



Enantiomeric composition of monoterpenes in conifer resins[†]

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Abstract: Resins from *Agathis* and *Araucaria* genera were collected to study the concentration as well as the enantiomeric compositions of selected monoterpenes, i.e. α -pinene, β -pinene, camphene and limonene. The compositions of the compounds were found to be species-specific and little variation was observed with season. The enantiomeric ratios for some compounds, i.e., α -pinene, vary tremendously between trees of different age but of the same species. The possible significance of this information to biological studies of conifers and to the study of amber are discussed. © 1997 Elsevier Science Ltd. All rights reserved.

Introduction

Resin is defined as a plant secretion that hardens on exposure to air.¹ It is a complex mixture composed primarily of terpenoids and/or phenolic compounds from living higher plants, particularly conifers. Primary (constitutive) resin is formed continuously and accumulates in the specialized secretory structures of these trees. Secondary (induced) resin is formed upon wounding or fungal infection. The main function of secondary resin in trees is for defense against herbivores and various pathogens (e.g., fungi). It is thought that secondary resin was the source for most ambers.²

Secondary resin in conifers is composed of non-volatile terpenoids and volatile monoterpenes such as α -pinene, β -pinene, camphene and limonene. The monoterpenes (particularly pinenes) give the resin some fluidity. They can constitute nearly 50% of the resin's mass. The hardening process for exposed resin involves vaporization of the monoterpenes, as well as time and environment dependent reactions of the terpenes.² The collection and processing of resins for commercially useful terpene products is a viable industry in some countries like New Zealand.³ Also the enzymes that produce terpenes can be isolated and used in the manufacture of industrially useful compounds.⁴ The smaller terpene compounds are used as solvents, and as reagents in organic synthesis. They are also used in the production of pharmaceuticals, insecticides and perfumes. The monoterpene compounds in resin are chiral compounds. Some of their commercial uses are dependent on their chirality. For example, reagents for asymmetric synthesis are made from pure or nearly pure enantiomers of α -pinene.^{5,6} The enantiomers of limonene have different fragrances and are used, enantioselectively, in different consumer products.⁷ Consequently it is not only important to know the relative and absolute concentration of the monoterpenes in resin, but their enantiomeric composition as well. In addition, it was demonstrated recently that the monoterpene compounds camphene, borneol and isoborneol in fossilized resin (i.e., amber) can have distinct enantiomeric ratios.⁸ The enantiomeric excess (ee) of some of these compounds was retained even after tens of millions of years. These ees can be used to distinguish among or identify pieces of amber. Furthermore, a greater understanding

[†] Dedicated to Professor Herbert C. Brown on the occasion of his 85th birthday.

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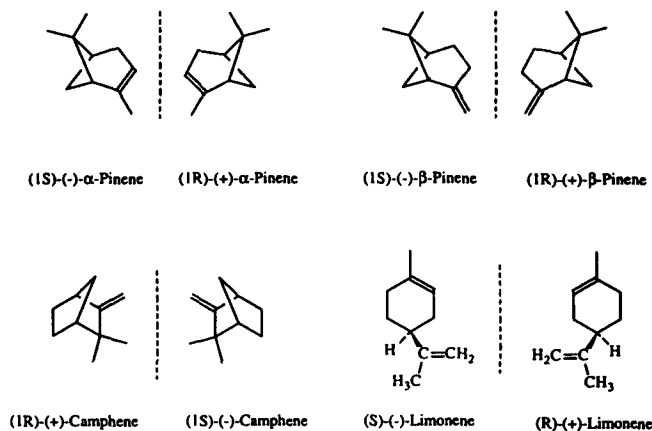


Figure 1. Structures and absolute configurations for the monoterpene compounds evaluated in this study.

of these compounds and their stereochemistry could be of value in future paleobotanical and paleoenvironmental studies involving amber. However, a better understanding of the monoterpenes in the resin of 'amber-producing trees' is needed. It is known that only a fraction of the trees can produce resin that forms amber. Many of these species are extinct. Some living trees of the *Agathis* and *Araucaria* genera are thought to produce resin of a type that can form amber. These trees are often located in the southern hemisphere.² Some work has been done on determining the composition of monoterpenes in barks,^{9,10} wood,⁹ buds,⁹ needles⁹ and leaves^{11,12} of gymnosperm species such as *Picea abies*⁹⁻¹¹ and *Quercus ilex*.¹² However, only a few reports concerning the enantiomeric composition of monoterpenes in conifers have been published.^{8,10,11} To our knowledge there have been no studies on the composition and stereochemistry of monoterpenes in the resins of *Agathis* and *Araucaria*.

