DOI: 10.1002/cbdv.201800411

FULL PAPER

CHEMISTRY & BIODIVERSITY

Synthesis and Biological Activity of New 4-*tert*-Butylcyclohexanone Derivatives

Agata Kozioł,[a, b] Mateusz Jasnowski,[a] Ewa Grela,[a] Maryla Szczepanik,[c] Beata Gabryś,[d] Katarzyna Dancewicz,[d] and Stanisław Lochyński*[a, b]

[a] Department of Bioorganic Chemistry, Wrocław University of Science and Technology, Wyb. Wyspiańskiego 27, 50-370 Wrocław, Poland, e-mail: stanislaw.lochynski@pwr.edu.pl

[b] Institute of Cosmetology, Wrocław College of Physiotherapy, Kościuszki 4, 50-038 Wrocław, Poland

[c] Department of Invertebrate Zoology, Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland

[d] Faculty of Biological Sciences, University of Zielona Gora, Szafrana, 65-516 Zielona Góra, Poland

In the synthesis performed in this study, derivatives of 4-*tert*-butylcyclohexanone **1** were obtained using typical reactions of organic synthesis. The bioactivity of the selected compounds was evaluated. 1-(Bromomethyl)-8-*tert*-butyl-2-oxaspiro[4.5]decan-3-one (**5**) was characterized by attractant properties against larvae and a weak feeding deterrent activity against adults of *Alphitobius diaperinus* PANZER. This bromolactone was a moderate antifeedant towards *Myzus persicae* SULZER. In addition, ethyl (4-*tert*-butylcyclohexylidene)acetate (**2**) and bromolactone **5** displayed antibacterial activity. The strongest bacteriostatic effect was observed against Gram-positive strains: *Bacillus subtilis* and *Staphylococcus aureus*. The bromolactone **5** also limited the growth of *Escherichia coli* strain.

Keywords: 4-*tert*-butylcyclohexanone, lactones, bromolactones, biological activity, antibacterial activity.

Introduction

The literature presents vast information on 4-*tert*-butylcyclohexanone **1** – an analog of monoterpenoids, containing the cyclohexane ring and 10 carbon atoms in its structure. This organic compound is commonly used in the air care, perfumes and fragrances, cosmetics and personal care products.[1] It was also examined by testing its ability to produce seizures or to inhibit seizures induced by pentylenetetrazol and maximal electroshock in CF-1 mice. In addition, this compound was tested for its ability to bind picrotoxin. Due to its interactions with the inhibitory neurotransmitter γ-aminobutyric acid, picrotoxin acts as a stimulant and convulsant and mainly impacts the central nervous system, causing seizures and respiratory paralysis when administered at high doses.[2] Anticonvulsant and antidepressant activity of the selected terpene derivatives was also proved in experimental tests in mice.[3] Monoterpenes – as the largest class of plant secondary metabolites – are generally associated with diverse biological activities, including antioxidant, anti-inflammatory and vast antimicrobial properties. They are known as dietary components, pharmaceuticals and insect repellants.[4] Previously, derivatives with cyclohexane unit were used as a substrate for active compounds with high local anesthetic activity.[5] The same substrate was subjected to the intramolecular modification of the bicyclo[4.1.0]heptane system to produce the bicyclo-[3.1.0]hexane unit.[6]

Due to the production of the undisclosed compounds in the 4-step synthesis reaction, we decided to study their antifeedant activity against dangerous pests, lesser mealworm *Alphitobius diaperinus* PANZER (Coleoptera: Tenebrionidae) and peach potato aphid *Myzus persicae* SULZER (Hemiptera: Aphididae). The lesser mealworm is one of the most important and widespread pests in commercial poultry production

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.201800411

around the world. In chicken house, the pest occurs in large numbers mainly in the bedding litter, especially under feeders and drinking troughs.[7] *M. persicae* is extremely polyphagous, highly effective in transmitting plant viruses, and resistant to several classes of insecticides.[8] These species differ in the feeding habits and food preferences. The lesser mealworm possesses the chewing mouthparts and consumes food as a whole, while the peach potato aphid has the sucking-piercing feeding apparatus and relies solely on plant sap in sieve elements that can be reached only after having penetrated outer plant tissues.

Additionally, screening tests were performed against three commonly chosen bacterial strains: Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*. *E. coli* is a known model microorganism in biological assays and, furthermore, has pathogenic properties, most often resulting in urinary tract infections.[9] *S. aureus*, another human pathogen, may cause bacteremia, endocarditis and – most commonly – skin infections.[10] On the other hand, most strains of *B. subtilis* are associated with food spoilage and even poisoning.[11] Due to structural diversity of these bacteria, their juxtaposition serves as a convenient approach for a basic antibacterial testing.

