Oxidation Products of Abietic Acid and Its Methyl Ester

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Abietic acid (**1**) and its methyl ester (**2**) were investigated under various storage conditions to provide an indication of their preferred oxidation mechanisms and to investigate the most susceptible positions for modification in the abietane skeleton. Six known compounds, methyl 7α,13*β*-dihydroxyabiet-8(14)-enoate (**4a**), methyl 7α,13α-dihydroxyabiet-8(14)-enoate (**4b**), methyl 12-oxoabietate (**6**), methyl 7-oxodehydroabietate (**7**), methyl 7α-hydroxydehydroabietate (**8**), and 13,14-*seco*-13,14-dioxoabiet-7(8)-enoic acid (**11**), were identified. Compounds **7** and **8** are regarded as potent allergens. In addition, six new oxidation products were isolated, methyl 13*β*-ethoxy-7α-hydroxyabiet-8(14)-enoate (**3a**), methyl 13α-ethoxy-7α-hydroxyabiet-8(14)-enoate (**3b**), methyl 7α-hydroperoxy-13α-hydroxyabiet-8(14)-enoate or methyl 13α-hydroperoxy-7α-hydroxyabiet-8(14)-enoate (**5**), 7α,13*β*-dihydroxyabiet-8(14)-enoic acid (**9a**), 7α,13α-dihydroxyabiet-8(14)-enoic acid (**9b**), and 7α,15-dihydroxydeydroabietic acid (**10**). Their structures were characterized on the basis of spectroscopic data interpretation. The cytotoxicity of several compounds against KB cells was evaluated, and weak activity was observed for **6**, **7**, and **8** with IC_{50} values of 12.5, 4.5, and 5.8 μg/mL, respectively.

Rosin is a natural resin obtained from conifer species. It consists mainly of resin acids[1] and plays an effective role in the wound healing of trees by hardening after evaporation of the essential oil, and it possesses antibacterial and antifungal activity.[2,3] A well-known product of rosin is colophony, the residue after distillation of the essential oils. Due to the extensive use of resin acids in several products of everyday life, e.g., in paper sizing, printing inks, adhesives, glues, technical resins, synthetic rubber, cosmetics, and dentistry, an increasing number of people suffer from contact dermatitis and asthma.[4,5] Several studies have revealed that it is not the resin acids themselves but mainly their oxidation products that cause allergenic reactions,[6−9] and colophony ranks within the top 10 international documented allergens.[10]

Due to their very wide application in technical products, the oxidation behavior of diterpenoid resin acids of the abietane and pimarane types has been investigated. The instability of resin acids against heat, air, and light, as well as their sensitivity to mineral acids, is partially understood, leading to isomerization and other reactions of resin acids, e.g., abietic acid (**1**) to dehydroabietic acid and dihydroabietic acid.[1,11] Investigations on the oxidative reactivity of abietane acids have revealed the rearrangement of the double bonds and the formation of endo- and hydroperoxides, epoxides, and hydroxyl and keto groups. Under photosensitizing conditions, these products have been obtained in good yields within a few hours.[12−14] Autocatalytic effects of the carboxylic protons have been assumed. Enoki et al. have described the autoxidation in the presence of sunlight and air within a few days.[15,16]

To the best of our knowledge, the oxidation of resin acids under normal storage conditions of rosin has not been reported. Compound **1**, as the most stable isomer, and its methyl ester **2** were chosen to investigate the oxidation behavior of resin acids. The oxidation of **2** in ethanolic solution was also investigated, as were the effects of mineral acid or air on **1** under different conditions. The isolation and structure elucidation of the resulting products give further indications of the preferred oxidation mechanisms of resin acids and the most susceptible positions.

Results and Discussion

The oxidation of **2** in ethanolic solution under normal atmospheric conditions resulted in a strong yellow discoloration within 48 h. The solvent was evaporated and the residue was subjected to column chromatography on silica gel with cyclohexane−ethyl acetate in a stepwise gradient. This led to the isolation of compounds **3a**, **3b**, **4a**, and **5**.

