

Synthesis and Analgesic-like Effect of (6*R*, 4*S*)-*p*-Mentha-1,8-dien-6-yl-methylene-*p*-toluenesulfonamide

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Z. Naturforsch. **2009**, *64b*, 351–355;
received October 19, 2008

The synthesis of a monoterpene-based *para*-toluenesulfonamide is reported starting from naturally occurring (*R*)-(–)-carvone (**1**), by 1,2-addition of HCN followed by reduction with lithium aluminum hydride to afford the amino alcohols **3a** and **3b**. Tosylation of this mixture with *p*-toluenesulfonyl chloride furnished sulfonamide **4** in 55% overall yield. Compound **4** was evaluated in behavior animal models to investigate its effects on the central nervous system. It showed low toxicity and sedative action in mice, indicating it to be psychoactive. It also caused a decrease in the spontaneous motor activity of mice. This depressant effect was confirmed in the acetic acid-induced writhing test, which demonstrated a significant antinociceptive response more potent than **1**. The present results provide evidence that sulfonamide **4** has analgesic-like psychopharmacological activity.

Key words: *p*-Toluenesulfonamide, Carvone, Monoterpene, Analgesic Activity, Antinociceptive Activity

Introduction

The total synthesis of naturally occurring terpenes has always been an important research area, with the use of simple, chiral, enantiopure monoterpenes as starting materials being a relevant option [1, 2]. These monoterpene starting materials are readily available, frequently in both enantiomeric forms, and contain significant scaffolds of the desired mono-, sesqui-, and

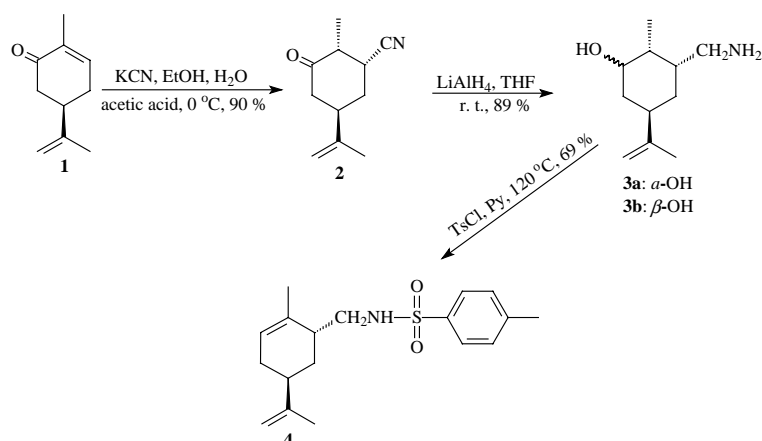
diterpene skeletons. Among the monoterpene starting materials, probably the (+)- and (–)-forms of carvone are the most versatile. The best source of (*R*)-(–)-carvone is spearmint oil, whereas the (*S*)-(+)-enantiomer is a constituent of dill and caraway oils. The cost of (*R*)-(–)-carvone is usually much lower than that of the (+)-isomer, but both enantiomers of carvone have been used as chirons in the synthesis of diverse intermediates and natural compounds, principally terpenoids [1–4].

The range of pharmacological activity that has been recorded for terpenes is remarkably wide. They are found to function as anticonvulsant, antinociceptive, sedative, and anxiolytic agents [5–11]. The range of pharmacological effects of this family of natural products is apparently due to a variety of action mechanisms. Terpene derivatives also have been shown to have several effects on the central nervous system (CNS), including antinociceptive [12, 13] and sedative [14] activity. These facts led us to verify in mice the psychopharmacological profile of (6*R*,4*S*)-*p*-mentha-1,8-dien-6-yl-methylene-*p*-toluenesulfonamide (**4**), an intermediate prepared from (*R*)-(–)-carvone (**1**) in our terpene total synthesis studies.

Results and Discussion

(*R*)-(–)-Carvone (**1**) is an excellent, cheap and commercially available, chiral enantiopure starting material for the synthesis of natural occurring compounds. We have already reported the synthesis of a chiral synthetic intermediate for perhydroazulene terpenoids, starting from (*R*)-(–)-carvone (**1**) with initial 1,2-addition of the nucleophile trimethylsilyl cyanide [3]. In the present paper we report the synthesis of a *p*-toluenesulfonamide beginning with 1,4-addition of cyanide to (*R*)-(–)-carvone (**1**), a reaction already known to furnish selectively the adduct **2** in 90% yield, isolated by crystallization [15, 16].