Materials and methods

Resin samples were taken from the trunks of 11 trees of different species and different ages at two localities. The first was a forest plantation (Botanical Gardens) close to Darlinghurst, NSW, Australia. The samples were collected five separate times from the same tree over the course of one year. The second group was made up of four 28-year-old white pine (*Pinus strobus*) trees located in Rolla, MO, USA.

All resin samples were stored in a freezer (-10°C) in sealed vials before use. For analysis, 0.1 g resin was transferred to a vial, and then 1 ml of diethyl ether was added to it. An internal standard (d_{12} -tetralin) was then added. After stirring with a spatula, the sample was put into an ultrasonic bath at room temperature for 20 minutes. It was then cleaned by passing through a solid phase extraction cartridge with Extract-clean[®] silica packing. A clear solution was obtained after this step and was then injected into the GC directly or after evaporating with nitrogen.

A QP-5000 GC-MS system (Shimadzu, Columbia, MA) was used in this study. The GC column (30 m \times 0.25 mm) containing a chiral stationary phase B-DM (di-*O*-methyl- β -cyclodextrin) was obtained from Advanced Separation Technologies Inc., Whippany, NJ. The enantio-enriched standards of α -pinene, β -pinene, camphene and limonene and d_{12} -tetralin were purchased from Aldrich (Milwaukee, WI). The diethyl ether was from Fisher (St Louis, MO) and the solid phase extraction cartridges were purchased from Alltech (Deerfield, IL). The structures of four monoterpene standards are shown in Figure 1.

The temperature of the injector of the GC and the mass transfer line to the MS was kept at 250°C . The temperature program for the GC was to hold isothermal at 60°C for 5 minutes, then increase to 140°C at $4^{\circ}\text{C}/\text{min}$, and hold at 140°C for 5 minutes. Helium (99.9999% grade) was used as the carrier gas. Both SCAN mode and SIM (selected ion monitoring) mode were used for the MS detector.

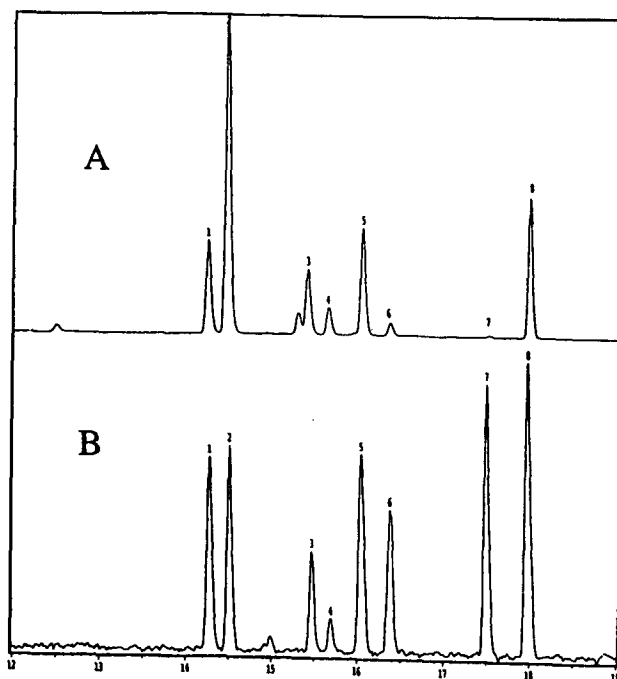


Figure 2. Enantioselective gas chromatograms showing: (A) the resolution of monoterpenes in the resin of 40-year-old *Agathis robustus*, SIM mode with M/S 93 was used in MS detector; (B) the separation and resolution of four monoterpene standards, SCAN mode was used in MS detector. The analysis was performed on a 30 m×0.25 mm ChiralDEX B-DM column. 1 (-)- α -pinene; 2 (+)- α -pinene; 3 (-)-camphene; 4 (+)-camphene; 5 (+)- β -pinene; 6 (-)- β -pinene; 7 (-)-limonene; 8 (+)-limonene.

