(C) *Methyl imbricatoloate* (II). The oily material from the acetone elution of the $AgNO_3$–Al_2O_3 column was chromatographed on silica using ether–benzene (1:15), yielding II in 99+% purity (established by GLC and TLC): $[\alpha]_D^{20}$ +39·8° (*c* 1·4, $CHCl_3$) (lit.[7] +45·3°). The NMR and IR of II and an authentic sample of methyl imbricatoloate were identical, as was the GLC on SE-30/EGiP.

II was oxidized to the aldehyde (I) using dicyclohexylcarbodiimide and dimethyl sulfoxide.[14] The product, after purification by chromatography on silica, showed a CD of $[\theta]_{306}^{25}$ — 700°. This product and I had identical spectral and GLC retention characteristics.

(D) *Isolation of a new diterpene alcohol.* A new diterpene alcohol was isolated by preparative GLC of cortex oleoresin using a column containing 5% Versamid 900 (70–80 ABS) at 220°. The product was 95% pure as determined by GLC on 5% Versamid 900 and 3% SE-30 columns. The alcohol had the following spectral characteristics: λ_{max} (isooctane) 240 nm (ϵ 26,200); ν_{max}^{film} 3580 (s) and 3430 (br) (hydroxyl), 1470, 1460, 1390, 1377, 1130, 1085, 975 and 940 cm^{-1}; NMR ($CDCl_3$) δ 0·90 (s, —CH_3), 0·91 (d, J = 7, isopropyl), 1·22 (s, —CH_3), 1·82 (s, —CH_3 gem to hydroxyl), 6·62 (d, J = 16, olefinic H), 5·66 (q, J = 16 and J = 10, one olefinic H) and 5·38 (t, J = 6, one olefinic H); mass spectrum *m/e* 290 (23%, M^+), 272 (18%), 257 (7%), 245 (8%), 235 (6%), 221 (6%), 195 (52%), 177 (100%) and 148 (37%).

(E) *Composition of slash pine cortex oleoresin.* Analysis of a typical slash pine cortex oleoresin using DEAE-Sephadex showed the presence of 71% acidic and 29% neutral materials. Chromatography of the methylated (CH_2N_2) oleoresin on neutral alumina (activity III) produced pure I, identical in all respects with the methyl imbricataloate as isolated from the needles.

Acknowledgements—We thank Dr. K. Bruns, Chemisch Therapeutische Gesellschaft, Düsseldorf, W. Germany, for the generous samples of methyl imbricatoloate and imbricadiol.

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Phytochemistry, 1971, Vol. 10, pp. 3292 to 3294. Pergamon Press. Printed in England.

THE *n*-HEXANE-SOLUBLE COMPONENTS OF *PSEUDOTSUGA MENZIESII* BARK*

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(*Received* 6 *May* 1971, *in revised form* 2 *July* 1971)

Abstract—β-Sitosterol and campesterol were identified in the *n*-hexane-soluble fraction of Douglas-fir bark and the presence of other 'steroid-like' compounds was demonstrated. GLC and MS showed the presence of terpenes.

* Presented at the Wood Extractives Symposium held as part of the 161st American Chemical Society Meeting, March 28–April 2, 1971, in Los Angeles under the sponsorship of the Division of Cellulose, Wood and Fiber Chemistry.

† Part is taken from the thesis submitted by Henry Hai-Loong Fang in partial fulfillment of the requirements for the MS degree, Oregon State University, Corvallis, Oregon (1971).

THE BARK of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] is noted for its *n*-hexane and benzene extractives—generally termed 'waxes'.[1–7] We partially resolved the *n*-hexane soluble components by column chromatography and recovered a prominent 'yellow band'. Improved resolution of the 'yellow band' components by TLC and GLC indicated the presence of as many as 19 compounds.

We present evidence that some of the above compounds are sterols and terpenes. Authentic β-sitosterol migrated the same distance on TLC as a spot from the yellow band in each of 14 different solvent systems. GLC resulted in peaks with the same retention times as β-sitosterol and campesterol on 4 individual liquid phases.

GLC–MS verified the presence of β-sitosterol and campesterol and indicated the presence of terpenes. An MS scan at the end of the GLC peak for β-sitosterol trimethylsilyl ether showed an *m/e* cracking pattern in good agreement with that reported by Eneroth, Hellstrom and Ryhage.[8] The base peak at *m/e* 129, which is a characteristic fragment for a 5-en-3-ol sterol trimethylsilyl ether,[9] was very intense as was a characteristic peak at *m/e* 255.

