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Unusual Isomerisation of Cubebene

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Dedicated to Dr Pedro Joseph Nathan

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Abstract. Some sesquiterpenes extracted from *Solidago canadensis L.* in methanol underwent apparent isomerisation and the addition of water or methanol when the Soxhlet extraction was replaced by a microwave heating method in polar solvents. The quantity of α-cubebene, one of these sesquiterpenes, during microwave extraction, was dramatically increased. In this study, we present the detailed comparison of two cubebenes subjected to this isomerisation, 6-epi-α-cubebene and α-cubebene, as well as their solvent microwave induced addition products obtained under microwave irradiation in polar solvents. The analysis is supported by high resolution bidimensional NMR (^{1}H, ^{13}C), mass spectroscopy and selected isotopic labelling experiments.
Key word. Microwave, soxhlet extraction, isomerisation of sesquiterpene, cubebenes, *Solidago canadensis*, NMR, tricyclic terpenes.

Resumen. Algunos sesquiterpenos extraidos de *Solidago canadensis* L. con metanol sufrieron aparente isomerización y la adición de agua o metanol cuando la extracción con soxhlet fue reemplazada por la extracción con disolventes polares en caliente usando microondas. La cantidad de α-cubebeno, uno de estos sesquiterpenos, se incrementó dramáticamente. En este estudio presentamos la comparación detallada de dos cubebenos sometidos a esta isomerización, 6-*epi*-α-cubebeno y α-cubebeno, así como sus productos de adición de disolvente obtenidos a partir de la irradiación con microondas en disolventes polares. El análisis se encuentra sustentado por evidencias de RMN bidimensional de alta resolución (^{1}H y ^{13}C), espectrometría de masas y experimentos selectos de marcaje isotópico.
Palabras clave. Microondas, extracción por soxhlet, isomerización de sesquiterpenos, cubebenos, *Solidago canadensis*, NMR, terpenos tricíclicos.

Introduction

The extracts of green parts of golden rod, *Solidago Canadensis L.*, an important and popular medicinal plant widely distributed in Europe and Americas, used sometimes as kidney and bladder medicine as well as more recently known for its antioxidant properties, are particularly rich in different terpenes [1]. These compounds extracted by classic Soxhlet or more recent methods, such as microwave assisted extractions (MAP), have been relatively well characterised [2, 3]. In particular the attention was focused on the rich sesquiterpene presence in essential oils [4]. Their analysis, using GC, LC with help of MS and NMR techniques enabled to identify them and in several cases to establish their absolute configuration, thanks in particular to seminal works of Konig [5] and that of Weyerstahl and Gora [6].

Some intriguing compounds isolated, cubebenes, have a tricyclic structure with the cyclopropane moiety squeezed in between two cyclohexane rings. The configuration of native 6-epi-α-cubebene **1** as well as that of its exocyclic double bond-bearing isomer and major component in this extract, 6-*epi*-β-cubebene **2** were established by comparison to several available sesquiterpenes products, e.g. commercially available α-cubebene **3**.

The rigid and hindered tricyclic cubebene skeleton renders this structure very much susceptible to rearrangement reactions. Searching for a possible relation between all these biosynthetically related compounds, several chemical photochemical transformations were attempted leading to the bicyclic cubenol **4** in particular, ylangene of copaene structures together with the already mentioned *exo-endo* double bond shift resulting compounds [5, 7-9].

The microwave assisted extraction of *Solidago* leaves in methanol produced some differences when compared to a traditional Soxhlet method. In particular, apart from the exocyclic cubebene **l** and **2**, two apparently isomeric cubebene structure compounds were isolated from the microwave heating extracts, compared to one only obtained via Soxhlet

extraction [10,12,13]. The striking difference lead to the design of the following experiments, where under GC-MS control, we observed the formation of bicyclic derivative of *epi*-cubenol **5** which could be the result of the double epimerisation of two of the four asymmetric centres found in this possible biological precursor of some of these compounds. The microwave heating extraction in polar solvents led to the isolation/formation of two cubebene isomer fractions, one of them (*ca*. 34%) corresponding to Konig's structure **1**. The second compound obtained (ca. 46%) in this extraction was compared to a-cubebene **3** with several striking resemblance. Prior to conclude on its identity we performed the microwave irradiation of commercial **3** in water and in D_2O, under base-catalyzed solvolysis, as well as in methanol and CD_3OD and recorded the GC-MS spectra of the resulting mixtures. Among the most interesting compounds observed in the two first cases, important quantities of methylated or the analogous deuterated sesquiterpene material were obtained. These reactions produced a minimum of 35 compounds, as detected by GC-MS.