The molecular formula of compound **3a** was established as $C_{23}H_{38}O_4$ by HREIMS, showing a fragment ion at *m*/*z* 335.2219 $[M - C_3H_7]^+$ (calcd 335.2217) and the molecular ion at *m*/*z* 378 in the LREIMS. The ^{13}C NMR data revealed typical signals of an abietane skeleton, but significant upfield shifts for carbons 7 (δ_C 72.7) and 13 (δ_C 75.2), compared to δ_C 120.6 (C-7) and δ_C 145.2 (C-13) in **2**,[17] indicated the hydroxylation of these positions accompanied by a loss of one double bond in **3a**. In an analogous manner, the ^1H NMR spectrum showed an upfield shift for H-7 resonating at δ_H 4.17 (J = 2.8 Hz) in **3a** instead of δ_H 5.35 in **2**. The small coupling constant permitted an equatorial assignment for H-7 and, therefore, an axial OH group. Two additional carbons at δ_C 56.4 (C-22, t) and 16.2 (C-23, q) pointed to an ethoxy-substituted abietane derivative. This was again confirmed by the ^1H NMR spectrum showing additional resonances at δ_H 3.35 (H-22a, dq, J = 8.4, 6.9 Hz), δ_H 3.28 (H-22b, dq, J = 8.4, 6.9 Hz), and δ_H 1.08 (H_3-23, t, J = 6.9 Hz). ^1H,^1H COSY, HSQC, and HMBC experiments were utilized extensively to complete the assignments of all ^1H and ^{13}C NMR signals. In particular, the HMBC correlations between C-13 and H_2-22 established the position of the ethoxy group. The relative stereochemistry at C-13 and C-7 was determined using 1D NOE experiments. Irradiation of H-12_{ax} enhanced the

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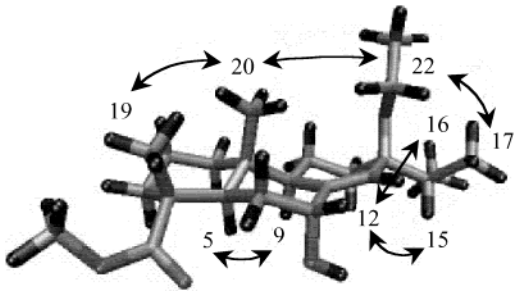
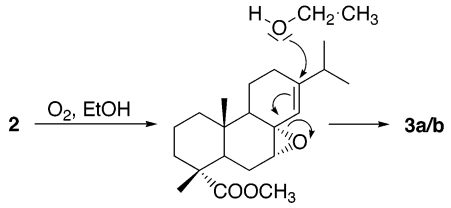


Figure 1. Selected NOE correlations of **3a**.

Scheme 1. Proposed Reaction Mechanism for the Conversion of **2** to **3a**/**b**



signals of H_3-16/H_3-17 and H-15, which permitted an equatorial assignment for the isopropyl group and an axial assignment for the ethoxy group (Figure 1). The H-9 signal was enhanced by an NOE from H-5, and H-14 from H-7. The HRMALDIMS of **3b** showed a pseudomolecular ion at *m*/*z* 401.2662 $[M + Na]^+$ (calcd 401.2667) and pointed to the same elemental formula for **3a** and **3b**. The EIMS of both compounds exhibited a base peak at *m*/*z* 335 $[M - C_3H_7]^+$ and a major fragment at *m*/*z* 317 $[M - C_3H_7 - H_2O]^+$. Analysis of their 1D and 2D NMR spectra revealed that both compounds differ only in the stereochemistry at C-13. For compound **3b** an NOE was observed from H_3-20 to H-15, demonstrating an axial arrangement of the isopropyl group. A possible reaction mechanism for the generation of **3a** and **3b** from **2** is shown in Scheme 1.

The known compound **4a** was identified by 1D and 2D NMR experiments as methyl 7α,13*β*-dihydroxyabiet-8(14)-enoate, showing spectroscopic and physical features identical to those reported in the literature.[18] Compound **5** was considered originally to be the C-13 diastereomer (**4b**) of compound **4a** from its NMR analysis, but its EIMS data revealed a molecular ion peak at *m*/*z* 366 instead of *m*/*z* 350, as observed for compound **4a**, indicating an additional oxygen and the presence of a hydroperoxide functional group. This was confirmed from the IR spectrum, which showed a weak hydroperoxide band at 888 cm^{-1},[19] and by reduction of **5** with $NaBH_4$, yielding methyl 7α,13α-dihydroxyabiet-8(14)-enoate (**4b**). The position of the OOH group in **5** could be either at C-7 or at C-13, as the chemical shifts in the ^{13}C NMR spectrum did not differ from the data of compound **4b**, the 7α,13α-diol, consistent with the literature.[18] On comparing the ^1H NMR data of compounds **5** and **9b** (both possessing the same relative configuration at C-7 and C-13), most significant differences were observed in the methyl groups of the isopropyl moiety (δ_H 0.95 and 0.85 in **5**, and 0.86 and 0.88 in **9b**). This seems to indicate that compound **5** is more likely to be the 13-hydroperoxy derivative, due to the fact that the presence of the hydroperoxy group at C-13 should hinder the free rotation of the isopropyl group and consequently effect larger differences in the chemical shifts of H_3-16 and H_3-17. Formation of hydroperoxides during storage is one possible explanation for the allergenic effects of resin acids. Over the past few years, mechanisms involving radicals have been suggested in the examination of hapten−protein binding, and previous studies indicate that radical reactions could be important in the case of haptens containing hydroperoxide groups.[20,21]