Thus, the reaction of (*R*)-(–)-carvone (**1**) with potassium cyanide and acetic acid in ethanol under the published conditions [16] produced the nitrile **2** (Scheme 1). Reduction of compound **2** with LiAlH₄ gave a 95:5 mixture of amino alcohols **3a** and **3b** in 89% yield. The constitution of the **3a/3b** mixture was confirmed by micro-analysis of the L-(+)-tartaric acid salts. Sulfonylation of these amino alcohols using *p*-



Scheme 1. Synthesis of (6*R*,4*S*)-*p*-mentha-1,8-dien-6-yl-methylene-*p*-toluenesulfonamide (**4**).

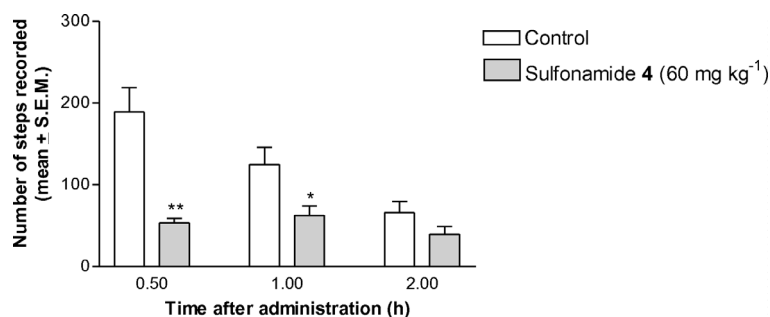


Fig. 1. Effect of sulfonamide **4** on spontaneous motoric activity in mice. $n = 8$; * $p < 0.05$, ** $p < 0.01$ significantly different from control.

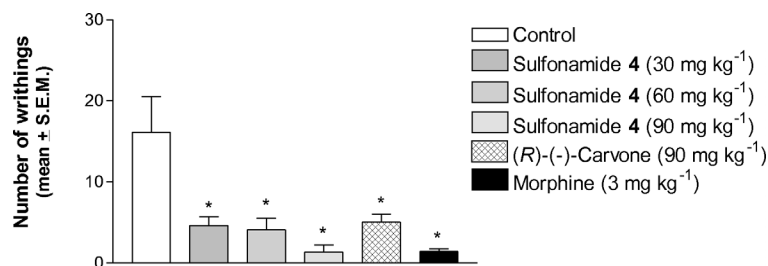


Fig. 2. Effect of sulfonamide **4**, (*R*)-(-)-carvone, morphine on acetic acid-induced writhing in mice. $n = 8$; * $p < 0.01$ significantly different from control.

toluenesulfonyl chloride afforded the easily purified *p*-toluenesulfonamide **4**, in 69% yield. TLC analysis of the reaction product suggested that both aminoalcohols, **3a** and **3b**, had reacted. Noteworthy in this reaction is the concomitant dehydration of the secondary alcohol under the reaction conditions, which was not entirely unexpected with the temperature used.

The pharmacological effects of the sulfonamide **4** on the CNS were then evaluated. The toxicological evaluation of compound **4** did not induce mortality up to a dose of 1000 mg/kg in mice, and no significant toxic effect was found during the observation period.

On the basis of animal observation, **4** (60 mg kg⁻¹) did not affect the motoric coordination and muscle tone. However, the parameters of the behav-

ioral screening were suggestive of a central depressant effect. The animals were found to show decreased locomotive activity and an increase in sedation at 0.5 h and 1 h (Fig. 1) after administration of compound **4** (60 mg kg⁻¹) indicating that the sulfonamide is psychoactive. In the evaluation of the antinociceptive profile, **4** (30, 60, 90 mg kg⁻¹) significantly decreased the incidence of acetic acid-induced writhing (Fig. 2). Compound **4** at 90 mg kg⁻¹ produced a near-maximal inhibition of the writhing response, similar to 3 mg kg⁻¹ of morphine. In comparison with the results found for unprotected terpenes, such as (*R*)-(-)-carvone (**1**) [6, 9], compound **4** was more potent and less toxic. The LD₅₀ value reported for (*R*)-(-)-carvone is 426.6 (389.0–478.6) mg kg⁻¹ [6]. Fig. 2 shows

that a sulfonamide group in the structure of **4** enhances the pharmacological effect and reduces the toxicity.

Conclusions

The data reported in this paper demonstrate the psychopharmacological activity of compound **4** in mice. The study shows that **4** has a CNS-depressant effect similar to some commonly used drugs. This effect of the sulfonamide was not different from that observed for other psychoactive terpenes [8, 9, 17]. However, **4** showed low toxicity, fewer side effects, and an improved pharmacological profile. Our experimental results also suggest that by appropriate structural modification of monoterpenes it should be possible to develop novel analgesic drugs.

