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Synthesis and characterization of abietadiene, levopimaradiene, palustradiene, and neoabietadiene: hydrocarbon precursors of the abietane diterpene resin acids

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Abstract—The abietane diterpenes—abietadiene, levopimaradiene, palustradiene, and neoabietadiene (**1b**–**4b**)—were prepared from the corresponding resin acids by diazomethane esterifications, $LiAlH_4$ reductions, tosylations, and Zn/NaI reductions. Abietadiene was also obtained less efficiently by catechol borane reduction of abietadienal tosylhydrazone and Li/NH_3 reduction of its 18-phenylthio derivative. These conjugated dienes were characterized by chromatographic properties (HPLC, TLC, GC), MS fragmentation patterns, optical rotations, and UV, IR, ^1H NMR, and ^{13}C NMR data. Assignments for the ^1H and ^{13}C NMR spectra were made by COSY, DEPT, HMQC, HMBC, NOE data, H–H coupling analysis, and comparisons with literature data. The four diterpenes were identified as cyclization products of recombinant abietadiene synthase, supporting their likely role in the biosynthesis of the abietane resin acids in conifer oleoresin.

1. Introduction

Abietic, levopimaric, palustric, and neoabietic acids (Fig. 1, **1a**–**4a**) are widely occurring tricyclic diterpene carboxylic acids which together with the biogenetically related pimaric acids comprise the majority of the C_{20} fraction of conifer oleoresins.[1,2] This viscous secretion serves an important defensive function in filling and sealing wounds by solidification and oxidative crosslinking.[3,4] The pine rosins are abundant natural chemicals which have many industrial applications including paper sizings, polymerization emulsifiers, adhesive tackifiers, printing ink resins, and waterproofing material as well as other uses.[5] The easily isolated abietic[6] and levopimaric[7] acids are common starting materials for synthesis of other natural products[8,9] and numerous diterpene derivatives.[1]

The abundance and widespread occurrence of the four abietane-type resin acids stand in marked contrast to the rarity and paucity of information in the literature concerning the corresponding hydrocarbons—abietadiene, levopimaradiene, palustradiene, and neoabietadiene (**1b**–**4b**)—despite their likely role as intermediates in resin acid biosynthesis. Abietadiene has been isolated from *Larix sibirica* rosin,[10a] *Pinus sibirica* resin,[10b] *Cedrus libani* cones,[11a] and *Cupressus arizonica* oil,[11b] and both abietadiene and palustradiene were separated from the essential oil of *Juniperus sabina* L. berries[12] and from the oleoresin of *Picea schrenkiana*.[13] Some characterization data are available for these more frequently isolated abietane hydrocarbons,[10–14] and partial syntheses of abietadiene by Wolff–Kishner reduction of abietadienal have been recorded.[15] The detection of these diterpenes, and occasionally neoabietadiene, by GC analyses of oleoresin or essential oils from *Cupressus* species,[11] *Pinus* species,[16] and *Abies nord-munnia* (Caucasian fir),[17] various commercial gum and wood rosins,[18] and southern pine tree oil have been reported.[1,19] Characteristic MS peaks used for GC/MS identification of abietadiene and palustradiene have been presented.[15] A plausible but tentative identification of all four isomers in *Cupressus arizonica* essential oil based on palmitic acid-catalyzed interconversions at 200°C and GC comparisons, in accord with characteristic thermal equilibrations of the corresponding resin acids,[1,20] was reported.[11]

Significant advances have been made recently in the characterization of the enzymes and genes responsible for resin acid biosynthesis in conifers. Abietadiene synthases from *Abies grandis* (grand fir),[21] *Pinus contorta* (lodgepole pine),[21] and *Pinus pinaster*[22] have been partially characterized. The gene encoding abietadiene synthase (AS) from grand fir has been cloned and the cDNA was functionally expressed as a single polypeptide bearing a presumed N-terminal plastidial targeting sequence.[23,24] Cytochrome P450 mixed function oxygenases and a dehydrogenase which carry out the sequential oxidation of abietadiene to abietadienol, abietadienal, and abietic acid have been

Keywords: abietane; terpenes and terpenoids; diterpenes; dienes; reduction; zinc.

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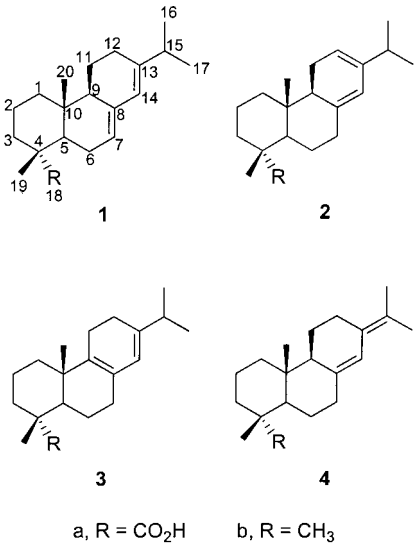


Figure 1. Structures of the abietane-type resin acids and the related hydrocarbons.

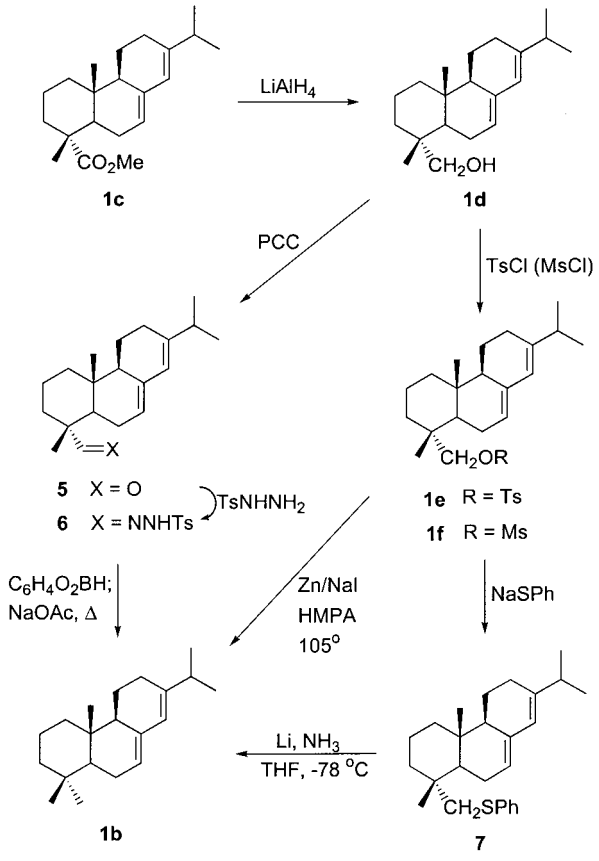
isolated from grand fir and lodgepole pine.[25] The regulation of induced oleoresinosis in grand fir has been studied by monitoring changes in cyclase activity levels and steady-state transcript abundances for monoterpene, sesquiterpene, and diterpene synthases.[4,26]

The recent discovery that cyclization of (*E*,*E*,*E*)-geranylgeranyl diphosphate catalyzed by native and recombinant AS from grand fir produces a mixture of abietadiene **1b** and three isomers presumed to be **2b**–**4b** based on MS data,[24] and the similarity of product ratios to those of **1a**–**4a** in fresh oleoresin,[27a] prompted the present investigation. Levopimaradiene has recently been identified as the cyclization product of a diterpene synthase isolated from *Gingko biloba*.[27b] In this paper we report the synthesis, ^{1}H NMR, ^{13}C NMR, and MS data and assignments, and chromatographic behavior of these abietane diterpene precursors to the resin acids.

2. Results and discussion

2.1. Conversion of resin acids to abietadiene isomers

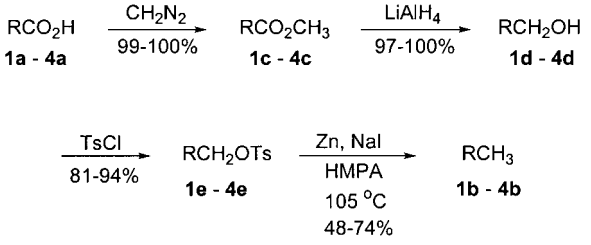
The conversion of the hindered carboxyl groups commonly found in natural diterpenes to methyl groups has been accomplished by Wolff–Kishner reductions of the corresponding aldehydes,[15,28,29] by catechol borane reduction of the aldehyde tosylhydrazone,[9] by Li/NH_3 reduction of phenylthiomethyl groups,[30,31] and by Zn/NaI reductions of tosylates and mesylates.[29,32] However, application of these or other methods to the resin acids **1a**–**4a** might be jeopardized by the sensitivity of the conjugated dienes to acids, oxidative reagents, reducing conditions, and free radical reactions. In the present work, three of these known deoxygenation methods were evaluated with abietic acid **1a** (Scheme 1), the most stable isomer, and the Li/NH_3 and Zn/NaI procedures were also applied to levopimaric acid **2a**, known to be the least stable member of the abietane resin acids.[20] We have found the Zn/NaI reduction[29,32] of



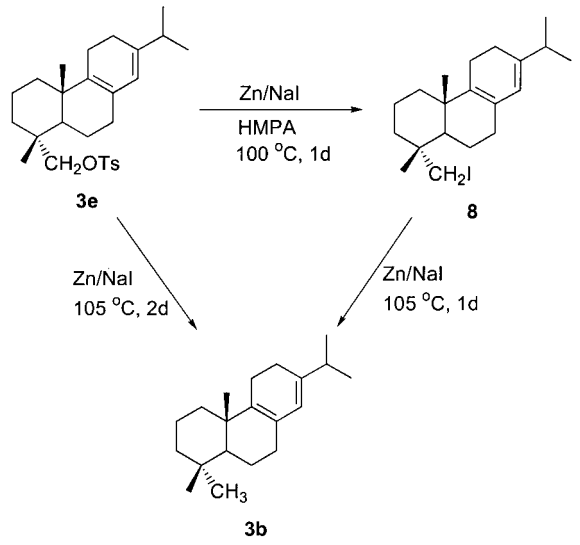
Scheme 1.

the tosylates to be a general and efficient approach (Scheme 2), and this method provided dienes **1b**–**4b** in high purity and satisfactory yields.

Diazomethane esterifications (CH_2N_2, ether, 0°C, 10 min) of abietic and levopimaric acids and hydride reductions ($LiAlH_4$, ether, 0°C, 1 h) of their esters provided abietadienol **1d** and levopimaradienol **2d** in high yields (99%) and purity. However, oxidations of **1d** with PCC (1.5 equiv., $CHCl_2$, rt, 6 h) or with $(COCl)_2$/DMSO (Swern oxidation: −78°C, 15 min; Et_3N) afforded abietadienal **5**[15a] in only fair yields (44 and 53% at 70% purity, respectively) and accompanied by side products that were difficult to separate. A plausible explanation for the low yields is competing oxidation of the conjugated diene by PCC since allylic oxidation of cycloalkenes by this reagent is well known.[31b,33] A similar PCC oxidation (1.0 equiv., $CHCl_2$, rt, 1 h) of levopimaradienol gave a 6:5:1 mixture (31%) of levopimaradienal/dehydroabietadienal/abietadienal (not shown) and a substantial recovered alcohol fraction of similar



Scheme 2.



Scheme 3.

