CSIRO PUBLISHING

Aust. J. Chem. **2013**, *66*, 1399–1405
http://dx.doi.org/10.1071/CH13306

Full Paper

Synthesis of Sabina δ-Lactones and Sabina δ-Lactams from (+)-Sabinene

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Two sabina δ-lactones (**3** and **4**) were obtained in a two-step synthesis from (+)-sabinene (**1**). The oxidation of (+)-sabinene (**1**) with potassium permanganate and sodium periodate to (−)-sabina ketone (**2**) was the first step. In the second step, the ketone obtained was subjected to chemical and microbial Baeyer–Villiger oxidation. Chemical Baeyer–Villiger oxidation of this ketone afforded two δ-lactones **3** and **4** whereas microbial Baeyer–Villiger oxidation afforded only 'abnormal' δ-lactone **4**. (−)-Sabina ketone was also the starting material for the synthesis of new δ-lactams (**7** and **8**). They were obtained by Beckmann rearrangement of sabina ketone oximes **5a** and **5b**. An attempt to separate (−)-sabina ketone oximes **5a** and **5b** is also presented.

Manuscript received: 13 June 2013.
Manuscript accepted: 15 July 2013.
Published online: 26 August 2013.

Introduction

Natural and synthetic terpenoid and sesquiterpenoid lactones exhibit many types of biological activity such as antifungal,[1,2] antibacterial,[1–4] and anticancer.[4–7] They are also known as good feeding deterrents towards insects.[8–11]

We are interested in the synthesis of terpenoid lactones because of their odoriferous properties and antifeedant activity. In recent years, starting from monoterpenes,[12–14] monoterpenoid alcohols,[15] and ketones[16,17] we have synthesized many terpenoid lactones. In most of the syntheses carried out, the Claisen rearrangement of allyl alcohols, halolactonization of γ- and δ-unsaturated acids and esters, Baeyer–Villiger oxidation of ketones, and addition of Meldrum acid to alkenes were essential steps leading to the final products. Some of the obtained lactones showed very high feeding-deterrent activity against insect pests.[17–20] Recently, we published the synthesis of four new lactones from (−)-α- and (+)-β-thujone.[21] Preliminary biological tests indicated that three of these possess antifeedant activity against peach aphid (*Myzus persicae*). Further, a lactone obtained from (−)-α-thujone by chemical Baeyer–Villiger oxidation exhibits strong antifeedant activity against the granary weevil (*Sitophilus granarius*).

Monoterpenoid ketones such as pinocamphone, caran-2-one, and menthone were also good starting materials for the synthesis of lactams via Beckmann rearrangement of the corresponding oximes.[22–24] Terpenoid lactams could be hydrolyzed to new chiral amino acids – potential analogues of γ-aminobutyric acid (GABA). Lochyński and coworkers showed that amino acids obtained from (−)-*cis*-caran-4-one and (−)-menthone via hydrolysis of corresponding ε-lactams were able to cross the blood–brain barrier and had neurological activity.[25] Furthermore, some natural lactams possess antifeedant activity.[26] To the best of our knowledge, synthetic terpenoid lactams have not been tested for antifeedant activity against insect pests so far.

In the present paper, the syntheses of two sabina δ-lactones (**3** and **4**) and two new sabina δ-lactams (**7** and **8**) from natural (+)-sabinene (**1**) are described.

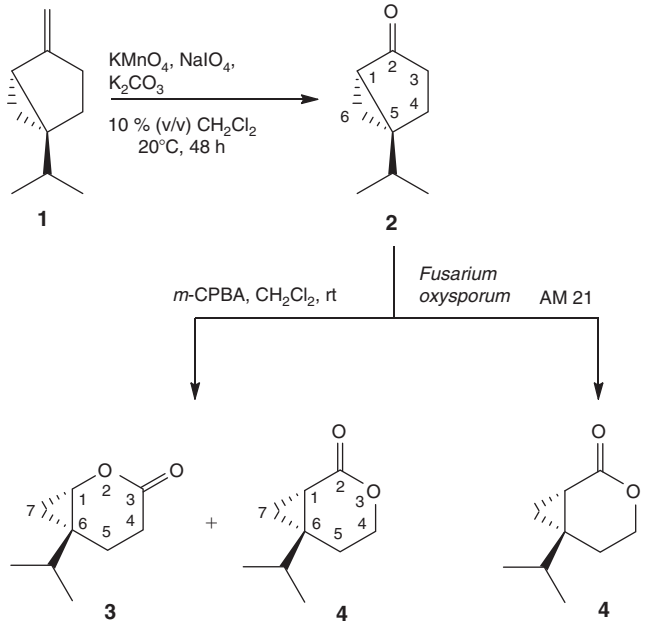
Results and Discussion

Synthesis of Sabina δ-Lactones

The syntheses of sabina δ-lactones **3** and **4** were carried out according to Scheme 1. The oxidation of natural (+)-sabinene (**1**, 70 % purity by GC) using an aqueous solution of sodium periodate with a catalytic amount of potassium permanganate[27] afforded, after purification by silica-gel column chromatography, pure (−)-sabina ketone **2** in 65 % isolated yield (Scheme 1). The optical rotation of this product ($[\alpha]_D^{20}$ −25.7, *c* 1.0 in $CHCl_3$) in comparison with data reported in literature for (+)-sabina ketone[28] ($[\alpha]_D^{20}$ +18.1, *c* 0.8 in $CHCl_3$, *enantiomeric excess* (*ee*) 80 %) indicates that (−)-sabina ketone **2** possesses *S* and *R* configuration on the C-1 and C-5 carbon atoms respectively. The spectral (^1H and ^{13}C NMR, mass spectrometry (MS)) data of ketone **2** are the same as presented in the literature.[28]

The Baeyer–Villiger oxidation of (−)-sabina ketone **2** with *meta*-chloroperbenzoic acid (*m*-CPBA) afforded a mixture of two δ-lactones (by GC): **3** (75 %) and **4** (25 %). After separation and purification of these products by silica-gel column chromatography, sabina lactones **3** and **4** were obtained in 43 and 20 % isolated yield respectively. The structures of these products were established on the basis of their spectral data (IR, ^1H, ^{13}C, heteronuclear single quantum correlation (HSQC), correlated spectroscopy (COSY) NMR, and high-resolution (HR)MS).

