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High-boiling Neutral Constituents from the Wood of *Pinus silvestris* L.*, **

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Extracts from fresh and from fungus infested (*Peridermium pini*) wood of *Pinus silvestris* L. have been investigated. The following compounds were shown to be present in both extracts (*cf.* Tables 2—4): α-longipinene, copaene, longicyclene, longifolene, β-ylangene, β-copaene, α-, γ- and ε-muurolene, γ- and δ-cadinene, calamenene, α-calacorene, δ-cadinol, pimaradiene, isopimaradiene, pimarinol, isopimarinol, abietinol, pimarinal, isopimarinal, abietinal, dehydroabietinal, β-sitosterol, and a mixture of fatty alcohols. Eugenol methyl ether, pinosylvin dimethyl ether, a new sesquiterpene alcohol and three unidentified diterpene hydrocarbons were isolated from the infested wood.

In spite of the great commercial importance of the wood of Scots pine (*Pinus silvestris* L.) our knowledge of its minor constituents is limited. The acidic and phenolic constituents, the resin acids (*e.g.* Ref. 1) and the heartwood phenols [2] have all been fairly well investigated. Although they are obtained in large quantities as by-products from the sulphate pulp industry, little is known about the neutral constituents other than the monoterpenes.[3]

In this and the following papers an extensive investigation of the sesquiterpenes and the neutral diterpenes from the wood of Scots pine and from Swedish sulphate turpentine is described. Some of the results have been published in three preliminary communications.[4–6]

The work was undertaken with the aim of finding out more about the constituents of the industrial by-products as well as of providing a careful chemical description of *Pinus silvestris*.

In 1929 Aschan found that an industrial extract of pine stumps (*P. silvestris*) contained two different sesquiterpene hydrocarbons.[7] With hydrogen chloride the fraction investigated yielded two dihydrochlorides, one of which was

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identical with the known cadinene dihydrochloride, the other being a new compound, the dihydrochloride derived from what Aschan called muurolene.

Investigations by Bergström [8] showed that Swedish sulphate turpentine and "tall oil" (*P. silvestris*) contained cadinenes and cadinols, as certain fractions on dehydrogenation gave cadalene. Recently, Pentegova and Lebedova [9] investigated Russian tall oil obtained from *Pinus silvestris* and isolated longifolene (*4*), "muurolene", γ-cadinene (*10*), δ-cadinene (*11*) and claimed the isolation of ylangene (*24*) from this oil.

A sesquiterpene fraction from *Pinus sibirica* has been reported to yield muurolene dihydrochloride.[10]

The diterpene aldehyde pimarinal ("cryptopinone") (*20*) has been isolated by Sörensen and Bruun [11] from twig roots of Scots pine and by Roberts and Lawrence [12] from *Pinus palustris*. The latter species contained isopimarinal (*21*) as shown by oxidation of the crude diterpene aldehydes to an acidic mixture, from which isopimaric acid was isolated. Similarly, the presence of abietinal (*23*) and levopimarinal (*25*) was demonstrated in *Pinus densiflora*.[13] The same species was found to contain neoabietinal (*26*) and palustrinal (*27*) by examination of the UV spectra of the oxidation products.

The present paper describes an investigation of neutral high-boiling constituents from fresh wood of Scots pine as well as from wood infested by the fungus *Peridermium pini*. This fungus induces the production of large amounts of "resin" in the wood (sometimes ten times the normal).

The extraction and the isolation of constituents from fresh and from infested wood were carried out under almost the same conditions. The milled wood was extracted with ether, the light petroleum soluble part of the extract was hydrolyzed and the neutral fraction was collected. Girard reagent P was used to isolate a carbonyl fraction. By vacuum distillation, the remaining neutral part of the extract was divided into five main fractions, monoterpenes, sesquiterpenes, diterpene hydrocarbons, diterpene alcohols and a final fraction containing high-boiling constituents.

The various fractions were analysed by gas-liquid chromatography (GLC) and argentative thin layer chromatography (Ag—TLC). Some of the components were isolated by chromatographic methods, mainly by preparative

Table 1. The occurrence of monoterpenes in the wood of *Pinus silvestris* (+ = isolated and characterized, × = presence indicated by GLC).

	Extract from fresh wood [3]	Extract from *Peridermium* infested wood
α-Pinene	+	×
Camphene	+	×
β-Pinene	+	×
Δ^3-Carene	+	×
Limonene	+	×
p-Cymene	+	×
Terpinolene	+	×

Table 2. The occurrence of sesquiterpenes in the wood of *Pinus silvestris* (+ = isolated and characterized, × = presence indicated by GLC and/or Ag—TLC).

