DOI 10.1007/s10600-016-1604-9
Chemistry of Natural Compounds, Vol. 52, No. 2, March, 2016

SYNTHESIS AND ANTICONVULSANT ACTIVITY OF MENTHYL γ-AMINOBUTYRATE

M. V. Nesterkina[1*] and I. A. Kravchenko[1,2]

The ester of l*-menthol and* γ*-aminobutyric acid, (1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 4-aminobutyrate hydrochloride was synthesized. Its anticonvulsant activity was studied* in vivo *by determining the minimum effective doses of pentylenetetrazole inducing clonic-tonic convulsions and tonic extension. Menthyl* γ*-aminobutyrate possessed prolonged anticonvulsant activity.*

Keywords: menthol, γ-aminobutyric acid, ester, anticonvulsant activity.

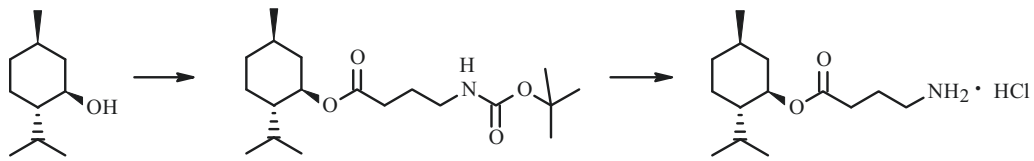
The chemistry of monocyclic terpenes has become especially crucial because of the discovery of new pharmacological targets for this class of compounds. Menthane-type alcohols, the most important representative of which is the *Mentha piperita* essential-oil constituent menthol, play significant roles in studies of the biological activity. Menthol exists as four stereoisomers, the principal one of which has all three cyclohexane substituents in equatorial positions and exists, in turn, as a pair of enantiomers. The isomer *l*-menthol has the highest biological activity and is widely used in the pharmaceutical industry [1].

Topical use of menthol causes feelings of coolness or heat due to its action on thermosensitive nerve endings by binding to TRPM8 receptors [2, 3]. Many recent studies of the mechanism of menthol action on the CNS showed that menthol is a positive allosteric modulator of γ-aminobutyric acid type-A receptor ($GABA_A$). Therefore, it exhibits sedative, anticonvulsant, and nootropic activity [4]. GABA itself exists at physiological pH values as a Zwitter-ion that penetrates poorly the blood–brain barrier and; therefore, has a low therapeutic effect [5]. Thus, it seemed worthwhile to prepare the ester of *l*-menthol and γ-aminobutyric acid and to study the anticonvulsant activity of the synthesized derivative.

Menthol esters are prepared by ordinary esterification using acid catalysts [6], acylation of the alcohol by acid chlorides [7], and reactions with various condensing agents [8]. A more common method for preparing menthyl esters is the condensation of the acid and alcohol by *N*,*N*′-dialkylcarbodiimides in the presence of 4-dimethylaminopyridine (DMAP). Herein, the synthesis of menthyl γ-aminobutyrate using the dicyclohexylcarbodiimide (DCC)–DMAP system is reported. The amine was blocked by the *tert*-butyloxycarbonyl (Boc) protecting group. *l*-Menthol (**1**) was acylated by *N*-Boc-γ-aminobutyric acid in CH_2Cl_2–MeCN (1:1) in the presence of a catalytic amount of DMAP at 0°C followed by warming the reaction mixture to 16–18°C. The Boc protecting group was removed by treatment of the resulting menthyl ester with HCl in AcOH.

The structure of the synthesized menthyl γ-aminobutyrate (**3**) was confirmed by mass, IR, and PMR spectra.

The mass spectrum of the isolated product contained a peak for the molecular ion with *m*/*z* 242 (80% of the base-peak intensity).



1 **2** **3**

1) I. I. Mechnikov Odessa National University, 2 Dvoryanskaya, Odessa, Ukraine 65026, e-mail: mashaneutron@gmail.com; kravchenko.pharm@gmail.com; 2) A. V. Bogatskii Physicochemical Institute, National Academy of Sciences of Ukraine, 86 Lyustdorfskaya Rd., Odessa, Ukraine 65080. Translated from *Khimiya Prirodnykh Soedinenii*, No. 2, March–April, 2016, pp. 208–210. Original article submitted June 21, 2015.