In the presented study, the starting compound 4-*tert*-butylcyclohexanone (**1**) was obtained from 4-(1,1-dimethylethyl)cyclohexan-1-ol in a reaction with bis(quinuclidine)bromine(I) bromide.[12] The mechanism of the reaction is based on the reaction of a carbonyl ketone group with an α-metal phosphonate, in this case triethyl phosphonoacetate, to obtain alkenes in the olefin form and readily soluble phosphate esters or acids.[13] The reaction involves the deprotonation of the triethyl phosphonoacetate, resulting in the carbonate of the phosphonoacetate. The ketone **1** is attached to the phosphorus atom to form a diethyl phosphate and ultimately is linked to the α,β-unsaturated isomer of the ester **2**. The C=O double bond is broken, and the carbon atom adopts the tetrahedral form. The tetrahedral form collapses by discarding the alcoholic group RO^-, in this case the EtO group, and generating an intermediate form of the aldehyde. In the next step, aldehyde reduction is performed by $LiAlH_4$, the C=O double bond is again broken, the electrons are transferred to oxygen, and the carbon is attached to the next hydrogen atom. The last step is the simple protonation reaction of the alkoxide.[14,15]

The allyl alcohol **3** obtained by the reduction of the ester was subjected to a rearrangement reaction described by Claisen.[16] The reaction mechanism consists of the protonation of the orthoacetate groups in the first stage. The resulting oxonium cation is attached to the hydroxy group of the alcohol. Due to its positive charge/protons, other alcoholic groups are protonated. After the reduction of the EtO groups, deprotonation occurs and a diene is formed in which the final form, e.g., γ,δ-unsaturated ester **4**,[17] is produced as a result of rearrangement of the charges.[18,19]

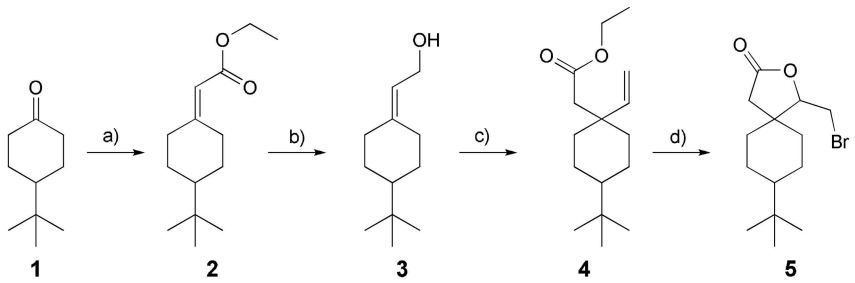
The mechanism of reduction of unsaturated esters to γ,δ-unsaturated carboxylic acids starts with the protonation of the carbonyl group of ester **4**,[17] causing it to become more electrophilic and resulting in the subsequent nucleophilic attack of the oxygen atom of water on the carbonyl carbon atom. The charge transfer results in the formation of a tetrahedral oxide ion.[19] The next step is the deprotonation of oxygen from the water. After deprotonation of the oxide ion and the removal of EtOH, a carboxy-terminal end product is formed.[20]

Another reaction was the lactonization of γ,δ-unsaturated acid **4**,[17] which consists of the formation of a positively charged halonium ion by the reaction of bromide with the alkene group. The nucleophilic hydroxy group is involved in the process of ring closure, thereby forming an intra-cyclic lactone **5**.[21]

The biological activity of 4-*tert*-butylcyclohexanone (**1**) derivatives is poorly described in the literature. Here, the synthesis method of novel 1-(bromomethyl)-8-*tert*-butyl-2-oxaspiro[4.5]decan-3-one (**5**)[21] was presented. Its antifeedant activity was evaluated against common pests. The antibacterial properties of the derivative **2** and the resulting lactone 5[21] were also presented and compared to the starting compound **1**.