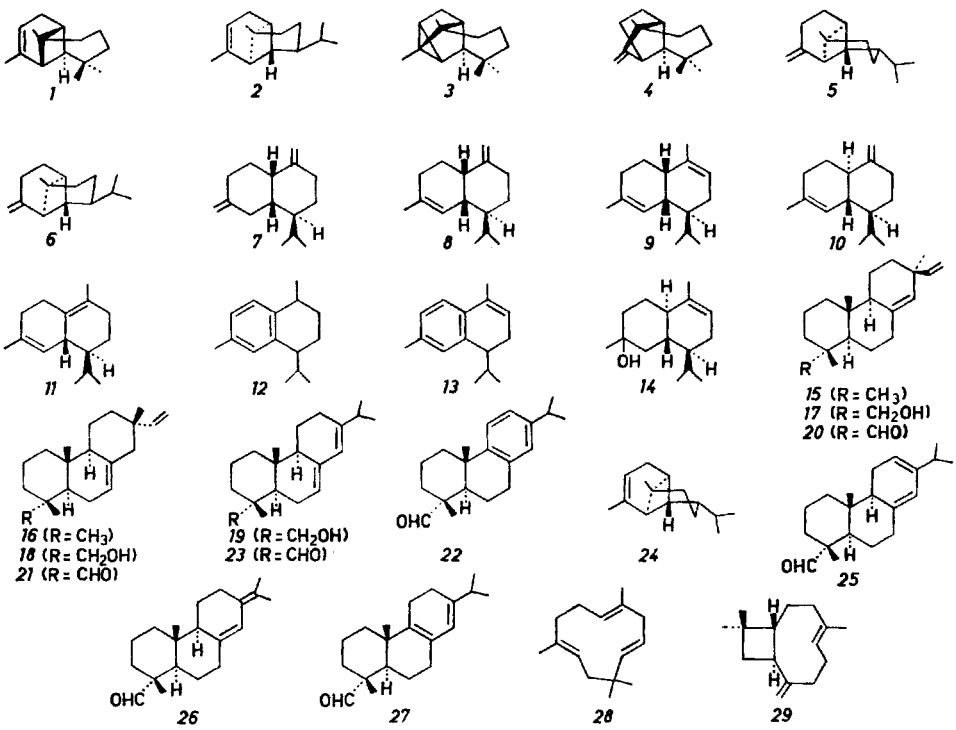
	Structure	Extract from fresh wood	Extract from *Peridermium* infested wood
α-Longipinene	(*1*)	×	×
Copaene	(*2*)	×	+
Longicyclene	(*3*)		×
Longifolene	(*4*)	×	+
β-Ylangene	(*5*)	×	×
β-Copaene	(*6*)	×	+
ε-Muurolene	(*7*)	×	+
γ-Muurolene	(*8*)	×	+
α-Muurolene	(*9*)	×	+
γ-Cadinene	(*10*)	×	+
δ-Cadinene	(*11*)	×	+
Calamenene	(*12*)		×
α-Calacorene	(*13*)		×
δ-Cadinol	(*14*)		×
New sesquiterpene alcohol (m.p. 48°[15])			×

Table 3. The occurrence of diterpenes in the wood of *Pinus silvestris* (+ and × same significance as in Table 2).

	Structure	Extract from fresh wood	Extract from *Peridermium* infested wood
Pimaradiene	(*15*)	+	+
Isopimaradiene	(*16*)	×	+
Hydrocarbon Y			+
Hydrocarbon Z			+
Hydrocarbon X			+
Pimarinol	(*17*)	×	+
Isopimarinol	(*18*)	×	+
Abietinol	(*19*)	×	+
Pimarinal	(*20*)	+	+
Isopimarinal	(*21*)	×	+
Dehydroabietinal	(*22*)	×	+
Abietinal	(*23*)	×	+

Table 4. Non-terpenoid high-boiling neutral compounds isolated from the wood of *Pinus silvestris*.

	Extract from fresh wood	Extract from *Peridermium* infested wood
Eugenol methyl ether		+
Pinosylvin dimethyl ether		+
β-Sitosterol	+	+
Mixture of fatty alcohols	+	+



GLC and argentative column chromatography. The results are summarized in Tables 1—4. As can be seen from these tables, there was no qualitative difference in composition between the extracts from fresh and from infested wood. Infested wood was therefore used when larger quantities of a certain pine wood constituent had to be isolated.

In *Pinus silvestris* the sesquiterpene fraction is small compared to the mono- and diterpene fractions. The sesquiterpenes could be divided into two groups, one including compounds of the cadalene type, and another consisting of longifolene (*4*) and compounds related to this hydrocarbon. Of the former group, γ-cadinene (*10*) and δ-cadinene (*11*) constitute only a minor part, the major part being composed of α-, γ-, and ε-muurolene (*9*), (*8*), and (*7*), respectively. The second, quantitatively smaller group contains longifolene (*4*) accompanied by small amounts of longicyclene (*3*) and α-longipinene (*1*).