Results and Discussion

First, the exhaustive microwave heated reactions in CH_3OH or CD_3OD solvents on pure **3**, enabled to identify in particular the cubinol **4**, or its methyl ether **5**. The analogous pair of solvent, water and D_2O, produced cubinol **4**-like or deuterated cubinol-like compounds. The identification of the double bond and deuteration position as well as the stereochemistry of the resulting solvolysis of the cubebene derivative of cubebene was helped by the following observations (Figure 1).

In methanol, as well as in heavy methanol, only one major compound corresponding to methanol addition was detected under the GC-MS control (search for *m/z* 236, 240, (239) Th masses respectively) with addition of CH_3O^- or CD_3O^- on the C-1 (compound **5**, **6c**, **6d**). The incorporation of one more deuterium in this case does not lead to the disappearance of the vinylic signal in the NMR spectrum, assuming that the double bond remains in 4,5-position or could be isomerise.

The optical rotation of this compound was modified, (cubebene **1** has $[\alpha]_D$-23°) to ca +20°, which indicated partial epimerisation or racemisation of at least some of the asymmetric centres present.

When the cubebene **3** is irradiated in water or heavy water the isomers of cubinol **4** were formed. Using single ion GC-MS at *m/z* 222 (or *m/z* 224 and *m/z* 223) Th, the ions corresponding to the addition of water or D_2O to **3,** two main water addition resulting products were detected. The catalytic methanolysis, with CH_3O^- and treatment of the cubebene **3** with sodium hydroxide in water produced some of the expected ions indicating that the addition of water of methanol takes place during the microwave irradiation.

The presence of aromatic signals in the crude post microwave-assisted mixture seems to be consistent with the transformation of some of these, either microwave by-products or the other *Solidago* sesquiterpenes into aromatic calamencene **7**-like structures [12,13]. The signals in the δ 4.95-5.10 area, corresponding to the major compounds **1** and **3**, respectively, also displayed small signals at δ 5.07 and 5.10, which could be due to the isomerisation of the double bond position after irradiation and recyclisation when polar solvents are used.

When under acidic anhydrous conditions, the acid isomerisation of **3** in methanolic solution (CH_3OH anh./HCl sat.) was attempted under microwave heating conditions both cyclopropane signals at δ 0.22 and 0.5-0.6 disappeared as well as vinylic H-3 was suppressed and a new signal reported in previous point (point 7), especially at δ 5.3, appeared. The