Results and Discussion

The synthesis reaction was performed as described in *Scheme 1* and started with the modification of 4-*tert*-butylcyclohexanone (**1**) by the Horner-Wadsworth-Emmons reaction. The resulting ethyl (4-*tert*-butylcyclohexylidene)acetate (**2**) was reduced using lithium aluminum hydride. The reaction product was an allyl alcohol: 2-(4-*tert*-butylcyclohexylidene)ethanol (**3**). The next step was to convert the resulting allyl alcohol to the unsaturated ester using the Claisen-Johnson rearrangement reaction. The reaction product was ethyl (4-*tert*-butyl-1-ethenylcyclohexyl)acetate (**4**).[17] The last step was the bromolactonization[22] of the previously obtained ester to yield the bromolactone,



Scheme 1. The synthesis of the bromolactone **5**. Reagents and conditions: a) DBU, LiCl, THF; b) $LiAlH_4$, THF; c) $MeC(OEt)_3$, $MeCH_2COOH$; d) NBS/THF, H_2O.

Table 1. Feeding deterrent activity of the lactone **5** against the lesser mealworm, *A. diaperinus*. The results were presented as the mean deterrence coefficients ± SD.

Compound	Deterrence coefficients[a] Larvae			Adult stage		
	A	R	T	A	R	T
5	1.57 ± 2.28	−27.97 ± 8.25	−26.40 ± 8.24	7.67 ± 1.58	23.55 ± 9.9	31.22 ± 7.69

[a] A – absolute deterrence coefficient (no-choice test). R – relative deterrence coefficient (choice test). T = A + R (total deterrence coefficient).

1-(bromomethyl)-8-*tert*-butyl-2-oxaspiro[4.5]decan-3-one (**5**).[21]

The antifeedant activity of lactone **5**[21] against the lesser mealworm varied, depending on the developmental stage of the pest (*Table 1*). In trials with larvae, this lactone stimulated higher food intake compared to the control, especially in choice test, and therefore it is defined as attractant (T = −26.40). The obtained total deterrence coefficient for adults (T = 31.22) indicates poor deterrence properties of the lactone **5**. For comparison, the value of this coefficient for the most potent antifeedant, azadirachtin, was close to 200, when determined using the same method.[23]

The activity of the studied compound **5**[21] was evaluated using aphid settling bioassay. Aphids only settle on a plant when they accept it as a food source after having probed plant tissues with their piercing-sucking mouthparts. During plant penetration with their stylet-like mouthparts, aphids collect samples of plant sap for gustatory purposes. Chemical cues in plant tissues determine the decision of aphids to accept or to reject the plant.[24] Aphids respond to deterrents by walking away from the potential food source.

In the present study, the application of compound **5**[21] caused aphids to reject the treated plants as early as 1 h after they had been granted access to the treated leaves (*Table 2*). Thus, compound **5**[21] exhibited deterrent properties. The deterrent effect of compound **5**[21] appeared to be relatively durable: aphids avoided the treated leaves for at least 24 h after exposure, although the potency of the effect decreased over time (*Table 2*). The indices of deterrence were relatively high (ID 0.38–0.59).

Table 2. Feeding deterrent activity of the tested lactone **5** on the peach potato aphid *M. persicae* (Sulzer). The results were presented as the mean number of aphids ± SD.

Time [h]	Number of aphids on control leaves	Number of aphids on treated leaves	*p*	ID
1	5.6 ± 0.7	2.6 ± 0.5	0.0016	0.38
2	6.8 ± 1.4	1.8 ± 0.5	0.0036	0.59
24	6.4 ± 1.7	2.3 ± 1.0	0.0530	0.48

The antibacterial activities of compounds **1**, **2** and **5**[21] were evaluated (*Table 3*). In our research, starting compound **1** was described with no antibacterial activity. Ester **2** did not display bacteriostatic activity toward *E. coli* and *B. subtilis*. However, it was effective against *S. aureus*, restricting the growth of the bacterial culture by more than 60% at a 200 μg/mL concentration. Further incubations allowed us to calculate the minimal inhibitory concentration required to limit bacteria growth by 50% (MIC_{50}) as 150 μg/mL. MIC_{90} was not recorded within examined range of concentrations (50–250 μg/mL). Compound **5**[21] was not characterized as displaying strong bacteriostatic activity with MIC values over 250 μg/mL.

Table 3. The antibacterial activities of compounds **1**, **2** and **5**.[21] Bacteria were cultured with 200 μg/mL compounds for 24 h. The assays were repeated minimum in triplicate on the separate bacterial cultures. The results were presented as the mean growth inhibition ± SD.

Compound	Growth inhibition [%][a]		
	Escherichia coli PCM 2057	*Bacillus subtilis* PCM 2021	*Staphylococcus aureus* PCM 2054
1	0	0	0
2	0	0	63.3 ± 2.5
5	20.4 ± 1.2	39.3 ± 1.8	19.4 ± 1.1

[a] After 24 h incubation with 200 μg/mL compound. The most active positive control – gentamicin – limited growth of the presented strains by 100%.

