Major flavonoid of Pityrogramma calomelanos. Pale silvery plates of 2′,6′-dihydroxy-4′-methoxydihydrochalcone (278 mg), m.p. 175–177°, crystallized from methanol after workup of an ether extract of dried fronds of *P. calomelanos* (30·5 g). NMR spectrum of the trimethylsilyl ether (except for a free 6′-OH) in CCl_4 (ppm, δ); five B-ring protons (H-2,3,4,5,6) appeared at 7·19(s); the two A-ring protons (H-3′,5′) came at 5·97(s); the 4′-methyl group at 3·72(s), the four C-ring protons at 2·92(s), and the 6′-hydroxyl proton at 11·02(s).

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GYMNOSPERMAE

PINACEAE

DITERPENOIDS OF CONES FROM TWO *CEDRUS* SPECIES

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Abstract—Ether extracts from the cones of *Cedrus atlantica* (Endl.) Carr and *C. libani* A. Rich have yielded resin acids of the abietane and pimarane types and related diterpenoids. The extract composition differed from that of the wood for both species but showed characteristic relationships to wood extractives of other species in the Pinaceae.

CHARACTERISTIC wood constituents of species in the family Pinaceae are resin acids of abietane and pimarane types. Thus such acids have been found in the wood of *Abies*,[1–3] *Larix*,[4–6] *Picea*,[7–11] *Pinus*[12] and *Pseudotsuga*.[13] Resin acids have also been found in the bark of Pinaceae species (*Picea*[14] and *Pinus*[15]). The wood and wood oil of different species belonging to the genus *Cedrus* have been studied by various investigators.[16–18] Although

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the wood of these species is rich in terpenoids (mainly sesquiterpenoids of himachalane type), no resin acids have been detected.

From the cones of *C. atlantica* (Endl.) Carr and of *C. libani* A. Rich a viscous ether-soluble oleoresin is exuded. In the present investigation we have studied the ether extracts of the cones from these two *Cedrus* species. For both species the extract composition was found to be different from that of the wood. The cone extracts contain large quantities of the common resin acids of abietane and pimarane types and related diterpenoids all of which are characteristic Pinaceae constituents.

RESULTS AND DISCUSSION

The resin acids present in the ether extract of the cones of *C. atlantica* are: sandaracopimaric, abietic, isopimaric, levopimaric, palustric, dehydroabietic and neoabietic acid. Other constituents are fatty acids, fatty alcohols, esterified resin and fatty acids. Small amounts of hydrocarbons were also detected. A significant amount of 13-epimanool was isolated together with smaller amounts of abieta-8,11,13-trien-7-one. To our knowledge the latter compound is a new natural product.* However, it may be an artefact formed by autoxidation of the secreted oleoresin.

The resin acids present in the cones of *C. libani* are: sandaracopimaric, abietic, neoabietic, dehydroabietic, levopimaric and palustric acid. Other constituents are fatty acids, esterified resin and fatty acids, alkanes, abieta-8,11,13-triene and abieta-7,13-diene. The optical rotation of abieta-8,11,13-triene ($[\alpha]_D$ + 52°, c 1·0 in $CHCl_3$) differed markedly from that previously reported (*lit.*[20] $[\alpha]_D$ + 5°, c 2·8 in $CHCl_3$; lit.[21] $[\alpha]_D$ + 0·49°; c 1·0 in MeOH). Rowe[19] observed a rotation of + 54° for abieta-8,11,13-triene isolated from the bark of western white pine (*Pinus monticola* Dougl. ex. D. Don).

13-Epimanool could not be detected in the extract from the cones of *C. libani*, whereas the amounts of alkanes and terpene hydrocarbons were much larger than in the cones of *C. atlantica*.

Chemically, the *Cedrus* species are unique in the Pinaceae because of their characteristic himachalane wood constituents.[16–18] Furthermore, resin acids and related diterpenoids, otherwise characteristic in the family, have not been found in the wood. The present result shows that the composition of the oleoresin from *Cedrus* cones exhibits a characteristic Pinaceae pattern.

The wood of most species in the Pinaceae have resin canals. However, such canals are normally not found in species belonging to the genus *Cedrus*. The presence of resin acids and related diterpenoids in the wood may thus be restricted to species possessing resin canals. *Tsuga* is another genus which does not have resin canals in its wood and which also lacks resin acids.