No hydrocarbons have been detected that do not belong to either of the above-mentioned groups. Sesquiterpenes of quite different type have been reported to occur in other pines. Humulene (*28*) and caryophyllene (*29*) have been isolated from *Pinus sibirica* [10] and the latter compound also from *Pinus maritima*.[14] Future investigations of the wood of pine species will show if such differences may be used for classification purposes within the genus *Pinus*.

The neutral diterpenes isolated from *Pinus silvestris* are all closely related to the resin acids occurring in the same species.[1] Cembrene, isolated from many pine species,[10,29] was not detected. The ratio between the amounts of neutral and acidic diterpenes present is about 1:11. Within the neutral diterpene fraction hydrocarbons, alcohols, and aldehydes occur in the approximate ratio 1:6:60.

Of the neutral diterpenes those with the pimarane skeleton predominate, whereas the resin acids are mainly of the abietane type.[1] Of the hydrocarbons, only compounds possessing the pimarane skeleton have been isolated. Hydrocarbons having the abietane skeleton do not seem to be present in any signifi cant amount as shown by examination of the UV spectra of all fractions obtained by chromatography.

It is of interest to note that the main components of the diterpene aldehyde fractions of *Pinus silvestris* as well as of *Pinus palustris* [12] are pimarinal and isopimarinal, but only abietic type aldehydes have been reported to occur in *Pinus densiflora*.[13] Again, more work on different pine species is needed to find out if this difference could have any taxonomic significance.

EXPERIMENTAL

Melting points were taken on a Kofler micro hot stage. IR spectra were recorded on a Perkin-Elmer No. 21 instrument (NaCl prism, sample as liquid film or, for solids, in KBr unless otherwise stated), UV spectra on a Beckman DK 2 recording spectrophotometer (solvent ethanol) and NMR spectra on a Varian A 60 instrument operating at 60 Mc/s (solvent carbon tetrachloride, internal standard tetramethylsilane). For analytical gas-liquid chromatography (GLC) a PYE argon chromatograph (column length 1.2 m, inner diameter 4 mm) and for preparative GLC an Aerograph A-700 "Autoprep" instrument (column length 20′, outer diameter 3/8″) were used.

Identifications of isolated compounds were made by comparison with authentic samples, when applicable, by mixed melting point determinations, by GLC and thin layer chromatography (TLC) and by comparison of IR spectra, UV spectra and optical rotations.

The procedure used to prepare columns for GLC, plates for argentative TLC (Ag–TLC) and adsorbent (Ag-silica) for argentative column chromatography have been described previously.[16]

Rotations were taken in chloroform. Light petroleum refers to the fraction b.p. 40–60°.

Extraction of wood of Pinus silvestris *L. infested by the fungus* Peridermium pini. The milled, air dried wood (64 kg) was continuously extracted (48 h) with ether (150 litres). Most of the ether was removed by distillation and the extract was allowed to stand at room temperature. A slow crystallization commenced, which appeared to have ceased after one week. The sticky product was filtered off to give solid material which, after washing with ice-cold light petroleum (3 l), consisted of almost colourless crystals (2420 g). The combined mother liquor and washings were slowly poured with vigorous stirring (vibrating stirrer) into light petroleum (75 l). The yellow precipitate formed (75 g, discarded) was collected by filtration and washed with light petroleum. The solvent was removed to give an oil, constituting the *light petroleum soluble products of the wood* (6470 g).

The light petroleum soluble fraction (3500 g) was treated with Girard reagent P (150 g) in refluxing absolute ethanol-glacial acetic acid (9:1, 1400 ml) for 2 h. The hot mixture was poured with stirring into a solution of sodium hydroxide (64 g) in water (2 l) mixed with ice (3 kg) and ether (4 l). The layers were allowed to separate, the aqueous layer was extracted with two portions of ether (500 ml each) which were combined with the organic phase. The carbonyl compounds were regenerated by adding hydrochloric acid

(conc., 200 ml) to the cooled aqueous phase and keeping it at +3° for 60 h and then at room temperature for three days. Extraction with four portions of ether (300 ml each), washing the combined ether solutions first with aqueous sodium hydroxide (10 %, 200 ml), then five times with small portions of water, gave after drying (sodium sulphate) and removal of the solvent *the carbonyl fraction A* (47.5 g).

