A Lymphotropic Prodrug of L-Dopa: Synthesis, Pharmacological Properties, and Pharmacokinetic Behavior of 1,3-Dihexadecanoyl-2-[(*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoyl]propane-1,2,3-triol

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A glyceride derivative of L-Dopa, 1,3-dihexadecanoyl-2-[(*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoyl]propane-1,2,3-triol (1), was synthesized and tested as an orally administrable prodrug endowed with lymphotropic properties. In the oxotremorine and reserpine tests, 1 exhibited an anti-Parkinsonian activity of longer duration than L-Dopa. The time course of concentration of 1 in the intestinal lymph of rat was determined and compared to that of L-Dopa. The results clearly demonstrate that 1 is selectively absorbed from the intestinal tract by the lymphatic route without any chemical or enzymatic degradation. In the blood of rats and mice, 1 functions as a prodrug to release L-Dopa by hydrolysis. In comparison with L-Dopa itself, higher L-Dopa levels for a longer period of time were observed as well as much more favorable L-Dopa/dopamine ratios. Ultimately, studies using mice show that the administration of 1 brings about a prolonged increase of L-Dopa and dopamine levels in the brain, without initial transient peak in concentration observed after an equimolecular dose of L-Dopa.

L-Dopa [(*S*)-(3,4-dihydroxyphenyl)alanine] represents undoubtedly one of the most clinically successful drugs in the treatment of Parkinson's disease. Substitution therapy with L-Dopa is, however, associated with a number of acute problems. The drug undergoes extensive decarboxylation in the gastrointestinal tract including the gastric mucosa, the gut wall, and the liver before entering the systemic circulation; inter- and intraindividual variability in the degree of this first-pass effect is probably one of the main causes of the well-known difficulty to find and to maintain an effective therapeutic regimen. In addition, the massive peripheral conversion of L-Dopa to dopamine and other related metabolites is responsible for most of the gastrointestinal (nausea, emesis) and cardiovascular (arrhythmia, hypotension) typical side effects. Although these peripheral side effects can be attenuated by coadministration of L-aromatic amino acid decarboxylase inhibitors, e.g. benserazide and carbidopa, that do not penetrate into the brain and markedly reduce the required dose of L-Dopa, other major central nervous side effects mainly dyskinesia and on–off phenomena still remain. Reasons for this are not clear, but recently[1,2] it has been suggested that both dyskinetic and end-of-dose deteriorations might be reduced by attenuating peaks and rapid fluctuations of L-Dopa plasma levels.

In an effort to improve the therapeutic value of L-Dopa, Bodor et al.[3] have reported the synthesis of various prodrugs of L-Dopa, obtained by protecting individually and in combination the three sensitive centers in the molecule: the carboxylic, the amino, and the catechol functions. A number of these derivatives produce a significantly higher bioavailability of L-Dopa and a ratio of L-Dopa/dopamine in plasma in favor of L-Dopa (1.16–1.87). However, the shape of the plasma concentration–time curves of free L-Dopa remains close to that obtained after administration of the parent drug itself: even higher transient peak levels (C_{max}) and shorter times to peak (t_{max}) are observed. In the present approach we have tested the possibility of shifting the mechanism of L-Dopa intestinal absorption from the normal venous route to the lymphatic route by incorporating the drug in a triacylglycerol moiety resembling natural neutral fats. By conferring lymphotropic properties on L-Dopa, it was expected (1) to reduce the overall first-pass effect and the consecutive production of dopamine, by protecting the drug in the gastrointestinal tract and by bypassing the liver and (2) to prolong in time the pharmacological action of L-Dopa and to avoid the initial sharp peak of plasma concentration. Thus, L-Dopa ester in the 2-position of 1,3-dihexadecanoylpropane-1,2,3-triol was synthesized and its anti-Parkinsonian effect, its lymphatic and plasma pharmacokinetics, and its uptake into mammalian brain were investigated. The results clearly demonstrate that the present compound is a lymphotropic prodrug of L-Dopa, resulting in a better systemic availability of the parent drug, in a decreased production of peripheral dopamine, in a longer duration of the pharmacological action, and accordingly, in L-Dopa plasma concentrations prolonged in time with flatter peak levels.

