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Esters of L-Dopa: Structure-hydrolysis Relationships and Ability to Induce Circling Behaviour in an Experimental Model of Hemiparkinsonism

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Abstract

A number of carboxylate esters of L-dopa, some of which are novel, were examined for their physicochemical and biological properties. A few esters of tyrosine and phenylalanine were included for comparison.

The compounds displayed great differences in their lipophilicity and stability towards chemical and enzymatic (human plasma) hydrolysis. Within subseries, relationships exist between structural properties and rate constants of chemical or enzymatic hydrolysis. In an experimental model of hemiparkinsonism (circling behaviour in rats), some of the L-dopa esters (the isopropyl, *sec*-butyl and 2-(tetrahydropyranyl)methyl esters) showed an activity distinctly greater than that of L-dopa, although the difference was not statistically significant.

L-3,4-Dihydroxyphenylalanine (L-dopa) is the immediate precursor of dopamine and is the most widely employed treatment of Parkinson's disease. However, the drug is characterized by an unsatisfactory pharmacokinetic behaviour such as irregular absorption and short half-life due to its physicochemical properties (e.g. poor water solubility) and its ease of chemical (oxidation) and enzymatic (L-aromatic amino acid decarboxylase and catechol-*O*-methyltransferase) breakdown (Nutt & Fellman 1984; Nakazato & Akiyama 1992).

In addition, long-term administration is often associated with the emergence of fluctuations in the clinical response to the drug, such as the wearing-off phenomenon, on-off fluctuations and the onset of involuntary movements in the form of dystonias or dyskinesias (Marsden & Parkes 1976; Nutt et al 1984; Gancher et al 1988). The pathogenesis of these alterations in clinical response to L-dopa is uncertain. The loss of drug efficiency does not appear to be causally linked to alterations in plasma L-dopa levels. Rather it may reflect decreased storage of L-dopa in brain as neural degeneration progresses. Dyskinesias are of unknown origin but appear at times of maximum plasma levels of L-dopa (peak dose dyskinesias) (Nutt et al 1984; Quinn et al 1984; Nutt & Woodward 1986; Mouradian & Chase 1988). However, control of plasma levels of L-dopa using continuous intravenous infusions can alleviate fluctuations in response to the drug and maintain patient mobility (Quinn et al 1984; Sage et al 1989). More recently, controlled release preparations of L-dopa have been used to produce prolonged plasma level of the drug, but large doses are required to maintain the clinical response.

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The poor water solubility of L-dopa limits its use as an intravenous or subcutaneous infusion. The chemical and enzymatic instability of L-dopa has also made it difficult to produce highly effective controlled-release preparations. Prodrug design offers one possibility to reduce some of these disadvantages. By displaying enhanced water solubility, lipophilicity, and chemical and enzymatic stability, prodrugs could improve the bioavailability and pharmacokinetic behaviour of L-dopa by increasing its absorption, reducing its metabolism and regulating its plasma concentrations. Several prodrugs of L-dopa have been prepared by acylating one or both phenolic groups or the amino function, or by alkylating the carboxylic group. However, none of these prodrugs appears to be an improvement, pharmacologically or clinically, on L-dopa itself (Lai & Mason 1973; Felix et al 1974; Barth 1977; Bodor et al 1977; Marrel et al 1985a, b; Garzon-Aburbeh et al 1986; Cooper et al 1987a, b; Fix et al 1989, 1990; Tye et al 1989).

Based on stability considerations and on quantitative structure-activity relationships previously published by us (van de Waterbeemd et al 1987), we have designed some novel L-dopa ester prodrugs. Here, we describe these compounds and their properties, simultaneously re-evaluating some known L-dopa esters. The properties examined here include basicity and lipophilicity, chemical and enzymatic hydrolysis, and behavioural activity in an experimental model of Parkinson's disease. A few prodrugs of L-tyrosine and L-phenylalanine, two amino acids which are precursors of L-dopa (Westerink & De Vries 1991), were also included for comparison.

Experimental

Chemicals

All chemicals purchased were of the best available purity. 2-Butanol, celite, *N,N'*-dicyclohexylcarbodiimide, dimethylsulphoxide, 3-(hydroxymethyl)pyridine, isopropanol, methanol, ninhydrine, 1-octanol, perchloric acid, 4-pyrrolidinopyridine, NaF, Na_2HPO_4, tetrahydrofurfuryl alcohol and thionyl chloride were purchased from Fluka (Buchs, Switzerland). Biphenyl, 3-hydroxytetrahydrofuran and sodium azide were obtained from Aldrich (Steinheim, Germany). Citric acid, ethanol, 3-morpholinopropane sulphonic acid (MPS buffer) and pyridine were supplied by Merck (Darmstadt, Germany). Potassium dichromate came from Siegfried (Zofingen, Switzerland) and 2-(*N*-morpholino)-ethanesulphonic acid monohydrate (MES buffer) from Calbiochem (Lucerne, Switzerland).

The following amino acids and derivatives were obtained from commercial sources in the highest available degree of purity and were used without further purification: L-dopa, L-phenylalanine, L-phenylalanine benzyl ester (**P2**), L-tyrosine, and L-tyrosine ethyl ester (**T2**) (Fluka, Buchs, Switzerland); N-BOC L-dopa dicyclohexylammonium salt (**D12**), and L-dopa methyl ester (**D1**) (Sigma, St Louis, USA); L-phenylalanine methyl ester (**P1**), L-tyrosine methyl ester (**T1**), N-BOC L-tyrosine *N*-succinimidyl ester (**T6**-BOC), N-BOC L-tyrosine *p*-nitrophenyl ester (**T7**-BOC), and N-BOC L-tyrosine (**T8**) (Bachem, Bubendorf, Switzerland). L-Dopa t-butyl ester (**D5**) was a gift from Chiesi Farmaceutici Spa (Parma, Italy).