The organic phase, from which the carbonyl compounds had been removed was washed five times with small portions of water to remove most of the acetic acid and then dried over sodium sulphate. The solvent was evaporated off to give a *viscous oil* (3280 g) free from carbonyl compounds. The oil was refluxed for 1.5 h with methanolic sodium hydroxide (20 %, 3.5 l) containing water (50 ml) and the hot mixture was poured with stirring into a separating funnel containing water (1 l), ice (3 kg) and ether (4 l). The layers were allowed to separate and the aqueous layer was extracted with three portions of ether (500 ml each). The combined ether solutions were washed with three portions of water (200 ml each) and these combined washings were in turn extracted with ether. All ether solutions were finally combined, extracted successively with two portions of aqueous sodium hydroxide (10 %, 200 ml each) and with three small portions of water, dried (sodium sulphate) and taken to dryness to yield a pale yellow oil containing *non-hydrolysable neutral compounds other than carbonyl compounds, fraction B* (417 g).

All aqueous phases obtained after the hydrolysis were combined and, after addition of ice (1 kg), were acidified with dilute sulphuric acid (1:1). Extraction with ether (four portions, 500 ml each), washing the combined ether phases with water until neutral, drying (sodium sulphate) and evaporation of the ether gave *acidic compounds* (2850 g). They were not further investigated.

Table 5. GLC (analytical) retention data for the sesquiterpenes occurring in *Pinus silvestris*.

	Relative retention time (longifolene 1.00)	
	non-polar column [a]	polar column [b]
α-Longipinene	0.75	0.61
Copaene	0.87	0.68
Longicyclene	0.89	0.68
Longifolene	1.00	1.00
β-Ylangene	1.13	1.06
β-Copaene	1.18	1.19
ε-Muurolene	1.30	1.58
γ-Muurolene	1.52	1.84
α-Muurolene	1.75	2.22
δ-Cadinene	1.85	2.61
γ-Cadinene	1.85	2.61
Calamenene		2.62 [c]
α-Calacorene		3.90
δ-Cadinol	3.52	12.7
New sesquiterpene alcohol (m.p. 48°[15])	2.92	11.2

[a] Column 1 % E 301 on Gas-Chrom P.[16] Longifolene had the retention time 6.0 min (temperature 101°, gas flow 53 ml/min). Suitable temperature for the sesquiterpene alcohols 120°.

[b] Column 1 % Reoplex 470 on Chromosorb W (80–100 mesh). Longifolene had the retention time 3.1 min (temperature 88°, gas flow 105 ml/min). Suitable temperature for the sesquiterpene alcohols 130°.

[c] Calamenene could be separated from γ- and δ-cadinene on a "polyethylene alkathene" (Perkin-Elmer), 5 %, on "Cilocel" (firebrick type) column at 110°. Retention times relative to longifolene: γ- and δ-cadinene 1.51, calamenene 2.04, respectively.

The neutral fraction B (417 g) was distilled *in vacuo* to give the following fractions. I, b.p. 53° (20 mm) to 87° (3.5 mm), 268 g; II, b.p. 87—157° (3.5 mm), 10.0 g; III, b.p. 157—200° (3.5 mm), 23.0 g; IV, b.p. 172—190° (0.9 mm), 16.5 g; V, b.p. 190—200° (0.9 mm), 5.0 g; VI, residue 18 g.

Fraction I, which contained monoterpenes, was only investigated by GLC. The same terpenes as Groth [3] has isolated from Swedish sulphate turpentine and which others have isolated from fresh wood of *Pinus silvestris* [3] appeared to be present, see Table 1.

Fraction II. Chromatography of fraction II (8.5 g) on alumina (basic, activity I, 250 g) gave the following fractions. Light petroleum (750 ml) eluted a colourless oil (II *a*, 6.0 g), containing sesquiterpene hydrocarbons. The oil (II *b*, 0.20 g) eluted with ether (10 %) in light petroleum (750 ml) was a complex mixture (GLC), and was not further investigated. Ether (50 %) in light petroleum (750 ml) gave an oil (II *c*, 0.52 g), which on rechromatography on alumina (basic, activity II, 45 g) using ether (10 %) in light petroleum as the eluent gave as the main fraction (0.32 g) *eugenol methyl ether, picrate* m.p. and mixed m.p. 113—115°. Fraction II *d* (0.50 g) was obtained on elution with methanol (1 %) in ether (250 ml), II *e* (0.20 g) with methanol (2 %) in ether (250 ml) and II *f* (1.00 g) with methanol (5 %) in ether (250 ml).

Sesquiterpene hydrocarbons from fraction II. Fraction II *a* (6.0 g) was distilled at 0.1 mm. The oil (2.95 g), b.p. 70—80°, was collected. GLC indicated the presence of all the sesquiterpene hydrocarbons included in Table 2. Chromatography on Ag-silica (90 g) was performed as shown in Table 7.

