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CHEMICAL AFFINITIES BETWEEN THE SOLVENT EXTRACTABLE AND THE BULK ORGANIC MATTER OF FOSSIL RESIN ASSOCIATED WITH AN EXTINCT PODOCARPACEAE

JOAN O GRIMALT, BERND R T. SIMONEIT* and PATRICK G HATCHER†

Department of Environmental Chemistry (CID-CSIC), Jordi Girona, 18, 08034-Barcelona, Spain, *Petroleum Research Group, College of Oceanography, Oregon State University, Corvallis, OR 97331 U.S A , † U S Geological Survey, National Center, Reston, VA 22092, U S A

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Abstract—Analyses by GC-MS and GC-IR of resin associated to *Dacridiumites mawsonii* deposits, an extinct species of Podocarpaceae occurring on the South Island of New Zealand during the Bortonian (Middle Eocene), have revealed that dehydroabietic acid is the predominant component of the solvent soluble fraction Accordingly, this diterpenoid has been selected as the principal component material for spectroscopic comparison with the bulk resin using IR and CP/MAS ^{13}C NMR

INTRODUCTION

Although ambers and fossil resins have been extensively investigated, their compositions and formation mechanisms are still largely unknown. They are generally constituted of insoluble macromolecules, so that diverse solid-state spectroscopic techniques have been used for their structural elucidation. IR [1–3], X-Ray Diffraction [4], ESR [5, 6], or, recently, Cross-Polarization ^{13}C NMR with Magic Angle Spinning (CP/MAS ^{13}C NMR) [7–10] They have been catalogued with respect to these analytical methods (i.e IR) [11] But, unfortunately, most ambers and fossil resins exhibit rather uniform patterns when examined by these techniques and this represents a major limitation for classification and differentiation purposes Furthermore, with these bulk methods the recognition of individual components is generally not possible, which severely limits the potential chemical and structural comparisons with the major terpenoid derivatives from modern higher plant precursors.

The chromatographic techniques, especially gas chromatography coupled with mass spectrometry (GC-MS) or with infrared spectrometry (GC-IR), help in overcoming this problem However, only the solvent soluble fraction can be studied and this represents a rather limited portion of the organic material present in the ancient resins The chemical information obtained with these two types of instrumental methods is therefore complementary but, surprisingly, few papers have reported their dual utilization in the study of these plant materials [12, 13] The combined use of these techniques is especially promising for samples containing a predominant component in the solvent soluble fraction Such a molecular constituent (identified by GC-MS or GC-IR) can be selected as the principal component material for comparison with the bulk resin

This approach is presented here for fossilized resin from the coalified wood of the Brunner Coal Measures (New Zealand), a series of coal fields of Bortonian Age (Middle Eocene) located at the West Coast of the South Island [14, 15]. These deposits contain large amounts of *Dacrydiumites mawsonii*, an extinct Podocarpaceae very common in the N Z. Westland during this geologic period [16]. Another characteristic species of these deposits is *Triorites harrisii* Couper [17], possibly an extinct Casuarinaceae or a Betulaceae [18] The resin was obtained from Burley's Mine (Buller Gorge). GC-MS and GC-IR analyses have shown that dehydroabietic acid was the predominant constituent in the solvent extractable fraction. Accordingly, a standard of this component was selected as target reference material for the structural analysis of the bulk resin by means of IR and CP/MAS ^{13}C NMR In addition, the predominant occurrence of dehydroabietic acid in this resin may be of interest for the controversy on the molecular composition of the main monomer structures undergoing polymerization during amber formation, either labdatriene compounds [9, 12] or abietic acid [19–21]