Synthesis

The following L-dopa esters have already been described and were freshly prepared (Marrel et al 1985a): ethyl ester (**D2**), 2-(tetrahydropyranyl)methyl ester (**D6**), benzyl ester (**D9**), *p*-methoxyphenylethyl ester (**D10**), and phenoxyethyl ester (**D11**).

The other L-dopa or L-tyrosine esters were prepared using three different synthetic methods.

Method A (thionyl chloride). Thionyl chloride (50 mmol) was added to 500 mmol of the alcohol cooled at −5°C in a nitrogen atmosphere (Jung & Rohloff 1985; Lai & Mason 1973; Marrel et al 1985a). To this mixture 25 mmol of L-dopa was added in one portion and the suspension was refluxed. After completion of the reaction (TLC monitoring, conditions see below), the solute was cooled and the solvent evaporated under vacuum. The product was recrystallized in acetonitrile. In two cases (**D7**, **D8**), the ester was precipitated with anhydrous ether and the solid dried in vacuum. The compounds were obtained as hydrochlorides.

The compounds prepared by this method were: L-dopa isopropyl ester (**D3**, yield 67%), L-dopa *sec*-butyl ester (**D4**, yield 54%), L-dopa 3-tetrahydrofuranyl ester (**D7**, yield 63%), L-dopa 2-(tetrahydrofuranyl)methyl ester (**D8**, yield 39%), L-tyrosine isopropyl ester (**T3**, yield 27%), and L-tyrosine *sec*-butyl ester (**T4**, yield 68%).

Method B (dicyclohexylcarbodiimide, DCC). A solution of the N-BOC amino acid (10 mmol) in 40 mL anhydrous pyridine was added at room temperature and under stirring to a mixture of 11 mmol of the alcohol, 4 mL pyridine, 11 mmol DCC and pyrrolidinopyridine in catalytic amounts (Dutta & Morley 1971). The reaction was monitored by TLC (conditions see below). The dicyclohexylurea so formed was filtered and 3 drops of glacial acetic acid were added to precipitate the remaining dicyclohexylurea. The solvent was evaporated, toluene being added to form an azeotrope with pyridine. The residue was dissolved in the minimum amount of dry ethyl acetate and the solution was filtered on Celite to remove the last traces of dicyclohexylurea, diluted with ethyl acetate, and washed successively with ice-cold water, aqueous 1 M $NaHCO_3$ and brine. The dried solution was evaporated under vacuum. The ester was separated from the product of dimerization by low-pressure chromatography (Lobar Lichroprep Si60 column, Merck; eluent, see TLC assays) and then recrystallized from acetonitrile.

This method was used to obtain the 3-pyridylmethyl ester of N-BOC L-tyrosine (**T5** N-BOC, yield 12%).

Method C (from N-BOC protected esters). A flow of gaseous HCl was passed through a suspension of 15 mmol N-BOC protected ester in 400 mL dried ether during one day. The product was filtered, dried under vacuum, and recrystallized as hydrochloride in acetonitrile.

This method was used to obtain the following esters of L-tyrosine with yields of 97–100%: 3-pyridylmethyl (**T5**), *N*-succinimidyl (**T6**), and *p*-nitrophenyl (**T7**).

Identity and purity

The identity and purity (>97%) of all compounds were checked by the following methods: ^1H NMR, ^{13}C NMR (Varian 200 MHz VXR-200, tetramethylsilane as reference), IR (Perkin Elmer 681), UV (Perkin Elmer 557), mass spectroscopy (Nermag R 10–10 C), elemental analysis (Microanalysis Laboratory, Institute of Organic Chemistry, University of Basel), melting points (Mettler FP5, non corrected, room temperature). The results were in agreement with the expected structures and are available from the authors as supplementary material.

The purity of the compounds was also checked by TLC on Kieselgel 60 F_{254} plates (Merck), eluent $CHCl_3$/isopropanol 60 : 40 or 50 : 50, or butanol/acetic acid/water 16 : 1 : 7.

pK_a measurements

The pK_a values of some L-dopa and L-tyrosine esters as hydrochlorides were determined by potentiometry at 37 ± 0·2°C and an ionic strength of 0·1. The method used for the potentiometric pK_a determination has been described previously (El Tayar et al 1985). Titration curves were recorded using the following equipment (Metrohm, Herisau, Switzerland): Dosimat 665, magnetic stirrer E649, titroprocessor 670 combined with a glass electrode (N 6.0204.10 PF), thermostat Haake FJ. The initial concentration of L-dopa esters was 10 mM, and that of L-tyrosine esters was 1 mM.

Measurement of distribution and partition coefficients

Distribution coefficients of the esters between 1-octanol and a pH 7·4 buffer were measured by centrifugal partition chromatography as described previously (Tsai et al 1992) and with the same equipment (Pharma-Tech Research

Corp., Baltimore, MD, USA). The aqueous phase was a 0·01 M MPS buffer of pH 7·4. The solutes were dissolved in 0·5 mL mobile phase for injection and detected at a wavelength of 280 nm. All measurements were performed at room temperature and in triplicate. The non-retained solutes used for the determination of the column deadtime were potassium dichromate (when the aqueous buffer was the eluent) and biphenyl (when octanol was the eluent).

