Phytochemistry, Vol. 23, No. 4, pp. 875–878, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00
Pergamon Press Ltd.

MINOR COMPONENTS OF PONDEROSA PINE OLEORESIN

RYOICHI FUJII and DUANE F. ZINKEL*†

Harima Chemicals Inc., 671-4 Mizuashi, Noguchi-Cho Kakogawa, Japan; *Forest Products Laboratory, Forest Service, USDA, P.O. Box 5130, Madison, WI, 53705, U.S.A.

(*Revised received* 9 *September* 1983)

Key Word Index—*Pinus ponderosa*; Pinaceae; ponderosa pine; oleoresin; resin acids; ferulic acid esters; acetylisocupressic acid; 12-acetoxyabietic acid.

Abstract—Esters of ferulic acid with monoterpene alcohols were found in ponderosa pine oleoresin. These colorless ferulic acid esters are responsible for a yellow band seen on DEAE-Sephadex fractionation of the oleoresin. Two diterpene resin acids not previously found in pines and several resin acid artifacts were identified.

INTRODUCTION

The DEAE-Sephadex method for quantitative separation of lipophilic neutrals from acids [1] has been used extensively in the analysis of extractives and oleoresins, and of commercial products such as rosin and tall oil. In many of these samples, components are present that are displaced ahead of the more acidic fatty acids and diterpene resin acids, forming sharp, yellow- to brown-colored bands. The fortuitous presence of such materials has served as a natural internal indicator for monitoring the practical ion-exchange capacity of DEAE-Sephadex columns during analytical separations, thus assuring that the acids are not eluted with the neutral fraction. The component of ponderosa pine oleoresin that forms a sharp yellow band on the DEAE-Sephadex column has now been isolated and identified. In the course of this work, two diterpene resin acids not previously found in pines and several resin acid oxidation artifacts were also identified.

RESULTS AND DISCUSSION

DEAE-Sephadex yellow band (YB) material

During an analysis of oleoresins of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws), it was noted that a number of the oleoresins were bright yellow, as reported by Smith [2]. The intensity of this yellow color of an oleoresin appeared to correlate with the intensity of a weakly acidic yellow band (YB) seen on the DEAE-Sephadex column when separating the oleoresin [1] for GC analysis. Subsequent work, however, showed that the components comprising the YB material were colorless (the yellow color of YB on the basic DEAE-Sephadex column is the result of a bathochromic shift) and were not responsible for the yellow color of the oleoresin.

On chromatography of oleoresin on silica gel, the YB was found in a nearly colorless fraction eluted with toluene. This fraction was carefully methylated with diazomethane and the methylated fraction separated on DEAE-Sephadex [1]. The resin acids, which were the predominant material in the fraction, were eluted as methyl esters in the neutral fraction. The YB remained on the DEAE-Sephadex and was recovered by elution with carbon dioxide saturated solvent.

The UV spectrum of the YB material showed a maximum at 325 nm ($\varepsilon = 15\,100$) with a bathochromic shift to 377 nm (yellow) on addition of sodium methoxide. IR, NMR, MS and GC data indicated the YB to be a complex mixture of $C_{10}H_{15}$ and $C_{10}H_{17}$ monoterpene alcohol esters of hydroxymethoxycinnamic acid(s). The characteristics and complexity of the GC of YB supported this interpretation. Menthyl and bornyl ferulates and menthyl isoferulate were synthesized as models. Comparison of the chemical shifts of the aromatic protons in YB with those of the model compounds clearly showed that the ester was a 3-methoxy-4-hydroxycinnamate (ferulate). Because the YB was isolated from a methylated (CH_2N_2) fraction, the possibility existed that the isolated YB was derived from a dihydroxycinnamate (caffeate) precursor. However, caffeate esters are seen as broad bands on the DEAE-Sephadex columns and not as the narrow bands seen for ferulic and isoferulic acid esters. Thus, a complex mixture of ferulic acid esters of monoterpene alcohols are responsible for the yellow band seen on DEAE-Sephadex fractionation of ponderosa pine oleoresin.

This is the first report of terpene ferulates in pine oleoresin. Bornyl ferulate, however has been reported in *Verbesina* species [3, 4] and in liverworts [5]. Alkyl ferulates have been reported in the barks of *Pinus banksiana* [6], *P. roxburghii* [7], *P. silvestris* [8] and Siberian pine [9].

