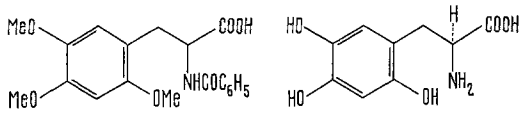
Preparation and Biological Properties of (—)- and (+)-6-Hydroxydopa

It has been demonstrated that 6-hydroxydopamine causes depletion of norepinephrine levels in various tissues[1] and degeneration of adrenergic neurons[2–4] and that (±)-3-(2,4,5-trihydroxyphenyl)alanine [(±)-6-hydroxydopa] also reduces tissue stores of norepinephrine[5]. We now report the preparation of the optical isomers of 6-hydroxydopa and their biological activities.

The preparation of (—)-6-hydroxydopa [(—)-II] and its enantiomer (+)-II (opposite stereochemistry) was accomplished via resolution of (±)-N-benzoyl-3-(2,4,5-trimethoxyphenyl)alanine[5] [(±)-I] with half an equivalent of either (—)- or (+)-α-methylbenzylamine (α-MBA). Treatment of (±)-I with the levo base in isopropanol provided the (—)-α-MBA salt of (—)-I: mp 147°, $[\alpha]_D^{25}$ — 25.7° (c 2, CH_3OH). Under the same conditions, (±)-I and the dextro base yielded the (+)-α-MBA salt of (+)-I: mp 147°, $[\alpha]_D^{25}$ + 25.0° (c 2, CH_3OH). Each of these diastereoisomers was neutralized to give the corresponding free acids (—)-I: mp 205–206° (from CH_3OH), $[\alpha]_D^{25}$ — 53.1° (c 1, CH_3OH) and (+)-I: mp 205–206° (from CH_3OH), $[\alpha]_D^{24}$ + 52.7° (c 1, CH_3OH).

Refluxing (—)-I with 48% HBr in a N_2 atmosphere for 4 h effected simultaneous cleavage of the amide and ether functions to afford, after neutralization with propylene oxide, (—)-6-hydroxydopa[6] [(—)-II]: mp 239–240° (from SO_2 saturated H_2O); $[\alpha]_D^{25}$ — 11.7° (c 2.1, CH_3OH: 1 *N* HCl, 1:1); CD (c 0.007 mole, CH_3OH:1 *N* HCl, 1:1) $[\theta]_{309}$ 0, $[\theta]_{289}$ + 860, and $[\theta]_{265}$ 0. Similarly, (+)-I was transformed to the dextrorotatory isomer (+)-II: mp 240 – 241°, $[\alpha]_D^{25}$ + 11.1° (c 2.1, CH_3OH:1 *N* HCl, 1:1), identical in IR, UV and NMR with (—)-II, CD mirror image of (—)-II.

All compounds gave acceptable elemental analyses. Thin layer chromatographic studies on (—)- and (+)-II indicated the absence of any significant amount of 6-hydroxydopamine (< 0.01%).



I (-)-II

For determination of the biological activities, (+)- and (—)-II were dissolved in saline acidified with several drops of 1 *N* HCl and injected i.p. into female Sprague Dawley rats (200 g). Brainstem and adrenals were analyzed fluorometrically for norepinephrine or serotonin after extraction by the method of MEAD and FINGER[7]. Heart norepinephrine was isolated by alumina adsorption as described by DE CHAMPLAIN et al.[8] and assayed spectrophotofluorometrically using a modification of the trihydroxyindole procedure described by CROUT[9]. 6-Hydroxydopa was analyzed fluorometrically by the same procedure as norepinephrine. The fluorescence attributable to 6-hydroxydopa was negligible (< 2% of an equivalent amount of norepinephrine). Although the norepinephrine fluorescence could be quenched as much as 25% by 6-hydroxydopa when the two were mixed in vitro, tissue samples from animals treated with 6-hydroxydopa exhibited no quenching of norepinephrine fluorescence.

When given once a day for 2 days, 100 mg/kg of (—)-II lowered the levels of norepinephrine in rat brainstem by 34% (from 0.83 μg/g of the controls to 0.55 ± 0.05). The same dosage of the (+)-isomer and 25 mg/kg of the (—)-isomer were without significant effect. Neither isomer depleted brainstem serotonin at either of the doses tested.

In contrast to the brain, both the (+)- and (—)-isomer of 6-hydroxydopa depleted peripheral tissues of catecholamines (Table). The (—)-isomer was the most effective in lowering the catecholamine content of the heart and adrenal. A dose of 25 mg/kg of (—)-6-hydroxydopa depleted heart norepinephrine more than 90% while (+)-6-hydroxydopa produced more than a 50% decline. Adrenals were significantly depleted by both isomers at the 100 mg/kg dose, 50% by the (—)-isomer and 20% by the (+)-isomer.

