The nature and fate of natural resins in the geosphere

Part XI.† Ruthenium tetroxide oxidation of a mature Class Ib amber polymer‡

Ken B. Anderson

Chemistry Division, Argonne National Laboratory, Argonne, IL, 60439, USA.
E-mail: kbanderson@anl.gov

Article

The results of ruthenium tetroxide (RuO_4) oxidation of a mature Class Ib amber polymer are reported and discussed. These data indicate that the residual double bond present in mature Class I ambers is not located in the A/B ring structure of these materials and that C17 of the original labdanoid precursors is retained in mature Class I ambers as a methyl group. These data also suggest that the reaction which results in formation of the residual unsaturated structure in mature ambers also results in a second covalent connection between the A/B ring system and the polymer backbone, probably through C8 of the original labdanoid structure.

Introduction

Fossil resins (ambers), are common sedimentary constituents in many areas, and occur in great abundance in some deposits, (*e.g.*, around the Baltic region, where amber has been commercially produced for centuries and in the Dominican Republic where commercial deposits are also mined). The majority of significant deposits are Tertiary but older deposits, stretching back to at least the Triassic, are known.

Ambers are classified chemically on the basis of their molecular structure.[2–4] Class I ambers are derived from higher plant resins based primarily on polymers of labdatriene diterpenes. When the plant exudes resin, (usually in response to injury or stress of some form), these compounds undergo polymerization across the terminal double bond located in the side chain to give a general 14,15-poly(labdatriene) polymer structure[5] (numbering used is the accepted numbering of labdanoid diterpenes, see Fig. 1). In many cases, a variety of related labdanoid precursors are incorporated into the developing polymeric structure resulting in a final copolymeric structure incorporating labdanoid carboxylic acids, alcohols and hydrocarbons. Ambers based on both *regular* (Fig. 1B) and *enantio* (Fig. 1C) labdanoids are well known in the geosphere,[6] and sub-classification of Class I ambers is based on this distinction (and on the incorporation, or lack thereof, of succininc acid).[2]

It has been found that maturation of Class I ambers results in regular changes in their structure, but details of the transformations involved remain elusive. It is known that maturation results (in some cases) in defunctionalization of susceptible labdanoid monomers,[6] but the primary maturation pathway of these materials involves changes in the nature and abundance of unsaturated structures. It has been shown that maturation results in a progressive loss of exomethylene character,[2,7] and also a net progressive loss of total unsaturation, approaching 1 double bond (*i.e.*, 2 olefinic C) per monomer in mature samples.[8,9] It has also been found that the structural characteristics of the residual double bond in mature Class I ambers are not comparable to either of the double bonds present in the initial poly(labdatriene), nor any of their

simple isomerization products.[10] This suggests that maturation of these materials involves reaction(s?) which transform both of the double bonds present in the initial polymeric structure. Since no obvious mechanism exists for reduction of either of these structures, it is likely that some form of cyclization and/or cross linking reaction is involved.

To further elucidate the details of this process, a mature Class Ib amber (Upper Cretaceous, Yantardakh Hill region, Siberia) has been subjected to ruthenium tetroxide (RuO_4) oxidation. This reagent aggressively cleaves carbon–carbon double bonds but does not attack saturated structures. In order to simplify interpretation of the resulting data, the sample was first extracted to isolate a soluble polymer phase[11] which was as free as possible of extraneous occluded materials. The results of these investigations are reported herein.

Experimental

Sample

The sample used for the analyses reported here was collected from Upper Cretaceous (Santorian) sediments in the Yantardakh Hill region (Khatanga depression) of Siberia. Analyses of the bulk sample have been described previously.[3]

Extraction

Amber (251.9 mg) was placed in a micro Gregar extractor and extracted with 1:1 (v/v) CH_2Cl_2:n-pentane to remove occluded materials. After 24 h, extraction was stopped and
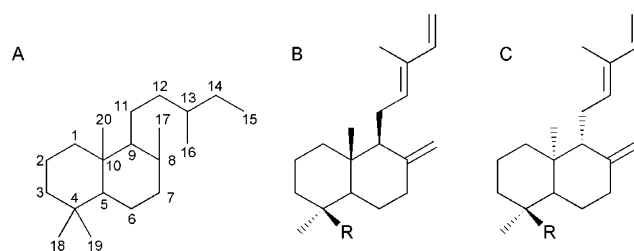

