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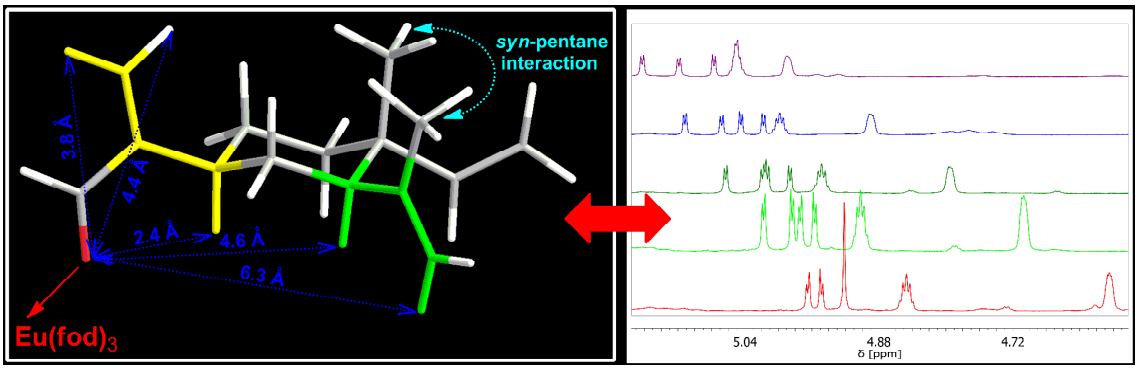
A new approach to the structural elucidation of constituent(s) of complex mixtures was developed based on the use of lanthanide-induced shift reagents. This methodology was successfully applied in the identification of a rare sesquiterpene elemenal.



# Lanthanide-induced shift reagents enable structural elucidation of natural products in inseparable complex mixtures - The case of elemenal from *Inula helenium* L. (Asteraceae)†

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† Electronic supplementary information (ESI) available: A comparison of experimental and simulated $^{1}$H NMR spectra of elemenal (the second order vinyl group spin system).

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## Abstract

The use of lanthanide complexes for resolving intricate NMR signals and, in the case of chiral ligands, for determining enantiomeric excess has progressively decreased in the last 30 years. Recently, a sesquiterpene aldehyde from *Inula helenium* with a possible potent antistaphylococcal activity remained unidentified due to the impossibility to separate the compound from its complex matrix available in very low amount (*ca.* 5 mg). Detailed analyses of 1D and 2D NMR spectra of this original complex sample allowed access to a very limited amount of structural data for the unknown aldehyde. We decided to investigate the potential usefulness of lanthanide-induced shift reagents for the resolution and assignation of overlapped $^{1}$H NMR signals originating from different components of this complex mixture (*i.e.* for a qualitative analysis). The incremental addition of *tris*(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III) (Eu(fod)$_3$) led to a simplification of NMR spectra in terms of signal overlap and removal of chemical shift degeneracy, allowing the mining of crucial data from the shifted NMR spectra. 2D-NMR spectra ($^{1}$H–$^{1}$H-COSY, NOESY, HSQC and HMBC) of the sample mixed with Eu(fod)$_3$ proved to be particularly valuable in this respect. The obtained additional information revealed that the compound in question was a rare sesquiterpene - elemenal (elema-1,3,11(13)-trien-12-al). Therefore, herein we report on a new chromatography-free methodology that could be of value in structure elucidation of unknown compounds even if they are not available in pure state.

## Introduction

Nuclear magnetic resonance spectroscopy is one of the most powerful analytical techniques for the elucidation of structures of organic compounds. Continuous efforts have been made to develop different 1D-, 2D- and multidimensional-NMR methods in order to obtain more information from NMR measurements that will facilitate and accelerate structure determination. In general, it is considered that the structure of a new organic molecule is established if total assignment of $^{1}$H and $^{13}$C NMR data could be achieved. However, the application of these sophisticated NMR methods can sometimes, even if one has a fairly good idea of the likely structure, be insufficient to remove concerns regarding, for example, the position of certain substituent(s) or a double bond, relative stereochemistry or unequivocal assignment of all carbon or hydrogen atoms from so called overlapping signals. Most frequently these difficulties occur in the interpretation of complex or poorly-functionalized

(*i.e.* having a small number of highly electronegative heteroatoms) organic molecules (e.g. sterols, terpenes or lipids) as the signals, especially in $^{1}$H NMR spectra, are bunched together in featureless clusters from which little definitive structural information can be obtained. This occurrence of overlapping resonances of non-equivalent protons is a consequence of relatively low sensitivity of proton chemical shifts to changes in the chemical and stereochemical environments.[1–3] Nowadays, this problem could be solved, to some extent, by the advent of high-field NMR equipment, but the cost of this is often beyond the means of many spectroscopic departments.

Another approach to NMR spectra simplification, initially reported in 1969 by Hinckley[4] and extensively employed mostly in the next two decades, is the introduction of a lanthanide shift reagent (LSR). The application of LSRs is based on their ability to selectively coordinate electron-donor functional groups in the substrates and induce shifts of signals in NMR spectra.[5] The most common practice is to successively add known amounts of LSR to the compound under study and record NMR spectra after each addition (the shifted spectra). The chemical shifts of some protons and carbons in the substrates alter, to a greater or lesser degree, with each addition of LSR and may result in the segregation of overlapping signals that could facilitate its assignments.[3] This approach has been successfully employed in structural and conformational analysis of many synthetic organic compounds and natural products, as well as in the study of their chirality, but it requires the substrate in pure state with known or almost resolved structure on the basis of data from regular NMR measurements.[1,5–11] In recent years experiments involving chiral LSRs have been successfully carried out for the enantiomeric discrimination of oxygenated bicyclic monoterpenes (bornyl acetate, fenchone and camphor) contained in essential oils, without isolation of the compounds.[12,13] Similar methodology was applied in enantiomeric ratio determination of atropine and hyoscyamine in the crude extract of *Datura stramonium* seeds.[14]

In the mentioned three quantitative studies,[12–14] where a particular compound was analyzed directly in a naturally occurring mixture, a standard of the compound in question was needed for the calibration curves set up and the methodologies were based on the existence of a set of non-overlapped (by the matrix molecules and the analytes) signals. Because of this, these researchers chose to track the lanthanide-induced shifts of $^{13}$C NMR signals, despite the loss of sensitivity and the onset of $^{13}$C NMR signal integration issues, since the proton decoupled $^{13}$C NMR spectrum is inherently less complex than the $^{1}$H NMR spectrum. Up to now, LSR methodology has not been used to resolve overlapped signals originating from different (non-enantiomeric) molecules (in both $^{1}$H and $^{13}$C NMR), let alone for the structural elucidation of individual unknown constituents in a mixture, *i.e.* for qualitative purposes. The resolution of accidently overlapped signals from two or more different molecules could be a plausible outcome of a gradual addition of a LSR directly to the mixture since a distinct conduct of these signals is expected due to molecular differences (differing spatial relationship with the LSR complexing functional group and its identity), competitive complexation (differing stabilities of the LSR complexes of different mixture constituents) and shifting of the complexation equilibrium by different amounts of the individual constituents.[2,3]

Furthermore, there is only a handful of studies that combined 2D NMR ($^{1}$H–$^{1}$H COSY, NOESY, HSQC and HMBC) with LSR experiments in an attempt to perform a complete assigment of $^{1}$H NMR and $^{13}$C NMR resonances of natural products.[15–17] Also, the presence of LSR, that are paramagnetic in nature, should result in the increased $^{13}$C NMR sensitivity and shorten the time needed to acquire good quality spectra even for samples available in low amount.[18] This could have a downside, since nuclear Overhauser effect decreases (but does not disappear) under such conditions and at high LSR concentrations this

could lead to signal broadening. Therefore, to avoid the shortcomings, a titration with LSR is advised.[2]

Previously, in search for antimicrobial constituents of *Inula helenium* L. (Compositae), the activity of the plant essential oil was allocated to a minor chromatographic fraction composed of a series of 3-methyl-2-alkanones of varying chain lengths ($C_{11}$–$C_{19}$) and an unidentified sesquiterpene.[19,20] The identity of the 3-methyl-2-alkanones was confirmed by a synthetic approach based on the creation of a combinatorial library of such compounds since the amount and complexity of the fraction did not permit further chromatographic separation. This synthetic approach also allowed us to verify whether the ketones were responsible for the high noted antistaphylococcal activity. Unfortunately, the synthetic compounds turned out to be poor antimicrobial agents, hence indicating that the activity of the fraction originated from the mentioned unknown sesquiterpene. This compound showed the highest *m/z* value at 218 (molecular weight of an oxygenated sesquiterpene, $C_{15}H_{22}O$) in its mass spectrum, accompanied by recognizable ions, $[M - 1]^+$ and $[M - 29]^+$, characteristic for aldehydes (Fig. 1), that resembled that of bicyclogermacrenal.[20]