Resin acids

The resin acids of ponderosa pine oleoresin and wood extractives consist primarily of the usual abietane, pimarane and isopimarane-type resin acids. Riffer *et al.* [10, 11] reported the presence of a tetrahydroabietic acid in ponderosa pine heartwood extractives and stated that little if any sandaracopimaric acid is present in either

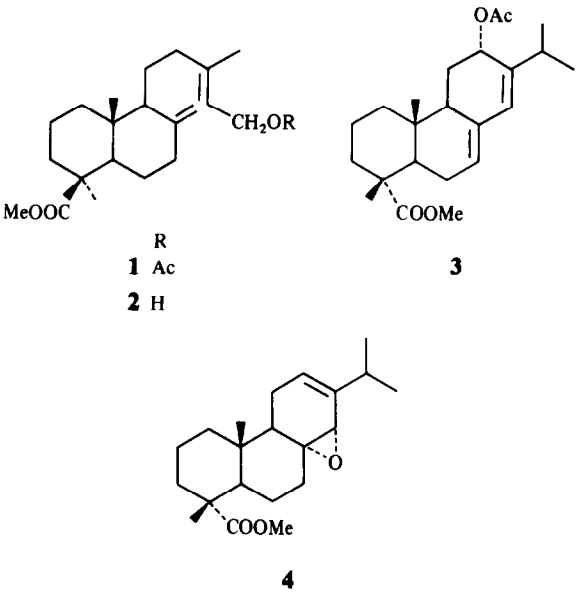
† Maintained in cooperation with the University of Wisconsin.

heartwood or sapwood extractives. The latter observation is contrary to other analyses [12, 13] of ponderosa pine oleoresins and extractives. Examination of our quantitative analytical data for the resin acids of ponderosa pine oleoresins as obtained by GC [14] with polar and nonpolar capillary columns confirmed that sandaracopimaric acid was an important (*ca* 5%) component. No evidence was found for the presence of the tetrahydroabietic acid.

In the course of this work, we observed unidentified components in the gas chromatograms of the methylated resin acids of ponderosa pine (Table 1), as have others [11–13]. Our unidentified resin acids were separated into the toluene-ether fractions on chromatography of the oleoresin on silica gel. The 9:1 toluene–ether eluate was enriched in the major unidentified compound. DEAE-Sephadex separation followed by silica gel chromatography of the methylated resin acids provided a single compound of 99% purity (GC). The spectral data are in accord with those reported for methyl isocupressate **1** [15] except that an additional acetyl group is present. The rotation agrees with that reported for methyl acetylisocupressate [methyl 15-acetoxy-8(17),*E*-13-labdadien-19-oate, **2**] isolated from *Araucaria cunninghami* [16]. Acetylisocupressic acid has been identified in *Araucaria* oleoresins [16, 17] and isocupressic acid has been shown to be present in *Pinus sibirica* [15]. The isocupressic acid in *P. sibirica* is probably an artifact from saponification of the acetate during isolation. Indeed, acetylisocupressic acid is partially hydrolysed during the DEAE-Sephadex separation of oleoresins for analysis.

The resin acids from the 7:3 toluene–ether fraction were chromatographed as the methyl esters on silica gel. Elution with petrol–ether gave a component having MW 374 and a UV spectrum typical of abietic acid. The NMR spectrum matched the data reported [18] for methyl 12α-acetoxyabietate (methyl 12α-acetoxyabieta-7,13-dien-18-oate, **3**). Further elution gave an unidentified, unstable acetate (MW 374).

The resin acids from the 1:1 toluene–ether fraction were also chromatographed on silica gel as methyl esters. Elution with petrol (9:1) gave methyl 8α,14α-epoxy-13-abieten-18-oate (**4**), a compound found in the air oxidation product of levopimaric acid [19]. Elution with petrol–ether (3:2) gave a component (probably a hydroxyabietadienoic acid methyl ester) that was unstable. Continued elution gave methyl 12α-hydroxyabietate. The 12α-hydroxyabietic acid could be either a natural product, an artifact from hydrolysis of 12α-acetylabietic acid, or an oxidation artifact of levopimaric acid [19] or another abietadienoic acid; the epoxylevopimaric acid and the unidentified hydroxy acid are probably oxidation artifacts.