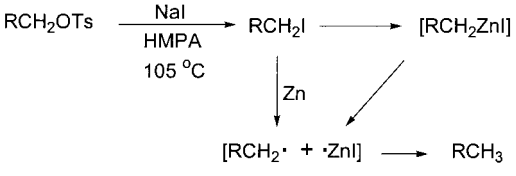
composition. Evidently double bond isomerization and aromatization compete with the oxidation of the CH_2OH group. Although abietadienal tosylhydrazone **6** was readily obtained ($TsNHNH_2$, EtOH, rt, 1 d; 77%) and reduced efficiently by the catechol borane procedure[34] (9 equiv., 4:1 $CHCl_3$/THF, rt, 12 h; NaOAc, reflux, 1 h) to abietadiene **1b** (81%), the low yields of the preceding oxidation step, the difficulty in purification of abietadienal, and the evident sensitivity of the cisoid diene to oxidation prompted the investigation of other methods.

Conversions of abietadienol to the tosylate **1e** (5 equiv. TsCl, pyridine, rt, 1 d) and mesylate **1f** (1.5 equiv. MsCl, Et_3N, CH_2Cl_2, 0°C, 30 min), and levopimaradienol **2d** to its tosylate **2e** (5 equiv. TsCl, pyridine, rt, 1 d) all proceeded in excellent yield (90–97%). However, the slow tosylation reaction necessitated the use of excess tosyl chloride and separation of the remaining reagent by either careful chromatography or better by reaction with 3-(dimethylamino)propylamine. The presence of small amounts (5–10%) of tosyl chloride in chromatographically purified tosylates **2e** and **4e** was readily detected in the ^1H NMR spectra. In one run, incomplete separation of tosyl chloride from palustradienyl tosylate **3e** resulted in decomposition during storage, presumably owing to hydrolysis and acid-induced reactions. S_N2 displacements of abietadienyl mesylate **1f** and levopimaradienyl tosylate **2e** with PhSNa (5 equiv., DMF, 115°C, 1 h) afforded 18-(phenylthio)abietadiene **7** and 18-(phenylthio)-levopimaradiene (structure not shown, 80% purity) in excellent yields (96 and 90%) despite the steric hindrance at the neopentyl positions.

Although the Li/NH_3 reduction of hindered phenyl sulfides has proven to be effective in carboxyl to methyl conversions in the literature,[30,31] the known capability of this reagent to reduce conjugated dienes was reason for concern. Reductions of the two phenylthio diterpenes with Li/NH_3 (2–3 equiv. Li, ~1:1 NH_3/THF, −78°C, ~5 min) were carried out by adding the metal last and rapidly quenching excess lithium with 3-hexyne and methanol. The yields of abietadiene **1b** from **7** varied from 59 (100% purity) to 86% (ca. 85% purity), and the presence of small amounts of abietene contaminants from over-reduction could be seen in the GC and ^1H NMR spectra in the latter case. Li/NH_3 reductions of levopimaradienyl sulfide afforded variable mixtures of levopimaradiene, abietadiene, and three unidentified abietene isomers. In the best run the product was a 2.6:1:3.1 mixture (36%) of **2b/1b/**abietenes. In another run the less polar abietene isomers resulting from over-reduction were separated by chromatography as a 1:1.3:1.7 mixture and partially characterized by GC/MS and ^1H NMR spectra. Evidently base-catalyzed isomerization and reduction of the cisoid diene were competitive with the reductive cleavage of the sulfide under the various conditions tried.

Reductive cleavages (Scheme 2) of the four tosylates **1e**–**4e** with Zn/NaI in HMPA at 105°C for 1–2 d according to the procedure of Fujimoto and Tatsuno[32] afforded all four diterpene hydrocarbons **1b**–**4b** in satisfactory yields (74, 68, 48, and 70%) and good purities (100, 88, ~95, and 100%) according to GC and/or NMR analyses. Recently levopimaradiene (**2b**) was synthesized by reduction of **2e** with $LiEt_3BH$ in THF at reflux.[27b] Unsuccessful trials to effect reduction of **1e** with Zn/NaI in DMF, diglyme, and diglyme-HMPA under similar conditions underscore the importance of the HMPA solvent. The reaction of palustradienyl tosylate **3e** with Zn/NaI HMPA (Scheme 3) under slightly milder conditions (100°C, 1 d) gave the corresponding iodide **8** (67%) which was converted to palustradiene **3b** (70%) at 105°C.

It therefore seems likely that these tosylate reductions proceed by S_N2 displacements forming the 18-iodo diterpenes followed by electron-transfer from zinc. The lack of deuterium incorporation into levopimaradiene **2b** after a reduction of **2e** and addition of methanol-d_4 implicates a free radical intermediate which abstracts a hydrogen atom from HMPA. (Eq. (1)) If an organozinc iodide is formed, it must undergo thermal decomposition under the high temperature conditions. The HMPA solvent evidently facilitates these reductions through its dipolar aprotic character which promotes the normally slow S_N2 displacement by iodide anion, and by serving as an efficient hydrogen atom donor for a free radical intermediate.



(1)

2.2. Characterization of abietadiene isomers

Synthesis of the diterpene hydrocarbons provided the opportunity to characterize the compounds fully by chemical, chromatographic, MS, optical, and NMR data. A Diels–Alder reaction of levopimaradiene with maleic anhydride (toluene, 25°C) (Eq. (2)) afforded the stable, crystalline adduct **9** (66%), previously prepared from abietadiene at

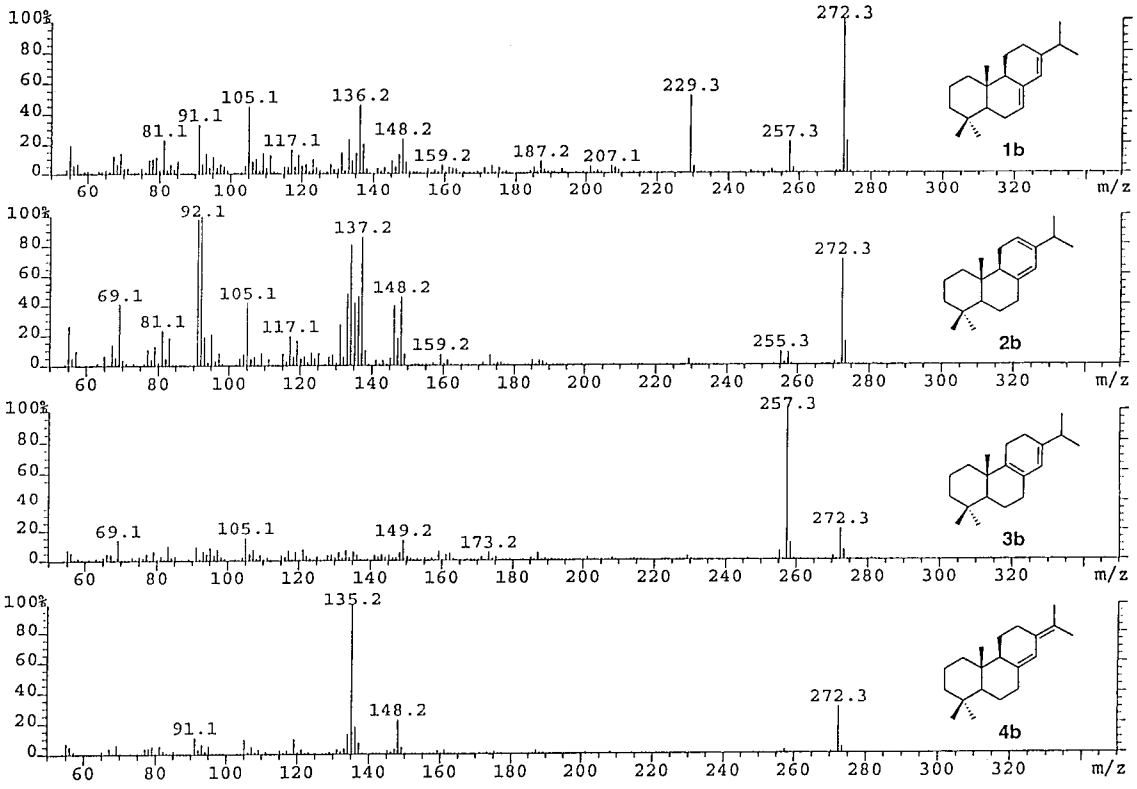
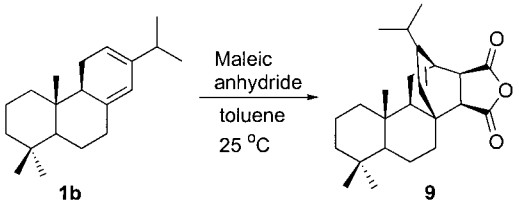


Figure 2. Electron impact mass spectra for GC/MS analyses of abietadiene (**1b**, top), palustradiene (**3b**), levopimaradiene (**2b**), and neoabietadiene (**4b**, bottom).

high temperature.[15c]



(2)

HPLC analysis of a mixture of the four isomers on a silica column showed only two peaks: one for **1b**+**2b**+**3b** and another for the slower eluting **4b**. TLC analyses on $AgNO_3$-silica gel (27–29%) showed significant mobility differences (R_f order **1b**>**4b**>**3b**>**2b**) for the transoid and cisoid dienes. TLC analysis of the resin acid esters on $AgNO_3$–alumina showed similar behavior except the order of **1c** and **4c** was reversed.[35,36] Separation of abietadiene and palustradiene by argentation chromatography has been reported.[12] Temperature-programmed capillary GC analyses of the mixture on a fused silica column displayed well-separated peaks in the R_t order, **3b**<**2b**<**1b**<**4b**, which is similar to the literature order for the tentatively identified hydrocarbons[11] with the exception of **2b/1b** reversal (SE-30 packed column). This GC retention order also corresponds to that usually observed for the corresponding resin acid esters **3c**≤**2c**<**1c**<**4c**, on non-polar stationary phases (fused silica and silicone gum rubber); however, the palustrate/levopimarate elution order is often opposite on polar columns.[27a,35,37]

Although the EI mass spectral fragmentation patterns for the isomeric dienes (Fig. 2) are similar, they show distinctive differences that may be useful for identification by GC/MS. The relative intensities of the *m/z* 272 (M^+), 257 ($M-CH_3$), and 229 ($M-C_3H_7$) appear to be diagnostic for **1b** (272>229>257), **2b** (272>257>229), and **3b** (257>272>229). The MS for **4b** exhibits much less intense peaks at *m/z* 272 (22) and 257 (4), no detectable peak at 229, and a base peak at 135. The MS data for abietadiene correspond reasonably well to the limited data in the literature.[15a] However, MS intensity patterns attributed to palustradiene[16] match better with those of levopimaradiene, indicating that this pine resin constituent may be mis-identified.

The optical rotations for abietadiene and levopimaradiene are strongly negative ($[\alpha]^{20}_D=-121$ and $-265°$) whereas those for palustradiene and neoabietadiene are positive ($[\alpha]^{20}_D=+59$ and $+145°$), and the signs and magnitudes ($\pm$ ~10%) match those of the esters (see Section 4). The -45 and $-68°$ optical rotations reported for palustradiene[12,13] are inconsistent with our measurements. The UV spectral data for the isomeric dienes are in good accord with existing literature data for **1a**[11] and **1d**[12] and with values for the related esters and alcohols.[38]

The ^1H and ^{13}C NMR spectral data for **1b**–**4b** (Tables 1 and 2) were assigned by means of DEPT, COSY, HMQC, HMBC, NOE data (Table 3 and Fig. 3), H–H coupling pattern analysis, and comparisons with ^{13}C NMR correlations in the literature.[14,39,40] The logic in deriving the assignments is illustrated for palustradiene **3b** in Section 4.2.20.