EXPERIMENTAL

Light petroleum refers to the fraction with boiling range 40–60°. NMR data are given in τ units (solvent, $CDCl_3$; internal standard, TMS).

* After the completion of this work Rowe[19] has communicated the occurrence of abieta-8,11,13-trien-7-one in the bark of *Pinus monticola* Dougl. ex. D. Don.

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Cedrus atlantica (Endl.) Carr. The ether extract (56·0 g) from fresh cones (1·11 kg) collected in Oxford, England, between October and December was separated into neutral (22·0 g) and acidic (34·0 g) fractions in the usual way. Part of the acid fraction was methylated (ethereal CH_2N_2) and analysed by GLC (1% E 301) and argentative TLC.[22] The following acids were detected: sandaracopimaric (54·3% of total acids), abietic, (32·5%), isopimaric (3·7%), levopimaric/palustric (3·6%), dehydroabietic (2·2%) neoabietic acid (1·1%) and fatty acids (2·6%) with palmitic acid as main component.

Part (10·5 g) of the neutral fraction was saponified with ethanolic NaOH (2M). The acid fraction obtained by the saponification consisted of resin and fatty acids with a composition similar to that of the free acids. The constituents of the unsaponifiable part (6·8 g) were separated on a silica gel column. Fraction 1 (0·12 g) which was eluted by light petroleum, was shown by its IR spectrum and TLC properties to consist of alkanes and terpenoid hydrocarbons among which abieta-8,11,13-triene could be detected. Ether (8%) in light petroleum was used to elute fraction 2 (0·26 g) from which abieta-8,11,13-trien-7-one (0·04 g; $[\alpha]_D$ + 13·5°, c 1·0 in $CHCl_3$; lit.[23] $[\alpha]_D$ + 19·0°, c 1·0 in acetone) could be isolated. This compound exhibited properties (IR, UV and NMR) similar to those given in literature[23]: characteristic IR bands (CCl_4) at 3070, 3020, 1673, 1600, 1480, 990, 920 cm^{-1}; UV, λ $EtOH_{max}$ 253 nm (log ϵ 3·91); λ $EtOH_{max}$ 301 nm (log ϵ 3·17); NMR (τ) 2·13 (1 H, broad signal, aromatic proton): 2·67 (2 H, multiplet, aromatic protons), 8·7–9·1 (five singlets, each 3 H, CH_3-groups). The identity of the compound was further confirmed by direct comparison with an authentic sample obtained from abieta-8,11,13-triene according to a method described by Wenkert.[24]

Further elution with ether in light petroleum gave fraction 3 (0·98 g) consisting mainly of 13-epimanool ($[\alpha]_D$ + 48·5, c 0·6 in $CHCl_3$) which, however, could not be induced to crystallize. This compound was purified by preparative TLC and identified by direct comparison (TLC, GLC, $[\alpha]_D$, IR and NMR) with an authentic sample.[25] Another diterpene alcohol was isolated in small quantities from fraction 3. This alcohol exhibited properties similar to those of 13-epimanool, however, the IR and NMR bands due to the exocyclic methylene group were missing indicating a $\Delta^{8(9)}$ structure instead of the $\Delta^{8(14)}$ structure in 13-epimanool. Additional elution with ether in light petroleum gave fraction 4 (1·08 g) which according to TLC and GLC consisted of fatty alcohols (C_{22}–C_{30} with C_{24} (27%), C_{26} (29%) and C_{28} (38%) as main components) and sterols (mainly β-sitosterol).

Elution with methanol yielded a brownish mixture (4·4 g) which was not further investigated.

C. libani A. Rich. The light petroleum soluble part (51·3 g) of the ether extract (68·3 g) from the fresh cones (1·37 kg) collected in Oxford, England, between October and December was separated into acid (32·8 g) and neutral (18·5 g) fractions in the usual way. The acid fraction was treated with cyclohexylamine. The cyclohexylamine salts of the resin acids were separated by filtration leaving fatty acids in solution. The liberated resin acids (87% of total acids) and the fatty acids (13%) were analysed separately by GLC and TLC as their methyl esters. The resin acid mixture had the following composition: sandaracopimaric (44·5% of total acids), abietic (28·5%), neoabietic (10·4%), dehydroabietic (2·3%) and levopimaric/palustric acid (1·4%). Among the fatty acids palmitic acid (4% of total acids) was found to be the main constituent.