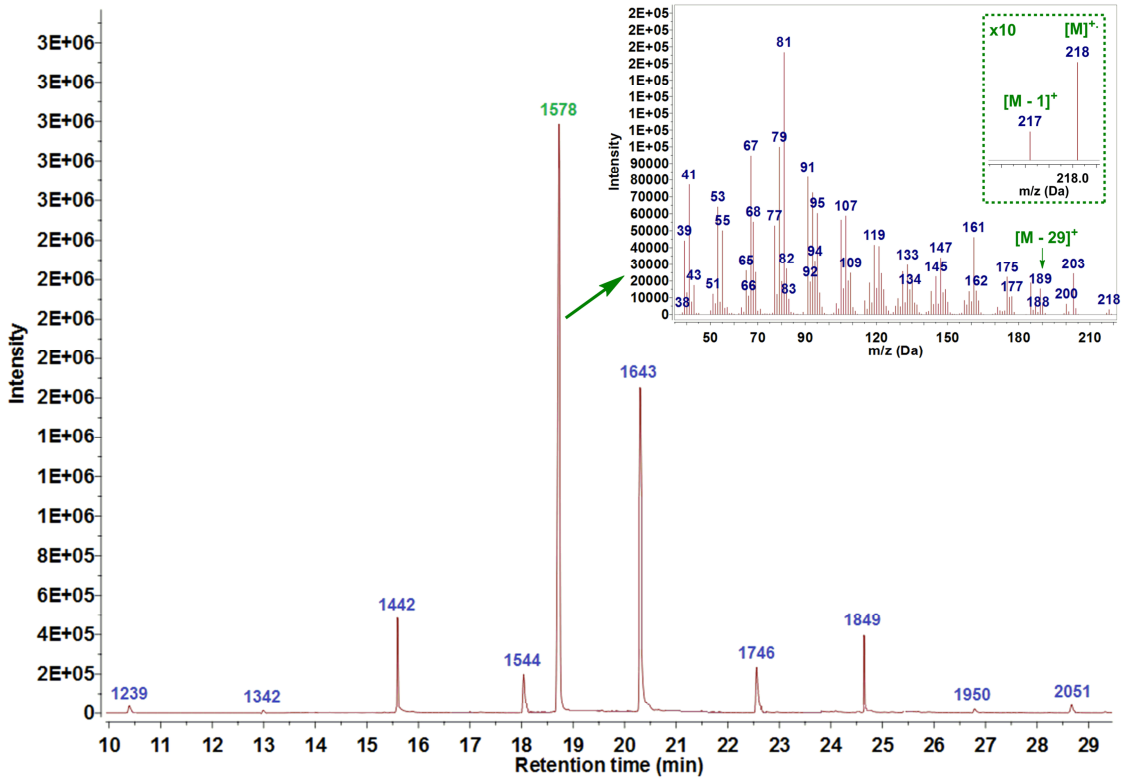


**Fig. 1.** The original TIC chromatogram of a GC–MS run of a fraction (5% diethyl ether in hexane) of *I. helenium* root essential oil showing peaks with retention indices (blue colored) corresponding to a series of 3-methyl-2-alkanones and an unidentified sesquiterpene aldehyde (green colored) and the mass spectrum of the aldehyde.

In order to identify this sesquiterpene aldehyde, a larger quantity of the essential oil was fractionated by chromatography on $SiO_2$. Repeated usage of a non-polar eluent led to sample **A**, which weighted *c.a.* 5 mg, enriched with the compound in question. The GC–MS analysis of this sample revealed that it contained roughly 85% of the sesquiterpene accompanied with geranyl (8%) and neryl isobutanoates (5%), as the main contaminants.

Furthermore, 1D and 2D NMR spectra of this sample were rather complex for interpretation and, hence uninformative, because of a number of overlapping signals originating from both the aldehyde and the contaminants. This mixture was an excellent candidate for the testing of the applicability of LSRs in structural elucidation of compounds in mixtures by the abovementioned simplification/resolution of 1D and 2D NMR spectra when further purification was not possible. Therefore, we decided to try to simplify the NMR spectra of this sample by an incremental introduction of $Eu(fod)_3$ in order to identify the mentioned sesquiterpene aldehyde. One can assume that the impact of LSR will be most obvious on the signals corresponding to this sesquiterpene as it is the major component of the mixture and has an aldehyde group that is very suitable for coordination of LSR.[3]

Thus, in this work, we report the successful identification and spectral characterisation of a sesquiterpene aldehyde from a complex sample representing a mixture of several compounds, without the isolation of the aldehyde in pure state, by the application of a new structural elucidation methodology based on the analyses of shifted 1D and 2D NMR spectra of the mentioned mixture.

## Results and discussion

Extensive NMR measurements of sample **A** were done in order to try to gain as much structural data on the sesquiterpene aldehyde as possible directly from the mixture (85% of the sesquiterpene accompanied with geranyl (8%) and neryl isobutanoates (5%)). Expectedly, $^1$H NMR spectrum of sample **A** in deuterated chloroform exhibited a distinguishing broad singlet for an aldehyde proton at $\delta_H$ 9.53 (assigned an integral value of 1). This spectrum contained seven olefinic protons, at $\delta_H$ 6.27 (1H, br d, $J$ = 0.7 Hz), 5.97 (1H, br s), 5.83 (1H, m), 4.82 (1H, pseudo-quintet), 4.58 (1H, m) and two-proton second-order signal in the region 4.88-4.95 ppm, that appeared to belong to the aldehyde according to the value of their integrals (Fig. 2a). Two low-intensity multiplets at 5.38-5.29 and 5.15-5.05 ppm were unambiguously confirmed to belong to the olefinic protons of the two main impurities, geranyl and neryl isobutanoates, by comparison with the corresponding chemical shifts in $^1$H NMR spectra of authentic standards (Figs. 2a and 2b). Surprisingly, a complete assignment of the $^1$H and $^{13}$C resonances of the isobutanoates was lacking in the literature. The region of $^1$H NMR spectrum up to 3 ppm was very complex to analyse since it contained a number of highly overlapped signals. The only peak that could be straightforwardly discerned was a singlet corresponding to a $CH_3$ group at $\delta_H$ 1.03 (3H) attached to a quaternary carbon atom.

Alongside the aldehyde carbon atom signal at $\delta_C$ 194.5, $^{13}$C NMR spectrum exhibited eight olefinic signals, four of which having significantly higher intensities than the rest. DEPT135 spectrum showed that three of them, at $\delta_C$ 132.9, 112.2 and 110.1, corresponded to methylene groups ($=CH_2$), while the remaining one at $\delta_C$ 150.0 was a methine olefinic carbon (=CH–). This matches the number of proton resonances that were associated with the aldehyde mentioned above. Consequently, the aldehyde should have (at least) three double bonds with two olefinic carbons being non-protonated. This assumption was confirmed by appropriate cross peaks observed in the gHMQC spectrum: (*i*) the methine carbon signal at 150.0 ppm correlated with the proton at $\delta_H$ 5.83 (*ii*) the methylene olefinic carbon at $\delta_C$ 132.9 with protons at $\delta_H$ 6.27 and 5.97; (*iii*) the methylene olefinic carbon at $\delta_C$ 112.2 with protons at $\delta_H$ 4.82 and 4.58; (*iv*) while the methylene olefinic carbon at $\delta_C$ 110.1 seemed to correlate with both protons in the region 4.88-4.95 ppm. All three pairs of methylene olefinic protons showed cross peaks in the $^1$H–$^1$H gDQCOSY spectrum, as well. Additionally, the DEPT135 spectrum revealed the presence of two methyl groups at 24.9 and 16.6 ppm that coupled in the gHMQC spectrum with the signals at $\delta_H$ 1.70 and 1.03, respectively. The singlet at 1.03 ppm was already proposed to correspond to a $CH_3$ group attached to a quaternary carbon

atom, but a closer inspection of $^1$H NMR spectrum in the region around 1.70 ppm allowed a perception of another $CH_3$ as a possible doublet of doublets ($J$ = 1.6, 0.8 Hz). Chemical shifts, $\delta_H$ 1.70 and $\delta_C$ 24.9, for the second deshielded methyl group implied that it was most probably attached to a double bonds.