Oxidation of **1** in the presence of sulfuric acid followed by methylation gave the known compounds methyl 12-oxoabietate (**6**), methyl 7-oxodehydroabietate (**7**), methyl 7-hydroxydehydroabietate (**8**), and **5**. Compound **6** was obtained by synthesis,[22,23] but literature NMR data of **6** are incomplete, and therefore full NMR signal assignments have been made (Tables 1 and 2). NMR data for **7** corresponded well with those reported by Krohn et al.,[24] who isolated **7** and **8** after photolysis of methyl dehydroabietate. Enoki et al.[16] obtained these compounds on the photolysis of **1**. Both compounds were also isolated from Portuguese gum rosin and have been described as strong allergens.[8]

Aerial exposure of resin is inevitable, either while processing or on storage of the final product. For a better understanding of resin acid-oxidation under normal atmospheric conditions, **1** was exposed to air and heated on an oil bath at 100 °C to result in oxidation within a reasonable time. During the first week, the fine colorless crystals melted to a yellow-brownish, sticky mass. Polar products were observed on TLC in increasing amounts, and after the fourth week approximately 30% of **1** was transformed. For exhaustive degradation, higher temperatures as well as more time would be required. Column chromatography was performed on silica gel with cyclohexane−EtOAc containing 0.1% glacial acetic acid in a stepwise gradient. The less polar fractions, in particular, showed intense yellow colors, revealed strong activity under UV light at 254 nm, and gave strong reactions with phosphomolybdic acid reagent. Column chromatography on Sephadex LH-20 with MeOH and further purification on silica gel yielded **9a** and **9b** in very small quantities. Their 1D and 2D NMR spectra resembled those of compounds **4a** and **4b**,[18] lacking only the signal of the methyl group C-21 near δ_C 52.0 and showing a significant downfield shift of the carboxyl group C-18 (δ_C 184.4 and 182.8, respectively). This indicated the presence of the corresponding free acids, which was confirmed by methylation of both compounds with diazomethane, yielding the ester derivatives **4a** and **4b**.

7α,15-Dihydroxydehydroabietic acid (**10**) was obtained from the further chromatography of the fractions with medium polarity. The ^1H NMR spectrum exhibited signals at δ_H 7.44 (d, J = 1.6 Hz, H-14), 7.31 (dd, J = 1.6 and 8.5 Hz, H-12a), and 7.20 (d, J = 8.5 Hz, H-11a), and the lack of the signal of H_3-21 near δ_H 3.60 pointed to an abietic acid derivative with an aromatic C ring. The ^{13}C and ^1H NMR spectra of **10** were similar to those at methyl 7-hydroxydehydroabietate (**8**), but the methyl groups H_3-16 and H_3-17 appeared as singlets at δ_H 1.54 (6H), indicative of a quaternary C-15. In turn, C-15 resonated as a quaternary carbon at δ_C 72.4 and pointed to substitution by an oxygenated functional group. The molecular ion peak in the EIMS at *m*/*z* 332 confirmed the presence of two OH groups. The structure of **10** was established by extensive 2D NMR spectroscopy**.** Compound **10** showed close structural similarities to 15-hydroxydehydroabietic acid, 7-oxodehydroabietic acid, 15-hydroxy-7-oxodehydroabietic acid, and 15-hydroperoxyabietic acid as well as to **7** and **8**, which have been identified as contact allergens in modified or unmodified rosin.[8,25] Therefore, **10** might also elicit allergenic effects. Structurally related alcohols and aldehydes with hydroxyl groups at C-7 and/or C-15 have been isolated from the cones of *Larix kaempferi*.[26,27]

Table 1. ^{1}H NMR Data of the Methyl Abietate Derivatives **3a**, **3b**, **5**, and **6** (δ ppm; m; *J* Hz)[a]