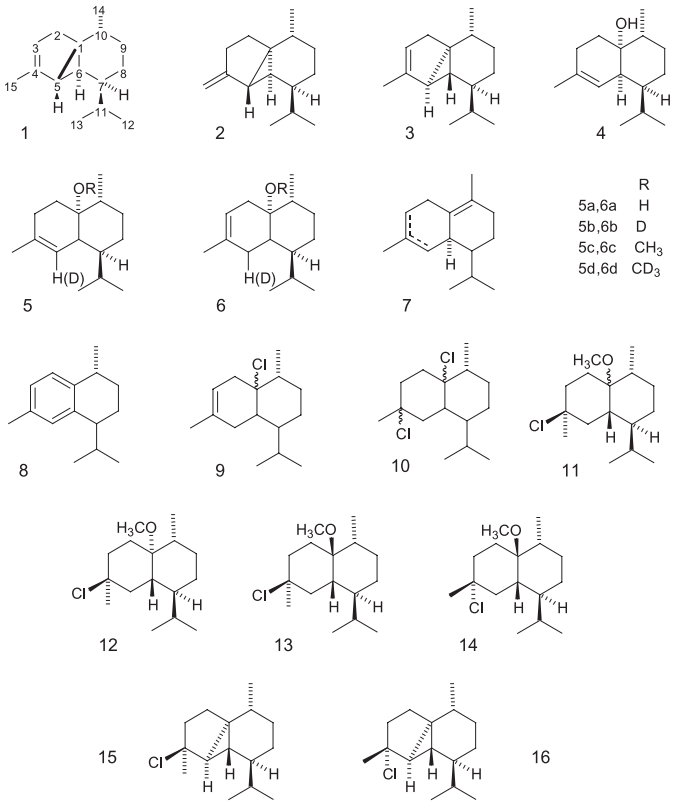

Fig. 1
6-*epi*-α-cubebene (**1**)
6-*epi*-β-cubebene (**2**)
α-cubebene (**3**)
6-*epi*-cubenol (**4**)
6-*epi*-cubenols and their methyl ethers, **a** and **e** protonated and **b** and **d** deuterated (**5**)
iso-6-*epi*-cubenols and their methyl ethers **a** and **c** protonated and **b** and **d** deuterated (**6**)
isomeric amorphenes (**7**)
calamenene (**8**)
HCl adducts to compound **3** (**9, 15, 16**)
2 mol of HCl adducts to compound **3** (**10**)
CH_3OH/HCl adducts to compound **3** (**11** to **14**)

characteristic exocyclic double bond proton double signal split at δ 4.8 and 5.1 were not observed. The hydrochloric acid single and double additions were easily monitored by the GC-MS experiments on this fraction. Moreover, this was indication of the formation of the tertiary carbocation at C-1 with the protonation at C-5, justifying however the loss of stereochemistry at the cyclopropane opening (on or after the eventual closure according to this pathway). Finally, the Markovnikov's addition of the HCl to both, the cyclopropane and the double bond (compound **9**, **16** or one of two quaternary structures **10** on C_1 (shown) or C_{10} carbons) and mixt addition of HCl to the double bond and methanol to the cyclopropane major compound **11** formation was observed.

The detailed 1D and 2D NMR analysis of two adducts **4** and **5** confirmed the double bond isomerisation, as both isomer $\Delta^{3,4}$ and $\Delta^{4,5}$ isomers could be both formed and the partially deuterated methylene C-5 is buried (CHD) in a highly overlapping area. It seems that the integration of vinylic proton at C-5 (for structure **4**) or the proton H-3 (for corresponding isomeric structure **5** or **6**), is reduced into 60% (integration) which could be an indication of partial deuteration of the C-5 carbon during the methanolysis or hydrolysis of **3** in perdeuterated solvents.

The molecular modelling of the sesquiterpenes in this series was performed [14]. The calculated total energy of cubebene **3** was slightly lower than **1**. The stereochemistry of the decaline junction for addition compounds was established as *trans* and the most stable isomers should have the chlorine in equatorial (α) position (for data on these calculations see Experimental), with the a-addition on the quaternary C_1 carbon of all nucleophile anions (OH, OCH_3 or Cl) (Table 4).

The strained cubebenes skeleton system confirms its propensity to rearrange. The microwave irradiation of these compounds in polar, microwave energy-absorbing solvents, produced the solvolysis and the formation of adducts as well as the isomerisation (epimerisation) of at least two (one protonated) most affected by solvolysis asymmetric centres. At this stage of the study it is however difficult to establish the sequence of the events: if the α-cubebene **3** isomer is then a result of direct epimerisation of **1** with help of microwave heating, or rather is one of many products coming from the derivatives **4** or **5** as already observed by Konig [5].

Table 1. NMR data for 1 and 3* (δ_C, δ_H).

