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CONSTITUENTS OF THE ESSENTIAL OIL OF *ARAUCARIA ARAUCANA*

L. H. BRIGGS* and G. W. WHITE
Department of Chemistry, University of Auckland, Auckland, New Zealand

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Abstract—The essential oil of *Araucaria araucana* has been shown to contain geraniolene, limonene, (+)-γ-cadinene, (−)-α-cadinol, (+)-hibaene, (−)-trachylobane, (−)-kaurene, (−)-atisirene and isokaurene/isoatisirene. This is the first record of the occurrence of geraniolene in Nature and the second for trachylobane and atisirene. A simple scheme depicting the biosynthesis of the tetra- and pentacyclic diterpenes is outlined.

Araucaria araucana (Mol.) K. Koch (syn. *A. imbricata*), a native of Chile, is grown around the world for ornamental purposes and is popularly known as "monkey puzzle" or Chile pine. No less than eleven botanical names have been given to this species. The only previous investigations have been on solvent extracts of the bark, which yielded several new oxygenated diterpenes with the labdane skeleton.[1]

Analysis of the Oil

Analytical GLC showed the presence of eight major constituents (referred to, for convenience, as Peaks 1–8). Crude distillation of the oil at reduced pressure yielded three fractions. The low boiling fraction was small and was shown by GLC to consist almost entirely of Peaks 1 and 2 which were then isolated by preparative GLC.

Peak 1 (1%†). Assignment of a structure with the normal molecular formula, $C_{10}H_{16}$, from the IR and NMR spectral data proved impossible. The mass spectrum, however, showed that it had the formula, C_9H_{16}, which immediately led to its identification as geraniolene. This was confirmed by comparison with a specimen synthesised by decarbonylation of citral.[2] This is the first record of its occurrence in Nature.

Peak 2 (9%). This was identified as limonene from its spectral data.

The second fraction from the distillation was also small. GLC showed that it contained a large number of sesquiterpene hydrocarbons but only one (Peak 3) could be isolated by preparative GLC in sufficient purity for identification.

Peak 3 (1%). This was identified as (+)-γ-cadinene from its spectral data and optical rotation.

Fraction 3, and the viscous residue from the distillation comprised about 80% of the total oil. They both exhibited the same five peaks on GLC but in slightly different proportions. Peaks 4–8 were isolated by preparative GLC.

Peak 4 (22%). A preliminary experiment showed that this compound was probably oxygenated as it was retained on chromatography of fraction 3 through deactivated alumina and elution with n-hexane. Further elution with n-hexane–chloroform (1:1) and chloroform, however, produced a semicrystalline solid which produced long, hair-like crystals, mp 72–4°, on evaporation of an acetone solution. The mass spectrum indicated a formula, $C_{15}H_{26}O$. The spectral data and optical rotation were in good agreement with those of (−)-α-cadinol[4] and this was confirmed by preparation of its *p*-nitrobenzoate.[4c,17]

Peak 5 (41%). The initial n-hexane fractions from the column chromatography of fraction 3 produced Peak 5, which was purified by preparative GLC. Its IR and NMR data, its positive rotation and comparison with an authentic specimen showed that it was (+)-hibaene **3**.

Peak 6 (3%). Because of the large proportion of hibaene present it was not possible to obtain a pure sample of Peak 6 by direct preparative GLC. Further investigation showed that most of the hibaene could be retained on dry column chromatography with silver nitrate-alumina. After α-cadinol had been removed from fraction 3 as above, a sample of the remaining diterpene fraction was chromatographed on silver nitrate-alumina and eluted with benzene–n-hexane (1:4). The initial fractions were rich in Peak 6 from which a sample was obtained in sufficient purity for identification. The IR and NMR spectra indicated that the compound was either saturated or contained a tetrasubstituted double bond. The only known saturated diterpene is trachylobane and comparison of the NMR spectrum of Peak 6 with that of authentic material, coupled with its negative rotation, showed that it was (−) trachylobane **8**.