Table 1. ^{1}H NMR (500 MHz, C_6D_6) Data and assignments for abietadiene (**1b**), levopimaradiene (**2b**), palustradiene (**3b**), and neoabietadiene (**4b**)

Position	1b	2b	3b	4b
1α	0.90 (td, 12.7; 3.8)	0.71 (td, 13.0; 3.8)	1.02 (td, 13.1; 3.7)	0.93 (td, 13.0; 3.6)
1β	1.75 (dq, 12.9; 3.2)	1.63 (dtd, 13.3; 3.2; 2.0)	1.75 (dtd, 12.6; 3.5; 1.3)	1.62 (dtd, 12.9; 4.3; 2.5)
2α	1.37–1.42 (m)	1.37 (dquin, 12.5; 3.4)	1.44 (dquin, 13.5; 3.6)	1.43–1.37 (m)
2β	1.53 (qt, 12.8; 3.7)	1.46 (qt, 13.5; 3.3)	1.59 (qt, 13.8; 3.4)	1.43–1.37 (m)
3α	1.14 (td, 13.2; 3.4)	1.09 (td, 13.5; 4.0)	1.14 (td, 13.5; 4.1)	1.16 (td, 14.5; 4.6)
3β	1.37–1.42 (m)	1.40–1.31 (m)	1.38 (dtd, 13.1; 3.3; 1.6)	1.47 (dtd, 14.2; 3.2; 0.8)
4	–	–	–	–
5	1.23 (dd, 12.4; 4.9)	0.91 (dd, 9.2; 6.6)	1.17 (dd, 12.4; 1.9)	0.99 (dd, 12.3; 2.3)
6α	2.09 (dtd, 18.3; 5.0; 1.8)	1.60 (ddt, 12.8; 5.3; 2.5)	1.65 (ddt, 12.3; 5.5; 3.0)	1.55 (ddt, 12.8; 5.8; 3.0)
6β	1.94 (dd, 19.0; 12.3)	1.12–1.01 (m)	1.47–1.40 (m)	1.33 (qd, 12.9; 5.0)
7α		2.04 (td, 12.9; 4.9)		2.19 (td, 13.6; 5.6)
	5.48 (dt, 5.1; 2.6)		2.12–2.08 (m)	
7β		2.41–2.29 (m)		2.40 (ddd, 14.6; 4.9; 1.8)
8	–	–	–	–
9	1.81 (dquin, 13.2; 3.4)	1.94 (dd, 12.6; 7.8)	–	1.81 (dd, 16.1; 7.1)
10	–	–	–	–
11α	1.27 (ddd, 14.1; 12.6; 8.1)			1.42 (qdd, 13.4; 3.2; 0.7)
		2.41–2.29 (m)	2.08–1.97 (m)	
11β	1.70 (dq, 11.9; 3.6)			1.75 (dt, 12.8; 4.2)
12α				1.96 (br t, 13.7)
	1.99 (m)	5.22 (m)	2.08–1.97 (m)	
12β				2.61 (dt, 13.9; 4.3)
13	–	–	–	–
14	5.96 (s)	5.66 (s)	5.56 (s)	6.43 (s)
15	2.20 (septet, 6.8)	2.21 (septet, 6.8)	2.27 (septet, 6.8)	–
16	1.02 (d, 7.0)[a]	1.06 (d, 7.0)	1.04 (d, 6.9)	1.78 (s)
17	1.03 (d, 6.8)[b]	1.06 (d, 7.0)	1.04 (d, 6.9)	1.72 (s)
18	0.85 (s)	0.83 (3H, s)	0.89 (s)	0.88 (s)
19	0.89 (s)	0.80 (3H, s)	0.86 (s)	0.84 (s)
20	0.84 (s)	0.93 (3H, s)	1.06 (s)	0.80 (s)

Chemical shifts (multiplicity, observed coupling(s) in Herz). Assignments based on COSY, HMBC, HMQC, and NOE (see text).
[a] Pro-*S*.
[b] Pro-*R*.

The ^{13}C NMR assignments for the A and B ring carbons of **3b** agree with those reported for the related diterpenes, pimara-8,15-diene[39] and podocarp-8-ene,[40] except for reversal of C8 and C9 in the former case.

Table 2. ^{13}C NMR (125 MHz, C_6D_6) Data and assignments for abietadiene (**1b**), levopimaradiene (**2b**), palustradiene (**3b**), and neoabietadiene (**4b**)

Position	1b	2b	3b	4b
1	39.5 (CH_2)	38.0 (CH_2)	36.1 (CH_2)	39.7 (CH_2)
2	19.2 (CH_2)	19.3 (CH_2)	19.28 (CH_2)[a]	19.3 (CH_2)
3	42.7 (CH_2)	42.4 (CH_2)	42.1 (CH_2)	42.4 (CH_2)
4	33.1 (C)	33.6 (C)[b]	33.37 (C)[c]	33.4 (C)
5	50.5 (CH)	55.3 (CH)	51.9 (CH)	54.7 (CH)
6	24.4 (CH_2)	24.1 (CH_2)	19.30 (CH_2)[a]	22.9 (CH_2)
7	121.7 (CH)	36.5 (CH_2)	31.0 (CH_2)	36.4 (CH_2)
8	135.7 (C)	139.2 (C)[d]	138.2 (C)	139.0 (C)
9	51.3 (CH)	49.9 (CH)	125.4 (C)	51.7 (CH)
10	35.2 (C)	41.0 (C)	37.9 (C)	38.4 (C)
11	23.0 (CH_2)	23.1 (CH_2)	23.0 (CH_2)	22.9 (CH_2)
12	27.7 (CH_2)	115.2 (CH)	26.6 (CH_2)	26.4 (CH_2)
13	144.2 (C)	139.4 (C)[d]	142.5 (C)	129.0 (C)[e]
14	123.7 (CH)	119.3 (CH)	121.3 (CH)	122.6 (CH)
15	35.3 (CH)	33.8 (CH)	34.7 (CH)	122.8 (C)[e]
16	21.7 (CH_3)	21.7 (CH_3)[f]	21.2 (CH_3)[g]	19.8 (CH_3)
17	22.1 (CH_3)	21.6 (CH_3)[f]	20.8 (CH_3)[g]	20.4 (CH_3)
18	33.5 (CH_3)	33.6 (CH_3)[b]	33.39 (CH_3)[c]	33.8 (CH_3)
19	21.1 (CH_3)	21.9 (CH_3)	21.7 (CH_3)[g]	22.2 (CH_3)
20	13.9 (CH_3)	14.3 (CH_3)	21.5 (CH_3)[g]	15.2 (CH_3)

Chemical shifts (hydrogen substitution). Assignments based on DEPT, HMBC, and HMQC (see text).
[a,c–g] These assignments may be reversed (see Section 4.2.20 for explanation).
[b] Superimposed signals (greater intensity relative to other signals).

The positional assignments for abietadiene, levopimaradiene, and neoabietadiene were deduced by similar means with appropriate consistency tests and confirmations. The better separation of the ^{13}C NMR signals for the ring A quaternary methyls avoided the overlap of HMQC and HMBC crosspeaks that occurred in the 2D plots for palustradiene. Consequently more definite assignments of the corresponding ^{1}H NMR peaks for these methyl groups could be made, despite their proximity in the proton spectra. The identification of the C16 (pro-*S*) and C17 (pro-*R*) methyl doublets from the isopropyl group of abietadiene with δ_H 1.02 and 1.03 was established previously by stereocontrolled deuterium labelling.[41] The vinyl methyl peaks (δ_H 1.72 and 1.78) in the ^{1}H NMR spectrum of neoabietadiene were readily associated with C16 (*Z*) and C17 (*E*), respectively, from the NOEs shown in Fig. 3. The ^{13}C NMR data and assignments shown for abietadiene agree with those in the literature[14] except for reversal of the close-lying signals for C4 and C10, and they correlate well with assignments for isopimaradiene.[39] The ^{13}C NMR assignments for the A and B ring carbons of levopimaradiene and neoabietadiene are consistent with those in the literature for pimara-8(14),15-diene,[13] all of which have an 8(14) double bond.

2.3. Enzymatic synthesis of 1b–4b mixture

Incubation of (*E*,*E*,*E*)-geranylgeranyl diphosphate with recombinant abietadiene synthase from grand fir afforded a mixture of all four abietadiene isomers (**1b–4b**) in a

Table 3. NOEs observed upon irradiation of quaternary methyl groups in abietadiene (**1b**), levopimaradiene (**2b**), and palustradiene (**3b**)

Compound no.	Methyl position	Signals irradiated δH (ppm)	NOE observed (%)
1b	18	0.85	ND[a]
	19	0.89[b]	H18 (2.4), H20 (3.3)
	20	0.84[b]	H19 (2.4)
2b	18	0.83	ND[a]
	19	0.80	H20 (9.0)
	20	0.93	H19 (4.8)
3b	18	0.89[b]	H5α (13.3), H6α (6.8)
	19	0.86[b]	H20 (11.8)
	20	1.06	H1β (3.2), H2β (2.1), H6β (4.9), H11β (3.7), H19 (7.8)

[a] Not determined.
[b] Irradiation caused some excitation of the proximal methyl and some uncertainty in assigning the NOE origin.

33:42:9:16 ratio according to GC analysis, in agreement with a recent report by Peters et al.[24] The identity of the individual components was established by comparison of their GC retention times and their mass fragmentation patterns in GC–MS recordings with those of mixture of synthetic samples of **1b**–**4b**. This finding confirms that abietadiene diterpene synthase is a multi-product cyclase which gives rise to mixture of abietane diterpenes, which in vivo is further oxidized to the corresponding resin acid mixture.

3. Conclusion

Abietadiene, levopimaradiene, palustradiene, and neoabietadiene (**1b**–**4b**) are now accessible by partial synthesis from the corresponding resin acids (**1a**–**4a**) in four steps (40–68% overall yield). The key step is the Zn/NaI reduction[32] of the C18 tosylates. These diterpene hydrocarbons are likely intermediates in resin acid biosynthesis, and the availability of reference samples and extensive characterization data will facilitate their identification as cyclase products, oxidase substrates, and oleoresin constituents. They may also be useful as enantiomerically pure starting materials for natural product syntheses and other purposes.

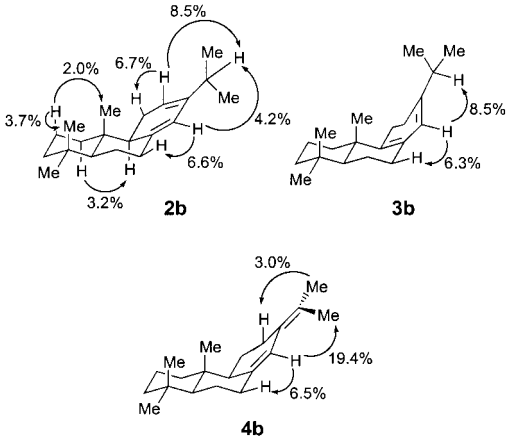


Figure 3. NOE intensities (500 MHz, C_6D_6) observed from the indicated irradiations of abietadiene (**1b**), levopimaradiene (**2b**), palustradiene (**3b**), and neoabietadiene (**4b**).

