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Hydroxyketones of *para*-Menthane Series as Promising Analgesic

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Abstract

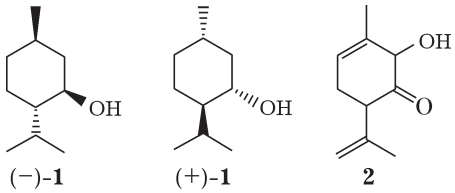
Data obtained in the investigation of the analgesic activity of monoterpenoid of the *para*-menthane series – 2-hydroxy-3-methyl-6-(1-methylethenyl)cyclohex-3-enone are presented. It is discovered that two its spatial isomers (2*R*,6*S* and 2*S*,6*R*) in the dose of 2.5 mg/kg exhibit high analgesic activity and to a substantial extent quench the development of pain syndrome caused by the introduction of acetic acid. With the hot plate model, a substantial effect of the absolute configuration of the terpenoid on the direction of action is demonstrated.

Key words: monoterpenoids of *para*-menthane series, analgesic activity, mice

INTRODUCTION

In spite of success achieved in the development of analgesic preparations, the problems connected with insufficient selectivity and undesirable side effects of analgesics remain unsolved yet. In this connection, the search for new low-toxic and highly efficient analgesics of new structural types is one of the significant problems [1].

It was demonstrated recently that (–)-menthol (–)-**1** (Scheme 1), a natural monoterpenoid of *para*-menthane series, after its intracerebroventricular introduction (directly into cerebral cavities), exhibits substantial analgesic activity in the tests of the hot plate and acetic convulsion [2]. It is interesting that the optical antipode (+)-menthol (+)-**1** did not exhibit significant analgesic activity in the mentioned tests. So, oxygen-containing terpenoids of *para*-menthane series can be considered as promising substances for the search of new analgesic agents.



(–)-**1** (+)-**1** **2**

Scheme 1.

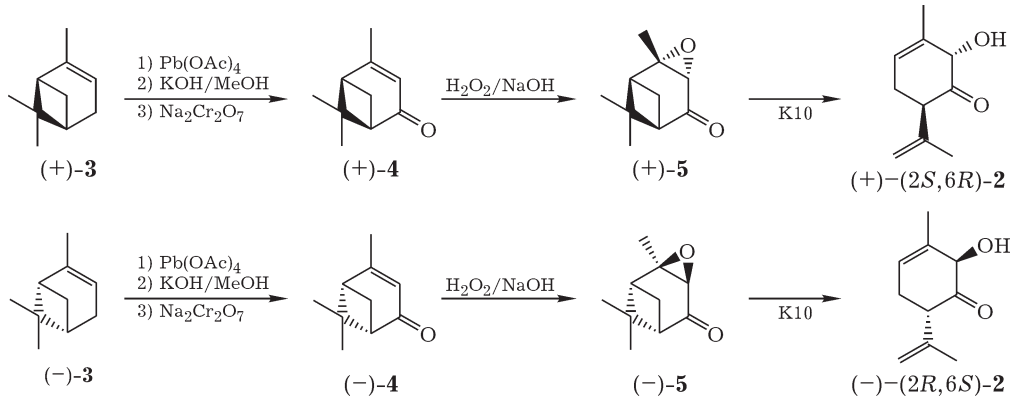
In the present work we studied for the first time the analgesic activity of hydroxy ketone **2**, a monoterpenoid of *para*-menthane series.

EXPERIMENTAL

Synthesis of (+)-(2S,6R)-2-hydroxy-3-methyl-6-(1-methylethenyl)cyclohex-3-enone (+)-(2S,6R)-2

Verbenone (+)-**4** was synthesized from (+)-α-pinene (+)-**3** (Aldrich, $[\alpha]_D^{20}$ +50.5 (oil), 97 % *ee* according to the procedure described in [3] (Scheme 2). The epoxide of verbenone (+)-**5** was obtained from verbenone (+)-**4** according to the procedure presented in [4].

To the suspension of 720 mg of K-10 clay (Fluka) annealed preliminarily for 3 h at 100–110 °C, in 5 mL of CH_2Cl_2 we added the solution of 300 mg (1.81 mmol) of the epoxide of (+)-verbenone (+)-**5** in 5 mL of CH_2Cl_2 under mixing. The reaction mixture was stirred for 1 h at room temperature. The catalyst was removed by filtering, washed with ethyl acetate, and the solution was evaporated. The residue



Scheme 2.

was separated by means of column chromatography on SiO_2 (60−200 μ; Merck), with the solution of diethyl ether in hexane 5 to 100 % as eluent. We obtained 50 mg (0.30 mmol, 17 %) of compound (+)-(2*S*,6*R*)-**2** ($[\alpha]_D^{29}$ +59.5 (*c* 1.13, $CHCl_3$)). The ^{1}H NMR spectrum of compound (+)-(2*S*,6*R*)-**2** was identical to the spectrum reported by the authors of [4].

Synthesis of (-)-2(2R,6S)-2-hydroxy-3-methyl-6-(1-methylethenyl)cyclohex-3-enone (−)-(2R,6S)-2

Verbenone (−)-**4** was synthesized from (−)-α-pinene (−)-**3** (Fluka, $[\alpha]_D^{20}$ −48.4 (oil), 94 % *ee*) according to the procedure described in [4] (see Scheme 2). The epoxide of verbenone (−)-**5** was obtained from verbenone (−)-**4** according to the procedure presented by the authors of [4].