The presence of two multiplets at 0.76 and 0.87 ppm of the CH_2-7 protons in the ^1H NMR spectrum of lactone **3** indicates that the cyclopropane ring was not affected during the oxidation process. In the same spectrum, a doublet of doublets (*J* 6.8, 2.8 Hz)



Scheme 1. Oxidation of (+)-sabinene (**1**) to sabina δ-lactones **3** and **4**.

at 3.81 ppm was observed. The value of the chemical shift of this multiplet and its integration indicate that only one proton (H-1) is bound to the carbon atom connected with the alkoxy oxygen atom. Furthermore, the chemical shift (61.20 ppm) of the carbon atom (C-1) coupled with this proton (H-1) in the HSQC NMR spectrum of lactone **3** is characteristic for a carbon atom directly connected with an oxygen atom. These data clearly prove that an oxygen atom was introduced between the carbonyl group and more substituted carbon atom (C-1) in ketone **2**.

Taking into account the possibility of migration of the less-substituted carbon atom to the electrophilic oxygen atom in a Criegee intermediate during Baeyer–Villiger rearrangement,[29,30] we expected the second, minor product to be the so-called 'abnormal' lactone **4**, in which the alkoxy oxygen atom is located between the carbonyl group and less-substituted carbon atom. Our expectation was fully confirmed by the spectral data of this product. The two doublets of doublets of doublets at 4.05 and 4.25 ppm in the ^{1}H NMR spectrum of product **4** are present. The chemical shifts of these multiplets indicate that an oxygen atom was inserted between the carbonyl group and less-substituted carbon atom (C-3) in (−)-sabina ketone **2**. Additionally, in the HSQC NMR spectrum of **4**, these two multiplets are coupled with the C-4 carbon atom, whose chemical shift (64.54 ppm) is characteristic for a carbon atom directly bound to an oxygen atom. It is worth noting that this type of lactone is usually obtained by microbial Baeyer–Villiger oxidation.[29] So (−)-sabina ketone **2** was also subjected to microbial Baeyer–Villiger oxidation using whole cells of *Fusarium oxysporum* AM21 as catalyst (Scheme 1). This microorganism was selected from three fungi strains tried in our previous studies.[21]

The microbial Baeyer–Villiger oxidation of (−)-sabina ketone **2** afforded only one product (**4**) in good (32 %) isolated yield. GC analysis and spectral data (IR, ^{1}H, ^{13}C, HSQC, and COSY NMR) confirmed that this product is 'abnormal' lactone **4**.

The configurations of the chiral centres of both obtained lactones were established taking into account the configuration of (−)-sabina ketone **2** as well as the mechanism of Baeyer–Villiger oxidation. It is very well documented in the literature that chemical[31] and microbial oxidation[32,33] of chiral ketones proceeds with the complete retention of configuration of migrating groups. So it can be concluded that lactones **3** and **4** possess a configuration of chiral centres identical to (−)-sabina ketone **2**.

In the literature, we found that compounds **3** and **4** were obtained as products of the ozonolysis of (+)-sabinene.[34] Griesbaum and Miclaus presented the spectral data of these lactones without assignment of signals in the ^{1}H and ^{13}C NMR spectra to corresponding protons and carbon atoms. For these reasons, we present in the Experimental section the complete spectroscopic (IR, ^{1}H, ^{13}C NMR, and HRMS) data for sabina lactones **3** and **4**.

Synthesis of Sabina δ-Lactams

(−)-Sabina ketone **2** was also the starting material for the synthesis of new δ-lactams (**7** and **8**). The reaction of this ketone (**2**) with hydroxylamine hydrochloride afforded a mixture of oximes (**5a** : **5b** 60 : 40 by GC) (Scheme 2).