EXPERIMENTAL

Yellow xylem oleoresins from several ponderosa pine trees were furnished by Dr. Harvey Alexander of the University of New Mexico. Common instrument parameters were: ^{1}H NMR 60 MHz, $CDCl_3$ solvent; UV MeOH solvent; EIMS at 70 eV with temp-prog probe, (supplementary CIMS were also obtained).

Yellow band (YB) material. Part of the resin acids was removed by filtration as crystals from a viscous, cold soln of *ca* 150 g of the oleoresin containing 10 ml MeOH. A 20-ml portion of the 110 ml filtrate was partitioned using 1 l. petrol and 800 ml

Table 1. Resin acid composition in three representative ponderosa pine oleoresins*

% of total resin acids									
Pimaric	Sandaraco-pimaric	Levopimaric	Palustric	Isopimaric	Abietic	Dehydro-abietic	Neobietic	1†	3
5.0	1.3	31.5	17.5	9.8	13.5	1.6	17.5	1.4	0.8
5.2	1.3	31.7	15.5	8.9	12.8	5.5	16.3	1.8	0.9
7.4	1.3	32.7	18.5	10.2	11.3	6.3	10.4	1.3	tr

*These analyses are from data for a large number of ponderosa pine oleoresins as part of a joint effort with Prof. R. Cates and H. Alexander of the Department of Biology, University of New Mexico, Albuquerque, in their study of interrelation of oleoresin characteristics and bark beetle attack on trees. Analysis was by GC using BDS and SP-2100 glass capillary columns [14]. The resin acids were separated from the oleoresin using the DEAE-Sephadex procedure [1] and methylated with CH_2N_2.

†These and all the oleoresin samples of the study with Cates and Alexander also contain about 0.1% isocupressic acid, identified by GC retention comparison with an authentic sample.

Table 2. Physical characteristics of model ferulic acid esters

Ester (MW)	^{1}H NMR* monoterpene methyls (δ)	MS m/z (%) [M]$^+$	194	177	GC† R_t (min)
Bornyl ferulate (330)	0.88, 0.88, 0.93	28	12	100	19.06
Menthyl ferulate (332)	0.80 (*d*), 0.92 (*d*, isopropyl)	12	100	21	17.96
Menthyl isoferulate (332)	0.79 (*d*), 0.91 (*d*, isopropyl)	27	100	31	20.96

*For ferulic hydrogen shifts, see ref. [6]; cf. bornyl caffeate [4].

†15 m DB-1 (bonded methyl silicone, 0.1 μM) fused silica capillary column, 190°, He flow 30 cm/sec; R_t Me ferulate = 1.0 min, R_t Me pimarate = 4.48 min.

Me_2CO–MeOH–1% aq. Na_2SO_4 (2:1:1) analogous to the Saltsman–Kuiken procedure [20] for tall oil in kraft black liquor. The yellow petrol extract, which contained most of the YB material, was then chromatographed on silica gel using petrol, toluene, Et_2O and MeOH to give 22 fractions: Fr. 1–5 (petrol–toluene, 2:1), 6–8 (toluene), 9–19 (toluene–Et_2O, from 19:1 to 1:4), 20 (Et_2O) and 21–22 (MeOH). Each fraction was monitored for the YB material by chromatography on a short (a Pasteur pipet) column of DEAE-Sephadex. The YB material was found in Fr. 7. The 4.2 g of Fr. 7 was methylated for 5 min with a slight excess of CH_2N_2 in Et_2O–MeOH (9:1). The YB material was removed from the methylated fraction using DEAE-Sephadex. The resin acid Me esters passed through the column and the retained YB material (8 mg) was recovered using CO_2-satd solvent. UV λ_{max} 325 nm (ε = 15000) with bathochromic shift to λ_{max} 377 nm on addition of NaOMe. IR ν_{max}^{film} cm^{-1}: 3420 (phenol), 1710 (ester), 1634 and 984 (*trans*-olefin), 1600 and 832. MS m/z (rel. int.): 328 [M]$^+$ (7), 330 [M]$^+$ (6). 194 [3-OMe, 4-OH-C_6H_3–CH=CH–COOH]$^+$ (59). 177 [194 – OH]$^+$ (100), 145 (20), 134 (27), 136 (10), 119 (26), 121 (10), 105 (7), 107 (5), 91 (22), 93 (20), and 79 (8). The ^{1}H NMR spectrum was superimposable with that of synthesized 1-bornyl ferulate except for a complex alkyl region in the NMR of YB. GC of YB (Table 2) showed numerous components (the largest being 13% at R_t = 20.34 min) in the region for model monoterpene ferulates.