Trachylobane has only recently been isolated for the first time from the essential oil of *Sideritis canariensis*.[5] However, it has been shown to be formed when mevalonic acid is fed to cell free extracts of castor bean seedlings.[6] The only other naturally occurring compounds with the trachylobane skeleton are oxygenated compounds from the bark of *Trachylobium verricosum*[7] and *Sideritis canariensis*.[8]

Peak 7 (1%). This constituent was inseparable from authentic isokaurene **6** when co-chromatographed. Since Peak 8 consists of (−)-kaurene **5** and (−)-atisirene **11** (see below) and Appleton and McCormick have shown[9] that it is not possible to separate isokaurene **6** and isoatisirene **12** derived from them, Peak 7 is considered to be this inseparable mixture of isokaurene **6** and isoatisirene **12**.

Peak 8 (8%). Preparative GLC of the diterpene fraction gave Peak 8, which, however, was shown by silver nitrate-silica gel TLC to be a mixture of ca. 50% of each of two compounds. Repeated preparative TLC on silver nitrate-alumina produced a small amount of each constituent. The two compounds were shown to be (−)-kaurene **5** and (−)-atisirene **11** by comparison of their NMR spectra with those of authentic samples and by specific rotation measurements.

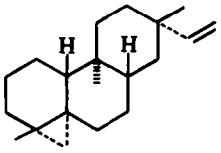
*Deceased 16/1/75.

†Approximate percentage of the total oil.

Atisirene has only been isolated previously from the essential oil of *Erythroxylon monogynum.*[10]

Biosynthesis of Tetra- and Pentacyclic Diterpenes

The essential oil of *A. araucana* is the first from which all of the basic types of the tetracyclic diterpenes and the one pentacyclic diterpene have been isolated from one species. The tetracyclic (+)-devadrene **14**[10] is regarded as atypical.



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This has prompted a reconsideration of the traditional methods of depicting the transformations involved in their biosynthesis.

(+) and (−)-Copalyl pyrophosphate **1** are regarded as intermediates in the biosynthesis of the tetra- and pentacyclic diterpenes. Further ring closure results in the formation of the commonly occurring tricyclic deterpenes, (+)-pimaradiene and (−)-isopimaradiene, their less common enantiomers, (−)-pimaradiene and (+)-isopimaradiene, as well as their respective double bond isomers.

In further cyclisation of the pimaradienes to the tetracyclic diterpenes and the rare pentacyclic diterpene, trachylobane **8**, there is no agreement of the structure of the transition ion which ranges from the face-protonated cyclopropyl non-classical carbonium ion of Wenkert,[11] an edge-protonated cyclopropyl species[12] and two different bridged ions.[13] None of these intermediates adequately explain the formation of all the isomers nor the preponderance of one or more isomers over the others.

The following scheme conveniently explains the relative abundance of the various structural types found in nature and produced in synthetic studies.[14] All the rearrangements occur via normal carbonium ions with the exception of the edge-protonated cyclopropyl species used to depict the formation of (−)-trachylobane **8**. There is strong evidence that the latter species is an intermediate transition state in transformations of the nortricyclene **15** series.[15]

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Loss of a proton from the original ion **2** to give preferentially (+)-hibaene **3** or Wagner–Meerwein transformation followed by proton loss to give (−)-kaurene **5**/(−)-isokaurene **6** are much more favoured pathways than the formation of the protonated cyclopropyl ion **7** or **9** by a 1,3-hydride shift. Thus the rare occurrence of (−)-trachylobane **8** and (−)-atisirene **11**/(−)-isoatisirene **12** and the non-isolation of the unknown diterpene **13** can be predicted by this scheme.

EXPERIMENTAL

M.Ps were determined on a Reichert "Kofler" block and are uncorrected. IR spectra were recorded on a Perkin-Elmer 237 spectrophotometer. NMR spectra were determined in carbon tetrachloride using TMS as the internal standard on a Varian A60 or T60 spectrometer. Optical rotations were measured on a JASCO ORD/UV5 instrument. Mass spectra were measured by Professor R. Hodges, Massey University, on an AE1 MS9 mass spectrometer. TLC was carried out on Silica Gel DG (Riedel de-Haen) and visualised with iodine vapour or by spraying with a saturated ceric sulphate solution in 70% sulphuric acid. Preparative TLC was carried out on Kieselgel $PF_{254+366}$ (E. Merck) layers, 1 mm in thickness, and the bands viewed in UV light. Analytical and preparative TLC was carried out as above with the adsorbent impregnated with 3% and 10% (w/w) silver nitrate unless otherwise stated. For column chromatography Spence Type H alumina was used, deactivated with 5% by volume of 10% acetic acid. For dry column chromatography the alumina was deactivated with 6% by volume of water. For silver nitrate dry column chromatography 3% by weight of silver nitrate was dissolved in the water used for deactivation.