According to Ag—TLC and GLC (conditions see Table 5) fraction 1_7 (Table 7) consisted of almost pure *longicyclene*.

Fraction 2_7 (Table 7) had $[\alpha]_D$ + 51.2° (*c* 2.3) and the IR spectrum was identical with that of *longifolene* from *Juniperus communis*.

From fraction 3_7 (Table 7), which, according to GLC, was a mixture of α-longipinene and copaene (ratio 1:6) a compound, $[\alpha]_D$ +5.7° (*c* 0.9), the IR spectrum of which was identical with that of *copaene* * was isolated by preparative GLC (conditions, see Table 8)

Table 6. GLC (analytical) retention data for the diterpenes occurring in *Pinus silvestris*.

	Relative retention time (pimaradiene 1.00)	
	non-polar column [a]	polar column [b]
Pimaradiene	1.00	1.00
Isopimaradiene	1.21	1.43
Hydrocarbon Y		0.95
Hydrocarbon Z		0.98
Hydrocarbon X		2.00
Pimarinol	2.24	10.2
Isopimarinol	2.80	13.6
Abietinol	3.70	19.5
Pimarinal	1.64	5.4
Isopimarinal	1.94	7.3
Dehydroabietinal	2.49	10.4
Abietinal	2.79	10.4

[a] Column 1 % E 301 on Gas-Chrom P.[16] Pimaradiene had the retention time 10.8 min (temperature 150°, gas flow 54 ml/min).

[b] Column 1 % Reoplex 470 on Chromosorb W (80—100 mesh). Pimaradiene had the retention time 3.7 min (temperature 144°, gas flow 98 ml/min). Suitable temperature for the diterpene aldehydes and alcohols 175°.

* I thank Professor G. Büchi for a generous gift of copaene.

Table 7. Chromatography of fraction II *a* on Ag-silica. Sesquiterpene hydrocarbons from *Peridermium* infested wood.

Fraction	Solvent [a]	Volume ml	Weight g
1_7	L.P.	150	0.05
2_7	—„—	150	0.82
3_7	—„—	300	0.54
4_7	—„—	375	0.71
5_7	—„—	150	0.12
6_7	—„—	300	0.14
7_7	E. (0.5 %) in L.P.	900	0.09
8_7	E. (1 %) in L.P.	900	0.34
9_7	E. (2 %) in L.P.	225	0.07
10_7	—„—	900	0.13
11_7	E. (5 %) in L.P.	2250	0.25

[a] Ether E., light petroleum L.P.

Table 8. GLC (preparative) retention data for sesquiterpene hydrocarbons occurring in *Pinus silvestris*.

Compound	Relative retention time [a] (longifolene 1.00)
α-Longipinene	0.76
Copaene	0.87
Longicyclene	0.88
Longifolene	1.00
β-Ylangene	1.15
β-Copaene	1.19
ε-Muurolene	1.56
γ-Muurolene	1.70
α-Muurolene	1.99
δ-Cadinene	2.18
γ-Cadinene	2.18

[a] Column 1 % SE 30 on Chromosorb W (60—80 mesh). Longifolene had the retention time 6.3 min (temperature 135°, gas flow 210 ml/min).

Fraction 4_7 (Table 7) appeared to be a mixture of *α-muurolene* [18] and *δ-cadinene* (ratio 9:1, GLC). This was supported by separation of the components by preparative GLC (conditions, Table 12) and comparison of their optical rotations, $[\alpha]_D^{23} -85°$ (*c* 1.5) and $+93°$ (*c* 0.8), respectively, and of their IR spectra (identical) with α-muurolene and δ-cadinene from Swedish sulphate turpentine.[15]

According to GLC fraction 5_7 (Table 7) was a mixture. One of the components (*ca.* 10 %) had the same retention time as *β-ylangene* isolated from sulphate turpentine.[15]

Fraction 6_7 (Table 7), $[\alpha]_D^{22} -12.1°$ (*c* 2.4), had an IR spectrum identical with that of *β*-copaene [17] obtained from sulphate turpentine.[15]

Fraction 11_7 (Table 7) was according to GLC a fairly complex mixture (about six components) containing *ε-muurolene* [18] (*ca.* 10 %). By preparative GLC (conditions see

Table 8) a compound, $[\alpha]_D$ +46° (*c* 0.5) was isolated, the IR spectrum of which was identical with that of *ε*-muurolene from sulphate turpentine.[15]

Fractions 8_7 and 10_7 (Table 7) were, according to GLC, not quite homogeneous samples of *γ-muurolene*[18] and *γ-cadinene*, respectively, fraction 9_7 (Table 7_7) being a mixture (ratio *ca.* 1:1) of the two hydrocarbons. Rechromatography of fraction 8_7 on Ag-silica (50 g) gave a pure compound, $[\alpha]_D$ −2.2° (*c* 3.6), with IR spectrum identical with that of γ-muurolene from sulphate turpentine.[15] In the same way, a compound, $[\alpha]_D$ +153° (*c* 2.3), obviously identical with γ-cadinene (IR spectra identical) was obtained from fraction 10_7.