Attempts were made to synthesize model hydroxymethoxycinnamic acid esters using dicyclohexylcarbodiimide as was done in the synthesis of hexacosyl ferulate [7], but the products appeared to be predominately the *N*-acylurea adducts rather than the sought-after esters even when *p*-toluene sulfonic acid was added [21]. Bornyl ferulate, menthyl ferulate and menthyl isoferulate were prepared by *p*-toluene sulfonic acid catalysed esterification. All esters were purified by silica gel chromatography and DEAE-Sephadex for comparative spectral purposes (Table 2).

Resin acids. A small portion of each fraction from the original silica gel column was analysed by GC [1] for unidentified resin acids (Table 3). Three fractions were selected for further study. The resin acids from each of these fractions were separated from neutral components by DEAE-Sephadex and were then methylated with CH_2N_2.

The 140 mg of methylated resin acids from one-half of Fr. 11 were chromatographed on silica gel. Elution with petrol–Et_2O (19:1) gave 110 mg of oily Me acetylisocupressate (**1**): $[\alpha]_D^{20}$ = + 50.5° ($CHCl_3$, *c* 1.2), lit. [15] + 51°; MS m/z (rel. int.) 376 [M]$^+$ (0.3), 316 [M – AcOH]$^+$ (18), 121 (100). ^{1}H NMR δ5.32 (1H, *t*, *J* = 7 Hz, H-14), 4.85 and 4.52 (2H, C=CH_2), 4.58 (2H, *d*, *J* = 7 Hz, CH_2OAc), 3.60 (3H, *s*, COOMe), 2.03 (3H, *s*, OOCMe), 1.70 (3H, *s*, H-16), 1.18 (3H, *s*, H-18) and 0.52 (3H, *s*, H-20).

The 260 mg of methylated resin acids from Fr. 14 were chromatographed on silica gel. Elution with petrol–Et_2O (19:1) gave 26 mg of Me 12α-acetoxyabietate (**3**) as an oil: MS m/z (rel. int.): 375 [M + 1]$^+$ (2), 314 [M – AcOH]$^+$ (9), 211 (12), 146 (100), 131 (24), 121 (14). NMR agreed with lit. [18]. Further elution with petrol–Et_2O (19:1) gave an unstable (GC, and during storage in $CDCl_3$ for several days) acetate of a resin acid Me ester: MS m/z (rel. int.): 374 [M]$^+$ (0.1), 314 [M – AcOH]$^+$ (40), 254 [$C_{19}H_{26}$]$^+$ (100). UV λ_{max} 250 nm (ε *ca* 18000). ^{1}H NMR: δ6.14 and 5.92 (2H, *s* and *br s*, respectively, olefinic H), 3.67 (COOMe), 2.02 (3H, *s*, OOCMe), 1.78 (6H, *s*, C=CMe_2), 1.22 (3H, *s*, H-19), and 0.77 (3H, *s*, H-20).

Table 3. GC retention data for methyl esters of minor and artifact resin acids of ponderosa pine oleoresin

Resin acid	r_{Pim}*
8α,14α-Epoxy-13-abieten-18-oic (**4**)	1.79
Isocupressic (**2**)	2.16
Unidentified hydroxy resin acid	2.23
12α-Hydroxyabietic	2.91
Acetylisocupressic (**1**)	3.0
12α-Acetoxyabietic (**3**)	3.30
Unidentified acetoxy resin acid	3.66

*DB-1 (methyl silicone) WCOT column and conditions as per Table 1. R_t relative to Me pimarate (r_{Pim}).