Analytical GLC was carried out with a Varian Aerograph series 1800 instrument using a 1/8 in aluminium column and nitrogen as carrier gas. For preparative GLC an Aerograph A-700 'Autoprep' instrument was used with a 3/8 in aluminium column and helium as carrier gas.

The branches of *Araucaria araucana* were collected from a mature tree in January 1939, at Hastings and steam distilled within a week to yield a pale yellow oil, 181 g (0·06%). This was dried and completely sealed in a brown glass bottle until studied.

GLC analysis of the oil

Relative retention times and approximate percentage composition of the eight major peaks (Table 1) were recorded under the following conditions: column, Carbowax 20 M (1/8 in × 8 ft, 5% in 100/120 Celite); flow rate, 15 ml/min.

The oil was distilled under reduced pressure without a fractionating column, the results being recorded in Table 2.

Preparative GLC of these fractions was carried out under the following conditions: column, Carbowax 20 M (3/8 in × 10 ft, 20% on 100/120 Celite); flow rate, 200 ml/min.

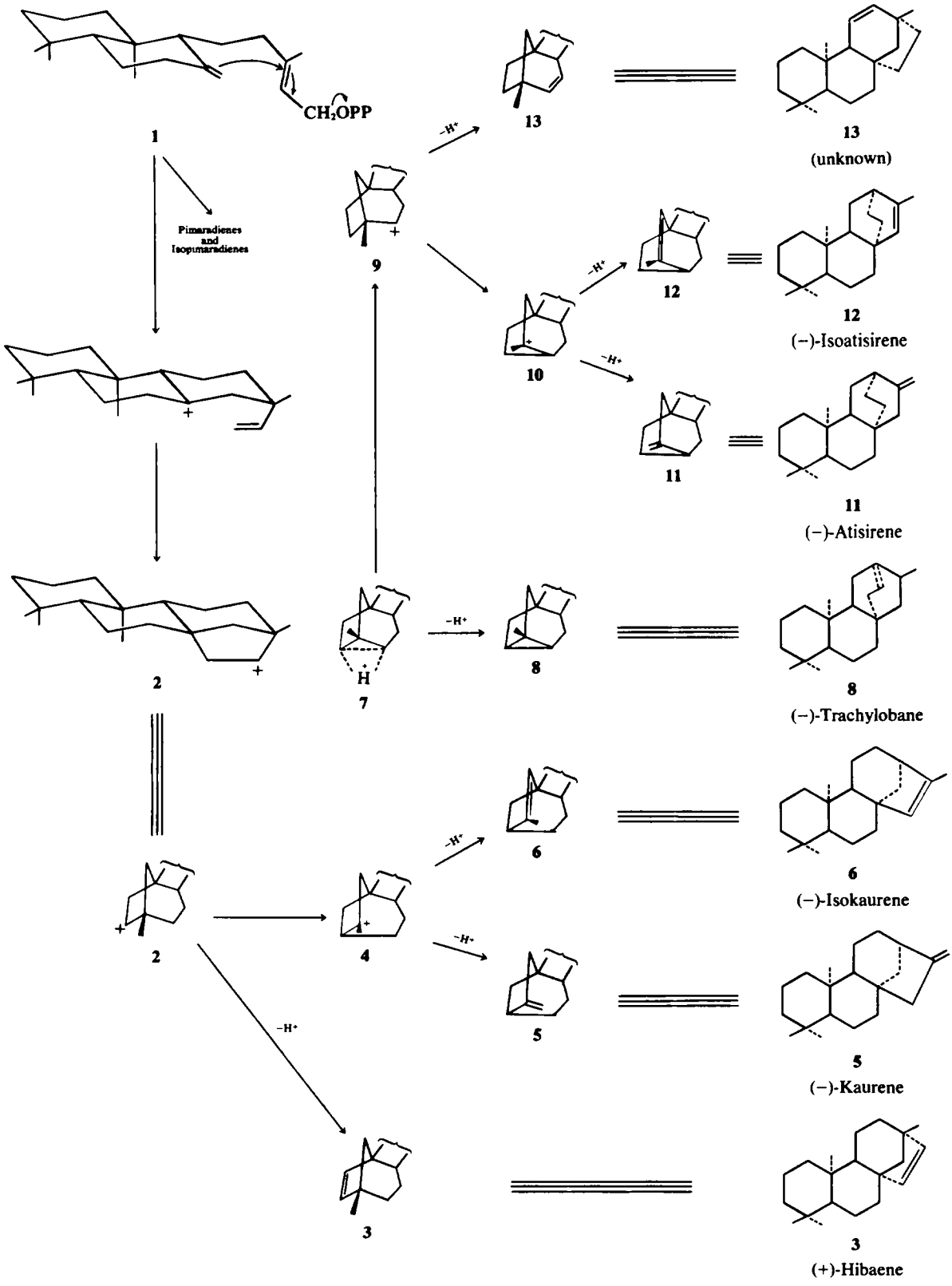
From fraction 1 two compounds were obtained corresponding to Peaks 1 and 2.