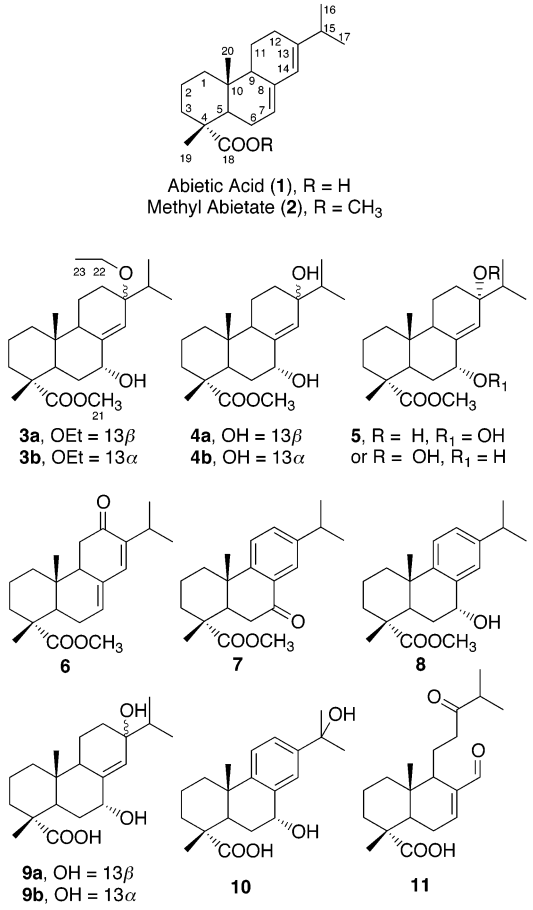
H	3a	3b	5	6
1a	1.67 m[c]	1.68 m[c]	1.64 m[c]	1.74 m[c]
1b	1.14 m[c]	1.14 m[c]	1.13 m[c]	1.09 m[c]
2	1.53 m[c] (2H)	1.50 m[c] (2H)	1.52 m[c] (2H)	1.53 m[c] (2H)
3a	1.74 m[c]	1.72 m[c]	1.72 m[c]	1.71 m[c]
3b	1.59 m[c]	1.56 m[c]	1.56 m[c]	1.62 m[c]
5	2.38 dd (13.3, 2.6)	2.40 dd (13.3, 2.5)	2.36 dd (13.2, 2.5)	2.01 dd (12.3, 3.9)
6_{ax}	1.71 td (13.3, 2.6)	1.64 m[c]	1.64 m[c]	2.11 m[c]
6_{eq}	1.35 dt (14.2, 2.6)	1.32 m[c]	1.29 m[c]	1.86 m[c]
7	4.17 t (2.8)	4.18 t (2.8)	4.11 t (2.7)	5.96 m[c]
9	2.18 ddd (10.0, 6.7, 1.4)	2.24 m[c]	2.22 m[c]	2.45 m[c]
11a	1.52 m[c] (2H)	1.60 m[c]	1.64 m[c]	2.41 m[c]
11b		1.50 m[c]	1.41 m[c]	2.18 m[c]
12a	1.55 m[c]	1.60 m[c]	1.84 m[c]	
12b	1.27 dd (14.3, 4.4)	1.52 m[c]	1.32 m[c]	
14	5.60 s	5.65 s	5.61 s	6.64 s
15	1.97 sept (6.9)	1.77 sept (6.9)	1.68 sept (6.9)	2.84 sept (6.9)
16[b]	0.86 d (6.9)	0.84 d (6.9)	0.95 d (6.9)	0.96 d (6.9)
17[b]	0.79 d (6.9)	0.88 d (6.9)	0.85 d (6.9)	0.99 d (6.9)
19	1.16 s	1.19 s	1.13 s	1.24 s
20	0.75 s	0.76 s	0.71 s	0.83 s
21	3.64 s	3.64 s	3.62 s	3.61 s
22	3.28 dq (8.4, 6.9) 3.35 dq (8.4, 6.9)	3.30 m[c] (2H)		
23	1.08 t (6.9)	1.08 t (6.9)		

[a] Spectra measured at 400 MHz, 295 K, $CDCl_3$. [b] Values interchangeable. [c] Multiplicity not determined due to overlapping signals.

