

Short Note

2-Propyl-*N'*-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]pentanehydrazide

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Abstract: 2-Propyl-*N'*-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]pentanehydrazide was obtained in 80% yield via the Einhorn variation of the Schotten–Baumann method by (+)-camphor hydrazide condensation with valproic acid (VPA) chloride. The structure of the titled compound was verified by Raman, FTIR, ¹H-NMR, and ¹³C-NMR spectral analysis along with FAB-mass spectrometry. Thermal properties of synthesized derivative were elucidated by DSC and its purity by HPLC. The compound was successfully tested as a potential anticonvulsant agent based on models of chemically- and electrically-induced seizures.

Keywords: hydrazone; (+)-camphor; valproic acid; technology; terpenoid; anticonvulsant activity

1. Introduction

Valproic acid (VPA) refers to extensively used antiepileptic drugs (AEDs) that have been found to be effective against all types of seizures [1]. However, clinical use of VPA is associated with a significant adverse effect such as hepatotoxicity [2], causing the need for chemical modification of the VPA molecule aimed at dose reducing VPA-containing derivatives. In this context, particular interest is focused on terpenoids, which have proven to be anticonvulsant agents and serve as versatile scaffolds for enhancing the permeability of drugs [3,4]. Recently, this strategy has been applied to facilitate penetration of gamma-amino butyric acid (GABA) through the blood-brain barrier (BBB) by its conjugation with *l*-menthol, resulting in an increase in anticonvulsant potency [5]. Notably, the binding of biologically active molecules to terpenoids is expedient by the formation of enzymatically degradable bonds such as -NH-N=C-, -CO-OR-, -CO-NH-, etc.

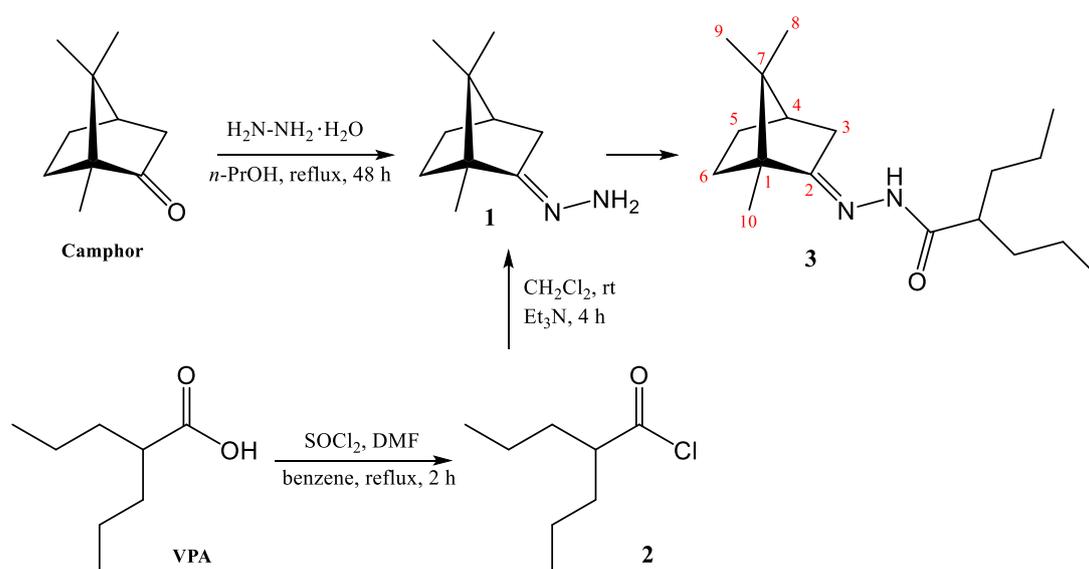
Bearing the aforementioned in mind, bicyclic terpenoid (+)-camphor possessing its own antiseizure action [6] has been used as a base for the synthesis of conjugates containing the VPA moiety. Thus, we report herein on the synthesis and detailed structural determination of 2-propyl-*N'*-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]pentanehydrazide comprising both (+)-camphor and VPA residues. The obtained compound was then investigated as a potential anticonvulsant agent on the models of maximal electroshock seizure (MES) and pentylenetetrazole (PTZ)-induced convulsions.

