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TERPENOIDS FROM OLEORESIN OF *PINUS TAIWANENSIS*

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Key Word Index—*Pinus taiwanensis*; Coniferae; Tawain red pine; oleoresin; terpenes; autoxidation.

Abstract—The neutral fraction of the oleoresin from *Pinus taiwanensis* contained 89% of monoterpene hydrocarbons (α-pinene, camphene, β-pinene, myrcene, limonene, β-phellandrene, *p*-cymene), 8·6% of sesquiterpene hydrocarbons (longicyclene, longifolene and caryophyllene) and 2·4% of oxygenated compounds (bornyl acetate, 4-terpineol, myrtenal, *trans*-pinocarveol, *trans*-verbenol, verbenone, myrtenol, caryophyllene oxide, 13-epimanoyl oxide, longiborneol and methyl dehydroabietate). Two diterpene hydrocarbons [pimara-8(14),15-diene, dehydroabietane], three norterpenoids [nopinone, longicamphenylone and 18-norpimara-8(14),15-dien-4-ol] and 13 *n*-alkanes (C_{22}–C_{34}) were also identified. The norterpenoids appear to be formed by autoxidation of hydrocarbons during the isolation process and the results of autoxidation of β-pinene and longifolene supported this view. The main constituent of the acidic part of the oleoresin was abietic acid (90%).

INTRODUCTION

The Taiwan red pine (*Pinus taiwanensis* Hayata), is native to Taiwan, growing frequently in Central Ranges at altitudes of 750–3000 m, and often forming large stands. The chemical constituents of this plant have not been studied, and the results of the analysis of the oleoresin are reported in this paper.

RESULTS AND DISCUSSION

The main constituents of the oleoresin were acidic (75% of total), and the main component was abietic acid (90% of acid fraction). By treatment with 2% NaOH solution, the neutral fraction was obtained. In order to prevent chemical changes to constituents and the loss of more volatile components during analysis, the investigation was carried out in two parts. First, large amounts of oleoresin were studied qualitatively by column chromatography and GLC methods. Thereafter, the small amounts of oleoresin were collected from several different sources and analyzed quantitatively by GLC.

The percentage composition of the oleoresin from five different sources is shown in Table 1. The oleoresin is characterized by large amounts of the monoterpene hydrocarbons, α-pinene and β-pinene (*ca* 82–87%) and the sesquiterpene hydrocarbon, longifolene. In addition to the mono- and sesquiterpenes, two diterpene hydrocarbons, 13-epimanoyl oxide and methyl dehydroabietate, three norterpenoids, nopinone, longicamphenylone and 18-norpimara-8(14),15-dien-4-ol; and an homologous series of *n*-alkanes (C_{22}–C_{34}) were isolated.

Of considerable interest is the isolation of norterpenoid components. Longicamphenylone also does not appear to have been previously isolated from a natural source. From the mixture of diterpenes, colorless crystals of 18-norpimara-8(14),15-dien-4-ol was obtained. This compound was first isolated from the benzene extract of lodgepole pine by Rowe *et al.* [1]. Recently, other workers [2] reported that norditerpenes are artifacts formed from the corresponding 4-axial aldehyde during the course of isolation. This was strongly supported by the results of autoxidation of pimara-

Table 1. Per cent composition of oleoresin of *Pinus taiwanensis*

Peak no.	Compound	RR_t*	Oleoresin sample No. 5	6	10	11	18	Ref.
1	Unidentified	0·32	0·30	0·21	0·51	1·14	0·12	
2	Unidentified	0·39	0·37	0·20	0·61	0·36	0·17	
3	(−)-α-Pinene	0·60	55·45	65·18	70·51	79·52	59·46	[3,4]
4	(Camphene)†	0·70	0·70	0·89	1·05	1·37	0·58	
5	(−)-β-Pinene	0·80	25·89	21·73	13·61	5·48	25·86	[3,4]
6	Myrcene	0·89	0·37	0·87	1·13	1·22	0·79	[3]
7	Limonene	1·00	0·52	0·87	1·05	1·18	0·84	[3]
8	β-Phellandrene‡	1·02	0·37	0·82	0·95	0·53	3·23	
9	(p-Cymene)†	1·18	trace	0·05	trace	0·10	0·12	
10	Unidentified	0·73	0·38	0·08	0·45	0·21	0·24	
11	Longicyclene‡	0·81	0·33	0·07	0·32	0·16	0·16	
12	Unidentified	0·90	0·29	0·07	0·32	0·10	0·16	
13	Nopinone‡	0·95	trace	trace	trace	trace	trace	
14a	(+)-Longifolene	1·00	11·40	4·51	6·80	3·61	7·11	[5]
14b	Bornyl acetate‡	1·00	trace	trace	trace	trace	trace	
15a	Caryophyllene‡	1·09	2·09	2·03	1·30	0·98	trace	
15b	4-Terpineol	1·09	trace	trace	trace	trace	trace	[3]
15c	Myrtenal‡	1·09	trace	trace	trace	trace	trace	
16	*trans*-Pinocarveol‡	1·24	trace	trace	trace	trace	trace	
17	*trans*-Verbenol‡	1·31	0·33	0·34	0·24	0·15	trace	
18	Verbenone‡	1·39	trace	trace	trace	trace	trace	
19	Myrtenol	1·93	trace	trace	trace	trace	trace	[7]
20	Caryophyllene oxide	2·33	0·17	0·23	trace	0·11	trace	[3]
21	Longicamphenylone‡	2·43	trace	trace	trace	trace	trace	
22	Longiborneol	2·92	0·17	0·13	0·24	trace	0·16	[3]
23	Pimara-8(14),15-diene	3·15	trace	0·13	trace	0·18	0·09	[6]
24	13-Epimanoyl oxide‡	3·63	trace	trace	trace	trace	trace	
25	Dehydroabietane‡	3·97	trace	trace	trace	trace	trace	
26	Unidentified	4·54	0·25	0·17	0·28	0·90	0·24	
27	Unidentified	4·66	0·42	0·96	0·45	1·80	0·49	
28	18-Norpimara-8(14),15-dien-4-ol	4·76	trace	trace	trace	trace	trace	[1]
29	Unidentified	4·96	0·04	0·48	0·16	0·75	0·16	
30	Methyl dehydroabietate‡	5·45	0·17	trace	trace	0·15	trace	

