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Short communication

Separation of 18α(H)-, 18β(H)-oleanane and lupane by comprehensive two-dimensional gas chromatography

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ABSTRACT

18α(H)-, 18β(H)-oleanane and lupane are angiosperm-derived biomarkers that are used as age indicators for the Late Cretaceous onwards when the first proliferation of angiosperms occurred. In addition, the 18α(H)-/18β(H)-oleanane ratio is employed as a thermal maturity parameter of crude oil. However, evidence has shown that accurate quantification of these compounds has been impeded by inadequate chromatographic separation by traditional one-dimensional gas chromatography. In this study, we present the separation of 18α(H)-, 18β(H)-oleanane and lupane with comprehensive two-dimensional gas chromatography (GC × GC). Furthermore, it was observed that 18β(H)-oleanane elutes earlier than 18α(H)-oleanane in second dimension (polarity) which we attribute to steric hindrance effects. Two GC conditions have been developed in order to achieve baseline separation of the triterpenoids of interest in complex mixtures such as sediment extracts and crude oils.

1. Introduction

18α(H)-, 18β(H)-oleanane and lupane are important age diagnostic biomarkers for petroleum as well as sediment geochemistry. They are derived from precursors (betulin, amyrin and other pentacyclic triterpanes [1,2], which are known to occur almost exclusively in angiosperms (flowering plants) [3 and references therein]. Angiosperms became prominent in the Late Cretaceous/Early Tertiary. Therefore, the presence of oleananes or lupane in an oil or bitumen provides valuable information about both the sample's terrestrial source and geologic age.

The separation and identification of biomarkers such as oleanane and lupane are challenging as a result of their usually low abundance in complex mixtures like crude oils. Particularly isomers like 18α(H)- and 18β(H)-oleanane, which are identical in chemical composition and just differ in their structure sterically usually elute at very similar retention times, further complicating their identification and quantification. 18α(H)-, 18β(H)-oleanane and lupane co-elute on traditional non-polar gas chromatographic (GC) columns (e.g. stationary phase of 5% phenyl-, 95% dimethylpolysiloxane) [4].

Comprehensive two-dimensional gas chromatography (GC × GC) is a very powerful technology for a broad range of applications. The separation power relies on the coupling of two gas chromatographic columns which separate based on different chemical characteristics, for instance boiling point on a non-polar column and polarity on a polar column (e.g. stationary phase of 50% phenyl-, 50% dimethylpolysiloxane). Commonly, the GC × GC system is connected to either a flame ionisation detection (FID) or a time-of-flight mass spectrometer (TOFMS) due to their ability to acquire data at a fast acquisition rate–typically 50–200 data points or spectra per second. Using a GC × GC–TOFMS system provides an extra dimension of separation.

The application of GC × GC to environmental problems emerged within the past ten years [5]. Nelson et al. [6] investigated the weathering of oil spills monitoring the continuous degradation of spilled crude oil over a period of six months. Reddy et al. [7] identified and quantified linear α-olefins (LAOs) and internal olefins (IOs) which are synthetic oil-based drilling fluids and can occur as contaminants in crude oils. GC × GC was also applied to fin-

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gerprinting of common petroleum biomarkers such as hopanoids, naphthalenes and phenanthrenes [8], biodegradation features [9] and separation of selected biomarkers [10] in petroleum. Lemkau et al. [11] identified the ruptured tank responsible for the M/V Cosco Busan oil spill using GC × GC. Furthermore, the high resolution separation was applied to assess the degree of similarity between oils and to elucidate even minute differences between oils that can give evidence, for example, of compartmentalisation within oil reservoirs [12,13].

In this study, we describe the separation of the two oleanane isomers from lupane by GC × GC (TOFMS + FID).

2. Experimental

18α(H)-, 18β(H)-oleanane and lupane have been identified by co-injection of standards. The standards 18α(H)-oleanane (100 μg mL^{-1}), 18β(H)-oleanane (100 μg mL^{-1}) and lupane (100 μg mL^{-1}) were purchased from Chiron AS (Trondheim, Norway).