RESULTS AND DISCUSSION

Solvent soluble fraction

The molecular components identified in the resin and their corresponding concentrations are given in Table 1. Some other unidentified components at concentrations below 0.1 mg/g are also present. The identifications are based on GC retention indices, mass spectra and, in some cases, IR spectra. In this respect, the presence of dehydroabietic acid (as the methyl ester derivative) was determined by comparison of the following mass spectrometric data MS (product), base peak *m/z* 239, M_r *m/z* 314 (10%), other significant peaks. *m/z* 240 (19%), 241 (2.2), 299 (16), 141 (5 4), 155 (5.4), 173 (4.4), 197 (4 9) and 255 (2 9) MS (standard [22]), base peak *m/z* 239, M_r *m/z* 314 (8 5%), other significant peaks: *m/z* 240 (19%), 241 (2.0), 299 (12),

Table 1 Components identified in the solvent soluble fraction of the resin from the Brunner Coal Measures

Compounds*	Concentration (mg/g)
Dehydroabietic acid	1 6
16,17-Bisnordehydroabietic acid	0 14
8-Pimaren-18-oic acid	0 13
1 2 3,7-Tetramethylbicyclo[4,4,0]-2-decen-7β-oic acid	0 11
Camphor	0 10
8-Isopimaren-18-oic acid	0 064
Ionene	0 058
13-Methylpodocarpa-8 11,13-triene	0 056
Fenchone	0 048
Calamenene	0 040
Dehydroabietin	0 032
Dihydro-*ar*-curcumene	0 030
19-Norabieta-8,11,13-triene	0 016
Cadalene	0 0096

*Identified as methyl ester derivatives

141 (8 5), 155 (5 9), 173 (6 4), 197 (5 9) and 255 (2 5) IR (product, gas phase). C–H stretching 2963, 2929 and 2881 cm^{-1}, C=O stretching 1742, aromatic skeletal vibrations 1609 and 1498, C–H deformations 1463 and 1387, C–O stretching 1237 and 1166, C–C stretching 1124, ArH deformation 1036 and 819 cm^{-1}. IR (standard, liquid solution), 2960, 2938, 2880, 1735, 1617, 1501, 1462, 1390, 1250, 1176, 1128, 1040, 822 cm^{-1}. The similarity between the data corresponding to the predominant resin component and standard methyl dehydroabietate is reinforced even more when considering that the instrumental resolution of gas phase IR spectra is 8 cm^{-1}.

Dehydroabietic acid is the major polycyclic diterpenoidal compound found in the geosphere [23]. It is derived from the rapid oxidative dehydrogenation of abietic acids [24]. The aromatic acid then degrades by decarboxylation to dehydroabietin or 19-norabieta-8,11,13-triene and further aromatization results in retene [23–25]. In the Brunner resin this transformation appears to have stopped with dehydroabietic acid and only minor amounts of tricyclic monoaromatic hydrocarbons and a total absence of retene. Thus, the unsaturated components were transformed during resin ageing, but defunctionalization has not occurred extensively.

At a much lower concentration, the second most abundant resin component is 16,17-bisnordehydroabietic acid, an homologue of dehydroabietic acid. This is derived from acids of the pimarane skeleton by the same oxidation processes described above [23, 25]. In this sense, 13-methylpodocarpa-8,11,13-triene is probably related to the occurrence of this C_{18} terpenoid acid. Resin aging is therefore reflected by the formation of the aromatic C-ring and the loss of the vinyl/ethyl side chain. However, in contrast with the abietane-derived terpenoids some unsaturated pimaranes are still present, such as 8-pimaren-18-oic acid and 8-isopimaren-18-oic acid. These components are in fact partially dehydrogenated derivatives of a group of diunsaturated pimarane acids found in abundance in more recent or better preserved resins pimaric, isopimaric, 8-isopimaric and, especially, sandaracopimaric acids [12, 13]. The presence of the monounsaturated components indicates that the exocyclic double bonds have not survived the maturation processes undergone by this resin, and also the endocyclic pimarane unsaturations are far more resistant to chemical transformation. In this respect, the only occurrence of Δ^8-15,16-dihydro components is in agreement with the pimarane double bond isomerization processes described elsewhere [26] as an early aging reaction leading to the more stable location at Δ^8.