TABLE 1. Dose–Effect Relationship for GABA Menthyl Ester 6 h After Oral Administration (M ± m)

MED of corazole, % of control	Dose, mg/kg					
	87	175	350	700	1350	Control
DCTC	147 ± 4.7	170 ± 4.0	195 ± 4.3	200 ± 5.7	200 ± 6.2	100 ± 5.3
DTE	144 ± 5.3	158 ± 2.8	186 ± 6.0	183 ± 4.0	217 ± 4.2	100 ± 5.1

TABLE 2. Time–Effect Relationship for GABA Menthyl Ester After Oral Administration at a Dose of 175 mg/kg (M ± m)

Time, h	MED of corazole, % of control		Time, h	MED of corazole, % of control	
	DCTC	DTE		DCTC	DTE
0.5	109 ± 3.3	114 ± 4.1	24	186 ± 4.5	189 ± 3.7
1	152 ± 2.8	167 ± 4.0	48	155 ± 2.7	161 ± 4.0
3	165 ± 3.0	167 ± 3.7	72	139 ± 3.1	142 ± 2.9
6	170 ± 4.0	158 ± 2.8	96	133 ± 4.0	142 ± 4.2
18	191 ± 2.9	196 ± 3.3	Control	100 ± 5.0	100 ± 4.2

The IR spectrum of **3** lacked an absorption band for O–H stretching vibrations, indicating that this group was substituted. The spectrum exhibited an ester C=O absorption band at 1721 cm^{-1} and a band at 1151–1201 cm^{-1} for C–O vibrations. The NH_3^+ stretching vibration band was observed at 3021 cm^{-1}. Bending vibrations of this group appeared as amino-acid band I at 1604 cm^{-1} and amino-acid band II at 1573 cm^{-1}.

The PMR spectrum of the synthesized compound showed at weakest field cyclohexane proton H-1 that was deshielded by the ester O atom as a triplet of doublets at 4.53–4.59 ppm. The C-5 methyl resonated as a doublet at 0.67 ppm with SSCC J = 6.53 Hz. The PMR spectrum also contained resonances for axial and equatorial ring protons, the positions and multiplicities of which corresponded to analogous resonances in the spectrum of *l*-menthol. Thus, the initial configuration of *l*-menthol was preserved in the obtained ester and the asymmetric C atom was unaffected according to PMR data.

Acute toxicity of the synthesized GABA ester was determined after oral administration. The obtained LD_{50} value was 2700 mg/kg. This allowed the synthesized compound to be regarded as having low toxicity. The anticonvulsant effect was studied for 0.5–96 h after oral administration at a dose of 87–1350 mg/kg. The compound exhibited anticonvulsant activity over the whole range of these doses. Table 1 shows that the menthyl ester caused a dose-dependent effect that manifested as increases of the doses inducing clonic-tonic (DCTC) and tonic extension (DTE) as the compound concentration was increased. The anticonvulsant activity was 170 and 158% for DCTC and DTE, respectively, 6 h after administration of the ester at a dose of 175 mg/kg.

The dynamics of the anticonvulsant activity at this dose were studied for 0.5–96 h after a single oral administration (Table 2). According to the results, the anticonvulsant activity of **3** reached a maximum 18 h after administration and was 191 and 196% for DCTC and DTE, respectively. The therapeutic activity persisted for long times (24–96 h) after administration and was indicative of its prolonged effect. A study of the time–effect relationship showed that the synthesized ester was not a classical prodrug and possessed its own pharmacological activity. This was confirmed by the rapid onset of the anticonvulsant activity, which was recorded already 30 min after oral administration.

EXPERIMENTAL

PMR spectra were recorded in DMSO-d_6 with TMS internal standard on a Bruker Avance DRX 500 instrument (500 MHz). Mass spectra were taken using FAB on a VG 70-70 EQ mass spectrometer, a beam of 8-kV Xe atoms, and *m*-nitrobenzylalcohol as the matrix. UV absorption spectra were recorded on a PerkinElmer Lambda 9 UV/Vis/NIR spectrophotometer. IR spectra were taken from KBr pellets on a PerkinElmer Frontier FT-IR spectrometer. The purity and identity of products were assessed using TLC on Kieselgel 60 F_{254} plates (Merck, Germany) using $CHCl_3$–MeOH–NH_4OH (50:14:2).

