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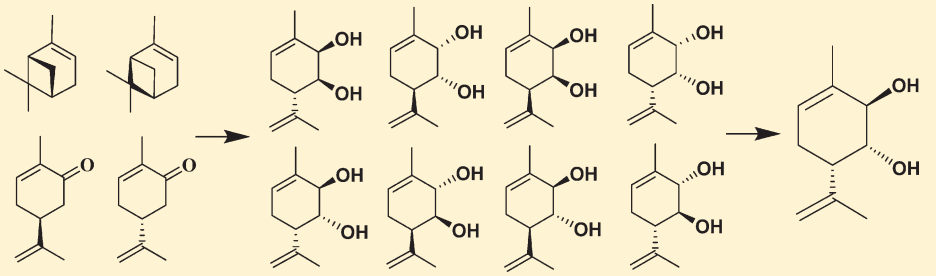
Highly Potent Activity of (1*R*,2*R*,6*S*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol in Animal Models of Parkinson's Disease

Oleg V. Ardashov, Alla V. Pavlova, Irina V. Il'ina, Ekaterina A. Morozova, Dina V. Korchagina, Elena V. Karpova, Konstantin P. Volcho,* Tat'yana G. Tolstikova, and Nariman F. Salakhutdinov

N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Lavrentiev Avenue, 9, 630090 Novosibirsk, Russian Federation

Supporting Information

ABSTRACT:



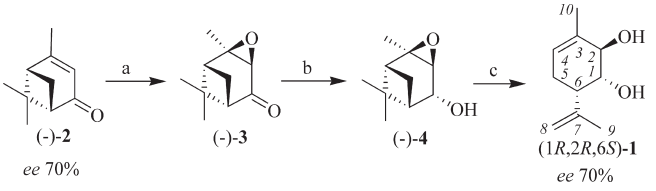
(1*R*,2*R*,6*S*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol **1** possesses potent antiparkinsonian activity in both MPTP and haloperidol animal models. The use of compound **1** resulted in nearly full recovery of the locomotor and exploratory activities and was as effective as the comparator agent (levodopa). All eight stereoisomers of compound **1** have been synthesized and the influence of the absolute configuration on the antiparkinsonian activity of compound **1** was shown.

INTRODUCTION

Parkinson's disease (PD) is one of the most common neurological diseases characterized mostly by motor disturbances and usually caused by the loss of the nigrostriatal path's dopamine-containing cells. The percent of people affected by PD at the age of 60−80 is around 1%.[1] While symptoms generally appear around the age of 60, they can be found in much younger people, which makes it a condition that does not recognize the boundaries of age, gender, or race.[2] Some of the symptoms associated with PD involve rigidity, bradykinesia, resting tremor, and postural instability along with cognitive and psychiatric complications.[3,4]

A number of medications are available to treat the movement-related (motor) symptoms of PD, including levodopa, dopaminergic agonists, anticholinergics, amantadine, and monoamine oxidase B inhibitors. Levodopa remains the most effective medicine, in particular for treating rigidity and slowness of movement. It is usually combined with a dopa decarboxylase inhibitor that prevents levodopa from being utilized on the periphery. Most patients initially do well on levodopa. However, the treatment is associated with several side effects that people with PD may experience, such as nausea and appetite loss, involuntary movements, twisting motions and abnormal postures (dystonia), hallucinations, and paranoia. Prolonged use of levodopa also gives rise to "on/off" episodes, resulting in additional complications.[2] Therefore, the search for new effective medications for the medical correction of PD remains important.

Scheme 1[a]



[a] Reagents and conditions: (a) H_2O_2/H_2O, NaOH, MeOH 10 °C, 2 h; (b) $LiAlH_4$, Et_2O, 0 °C, 3.5 h; (c) K10 clay, CH_2Cl_2, 1 h.

We have recently discovered[5] that compound (1*R*,2*R*,6*S*)-**1** (Scheme 1) possesses a high antiparkinsonian activity expressed in the elimination of oligokinesia in C57Bl/6 mice caused by single or systematic injections of the neurotoxin MPTP. The parkinsonian syndrome was caused by intraperitoneal administrations of 0.17 mmol/kg (30 mg/kg) doses of MPTP daily for 3 days. A 0.12 mmol/kg (20 mg/kg) dose of (1*R*,2*R*,6*S*)-**1** (70% ee) was administered per os 24 h after the final injection of MPTP. Hypokinesia was evaluated 1.5 h after the injection of MPTP on the basis of the locomotor-orientational activity. The studied agent, (1*R*,2*R*,6*S*)-**1** (70% ee), distinctly improved the markers of the locomotor and exploratory activities of the animals (demonstrated

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Table 1. Study of the Antiparkinsonian Activity of (1*R*,2*R*,6*S*)-1 (70% ee) in C57Bl/6 Mice[a]

group, dose	*A*	*B*	*C*	*D*	*E*	*F*
saline	73.3 ± 2.3**	90.0 ± 1.4**	556.7 ± 19.6**	4.6 ± 0.2**	4.1 ± 1*	24.9 ± 3.3**
MPTP[b]	89.3 ± 1.8	64.1 ± 4.1	286.6 ± 36.5	2.5 ± 0.2	1.6 ± 0.6	8.5 ± 2.2
MPTP[b] and (1*R*,2*R*,6*S*)-**1**, 0.12 mmol/kg (20 mg/kg)	80.5 ± 3.9	74.8 ± 6.4*	439.6 ± 32.9**	3.6 ± 0.3**	6.5 ± 1.6*	14.8 ± 3.4*

[a] *A*, general locomotor activity (number of acts); *B*, time of locomotor activity (s); *C*, movement distance (cm); *D*, movement speed (cm/s); *E*, number of explored holes; *F*, number of upright postures. (*) $P < 0.05$, (**) $P < 0.01$ reliability in comparison with MPTP group. [b] MPTP (4 × 20 mg/kg).

Table 2. Study of the Antiparkinsonian Activity of (1*R*,2*R*,6*S*)-1 (70% ee) in Rats during a 30-Day Administration of the Agents and MPTP (0.23 mmol/kg (40 mg/kg))[a]

group, dose	*A*	*B*	*C*	*D*	*E*	*F*
saline	80.3 ± 2.5**	69.7 ± 2.1**	367.5 ± 21.2**	3.0 ± 0.2**	4.6 ± 0.9	14.6 ± 1.8**
MPTP	50.4 ± 7.9	27.8 ± 4.8	110.4 ± 19.5	0.9 ± 0.2	2.7 ± 1.0	2.6 ± 0.8
MPTP and (1*R*,2*R*,6*S*)-**1**, 0.12 mmol/kg (20 mg/kg)	81.9 ± 5.5**	62.6 ± 5.2**	305.6 ± 30.4**	2.5 ± 0.3**	4.3 ± 0.7	10.7 ± 1.2**
MPTP and levodopa, 0.05 mmol/kg (10 mg/kg)	86.0 ± 3.9**	65.1 ± 2.9**	316.8 ± 16.2**	2.6 ± 0.13**	4.6 ± 0.6	9.6 ± 1.2**

[a] *A*, general locomotor activity (number of acts); *B*, time of locomotor activity (s); *C*, movement distance (cm); *D*, movement speed (cm/s); *E*, number of explored holes; *F*, number of upright postures. (*) $P < 0.05$, (**) $P < 0.01$ reliability in comparison with MPTP group.

by the number of the explored holes and the time of exploratory reactions); in other words, it removed the symptoms of the PD induced by the thrice-repeated administration of the neurotoxin.