Table 2. ^{13}C NMR Data of Compounds **3a**, **3b**, **5**, **6**, **9a**, **9b**, and **10** (δ ppm; m[a])[b]

C	3a	3b	5	6	9a	9b	10
1	37.8 t	37.9 t	37.8 t	37.6 t	37.6 t	37.9 t	36.7 t
2	16.7 t	18.0 t	18.0 t	17.7 t	18.0 t	18.0 t	17.9 t
3	36.9 t	37.0 t	36.8 t	37.0 t	36.8 t	36.8 t	35.9 t
4	46.9 s	45.6 s	47.0 s	46.0 s	46.6 s	46.7 s	46.9 s
5	42.0 d	41.9 d	42.1 d	43.5 d	41.6 d	41.8 d	39.1 d
6	31.9 t	32.1 t	31.9 t	26.2 t	31.5 t	31.8 t	30.3 t
7	72.7 d	72.7 d	72.2 d	131.8 d	72.7 d	72.9 d	67.9 d
8	145.0 s	142.8 s	140.5 s	133.8 s	143.7 s	143.2 s	125.8 s
9	47.0 d	46.9 d	45.6 d	48.5 d	47.1 d	45.6 d	147.5 s
10	38.1 s	38.5 s	38.3 s	34.6 s	37.8 s	38.5 s	33.5 s
11	18.0 t	18.2 t	18.1 t	37.4 t	18.1 t	18.1 t	124.1 d
12	26.6 t	26.8 t	31.9 t	199.5 s	28.8 t	25.9 t	124.2 d
13	75.2 s	76.0 s	72.0 s	142.0 s	71.4 s	76.4 s	146.9 s
14	130.2 d	131.7 d	132.5 d	140.9 d	131.6 d	131.3 d	124.6 d
15	32.8 d	34.6 d	35.7 d	26.0 d	38.0 d	34.3 d	72.4 s
16[c]	16.0 q	16.3 q	16.6 q	21.5 q	17.0 q	16.7 q	31.6 q
17[c]	17.8 q	17.1 q	16.9 q	22.0 q	17.0 q	17.1 q	31.6 q
18	178.9 s	178.9 s	179.0 s	178.5 s	184.4 s	182.8 s	182.8 s
19	16.8 q	16.8 q	16.7 q	16.8 q	16.7 q	16.7 q	15.7 q
20	13.8 q	14.6 q	14.4 q	14.4 q	14.6 q	14.5 q	24.1 q
21	52.0 q	52.0 q	52.0 q	51.9 q			
22	56.5 t	56.9 t					
23	16.2 q	16.7 q					

[a] Multiplicities obtained from HSQC/DEPT experiments. [b] Spectra measured at 100 MHz, 295 K, in $CDCl_3$. [c] Values are interchangeable.



For further stability testing, **1** was absorbed on silica gel and exposed to air at room temperature. After five months the mixture was desorbed from the silica gel and purified. HPLC separation over RP-18 was performed with MeOH−H_2O according to Sadhra et al.[9] and gave the known **11**. Buchbauer et al. obtained **11** by oxidation of 13,14-dihydroxyabietic acid with $NaIO_4$ during the synthesis of desmethylambraoxide.[28] The physical and spectral data of **11** corresponded well to those given in the literature.[29]

Investigations on the cytotoxicity of these oxidative products revealed weak cytotoxicity for compounds **6**, **7**, and **8** against KB cells (HeLa cells, ATCC CCL17), which exhibited IC_{50} values of 12.5, 4.5, and 5.8 μg/mL, respectively. All other compounds were considered inactive. Due to the small quantities of compounds **3b**, **4b**, **9a**, and **9b** obtained, these substances were not tested.

In conclusion, the work described herein has demonstrated the high sensitivity of **1** and **2** to oxidation in rings B and C, whereas their A rings remained unaffected under all conditions used. It was found that C-7 and C-13 were the most susceptible positions for oxidation. The isolation of **7** and **8**, two known potent allergens, verified that compounds with strong sensitizing properties can be obtained under the reaction conditions utilized. Since it has been assumed for a long time that hydroperoxides are

involved in allergenic processes, compound **5** is an interesting candidate for future dermatological screening. This is also true for compound **10**, which has close structural similarities with known allergens, even though it does not bear a hydroperoxy group.