Still, it exhibited a slight antibacterial activity against all examined bacterial strains with the strongest bacteriostatic activity towards *B. subtilis*. Here, an incubation with 200 μg/mL compound **5**[21] limited bacterial growth by approximately 40%. In comparison to the starting compound **1** – a significant improvement of the antibacterial activity was achieved. In our previous article, a similar enhancement of monoterpene antibacterial activity was observed after incorporation of bromolactone moiety into the molecule.[25] Monoterpenes tend to exhibit weaker antibacterial activity towards Gram-negative strains,[26] in contrast to bromolactones that may affect the growth of both Gram-positive and Gram-negative bacteria.[27] It is assumed that it is the effect of the compounds interaction with cellular membranes, resulting in their permeabilization and disruption.[4][28] Wider spectrum of bromolactone derivatives activity confirmed in our studies may be the basis for a further search for novel antimicrobials, as the growing drug resistance of common pathogens is a known matter of concern.[29]

Conclusions

In the presented study, 1-(bromomethyl)-8-*tert*-butyl-2-oxaspiro[4.5]decan-3-one (**5**)[21] was synthesized via four step reaction. This compound was attractant for larvae and a weak feeding deterrent against adults of *A. diaperinus*. Contrarily, compound **5**[21] exhibited relatively high deterrent effect (ID 0.38–0.59) against the peach potato aphid *M. persicae*.

A moderate bacteriostatic activity of derivatives **2** and **5** was observed (up to 60% bacterial growth limitation at the concentration of 200 μg/mL), in the contrast to the starting compound **1** that was inactive under the standard incubation conditions. Notably, the examined structures were more effective against Gram-positive bacteria. Additionally, the bromolactone **5** was the only compound in the presented study that was effective against *E. coli* strains.

Experimental Section

Feeding Deterrent Activity

A previously described standard method employing choice and no-choice tests was used to determine the feeding deterrent activity of the studied compound against *A. diaperinus*.[30] Oat flakes purchased from Melvit S. A. (Warsaw, Poland) were used as the test food. For the feeding assays, acetone solutions of the test compounds were prepared at a concentration of 10 mg/mL. One ml of solution or acetone alone as a control was applied to one gram of flakes using a micropipette. After evaporation of the solvent (30 min of air-drying), the flakes were weighted and placed in Petri dishes (15 cm in diameter) together with 10 approximately 25–30-day-old larvae or 10 unsexed 7–10-day-old adults. In choice tests (insects could choose either the control or treated food), control and treated flakes were placed in Petri dishes and separated by a thin glass capillary. In the no-choice test, insects were exposed to only one type of food – treated or control. Four replicates for each type of test and each compound were conducted on insects at each life stage. Dishes were maintained in the rearing chamber at 29 ± 1 °C in the dark for 3 days. After this period, the remaining uneaten oat flakes were reweighed and the average weight of food eaten was calculated. This value was the basis for calculating the deterrence coefficients.

The peach potato aphid *M. persicae* was used to evaluate the deterrent potential of the bromolactone against insects. Aphids (maintained as a multiclonal colony) and plants (Chinese cabbage *Brassica pekinensis*) were reared in a laboratory at 20 °C with 65% r.h. and a 16:8 (L/D) photoperiod. One- to seven-day-old apterous female *M. persicae* and 3-week-old plants with 4–5 fully developed leaves were used for the experiments. All experiments were performed under the same temperature, relative humidity, and photoperiod conditions, described above. The procedures have been described in detail by Grudniewska et al.[8] Basically, this bioassay allows us to study aphid host preferences under semi-natural conditions, where

aphids are given a free choice between control and treated leaves. Compound **5**[21] was applied to one leaf of a plant by immersing it in a 0.1% ethanolic solution of the indicated compound for 30 seconds. Control leaves of similar size were immersed in 70% ethanol, the solvent for compound **5**.[21] Treated and control leaves were placed in a Petri dish and allowed to dry for 1 h before the start of the experiment to permit the evaporation of the solvent. Next, aphids were placed in the dish along the line that divided the arena into two halves and could choose between treated (on one half of a Petri dish) and control leaves (on the other half of the dish). Aphids that settled, e.g., they did not move and the position of their antennae indicated feeding, on each leaf were counted at 1, 2 and 24 h intervals after the insects were provided access to the leaf (8 replicates, 20 *Viviparous apterous* females/replicate). Aphids that were moving or not settled on any of the leaves were not counted.