It was shown[6] that (1*R*,2*R*,6*S*)-**1** (70% ee) enhanced the inhibitory effect of high doses of levodopa (1.0 mmol/kg (200 mg/kg)) and thereby potentiated the dopaminergic system. Moreover, in the arecoline tremor test, which makes it possible to estimate the effect on muscarinic cholinergic receptors, the compound decreased the duration of arecoline induced tremor, exhibiting an M-anticholinergic activity. The combination of these properties is probably responsible for the antiparkinsonian activity of (1*R*,2*R*,6*S*)-**1**.

We also note the low acute toxicity of compound (1*R*,2*R*,6*S*)-**1**, the LD_{50} of which amounts to 4250 mg/kg.[6]

Because of this combination of traits, further study of the antiparkinsonian activity of this substance holds much promise.

RESULTS

Study of the Antiparkinsonian Activity of (1*R*,2*R*,6*S*)-1 with 70% ee. Compound (1*R*,2*R*,6*S*)-**1** was previously synthesized from verbenone (−)-**2** by epoxidation with hydrogen peroxide, reduction of verbenone epoxide (−)-**3** to *cis*-verbenol epoxide (−)-**4** using $LiAlH_4$, and subsequent rearrangement of this epoxide into the desired substance in the presence of montmorillonite clay (Scheme 1).[7,8] Because of the commercial availability of verbenone with up to 70% ee, we also used (1*R*,2*R*,6*S*)-**1** with 70% ee in our previous work.[5] Consequently, we also studied the possible antiparkinsonian activity of (1*R*,2*R*,6*S*)-**1** with the same 70% ee in this work.

In animal studies, neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine (MPTP) and neuroleptic drugs (e.g., haloperidol) are commonly used to create experimental models of PD, which may be used to model certain aspects of the disease, such as catalepsy, motor imbalance, and slowing of movement.[9−11]

In our previous article[5] we used the MPTP model of PD in accordance with the Russian "Guide for Preclinical Trials of New Pharmacological Substances".[12] The model includes the intraperitoneal injection of MPTP (0.17 mmol/kg (30 mg/kg)) to mice 15 min before the administration of the studied substances. Hypokynesia was evaluated with the "open field" test 1.5 h after the injection of MPTP.

To confirm the results obtained earlier in this work, we additionally used the protocol for the MPTP mouse model of Parkinson's disease published in *Nature Protocols*.[13] It involves one injection of MPTP ((0.12 mmol/kg (20 mg/kg) per dose) to mice of C57Bl/6 line every 2 h for a total of four doses over an 8 h period in 1 day. The studied agent was administered per os 24 h after the last injection of MPTP in a dose of 0.12 mmol/kg (20 mg/kg). Hypokynesia was measured 2 h after the administration of the agent with the "open field" test for 2 min. The effectiveness of the studied medication was evaluated according to its ability to reduce the symptoms of hypokinesia induced by MPTP.

As can be seen from Table 1, (1*R*,2*R*,6*S*)-**1** in a dose of 0.12 mmol/kg (20 mg/kg) sufficiently improved both the locomotor (columns B−D) and exploratory (columns E, F) activities. Thus, (1*R*,2*R*,6*S*)-**1** (70% ee) demonstrated a reliable antiparkinsonian activity in this model, too.

A lengthier experiment, which involved a 30-day administration of MPTP, (1*R*,2*R*,6*S*)-**1**, and a reference agent (levodopa), was performed on rats of the Wistar line. Rats are less sensitive to the MPTP effect, so it is reasonable to use them in a prolonged experiment. The parkinsonian syndrome was induced via intraperitoneal administrations of MPTP (0.23 mmol/kg (40 mg/kg) doses daily) for 30 days, while the control group received saline. Compound (1*R*,2*R*,6*S*)-**1** (70% ee) in a dose of 0.12 mmol/kg (20 mg/kg) or the levodopa reference in a dose of 0.05 mmol/kg (10 mg/kg) was administered per os every day 4 h after the MPTP injections. Hypokinesia was evaluated from the locomotor and exploratory activity on the 30th day after the start of the experiment with the "open field" test for 2 min; the experimental data are presented in Table 2.

The experiment showed that the 0.12 mmol/kg (20 mg/kg) dose of (1*R*,2*R*,6*S*)-**1** (70% ee) exhibited a distinct antiparkinsonian activity after a 30-day administration of the (1*R*,2*R*,6*S*)-**1** and MPTP, which was demonstrated by a nearly full recovery of the animal's locomotor (general locomotor activity, movement distance, movement speed, time of locomotor activity) and exploratory (an increase in the number of explored holes and assumption

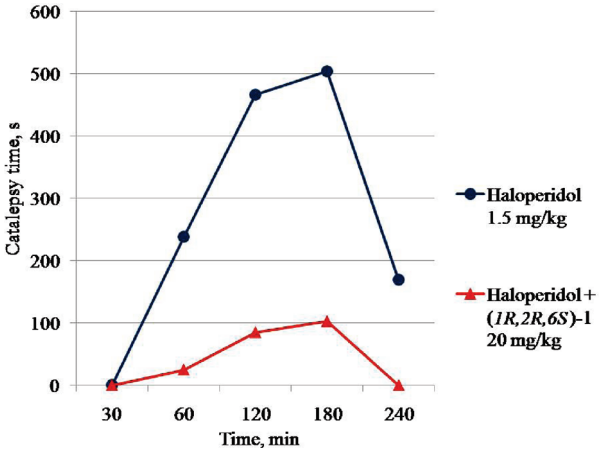


Figure 1. Duration of catalepsy evoked by haloperidol.

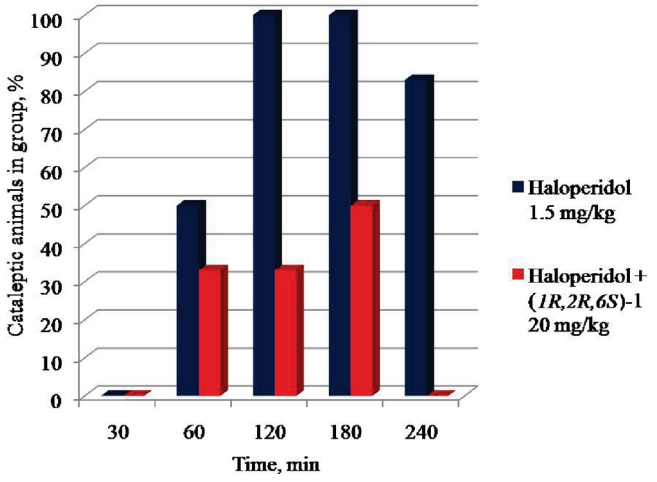


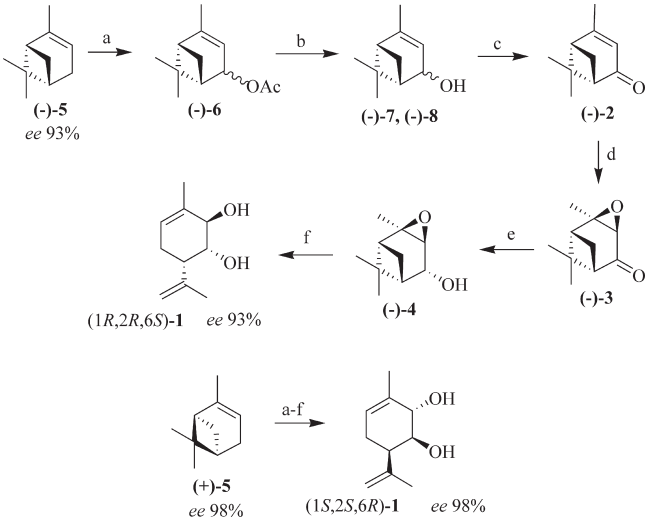
Figure 2. Percent of animals in the group with catalepsy evoked by haloperidol.

of vertical poses) activities. Compound (1*R*,2*R*,6*S*)-**1** (70% ee) works as effectively as the reference agent (levodopa) in this test.