4. Experimental

4.1. General

The following solvents and reference values (ppm) were used for NMR spectroscopy: $CDCl_3$ (^{1}H: 7.27, ^{13}C: 77.0), C_6D_6 (^{1}H: 7.15, ^{13}C: 128.0). Coupling constants are given in Hz. Mass spectra (MS) were obtained on a 70-VSE or TofSpec mass spectrometer. Electron impact (EI) MS ion analysis was performed with the attached HP 5970 quadrapole MS and related data station (Fig. 2). GC–MS spectra were obtained on a Quattro instrument. HPLC was conducted with a Waters Model M-6000A delivery system using a Schoeffel SF 770 variable wavelength UV detector at 250 nm and an analytical silica column (0.46×25 cm) with a flow rate of 1 mL min^{-1}. GC analyses were carried out with a Shimadzu Model 14A-GC on Rt_x-5 30 m fused silica capillary column (5% diphenyl- and 95% dimethylpolysiloxane; ID 0.32 mm, 0.25 mm film thickness) with a split ratio of 100:1. $AgNO_3$-impregnated silica gel (27–29% w/w) TLC plates were freshly prepared by immersing silica gel TLC plates (Merck, 0.25 mm 60 F-254 silica gel) in a solution of 10% $AgNO_3$ in acetonitrile and drying for 3 h at rt.[31b] After development $AgNO_3$–SiO_2 TLCs were visualized by spraying with $KMnO_4$ (5% $KMnO_4$ and 10% $NaHCO_3$). HMPA was distilled from CaH_2 and stored over 4 Å sieves. Li metal surface was cleaned by rinsing in hexane, submerging in EtOH, and rinsing in fresh hexane before use. NaH (60% dispersion in mineral oil) was rinsed under N_2 with hexane three times before use. Anhydrous NH_3 was vapor transferred directly from the compressed gas cylinder. $CDCl_3$ was passed through a pipette filled with basic alumina immediately before use. Flash column chromatography was performed on 230–400 mesh silica gel (Merck 60 Å, grade 9385).[42] Solvents (EA=ethyl acetate) were removed by rotary evaporation. The purity of all products was judged to be >95% by ^{1}H NMR analysis unless specified otherwise. All products were characterized by UV, IR, ^{1}H NMR and ^{13}C NMR spectra. If satisfactory spectral data for known compounds are available in the literature, only optical rotations and mp are reported. (*E*,*E*,*E*)-Geranylgeraniol, prepared from (*E*,*E*)-geranyllinalool (Takasago Fine Chemicals, Japan) by PCC oxidation[43] and $NaBH_4$ reduction (MeOH, 0°C), was converted to (*E*,*E*,*E*)-geranylgeranyl pyrophosphate (GGPP) by procedures similar to those in the literature for geranyl pyrophosphate.[44]

Truncated recombinant abietadiene synthase was kindly provided by Dr Reuben Peters.[24]

4.2. Resin acid sources and properties

Abietic acid (Aldrich) was purified according to a literature procedure:[6] mp 170–171°C [lit.[38b] mp 172–174°C], $[\alpha]^{20}_D=-101°$ (*c* 1.60, EtOH) [lit.[38b] $[\alpha]^{20}_D=-106°$, EtOH]. Levopimaric, palustric, and neoabietic acids are commercially available from Helix BioTech. Levopimaric acid was generously provided from USDA collection at Forest Products Laboratory, Madison, WI, with the help of Dr Duane Zinkel. These air- and acid-sensitive materials were stored as solids at −20°C under nitrogen.

4.2.1. Methyl abietadien-18-oate (methyl abietate, 1c). A solution of abietic acid (2.00 g, 6.62 mmol) in ether (10 mL) was stirred and cooled at 0°C under N_2 as excess diazomethane in ether (40 mL) generated from *N*-methyl-*N*-nitrosotoluenesulfonamide according to the Aldrich Bulletin and kit 210,025-*O* was added. After 10 min, solvent and unreacted diazomethane were removed by rotary evaporation. Purification of the residue by flash column chromatography on silica gel (20% diethyl ether/pentane) afforded methyl ester **1c** (2.07 g, 99%) as a colorless oil: $[\alpha]^{20}_D=-96°$ (*c* 0.54, $CHCl_3$) [Lit.[38a,b] $[\alpha]^{20}_D=-96°$ (95% EtOH)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.76 (1H, s), 5.35 (1H, m), 3.61 (3H, s), 2.21 (1H, septet, *J*=6.6 Hz), 2.10–2.00 (4H, m), 1.93 (1H, d, *J*=12.9 Hz), 1.87 (1H, d, *J*=12.9 Hz), 1.82–1.68 (3H, m), 1.64–1.52 (3H, m), 1.24 (3H, s), 1.22–1.10 (2H, m), 1.00 (3H, d, *J*=6.8 Hz), 0.98 (3H, d, *J*=6.8 Hz), 0.81 (3H, s). UV and ^{13}C NMR (50 MHz) data are available in the literature.[14,38]

4.2.2. Abietadien-18-ol (1d). A suspension of $LiAlH_4$ (46.8 mg, 1.23 mmol) in ether (4 mL) under N_2 was stirred and cooled at 0°C as methyl ester **1c** (130 mg, 0.41 mmol) in ether (1 mL) was added. After 1 h at 0°C, water (47 μL), aqueous 15% NaOH (47 μL), and water (141 μL) were added.[45] The white salts were filtered and washed thoroughly with ether (20 mL). Concentration of the filtrate and purification by flash column chromatography on silica gel (20% diethyl ether/pentane) afforded alcohol **1d** (117 mg, 99%) as a white solid. Recrystallization from pentane/ether gave the analytical sample: mp 88–89°C [Lit.[15a] mp 80–84°C; Lit.[46] mp 85.5–87°C]; $[\alpha]^{20}_D=-149°$ (*c* 0.54, $CHCl_3$); UV (hexane) λ_{max} (log ε) 234 (4.32), 242 (4.34), 250 (4.17) nm. IR, ^{1}H NMR (60 MHz), ^{13}C NMR (15 and 50 MHz), and EIMS data are available in the literature.[14,15a]

4.2.3. Abietadien-18-al (5). *(A) By Swern oxidation*. Swern oxidation of abietadienol **1d** (116 mg, 0.4 mmol) in CH_2Cl_2 (1 mL) was carried out with DMSO (88 μL, 1.24 mmol) and oxalyl chloride (52 μL, 0.6 mmol) in CH_2Cl_2 (5 mL) under N_2 for 15 min at −78°C after which Et_3N (0.2 mL, 1.5 mmol) was added.[47] Isolation of the product by extraction and purification by flash column chromatography (20% EA/hexane) afforded aldehyde **5** (60 mg, 53% yield, 70% purity based upon ^{1}H NMR analysis) and a more polar unidentified byproduct (24.9 mg, 22%) as colorless oils. Characteristic ^{1}H NMR (500 MHz, $CDCl_3$) data for the byproduct are 9.20 (1H, s), 5.83 (1H, s), 5.53 (1H, m).

(B) By pyridinium chlorochromate (PCC) oxidation. Oxidation[15a] of **1d** (68 mg, 0.24 mmol) with a suspension of PCC (77 mg, 0.36 mmol) in CH_2Cl_2 (2 mL) with TLC monitoring was carried out for 6 h at 25°C under N_2. Isolation of the product by ether extraction and purification by flash column chromatography (20% diethyl ether/pentane) afforded known[15a] aldehyde **5** (30.4 mg, 44%) as a colorless oil: UV (MeOH) λ_{max} (log ε) 234 (4.28), 242 (4.31), 250 (4.14) nm; IR (neat) ν_{max} 2930, 2838, 2691 (CH═O), 1724 (C═O), 1626 (C═C), 1458, 1382, 1234, 1032, 899 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (1H, s), 5.76 (1H, s), 5.33 (1H, m), 2.21 (1H, septet, *J*=6.9 Hz), 2.10–1.89 (5H, m), 1.81 (1H, dq, *J*=12.0, 3.2 Hz), 1.76 (1H, dd, *J*=12.2, 4.5 Hz), 1.66–1.59 (3H, m), 1.47–1.40 (1H, m), 1.30 (1H, d, *J*=13.1 Hz), 1.25–1.16 (1H, m), 1.12 (3 H, s), 1.11–1.05 (1H, m), 1.00 (3H, d, *J*=6.9 Hz), 0.99 (3H, d, *J*=6.9 Hz), 0.83 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.3, 145.6, 135.7, 122.3, 120.1, 50.5, 49.0, 42.4, 38.3, 34.8, 33.8, 32.9, 27.4, 25.4, 22.5, 21.4, 20.8, 17.2, 14.3, 14.0; EIMS *m*/*z* 286.2 $[M]^+$ (41.5), 271.2 (9.5), 255.2 (23.0), 243.2 (15.9), 225.1 (3.6), 215.2 (7.9), 199.1 (5.9), 187.1 (100.0), 171.1 (5.8), 159.1 (9.4), 145.1 (23.2), 131.1 (59.1), 105.1 (24.6), 93.0 (17.5), 83.9 (23.8), 69.1 (20.6); HREIMS *m*/*z* 286.2300 (calcd for $C_{20}H_{30}O$, 286.2297). The ^{1}H NMR (60 MHz) and EIMS data agree with those reported for impure aldehyde **5**.[15a]

4.2.4. Abietadien-18-al *p*-toluenesulfonylhydrazone (6). A solution of aldehyde **5** (60 mg, 0.21 mmol) and $TsNHNH_2$ (39 mg, 0.21 mmol) in absolute ethanol (3 mL) was stirred for 1 d at 25°C under N_2.[48] Evaporation of solvent and purification by flash column chromatography (20% EA/Hexane) afforded the tosylhydrazone **6** (67.3 mg, 71%) as a white solid. Recrystallization from pentane/ether gave the analytical sample: mp 84–85°C; UV (MeOH) λ_{max} (log ε) 234 (4.43), 244 (4.41), 250 (4.20) nm; IR (thin film) ν_{max} 2915, 2868, 1737, 1641, 1598, 1468, 1383, 1166, 619 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, *J*=8.2 Hz), 7.29 (2H, d, *J*=8.6 Hz), 7.15 (1H, br s), 6.79 (1H, s), 5.72 (1H, s), 5.15 (1H, m), 2.43 (3H, s), 2.20 (1H, septet, *J*=6.8 Hz), 2.10–2.01 (2H, m), 1.84 (1H, d, *J*=13.0 Hz), 1.81–1.73 (3H, m), 1.59–1.48 (5H, m), 1.37 (1H, d, *J*=14.3 Hz), 1.33–1.25 (2H, m), 1.23–1.12 (2H, m), 1.08 (3H, s), 1.00 (3H, d, *J*=6.8 Hz), 0.99 (3H, d, *J*=6.8 Hz), 0.87–0.81 (2H, m), 0.78 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.7, 145.3, 143.9, 135.4, 134.9, 129.4, 128.0, 122.3, 120.5, 50.8, 45.9, 41.1, 38.3, 37.3, 34.8, 34.3, 27.3, 24.4, 22.4, 21.5, 21.3, 20.8, 17.7, 16.8, 14.1, 14.0; EIMS *m*/*z* 454.3 $[M]^+$ (3.1), 299.3 (33.6), 284.2 (12.4), 270.2 (84.1), 255.2 (55.1), 241.2 (14.0), 227.2 (44.5), 199.1 (36.7), 185.1 (26.8), 171.1 (14.4), 156.0 (19.4), 135.1 (36.3), 105.1 (45.7), 91.1 (100.0), 65.0 (37.3); HREIMS *m*/*z* 454.2662 (calcd for $C_{27}H_{38}N_2O_2S$, 454.2654).