Solubility

The solubility of three branched-alkyl esters of L-dopa (**D3**, **D4** and **D5**) was measured by UV spectrometry with a Perkin-Elmer 557 apparatus at 280 nm, in an isotonic solution of 0·05 M MPS at pH 7·4 containing 9 g L^{-1} NaCl. The saturated solutions were prepared by filtering (Millipore 0·45 μm) a suspension of the compound ultrasonicated for 15 min.

Chemical hydrolysis

Incubations. All incubations were carried at 37°C and pH 7.4. A stock solution of each ester of L-dopa or L-tyrosine ($3{\cdot}37 \times 10^{-2}$ M) was prepared in 0·05 M MPS buffer containing 9 g L^{-1} NaCl and 3% DMSO as cosolvent. It had been verified that the cosolvent is without detectable influence on the rate of hydrolysis. The hydrolysis began when 3 mL stock solution was added to 97 mL degassed buffer at 37°C. The experiments were conducted in triplicate in an agitating water-bath (129 rev min^{-1}) (Infors WTR-1, Zürich, Switzerland). In each experiment, sampling was continued until 90% hydrolysis had occurred, 10 samples or more being taken at regular intervals and analysed as described below.

Analysis. The internal standards for L-dopa and L-tyrosine were a saturated and filtered solution of L-tyrosine (ca. 10^{-3} M) or L-dopa ($1{\cdot}2 \times 10^{-4}$ M), respectively, in 0·1 M citric acid/0·2 M Na_2HPO_4 buffer of pH 4.1.

Each sample (0·8 mL) was mixed with 0·2 mL of internal standard and the solution eluted with 1 mL of the same buffer on a Baker C18 SPE disposable column (1 mL) (Philipsburg, NJ, USA) to separate the unreacted ester from the product of hydrolysis (L-dopa or L-tyrosine). The columns had been conditioned successively with 2 × 1 mL methanol, 2 × 1 mL water and 2 × 1 mL of the same buffer. A preliminary investigation had demonstrated that this method allows a quantitative and reproducible recovery of L-dopa and L-tyrosine with complete elimination of esters.

The analyses were performed by HPLC using a Kontron Data System 450 (Kontron AG, Zürich, Switzerland) equipped with an autosampler 460, a precolumn LiChroCart 4–4 (filled with Lichrosphere 100 RP18 5 μm, Merck), a column LiChroCart 125–4 (5 μm Lichrosphere 100, Merck), and a type 430 UV detector set at 280 nm for all compounds. The mobile phase was 0·1 M MES buffer of pH 5.1 containing 5% methanol and 0·01% sodium azide as antibacterial; it was filtered (Millipore 0·45 μm) and degassed by ultrasonication prior to use. The flow rate was fixed at 0·8 mL min^{-1}. Triplicate calibration curves were established for L-dopa and L-tyrosine (range 10^{-2} to 10^{-5} M and 10^{-3} to 10^{-5} M, respectively). Excellent linearity was obtained ($r^2 > 0{\cdot}99$), with s.e.m. always < 3%.

The chemical hydrolysis under physiological conditions (pH 7·4; 37°C) of all investigated esters followed pseudo-first-order kinetics. For each compound, three separate investigations yielded the reported rate constant and s.e.m.

Stability in human plasma

Preparation of human plasma. The blood of five human male volunteers was collected in 500-mL heparinized blood-pack units (Baxter, Thetford, UK) and centrifuged for 15 min at 930 *g*. The plasma was kept in Eppendorf tubes (0·8 mL portions) at −20°C and retained more than 90% of enzymatic activity for up to six months. The plasma protein content was determined according to Lowry et al (1951) with bovine serum albumin as the standard.

Incubations. The plasma samples in Eppendorf tubes were diluted to 80% and pH 7·4 with a 0·05 M MPS buffer. Aliquots (30 mL) of a stock solution of the substrates (3×10^{-2} M in DMSO) were added to the diluted plasma (final ester concentration 9×10^{-4} M, 3% DMSO) and the tubes were incubated in a water bath at 37°C. At various time intervals (0, 15, 60, 120, 150, 180, 210, 240 min), tubes were removed from the water bath and completed with 0·2 mL internal standard (0·1 mL for L-tyrosine esters) prepared as described for chemical hydrolysis.

Analysis. The esterase activity was then stopped with 0·2 mL NaF 3% and the proteins precipitated with 0·05 mL $HClO_4$ > 70%. The pH reached 2·0. The solution was vortexed briefly and centrifuged for 2 min at 14 000 *g* (Eppendorf centrifuge model 3200). The supernatant was transferred to another Eppendorf tube and the pH raised to 4 with 0·14 mL 2 M NaOH, vortexed again and centrifuged again. An aliquot (1 mL) of the supernatant was passed through a Baker column as described for chemical hydrolysis.

The same HPLC system was used as described for chemical hydrolysis. A third peak appeared in the chromatograms and was due to plasma uric acid. The ideal eluent pH for separating the three peaks was pH 5·1.

Plasma hydrolysis also followed pseudo-first-order kinetics. To take chemical hydrolysis into account, control experiments were carried out in parallel (0·05 M MPS buffer + 9 g L^{-1} NaCl, pH 7·4). The rate constants and half-lives were calculated for the enzymatic hydrolysis (i.e. total observed plasma hydrolysis minus chemical hydrolysis).