Fig. 1 (A) Labdanoid carbon skeleton (accepted numbering convention indicated). (B) *Regular* configuration labdanoid diterpene. R = CO_2H, communic acid; R = CH_2OH, communol; R = CH_3, biformene. (C) *Enantio* configuration labdanoid diterpene. R = CO_2H, ozic acid; R = CH_2OH, ozol; R = CH_3, *enantio* biformene.

---

the CH_2Cl_2–n-pentane solution removed and replaced with CH_2Cl_2 : CH_3OH (1 : 1 v/v) and the extraction recommenced. After a further 24 h, the CH_2Cl_2–CH_3OH soluble phase was rotary evaporated to near dryness, then taken up to the extent possible in 10 mL CH_3OH and fully precipitated by slow addition of cold H_2O. The resulting precipitate was recovered by filtration and washed with cold H_2O. Residual insoluble materials were also collected, and washed with CH_3OH–H_2O. Both products were then dried overnight *in vacuo* at 60 °C to give 149.6 mg soluble polymer (60% yield relative to original amber) and 15.2 mg insoluble residue (6% yield relative to original amber).

RuO$_4$ oxidation

Soluble polymer (24.8 mg) was placed together with $NaIO_4$ (220 mg) and $RuO_2 \cdot xH_2O$ (9.6 mg) in a 25 mL round-bottom flask to which CCl_4 (1.0 mL), CH_3CN (5 mL) and H_2O (4 mL) were then added. The resulting mixture rapidly yellowed and was allowed to stir for 4 d, after which time the reaction was quenched by addition of CH_3OH (0.5 mL). The entire crude product was then evaporated to dryness, then taken up in CH_3OH (4 × 10 mL washes) and filtered to remove insoluble byproducts (largely RuO_2, $NaIO_3$ and any unreacted $NaIO_4$). To this $MgSO_4$ was added to remove residual H_2O and the resulting mixture refiltered to give a clear CH_3OH solution which was then evaporated to *ca.* 20 mL. To this solution 2 mL of BF_3–CH_3OH complex was then added and the reaction was refluxed for 2 h, then allowed to stand overnight, after which 5 mL saturated aqueous $NaHCO_3$ was added to terminate the reaction. This product was then taken up in diethyl ether–CH_3OH (1 : 3 v/v 40 mL) filtered over $MgSO_4$, evaporated to dryness, washed again with H_2O, then finally recovered by filtration and dried at 60 °C overnight. Yield *ca.* 70% (by mass).

^{1}H and ^{13}C NMR spectroscopy

NMR experiments were performed on a Bruker model DMX 500 NMR spectrometer (11.7 T). With the use of the nitrogen pre-cooler, heater coil, and the variable temperature controller, the temperature was stable at 294.3 × 0.1 K. ^{13}C NMR spectra were recorded with 22,000 acquisitions, 10 s recycle delay, 9 ms pulse width (70°) and 33 kHz sweep width using a two channel 10 mm broadband, direct detection, variable temperature probe with proton decoupling and ^{2}H lock at 76.773 MHz. ^{1}H experiments were carried out using a three channel 5 mm inverse detection, three axis gradient, variable temperature probe with ^{2}H lock at 76.773 MHz. ^{1}H spectra were recorded with 64 acquisitions, 4 s recycle delay, 4 ms pulse width (70°) and 6 kHz sweep width. Solvent used for both ^{13}C and ^{1}H experiments was CD_2Cl_2–CD_3OD *ca.* 1 : 1 vol.

Pyrolysis GC-MS and GC-MS analyses

Both the isolated soluble polymer and the product of its RuO_4 oxidation (which was found to be unamenable to analysis by gas chromatography—no significant products observed) were further analyzed by Py-GC-MS. Details of the analytical system used have been reported elsewhere.[12] Briefly, T_{pyrol} = 480 °C (10 s). Chromatographic conditions: 60 m DB-XLB (0.25 mm id, 0.25 μm phase thickness), T_{init} = 40 °C (4 min), ramp at 4° min^{-1}, T_{final} = 310 °C (16 min). For analysis of the isolated polymer, TMAH was added as described elsewhere.[12] No TMAH was used in Py-GC-MS analyses of the RuO_4 oxidation product of the soluble polymer.