The MS scans taken at the GLC peak attributed to campesterol were not as clear as those for β-sitosterol. However, the characteristic peaks at *m/e* 129 and 255 were very distinct. Difficulty in obtaining a good spectrum for campesterol in the presence of β-sitosterol was also experienced by Eneroth, Hellstrom and Ryhage.[8]

The evidence for terpenes is based on GLC and MS data. MS scans of early peaks to elute from the GC showed fragmentation patterns characteristic of terpenes.[10–13]

EXPERIMENTAL

n-Hexane extraction. Bark samples, collected near Corvallis, Oregon were ground to pass a 10-mesh screen and extracted with *n*-hexane for 48 hr. The solvent was evaporated leaving a 'wax-like' solid.

Column chromatographic separation. A portion (2·00 g) of the above solid was dissolved in $CHCl_3$ (15·0 ml) and fractionated on a Silica Gel G column using $CHCl_3$–*n*-hexane (3:1 v/v) as developer. Of the seven bands observed under UV light, the two fastest moving were stored, and the next two (fluoresced bright-blue and light-bluish green) were collected together (appeared as a 'yellow band' in ordinary light).

TLC separation. The 'yellow band' eluate was subjected to TLC on Silica Gel G simultaneously with authentic β-sitosterol using the following solvent systems: $CHCl_3$–CCl_4 (6:1 v/v); $CHCl_3$–CCl_4 (1:1 v/v); $CHCl_3$–CCl_4 (1:4 v/v); $CHCl_3$–*n*-C_6H_{14} (4:1 v/v); C_6H_6–*n*-C_6H_{14} (2:1 v/v); Et_2O–*n*-C_6H_{14} (3:1 v/v); Et_2O–*n*-C_6H_{14} (1:4 v/v); $CHCl_3$–C_6H_6 (6:1 v/v); $CHCl_3$–C_6H_6 (1:1 v/v); $CHCl_3$–C_6H_6 (1:4 v/v); C_6H_6–MeOH–HOAc (45:8:4 by vol.); *n*-C_6H_{14}–Et_2O–HOAc (70:30:1 by vol.); *n*-C_6H_{14}–Et_2O–HOAc (85:15:1 by vol.); $CHCl_3$–Et_2O–formic acid (5:4:1 by vol.). The spots were detected by exposure to iodine vapors.

GLC separations. The instrument used was a Hewlett–Packard 5751B with flame ionization detectors. The following column systems were used: 10% SE-52 on 60/80 mesh Gas-Chrom W; 3% OV-17 on 100/120

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mesh Gas-Chrom Q; 10% UC W-98 on 80/100 mesh Gas-Chrom S; 3% Hi-Eff 8 BP on 100/120 mesh Gas Chrom Q. All columns were stainless steel, 1/8 in. o.d. and 6 ft long. Typical conditions were: injection port 235°, detector 255°, column temp. held at 148° for 10 min, then programmed at 2°/min to 300°; helium flow rate 28 ml/min. In most cases the 'yellow band' eluate was injected directly into the GC but in some instances it was silylated[14] prior to injection.

Authentic samples of β-sitosterol and campesterol were subjected to GLC for comparison purposes, and small quantities were added to the 'yellow band' eluate for 'peak enhancement' studies.

GLC–MS. An F&M 810 GC was hooked in tandem to an Atlas MAT CH-4, Nier-type single focusing, 70 eV, MS. The 'yellow band' eluate was silylated,[14] and injected into the GC. An MS scan at the β-sitosterol peak showed *m/e* data at 486, 396, 381, 357, 255 and 129. A scan at the campesterol peak resulted in prominent *m/e* fragments at 129 and 255. MS scans of early peaks to elute from the GC showed prominent *m/e* fragments at 136, 121, 112, 93, 91, 80, 70, 69, 55, 43 and 41.

Acknowledgements—We wish to acknowledge Dr. Leonard M. Libbey, Associate Professor, Food Science and Technology, Oregon State University, for his assistance with the mass spectra. We also gratefully acknowledge financial support from the Environmental Protection Agency, Bureau of Solid Waste Management, Grant Number EC-00276-03.

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Phytochemistry, 1971, Vol. 10, pp 3294 to 3295 Pergamon Press. Printed in England.

ANGIOSPERMAE DICOTYLEDONAE

ANONACEAE

DITERPENES FROM *ANNONA SENEGALENSIS*

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(*Received* 9 *December* 1970)

Abstract—The bark of *Annona senegalensis* Pers. has yielded kauran-16α-ol, kaur-16-en-19-oic acid, kauran-19-al-17-oic acid, and 19-norkauran-4α-ol-17-oic acid.

Annona senegalensis Pers. (Yoruba—abo) is a shrub common in the West African savannah. The bark is used in Nigeria for the treatment of convulsions in children.

Light petroleum extraction of the bark gave acidic and neutral fractions, the latter gave a solid m.p. 217°, which was identified as kauran-16α-ol[1](I). The acid fraction after chromatography over silica gel gave mainly kaur-16-en-19-oic acid[2](II) together with a little kauran-19-al-17-oic acid (IIIa).[2]

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