Chemistry of Natural Compounds, Vol. 42, No. 5, 2006

CHANGES IN THE COMPOSITION OF VOLATILE MONOTERPENES AND SESQUITERPENES of *Pinus armandi, P. tabulaeformis,* AND *P. bungeana* IN NORTHWEST CHINA

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The volatile mono- and sesquiterpenes obtained from the needles and resin of Pinus armandi, P. tabulaeformis, *and* P. bungeana *growing in the Qinling, Taibai, and Huanglong Mountain forest ecosystem were analyzed by means of GC-MS. Forty-eight constituents were identified, and* α*-pinene,* β*-pinene, 1*R-α*-pinene,* β*-caryophyllene, cadindiene,* α*-caryophyllene, D-limonene, and 1*S-β*-pinene were the major components of the mono- and sesquiterpenes in the needles and resin. The components of the volatile mono- and sesquiterpenes from the needles and resin at Qinling, Taibai, and Huanglong Mountains had remarkable differences in three pine species, whereas the monopertene content such as* α*-pinene,* β*-pinene, D-limonene, and camphene were mostly changed in the growing stage. The intraspecies variation in the different ecosystems can be attributed to the species' geography and genetic variation, and even the adaptation of the pine species to different ecological environments. Moreover, monoterpenes and sesquiterpenes can be induced by the attack of bark beetles, of which the* α*-pinene,* β*-pinene, 1*R-α*-pinene, 1*S-α*-pinene,* β*-myrecene, and* β*-caryophyllene contents had positive relations with the attacking* Dendroctonus armandi *and* D. valens*.*

Key words: *Pinus armandi, P. tabulaeformis, P. bungeana*, volatile compounds, monoterepenes, sesquiterpenes, bark beetles.

Pinus armandi, *Pinus tabulaeformis*, and *Pinus bungeana* are the most important pine species in Northwest China, which grow on arid calcareous areas and has excellent resistance to drought, saline, and poor soil. Therefore, *P. armandi*, *P. tabulaeformis*, and *P. bungeana*, as the native species with extensive ecological adaptability, were widely employed in reafforestation in Northwest China. But since 1953, *P. armandi* has been seriously damaged by *Dendroctonus armandi* Tsai et Li and other secondary bark beetles. *D. armandi* primarily attacks healthy *P. armandi* at age over 30 years, and in rare cases it may also attack *Pinus tabulaeformis* Carr. in the Qinling and Bashan Mountains [1]. Meanwhile, an outbreak of *Dendroctonus valens* (LeConte), as an exotic forest pest, was seen in the Zhongtiao, Huanglong, and Qiaoshan Mountains of Shaanxi, Shanxi, and Hebei provinces in 1998; this mainly attacked the weak *P. tabulaeformis* by all kinds of factors, and the epidemic areas expanded attributed to the bark beetles, ecological adaptability and sensitivity to terpenoid volatiles of the host trees.

Plant terpenoid volatiles are a complex chemically diverse mixture of monoterpenes, sesquiterpenes, and diterpenes that serve many functions. All living organisms contain terpenoids, but plants contain the highest diversity of terpenoids. Furthermore, the terpenoid volatiles of different pine species change with genetic and geographic diversity and variation [2–5], and also the same terpenes that are produced for defense can be used by plant herbivores in host location and selection [6–8]. In contrast, the monoterpenes also have ecological functions that are advantageous to the pest insect: they can act as precursors in pheromone biosynthesis [9–11] or as primary attractants for opportunistic herbivores, and even fungal symbionts and beetle gut bacteria converted tree monoterpenes such as α-pinene and myrcene to the corresponding pheromones [12–16].We were studied 3 species of Pinus (Table 1).

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 0009-3130/06/4205-0534 ©2006 Springer Science+Business Media, Inc.