The relative deterrence coefficient R was calculated using the following formula:

$$R = \frac{(C - E)}{(C + E)} \times 100$$

where C and E are the weights of the control and treated foods consumed by the insects in the choice test, respectively. The absolute deterrence coefficient A was calculated using the same formula, but C and E were obtained from the no-choice test. The total coefficient of deterrence (T = A + R), which ranged from −200 to 200 served as the index activity. Compounds with T-values ranging from 151 to 200 are very good deterrents, those with values ranging from 101–150 are good deterrents, and those with values of 51–100 are only moderate antifeedants. T-values less than 50 indicate a weak deterrent activity. Negative T values indicate attractant properties of the compound.

Antibacterial Activity

The examined bacterial strains (*Escherichia coli* PCM2057, *Bacillus subtilis* PCM 2021 and *Staphylococcus aureus* PCM2054) were obtained from the collection of Polish Academy of Sciences.

Tests of antibacterial activity were performed in 96-well microplates containing Mueller-Hinton broth. Cultures were inoculated with bacterial suspensions adjusted to 5×10^5 CFU/mL using 0.5 McFarland standard. Stock solutions of examined compounds were prepared in ethanol and added to bacterial suspensions at final concentrations of 250–50 μg/mL. Ethanol was added to the untreated control. Cultures were incubated overnight at 37 °C with gentle shaking. The optical density was recorded at 650 nm using a Tecan Sunrise microplate reader equipped with Magellan software. The minimal inhibitory concentration (MIC_{50} and MIC_{90}) was calculated as the lowest concentration that restricted the growth of microorganism by 50% or 90%, respectively.[31]

Chemistry

4-*tert*-Butylcyclohexanone (**1**) was obtained from Sigma-Aldrich®. Macherey–Nagel, ALUGRAM SIL G/UV254 tiles were used for the rapid analysis of thin-layer chromatography. A CombiFlash Rf + Lumen with a RediSept 12 gr column of silica gel flash column chromatography or a 50 cm classical chromatography column of Macherey-Nagel with a pore size of 0.04–0.063 mm was used to clean the compounds.

Gas chromatography measurements were performed on an Agilent 7890A GC. Mass spectrometry was performed on the Waters GCT Premier system, consisting of a high resolution mass spectrometer with a flight time (TOF) spectrometer. Infrared spectra were recorded on a Bruker VERTEX 70 V equipped with ATR Platinum and the results were analyzed using Bruker OPUS; $\tilde{\nu}$ in cm^{-1}. The optical rotation was measured with a polAAr 31 polarimeter. Measurements were performed in a methanol solution at 24 °C and a wavelength of 589 nm, and the cuvette length was 100 mm. The NMR analysis was performed on a Bruker Avance DRX 600 nuclear magnetic resonance spectrometer; δ in ppm relative to Me_4Si as internal standard, J in Hz. MestReNova version 6.0.2 was used to describe the resulting NMR spectra. In the *Supporting Information*, the spectra of novel compounds **4**[17] and **5**[21] were included.

Ethyl (4-*tert*-Butylcyclohexylidene)acetate (2). LiCl (0.0134 M) was dissolved in acetonitrile. The mixture was stirred for 10 min. Then, a mixture of triethyl phosphonoacetate (0.0133 M) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 0.00834 M) was dissolved in 15 mL of acetonitrile. The mixture was added dropwise to the solution. After stirring for 40 min at 0 °C, 4-*tert*-butylcyclohexanone (**1**; 0.0063 M) in 5 mL of acetonitrile was added to the mixture dropwise. The

solution was mixed for 1 h in an ice bath. The reaction was controlled by thin layer chromatography. After 72 h, the synthesis reaction was discontinued, then 30 ml of distilled water and 40 ml of distilled hexane were added. The product was extracted with hexane, and then the combined organic layer was dried with anhydrous $MgSO_4$. The crude product was purified by flash column chromatography (hexane/ethyl acetate, 4:1) to yield compound **2**. IR (ATR): 3341 (m), 2953 (vs), 2852 (vs), 1261 (vs), 1029 (m). ^{1}H-NMR ($CDCl_3$, 600 MHz): 1.16 (s, 9H, H-8, H-9, H-10), 1.22 (t, J=7.2, 3H, H-3, H-4, H-5), 1.28 (t, J=3.9, 3H, H-14), 1.95–2.00 (m, 2H, H-3, H-5), 2.50 (dd, J=11.7, 9.3, 4H, H-2, H-6), 4.05 (q, J=4.8, 2H, H-13), 5.97 (s, 1H, H-11). ^{13}C-NMR ($CDCl_3$, 150 MHz): 14.01 (C-14), 16.20 (C-3, C-5), 20.86 (C-8, C-9, C-10), 33.82 (C-2, C-6), 34.71 (C-7), 41.41 (C-4), 60.23 (C-13), 114.00 (C-11), 165.71 (C-1), 170.94 (C-12). HR-TOF-MS (pos.): 224.1845 (M^+; calc. 225.1850).