Cataleptic immobility induced in rodents by typical neuroleptics (e.g., haloperidol, chlorpromazine, fluphenazine) is a behavioral method to study the nigrostriatal function and its modulation by other neurotransmitters.[14] Haloperidol blocks dopamine D2 receptors in the striatum and is used to evoke drug-induced parkinsonism.[15] Drugs that attenuate haloperidol-induced motor disorders might reduce the extrapyramidal signs of PD.[16]

After the administration of the drugs (10 min) to rats of the Wistar line, catalepsy was induced via the intraperitoneal administration of haloperidol in a dose of 4.0 μmol/kg (1.5 mg/kg) of body weight. The severity of catalepsy was measured 30, 60, 120, 180, and 240 min after the administration of haloperidol using the parallel bars method. We recorded the time spent by an animal in a cataleptic state and evaluated the general duration of catalepsy and the percent of cataleptic animals in the group. Compound (1*R*,2*R*,6*S*)-**1** (70% ee) significantly blocks the development of catalepsy evoked by haloperidol, which showed itself as a decrease in catalepsy time, haloperidol's time course (Figure 1), and the percent of cataleptic animals (Figure 2). Thus, compound (1*R*,2*R*,6*S*)-**1** (70% ee) demonstrated potent

Scheme 2[a]



[a] Reagents and conditions: (a) $Pb(OAc)_4$, C_6H_6, 65 °C, 1 h; (b) KOH, MeOH, H_2O, 24 h; (c) $Na_2Cr_2O_7$, H_2SO_4, Et_2O, 0 °C (1 h), rt (24 h); (d) H_2O_2, NaOH, MeOH, H_2O, 10 °C, 2 h; (e) $LiAlH_4$, Et_2O, 0 °C, 3.5 h; (f) K10 clay, CH_2Cl_2, 1 h.

antiparkinsonian activity during in vivo experiments on mice and rats in models induced by MPTP and haloperidol.

Synthesis of All Stereoisomers of Compound 1 with High Optical Purity. As is known, the absolute configuration of chiral compounds tends to be critical for the manifestation of various biological activities. Compound **1** has three asymmetric centers and hence eight stereoisomers. Synthesis of all stereoisomers with high (no less than 90% ee) optical purity is necessary for an in-depth study of the influence of the absolute configuration of compound **1** on its antiparkinsonian activity.

Synthesis of the first enantiomers, compounds (1*R*,2*R*,6*S*)-**1** and (1*S*,2*S*,6*R*)-**1**, was based on the commercially available (−)- and (+)-α-pinenes (−)- and (+)-**5** with high optical purity (Scheme 2).

According to the methods suggested in the article,[17] (−)- and (+)-verbenones **2** were obtained by the interaction of α-pinenes **5** with $Pb(OAc)_4$, subsequent saponification of acetates **6**, and the oxidation of *trans*- and *cis*-verbenols **7** and **8**. The following transformations of verbenones **2** into compounds (1*R*,2*R*,6*S*)-**1** and (1*S*,2*S*,6*R*)-**1** were performed according to the previously developed methods.[7,18] Thus, compound (1*R*,2*R*,6*S*)-**1** was synthesized from α-pinene **5** in six steps with a 12% overall yield; the yield of compound (1*S*,2*S*,6*R*)-**1** amounted to 11%. The enantiomeric excess of products **1** was defined by GC−MS using a chiral column; it was caused by the optical purity of the starting pinenes and amounted to 93% for (1*R*,2*R*,6*S*)-**1** and 98% for (1*S*,2*S*,6*R*)-**1**.

The key stage of the synthesis of the second enantiomer pair, (1*S*,2*R*,6*S*)-**1** and (1*R*,2*S*,6*R*)-**1**, was synthesis of *trans*-verbenol epoxides (+)- and (−)-**9** and their further rearrangement into the corresponding isomers of diol **1** (Scheme 3); *trans*-verbenols (+)- and (−)-**7** were obtained by the literature procedure.[19] The main difficulty in the synthesis of epoxides **9** was the low stability of this compound even in weak acid media, which made it impossible to use peracids, generally used for similar oxidations. For the epoxidation of *trans*-verbenol **7**, we used the

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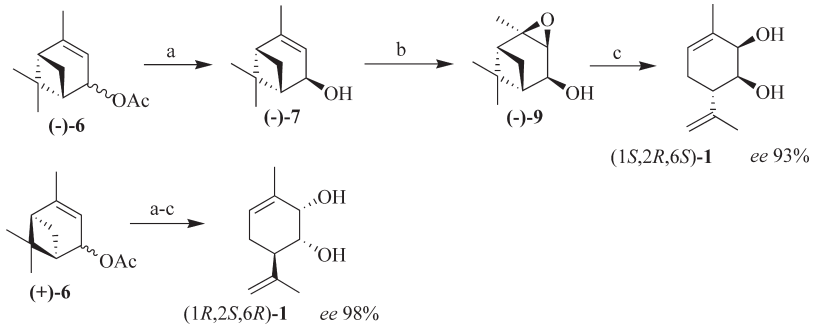
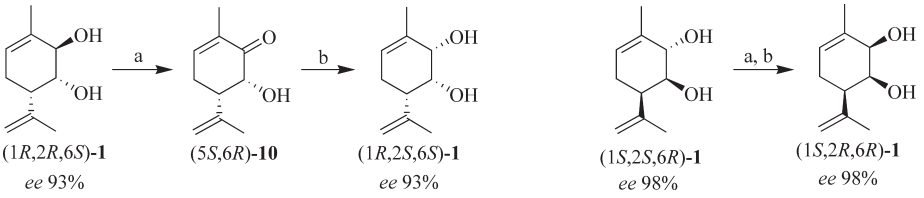
Scheme 3[a]



[a] Reagents and conditions: (a) (1) KOH, MeOH, H_2O, 24 h; (2) separation on SiO_2; (b) *t*-BuOOH, $VO(acac)_2$, toluene, reflux, 40 min; (c) K10 clay, CH_2Cl_2, 1 h.

Scheme 4[a]



[a] Reagents and conditions: (a) (1) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −25 °C, 40 min; (2) separation on SiO_2; (b) (1) $LiAlH_4$, Et_2O, 0 °C, 5 h; (2) separation on SiO_2.

$VO(acac)_2$/*t*-BuOOH system, which had previously been successfully used for the epoxidation of allylic alcohols.[20] The rearrangement of epoxides **9** in the presence of K10 clay resulted in the obtaining of the desired diol of (1*S*,2*R*,6*S*)-**1**. After the rearrangement of *trans*-verbenol epoxides **9**, the yield of compound **1** was significantly smaller than that obtained for *cis*-verbenol epoxides **4** (12−17% versus 40−48%).

Attempts to obtain the *cis*-epoxides of verbenone and verbenol, which could serve for the synthesis of the remaining four isomers of compound **1** through the corresponding bromohydrins, failed because of the ring-opening of cyclobutane under the reaction conditions.[21]

One of the most effective methods of the configuration inversion of the hydroxy group is the Mitsunobu reaction.[22,23] It involves the interaction with diethyl azodicarboxylate, *p*-nitrobenzoic acid, and triphenylphosphine to form nitrobenzoate, with the inversion at the hydroxyl-bearing carbon atom and hydrolysis at the second stage. Unfortunately, using the Mitsunobu reaction for altering the configuration of the hydroxy group in (1*R*,2*R*,6*S*)-**1** resulted, after all the necessary manipulations, in the obtaining of the initial compound with no changes in the configuration.