2. Results and Discussion

2.1. Chemistry

2-Propyl-*N'*-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]pentanehydrazide **3** was synthesized via the Einhorn variation of the Schotten–Baumann method, as shown in Scheme 1. For this purpose,

camphor hydrazine **1** was acylated with valproic acid chloride **2** in the presence of triethylamine (TEA) both as an acid scavenger and nucleophilic acylation catalyst. Initial hydrazine **1** was obtained by camphor treatment with hydrazine hydrate and acid chloride **2** through VPA reflux with SOCl_2 [7,8]. Target compound was isolated in an 80% yield as white crystals well soluble in organic solvents such as chloroform, hexane, acetonitrile, and benzene. The structure of hydrazone **3** was verified by ^{13}C -NMR, ^1H -NMR, FT-IR, Raman spectroscopy, and FAB-mass spectrometry. Thermal transition of compound **3** was carried out by differential scanning calorimetry (DSC) along with the determination of melting enthalpy (ΔH_m) as 120.2 J/g. The purity was assessed by HPLC analysis using the reversed-phase method with isocratic elution by a system composed of acetonitrile: 0.01% formic acid aqueous solution (70:30). According to the HPLC assay, hydrazone purity was established by internal normalization with detection at ultraviolet wavelengths (230 nm and 260 nm) as 100% and 99%, respectively.



Scheme 1. Synthesis of 2-propyl-*N'*-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]pentanehydrazide.

Fast-atom bombardment (FAB) has been applied as an ionization method in mass spectrometry investigation; the FAB-MS spectrum of derivative **3** displays the protonated molecular ion peak $[\text{M} + \text{H}]^+$ at m/z 293. The FT-IR spectrum exhibits absorption bands of N–H bonds (3179 cm^{-1}), C=O groups (1697 cm^{-1}), and alkyl C–H ($2970\text{--}2834\text{ cm}^{-1}$). Taking into account the overlapping of C=N and C=O vibrations in the FT-IR spectrum, the formation of the imine (C=N) group was confirmed by Raman spectroscopy as an intense peak at 1646 cm^{-1} . The ^1H -NMR spectrum corroborates the proposed structure of compound **3** by chemical shift, integration, and multiplicity of resonance signals. The characteristic proton signal of the -NH group was observed as a singlet at 9.51 ppm. Likewise, the ^{13}C -NMR spectrum detected the presence of all carbon atoms in the molecule.

2.2. Anticonvulsant Activity

Anticonvulsant activity of the title compound was determined by two pharmacological seizure models including intravenous pentylenetetrazole (i.v. PTZ) and maximal electroshock seizure (MES) tests. In the PTZ-induced model, the anticonvulsant effect was established by the assessment of PTZ minimum effective doses (MED) that provoke clonic-tonic convulsions (DCTC) and tonic extension (DTE). As illustrated in Figure 1, camphor, hydrazone **3**, along with VPA, demonstrated protection against PTZ-induced seizures at 3 h after administration, as validated by the increase of DCTC and DTE values to 165–187% and 170–197%, respectively, compared with the control (100%). However, at this time point, there were no statistically significant differences between the afore-mentioned experimental groups, indicating comparable activity of the investigated compounds. In marked

contrast, synthesized derivative **3** was found to possess significant antiseizure action over a long time period (24 h after administration) with the average values of 277% for DCTC and 238% for DTE. As seen, hydrazone **3** exhibited higher potency versus initial camphor and VPA ($p < 0.01$), which highlighted its prolonged effect.