GC-MS analyses were conducted using an HP 5972 MSD coupled with an HP 5890 (II) equipped with an automated direct on-column injector. GC-MS analyses reported herein (data set 1) were carried out using a 60 m DB-5 MS column (0.25 mm id, 0.25 μm phase thickness) programmed as follows:
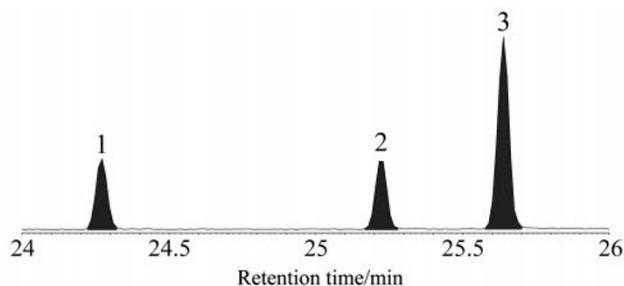
Data set 1. GC-MS data for the crude product of hydrolysis–dehydration/methylation of sclareolide by oxalylchloride. MS data and structural assignments for these eluants are presented in pdf format as supplementary data.§

T_{init} = 140 °C (4 min), ramp at 4° min^{-1}, T_{final} = 300 °C (10 min).

Synthesis of (2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydro-naphthalen-1-yl)-acetic acid methyl ester (1S,4aS,8aS), (5,5,8a-trimethyl-2-methylene-decahydro-naphthalen-1-yl)-acetic acid methyl ester (1S,8aS) and (2,5,5,8a-tetramethyl-3,4,4a,5,6,7,8,8a-octahydro-naphthalen-1-yl)-acetic acid methyl ester (4aS,8aS)

In order to confirm the assignments of products identified in Py-GC-MS analyses of the RuO_4 oxidation product described above, these compounds were synthesized by hydrolysis–dehydration of 3aR(+)-sclareolide (Aldrich Chemical Co.). These products have been previously identified by Hinder and Stoll[13] as products of the H_2SO_4 hydrolysis of 3aR(+)-sclareolide. In the present study it was found that the synthesis described by these authors[13] could be considerably simplified (reduced to a single step) as follows: 3aR(+)-sclareolide (103.2 mg) was dissolved in oxalylchloride (2 M in CH_2Cl_2) (8 mL) and the mixture brought to reflux for 45 min. After this time, the reaction mixture was allowed to cool and quenched with CH_3OH (3 mL) to give quantitative (GC) conversion to the mixture of products described in data set 1. The observed distribution of products could be affected (slightly) by varying reaction conditions, but the same qualitative mixture was obtained in all cases.

Results and discussion

^{13}C NMR analysis of the soluble polymer prior to oxidation indicates terminal CH_2 : total other CC (excluding aromatic C) of approximately 1 : 7.5, indicating that maturation related transformation of the initial exomethylene double bond is advanced to essentially complete in this sample. ^{1}H NMR spectra for this sample are also consistent with this conclusion (NMR data are illustrated in the supplementary data§).

Results of Py-GC-MS analyses of the isolated soluble polymer of the amber used in this study are given in data set 2.