Sesquiterpene alcohols from fraction II. The IR spectrum of fraction II *d* was almost identical with that of the new sesquiterpene alcohol, m.p. 48°, isolated from sulphate turpentine.[15] Further evidence for the occurrence of this alcohol in fraction II *d* was gained by GLC and TLC comparisons.

According to IR spectrum and GLC fraction II *f* contained *δ*-cadinol and the same main component as a sesquiterpene alcohol fraction from sulphate turpentine (fraction 4_{12}, Table 12[15]). As shown by GLC fraction II *e* was a mixture of the components of II *d* and II *f*.

Fraction III, diterpene hydrocarbons. The hydrocarbon components of fraction III (10 g) were isolated by filtration through a short alumina column (200 g) eluting with light petroleum (500 ml). The colourless oil (5.2 g) obtained was distilled at 1.2 mm and the main fraction (3.0 g, b.p. 140−160°) was chromatographed on Ag-silica (200 g). Fractions were collected as shown in Table 9. Rechromatography of fraction 2_9 on the same adsorbent (25 g), eluting with light petroleum, afforded as the only pure fraction an unidentified *hydrocarbon X* (0.10 g, eluted with 50 ml after 160 ml had been collected), $[\alpha]_D$ −162° (*c* 2.0), IR bands at 1635 and 890 cm^{-1}. (Found: C 87.2; H 12.7. $C_{20}H_{34}$ requires C 87.5; H 12.5). The purity was checked by Ag−TLC and GLC.

Table 9. Chromatography of fraction III (passed through alumina and distilled) on Ag-silica. Diterpene hydrocarbons.

Fraction	Solvent	Volume ml	Weight g
1_9	L.P.	1800	0.40
2_9	−,,−	1400	0.42
3_9	−,,−	2800	0.89
4_9	E. (1 %) in L.P.	2600	0.47
5_9	E. (2 %) in L.P.	600	0.18
6_9	−,,−	1000	0.22
7_9	E. (5 %) in L.P.	1600	0.19
8_9	−,,−	1500	0.08

The main component of fraction 3_9 (Table 9) was isolated by chromatography on Ag-silica (50 g) using light petroleum as the eluent. The distilled compound (0.32 g) crystallized when left in the refrigerator overnight. The crystals had m.p. 24.5−27°, $[\alpha]_D^{22}$ +100° (*c* 2.4) and were shown to consist of *pimaradiene*.[19] For comparison pimaradiene was prepared by Wolff-Kishner reduction of primarinal. GLC of the crude product indicated that it contained *ca.* 80 % pimaradiene. The hydrocarbon was purified by argentative column chromatography.

Fraction 4_9 (Table 9) gave on rechromatography on Ag-silica (30 g, eluent 0.5 % ether in light petroleum) an oil (0.15 g, eluted with 250 ml after 500 ml had been collected). This was identified as *isopimaradiene*,[20] $[\alpha]_D^{22}$ −31.3° (*c* 1.8), by direct comparison with a sample obtained by Wolff-Kishner reduction of isopimarinal.

Rechromatography of fraction 6_9 (Table 9) on Ag-silica (10 g) using ether (1 %) in light petroleum as the eluent afforded as the main fraction an unidentified *hydrocarbon Y* (0.12 g), $[\alpha]_D^{20}$ +53° (*c* 1.0), IR bands at 3080, 1647, 1421, 1000, and 910 cm^{-1}. It was a pure compound according to GLC and Ag−TLC. (Found: C 88.3; H 11.5. $C_{20}H_{32}$

requires C 88.2; H 11.8). Although the IR spectra of compound Y and sandaracopimaradiene were similar, the compounds were shown not to be identical by direct comparison (GLC).

Rechromatography of fraction 8_9 (Table 9) gave an unidentified *hydrocarbon Z* (0.03 g), $[\alpha]_D^{20}$ +88.5° (*c* 1.1), IR bands at 3120, 1650, 1410, 997, 912, and 890 cm^{-1}. According to GLC and Ag—TLC it was a pure compound although it did not give a satisfactory analysis. (Found: C 87.0; H 11.3. $C_{20}H_{32}$ requires C 88.2; H 11.8).

