Phytochemistry, 1974, Vol. 13, pp. 467 to 470. Pergamon Press. Printed in England.

DITERPENES FROM *ARAUCARIA BIDWILLI**

ROMUALDO CAPUTO and LORENZO MANGONI

Institute of Organic Chemistry, University of Naples, Italy

(*Received* 5 *August* 1973. *Accepted* 10 *September* 1973)

Key Word Index—*Araucaria bidwilli*; Araucariaceae; conifers oleoresins; diterpene acids; labdanes; clerodanes.

Abstract—Eight diterpene acids of the labdane or clerodane type were obtained from the oleoresin of *Araucaria bidwilli* and characterized as their methyl esters.

INTRODUCTION

WHILE the oleoresins of *Agathis* have been extensively studied, those of *Araucaria*, the only other genus of the Araucariaceae, have been very little investigated. Only the isolation of the diterpene imbricatolic acid from the resin of *A. imbricata* has been reported.[1]

In connection with our interest in the oleoresins exuded from conifers, we began a systematic study of *Araucaria*, a genus with relatively few species in it.[2] This paper describes the examination of the oleoresin of *A. bidwilli* which was actually restricted to the acid components since the neutral fraction was present in the resin as a complex mixture and in a very small amount.

RESULTS

The fresh resin collected at the end of summer, when treated with cold ether, gave an extremely viscous yellow oil which turned out to be almost completely soluble in dilute alkali. In fact, the acid fraction was about 90% of the extract. All attempts to separate the acid components by chromatography were unsatisfactory; the acid fraction was therefore treated *in toto* with ethereal diazomethane and the resulting esters subsequently separated through repeated chromatography. In this way the methyl esters listed in Table 1 were obtained.

The known ester (**1**a) was identified on the basis of the spectral characteristics and conversion into the alcohol (**1**b) (cryst. *p*-nitrobenzoate m.p. 107–108°). The isomeric esters (**2**a) and (**3**) could be separated only by $AgNO_3$ silica TLC. The first was the known methyl kolavenate whose identity was confirmed by $LiAlH_4$ reduction to the oily alcohol (**2**b) whose crystalline 3,5-dinitrobenzoate (**2**c) had m.p. 104–105°. The second was a slightly dextrorotatory oil which was assigned structure (**3**) on the basis of the spectral properties very close to those of the ester (**2**a), except for the lack of one vinyl methyl and for the presence of two vinyl hydrogens as a singlet at 4·57 δ in place of the vinyl hydrogen at 5·18 δ. Additional evidence for structure (**3**) was obtained as follows. Treatment of (**3**) with

---

* Part I in the projected series "Diterpenoids of the Araucariaceae". A short communication concerning part of this work has already been published.[3]

[1] WEISSMAN, G., BRUNS, K. and GRUTZMAKER, H. FR. (1965) *Tetrahedron Letters* 4623.

[2] HEGNAVER, R. (1966) *Chemotaxonomie der Pflanzen*, Birkäuser, Berlin.

[3] CAPUTO, R. and MANGONI, L. (1969) *La Chimica e l'Industria* (*Milano*) **51**, 1382.

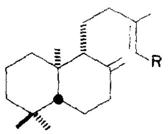
monoperphthalic acid led to the β-epoxide (**4**) because of the steric hindrance at the α-face of the molecule. $LiAlH_4$ reduction of (**4**) then yielded the diol (**5**a) having an axial tertiary hydroxyl group. Conversion of (**5**a) into its mono-3,5-dinitrobenzoic ester (**5**b) and dehydration of the latter to give the endocyclic Δ^3 double bond[8] then afforded a crystalline compound m.p. 104–105°, $[\alpha]_D$ − 36° identical with the above 3,5-dinitrobenzoate (**2**c).