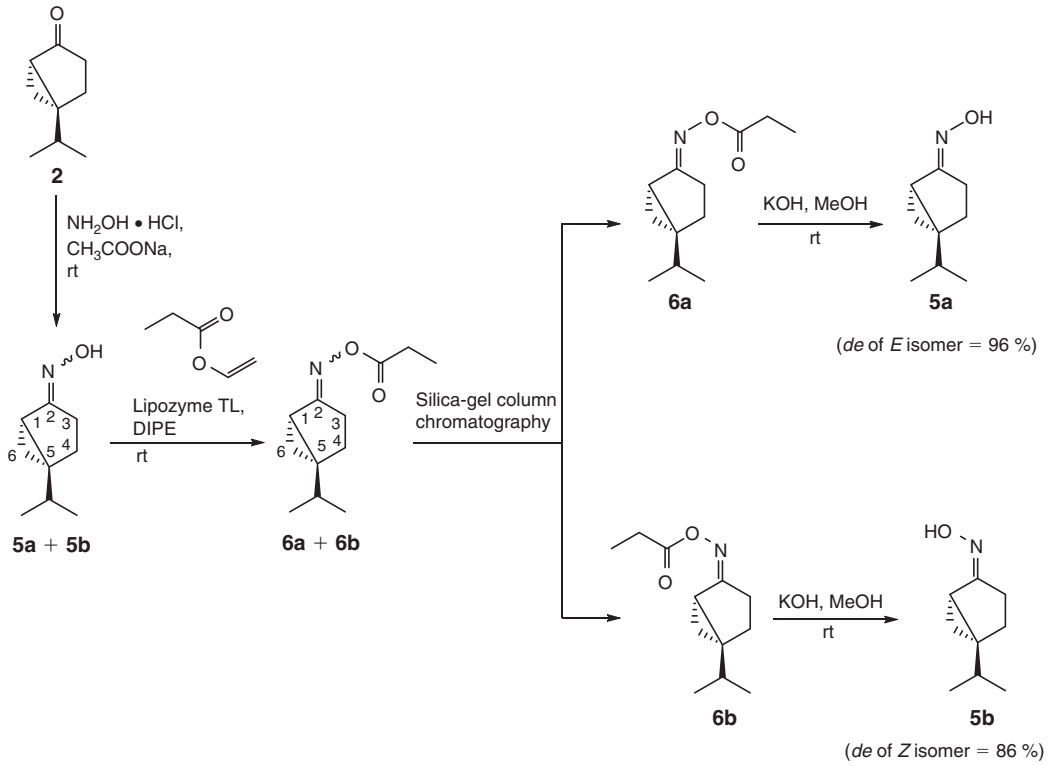
Difficulties with the separation of the sabina ketone oximes (**5a** + **5b**) by silica-gel column chromatography pushed us to separation of their esters. So the mixture of oximes (**5a** : **5b**, 60 : 40 by GC) was converted by an enzymatic esterification process into mixture of propionic esters. The lipase from *Thermomyces lanuginosus* (Lipozyme TL, Novozymes) was used as a catalyst with vinyl propionate as an acyl group donor. As a result of this reaction, a mixture of oxime propionates (**6a** : **6b**, 60 : 40 according to GC) was obtained. The products (**6a** + **6b**) were easily separated by silica gel-column chromatography, giving pure oxime propionates **6a** and **6b** in 30 and 27 % isolated yields respectively (Scheme 2).

The structures of these compounds were confirmed by their spectral data (^{1}H, ^{13}C, COSY, and HSQC NMR). The signals of the carbonyl carbon atoms of the ester group in the ^{13}C NMR spectra of oxime propionates **6a** and **6b** are located at 174.44 and 173.46 ppm respectively. Triplets at 1.10 and 1.11 ppm of methyl group protons and quartets at 2.35 and 2.38 ppm of methylene group protons for **6a** and **6b** respectively observed in the ^{1}H NMR spectra of the oxime propionates proved the presence of the propionyl group in both (**6a** and **6b**) products.

The *E*/*Z* configuration of the C=N double bond was assigned taking into account the chemical shifts of the CH_2-3 group and H-1 protons. In the ^{1}H NMR spectrum of **6a**, the multiplets of the CH_2-3 protons are located at 2.79 ppm and in the range of 1.80–2.11 ppm, whereas the multiplet of the H-1 proton appears in the same region as one of the CH_2-3 protons (1.80–2.11 ppm). The difference in chemical shift of CH_2-3 protons ($\Delta\delta = 0.9$ ppm) indicates that one of these, located closer to the C=N bond plane, is strongly deshielded by the *cis*-oriented alkoxycarbonyl group. These data indicate that (+)-sabina ketone oxime propionate **6a** is the *E*-isomer.

In the case of oxime propionate **6b**, the multiplets of the CH_2-3 protons in the ^{1}H NMR spectrum are located closer to each other ($\Delta\delta = 0.2$ ppm). The signal of one of these protons is located at 2.12–2.26 ppm (together with the multiplets of the H-1 proton) and the second at 2.37 ppm. These data indicate that it is a *Z*-isomer of sabina ketone oxime.

The pure *E*-oxime propionate (**6a**) was hydrolyzed with a 2.5 % methanolic solution of potassium hydroxide at room temperature (Scheme 2). As a result of this reaction, *E*-oxime **5a** was obtained in 94 % yield (96 % purity according to GC;



Scheme 2. Resolution of sabina ketone oximes **5a** and **5b**.

diastereoisomeric excess (*de*) of *E* isomer = 96 %). The structure of this compound was established on the basis of its spectral (^{1}H, ^{13}C, HSQC, COSY, distortionless enhancement by polarization transfer (DEPT) 135 NMR, and HRMS) data.

The two multiplets at 0.55 and 0.78 ppm in the ^{1}H NMR spectrum of **5a** confirm that the cyclopropane ring was not affected during hydrolysis. The value of the chemical shift (2.75 ppm) of one of the CH_2-3 protons in the ^{1}H NMR spectrum of **5a** indicates that it is strongly deshielded by the hydroxy group. The second CH_2-3 proton is located between 1.71 and 1.85 ppm. Therefore, the first, similarly to one of the CH_2-3 protons in **6a**, is located closer to the plane of the C=N moiety. These facts prove that the configuration of the C=N double bonds in oxime **5a** is the same as in the oxime propionate **6a**. The value (9.16 ppm) of the chemical shift of the proton of the hydroxy group in the ^{1}H NMR spectrum of **5a** indicates its acidic character.

During the workup of the reaction mixture and purification of *E*-sabina ketone oxime **5a**, *E*/*Z* isomerization in an aprotic solvent (tetrahydrofuran) to a mixture of sabina ketone oximes (**5a** : **5b** 60 : 40 by GC) was observed. It is well documented in the literature[35] that compounds containing a carbon–nitrogen double bond undergo acid-catalyzed *E*/*Z* isomerization. It can be suggested that the acidic proton of the hydroxy group catalyzes the *E*/*Z* isomerization of **5a**. Moreover, we did not observe *E*/*Z* isomerization of *E*-oxime propionate **6a** in aprotic solvents. However, in chloroform solution, this ester (**6a**) very quickly undergoes *E*/*Z* isomerization to a mixture of both oxime propionates (**6a** : **6b** 60 : 40 by GC).