Peak 1, geraniolene, IR (CS_2) 3078, 2970, 2960, 2852, 2723, 1775, 1670, 1650, 1374, 1273, 1230, 1208, 1105, 1030, 980, 885, 815, 745 cm^{-1}; NMR δ 1·61 (3H, s, C=CMe), 1·71 (6H, s, $C{=}CMe_2$),

Table 1

Peak No.	Column temperature (°C)	Relative retention time*	%
1	80	0·35	1
2	80	1·00	9
3	160	0·21	1
4	160	0·92	22
5	160	1·00	41
6	160	1·16	3
7	160	1·25	1
8	160	1·70	8

*At 80°, relative to limonene and at 160°, relative to hibaene.


CH₂OPP
1
Pimaradienes and Isopimaradienes
2
9
13
13
(unknown)
12
12
(−)-Isoatisirene
10
11
11
(−)-Atisirene
7
8
8
(−)-Trachylobane
6
6
(−)-Isokaurene
4
5
5
(−)-Kaurene
3
3
(+)-Hibaene
-H⁺

Scheme 1.

Table 2

Fraction	B.p. °C	Pressure (mm)	Wt. (g)
1*	—	—	1·4
2	60–100	0·5	3·4
3	100–115	0·5	15·7
Residue	—	—	16·6

*Cold trap.

4·63 (2H, s, C=CH_2), and 5·02 br (1H, m, CH_2CH=C) ppm; M (mass spectrum) 124.

Synthesis of geraniolene. Citral (2·05 g) was refluxed with 10% Pd/C (30 mg) under CO_2 atmosphere for 6 h to produce geraniolene in 60% yield (GLC). Preparative GLC yielded pure geraniolene, the IR and NMR spectra of which were identical with those of Peak 1.

Peak 2, limonene, $[\alpha]_D$ + 40° (*c* 1·1 in CCl_4); IR (CCl_4) 3080, 1645, 890 (C=CH_2); IR (CS_2) 798 (C–CH=C) cm^{-1}; NMR: δ 1·65 and 1·73 (6H, 2s, C=CMe_2), 4·79 (2H, s, C=CH_2), and 5·39 (1H, m, CH_2CH=C) ppm.

From fraction 2 only one compound was obtained by preparative GLC corresponding to Peak 3.

Peak 3, γ-cadinene, $[\alpha]_D$ + 108° (c 2·3 in CCl_4): IR (CCl_4) 3085, 1645, 885 (C=CH_2), 1383 and 1373 ($CHMe_2$), 1665 and 840 (C–CH=C) cm^{-1}; NMR δ 0·74 and 0·93 (6H, d, *J* 7Hz, $CHMe_2$), 1·65 (3H, s, C=CMe), 4·50 (2H, d, *J* 6Hz, C=CH_2), and 5·46 (1H, m, CH_2–CH=C) ppm.