All of the products observed in these data belong to one of the series of *regular* bicyclic compounds derived from the A/B ring system of various *regular* labdanoid monomers (see Fig. 2). These compounds are characteristic of the pyrolysates of Class Ia and Ib ambers.[2,6] Structures in which C11 is retained (Fig. 2, C and D) predominate over those in which this carbon is absent (Fig. 2, A and B), as is typical for mature Class I ambers.[2] These data are, not surprisingly, closely comparable with data from analyses of the whole amber from this site,[3] the only significant difference being the presence in significant abundance of bicyclic methyl ethers, which are undoubtedly an artefact of the isolation procedure used. These data also show
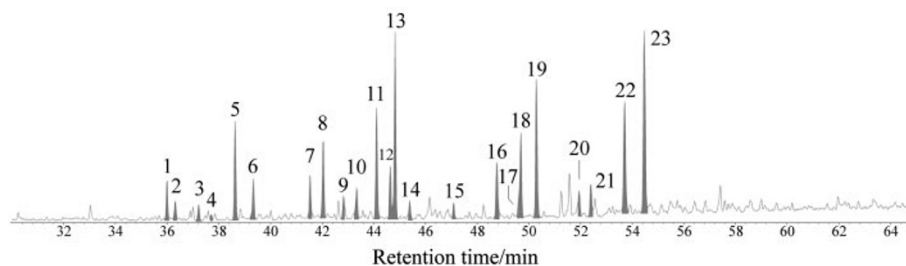
Data set 2. Py-GC-MS data for the isloated (soluble) polymer fraction of Yantardakh Hill (Siberia) Cretaceous amber. MS data and structural assignments for these eluants are given in the supplementary data§.
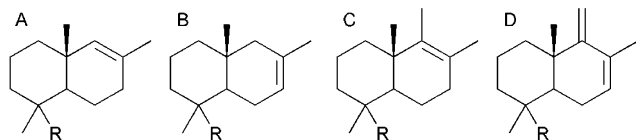

Fig. 2 Bicyclic products characteristic of the pyrolysates of Class Ia and Ib ambers. R = H(α or β), CH$_3$, CH$_2$OH, CH$_2$OCH$_3$, CO$_2$CH$_3$.

that this product is free of occluded materials, especially abietane-type diterpenes, which are observed in the pyrolysate of the whole amber[3] and which may have complicated interpretation of subsequent data from RuO$_4$ oxidation studies.

Data from Py-GC-MS analysis of the RuO$_4$ oxidation product of the amber polymer described above, are given in data set 3.

Although not all of the observed products can be unambiguously assigned at this time, data from the hydrolysis–methylation of sclareolide (data set 1) and comparison of the data given in data set 3 with literature data[14] do allow the majority of products, including most of the major products, to be assigned with a high degree of confidence. All of these compounds (data set 3, 1–3 and 6–8) belong to two homologous series of compounds which are clearly derived from, and which preserve the A/B ring of the original labdanoids (see Fig. 3).

The structural characteristics of these compounds are very informative in a number of ways. Firstly, as just noted, these compounds all preserve intact the original labdanoid A/B ring structure. Given the high yield recovered from the oxidation procedure, this indicates that the residual double bond present in this amber is not located in this ring system, since had it been so, these rings would have been cleaved by the oxidation procedure. It can also be concluded that the final double bond is not directly attached to this ring system since if it were then one would anticipate keto products at the point of attachment, and no such products have been identified.

It is also informative that all of these products are themselves unsaturated. Clearly this is a result of the pyrolytic analyses used in this investigation since olefinic structures are very

susceptible to cleavage by RuO$_4$ and NMR analyses of this product prior to pyrolysis confirm the absence of C=C unsaturation. All of these products are unsaturated around C8. This suggests that prior to pyrolysis (and after oxidation) this carbon is likely to be functionalized and that on pyrolysis this functionality is being lost, probably through dehydration or decarboxylation or a similar mechanism.

In all of these products C17 of the original labdanoid is retained. In the original labdanoid structure this carbon is present in an exomethylene structure. Progressive 'loss' of exomethylene character is one of the defining characteristics of maturation of these polymers,[2,7–9] and it might be (and in fact has been[8,9,15]) postulated that this reflects cross-linking or condensation reactions directly involving this structure. However, the preservation of this carbon in all cases as a methyl group argues against this possibility since such reactions would retain this carbon as a methylene structure and it is difficult to postulate any mechanism by which such a structure can be universally converted to a methyl group upon pyrolysis. It is also noteworthy that all of the characteristic bicyclic structures observed in the pyrolysates of Class I ambers also retain this carbon as a methyl group, which supports the view that this carbon is retained as a methyl group in mature Class I ambers.