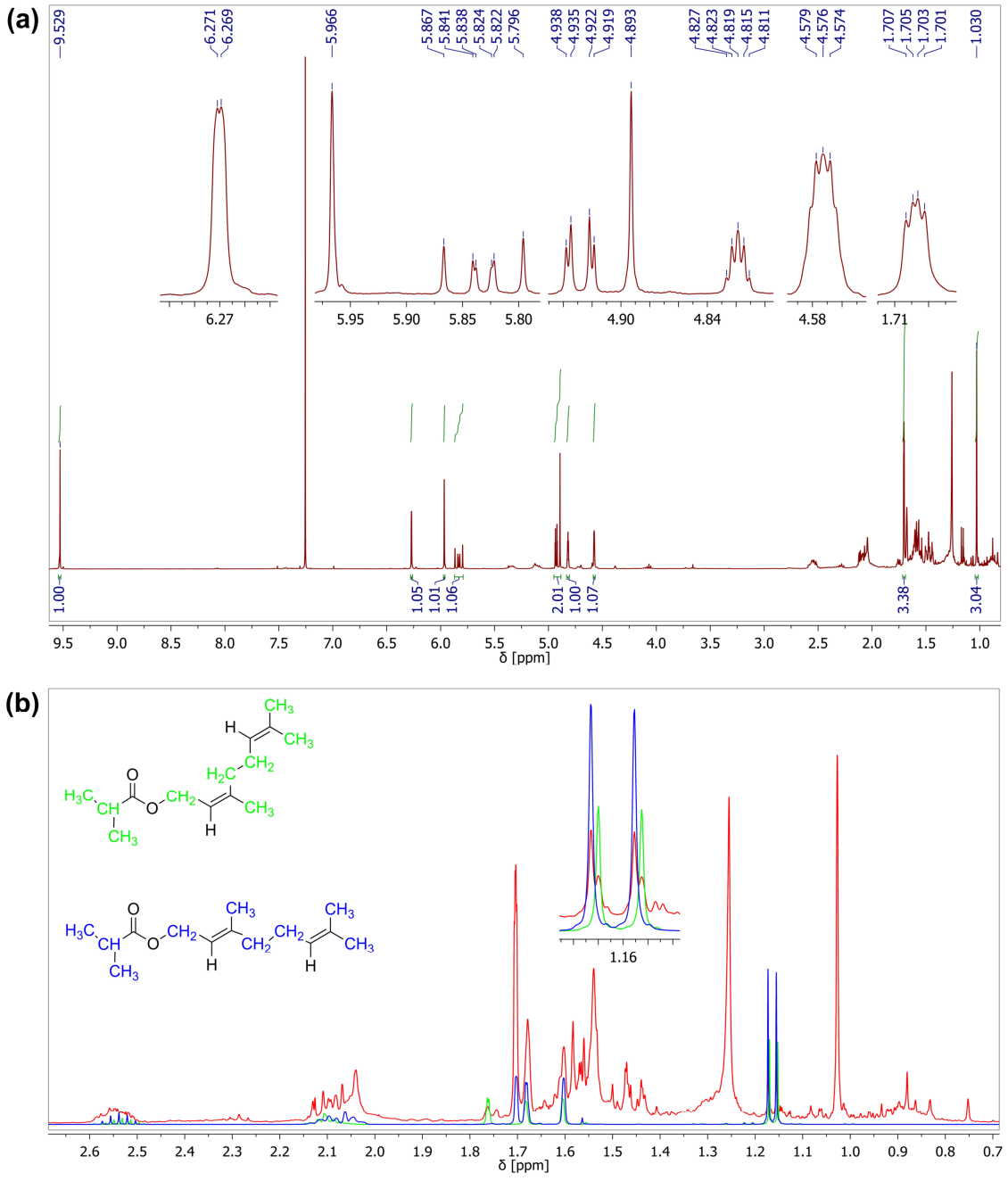


**Fig. 2. (a)** $^1$H NMR spectrum of sample **A** with signals assigned to unknown sesquiterpene aldehyde. **(b)** Overlapping of $^1$H NMR spectrum (high field region) of sample **A** (marked red) with $^1$H NMR spectra of two main contaminants neryl isobutanoate (marked green) and geranyl isobutanoate (marked blue).

The correlations observed in $^{1}H–^{13}C$ gHMBC spectrum of sample **A** were very informative and enabled the construction of two structural moieties presented in Fig. 3. The geminal methylene protons at $\delta_H$ 6.27 and 5.97 had significantly different chemical shifts due to an anisotropic effect of the aldehyde group. The proton Ha that resonated at lower field was readily assigned to the proton *cis* to the aldehyde group. It demonstrated a relatively resolved long-range coupling constant of 0.7 Hz, and most probably additional ones that led to signal broadening. In this way an α-substituted acrolein moiety was established (Fig. 3b). The structure of the second fragment was deduced from a combination of data inferred from $^{1}H–^{13}C$ gHMBC and $^{1}H–^{1}H$ gDQCOSY spectra related to the methylene olefinic protons at $\delta_H$ 4.82 and 4.58, and two methyl groups at $\delta_H$ 1.70 and 1.03. $^{1}H–^{13}C$ long-range correlations of protons at 4.82, 4.58 and 1.70 ppm revealed the presence of an isopropenyl spin system linked to a methyne group at $\delta_C$ 52.5 (Fig. 3c). The second $CH_3$ group ($\delta_C$ 16.6 and $\delta_H$ 1.03) expectedly showed correlations with quaternary carbon atom at $\delta_C$ 39.5 ppm, while further extension of this structural fragment was made possible by the cross peaks with an =CH– group ($\delta_C$ 150.0 and $\delta_H$ 5.83), as well as, with the mentioned methine carbon at $\delta_C$ 52.5. Unfortunately, the proton of the =CH– group showed long-range $^{1}H–^{13}C$ coupling to only a quaternary carbon atom at $\delta_C$ 39.5 ppm. However, this proton coupled to a proton ($^{1}H–^{1}H$ gDQCOSY) in the range 4.88-4.95 ppm corresponding to both remaining methylene olefinic protons.

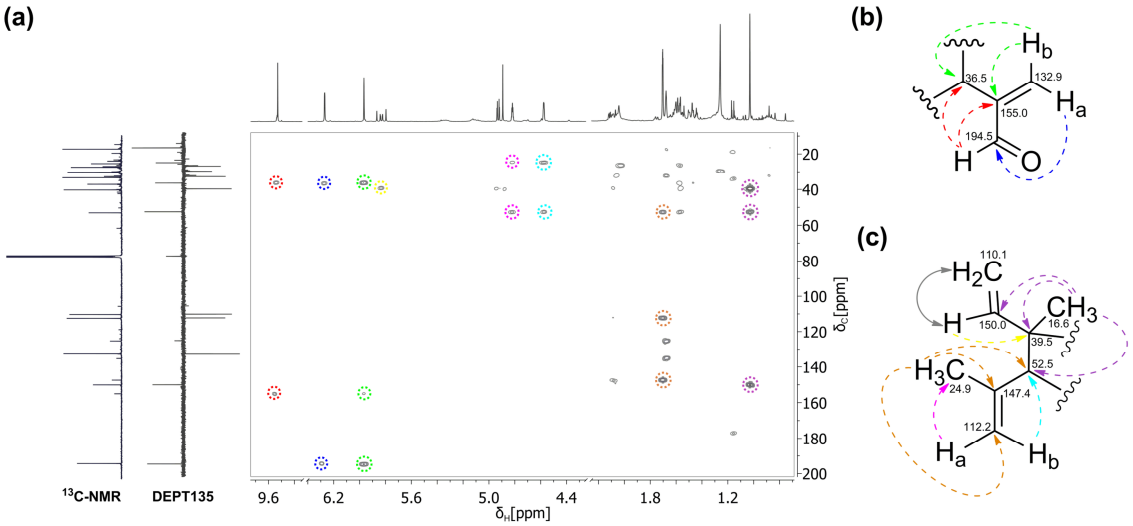


**Fig. 3**. (**a**) Expansion of the $^{1}H–^{13}C$ gHMBC spectrum of sample **A** with key cross peaks marked with appropriate coloring to match that in **b** and **c**. (**b**) and (**c**) structural fragments with marked observed $^{1}H–^{13}C$ gHMBC interactions (dashed colored arrows). Gray double ended arrow represents a $^{1}H–^{1}H$ gDQCOSY interaction.

Thus, one should expect a vinyl group in the structure of the sesquiterpene aldehyde, *i.e.* it should be expected to display a characteristic ABX spin system. The chemical shifts and coupling constants of the multiplets that appeared at 5.83 and 4.88-4.95 ppm were solved by the use of WinDNMR software,[21] and the simulated spectrum is given as a supplementary file (Fig. S1). The established 2,4-dimethylhexa-1,5-diene-3,4-diyl fragment is frequently encountered in sesquiterpenes of elemene and related skeletons.

Judging from the molecular formula of the aldehyde, a total of five unsaturations should be accounted for. This means that the fifth unsaturation (alongside the three C=C and one C=O) should correspond to a ring. The up to now assigned carbon and hydrogen

resonances make up a total of 12 carbon and 14 hydrogen atoms, while 2 additional protons from CH groups ($\delta_C$ 52.5 and $\delta_H \approx 2.10$, and $\delta_C$ 36.5 and $\delta_H \approx 2.55$) located only by the data from 2D spectra. Thus, the identity-chemical shifts of 3 carbon and 6 hydrogen atoms still remained undetermined. The only unassigned intense peaks in the DEPT135 spectrum were those from $CH_2$ groups at $\delta_C$ 39.7, 32.8, 29.7 and 26.8. Thus, it appeared likely that the missing carbons and protons of the aldehyde were three $CH_2$ groups. However, as these methylene protons resonated at high field, their precise chemical shift assignment was rendered impossible due to severe overlap, both mutual and with impurity signals (Fig. 2b).

Although the above given detailed analysis of the NMR spectra of mixture **A** provided valuable data it did not result in a specific complete proposition of the structure of the aldehyde. Next, having in mind that the sesquiterpene contains an aldehyde group, which is considered a good Lewis base, it was decided to try to simplify the proton spectrum in the high field region by an NMR-monitored titration with LSR. The formation of an adduct with LSR could possibly enable the assignment of the proton from the CH group at $\delta_C$ 36.5 ($\delta_H \approx$ 2.55) attached to the acrolein moiety-the coordination site of LSR, *i.e.* a significant downfield shift should be expected. $Eu(fod)_3$ was chosen as LSR because it combines the maximum shift capacity with minimum broadening of the shifted resonances, good solubility in chloroform with absence of interfering chelate resonances in the usual range of NMR frequencies.[8] The incremental addition of $Eu(fod)_3$ resulted in a great simplification of the $^1H$ NMR spectrum of sample **A** as a number of signals were observed to move to lower field. While the shifts of the first fragment (Fig. 3b) proton signals, located near the coordination site, were expected, the effect of $Eu(fod)_3$ on the protons from the vinyl, isopropenyl and the second CH group ($\delta_C$ 52.5 and $\delta_H \approx 2.10$) from the second fragment were unforeseen (Fig. 4).

