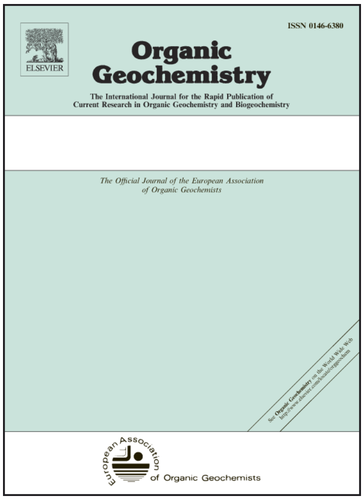

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Dehydroicetexanes in sediments and crude oils: Possible markers for Cupressoideae

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Abstract

Two previously unidentified dehydroabietane isomers were isolated from Miocene Serbian lignite and Rhaetian (Late Triassic) coaly mudstones from South Sweden and characterized using NMR-spectroscopy as *cis*- and *trans*-dehydroicetexane. Both have a 9(10→20)-*abeo*-abietane or icetexane skeleton consisting of a 6-7-6 tricyclic framework with seven carbons in ring B instead of the usual six in common diterpanes of the abietane-type. Dehydroicetexanes can be detected using GC-MS-MS in m/z 270 → 146 chromatograms without interference from dehydroabietane or other isomers. Dehydroicetexanes are often abundant in high latitude coals and mudstones ranging from

Triassic to Miocene, and in high latitude oils (Canada and Greenland) sourced from terrigenous organic matter. The *trans*/(*cis*+ *trans)* dehydroicetexane ratio is low in immature sediments, but usually around 0.83 in oils and mature sediments with vitrinite reflectance (%R_r) above 0.5 suggesting an equilibrium from the start of the oil window. Dehydroicetexanes are more stable than dehydroabietane and some oils, rich in dehydroicetexanes, contain no dehydroabietane. Precursors could be plant diterpenoids having the icetexane structure, which have been known for more than 40 years and isolated from a variety of higher plant sources, including some angiosperms. Many of the relatively simple icetexanes were isolated for the first time from *Chamaecyparis pisifera* and related species where they seem to be particularly abundant, suggesting that dehydroicetaxanes may be used as markers for the genus *Chamaecyparis* or for Cupressoideae in general.

1. Introduction

Dehydroabietane is a monoaromatic diterpane ($C_{20}H_{30}$; Structure in Fig. 1). Synthetic dehydroabietane was known long before it was isolated from a natural source (Vlad et al., 1971). It can be found in fresh and fossil wood, and is often abundant in low rank coal and in some oils having a terrigenous origin (Simoneit et al., 1986; Heppenheimer et al., 1992; Otto et al., 1997; Stefanova et al., 2005; Tuo and Philp, 2005; Haberer et al., 2006). Lists of compounds identified in fossil wood and coal (Otto and Simoneit, 2001, 2002; Otto et al., 2003, Stefanova et al., 2005; Tuo and Philp, 2005; Haberer et al., 2006) have dehydroabietane as the only non-functionalized monoaromatic diterpane compound with a molecular weight (MW) of 270.

Recently, Williford et al. (2014) found anomalously high concentrations of an isomeric monoaromatic diterpane with a characteristic mass spectrum in extracts from the fluvial Triassic-Jurassic boundary section at Astartekløft, East Greenland. A compound with a similar mass spectrum had been noted a few times before (Hazai et al., 1986; ten Haven et al., 1992). At the end of the Triassic large environmental changes took place leading to mass extinctions in the marine and terrestrial realms (Williford et al., 2014 and refs. therein). It was suggested that environmental stress factors leading to peak plant extinction stimulated increased resin production and hence an increased abundance of the unidentified compound (Williford et al., 2014). A very small amount of the unidentified compound was isolated using preparative gas chromatography, GC (Williford et al., 2014). Nuclear magnetic resonance (NMR) spectroscopy was used to determine that it was an isomer of dehydroabietane also having isopropyl on the aromatic ring, but the complete structure could not be determined.

We first noticed a compound with an identical mass spectrum in an extract from a Baffin Bay core (Cretaceous), but the sample was too small for isolation of the unidentified compound for NMR spectroscopy. Later, we found the same compound and several others with similar mass spectra in other samples. Large amounts of some samples could be obtained allowing us to isolate the two most important compounds using preparative high pressure liquid chromatography (HPLC) in quantities and in sufficient purity for NMR. They were found to have an icetexane structure (Figs. 1, 2).

2. Experimental

2.1. Isolation of dehydroabietanes

Cis-dehydroicetexane eluting immediately before dehydroabietane on GC (Fig. 3) was isolated from Upper Miocene (Pontian) lignite from the Kostolac Basin, Serbia (Đoković et al., 2018) having a very high concentration of saturated diterpanes. Simonellite and dehydroabietane were major compounds in the aromatic fraction whereas *cis*-dehydroicetexane was a minor compound. Lignite (3 kg) was pulverized and extracted (Soxhlet) with dichloromethane/methanol azeotrope (15:2, v:v) for 42 h. Copper was added to remove elemental sulphur during the extraction process. Asphaltenes were precipitated using *n*-hexane. Asphaltene-free bitumen (maltenes) was chromatographed on open columns with silica-gel/*n*-hexane/benzene yielding a fraction containing saturated and aromatic compounds, which were then separated into two saturated and three aromatic fractions by medium pressure liquid chromatography (MPLC) following procedures modified from Radke et al. (1980). The least polar aromatic fraction containing the target compound was separated using reverse phase HPLC (Phenomenex C_{18}, 250 × 10 mm, 5 µm particle size; mobile phase: acetone/acetonitrile, 60:40, v:v, at 2 cm^3/min; ~ 10 mg/injection) giving the dehydroabietane isomer *cis*-dehydroicetexane (1 mg) in more than 50% purity. Dehydroabietane (5 mg) was isolated from the same sample at 70 % purity.

Trans-dehydroicetexane eluting after dehydroabietane on GC (Fig. 3) was isolated from Rhaetian (Late Triassic) coaly mudstones from the Norra Albert quarry, South Sweden (Petersen et al., 2013; Lindström et al., 2015). Crushed mudstone (387 g having ca. 10% total organic carbon, TOC) was extracted (Soxtec, 36h, dichloromethane/methanol, 95:5, v:v). The combined extract (5.06 g) was deasphaltened using *n*-pentane and the maltenes (1.44 g) were separated using MPLC yielding two saturated, three aromatic and one polar fraction. The least polar aromatic fraction (55.7 mg) containing ca. 10% dehydroicetexanes (GC-MS, total ion current - TIC, full scan) was separated using reverse phase HPLC as described earlier, giving *trans*-dehydroicetexane (1.9 mg) of ca. 60% purity. An unidentified isomer U1 (ca. 10%) and an unresolved hump with no individual compounds above 1% accounted for the rest.

2.2. Gas chromatography-mass spectrometry (GC-MS and GC-MS-MS)

GC-MS was carried out using an Agilent 6890N gas chromatograph connected to a Waters (Micromass) Quattro Micro GC tandem quadrupole mass spectrometer. An Agilent HP-5 or Phenomenex ZB-5 column (30 m x 0.25 mm i.d., film thickness 0.10 µm) was used. The injection temperature was 70 °C (2 min hold). The temperature program was: 30 °C/min from 70 to 100 °C and 4 °C/min from 100 to 308 °C (held 8 min). Argon was used as collision gas for MS-MS experiments. For M^+ daughter scans and multiple reaction monitoring (MRM) experiments, collision energy was 6eV.