If it is correct that C17 is retained in mature Class I ambers as a methyl group, then this implies that isomerization of the initial exomethylene structure is an early step in the maturation of these materials. However, as noted above, the data given in data set 3 preclude the presence of a double bond in, or on the labdanoid A/B ring system in mature samples. How then, can these observations be reconciled? One additional observation is helpful in this regard—the product of RuO$_4$ oxidation of this
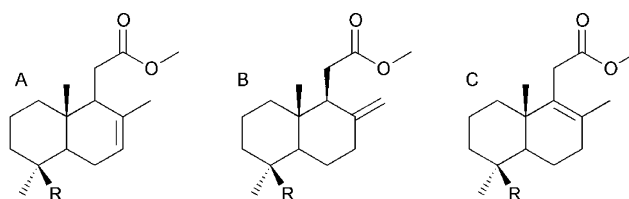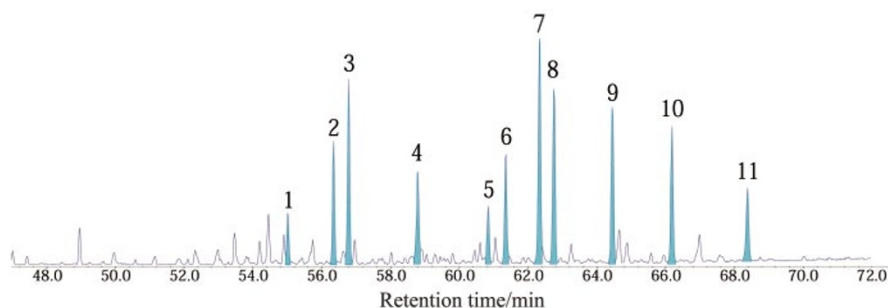

Fig. 3 Major products observed in pyrolysate of RuO$_4$ oxidation product of Siberian amber polymer. R = CH$_3$ or CO$_2$CH$_3$.

Data set 3. Py-GC-MS data for the RuO$_4$ oxidation product of the (soluble) polymer fraction of Yantardakh Hill (Siberia) Cretaceous amber. MS data and structural assignments for these eluants are given in the supplementary data§.

polymer was not amenable to analysis by GC-MS. Although it was not possible to determine absolute molecular weights for the RuO$_4$ oxidation products described herein, this observation suggests that this product is still a high molecular weight material.

If maturation of polymers with an initial 14,15-poly(labda-triene) structure results in a structure with one double bond, which is not located in nor directly attached to the A/B ring systems of the labdanoid monomers, then it follows *ex necessitate rei*, that the double bond must be located somewhere in the side chain structure. However, one would expect oxidation of such a structure to give volatile, low molecular weight products due to cleave of the linkage between the polymer backbone and the A/B ring system, and this is observed on oxidation of immature samples (Anderson, unpublished results). The absence of volatile products from the RuO$_4$ oxidation of this mature sample therefore suggests that covalent connection still exists between the polymer backbone and the A/B ring system after oxidation.

Hence, we are left with the following:

(i) Maturation of Class I ambers results in a structure with ~1 double bond per original monomer.[8,9]

(ii) This residual double bond is not structurally equivalent to either of the initial double bonds,[10] nor is it located in, or directly attached to the labdanoid A/B ring system of the original monomer.

(iii) C17 of the original labdanoid structure is retained in the mature structure as a methyl group.

(iv) In addition to the connection through the original side chain, maturation results in a new covalent connection between the polymer backbone and the labdanoid A/B ring system.

Conclusions

On the basis of the data presented and discussed above, the following conclusions may be drawn: During the early stages of maturation of Class I ambers, the (8,17) exomethylene structure undergoes a slow isomerization into the B ring of the original monomer. This process sets up a (relatively) rapid second reaction involving both this isomerized double bond and the second double bond located in the side chain of the monomer. This second reaction results in a new covalent linkage between C8 and the polymer backbone and leaves the residual double bond in a structural environment dissimilar to that of either of the original double bonds (or their simple isomerization products) as shown by NMR data.[10] This reaction may occur between double bonds present within a single monomer unit, but is more likely to involve reaction between double bonds originally located in different monomers.