At first glance, the most striking changes were: the mutual separation achieved for the protons from the $=CH_2$ end of the vinyl group and the clean detachment of two CH groups from the complex upfield region ($\delta_H < 3$ ppm) in the unshifted reference spectrum. At the approximate molar ratio $[Eu(fod)_3]/[aldehyde] = 0.45$, two well separated doublet of doublets at $\delta_H$ 5.06 ($J$ = 17.5, 1.3 Hz) and $\delta_H$ 5.00 ($J$ = 10.8, 1.3 Hz), that together with a doublet of doublets at $\delta_H$ 6.03 ($J$ = 17.5, 10.8 Hz) formed the ABX spin system of the vinyl group, could be observed (Fig. 4, Table 1). In alkenes, *trans* coupling generally results in larger coupling constants compared to *cis* coupling and with geminal coupling being by far the smallest. Thus, the proton (labelled Hb) that had a slightly higher $\delta_H$ occupied the *trans* position relative to the =CH- proton. The location of protons from the two CH groups was facilitated by the fact that the effect of LSR is approximately equal for a $^{13}C$ nucleus as for a proton in approximately the same location (the same $\Delta\delta$ values in ppm), *i.e.* directly attached to the carbon.[6] The CH groups could be easily located from the shifted gHMQC spectrum (at molar ratio 0.45) as $^{13}C$ NMR spectra changed much less dramatically than the proton spectra did, since the chemical shift range of the $^{13}C$ nucleus is much larger. At the molar ratio 0.45, the proton from the CH group closer to the coordination site appeared as a broad triplet of triplets at $\delta_H$ 4.74 ($J \approx 12$, 3 Hz), while the proton from the more remote CH group resonated at $\delta_H$ 2.68 as a broad doublet of doublets ($J$ = 12.8, 3.0 Hz).

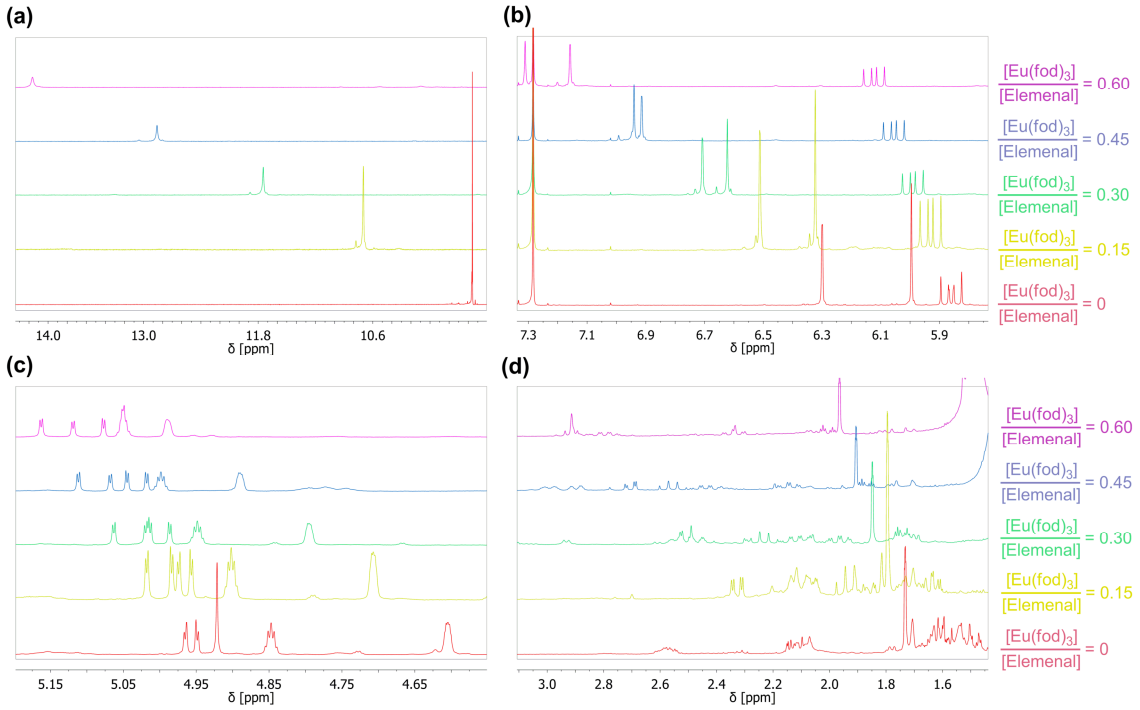


**Fig. 4.** The shifts of signals after incremental additions of Eu(fod)$_3$ corresponding to: (**a**) the aldehyde proton, the olefinic protons with (**b**) $\delta_H$ > 5.8 ppm and (**c**) $\delta_H$ < 5 ppm in the unshifted spectrum, and (**d**) the enlargement of the area with the aliphatic protons with $\delta_H$ between 1.45 and 2.60 ppm in the unshifted spectrum.

Furthermore, the shifted gHMQC spectrum (at the molar ratio of 0.45) revealed the presence of three pairs of diastereotopic protons, *i.e.* three $CH_2$ groups as: (*i*) protons at $\delta_H$ 2.95 and 2.41 that correlated with the carbon at $\delta_C$ 28.0, (*ii*) protons at $\delta_H$ 2.87 and 2.53 that correlated with the carbon at $\delta_C$ 33.9, and (*iii*) protons at $\delta_H$ 2.12 and 1.84 attached to the carbon with $\delta_C$ 40.3 (Table 1). Such a segregation of the mentioned diastereotopic protons was crucial for the completion of the structure of the sesquiterpene aldehyde since the presence of a $(C)_2CH\text{-}CH_2\text{-}CH(C)\text{-}CH_2\text{-}CH_2\text{-}C(C)_3$ closed spin system was finally clearly evident from the $^1H–^1H$ gDQCOSY spectrum (molar ratio 0.45; Fig. 5a). The established connectivity was also sustained by appropriate correlations in $^1H–^{13}C$ gHMBC spectrum (molar ratio 0.45). This system linked fragments 1 and 2, making up a cyclohexane ring and completing the elemane skeleton of the aldehyde. The observed multiplicity of the $CH_2$ group signals was in agreement with the existence of an 1,3,4,4-tetrasubstituted cyclohexane ring: geminal and axial-axial couplings on one side, and equatorial-equatorial and axial-equatorial on the other, are both of very similar magnitude ($J_{gem} \approx J_{aa}$ and $J_{ee} \approx J_{ae}$) giving rise to very complex pseudo-shaped signals (Table 1).

**Table 1.** $^{1}H$ and $^{13}C$ NMR data for elemenal and $Eu(fod)_3$–elemenal complex at molar ratio 0.45 and experimental ΔEu values for certain protons

| Position | Elemenal | | [Eu(fod)$_3$]/[Elemenal] = 0.45 | | ΔEu |
|---|---|---|---|---|---|
| | $\delta_H$ [ppm] | $\delta_C$ [ppm] | $\delta_H$ [ppm] | $\delta_C$ [ppm] | |
| 1 | 5.83 (1H, m) | 150.0 | 6.03 (1H, dd, *J* = 17.5, 10.8 Hz) | 150.2 | 0.41 |
| 2 | 4.92[a] | 110.1 | **a**: 5.00 (1H, dd, *J* = 10.8, 1.3 Hz) | 110.2 | 0.23 |
| | 4.93[a] | | **b:** 5.06 (1H, dd, *J* = 17.5, 1.3 Hz) | | 0.27 |
| 3 | **a**: 4.82 (1H, pseudo-quint, *J* = 1.6 Hz) | 112.2 | **a**: 4.97 (1H, m) | 112.5 | 0.32 |
| | **b**: 4.58 (1H, pseudo-dq, *J* = 1.6, 0.8 Hz) | | **b:** 4.86 (1H, m) | | 0.61 |
| 4 | / | 147.4 | / | 147.8 | / |
| 5 | 2.14[a] | 52.5 | 2.68 (1H, br dd, *J* = 12.8, 3.0 Hz) | 53.1 | 1.23 |
| 6 | ≈ 1.59 (2H)[b] | 32.8[c] | **e:** 2.87 (1H, pseudo-dt, *J* = 12.9, 3.2 Hz) | 33.9 | / |
| | | | **a**: 2.53 (1H, pseudo-q, *J* = 12.5 Hz) | | / |
| 7 | 2.58[a] | 36.5 | 4.74 (1H, br tt, *J* ≈ 12, 3 Hz) | 38.6 | 4.95 |
| 8 | **e:** ≈ 1.62[b] | 26.8 | **e:** 2.95 (1H, br pseudo-d quint *J* = 13.5, 3 Hz) | 28.0 | / |
| | **a:** ≈ 1.46[b] | | **a**: 2.41 (1H, pseudo-qd, *J* = 13.5, 3.4 Hz) | | / |
| 9 | **e:** ≈ 1.47[b] | 39.7 | **e:** 1.84 (1H, pseudo-dt, *J* = 13.4, 3.4 Hz) | 40.3 | / |
| | **a:** ≈ 1.56[b] | | **a**: 2.12 (1H, pseudo-td, *J* = 13.4, 3.6 Hz) | | / |
| 10 | / | 39.5 | / | 40.0 | / |
| 11 | / | 155.0 | / | /[d] | / |
| 12 | 9.53 (1H, br s) | 194.5 | 12.83 (1H, s) | 207.2 | 7.55 |
| 13 | **a**: 6.27 (1H, br d, *J* = 0.7 Hz) | 132.9 | **a:** 6.89 (1H, br d, *J* = 0.7 Hz) | 135.3 | 1.39 |
| | **b**: 5.97 (1H, br s) | | **b:** 6.91 (1H, s) | | 2.13 |
| 14 | 1.03 (3H, br s) | 16.6 | 1.38 (3H, br s) | 17.0 | 0.74 |
| 15 | 1.70 (3H, dd, *J* = 1.6, 0.8 Hz) | 24.9 | 1.88 (3H, dd, *J* = 1.6, 0.8 Hz) | 25.0 | 0.36 |

[a] Chemical shift is determined by extrapolation of proton plot to [Eu(fod)$_3$]/[Elemenal] = 0;
[b] Chemical shift is estimated from a cross-peak in the gHMQC spectrum;
[c] Chemical shift were assigned based on the value of appropriate induced chemical shift;
[d] The determination of this chemical shift value was hindered due to extensive signal broadening.