TABLE 1. Site Details for Samples of Three Pine Species in China

Species	Site	Altitude	Age	Situation	Species	Site	Altitude	Age	Situation
P. armandi (PA1)	Qinling	1900	36	Healthy	*P. tabulaeformis* (PT2)	Getai	1200	39	Healthy
P. armandi (PA2)	Qinling	1900	41	Infected	*P. tabulaeformis* (PT3)	Qinling	1900	36	Healthy
P. armandi (PA3)	Qinling	1900	38	Healthy	*P. tabulaeformis* (PT4)	Dalin	1200	39	Healthy
P. armandi (PA4)	Taibai	1200	37	Healthy	*P. bungeana* (PB1)	Huanglong	1200	35	Healthy
P. tabulaeformis (PT1)	Qinling	1900	37	Healthy	*P. bungeana* (PB2)	Huanglong	1200	34	Healthy

Table 2 lists the volatile mono- and sesquiterpenes of needles of *P. armandi*, *P. tabulaeformis*, and *P. bungeana* from GC-MS analysis; 32 compounds have been identified from needles of *P. armandi*, and the principal constituents were β-caryophyllene (29.09%), followed by cadindiene (26.10%), α-caryophyllene (5.34%), α-pinene (4.27%), and D-limonene (1.00%), respectively.

But α-pinene (74.21%), β-pinene (39.27%), and 1*R*-α-pinene (52.39%) were mainly constituents among 25 in the resin compounds of *P. armandi* from different forest ecosystem; α-pinene was the predominant compound in the resin, while β-caryophyllene, cadindiene, and α-caryophyllene dropped to 0.33, 0.44, and less, than 0.05%, respectively. The volatile mono- and sesquiterpenes in needles of *P. tabulaeformis* included 29 compounds, of which β-caryophyllene (23.62%), cadindiene (13.99%), α-caryophyllene (5.97%), 1*R*-α-pinene (5.87%), and 1*S*-β-pinene (5.30%) were the dominant constituents. The predominant constituents in the resin of *P. tabulaeformis* were α-pinene (75.60%, 78.52%), β-pinene (16.59, 3.55%), and D-limonene in the 24 compounds identified for this species at Qinling and Huanglong Mountains, and the variation was very limited between Qinling and Huanglong Mountains besides β-pinene and D-limonene. But the volatile mono- and sesquiterpenes in the needles and resin of *P. bungeana* consisted of 18 and 22 compounds, of which α-pinene (37.70, 40.92%), β-pinene (54.92, 7.42%), D-limonene (2.02, 11.33%), camphene (0.64, 9.45%), and cadindiene (0.24, 6.73%) exceeded 95.52 and 85.85% of all constituents in the volatile mono- and sesquiterpenes, respectively.

By comparison, the predominant monoterpenes and sesquiterpenes between the needles and resin of *P. armandi*, *P. tabulaeformis*, and *P. bungeana* had obvious differences (Table 2). The general trend of monoterpene contents was higher in the resin of the stem than in the needles, and the content of monoterpene compounds, such as α-pinene, β-pinene, D-limonene, and camphene, was the most remarkable variation between the needles and resin of the three pine species. For example, the content of β-pinene was 0.33, 39.27, and 0% and 16.59, 54.92, and 7.42% among the needles and stem resin of *P. armandi*, *P. tabulaeformis*, and *P. bungeana*, but the α-pinene content between the needles and resin of *P. armandi*, *P. tabulaeformis*, and *P. bungeana* was 4.27 and 0%, 0 and 75.6%, and 37.7 and 40.92%, respectively. However, the sesquiterpene content was reduced quite sharply in the resin of *P. armandi*, *P. tabulaeformis*, and *P. bungeana*.

The mono- and sesquiterpene constituents in the different forest ecosystem between the needles and resin had obvious interspecies variations. For example, the mono- and sesquiterpene constituents and content changed extremely among the needles of *P. armandi* (PA1), *P. tabulaeformis* (PT1), and *P. bungeana* (PB1). Moreover, the intraspecies growth variation in the different ecosystems are mainly represented in the geography, except *P. armandi* which was distributed between Qinling and Taibai Mountains (Table 2). There are extreme differences found in *P. tabulaeformis* between Qinling Mountains and Huanglong Mountains due to gene and geographic diversity. Needles of *P. tabulaeformis* (PT1, PT2) located in the Qinling Mountains came from seeds and seedlings that originated from natural distribution, but those located in the Huanglong Mountains came from seeds and seedlings imported from the Zhongtiao Mountains of Shanxi province. The original genetic variation was the primary factor that resulted in the difference in interspecies needles yielding mono- and sesquiterpenes between the Qinling Mountains and Huanglong Mountains. On the other hand, mono- and sesquiterpenes in the resin of *P. tabulaeformis* (PT3, PT4) were very similar because the seeds and seedlings in the Huanglong Mountains were imported from the Qinling Mountains.