The second oxime (**5b**) was obtained after hydrolysis of **6b** in 84 % yield (95 % purity by GC, *de* of *Z* isomer = 86 %). The structure of this compound was also proved by spectral (^{1}H, ^{13}C, HSQC, COSY, DEPT 135 NMR) data. The two multiplets at 0.72 and 0.87 ppm in the ^{1}H NMR spectrum of **5b** indicate that the cyclopropane moiety is present in the molecule. The one-proton doublet of doublets (*J* 8.7 and 3.4 Hz) at 2.25 ppm in the ^{1}H NMR spectrum of **5b** was assigned to the H-1 proton. The value of its chemical shift compared with the chemical shift of the same proton (1.68 ppm) in the ^{1}H NMR spectrum of **5a** indicates the deshielding effect of the *cis*-oriented hydroxy group. This fact clearly confirms the *Z*-configuration of the C=N double bond in **5b**.

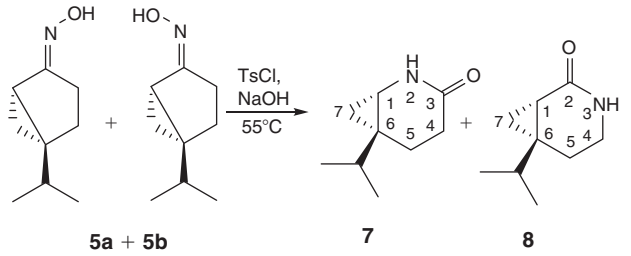
The signal of the hydroxy group proton is situated at 9.13 ppm in the ^{1}H NMR spectrum of **5b**. As in the case of oxime **5a**, *E*/*Z* isomerization of **5b** to a mixture of oximes (**5a** : **5b** 60 : 40 by GC) was observed in tetrahydrofuran. The corresponding oxime propionate (**6b**) did not undergo *E*/*Z* isomerization in aprotic solvents but quickly isomerized to a mixture of both oxime propionates (**6a** : **6b** 60 : 40 by GC) in chloroform solution.

These facts prove that the acidic proton of the hydroxy group in *Z*-sabina ketone oxime **5b** also catalyzes *E*/*Z* isomerization.

It can be concluded that sabina ketone oximes **5a** and **5b** are unstable in protic and aprotic solvents. Such isomerization also caused the Beckmann rearrangement of pure *Z*-oxime propionate **6b** with *p*-toluenesulfonyl chloride in alkaline solution to give a mixture of lactams (**7** : **8** 39 : 61 by GC).

For these reasons, the inseparable mixture of sabina ketone oximes (**5a** : **5b** 60 : 40 by GC) was subjected to Beckmann rearrangement with *p*-toluenesulfonyl chloride in alkaline solution.[36] As a result of this reaction, a mixture of two lactams: **7** (70 %) and **8** (30 %) was obtained (Scheme 3). These compounds were separated by silica-gel column chromatography and obtained in 23 % (**7**) and 8 % (**8**) yields.

The structures of lactams **7** and **8** were established on the basis of their spectral (^{1}H, ^{13}C, HSQC, COSY, DEPT 135 NMR,



Scheme 3. Beckmann rearrangement of sabina ketone oximes **5a** and **5b**.

and HRMS) data. In the ^{1}H NMR spectrum of **7**, a broad singlet at 6.95 ppm and multiplet at 2.42 ppm were observed. The first was assigned to the proton of the NH group and the second to the H-1 proton. The chemical shift of the H-1 proton indicates that it is bonded to the carbon atom directly connected to the nitrogen atom. Therefore, the nitrogen atom was introduced between the carbonyl group and C-1 carbon atom in (−)-sabina ketone **2**. Furthermore, the H-1 proton is coupled in the COSY NMR spectrum of product **7** with two protons giving doublets of doublets at 0.58 ppm (*J* 5.4, 3.5 Hz) and 0.66 ppm (*J* 6.5, 5.4 Hz) in the ^{1}H NMR of this product. These two multiplets were assigned to the protons of the CH_2-7 methylene group and indicate that the cyclopropane ring was not affected during the Beckmann rearrangement.

Two multiplets at 3.03 and 3.17 ppm were observed in the ^{1}H NMR spectrum of **8**. They are coupled with carbon atom at 37.43 ppm (C-4) in the HSQC NMR spectrum of **8**. These facts allowed the assignment of these signals to the protons of the CH_2-4 methylene group in this lactam (**8**). The chemical shifts of the signals of CH_2-4 protons indicate that they are bonded to the carbon atom directly connected with the nitrogen atom. Therefore, the nitrogen atom was inserted between the carbonyl group and C-3 carbon atom in (−)-sabina ketone **2**. Two doublets of doublets located at 0.81 ppm (*J* 9.3, 5.4 Hz) and 1.34 ppm (*J* 5.4 and 4.2 Hz) in the ^{1}H NMR spectrum of **8** are coupled with the H-1 proton (1.43 ppm, dd, *J* 9.3, 4.2 Hz) in the COSY NMR spectrum of this compound. The chemical shifts of these protons confirm the presence of the cyclopropane ring in the molecule.

The configurations of the chiral centres of lactams **7** and **8** were assigned taking into account the configuration of (−)-sabina ketone **2** as well as the mechanism of Beckmann rearrangement. It is well documented that Beckmann rearrangement of enantiomerically pure oximes proceeds with the retention of configuration of a migrating group.[22–24,37,38] It can be concluded that the configurations of the chiral centres in products **7** and **8** have identical configurations to (−)-sabina ketone **2**. Taking into consideration the mechanism of Beckmann rearrangement, the amounts of lactams **7** and **8** obtained, and their spectral data, it can be seen that compound **7** is a product of the rearrangement of the *trans* oxime (**5a**) whereas **8** must be a product of the rearrangement of the *cis* oxime (**5b**) (Scheme 3).

