NPC Natural Product Communications

2018
Vol. 13
No. 4
387 - 388

Spatial Structure and Antimicrobial Activity of Cyclopropane Derivative of Limonene

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Received: February 7th, 2018; Accepted: February 16th, 2018

A cyclopropane derivative of limonene, (1*S*, 4*S*, 6*R*)-7,7-dichloro-4-[(1*S*)-2,2-dichloro-1-methylcyclopropyl]-1-methylbicyclo [4.1.0] heptane (compound **2**), was synthesized and its structure was determined by NMR and X-ray crystallographic methods. In addition, an antimicrobial activity of the compound against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacterial strains was also scrutinized.

Keywords: Limonene, Cyclopropane derivative, Structure, X-ray, Antimicrobial activity.

Limonene is one of well-known cyclic monoterpenes, and its isomers are widely represented in citrus fruits. This compound has been approved by FDA and extensively utilized as a flavour in food and chemical industries. It was shown that derivatives of D-limonene exhibit antimicrobial [1,2], anticancer [3], anti-inflammatory and antioxidant [4] properties. In order to optimize synthesis and improve biological properties of limonene, a range of limonene derivatives has been proposed in the last decades [5]. Here we report on the synthesis of tetrachlorocarbon derivative of limonene (compound **1**), 7,7-dichloro-4-(2,2-dichloro-1-methyl-cyclopropyl)-1-methyl-bicyclo[4.1.0]heptane (compound **2**) (Figure 1). The spatial structure of newly synthesized **2** and its antimicrobial activity were analysed using standard protocols.

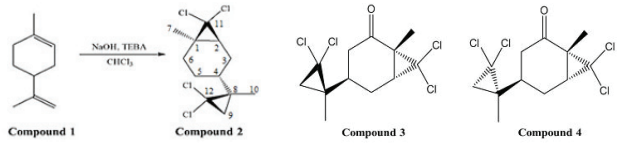

Figure 1: Synthesis of cyclopropane derivative (**2**) of limonene (**1**) and structures of compounds **3** and **4**.

The limonene was isolated from the essential oil obtained by hydro-distillation of air-dried crushed raw material (*Citrus sinensis*). The content of limonene in the resulting oil was 96% according to the results of gas-liquid chromatography (GLC). For further refining, limonene was purified by column chromatography. The final structure of the molecule **2** and its crystal structure were established based on X-ray diffraction data. In this case, the lengths of bonds in molecule **2** correspond to the statistical average [6]. The conformation of the six-membered ring is close to the envelope, the 0.708 (3) Å. Among intermolecular contacts, we observed a slightly reduced contact Cl_1 ... Cl_3 3.442 (1) Å [7] (Figure 2).

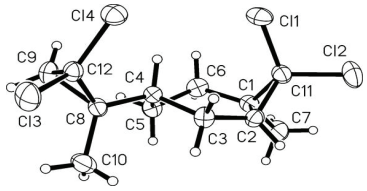

Figure 2: The schematic structure of the molecule of compound **2** (as a crystal). 30% of thermal ellipsoids are shown.

It is interesting to note that we found two very close compounds in the Cambridge Structural Database (CSD) [8]: 1) (1*R*, 4*R*, 6*R*, 9*S*)-7,7-dichloro-1-methyl-4-(2', 2'-dichloro-1' methylcyclopropyl) bicyclo[4.1.0]heptane-2-one (compound **3**), and 2) (1*R*, 4*R*, 6*R*, 9*R*,)-7,7-dichloro-1-methyl-4-(2',2'-dichloro-1'-methylcyclopropyl)-bicyclo[4.1.0]heptan-2-one (compound **4)** [9] (Figure 1). The conformation of the six-membered ring in these compounds is close to the shape of the half-arm with the deviation of the atoms C3, C4 by +0.422,-0.248 and +0.362,-0.299 Å, respectively.

Compared to **1**, its semi-synthetic derivative (**2**) demonstrated similar antibacterial activity (Table 1). The highest activity of **2** was observed against *Staphylococcus aureus* (18 ± 0.2), and the lowest was against *Pseudomonas aeruginosa* (13 ± 0.1). These findings indicate a potential usage of cyclopropane limonene derivative in medicine and food industry.