TABLE 1. PROPERTIES OF ACIDIC DITERPENES

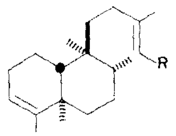
Methyl esters*		$[\alpha]_D$	Ref.
ent-8(17),*E*-13-Labdadien-15-oate	(1a)	−42°	4
ent-3,*E*-13-Clerodadien-15-oate	(2a)	−55°	5
ent-4(18),*E*-13-Clerodadien-15-oate	(3)	+ 9°	—
ent-8β-Hydroxy-labd-*E*-13-en-15-oate	(6a)	−36°	4
19-Hydroxy-8(17),*E*-13-labdadien-15-oate (agatholate)	(7a)	+45°	6
19-Acetoxy-8(17),*E*-13-labdadien-15-oate (acetyl agatholate)	(7b)	+39°	—
19-Aldehydo-8(17),*E*-13-labdadien-15-oate	(7c)	+33°	—
8(17),*E*-13-Labdadien-15,19-dioate (dimethyl agathate)	(7d)	+52°	7

* Nomenclature is according to J. W. Rowe (Forest Prod. Lab., Madison–Wisconsin), private communication. All compounds were oils.

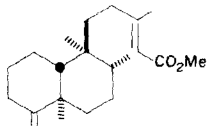
The structures of the hydroxyester (**6**a), of the hydroxyester (7a), of its acetyl derivative (7b), of the aldehydoester (7c)* and of the diester (7d) were all assigned by identification of their $LiAlH_4$ reduction products. Indeed, the hydroxyester (**6**a) gave the known[4] crystalline diol (**6**b) m.p. 126–127° and all the others gave agathadiol[6] m.p. 106–107° identical with authentic material.



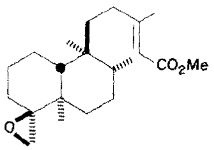
(1a) R = CO_2Me
(1b) R = CH_2OH



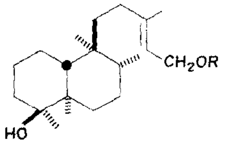
(2a) R = CO_2Me
(2b) R = CH_2OH
(2c) R = $CH_2OCOC_6H_3(NO_2)_2$



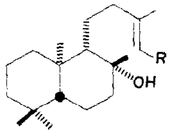
(3)



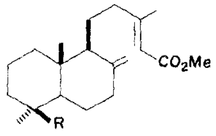
(4)



(5a) R = H
(5b) R = $COC_6H_3(NO_2)_2$



(6a) R = CO_2Me
(6b) R = CH_2OH



(7a) R = CH_2OH
(7b) R = CH_2OAc
(7c) R = CHO
(7d) R = CO_2Me

---

* Contemporaneously with our short communication it was also described by Carman *et al.*[9]

[4] HUGEL, C., OEHLSCHLAGER, A. C. and OURISSON, G. (1966) *Tetrahedron* Suppl. **8**, 203.

[5] MISRA, R., PANDEY, R. C. and SUKH, D. (1964) *Tetrahedron Letters* 3751.

[6] ENZELL, C. (1961) *Acta Chem. Scand.* **15**, 1303.

[7] RUZICKA, L. and HOSKING, J. R. (1929) *Annalen* **469**, 147.

[8] BARTON, D. H. R. and MORRIS, G. A. (1961) *Fort. Chem. Org. Natur.* **19**, 202.

[9] CARMAN, R. M. and MARTY, R. A. (1968) *Australian J. Chem.* **21**, 1923.

As far as we know, this is the first time that diterpenes with the clerodane skeleton [(**2a**) and (**3**)] have been found to co-occur with diterpenes with the labdane skeleton, which supports the hypothesis that both types have a common biogenesis. In addition, this is the second[10] example of the co-occurrence in the same plant of labdanes belonging to both the normal and the enantiomeric series. Furthermore, it is noteworthy that compounds carrying an oxygenated function at C_{19} have the normal labdane configuration whereas those having a C_{19} methyl group possess the enantiomeric configuration. This suggests the existence in the plant of two different enzymes alternatively acting in the cyclization according to the substitution at C_{19}.

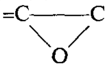
EXPERIMENTAL

M.ps are uncorrected. IR spectra were determined in CCl_4. NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard. Rotations were taken for $CHCl_3$ solns at r.t. TLC were performed on silica-gel F_{254} (Merck). Silica-gel 0·05–0·20 mm (Merck) or alumina (Woelm, neutral) were used for column chromatography. Silica-gel–$AgNO_3$ 38% was prepared by treating silica-gel with 38% $AgNO_3$ aq. (1:1 w/w), keeping the mixture in the dark for 1 hr and drying it at 110° for 24 hr. Petrol refers to the fraction with b.p. 40–70°. The oleoresin examined was collected from only one plant (Botanical garden of the University of Naples, Italy).