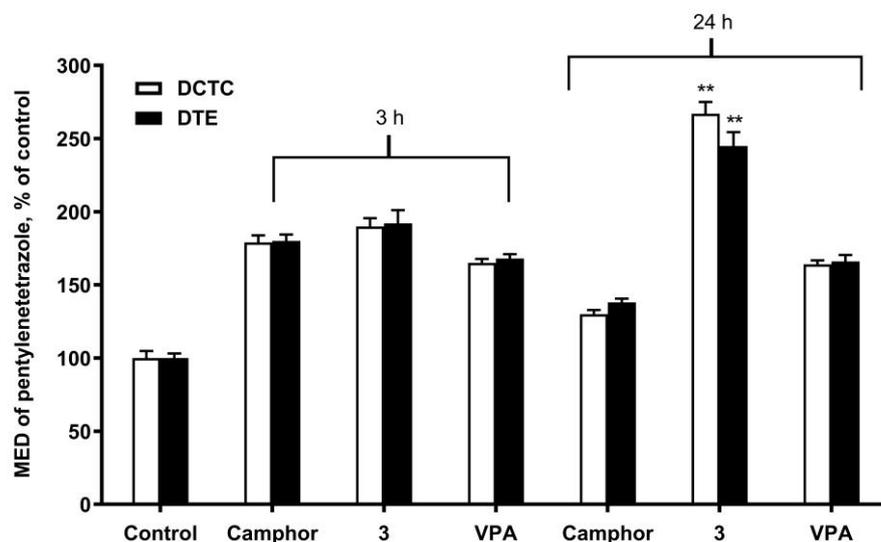


Figure 1. At 3 h and 24 h after oral administration. Values are given as mean \pm SEM, $n = 5$ mice; for all groups $p < 0.01$ compared with the control; ** $p < 0.01$ compared with VPA.

In the MES test, camphor hydrazone **3** substantially prevented the animals' mortality at 3 h after administration, manifesting 80% protection that is equivalent to the VPA effect (80%), whereas moderate antiseizure action was observed for the initial camphor (60%) (Table 1). Conversely, the activity of the titled compound was maintained at a long time period (24 h) with 100% of mortality protection, which confirms the idea toward enzymatic cleavage of labile bonds (C=N, N-NH, or CO-NH) in hydrazone molecules, followed by gradual release of pure terpenoid and VPA.

Table 1. Against maximal electroshock (MES)-induced seizures.

Compound	Control	Camphor	VPA	Compound 3
3 h after single oral administration				
% Mortality protection	0	60	80	80
24 h after single oral administration				
% Mortality protection	0	20	60	100

Thus, camphor derivative **3** protects against seizures induced by chemical and electrical stimuli both in short (3 h) and long (24 h) time periods.

3. Materials and Methods

3.1. General Information

(+)-Camphor and valproic acid (VPA) were obtained from commercial sources. Hydrazine **1** and acid chloride **2** were synthesized according to the standard procedure [7,8]. The progress of reaction was monitored by TLC on Merck-made (TLC Silica gel 60 F₂₅₄) plates (Darmstadt, Germany) visualized by UV light using ethyl acetate-benzene (1:1) as the eluent system. Structure of the obtained compound was established by ¹H-NMR spectroscopy on a Varian VXR-300 (300 MHz) instrument (Varian Inc., Palo Alto, CA, USA) and by ¹³C-NMR spectroscopy on a Varian-Mercury 400 spectrometer

(Varian Inc., Palo Alto, CA, USA) using DMSO- d_6 as a solvent and TMS as an internal standard. FAB mass spectra were obtained on a VG 70-70EQ mass spectrometer (VG Analytical Ltd., Manchester, UK) equipped with a Xe ion gun (8 kV); the sample was mixed with the *m*-nitrobenzyl-alcohol matrix. The purity of the compound was checked by high-performance liquid chromatography on an Agilent 1260 Infinity HPLC system (Agilent, Santa Clara, CA, USA). IR spectra were measured with a Frontier FT-IR spectrometer (Perkin-Elmer, Hopkinton, MA, USA) using KBr pellets. Raman spectra were undertaken with a DXR Raman Microscope (Thermo Fisher Scientific, Madison, WI, USA). DSC curves were recorded in a Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE, USA) using aluminum crucibles containing approximately 2 mg of samples, under a dynamic nitrogen atmosphere and a heating rate of 5 °C min⁻¹ in the temperature range of 20 to 200 °C.