Acknowledgements

References

1 K. B. Anderson and J. V. Muntean, The Nature and Fate of Natural Resins in the Geosphere. Part X. Structural Characteristics of the Macromolecular Constituents of Modern Dammar Resin and Class II Ambers, *Geochem. Trans.*, 2000, 1.

2 K. B. Anderson, R. E. Winans and R. E. Botto, The Nature and Fate of Natural Resins in the Geosphere. II. Identification, Classification, and Nomenclature of Resinites, *Org. Geochem.*, 1992, **18**(6), 829.

3 K. B. Anderson, The Nature and Fate of Natural Resins in the Geosphere. IV. Middle and Upper Cretaceous Amber from the Taimyr Peninsula, Siberia - Evidence for a New Form of Polylabdanoid of Resinite and Revision of the Classification of Class I Resinites, *Org. Geochem.*, 1994, **21**(2), 209.

4 K. B. Anderson and J. C. Crelling, Introduction (Amber, Resinite, and Fossil Resins), *ACS Symp. Ser.*, 1995, 617, xi–xvii.

5 R. M. Carman, D. E. Cowley and R. A. Marty, Diterpenoids. XXV. Dundathic acid and Polycommunic acid, *Aust. J. Chem.*, 1970, **23**(8), 1655.

6 K. B. Anderson, The Nature and Fate of Natural Resins in the Geosphere. Part V. New Evidence Concerning the Structure, Composition, and Maturation of Class I (Polylabdanoid) Resinites. *ACS Symp. Ser.*, 617, 1995, Amber, Resinite, and Fossil Resins, 105 and errata thereto; *Org. Geochem.* 1997, **25**(3/4), v–vi.

7 J. B. Lambert, J. S. Frye and G. O. Poinar, Amber from the Dominican Republic: Analysis by Nuclear Magnetic Resonance Spectroscopy, *Archaeometry*, 1985, **27**(1), 43.

8 D. J. Clifford and P. G. Hatcher, Structural Transformations of Polylabdanoid Resinites During Maturation, *Org. Geochem.*, 1995, **23**(5), 407.

9 D. J. Clifford and P. G. Hatcher, Maturation of Class Ib (Polylabdanoid) Resinites (Amber, Resinite, and Fossil Resins), *ACS Symp. Ser.*, 1995, 617, 92.

10 D. J. Clifford, An Investigation of Polylabdanoid (Class I) Resinite Maturation by Characterization of Olefinic Functional Groups, 1996, PhD Thesis, Pennsylvania State University, ch. 7.

11 D. J. Clifford, P. G. Hatcher, R. E. Botto, J. V. Muntean, B. Michels and K. B. Anderson, The Nature and Fate of Natural Resins in the Geosphere. VIII. NMR and Py-GC-MS Characterization of Soluble Labdanoid Polymers, Isolated from Holocene Class I Resins, *Org. Geochem.*, 1997, **27**(7/8), 449.

12 K. B. Anderson and R. E. Winans, The Nature and Fate of Natural Resins in the Geosphere. I. Evaluation of Pyrolysis-Gas Chromatography-Mass Spectrometry for the Analysis of Natural Resins and Resinites, *Anal. Chem.*, 1991, **63**, 2901.

13 M. Hinder and M. Stoll, Odeur et Constitution XI. Etude de la transesterification-deshydration de la lactone de l'acide tetra-methyl-1,1,7,10-hydroxy-6-decalyl-5-acetique, *Helv. Chim. Acta*, 1954, **37**, 1859.

14 R. C. Cambie, G. R. Clark, M. E. Goeth, C. E. F. Rickard, P. S. Rutledge, G. R. Ryan and P. D. Woodgate, Chemistry of the podocarpaceae. LXXIV. The conversion of podocarpic acid into g-bicyclohomofarnesals, *Aust. J. Chem.*, 1989, **42**(4), 497.

15 M. A. Wilson, J. C. Hanna, P. A. Cole-Clarke, P. F. Greenwood and G. D. Willett, Structure and Reactivity of Latrobe Valley Resinites: Characterization of Fractions Separated on the Basis of Color, *Fuel*, 1992, **71**(10), 1097.