Another approach to the inversion of the allylic hydroxyl group can be its oxidation into corresponding hydroxy ketone **10** followed by its reduction. Considering the steric hindrance caused by the isopropenyl group, one can expect a preferential formation during the reduction of ketone **10** of the desired isomer. Indeed, it is known that the reduction of carvone to carveol occurs mostly on the side with lesser steric hindrances, giving a hydroxyl group in the cis-position in relation to the isopropenyl group.[24]

According to the literature, the oxidation of vicinal diols often does not stop at the stage of hydroxy ketone or diketone formation but proceeds further and is followed by C−C bond cleavage, ultimately resulting in carbonyl compounds and carboxylic acids. This behavior is typical not only for the rather stringent oxidants such as sodium dichromate, chromic anhydride, and potassium permanganate but also for the well-known mild oxidants, such as iodoxybenzoic acid (IBX), Dess−Martin periodinane (DMP), pyridinium chlorochromate (PCC), and manganese dioxide.[25−28]

We tested the oxidation of (1*R*,2*R*,6*S*)-**1** using a broad set of oxidizing systems: Fétizon's reagent,[29] manganese dioxide (Attenborough method),[30] sodium dichromate in the presence of sulfuric acid, Collins reagent ($CrO_3 \cdot 2Py$),[31] pyridinium chlorochromate (PCC) applied to alumina,[32] NaOCl in the presence of TEMPO nitroxide,[33] and *t*-BuOOH with $VO(acac)_2$, IBX,[34] DMP,[35] and the Swern oxidation.[36] In all cases, the yield of (5*S*,6*R*)-**10** was low; the best result was obtained with the Swern system. The preparative yield of (5*S*,6*R*)-**10** during the Swern oxidation amounted to 37%. Using the reduction of (5*S*,6*R*)-**10** with $LiAlH_4$ followed by column chromatography, we obtained the desired full cis-isomer of (1*R*,2*R*,6*S*)-**1** with a 51% yield. In addition, the initial (1*R*,2*R*,6*S*)-**1** isomer was also isolated (11%). Similarly, we synthesized (1*S*,2*R*,6*R*)-**1** with an overall yield of 21% in two steps (Scheme 4).

Two more stereoisomers, (1*S*,2*S*,6*S*)- and (1*R*,2*R*,6*R*)-**1**, were synthesized from (+)- and (−)-carvones **11** according to Scheme 5. Synthesis of (5*S*,6*S*)-**10** via the intermediate formation of (−)-**12** and (−)-*trans*-**13** is described in literature.[37] Note that ref 38 suggested a modified version of the method in ref 37, offering simplified handling and processing and higher yields of the desired products. When we attempted to use it, however, most of its steps turned out to be irreproducible. As a result, we synthesized (−)-*trans*-**13** by the procedure described in ref 37. We used $NH_4F \cdot HF$ instead of the previously suggested HF to

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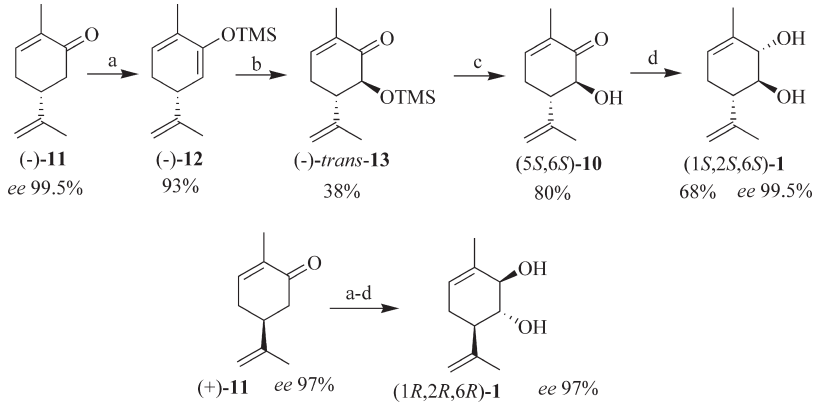
Scheme 5[a]



[a] Reagents and conditions: (a) LDA, TMSCl, THF, −80 °C (20 min), 0 °C (1.5 h); (b) 1) *m*-CPBA, CH_2Cl_2, 0 °C, 1.5 h, (2) separation on SiO_2; (c) $NH_4F \cdot HF$, EtOH, H_2O, 6 h; (d) (1) $LiAlH_4$, Et_2O, 0 °C, 4 h; (2) separation on SiO_2.

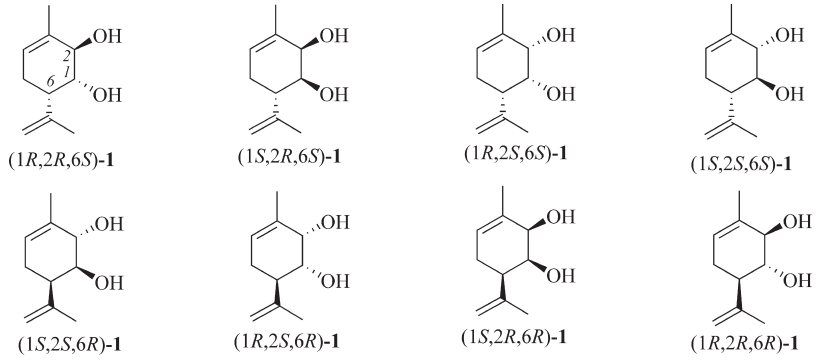


Figure 3

Table 3. Study of the Antiparkinsonian Activity of the Stereoisomers of Compound 1 (0.12 mmol/kg (20 mg/kg)) in C57Bl/6 Mice after a Single Administration of MPTP (0.17 mmol/kg (30 mg/kg))[a]

no.	group, dose	*A*	*B*	*C*	*D*	*E*	*F*
1	saline	84.1 ± 1.9**	58.3 ± 3.9**	276.8 ± 27.8**	2.3 ± 0.2**	15.6 ± 3.3	6.5 ± 1.3**
2	MPTP	50.6 ± 8.1	27.8 ± 4.8	107.8 ± 20.6	0.9 ± 0.2	2.6 ± 0.8	2.8 ± 0.9
3	MPTP and (1*R*,2*R*,6*S*)-**1**	78.6 ± 5.0**	64.0 ± 5.5**	332.6 ± 39.6**	2.7 ± 0.3**	13.1 ± 2.5**	3.2 ± 0.9
4	MPTP and (1*S*,2*S*,6*R*)-**1**	57.9 ± 6.0	34.9 ± 4.3	147.8 ± 20.3	1.1 ± 0.2	4.4 ± 1.4	2.5 ± 0.9
5	saline	84.1 ± 1.9**	58.3 ± 3.9**	276.8 ± 27.8**	2.3 ± 0.2**	15.6 ± 3.3**	6.5 ± 1.3**
6	MPTP	71.8 ± 6.4	47.9 ± 4.5	218.1 ± 26.2	1.8 ± 0.2	8.9 ± 1.5	1.9 ± 0.5
7	MPTP and (1*S*,2*R*,6*S*)-**1**	64.9 ± 8.7	45.5 ± 8.2	212.4 ± 48.7	1.7 ± 0.4	3.5 ± 1.0	4.0 ± 1.3
8	MPTP and (1*R*,2*S*,6*R*)-**1**	80.2 ± 6.2*	55.6 ± 6.9	245.9 ± 34.6*	1.2 ± 0.2	14.4 ± 3.6**	3.1 ± 0.6*
9	Saline	83.0 ± 2.4**	75.9 ± 1.2**	417.1 ± 21.2**	3.4 ± 0.2**	17.6 ± 5.4	5.4 ± 1.0
10	MPTP	69.9 ± 4.7	46.8 ± 4.4	211.8 ± 29.1	1.7 ± 0.3	11.3 ± 3.8	6.3 ± 1.8
11	MPTP and (1*R*,2*S*,6*S*)-**1**	28.5 ± 10.1*	15.0 ± 5.4**	55.9 ± 20.9**	0.4 ± 0.2**	0.9 ± 0.6*	1.7 ± 0.6*
12	MPTP and (1*S*,2*R*,6*R*)-**1**	82.4 ± 5.8	56.6 ± 6.2	257.7 ± 36.6	2.1 ± 0.3	8.1 ± 2.0	7.9 ± 1.6
13	MPTP and (1*S*,2*S*,6*S*)-**1**	77.4 ± 8.2	55.7 ± 7.4	253.3 ± 38.1	2.1 ± 0.3	8.1 ± 1.6	10.3 ± 1.0
14	MPTP and (1*R*,2*R*,6*R*)-**1**	65.5 ± 8.8	46.9 ± 6.0	158.8 ± 32.7	1.3 ± 0.3	3.9 ± 1.4	4.7 ± 2.1

