Phytochemistry, 1975, Vol. 14, pp. 2233–2235. Pergamon Press. Printed in England.

VOLATILE CONSTITUENTS OF *CUPRESSUS STEPHENSONII* HEARTWOOD

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(*Received* 17 *March* 1975)

Key Word Index—*Cupressus stephensonii*; Cupressaceae; volatile heartwood extractives; terpenoids; carvacrol; tropolones; methyl 4-*trans*-dehydrogeranate.

Abstract—Nonpolar volatile extractives of *Cupressus stephensonii* heartwood amounting to 1·3% (drywood weight basis) were analyzed for their constituents and the main component was found to be carvacrol (78%). Tropolones (17%) were composed largely of β-thujaplicin and nootkatin with γ-thujaplicin in secondary quantities. Acids were low (1·7%). Neutral constituents (3·4%) contained α-pinene (8%), 4-terpinenol (27%), and methyl 4-*trans*-dehydrogeranate (45%).

INTRODUCTION

Cupressus stephensonii C. B. Wolf is one of the rarest cypress species, since it is known in the United States from only a single grove on the southwest side of Cuyamaca Peak in San Diego County of California [1] and from one site in Baja California [2]. It is considered to be closely allied to the *C. arizonica* group, and in fact a recent revision [3] transfers it to *C. arizonica* var. *stephensonii*. Ecologically and morphologically, however, *C. stephensonii* greatly resembles *C. forbesii*, a stand of which occurs on Guatay Mountain only 10 km to the south at a similar elevation. The chief superficial differences between the two species are the presence in *C. stephensonii* of harsher foliage and active foliar resin glands, the foliar resin producing a grayish-green cast which contrasts with the bright green foliage of *C. forbesii*. These species share a generally low spreading crown form, exfoliating bark and the closed cone habit in which seed dispersal is deferred for many years after maturation of the cones. Both occur as small, nearly pure stands in the midst of extensive chaparral vegetation, and appear to respond similarly to fire and competition from the associated plants. There are also some similarities in the composition of the essential oils of the foliage [4]. However, genetic interchange between the species seems unlikely in view of the six month separation of pollen shedding, *C. forbesii* doing so in winter and *C. stephensonii* in mid-summer [5].

RESULTS AND DISCUSSION

The heartwood sample of *C. stephensonii* came from an old log and was collected in Cuyamaca Peak grove. Acetone extraction of the sawdust resulted in separation of 5·0% (drywood weight basis) of extractives; these were composed of 73·7% of phlobaphenes and related polar phenols insoluble in light petrol, and of 26·3% of less polar, largely distillable materials composed of phenols, tropolones, organic acids and neutrals.

Phenols represented 70·0% of volatiles, and were composed of a 99% pure carvacrol with the rest of materials in amounts too small for identification. Carvacrol has been identified on many occasions in heartwood of various *Cupressus* species and other genera of Cupressaceae [6]. Organic acids represented a minor (1·7%) fraction and were disregarded.

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Tropolones, which represented 16·9% of the nonpolar materials, were analyzed by paper chromatography [7–9]. To obtain some idea about tree-to-tree variability, three additional heartwood samples obtained from different trees in the area were processed for tropolones and analyzed separately. The main difference in tropolone composition between the *C. arizonica* group of cypresses (comprising acc. to Wolf *C. arizonica*, *C. glabra*, *C. nevadensis*, *C. montana* and *C. stephensonii*—the latter two not investigated for tropolones) and *C. forbesii* group of cypresses (comprising *C. forbesii* and *C. guadalupensis*) is the presence of γ-thujaplicin as a primary or secondary constituent in the latter group vs its absence or (less commonly) presence in secondary quantities in the former group [9,10]. On the basis of tropolones, *C. stephensonii* fell between the two cypress groups; all four trees contained nootkatin (identified by IR) and β-thujaplicin as main constituents, with γ-thujaplicin as a secondary constituent in three samples, and in traces in one sample.

The neutral fraction (3·4%) gave a series of peaks in GLC and was composed mostly of 8% α-pinene (identified by its characteristic retention time in GLC), 27·0% 4-terpinenol (identified by IR), and 45·0% of methyl 4-*trans*-dehydrogeranate. α-Pinene has been identified only in wood of a few *Chamaecyparis* species, while 4-terpinenol apparently has not been reported in wood of any species of this family; both compounds are, however, common in Cupressaceae volatile oils derived from foliage [6].