The SCAN mode was used for the identification and quantitation and SIM mode can increase the sensitivity in cases of samples having very low levels of target compounds. The fragment ion with M/S 93 was used for detection in SIM mode. Figure 2 shows the separation of these standards and the monoterpenes in an authentic resin sample on the Astec (Whippany, NJ) B-DM column. All the compounds and their enantiomers were baseline resolved from one another.

Results and discussion

Table 1 gives the concentrations and enantiomeric ratios of four monoterpenes found in conifer resins. It is quite obvious that the absolute concentrations of the four compounds vary dramatically with the season. Though it may be true that there is an optimal season for the trees to produce resin, it is hard to draw any conclusion from the present data. It has been reported that emission of terpenes from conifers varies considerably with environmental factors,¹² season,¹⁰ and even with different times during the day.¹³ In order to study the variation in concentration with the season, all these parameters should be considered. Moreover, these monoterpenes are sufficiently volatile that the absolute concentrations are dependent, to a large extent, upon the freshness of the resin samples. A more controlled study is needed to determine when or whether there is an optimal season for the output of terpenes in resins. However, it is interesting to note that the relative concentrations of the monoterpenes (which is defined as the concentration of the individual monoterpene divided by the total concentration of all four compounds) are fairly constant over time, as shown in Table 2. Table 3 gives the averaged relative concentrations of the monoterpenes for each tree. Note that α -pinene was the dominant monoterpene for all species except *Agathis moorei*. Limonene was far and away the dominant monoterpene (>90%) in this particular tree.

The enantiomeric ratios of the monoterpenes produced by an individual tree do not vary a lot over

Table 1. Concentration (%) and enantiomeric ratio of monoterpenes in conifer resins