Conclusions

1. One can observe the analogy of biotransformation of (−)-sabina ketone **2** and microbial transformation of (+)-β-thujone[21] in *Fusarium oxysporum* AM21 culture. Both ketones were oxidized only to the 'abnormal' lactones, in which less-substituted carbon atoms migrated to the electrophilic oxygen atom in Criegee intermediates.
2. The *E*-isomer of (−)-sabina ketone oxime (**5a**, *de* of *E* isomer = 96 %) and *Z*-isomer oxime of the same ketone (**5b**, *de* of *Z* isomer = 86 %) were obtained. Unfortunately, these compounds were unstable and underwent acid-catalyzed *E*/*Z* isomerization in both aprotic and protic solvents. It is noteworthy that this isomerization in aprotic solvents was catalyzed by the acidic proton of the hydroxy group of the oxime.
3. The (−)-sabina ketone oximes **5a** and **5b** are a good substrates for enzymatic esterification catalyzed by lipase from *Thermomyces lanuginosus*.
4. The two new δ-lactams **7** and **8** were obtained from (+)-sabinene **1**. Their physical and spectral data are presented.

Experimental

General

Analytical TLC was performed on aluminium plates coated with silica gel (Fluka, Kieselgel 60, F_{254}). Most compounds were detected by spraying plates with solution of: 1 % $Ce(SO_4)_2$, 2 % $H_3[P(Mo_3O_{10})_4]$ in 10 % H_2SO_4, followed by heating at 120°C. Lactams **7** and **8** were detected by spraying the plates with a solution of: ninhydrin (0.3 g) in *n*-butanol (100 mL) and acetic acid (3 mL), followed by heating at 100°C.

Liquid column chromatography was carried out on silica gel (Kieselgel 60, 230–400 mesh ASTM, Merck) with suitable mixtures of hexane, ethyl acetate, dichloromethane, diethyl ether, acetone, chloroform, and methanol as eluents.

Gas chromatography analyses were performed with a flame ionization detector (FID) on an Agilent Technologies 7890A. The capillary columns used were: for **1**–**4**, HP-5 (30 m × 0.32 mm ID × 1.0 μm film); oven temperature: start 90°C; programmed from 90 to 150°C at 5°C min^{-1}, hold 2 min; from 150 to 300°C at 40°C min^{-1}, hold 3 min; as carrier gas, H_2 was used at a flow rate of 1 mL min^{-1}; split ratio 30 : 1; for **5a**, **5b**, **6a**, **6b**, **7**, **8**: CP-ChiralSil-L-Val (25 m × 0.25 mm ID × 0.12 μm film); oven temperature: start 100°C; programmed from 100 to 200°C at 15°C min^{-1}, hold 3 min; as carrier gas, H_2 was used at a flow rate of 2 mL min^{-1}; split ratio 15 : 1. The injection temperature was kept at 250°C and FID temperature at 300°C for all analyses.

HRMS (electrospray ionization (ESI)) was carried out on a high-resolution mass spectrometer micrOTOF-Q II (Bruker).

Optical rotation was measured on a Jasco P-2000-Na with iRM automatic polarimeter in chloroform solution for compounds **1**–**4**, **7**, and **8**, and in tetrahydrofuran solution for compounds **6a** and **6b**. Concentration is denoted in g per 100 mL.

^{1}H, ^{13}C, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C HSQC NMR spectra for products **2**, **3**, and **4** were recorded in $CDCl_3$ solution on a Bruker Avance DRX 300 MHz; spectra for products **7** and **8** were recorded in $CDCl_3$ solution on a Bruker Avance 600 MHz; spectra for products **5a**, **5b**, **6a**, and **6b** were recorded in [D8] tetrahydrofuran solution on a Bruker Avance DRX 300 MHz. Chemical shifts were referenced to the residual signal of $CHCl_3$ (δ 7.26 ppm for ^{1}H NMR, 77.0 ppm for ^{13}C NMR) or THF (δ 3.58 ppm for ^{1}H NMR, 67.57 ppm for ^{13}C NMR).

Chemical Synthesis of Sabina Lactones

A solution of (+)-sabinene (**1**, 500 μL, mixture of sabinene and β-pinene 70 : 30 by GC, Treatt PLC) in dichloromethane (20 mL) was added to 200 mL of an aqueous solution of sodium

periodate (6.1 g, 28.5 mmol), potassium permanganate (170 mg, 1.08 mmol), and potassium bicarbonate (1.2 g, 9 mmol). The reaction mixture was stirred for 3 days at 20°C. Progress of the oxidation of (+)-sabinene (**1**) was monitored by GC. After this time, the product was extracted with dichloromethane (3 × 100 mL), dried over anhydrous $MgSO_4$, and concentrated under vacuum. The crude product (**2**) was separated and purified by silica-gel column chromatography (hexane/ethyl acetate 10 : 1) to give pure (−)-sabina ketone **2** (196 mg, 1.4 mmol, 65 % yield).

A solution of *meta*-chloroperbenzoic acid (166 mg, 0.96 mmol, 77 %, Aldrich) in dichloromethane (5 mL) was dried over anhydrous $MgSO_4$ and then evaporated under nitrogen. This prepared peracid in a small amount of dichloromethane (100 μL) was added to (−)-sabina ketone **2** (53 mg, 0.38 mmol) and the mixture was stirred for 72 h at room temperature. The progress of the reaction was monitored by TLC and GC. When the reaction was complete, dichloromethane (5 mL) was added to the reaction mixture. The solution was washed with sodium sulfite and sodium bicarbonate, dried ($MgSO_4$), and the solvent was evaporated under vacuum. The crude products (**3** and **4**) were separated and purified by silica-gel column chromatography (hexane/ethyl acetate/dichloromethane 10 : 1 : 1) to give pure lactone **3** (25 mg, 0.16 mmol, 43 % yield) and pure lactone **4** (12 mg, 0.08 mmol, 20 % yield). It is noteworthy that lactone **4** was invisible on TLC plates after spraying with cerium–molybdenum solution and heating at 120°C. For this reason, product **4** was detected by GC. The physical and spectral data of all synthesized products are given below.