2.1. GC × GC–TOFMS/FID analysis

Two GC × GC systems were used, a GC × GC–TOFMS and a GC × GC–FID. Both were Leco Pegasus 4D systems equipped with a Hewlett-Packard 6890 GC (TOF-system) and a 7890 GC (FID-system), respectively. They were configured with a split/splitless auto-injector (7683B series), two capillary gas chromatography columns and a dual stage cryogenic modulator (Leco, Saint Joseph, Michigan). The modulator cold jet gas was dry N_2, chilled with liquid N_2. The thermal modulator hot jet air was heated to 55 °C above the temperature of the main GC oven. The first-dimension column was a non-polar Restek Rtx-1MS Crossbond, (25 m length (TOF)/20 m (FID), 0.20 mm I.D., 0.2 μm film thickness), whereas the second-dimension separations were performed on a 50% phenyl polysilphenylene-siloxane column (SGE BPX50, 1.25 m length (TOF)/1 m (FID), 0.10 mm I.D., 0.1 μm film thickness).

Tertiary crude oil samples for analysis on the GC × GC–TOFMS were dissolved in hexane. 3 μL of a 50 mg mL^{-1} solution were injected into a 300 °C splitless injector (0.5 min purge time). For GC × GC -FID analysis, 1 μL of a 4.5 mg mL^{-1} solution was injected under the same conditions. The first-dimension column and the dual stage cryogenic modulator resided in the main oven. The second-dimension column was housed in a separate oven. With this configuration temperature profiles of all three parts could be programmed independently.

Two GC × GC conditions have been developed and applied. Condition A was optimised for the best possible separation of the oleanane isomers and lupane while condition B was optimised for a more general application and screening of the crude oils without losing too much resolution of the three biomarkers in focus. In condition A, the temperature program in the main oven was ramped from 130 °C (10 min) to 315 °C at 1 °C min^{-1}. The modulation temperature offset was 55 °C with a modulation period of 6 s, a hot pulse time of 0.66 s and 2.34 s cooling time between stages. The second oven was programmed at 145 °C (10 min) to 330 °C at 1 °C min^{-1}. The carrier gas was helium (hydrogen for the FID system) at a constant flow rate of 1.05 mL min^{-1} (0.95 mL min^{-1} for the FID system).

The general temperature program of the second GC × GC condition (referred to as condition B) started isothermal at 45 °C (10 min) and then ramped from 45 to 317 °C at 1.25 °C min^{-1}. The hot jet was pulsed for 0.75 s every 10 s with a 4.25 s cooling period between stages. The second dimension oven was programmed from 68 °C (10 min) to 340 °C at 1.25 °C min^{-1}.

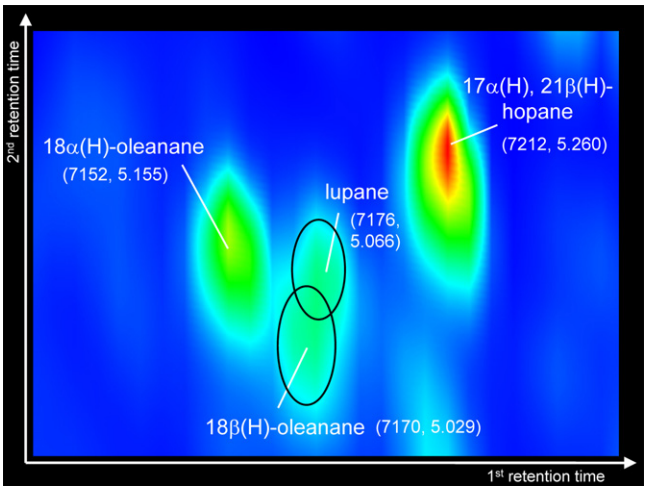

Fig. 1. GC × GC–FID chromatogram (section) of a Tertiary crude oil showing the separation of 18α(H)-oleanane, 18β(H)-oleanane and lupane applying GC × GC condition A. Different colours represent the peak abundance. With increasing intensity the peak colour changes from blue to green, to yellow, to red. Numbers in parentheses are first and second retention times in seconds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

The Leco TOFMS detector signal was sampled at a data rate of 50 spectra per second. The transfer line from the second oven to the TOFMS was deactivated fused silica (0.5 m length, 0.18 mm I.D.) which was held at a constant temperature of 280 °C. The TOF source temperature was 225 °C and the detector voltage was 1525 V. The mass spectrometer employs 70 eV electron ionisation and operates at a push pulse rate of 5 kHz, allowing sufficient signal averaging time to ensure good signal-to-noise ratios while still operating at a high enough data acquisition rate to accurately process (signal average) spectra from the peaks eluting from the second dimension column in this high resolution separation technique with second dimension peak widths on the order of 50–200 ms. The FID signal was sampled at 100 Hz.