Similarly to the procedure of obtaining (+)-(2*S*,6*R*)-**2**, from 300 mg of the epoxide of (−)-verbenone (−)-**5** we obtained 30 mg (10 %) of (−)-(2*R*,6*S*)-**2** (94 % *ee* (GLC-MS); $[\alpha]_D^{29}$ −50.7 (*c* 0.52, $CHCl_3$)). The ^{1}H NMR spectrum of compound (−)-(2*R*,6*S*)-**2** coincided with the spectrum published in [4].

Examination of the analgesic activity

The experiment was carried out with male outbred mice with the body mass 22−25 g. Experimental groups were formed to include eight animals in each group.

Acetic convulsions were reproduced by means of the intraperitoneal introduction of 0.75 % acetic acid in the amount of 0.1 mL per one animal. The agents under examination were introduced once intragastrically 1 h before the reproduction of the model in the dose of 2.5 mg/kg. The reference group included animals into which only acetic acid was introduced. Estimation of the activity was carried out on the basis of the number of convulsions within 3 min.

In the hot plate test, the animals were placed onto a copper plate (54 °C). The agents under investigation were introduced once intragastrically 1 h before the reproduction of the model in the dose of 2.5 mg/kg. The corresponding solvent was introduced into the animals of the reference group. The effect was estimated on the basis of the time within which an animal remained on the hot plane before the first vocalization (in seconds).

The check standard (sodium diclofenac) was introduced once intragastrically 1 h before the reproduction of the model in the dose of 10 mg/kg.

Acute toxicity of compound **2** was determined by means of a single intragastrical introduction according to Kerber's method.

RESULTS AND DISCUSSION

In the course of directed screening of the pharmacological activity of monoterpenoids of *para*-menthane series, we chose hydroxy ketone **2** for the examination of the analgesic activity. Compound (−)-(2*R*,6*S*)-**2** with the enantiomer excess of 60 % was obtained previously and described in [4]; no data on its pharmacological activity were published.

TABLE 1

Analgesic action of compounds (−)-(2*R*,6*S*)-**2** and (+)-(2*S*,6*R*)-**2**

Groups/agents	Dose, mg/kg	Acetic convulsions, number	Hot plate, s
Reference		10.0±0.8	12.3±1.4
(−)-(2*R*,6*S*)-**2**	2.5	3.8±1.5*	14.9±1.5
Reference		9.0±0.9	20.5±2.7
(+)-(2*S*,6*R*)-**2**	2.5	3.7±1.1*	14.6±1.7
Reference		8.4±0.84	20.4±2.2
Sodium diclofenac	10	0.75±0.41*	33.4±2.3*

*$p < 0.05$ with respect to the reference.

The synthesis of both enantiomers of compound **2** with high optical purity was performed using (+)- and (−)-α-pinenes **3** according to the known procedures [3, 4] (see Scheme 2).

In the interaction of (+)- and (−)-α-pinenes **3** with lead tetraacetate followed by saponification and oxidation, we obtained the corresponding (+)- and (−)-verbenones **4**. Epoxidation of verbenones with the aqueous solution of hydrogen peroxide and isomerisation of the synthesized epoxides (+)- and (−)-**5** in the presence of montmorillonite clay K10 resulted in the formation of the target ketoalcohols (+)-(2*S*,6*R*)-**2** and (−)-(2*R*,6*S*)-**2**, respectively.

The analgesic activity of compounds (+)-(2*S*,6*R*)-**2** and (−)-(2*R*,6*S*)-**2** was studied for the dose of 2.5 mg/kg after peroral introduction using the model of visceral pain "acetic convulsions" and in the hot plate test characterizing thermal stimulation.

It was discovered that compounds (+)-(2*S*,6*R*)-**2** and (−)-(2*R*,6*S*)-**2** in the dose of 2.5 mg/kg quench to a substantial extent (59–72 %) the development of pain effect caused by the introduction of acetic acid, and exhibit high analgesic activity comparable with that of the reference preparation sodium diclofenac, the dose of which was 4 times as high as the dose of the agents under examination (Table 1).

In the hot plate test, compound (−)-(2*R*,6*S*)-**2** in the dose of 2.5 mg/kg causes an increase in the time of staying on the hot plate before the first vocalization of the animals by 21 % thus exhibiting not very high analgesic effect (see Table 1). Its optical antipode, compound (+)-(2*S*,6*R*)-**2**, quite contrary, decreases the time of staying on the hot plate before the first vocalization of the animal by 29 % thus exhibiting insignificant hyperalgesia.

Investigation of the acute toxicity of compound **2** showed that its LD_{50} exceeds 1000 mg/kg, that is, it possesses substantially lower acute toxicity than sodium diclofenac does (LD_{50} 370 mg/kg) [3].

CONCLUSION

Thus, we discovered that hydroxy ketone **2** with *para*-menthane framework after oral intake combines low toxicity with high analgesic activity in the test of acetic convulsions modelling visceral pain. It was shown in the hot plate test that the direction of action depends on the absolute configuration of compound **2**: compound with 2*R*,6*S*-configuration possesses not very high analgesic activity, while its optical antipode, quite contrary, causes insignificant hyperalgesia.

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