Experimental Section

General

GLC analyses were performed on a Shimadzu GC-17A instrument equipped with a flame-ionization detector, using a DB-1 (30 m × 0.25 mm) glass column. Column chromatography was performed on silica gel 60 (70–230 mesh ASTM Merck). Radial thin-layer chromatography was carried out on a Chromatotron model 8924 (silica gel 60PF274 Merck). Melting points were determined on a Microquímica MQWAPF-301 apparatus and are uncorrected. Infrared spectra were recorded with a Bomen Hartman & Braun MB-Series spectrometer. ^1H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, on a Bruker ARX-200 spectrometer or at 400 and 100 MHz on a Bruker DRX-400 spectrometer, in CDCl_3 with TMS as internal standard. The mass spectra were recorded on a Micromass mass spectrometer Quattro LC, coupled with a chemical ionization source (reagent MeOH) under atmospheric pressure (APCI). Microanalyses were performed on a Fisons EA 1108 CHNS-O analyzer, at the Chemistry Department, Universidade Federal de São Carlos. Solvents were purified prior to use: ethyl acetate and hexane were refluxed over P_2O_5 , distilled and stored over molecular sieves; pyridine was stirred and refluxed over KOH, distilled and stored over KOH. Tetrahydrofuran (THF) was distilled from sodium under nitrogen.

(–)-(1*R*,2*R*,5*R*)-2-Methyl-5-(1'-methylvinyl)-3-oxocyclohexanecarbonitrile (**2**)

Reaction of (*R*)-(–)-carvone (**1**), (12.500 g), with hydrogen cyanide gave nitrile **2** in 90% yield, m.p. 91.5–92.2 °C. – $[\alpha]_{\text{D}}^{30} = -3.8$ ($c = 1.20$ in CHCl_3) (lit. [16]: 93–94 °C. – $[\alpha]_{\text{D}}^{18} = -4.0$ ($c = 1.04$ in CHCl_3). – ^1H and ^{13}C NMR data were in agreement with the literature [16].

(1*S*,2*S*,4*R*,6*S*)-6-Aminomethyl-8-*p*-menthen-2-ol (**3a**) and (1*S*,2*R*,4*R*,6*S*)-6-aminomethyl-8-*p*-menthen-2-ol (**3b**)

A dry, nitrogen-purged, 100 mL three-necked flask with a magnetic stirrer was charged with a suspension of lithium aluminum hydride (1.189 g, 31.3 mmol) in anhydrous tetrahydrofuran (76 mL). A solution of 8.0 g (45.19 mmol) of nitrile **2** in tetrahydrofuran (12.5 mL) was added dropwise over 20 min to this suspension and the stirring continued for a further 1 h. Destruction of the excess lithium aluminum hydride was effected by cautious dropwise addition of water (10 mL), followed by dropwise addition of 15% NaOH (10 mL), and subsequent addition of water (30 mL). Stirring was continued until a granular white precipitate was formed. Filtration yielded a clear tetrahydrofuran solution which was dried over anhydrous sodium sulfate. The aminoalcohols **3a** and **3b**, in a 95:5 ratio according to gas chromatographic analysis, were isolated as free amines by removal of the tetrahydrofuran under reduced pressure (7.341 g, 40.11 mmol, 89% yield). The **3a/3b** mixture (1.0 g) was subjected to column chromatography over neutral alumina, eluting with hexane-EtOAc (1:1) to afford aminoalcohol **3a** (0.792 g, 79% yield). Compound **3a**: M.p. 72.8–73.5 °C. – $[\alpha]_{\text{D}}^{30} = +7.0$ ($c = 1.0$; CHCl_3). – IR (film): $\nu = 3489, 2933, 1647, 1037, 993, 887 \text{ cm}^{-1}$. – ^1H NMR: $\delta = 4.73$ (2H, s), 3.75 (1H, q, $J = 3.2$ Hz), 2.92 (2H, s), 2.84 (2H, d, $J = 3.4$ Hz), 2.35 (1H, tt, $J = 3.6$ Hz; 11.6 Hz), 2.00–1.93 (1H, m), 1.92–1.89 (1H, m), 1.87–1.84 (1H, m), 1.74 (3H, s), 1.68 (1H, s), 1.65–1.59 (1H, m), 1.46–1.20 (2H, m), 1.05 (3H, d, $J = 6.8$ Hz). – ^{13}C NMR: $\delta = 149.9, 108.6, 68.9, 41.5, 39.3, 39.0, 37.6, 35.7, 34.3, 21.0, 14.9$. – MS: $m/z = 184$ $[\text{M}+1]^+$.