A literature survey revealed that an aldehyde with the elemane skeleton like the one just established had been previously described but has a rather restricted occurrence in nature. This sesquiterpene aldehyde named elemenal had been previously isolated in pure state only from *Thujopsis dolabrata* Stieb. et Zucc.[22] and *Saussurea lappa* Clarke[23] root essential oils. The structure of elemenal was initially proposed based on limited spectral data (UV-Vis, IR and $^1$H NMR (at 90 Hz); available at the time) and afterwards confirmed, along with the determination of its absolute configuration, by comparison with an authentic semisynthetic sample obtained from (–)-β-elemene. The relative stereochemistry of our elema-1,3,11(13)-trien-12-al was inferred to be the same as that of the previously reported elemenal from the very informative shifted NOESY spectrum of sample A (Fig. 5b). The presence of Eu(fod)$_3$ (at 0.45 molar ratio) did not result in the disappearance of nOe, most probably because the main *modus operandi* of this LSR was a pseudocontact or dipolar interaction (a through space effect) - which originates from a secondary magnetic field, that is usually anisotropic, generated by the paramagnetic cation (as opposed to contact shifts (*e.g.* a through-bonds effect) - which arise from delocalization of the unpaired electron-spin through bonds to the nuclei affected).[3]

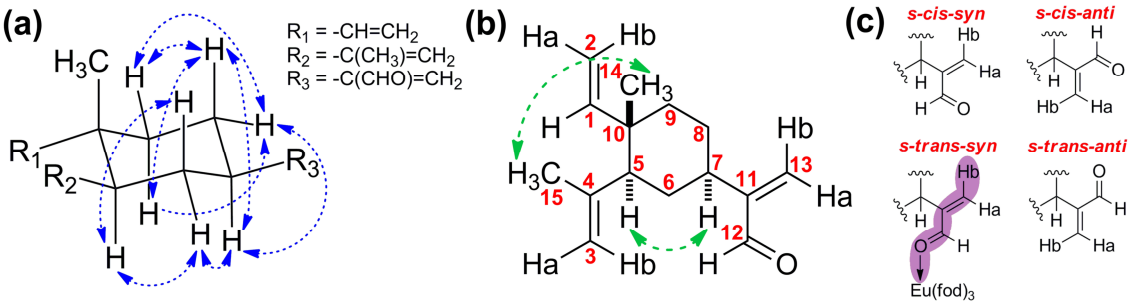


**Fig. 5. (a)** The most important $^1$H–$^1$H gDQCOSY cross-peaks used for the structure elucidation of the sesquiterpene aldehyde. **(b)** The structure of elemenal with a numberings scheme of its carbon atoms and the crucial nOe cross-peaks used for the determination of its relative stereochemistry. **(c)** Four possible conformations of the methacrolein moiety. The zig-zag oriented σ-system in the *s-trans-syn* conformation, through which the largest extent of electron spin density transfer could be expected, is denoted by violet shading.

Furthermore, elemenal had also been tentatively identified (based solely on the fragmentation pattern visible in its mass spectrum, since there was no MS published till de Kraker *et al.*[24]) as a minor constituent of essential oils obtained from *Origanum onites* L.,[25] *Teucrium pestalozzae* Bois[26] and *Perovskia scrophulariifolia* Bunge[27] aerial parts, and *Abies cilicica* subsp. *cilicica* (Ant. et Kotschy) Carr. young shoots.[28] It was claimed to be identified by mass spectral comparison (GC–MS, but no isolation) as a constituent of essential oils from *Mentha pulegium* L. aerial parts,[29] *Zingiber neesanum* (Graham) Ramamoorthy[30] and *Zingiber zerumbet* (L.) Smith rhizome.[31] However, the latest reinvestigation of *Saussurea lappa* Clarke fresh root essential oil and extract composition indicated that (–)-elemenal, along with (–)-β-elemene and (–)-elema-1,3,11(13)-trien-12-ol, is most possibly a heat-induced artefact formed from the corresponding germacrane derivatives by Cope rearrangement during drying of the roots, and/or manufacture of the oil and/or GC analysis.[24]

Although a large amount of information can be gleaned by a visual analysis of a series of spectra obtained from incremental additions of LSR, the information could be sometimes more conveniently expressed in graphical form, usually as a plot of induced shift *vs.* the ratio of [LSR]/[substrate] and generally good linear correlation is noted for the range 0.2-0.6 mole

ratio.[2] The slope of the plot is called shift gradient (ΔEu) and its value is generally greater if a proton is near to the coordination site of LRS. In our case, the highest value of the induced chemical shift was observed for the protons from the aldehyde group and C(7)-H group (at $\delta_C$ 36.5 and $\delta_H \approx 2.58$) with ΔEu of 7.55 and 4.95, respectively, followed by the protons from the conjugated $=CH_2$ group with ΔEu 1.39 and 2.13 for H-13a and H-13b protons, respectively (Fig. 6a, Table 1).

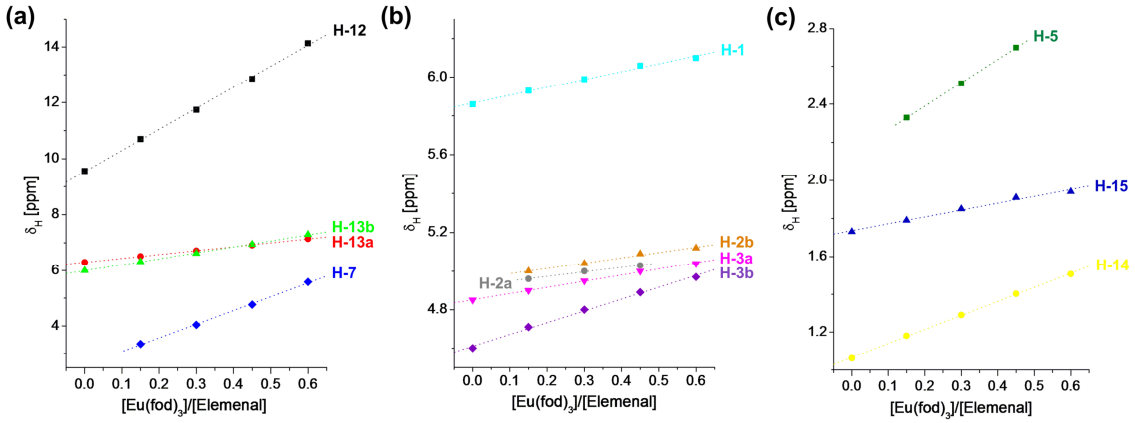


**Fig. 6**. The chemical shift *vs.* the ratio of the shift reagent and elemenal plots obtained from the incremental addition of $Eu(fod)_3$ to elemenal for: **(a)** the protons closest to the coordination site, **(b)** the remaining olefinic protons and **(c)** for the protons that resonated at the high field region.

The geminal protons H-13a and H-13b displayed a so-called "signal crossover" phenomenon, since the induced shift plot of H-13b crossed over the plot of H-13a (Fig. 6a). Usually this occurrence could be explained by the fact that H-13b is closer to the binding site of $Eu(fod)_3$ when compared to H-13a.[1] However, according to its greater $\delta_H$, H-13a has already been assigned to be *cis* to the aldehyde group, and thus should be closer to the coordinating site of the paramagnetic cation and, thus, should feel a stronger secondary magnetic field and have a greater lanthanide-induced shift. Moreover, previous studies on methacrylaldehyde indeed showed that a proton *cis* to the aldehyde group had a somewhat greater lanthanide-induced shift.[32,33] As there are two possible conformers for this system (*s-cis* and *s-trans* around C-11-C-12), if the methacrylic moiety were to adopt the *s-trans* conformation, $Eu(fod)_3$ bonded to the oxygen atom would be placed further away and this would result in a lesser effect of the secondary magnetic field on H-13a (this orientation of the aldehyde group is in agreement with the NOESY cross-peak observed for CHO proton and H-13a in the shifted spectra).

Another conformational issue that can be inferred from the shifted spectra is the relative orientation around C-11-C-7 bond. Since the influence of the secondary magnetic field falls away sharply with distance, the drastically higher ΔEu value (4.95) of H-7 compared to the methylene protons H-13a and H-13b (1.39 and 2.13; Fig. 6a, Table 1) suggests that elemental-$Eu(fod)_3$ complex should adopt such a conformation (*s-trans* around C-11-C-12 and a *syn* orientation of H-7 and CHO around C-11-C-7) in which this proton (H-7) is in very close proximity to the paramagnetic ion (Fig. 5c). Moreover, another pro argument for this conformation is that the methylene proton H-3b also felt a stronger influence of the paramagnetic ion than its geminal proton H-3a (Fig. 6b; Table 1), having almost two-fold higher ΔEu value, and being possible only in the *s-trans-syn* conformation

orienting $Eu(fod)_3$ towards the mentioned isopropenyl group. However, these conclusions are strictly valid only for the europium complex, not for the free aldehyde since the observed anisotropic influence of CHO on H-13a is only possible in the *s-cis* conformation.