Results and Discussion

Chemistry. When designing 1, the 2-position of glycerol was selected to incorpotate L-Dopa in order to protect the resulting ester bond from the action of the pancreatic lipase that hydrolyzes triacylglycerols in their 1- and 3-positions. The choice of the palmitoyl chain was based on the results of previous studies[4] that demonstrate the ability of 1,3-dipalmitoylglycerol moiety to endow orally administrable drugs with lymphotropic properties. Scheme I provides a synopsis of the route followed for synthesizing the acylglycerol of L-Dopa, namely 1,3-dihexadecanoyl-2-[(*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoyl]-propane-1,2,3-triol (1). The catechol and amine functions had first to be protected, and carbobenzoxylation of L-Dopa was carried out according to Felix et al.[5] to afford *N,O,O'*-tricarbobenzoxy-L-Dopa (2). Esterification of 2 with 1,3-dihexadecanoylpropane-1,2,3-triol (3) in solution in dichloromethane using dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine as activating system yielded 4, which was deprotected by hydrogenolysis over palladium

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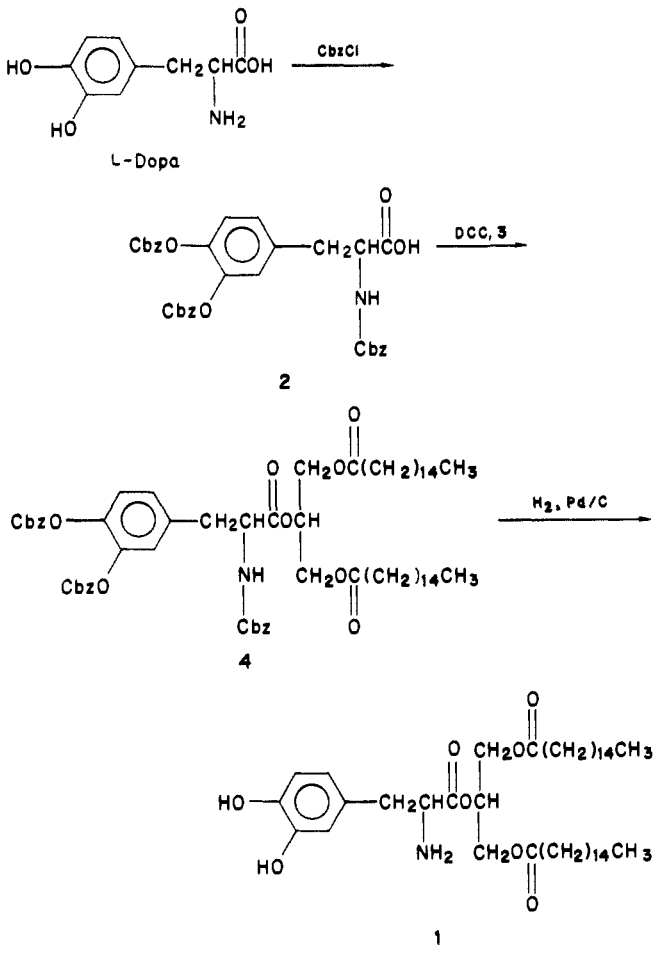
Scheme I



on charcoal to afford the target compound in 20% overall yield.

Pharmacological Properties. The comparative evaluation of the anti-Parkinsonian activity of L-Dopa and 1 was performed in mice using the two standard tests: reserpine and oxotremorine tests, as described by Horst et al.[6] In the reserpine test, the ED_{50} is the dose that reverses in half of the treated animals the catatonia and ptosis induced by that drug; in the oxotremorine test, the ED_{50} is the dose that protects half of the treated animals against both hypothermia and tremors. ED_{50} values were determined at different times after oral administration of L-Dopa and 1. These ED_{50} vs. time are shown in Figures 1 and 2.

It is obvious that 1 has fully retained the pharmacological activity of L-Dopa. Indeed, ED_{50} values lie in the same range for both these compounds. This suggests that 1 functions as prodrug to release L-Dopa, inasmuch its structure is by its own unlikely compatible with an anti-Parkinsonian activity. Very interestingly, 1 by comparison with L-Dopa is acting during a prolonged period of time: in the test with reserpine, L-Dopa activity completely disappears after 60 min, whereas the activity observed for 1 lasts about twice as long. Although less pronounced, a similar effect is observed with the oxotremorine test. Incidentally, it should be pointed out that this last test is a less specific predictor of central anti-Parkinsonian efficacy than the reserpine test.[6]