2.3. Stable isotopes, GC-IRMS

Carbon isotope determination of aromatic fractions from Serbian lignite and four HPLC-fractions from two samples of Swedish mudstone was performed using a Trace GC instrument attached to a ThermoFisher DELTA-V isotope ratio mass spectrometer via a combustion interface (GC Isolink, ThermoFisher). A DB-5MS fused silica column (30 m length; i.d. 0.25 mm; 0.25 µm film thickness) was used. The oven temperature gradient was programmed from 70 ºC to 300 ºC at 4 ºC/min, followed by an isothermal period of 15 min at 300 ºC. Helium (flow 1.2 cm^3/min) was used as carrier gas. Commercial CO_2 was injected at the beginning and end of each analysis as monitoring gas, calibrated against the NBS-19 reference material. Each sample was analysed in duplicate. Stable isotope ratios are reported in delta notation (δ13C; Coplen, 2011) relative to the Vienna-Pee Dee Belemnite (V-PDB) standard (δ13C=[(13C/12C)sample/(13C/12C)standard -1]). Delta notation is expressed in parts per thousand (‰). In order to monitor the system performance, an *n*-alkane standard mixture of known isotopic composition (C3-Mix from Arndt Schimmelmann, Indiana University) was analysed at the beginning and end of the sequence. The analytical error was in the range of 0.2-0.3 ‰.

2.4. NMR spectroscopy

NMR spectra of the isolated monoaromatic diterpanes were recorded on a Bruker Avance 600 FT-NMR spectrometer operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C, and at a sample temperature of approximately 298K. The instrument was equipped with a 5 mm TCI CryoProbe^{TM}. The samples were dissolved in approximately 0.5 cm^{3} $CDCl_3$ (99.8% D). For all compounds, a sequence of 1D and 2D experiments was performed, comprising 1D ^{1}H NMR, ^{13}C Z-restored spin-echo (Xia et al., 2008), DEPT135, 2D NOESY, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HSQC-TOCSY and ^{1}H-^{13}C HMBC. ^{1}H and ^{13}C chemical shifts in ppm were calibrated against residual $CHCl_3$ (^{1}H, 7.26 ppm) or $CDCl_3$ (^{13}C, 77.00 ppm). For one of the compounds, we were unable to obtain good quality 1D spectra using direct ^{13}C observation (see below). Neither increasing the temperature to 308K or using regular decoupled single-pulse ^{13}C experiments improved the spectral quality. The two ^{13}C chemical shifts of this compound in Table 1 reported down to only one decimal place are therefore obtained from HSQC and HMBC spectra, and not observed in the 1D spectra.

3. Results and discussion

3.1. NMR spectral assignment of cis- and trans-dehydroicetexane

From the spectral data of the isomer isolated from the Swedish mudstone, we identified four methyl groups, six methylene groups, three aliphatic methines and one aliphatic quaternary carbon atom. In addition, a trisubstituted benzene ring was identified. It was apparent from HMBC data that the methyl groups belonged to two different geminal pairs. One pair gave long-range correlations to one quaternary carbon, a methylene and a methine, whereas the other pair correlated to a methine and a substituted aromatic carbon, thus representing an isopropyl substituent on the benzene ring. A 1,2,4-substitution pattern was concluded from long-range correlations from the isopropyl methine proton to C-12, C-13 and C-14, and from the benzylic methylene protons H-7 and H-20 to C-8, C-9, C-14, and C-8, C-9, C-11 respectively. The remaining carbon framework could be constructed from the HMBC and COSY spectra, resulting in the assigned dehydroicetexane structure, with the characteristic seven-membered B ring (Fig. 1), and with the complete shift assignment as shown in Table 1. The relative orientation of all diastereotopic methylene protons, as well as the geminal methyl groups in ring A, could be determined by way of NOE correlations from the NOESY spectrum (Fig. 2a). Based on the same data it was concluded that the relative configuration of the C-5 – C-10 ring junction was *trans*, and thus this new diterpane is *trans*-dehydroicetexane, with a note of caution that the absolute configuration is not established and the compound could be either partly or fully racemized.

For reasons explained below, the diterpane isolated from the Serbian coal was assigned mainly based on ^{1}H-detected experiments. The carbon-carbon bond framework, including substitution pattern of the benzene ring, could be established from HMBC and COSY spectral data in an analogous manner as for the *trans*-dehydroicetexane. It was concluded that this compound has a similar dehydroicetexane carbon framework. Taken together with the HSQC spectrum, all chemical shifts could be assigned, as presented in Table 1. In a similar manner, diastereotopic methylene protons and geminal methyl groups were readily assigned based on NOE correlations from the NOESY spectrum (Fig. 2b). Likewise, this also supported the assignment of the C-5 – C-10 ring junction as *cis*, and thus this diterpane could be named *cis*-dehydroicetexane. Also in this case, the configuration should be considered as relative and the extent of racemization, if any, is not known.

In the case of *cis*-dehydroicetexane, we observed increased line-widths of several signals in all experiments with direct ^{13}C observation, to the extent that C-5 and C-8 could not be detected at all, whereas C-1, C-6 and C-7 had broad, indistinct appearances. The *cis* ring junction provides both ring A and B with great conformational flexibility (Jensen and Beck, 1966), moreover the benzocycloheptane moiety itself is known to produce temperature dependent NMR-spectra, with coalescence temperatures for ^{1}H NMR signals approaching room temperature (Grunwald and Price, 1965). We could offer no proof of line-broadening caused by dynamics, or multiple signals from different conformers in our 1D ^{1}H spectrum, but are aware that broadening phenomena caused by conformational interconversions are highly dependent on, among other things, magnetic field strength and nuclei of observation. On the other hand, it cannot be ruled out that the reported ^{1}H data herein corresponds to only one particularly stable conformer, in contrast to the time-averaged data arising from an ensemble of relatively stable conformers in rapid equilibrium which we would normally see. The appearance of the spectrum is more complex than the GC-MS impurity profile would suggest, which is in support of this hypothesis. It is therefore also our opinion that the anomalous signals in the ^{13}C spectra of the *cis*-isomer is caused by conformational equilibria, for which some of the rate constants are in the so-called "intermediate exchange" timescale for the herein employed experimental conditions. Furthermore, it seems that the more "locked" configuration of the *trans*-isomer only allows for slight and rapid conformational equilibria, giving sharp signals for all nuclei, evident of being in the "fast exchange" region of the NMR timescale. Thus, additional studies of the *cis*-dehydroicetexane ring system are of interest from a physical organic chemistry viewpoint, but outside the scope of this work.

We have also included the full ^{1}H and ^{13}C NMR data for dehydroabietane (Table 2). Our data for ^{13}C agrees with literature values (Nishida et al., 1977) in every aspect, except for the assignment of the very close shifts of C-4 and C-15, which we were able to establish only from comparison of Z-restored spin-echo and DEPT135 spectra recorded at very high resolution. On the other hand, our data for ^{1}H disagrees with recent literature values (Mothana et al., 2014), even when a few misassignments are sorted out. Experimental details of their 2D NMR spectroscopy are scarce, making it difficult to judge the accuracy of their data. We have therefore taken care to report in detail the experimentally determined coupling constants and chemical shifts from our 1D ^{1}H spectrum for future reference. In contrast, only a limited number of coupling constants could be extracted from the ^{1}H spectra of either dehydroicetexane isomer (Table 1), and were of little diagnostic use, albeit in agreement with the suggested structures.