	1	**3**		**1**	**3**
C_1	32.73	34.50	$H_{2\alpha}$	2.30	2.151
C_2	40.53	40.20	$H_{2\beta}$	2.66	2.522
C_3	120.70	120.00	H_3	4.98	4.905
C_4	145.20	145.80	H_5	1.28	1.155
C_5	35.86	35.90	H_6	0.57	0.224
C_6	35.28	34.50	H_7	1.42	1.082
C_7	41.16	44.35	$H_{8\alpha}$	0.68	0.885
C_8	23.99	26.30	$H_{8\beta}$	1.42	1.383
C_9	32.98	31.17	$H_{9\alpha}$	0.97	0.523
C_{10}	31.88	31.00	$H_{9\beta}$	1.50	1.610
C_{11}	33.85	33.20	H_{10}	1.87	1.824
C_{12}	21.12	19.60	H_{11}	1.42	1.587
C_{13}	21.08	19.60	H_{12}	0.92	0.899
C_{14}	20.19	19.40	H_{13}	1.02	0.932
C_{15}	16.86	16.50	H_{14}	1.07	0.932
			H_{15}	1.79	1.769

*All detailed spectra are available on request from CKJ, Canada (Table 1-3). The compound **1** 400 MHz spectrum quoted according to reference [5], compound **3** as from 600 MHz ^1H and 125 MHz ^{13}C spectrum.

Table 2. Key proton chemical shifts differences for two cubebene isomers from COSY experiments (δ_H, ppm).

	1*	**3**
$H_{5\alpha}$	—	1.156
$H_{5\beta}$	1.26-1.30	—
$H_{6\beta}$	—	0.227
$H_{6\alpha}$	0.55-0.59	—
$H_{7\alpha}$	1.37-1.46	1.100
$H_{10\beta}$	1.80-1.90	1.270
$(CH_3)_{-13}$	1.020	0.932
$H_{8\alpha}$	0.64-0.72	0.892
$H_{8\beta}$	1.37-1.46	1.383

*according to reference [5]

NMR assignment of structure 3

The assignment of all proton and carbon signals of **3** was done on 600 MHz with help of 1D and 2D mono and heterocorrelated spectra (COSY, HSQC, HMBC experiments; see Experimental for details) as well as for selected key protons for comparison of **1** and **3**.

The cubebene's two isomeric structure identifications were based on the analysis of the sequence of H-5, H-6 and H-7 α-proton orientations. Protons, H-5 and H-6 are placed on the cyclopropane moiety of the molecule in *trans* position respectively for α-cubebene **3**, H-6 and H-7 α being *trans* (the isopropyl-7 is placed in β position. The signal of two cyclopropane protons at δ 0.5-0.6 (H-6) and δ 1.25-1.35 (H-5) should demonstrate completely different splitting (two consecutive *trans* couplings for 3).

The examination of the high-resolution NMR data of these two signals led to the conclusion that unfortunately H-7 α still unavailable as a source of $J_{6,7}$ coupling constant and H-5 (dd, 2.7 Hz, ^3J, 2.7 Hz, $^4J_{3,5}$) and still remain in relatively crowded area, even at 600 MHz and after proton decoupling. However the 600 MHz spectra enabled unambiguously assign all proton and carbon signals of **3**. From the complete analysis it seems to appear that the isopropyl bearing cycle B is not adopting the perfect chair conformation, which is however understandable in view of the attached strained condensed cyclopropane.

The identification of structures was also based on two parallel observation of large coupling constants $^3J_{H,H}$ for *trans*

Table 3. NOE spectra assignment for key protons.

Proton \ Compound	Observed NOE effect	
Proton	3	1*
$H_{5\alpha}$	$H_{7\alpha}$, $H_{8\alpha}$, $H_{9\alpha}$, $H_{2\alpha}$**	—
$H_{5\beta}$	—	$H_{9\beta}$, $H_{8\beta}$, $H_{10\beta}$, $H_{2\beta}$
$H_{6\beta}$	$H_{8\beta}$, $H_{9\beta}$, H_{10}, $H_{2\beta}$	—
$H_{6\alpha}$	—	$H_{2\alpha}$, $H_{7\alpha}$, $H_{8\alpha}$, $H_{9\alpha}$, H_{11}, CH_3-iPr, H_{13}
$H_{7\alpha}$	$H_{5\alpha}$, $H_{8\alpha}$, $H_{9\alpha}$, $H_{2\alpha}$	$H_{6\alpha}$, $H_{8\alpha}$, $H_{9\alpha}$, $H_{2\alpha}$

*according to reference (5), ** absence of NOE to H_6

Table 4. Calculated energy for some selected cubebene derivatives*.