Experimental Section

General Experimental Procedures. Melting points were determined with an Electrothermal IA 9200 digital melting point apparatus and are not corrected. Optical rotations were measured with a 241 MC polarimeter (Perkin-Elmer). IR spectra were obtained using a 2000 FTIR infrared spectrometer system (Perkin-Elmer). NMR spectra were recorded on Varian Unity Inova 400, Bruker AMX-300, and Bruker DRX-500 instruments. Chemical shifts were reported with reference to the respective residual solvent peaks (δ_H 7.24 and δ_C 77.0 ppm for $CDCl_3$). EIMS data were recorded on a Varian MAT 711 spectrometer, at 70 eV, and ESIMS on a TSQ 7000 spectrometer. HREIMS and HRMALDIMS were performed on a VG-TRIBRID spectrometer and an IonSpec Ultima FT mass spectrometer, respectively. HPLC was performed with a Merck-Hitachi L6200 Intelligent Pump connected to a Rheodyne 7125 injector, a Merck-Hitachi L-4000 UV detector, a Merck-Hitachi D-2500 Chromo-Integrator, a Kauer HPLC precolumn (4 × 4 mm, LiChrosorb RP-18, 10 μm, Merck), and a Knauer HPLC column (250 × 8 mm, Spherisorb ODS 2, 5 μm, Waters Spherisorb). Materials for column chromatography: silica gel 60 (64−200 μm, pore diameter 60 Å, Merck) and Sephadex LH-20 (Pharmacia). Materials for thin-layer chromatography (TLC): TLC aluminum sheets silica gel 60 F_{254} (0.2 mm, 200 × 200 mm, Merck) and TLC RP-18 F_{254} aluminum sheets (0.2 mm, 200 × 200 mm Merck). Materials for preparative TLC: PSC plates and silica 60 F_{254} (1 mm, 200 × 200 mm, Merck). Compounds were detected under UV light at 254 nm, as well as by spraying with phosphomolybdic acid reagent [12 MoO_3 × H_3PO_4, 20% (w/w) in ethanol] and subsequent heating to 105 °C. Solvents for column chromatography were distilled prior to use, and HPLC grade solvents were used for HPLC. Sacotan 90 was obtained from Krems Chemie AG (Krems an der Donau, Austria).

Isolation of Abietic Acid (1) and Synthesis of Methyl Abietate (2). Compound **1** was isolated from Sacotan 90, a fraction of tall oil, one of the byproducts of cellulose manufacturing of softwood (from *Pinus* species). Altogether 55 g of dipentylamine were added dropwise within 1 h to a refluxing solution of 100 g of Sacotan 90 in 400 mL of acetone. After stirring for an additional hour under reflux the reaction was allowed to cool to room temperature. The precipitated ammonium salt was recrystallized six times from acetone, dried in vacuo, and suspended in diethyl ether, and **1** was liberated with 2 N H_2SO_4[30] and crystallized from EtOH−H_2O.[24] Compound **2** was prepared using the method of Abad et al.[31]

Oxidation of 2 in Ethanolic Solution. Compound **2** (1.99 g) was dissolved in 30 mL of EtOH and stirred vigorously at room temperature under air. After 48 h the solvent was evaporated and the obtained residue subjected to column chromatography on silica gel with cyclohexane−EtOAc (68:32), leading to the isolation of **3a/3b** (317 mg/48 mg), **4a** (46 mg), and **5** (40 mg).

Oxidation of 1 in the Presence of H_2SO_4 and Subsequent Methylation. Compound **1** (30 g) was crystallized as the dipentylammonium salt in acetone, and then 300 mL of 2 N H_2SO_4 was added. The volume was reduced on a rotary evaporator at ca. 50 °C within 2−3 h. The liberated acids were extracted with diethyl ether and converted into their methyl esters. The orange oil (14 g) was purified by column chromatography with stepwise gradients of cyclohexane−EtOAc (9:1−0:10) and EtOAc−EtOH (5:1−2:1) and gave **6** (49 mg), **7** (28 mg), **8** (65 mg), and **5** (52 mg).

Oxidation of Powdered 1. Compound **1** (10 g) was heated on an oil bath at 100 °C in a crystallizing dish for 5 weeks. The sticky, yellow-brownish mixture was subjected to column chromatography on silica gel, eluting with cyclohexane−EtOAc−glacial acetic acid (1:9:0.01−0:10:0.01) to obtain seven fractions. An aliquot (0.5 g) of fraction 2 (ca. 6 g) was separated by column chromatography on Sephadex-LH 20 with MeOH as solvent (no polymerization products were detected). Further purification of 240 mg of fraction 2 [R_f 0.47−0.72, cyclohexane−EtOAc−glacial acetic acid (1:9:0.01)] on silica gel yielded **9a** and **9b** (2 mg each). Another 2.5 g quantity of fraction 2 was absorbed on silica gel and eluted with cyclohexane−EtOAc−glacial acetic acid (6:4:0.01) and CH_2Cl_2−MeOH (1:1) to give eight fractions. Purification of 1.5 g of the most nonpolar fraction 1 [R_f 0.11−0.51, cyclohexane−EtOAc−glacial acetic acid (4:6:0.01)] with column chromatography on silica gel with cyclohexane−EtOAc−glacial acetic acid (1:9:0.01 and 6:4:0.01) as solvents afforded **10** (6 mg).