3.2. Mass spectra and relative retention times of dehydroictexanes and isomers

Mass spectra of dehydroabietane and *cis*- and *trans*-dehydroicetexane are shown in Fig. 4. The mass spectra of the two major dehydroicetexanes are almost identical, with a characteristic fragment at *m/z* 146 originating from cleavage of ring B at the benzylic carbons and *m/z* 227 from loss of the isopropyl-group. The Norra Albert samples contain an additional unidentified compound (U1) eluting slightly later on GC than *cis*-dehydroicetexane (Fig. 3) and only a few seconds before dehydroabietane. It is absent from other samples. It was obtained free of compounds coeluting on GC in an HPLC-fraction but not in quantities for NMR. Its mass spectrum is similar to that of the *cis*- and *trans*-dehydroicetexanes, but has *m/z* 147/146 and 255/270 > 1 and a more intense fragment at *m/z* 159 (Fig. 4). Numerous minor compounds having dehydroicetexane-like spectra were also observed in the HPLC-fractions during isolation of icetexanes. One of them (U2) is clearly visible in Fig. 3. They probably all have a seven carbon B-ring like the dehydroicetexanes, but the isopropyl group could be attached to other carbons in the aromatic ring.

Two unidentified isomers of dehydroabietane and dehydroicetexane (U3 and U4) having similar mass spectra with *m/z* 173 as base peak (Figs. 3, 5) were found in all samples with dehydroicetexanes except in very immature samples such as the Danish and Serbian lignites. Compound U3 coelutes with fluoranthene under our GC conditions. The *m/z* 171/173 fragments could correspond to loss of the seven membered ring A from a 1(10→20)-*abeo*-abietane structure.

3.3. Demethylated dehydroicetexanes

Small amounts of early eluting C_{19} compounds were also observed in HPLC-fractions. The major compounds had icetexane-like mass spectra with important fragments at *m/z* 270, 255 and 227 shifted to 256, 241 and 213, indicating loss of methyl from ring A and the presence of an isopropyl-group in the aromatic ring. However, clean mass spectra were not obtained for any of them.

3.4. Dehydroicetexanes in sediments and crude oils

Dehydroicetexanes occur at the Triassic-Jurassic boundary in East Greenland (Williford et al., 2014) and South Sweden (this work) but are not restricted to such samples. In addition to samples obtained specifically for this study, a large number of aromatic fractions from previous studies were reanalyzed for dehydroicetexanes. They were found in Jurassic coal and Lower Cretaceous mudstone from Denmark (Petersen, 1994; Petersen et al., 1996), Cretaceous coal and mudstone from West Greenland (Bojesen-Koefoed et al., 2001; Pedersen et al., 2006), Cretaceous black shale (Albian Hassel Formation, Sverdrup Basin, Ellesmere Island, Canada), Miocene lignite from Søby, Denmark, Upper Miocene lignite from the Kostolac Basin, Serbia (Đoković et al., 2018), and the Kovin deposit, Serbia (Mitrović et al., 2016). Strangely, only one of 34 samples from 6 Cretaceous cores obtained during the M/V Joides Resolution cruise in 2012 to Melville Bay, N.W. Greenland (GEUS unpublished) contained dehydroicetexanes whereas all samples were rich in other aromatic diterpanes such as dehydroabietane, simonellite, 6-isopropyl-2-methyl-1-(4-methylpentyl) naphthalene and retene. This particular sample was also very rich in saturated diterpanes. Most were abietane and its isomers (*m/z* 276). Abietane (Livsey et al., 1984; Stefanova et al., 1995) accounted for 3% of the saturated hydrocarbons and half of the saturated diterpanes. Isopimarane (Noble et al., 1986; Blunt et al., 1988) was also present, but the rest appeared to be novel compounds. They could be saturated icetexanes. They were not discovered in saturated fractions from other samples.

So far, we have only found dehydroicetexanes in relatively high latitude samples (Serbia, Denmark, Sweden, Canada and Greenland). A collection of worldwide coals and mudstones have previously been analyzed for other compounds (Nytoft, 2011; Nytoft et al., 2015 and refs. therein). Several of them from Colombia, Nigeria, Indonesia, and Vietnam (tropic and subtropic) were reanalyzed for icetexanes without finding any, although other aromatic diterpanes were sometimes abundant. Chromatograms in Williford et al. (2014) show only one compound having a molecular weight of 270 and only one mass spectrum is presented. We could not determine from their figures if it is the *cis*- or *trans*-dehydroicetexane.

The most prolific resin producers of the northern temperate climatic zones are the conifers (Simoneit, 1977), and since dehydoicetexanes are abundant in shales and coal from Canada and Greenland it was obvious first to search for them in high latitude oils. Five different oil types were detected in West Greenland (Christiansen et al., 1996; Bojesen-Koefoed et al., 1999; Nytoft et al., 2002). Three of them have a terrestrial origin and one of them (the Marraat type) is rich in saturated angiosperm markers such as oleanane and lupane. *Cis*- and *trans*-dehydroicetexanes are found in all 11 of the West Greenland oils reanalyzed during this study, together with simonellite, retene and 6-isopropyl-2-methyl-1-(4'-methylpentyl) naphthalene (Ellis et al., 1996). *Trans*-dehydroicetexane is the major monoaromatic diterpane in all cases. Dehydroicetexanes are also major compounds in the Amauligak oil (Fig. 6) from the Mackenzie Delta-Beaufort region, Canada (Curiale, 1991). Dehydroicetexanes were not detected in Danish North Sea crude oils having a Jurassic source although all of them contained retene which is the endpoint in aromatization of abietane type diterpenoids (Marchand-Geneste and Carpy, 2003). As mentioned previously, dehydroicetexanes occurred in oil from North Canada and Greenland but they were absent from the rest of the oils in a collection of worldwide oils analyzed earlier for unusual hopanes (Nytoft, 2011; Nytoft et al., 2015).

3.5. Trans- and cis dehydroicetaxane vs. thermal maturity

An immature Miocene lignite from Denmark (huminite reflectance unknown) contained only the *cis*-isomer. Lignite samples from the Kostolac Basin, Serbia used for isolation of dehydroicetexanes are immature with a huminite reflectance of 0.30 ± 0.03% (Đoković et al., 2018). Two of them have *trans/ (cis + trans)* dehydroicetexane ratios of 0.06 and 0.20 respectively. Eight samples of Jurassic carbonaceous claystone from Bornholm, Denmark with a huminite reflectance value of 0.24% Rm have ratios from 0.27 to 0.52 (Petersen et al., 1996). Two coal samples from the Øresund-7 and Øresund-18 wells (Petersen, 1994) with huminite reflectances at 0.41 and 0.51% Rm have *trans/(cis+trans)* ratios at 0.72 and 0.86 respectively. The average *trans /(cis + trans)* dehydroicetexane ratio for eleven West Greenland oils is 0.83 (range: 0.80-0.86) and this is probably the equilibrium value obtained slightly before the "oil window". A Cretaceous black shale (Albian Hassel Formation, Sverdrup Basin, Ellesmere Island, Canada) had previously been subjected to hydrous pyrolysis for 72 h at 220, 250, 270, 285 and 300 °C (GEUS unpublished) and the aromatic fractions could be used for this study. The *trans/(cis + trans)* ratio for the untreated sample was 0.85 and the five pyrolysed samples ranged from 0.75 to 0.81 with no clear trend, suggesting that an equilibrium had already been obtained. The biodegraded Amauligak oil from Canada (Fig. 6) had a *trans/ (cis + trans)* ratio of only 0.75. It also had a high concentration of oleanenes and other unsaturated compounds that could have been picked up during migration or in the reservoir.

3.6. Dehydroicetaxanes vs. dehydroabietane

Dehydroicetexanes appear to be more thermally stable than dehydroabietane. Alternatively, they are generated at higher maturities or are formed by isomerization of dehydroabietanes. Further aromatization of dehydroabietane will lead to simonellite and finally to retene (Marchand-Geneste and Carpy, 2003; Hautevelle et al., 2006) "removing" dehydroabietane from the samples, whereas the seven carbon B-ring may prevent further aromatization of dehydroicetexanes. Compounds having mass spectra that could correspond to ring A and C diaromatic icetexanes were not detected.