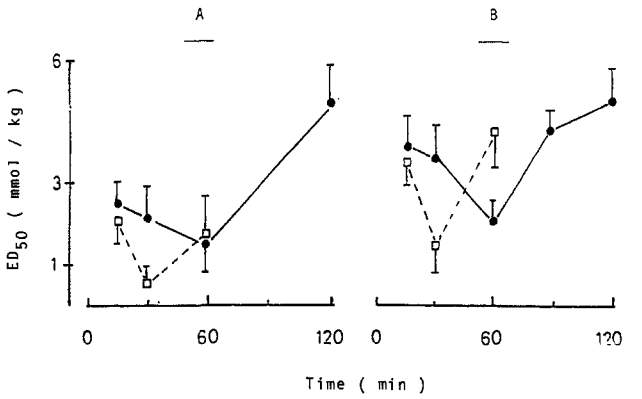


Figure 1. Antagonist effect of L-Dopa (□) and its glyceride derivative 1 (●) at different times (15, 30, 60, 90, 120 min) after their oral administration to mice on ptosis (A) and catataonia (B) induced by reserpine. ED_{50} values were calculated by the method of Litchfield and Wilcoxon and the corresponding SE values as described by Hayes.[7] Five animals per dose and at least five doses were used to determine each ED_{50}. The last point of each curve corresponds to the longest time beyond which no ED_{50} can be determined any longer. Test compounds were administered by gastric intubation in a 5% tragacanth gum suspension representing 1% of the body weight of overnight-fasted male NMRI mice (22–25 g). Reserpine (5 mg/kg) was given by intraperitoneal injection. Each test started in the morning.

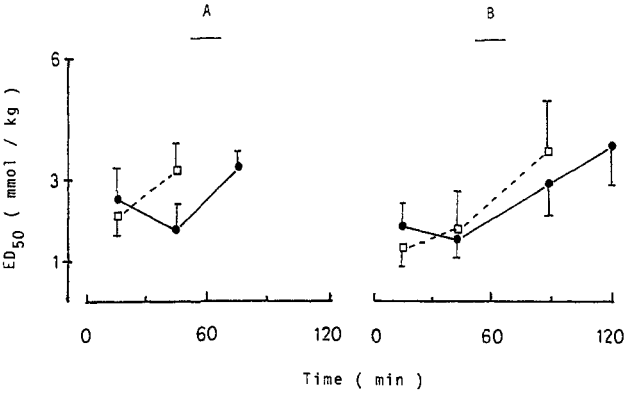


Figure 2. Protective effect of L-Dopa (□) and its glyceride derivative 1 (●) at different times (15, 45, 75, 90, 120 min) after their oral administration to mice, on tremors (A) and hypothermia (B) induced by oxotremorine. The ED_{50} method of calculation and experimental conditions are described in the legend of Figure 1. Oxotremorine (0.5 mg/kg) was given by intraperitoneal injection. Each test was carried out in the afternoon.

Pharmacokinetic Studies. Intestinal Lymphatic Uptake. Compound 1 was then tested for its lymphotropic properties. Thus, we investigated comparatively the intestinal lymph content at various times after oral administration to rats of an equivalent dose (0.5 mmol/kg) of 1 and L-Dopa. The intestinal lymphatic was cannulated; lymph samples were collected at 30-min intervals for as long as 6 h and analyzed by HPLC using a fluorometric method of detection. After L-Dopa administration, only very small concentrations of L-Dopa and its main metabolite, dopamine, were present in the lymph. In contrast to L-Dopa itself, the lymph content of 1 was higher of more than 2 orders of magnitude while neither free L-Dopa nor dopamine could be detected. In addition to the peak of 1, HPLC chromatograms presented four partially resolved peaks. These peaks had the same fluorescence characteristics as 1 and comparable capacity factors; moreover, their evolution with time paralleled that of 1. Therefore, they were tentatively ascribed to hydrolyzed or trans-