Pharmacological assays

Unilateral 6-hydroxydopamine (6-OHDA)-induced lesions of the nigrostriatal pathway. Male Wistar rats (180–220 g, Bantin and Kingman Ltd, UK) were lesioned in the left substantia nigra using standard stereotaxic techniques. Animals were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and positioned in a Kopf stereotaxic frame. 6-Hydroxydopamine HBr (8 mg free base in 4 mL 0·9% saline containing 0·05% ascorbic acid, Sigma) was injected over a 4-min period using a 10-mL Hamilton syringe (gauge 22S) into the left substantia nigra (coordinates A = +2·2 mm and L = +2·0 mm from lambda, V = −8·0 mm from dura (König & Klippel 1963)). Following surgery, the rats were treated with sodium ampicillin (5 mg kg^{-1}, s.c.) on recovery and for a further two days.

Measurement of circling behaviour. Circling behaviour was examined at least two weeks following surgery. Rats were placed into individual perspex observation boxes (35 × 25 × 16 cm). Thirty minutes later they were monitored for 1 min for spontaneous rotational behaviour. Initial lesion placement was verified by the administration of apomorphine HCl (0·5 mg kg^{-1}, s.c.) dissolved in distilled water containing 0·1% $Na_2S_2O_5$. The number of full rotations was noted for a 4-min observation period, 20 min after the administration of apomorphine. Only those animals that rotated at least 20 times in the contralateral direction were used in subsequent studies.

Administration of prodrugs. In subsequent experiments the effect of administration of L-dopa, L-tyrosine, L-phenylalanine, or their prodrugs on circling behaviour was measured at least 28 days following apomorphine treatment. Rats received carbidopa (25 mg kg^{-1}, i.p.) as a suspension in 1% methylcellulose and were placed in the observation boxes. After 45 min, circling behaviour was monitored for a period of 1 min. One hour after carbidopa administration, L-dopa or its prodrugs (0·25 mmol kg^{-1}, p.o. or i.p.) as a suspension in 1% methyl cellulose, or L-tyrosine, or L-phenylalanine and their respective prodrugs (2·75 mmol kg^{-1}, p.o. or i.p.), as a suspension in 1% methylcellulose were administered. Circling behaviour was measured for 1 min every 15 min for a period of 5 h. Data were expressed as total number of turns (± s.e.m.) for the 5-h period of observation.

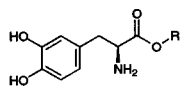
Acute treatment studies. L-Dopa and L-tyrosine, and their respective prodrugs were studied on a single occasion, each rat receiving the parent drug, up to two prodrugs and vehicle in a random cross-over design with one week between each treatment.

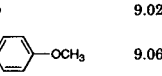
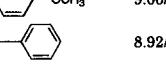
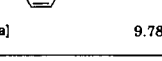
Subacute treatment studies. The effect of a 5-day pretreatment with L-dopa (0·25 mmol kg^{-1} day^{-1}), L-tyrosine (2·75 mmol kg^{-1} day^{-1}) and L-phenylalanine (2·75 mmol kg^{-1} day^{-1}) or equimolar amounts of their respective prodrugs were studied on the ability of the drug to produce circling behaviour. Circling behaviour was tested on the 6th day as described above.

Computations

n-Octanol/water partition coefficients (log P values) were calculated from the molecular lipophilicity potential (MLP) of the compounds as described by Gaillard et al (1994 a, b), using an arbitrary conformation optimized using the molecular modeling package SYBYL (Tripos Associates, St Louis, MO, USA). Log P values were also calculated using the ClogP program (Leo 1993). Molecular volumes were calculated with the software MOLSV (QCPE No 509) using the atomic radii of Gavezzotti (1983). Statistical analyses were performed with the QSAR module in the SYBYL software and with the TSAR program (Oxford Molecular, Oxford, UK). All calculations were run on an Indigo R4000 workstation.

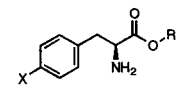
Table 1. Physicochemical properties of L-dopa esters.



Code	—R	pK$_a$[a]	log D[b]	log P$_{MLP}$[c]
L-Dopa	—H	9.63/8.46/2.41	-2.38	-1.26
D1	—CH_3	8.91/7.17/-	-0.28	0.14
D2	—CH_2CH_3	8.91/7.14/-	0.17	0.60
D3	—$CH(CH_3)_2$	8.83/7.33/-	0.52	1.06
D4	—$CH(CH_3)CH_2CH_3$	ND[d]	0.93	1.47
D5	—$C(CH_3)_3$	9.90/7.92/-	0.81	1.16
D6		9.29/7.14/-[e]	0.37	1.12
D7		ND	-0.45	0.09
D8		ND	0.01	0.09
D9		9.02/6.93/-[e]	1.42	1.23
D10		9.06/7.02/-[e]	1.66	2.09
D11		8.92/7.11/-	1.50	1.54
D12	[N-BOC L-Dopa]	9.78/-/3.65	-1.86	1.43

[a] At 37°C, μ = 0·1, pK$_a$(OH)/pK$_a$(NH_3^+)/pK$_a$(COOH), s.d. ≤ 0·05. [b] 1-Octanol/water distribution coefficient at pH 7.4, s.d. ≤ 0·04. [c] Log P (neutral species) calculated from the molecular lipophilicity potential. [d] Not determined. [e] Marrel et al (1985a).

Table 2. Physicochemical properties of L-tyrosine (X = OH) esters and L-phenylalanine (X = H) esters.