Generally, it is believed that with lanthanides a small degree (*ca*. 1%) of contact shift (*e.g*. a through-bonds effect) is usually possible, particularly for protons attached to the carbons nearest the lone-pair-bearing atoms.[3] Thus, the second part of the explanation of this unusual phenomenon (H-13b *trans* to the aldehyde group in an α,β-unsaturated carbonyl system has a higher ΔEu value than the *cis* H-13a) could be the greater degree of contact shift for H-13b in our LSR adduct. It has been suggested that a contact shift is significant for aromatic systems where the presence of conjugation may increase the electron delocalization, thus increasing the degree of contact contribution to the observed shift of resonances for the protons throughout the molecule.[3] In a similar manner, electron spin density could be transferred through a properly oriented (zig-zag) σ-system electrons of an α,β-unsaturated carbonyl system, as present in the *s-trans* conformation in our case, and affect more the proton *trans* to the aldehyde group due to better orbital overlap (Fig. 5c).

Plotting the spectral data obtained during the incremental addition studies turned out to be additionally advantageous. For example, $\delta_H$ values from clustered, highly overlapped signals (no LSR present) could be estimated by extrapolation of proton plots to $[Eu(fod)_3]/[substrate] = 0$.[1] In fact, $\delta_H$ values for protons H-2a, H-2b, H-5 and H-7 could probably be determined more accurately from the *y*-axis cut-off of the proton plots (Fig. 6; Table 1) than from $^1H$ NMR spectrum shown in Fig. 2a, or from 2D spectra, since the precise chemical shift positions of these proton signals are uncertain either due to mutual overlap of these signals or the presence of signals arising from the impurities (Fig. 2b).

As mentioned above, elemanes are believed to be formed by a Cope rearrangement of the corresponding germacranes. In solution, germacranes could adopt any of the four distinct conformations that allow a [3,3]-sigmatropic rearrangement to occur, namely UU, UD, DU, and DD with an assumption that the isopropenyl or related substituent is large enough to ensure its equatorial or pseudo-equatorial position on the cyclodecadiene ring (Fig. 7).[34] Cope rearrangement is a stereospecific reaction that generally proceeds *via* a chair-like transition state and this geometric requirement is fulfilled in UU or DD forms of the specific germacranes.[24] Both experimental data and computational studies point to the UU (up-up) conformation, in which the two methyl groups and the pseudo-equatorial substituent adopt positions on the top face of the crossed cyclodecadiene ring, as the most stable one and predominant in the conformational equilibrium.[34]

Thus, the Cope rearrangement should preferably proceed *via* this conformation and it is considered that the "naturally occurring" β-elemene (and its derivatives as well) adopt a chair conformation with the relative stereochemistry of the groups on the cyclohexane ring that was governed by the stereochemistry in the starting germacrene and the geometric demands of the cyclic transition state (Fig. 7).[24,35] The main destabilizing factor in this proposed conformation (designated as **Conf-1**) for β-elemene and its derivatives is the *syn*-pentane interaction between the angular methyl group and the methyl group from the isopropenyl substituent (Fig. 8a). Our MM2 calculations for elemenal revealed that the destabilizing effect of the *syn*-pentane interaction would be decreased for *ca*. 2 kcal $mol^{-1}$ if $=CH_2$ end of the isopropenyl group was oriented in parallel to the angular methyl group (as in conformer **Conf-2**; Fig. 8b). Conversely, NOESY cross-peaks between the mentioned methyl groups, as well as the occurrence of a four-bond vinyl-allylic proton spin-coupling, between H-5 and H-3b (H-3b is a pseudo-doublet quintets; Fig. 5b; Fig. 8d; Table 1), and not H-5 and H-3a (H-3a is a pseudo-quintet), clearly point to the orientation of the isopropenyl group as in conformer **Conf-1**, *i.e.* a U relationship between H-5 and H-3b. Furthermore, in the more stable conformer **Conf-2** only a W-type coupling of the allylic H-5 and the vinyl H-3a proton

is expected ($^4J_\sigma$ > 0 Hz). Conformer **Conf-1** does not permit this coupling to be observed ($^4J_{\sigma,\pi} \approx 0$ Hz) as the dihedral angle θ between these vinyl and allylic C-H bonds is very close to 180º (175.7º).[36]

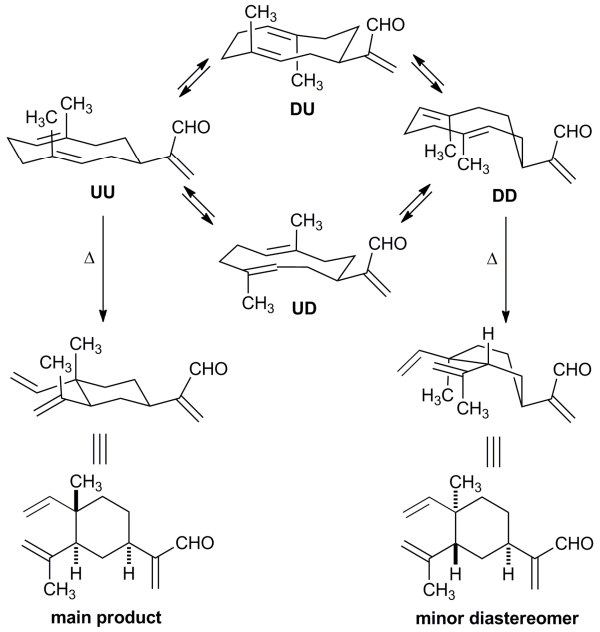


**Fig. 7.** Possible conformations of germacradienal fixed by the equatorial or pseudo-equatorial position of the relatively large substituent in the position 7 of the 10-membered ring. The conformers are denoted as UU, UD, DU, and DD in reference to the U (up) and D (down) orientations of C-14 and C-15 methyl groups. Both UU and DD conformations could undergo Cope rearrangement, however, the predominant UU conformation would yield the main stereoisomer of elemenal, whereas the less stable DD conformation would give the minor diastereoisomer of elemenal.

Interestingly, in the both mentioned conformers so far (**Conf-1** and **Conf-2**; Figs. 8a and 8b), MM2 calculations predict, as the most energetically favorable, the *s-trans* conformation of the methacrylic fragment and the *anti*-orientation of the CHO group with respect to H-7 on the cyclohexane ring. However, a noted small vinyl-allylic coupling ($J$ = 0.7 Hz) between H-13a (*cis* to the aldehyde group) and H-7 in both shifted and non-shifted spectra implies that the aldehyde group is oriented *syn* to H-7 (conformer **Conf-3**; Fig. 8c) in both the free aldehyde and the europium complex. **Conf-3** displays a value of the dihedral angle θ between the vinyl and allylic C-H bonds very close to zero ($\theta$ = 0.9º), so a characteristic W-coupling should be expected. On the other hand, conformers **Conf-1** and **Conf-2**, with the corresponding dihedral angle close to 180º (179.4º), do not support this coupling (the value of $^4J_{\sigma,\pi}$ should be close to zero in this case). Additionally, conformers **Conf-1** and **Conf-2** should give rise to U-coupling of H-13b (*trans* to the aldehyde group) and H-7 (Figs. 8a and 8b) which is not observed. Furthermore, in the *anti*-orientation of the aldehyde group (**Conf-1** and **Conf-2**), the distance between H-13a and H-7 from the aldehyde oxygen atom are almost the same, (3.8 Å and 3.9 Å, respectively), and these protons should, thus, experience a very similar effect of the secondary magnetic field. However, as mentioned above the very opposite was noted since the experimentally determined ΔEu values for these protons differed significantly. As previously put forward, the greater magnitude of the induced shift for H-7 could be only explained by the *s-trans-syn* conformation of the

elemenal-Eu(fod)$_3$ complex in which this proton is in close proximity to the paramagnetic ion and, indeed, according to MM2 calculations, the proton H-7 should be considerably closer than H-12a to the coordinating site (2.3 Å compared to 3.8 Å; **Conf-3**, Fig. 8c).