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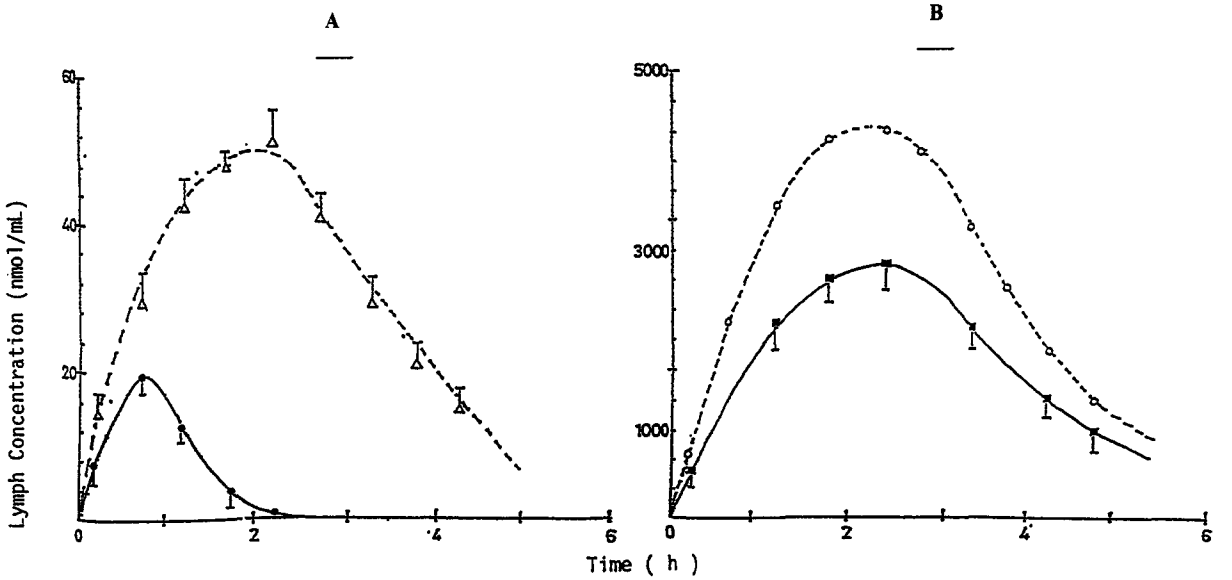

Figure 3. L-Dopa (●), dopamine (△), L-Dopa glyceride derivative 1 (■), and related glycerides (○) lymph levels after oral administration to rats of a 0.5 mmol/kg dose (A) of L-Dopa and (B) of its glyceride derivative 1. Each point represents the mean value obtained from five animals. Standard errors are indicated. Note the difference of scale for the ordinate between parts A and B.

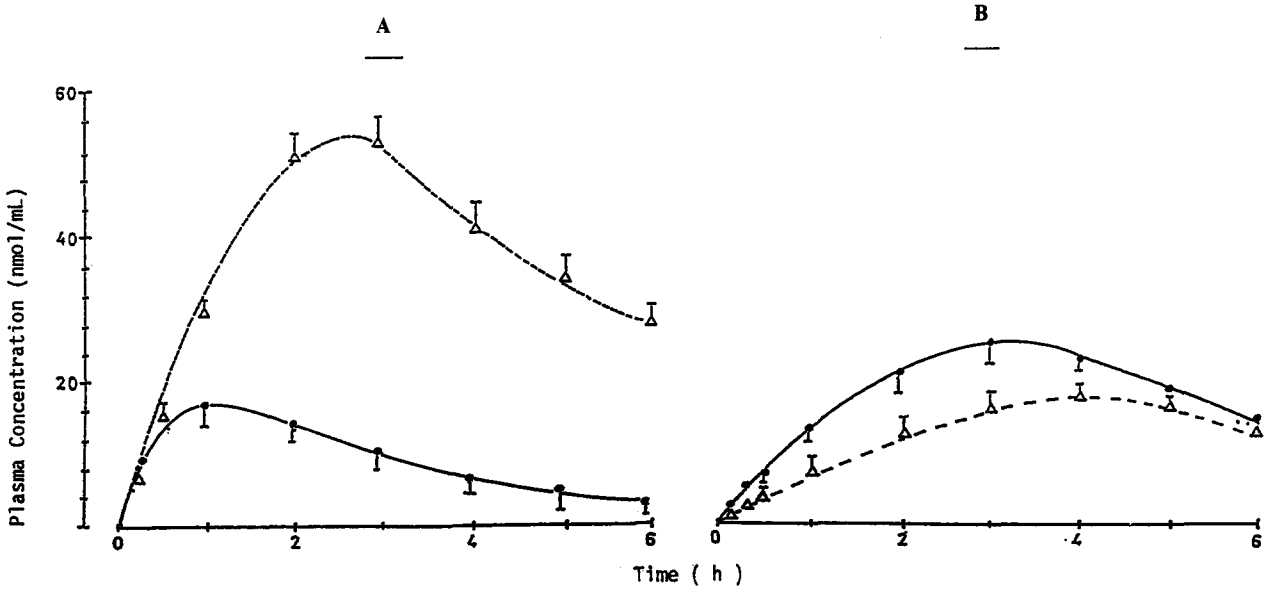

Figure 4. L-Dopa (●) and dopamine (△) plasma levels after oral administration to rats of a 0.5 mmol/kg dose (A) of L-Dopa and (B) of its glyceride derivative 1. Each point represents the mean value obtained from five animals. Standard errors are indicated.

esterified forms of 1 (related glycerides), produced mainly in the lumen of the small intestine by the action of pancreatic lipases and in the intestinal mucosal cells.