Immature samples have dehydroicetexanes/dehydroabietane ratios below one (from 270 → 146 and 270 → 173 respectively). The ratio for the Canadian Amauligak oil is 11.2 and seven of eleven oils from West Greenland range from 14.5 to 130. The remaining four oils contain no dehydroabietane. Hydrous pyrolysis of black shale from Ellesmere Island at 220, 250, 270, 285 and

300°C increased the ratio from 0.40 in the untreated sample to 1.2, 3.8, 7.0, 9.9 and 8.0, respectively.

The dehydroicetexanes/dehydroabietane ratio in sediments is also influenced by the depositional environment. A thin coal seam at Asuk, West Greenland has previously been described in detail (Bojesen-Koefoed et al., 2001). The depositional environment went from shallow marine conditions, through fresh water mire back to shallow marine conditions (Bojesen-Koefoed et al., 2001). TOC is high in the middle of the seam, but low at the top and bottom (Table 3). The *trans/(cis + trans)* dehydroicetexane ratio is almost constant throughout and close to equilibrium, whereas the dehydroicetexanes/dehydroabietane ratio increases almost two orders of magnitude from the center with a high TOC to the edges with a low TOC indicating that a high content of mineral matter could favour the formation of dehydroicetexanes. Alternatively, the difference could be caused by a change in vegetation.

3.7. Precursors of dehydroicetexanes

A 9(10→20)-*abeo*-abietane or icetexane skeleton structure (Fig. 1) for the novel compounds could be assigned using NMR. Icetexane diterpenoid natural products have been isolated from a variety of terrestrial sources (Simmons and Sarpong, 2009). Their review article about icetexane diterpenoids shows the structure of several of them. Four of which are reproduced here in Fig. 7. The natural products are divided into five subclasses based on the presence or absence of oxygenation at the C-3, C-11, C-14 and C-19 positions. The simplest subclass of icetexanes is the pisiferins which are oxygenated at C-12 but not at C-3, C-11, C-14 and C-19. The parent compound, pisiferin, was isolated from *Chamaecyparis pisifera* (Yatagai and Takahashi, 1980). The original structure was revised by Hasegawa et al. (1984, 1985) who showed that it had a 9(10→20)-abeo-abietane or icetexane skeleton consisting of a 6-7-6 tricyclic framework instead of 7-6-6 as first believed.

Pisiferins appear to be particularly abundant in *Chamaecyparis pisifera* which has been intensively studied (Yatagai and Takahashi 1979, 1980; Ahn et al., 1984; Hasegawa et al., 1985; Xiao et al., 2001). However, two new pisiferins were first identified in the related *Chamaecyparis formosensis.* The genus *Chamaecyparis* includes five or six living species which are native to Eastern Asia, Japan and North America. Early members of *Chamaecyparis* were widely distributed in the mid to high latitudes of North America and Europe during the Paleogene (McIver, 1994;

Kotyk et al., 2003; Liu, et al., 2009). *Chamaecyparis* disappeared from Europe in the Plio-Pleistocene (Liu et al., 2009). It is, therefore, likely that a large part of the dehydroicetexanes in older sediments and oil originate from various *Chamaecyparis* species. However, other *Cupressoideae* could also be the source. Recently, the phenolic icetexane-diterpenoid *abeo*-carnosol was found in fossil *Glyptostrobus nordenskioldii* and in extant *Glyptostrobus pensilis* (Simoneit et al., 2016). Extant *Metasequoia glyptostroboides* contained *abeo*-pisiferol which is another compound with the icetexane skeleton (Simoneit et al., 2016). Forests containing such deciduous conifers existed north of the Arctic Circle during the Eocene. Little is known about diterpanes in early Cupressaceae but it is likely that they also could produce icetexanes. We noted that the Triassic/Jurassic boundary samples from Sweden used for isolation of *trans*-dehydroicetexane were rich in *Perinopollenites elatoides* pollen. *Perinopollenites elatoides* belongs to the Family Cupressaceae (Riding et al., 2013; Shevchuk et al., 2018).

The majority of icetexane natural products discovered to date have been found in plant species that also produce abietane diterpenoids. Expansion of ring B in abietane diterpenoids could be enzyme-mediated (Gonzales et al., 1991; Fraga et al., 2005) but is also possible by “chemical means” such as clay catalyzed isomerization. Yatagai et al. (1980) thus obtained pisiferin from 20-hydroxyferruginol (pisiferol) which has been isolated from *Sequoia sempervirens* (Son et al., 2005). Formation of icetexanes in sediments from abietane diterpenoids during diagenesis has, to our knowledge, not been described but might also be possible. Compounds having the icetexane skeleton are not restricted to conifers but have also been found in angiosperms. Several of them have been isolated from *Salvia* species (Simmons and Sarpong, 2009 and references therein).

So far, we have not detected dehydroicetexanes in plant material. High concentrations of dehydroabietane were found in rotten wood (*Pinus sylvestris* and *Picea omorica*). We also found dehydroabietane in twigs of *Chamaecyparis pisifera* obtained from a botanical garden near Copenhagen in 2016. In all cases, dehydroabietane was the only monoaromatic C_{20} diterpane.

During isolation of *trans*-dehydroicetexane from Swedish mudstones by way of HPLC several fractions were collected. Fractions having the highest concentrations of the target compound were used for NMR spectroscopy. Fractions eluting before and after “pure” *trans*-dehydroicetexane were used for measurement of $\delta^{13}C$ values for dehydroabietane and both dehydroicetexanes (Table 4). All three compounds were obtained free from compounds coeluting on GC. They had very similar $\delta^{13}C$ values (Table 4). Front and tail of the *trans*-dehydroicetexane peaks yielded similar values. The $\delta^{13}C$ values of several diterpanes in lignites from the Kovin deposit, Serbia have been measured (Mitrović et al., 2016, 2017). The $\delta^{13}C$ values for *cis*-dehydroicetexane in 15 of the samples were also measured but not listed. In these samples, *cis*-dehydroicetexane was more depleted in $\delta^{13}C$ than dehydroabietane (average difference: 1.33 ‰; range: 0.80 – 1.96 ‰) which could indicate different sources for the two compounds.

4. Conclusion

Two isomers of dehydroabietane ($C_{20}H_{30}$) were isolated and characterized using NMR as having a 7-carbon B-ring instead of the usual 6-carbon B ring in abietane deived diterpanes and identified as *cis*- and *trans*-dehydroicetexanes. This is probably the first identification of dehydroicetexanes in geological samples. Dehydroicetexanes can be detected using GC-MS-MS in *m/z* 270 → 146 chromatograms without interference from isomers. Dehydroicetexanes have, so far, only been detected in high latitude sediments ranging in age from Triassic to Miocene and in high latitude oils. The *trans*/*(cis + trans)* ratio is low in immature sediments, but usually around 0.83 in oils and mature sediments with vitrinite reflectance (%R_o) above 0.5, suggesting that equilibrium has already been reached at the start of the oil window. Dehydroicetexanes are more stable than dehydroabietane. Precursors for dehydroicetexanes could be higher plant compounds having the icetexane skeleton. They are abundant in the *Chamaecyparus* genus, but they could also occur in some angiosperms. Alternatively, dehydroicetexanes may be formed from rearrangement of the more abundant abietane dieterpenoids in immature sediments during diagenesis.

Acknowledgements

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Figure captions

Fig. 1. Structures of dehydroabietane and dehydroicetexane with numbering of carbon atoms.