2-(4-*tert*-Butylcyclohexylidene)ethanol (**3**). Ethyl (4-*tert*-butylcyclohexylidene)acetate (**2**; 0.0044 M) was dissolved in 10 mL of anhydrous THF. A 10% solution of ester in solvent was placed in an ice bath and stirred slowly to a temperature of 0–2 °C. After 15 min, $LiAlH_4$ (0.0087 M) was added to the flask. The reaction was conducted for 80 min. The solution from the precipitate was decanted by adding 6 mL of distilled water and the product was extracted with hexane. The organic phase was dried with anhydrous $MgSO_4$. Product **3** was purified by flash column chromatography (hexane/ethyl acetate, 4:1). IR (ATR): 3341 (m), 2953 (vs), 2852 (vs), 1261 (vs), 1029 (m). ^{1}H-NMR ($CDCl_3$, 600 MHz): 0.91 (s, 9H, H-8, H-9, H-10), 0.97–1.02 (m, 2H, H-3, H-5), 1.16–1.21 (m, 1H, H-4), 1.16–1.21 (m, 2H, H-3, H-4), 1.51–1.61 (m, 2H, H-3, H-5), 1.63–1.75 (m, 2H, H-2, H-6), 1.76–1.88 (m, 2H, H-2, H-6), 2.08 (s, 1H, OH), 4.20–4.31 (m, 2H, H-12), 5.31–5.35 (m, 1H, H-11). ^{13}C-NMR ($CDCl_3$, 150 MHz): 22.71 (C-3, C-5), 29.05 (C-8, C-9, C-10), 29.34 (C-2), 31.91 (C-6), 31.95 (C-7), 42.34 (C-4), 59.46 (C-12), 122.93 (C-11), 141.45 (C-1). HR-TOF-MS (pos.): 183.1400 (M^+; calc. 183.1670).

Ethyl (4-*tert*-Butyl-1-ethenylcyclohexyl)acetate (**4**). Compound **3** (0.00158 M) was charged in a solution containing 14.5 mL of triethyl orthoacetate and 0.05 mL of propanoic acid. The reaction was performed for 5 h at 135 °C, and TLC (hexane/acetone 4:1) was used to monitor the reaction. After 5 h, the reaction was removed from heat and allowed to proceed for an additional 19 h. The synthesis reaction was terminated after 24 h and triethyl orthoacetate was distilled off. The crude product **4** was purified by flash column chromatography (hexane/ethyl acetate, 4:1).

1-(Bromomethyl)-8-*tert*-butyl-2-oxaspiro[4.5]-decan-3-one (**5**). Ethyl (4-*tert*-butyl-1-ethenylcyclohexyl)acetate (**4**; 0.001 M) in 5.5 ml of THF was mixed with 2 ml of distilled water. After 10 min of stirring, NBS (0.025 M) was added to the mixture and then stirred at 0–5 °C until the reaction was complete; the reaction was monitored by TLC. The mixture was then diluted with Et_2O and sequentially washed with a saturated $NaHCO_3$ solution and water. The organic layer was dried with anhydrous $MgSO_4$. The crude product was purified by column chromatography (hexane/isopropanol/acetone/ethyl acetate 60:5:3:1) to yield compound **5**.

Acknowledgements

English editing was funded by the Wroclaw Centre of Biotechnology Program, The Leading National Research Centre (KNOW) for the years 2014–2018 (know.wroc.pl).

Author Contribution Statement

A. K. and M. J. synthesized the 4-*tert*-butylcyclohexanone derivatives. E. G. performed the antibacterial studies. M. S., B. G. and K. D. evaluated the feeding deterrent activity. A. K., E. G. and S. L. wrote the article. A. K. obtained the funding for the English editing.

