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NEUTRAL DITERPENES FROM *ARAUCARIA BIDWILLI**

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Key Word Index—*Araucaria bidwilli*; Araucariaceae; diterpenes; *ent*-labdane.

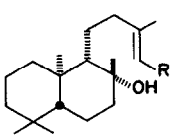
Abstract—Five *ent*-labdane diterpenes were isolated from the neutral fraction of the resin of *Araucaria bidwilli*. Three of them, previously unknown, were assigned the structures *ent*-15-acetoxy-labda-8,*E*-13-diene, *ent*-labda-8,E-13-dien-15-ol and methyl *ent*-8α-hydroxy-labd-*E*-13-en-15-oate.

INTRODUCTION

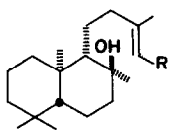
In a previous paper [1] we reported a chemical study of the resin exuded from the trunk of *Araucaria bidwilli*. Eight diterpene acids were isolated, some of which had not been found in nature before. Unfortunately we were unable to examine the neutral fraction, owing to the relatively small amount of oleoresin which was available. With a much larger quantity of resin available we have now investigated the diterpene compounds present in the neutral fraction. These compounds constitute less than 5% of the crude starting oleoresin and the results are reported in this paper.

RESULTS

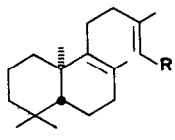
The alkali insoluble fraction of the resin of *A. bidwilli*, obtained as previously reported [1], was first submitted to a rough chromatographic separation which afforded a complex hydrocarbon mixture, not yet examined, besides several enriched fractions whose further purification then gave the five compounds which are listed in Table 1. Two of the compounds were the already known methyl *ent*-8β-hydroxy-labd-*E*-13-en-15-oate (**1a**) and *ent*-8β,15-labd-*E*-13-ene-diol (**1b**) respectively, both identified on the basis of their spectral characteristics and by comparison with authentic samples.



(**1a**) R = CO_2Me
(**1b**) R = CH_2OH



(**2a**) R = CO_2Me
(**2b**) R = CO_2H



(**3a**) R = CH_2OAc
(**3b**) R = CH_2OH
(**3c**) R = CO_2Me

The first unknown substance was a crystalline hydroxy ester mp 95–97°, $[\alpha]_D$ −10° which was assigned structure **2a**. Spectral evidence indeed suggested it could be the C-8 epimer of the hydroxy ester **1a** and in addition all its physical characteristics, excepting the specific rotation, were identical with those of an already known [5] synthetic methyl 8α-hydroxy-labd-13-en-15-oate† mp 97–98°, $[\alpha]_D$ +8°. Thus, the compound **2a** could be expected to be the enantiomer of this latter compound. However, the low absolute values of the specific rotations could not be considered quite diagnostic in this case. Rotations of both compounds were then measured at four different wavelengths and the results obtained (see Experimental) always showed good agreement of the absolute values but of opposite signs thus confirming the assignment to our hydroxy ester of the structure and configuration shown in **2a**.

The co-occurrence in the neutral fraction of both the C-8 epimeric hydroxy esters, **1a** and **2a**, prompted us to check for the presence of the acid **2b** which was not reported previously to occur in the acidic fraction of the resin. Compound **2b**, identified by conversion into the methyl ester **2a**, was isolated from the acid fraction in a 1.6% yield.

The other two unknown compounds, **3a** and **3b**, were both oily, $[\alpha]_D$ −18° and −20° respectively, elemental composition $C_{22}H_{36}O_2$ and $C_{20}H_{34}O$ respectively. They were clearly related because of their very close spectral characteristics and since the former afforded the latter quantitatively by hydrolysis under mild alkaline conditions, thus showing that **3b** was an alcohol and **3a** the corresponding acetyl derivative. The NMR spectrum of **3b** showed two three proton singlets at δ 1.68 and 1.55 respectively, both attributable to the resonance of methyl groups on double bonds. In addition, a vinyl proton resonance at δ 5.40 and a two proton doublet centered at 4.19, were assigned to the resonance signal of an allylic-CH_2OH group. All the above data were consistent with the structure labda-8,*E*-13-dien-15-ol but the absolute stereochemistry could not be defined at this stage. However by analogy with the other neutral and acidic diterpenes from the same resin it could be expected to have the *ent*-configuration.

*Part 6 in the series "Araucariaceae". For parts 5 and 7 see refs. [2] and [3].

†Actually, the configuration at the Δ^{13} double bond for this methyl ester has not been reported. However, the δ values of the carbomethoxyl and vinyl protons signals [6] allowed a safe assignment of the *E*-configuration.

Table 1. Neutral diterpenes isolated from the resin of *Araucaria bidwilli*

Compounds*	mp	$[\alpha]_D$	% Amount†	Ref.
ent-8β,15-Labd-*E*-13-ene-diol (**1b**)	126–127°	−33°	1.3	4
Methyl *ent*-8β-hydroxy-labd-*E*-13-en-15-oate (**1a**)	oily	−36°	1.2	4
Methyl *ent*-8α-hydroxy-labd-*E*-13-en-15-oate (**2a**)	oily	−10°	1.0	—
ent-15-Acetoxy-labda-8,*E*-13-diene (**3a**)	oily	−18°	0.8	—
ent-Labda-8,*E*-13-dien-15-ol (**3b**)	oily	−20°	0.7	—

* Nomenclature is according to J. W. Rowe, Forest Products Lab., Madison, Wisconsin. † Referred to the total starting oleoresin.