Several sesquiterpenoids have been also identified in the resin extract (Table 1). Similarly to the diterpenoids some of them are probable aromatization products of unsaturated or functionalized precursors. Thus, calamenene and cadalene may be derived from cadinenes [25], cadinols [25] or muurolenes and dihydro-ar-curcumene from bisabolenes. Their occurrence may therefore reflect maturation reactions with regards to sesquiterpenoids as described above.

The solvent extractable fractions of several fossil resins from New Zealand have already been studied by Thomas [27], who related their composition with that of Kauri resin, especially *Agathis australis*. In that study it was reported the unusual composition, not compatible with that of Kauri resin (i.e. absence of agathic acid), of two fossil resins of Eocene age, Maramarua and Ohai, collected in the southern area of the South Island. Unfortunately, the constituents of these two resins could not be identified at that time. However, the Ohai coal measures, although they are older than Bortonian, contain a floral distribution in their upper beds which is rather similar to that of the Brunner coal [14, 17]. It is therefore possible that the distinct composition of these two resins could be due to a relationship to different plants (i.e. *Dacridiumites mawsoni*) and not to additional geochemical transformations of Kauri resin as suggested [27].

Bulk resin

The elemental composition of the bulk resin is 74 4% carbon, 10 6% hydrogen, 4 05% sulphur, and nitrogen is below 0 1%. The most significant aspect is the high H/C ratio (1 71) when compared with that of the coalified wood to which the resin is attached (0.61–1.26) [7], suggesting that major structural differences exist between