The predominant monoterpenes and sesquiterpenes, such as α-pinene, β-pinene, 1*R*-α-pinene, 1*S*-α-pinene, β-myrecene, and β-caryophyllene, between the needles of healthy trees and attacked trees of *P. armandi* also had obvious differences, but the camphene, D-limonene, γ-elemene, cadindiene, and α-caryophyllene were not quite variable (Table 2). On the other hand, the resistance of the host trees declines following attacked by *D. armandi*, resulting in resin metabiotic disorder and death of the host trees, so that the resin in the stem of the weakened trees was not collected.

TABLE 2. Constituents of Essential Oils of Three *Pinus* Species under Different Conditions, %

Constituent	*P. armandi*				*P. tabulaeformis*				*P. bungeana*	
	PA1	PA2	PA3	PA4	PT1	PT2	PT3	PT4	PB1	PB2
α-Pinene	4.27	-	-	74.21	-	21.24	75.60	78.52	37.70	40.92
Camphene	0.44	0.45	0.75	1.43	0.61	4.87	1.25	1.48	0.64	9.45
4-Methylene-1-isopropylbicyclo[3.1.0]hex-2-ene	-	-	-	-	-	0.09	-	0.10	-	0.06
1*S*-*α*-Pinene	-	Tr.	0.10	-	Tr.	-	-	-	-	-
p-Menthatriene	Tr.	-	-	Tr.	-	-	-	-	-	-
β-Pinene	0.33	-	39.27	6.47	-	9.89	16.59	3.55	54.92	7.42
β-Phellandrene	-	-	-	-	1.06	-	-	Tr.	-	-
1*R*-*α*-Pinene	-	5.07	52.39	-	5.87	-	-	-	-	-
β-Myrcene	0.37	-	0.64	0.52	-	2.82	0.90	0.77	0.86	1.56
α-Phellandrene	Tr.	-	1.30	Tr.	Tr.	Tr.	Tr.	0.14	-	Tr.
δ-Carene	0.40	Tr.	Tr.	-	0.08	Tr.	0.025	2.45	Tr.	-
p-Cymenene	0.08	0.10	Tr.	Tr.	Tr.	0.43	Tr.	0.19	Tr.	0.30
3-Menthene	-	-	-	-	-	Tr.	-	-	-	-
1*S*-*β*-Pinene	-	0.41	-	-	5.30	-	-	-	-	-
Menthene	-	-	-	Tr.	-	-	-	-	-	-
Limonene	1.00	0.73	-	1.31	-	6.74	1.89	1.26	2.02	11.33
1*R*-*β*-Pinene	-	0.60	-	-	0.78	-	-	-	-	-
Terpinolene	-	Tr.	Tr.	Tr.	Tr.	-	Tr.	Tr.	-	Tr.
β-Ocimene	-	-	-	-	-	-	Tr.	-	-	-
γ-Terpinene	Tr.	-	-	-	0.97	-	-	-	-	-
Naphthalene	0.06	0.21	-	-	-	-	-	-	-	-
γ-Elemene	0.10	-	-	-	3.37	-	-	-	-	-
Eudesmadiene	-	-	-	-	-	-	-	-	-	0.08
α-Cubebene	0.21	0.72	0.15	0.38	0.31	0.16	-	0.07	Tr.	0.24
Cycloisosativene	-	-	Tr.	0.09	-	-	Tr.		-	-
α-Ylangene	0.17	0.21	-	-	-	-	Tr.	-	Tr.	0.21
α-Copaene	0.59	-	0.33	0.70	0.11	-	-	-	Tr.	0.37
β-Bourbonene	-	-	-	-	-	-	-	-	Tr.	0.29
Tricycloterpene	0.18	0.23	-	-	-	-	-	-	-	-
Iso-*β*-Caryophyllene	-	-	-	-	-	-	Tr.	-	0.61	3.62
β-Caryophyllene	29.09	-	-	0.33	23.62	6.47	Tr.	0.07	-	Tr.
Cadindiene	26.10	28.61	0.38	0.44	13.99	2.85	-	Tr.	0.24	6.73
Iso-Cubebene	0.37	-	-	-	-	-	-	-	-	-
α-Caryophyllene	5.34	3.93	Tr.	Tr.	5.97	1.59	-	Tr.	0.12	0.82
Farnesene	-	-	-	0.20	-	-	Tr.	Tr.	-	-
Eudesma-4(14),11-diene	-	-	-	-	-	0.35	Tr.	-	-	0.12
Iso-Ledene	-	-	Tr.	-	-	-	-	-	-	-
α-Calacorene	-	0.63	-	Tr.	0.06	0.12	-	Tr.	-	-
Calacorene	0.24	Tr.	-	-	-	-	-	-	-	-
β-Calacorene	-	-	-	-	-	-	-	Tr.	-	-
Cadalene	-	-	-	-	-	-	-	-	-	Tr.