[a] *A*, general locomotor activity (number of acts); *B*, time of locomotor activity (s); *C*, movement distance (cm); *D*, movement speed (cm/s); *E*, number of explored holes; *F*, number of upright postures. (*) $P < 0.05$, (**) $P < 0.01$ reliability in comparison with MPTP group.

remove the trimethylsilyl protection in (−)-*trans*-**13**, which allowed us to increase the yield from 40% to 80% at this stage. Compound (1*S*,2*S*,6*S*)-**1** (99.5% ee) was obtained by the reduction of the ketone group into the alcohol group in (5*S*,6*S*)-**10** with $LiAlH_4$ followed by the separation of the diastereomers.

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Similarly, from (+)-carvone **11** (97% ee) we synthesized (1*R*,2*R*,6*R*)-**1** (97% ee) with an overall yield of 17% over the sequence (Scheme 5).

Thus we managed to successfully synthesize all eight stereoisomers of compound **1** with no less than 93% ee.

Antiparkinsonian Activity of the Stereoisomers of Compound 1. We studied the antiparkinsonian activity of the stereoisomers of compound **1** (Figure 3) on a model with a single administration of MPTP neurotoxin to mice of C57Bl/6 line, which was injected intraperitoneally in a 0.17 mmol/kg (30 mg/kg) dose 15 min before the administration of the studied isomer (0.12 mmol/kg (20 mg/kg)) by the method described in the literature.[12] Hypokinesia was evaluated 1.5 h after the injection of MPTP on the basis of the locomotor and exploratory activities with the "open field" test for 2 min.

According to our study of the antiparkinsonian activity of (1*R*,2*R*,6*S*)-**1** (93% ee), which amounts to 85% of the previously studied (1*R*,2*R*,6*S*)-**1** (70% ee), the compound is characterized by high activity and restores the locomotor and exploratory activities almost completely to the rates of the saline group except the number of the explored holes (Table 3, no. 3). The minor (1*S*,2*S*,6*R*)-**1** isomer demonstrated an unreliable, although visible, antiparkinsonian activity (Table 3, no. 4).

The inversion of the configuration of the hydroxy group in position 1 during the transfer from (1*R*,2*R*,6*S*)-**1** to (1*S*,2*R*,6*S*)-**1** resulted in a complete disappearance of the antiparkinsonian activity (Table 3, no. 7). Its optical antipode, (1*R*,2*S*,6*R*)-**1**, however, exhibited a significant activity (Table 3, no. 8), although it was slightly less than that of the (1*R*,2*R*,6*S*)-**1** isomer.

An unexpected result was obtained after the configuration of the hydroxy group in position 2 was changed. The transfer to a complete cis-isomer of (1*R*,2*S*,6*S*)-**1** led to a sudden and nearly full inversion of activity, namely, to a strong increase in hypokinesia and a decrease in the animals' exploratory activity (Table 3, no. 11). Compound (1*S*,2*R*,6*R*)-**1**, the enantiomer of (1*R*,2*S*,6*S*)-**1**, did not display any reliable activity (Table 3, no. 12), yet it significantly increased the general locomotor activity.

The (1*S*,2*S*,6*S*)-**1** and (1*R*,2*R*,6*R*)-**1** enantiomer pair produced no reliable effect on the locomotor-orientational activity in these tests (Table 3, nos. 13 and 14), but (1*R*,2*R*,6*R*)-**1** led to a visible decrease in the animals' exploratory activity.

Thus, it can be said that the absolute configuration of compound **1** greatly influences the antiparkinsonian activity of the compound in the test with MPTP, from nearly full removal to a sharp increase in the symptoms of the parkinsonian syndrome.

CONCLUSION

Our study demonstrates that (1*R*,2*R*,6*S*)-**1** displays a potent antiparkinsonian activity in vivo on models with MPTP in mice and rats and is not inferior to the reference agent (levodopa). Compound (1*R*,2*R*,6*S*)-**1** clearly improves the markers of the locomotor and exploratory activities, thus removing the signs of the parkinsonian syndrome induced by neurotoxin injection. The described activity is proved by a number of tests with varying duration of toxin and agent administration (1−30 days).

Compound (1*R*,2*R*,6*S*)-**1** almost completely prevents the development of catalepsy caused by haloperidol, which is demonstrated by a notable decrease in the catalepsy duration in animals, the duration of haloperidol time course, and the percent of cataleptic animals.

We synthesized all eight stereoisomers of compound **1** from the available monoterpenoids (+)- and (−)-α-pinenes and (+)- and (−)-carvones for the first time. According to our observations, the absolute configuration of compound **1** greatly influences its antiparkinsonian activity in the test with MPTP, from nearly full removal to a sharp increase in the symptoms of the parkinsonian syndrome.

EXPERIMENTAL SECTION

Reagents and solvents were purchased from commercial suppliers and used as received. Dry solvents were obtained according to the standard procedures. The K10 clay (Merck) was calcinated at 110 °C for 3 h immediately before use. GC parameters were as follows: 7820A gas chromatograph (Agilent Technologies, U.S.); flame-ionization detector; HP-5 capillary column (0.25 mm ∅ × 30 m × 0.25 μm), He as carrier gas (flow rate 2 mL/min, flow division 99:1). Chirospecific GC−MS parameters were as follows: 6890N gas chromatograph (Agilent Technologies, U.S.); 5973 INERT mass-selective detector (Agilent Technologies, U.S.); Cyclosil-B capillary column (0.32 mm ∅ × 30 m × 0.25 μm, Agilent Technolgies, U.S.); temperature of the column thermostat was 50 °C/2 min; temperature gradient from 2 °C/min to 220 °C/5 min; evaporator and interface temperature 250 °C; He as carrier gas (flow rate 2 mL/min, flow division 99: 1); sweep from *m*/*z* 29 to *m*/*z* 500; 1 μL sample. Optical rotation parameters were as follows: polAAr 3005 spectrometer; $CHCl_3$ solution. ^1H and ^{13}C NMR parameters were as follows: Bruker DRX-500 apparatus at 500.13 MHz (^1H) and 125.76 MHz (^{13}C) in $CCl_4/CDCl_3$ 1:1 (v/v); chemical shifts δ in ppm relative to residual $CHCl_3$ [δ(H) 7.24, δ(C) 76.90 ppm], *J* in Hz. The structure of the products was determined by analyzing the ^1H and ^{13}C NMR spectra, $^1H,^1H$ double-resonance spectra, and $^{13}C,^1H$-type 2D-COSY (*J*(C,H) = 135 Hz). HR-MS parameters were as follows: DFS Thermo Scientific spectrometer in a full scan mode (0−500 *m*/*z*, 70 eV electron impact ionization, direct sample administration). Column chromatography (CC) was performed on silica gel (60−200 μm, Macherey-Nagel). The purity of the target compounds was determined by gas chromatography methods. All of the target compounds reported in this paper have a purity of ≥95%.