Figure 3 shows the results of this study. Such results clearly demonstrate the highly selective absorption of 1 by the intestinal lymphatic system as compared to that of L-Dopa. Five hours after oral administration of 1, 8.3 ± 0.5% of unchanged 1 as well as 14.9 ± 0.9% of related glycerides were recovered in the lymph; this is to be compared with the 0.19% of unchanged L-Dopa plus dopamine found in the same conditions after administration of L-Dopa. A second interesting aspect of the absorption process of 1 is that neither L-Dopa nor dopamine appeared in the lymph after oral administration of 1. This situation indicates the absence of degradation of 1 at the level of the small intestine. This protection effect is likely to be associated with the inclusion of 1 in micellar structures, which prevents the contact of 1 with esterases and L-aromatic amino acid decarboxylase of the gut content and wall.

Table I. Comparison of Pharmacokinetic Parameters after Oral Administration of an Equivalent Dose (0.5 mmol/kg) of L-Dopa or 1 to Different Animal Species

species	admin compd	L-Dopa		dopamine	
		C_{max}[a]	t_{max}[b]	C_{max}[a]	t_{max}[b]
mouse	L-Dopa	1.12 ± 0.32	30	4.73 ± 0.49	30
	1	1.60 ± 0.21	120	3.54 ± 0.19	120
rat	L-Dopa	16.2 ± 2.6	60	52.0 ± 1.7	180
	1	24.6 ± 3.2	180	17.1 ± 2.2	240
monkey[c]	L-Dopa	9.5	25	8.1	90
	1	18.3	110	6.1	150

[a] Peak plasma concentration (nmol/mL). [b] Time of maximum plasma concentration (min). [c] One male Cynomolgus (2.7 kg) fasted for 12 h. Blood samples for analysis were withdrawn from the femoral vein. For other details, see the Experimental Section.

Fate in the Plasma. The ability of 1 to act as an orally administrable prodrug was evaluated by measurement of the released L-Dopa in the plasma of mouse, rat, and monkey. In a comparative study, we determined both L-Dopa and dopamine plasma concentrations at various

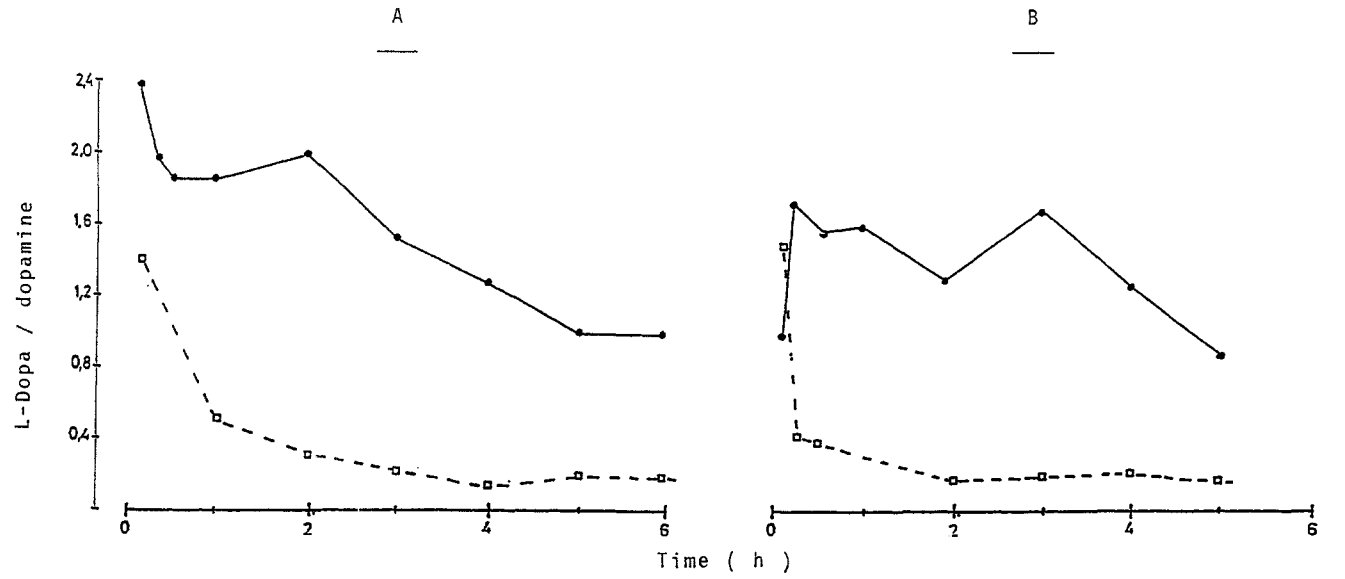

Figure 5. Time course of the plasma L-Dopa/dopamine ratio after a single oral dose (0.5 mmol/kg) of L-Dopa (□) or 1 (●) (A) to rats (n = 5) and (B) to mice (n = 5 for each time point). Each point represents the mean value of five determinations. For other details, see the Experimental Section.