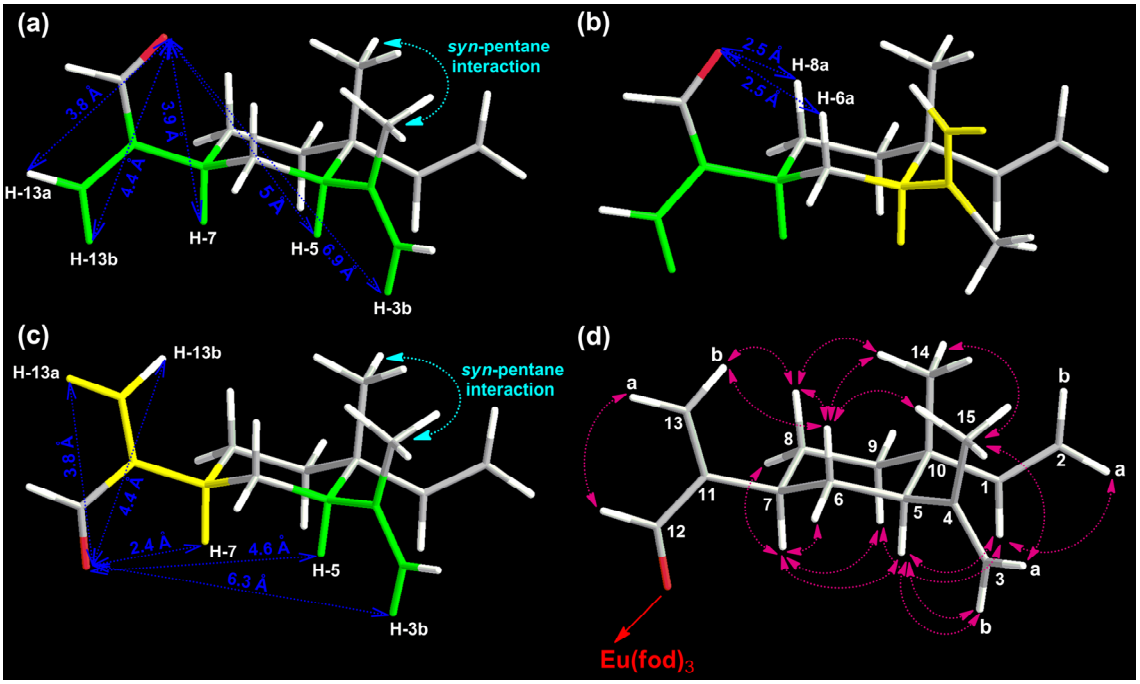


**Fig. 8.** Plausible conformations of elemenal denoted throughout the text as **Conf-1** (**a**), **Conf-2** (**b**) and **Conf-3** (**c**) with the indicated distances of certain protons to the aldehyde oxygen atom, as well as, the observed NOESY cross-peaks presented on conformation **Conf-3** that is most likely adopted in the elemenal-Eu(fod)$_3$ complex (**d**). The possible vinyl-allylic W-couplings are marked in yellow, while U-couplings are marked in green color.

MM2 calculations also predict that conformer **Conf-3** should be only 0.5-0.6 kcal mol$^{-1}$ higher in strain energy than conformer **Conf-1**. Another fact which does not favor the predominance of **Conf-1**, in our case, is the relatively small magnitude of lanthanide-induced shift detected for axial H-6 and H-8. These shifts should be quite higher since the distance of these protons from the Eu(fod)$_3$ binding site is around 2.5 Å in **Conf-1** (Fig. 8a). Similar considerations stand for other protons (Table 1; Fig. 8), as well. Thus, the herein presented experimental data (vinyl-allylic couplings, NOESY cross-peaks and ΔE values) unequivocally support **Conf-3** as the major conformer of elemenal-Eu(fod)$_3$ complex.

In order to additionally justify the conclusions regarding the stereochemistry of the elemenal-Eu(fod)$_3$ complex, we performed a conformational analysis using the lanthanide probe method.[37,38] An internal Cartesian coordinate was set up with the carbonyl oxygen at the origin while the C=O bond defined the negative *z*-axis. Subsequently, the location of the europium ion could be specified by the bond length Eu–O ($r_o$), the bond angle Eu–O–C(12), ($\alpha_o$) and the dihedral angle Eu–O–C(12)–C(13) ($\beta_o$).[37] Throughout the calculation, the optimal europium ion position, the substrate coordinates, as well as its geometry (as in **Conf-3**), were kept constant and the europium ion was allowed to move (to give the best match with the experimental data), *i.e.* its coordinates were changed incrementally using the ChemBio 3D Ultra 12.0 software package. The value of the dihedral angle was set to 180° in order to ensure the co-planarity of europium and the methacrolein moiety (that was mandatory for a good electron spin density transfer), while the bond angle $\alpha_o$ was varied from

110° to 130° (to provide minimal deviations from the properly zig-zag oriented σ-system) and the bond length $r_o$ was in the range between 2.30 and 2.56 Å. For each location of the lanthanide ion, the variable geometrical factors $(3\cos^2\theta_i - 1)/r_i^3$ in the McConnell-Robertson equation were calculated for all the observed nuclei ($i$) of the substrate (where $r_i$ is the distance between the lanthanide ion and the $i$-th nucleus, and $\theta_i$ is the angle between the vector corresponding to $r_i$ and the vector $r_o$ representing the Ln–coordination center bond). Then the calculated values ($\Delta_{cal}$) for all tested europium positions were plotted against the observed values ($\Delta$Eu).[38] The highest correlation coefficient ($R^2 = 0.9980$; Fig. S2) was found when europium was located at $r_o$ = 2.328 Å, $\alpha_o$ = 120° and $\beta_o$ = 180° (Fig. S3). Since the McConnell-Robertson equation can be applied only to nuclei where contact interactions are negligible,[38] the values for H-12, H-13a and H-13b were excluded from these fittings (*e.g.* this pseudocontact model predicted a higher induced shift value ($\Delta_{cal}$) for H-13a when compared to H-13b, while the experimental values were the other way around). The observed induced shift values ($\Delta$Eu) for protons H-12, H-13a and H-13b could be regarded as to represent the combination (in the first approximation, a simple sum) of the dipolar or pseudocontact ($\Delta Eu_{dip}$) and contact ($\Delta Eu_{contact}$) terms. Thus, an estimation of the share of the contact and dipolar contributions for these protons could be performed based on their $\Delta_{cal}$ values and the linear regression equation $\Delta Eu_{dip} = -0.28 + 251.82 \times \Delta_{cal}$ obtained for the most likely conformation (Fig. S3). In this way, we found that, for example, for proton H-13b a significant fraction of the observed induced chemical shift ($\Delta Eu_{total} = 2.13$) could be attributed to the contact interaction ($\Delta Eu_{contact} \approx 0.62$).

The instability of germacrenes, *i.e.* its susceptibility to heat-induced (*e.g.* steam distillation or high temperature drying of plant material) Cope rearrangement which yield the corresponding elemenes, is one of the main reasons terpenes with an elemane skeleton are considered to have an artefactual origin and not a natural one.[24] We found that elemenal, isolated from *I. helenium* root essential oil, did not adopt the most stable conformation (**Conf-2**). The established conformation (**Conf-3**) displays the orientation of the angular methyl group and methyl group from the isopropenyl substituent on the cyclohexane ring which strictly reflects the spatial relationship of these groups on the 10-membered ring in the most stable conformation (UU) of the corresponding germacradiene from which Cope rearrangement most probably occurred. Interestingly, there were no peaks in NMR spectra that supported the existence of elemenal (even in a small percentage) in the most stable conformation (**Conf-2**), most probably because of the high energy barrier for the rotation around C-4–C-5 bond. Furthermore, de Kraker and co-workers[24] found that during the heating of germacradienal, a small amount of another artefact which is a diastereomer of elemenal was also formed and this was explained by the fact that Cope rearrangement had also occurred through the less stable DD conformation. This diastereomer of elemenal was also noted in sample **A** after a closer inspection of the TIC chromatogram (RI = 1546). All these facts go in favor that elemenal is highly related to the corresponding germacradienal but these do not exclude either a thermal or a biosynthetic link.

Interestingly, β-elemene is widely considered as a potential novel natural anticancer plant drug and some formulations for pharmacological uses based on this compound have been patented and are currently in application for clinical studies in the United States.[39] A recent study revealed that β-elemenal was appreciably more potent than β-elemene in suppressing nonsmall cell lung cancer growth and proliferation. Thus, β-elemenal may have great potential as an anticancer alternative to β-elemene in treating lung cancer and other tumors.[40]

## Conclusion

In conclusion, herein, we reported on the identification of a rare sesquiterpene elemenal directly from minute amounts of a complex mixture (without the availability of a pure sample of the aldehyde) achieved by the aid an NMR titration of the mixture with a lanthanide-induced shift reagent. The incremental addition of $Eu(fod)_3$ led to a simplification of NMR spectra in terms of signal overlap and removal of chemical shift degeneracy, allowing the mining of crucial data from the shifted NMR spectra. 2D-NMR spectra ($^1H$–$^1H$-COSY, NOESY, HSQC and HMBC) of the sample mixed with $Eu(fod)_3$ proved to be particularly valuable in this respect. The addition of the shift reagent shortened the time needed to acquire $^{13}C$ NMR and 2D spectra from 5 mg of the mixture, while it did not cause any serious line broadening or loss of nOe. Alongside the total NMR assignments of elemenal, the data obtained from the shifted spectra enabled a detailed assessment of the conformation that this sesquiterpene aldehyde adopts in its complex with $Eu(fod)_3$. Providing this much data, this new approach in structure elucidation promises to be of great help in the NMR analysis of complex samples comprising of several compounds.

## Experimental section

### Chemicals and reagents

All chemicals and solvents were of analytical grade and used without further purification. Diethyl ether, *n*-hexane, anhydrous magnesium sulphate, neryl isobutanoate, geraniol, isobutanoic acid, *tris*(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III) ($Eu(fod)_3$), tetramethylsilane and deuterated chloroform were purchased from Sigma-Aldrich (St Louis, MS, USA). Dicyclohexylcarbodiimide and 4-dimethylaminopyridine were supplied by Merck (Darmstadt, Germany). Geranyl isobutanoate was synthesized from geraniol and isobutanoic acid by a Steglich procedure, utilizing dicyclohexylcarbodiimide and 4-dimethylaminopyridine.[41] Chromatographic separations were carried out using silica gel 60 (particle size distribution 20–45 μm) obtained from Carl Roth GmbH + Co.KG (Karlsruhe, Germany).

### Plant material

Roots of *I. helenium* were collected in the beginning of April 2014 in vicinity of the town Svrljig in South-eastern Serbia. A voucher specimen was deposited with the Herbarium of the Faculty of Science and Mathematics, University of Niš, under the accession number GM0114.