(1S,6R)-6-(Propan-2-yl)-2-oxabicyclo[4.1.0] heptan-3-one ***3***

Colourless liquid. $[\alpha]_D^{20}$ 40.8 (*c* 1.2 in $CHCl_3$). ν_{max} (film)/cm^{-1} 1740, 1468, 1174, 757. δ_H (300 MHz, $CDCl_3$) 0.76 (t, *J* 6.9, 1H, one of the CH_2-7), 0.87 (dd, *J* 7.0, 2.8, 1H, one of the CH_2-7), 0.91 and 1.00 (two d, *J* 6.5, 6H, $(CH_3)_2CH$–), 1.11 (m, 1H, $(CH_3)_2CH$–), 1.74 (ddd, *J* 13.9, 8.5, 5.7, 1H, one of the CH_2-5), 2.18 (ddd, *J* 13.9, 6.6, 6.2, 1H, one of the CH_2-5), 2.31 (ddd, *J* 16.6, 6.6, 5.7, 1H, one of the CH_2-4), 2.40 (ddd, *J* 16.6, 8.5, 6.2, 1H, one of the CH_2-4), 3.81 (dd, *J* 6.8, 2.8, 1H, H-1). δ_C (75.5 MHz, $CDCl_3$) 17.94 and 18.88 ($(CH_3)_2CH$–), 18.93 (C-7), 21.66 (C-5), 24.02 (C-6), 28.73 (C-4), 34.22 ($(CH_3)_2CH$–), 61.20 (C-1), 171.46 (C-3). *m/z* (ESI) 177.0882 $[M + Na]^+$ (calc. for $C_9H_{14}NaO_2$ 177.0891).

(1S,6S)-6-(Propan-2-yl)-3-oxabicyclo[4.1.0] heptan-2-one ***4***

Colourless liquid. $[\alpha]_D^{20}$ 18.4 (*c* 0.5 in $CHCl_3$). ν_{max} (film)/cm^{-1} 1717, 1216, 1085, 770, 750, 668. δ_H (300 MHz, $CDCl_3$) 0.93 (dd, *J* 9.5, 5.6, 1H, one of the CH_2-7), 0.98 and 0.99 (two d, *J* 6.8, 6H, $(CH_3)_2CH$–), 1.23 (septet, *J* 6.8, 1H, $(CH_3)_2CH$–), 1.58 (dd, *J* 5.6, 4.3, 1H, one of the CH_2-7), 1.65 (dd, *J* 9.5, 4.3, 1H, H-1), 1.82 (dd, J 13.9, 3.6, 1H, one of the CH_2-5), 2.04 (ddd, J 13.9, 13.5, 6.0, 1H, one of the CH_2-5), 4.05 (ddd, J 13.5, 12.0, 3.6, 1H, one of the CH_2-4), 4.25 (ddd, J 12.0, 6.0, 1.4, 1H, one of the CH_2-4). δ_C (75.5 MHz, $CDCl_3$) 14.96 (C-7), 18.97 and 19.09 ($(CH_3)_2CH$–), 20.64 (C-5), 23.07 (C-1), 29.81 ($(CH_3)_2CH$–), 31.05 (C-6), 64.54 (C-4), 171.94 (C-2). *m/z* (ESI) 177.0873 $[M + Na]^+$ (calc. for $C_9H_{14}NaO_2$ 177.0891).

Microbial Synthesis of Sabina Lactone ***4***

Fusarium oxysporum AM21 was obtained from the Collection of the Institute of Biology and Botany, Wroclaw Medical University. The microorganism was maintained at 4°C on Sabouraud agar slants containing peptone (10 g L^{-1}), glucose (40 g L^{-1}), and agar (15 g L^{-1}). A loop of fungal strain was used to inoculate 100 mL of sterile medium (10 g L^{-1} peptone, 30 g L^{-1} glucose) in a 300-mL Erlenmeyer flask. After 3 days of agitation on a rotary shaker at 150 rpm at 25°C, the culture was used to inoculate 400 mL of the same medium in a 2-L flat-bottomed flask. The fungal strain was incubated on a rotary shaker at 150 rpm at 25°C. After 3 days, (−)-sabina ketone **2** (83 mg, 0.6 mmol) dissolved in acetone (1 mL) was added to the grown culture. The reaction mixture was shaken for 11 days and progress of transformation of **2** was monitored by GC. After that time, the product was extracted with dichloromethane (3 × 150 mL). The organic solution was dried over anhydrous $MgSO_4$ and concentrated under vacuum. Crude product was purified by silica-gel column chromatography (hexane/ethyl acetate/diethyl ether 3 : 1 : 1) to give pure lactone **4** (30 mg, 0.19 mmol, 32 % yield).

Enzymatic Esterification of Sabina Ketone Oximes

The (−)-sabina ketone **2** (65 mg, 0.5 mmol) dissolved in methanol (3 mL) was added to 5 mL of an aqueous solution of hydroxylamine hydrochloride (75 mg, 1.1 mmol) and sodium acetate (150 mg, 1.9 mmol). The reaction mixture was stirred for 48 h at room temperature. After that time, the products (**5a** + **5b**) were extracted with diethyl ether (3 × 25 mL). The organic solution was washed with sodium bicarbonate, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The mixture of the two diastereoisomers of sabina ketone oximes obtained (**5a** : **5b** 60 : 40 according to GC, 83 mg, 0.5 mmol) was dissolved in diisopropyl ether (1 mL). Lipase from *Thermomyces lanuginosus* (160 mg, Lipozyme TL) and vinyl propionate (184 mg, 1.8 mmol) were added to this solution. The reaction mixture was stirred overnight at room temperature. The progress of the reaction was monitored by GC. After 14 h, the reaction mixture was filtered through Celite 560 (Sigma–Aldrich) and washed with diisopropyl ether (20 mL). Then, the organic solution was washed with sodium bicarbonate, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The crude products (**6a** and **6b**) were separated and purified by silica-gel column chromatography (hexane/acetone 5 : 1) to give pure *E*-oxime propionate **6a** (29 mg, 30 % yield) and pure *Z*-oxime propionate **6b** (26 mg, 27 % yield).