3.2. Synthesis of 2-Propyl-*N'*-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]pentanehydrazide (3)

To a stirred solution of (+)-camphor hydrazine **1** (0.8 g, 5.26 mmol) in CH₂Cl₂ (25 mL) at room temperature, valproic acid chloride (0.897 g, 5.52 mmol) was added. The reaction mixture was cooled to 0 °C, stirred for 10 min, and triethylamine (TEA) was added dropwise (0.77 mL, 5.52 mmol). Stirring was continued for 30 min, then the flask was gradually warmed to room temperature and the stirring continued for an additional 4 h. The reaction completion was monitored by TLC. The reaction mixture was filtered, the filtrate was diluted with CH₂Cl₂ to 100 mL, and washed with 1 M aqueous HCl, 10% aqueous NaHCO₃, and water. The combined organic phases were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by recrystallization from methanol.

White crystals (80%). ¹H-NMR (300 MHz, DMSO- d_6) δ : 0.65 (s, 3H, CH₃), 0.82 (s, 6H, 2CH₃), 0.86 (s, 3H, CH₃), 0.90 (s, 3H, CH₃), 1.21 (m, 8H, 2CH₂-CH₂), 1.46-1.51 (m, 2H, CH₂), 1.65 (t, 1H, CH), 1.77 (m, 1H, CH), 1.89 (m, 2H, CH₂), 2.30 (m, 1H, CH), 9.51 (s, 1H, NH). ¹³C-NMR (100 MHz, DMSO- d_6) δ : 177.4 (C=O), 171.3 (C-2), 52.3 (C-1), 47.8 (C-7), 47.6 (C-4), 43.9 (CH), 35.1 (C-3), 34.5 (CH₂), 32.7 (C-6), 27.3 (C-5), 20.5 (CH₂), 18.8 (C-8,9), 14.2 (C-10), 11.5 (CH₃). FT-IR (ν_{\max} , CM⁻¹): 3179 (N-H), 2970–2834 (C-H), 1697 (C=O). Raman (ν_{\max} , CM⁻¹): 2943-2872 (C-H), 1646 (C=N). MS (FAB) *m/z*: 293 [M + H]⁺. HPLC: *t*_r = 23.43 min. M.p. (DSC) onset: 154.26 °C, peak max: 155.46 °C (Supplementary Materials).

3.3. Anticonvulsant Screening

Anticonvulsant activity was studied using outbred male white mice (18–22 g) as experimental animals. Animals were maintained under a 12 h light regime and in a standard animal facility with free access to water and food. All the animals were purchased from Odessa National Medical University, Ukraine. The Animal Ethics Committee (agreement No. 03/2020) of Odessa National Polytechnic University (Ukraine) approved the study. (+)-Camphor, VPA, and compound **3** were administered orally (preliminarily dissolved in Tween 80/water emulsion): camphor at a dose of 50 mg/kg; VPA and hydrazine **3** in equimolar amounts. Antiseizure evaluation was carried out at 3 h and 24 h after administration both on MES and PTZ-induced convulsions according to the earlier reported procedures [9,10].

4. Conclusions

Einhorn variation of the Schotten–Baumann method was successfully applied for the synthesis of 2-propyl-*N'*-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]pentanehydrazide via (+)-camphor hydrazide condensation with valproic acid (VPA) chloride, followed by structure confirmation using Raman, FT-IR, ¹H-NMR, and ¹³C-NMR spectral analysis along with FAB-mass spectrometry. The title compound was found to demonstrate prolonged anticonvulsant activity both on PTZ- and MES-induced seizures.

