; H + D, 4.48; N, 11.34. Found: C, 58.03; H + D, 4.69; N, 11.68.

DISCUSSION

The availability of metabolites containing deuterium atoms in nonexchangeable positions makes possible the preparation of a wide variety of pharmacologically active derivatives in which a substantial number of hydrogen atoms are replaced by deuterium atoms. For this investigation L-deuterio-glutamic acid and L-deuterio-aspartic acid were selected as starting compounds because of their relative abundance in the ionic fraction of the algae hydrolysate and the ease with which they can be fractionated by ion-exchange chromatography. In addition, there are a number of physiologically active compounds which can be synthesized conveniently from these amino acids. Partially deuteriated thalidomide (VIII) and its aspartic acid analog (VII) which contain deuterium atoms in the amino acid moiety where hydrogen is normally present were prepared. The methods of synthesis of the partially deuteriated drugs were identical to those employed in preparing the hydrogen compounds. These are illustrated in Scheme I. Comparable percentage yields were obtained for each in the synthesis. Characterization of the deuteriated intermediates and end products was based on analytical data (carbon, hydrogen + deuterium, nitrogen), infrared spectral data, melting points, and NMR spectral analysis.

Compounds containing a significant number of deuterium-to-carbon bonds should provide for a number of interesting studies. Since both 2,6-dioxo - 3 - phthalimidopiperidine and 2,5 - dioxo-3-phthalimidopyrrolidine elicit sedative and teratogenic activity (9, 10), the effect of deuterium substitution on these activities can be evaluated.

Such studies may provide information concerning the mechanism by which these agents produce their effects. It has been indicated that the rupturing of a carbon-to-deuterium bond is considerably more difficult than a carbon-to-hydrogen bond. Accordingly, if oxidation of carbon-to-deuterium bonds is involved in the metabolism of the drugs synthesized in this study, a pronounced effect on the pharmacological activity should be observed. Such studies are currently being undertaken.

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Effect of Certain Drugs on Perfused Human Placenta V

Use of α and β Adrenergic Blocking Agents to Determine the Specific Effector Site of Vasoactive Substances

By RALPH T. MANCINI and RONALD F. GAUTIERI

In this investigation pretreatment of placental vessels with both an α (phentolamine) and a β (dichloroisoproterenol, DCI) adrenergic blocking agent was performed to determine the specific nature of the effector site upon which epinephrine and isoproterenol act. The results of this combination of blocking agents in producing an almost complete inhibition of the diphasic action of epinephrine provided further support to the hypothesis that both α and β adrenergic receptors exist in placental vessels. In addition, DCI alone had relatively little effect on the action of large doses of isoproterenol in these vessels, but did reverse the vasodilation produced by small doses of this agent. The effects of DCI on the activity of vasodilators and phentolamine on vasoconstrictors were also investigated. DCI appeared to have only a slight, if any, blocking action on the vascular responses to nitroglycerin, papaverine, isosorbide dinitrate, sodium nitrite, sodium cobaltinitrite, and cyclandelate. Likewise, phentolamine had relatively little blocking action on the vasoconstricting effect of vasopressin and oxytocin.

THE HUMAN placenta is a flat discoid organ which at parturition weighs approximately one-sixth the weight of the fetus. From the onset of its development in pregnancy, this highly vascular organ is constantly increasing in size and structure, in order to meet the metabolic demands of its growing fetus, and it acts as the major organ of transfer between the maternal and fetal circulation.

Because it has been shown in many investigations that the placenta and all of the umbilical cord except that portion immediately adjacent to the fetus are devoid of nervous tissue (1–4), the placental vessels respond to enviromental influences without the endogenous release of the normal mediators. However, even without innervation, the placental vessels have been shown to be extremely sensitive to the action of certain drugs (such as 5-hydroxytryptamine) by a direct musculotropic action and/or stimulation of specific receptor sites (5). Poulson *et al.* have demonstrated that 5-hydroxytryptamine possesses teratogenic properties and suggested that its teratogenicity might be due to an action on placental function and blood supply (6). This research in the field of teratology has pointed to the obvious need for enlarging present knowledge concerning the action of all drugs on placental vasculature with particular emphasis on discerning the precise manner in which drugs are capable of eliciting responses in this vascular system.

Recently, in our laboratory human placental preparations were perfused to try to evaluate

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