Oxidation of 1 Adsorbed on Silica Gel. Compound **1** (0.8 g) was dissolved in ethanol, adsorbed on silica gel (4 g), and exposed to light and air for 5 months. Column chromatography on silica gel using the solvent systems cyclohexane−EtOAc−glacial acetic acid (9:1:0.01−0:10:0.01) and EtOAc−EtOH−glacial acetic acid (5:5:0.1−1:9:0.1) gave seven fractions of orange-yellow oils. Fraction 4 (30 mg) [R_f 0.77−0.87, cyclohexane−EtOAc−glacial acetic acid (1:9:0.01)] was purified by HPLC on RP-18 eluting with MeOH−H_2O ((8:2) flow rate 1 mL/min, detection at 254 nm) to afford 2.3 mg of **11**.

Methyl 13*β*-ethoxy-7α-hydroxyabiet-8(14)-enoate (methyl [1*R*-(1α,4a*β*,4bα,7*β*,9α,10aα)]-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-7-ethoxy-9-hydroxy-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-carboxylate,3a): colorless spherolites (cyclohexane−EtOAc); mp 95−96 °C; $[\alpha]^{24}_D$ −3.3° (*c* 0.51, CH_2Cl_2); IR (KBr) ν_{max} 3431, 2941, 1728, 1670, 1460, 1388, 1254, 1186, 1149, 1073, 1048, 964, 872, 816, 744 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 378 $[M]^+$ (1), 335 $[M - C_3H_7]^+$ (100), 317 $[335 - H_2O]^+$ (6), 289 (1), 275 (4), 257 (3), 247 (14), 229 (10), 201 (4), 173 (1), 159 (3), 123 (6), 107 (6), 55 (3), 43 (4), 32 (18); HREIMS (pos) *m*/*z* 335.2219 $[M - C_3H_7]^+$ (calcd 335.2217).

Methyl 13α-ethoxy-7α-hydroxyabiet-8(14)-enoate (methyl [1*R*-(1α,4a*β*,4bα,7*β*,9α,10aα)]-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-7-ethoxy-9-hydroxy-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-carboxylate, 3b): colorless oil; $[\alpha]^{24}_D$ −33.9° (*c* 0.049, CH_2Cl_2); IR (KBr) ν_{max} 3436, 2948, 2871, 1726, 1459, 1387, 1249, 1191, 1104 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 335 $[M - C_3H_7]^+$ (100), 317 $[335 - H_2O]^+$ (10), 289 (2), 275 (3), 257 (2), 247 (9), 229 (7), 201 (3), 181 (2), 159 (2), 139 (2), 123 (5), 100 (6), 81 (3), 55 (4), 43 (8), 32 (3); HRMALDIMS (pos) *m*/*z* 401.2662 $[M + Na]^+$ (calcd 401.2662).

Methyl 7α-hydroperoxy-13α-hydroxyabiet-8(14)-enoate or methyl 13α-hydroperoxy-7α-hydroxyabiet-8(14)-enoate (methyl [1*R*-(1α,4a*β*,4bα, 7α,9α,10aα)]-1,2,3,4,4a,4b,5,6,7,9,-10,10a-dodecahydro-7-hydroperoxy-9-hydroxy-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-carboxylate or methyl [1*R*-(1α,4a*β*,4bα,7α,9α,10aα)]-1,2,3,4,4a,4b,5,6,7,9,-10,10a-dodecahydro-9-hydroperoxy-7-hydroxy-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-carboxylate, 5): colorless needles (cyclohexane−EtOAc); mp 83−84 °C; $[\alpha]^{24}_D$ −54.5° (*c* 0.23, CH_2Cl_2); IR (KBr) ν_{max} 3545, 3393, 2954, 2867, 1706, 1661, 1466, 1436, 1387, 1305, 1257, 1193, 1149, 1132, 1104, 1080, 1057, 1041, 991, 968, 948, 888 (w, *ν* O−O, weak hydroperoxide bond),[19] 851 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR spectral data, see Table 2; EIMS *m*/*z* 366 $[M]^+$ (3), 348 $[M - H_2O]^+$ (7), 333 $[M - OOH]^+$ (9), 332 $[M - H_2O_2]^+$ (29), 315 $[m/z\ 333 - H_2O]^+$ (8), 314 (22), 307 $[M - COOCH_3]^+$ (7), 289 (11), 263 (11), 255 (18), 247 (100), 229 (25), 211 (13), 199 (11), 183 (17), 165 (11), 159 (12), 146 (17), 123 (40), 121 (29), 109 (28), 107 (28), 95 (18), 81 (18), 79 (18), 55 (18), 43 (28), 41 (14); HRMALDIMS (pos) *m*/*z* 389.2295 $[M + Na]^+$ (calcd 389.2298).