References

[1] S. Telalović, A. Ramanathan, J. F. Ng, R. Maheswari, C. Kwakernaak, F. Soulimani, H. C. Brouwer, G. K. Chuah, B. M. Weckhuysen, U. Hanefeld, 'On the synergistic catalytic properties of bimetallic mesoporous materials containing aluminum and zirconium: the Prins cyclisation of citronellal', *Chem. Eur. J.* **2011**, *17*, 2077–2088.

[2] K. D. Holland, D. K. Naritoku, A. C. McKeon, J. A. Ferrendelli, D. F. Covey, 'Convulsant and anticonvulsant cyclopentanones and cyclohexanones', *Mol. Pharmacol.* **1990**, *37*, 98–103.

[3] K. Gajcy, J. Pekala, B. Frackowiak-Wojtasek, T. Librowski, S. Lochyński, 'Stereochemistry of terpene derivatives. Part 7: Novel rigidified amino acids from (+)-3-carene designed as chiral GABA analogues', *Tetrahedron: Asymmetry* **2010**, *21*, 2015–2020.

[4] A. Kozioł, A. Stryjewska, T. Librowski, K. Sałat, M. Gaweł, A. Moniczewski, S. Lochyński, 'An overview of the pharmacological properties and potential application on natural

monoterpenes', *Mini-Rev. Med. Chem.* **2014**, *14*, 1156–1168.
[5] T. Librowski, R. Czarnecki, S. Lochyński, B. Frąckowiak, M. Pasenkiewicz-Gierula, J. Grochowski, P. Serda, 'Comparative investigations of hydroxyamine carane derivative and its R,S-diastereoisomers with strong local anesthetic activity', *Pol. J. Pharmacol.* **2001**, *53*, 535–539.
[6] M. Walkowicz, B. Laczyńska-Przepiórka, 'Stereochemistry of bicyclo[3.1.0]hexane derivatives. Part XVI. Stereochemistry of reduction of ketones, analogs of thujone', *Pol. J. Chem.* **1985**, *59*, 801–810.
[7] R. C. Axtell, J. J. Arends, 'Ecology and management of arthropod pests of poultry', *Annu. Rev. Entomol.* **1990**, *35*, 101–126.
[8] A. Grudniewska, K. Dancewicz, A. Białońska, C. Wawrzeńczyk, B. Gabryś, 'Piperitone-derived saturated lactones: Synthesis and aphid behavior-modifying activity', *J. Agric. Food. Chem.* **2013**, *61*, 3364–3372.
[9] M. Q. Alanazi, F. Y. Algahtani, F. S. Aleanizy, 'An evaluation of *E. coli* in urinary tract infection in emergency department at KAMC in Riyadh, Saudi Arabia: retrospective study', *Ann. Clin. Microbiol. Antimicrob.* **2018**, *17*, 3.
[10] S. Y. Tong, J. S. Davis, E. Eichenberger, T. L. Holland, V. G. Fowler Jr., '*Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management', *Clin. Microbiol. Rev.* **2015**, *28*, 603–61.
[11] C. Apetroaie-Constantin, R. Mikkola, M. A. Andersson, V. Teplova, I. Suominen, T. Johansson, M. Salkinoja-Salonen, '*Bacillus subtilis* and *B. mojavensis* strains connected to food poisoning produce the heat stable toxin amylosin', *J. Appl. Microbiol.* **2009**, *106*, 1976–1985.
[12] L. K. Blair, S. Hobbs, N. Bagnoli, L. Husband, N. Badika, 'A new positive halogen reagent. Oxidation of secondary alcohols to ketones by bis(quinuclidine)bromine(I) bromide in the presence of pyridinium catalyst', *J. Org. Chem.* **1992**, *57*, 1600–1603.
[13] W. S. Wadsworth, 'Synthetic applications of phosphoryl-stabilized anions', *Org. React.* **2005**, *25*, 73–253.
[14] M. Edmonds, A. Abell, 'The Wittig Reaction – modern carbonyl olefination', Wiley-VCH, Weinheim, 2004, pp. 1–17.
[15] T. Kapferer, R. Brückner, 'Asymmetric dihydroxylation of β,γ-unsaturated carboxylic esters with trisubstituted C=C bonds – Enantioselective syntheses of trisubstituted γ-butyrolactone', *J. Org. Chem.* **2006**, *9*, 2119–2133.