Methyl 4-*trans*-dehydrogeranate (methyl 3,7-dimethylocta-2-*trans*: 4-*trans*: 6-trieonate) was isolated by preparative GLC and identified by its UV, IR, mass, and NMR [11–14] spectra. Depending upon *cis/trans* isomerism of double bonds, four stereoisomers of 3,7-dimethylocta-2,4,6-trienoic acid are possible. The all-*trans* acid has been isolated from the wood of both *Callitris glauca* (as such) [15] and *Callitropsis araucarioides* (as the geraniol ester) [16], and has been synthesized on several occasions [11,17–22]. 4-*trans*-Dehydronerylic acid (2 *cis*) has been obtained only synthetically [11,12].

Monoterpenoid acids are common in Cupressaceae and include, other than 4-*trans*-dehydrogeranic acid, thujic acid, dihydrothujic acid (shonanic acid), chamic acid, chaminic acid, and citronellic acid [6].

EXPERIMENTAL

A 437 g portion of *C. stephensonii* heartwood sawdust (drywood weight) was exhaustively extracted with acetone. Non-polar constituents were then removed from the distn residue of the extract by repeated extractions with light petroleum and separated into neutrals (insoluble in aq NaOH), phenols (acidic fraction insoluble in aq NH_3), tropolones (fraction of acids precipitated from aqueous ammonia as copper complexes) [9], and stronger acids (pptd by acidification with mineral acid). Phenols were analyzed by analytical GLC using 3·2 mm o.d. × 3·0 m 1·0% Silicone Dow 550 on Chromosorb P 60/80 column at 160°, with carvacrol identified by IR (neat) using the entire fraction. Tropolones were analyzed by paper chromatography as before [9], with nootkatin identified by IR (KBr disc) as copper complex, obtained by MeOH crystallization. Neutrals were analyzed by analytical GLC using with α-pinene a 3·2 mm o.d. × 3·0 m. 10% 1,2,3-*tris*-(2-cyanoethoxy) propane on Chromosorb P 100/120 column at 72°, and with oxygenated terpenoids a 3·2 mm o.d. × 9·25 m. 1·0% Silicone OV-17 on Chromosorb G 100/120 column at 152°; preparative GLC separations of oxygenated terpenoids were performed using a 6·4 mm o.d. × 18·4 m 1·5% Silicone OV-17 on Chromosorb G 100/120 column at 200°. IR spectra were taken on Perkin–Elmer 457 (beam condenser with Barnes micro 0·5 mm cell), NMR spectra on Varian 60 mc EM 360, and UV spectra on Beckman Acta III instrument.

Spectral and GLC data for methyl 4-trans-dehydrogeranate. UV (EtOH): 311 nm; log ε = 4·54. NMR (CCl_4): δ = 1·90 (6·0); 2·35 (3·0); 3·70 (3·0); a multiplet at δ = 5·70, 5·82, 6·00 6·25, 6·60, 6·77–6·86, 7·00 (total intensity 4·0), identical to the published spectrum [12]. MS: 180 (parent), 165 (M-Me), 154, 149 (M-OMe); 125, 121 (M-CO_2Me), 105, 95. IR (In CCl_4/CS_2, 4000–600 cm^{-1}, in order of decreasing intensity, *s*-shoulder): 1150, 1600, 1710, 1238, 1430, 1516, 1345, 952, 1355, 1440*s*, 2940, 2920, 2910*s*, 1385, 1033, 1182, 878, 1375*s*, 1270, 2983, 2990*s*, 980, 823, 2845, 1635, 3040, 920, 855, 735. GLC *retention times*. Carvacrol, 3·21 to *p*-cresol; methyl 4-*trans*-dehydrogeranate, 3·47 to bornyl acetate.

Acknowledgements—We are indebted to Mr. Karel Snajberk for his help in conducting some of the experiments, to Mr. I. Papiashivili, Division of Natural Science, University of California, Santa Cruz, for determination of mass spectra, and to Mr. James Popenoe for assistance in the collection of specimens.

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