Tartrate salts of amino alcohol **3a** and **3b**

A 50 mL flask with a magnetic stirrer was charged with distilled water (4.55 mL). L-(+)-Tartaric acid (1.025 g, 6.83 mmol) was added with stirring in one portion. The solution was stirred as 1.0 g (5.46 mmol) of the aminoalcohols **3a–b** was carefully added in one portion. A slurry was formed initially, but complete dissolution was observed once the addition was complete. The solution was heated to 90 °C. Glacial acetic acid (0.75 mL) was then added in one portion. The product began to precipitate during the addition and continued to precipitate while the reaction mixture was allowed to cool from 90 °C to 5 °C, with stirring, over 3 h. The temperature was maintained at 5 °C for an additional hour, and the product was isolated by filtration. The filter cake was washed with cold (5 °C) water (5 mL). The product was dried at 40–45 °C under reduced pressure to give the aminoalcohols **3a, b** as tartrate salts (1.456 g, 4.37 mmol, 80% yield) as a white amorphous solid. – Anal. for $\text{C}_{15}\text{H}_{27}\text{NO}_7$: calcd. C 54.04, H 8.16, N 4.20; found C 54.10, H 8.14, N 4.21.

(6*R*, 4*S*)-*p*-Mentha-1,8-dien-6-yl-methylene-*p*-toluenesulfonamide (**4**)

To a stirred solution of the aminoalcohols (**3a**, **b**) (0.300 g, 1.63 mmol) in dry pyridine (0.8 mL, 9.78 mmol) under a nitrogen atmosphere was added *p*-toluenesulfonyl chloride (0.622 g, 3.26 mmol) at r. t. The solution was heated to 120 °C for 3 h, and then cooled to r. t. After addition of water (10 mL) the reaction mixture was extracted with ethyl acetate, washed with saturated copper sulfate, water, sodium bicarbonate, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by radial chromatography (90:10 hexane-EtOAc) on silica gel to yield the pure product **4** (0.375 g, 1.12 mmol, 69% yield). – $[\alpha]_{\text{D}}^{29} = +100.8$ ($c = 2.1$; C₂H₅OH). – IR (film): $\nu = 3520, 3283, 2933, 1645, 1325, 887, 660 \text{ cm}^{-1}$. – ¹H NMR: $\delta = 7.75$ (2H, d, $J = 8.1$ Hz), 7.30 (2H, d, $J = 8.1$ Hz), 5.52–5.48 (1H, m), 4.70 (1H, t, $J = 1.8$ Hz), 4.64–4.62 (1H, m), 4.39 (1H, t, $J = 6.4$ Hz), 3.17–3.06 (1H, m), 2.92–2.79 (1H, m), 2.42 (3H, s), 2.17–2.10 (1H, m), 2.09–2.05 (1H, m), 2.04–1.87 (2H, m), 1.68 (3H, s), 1.63–1.56 (2H, m), 1.59 (3H, s). – ¹³C NMR: $\delta = 149.0, 143.2, 136.8, 132.2, 129.6, 127.0, 124.7, 108.7, 44.8, 39.1, 35.6, 30.6, 29.7, 21.7, 21.4, 20.7$. – MS: $m/z = 320$ [M+1]⁺. Anal. for C₁₈H₂₅NO₂S: calcd. C 67.68, H 7.89, N 4.38, S 10.04; found C 67.61, H 7.91, N 4.37, S 10.06.

Animals

Male Swiss mice (28–34 g) were obtained from the research animal facility of the Laboratório de Tecnologia Farmacêutica. The animals were maintained at constant r. t. (26 ± 1 °C) and on a 12/12-h light-dark cycle (light from 06:00 to 18:00), with free access to food and water. All behavioral observations were conducted between 08:00 and 17:00 h and carried out in accordance with ethical committee approvals.

Statistical analysis

The statistical analysis was performed using analysis of variance, followed by Dunnett's test. A probability level of 0.05 was regarded as significant.

Acute toxicity and behavioral effects

Different doses of **4** were administered intraperitoneally (i. p.) to groups of mice ($n = 10$), and mortality was recorded for 48 h for the determination of LD₅₀ [18]. The behavioral screening of the mice was performed at 0.5 and 1 h after injection of **4** (60 mg kg⁻¹, i. p.) [19].

Locomotor activity

Mice were divided into four groups of eight each. Vehicle (control) and **4** (60 mg/kg, i. p.) were injected. The spontaneous motor activity of the animals was assessed in an activity cage (controller model 7441 and Grid-Floor Detecting Arrangement Cage model 7432; Ugo Basile, Italy) at 0.5, 1, and 2 h after administration [20].

Acetic acid-induced writhing

The mice were divided into five groups ($n = 8$). The first group was pretreated with saline 0.9% (control). Compound **4** (30, 60, and 90 mg kg⁻¹ i. p.) (*R*)-(-)-carvone **1** (90 mg/kg, i. p.) and morphine (3 mg kg⁻¹ i. p.) were administered. After 30 min an acetic acid solution (0.8%; 0.1 mL/10 g i. p.) was injected. After a further 10 min, the number of constrictions was recorded for 10 min [17, 21].

Acknowledgements

We thank FAPESP, CNPq and CAPES for financial support. The (*R*)-(-)-carvone was generously donated by Firmenich S.A.

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