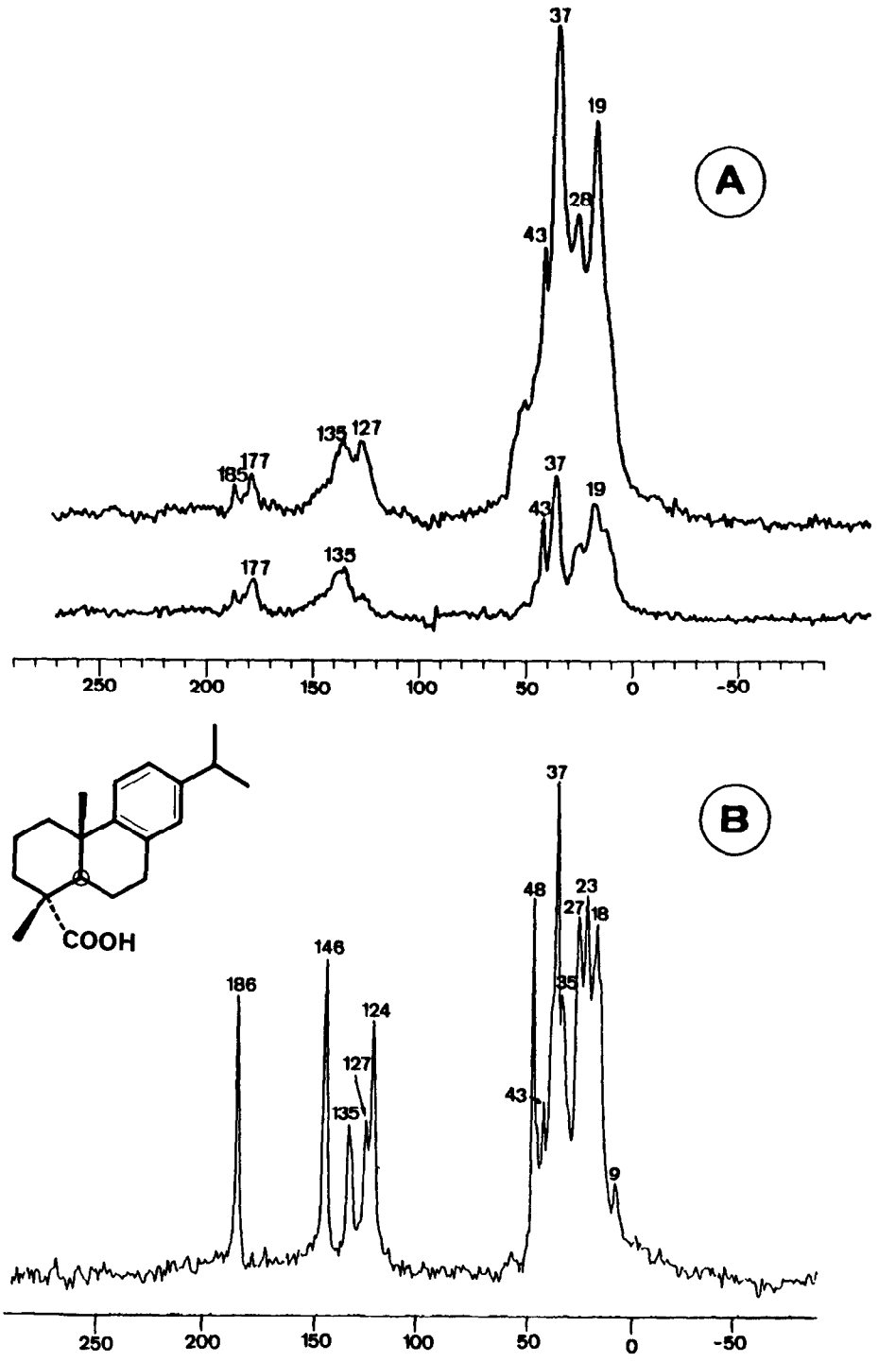

Fig 1 Solid state NMR spectra of (A) the resin studied and (B) dehydroabietic acid, the predominant component in the solvent soluble fraction of the resin. The lower trace in (A) corresponds to a dipolar dephasing time of 50 μsec

the resins and the hard constituents of their plants of origin. The difference is even more striking when considering the geological age of the resin examined here. Coalification of wood involves a significant depletion in the content of hydrogen versus carbon [10], a trend which is also observed in natural oxidation processes [28] or gelification [29] of coals. The hydrogen content of the resin is even higher than that corresponding to the elemental composition of dehydroabietic acid ($C_{20}H_{28}O_2$; H/C = 1.4). In fact, it corresponds to the empirical formula of a fully saturated tricyclic diterpenoid acid, suggesting that other more aliphatic materials have also contributed to the polymer structure of the resin

The IR spectrum of the whole resin exhibits a rather common pattern. A broad absorption band at 3500 cm^{-1} representing the hydroxyl functions (stretching), a group of more or less well resolved bands in the 2840–2950 cm^{-1} range due to carbon–hydrogen bond stretching, the corresponding bending motions being at 2430–2520 and 1370–1380 cm^{-1}, and the remaining prominent band between 1680 and 1730 cm^{-1} produced by stretching of carbon oxygen double bonds, the 'carbonyl band' All of them correspond with the IR spectrum of dehydroabietic acid, but the profile of the whole resin does not show distinct features with respect to other resins reported elsewhere [1, 2, 3, 11] In this regard, the three bands near 880, 1640 and 3070 cm^{-1} characteristic of methylene exocyclic groups [30] are absent, which is in agreement with the observed lack of exocyclic unsaturated components in the solvent soluble fraction

The CP/MAS ^{13}C NMR spectra of Brunner resin and dehydroabietic acid are displayed in Fig 1. Three regions are defined in each spectrum One corresponding to the saturated carbons (δ0–50), another for the olefinic and/or aromatic carbons (δ120–150) and a third for the carbonyl groups (above δ160)

As described for diterpenoids [31], the peaks at δ19, 28, 37 and 43 of the Brunner resin correspond to diverse methyl, methylene, methine and quaternary carbons Specific assignment is difficut due to overlapping resonances but chemical shift considerations and the use of dipolar dephasing techniques allow differentiation of methyl and quaternary carbons [9] Thus, the peak at δ19 has a contribution from some methyl groups, and quaternary carbons most likely contribute to the peak at δ37 ppm (in part) and 43 (entirely). Dehydroabietic acid also has some NMR bands corresponding to these saturated carbons (i.e δ9, 18, 23, 27, 35, 37) However, it contains a large band at δ48 not observed in the resin which corresponds to the quaternary carbon attrached to the carboxylic acid group