Methyl 12-oxoabietate (methyl [1*R*-(1α,4a*β*,4bα,10aα)]-1,2,3,4,4a,4b,5,6,10,10a-decahydro-6-oxo-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-carboxylate, 6): colorless oil; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2. Other physical and spectral data, consistent with literature values.[22,23]

Table 3. ^{1}H NMR Data of **9a**, **9b**, and **10** (δ ppm; m; *J* Hz)[a]

H	9a	9b	10
1a	1.71 m[c]	1.70 m[c]	2.29 bd (12.9)
1b	1.17 m[c]	1.16 m[c]	1.45 m[c]
2	1.55 m[c] (2H)	1.55 m[c] (2H)	1.76 m[c] (2H)
3a	1.79 m[c]	1.80 m[c]	1.78 m[c]
3b	1.65 m[c]	1.63 m[c]	1.68 m[c]
5	2.39 dd (13.4, 2.4)	2.43 dd (13.2, 2.8)	2.46 bd (13.4)
6a	1.72 m[c]	1.68 m[c]	2.08 m[c]
6b	1.48 m[c]	1.49 m[c]	1.69 m[c]
7	4.21 t (2.7)	4.27 t (2.7)	4.75 d (3.5)
9	2.17 m[c]	2.25 dt (6.2, 1.7)	
11a	1.66 m[c]	1.69 m[c]	7.20 d (8.5)
11b	1.54 m[c]	1.47 m[c]	
12a	1.59 m[c]	1.58 m[c] (2 H)	7.31 dd (8.5, 1.6)
12b	1.35 m[c]		
14	5.69 bs	5.66 d (1.6)	7.44 d (1.6)
15	1.69 sept (6.9)	1.82 sept (6.9)	
16[b]	0.87 d (6.9)	0.86 d (6.9)	1.54 s
17[b]	0.92 d (6.9)	0.88 d (6.9)	1.54 s
19	1.25 s	1.18 s	1.26 s
20	0.75 s	0.76 s	1.14 s

[a] Spectra measured at 400 MHz, 295 K, $CDCl_3$. [b] Values interchangeable. [c] Multiplicity not determined due to overlapping signals.

7α,13*β*-Dihydroxyabiet-8(14)-enoic acid ([1*R*-(1α,4a*β*,-4bα,7*β*, 9α,10aα)]-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-7,9-dihydroxy-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-carboxylic acid, 9a): pale yellow oil; $[\alpha]^{21}_D$ −10.5° (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max} 3441, 2934, 1771, 1698, 1458, 1418, 1387, 1266, 1167, 1017, 982, 912, 736, 702 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; ESIMS (neg) *m*/*z* 335 $[M - H]^-$, (pos) 359 $[M + Na]^+$; HRESIMS (pos), no molecular or pseudomolecular ion peak observed.

7α,13α-Dihydroxyabiet-8(14)-enoic acid ([1*R*-(1α,4a*β*,-4bα,7*β*,9α, 10aα)]-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-7,9-dihydroxy-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-carboxylic acid, 9b): pale yellow oil; $[\alpha]^{21}_D$ −6.9° (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 3375, 2932, 2871, 2348, 2252, 1696, 1552, 1461, 1386, 1248, 1187, 1152, 1104, 1076, 1027, 967, 944, 911, 873, 848, 830, 731 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; ESIMS (neg) *m*/*z* 335 $[M - H]^-$, (pos) 359 $[M + Na]^+$; HRESIMS (pos), no molecular or pseudomolecular ion peak observed.

7,15-Dihydroxydehydroabietic acid ([1*R*-(1α,4a*β*,10aα)]-1,2,3,4,4a,9,10,10a-octahydro-7-hydroxy-1,4a-dimethyl-7-(1-hydroxy-1-methylethyl)phenanthrene-1-carboxylic acid 10): pale yellow oil; $[\alpha]^{21}_D$ −7.7° (*c* 0.27, $CHCl_3$); IR (KBr) ν_{max} 3391, 3055, 2934, 2631, 1704, 1497, 1461, 1387, 1265, 1180, 1072, 1044, 984, 958, 902, 861, 828, 737, 703 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 332 $[M]^+$ (6), 317 $[M - CH_3]^+$ (100), 281 (4), 253 (16), 211 (7), 195 (10), 155 (17), 149 (15), 109 (7), 81 (6), 71 (10), 43 (31); HRDEIMS (pos) *m*/*z* 317.1742 $[M - CH_3]^+$ (calcd 317.1747).

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