then dissolved in H_2O. The filtered solution was allowed to stand during which time a light brown solid (2·49 g) separated.

(B) The spores (750 g) were extracted (Soxhlet) for 10 hr with MeOH and the extract evaporated nearly to dryness. The residue was taken up in petroleum and extracted with H_2O. After concentration of the combined aq. layers yellow-brown needles (2·17 g) separated. Extraction of the spores with methanol for a further 10 hr followed by the same work-up gave a further 1·10 g of yellow-brown needles.

Paper chromatography using Whatman No. 3 paper and 5% HOAc or *n*-BuOH–HOAc–H_2O (4:1:5) showed that the yellow-brown solids were almost pure but the aq. solutions from which they separated were complex mixtures of up to six components. In the former solvent, equisporoside scarcely moved from the baseline while in the latter it had an R_f of 0·43.

Purification. Equisporoside (788 mg) was dissolved in MeOH–H_2O (1:1, 40 ml) and applied to a column of polyamide–celite (10 g) which had been packed with H_2O. Elution with MeOH–H_2O (1:1) yielded equisporoside (608 mg, 77% recovery). Equisporoside crystallized as small yellow needles from aq. HOAc. After drying at 85° under vacuum for 12 hr, it melted at 202–204°. An authentic sample of gossypitrin (obtained from Geissman), melted at 199–201°; mixed m.p. 199–201°; ν_{max} (KBr): 3400, 2900 (shoulder), 1650, 1605, 1557 and 1510 cm^{-1}; superimposable on that of gossypitrin.

Hydrolysis of equisporoside. Equisporoside (66·7 mg) in MeOH (20 ml) and 2 N H_2SO_4 (20 ml) was refluxed for 4 hr, cooled and the solution extracted with EtOAc. Evaporation of the combined EtOAc extracts yielded the aglycone, equisporol, (43 mg). It crystallized from aq. HOAc as yellow needles, m.p. 301–304° (dec., Kofler preheated to 290°). There was no depression of the m.p. when mixed with authentic gossypetin.

Equisporol hexamethyl ether. The procedure used here was essentially that of Geissman.[4] The product obtained after crystallization from MeOH–$CHCl_3$ melted at 170–171·5°; gossypetin hexamethyl ether (obtained from Geissman), 166–168°; mixed m.p. 166–169°. IR spectra (KBr) of both compounds were superimposable.

Equisporol hexaacetate. The hexaacetate obtained via the previously described procedure[4] had an unusual behaviour during the m.p. determination. The compound first sintered at 190°, again at 216° and finally melted at 226–230°. Again comparison with authentic sample from Geissman showed no depression.

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GYMNOSPERMAE

PINACEAE

NEW LABDANE RESIN ACIDS FROM *PINUS ELLIOTTII**

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Abstract—Slash pine needles and cortex oleoresin have been found to contain a new major diterpene constituent, imbricataloic acid. The closely related imbricatoloic acid, previously reported only in *Araucaria imbricata*, was found to be present in small amounts in slash pine needle extract. Spectral data are given for an unidentified diterpene alcohol isolated from the cortex oleoresin.

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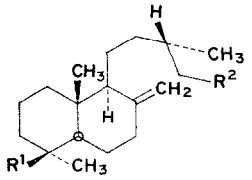
INTRODUCTION

ONLY four resin acids having the labdane skeleton have been isolated from the genus *Pinus*: communic (elliotinoic),[1] pinifolic,[2] antidaniellic (lambertianic),[3,4] and anticopalic.[5] We now report the isolation of a new labdane resin acid, imbricataloic [15-oxo-8(17)-labden-19-oic][6] acid, as the methyl ester (I) from the needles and cortex oleoresin of slash pine (*Pinus elliottii* Engelm.). In addition, imbricatolic [15-hydroxy-8(17)-labden-19-oic] acid was isolated as its methyl ester (II) from the needle extract. This is the first reported occurrence of this acid in the Pinaceae although it has been found[7] in *Araucaria imbricata*. Bruns also reported two neutral compounds, imbricadiol (III) and imbricatolal but did not find the corresponding di-acid, dihydroagathic acid; this acid was not found in our study of slash pine nor was its C-4 epimer, pinifolic acid, which is present in the needles of *Pinus sylvestris*.[2]

RESULTS

GLC analysis showed that methyl imbricataloate (I) comprises 24–64 per cent of the methylated resin acids of the needle extracts and 11–27 per cent of the resin acids of cortex oleoresins from a series of trees selected from different parts of the typical slash pine range. These figures have been corrected for the amounts of (I) that had been converted (by the reaction of diazomethane with the aldehyde function) to the artifact methyl ketone (IV): this ketone was isolated and characterized. Methyl imbricatoloate (II) was present as only 1 per cent of the resin acids of the needle extracts but was not observed in the cortex oleoresins.