Table 1: Antimicrobial activity of compounds.

Bacterial strains	Control	Gentamicin	Compound **1**	Compound **2**
Staphylococcus aureus	14 ± 0.1	24 ± 2.0	16 ± 0.2	18 ± 0.2
Bacillus subtilis	14 ± 0.1	25 ± 2.0	18 ± 0.1	17 ± 0.1
Escherichia coli	13 ± 0.2	20 ± 4.0	16 ± 0.2	15 ± 0.2
Pseudomonas aeruginosa	11 ± 0.2	18 ± 3.0	12 ± 0.9	13 ± 0.1

The experimental data, zone of inhibitions (mm), are represented as means from at least 3 independent samples (± SD).

Experimental

Synthesis of limonene derivative: The synthesis of **2** was conducted according to the method previously described by Julia and Ginebreda [10]. Briefly, a phase transfer catalyst TEABAC (benzyltriethylammonium chloride) (0.34 g, 1.5 mmol) was added to limonene (20.4 g, 0.15 mol) dissolved in chloroform (100 mL). Then, powdered sodium hydroxide NaOH (20 g, 0.5 mol) was added. The reaction was conducted with a reflux condenser. After 15 min of stirring, the mixture is cooled with ice bath, filtered, washed with chloroform (50 mL) and evaporated. The residue recrystallized from methanol gave **2** as colourless crystals, MP: 121–122° C, with 60% yield (27.1 g). NMR: Table 2.

Table 2: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for compound **2** in $CDCl_3$.

Position	^{13}C	^{1}H
1	33.5	–
2	38.7	2.16 (dd, 1H, *J*=14.2, 6.2 Hz, methine)
3	25.2	1.89-1.92 (m, 1H, 3-CH_e); 1.11-1.23 (m, 1H, 3-CHa)
4	33.1	1.54 (br. s, 1H, 4-CH)
5	20.7	1.72-1.78 (m, 1H, 5-CH_e); 1.11-1.23 (m, 1H, 5-CHa)
6	28.6	1.33-1.39 (m, 1H, 6-CH_e); 1.11-1.23 (m, 1H, 6-CHa)
7	25.0	1.40 (s, 3H)
8	27.7	–
9	33.1	1.72-1.78 (m, 1H, 9-CH_a); 1.33-1.39 (m, 1H, 9-CHb)
10	15.5	1.16 (s, 3H)
11	72.6	–
12	67.7	–

X-ray crystallography: X-ray diffraction studies of compound **2** were performed on a Bruker APEX-II CCD diffractometer (MoKα radiation, graphite mono-chromator, φ, ω-scanning) at room temperature. Adjustments have been made for absorption by the empirical method according to the SADABS programme [11]. The structure was deciphered by a direct method using the SHELXS-97 programme and refined by the least-squares method in the anisotropic-isotropic approximation according to the SHELXL-97 programme [12]. The positions of the hydrogen atoms were set geometrically and refined in the model of the rider.

Crystallographic data: orthorhombic system, space group $P2_12_12_1$, a = 10.3624 (6), b = 10.7618 (6), c = 12.5599 (8) Å, V = 1400.7 (1) Å3, $C_{12}H_{16}Cl_4$, Z = 4, D_{calc} = 1.432 g • cm-3, μ = 0.817 cm^{-1}, transmission Tmin / Tmax = 0.493 / 0.647, scanning region 2θ < 54°, sample size 0.03 × 0.3 × 0.3 mm. The refinement parameters are wR_2 = 0.0925, S = 1.134 (over all 2952 reflections), R_1 = 0.0350 (2624 I ≥ 2σ (I)), 145 refined parameters. The absolute structure parameter is 0.06 (8) that allow determining an absolute configuration. The results were deposited at the Cambridge Crystallographic Centre (deposit number CCDC 1586136), and they can be obtained free of charge at http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

Antimicrobial activity: The reference bacterial cultures of Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633) and Gram-negative strains (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) were provided by American Type Culture Collection (ATCC, USA). Agar diffusion test (wells) was performed to study antimicrobial activity of **2** against above-mentioned strains. The method has been previously described by Balouiri *et al*. [13]. The antibiotic gentamicin (Sigma-Aldrich, Merck) was used as a positive control, whilst ethanol was used as a negative one. **2** and gentamicin were tested in the dosage of 10 μL (possessing antimicrobial activity). The prepared plates were incubated at 37°C for 48 h. Then, inhibition zones were measured (mm) and analyzed. All values are expressed as mean ± SD of at least 3 separate experiments.