Fig. 2. Diagnostic ^{1}H-^{1}H-NOESY correlations used for determining relative configurations of diastereotopic protons and methyl groups. Correlations with ambiguous interpretation and geminal correlations are omitted for clarity. The illustrated conformations were obtained by energy minimization using MM2 methods available in the Chem3D Pro software (PerkinElmer). a) *trans*-dehydroicetexane, b) *cis*-dehydroicetexane.

Fig. 3. Dehydroabietane, dehydroicetexanes and unidentified compounds in an extract of a Triassic/Jurassic boundary mudstone from Sweden.

Fig. 4. EI and *m/z* 270 daughter spectra of dehydroabietane, *cis*-dehydroictexane, *trans*-dehydroicetexane and an unidentified isomer.

Fig. 5. EI and *m/z* 270 daughter spectra of two unidentified dehydroabietane isomers.

Fig. 6. Aromatic hydrocarbons in the Amauligak oil, Canada (GC-MS, TIC full scan). Inset shows dehydroabietane and dehydroicetexanes from GC-MS-MS (270 → 255 and 270 → 146).

Fig. 7. Some natural products having the icetexane skeleton. Structures were reproduced from Simmons and Sarpong (2009) with corresponding numbers.

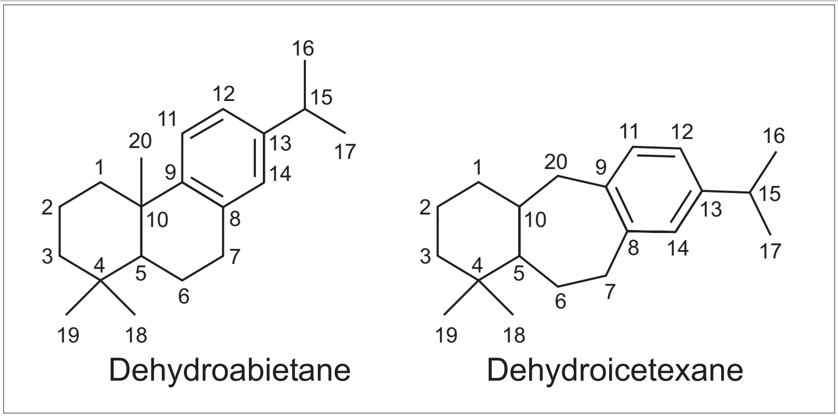
Table 1. ^{1}H and ^{13}C NMR chemical shifts (ppm) for *trans*-dehydroicetexane, and *cis*-dehydroicetexane (600/150 MHz, $CDCl_3$). Multiplet structures are provided in italics and coupling constants given in Hz. Integrals are given for clarity reasons and are not experimentally determined.

Table 2. ^{1}H and ^{13}C NMR chemical shifts (ppm) for dehydroabietane (600/150 Mhz, $CDCl_3$). Multiplet structures are provided in italics and coupling constants given in Hz.

Table 3. Mudstones and coals in a ca. 25 cm thick seam from Asuk, West Greenland (Data in first seven columns: Bojesen-Koefoed et al., 2001). TOC: total organic carbon. TS: total sulphur. R_o measured on ulminite B and ulminite A respectively. T_{max}: temperature corresponding to S2 (pyrolysate hydrocarbons) peak maximum from the Rock-Eval analysis. Diaster.: C_{29} diasteranes/(diasteranes + reg. steranes) from 400 → 217. *Trans/(cis + trans)*: *Trans/(cis + trans)* dehydroicetexane from 270 → 146. DHXs/DHA: Sum of *cis*- and *trans*-dehydroicetexanes/dehydroabietane from 270 → 146 and 270 → 173, respectively.

Table 4. δ^{13}C values of dehydroicetexanes and dehydroabietane from two Swedish mudstones. P03 and P10: HPLC-fractions collected before pure *trans*-dehydroicetexane for NMR. P05 and P12: HPLC-fractions collected after pure *trans*-dehydroicetexane.

Figure


16
15
17
12
11
13
14
20
9
1
2
10
8
7
3
4
5
6
19
18
Dehydroabietane
11
12
16
20
9
1
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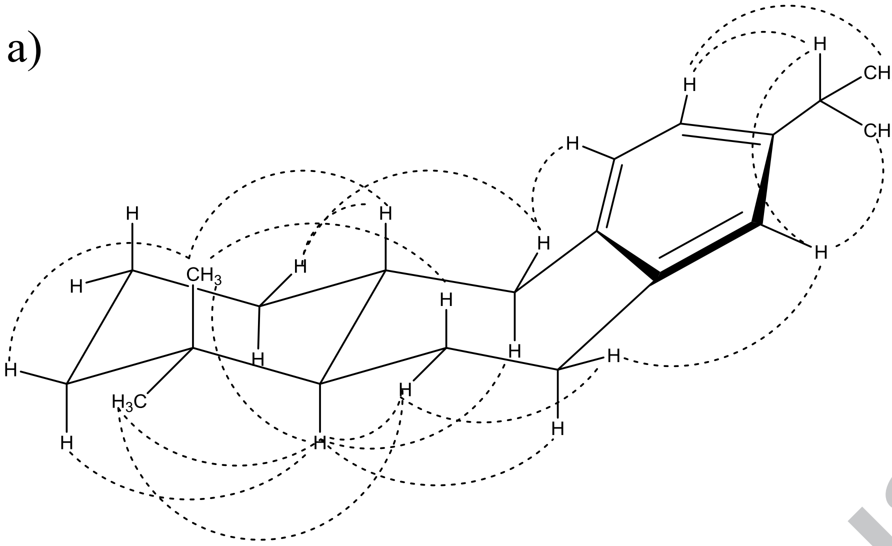
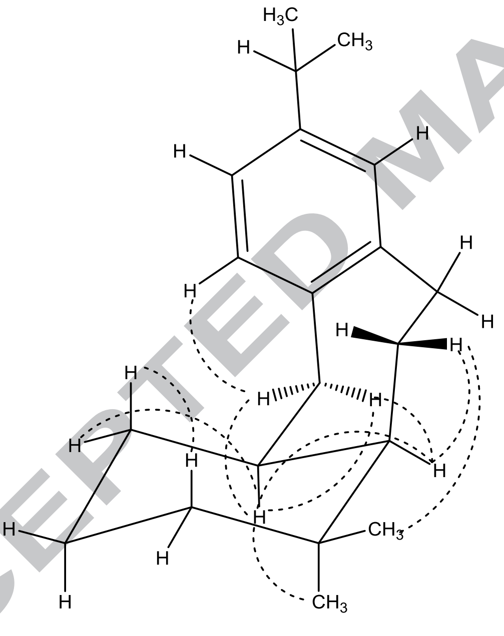
3
4
5
6
7
19
18
Dehydroicetexane

a)


H
CH$_3$
H
CH$_3$
H
H
H
H
H
CH$_3$
H
H
H
H
H
H
H
H
H
H$_3$C
H
H
H

b)