A part (10 g) of the neutral fraction was chromatographed on a silica gel column. Light petroleum eluted a hydrocarbon fraction (2·5 g). Ether in light petroleum (1–50%) was used to elute a more polar fraction (6·0 g). The rest of the neutral fraction (1·4 g) could be eluted by ether and by methanol. This product was not further investigated.

The hydrocarbon fraction (2·5 g) was further separated on silver nitrate impregnated silica gel. On elution with light petroleum, an alkane fraction (0·5 g) was obtained. Analysis by GLC showed it to contain C_{21}–C_{29} *n*-alkanes with odd numbers of carbon atoms dominating, *n*-C_{23} (28% of total alkanes), *n*-C_{25} (49%) and *n*-C_{27} (16%) as main constituents. Ether in light petroleum was used to elute abieta-8,11,13-triene (0·3 g; $[\alpha]_D$ + 52°, c 1·0 in $CHCl_3$) followed by abieta-7,13-diene (1·1 g; $[\alpha]_D$ − 84°, c 2·0 in $CHCl_3$; lit.[26] $[\alpha]_D$ − 86°).

These two compounds were identified by their characteristic UV, IR, NMR and mass spectra. Abieta-8,11, 13-triene was further characterized by the preparation of its crystalline dinitro-derivative, m.p. 188–189·5°; $[\alpha]_D$ + 62°, c 0·5 in $CHCl_3$ (lit.[20] m.p. 189–190°; $[\alpha]_D$ + 58°, c 0·7 in $CHCl_3$). The optical rotation of abieta-8,11,13-triene was, however, different ($[\alpha]_D$ + 52°, c 1·0 in $CHCl_3$) from that previously reported[20] ($[\alpha]_D$ + 5°, c 2·8 in $CHCl_3$). Abieta-7,13-diene was dehydrogenated with bromine according to Dupont *et al.*[27] yielding abieta-8,11,13-triene ($[\alpha]_D$ + 50·5°, c 1·0 in $CHCl_3$) identical in all respects (IR, UV, NMR and mass spectra) with the sample described above. The residual hydrocarbons were eluted as complex mixtures which were not further investigated.

The polar fraction (6·0 g) from the chromatography of the neutral fraction, eluted by ether–light petroleum mixtures, was saponified (boiling 2N methanolic NaOH). The acidic part (0·8 g) from this saponification

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contained fatty and resin acids in the proportion 2:1 and with similar constituents as found for the free acids. The unsaponifiable part (5·2 g) was analysed by TLC (preparative and analytical). Fatty alcohols, sterols (mainly β-sitosterol) and terpene aldehydes (mixture of abietinal and dehydroabietinal according to NMR) were detected.

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PODOCARPACEAE

ANTHOCYANINS FROM FIVE SPECIES OF THE PODOCARPACEAE

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Abstract—Cyanidin 3-glucoside has been identified in five species of Podocarpaceae. In addition, pelargonidin 3-glucoside was isolated from *Microcachrys tetragyna* Hook., and cyanidin 3-rutinoside from *Podocarpus lawrencii* Hook.

THE DISTRIBUTION of anthocyanins in the Gymnospermeae has not been widely reported. Santamour[1] has demonstrated the presence of cyanidin and delphinidin 3-glucoside and cyanidin 3-rhamnoside in conelets of Pinaceae species, and Lowry[2] has recently reported the occurrence of delphinidin 3,5-diglucoside in young leaves of *Podocarpus polystachus* (Podocarpaceae).

We have investigated the distribution of anthocyanins in five Tasmanian species of the Family Podocarpaceae. Our results are set out in Table 1.

The family Podocarpaceae is represented in the Tasmanian flora by five species distributed amongst the three subfamilies, Phyllocladoideae, Pherosphaeroideae and Podocarpoideae, the former two being monogeneric. Only in the subfamily Podocarpoideae was any significant variability in anthocyanin content encountered. Thus (including Lowry's data)[2] anthocyanin structures based on the three common non-methylated anthocyanidins, and with both 3- and 5-substituted glycosides have now been identified in this subfamily.

EXPERIMENTAL

Isolation and identification of anthocyanins was carried out using the procedures described by Harborne.[3] Characterization of purified compounds was confirmed by co-chromatography with authentic reference compounds.

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