Acknowledgments – The authors are grateful for the support through the grant: "Biologically active low-molecular compounds of essential plant oils. Synthesis of new derivatives of terpenoids and study of the relationship ‘structure-biological activity" (Programme of Fundamental Research; F.0500; Republic of Kazakhstan).

References

[1] Umagiliyage AL, Becerra-Mora N, Kohli P, Fisher DJ and Choudhary R. (**2017**) Antimicrobial efficacy of liposomes containing D-limonene and its effect on the storage life of blueberries. *Postharvest Biology and Technology*, ***128***, 130 –137.

[2] Erasto P, Viljoen AM. (**2008**) Limonene - A review: Biosynthetic, ecological and pharmacological relevance. *Natural Product Communications*, ***3***, 1193–1202.

[3] Murthy KNC, Jayaprakasha GK and Patil BS. (**2012**) D-limonene rich volatile oil from blood oranges inhibits angiogenesis, metastasis and cell death in human colon cancer cells. *Life Sciences*, ***91***, 429–439.

[4] Yu LH, Yan J and Sun ZG. (**2017**) D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. *Molecular Medicine Reports*, ***15***, 2339–2346.

[5] Ardashov OV, Volcho KP and Salakhutdinov NF. (**2014**) Synthesis of hydroxy derivatives of limonene. *Russian Chemical Reviews*, ***83***, 281–298.

[6] Allen FH, Kennard O, Watson DG, Brammer L, Orpen AG and Taylor R. (**1987**) Tables of Bond Lengths Determined by X-Ray and Neutron-Diffraction .1. Bond Lengths in Organic-Compounds. *Journal of the Chemical Society-Perkin Transactions 2*, S1-S19.

[7] Rowland RS and Taylor R. (**1996**) Intermolecular nonbonded contact distances in organic crystal structures: Comparison with distances expected from van der Waals radii. *Journal of Physical Chemistry*, ***100***, 7384–7391.

[8] Allen FH. (**2003**) The Cambridge structural database (CSD) and its research applications in structural chemistry. *Abstracts of Papers of the American Chemical Society*, ***226***, U302–U302.

[9] Zukermanschpector J, Castellano EE, Oliva G, Brocksom TJ and Canevarolo ET. (**1984**) Structural studies on the dichlorocarbene adducts with (-)-*R*-carvone .1. The crystal-structures of [1*R*, 4*R*, 6*R*, 9*R*]-7,7-dichloro-1-methyl-4-[2',2'-dichloro-1'-methylcyclopropyl]-bicyclo[4.1.0]-heptan-2-one and [1*R*, 4*R*, 6*R*, 9*S*]-7,7-dichloro-1-methyl-4-[2',2'-dichloro-1'-methylcyclopropyl]-bicyclo[4.1.0]-heptan-2-one. *Canadian Journal of Chemistry-Revue Canadienne De Chimie*, ***62***, 570–573.

[10] Julia S and Ginebreda A. (**1977**) New method for generation of dichlorocarbene using solid-liquid phase-transfer catalysis. *Synthesis-Stuttgart*, 682–683.

[11] Krause L, Herbst-Irmer R, Sheldrick GM and Stalke D. (**2015**) Comparison of silver and molybdenum microfocus X-ray sources for single-crystal structure determination. *Journal of Applied Crystallography*, ***48***, 3–10.

[12] Sheldrick GM. (**2008**) A short history of SHELX. *Acta Crystallographica Section A*, ***64***, 112–122.

[13] Balouiri M, Sadiki M and Ibnsouda SK. (**2016**) Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, ***6***, 71–79.