### Essential oil – extraction and fractionation

*Inula helenium* root essential oil was obtained by hydrodistillation (air-dried plant material) using the original Clevenger-type apparatus according to a previously described method.[16] The yield of the essential oil was 1.3% (w/w).

A sample of the oil (2.1 g) was subjected to "dry flash" column chromatography on silica gel (particle size 20–45 μm). Pure *n*-hexane (100 mL) was used as the eluent for the first three fractions (**I-III**), followed by 1% (v/v) diethyl ether in *n*-hexane (fraction **IV**, 100 mL), 2% (v/v) diethyl ether in *n*-hexane (fractions **V** and **VI**, 100 mL) and finally pure diethyl ether (fraction **VII**, 200 mL). The solvent was removed *in vacuo* and the obtained fractions submitted to GC–MS analyses. Fractions **III** and **IV** contained the unknown sesquiterpene aldehyde as a major component and were polled together (sample **A**, 4.8 mg).

**Elemenal**: RI (DB-5) = 1578; MS (EI, 70 eV), *m/z* 218 ($M^{+\cdot}$, 2.1%), 217 (0.6, M – H), 203 (14.9, M – $CH_3$), 200 (3.5, M – $H_2O$), 189 (9.6, M –CHO), 175 (15.5), 161 (28.7), 147 (21.7), 133 (18.8), 121 (27.1), 119 (28), 107 (37.7), 105 (34.6), 95 (38.6), 93 (47), 91 (52), 81 (100), 79 (64.3), 77 (34.1), 68 (35), 67 (60.6), 55 (32.7), 53 (40.9), 41 (49.7), 39 (28.2). For $^{1}H$ and $^{13}C$ NMR data see Table 1.

**Geranyl isobutanoate**: RI (DB-5) = 1506; MS (EI, 70 eV), *m/z* 224 ($M^{+\cdot}$, 0.2%), 181 (0.1, M – $C_3H_7$), 154 (2.3), 136 (16.7), 121 (29), 107 (8.2), 93 (46.1), 80 (19.8), 69 (100, $(CH_3)_2C{=}CCH_2$), 53 (9.4), 41 (41); $\delta_H$ (400 MHz; $CDCl_3$; $(CH_3)_4Si$) 1.16 (6H, d, $J$ = 7.0 Hz, $(CH_3)_2CHC{=}O$), 1.60 (3H, br s, -$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 1.68 (3H, br d, $J$ = 1.0 Hz, -$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 1.70 (3H, br s, -O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 2.01– 2.07 (2H, m, -O-$CH_2$-CH=C($CH_3$)-$CH_2$), 2.07–2.15 (2H, m, -$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 2.54 (1H, hept, $J$ = 7.0 Hz, $(CH_3)_2CHC{=}O$), 4.58 (2H, br d, $J$ = 7.0 Hz, -O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 5.08 (1H, pseudo-t hept, $J$ = 6.8, 1.4 Hz, -$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$) and 5.33 (1H, pseudo-t sext, $J$ = 7.0, 1.3 Hz, -O-$CH_2$-CH=C($CH_3$)-$CH_2$-); $\delta_C$ (101 MHz; $CDCl_3$; $(CH_3)_4Si$) 177.3 ($(CH_3)_2CHC{=}O$), 142.0 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 131.9 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 124.0 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 118.8 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 61.4 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 39.7 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 34.2 ($(CH_3)_2CHC{=}O$), 26.5 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 25.8 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 19.2 ($(CH_3)_2CHC{=}O$), 17.8 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$) and 16.6 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-).

**Neryl isobutanoate**: RI (DB-5) = 1482; MS (EI, 70 eV), *m/z* 224 ($M^{+\cdot}$, 0.2%), 181 (0.1, M – $C_3H_7$), 154 (2.2), 136 (13.9), 121 (34.1), 107 (8.6), 93 (68.3), 80 (26.8), 69 (100, $(CH_3)_2C{=}CCH_2$), 53 (9.9), 41 (53.1); $\delta_H$ (400 MHz; $CDCl_3$; $(CH_3)_4Si$) 1.16 (6H, d, $J$ = 7.0 Hz, $(CH_3)_2CHC{=}O$), 1.60 (3H, br s, -$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 1.68 (3H, br s, -$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$ ), 1.76 (3H, pseudo-q, $J$ = 1.3 Hz, -O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 2.03–2.15 (4H, m, -$CH_2$-$CH_2$-), 2.53 (1H, hept, $J$ = 7.0 Hz, $(CH_3)_2CHC{=}O$), 4.56 (2H, br d, $J$ = 7.2 Hz, -O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 5.10 (1H, pseudo-t hept, $J$ = 6.9, 1.4 Hz, -$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$) and 5.35 (1H, pseudo-t sext, $J$ = 7.2, 1.3 Hz, -O-$CH_2$-CH=C($CH_3$)-$CH_2$-); $\delta_C$ (101 MHz; $CDCl_3$; $(CH_3)_4Si$) 177.3 ($(CH_3)_2CHC{=}O$), 142.4 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 132.2 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 123.8 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 119.6 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 61.1 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 34.2 ($(CH_3)_2CHC{=}O$), 32.4 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 26.8 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 25.8 (-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$), 23.6 (O-$CH_2$-CH=C($CH_3$)-$CH_2$-), 19.2 ($(CH_3)_2CHC{=}O$) and 17.8(-$CH_2$-CH=C($CH_3^{cis}$)$CH_3^{trans}$).

## Analytical and spectral analyses

**GC–MS analyses.** The GC–MS analyses of all samples (the essential oil and chromatographic fractions, standards) were repeated three times using a Hewlett-Packard 6890N gas chromatograph. The gas chromatograph was equipped with a fused silica capillary column DB-5 (5% phenylmethylsiloxane, 30m × 0.25 mm, film thickness 0.25 μm; Agilent Technologies, Santa Clara, CA, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 and 300 °C, respectively. The oven temperature was raised from 70 to 290 °C at a heating rate of 5 °C/min and then isothermally held for 10min. Helium at 1.0 mL/min was used as a carrier gas. The samples, 1 μL of the corresponding solutions in diethyl ether (1:10, w/v), were injected in a pulsed split mode (the flow was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40:1). The mass selective detector was operated at the ionisation energy of 70 eV, in the 35–500 amu range, with a scanning speed of 0.34 s.

**NMR measurements.** All NMR spectra were recorded at 27 °C in deuterated chloroform with tetramethylsilane as the internal standard. Chemical shifts ($\delta$) are reported in parts per million and referenced to tetramethylsilane ($\delta_H$ = 0 ppm) in $^1$H NMR spectra and/or to solvent protons (deuterated chloroform: $\delta_H$ = 7.25 ppm and $\delta_C$ = 77 ppm) in heteronuclear 2D spectra. Scalar couplings are reported in hertz (Hz). Samples (10 mg for geranyl and neryl isobutanoates, and a 4.8-mg fraction containing elemenal) were dissolved in 1 mL of deuterated chloroform, and 0.7 mL of the solution transferred into a 5 mm Wilmad, 528-TR-7 NMR tube.

The $^1$H- and $^{13}$C NMR spectra were recorded on a Bruker Avance III 400 MHz NMR spectrometer (Fällanden, Switzerland; $^1$H at 400 MHz, $^{13}$C at 101 MHz), equipped with a 5-mm dual $^{13}$C/$^1$H probe head. The $^1$H spectra were recorded with 16 scans, 1 s relaxation delay, 4 s acquisition time, 0.125 Hz digital FID resolution, 51 280 FID size, with 6410 Hz spectral width, and an overall data point resolution of 0.0003 ppm. The $^{13}$C spectra were recorded with Waltz 161H broadband decoupling, 12000 scans, 0.5 s relaxation delay, 1 s acquisition time, 0.5 Hz digital FID resolution, 65536 FID size, 31850 Hz spectral width, and an overall data point resolution of 0.005 ppm. Standard pulse sequences were used for 2D spectra. $^1$H–$^1$H gDQCOSY and NOESY spectra were recorded at spectral widths of 5 kHz in both F2 and F1 domains; 1 K × 512 data points were acquired with 32 scans per increment and the relaxation delays of 2.0 s. The mixing time in NOESY experiments was 1 s. Data processing was performed on a 1 K × 1 K data matrix. Inverse detected 2D heteronuclear correlated spectra were measured over 512 complex points in F2 and 256 increments in F1, collecting 128 (gHMQC) or 256 ($^1$H–$^{13}$C gHMBC) scans per increment with a relaxation delay of 1.0 s. The spectral widths were 5 and 27 kHz in F2 and F1 dimensions, respectively. The gHMQC experiments were optimized for C–H couplings of 125 Hz; the $^1$H–$^{13}$C gHMBC experiments were optimized for long-range C–H couplings of 10 Hz. Fourier transforms were performed on a 512 × 512 data matrix. $\pi/2$ Shifted sine-squared window functions were used along F1 and F2 axes for all 2D spectra.

Eu(fod)$_3$ was used as the lanthanide shift agent and four equimolar increments of Eu(fod)$_3$ were added to a 0.015 mol/dm$^3$ solution of the substrate in deuterated chloroform. The molar ratio of Eu(fod)$_3$ to elemenal was estimated to be in the range from 0 to 0.6. The reagents were dissolved by shaking and the spectra were recorded at 27 °C after dissolution.

## Computational methods

Geometry optimizations and calculation of the (thermodynamic) properties of elemenal were performed using the MM2 molecular mechanics force field method incorporated in ChemBio 3D Ultra 12.0 software package.

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