Species	Age (years)	Collect date	Concentration ^a , %(-/+ ratio ^b)			
			α -pinene	β -pinene	camphene	limonene
Agathis robustus	40	11/29/95	2.58(17/83)	0.55(9/91)	0.51(67/33)	1.47(1/99)
		1/30/96	8.42(20/80)	1.30(10/90)	1.00(74/26)	3.81(1/99)
		4/30/96	11.44(16/84)	1.98(11/89)	3.02(61/39)	4.81(1/99)
		7/30/96	6.41(23/77)	0.88(8/92)	2.07(74/26)	2.92(2/98)
		11/1/96	7.68(20/80)	1.16(10/90)	1.51(73/27)	2.83(1/99)
	100	11/29/95	3.62(26/74)	0.26(20/80)	1.13(75/25)	1.17(0/100)
		1/30/96	11.02(36/64)	2.00(19/81)	6.63(75/25)	4.14(3/97)
		4/30/96	7.28(34/66)	0.77(20/80)	3.21(74/26)	2.66(2/98)
		7/30/96	1.53(32/68)	0.17(20/80)	1.94(76/24)	0.77(1/99)
		11/1/96	7.95(33/67)	1.06(19/81)	6.32(74/26)	2.47(1/99)
Agathis moorei	8	11/29/95	0.27(65/35)	0.21(74/26)	0.06(75/25)	5.63(1/99)
		1/30/96	0.28(82/18)	0.08(90/10)	0.05(87/13)	5.86(0/100)
		4/30/96	0.06(48/52)	<0.01(90/10)	<0.01(94/6)	4.99(0/100)
		7/30/96	0.18(51/49)	0.19(73/27)	0.10(89/11)	6.29(0/100)
		11/1/96	0.07(13/87)	0.03(79/21)	0.01(74/26)	2.29(0/100)
	100	11/29/95	0.01(11/89)	0.01(63/37)	0.04(82/18)	6.48(0/100)
		1/30/96	0.07(12/88)	0.01(66/34)	0.03(79/21)	12.10(0/100)
		4/30/96	0.05(11/89)	0.01(65/35)	0.04(75/25)	11.76(0/100)
		7/30/96	0.07(13/87)	0.02(66/34)	0.09(64/36)	5.48(0/100)
		11/1/96	0.12(9/91)	0.08(72/28)	0.01(65/35)	6.32(0/100)
Araucaria cunninghamii	10	11/29/95	0.54(92/8)	0.06(96/4)	0.01(85/15)	0.01(15/85)
		1/30/96	1.09(88/12)	0.16(95/5)	0.01(84/16)	0.05(13/87)
		4/30/96	0.78(89/11)	0.06(97/3)	<0.01(84/16)	0.08(14/86)
		7/30/96	1.70(90/10)	0.25(97/3)	<0.01(82/18)	0.16(16/84)
		11/1/96	1.43(88/12)	0.19(97/3)	<0.01(80/20)	0.09(17/83)
	90	11/29/95	<0.01(21/79)	<0.01(66/34)	<0.01(94/16)	<0.01(0/100)
		1/30/96	0.01(21/79)	0.01(93/7)	<0.01(90/10)	0.02(0/100)
		4/30/96	0.01(17/83)	0.01(81/19)	0.02(90/10)	0.06(0/100)
		7/30/96	0.04(20/80)	0.01(83/17)	0.01(98/2)	0.03(2/98)
		11/1/96	0.02(32/68)	<0.01(71/29)	<0.01(85/15)	0.03(3/97)
Agathis macrophylla	80~150	11/29/95	2.55(13/87)	0.49(95/5)	0.06(73/27)	0.60(1/99)
		1/30/96	10.59(10/90)	2.31(95/5)	0.14(73/27)	2.58(1/99)
		4/30/96	9.11(12/88)	2.59(96/4)	0.13(70/30)	2.56(1/99)
		7/30/96	9.92(11/89)	2.07(94/4)	0.13(72/28)	2.59(1/99)
		11/1/96	10.38(9/91)	2.25(97/3)	0.11(74/26)	2.56(0/100)
Pinus strobus	(#1) 28	10/2/97	2.66(29/71)	0.93(5/95)	0.17(76/24)	0.22(38/62)
	(#2) 28	10/2/97	2.32(23/77)	1.07(4/96)	0.09(62/38)	0.20(28/72)
	(#3) 28	10/2/97	3.38(35/65)	1.54(4/96)	0.16(69/31)	0.27(30/70)
	(#4) 28	10/2/97	3.69(31/69)	2.12(4/96)	0.16(74/26)	0.22(19/81)

^aThe % average deviation in these results is $\pm 5\%$ as determined from 3 replicate analyses. Individual standard curves were made in all cases.

^bThe relative error in the ratio is $\pm 2\%$ as determined from 3 replicate analyses.

the course of a year (see Table 1). Occasionally there would be a larger change (usually within 10–20% of the major enantiomer). The greatest yearly variations in the enantiomeric ratios of monoterpenes occurred in *Agathis moorei*, particularly in the younger tree (Table 1). The average enantiomeric excess (ee) of the monoterpenes produced by each tree are given in Table 4.

It is clear from the data in Tables 3 and 4 that the relative concentration of the monoterpenes and