Code	—R	pK$_a$[a]	log D[b]	log P$_{MLP}$[c]
L-Tyr	—H	10.02/9.11/2.20	-2.11[e]	-0.80
T1	—CH_3	9.95/7.21/-	0.18	0.60
T2	—CH_2CH_3	9.36/7.31/-	0.60	1.05
T3	—$CH(CH_3)_2$	ND[d]	0.99	1.46
T4	—$CH(CH_3)CH_2CH_3$	ND	1.59	1.75
T5		ND	ND	0.51
T6		ND	NM[f]	-0.28
T7		ND	1.62	1.14
T8	[N-BOC L-Tyr]	9.68/-/3.57	-1.44	1.92
L-Phe	—H	-/9.13/1.83	-1.45[e]	-0.24
P1	—CH_3	ND	0.83	1.17
P2	—CH_2 	ND	2.48	2.22

[a] At 37°C, μ = 0·1, pK$_a$(OH)/pK$_a$(NH_3^+)/pK$_a$(COOH), s.d. ≤ 0·05. [b] 1-Octanol/water distribution coefficient at pH 7.4, s.d. ≤ 0·04. [c] Log P (neutral species) calculated from the molecular lipophilicity potential. [d] Not determined. [e] Data from El Tayar et al (1992). [f] Not measurable due to fast hydrolysis.

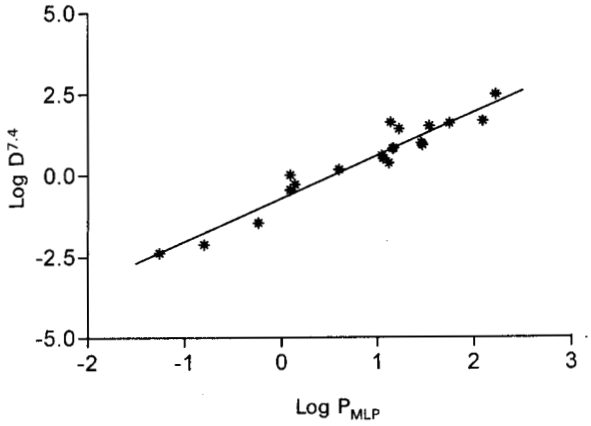


FIG. 1. Relationship between experimental distribution coefficients at pH 7·4 (log $D^{7.4}$) and partition coefficients computed from the molecular lipophilicity potential (log P_{MLP}).

Results and Discussion

Physicochemical properties

Ionization. The L-dopa esters investigated in this study, be they commercially available, already described in previous studies, or novel (**D4**, **D5**, **D7** and **D8**), are shown in Table 1 which reports their chemical structure, pK_a values, and lipophilicity parameters (distribution coefficients at pH 7·4 and calculated partition coefficients). The L-tyrosine and L-phenylalanine esters, be they commercially available or not (**T3**, **T4**, **T5**, **T6** and **T7**), are described in Table 2. The synthetic methods are well-known and need no comment.

The pK_a values of L-dopa, L-tyrosine and L-phenylalanine were determined for comparison purposes and are in agreement with literature values (Albert 1952). With the exception of L-dopa *t*-butyl ester (**D5**), the esters consistently show the expected increase in acidity of the phenolic group (by 0·1 to 0·8 pK_a units) relative to the free amino acid. However, the differences between the various esters cannot be easily explained. For the two L-tyrosine esters, the basicity of the amino group is decreased by 1·8 to 1·9 pK_a units relative to the free amino acid, in agreement with literature data (Ishimitsu & Sakurai 1983). For the L-dopa esters, the decrease in basicity is of 1·1 to 1·5 pK_a units, with the exception again of the *t*-butyl ester which would warrant a deeper study.

Solubility. Poor water solubility is one of the pharmaceutical limitations of L-dopa, literature values being around 2–3 mg mL^{-1}. In an isotonic buffer of pH 7.4, the three branched-alkyl esters of L-dopa showed the following solubilities: isopropyl ester (**D3**) 745 mg mL^{-1} (2·7 M); *sec*-butyl ester (**D4**) 666 mg mL^{-1} (2·3 M); *t*-butyl ester (**D5**) 783 mg mL^{-1} (2·4 M). The value for the isopropyl ester compares well with that reported by Fix et al (1989) for the compound as its hydrochloride in pure water (782 mg mL^{-1}). Clearly these simple esters are 200- to 300-fold more water-soluble than L-dopa itself.

Lipophilicity. The distribution coefficients (log D) measured by centrifugal partition chromatography in a 1-octanol/(pH 7·4 buffer) are reported in Tables 1 and 2. The values measured here for **D1** and **D3** compare very well with those reported by Fix et al (1989) by the shake-flask method (-0·20

Table 3. Rate constants (k, $min^{-1} \pm$ s.d.) and half-lives ($t_{½}$, min ± s.d.) of hydrolysis of esters of L-dopa and L-tyrosine at 37°C.10^{-3}