The relaxed geometries for 18α(H)-, 18β(H)-oleanane were calculated from first principles with the program GAUSSIAN09 [14]. The molecules were relaxed in vacuum using the Hartree-Fock exchange and the 6-31G** basis set for all the atoms.

3. Results and discussion

In a GC × GC chromatogram, compounds are grouped along the *x*-axis according to their carbon number and along the *y*-axis according to their polarity. Clear groupings of compound classes like alkanes, hopanoids, steroids or mono-, di- and triaromatics within the chromatogram plane spanned by the first and second retention time can be observed. Additionally, members of a homologous series can be easily identified as they form a line within the plane of the chromatogram. This way, simply knowing the relative retention times for the first and second dimension of an unknown compound provides first information about the structure of that compound.

Baseline separation of 18α(H)-, 18β(H)-oleanane and lupane in crude oils or rock extracts was achieved using both techniques, GC × GC–FID and GC × GC–TOFMS (Fig. 1). No liquid chromatographic separation of the oil into saturated and aromatic fraction prior to the analysis was required.

Two optimised conditions have been developed for two different applications: (1) the separation of the two oleanane isomers and lupane for quantification of all three compounds in one analysis

and (2) the separation of crude oils for fingerprinting with containing a reasonable separation of the plant biomarkers. Condition A is tailored to only those three biomarkers while the separation of all other compounds was sacrificed. Hence, another condition was developed to optimise the separation of all compounds. Both GC × GC conditions have been applied on a GC × GC–TOFMS as well as a GC × GC–FID system. It is advisable to use both systems – GC × GC–FID and GC × GC–TOFMS – since they complement each other. GC × GC–FID provides very clear and clean chromatograms with excellent peak shape due to the use of hydrogen as a carrier gas. Additionally, GC × GC–FID results in very similar responses for all hydrocarbons, allowing for reliable quantification without the availability of standards, given that the retention times of the compounds are known. The power of the GC × GC–TOFMS system relies on the accessibility of the mass spectrum for each peak, adding a fourth dimension to the separation. The mass spectrum of a compound in combination with the retention time information allows identification. However, the vacuum requirements of the mass spectrometer degrade the chromatographic peak shape. When comparing GC × GC–FID with GC × GC–TOFMS chromatograms, the GC × GC–TOFMS chromatogram appears to produce vertical peak tailing which is not noticeable in GC × GC–FID chromatography. These vertical tails are not chromatographic artefacts but instead are artefacts produced in the ionisation chamber. When a chromatographic analyte enters an FID that component is ionised instantaneously producing a Gaussian shaped chromatographic peak, however, the same is not true when the same component enters the ionisation chamber of a TOFMS system. In TOFMS, the chromatographic analytes are ionised *via* an electron ionisation source (filament) and the resulting cloud of ions is then electromagnetically focused towards the flight tube of the TOFMS. This is not as "instantaneous" as in an FID. In order to help clear the ionisation chamber as quickly as possible, a large turbo pump is positioned directly over the top of the ionisation chamber on GC × GC–TOFMS systems. This helps reduce the residence/clearance time of the cloud of ions in the ionisation chamber and improves the vertical peak tailing phenomenon dramatically. This phenomenon occurs in all mass spectrometric detectors but is not problematic or even noticeable in bench top GC–MS systems because in these systems, chromatographic peaks are generally on the order of 20–25 s in width. This phenomenon is much more noticeable in GC × GC systems because the second dimension peak width on these systems is generally on the order of 50–200 ms. Furthermore, GC × GC–FID is approximately five times more sensitive than the GC × GC–TOFMS. In order to obtain full mass spectra for relatively low abundant biomarkers like oleanane and lupane, compared to more dominant biomarkers such as *n*-alkanes, more material has to be injected onto the chromatographic column in GC × GC–TOFMS systems. Targeting the biomarkers that are present in very low abundances, the concentration used for the GC × GC–TOFMS analysis was about 30 times higher compared to the GC × GC–FID analysis. The increased concentration of sample material can further intensify the above described peak shape difference between FID and TOFMS systems. A decision on the system used should always be based on the application needed.