Compound	Energy (kcal/mol) A/B ring junction**
1	34.27
3	28.88
6c (*cis*)	34.70
6c (*trans*)	33.01
11	36.38
12	37.95
13	40.47
14	39.07
15	36.70
16	36.27

*from HyperChem Mm+ (12), **modelled on *cis* and *trans* decalin respectively

protons combined to small NOE between these protons and a smaller coupling constant $^3J_{H,H}$ for *cis* protons with larger NOE between these nuclei.

This distinction between two diastereomeric isomeric cubebenes *trans*, *trans* **3** and *trans*, *cis* **1** was then possible using the combined analysis of high resolution H-1 spectra and the homonuclear proton, proton COSY and in particular the unambiguous assignment of the cyclopropane moiety signals, then confirmed by COSY DFQ and by NOE experiments.

According to COSY DFQ experiments, both isomeric compounds **1** and **3** displayed some important differences. The key cyclopropane part of the molecule showed the coupling of unusually high field proton H-6β (at ΔH 0.227) to H-7 and H-5 as well as to the methyl H-13 or H-14 (being *syn* to the H-5). The signals of H-13 and H-14 protons, are displaying a very close resonance at ΔH 0.932 for H-13 and 0.935 for H-14, they are difficult to separate even at 600 MHz. The second cyclopropane protonated carbon resonance, at C-5, is showing at much lower field (at 1.156 ppm). The remaining signals of the system are in the expected area.

The most important differences between the two isomers were the strong methyl proton coupling for H-13, as well as some differences in C-8 and C-9, as seen through NOE experiments.

NOE experiments

NOESY as well as ROESY experiments were performed at 600 MHZ. The NOESY, carried out on product **3**, showed several cross-peak between key protons to this assignment. NOE experiments analysis of cubebene enabled to show that H-5, H-6 and H-7 proton system is in *trans*, *trans* configuration of these nuclei. The detailed examination of NOESY showed also some additional features, particularly if compared to the same sequence of protons for isomeric Konig's cubebene **1** (Table 3). NOE spectra, together with other 2D experiments as well as the vicinal $^3J_{H,H}$ coupling constants of the cyclopropane moiety are consistent with the geometry of the molecule **3**.

Conclusions

In this study it was observed that when the methanol extraction of *Solidago canadensis L*. is performed using microwave heating, some of sesquiterpenes underwent apparent isomerisation and easy addition of water or methanol. The pure α-cubebene **3**, under microwave in methanol or in water, is easily forming the addition products (together with at least thirty other compounds detected by GC-MS) both solvents act in fact as the reagent to these strained structures. The re-examination of Soxhlet (classical) extract lead to conclusion that during these extraction isomers **3-5** were probably also produced, however the microwave assisted extraction simply enhanced their concentration. This confirms our previous work demonstrating the risks associated with performing first microwave-assisted extraction in polar solvents where high temperatures prevail [9, 10, 12] then to identify the structure of natural products in general using this appealing extraction.

The structures of methanol or water adducts **4** and **5** are perhaps missing biosynthetic links between the transformation between the highly strained cubebene and decaline type sesquiterpenes or cyclodecane skeleton bearing (e.g. germacene) or copaene (tricyclic sesquiterpenes) compounds prior to their successive aromatisation. In this respect, the formation of two cubenols and their methyl ether products seems to be formally solvent assisted cyclopropane ring opening, the reaction observed sometimes under photochemical conditions for solvolysis of steroids for example [11].

Experimental

Material and Methods

Vortex dried green part material of *Solidago canadensis* L., crop 1999 collected in Southern Poland, were purchased from Herbapol via Bio-Chic, SA (Warsaw, Poland). Soxhlet or MAP methanolic extracts showed the presence of 40-70 dif-

ferent terpenes. Among them, in order, germacrene-D, limonene, α-pinene, mycrene, β-sesquiphellandrene and β-sesquiphellandren-9-one accounted for 73-5% of essential oils extraction the via Soxhlet method. All GC analysis were performed on Hewlett-Packard system described in the following section. The model α-cubebene **3** (α_D = +23°), all usual solvents as well as the essential reagents were purchased from Aldrich Chemicals. The deuterated solvents methanol-d_4 or heavy water, were from Euroiso-Top (France).