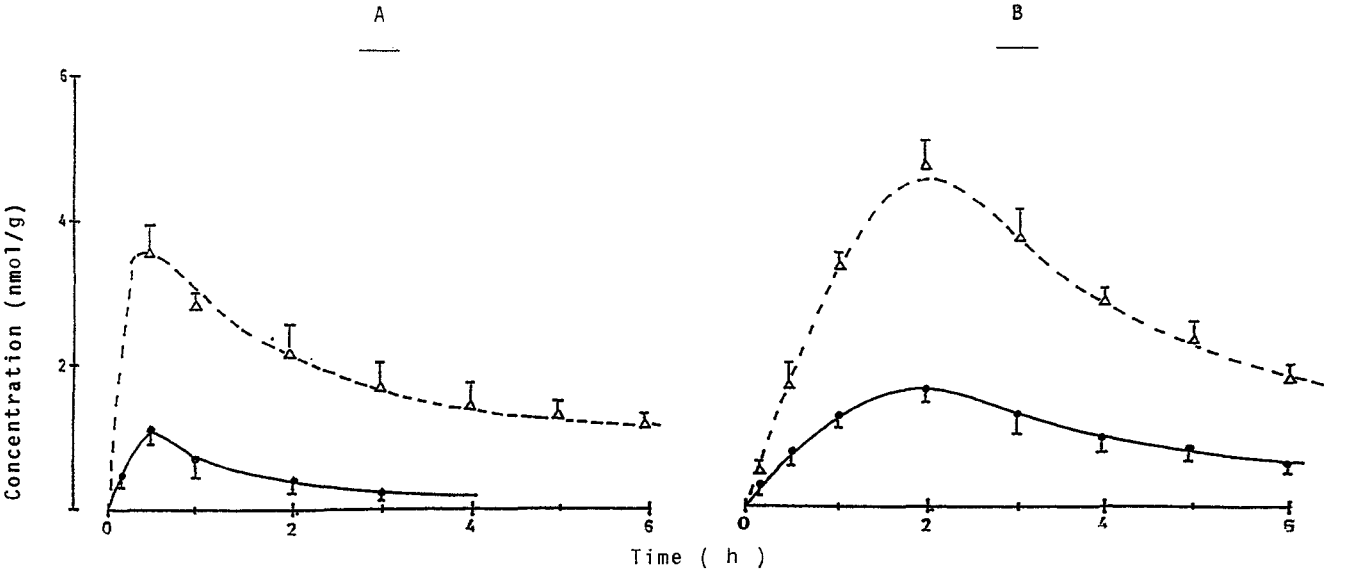

Figure 6. L-Dopa (●) and dopamine (△) brain levels after oral administration to mice of a 0.5 mmol/kg dose (A) of L-Dopa and (B) of its glyceride derivative 1. Each time point represents the mean value obtained from five animals. Standard errors are indicated.

times following the administration of an equivalent dose (0.5 mmol/kg) of 1 or L-Dopa. The plasma level curves obtained with the rat are shown in Figure 4. Similar pharmacokinetic characteristics were obtained with the two other animal species (Table I). From all these results it is obvious that 1, once arrived in the blood is hydrolysed to liberate L-Dopa. As compared with administered L-Dopa, higher L-Dopa plasma concentrations are observed when 1 is given and remain for a longer time. This is in agreement with the different time course of the anti-Parkinsonian activity presented by the two compounds (Figures 1 and 2). Another interesting feature is concerned with the plasma levels of dopamine. Following the administration of 1 these levels are at all times significantly lower than those of L-Dopa. This finding is in sharp contrast to that it is observed after an equivalent dose of L-Dopa and as shown in Figure 5 results in highly favorable plasma L-dopa/dopamine ratios. From these data, it may be anticipated that the therapeutic use of 1 would allow the peripheral side effects due to dopamine to be markedly reduced.