(1*R*,2*R*,6*S*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*R*,2*R*,6*S*)-1 (70% ee) was obtained from (−)-verbenone (−)-**2** (Aldrich) (70% ee) according to previously described methods.[6,7]

(−)-Verbenone (−)-2 and (+)-verbenone (+)-2 were synthesized from (−)-α-pinene (−)-**5** (93% ee) and (+)-α-pinene (+)-**5** (98% ee) in accordance with procedure.[17]

(−)-Verbenone Epoxide (−)-3. (−)-Verbenone (−)-**2** (42.0 g, 280 mmol) was dissolved in methanol (420 mL) and cooled to 10 °C; 33% H_2O_2 (89 mL) and 6 N NaOH (21 mL) were added. The mixture was stirred for 2 h at 12−15 °C. EtOAc (4 × 300 mL) was used for extraction, H_2O (2 × 300 mL) for washing, and Na_2SO_4 for drying. The solvent was distilled off, and 33.0 g of (−)-verbenone epoxide (199 mmol, 71%) (−)-**3** ($[\alpha]_D^{25}$ −106.0 (*c* 1.74, $CHCl_3$)) was obtained. The spectral characteristics of compound (−)-**3** corresponded to those described in ref 7.

(+)-Verbenone Epoxide (+)-3. Similar to the synthesis of (−)-**3**, 2.31 g of (+)-verbenone epoxide (+)-**3** (13.9 mmol, 70%) was obtained from 3.00 g (20.0 mmol) of (+)-verbenone (+)-**2**.

(−)-*cis*-Verbenol Epoxide (−)-4. A solution of (−)-verbenone epoxide (−)-**3** (32.6 g, 196 mmol) in ether (420 mL) was added to a suspension of $LiAlH_4$ (8.08 g, 213 mmol) in ether (300 mL) at 0 °C. The mixture was stirred for 3.5 h at 0 °C, after which H_2O (20 mL) was added. The sediment was filtered, and the filtrate was washed with H_2O (2 × 300 mL) and dried with Na_2SO_4. The solvent was distilled off to get 25.0 g (149 mmol, 76%) of (−)-*cis*-verbenol epoxide (−)-**4** ($[\alpha]_D^{25}$ −76.3 (*c* 0.92,

$CHCl_3$). The spectral data of compound (−)-**4** corresponded to those described in ref 7.

(+)-*cis*-Verbenol Epoxide (+)-4. Similar to the obtaining of compound (−)-**4**, 0.873 g of (+)-*cis*-verbenol epoxide (+)-**4** (5.20 mmol, 75%) was obtained from 1.16 g (6.98 mmol) of (+)-verbenone epoxide (+)-**3**.

(1*R*,2*R*,6*S*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*R*,2*R*,6*S*)-1. A solution of (−)-*cis*-verbenol epoxide (−)-**4** (23.6 g, 140 mmol) in CH_2Cl_2 (200 mL) was added to a suspension of K10 clay (51 g) in CH_2Cl_2 (300 mL). It was stirred for 1 h 10 min at rt. EtOAc (150 mL) was added, the catalyst was filtered, and the solvent was distilled off to furnish 23.6 g of mixture. It was separated on a column with SiO_2 (150 g), with a gradient of EtOAc in hexane from 0% to 100% as eluent, to obtain 9.44 g (56.2 mmol, 40%) of (1*R*,2*R*,6*S*)-**1** ($[\alpha]_D^{29}$ −84.0 (*c* 3.47, $CHCl_3$), 93% ee), 5.54 g (33.0 mmol, 24%) of 2-hydroxy-1-[(1*S*)-2,2,3-trimethylcyclopent-3-en-1-yl]ethanone ($[\alpha]_D^{27}$ −25.0 (*c* 1.07, $CHCl_3$)), 2.02 g (12.0 mmol, 9%) of (−)-2-(2,2-dimethylcyclopent-3-enyl)-2-hydroxypropanal ($[\alpha]_D^{24}$ −5.4 (*c* 0.48, $CHCl_3$)), and 1.04 g (7.76 mmol, 6%) of *p*-cymene. The spectral characteristics of (1*R*,2*R*,6*S*)-**1**, 2-hydroxy-1-[(1*S*)-2,2,3-trimethylcyclopent-3-en-1-yl]ethanone and (−)-2-(2,2-dimethylcyclopent-3-enyl)-2-hydroxypropanal corresponded to those found in refs 7 and 8.

(1*S*,2*S*,6*R*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*S*,2*S*,6*R*)-1. Similar to the obtaining of compound (1*R*,2*R*,6*S*)-**1**, 0.333 g (1.98 mmol, 40%) of (1*S*,2*S*,6*R*)-1 **1** ($[\alpha]_D^{29}$ +82.7 (*c* 0.73, $CHCl_3$), 98% ee) and 0.095 g (0.56 mmol, 11%) 2-hydroxy-1-[(1*R*)-2,2,3-trimethylcyclopent-3-en-1-yl]ethanone ($[\alpha]_D^{27}$ +27.6 (*c* 1.20, $CHCl_3$)) were obtained from 0.850 g (5.05 mmol) (+)-*cis*-verbenol epoxide (+)-**4**.[39]

(−)-*trans*-Verbenol Epoxide (−)-9. A solution of 5 mg of $VO(acac)_2$ and 3.0 mL (16.5 mmol) of 5.5 M *t*-BuOOH in hexane was added at rt to a solution of 2.00 g (13.2 mmol) of (−)-*trans*-verbenol (−)-**7** in dry toluene (100 mL). The reaction mixture was boiled for 40 min and washed with a saturated solution of $NaHCO_3$ (100 mL) and H_2O (2 × 100 mL). Na_2SO_4 was used for drying. The solvent was distilled off to get 1.97 g (11.7 mmol, 89%) of (−)-*trans*-verbenol epoxide (−)-**9** ($[\alpha]_D^{29}$ −118.6 (*c* 6.67, $CHCl_3$)). ^{1}H NMR (500 MHz, $CCl_4/CDCl_3$): δ 0.87 (s, C^9H_3), 1.27 (s, C^8H_3), 1.31 (s, $C^{10}H_3$), 1.66 (d, H^{7an}, $J_{7an,7sin}$ = 9 Hz), 1.79−1.90 (m, H^5, H^{7sin}), 1.92 (dd, H^1, $J_{1,7sin}$ = 6 Hz, $J_{1,5}$ = 5 Hz), 2.41 (br.s, OH), 3.20 (dd, H^{3sin}, $J_{3sin,4sin}$ = 4 Hz, $J_{3sin,5}$ = 1.2 Hz), 3.91 (m, H^{4sin}). ^{13}C NMR (125 MHz, $CCl_4/CDCl_3$): δ 45.50 (d, C^1), 61.48 (s, C^2), 58.87 (d, C^3), 66.87 (d, C^4), 46.11 (d, C^5), 42.96 (s, C^6), 21.78 (t, C^7), 26.66 (q, C^8), 19.45 (q, C^9), 21.89 (q, C^{10}).