[16] L. Claisen, 'Über Umlagerung von Phenol-allyläthern in C-Allyl-phenole', *Ber. Dtsch. Chem. Ges.* **1912**, *45*, 3157–3166.
[17] S. Lochyński, A. Kozioł, M. Jasnowski, 'Kwas (4-*tert*-butylo-1-etenylocykloheks-1-ylo)octowy i sposób jego wytwarzania', Polish Patent Application, P-419800, 2016.
[18] R. S. Huber, G. B. Jones, 'Acceleration of the orthoester Claisen rearrangement by clay catalyzed microwave thermolysis: expeditious route to bicyclic lactones', *J. Org. Chem.* **1992**, *57*, 5778–5780.
[19] A. Srikrishnaa, S. Nagarajua, P. Kondaiahb, 'Application of microwave heating technique for rapid synthesis of γ,δ-unsaturated esters', *Tetrahedron* **1995**, *51*, 1809–1816.
[20] W. S. Johnson, L. Werthemann, W. R. Bartlett, T. J. Brocksom, T.-T. Li, D. J. Faulkner, M. R. Petersen, 'Simple stereoselective version of the Claisen rearrangement leading to *trans*-trisubstituted olefinic bonds. Synthesis of squalene', *J. Am. Chem. Soc.* **1970**, *92*, 741–743.
[21] S. Lochyński, A. Kozioł, M. Jasnowski, '1-(Bromometylo)-8-*tert*-butylo-2-oksapiro[4.5]dekan-3-on i sposób jego wytwarzania', Polish Patent Application, P-419799, 2016.
[22] K. Moriyama, C. Nishinohara, T. Sugiue, H. Togo, 'Oxidative oxygen-nucleophilic bromo-cyclization of alkenyl carbonyl compounds without organic wastes using alkali metal reagents in green solvent', *RSC Adv.* **2015**, *5*, 85872.
[23] C. Wawrzeńczyk, I. Dams, A. Szumny, M. Szczepanik, J. Nawrot, A. Prądzyńska, B. Gabryś, K. Dancewicz, E. Magnucka, A. Wzorek, 'Synthesis and evaluation of antifeedant, antifungal and antibacterial activity of isoprenoid lactones', *Pol. J. Environ. Stud. (Supl. III)* **2005**, *14*, 69–84.
[24] B. Gabryś, K. Dancewicz, A. Gliszczyńska, B. Kordan, C. Wawrzeńczyk, 'Systemic deterrence of aphid probing and feeding by novel β-damascone analogues', *J. Pest. Sci.* **2015**, *88*, 507–514.
[25] A. Kozioł, K. Macegoniuk, E. Grela, A. Grabowiecka, M. Biernat, S. Lochyński, 'Synthesis of terpenoid oxo derivatives with antiureolytic activity', *Mol. Biol. Rep.* **2018**, DOI: 10.1007/s11033-018-4442-y.
[26] D. Trombetta, F. Castelli, M. G. Sarpietro, V. Venuti, M. Cristani, C. Daniele, A. Saija, G. Mazzanti, G. Bisignano, 'Mechanisms of antibacterial action of three monoterpenes', *Antimicrob. Agents Chemother.* **2005**, *49*, 2474–2478.
[27] A. Kamizela, B. Gawdzik, M. Urbaniak, Ł. Lechowicz, A. Białońska, W. Gonciarz, M. Chmiela, 'Synthesis, characterization, cytotoxicity, and antibacterial properties of *trans*-γ-halo-δ-lactones', *ChemistryOpen* **2018**, *7*, 543–550.
[28] M. Cristani, M. D'Arrigo, G. Mandalari, F. Castelli, M. G. Sarpietro, D. Micieli, V. Venuti, G. Bisignano, A. Saija, D. Trombetta, 'Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity', *J. Agric. Food Chem.* **2007**, *55*, 6300–6308.
[29] E. Grela, A. Dziełak, K. Szydłowska, A. Mucha, P. Kafarski, A. M. Grabowiecka, 'Whole-cell *Proteus mirabilis* urease inhibition by aminophosphinates for the control of struvite formation', *J. Med. Microbiol.* **2016**, *65*, 1123–1129.
[30] M. Szczepanik, I. Dams, C. Wawrzeńczyk, 'Terpenoid lactones with the *p*-menthane system as feeding deterrents to the lesser mealworm', *Entomol. Exp. Appl.* **2008**, *128*, 337–354.
[31] CLSI, 'Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard', Ninth Edition, CLSI document M07-A9, Wayne, PA, Clinical and Laboratory Standards Institute, 2012.

Received August 16, 2018
Accepted November 27, 2018