Brain Uptake Studies. In order to obtain immediate evidence of the capability of 1 to deliver L-Dopa to the brain, L-Dopa and dopamine were investigated after the oral administration to mice of a 0.5 mmol/kg dose and compared with those resulting from the administration of an equimolar dose of L-Dopa. Significant elevations of L-Dopa and dopamine brain concentrations were observed with both these compounds (Figure 6). However, the time course of the brain levels presented a quite different profile. The data show a rapid but short-lasting increase in L-Dopa and dopamine concentrations (t_{max} approximately 30 min) after the oral administration of L-Dopa. In contrast, compound 1 results in L-Dopa and dopamine concentrations that increase much less rapidly (t_{max} approximately 120 min) and remain at elevated levels for longer periods of time; thus, an effect of slow release is obtained while the initial transient peaks in concentration produced by orally administered L-Dopa are avoided. These findings are in the line of the prolonged pharmacological activity shown by 1 and may constitute significant improvements in L-Dopa therapy for Parkinson's disease.

During the present study the question arised whether 1 can penetrate the blood–brain barrier and play its role of prodrug once in the brain. Actually no trace of 1 could be detected in that tissue, and it may be concluded that the release of L-Dopa from 1 takes place predominantly, if not exclusively, in the vascular system.

Conclusion. In the present study we described the synthesis and the in vivo evaluation of a L-Dopa–dihexadecanoylglycerol conjugate (1). Owing to its similarity with natural lipids, this compound is absorbed via an enteral route through the intestinal lymphatic system and protects the L-Dopa moiety from the well-known extensive metabolization that takes place for the free parent drug predominantly in the gastrointestinal tract. Once in the blood the conjugate functions as a prodrug that slowly releases L-Dopa by hydrolysis. As compared with the administration of L-Dopa itself, such a prodrug brings about higher L-Dopa plasma levels for a longer time and leads to much more favorable L-Dopa/dopamine ratios. In the brain, a marked and prolonged increase in L-Dopa and dopamine concentrations is observed while the initial transient peak in concentrations that follows the administration of an oral dose of L-Dopa is considerably attenuated. This pharmacokinetic and metabolic profile correlates with the results of oxotremorine and reserpine tests for anti-Parkinsonian activity. In conclusion, 1 appears to be an orally administerable prodrug of L-Dopa absorbed from the gastrointestinal tract via the intestinal lymphatic system without any chemical or enzymatic degradation that, in comparison with the parent drug combines a slow-release effect and an anti-Parkinsonian activity prolonged in time with a highly significant reduction of peripheral dopamine levels; for all these reasons it represents a valuable candidate in the treatment of Parkinson's disease.

Experimental Section

L-Dopa, DCC, benzyl chloroformate (CbzCl), and 4-pyrrolidinopyridine were purchased from Aldrich. 3 was synthesized according to Bentley et al.[8] Melting points (uncorrected) were determined in open capillaries with a Thomas Hoover Uni-Melt apparatus. Mass spectra were recorded on a LKB900 GC-MS instrument using the direct way of sample introduction. ^1H and ^{13}C NMR spectra were recorded on Bruker WP-80/SY and WM-250 spectrometers. Chemical shifts (δ) are expressed in ppm downfield from an internal reference of tetramethylsilane. Elemental analyses, performed by Continental Pharma (Louvain-la-Neuve, Belgium), are indicated only by symbols of the analyzed elements and are within 0.4% of the theoretical values. Optical rotations were measured with a Adam Hilger Ltd. polarimeter. HPLC analyses were carried out on a Perkin-Elmer Series 4 solvent delivery system, a fluorometric LS-4 detector, and Chromatographic 2 data system.

***N,O,O'*-Tricarbobenzoxy-L-Dopa (2).** This compound was synthesized according to Felix et al.[5] in 85% yield: mp 80–82 °C (lit.[5] 82–84 °C); $[\alpha]_D$ −0.95° (*c* 2.93, CH_3OH).