Table 2. Relative concentration of monoterpenes in conifer resins

Species	Age (years)	Collect Date	Relative Concentration(%) ^a				
			α -Pinene	β -Pinene	camphene	limonene	
Agathis robustus	40	11/29/95	50	11	10	29	
		1/30/96	58	9	7	26	
		4/30/96	54	9	14	23	
		7/30/96	52	7	17	24	
		11/1/96	58	9	11	22	
		11/29/95	59	4	18	19	
		1/30/96	46	8	28	18	
		4/30/96	52	6	23	19	
		7/30/96	35	4	44	17	
		11/1/96	45	6	35	14	
	Agathis moorei	8	11/29/95	4	3	1	92
			1/30/96	4	1	1	94
			4/30/96	1	0	0	99
			7/30/96	3	3	1	93
11/1/96			3	1	0	96	
100		11/29/95	0	0	1	99	
		1/30/96	1	0	0	99	
		4/30/96	0	0	0	100	
		7/30/96	1	0	2	97	
		11/1/96	2	1	0	97	
Araucaria cunninghamii	10	11/29/95	87	9	12	2	
		1/30/96	83	12	1	4	
		4/30/96	85	6	0	9	
		7/30/96	81	12	0	7	
		11/1/96	84	11	0	5	
Agathis macrophylla	80-150	11/29/95	69	13	2	16	
		1/30/96	68	15	1	16	
		4/30/96	63	18	1	18	
		7/30/96	67	14	1	18	
		11/1/96	68	15	1	16	
Pinus strobus	#1 28	10/2/97	67	23	4	6	
		10/2/97	63	29	2	5	
		10/2/97	63	29	3	5	
		10/2/97	60	33	3	4	

^aThe relative concentration (%) is the concentration of each compound divided by the total concentration of the four compounds.

their enantiomeric ratios, vary considerably with the species of tree. The one exception to this is the enantiomeric composition for limonene for *Agathis* and *Araucaria* trees. In all cases the (*R*)-(+)-enantiomer is dominant with ees >97%. In general, (*R*)-(+)-limonene is the dominant enantiomer in most plants that produce it.⁷ Within a species, the enantiomeric composition of α -pinene, β -pinene and camphene can vary considerably with age (Tables 1 and 4). In some cases the enantiomeric composition of α -pinene is essentially reversed in trees of the same species but of different ages (see *Agathis moorei* and *Araucaria cunninghamii* in Table 4).

Table 3. Relative concentration of monoterpenes in resins of different species

Species	Age (years)	Relative concentration(%) ^a			
		α -pinene	β -pinene	camphene	limonene
<i>Agathis robustus</i>	40	54.4 \pm 3.6	9.0 \pm 1.4	11.8 \pm 3.8	24.8 \pm 2.8
	100	47.4 \pm 8.9	5.6 \pm 1.7	29.6 \pm 10.2	17.4 \pm 2.1
<i>Agathis moorei</i>	8	3.0 \pm 1.2	1.6 \pm 1.3	0.6 \pm 0.4	94.8 \pm 2.8
	100	0.8 \pm 0.8	0.2 \pm 0.4	0.6 \pm 0.9	98.4 \pm 1.3
<i>Araucaria-cunninghamii</i>	10	84.0 \pm 2.2	10.0 \pm 2.5	0.6 \pm 0.9	5.4 \pm 3.1
<i>Agathis microphylla</i>	80~150	67.0 \pm 2.3	15.0 \pm 1.9	1.2 \pm 0.5	16.8 \pm 1.1
<i>Pinus strobus</i>	28	63.2 \pm 2.5	28.5 \pm 4.1	3.0 \pm 0.8	5.0 \pm 0.8

^aFor *Agathis* and *Araucaria* species this is the average of relative concentration for 5 samples collected from different seasons; for *Pinus strobus*, it is the average for 4 samples collected from different trees of the same age at the same time.

Table 4. Enantiomeric excess (ee) of monoterpenes in conifer resins of different species

Species	Age (years)	ee % of dominant enantiomers ^a			
		α -pinene	β -pinene	camphene	limonene
<i>Agathis robustus</i>	40	61.6 \pm 5.5(+)	80.8 \pm 2.3(+)	39.6 \pm 11.4(-)	97.6 \pm 0.9(+)
	100	35.6 \pm 7.5(+)	60.8 \pm 1.1(+)	49.6 \pm 1.7(-)	97.2 \pm 2.3(+)
<i>Agathis moorei</i>	8	26.0 \pm 33.1(-)	62.4 \pm 17.6(-)	67.2 \pm 17.6(-)	99.6 \pm 0.9(+)
	100	77.6 \pm 3.0(+)	32.8 \pm 6.7(-)	46.0 \pm 16.3(-)	100.0 \pm 0.0(+)
<i>Araucaria-cunninghamii</i>	10	80.8 \pm 5.2(-)	92.8 \pm 1.8(-)	66.0 \pm 4.0(-)	100.0 \pm 0.0(+)
	90	59.6 \pm 3.8(+)	57.6 \pm 21.2(-)	78.8 \pm 11.1(-)	98.0 \pm 2.8(+)
<i>Agathis microphylla</i>	80~150	78.0 \pm 3.2(+)	91.6 \pm 1.7(-)	44.8 \pm 3.0(-)	98.4 \pm 0.9(+)
<i>Pinus strobus</i>	28	41.0 \pm 10.0(+)	91.5 \pm 1.0(+)	40.5 \pm 12.4(-)	42.5 \pm 15.6(+)