4.2.5. Abietadien-18-yl *p*-toluenesulfonate (1e). A solution of alcohol **1d** (90 mg, 0.31 mmol) and tosyl chloride (296 mg, 1.56 mmol) in pyridine (0.5 mL) was stirred for 1 d at 25°C under N_2[49] with monitoring by TLC. CH_2Cl_2 (30 mL) was added and the resulting solution was washed with 5% aqueous $NaHCO_3$ (2×20 mL) and satd. NaCl (2×20 mL). Concentration and purification by flash column chromatography on silica gel (20% diethyl ether/pentane) afforded the known[15a] tosylate **1e** (129 mg, 94%) as a colorless

oil: $[\alpha]^{20}_D=-61°$ (*c* 0.46, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 228 (4.20), 242 (3.87), 250 (3.83) nm; IR (neat) ν_{max} 2929, 1599, 1464, 1358, 1187, 1099, 965, 844, 812, 666 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.74 (2H, d, *J*=8.3 Hz), 7.31 (2H, d, *J*=8.4 Hz), 5.74 (1H, s), 5.25 (1H, dt, *J*=5.1, 2.4 Hz), 3.65 (1H, d, *J*=9.4 Hz), 3.52 (1H, d, *J*=9.4 Hz), 2.44 (3H, s), 2.21 (1H, septet, *J*=6.8 Hz), 2.22–2.07 (2H, m), 1.87–1.68 (5H, m), 1.55–1.43 (3H, m), 1.38–1.26 (2H, m), 1.21–1.11 (1H, m), 1.00 (3H, d, *J*=6.8 Hz), 0.99 (3H, d, *J*=6.8 Hz), 0.86 (3H, s), 0.86–0.81 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.3, 144.5, 136.4, 132.9, 129.8, 127.9, 122.3, 120.2, 78.0, 50.5, 43.5, 38.4, 36.8, 35.7, 34.8, 34.6, 27.4, 23.6, 22.5, 21.6, 21.4, 20.8, 17.8, 17.4, 14.1; EIMS *m/z* 442.3 $[M]^+$ (21), 270.3 (95), 255.2 (44), 227.2 (42), 199.2 (18), 185.2 (28), 155.0 (33), 136.1 (21), 105.1 (32), 91.1 (100), 71.1 (19); HREIMS *m/z* 442.2538 (calcd for $C_{27}H_{38}O_3S$, 442.2542). The ^1H NMR and EIMS data agree with the less complete 60 MHz ^1H NMR and MS data listings in the literature.[15a]

4.2.6. Abietadien-18-yl methanesulfonate (1f). A solution of alcohol **1d** (1.30 g, 4.5 mmol) and triethylamine (1.88 mL, 13.6 mmol) in CH_2Cl_2 (20 mL) under N_2 was stirred and cooled at 0°C as CH_3SO_2Cl (0.52 mL, 6.8 mmol) was added.[31b] After 30 min at 0°C, the solution was diluted with CH_2Cl_2 (20 mL), washed with 10% NH_4Cl (3×50 mL), and concentrated. Purification by flash column chromatography (25% EA/hexane) afforded the known[15a] mesylate **1f** (1.60 g, 97%) as a colorless oil: IR (neat) ν_{max} 1356, 1176 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.75 (1H, s), 5.38 (1H, q, *J*=3.5 Hz), 3.90 (1H, d, *J*=9.3 Hz), 3.74 (1H, d, *J*=9.3 Hz), 2.96 (3H, s), 2.20 (1H, septet, *J*=6.8 Hz), 2.1–2.0 (4H, m), 1.9–1.8 (2H, m), 1.78 (1H, dq, *J*=12.4, 3.7 Hz), 1.6–1.5 (3H, m), 1.5–1.4 (1H, m), 1.43 (1H, d, *J*=3.8 Hz), 1.18 (1H, tdd, *J*=12.4, 10.4, 6.6 Hz), 1.1–0.95 (1H, m), 0.99 (3H, d, *J*=6.8 Hz), 0.98 (3H, d, *J*=6.8 Hz), 0.96 (3H, s), 0.81 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.8, 135.8, 122.5, 120.5, 77.8, 50.9, 44.0, 38.7, 37.4, 37.1, 36.1, 35.1, 35.0, 27.7, 24.1, 22.8, 21.6, 21.1, 18.1, 17.6, 14.4. The ^1H NMR and EIMS data agree with the less complete 60 MHz ^1H NMR and MS values given in the literature for impure **1f**.[15a]

4.2.7. 18-(Phenylthio)abietadiene (7). A suspension of oil free NaH (524 mg, 21 mmol) in DMF (4 mL) under N_2 was stirred at rt as thiophenol (2.32 mL, 22 mmol) was added.[31b,50] After 1 h at 25°C, mesylate **1f** (1.60 g, 4.3 mmol) was added to the homogenous solution of NaSPh.[50] The solution was heated at 115°C for 1 h, cooled to rt, and diluted with 5% NaOH (150 mL). The product was extracted with hexane (3×75 mL). Solvent evaporation and purification by flash column chromatography on silica gel (5% EA/hexane) afforded sulfide **7** (1.58 g, 96%) as a colorless oil: IR (neat) ν_{max} 2956, 2923, 2866, 1583, 1479, 1439, 1382, 1089, 1025, 885, 736, 689 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.5–7.1 (5H, m), 5.79 (1H, s), 5.39 (1H, dt, *J*=5.1, 2.6 Hz), 2.95 (1H, ABd, *J*=12.1 Hz), 2.85 (1H, ABd, *J*=12.1 Hz), 2.23 (1H, septet, *J*=6.8 Hz), 2.2–1.9 (5H, m), 1.85 (1H, d, *J*=12.8 Hz), 1.81 (1H, dq, *J*=12.3, 3.5 Hz), 1.62 (1H, dd, *J*=11.5, 4.9 Hz), 1.6–1.5 (4H, m), 1.21 (1H, tdd, *J*=12.6, 9.7, 7.2 Hz), 1.1–1.0 (1H, m), 1.07 (3H, s), 1.02 (3H, d, *J*=6.8 Hz), 1.01 (3H, d, *J*=6.8 Hz), 0.83 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.5, 138.6, 135.7, 129.4, 129.0, 125.8, 122.7, 121.0, 51.1, 49.2, 47.2, 39.0, 38.2, 37.8, 35.2, 35.1, 27.7, 24.0, 22.9, 21.7, 21.1, 21.0, 18.7, 14.3. The aqueous solution should be kept basic during the extractive product isolations. The generation of several side products during isolation of abietadienyl sulfide **7** is attributed to the presence of thiophenol (or other thiol byproducts) and the likelihood of radical reactions of the transoid diene.

4.2.8. Abieta-7,13-diene (1b). *(A) By Catechol borane reduction*. A solution of tosylhydrazone **6** (34 mg, 0.08 mmol) in $CHCl_3$ (3 mL) under N_2 was stirred at 25°C as catechol borane in THF (0.75 mL, 1 M, 0.75 mmol) was added.[31b,34] After 12 h at 25°C anhyd. NaOAc (204 mg, 1.5 mmol) was added. The resulting suspension was stirred at reflux for 1 h and cooled to rt. Water (10 mL) was added, the product was extracted with ether (3×10 mL), and the combined extracts were concentrated. Purification by flash column chromatography (100% pentane) afforded abietadiene **1b** (16.6 mg, 81% yield, 93.4% purity by GC) as a colorless oil.

(B) By Li/NH_3 reduction. NH_3 gas was condensed into a stirred solution of sulfide **7** (30 mg, 0.08 mmol) in THF (1 mL) at −78°C until the cloud point was reached (ca. 1:1 NH_3/THF).[31b] A single piece of hexane-washed lithium (2.9 mg, 0.40 mmol) was added, and the reaction mixture was stirred and cooled at −78°C for 5 min. The dark blue color developed was discharged by adding 3-hexyne (0.5 mL) and methanol (0.5 mL). After evaporation of excess NH_3, the remaining THF solution (ca. 1 mL) was diluted with 5% aqueous NaOH (20 mL), and the product was extracted with hexane (3×20 mL). The combined extracts were concentrated. Purification by flash column chromatography (100% pentane) afforded abietadiene **1b** (13.2 mg, 59% yield, 99.9% purity by GC) as a colorless oil. When too much NH_3 was used causing precipitation of starting material, reductions were incomplete. Longer reaction times or inadequate mixing led to increased amounts of the abietene over-reduction byproducts.

(C) By Zn/NaI reduction. A solution of tosylate **1e** (23.7 mg, 0.053 mmol) in HMPA (1 mL) was purged with N_2 and NaI (40 mg, 0.27 mmol) and zinc dust (35 mg, 0.53 mmol) were added.[29,32] The suspension was stirred and heated at 105°C for 1 d, cooled to rt, and filtered. Water (10 mL) was added, the product was extracted with pentane (3×10 mL), and the combined extracts were concentrated. Purification by flash column chromatography (100% pentane) afforded abietadiene **1b** (10.6 mg, 74% yield, 99.9% purity by GC) as a colorless oil: R_f (100% hexane) 0.78; $[\alpha]^{20}_D=-121°$ (*c* 0.80, $CHCl_3$) [Lit.[51] $[\alpha]^{20}_D=-137°$ ($CHCl_3$); Lit.[11b] −166.0° (*c* 0.4); Lit.[13] −78° (*c* 4.0); Lit.[10b] −75° (*c* 2.9, $CHCl_3$); Lit.[10a] −79.6°]; UV (hexane) λ_{max} (log ε) 234 (4.36), 242 (4.35), 250 (4.22) nm [Lit.[11b] λ_{max} (log ε) (EtOH) 233 (4.34), 241 (4.36), 249 (infl.) nm]; IR (neat) ν_{max} 2925, 1625 (C=C), 1462, 1380, 1363, 884 cm^{-1}; EIMS *m/z* 272.2 $[M]^+$ (100.0), 257.2 (48.6), 244.0 (3.3), 229.2 (74.8), 215.2 (2.6), 201.1 (11.0), 187.1 (22.9), 159.1 (15.3), 148.1 (56.7), 133.1 (5.7), 105.1 (82.6), 83.9 (62.6), 69.1 (40.4). See Tables 1 and 2 for NMR data.[11b,14]

Levopimaric, palustric, and neoabietic acids were converted

to the respective methyl esters, alcohols, tosylates, and hydrocarbons by procedures similar to those described above for abietic acid. In the case of identical procedures, only yields, purity, and characterization data for the products are provided below. Abbreviated or complete procedures are provided if significant changes were made.