Code	Buffer pH 7·4[a]		80 % Human plasma pH 7·4[b])	
	k	$t_{½}$	k	$t_{½}$
D1	$1·1 \times 10^{-3} \pm 1·0 \times 10^{-4}$	620[c]	$8·7 \times 10^{-4} \pm 1·8 \times 10^{-4}$	800
D2	$2·8 \times 10^{-4} \pm 5·0 \times 10^{-5}$	2500	$9·0 \times 10^{-4} \pm 1·0 \times 10^{-5}$	770
D3	$8·2 \times 10^{-5} \pm 2·0 \times 10^{-6}$	8500	$3·5 \times 10^{-4} \pm 2·0 \times 10^{-5}$	2000
D4	$5·0 \times 10^{-5} \pm 3·0 \times 10^{-6}$	14000	$8·7 \times 10^{-4} \pm 2·0 \times 10^{-5}$	1200
D5	$9·4 \times 10^{-5} \pm 1·0 \times 10^{-6}$	7400	$(7·6 \times 10^{-5} \pm 2·0 \times 10^{-5}$	9100[h])
D6	$7·3 \times 10^{-4} \pm 7·0 \times 10^{-5}$	950[c]	$2·8 \times 10^{-3} \pm 1·0 \times 10^{-4}$	250
D7	[d]		[d]	
D8	$1·0 \times 10^{-3} \pm 5·0 \times 10^{-5}$	670	$5·1 \times 10^{-3} \pm 2·0 \times 10^{-4}$	140
D9	$3·3 \times 10^{-4} \pm 1·0 \times 10^{-5}$	2100[c]	$1·3 \times 10^{-3} \pm 3·0 \times 10^{-4}$	530
D10	$4·5 \times 10^{-4} \pm 1·0 \times 10^{-5}$	1500[c]	$4·0 \times 10^{-3} \pm 2·0 \times 10^{-4}$	170
D11	$1·1 \times 10^{-3} \pm 1·0 \times 10^{-4}$	600	$1·4 \times 10^{-2} \pm 2·0 \times 10^{-3}$	50
D12	[e]		[d]	
T1	$1·2 \times 10^{-3} \pm 2·0 \times 10^{-4}$	550	$5·2 \times 10^{-3} \pm 6·0 \times 10^{-4}$	130
T2	$4·8 \times 10^{-4} \pm 3·0 \times 10^{-5}$	1400	$4·6 \times 10^{-3} \pm 1·0 \times 10^{-4}$	150
T3	$7·6 \times 10^{-5} \pm 4·0 \times 10^{-6}$	9100	$1·1 \times 10^{-3} \pm 1·0 \times 10^{-4}$	630
T4	$5·0 \times 10^{-5} \pm 3·0 \times 10^{-6}$	14000	$1·9 \times 10^{-3} \pm 1·0 \times 10^{-4}$	360
T5	$1·5 \times 10^{-3} \pm 1·0 \times 10^{-4}$	460	$4·0 \times 10^{-3} \pm 9·0 \times 10^{-4}$	170
T6	[f]		[f]	
T7	[d]		[d]	
T8	[g]		[g]	

[a] MPS buffer, m = 0·17; n = 3. [b] Human plasma diluted to 80 % with MPS buffer; n = 3–6. [c] Marrel et al (1985b). [d] Not measurable due to competitive oxidation or breakdown. [e] No hydrolysis detectable after 2600 min; oxidation seen. [f] Too fast to be quantifiable (25 % after 3 min). [g] Too slow to be measurable. [h] This is the **total** plasma hydrolysis, which is slower than chemical hydrolysis; enzymatic hydrolysis is effectively nil.

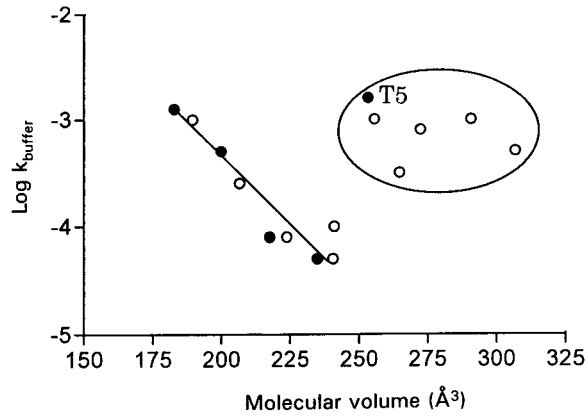


FIG. 2. Relationship between rate constants of chemical hydrolysis at pH 7.4 (log k_{buffer}; min^{-1}) and calculated molecular volumes (MV in Å^3). L-Dopa esters ○; L-tyrosine esters: ●.

and 0·57, respectively). The high polarity of the free amino acids is markedly reduced by esterification, the increase in log D upon formation of the methyl ester being 2·1 for L-dopa, and 2·3 for L-tyrosine and L-phenylalanine. Globally, the increases in log D values span 2–4 orders of magnitude.

It would be possible from these distribution coefficients to calculate partition coefficients (i.e. log P values which characterize neutral species) using equations which correct for ionization (Van de Waterbeemd & Testa 1987). Because the esters examined here generally show only limited variations in their pK_a values, their correction for ionization would be comparable, meaning their log D and log P values must be highly correlated and thus contain the same information. This has indeed been verified (results not shown). Because of their interest in medicinal chemistry to estimate lipophilicity prior to synthesis, computational methods were used to calculate log P values directly from the molecular structures. The ClogP method (Leo 1993) yields log P values that correlate well with the experimental log D values (results not shown). The same is true of the molecular lipophilicity potential procedure of Gaillard et al (1994a, b). The log P values so calculated are reported in Tables 1 and 2, while the correlation is shown in Fig. 1 and equation 1:

$$\log D_{7\cdot4} = (1\cdot33 \pm 0.18) \log P_{MLP} - (0\cdot69 \pm 0\cdot22) \quad (1)$$
$$n = 21;\ r^2 = 0\cdot93;\ s = 0\cdot34,\ F = 241$$

The two N-BOC amides were not included in the correlation since their ionic behaviour is completely different from that of the esters. In equation 1, the non-zero intercept and the slope difference from one are explained by the different ionic species being taken into account, namely neutral species in log P and a mixture of neutral and cationic species in the case of log D.