Application of GC × GC condition A results in excellent separation of the three compounds of interest, namely 18α(H)-, 18β(H)-oleanane and lupane (Fig. 1). Clean mass spectra of all three compounds from the GC × GC–TOFMS (Fig. 2) support baseline separation. The spectra show no interference from co-eluting peaks or column bleed in the form of additional fragments that are not genuine to the compounds. Separation and identification of 18α(H)- and 18β(H)-oleanane by GC–MS were reported previously [15,16]. However, the additional presence of the co-eluting lupane in many samples was overseen in traditional GC–MS chromatograms due to its similar retention time and mass spectrum with only one additional fragment of *m*/*z* 369, which is derived from the *iso*propyl side chain (Fig. 2). Nytoft et al. [4] showed that many of the identifications and quantifications of oleanane in Tertiary deltaic oils have to be reconsidered as the peak eluting adjacent to 17α(H), 21β(H)-hopane had been attributed to oleanane. The overseen presence of lupane has falsified the results of α/β-oleanane ratios in the past. Nytoft et al. [4] presented the separation of lupane from the oleanane isomers using a polar column (polyethylene glycol phase) but the 18α(H)- and 18β(H)-oleanane could not be separated in the same analysis. Application of GC × GC–TOFMS coupling a non-polar column and a polar column enables the separation of all three compounds to baseline in one analysis and to assess their abundance in complex mixtures such as crude oils and rock extracts.

The 18α(H)-/18β(H)-oleanane ratio is a thermal maturity parameter with 18β(H)-oleanane being the naturally occurring but thermally less stable isomer, which gradually undergoes β → α isomerisation [16,17]. Equilibrium probably occurs before peak oil generation. Riva et al. [16] observed a maximum value for the 18α(H)-/18β(H)-oleanane ratio of 2 which suggests an equilibrium mixture with twice as much 18α(H)-oleanane as 18β(H)-oleanane. However, Armanios [18] suggested that 18α(H)-oleanane is derived *via* hydrogenation of 18α(H)-olean-12-ene in rather immature samples. He found olean-18-ene to be reduced at a faster rate forming 18β(H)-oleanane, while 18α(H)-olean-12-ene reacts at a slower rate yielding 18α(H)-oleanane. Upon further maturation 18β(H)-oleanane may either be preferentially destroyed, or may isomerise to the more thermally stable 18α(H)-oleanane. The separation of lupane and the oleanane isomers by applying GC × GC allows further study of the oleanane ratio as a maturity parameter in geologic samples without the error posed by the underlying lupane.

For the development of our second GC × GC condition, condition B, we focused on a better separation of crude oil for a thorough identification of major compound classes, detailed fingerprinting of oils, and identification and quantification of specific biomarkers of interest. Aspects such as "wrapping" of highly polar compounds into the next modulation period had to be considered in the development of this condition. A shorter modulation period improves the separation of compounds. However, highly polar (5 or 6 aromatic rings) compounds require a minimum time to elute from the second chromatographic column or they will be carried over into the next modulation period. This falsifies their first and second dimension retention times, their position within the chromatogram plane, and can cause co-elution. Applying condition B, 18α(H)- and 18β(H)-oleanane are separated to baseline. 18β(H)-oleanane and lupane are not baseline separated but only co-elute slightly (Fig. 3). This condition is very suitable as a standard condition for crude oils as it results in good separation of most biomarkers. Separation of a number of isomers could also be achieved that was not possible using other techniques like traditional GC–MS. Relatively long retention times apply to the three compounds for both conditions (Figs. 1 and 3) increasing the time for each analysis as well as the costs for consumables such as liquid nitrogen.

The improved separation of the biomarkers of interest in this study, 18α(H)-and 18β(H)-oleanane and lupane, is based on the two-dimensional features of GC × GC. That is, they are not just separated in the first, non-polar dimension but additionally in the second, polar dimension. Hence, this technique is called "comprehensive"; all material injected onto the first column is separated on the second dimensional column, too. This is especially true for 18β(H)-oleanane, which elutes earlier in the second dimension than 18α(H)-oleanane. This is interesting as 18α(H)- and 18β(H)-oleanane, being diastereomers, are both considered non-polar compounds and were therefore not expected to behave