The physical and spectral data of the oxime propionates obtained are given below.

(+)-(E)-Sabina Ketone Oxime Propionate ***6a***

Colourless liquid. $[\alpha]_D^{20}$ 73 (*c* 1.355 in THF). δ_H (300 MHz, [D8] THF) 0.76 (dd, *J* 4.7, 3.3, 1H, one of the CH_2-6), 0.92 and 0.98 (two d, *J* 6.8, 6H, $(CH_3)_2CH$–), 1.01 (m, 1H, one of the CH_2-6), 1.10 (t, *J* 7.5, 3H, CH_3CH_2COO–), 1.57 (septet, *J* 6.8, 1H, $(CH_3)_2CH$–), 1.80–2.11 (m, 4H, one of the CH_2-3, CH_2-4, and H-1), 2.35 (q, *J* 7.5, 2H, CH_3CH_2COO–), 2.79 (m, 1H, one of the CH_2-3). δ_C (75.5 MHz, $CDCl_3$) 9.55 (CH_3CH_2COO–), 17.75 (C-6), 19.86 and 20.02 ($(CH_3)_2CH$–), 25.12 (C-3), 26.71 (CH_3CH_2COO–), 26.82 (C-4), 27.69 (C-1), 33.30 ($(CH_3)_2CH$–), 38.52 (C-5), 171.59 (C-2), 174.44 (CH_3CH_2COO–). *m/z* (ESI) 232.1293 $[M + Na]^+$ (calc. for $C_{12}H_{19}NNaO_2$ 232.1313).

(+)-(Z)-Sabina Ketone Oxime Propionate ***6b***

Colourless liquid. $[\alpha]_D^{20}$ 39 (*c* 0.610 in THF). δ_H (300 MHz, [D8] THF) 0.92 and 0.97 (two d, *J* 6.8, 6H, $(CH_3)_2CH$–), 0.95 (m, 1H,

one of the CH_2-6), 1.06 (m, 1H, one of the CH_2-6), 1.11 (t, *J* 7.5, 3H, CH_3CH_2COO–), 1.53 (septet, *J* 6.8, 1H, $(CH_3)_2CH$–), 1.76–1.95 (m, 2H, CH_2-4), 2.12–2.26 (m, 2H, one of the CH_2-3 and H-1), 2.37 (m, 1H, one of the CH_2-3), 2.38 (q, J 7.5, 2H, CH_3CH_2COO–). δ_C (75.5 MHz, $CDCl_3$) 9.56 (CH_3CH_2COO–), 18.21 (C-6), 19.85 and 19.97 ($(CH_3)_2CH$–), 25.15 (C-1), 25.73 (C-4), 26.72 and 27.14 (CH_3CH_2COO– or C-3), 33.52 ($(CH_3)_2CH$–), 40.08 (C-5), 171.98 (C-2), 173.46 (CH_3CH_2COO–). *m/z* (ESI) 232.1304 $[M + Na]^+$ (calc. for $C_{12}H_{19}NNaO_2$ 232.1313).

Hydrolysis of Sabina Ketone Oxime Propionates

A methanolic solution of potassium hydroxide (1 mL of 2.5 % solution) was added to the oxime propionate (**6a**, 29 mg, 0.14 mmol; **6b**, 26 mg, 0.12 mmol). The reaction mixture was stirred for 30 min at room temperature and then concentrated under vacuum. The ether solution of crude oxime (3 mL) was transferred to a flask containing a saturated aqueous solution of sodium bicarbonate (5 mL). The product was extracted with diethyl ether (4 × 3 mL). The ether extracts were combined, dried over anhydrous $MgSO_4$, and concentrated under vacuum. As a result, **5a** (20 mg, 94 % yield), (96 % purity according to GC, *de* of *E* isomer = 96 %) and **5b** (16 mg, 84 % yield), (95 % purity according to GC; *de* of *Z* isomer = 86 %) were obtained.

The spectral data of the oximes obtained are given below.

(E)-Sabina Ketone Oxime *5a*

Colourless liquid (20 mg, yield 94 %, *de* of *E* isomer = 96 %). $[\alpha]_D^{20}$ 61 (*c* 1.105 in THF). δ_H (300 MHz, [D8]THF) 0.55 (dd, *J* 4.5, 3.4, 1H, one of the CH_2-6), 0.78 (dd, J 8.6, 4.5, 1H, one of the CH_2-6), 0.90 and 0.96 (two d, *J* 6.9, 6H, $(CH_3)_2CH$–), 1.50 (septet, *J* 6.9, 1H, $(CH_3)_2CH$–), 1.68 (dd, *J* 8.6, 3.4, 1H, H-1), 1.71–1.85 (m, 3H, one of the CH_2-3 and CH_2-4), 2.75 (m, 1H, one of the CH_2-3), 9.16 (s, 1H, –OH). δ_C (75.5 MHz, $CDCl_3$) 16.99 (C-6), 19.99 and 20.14 ($(CH_3)_2CH$–), 22.97 (C-3), 26.82 (C-4), 27.63 (C-1), 33.54 ($(CH_3)_2CH$–), 36.70 (C-5), 164.57 (C-2). *m/z* (ESI) 176.1041 $[M + Na]^+$ (calc. for $C_9H_{15}NNaO$ 176.1051).