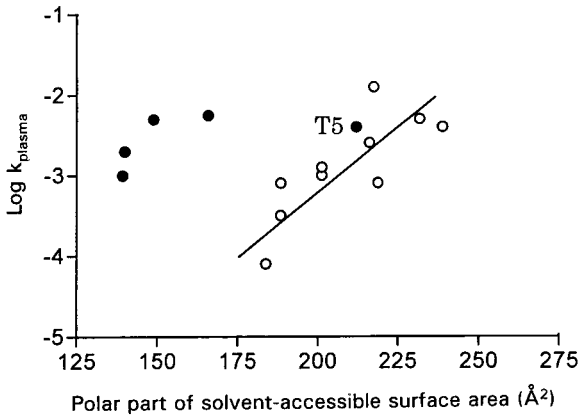


FIG. 3. Relationship between rate constants of enzymatic hydrolysis in 80% human plasma at pH 7·4 (log k_{plasma}; min^{-1}) and the polar part of the solvent-accessible surface areas (SAS in Å^2). L-Dopa esters ○; L-tyrosine esters ●.

Chemical hydrolysis

Chemical hydrolysis of L-dopa and Tyr esters was monitored in an MPS buffer under biomimetic conditions (pH 7·4, 37°C). For a few esters, the rate of hydrolysis was not measurable either because it was too fast (**T6**), or due to competitive reactions of oxidation or breakdown (**D7** and **T7**). In all other cases, the reaction obeyed pseudo-first-order kinetics, the straight lines in the semi-log plots in most cases having $r^2 > 0\cdot99$. The results are reported in Table 3.

When comparing pairs of L-dopa and Tyr esters with the same substituent, little differences are seen, i.e. **D1-T1**, **D2-T2**, **D3-T3** and **D4-T4**. In contrast, and as expected, the rate of reaction was highly dependent on the nature of the substituent. Thus, the rate of hydrolysis decreased in the series methyl > ethyl > isopropyl ≈ *sec*-butyl ≈ *t*-butyl. Esters **D6** and **D8** had similar substituents and comparable rates of hydrolysis. Benzyl and phenylethyl substituents (**D9** and **D10**, respectively) had a comparable influence. Interestingly, a phenoxyethyl and a pyridylmethyl substituent (**D11** and **T5**) had the same influence. The two N-BOC derivatives (**D12** and **T8**) were very stable at neutral pH.

An extensive search for quantitative structure-property relationships using a variety of structural descriptors (steric, electronic, lipophilic) yielded only one interesting relation. As shown in Fig. 2, a linear correlation exists for the 9 alkyl esters (**D1-D5**, **T1-T4**) between log k_{buffer} and molecular volume (eqn 2), such that the greater the substituent the slower the hydrolysis:

$$\log k_{buffer} = (-2\cdot4 \times 10^{-2} \pm 0\cdot8 \times 10^{-2})\ MV + (1\cdot45 \pm 1\cdot65) \quad (2)$$
$$n = 9;\ r^2 = 0\cdot89;\ s = 0\cdot20,\ F = 55$$

The other esters (**D6**, **D8-D11**, **T5**) are those containing an aromatic or polar substituent. They cluster together on the right-hand side of the figure, indicating that they are hydrolysed much faster than predicted from their molecular volume. Preliminary results suggest that these esters, in contrast to those included in equation 2, exist as folded conformers exposing their ester bridge.

Hydrolysis in human plasma

The ability of diluted, buffered human plasma to hydrolyse L-dopa and L-tyrosine esters was investigated under conditions of a very large excess of enzyme over substrate (initial substrate concentration = 9×10^{-4} M). The total protein concentration in 80% plasma was 57 ± 9 mg mL^{-1}. The

Table 4. Circling behaviour in rats following administration of equimolar doses of L-dopa (0·25 mmol kg^{-1}), L-tyrosine (2·75 mmol kg^{-1}) and L-phenylalanine (2·75 mmol kg^{-1}) and their respective prodrugs. All animals received carbidopa (25 mg kg^{-1}, i.p., 1 h before). Drug administration followed either an acute or sub-acute administration regimen. Data are expressed as total number of turns over the 5-h period (mean ± s.e.m., n = 6).

Administration		Compound (amino acid)	Control	Prodrug	Relative activity of prodrug
Acute	i.p.	**D3**	610 ± 213	500 ± 189	82
		D5	769 ± 188	910 ± 461	118
		D6	769 ± 188	1030 ± 312	134
		D11	610 ± 213	349 ± 174	57
		D12	828 ± 221	0	0
	p.o.	**D1**	1755 ± 283	1586 ± 284	90
		D3	1997 ± 179	2042 ± 103	102
		D4	1755 ± 283	1957 ± 367	112
		D7	1772 ± 344	1587 ± 382	90
Subacute	i.p.	**D3**	821 ± 349	1360 ± 456	166
		D4	821 ± 349	1246 ± 431	152
	p.o.	**D12**	1509 ± 504	0	0
Acute	p.o.	**T2**	0	0	—
		T3	0	0	—
		T4	0	0	—
Subacute	p.o.	**T1**	0	0	—
		T7	0	0	—
		T8	0	0	—
Subacute	i.p.	**P1**	0	0	—
		P2	0	0	—

concentrations of products monitored over time were corrected for chemical hydrolysis and thus represent the enzymatic hydrolysis alone. In all cases where the reaction could be monitored, it proved to be pseudo-first order, the lines in the semi-log plots often having $r^2 > 0{\cdot}97$ and always $> 0{\cdot}90$. This confirms that the effective enzymatic concentrations largely exceeded substrate concentrations. Table 3 reports the rate constants and half-lives of enzymatic hydrolysis.