%, Relation percentage obtained from peak area. PA1 obtained from healthy tree needles of *P. armandi* at Qinling Mountains, PA2 was the attacked trees of *P. armandi* by *D. armandi* at Qinling Mountains; PA3 obtained from healthy tree resin of *P. armandi* at Qinling Mountains; PA4 obtained from healthy tree resin of *P. armandi* at Taibai Mountains; PT1 obtained from healthy tree needles of *P. tabulaeformis* at Qinling Mountains; PT2 obtains from healthy tree needles of P. *tabulaeformis* at Huanglong Mountains; PT3 obtained from healthy tree resin of *P. tabulaeformis* at Qinling Mountains; PT4 obtains from healthy tree resin of *P. tabulaeformis* at Huanglong Mountains; PB1 obtains from healthy tree needles of *P. bungeana* at Huanglong Mountains; PB2 obtains from healthy tree resin of *P. bungeana* at Huanglong Mountains.

EXPERIMENTAL

The needles (leaves of healthy trees and trees infested by *D. armandi*) and resin samples of *P. armandi* were collected from the Qinling and Taibai Mountains, located 1900 and 2300 m above sea level, respectively; samples (leaves and resin) of *P. tabulaeformis* were collected from the Qinling and Huanglong Mountains, 1900 m and 1200 m above sea level, respectively; samples of needles and resin of *P. bungeana* were collected from the Huanglong Mountains during April 2004. One- and two-year-old needles collected from four directions and three levels of the crown, 500 g, and fresh needles collected from 10 different individual trees were air dried at room temperature to constant weight and cut into small pieces; 400 g of the powder and 500 mL water were placed in a round-bottomed flask and steam distilled for 4 hours. The essential oil samples were extracted with an equal volume of ether and dried over anhydrous sodium sulfate. After evaporation, the essential oils were obtained.

Healthy, dominant trees were selected from the Qinling Mountains at 1900 m above sea level, the Taibai Mountains at 2300 m above sea level, and the Huanglong Mountains at 1200 m above sea level. A slightly sloping hole was drilled into the stem at breast height and a tightly fitting 30 mL glass vial inserted. The vial, containing accumulated resin, was removed the next day and stored in the refrigerator until required for analysis. Needles and resin samples were submitted to hydrodistillation for 4 h using a round-bottomed flask. The essential oil samples were separated and dried using anhydrous sodium sulfate before analysis; the oil yields were expressed as mL/100 g dried leaves or fresh resin samples (Table 1).