6-Hydroxydopamine is capable of depleting norepinephrine from peripheral tissues[1,4,10–12]. It also has the potential of reducing brain norepinephrine levels if administered directly into the central nervous system[2,3]. The precursor of this amine is very probably the amino acid (—)-6-hydroxydopa[5]. The marked effects reported in these studies by (—)-6-hydroxydopa on the norepinephrine content are understandable as it is easily decarboxylated enzymatically[5,13]. However, the mechanism by which (+)-6-hydroxydopa also lowers the

Effect of (+)- and (—)-6-hydroxydopa on the catecholamine levels in heart and adrenals of the rat[a]

Isomer	Dose (mg/kg)	Catecholamines[b]	
		Heart (μg/g)	Adrenal (μg/pair)
Control		1.15 ± 0.05	27.1 ± 1.9
(+)-	25	0.38 ± 0.15[c]	24.7 ± 0.7
(+)-	100	0.55 ± 0.05[c]	20.5 ± 1.5[c]
(—)-	25	0.10 ± 0.05[c]	24.0 ± 0.6
(—)-	100	0.11 ± 0.02[c]	14.6 ± 2.0[c]

[a] Drugs were injected i.p. for two days and rats sacrificed on the third day. Results are the mean ± S.E. of 6 animals per dose. [b] Heart catecholamine is norepinephrine, the adrenal catecholamine was calculated as norepinephrine equivalents per adrenal pair. [c] Statistically significant $p < 0.05$ (calculated by Student *t*-test).

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heart and adrenal catecholamine concentration is as yet to be determined.

The heart is the most sensitive tissue to the depleting action of the (—)-isomer with greater than 90% reduction of norepinephrine at the 25 mg/kg dose. Brainstem and adrenal glands were lowered 30 and 50% respectively at the 100 mg/kg dose. This depleting action on the adrenals has been confirmed by DAIRMAN[13] and has not been observed with 6-hydroxydopamine[14]. In contrast, both isomers failed to lower brainstem serotonin concentration which is similar to the effects of 6-hydroxydopamine[2].

The results with the optical isomers of 6-hydroxydopa suggest some interesting possibilities in utilizing one of these compounds as experimental pharmacological tool. First, (—)-6-hydroxydopa at a dose of 25 mg/kg for 2 days can markedly deplete peripheral stores of norepinephrine without altering central levels. Alternatively, utilizing (—)-6-hydroxydopa with a peripheral decarboxylase inhibitor such as N^1-(DL-seryl)-N^2-(2, 3, 4-trihydroxybenzyl)hydrazine[12], peripheral depletion of catecholamines can be kept at minimal levels while central depletion is pronounced. Finally, (—)-6-hydroxydopa (100 mg/kg) administered peripherally can deplete brain norepinephrine without altering brain serotonin content.

Zusammenfassung. Die optischen Isomeren von 6-Hydroxydopa wurden synthetisiert und ihr Effekt auf den Katecholaminspiegel verschiedener Organe in der Ratte bestimmt. Dem (—)-Isomeren gegenüber waren Herz und Nebennieren äusserst empfindlich und im Gehirn wurde der Norepinephrinspiegel erniedrigt.

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Electrocardiographic and Behavioral Effects of L-DOPA in the Guinea-Pig

Since 1960[1] the guinea-pig has been shown to be susceptible to psychopharmacological procedures, particularly to the deconditioning effect of psychotropic drugs[2–12].

It is known that L-DOPA, beside representing a new and very effective agent for the treatment of parkinsonism, may influence human and animal behavior[13]. Perhaps the behavioral effects of L-DOPA may contribute in some degree to the efficacy of the drug in parkinsonism. Therefore, in the present note we report some results of research in progress on the behavioral effects of L-DOPA in the guinea-pig. A specific deconditioning effect[6,11,12] of the drug in this animal could support the hypothesis that L-DOPA has psychotropic properties, in addition to antiparkinson action.

On the other hand, many Parkinson patients treated with L-DOPA develop side effects, including psychoneurological and cardiological alterations[13]. Thus the purpose of our research was also to investigate and compare the effects of L-DOPA on spontaneous behavior and ECG in guinea-pigs[2,3]. Since the side-effects and toxicity of L-DOPA per os in man seem to be mainly connected with impurities contained in a 'preparation of doubtful quality'[13], in our experiments we have used L-DOPA supplied by Roche of Switzerland (LARODOPA), which 'until recently was the only L-DOPA approved by the Committee' on the Safety of Drugs[13].

For the clinical implications of our experimental results we have used the same route of administration and a dose pro kg close to the daily dose pro kg usually employed in Clinics for the L-DOPA treatment of parkinsonism.

Methods and results. Effects on spontaneous behavior and ECG. In 15 male guinea-pigs weighing 420–650 g, L-DOPA in single oral dose of 10-100-1000 mg/kg (5 animals for each dose) did not induce any changes in spontaneous behavior or ECG recorded before and 1–24 h after the administration.

In another experiment, 4 male guinea-pigs, each weighing about 500 g, were subjected first to administration of 1000 mg/kg per os of L-DOPA followed 24 h later by a second administration of 3000 mg/kg per os of L-DOPA. After the first administration no behavioral changes were observed, while increased diuresis, tremors and depression were found in all the 4 guinea-pigs after the second administration. 1 guinea-pig died 40 h after the second administration, while the other 3 animals survived, recovering from the behavioral changes in a few hours following the second L-DOPA administration.

No significant changes were found in the electrocardiographic records made in D_2 before, 1 h and 24 h after each administration of L-DOPA. No more deaths or behavioral changes occurred in the 18 guinea-pigs during the week following both experiments.

Effects on avoidance conditioning. 12 male guinea-pigs weighing about 500 g each were conditioned to an avoidance situation by the method described by MA-

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