Supplementary Materials: Copies of the ¹H-NMR ¹³C-NMR, FT-IR, Raman, FAB mass spectra, DSC thermograms, and HPLC chromatograms are available online.

Author Contributions: I.K. conceived and designed the experiments; M.N. performed the synthesis and analyzed the NMR spectral data; D.B. performed the analysis of FT-IR, Raman, DSC, and HPLC experiments; I.R. carried out FAB characterization of the compound. All authors contributed in manuscript writing. All authors have read and agreed to the published version of the manuscript.

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References

1. Romoli, M.; Mazzocchetti, P.; D'Alonzo, R.; Siliquini, S.; Rinaldi, V.E.; Verrotti, A.; Calabresi, P.; Costa, C. Valproic acid and epilepsy: From molecular mechanisms to clinical evidences. *Curr. Neuropharmacol.* **2019**, *17*, 926–946. [[CrossRef](#)]
2. Gayam, V.; Mandal, A.K.; Khalid, M.; Shrestha, B.; Garlapati, P.; Khalid, M. Valproic acid induced acute liver injury resulting in hepatic encephalopathy- a case report and literature review. *J. Community Hosp. Intern. Med. Perspect.* **2018**, *8*, 311–314. [[CrossRef](#)] [[PubMed](#)]
3. De Almeida, R.N.; Agra, M.; Maior, F.N.; De Sousa, D.P. Essential oils and their constituents: Anticonvulsant activity. *Molecules* **2011**, *16*, 2726–2742. [[CrossRef](#)] [[PubMed](#)]
4. Chen, J.; Jiang, Q.D.; Chai, Y.P.; Zhang, H.; Peng, P.; Yang, X.X. Natural terpenes as penetration enhancers for transdermal drug delivery. *Molecules* **2016**, *21*, 1709. [[CrossRef](#)] [[PubMed](#)]
5. Nesterkina, M.V.; Kravchenko, I.A. Synthesis and anticonvulsant activity of menthyl γ -aminobutyrate. *Chem. Nat. Compd.* **2016**, *52*, 237–239. [[CrossRef](#)]
6. Agrawal, S.; Jain, J.; Kumar, A.; Gupta, P.; Garg, V. Synthesis molecular modeling and anticonvulsant activity of some hydrazone, semicarbazone, and thiosemicarbazone derivatives of benzylidene camphor. *Res. Rep. Med. Chem.* **2014**, *4*, 47–58. [[CrossRef](#)]
7. Da Silva, E.T.; da Silva Araújo, A.; Moraes, A.M.; de Souza, L.A.; Silva Lourenço, M.C.; de Souza, M.V.; Wardell, J.L.; Wardell, S.M. Synthesis and biological activities of camphor hydrazone and imine derivatives. *Sci. Pharm.* **2016**, *84*, 467–483. [[CrossRef](#)] [[PubMed](#)]
8. Wang, Z.; Li, J.; Zeng, X.D.; Hu, X.M.; Zhou, X.; Hong, X. Synthesis and pharmacological evaluation of novel benzenesulfonamide derivatives as potential anticonvulsant agents. *Molecules* **2015**, *20*, 17585–17600. [[CrossRef](#)] [[PubMed](#)]
9. Nesterkina, M.V.; Alekseeva, E.A.; Kravchenko, I.A. Synthesis, physicochemical properties, and anticonvulsant activity of the gaba complex with a calix[4]arene derivative. *Pharm. Chem. J.* **2014**, *48*, 82–84. [[CrossRef](#)]
10. Nesterkina, M.; Barbalat, D.; Konovalova, I.; Shishkina, S.; Atakay, M.; Salih, B.; Kravchenko, I. Novel (–)-carvone derivatives as potential anticonvulsant and analgesic agents. *Nat. Prod. Res* **2020**, (in press). [[CrossRef](#)] [[PubMed](#)]

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