GC analysis was performed using an HP-6890 N instrument equipped with HP-Wax and a capillary column (HP-5MS, crosslined 5% PH ME siloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness), and an FID detector. The flow rate for the helium carrier gas was 1.2 mL/min. The injector temperature was 250°C. A 1 mL sample was injected under the split condition (75:1). The temperature program was 40° for 4 min, then 40–290°C at a rate of 4°/min, and subsequent isothermal hold for 20 min.

GC-MS analysis of the oil was performed on a Hewlett- Packard 6890 GC-MS system operating in EI mode at 70 eV, equipped with a Hewlett-Packard 6890 series injector, and a mass spectrometer selective detector 5973 (N) (Hewlett- Packard Corporation, GA, USA). GC conditions: flow rate of helium carrier gas 1.0 mL/min; injector temperature 250°C. A 1 mL sample was injected under the split condition (8:1). MS conditions: ion source temperature 230°C; scan mass range 25–400 *m/z*; solvent delay time 3 min; the column used and other operating conditions were the same as those of GC; transfer-line temperature was 250°C. Volatiles were identified by comparison of the retention data and mass spectra with those of the available authentic synthetic compounds, with the computerized data library, NIST02L, and/or by comparison of mass spectra with those in the data system libraries. Absolute amounts were obtained by comparison with the internal standard, the stabilizer (butylated hydroxytoluene; BHT) of diethyl ether.

ACKNOWLEDGMENT

We would like to thank professor Hu Jinjiang and professor Harald Scherm for their useful suggestions for improving the experimental protocol. We are grateful to Meng Qianxiang (Lanzhou geological institute) for providing the Hewlett-Packard 6890 and the necessary experimental reagents. This work was supported by the National Natural Science Foundation of China (30271090).

REFERENCES

1. H. Chen, M. Tang, and H. Ye, *Scientia Silvae Sinicae*, **35**, 40 (1999).
2. F. Macchioni, P. L. Cioni, G. Flamini, I. Morelli, S. Maccioni, and M. Ansaldi, *Flavour Fragr. J.*, **18**, 139 (2003).
3. R. Schonwitz, L. Merk, and H. Ziegler, *Flavour Fragr. J.*, **4**, 149 (1989).
4. P. Tammela, M. Nygren, I. Laakso, A. Hopia, H. Vuorela, and R. Hiltunen, *Flavour Fragr. J.*, **18**, 290 (2003).
5. P. V. Petrakis, C. Tsitsimpikou, O. Tzakou, M. Couladis, C. Vagias, and V. Roussis, *Flavour Fragr. J.*, **16**, 249 (2001).
6. L. Delorme and F. Lieutier, *Eur. J. Forest Pathol.*, **20**, 304 (1990).
7. K. F. Raffa and E. B. Smalley, *Oecologia*, **102**, 285 (1995).
8. K. D. Klepzig, J. M. Riba, E. B. Smalley, and K. F. Raffa, *J. Chem. Ecol.*, **22**, 1367 (1996).

9. M. Lindstrom, T. Norin, G. Birgersson, and F. Schlyter, *J. Chem. Ecol.*, **15**, 541 (1989).
10. S. J. Seybold, *J. Chem. Ecol.*, **19**, 1809 (1993).
11. J. A. Byers, *Host-tree Chemistry Affecting Colonization in Bark Beetles* in: *Chemical Ecology of Insects* (Ed. R. T. Carde and W. J. Bell) New York, Chapman and Hall, 1995.
12. J. A. Byers, B. S. Lanne, F. Schlyter, J. Lofquist, and G. Bergstrom, *Naturwissenschaften*, **75**, 324 (1985).
13. D. A. Tilles, K. Sjodin, G. Nordlander, and H. H. Eidmann, *J. Econ. Entomol*., **79**, 970 (1986).
14. H. Jactel, M. Kleinhentz, A. Marpeau-Bezard, F. Marion-Poll, P. Menassieu, and C. Burban, *J. Chem. Ecol.*, **22**, 1037 (1996).
15. M. Kleinhentz, H. Jactel, and P. Menassieu, *J. Chem. Ecol.*, **25**, 2741 (1999).
16. S. J. Seybold, J. Bohlmann, and K. F. Raffa, *Can. Entomol*., **132**, 697 (2000).