4.2.9. Methyl levopimaradien-18-oate (methyl Levopimarate, methyl abieta-12(13),8(14)-diene-18-oate 2c). Yield, 2.07 g (99%); mp 62°C [Lit.[9] mp 60–62°C]; $[\alpha]^{20}_D=-238°$ (*c* 0.51, $CHCl_3$) [Lit.[38a,b] $[\alpha]^{20}_D=-269°$ (95% EtOH)]; UV (hexane) λ_{max} (log ε) 270 (3.97) nm [Lit.[38a] λ_{max} (log ε) 272.8 (3.76); Lit.[38b] 272 (3.76) nm]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.0, 145.3, 135.5, 122.3, 120.6, 51.8, 50.9, 46.6, 45.1, 38.3, 37.1, 34.9, 34.5, 27.4, 25.7, 22.4, 21.4, 20.8, 18.1, 17.0, 14.0. ^{1}H NMR (200 MHz) and HRMS data agree with the literature values.[9]

4.2.10. Levopimaradien-18-ol (2d). Yield, 542 mg (100%); $[\alpha]^{20}_D=-184°$ (*c* 0.47, $CHCl_3$); UV (hexane) λ_{max} 268 nm; IR (neat) ν_{max} 3380 (br OH), 2923, 1636 (C=C), 1489, 1403, 1039, 889 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.77 (1H, s), 5.39 (1H, m), 3.35 (1H, d, *J*=10.8 Hz), 3.13 (1H, d, *J*=11.0 Hz), 2.21 (1H, septet, *J*=6.8 Hz), 2.08–1.95 (3H, m), 1.86 (2H, t, *J*=13.2 Hz), 1.80 (1H, dq, *J*=1.21, 3.5 Hz), 1.59 (1H, dt, *J*=13.2, 3.7 Hz), 1.56–1.49 (3H, m), 1.45 (1H, broad s), 1.37 (1H, dd, *J*=12.7, 3.7 Hz), 1.36–1.28 (1H, m), 1.23–1.10 (1H, m), 1.00 (3H, d, *J*=6.8 Hz), 0.99 (3H, d, *J*=6.8 Hz), 0.87 (3H, s), 0.87–0.80 (1H, m), 0.81 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.3, 135.5, 122.4, 120.9, 72.2, 50.8, 43.7, 38.9, 37.5, 35.7, 34.9, 34.6, 27.5, 23.8, 22.7, 21.4, 20.8, 18.2, 17.7, 14.2; EIMS *m*/*z* 288.3 $[M]^+$ (74), 271.2 (26), 253.2 (32), 241.2 (9), 225.2 (2), 213.2 (10), 199.2 (13), 185.2 (23), 173.2 (28), 159.1 (25), 148.1 (39), 133.1 (99), 105.1 (56), 91.1 (100); HREIMS *m*/*z* 288.2463 (calcd for $C_{20}H_{32}O$, 288.2453).

4.2.11. Levopimaradien-18-yl tosylate (2e). A solution of alcohol **2d** (36 mg, 0.13 mmol) and TsCl (119 mg, 0.63 mmol) in pyridine (1 mL) under N_2 was stirred for 1 d at 25°C and cooled to 0°C before adding 3-(dimethylamino)propylamine (153 mg, 1.5 mmol). After 30 min at 0°C, solvent and excess amine were removed under reduced pressure (0.15 mm Hg), and the residue was dissolved in CH_2Cl_2 (10 mL). The solution was washed with satd $NaHCO_3$ (2×10 mL) and satd NaCl (2×10 mL) and concentrated to dryness. The ^{1}H NMR spectrum showed the absence of alcohol, amine, pyridine, and TsCl. Purification by flash column chromatography (15% diethyl ether/pentane) afforded tosylate **2e** (54 mg, 94%) as a white solid. Further elution with 30% MeOH/CH_2Cl_2 gave *N*-(3-dimethylaminopropyl)toluenesulfonamide (55 mg, 44%) as a colorless oil. Recrystallization of the tosylate from pentane/ether gave the analytical sample: mp 82–83°C; $[\alpha]^{20}_D=-129°$ (*c* 0.52, $CHCl_3$); UV (hexane) λ_{max} (log ε) 268 (3.97) nm; IR (thin film) ν_{max} 2954, 2881, 1671, 1616 (C=C), 1459, 1364, 1189, 1118, 965, 848, 814, 665 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, *J*=8.3 Hz), 7.33 (2H, d, *J*=8.1 Hz), 5.49 (1H, s), 5.11 (1H, m), 3.71 (1H, d, *J*=9.4 Hz), 3.47 (1H, d, *J*=9.4 Hz), 2.44 (3H, s), 2.31–2.26 (1H, m), 2.27 (1H, d, *J*=4.4 Hz), 2.20 (1H, dd, *J*=14.3, 3.3 Hz), 2.12 (1H, septet, *J*=6.8 Hz), 2.02 (2H, dd, *J*=12.7, 7.2 Hz), 1.96 (1H, m), 1.70 (1H, d, *J*=3.0 Hz), 1.48 (1H, m), 1.41–1.23 (6H, m), 0.95 (6H, d, *J*=6.8 Hz), 0.84 (3H, s), 0.75 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.6, 138.8, 138.4, 132.9, 129.8, 127.9, 119.0, 114.8, 77.8, 49.3, 47.9, 40.5, 37.2, 36.9, 35.5, 35.4, 33.2, 23.4, 22.6, 21.6, 21.4, 21.3, 17.9, 17.2, 14.4; EIMS *m*/*z* 442.3 $[M]^+$ (14), 427.3 (6), 270.3 (75), 255.3 (84), 241.2 (28), 227.2 (36), 213.2 (8), 199.2 (19), 172.1 (37), 149.2 (18), 135.1 (40), 107.1 (42), 91.1 (100), 77.1 (27); HREIMS *m*/*z* 442.2538 (calcd for $C_{27}H_{38}O_3S$, 442.2542). Tosylation of **2d** (439.3 mg, 1.52 mmol) with tosyl chloride (1.44 g, 7.6 mmol) in pyridine (2.5 mL) as described above without adding 3-(dimethylamino)propylamine and with chromatographic purification afforded tosylate **2e** (605 mg, 90%). The ^{1}H NMR spectrum showed the presence of 5–10% of residual tosyl chloride.

4.2.12. 18-(Phenylthio)levopimaradiene. Yield, 207 mg (90%); purity, 80% (GC); IR (neat) ν_{max} 3010, 2917, 2847, 1670, 1583, 1479, 1438, 1382, 1090, 1024, 736, 690 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (2H, dd, *J*=8.6, 1.3 Hz), 7.30 (2H, tt, *J*=7.7, 1.1 Hz), 7.19 (1H, tt, *J*=7.3, 1.5 Hz), 5.56 (1H, q, *J*=1.7 Hz), 5.20 (1H, t, *J*=3.6 Hz), 3.08 (1H, ABd, *J*=12.2 Hz), 2.80 (1H, ABd, *J*=12.2 Hz), 2.39 (1H, dd, *J*=9.0, 3.6 Hz), 2.36 (1H, t, *J*=4.9 Hz), 2.30 (1H, ddd, *J*=13.5, 4.3, 2.4 Hz), 2.20 (1H, septet d, *J*=6.9, 0.9 Hz), 2.12 (1H, dd, *J*=12.2, 7.3 Hz), 2.03 (1H, td, *J*=12.9, 5.1 Hz), 1.79 (1H, dt, *J*=13.1, 3.4 Hz), 1.64–1.55 (4H, m), 1.51–1.45 (2H, m), 1.42 (1H, dd, *J*=12.4, 4.5 Hz), 1.03 (6H, d, *J*=6.9 Hz), 1.02 (3H, s), 0.95 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9, 138.8, 138.3, 129.4, 128.8, 125.6, 118.9, 114.8, 50.9, 49.5, 48.9, 40.7, 38.0, 37.5, 37.2, 35.5, 33.2, 23.6, 22.6, 21.4, 21.3, 20.9, 18.5, 14.3; EIMS *m*/*z* 380.2 $[M]^+$ (43.0), 271.2 (6.6), 257.2 (40.2), 218.0 (40.6), 187.1 (12.7), 173.1 (31.0), 147.1 (22.4), 133.1 (100.0), 109.1 (90.1), 91.1 (42.4); HREIMS *m*/*z* 380.2537 (calcd for $C_{26}H_{36}S$, 380.2538). The ^{1}H NMR spectrum showed some extra peaks attributed to dehydroabietadienyl sulfide [δ_H 7.22 (2H, d, *J*=8.4 Hz), 7.04 (2H, dd, *J*=7.9, 1.9 Hz), 6.92 (1H, br s)] and diphenyl disulfide [δ_H 7.54 (2H, dd, *J*=8.6, 1.3 Hz), 7.34 (2H, t, *J*=7.7 Hz), 7.26 (2H, tt, *J*=7.5, 1.3 Hz)].

4.2.13. PCC oxidation of levopimaradienol. Oxidation of alcohol **2d** (53.2 mg, 0.18 mmol) with PCC (40 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) for 1 h at 25°, isolation of the crude product, and purification afforded two fractions containing aldehydes (16.5 mg, 31%) and alcohols (21 mg, 39%). The ^{1}H NMR spectra showed the fractions were 6:5:1 (levopimaradienal/dehydroabietadienal/**5**) and 4.7:1.7:1 (dehydroabietadienol/levopimaradienol/**1d**) mixtures.

4.2.14. Levopimaradiene (abieta-12(13),8(14)-diene, 2b). *(A) By Li/NH_3 reduction*. Reduction of levopimaradienyl sulfide (36.5 mg, 0.1 mmol) with Li/NH_3 in THF as described above for **1f** and purification by chromatography gave 9.8 mg (36%) of a 2.6:1:3.1 mixture of **2b**, **1b**, and 3 abietene isomers according to GC. In another run the abietene mixture was obtained as a separate, less polar fraction: GC–EIMS: *m*/*z* 274.3 $[M]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.47 (m), 5.31 (s), and 5.09 (s).

(B) By Zn/NaI reduction. Yield, 52 mg (68%); purity, 88.2% (GC); $[\alpha]^{20}_{D}=-265°$ (*c* 0.52, $CHCl_3$); UV (hexane) λ_{max} (log ε) 268 (3.93) nm; IR (neat) ν_{max} 2956, 2868, 1669, 1617 (C=C), 1458, 1388, 1364, 862 cm^{-1}; EIMS *m/z* 272.3 $[M]^+$ (90), 257.3 (48), 229.2 (28), 215.2 (2), 201.2 (6), 187.2 (14), 175.2 (8), 159.1 (15), 148.1 (56), 137.2 (88), 117.1 (30), 105.1 (58), 91.1 (100), 69.1 (57); HREIMS *m/z* 272.2503 (calcd for $C_{20}H_{32}$, 272.2504). See Tables 1 and 2 for NMR data.