^aFor *Agathis* and *Araucaria* species, this is the average of ee% for 5 samples collected from different seasons; for *Pinus strobus*, it is the average of ee% for 4 samples collected from different trees of the same age at the same time. The dominant configuration is shown in the bracket.

One experiment that could not be performed was to compare the monoterpene's ees for several trees of the same age. Only a few of the *Agathis* and *Araucaria* were available and none were of the same age. However, we were able to find four white pine (*Pinus strobus*) of the same age, locally. The monoterpenes in the resin of the 28-year-old *Pinus* varied somewhat in their enantiomeric excess (Table 1). The ee range was approximately 12 for α -pinene and camphene and 19 for limonene. Hence, trees of the same species, age and location can produce monoterpenes of somewhat different ees. The wide variation in the monoterpene ees of resin-producing trees would help explain the wide variation of the monoterpene ees found in amber.⁸ Previously it was found that the absolute configurations of the dominant enantiomer of the monoterpenoids in any amber sample were the same. This indicates

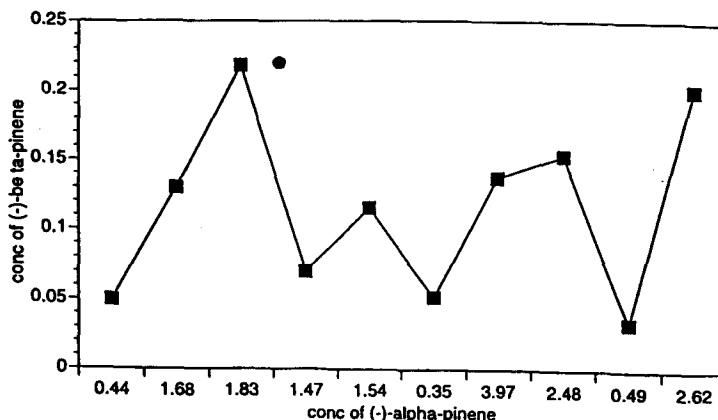


Figure 3. Variation of the concentrations of (-)- β -pinene with the concentrations of (-)- α -pinene in the resin of *Agathis robustus*. No correlation was observed between them.

that they may have come from a common source.⁸ In addition, information on the uses of monoterpenes in resin may be useful to the above-mentioned industries that isolate and purify these materials.

The enantiomeric composition of monoterpenes in resin are relevant in two other areas of scientific investigation. One area is the enzymology of the conifers that produce resin. The other involves the effect of monoterpene uses in the tree's defense against pathogens and herbivores.

The mechanism by which conifers synthesize monoterpenes is an area of biochemical and ecological study.¹⁴⁻¹⁶ There is evidence that each monoterpene (and each enantiomer) is synthesized by a specific enzyme (i.e., synthase or cyclase). Different conifer species may have different levels of cyclases. Also the levels of cyclases may change over time for individual species and trees. This phenomenon is analogous to the enzyme and hormonal changes that occur in animals as they age. Our results (Tables 1-4) indicate that: (1) there can be large differences in the relative amounts of monoterpene produced by different genus and species of conifer; (2) there are smaller but measurable age-related differences in the relative concentration of monoterpenes within a species; and (3) the enantiomeric composition of the monoterpenes in resin can vary tremendously both with the species of the tree and with its age. It would be useful to test and correlate the specific cyclase activities to the monoterpene composition in the resin. It was reported in one study that some conifer species use the same cyclase for both (-)- α -pinene and (-)- β -pinene.¹⁷ However, our studies have shown no correlation in the concentration of (-)- α -pinene and (-)- β -pinene for any of the trees in this study (see Figure 3 and Table 1).