H$_3$C
CH$_3$
H
H
H
H
H
H
H
H
H
H
H
H
H
H
H
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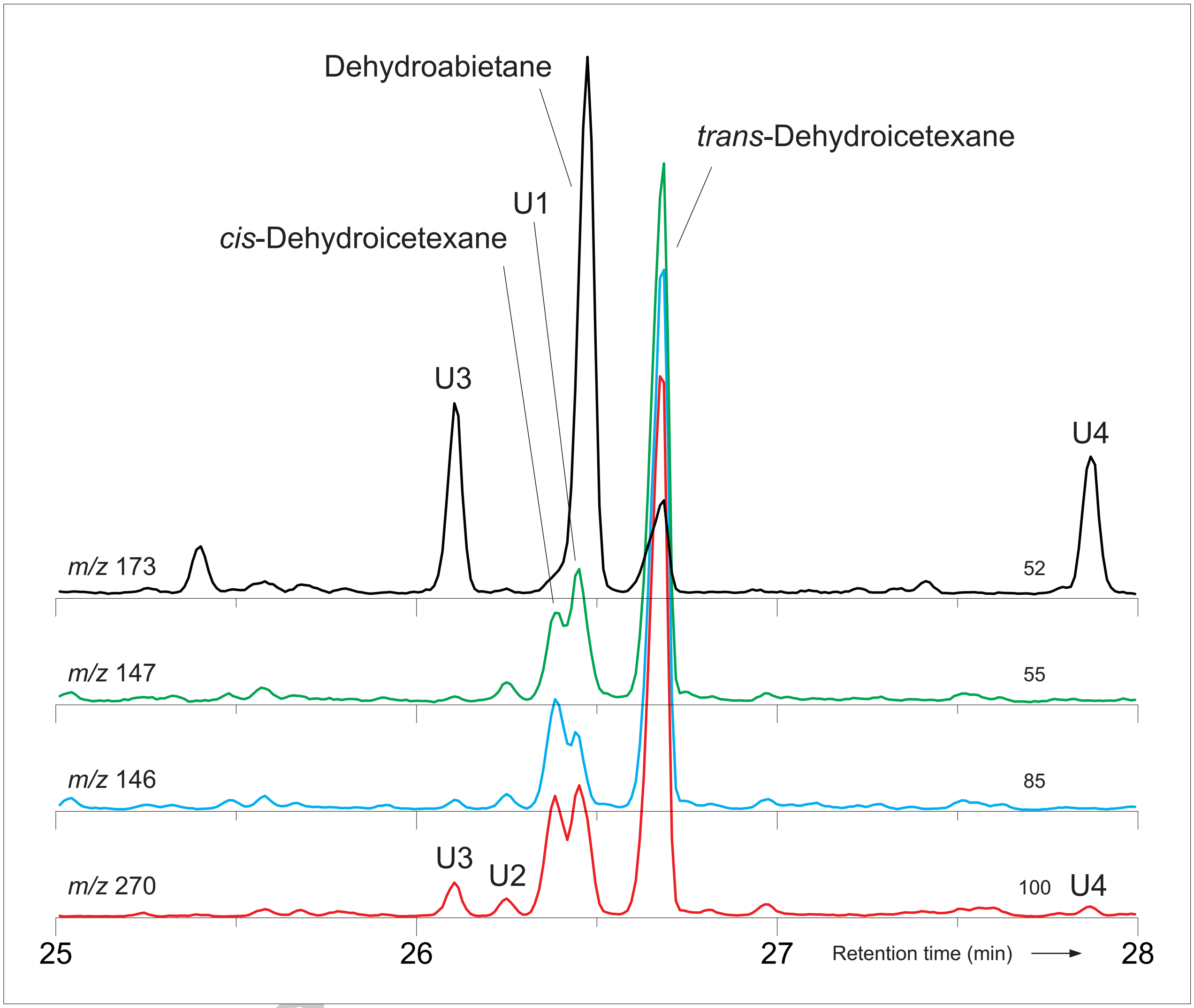
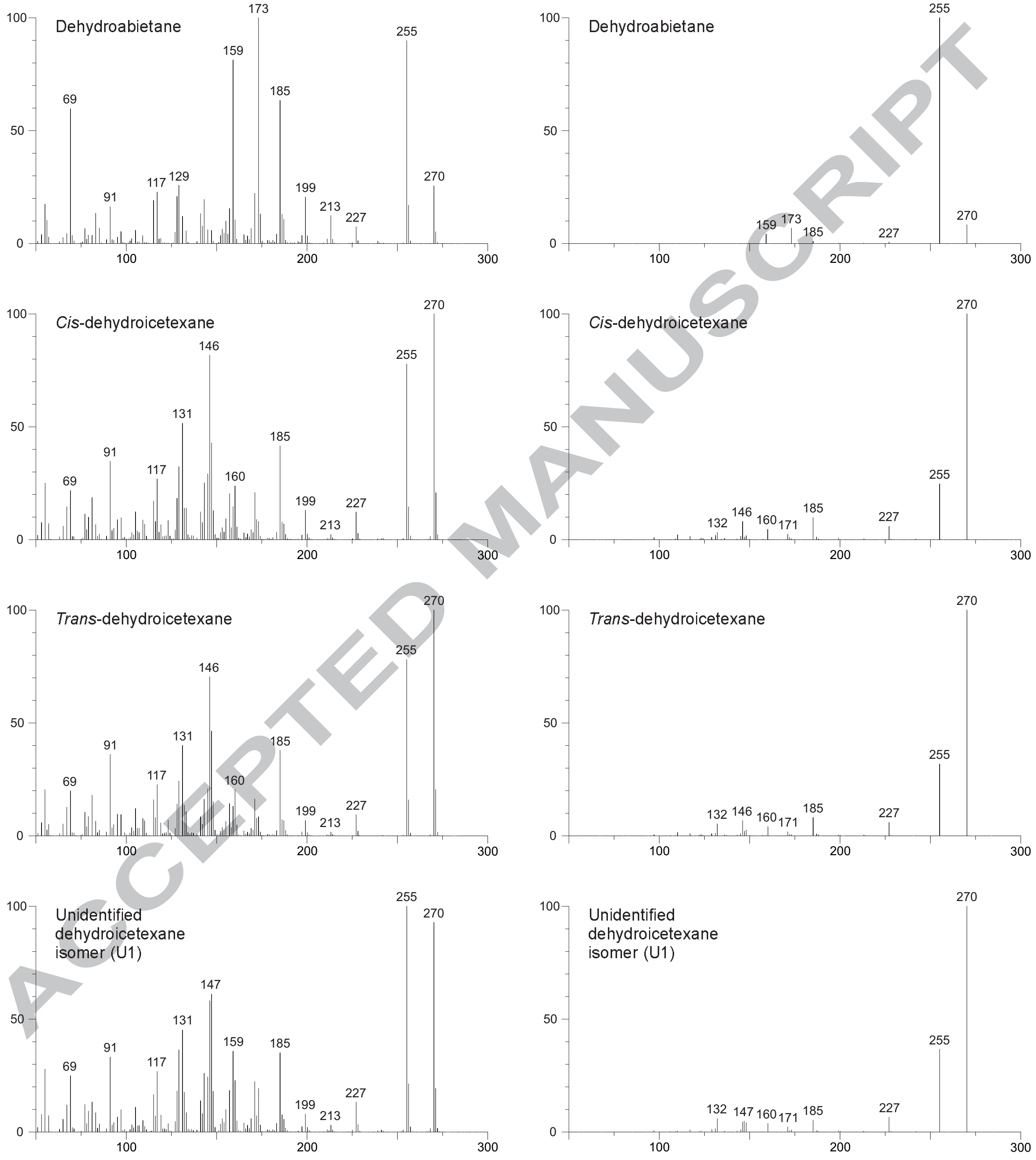
CH$_3$
H
H
CH$_3$

Figure


Dehydroabietane
trans-Dehydroicetexane
U1
cis-Dehydroicetexane
U3
U4
m/z 173
52
m/z 147
55
m/z 146
85
U3
U2
m/z 270
100
U4
25
26
27
Retention time (min)
28