4.2.15. Methyl palustradien-18-oate (methyl palustrate, methyl abieta-8,13-dien-18-oate, 3c). Yield, 209 mg (100%); Lit.[38b] mp 24–27°C; $[\alpha]^{20}_{D}=+65°$ (*c* 1.60, $CHCl_3$) [Lit.[52] $[\alpha]^{20}_{D}=+69.9°$ (*c* 2.09, $CHCl_3$); Lit.[38a,b] +67°]; UV (hexane) λ_{max} (log ε) 268 (3.95) nm; IR (neat) ν_{max} 2929, 2867, 1726 (C=O), 1660 (C=C), 1458, 1386, 1249, 1170, 1112, 1038, 862 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.37 (1H, s), 3.64 (3H, s), 2.27 (1H, septet, *J*=6.8 Hz), 2.12–1.98 (7H, m), 1.83 (1H, d, *J*=13.4 Hz), 1.72 (1H, dd, *J*=11.5, 4.6 Hz), 1.66–1.53 (4H, m), 1.24–1.19 (1H, m), 1.18 (3H, s), 1.15 (1H, td, *J*=12.5, 4.2 Hz), 1.04 (3H, s), 1.01 (3H, s), 1.00 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.2, 143.4, 137.6, 124.9, 120.2, 51.8, 47.7, 46.2, 37.1, 36.6, 35.2, 34.3, 30.1, 26.2, 22.4, 21.6, 21.2, 21.0, 20.7, 18.2, 16.3; EIMS *m/z* 316.3 $[M]^+$ (87), 301.3 (100), 273.2 (4), 257.3 (21), 241.2 (90), 227.2 (4), 213.2 (19), 199.2 (9), 185.2 (26), 173.2 (8), 149.1 (34), 128.1 (12), 117.1 (16), 105.1 (34), 91.1 (29), 79.1 (13); HREIMS *m/z* 316.2397 (calcd for $C_{21}H_{32}O_2$, 316.2394). The UV data agree with the literature values.[38,52]

4.2.16. Palustradien-18-ol (3d). Yield, 164 mg (99%); mp 130–131°C [Lit.[51] mp 131–132°C]; $[\alpha]^{20}_{D}=+50°$ (*c* 0.69, $CHCl_3$) [Lit.[51] $[\alpha]^{20}_{D}=+53°$ (*c* 2.64, $CHCl_3$)]; ^1H NMR (500 MHz, $CDCl_3$) δ 5.39 (1H, s), 3.43 (1H, d, *J*=10.3 Hz), 3.17 (1H, dd, *J*=10.3, 4.5 Hz), 2.27 (1H, septet, *J*=6.6 Hz), 2.12–1.94 (5H, m), 1.82 (1H, broad t, *J*=12.6 Hz), 1.65 (1H, dt, *J*=13.5, 3.9 Hz), 1.63–1.59 (2H, m), 1.55 (1H, dt, *J*=14.1, 3.9 Hz), 1.46–1.42 (2H, m), 1.38–1.29 (4H, m), 1.05 (3H, s), 1.02 (3H, s), 1.00 (3H, s), 0.80 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.2, 138.2, 124.8, 120.2, 72.3, 45.3, 37.6, 37.5, 35.5, 35.0, 34.2, 30.3, 26.2, 22.6, 21.2, 21.0, 20.9, 18.6, 18.3, 17.3; EIMS *m/z* 288.3 $[M]^+$ (56), 273.2 (100), 255.2 (20), 227.2 (9), 213.2 (6), 199.2 (10), 188.2 (5), 173.1 (13), 161.2 (16), 149.1 (38), 133.1 (21), 105.1 (30), 91.1 (26), 67.1 (13); HREIMS *m/z* 288.2454 (calcd for $C_{20}H_{32}O$, 288.2453). UV, IR, and limited ^1H NMR data are available in the literature.[51]

4.2.17. Palustradien-18-yl *p*-toluenesulfonate (3e). The tosylate was prepared by the procedure described for **2e**. Data for **3e**: yield, 189 mg (85%); mp 68–69°C; $[\alpha]^{20}_{D}=+61°$ (*c* 0.33, $CHCl_3$); UV (hexane) λ_{max} (log ε) 266 (4.01) nm; IR (thin film) ν_{max} 2956, 2871, 1658 (C=C), 1599, 1459, 1358, 1189, 1099, 965, 844, 814 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, *J*=8.4 Hz), 7.33 (2H, d, *J*=8.6 Hz), 5.36 (1H, s), 3.73 (1H, d, *J*=9.2 Hz), 3.55 (1H, d, *J*=9.2 Hz), 2.45 (3H, s), 2.27 (1H, septet, *J*=6.9 Hz), 2.07–1.95 (6H, m), 1.78 (1H, dt, *J*=12.6, 3.0 Hz), 1.59 (1H, dt, *J*=13.5, 3.9 Hz), 1.51 (1H, dt, *J*=13.9, 3.6 Hz), 1.46–1.26 (6H, m), 1.02 (3H, s), 1.00 (6H, s), 0.79 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.6, 143.4, 137.6, 132.9, 129.8, 127.9, 124.8, 120.1, 78.0, 44.9, 37.5, 36.9, 35.1, 35.0, 34.2, 29.9, 26.2, 22.5, 21.6, 21.2, 20.9, 20.8, 18.6, 17.9, 16.9; EIMS *m/z* 442.3 $[M]^+$ (25), 255.2 (100), 227.2 (9), 213.2 (6), 185.1 (17), 149.1 (26), 91.1 (52); HREIMS *m/z* 442.2542 (calcd for $C_{27}H_{38}O_3S$, 442.2542).

4.2.18. 18-Iodopalustradiene (8). Reaction of tosylate **3e** (63.8 mg, 0.14 mmol) with Zn (94 mg, 1.44 mmol) and NaI (108 mg, 0.72 mmol) for 16 h at 100°C, as described above for **1b** and similar purification by chromatography with pentane as eluant afforded iodide **8** (37.6 mg, 67%) as a colorless oil: UV (hexane) λ_{max} (log ε) 268 (3.96) nm; IR (neat) ν_{max} 2955, 2930, 2868, 1658 (C=C), 1467, 1380, 1212, 1177, 861 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.40 (1H, s), 3.30 (1H, d, *J*=9.9 Hz), 3.19 (1H, d, *J*=9.9 Hz), 2.28 (1H, septet, *J*=6.9 Hz), 2.22–1.97 (6H, m), 1.80 (1H, dt, *J*=12.9, 3.2 Hz), 1.64–1.50 (4H, m), 1.48 (1H, d, *J*=12.2 Hz), 1.41 (1H, td, *J*=11.8, 5.6 Hz), 1.32 (2H, dd, *J*=8.8, 3.9 Hz), 1.03 (3H, s), 1.02 (3H, s), 1.01 (3H, s), 1.00 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.3, 137.7, 124.9, 120.2, 48.5, 38.6, 37.7, 35.4, 35.3, 34.2, 30.1, 28.5, 26.2, 22.6, 21.2, 20.9, 20.3, 18.6, 18.4, 18.3; EIMS *m/z* 398.2 $[M]^+$ (71), 383.1 (100), 369.0 (5), 306.2 (3), 291.2 (12), 271.3 (16), 256.2 (30), 241.2 (7), 213.2 (5), 188.2 (34), 173.1 (17), 149.1 (56), 123.1 (18), 105.1 (26), 91.1 (30), 69.1 (15); HREIMS *m/z* 398.1465 (calcd for $C_{20}H_{31}I$, 398.1470).

4.2.19. Palustradiene (abieta-8,13-diene, 3b). Reductions of iodide **8** (37.6 mg, 0.09 mmol) and tosylate **3e** (85.1 mg, 0.19 mmol) with Zn/NaI in HMPA at 105°C for 1 and 2 d, respectively, were carried out as described in Section 4.2.8. The yields and purities were 18 mg (70% from **8**, 95% purity by GC) and 25 mg (48% from **3e**, ca. 95% purity by ^1H NMR estimate): $[\alpha]^{20}_{D}=+59°$ (*c* 0.72, $CHCl_3$) [Lit.[13] $[\alpha]^{20}_{D}=-68°$ (*c* 5.00), Lit.[12] −45.5° ($CHCl_3$)]; UV (hexane) λ_{max} (log ε) 268 (3.95) nm [Lit.[13] λ_{max} 267 (3.92, heptane) nm]; IR (neat) ν_{max} 2958, 2933, 2865, 1673 (C=C), 1457, 1370, 862 cm^{-1}; EIMS *m/z* 272.3 $[M]^+$ (33), 257.3 (100), 243.1 (2), 229.2 (9), 214.2 (4), 188.2 (5), 173.2 (7), 161.2 (12), 149.2 (23), 133.1 (13), 117.1 (9), 107.1 (11), 91.1 (16), 81.1 (8), 69.1 (25); HREIMS *m/z* 272.2510 (calcd for $C_{20}H_{32}$, 272.2504). See Tables 1 and 2 for NMR data.

4.2.20. Logic used to assign ^1H and ^{13}C NMR data for palustradiene (3b). The ^1H NMR singlets for the three quaternary methyl groups on ring A (δ_H 0.89, 0.86, 1.06) were identified with C18, C19, and C20, respectively, by the NOE results presented in Table 3. Although irradiations intended for one of the gem dimethyl groups inevitably excited both, the distinctive NOEs observed at δ_H 1.06–2.08 (H11β, 3.2%) and 0.86 (H19, 7.8%) from selective irradiation at 1.06 (H20) allowed unambiguous assignments of C20 and C19, and by process of elimination C18. These methyl peaks were correlated with ^{13}C NMR signals at δ_C 33.4 and 21.5–21.7 by HMQC cross peaks, but overlap of the higher field pair left the assignments to C19 and C20 ambiguous. The low field position of the equatorial methyl (C18, 33.4) in palustradiene is consistent with its location in the ^{13}C NMR spectra of the other 3 dienes (δ_C 33.5–33.8). However, its proximity ($\Delta\delta_C$ 0.02) to the signal for C4

precluded unique assignments for these close lying resonances. Similarly the clustering of the four peaks for the C16, C17, C19, and C20 methyls and the resulting overlap of HMBC correlations prevented their individual ^{13}C NMR assignment.

The proton peaks arising from the isolated C1–C2–C3, C5–C6–C7, and C11–C12 segments were assigned by COSY plots, and also were correlated to the assigned carbon resonances by HMQC crosspeaks. The equatorial proton H1β (δ_H 1.75) was identified, and clearly distinguished from the H3β proton (δ_H 1.38), by the NOE arising from irradiation at C20 and density at the C9↔H1 intersection in the HMBC plot. The assignments for all of the A ring methylene protons are consistent with the multiplicities and the usual magnitudes for axial–axial, axial–equatorial, and equatorial–equatorial coupling on a chair cyclohexane conformation. In addition, long-range W coupling between H1β and H3β (J ~1.4 Hz) was evident.

Identification of H5 (δ 1.17) was apparent from the NOE by C18 irradiation, its multiplicity and coupling (dd, ^3J=12.4, 1.9 Hz), and the HMQC crosspeak to the CH peak at δ_C 51.9. Although severe peak overlap precluded coupling analysis for H6β, H7α, H7β, H11α, H11β, H12α, and H12β, chemical shift range assignments could be made by NOEs observed at H6β and H11β (irrad. H20) and at H7β (irrad. H14), and by the HMQC crosspeaks which identify both attached protons of the four CH_2 groups. The assignments for the C11 and C12 methylene carbons and for the five quaternary carbons were deduced from the following HMBC crosspeaks: C12↔H14, H15; C4↔H5, H18/H19; C10↔H5, H20; C8↔H7, H11, H14; C9↔H1, H7, H14; C13↔H12, H15.