(1*S*,2*R*,6*S*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*S*,2*R*,6*S*)-1. A solution of 1.57 g (9.35 mmol) of (−)-*trans*-verbenol epoxide (−)-**9** in CH_2Cl_2 (15 mL) was added to a suspension of K10 clay (3.10 g) in CH_2Cl_2 (15 mL). It was stirred for 1 h at rt. EtOAc (10 mL) was added, clay was filtered, and the solvent was distilled off. The residue was separated on a column with 17 g of SiO_2, with a gradient of EtOAc in hexane from 0% to 100% as eluent, to get 0.192 g (1.14 mmol, 12%) of (1*S*,2*R*,6*S*)-**1** ($[\alpha]_D^{29}$ −90.1 (*c* 6.07, $CHCl_3$), 93% ee). The spectral data of (1*S*,2*R*,6*S*)-**1** corresponded to those described in the literature.[40]

(1*R*,2*S*,6*R*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*R*,2*S*,6*R*)-1. Similar to the synthesis of (1*S*,2*R*,6*S*)-**1**, 0.278 g (1.65 mmol, 17%) of (1*R*,2*S*,6*R*)-**1** ($[\alpha]_D^{22}$ +87.9 (*c* 1.70, $CHCl_3$), 98% ee) was obtained from 1.50 g (9.87 mmol) of (+)-*trans*-verbenol (+)-**7**.

(5*S*,6*R*)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl)-2-cyclohexene-1-one (5*S*,6*R*)-10. A mixture of DMSO (0.63 mL, 8.8 mmol) and CH_2Cl_2 (4 mL) was added to a solution of oxalyl chloride (0.38 mL, 4.5 mmol) in CH_2Cl_2 (8 mL) at −25 °C. Then a solution of 0.377 g (2.24 mmol) of (1*R*,2*R*,6*S*)-**1** (93% ee) in DMSO (0.94 mL, 13 mmol) and CH_2Cl_2 (8 mL) was added. The reaction mixture was stirred for 25 min at −25 °C. Then triethylamine (2.51 mL, 18 mmol) was added, and the mixture was stirred for 15 min at −25 °C. The reaction mixture was warmed to rt. H_2O (20 mL) was added, and the water phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with 3% HCl (2 × 20 mL), 5% $NaHCO_3$ (2 × 15 mL), and H_2O (2 × 20 mL). Na_2SO_4 was used for drying. The solvent was distilled off. The obtained mixture was separated on a column with SiO_2 (17 g), with a gradient of EtOAc in hexane from 0% to 100% as eluent, to get 0.138 g (0.83 mmol, 37%) of (5*S*,6*R*)-**10** ($[\alpha]_D^{24}$ −56.4 (*c* 1.50, $CHCl_3$)). The spectral data of (5*S*,6*R*)-**10** corresponded to those described in the literature.[38]

(5*R*,6*S*)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl)-2-cyclohexene-1-one (5*R*,6*S*)-10. Similar to the synthesis of (5*S*,6*R*)-**10**, 0.074 g (0.45 mmol, 40%) of (5*R*,6*S*)-**10** was obtained from 0.188 g (1.12 mmol) of (1*S*,2*S*,6*R*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*S*,2*S*,6*R*)-**1** (98% ee).

(1*R*,2*S*,6*S*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*R*,2*S*,6*S*)-1. A solution of (5*S*,6*R*)-**10** (0.065 g, 0.39 mmol) in diethyl ether (7 mL) was added to a suspension of $LiAlH_4$ (0.015 g, 0.39 mmol) in diethyl ether (3 mL) at 0 °C. The mixture was stirred for 5 h at 0 °C, after which 4 drops of H_2O were added and the residue was filtered. The filtrate was dried with Na_2SO_4. The solvent was distilled off to obtain 0.062 g of the product mixture. It was separated on a column of SiO_2 (9 g), with a gradient of EtOAc in hexane from 0% to 100% as eluent, to get 0.033 g (0.20 mmol, 51%) of (1*R*,2*S*,6*S*)-**1** ($[\alpha]_D^{21}$ +14.3 (*c* 0.63, $CHCl_3$), 93% ee) and 0.007 g (0.042 mmol, 11%) of (1*R*,2*R*,6*S*)-**1**.

Data for (1*R*,2*S*,6*S*)-**1**. ^{1}H NMR (500 MHz, $CCl_4/CDCl_3$): δ 1.73 (m, $C^{10}H_3$), 1.80 (br s, C^9H_3), 1.87−1.96 (m, H^{5e}), 2.00 (br s, OH), 2.21−2.30 (m, H^6, H^{5a}), 2.43 (br s, OH), 4.04 (br s, H^1, H^2), 4.84 (br s) and 4.90 (m, $2H^8$), 5.48 (dm, H^4, $J_{4,5e}$ = 5.5 Hz). ^{13}C NMR (125 MHz, $CCl_4/CDCl_3$): δ 69.30 (d, C^1), 71.90 (d, C^2), 133.64 (s, C^3), 122.99 (d, C^4), 24.76 (t, C^5), 44.80 (d, C^6), 145.69 (s, C^7), 111.46 (t, C^8), 22.46 (q, C^9), 19.16 (q, C^{10}). HR-MS: 150.1041 ($[M^+ - H_2O]$, $C_{10}H_{14}O$; calcd 150.1045).

(1*S*,2*R*,6*R*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*S*,2*R*,6*R*)-1. Similar to the obtaining of (1*R*,2*S*,6*S*)-**1**, 0.065 g of product mixture was obtained from 0.072 g (0.43 mmol) of (5*R*,6*S*)-6-hydroxy-2-methyl-5-(prop-1-en-2-yl)-2-cyclohexene-1-one (5*R*,6*S*)-**10**. It was separated on a column with SiO_2 (9 g), with a gradient of EtOAc in hexane from 0% to 100% as eluent, to get 0.038 g (0.23 mmol, 53%) of (1*S*,2*R*,6*R*)-**1** ($[\alpha]_D^{18}$ −40.8 (*c* 1.23, $CHCl_3$), 98% ee) and 0.016 g (0.095 mmol, 22%) of (1*S*,2*S*,6*R*)-**1**.

(5*S*,6*S*)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl)-2-cyclohexen-1-one (5*S*,6*S*)-10 and (5*R*,6*R*)-6-hydroxy-2-methyl-5-(prop-1-en-2-yl)-2-cyclohexen-1-one (5*R*,6*R*)-10 were synthesized from (−)-carvone (-)-**11** (99.5% ee) and (+)-carvone (+)-**11** (97% ee) by the procedure described in ref 37.

(1*S*,2*S*,6*S*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*S*,2*S*,6*S*)-1. A solution of (5*S*,6*S*)-**10** (0.067 g, 0.40 mmol) in ether (4 mL) was added to a suspension of $LiAlH_4$ (0.015 g, 0.39 mmol) in ether (3 mL) at 0 °C. The mixture was stirred for 4 h at 0 °C, after which 15 drops of H_2O were carefully added. The residue was filtered through a filter with a SiO_2 layer and washed with 35 mL of EtOAc. Na_2SO_4 was used for drying and the solvent was distilled off to get 0.070 g of a product mixture, which was separated on a column with SiO_2 (9 g), with a gradient of EtOAc in hexane from 0% to 100% as eluent, to obtain 0.046 g (0.27 mmol, 68%) of (1*S*,2*S*,6*S*)-**1** ($[\alpha]_D^{23}$ +35.0 (*c* 1.52, $CHCl_3$), 99.5% ee) and 0.022 g (0.13 mmol, 32%) of (1*S*,2*R*,6*S*)-**1**.