Figure


Dehydroabietane
100
50
0
69
91
117
129
159
173
185
199
213
227
255
270
100
200
300
Dehydroabietane
159
173
185
227
255
270
Cis-dehydroicetexane
69
91
117
131
146
160
185
199
213
227
255
270
Cis-dehydroicetexane
132
146
160
171
185
227
255
270
Trans-dehydroicetexane
69
91
117
131
146
160
185
199
213
227
255
270
Trans-dehydroicetexane
132
146
160
171
185
227
255
270
Unidentified dehydroicetexane isomer (U1)
69
91
117
131
147
159
185
199
213
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255
270
Unidentified dehydroicetexane isomer (U1)
132
147
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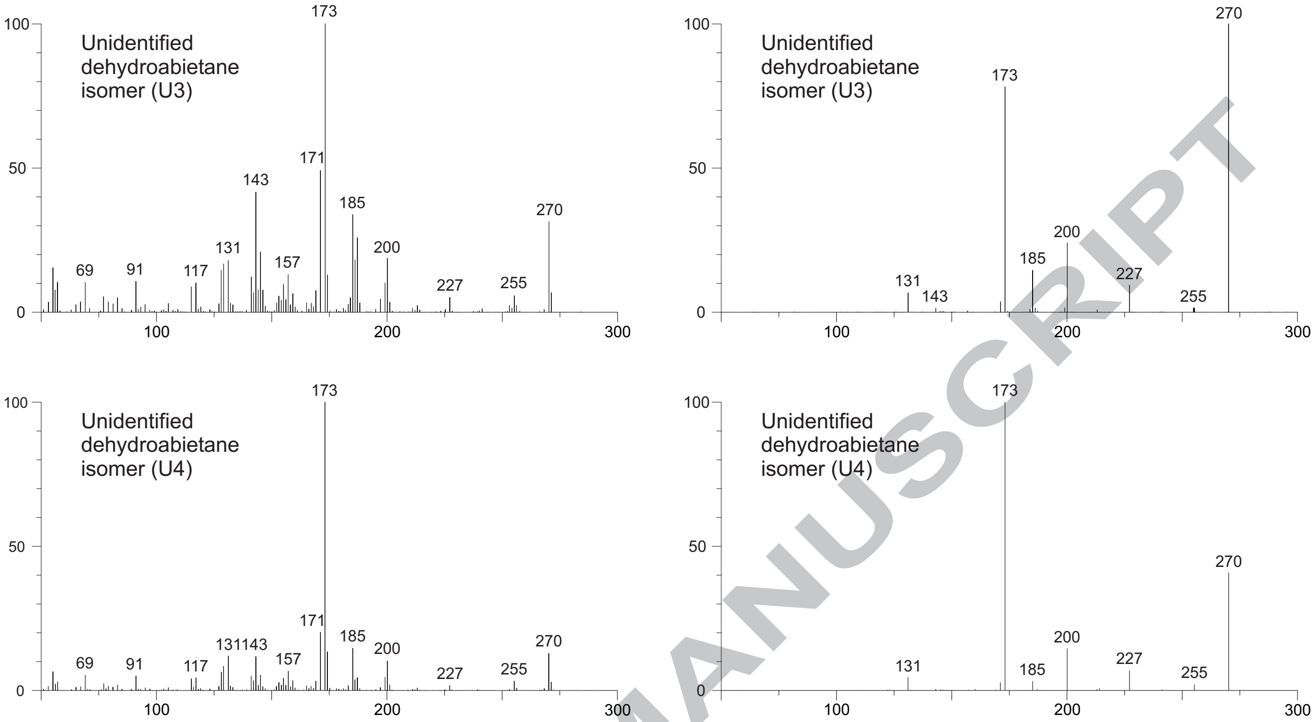
227
255
270

Figure


Unidentified dehydroabietane isomer (U3)
100
50
0
69
91
117
131
143
157
171
173
185
200
227
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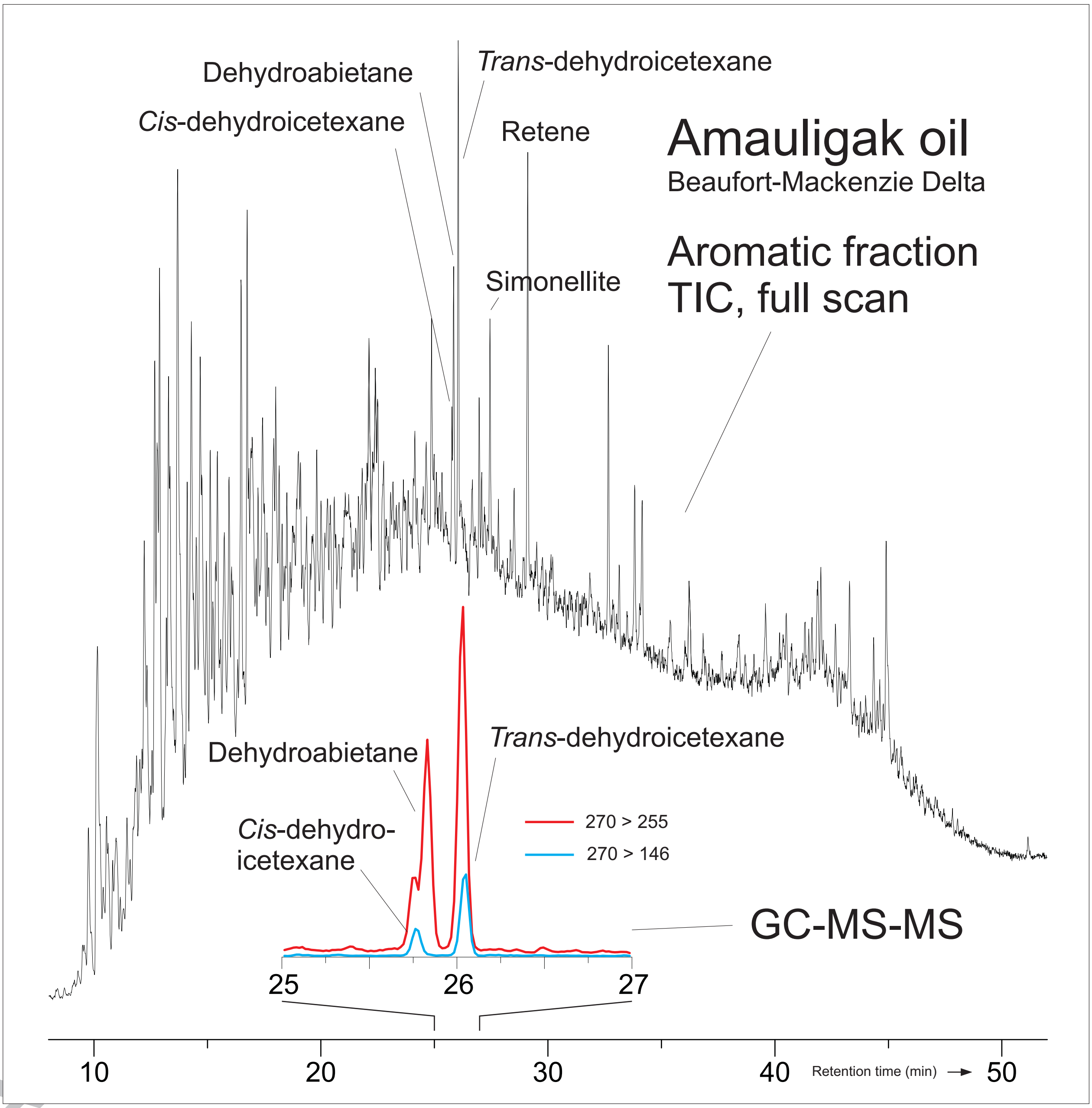
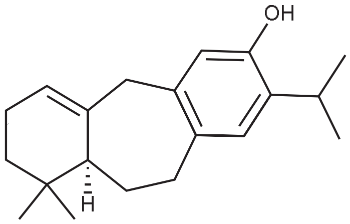
270
100
200
300
Unidentified dehydroabietane isomer (U3)
131
143
173
185
200
227
255
270
Unidentified dehydroabietane isomer (U4)
69
91
117
131143
157
171
173
185
200
227
255
270
Unidentified dehydroabietane isomer (U4)
131
173
185
200
227
255
270