Extraction and fractionation of the resin. Fresh resin (87 g) was continuously extracted with cold Et_2O for 6 hr in order to separate it from extraneous matter and polymer. Evaporation of the ethereal extract gave a very viscous yellow oil (61 g) which was redissolved in Et_2O (1 l.) and extracted with N Na_2CO_3 aq. (6 × 150 ml). Acidification with 12N H_2SO_4 of the combined alkaline layers then gave the acid fraction (56 g; 93%).

Separation of the acid components. The acid fraction (20 g) was treated with excess ethereal CH_2N_2 and the crude resulting esters were adsorbed on alumina (600 g; grade III). Elution with petrol containing increasing percentages of Et_2O gave 25 fractions then collected in four groups on the basis of TLC and spectral evidences: A (fractions 1–3; 11·2 g eluent petrol); B (fractions 4–11; 3·9 g, petrol–Et_2O, 9:1); C (fractions 12–16; 1·6 g, petrol–Et_2O, 17:3); D (fractions 17–25; 2·2 g, petrol–Et_2O, 7:3).

Methyl esters (**1***a*), (**2***a*) *and* (**3**). Fraction A (5 g) was adsorbed on silica-gel (500 g; petrol–Et_2O, 99:1). The first 6·5 l. of eluent gave the crude methyl ester (**1a**) (900 mg) while further 3 l. of eluent gave first a mixture (1·3 g) of **1a**, **2a** and **3** and then a mixture (2·3 g) of the isomers **2a** and **3**. The crude methyl ester (**1a**) was purified through alkaline hydrolysis (KOH–MeOH, 10%) followed by chromatography of the resulting acid (HCl washed silica-gel; petrol–Et_2O, 19:1). Treatment of the acid with excess CH_2N_2 then afforded the pure methyl ester (**1a**) as a colourless oil $[\alpha]_D$ − 42° (*c* 1). $LiAlH_4$ reduction of **1a** followed by treatment of the resulting alcohol (**1b**) with *p*-nitrobenzoyl chloride afforded the crystalline *p*-nitrobenzoate m.p. 107–108° (from hexane). The mixture of esters (**2a**) and (**3**) was adsorbed on silica-gel containing 38% $AgNO_3$ in a dark glass column. Elution with petrol–Et_2O (49:1) afforded the pure oily **2a** (1·8 g), $[\alpha]_D$ − 55° (*c* 1·1). $LiAlH_4$ reduction of **2a** followed by treatment of the resulting alcohol (**2b**) with 3,5-dinitrobenzoyl chloride yielded the crystalline 3,5-dinitrobenzoate (**2c**) m.p. 104–105° (from hexane), $[\alpha]_D$ − 36° (*c* 0·9). Further elution with petrol–Et_2O (19:1) then gave the ester (**3**) which, after purification by chromatography on alumina (grade III; eluent petrol) was a colorless viscous oil $[\alpha]_D$ + 9° (*c* 2) (Found: C, 78·99; H, 10·76. $C_{21}H_{34}O_2$ requires: C, 79·19; H, 10·76 %). ν_{max} 1725, 1225, 1150, 890 cm^{-1}. δ 4·57 (*s*, 2H, *exo*CH_2), 5·72 and 2·17 [*s*, 1H and *s*, 3H, –C(Me)=CH–], 0·85 (*br*, 3H, *sec*Me).

Epoxy-ester (**4**). Pure **3** (228 mg) in Et_2O (5 ml) was added of 1·5% ethereal monoperphthalic acid cooling the soln in ice. After 24 hr at r.t., working up gave crude **4** (240 mg) which after chromatography on silica-gel (6 g, hexane–Et_2O, 19:1) was an oil $[\alpha]_D$ + 26° (*c* 1) (Found: C, 75·49; H, 10·18. $C_{21}H_{34}O_3$ requires: C, 75·40; H, 10·25%). ν_{max} 920 cm^{-1}; δ 2·54 (*q*, 2H, *J* 5 Hz,  $=C\underset{O}{\text{——}}CH_2$).