Final assignment of the structure and absolute configuration came by comparison of the natural **3a** with a sample prepared from the known hydroxy ester **1a** which was available in appreciable amounts from the acid fraction of the same resin. The hydroxy ester **1a** was dehydrated with iodine in anhydrous benzene to give the unsaturated ester **3c**. The subsequent reduction of **3c** by $LiAlH_4$ then led to the corresponding oily alcohol, $[\alpha]_D$ −19°, identical in every respect with the natural **3b**.

DISCUSSION

The diterpene distribution in the oleoresin of *A. bidwilli* requires some comments. Clerodane compounds, which represent more than 50% of the acidic fraction of the resin, are completely absent in the neutral fraction. In addition, the labdane compounds present in this neutral fraction have only the *ent*-configuration whereas *ent*-labdane compounds were found in the acidic fraction together with other labdanes having the normal absolute configuration. On the other hand, we had already observed that derivatives of the acids with an *ent*-labdane configuration did not occur with any oxygenated function at either C-18 or C-19. This contrasts with the labdanes of normal configuration, all of which had a C-19 function. Likewise, all the neutral compounds we have isolated had the *ent*-labdane configuration and no functional groups at C-19. This fact seems to support the hypothesis that functionalization at either C-18 or C-19 cannot take place after cyclization of the acyclic geranyl geraniol and that compounds having such functions may be best considered as arising by the cyclization of an acyclic pre-functionalized precursor. In fact, in *A. bidwilli* there may exist two different enzymatic systems which perhaps act in the cyclization of acyclic precursors according to their functionalization.*

In the light of these results, this plant appears to be quite different from all the other *Araucaria* species we have so far examined [2,3,7–9]. Their oleoresins present in fact marked affinities in the chemical composition and in addition resemble very closely the oleoresins of plants belonging to the *Agathis* genus which are also Araucariaceae.

EXPERIMENTAL

General experimental methods have been reported elsewhere [1]. The fresh oleoresin (100g) was collected from only one plant which grows in the Botanical Garden of the University in Napoli. The crude alkali insoluble fraction of the resin (6 g), obtained by conventional methods of extraction, was directly adsorbed on Si gel (150 g) and eluted with petrol–Et_2O mixtures to afford a complex (GLC shows 18 peaks) less polar hydrocarbon fraction (1 g) besides 21 enriched fractions whose further purification then afforded the compounds listed in Table 1.

ent-15-Acetoxy-labda-8,E-13-diene (**3a**). Eluted with petrol. Colourless oil, $[\alpha]_D$ −18° (c 1.5). (Found: C, 78.85; H, 11.07. $C_{22}H_{36}O_2$ requires: C, 79.46; H, 10.92%). MW 332 (MS); ν_{max} cm^{-1}: 1725, 1230; NMR: δ 5.10 (*br*, 1H, vinyl proton), 4.59 (*d*, *J* 7 Hz, 2H, allylic-CH_2OAc), 2.05 (*s*, 3H, Me–CO_2–), 1.72 and 1.61 (2*s*, 3H, vinyl methyls).

ent-Labda-8,E-13-dien-15-ol (**3b**). Eluted with petrol–Et_2O (95:5). Colourless glassy oil, $[\alpha]_D$ −20° (*c* 1.3). (Found: C, 82.75; H, 11.76. $C_{20}H_{34}O$ requires: C, 82.69; H, 11.80%). MW 290 (MS); ν_{max} cm^{-1}: 3330; NMR: δ 5.40 (*br*, 1H, vinyl proton), 4.19 (*d*, *J* 7 Hz, 2H, allylic-CH_2OH), 1.68 and 1.55 (2*s*, 3H, vinyl methyls).

Methyl ent-8α-hydroxy-labd-E-13-en-15-oate (**2a**). Eluted with petrol–Et_2O (9:1). Crystalline solid mp 95–97° (from petrol), $[\alpha]^{20}$ (*c* 1 in $CHCl_3$) −10° (589 A), −11° (578 A), −12° (546 A), −24° (436 A). [A pure sample of the synthetic enantiomer [5] under the same conditions, exhibited the following rotations: +8°, +10°, +11°, +22°.] (Found: C, 75.21; H, 10.80. $C_{21}H_{36}O_3$ requires: C, 74.95; H, 10.78%). MW 336 (MS); ν_{max} cm^{-1}: 3580, 1725, 1650; NMR: δ 5.60 (*s*, 1H, vinyl proton), 3.58 (*s*, 3H, $-CO_2Me$), 2.13 (*s*, 3H, vinyl methyl), 1.10 (*s*, 3H, C-17 methyl).

Preparation of **3a** *from the hydroxy ester* **1a**. Pure oily **1a**, $[\alpha]_D$ −36° (300 mg) was refluxed for 3 hr in dry C_6H_6 (10 ml) containing a small iodine crystal. The C_6H_6 soln was then washed with N aq sodium thiosulphate, dried and evaporated to give the crude ester **3c** (260 mg) which was directly reduced by excess $LiAlH_4$ in dry Et_2O. Workup of the reduction mixture in the usual way then afforded the alcohol **3b** (220 mg) which, after chromatographic purification, had spectra superimposible to those of the natural **3b**. Acetylation of **3b** in the usual way yielded the expected acetate (**3a**), oily, $[\alpha]_D$ −19° (*c* 1.3) identical in every respect with the natural **3a**.

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*A Referee has observed that our results may suggest that *A. bidwilli* does not possess an enzyme system capable of oxidising the *ent*-labdane series at C-18 or C-19 and that both the *ent*- and normal labdanes may arise by cyclisation of a common acyclic precursor.