Fraction IV (7.7 g) was chromatographed on alumina (neutral, activity I, 250 g). Benzene (1500 ml) eluted an oil (IV *a*, 2.05 g). Rechromatography of IV *a* on alumina (100 g) furnished as the main component (eluted with light petroleum-benzene 1:1) *pinosylvin dimethyl ether*,[21] which, after recrystallisation from light petroleum, had m.p. 54—55°. Ether (750 ml) eluted an oil (IV *b*, 0.57 g), which was not investigated. Methanol (2 %) in ether (750 ml) eluted a viscous oil (IV *c*, 4.67 g).

Diterpene alcohols from fraction IV. Fraction IV *c* (4.67 g) was chromatographed on Ag-silica (200 g, Table 10).

Table 10. Chromatography of fraction IV *c* on Ag-silica. Diterpene alcohols.

Fraction	Solvent	Volume ml	Weight g
1_{10}	E. (5 %) in L.P.	4000	0.86
2_{10}	E. (10 %) in L.P.	600	0.93
3_{10}	—„—	300	0.67
4_{10}	—„—	300	0.18
5_{10}	E. (20 %) in L.P.	800	0.45
6_{10}	—„—	1200	0.39

Fraction 2_{10} consisted of almost pure *pimarinol*,[22] m.p. 87.5—89.5° (not previously obtained crystalline), $[\alpha]_D^{22}$ +94° (*c* 2.5) after sublimation *in vacuo*. For comparison, pimarinol was prepared by sodium borohydride reduction of pimarinal. The crude product crystallized on storage in the refrigerator and was purified by vacuum sublimation.

Rechromatography of fraction 4_{10} (Table 10) on Ag-silica (25 g, elution with ether, 2 %, in light petroleum) gave *abietinol* [23] (0.034 g) as the main fraction. After purification by chromatography on alumina (eluent 1 % methanol in ether) followed by sublimation *in vacuo* the compound had m.p. 85.5—87°, $[\alpha]_D$ −130° (*c* 2.0), λ_{max} 234 (ε 17 900) and 241 mμ (ε 19 000). Lithium aluminum hydride reduction [23] of abietic acid afforded an authentic sample of abietinol.

Fraction 6_{10} (Table 10) was rechromatographed on Ag-silica (25 g). Ether (20 %) in light petroleum (1200 ml) eluted a mixture (0.25 g). The next portion (1200 ml, the same solvent) eluted a viscous oil (0.15 g), which crystallized on seeding with isopimarinol obtained by sodium borohydride reduction of isopimarinal. Sublimation *in vacuo* gave pure *isopimarinol*,[24] m.p. 85—86°, $[\alpha]_D$ −24.6° (*c* 1.8).

Fraction V (5.0 g) was chromatographed on alumina (basic, activity II, 200 g). Benzene (600 ml) eluted an oil (0.66 g). After elution with methanol (1 %) in ether (600 ml) the next portion of the same solvent (200 ml) eluted a crystalline material (0.53 g), which after several crystallizations from light petroleum (b.p. 60—70°) had m.p. 69—70° probably being a mixture (GLC) of *fatty alcohols*, IR bands at 3280, 1060, 730, and 720 cm^{-1}. After further elution with the same solvent (100 ml) the next portion (300 ml) of the same solvent eluted a crystalline material, which on recrystallization from ether-light petroleum gave almost pure *β-sitosterol*, m.p. 137—138°.

β-Sitosterol was also obtained when the distillation residue fraction VI was treated with methanol and the precipitate formed was recrystallized from ether-light petroleum.

Fraction A, diterpene aldehydes. Fraction *A* (12 g) containing carbonyl compounds was distilled *in vacuo*. The main fraction (10.5 g) had b.p. 165—185° at 0.9 mm and

Table 11. Chromatography of fraction *A* (distilled and chromatographed on alumina) on Ag-silica. Diterpene aldehydes.

Fraction	Solvent	Volume ml	Weight g
1_{11}	L.P.	3500	0.61
2_{11}		500	0.34
3_{11}	linearly	500	0.40
4_{11}		250	0.21
5_{11}	changed to	1500	2.85
6_{11}		1500	1.16
7_{11}	E. (4 %) in L.P.	2200	1.78

showed strong IR absorption (carbon tetrachloride) due to aldehyde functions at 2680 and 1720 mc^{-1}. It was chromatographed on alumina (neutral, activity I, 250 g), the main fraction (10.0 g) was eluted with ether (2 %) in benzene. Chromatography of this fraction on Ag-silica (300 g) was performed as shown in Table 11.