The main function of resin in trees is thought to be for defense from pathogens, herbivores and adverse environmental conditions. Resins can cover wounds thereby preventing water loss, infection and other damage. The volatile compounds in resin (e.g., monoterpenes and other smaller terpenes) are toxic to many of the microbes and insects that infest conifers. This is a direct defense mechanism. The types of monoterpene and their stereochemistry control can affect the toxicity of the resin.^{18,19} In addition, there is an indirect defense mechanism in which some volatile terpenes act as attractants to insects that are predators of conifer pests.² Insect pheromone and attractants are known to be enantioselective.²⁰ However, the enantio-biological activity of some of the monoterpenes in this study have not been evaluated in that regard. This is an area of future study that should expand prior findings involving the role of monoterpenoids in the relationship between plants and insects.^{10,21,22}

Acknowledgements

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References

1. J. H. Langenheim, *American Scientist*, **1990**, *70*, 16–24.
2. J. H. Langenheim, *Amber, Resinite & Fossil Resins*, ACS Symposium Series 617, **1994**, pp. 1–31.
3. J. McNeil, *New Zealand Geographic*, **1991**, *10*, 18–45.
4. R. B. Croteau, D. M. Satterwhite, C. J. Wheeler, N. M. Felton, *J. Bio. Chem.*, **1989**, *264*, 2075–2080.
5. H. C. Brown and P. V. Ramachandran, *Pure & Appl. Chem.*, **1994**, *66*(2), 201–212.
6. H. C. Brown and P. V. Ramachandran, *Adv. Asymmet. Syn.*, **1995**, *1*, 147–210.
7. R. J. Braddock, K. R. Cadwallader, *Fruit Flavors, Biogenesis Characterization and Authentication*. ACS Symposium Series 596, **1995**, pp. 142–148.
8. D. W. Armstrong, E. Y. Zhou, J. Zukowski, B. K. Ceranowicz, *Chirality*, **1996**, *8*, 39–48.
9. U. Bufler, G. Seufert, F. Juttner, *Environ. Pollu.*, **1990**, *68*, 367–375.
10. M. Lindström, T. Norin, G. Birgersson, F. Schlyter, *J. Chem. Ecol.*, **1989**, *15*(2), 541–548.
11. D. Kotzias, C. Spartà, M. Duane, *Naturwissenschaften*, **1992**, *79*, 24–26.
12. F. Loreto, P. Ciccioli, A. Cecinato, E. Brancaleoni, M. Frattoni, D. Tricoli, *Plant Physio.*, **1996**, *110*, 267.
13. M. Lu, Y. Wang, *Huanjing Huaxue*, **1992**, *11*(2), 21–25.
14. R. B. Croteau, *Chem. Rev.*, **1987**, *87*, 929–954.
15. K. Wagschal, T. Savage, R. B. Croteau, *Tetrahadron*, **1991**, *47*, 5933–5944.
16. H. J. Pyun, R. M. Coates, K. C. Wagschal, P. McGeady, R. B. Croteau, *J. Org. Chem.*, **1993**, *58*, 3998–4009.
17. R. B. Croteau, C. J. Wheeler, *Biochem.*, **1987**, *26*, 5383–5389.
18. S. P. Cook, F. P. Hain, *J. Entomol. Sci.*, **1988**, *23*, 1287–1292.
19. K. F. Raffa, A. A. Berryman, J. Simasko, W. Teal, B. L. Wong, *Environ. Entomol.*, **1985**, *14*, 552–556.
20. C. Everaerts, O. Bonnard, J. M. Pasteels, Y. Roisin, W. A. König, *Experientia*, **1990**, *46*(2), 227–230.
21. T. S. Aronen, *European J. Forest Pathol.*, **1997**, *27*(1), 55–67.
22. M. Gijzen, E. Lewinsohn, T. J. Savage, R. B. Croteau, *Bioactive Volatile Compounds from Plants*. ACS Symposium Series 525, **1993**, pp. 8–22.

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