***N,O,O'*-Tricarbobenzoxy-1,3-dihexadecanoyl-2-[(*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoyl]propane-1,2,3-triol (4).** A solution of 2 (30.1 g, 50 mmol) and 3 (28.4 g, 50 mmol) in 250 mL of CH_2Cl_2 was treated at 0 °C by DCC (10.3 g, 50 mmol) and 4-pyrrolidinopyridine (100 mg, 0.67 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 25 h. Some insoluble material was filtered off, and the filtrate was washed with water, 5% $NaHCO_3$, and 0.05% M HCl. The organic layer dried over $MgSO_4$ was filtered and evaporated in vacuo to give a residue that was chromatographed over silica gel using a mixture of *n*-hexane/ether (70/30, v/v) as the eluting system: yield 35%; mp 75 °C (from C_2H_5OH); $[\alpha]_D$ −3.14° (*c* 3, CH_3OH); ^{13}C NMR ($CDCl_3$, 0.15 M) (Cbz carbons) δ 155.88, 152.51 (CO), 135.31, 134.89 (i), 128.68, 128.56, 128.37 (o, m, p), 70.67 (CH_2), (L-Dopa carbons)[10] δ 169.69 (CO), 142.49 (3), 141.67 (4), 136.43 (1), 127.81 (6), 123.10 (2), 122.77 (5), 54.46 (8), 37.20 (7), (1,3-dihexadecanoyl carbons)[4] δ 173.78 (CO), 34.20, 33.91, 31.98, 29.71 (unresolved m), 24.99, 22.70 (CH_2), 14.05 (CH_3), (1,2,3-propanetriol carbons) δ 68.49 (CH), 65.16 (CH_2).

1,3-Dihexadecanoyl-2-[(*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoyl]propane-1,2,3-triol (1). A solution of 4 (10 g, 8.55 mmol) in 100 mL of THF was hydrogenolyzed over 10% palladium on charcoal (300 mg) at an initial pressure of 250 kPa for 18 h. After the catalyst was filtered off, the filtrate was evaporated in vacuo to give a residue that was chromatographed over silica gel with a mixture of *n*-hexane/ether (70/30, v/v) as eluting solvent: yield 63%; mp 78 °C (from C_2H_5OH); $[\alpha]_D$ −6.23° (*c* 3.2, DMF). Anal. ($C_{44}H_{77}NO_8$) C, H, N.

Pharmacokinetic Studies. Lymph. All studies were carried out in overnight-fasted male Wistar rats weighing 230–250 g. Following anesthesia with 35 mg/kg of sodium pentobarbital (ip), they were positioned on a surgical board. The intestinal lymphatic was cannulated according to the method described by Warshaw.[9] Oral doses (0.5 mmol/kg) of L-Dopa and 1 were administered by gastric intubation of a 5% tragacanth gum suspension just before the rat woke up. The animals were kept in restraining cages with free access to water. Lymph samples (0.95 ± 0.15 mL/h) were collected at 30-min intervals for 6 h and deproteinized by the addition of a 3% volume of 70% perchloric acid. After centrifugation, the supernatant was analyzed for L-Dopa and dopamine by HPLC on an RP 18 column (Lichrosorb, Merck, 5 μm, 25 cm × 4.6 mm); the eluent was a CH_3OH/H_2O (95/5) mixture containing NaH_2PO_4, $C_5H_{11}SO_3H$, and Na_2EDTA in 0.15, 5×10^{-3}, and 1×10^{-4} M concentrations, respectively. The pH was adjusted to 2.7 with H_3PO_4. The flow rate was 0.9 mL/min. Fluorometric detection was performed on underivatized compounds at the activation wavelength of 282 nm and emission wavelength of 322 nm (k'(L-Dopa) = 3.92; k'(dopamine) = 5.92). The separation and quantitation of 1 (k' = 6.16) were similarly carried out by HPLC using the same conditions with exception for the eluent, which was a mixture of acetonitrile/acetone (60/40, v/v). The pool of the four glycerides related to 1 (k' = 7.02, 7.45, 7.8, 8.0) was also assayed by HPLC using the same eluting system and assuming identical quantum efficiency with compound 1. Peak areas were used for quantitation of L-Dopa, dopamine, 1, and related glycerides.

Plasma. Oral doses (0.5 mmol/kg) of L-Dopa and compound 1 were administered to overnight-fasted male Wistar rats (five rats per compound) weighing 230–250 g. Blood samples (±100 μL) were obtained from tail blood vessels at various time intervals for 6 h (10, 20, 30 min; 1, 2, 3, 4, 5, 6 h), and the plasma was treated as above.

Brain. Male NMRI mice (five animals per time and compound) weighing 20–25 g were used for this study. At various time intervals after oral administration of the compounds (10, 20, 30 min; 1, 2, 3, 4, 5, 6 h), the mice were sacrificed by decapitation, and the whole brain was removed from the skull and frozen immediately on dry ice. Their blood was collected and analyzed as described above. The mouse whole brain was homogenized with an equal volume of 3% $HClO_4$ solution. After centrifugation the clear supernatant was analyzed by HPLC as described above.

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Registry No. 1, 100858-16-0; 3, 502-52-3; 4, 100858-15-9; L-dopa, 59-92-7.

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