The 240 mg of methylated resin acids from Fr. 16–17 were chromatographed on silica gel. Elution with petrol–Et_2O (9:1) gave 22 mg Me 8α,14α-epoxy-13-abieten-18-oate (**4**): MS m/z (rel. int.): 332 [M]$^+$ (86), 317 [M – Me]$^+$ (42), 121 (100). ^{1}H NMR δ5.43 (1H, *m*, H-12), 3.67 (3H, *s*, COOMe), 2.98 (1H, *d*, *J* = 2 Hz, H-14), 1.18 (3H, *s*, H-19), 1.06 (6H, *d*, isopropyl Me's), and 0.80 (3H, *s*, H-20), cf. [22]. ^{1}H NMR (CCl_4) δ5.32, 3.61, 2.76, 1.12, 1.05, and 0.78, cf. [19]. Elution with petrol–Et_2O (3:2) gave a compound that was unstable on recovery from $CDCl_3$–D_2O. ^{1}H NMR: δ5.72 (1H, *s*), 5.45 (1H, *m*), 3.65 (3H, *s*, COOMe), 1.32 (6H, *s*, 2 × Me), 1.16 (3H, *s*, Me), and 0.89 (3H, *s*, Me). MS m/z (rel. int.): 332 [M]$^+$ (12). IR ν_{max}^{film} 3440 cm^{-1} (OH). Further elution with petrol–Et_2O (3:2) gave an impure material that on additional silica gel chromatography provided 9 mg of oily Me 12α-hydroxyabietate (**2**). ^{1}H NMR: δ5.85 (1H, *s*, H-14), 5.48 (1H, *m*, H-7), 4.23 (1H, *m*, H-12), 3.64 (3H, *s*, COOMe), 1.27 (3H, *s*, H-19), 1.10, 1.08 (6H, *dd J* = 7 Hz, isopropyl Me's), 0.82 (3H, *s*, H-20); cf. [18] but the δ5.58 reported for H-14 in the Experimental section should read δ5.85 [W. Herz, personal communication].

REFERENCES

1. Zinkel, D. F. and Rowe, J. W. (1964) *Analyt. Chem.* **36**, 1060.
2. Smith, R. H. (1977) *Monoterpenes of Ponderosa Pine Xylem Resin in Western United States.* USDA Forest Service Tech. Bull. 1532, p. 35.
3. Box, V. G. S. and Chan, W. R. (1975) *Phytochemistry* **14**, 583.
4. Bohlmann, F. and Zdero, C. (1976) *Phytochemistry* **15**, 1310.
5. Suire, C., Asakawa, Y., Toyota, M. and Takemoto, T. (1982) *Phytochemistry* **21**, 349.
6. Rowe, J. W., Bower, C. L. and Wagner, E. R. (1969) *Phytochemistry* **8**, 235.
7. Chatterjee, A., Dhara, K. P., Rej, R. N. and Ghosh, P. C. (1977) *Phytochemistry* **16**, 397.
8. Norin, T. and Winell, B. (1972) *Acta Chem. Scand.* **26**, 2297.
9. Leont'eva, V. G., Gromova, A. S., Lutskii, V. I., Modonova, L. D. and Tyukavkina, N. A. (1974) *Khim. Prir. Soedin* **2**, 240.
10. Riffer, R. and Anderson, A. B. (1966) *Holzforschung* **20**, 36.
11. Anderson, A. B., Riffer, R. and Wong, A. (1969) *Phytochemistry* **8**, 873.
12. Joye, N. M., Jr. and Lawrence, R. V. (1967) *J. Chem. Eng. Data* **12**, 279.
13. Conner, A. H., Diehl, M. A. and Rowe, J. W. (1980) *Wood Sci.* **12**, 183.
14. Foster, D. O. and Zinkel, D. F. (1982) *J. Chromatogr.* **248**, 89.
15. Lisina, A. I., Yasnetskaya, S. M. and Pentagova, V. A. (1972) *Khim. Prir. Soedin* **3**, 300.
16. Caputo, R., Dovinola, V. and Mangoni, L. (1974) *Phytochemistry* **13**, 475.
17. Caputo, R., Mangoni, L., Monaco, P. and Previtera, L. (1974) *Phytochemistry* **13**, 471.
18. Herz, W., Wahlborg, H. J., Lloyd, W. D., Schuller, W. H. and Hedrick, G. W. (1965) *J. Org. Chem.* **30**, 3190.
19. Enoki, A. and Kitao, K. (1974) *Mokuzai Gakkaishi* **20**, 600.
20. Saltsman, W. and Kuiken, K. A. (1959) *Tappi* **42**, 873.
21. Holmberg, K. and Hansen, B. (1979) *Acta Chem. Scand.* **B33**, 410.
22. Herz, W., Ligon, R. C., Kanno, H., Schuller, W. H. and Lawrence, R. V. (1970) *J. Org. Chem.* **35**, 3338.