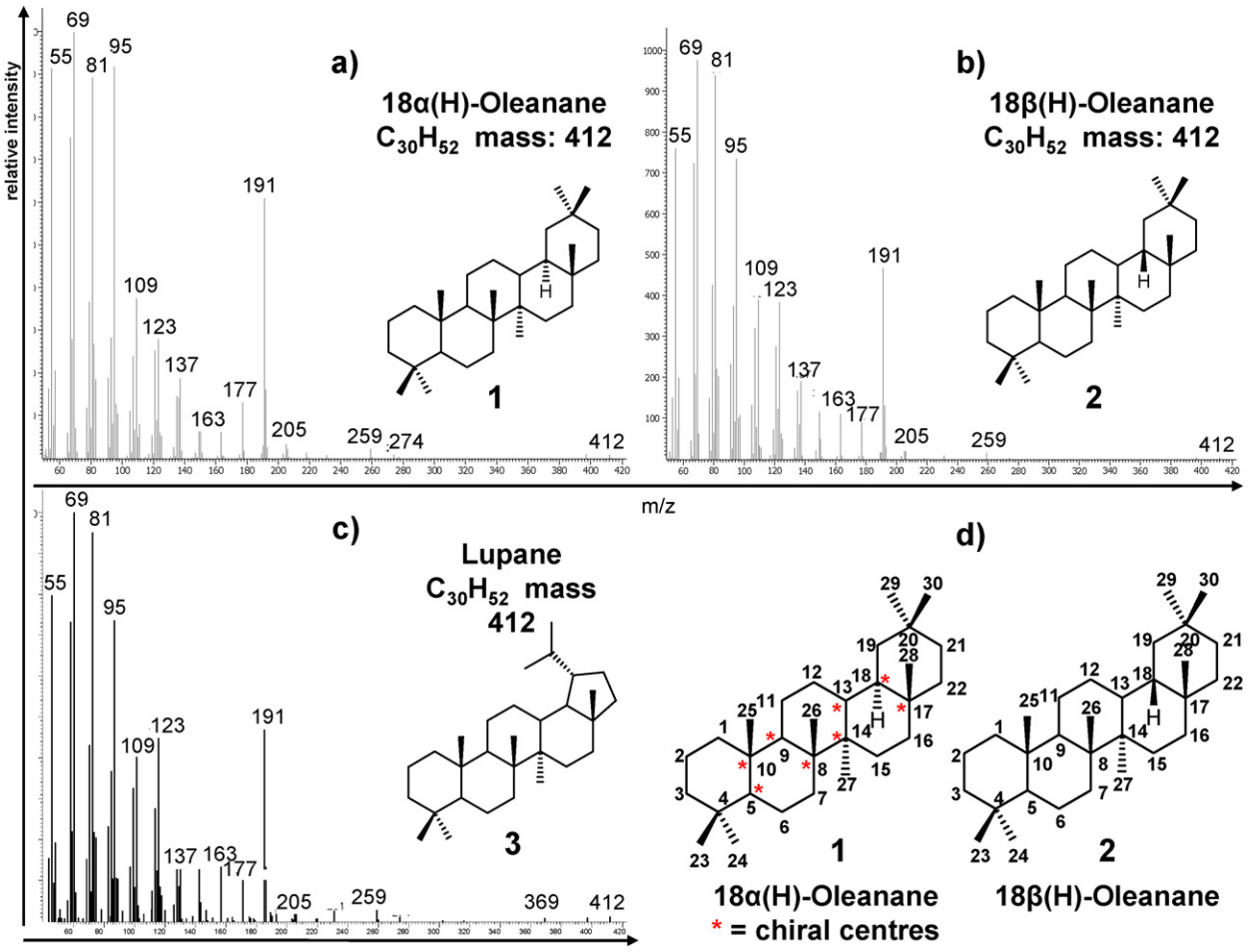

Fig. 2. GC × GC–TOFMS mass spectra of the separated peaks of (a) 18α(H)-oleanane, (b) 18β(H)-oleanane and (c) lupane. The numbering of the carbon atoms in oleanane is shown in (d). Asterisks indicate chiral centres. Note the only significant difference in the mass spectra of oleanane and lupane is the small *m*/*z* 369 fragment which is attributed to the loss of the *iso*propyl group in the lupane structure. This *iso*propyl group does not exist in oleanane.