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl-4-aminobutyrate Hydrochloride. A solution of *l*-menthol (0.5 g, 3.2 mmol) in CH_2Cl_2–MeCN (1:1, 10 mL) was treated with *N*-Boc-γ-aminobutyric acid (0.662 g, 3.26 mmol) and DMAP

(0.097 g, 0.794 mmol), cooled to 0°C, treated dropwise with DCC (0.727 g, 3.53 mmol) in CH_2Cl_2 (2 mL), heated gradually to 16–18°C, stirred vigorously for 15 h, and diluted with CH_2Cl_2. The precipitate of dicyclohexylurea was filtered off. The filtrate was washed successively with HCl (1 M), $NaHCO_3$ (10%), and H_2O. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*. The *N*-protecting group was removed using the literature method [9]. Yield 96%, $C_{14}H_{27}NO_2 \cdot HCl$. IR spectrum (KBr, ν_{max}, cm^{-1}): 3021 (NH_3^+), 2957–2868 (C–H), 1721 (C=O), 1573, 1604 (NH_3^+). UV spectrum (MeOH, λ_{max}, nm): 284. Mass spectrum *m*/*z* 242 (M^+). ^1H NMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 0.67 (3H, d, J = 6.53, CH_3-7), 0.81–0.84 (7H, m, CH_3-9, 10, H-4a), 0.87–0.93 (1H, m, H-6a), 0.96–1.01 (1H, m, H-2), 1.27–1.32 (1H, m, H-5), 1.40 (1H, m, H-3a), 1.59 (2H, m, H-3e, 4e), 1.76 (2H, t, J = 7.03, γ-CH_2), 1.82 (1H, d, J = 14.55, H-6e), 2.37 (2H, m, β-CH_2), 2.74 (2H, t, J = 7.28, α-CH_2), 4.53–4.59 (1H, td, J = 2.67, H-1).

Anticonvulsant activity of menthyl γ-aminobutyrate was studied using white laboratory mice (18–20 g). Animals were maintained under standard vivarium conditions with a 12-h light cycle and standard ration. Animal tests were conducted according to rules of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* [10].

Anticonvulsant activity of the synthesized compound was studied using acute generalized convulsion induced by i.v. injection of pentylenetetrazole (PTZ) solution (1%) at 0.01 mL/s. The minimum effective doses (MED) of PTZ inducing clonic-tonic convulsions (DCTC) and tonic extension (DTE) were recorded. The threshold PTZ dose required to induce these convulsions was calculated for each animal (mg/kg) and expressed as percent relative to the control. These effects were quickly reversible and concentration-dependent. This enabled the anticonvulsant activity of the tested compound to be correctly assessed.

REFERENCES

1. V. V. Plemenkov, *Introduction to the Chemistry of Natural Compounds* [in Russian], Kazan′, 2001, 376 pp.
2. D. D. McKemy, *Mol. Pain*, **16**, 2 (2005).
3. R. Eccles, *J. Pharm. Pharmacol.*, **46**, 624 (1994).
4. B. K. Lau, S. Karim, A. K. Goodchild, C. W. Vaughan, and G. M. Drew, *Br. J. Pharmacol.*, **171**, 2804 (2014).
5. V. V. Bagmetova, L. E. Borodkina, I. N. Tyurenkov, V. M. Berestovitskaya, and O. S. Vasil′eva, *Fundam. Issled.*, **10**, 467 (2011).
6. P. M. Dewang, V. P. Nikumbh, V. S. Tare, and P. P. Mahulikar, *J. Sci. Ind. Res.*, **62**, 990 (2003).
7. T. S. Raikova, G. V. Kalechits, E. N. Manukov, T. K. Vyalimyae, and S. A. Makhach, *Chem. Nat. Compd.*, **17**, 526 (1981).
8. V. F. Pozdnev, *Bioorg. Khim.*, 725 (1985).
9. A. A. Gershkovich and V. K. Kibirev, *Peptide Synthesis. Reagents and Methods* [in Russian], Naukova Dumka, Kiev, 1987, 264 pp.
10. *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*, Council of Europe, Strasbourg, **123**, 51 (1986).