Rechromatography of fraction 2_{11} (0.34 g) on the same adsorbent (30 g, eluent 1 % ether in light petroleum) followed by sublimation of the main fraction *in vacuo* gave pure *dehydroabietinal*,[26] m.p. 53–55°, $[\alpha]_D^{22}$ +52°, IR bands at 2720, 1728, 1509, and 825 cm^{-1}, λ_{max} 268 mμ (ε 628) and 276 mμ (ε 725). For comparison dehydroabietinal was also prepared from dehydroabietic acid by Rosenmund reaction.[25] The reaction product was sublimed at 0.1 mm to give pure dehydroabietinal.

Rechromatography of fraction 3_{11} (Table 11) on Ag-silica afforded as the main fraction an oil (0.30 g), which crystallized after distillation at 0.1 mm. Traces of oily material were removed by suction on a sintered glass filter. The *abietinal* [26,27] thus obtained (not previously obtained crystalline) had m.p. 45–48°, $[\alpha]_D^{22}$ −77°, IR bands at 2730 (infl.) and 1720 cm^{-1}, λ_{max} 241 mμ (ε 14 000). It was identified by conversion to abietinol by sodium borohydride reduction. The crude product obtained was chromatographed on alumina. By sublimation *in vacuo* pure abietinol, m.p. 85–87°, was obtained from the main fraction.

Fraction 5_{11} (Table 11) consisted of almost pure *pimarinal* [11] which after recrystallization from acetone-water had m.p. 52–54°, $[\alpha]_D^{22}$ +99° (*c* 3.0); *2,4-dinitrophenylhydrazone*, m.p. 194–195°.[11]

Fraction 7_{11} (Table 11) was purified by distillation at 0.1 mm followed by chromatography on alumina (neutral, activity I, 40 g). Light petroleum (200 ml) eluted a viscous oil (1.51 g), which crystallized on standing overnight in the refrigerator. The crystals were collected and recrystallized from ethyl acetate to give a compound, m.p. 35–37°, identical with *isopimarinal*,[11] $[\alpha]_D^{22}$ −15.0° (*c* 2.0) (not previously obtained crystalline). IR bands at 3090, 2710, 1725, 1643, 995, and 905 cm^{-1}; *2,4-dinitrophenylhydrazone*, m.p. 182–183°.[11] For identification, the aldehyde (20 mg) in acetone (0.5 ml) was treated at 0° with a solution of chromium trioxide (6 mg) in dilute sulphuric acid (7 %, 0.2 ml) with stirring, the mixture was allowed to reach room temperature, stirred for one more hour, diluted with water (5 ml) and extracted with ether. The organic phase was then shaken with four portions of aqueous sodium hydroxide (10 %, 1 ml each), the alkaline solution was acidified, extracted with ether, the ether solution was dried and taken to dryness. The residue (15 mg) crystallized when treated with aqueous methanol, yielding *isopimaric acid*.[28]

Extraction of fresh wood of Pinus silvestris. The neutral fraction of the light petroleum soluble part of the ether extract was obtained essentially in the same way as from the infested wood. The light petroleum soluble part of the ether extract of milled wood (60 kg) was hydrolyzed and the neutral fraction (85 g) was isolated. From this, carbonyl compounds were removed by treatment with Girard reagent P and the residue was distilled.

A fraction (5 g, b.p. 110°/10 mm to 160°/3.5 mm) corresponding approximately to fractions II + III from infested wood was chromatographed on alumina (eluent light

petroleum) to give a mixture of hydrocarbons. The presence of sesquiterpene hydrocarbons in this mixture, as indicated by GLC, is apparent from Table 2. Furthermore, GLC and Ag–TLC indicated the presence of pimaradiene and isopimaradiene (Table 3). Almost pure *α-muurolene*, $[\alpha]_D$ −76° and *pimaradiene*, $[\alpha]_D$ +88° were isolated by chromatography on Ag-silica (eluent 1 % ether in light petroleum).

By GLC and Ag–TLC analysis pimarinol, isopimarinol, and abietinol were detected in the fraction (8.8 g, b.p. 160–210°/3.5 mm) roughly corresponding to fraction IV from infested wood.

The fraction (3.5 g, b.p. 210–220°/3.5 mm), roughly corresponding to fraction V (infested wood), was chromatographed on alumina. A *mixture of fatty alcohols* approximately of the same composition as that from fraction V (GLC) was obtained as well as *β-sitosterol*. After recrystallization from ethanol the latter had m.p. 137–138°, $[\alpha]_D$ −25.6° and IR bands at 3380 and 1055 cm^{-1}.

The acidified aqueous phase containing carbonyl compounds was extracted with ether to give an oil (9.0 g). GLC and Ag–TLC indicated the presence of pimarinal and isopimarinal. Dehydroabietinal and abietinal were also present as indicated by GLC (Table 3). By alumina chromatography (neutral, activity I, eluent benzene-light petroleum 1:1) *pimarinal*, m.p. 52–53°, was obtained as the main fraction.

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