Mass spectroscopy of compound **3** (Riber 1010, EI, PI). Mass spectrum m/z (I,%): 204 ($M^{\bullet+}$, 17), 120 (100), 91 (53), 41 (47), 161(45), 55(32), 93(30) and 119(21).

The NMR analysis was performed on Bruker Avance 600 (600 MHz) apparatus equipped with cryoprobe. The xwinnmr program was used for the assignment of signals from 1D or 2D experiments (COSY, HSQC, HMBC, NOESY, ROESY). The printing of spectra was done with Bruker nmrview program. All spectra were recorded in $CDCl_3$ at 20° C, the 7.27 ppm and 77.0 ppm signals were used for calibration purposes. 2D experiments were recorded using respectively for COSY F1 1024 and F2 2048, HSQC 256 (^{13}C) and 1024 (^{1}H), HMBC 256 (^{13}C) and 2048 (^{1}H), then NOESY F1 512 and F2 2048 finally ROESY F1 512 and F2 2048.

All NMR and MS spectral material in this series is available on request in paper version from CKJ (U de M, Canada) and electronically from OM (Evry, France) and the GC-MS from JMRB (Ottawa, Canada).

Isomerisation of 3 Microwave irradiation

Microwave experiments were carried out at atmospheric pressure using a focused microwave reactor (CEM *Discover*™). The instrument consists of a continuous focused microwave power output from 0 to 300W. Reactions were performed in a glass vessel prolonged by a condenser; it is also possible to work under dry atmosphere, in vacuo, or under pressure (0-20 bar, tubes of 10 mL, sealed with a septum). The temperature content of a vessel is monitored using calibrated infrared sensor mounted under the vessel. All the experiments were performed using stirring option whereby the contents of a vessel are stirred by means of a rotating plate located below the floor of the microwave cavity and a Teflon-coated magnetic stir bar in the vessel. In all experiments a power of 300 W was selected and the reflux temperature was reached with a ramp of about 1 min. The time of the reaction does not include the ramp period.

The sample of 10 mg of α-cubebene **3** was irradiated in 3 ml of methanol (or methanol-d4) on the CEM *Discover* or Syntewave 402 Probablo, until the reflux, then maintained at reflux for 10 min and analysed by GC-MS after the evaporation of solvents in vacuo (at 30° C), using the following 25m columns CPSIL-5, SE-30 or grafted SE-30 modeled on Konig's methodology (5).

Under these conditions the α-cubebene **3** Rt 7.22 and its isomer **2** 7.28 corresponded to ca 1400 RRI (6) Kovac indexes used in this study indicated, for alcohols Rt 7.60 min. (1475) and methoxy derivatives Rt 9.0 min. (1747) where usually the natural limonene (1000) and, germacrene D (1500) were the references.

Sodium methoxide or sodium hydroxide catalyzed isomerisation

A sample of α-cubebene (**3**), (50 mg 0,24 mmol), was added to a solution of sodium methoxide or hydroxide. Water or heavy water was added to the solution and then brought to boil for 1h. The mixture then was extracted with CH_2Cl_2 and dried over $MgSO_4$, then analyzed by GC-MS, then through 1D and 2D NMR experiments.

HCl/DCl addition to 3

α-Cubebene (0.05 mmol, 9 mg, **3**) in methanol (or methanol-d_4) 2 mL saturated with HCl (DCl) at 0° C was treated for 5 min under microwave (300 W, 100 W) in a sealed tube. The resulting mixture was analysed with help of GC/MS and NMR.

GC-MS spectroscopy

GC-MSD Conditions:

All samples were runned on a Hewlett-Packard GC/Mass Selective Detector (6890N/5973N) with Automated Liquid Sampler (7683 Series). GC column flow rate was 1.0 mL/min regulated by Electronic Pressure Controller (Hewlett Packard, Palo Alto, CA, USA). Universal injection port set to 250° C, in splitless mode with a purge of 3 mL/min after 2 min. Capillary direct MS interface temperature was 280° C, ion source temperature 176° C. Ionization voltage 70 eV, and electron multiplier 1740 V. Capillary column used HP-5 MS (Hewlett Packard, USA). GC oven programming started at 45 °C, hold 2 min, increase to 100° C with rate 10° C/min, then increase to 200° C with rate 20° C/min and then hold for 2 min.