Data for (1*S*,2*S*,6*S*)-**1**. ^{1}H NMR (500 MHz, $CCl_4/CDCl_3$): δ 1.71 (m, $C^{10}H_3$), 1.72 (br s, C^9H_3), 2.02 (dddm, H^{5e}, ^2J = 17.3, $J_{5e,6a}$ 6.0, $J_{5e,4}$ = 5.0 Hz), 2.02 (dddm, H^{5a}, ^2J = 17.3, $J_{5a,6a}$ = 11.2, $J_{5a,4}$ = 2.5 Hz), 2.36 (ddd, H^{6a}, $J_{6a,1a}$ = 11.2, $J_{6a,5a}$ = 11.2, $J_{6a,5e}$ = 6.0 Hz), 2.71 (br s, OH), 3.17 (br s, OH), 3.55 (dd, H^{1a}, $J_{1a,6a}$ = 11.2 Hz, $J_{1a,2a}$ = 7.3 Hz), 3.97

(br d, H^{2a}, $J_{2a,1a}$ = 7.3 Hz), 4.84 (br s) and 4.87 (m, $2H^8$), 5.34 (m, H^4). ^{13}C NMR (125 MHz, $CCl_4/CDCl_3$): δ 74.53 (d, C^1), 76.16 (d, C^2), 134.55 (s, C^3), 122.10 (d, C^4), 30.29 (t, C^5), 48.14 (d, C^6), 145.15 (s, C^7), 113.60 (t, C^8), 18.85 (q, C^9), 18.66 (q, C^{10}). HR-MS: 168.1149 (M^+, $C_{10}H_{16}O_2$; calcd 168.1145).

(1*R*,2*R*,6*R*)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (1*R*,2*R*,6*R*)-1. A solution of (5*R*,6*R*)-**10** (0.057 g, 0.34 mmol) in ether (4 mL) was added for 3 min to a suspension of $LiAlH_4$ (0.013 g, 0.34 mmol) in ether (3 mL) stirred at 0 °C. The mixture was stirred for 4 h at 0 °C, after which 15 drops of H_2O were added and the mixture was immediately filtered through a filter with a SiO_2 layer, washed with 30 mL of EtOAc, and dried over sodium sulfate. The solvent was distilled off. The obtained product mixture (0.053 g) was separated on a column with SiO_2 (4.5 g, 60−200 μm; Macherey-Nagel), with a gradient of EtOAc in hexane from 0% to 100% as eluent, to get 0.029 g (0.17 mmol, 50%) of (1*R*,2*R*,6*R*)-**1** ($[\alpha]_D^{23}$ −38.1 (*c* 0.93, $CHCl_3$), 97% ee) and 0.015 g (0.089 mmol, 26%) of (1*R*,2*S*,6*R*)-**1**.

Animals. The experiments were performed on Wistar rats (male) weighing 200−220 g and C57BL/6 mice (male) weighing 25−30 g (SPF-vivarium of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences). The animals were maintained at 22−25 °C on a 12 h light−dark cycle with food and water available ad libitum. All work with animals was performed in strict accordance with the legislation of the Russian Federation, the regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the requirements and recommendations of the Guide for the Care and Use of Laboratory Animals.

MPTP Mouse Model of Parkinson's Disease Induced by Single Administration of MPTP Neurotoxin[12]. MPTP (0.17 mmol/kg (30 mg/kg)) was injected intraperitoneally to mice of the C57Bl/6 line 15 min prior to the administration of the studied substances. The compound under study was administrated per os in a dose of 0.12 mmol/kg (20 mg/kg). Normal saline was injected to the control group. The effectiveness of the studied medications was evaluated according to their ability to reduce the symptoms of hypokinesia induced by MPTP. Hypokinesia caused by neurotoxin administration was evaluated with the "open field" test performed for 2 min using Tru Scan (U.S.) 1.5 h after the injection of MPTP, registering the main markers of the locomotor and exploratory activities: general locomotor activity (number of acts), time of locomotor activity (s), movement distance (cm), movement speed (cm/s), number of upright postures, and number of explored holes.

MPTP Mouse Model of Parkinson's Disease Induced by 4-Fold Administration of MPTP Neurotoxin[13]. MPTP was injected intraperitoneally to mice of C57Bl/6 line every 2 and 8 h period in 1 day in a dose of 0.12 mmol/kg (20 mg/kg) for a total of four doses. The studied agent was administrated per os 24 h after the last injection of MPTP in a dose of 0.12 mmol/kg (20 mg/kg). The effectiveness of the studied medications was evaluated according to their ability to reduce the symptoms of hypokinesia induced by MPTP. Hypokinesia caused by neurotoxin administration was evaluated with the "open field" test performed for 2 min using Tru Scan (U.S.) 2 h after the administration of the studied agent, registering the main markers of the locomotor and exploratory activities: general locomotor activity (number of acts), time of locomotor activity (s), movement distance (cm), movement speed (cm/s), number of upright postures, number of explored holes.

MPTP Rat Model of Parkinson's Disease Induced by Administration of MPTP Neurotoxin[12]. MPTP (0.23 mmol/kg (40 mg/kg)) was injected intraperitoneally to rats 4 h prior to the administration of the studied substances. Compound (1*R*,2*R*,6*S*)-**1** was administrated per os in a dose of 0.12 mmol/kg (20 mg/kg). Normal saline was injected to the control group. The effectiveness of the studied medications was evaluated on the 30th day after the start of the experiment according to their ability to reduce the symptoms of hypokinesia induced by MPTP. Hypokinesia caused by neurotoxin administration was evaluated with the "open field" test performed for 2 min using Tru Scan (U.S.) 2 h after the administration of the studied agent, registering the main markers of the locomotor and exploratory activities: general locomotor activity (number of acts), time of locomotor activity (s), movement distance (cm), movement speed (cm/s), number of upright postures, and number of explored holes.

Catalepsy Mouse Model Induced by Haloperidol[41]. Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for parkinsonism.[42] Adult male Wistar rats were divided into two groups each containing six animals. Group I received haloperidol 4.0 μmol/kg (1.5 mg/kg) in normal saline and served as the cataleptic control without any drug treatment. Group II received (1*R*,2*R*,6*S*)-**1** (0.12 mmol/kg (20 mg/kg) per os); 10 min after the administration of this drug, catalepsy was induced by the intraperitonial administration of haloperidol in a dose of 4.0 μmol/kg (1.5 mg/kg) body weight in normal saline. All the behavioral studies were performed at rt in a calm room without any external interference. The severity of catalepsy was measured 30, 60, 120, 180, and 240 min after haloperidol administration using the method of parallel bars. The fore and hind limbs of a rat were placed on parallel bars (walls) to ensure that the animal's back was straight, and the time spent by the animal in an immobilized state was recorded. The general duration of catalepsy and the percent of cataleptic animals in the group were evaluated.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and spectra for compounds **2**, **6**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone +73833308870. Fax: +73833309752. E-mail: volcho@nioch.nsc.ru.

■ ABBREVIATIONS USED

CC, column chromatography; DMP, Dess−Martin periodinane; IBX, iodoxybenzoic acid; ee, enantiomeric excess; GC−MS, gas chromatography−mass spectrometry; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine; PCC, pyridinium chlorochromate; PD, Parkinson's disease; rt, room temperature

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