Fraction 3 (3 g) was chromatographed on deactivated alumina (150 ml). Elution with n-hexane produced a diterpene fraction (rich in Peaks 5 to 8). Elution with n-hexane-chloroform (1:1) and with chloroform yielded a semi-crystalline solid (1·19 g). GLC showed that this was Peak 4.

Peak 4, α-cadinol, m.p. 72–74°, from evaporation of an acetone solution (lit.[4c] m.p. 74–74·5°), $[\alpha]_D$ – 43° (c in $CHCl_3$), IR (CCl_4) 3605, 3380, and 1120 (OH), 1385, 1378, and 1370 ($CHMe_2$), 1665 and (CS_2) 810 (C–CH=C) cm^{-1}; NMR δ 0·75 and 0·91 (6H, 2d, *J* 7Hz, $CHMe_2$), 1·01 (3H, s, C(OH)Me), 1·62 (3H, s, C=CMe), and 5·38 br (1H, s, CH_2–CH=C) ppm; M (mass spectrum) 222; p-nitrobenzoate, m.p. 138–138·5° (lit.[4c] m.p. 137–138°; lit.[16] m.p. 139°) (Found: C, 71·4; H, 7·9; N, 3·9. $C_{22}H_{29}NO_4$ requires C, 71·7; H, 7·9; N, 3·8%).

Preparative GLC of the initial n-hexane fractions from the column chromatography of fraction 3 yielded Peak 5.

Peak 5, hibaene, $[\alpha]_D$ + 12° (c 1·0 in $CHCl_3$); IR (NaCl) 1388 and 1365 (CMe_2) and 750 (C–CH=CH–C) cm^{-1}; NMR δ 0·75, 0·81, 0·85 and 0·99 (12H, 4s, CMe), 5·36 and 5·62 (2H, AB q J 5·5Hz, C–CH=CH–C) ppm.

Dry column chromatography of the diterpene fraction on silver nitrate-alumina and elution with benzene-n-hexane (1:4) yielded initial fractions rich in Peak 6, which was purified by preparative GLC.

Peak 6, trachylobane, $[\alpha]$ – 5·5° (c in $CHCl_3$); IR (CCl_4) 3020, 2995, 2925, 2860, 1480, 1460, 1440, 1385, 1367, 1290, 1215, 1195, 1160, 1117, 1040, 1010, 965 and 845 cm^{-1}; NMR: δ 0·78, 0·82, 0·93 and 1·10 (12H, 4s, CMe) ppm (in agreement with that of an authentic specimen provided by Professor G. Ourisson, University of Strasbourg).

Simultaneous injection of an authentic specimen showed that isokaurene was inseparable from Peak 7 by GLC on both Carbowax 20M and SE-30 columns.

Preparative GLC of the diterpene fraction yielded Peak 8. Analytical TLC on silver nitrate-silica gel with benzene-n-hexane (1:4) showed that this was a mixture of approximately equal amounts of two constituents (R_F 0·35 and 0·40). Preparative GLC of the diterpene fraction under comparable conditions yielded a sample (110 mg) of the mixture and subsequent preparative TLC [benzene-n-hexane (9:1), 55 mg per plate, each plate run three times] of this mixture yielded pure samples of each constituent, (–)-kaurene and (–)-atisirene.

(–)-Kaurene, $[\alpha]_D$ – 30·6° (c 3·0 in $CHCl_3$); IR (CCl_4) 3070, 1655 and 872 (C=CH_2), 1385 and 1365 (CMe_2) cm^{-1}; NMR δ 0·80, 0·85 and 1·01 (9H, 3s, CMe), and 4·65 br (2H, s, C=CH_2) ppm, in agreement with the spectrum of an authentic specimen.

(–)-Atisirene, $[\alpha]_D$ – 43° (c 3·5 in $CHCl_3$), IR: (CCl_4) 3081, 1650 and 875 (C=CH_2), 1386 and 1368 (CMe_2) cm^{-1}; NMR: δ 0·82, 0·85 and 0·95 (9H, 3s, 3 × CMe), 4·40 (1H, d, *J* 2Hz, C=CH_2), and 4·60 br (1H, s, C=CH_2) ppm (IR and NMR values in agreement with authentic spectra provided by Dr. S. Dev, National Chemical Laboratory, Poona, India).

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