Diol (**5***a*). The epoxy ester (**4a**) (200 mg) in anhyd. Et_2O was added of excess $LiAlH_4$ and refluxed for 4 hr. Working up of the reaction followed by chromatography on silica-gel (6 g, hexane–Et_2O, 9:1) of the crude product afforded the pure glassy diol (**5a**) (160 mg), $[\alpha]_D$ +9·5° (*c* 2) (Found: C, 77·90; H, 11·79. $C_{20}H_{36}O_2$ requires: C, 77·86; H, 11·76%). ν_{max} 3550, 3350 cm^{-1}. δ 1·12 (*s*, 3H, =C(OH)–Me).

3,5-*Dinitrobenzoate* (**2***c*). The diol (**5a**) (150 mg) in anhyd. C_6H_6 (8 ml) and pyridine (1·6 ml) was added of 3,5-dinitrobenzoyl chloride (350 mg) and kept at r.t. over the night. The crude unstable hydroxy ester (**5b**) formed was directly dissolved in anhyd. pyridine (9 ml) and freshly dist. $SOCl_2$ added (1·3 ml) and kept at 0° for 1 hr. The reaction mixture was then diluted with cold H_2O and extracted with Et_2O. The organic layer was then washed with 2N HCl and after with 2N Na_2CO_3 and evaporated to give the crude product (**2c**) (130 mg) which spontaneously crystallized. After crystallization from hexane the pure (**2c**) had m.p. 104–105°, $[\alpha]_D$ − 36° (*c* 0·9) and was identical with the 3,5-dinitrobenzoyl derivative of **2b**.

---

[10] EKONG, D. E. U. and OKOGUN, J. I. (1967) *Chem. Commun.* 72.

Methyl diagathate (*7d*). Fraction B (500 mg) was adsorbed on alumina (50 g; grade III). Elution with petrol–Et_2O (99:1) gave pure oily **7d** (260 mg), $[\alpha]_D + 52°$ (*c* 1·1) identical with authentic material. $LiAlH_4$ reduction of **7d** gave the crystalline agathadiol m.p. and m.m.p. 108–109°.

Methyl esters (*7b*), (**6***a*) *and* (*7c*). Fraction C (1·6 g) was adsorbed on alumina (160 g; grade II). Elution with petrol–Et_2O (49:1; 800 ml) gave the crude oily 7b (515 mg) which after further chromatographic purification had $[\alpha]_D +39°$ (*c* 1). Alkaline hydrolysis (KOH–MeOH, 10%) of **7b** gave the crystalline agatholic acid m.p. 185°, $[\alpha]_D + 42°$ (*c* 0·9). Further elution with petrol–Et_2O (97:3, 700 ml) gave the crude oily **6**a (480 mg) which after chromatographic purification had $[\alpha]_D - 36°$ (*c* 1·3). $LiAlH_4$ reduction of **6**a gave the crystalline diol **6**b m.p. 126–127°, $[\alpha]_D -33°$ (*c* 1). Finally, further elution with petrol–Et_2O (19:1, 600 ml) afforded the crude extremely unstable aldehydoester (7c), $[\alpha]_D + 33°$ (*c* 1). MW 332 (MS); ν_{max} 2700, 1725, 895 cm^{-1}; δ 9·75 (*s*, 1H, –CHO), 4·89 and 4·52 (two *s*, 2H, *exo*CH_2), 5·64 and 2·14 (*s*, 1H and *s*, 3H, –C(Me)=CH–). $NaBH_4$ reduction of 7c followed by alkaline hydrolysis of the reduction product gave the crystalline agatholic acid m.p. 185–186° identical with authentic material.

Methyl agatholate (*7a*). Fraction D (550 mg) was adsorbed on silica-gel (16 g). Elution with petrol–Et_2O (4:1) afforded pure methyl agatholate (7a) (350 mg) m.p. 75–76°, $[\alpha]_D + 45°$ (*c* 0·8) whose alkaline hydrolysis (KOH–MeOH, 10%) gave agatholic acid m.p. and m.m.p. 184–185°.

Acknowledgements—The authors thank Professor A. Merola of the Botanical Garden of the University in Naples for his interest in this work. Financial support from the Italian National Research Council (CNR) is gratefully acknowledged.