* RR_t values (a) monoterpenes (peaks 1–9) measured relative to limonene, on a 20% PEG 20 M column (180 × 0·2 cm), temp. programmed from 50° to 220° at 4° per min. (b) others relative to longifolene, on a 5% PEG 20 M column (180 × 0·2 cm), temp. programmed from 50° to 220° at 4° per min.

† Names in parentheses represent compounds tentatively identified.

‡ Identified by comparison with IR of authentic sample.

a,b,c Peaks were resolved on a QF-1 column.

8(14),15-dien-19-al [2a] and torulosal [2b,c]. Since Taiwan pine oleoresin contains large amounts of β-pinene and longifolene, it is probable that nopinone and longicamphenylone are also secondary products derived from the corresponding hydrocarbons. Longicamphenylone could be produced in 70% yield from the autoxidation of longifolene at 97° for 100 hr. Nopinone was also one of the products formed by the autoxidation of β-pinene.

EXPERIMENTAL

M.ps are uncorrected. IR spectra were obtained from a thin film or in nujol mull. Optical rotations were measured at room temp. in EtOH.

Extraction and prefractionation. Oleoresin from *P taiwanensis* Hayata (2·86 Kg) was extracted × 3 with *n*-hexane at room temp. The combined *n*-hexane soln was filtered and the vol. reduced, then extracted with 2% NaOH to remove the acidic components (2·115 kg). The acidic components (10 g) were subjected to silica gel (320 g) column chromatography and pure abietic acid (m.p. 174–175°) was obtained in 90% yield. The *n*-hexane fraction was thoroughly washed, dried and the solvent evaporated. The residue (495 g) was steam distilled, and the steam-volatile fraction (363 g) was separated into a monoterpene hydrocarbon fraction A, mid-fraction B and a residual fraction C by reduced fractional distillation.

Separation and identification of neutral components. Fraction A was separated on a 90 × 0·6 cm 20% SE-30 column (temp. programmed from 50° to 220°) and each isolated fraction was further purified by GLC on a 20% PEG 20 M column (isothermal at 80° and 110°). (−)-α-Pinene, (−)-β-pinene, myrcene, limonene and β-phellandrene were identified (IR, NMR, GLC R_t data). Fraction B was separated into a homologous series of

straight chain hydrocarbons, sesquiterpene hydrocarbons and an oxygenated monoterpene fraction by column chromatography on neutral alumina. The hydrocarbon fraction was chromatographed on a column of neutral alumina containing 15% $AgNO_3$ and eluting with *n*-hexane followed by *n*-hexane–C_6H_6 mixtures (9:1, 1:1, 1:9) then C_6H_6. Final purification of each compound was achieved by GLC, and caryophellene, (+)-longifolene and longicyclene were identified. The compounds present in the oxygenated fractions were further separated on a 180 × 0·4 cm 20% PEG 20 M column (117° isothermal) and the following components were identified by IR and PMR: nopinone, bornyl acetate, (+)-4-terpineol, myrtenal, (+)-*trans*-pinocarveol, (+)-*trans*-verbenol, (+)-verbenone and (−)-myrtenol. Fraction C was chromatographed on neutral alumina and then purified on a 90 × 0·4 cm 20% PEG 20 M column resulting in the isolation of pimara-8(14),15-diene, dehydroabietane, methyl dehydroabietate, caryophellene oxide, 13-epimanoyl oxide (m.p. 93–94°), longicamphenylone (m.p. 50–51°), longiborneol (m.p. 108–109°) and (+)-18-norpimara-8(14), 15-dien-4-ol (m.p. 119–120°). Detailed comparison of IR, MS and NMR spectra with lit or that of authentic samples was used for identification.

Autoxidation of longifolene. Air was passed through longifolene (2 ml) at 97° at 120 ml/min for 100 hr. GLC of the reaction mixture showed that 70% of the compound was oxidized. The reaction mixture was purified on a 20% PEG 20 M column and longicamphenylone was obtained as the major product (71%). Using the same temp. and CO_2-free air at the same flow rate, only 40% of the reactant was oxidized after 100 hr although longicamphenylone was still the major product. In order to check that the reaction was not pyrolysis, 2 ml of longifolene was heated at 97° under N_2 for 100 hr. GLC showed that no reaction occurred in the absence of air.

Autoxidation of β-pinene. Air was passed through β-pinene (2 ml) at 96° at 120 ml/min for 41 hr. GLC analysis of the reaction mixture gave 4 peaks and one peak was shown to be nopinone by co-injection with authentic material.

Determination of composition of resin samples. Oleoresins were collected from several different trees at altitudes of 750 m at Chieu Liangsi, Hoping Villiage, Taichung County. The samples were macerated in *n*-hexane and the extractives were treated with 2% NaOH to remove the acidic components. The neutral components were analyzed by GLC, and the percentage composition is shown in Table 1.

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