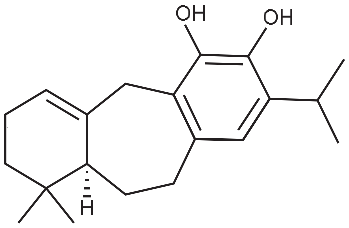
Figure


Dehydroabietane
Cis-dehydroicetexane
Trans-dehydroicetexane
Retene
Simonellite
Amauligak oil
Beaufort-Mackenzie Delta
Aromatic fraction
TIC, full scan
Dehydroabietane
Trans-dehydroicetexane
Cis-dehydro-icetexane
270 > 255
270 > 146
GC-MS-MS
25
26
27
10
20
30
40
Retention time (min) →
50

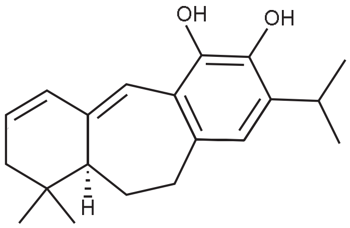
Figure



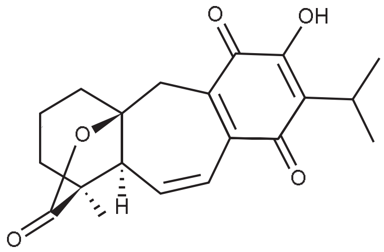
Pisiferin (1)



Barbatusol (11)



Przewalskin D (20)



Icetexone (39)

Structure of two dehydroabietane isomers (dehydroicetexanes) determined using NMR
Dehydroicetexanes are often abundant in high latitude coal and oil
Dehydroictexanes are more stable than dehydroabietane
The ratio between isomers (trans/cis) reaches 83:17 at the start of the oil-window
Functionalized precursors having the icetexane skeleton are abundant in Cupressoideae.

Table 1. ^{1}H and ^{13}C NMR chemical shifts (ppm) for *trans*-dehydroicetexane, and *cis*-dehydroicetexane (600/150 MHz, $CDCl_3$). Multiplet structures are provided in italics and coupling constants given in Hz. Integrals are given for clarity reasons and are not experimentally determined.

	***trans*-Dehydroicetexane**		***cis*-Dehydroicetexane**	
	δ_C	δ_H, *mult.*, *J* (Hz)	δ_C	δ_H, *mult.*, *J* (Hz)
1	36.88	1.14 (α-H), *m* 1.74 (β-H), *d**, 12.8	26.00	1.14 (α-H), *m* 0.71 (β-H), *m*
2	22.27	1.49 (2H), *m*	22.49	1.42 (2H), *m*
3	42.52	1.24 (α-H), *m* 1.33 (β-H), *m*	33.96	1.03 (α-H), *m* 1.19 (β-H), *m*
4	34.18	-	33.43	-
5	56.95	1.12, *m*	52.1	1.46, *m*
6	28.38	2.12 (α-H), *dd**, 8.0, 13.5 1.05 (β-H), *m*	23.51	1.90 (α-H), *m* 1.36 (β-H), *m*
7	35.52	2.79 (α-H), *m* 2.73 (β-H), *m*	35.15	2.70 (2H), *m*
8	143.67	-	142.8	-
9	139.21	-	137.11	-
10	38.56	1.31, *m*	32.88	2.24, *m*
11	128.88	7.02, *d*, 7.5	129.50	6.94, *m*
12	123.57	6.95, *m*	123.26	6.92, *m*
13	146.49	-	146.48	-
14	126.24	6.94, *m*	126.16	6.91, *m*
15	33.64	2.85, *sept.*, 6.9	33.61	2.84, *sept.*, 6.9
16/17	24.07/24.13	1.234, *d*, 6.9 1.235, *d*, 6.9	24.08/24.15	1.23 (6H), *d*, 6.9
18	30.90	0.94, *s*	27.57	1.04, *s*
19	20.45	0.71, *s*	30.41	0.87, *s*
20	44.12	2.70 (α-H), *m* 2.42 (β-H), *dd*, 1.4, 14.0	40.78	2.96 (α-H), *dd*, 2.2, 14.0 2.61 (β-H), *dd*, 6.6, 14.0

*Additional unresolved couplings ($J < 2.0$ Hz)

Table 2. ^{1}H and ^{13}C NMR chemical shifts (ppm) for dehydroabietane (600/150 MHz, CDCl$_3$). Multiplet structures are provided in italics and coupling constants given in Hz.

	δ_C	δ_H, *mult.*, *J* (Hz)
1	38.83	1.40 (α-H), *ddd**, 2.8, 13.4, 13.4 2.27 (β-H), *dddd*, 1.5, 3.4, 3.4, 12.6
2	19.32	1.60 (α-H), *m* 1.75 (β-H), *m*
3	41.72	1.21 (α-H), *m* 1.47 (β-H), *dddd*, 1.6, 3.3, 3.3, 13.2
4	33.43	-
5	50.41	1.35, *dd*, 2.3, 12.5
6	19.10	1.87 (α-H), *dddd*, 2.1, 2.1, 7.5, 13.3 1.70 (β-H), *m*
7	30.49	2.86 (α-H), *m* 2.92 (β-H), *ddd*, 1.6, 7.0, 17.2
8	134.93	-
9	147.61	-
10	37.51	-
11	124.26	7.18, *d*, 8.2 Hz
12	123.76	6.99, *dd*, 2.1 Hz, 8.2 Hz
13	145.38	-
14	126.79	6.89, *d*, 1.7 Hz
15	33.42	2.82, *sept.*, 6.9 Hz
16/17	23.97/23.99	1.23, *d*, 6.9 Hz
18	33.31	0.95, *s*,
19	21.61	0.93, *s*,
20	24.88	1.18, *d*, 0.7 Hz

*Additional unresolved couplings to CH$_3$-20.

Sample No.	cm. above datum	Lithology	TOC (%)	Ts (%)	R_o	Tmax (°C)	Diaster.	*Trans/ (cis + trans)*	DHXs/ DHA
440122	15 - 25	Sandy mudstone	3.61	0.08		430	0.61	0.81	10.1
440121	12 – 15	Coal	59.91	0.96	0.55/0.48	426	0.56	0.77	0.6
440120	9 - 12	Coal	69.18	0.81		425	0.55	0.85	0.14
440119	6 - 9	Coal	64.18	0.69	0.55/0.48	427	0.52	0.80	1.1
440118	3 - 6	Coaly mudstone	30.00	0.27	0.57/0.48	429	0.61	0.82	4.4
440117	0 – 3	Carbonaceous mudstone	8.52	0.12		434	0.67	0.84	5.1

Table 4. $\delta^{13}C$ values of dehydroicetexanes and dehydroabietane from two Swedish mudstones. P03 and P10: HPLC-fractions collected before pure *trans*-dehydroicetexane for NMR. P05 and P12: HPLC-fractions collected after pure *trans*-dehydroicetexane.

Sample	*Cis*-dehydroicetexane	Dehydroabietane	*Trans*-dehydroicetexane
	$\delta^{13}C$ (‰, PDB)	$\delta^{13}C$ (‰, PDB)	$\delta^{13}C$ (‰, PDB)
519546-P03		-24.7	-24.6
519546-P05	-24.4		-24.8
519550-P10		-25.0	-24.4
519550-P12	-24.1		-25.0