(I) R^1=$COOCH_3$, R^2=CHO
(II) R^1=$COOCH_3$, R^2=CH_2OH
(III) R^1=R^2=CH_2OH
(IV) R^1=$COOCH_3$, R^2=$COCH_3$

The IR and NMR spectra of (I) were typical for an 8(17)-labdene having an axial carbomethoxy group at C-4. The NMR spectrum showed an aldehyde proton at δ 9·74 as a triplet which establishes the aldehyde function at C-15. The configuration at C-13 was determined to be *S* by comparison of the negative circular dichroism curve with the Cotton effects observed in the optical rotatory dispersion curves for a series of methyl-substituted, aliphatic aldehydes.[8] The assignment of structure (I) was confirmed by $LiAlH_4$ reduction of (I) to imbricadiol (III), which was compared with an authentic sample.

The identification of the hydroxy ester as (II) was confirmed by comparison with an authentic sample of methyl imbricatoloate. The C-13 configuration[9] of (II) was reconfirmed as *S* by oxidation of (II) to (I), for which a negative CD curve was obtained.

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A new diterpene alcohol, mol. wt. 290, was obtained from the cortex oleoresin by preparative GLC; this alcohol was not present in the needles. The UV spectrum shows the presence of a conjugated diene, similar to abietic acid. The molecular weight is best accommodated by a bicyclic structure having two double bonds. The NMR spectrum contains many similarities to the spectra for thunbergene and thunbergol.[10] Our diterpene alcohol probably has a similar macrocyclic skeleton except for having an additional intramolecular C–C bridge. Sufficient amounts of the alcohol were not available to complete the characterization.

TABLE 1

	GLC (r_{pim})*	
	DEGS	SE/30-EGiP
Methyl imbricataloate (I)	3·63	1·58
Methyl imbricatoloate (II)	—	2·01
IV	4·20	1·97
Dimethyl dihydroagathate†	3·64	2·03

* GLC conditions as described by Nestler and Zinkel.[12]

† Prepared by oxidation of (I).

EXPERIMENTAL

(A) *Fractionation of slash pine needle extract.* Slash pine needles were cut into small pieces (< 1 cm) and extracted with anhydrous Et_2O. The non-volatile portion of a typical needle extract contains 49% neutrals and 51% acids as determined on an aliquot by means of the DEAE-Sephadex separation.[11] The total needle extract was methylated (CH_2N_2) and chromatographed on neutral alumina. Elution with benzene–petroleum (1:1) yielded a green oil containing about 20% of I. (GLC of all the previously eluting fractions did not indicate even trace amounts of dihydroagathic or pinifolic acid methyl esters.) The fraction containing 20% (I) was chromatographed on a 40% $AgNO_3$–Al_2O_3 column. Elution with ether–petroleum (4:1) gave a clear oil, rich in I. Further elution with acetone produced an oil rich in II.

(B) *Methyl imbricataloate* (I). The fraction rich in I was chromatographed on silica using petroleum with a 15–50% benzene gradient to yield I in 99⁺% purity (established by GLC[12] and TLC): $[\alpha]_D^{20}$ +38·5° (*c* 2·3, $CHCl_3$); NMR ($CDCl_3$) δ 9·74 (triplet, $J = 2$, —CHO), 4·46 and 4·83 (s, $=CH_2$), 3·59 (s, $COOCH_3$), 0·96 (d, $J = 6·5$, $-CH_3$ at C-13), 1·17 (s, $-CH_3$ at C-4) and 0·50 (s, $-CH_3$ at C-10); ν_{max}^{film} 3080, 1645, 890 (exocyclic $=CH_2$), 1730, 1228, 1152 (axial ester[13]), and 1730, 2715 cm^{-1} (—CHO); CD $[\theta]_{306}^{25}$ −670°. (Found: C, 75·53; H, 10·15. $C_{21}H_{34}O_3$ required: C, 75·41; H, 10·25.)

$LiAlH_4$ reduction of I in anhydrous ether produces imbricadiol (III) which was purified by column chromatography on silica, followed by recrystallization from heptane: m.p. 110–110·5° (evac. capillary) (lit.[7] m.p. 113–114·5°); $[\alpha]_D^{25}$ +25·9° (*c* 0·5, $CHCl_3$) lit.[7] +25·4°). The NMR and IR spectra of III and an authentic sample of imbricadiol were identical; both had the same GLC retention times on 3% SE-30 and on 3% Dexsil 300GC columns.

Also isolated in the silica column chromatography of I by further elution was the methyl ketone, IV: $[\alpha]_D^{20}$ +45·1° (*c* 2·3, $CHCl_3$); NMR ($CDCl_3$) δ 4·46 and 4·83 (s, $=CH_2$), 3·59 (s, $COOCH_3$), 2·10 (s, $COCH_3$), 1·17 (s, $-CH_3$ at C-4), 0·89 (s, $-CH_3$ at C-13), and 0·50 (s, $-CH_3$ at C-10); ν_{max}^{film} 1730 cm^{-1}; mass spectrum *m/e* 348 (19%, M^+) and 121 (100%); CD $[\theta]_{297}^{25}$ −397°. (Found: C, 75·90; H, 10·40. $C_{22}H_{36}O_3$ required: C, 75·81; H, 10·42.) Treatment of 10 mg of I in 3 ml of ether–MeOH (9:1) with an excess of CH_2N_2 for 15 hr gave IV in 99% yield.

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(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

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

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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.

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