As for its chemical hydrolysis, the plasma hydrolysis of **T6** was too fast to be assessed. The *t*-butyl ester of L-dopa (**D5**) was hydrolysed more slowly in plasma that in buffer under identical conditions of temperature and pH. This result may seem paradoxical but is believed to reflect binding to plasma proteins which in effect protects part of the substrate molecules from hydrolytic attack. Such an effect has been reported for aspirin (Aarons et al 1980) and timolol esters (Bundgaard et al 1986). No relationship exists between the rate constants of chemical and enzymatic hydrolysis.

A pairwise comparison of L-dopa and Tyr esters with the same alkyl substituent (**D1-T1**, **D2-T2**, **D3-T3** and **D4-T4**) showed that the latter are hydrolysed faster than the former by a factor of 4·5 ± 1·4. For a given amino acid, esters with primary alkyl groups (methyl and ethyl) differ negligibly in their hydrolysis, whereas esters with secondary alkyl groups are comparably more resistant. As mentioned above, the *t*-butyl ester did not appear to be hydrolysed. The hydrolysis of L-dopa esters containing a cyclic ether (**D6** and **D8**) or an aromatic ring (**D9**, **D10** and **D11**) in their substituent is difficult to rationalize at this stage.

Again relationships were sought with physicochemical properties and structural descriptors, but no clear correlation emerged. For all L-dopa esters but not Tyr esters, a trend was seen (Fig. 3) such that the rate of enzymatic hydrolysis increased with increasing polar surface. This suggests that electronic effects play a marked role in influencing the rate constant of hydrolysis and in making the phenoxyethyl ester the best substrate among all investigated compounds. For Tyr ester, no trend is apparent, this being in itself an interesting difference with L-dopa esters. Only **T5** appears to behave like an L-dopa ester, while the alkyl esters **T1-T4** are better substrates than predicted from their polar surface. Such an effect may be due to a more favourable positioning in the catalytic site as compared with L-dopa alkyl esters.

Ability to induce circling behaviour in an experimental model of hemiparkinsonism

Acute administration of L-dopa plus carbidopa induced a tight contraversive posture and rapid contraversive circling behaviour in rats with a prior 6-hydroxydopamine lesion of the substantia nigra. Circling commenced between 15 and 30 min after L-dopa administration and continued for 3–5 h. Acute intraperitoneal or oral administration of **D1**, **D3-D7** and **D11** also produced contraversive turning behaviour in rats. The overall activity of these prodrugs was not statistically different from that of L-dopa (Table 4). Two *sec*-alkyl esters of L-dopa (**D3** and **D4**) were also tested subacutely and produced a marked contraversive turning behaviour in rats on the 6th day of administration. Total number of turns on the 6th day tended to be greater than that achieved for L-dopa although the difference did not reach statistical significance (2-way analysis of variance) (Table 4). N-BOC L-dopa (**D12**) was inactive after both acute and subacute administration (Table 4).

Neither L-tyrosine nor any of its prodrugs induced circling in 6-hydroxydopamine-lesioned rats following acute or subacute treatment (Table 4). Administration of L-phenylalanine or its prodrugs for 5 days followed by a final challenge

on the 6th day did not induce circling behaviour in 6-hydroxydopamine-lesioned rats (Table 4).

To the best of our knowledge, the L-dopa esters **D3**, **D4**, **D5** and **D7** had never before been examined for their in-vivo dopaminergic activity. In a previous study (Cooper et al 1987a), **D6** and **D11** were found to be somewhat more and less active, respectively, than shown in the present work.

Conclusion

Previous studies (Cooper et al 1987a; van de Waterbeemd et al 1987) suggested that L-dopa esters with small substituents of moderate hydrophobicity or polarity might improve on the pharmacological activity of L-dopa. Based on this hypothesis, the esters **D3**, **D4**, **D5** and **D7** were synthesized and examined in an improved model of hemiparkinsonism. Other esters already described by us (**D1**, **D6** and **D11**) were used for comparison. These and other esters (**D2**, **D8-D10**) were also examined for their rate of hydrolysis in human plasma, an important property when it comes to evaluating prodrug candidates.

It is difficult to make conclusions from the present study on the advantages and limitations of L-dopa prodrugs. The compounds reported here differed vastly in their lipophilicity and rates of chemical and enzymatic hydrolysis, yet their absorption and distribution characteristics in rats, as deduced from the onset of rotation and general pharmacological profile, were comparable with those of L-dopa itself. Indeed, none of the tested prodrugs were longer acting or markedly more active in rats than L-dopa itself. However, the rat has carboxylesterase activity in plasma that does not exist in man or in monkey, making our results difficult to extrapolate to humans.

One clear advantage of L-dopa prodrugs over L-dopa itself is their much greater water solubility. This could allow alternative modes of administration to be explored, such as subcutaneous and intravenous infusions, the development of which calls for prior knowledge of lipophilicity and hydrolytic behaviour. Furthermore, L-dopa prodrugs may soon attract increased interest due to the recent finding that L-dopa itself may act as an endogenous neurotransmitter in addition to being the precursor of dopamine (Misu & Goshima 1993).

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