(Z)-Sabina Ketone Oxime *5b*

Colourless liquid (16 mg, yield 84 %, *de* of *Z* isomer = 86 %). $[\alpha]_D^{20}$ 19 (*c* 0.755 in THF). δ_H (300 MHz, [D8]THF) 0.72 (dd, *J* 4.4, 3.4, 1H, one of the CH_2-6), 0.87 (ddd, J 8.7, 4.4, 1.3, 1H, one of the CH_2-6), 0.90 and 0.95 (two d, *J* 6.9, 6H, $(CH_3)_2CH$–), 1.50 (septet, *J* 6.9, 1H, $(CH_3)_2CH$–), 1.73–1.83 (m, 2H, CH_2-4), 1.97 (dd, J 16.2, 9.3, 1H, one of the CH_2-3), 2.16 (ddd, J 16.2, 8.4, 2.4, 1H, one of the CH_2-3), 2.25 (dd, *J* 8.7, 3.4, 1H, H-1), 9.10 (s, 1H, –OH). δ_C (75.5 MHz, $CDCl_3$) 16.60 (C-6), 19.98 and 20.12 ($(CH_3)_2CH$–), 23.29 (C-1), 26.00 (C-4), 26.84 (C-3), 33.70 ($(CH_3)_2CH$–), 37.88 (C-5), 163.47 (C-2). *m/z* (ESI) 176.1045 $[M + Na]^+$ (calc. for $C_9H_{15}NNaO$ 176.1051).

Beckmann Rearrangement of Sabina Ketone Oximes

The crude mixture of sabina ketone oximes (**5a** : **5b** 60 : 40 by GC, 287 mg, 1.7 mmol, obtained from 196 mg of **2**) was dissolved in acetone (1.5 mL) and added dropwise to an aqueous solution (2 mL) of sodium hydroxide (134 mg, 3.4 mmol). To this reaction mixture, stirred at room temperature, *p*-toluenesulfonyl chloride (359 mg, 1.9 mmol) in acetone (1 mL) was added. The reaction mixture was stirred 4 h at 55°C. After that time, the reaction mixture was concentrated under vacuum and the products (**7** and **8**) were extracted with diethyl ether (3 × 25 mL). The ether extracts were combined, washed with sodium bicarbonate and brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The products (**7** and **8**) were separated and purified by silica-gel column chromatography (hexane/acetone 2 : 1). The pure lactam **7** (50 mg, 23 % yield with respect to the ketone) and impure lactam **8** (50 mg) were obtained. Therefore, compound **8** was additionally purified by preparative TLC (chloroform/methanol 20 : 1) to give 17 mg (8 % yield with respect to the ketone) of pure **8**. The physical and spectral data of the lactams obtained are given below:

(1S,6R)-6-(Propan-2-yl)-2-azabicyclo[4.1.0] heptan-3-one *7*

Colourless liquid. $[\alpha]_D^{20}$ 76.4 (*c* 1.1 in $CHCl_3$). ν_{max} (film)/cm^{-1} 3210, 1670, 1468. δ_H (600 MHz, $CDCl_3$) 0.58 (dd, *J* 5.4, 3.5, 1H, one of the CH_2-7), 0.66 (dd, J 6.5, 5.4, 1H, one of the CH_2-7), 0.90 and 0.97 (two d, *J* 6.7, 6H, $(CH_3)_2CH$–), 1.05 (septet, *J* 6.7, 1H, $(CH_3)_2CH$–), 1.60 (m, 1H, one of the CH_2-5), 2.08–2.14 (m, 2H, one of the CH_2-5 and one of the CH_2-4), 2.24 (m, 1H, one of the CH_2-4), 2.42 (m, 1H, H-1), 6.95 (bs, 1H, NH). δ_C (75.5 MHz, $CDCl_3$) 18.29 and 19.09 ($(CH_3)_2CH$–), 22.61 (C-7), 23.23 (C-5), 24.44 (C-6), 30.62 (C-4), 34.36 (C-1), 35.68 ($(CH_3)_2CH$–), 174.11 (C-3). *m/z* (ESI) 176.1040 $[M + Na]^+$ (calc. for $C_9H_{15}NNaO$ 176.1051).

(1S,6S)-6-(Propan-2-yl)-3-azabicyclo[4.1.0] heptan-2-one *8*

Colourless liquid. $[\alpha]_D^{20}$ 34 (*c* 1.0 in $CHCl_3$). ν_{max} (film)/cm^{-1} 3213, 1658, 1495. δ_H (600 MHz, $CDCl_3$) 0.81 (dd, *J* 9.3, 5.4, 1H, one of the CH_2-7), 0.97 and 0.98 (two d, *J* 6.8, 6H, $(CH_3)_2CH$–), 1.17 (septet, *J* 6.8, 1H, $(CH_3)_2CH$–), 1.34 (t, *J* 4.8, 1H, one of the CH_2-7), 1.43 (dd, *J* 9.3, 4.2, 1H, H-1), 1.78–1.82 (m, 2H, CH_2-5), 3.11 (m, 1H, one of the CH_2-4), 3.17 (m, 1H, one of the CH_2-4), 6.51 (bs, 1H, NH). δ_C (151 MHz, $CDCl_3$) 14.19 (C-7), 19.07 and 19.31 ($(CH_3)_2CH$–), 20.00 (C-5), 24.00 (C-1), 30.75 (C-6), 35.71 ($(CH_3)_2CH$–), 37.43 (C-4); 174.48 (C-2). *m/z* (ESI) 176.1042 $[M + Na]^+$ (calc. for $C_9H_{15}NNaO$ 176.1051).

Supplementary Material

The NMR spectra of all obtained compounds are available on the Journal's website.

Acknowledgements

This research was supported financially by the European Union through the European Regional Development Fund (grant No. POIG.01.03.01–00–158/09–05). We thank Dr Anna Poliwoda (Opole University, Poland) for performing HRMS measurements.

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