Sample preparation:

Each dried sample received was diluted in 200 µL of HPLC grade Methanol, vortexed for 1 min and 1 µL was injected on GC.

Molecular modelling

The molecular modelling calculations were performed using HyperChem 6 and 7 Mm^+, according to the methodology developed in our laboratory [14] in gas phase and in solvent box modes. The minimization of energy was performed according to Polak-Riblere iteration two-step method (10 000 cycles at 0,005 kcal/mole, then 0,001 ps for 1 ps) at 370° K.

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References

1. Arctander, S. *Perfume and Flavor Materials of Natural Origin,* **1960**, S. Arctander, Elsbth, N.Y. pp. 281-5
2. a) Guterman, I. *The Plant Cell*, **2002**, *14*, 325-38. b) Kalemba, D.; Gora, J.; Kurowski, A.; Majda, T. *Zesz. Nauk PL Technol, Chem. Spoz.* **1990,** *47*, 92-97.
3. Niwa, M.; Iguchi, M.; Yamamura, S. *Chem. Pharm. Bull.* **1980**, *28*, 997-999.
4. Kalemba, D.; Marschall, H.; Brandesi. P. *Plav. Fragr. J.* **2001**, *16*, 19-26.
5. Kasali, A. A.; Ekundayo, O.; Paul, C.; Konig, W.A. *Phytochemistry* **2002**, *59*, 805-810.
6. a) Weyerstahl, P.; Marschall, H.; Christansen, C.; Kalemba, D.; Gora, J. *Planta Med.* **1993**, *59*, 281-282; b) Kalemba, D.; Gora, J.; Kurowska, A. *Planta Med*. **1990**, *56*, 222-223.
7. Bulow, N.; Konig, W.A. *Phytochemistry* **2000**, *55*, 141-168.
8. Julain, D.; Konig, W.A. *Atlas of Spectral Data of Sesquiterpene Hydrocarbons*. **1998**, EB Verlag Hamburg, Germany.
9. a) Savoie, A. M..Sc. thesis, Université de Moncton, Moncton, NB, Canada, 2005b) Paré, J. R. J.; Sigouin, M.; Lapointe, J. *Microwave Assisted Natural Products Extraction*, U.S. Patent No. 5 002 784, March 26, **1991**.
10. Paré, J. R. J.; Bélanger, J. M. R. *Microwave-Assisted Process (MAP™):* Principles and Applications, in: *Instrumental Methods in Food Analysis*, Paré, J. R. J. ; Bélanger, J. M. R. Eds., Elsevier Science, Amsterdam, pp. Chapter 10, **1997**, 395-420.
11. Zimmerman, H.E.; Epling, G.A. *J. Am. Chem. Soc.* **1972**, *94*, 3245; Darben, W.G.; Loder, G.; Ipastski, J. *Topres Curr. Chem.* **1975**, *54*, 23.
12. Detailed descriptions of single-mode microwave reactors with integrated robotics were recently published: For *CEM corp.*: Ferguson, J. D. *Mol. Diversity* **2003**, *7*, 281-286; For *Biotage (Personal Chemistry AB)*: Schanche, J.-S. *Mol. Div*ersity **2003**, *7*, 293-300.
13. For a recent review in the area see: C.O. Kappe, *Angew. Chem. Int. Ed*. **2004**, *43*, 6250 6284; For the most cited book on microwaves in chemistry see: *Microwaves in organic synthesis*, A. Loupy, Ed. Wiley-VCH Verlag Gmbh & Co. KGaA, Weinhein, **2002**; Mesangeau, C.; Yous, S.; Peres, B.; Lesieur, D.; Besson, T. *Tetrahedron Lett.* **2005**, 2466.
14. Jankowska, A.; Jankowski, C. K.; Chiasson. J. B. *J. Inclusion Phenom.* (accepted 2004); Wagner, B.; Stojanovic, N.; Jankowski, C. K.; LeClair, G. *J. Inclusion Phenom.* **2003**, *45,* 273.