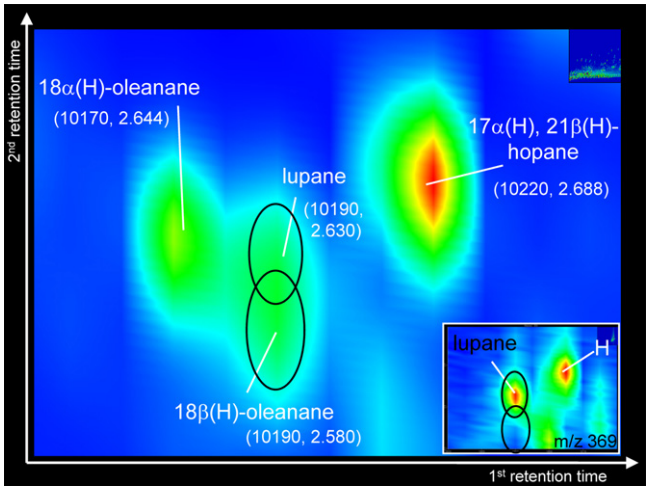

Fig. 3. Section of a comprehensive two-dimensional GC × GC–FID chromatogram of a crude oil applying GC × GC condition B. The insert shows the chromatogram of mass fragment 369 of this same section of the same sample in the equivalent GC × GC–TOFMS analysis. The mass fragment 369 is present in lupane and hopane, but not in the two oleanane isomers. Numbers in parentheses are first and second dimension retention times in seconds. Peak abundance is represented as in Fig. 1. 18α-Ol = 18α(H)-oleanane; 18β-Ol = 18β(H)-oleanane; H = 17α(H), 21β(H)-hopane; HH (*S*) = 17α(H), 21β(H)-22*S*-homohopane; HH (*R*) = 17α(H), 21β(H)-22*R*-homohopane.

differently on a polar gas chromatographic stationary phase. The main difference between these two diastereomers is their steric hindrance caused by their different configurations which can result in increased interaction with the stationary phases and therefore longer retention times. In order to verify our hypothesis we calculated the relaxed geometries for the two oleanane isomers (Fig. 4). While 18α(H)-oleanane appears almost planar in its shape, a distinct bend away from the plane created by the cyclohexane rings can be observed at C_{18} in 18β(H)-oleanane extending the distance from C_{28} to C_{29} by 0.55 Å compared to 18α(H)-oleanane. This flip in the orientation of the E-ring explains the slightly longer retention time for 18β(H)-oleanane in first dimension caused by the increased steric hindrance with the methyl groups in the dimethyl polysiloxane of the stationary phase in the non-polar column additionally to potential minimal differences in boiling point. Interestingly, this elution order is reversed on the polar column. The sterically more hindered 18β(H)-oleanane has slightly less interaction with the phenyl groups replacing the common methyl groups at the polysiloxane backbone of the stationary phase than 18α(H)-oleanane, respectively, for condition A and B. However, the exact mechanism causing this phenomenon is still unclear. From the steric hindrance point of view the opposite effect would be expected with 18β(H)-oleanane experiencing a longer retention time due to an increase in steric interaction with the larger phenyl groups in polar columns as opposed to the methyl groups in non-polar columns. The observed reduced retention time compared to the less hindered 18α(H)-oleanane warrants further investigation.

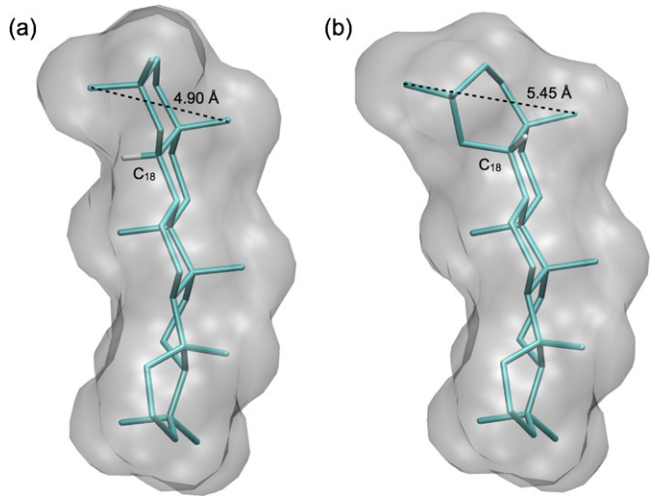

Fig. 4. Geometries of a) 18α(H)-oleanane and b) 18β(H)-oleanane. For clarity we removed the gain hydrogen atoms except for the one on the C_{18} chiral centre indicated in white. To better evidence the different hindrances of the molecules we show the surface resulting from the convolution of the atoms' van der Waals exclusion spheres (hydrogen atoms included) as a grey transparent cloud. The distance between C_{28} and C_{29} as the one controlling the difference in steric hindrance of these two isomers is given in Angstrom.

4. Conclusions

GC × GC is a very powerful technique for separation of petroleum biomarkers. The first reported baseline separation of 18α(H)-, 18β(H)-oleanane and lupane in one analysis is achieved to full satisfaction. Reassessment of previous studies involving these compounds is advisable in order to study the influence of overseen lupane in the maturity ratio of 18α(H)-/18β(H)-oleanane.

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