4.2.21. Methyl neoabietadien-18-oate (methyl abieta-8(14),13(15)-dien-18-oate, 4c). Yield, 205 mg (100%); mp 62°C [Lit.[53] mp 61.5–62°C]; $[\alpha]^{20}_D$=+133° (*c* 1.56, $CHCl_3$) [Lit.[53] $[\alpha]^{20}_D$=+147.8° (2% EtOH); Lit.[38a,b] $[\alpha]^{20}_D$=+148.0 (95% EtOH)]; IR (thin film) ν_{max} 2930, 2867, 1725 (C=O), 1623 (C=C), 1444, 1384, 1242, 1187, 1144, 1101, 866 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.17 (1H, s), 3.65 (3H, s), 2.50 (1H, dt, J=14.2, 4.2 Hz), 2.31 (1H, dd, J=14.4, 3.2 Hz), 2.19 (1H, td, J=13.4, 4.9 Hz), 1.95 (1H, br t, J=7.2 Hz), 1.92 (1H, dd, J=12.5, 2.4 Hz), 1.84 (1H, br t, J=13.2 Hz), 1.78–1.68 (3H, m), 1.72 (3H, s), 1.68 (3H, s), 1.58–1.50 (3H, m), 1.46 (1H, dd, J=12.7, 3.7 Hz), 1.34 (1H, dt, J=13.2, 3.7 Hz), 1.19 (3H, s), 1.17–1.10 (2H, m), 0.77 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 138.5, 128.2, 123.5, 122.1, 51.9, 51.4, 48.9, 47.5, 38.5, 37.7, 36.9, 35.5, 25.7, 24.8, 22.3, 20.3, 19.7, 18.2, 17.0, 15.3; EIMS *m/z* 316.3 $[M]^+$ (47), 257.2 (7), 181.1 (11), 148.1 (29), 135.1 (100), 121.1 (41), 105.1 (15), 91.1 (22), 79.1 (13); HREIMS *m/z* 316.2399 (calcd for $C_{21}H_{32}O_2$, 316.2394). UV data are available in the literature.[38,53]

4.2.22. Neoabietadien-18-ol (4d). Yield, 159 mg (97%); mp 99–100°C [Lit.[53] mp 98–99.5°C]; $[\alpha]^{20}_D$=+180° (*c* 0.19, $CHCl_3$) [Lit.[53] $[\alpha]^{20}_D$=+187° (1% $CHCl_3$)]; IR (thin film) ν_{max} 3375 (br OH), 2931, 2863, 1628 (C=C), 1448, 1440, 1040, 870 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.18 (1H, s), 3.41 (1H, d, J=10.9 Hz), 3.12 (1H, d, J=10.7 Hz), 2.51 (1H, dt, J=13.9, 4.3 Hz), 2.35 (1H, d, J=15.9 Hz), 2.19 (1H, broad t, J=12.2 Hz), 1.91 (1H, t, J=8.1 Hz), 1.84 (1H, t, J=12.9 Hz), 1.73 (3H, s), 1.69 (3H, s), 1.59 (1H, s), 1.54–1.49 (3H, m), 1.44–1.28 (7H, m), 1.03 (1H, td, J=12.4, 4.9 Hz), 0.80 (3H, s), 0.78 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0, 128.3, 123.2, 121.8, 72.2, 51.3, 47.6, 39.0, 38.1, 37.8, 35.7, 35.4, 25.8, 22.5, 22.3, 20.3, 19.6, 18.3, 17.9, 15.7; EIMS *m/z* 288.3 $[M]^+$ (29), 273.3 (2), 257.3 (6), 173.2 (5), 161.2 (7), 148.1 (29), 135.1 (100), 119.1 (8), 91.1 (6), 79.1 (4); HREIMS *m/z* 288.2449 (calcd for $C_{20}H_{32}O$, 288.2453). UV and elemental analysis data are available in the literature.[53]

4.2.23. Neoabietadien-18-yl *p*-toluenesulfonate (4e). The tosylate **4e** was prepared as described above for **2e**. Data for **4e**: yield, 45.5 mg (63%); mp 112–114°C; $[\alpha]^{20}_D$=+105° (*c* 0.73, $CHCl_3$); UV (hexane) λ_{max} (log ε) 254 (4.45) nm; IR (thin film) ν_{max} 2933, 2867, 1614 (C=C), 1462, 1365, 1189, 1099, 965, 846, 814 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, J=8.6 Hz), 7.34 (2H, d, J=7.9 Hz), 6.16 (1H, s), 3.72 (1H, d, J=9.2 Hz), 3.50 (1H, d, J=9.4 Hz), 2.50 (1H, dt, J=14.1, 4.1 Hz), 2.45 (3H, s), 2.23 (1H, ddd, J=14.6, 4.3, 1.9 Hz), 2.04 (1H, td, J=14.2, 4.3 Hz), 1.83 (2H, q, J=13.7 Hz), 1.76–1.71 (1H, m), 1.72 (3H, s), 1.68 (3H, s), 1.67 (1H, dq, J=13.9, 1.3 Hz), 1.49–1.38 (3H, m), 1.34–1.24 (5H, m), 1.00 (1H, ddd, J=12.9, 9.9, 7.1 Hz), 0.79 (3H, s), 0.73 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.6, 138.3, 133.0, 129.8, 128.2, 127.9, 123.5, 122.0, 78.0, 51.1, 47.3, 38.6, 38.0, 37.1, 35.3, 25.7, 22.4, 22.2, 21.6, 20.3, 19.6, 17.9, 17.6, 15.6; EIMS *m/z* 442.3 $[M]^+$ (20), 270.3 (21), 255.2 (7), 241.2 (4), 187.2 (7), 173.1 (6), 155.0 (25), 135.1 (100), 119.1 (16), 105.1 (12), 91.1 (58), 71.1 (21); HREIMS *m/z* 442.2533 (calcd for $C_{27}H_{38}O_3S$, 442.2542). Tosylation of **4d** (160 mg, 0.55 mmol) with tosyl chloride (526 mg, 2.77 mmol) in pyridine (1 mL) as described above without the addition of 3-(dimethylamino)-propylamine afforded tosylate **4e** (197 mg, 81%). The ^1H NMR spectrum showed the presence of 5–10% of residual tosyl chloride. The standard Zn/NaI reduction of this contaminated tosylate resulted in the decomposition.

4.2.24. Neoabietadiene (abieta-8(14),13(15)-diene, 4b). Reaction of tosylate **4e** (20.7 mg, 0.05 mmol) with Zn (30.1 mg, 0.46 mmol) and NaI (35 mg, 0.23 mmol) in HMPA (1 mL) for 1 d at 105°C, as described above for **1b**, gave neoabietadiene **4b** (9 mg, 70% yield, 99.9% purity by GC) as a white solid: mp 62°C; $[\alpha]^{20}_D$=+145° (*c* 0.57, $CHCl_3$); UV (hexane) λ_{max} (log ε) 254 (4.30) nm; IR (thin film) ν_{max} 2936, 2865, 2842, 1622 (C=C), 1455, 1386, 1364, 1114, 880 cm^{-1}; EIMS *m/z* 272.3 $[M]^+$ (22), 257.2 (4), 187.2 (3), 161.1 (4), 148.1 (26), 135.1 (100), 117.1 (5), 105.1 (13), 91.1 (18), 69.1 (13); HREIMS *m/z* 272.2509 (calcd for $C_{20}H_{32}$, 272.2504). See Tables 1 and 2 for NMR data.

4.2.25. Maleic anhydride adduct (9) of levopimaradiene. The procedure was based on those in the literature.[54] A solution of levopimaradiene **2b** (58.5 mg, 0.21 mmol) and maleic anhydride (84 mg, 0.86 mmol) in toluene (0.2 mL) was stirred for 1 d at rt under N_2. The solution was diluted with $CHCl_3$ (20 mL), washed with water (3×20 mL), dried ($MgSO_4$), and concentrated to dryness. Purification by flash column chromatography (20% ether/pentane) and

recrystallization from 2% ether/pentane afforded the known[15c] adduct **9** (51.5 mg, 66%) as a colorless crystals: mp 105–107°C [Lit.[15c] mp 110–111°C]; $[\alpha]^{20}_{D}=-17°$ (*c* 1.48, $CHCl_3$); IR (thin film) ν_{max} 2930, 2881, 1857 (C=O), 1775 (C=O), 1465, 1386, 1227, 1085, 939, 908, 852 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.53 (1H, s), 3.08 (1H, m), 3.07 (1H, dd, *J*=8.6, 3.2 Hz), 2.68 (1H, d, *J*=8.4 Hz), 2.54 (1H, dt, *J*=13.7, 3.2 Hz), 2.23 (1H, septet, *J*=6.9 Hz), 1.69–1.61 (2H, m), 1.55 (1H, td, *J*=13.7, 4.7 Hz), 1.47 (1H, qt, *J*=13.3, 3.0 Hz), 1.39–1.29 (4H, m), 1.26–1.20 (2H, m), 1.11 (1H, td, *J*=13.5, 3.9 Hz), 0.98 (3H, s), 0.97 (3H, s), 0.88 (3H, s), 0.87–0.77 (2H, m), 0.79 (3H, s), 0.54 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 171.2, 147.9, 125.4, 55.1, 53.3, 53.2, 45.6, 41.8, 40.3, 38.9, 38.2, 35.7, 35.3, 33.5, 32.8, 32.7, 27.4, 21.8, 20.5, 19.9, 18.9, 17.8, 15.1; EIMS *m/z* 370.3.3 $[M]^+$ (7.8), 342.3 (23.7), 327.3 (11.4), 272.3 (100), 255.2 (11.6), 229.2 (2.6), 187.2 (3.1), 173.2 (5.2), 161.1 (2.5), 148.1 (26.9), 134.1 (48.7), 105.1 (14.5), 91.1 (29.9), 67.1 (11.3); HREIMS *m/z* 370.2508 (calcd for $C_{24}H_{34}O_3$, 370.2508). IR and elemental analysis data are available in the literature.[15c]

4.3. Incubation of GGPP with rAS and GC–MS analysis of enzymatic products

GGPP (2 μmol, 200 μL) was added to truncated recombinant abietadiene synthase[24] (rAS, 0.4 mL) in 3.6 mL of buffer (30 mM HEPES, pH 7.2, 7.5 mM $MgCl_2$, 20 μM $MnCl_2$, 5% (v/v) glycerol, 5 mM DTT) and then were gently mixed. After the mixture was incubated at 33°C for 3 h, 2 mL of hexane containing 1.6×10^{-4} M BHT was added. The phases were vigorously mixed on a vortex genie and centrifuged to separate the emulsion. The hexane layer was carefully concentrated and dissolved in 10 μL of hexane containing 1.6×10^{-4} M BHT under nitrogen. GC–MS spectra were obtained on a Quattro instrument. GC–MS analysis of a mixture of the synthetic samples **1b**–**4b** was compared with that of abietadiene (**1b**, 33%, R_t=16.4 min), levopimaradiene (**2b**, 42%, R_t=14.1 min), palustradiene (**3b**, 9%, R_t=13.8 min) and neoabietadiene (**4b**, 16%, R_t=19.7 min) of the hexane extract obtained from incubation. GC conditions were as follows: Rt_x-5 30-m fused silica capillary column with a split ratio of 100:1; initial temperature 190°C for 20 min followed by an increase of 15°C min^{-1} up to 250°C. Although the GC peaks for **2b** and **3b** are very close, it proved possible to obtain mass spectra with very little cross contamination, thus confirming the identity of these two products. No attempt was made to identify other minor components.

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