In the olephinic/aromatic region, comparison of the conventional and dipolar dephasing NMR spectra of the Brunner resin shows that the peaks at δ127 and 135 correspond to protonated and quaternary carbons, respectively. Integration of these peaks indicates that only 11% of the total carbons are olephinic which, assuming that the resin is composed by diterpenoid molecules, represents one double bond per unit The lack of a peak at δ120 is consistent with the IR data and rules out methylene exocylic groups In the spectrum of dehydroabietic acid the 124–146 peaks account for about 30% of the total area which is in agreement with its known structure These NMR features imply that dehydroabietic acid is not a major subunit of the resin polymer The ratio of unsaturated-to-saturated bands, is in agreement, with the high H/C ratio of the resin and suggest that polymerization and not aromatization has been the major process during resin ageing

Finally, the total intensity of carbonyl groups in the NMR spectrum of the Brunner resin (δ177–185) is 2 3% corresponding to approximately one carbonyl atom per two diterpenoid subunits Dehydroabietic acid shows about 5% carbonyl carbon which is consistent with its known composition The carbonyl groups of the Brunner resin may have also been modified during ageing, as their corresponding NMR bands appear depleted and poorly resolved Extensive defunctionalization has not occurred since the IR spectrum of the resin exhibits an important carbonyl band and the solvent extractable fraction is dominated by acidic components.

EXPERIMENTAL

Bulk analyses The resin was pulverized to pass a 100-mesh screen prior to analysis The elemental composition was determined using two Carlo Erba Elemental Analyzers, models 1106 and 1500 Five or more replicates per sample were analysed until the observed dispersion of the results had comparable values to instrumental precision (*ca* 0 1–0 3%) IR KBr pellet

Solid state ^{13}C NMR spectra (conventional and dipolar dephasing) were obtained as described previous [7] using cross polarization with magic-angle spinning (CP/MAS) on a Chemagnetics Model 100S/200L spectrometer, operating at a field strength of 2 35 Tesla (25 2 MHz for carbon) Ca 10 000–50 000 scans were obtained with a 1 sec delay and 1 msec contact time each Spinning speeds of 3 to 3 5 KHz were maintained to minimize interference from spinning sidebands Dipolar dephasing time was 50 μsec

Extraction and fractionation The resin was extracted with $CHCl_3$–MeOH (3 1), concentrated and treated with CH_2N_2 dissolved in Et_2O to esterify the free fatty acids The extracts were then subjected to TLC using hexane–Et_2O (9 1) as eluent The bands corresponding to hydrocarbons, esters and ketones were scrapped off the TLC plate after visualization with I_2 vapour, and eluted with CH_2Cl_2 The fractions enriched in hydrocarbons (F1) and esters plus ketones (F2) were analysed by GC-MS as well as GC-IR (F2 only)

Chromatographic analyses GC analyses were performed with a Carlo Erba 4160 GC instrument equipped with a 20 m × 0 25 mm i d glass capillary column coated with SE-54 Hydrogen was the carrier gas The temp was programmed from 60 to 310 at 6 /min (injector temp 260 , detector temp 340) The injection was in the splitless mode (solvent *i*-octane), keeping split and sweep valves closed for 40 sec

The chromatographic conditions for GC-MS were similar to those described above except that He was the carrier gas The mass spectrometer temperatures were transfer oven 300 , ion source 200 and multiplier 230 , and the quadrupole was scanned from m/z 40 to 540 at 1 sec per decade

GC-IR 25 m × 0 30 mm i d SE-54 fused silica column and He was used as carrier gas The temp was programmed from 60 to 280° at 6°/min (injector, transfer lines and flow cell temp were 275) Injection was in the splitless mode as described above Spectra were collected between 4000 and 800 cm^{-1} at 3 scans sec Spectral resolution was 8 cm^{-1}

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