

NEW DITERPENES FROM *ARAUCARIA CUNNINGHAMI**

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Key Word Index—*Araucaria cunninghami*; Araucariaceae; conifers oleoresins; labdane diterpenes.

Abstract—Eleven labdane diterpenes, nine of which are new compounds, were identified in the oleoresin of *Araucaria cunninghami*.

INTRODUCTION

IN CONTINUING our interest in the oleoresins of the *Araucaria* genus,¹ we have examined the oleoresin of *A. cunninghami*. This paper describes the isolation from this source of a number of acidic and neutral diterpenes with labdane skeleton, most of which have not been found in nature before.

RESULTS

The fresh resin spontaneously exuded from the plant as large milky white drops was collected during the summer. The crude material, when treated with cold ether to remove a rather large polymeric fraction, gave a clear colourless oil. Treatment of the ethereal extract with dilute alkali then afforded an acid fraction (75%) and a neutral fraction (20%). The acid fraction was separated by repeated chromatography, which gave the acids (in some instances characterized as their methyl esters) listed in Table 1.

The already known methyl communate (1) and methyl isocupressate (2a) were both identified by comparison with authentic samples. The structure of the unknown hydroxy

TABLE 1. PROPERTIES OF ACIDIC DITERPENES

Compounds†		m.p.	$[\alpha]_D$	Ref.
Methyl 8(17),E-12,14-labdatrien-19-oate (Methyl communate)	(1)	107–108°	+48°	3
Methyl 15-hydroxy-8(17),E-13-labdadien-19-oate (Methyl isocupressate)	(2a)	oil	+50°	4
15-hydroxy-8,E-13-labdadien-19-oic acid	(3a)	124–125°	+128°	—
Methyl 15-acetoxy-8(17),E-13-labdadien-19-oate (Methyl acetyl-isocupressate)	(2b)	oil	+51°	—
Methyl 15-acetoxy-8,E-13-labdadien-19-oate	(3c)	oil	+109°	—
Methyl 7-oxo-19-acetoxy-8,E-13-labdadien-15-oate	(4)	oil	+132°	—

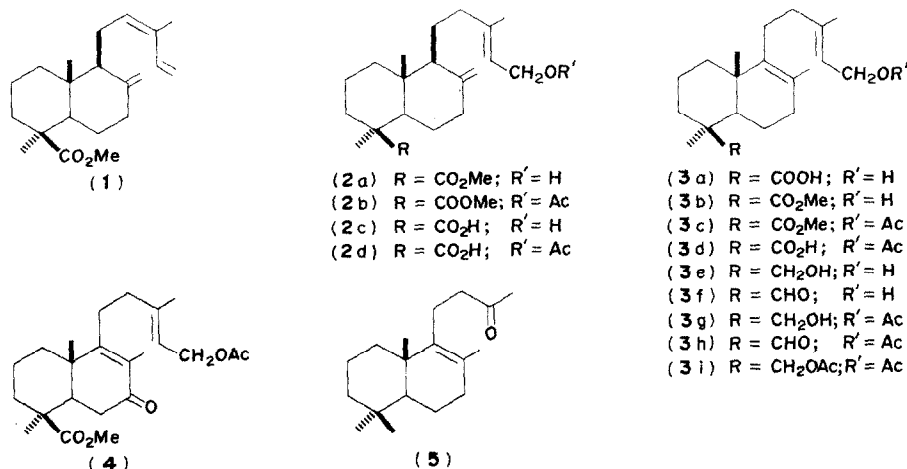
† Nomenclature is according to J. W. Rowe (Forest Prod. Lab., Madison-Wisconsin), private communication.

* Part II in the series "Diterpenoids of the Araucariaceae". A short communication concerning part of this work has already been published.²

¹ CAPUTO, R. and MANGONI, L. (1974) *Phytochemistry* **13**, 467.

² CAPUTO, R., DOVINOLA, V. and MANGONI, L. (1969) *La Chimica e l'Industria (Milano)* **51**, 1383.

acid (3a) was assigned on the basis of the spectral characteristics of its methyl ester. Beside IR indication for a hydroxyl group, NMR evidence for only one vinylic proton (t at 5.46 δ), two allylic protons geminal with an hydroxyl group (d at 4.16 δ) and two vinyl methyls at 1.68 and 1.53 δ was obtained. The assigned structure was then confirmed by identity of 3a with the acid isomerization product of the exocyclic isomer (2c).



The acetoxy esters (2b) and (3c) were easily identified by mild alkaline hydrolysis that gave methyl isocupressate (2a) and the methyl ester (3b) respectively. Finally, the structure of the 7-oxo acetoxy ester (4)* suggested by UV, IR and NMR evidence was confirmed through its partial synthesis by chromic oxidation of the acetoxy ester (3c). Elaboration of the neutral fraction then gave six labdane diterpenes none of which has been reported before (Table 2).

TABLE 2. PROPERTIES OF NEUTRAL DITERPENES

Compounds		m.p.	$[\alpha]_D$	Ref.
8,E-13-Labdadien-15,19-diol	(3e)	141–143°	+ 54°	---
15-Hydroxy-8,E-13-labdadien-19-al	(3f)	oil	+ 68°	---
15-Acetoxy-8,E-13-labdadien-19-ol	(3g)	oil	+ 51°	---
15-Acetoxy-8,E-13-labdadien-19-al	(3h)	oil	+ 67°	---
8,E-13-Labdadien-15,19-diacetate	(3i)	oil	+ 49°	--

The structure of the diol (3e), assigned essentially on spectroscopic grounds, was confirmed by identity of 3e with the LAH reduction product of the esters (3b). The hydroxyaldehyde (3f) was converted by NaBH₄ into the above diol (3e). The unstable acetoxy aldehyde (3h), characterized as the crystalline semicarbazone m.p. 126–128°, was also converted into the same diol (3e) by LiAlH₄ reduction. Structures of the monoacetate (3g) and of the diacetate (3i) were both supported by obtaining 3e by alkaline hydrolysis.

* The possibility that 4 may be an artefact, formed via allylic oxidation of 3c by the atmospheric oxygen, cannot be excluded. In fact, when left few days in contact with air, 14,15-bisnorlabd-8-en-13-one (5)⁶ was found to contain an appreciable amount of the corresponding 7-oxo derivative.⁶

³ ARYA, P. V., ERDTMAN, H. and KUBOTA, T. (1961) *Tetrahedron* **16**, 255.

⁴ MANGONI, L. and BELARDINI, M. (1964) *Gazz. Chim. Ital.* **94**, 1108.

⁵ BIGLEY, D. B., ROGERS, N. A. J. and BALTRUP, J. A. (1960) *J. Chem. Soc.* 4613.

⁶ MANGONI, L. and BELARDINI, M. unpublished results.

The chemical composition of the oleoresin of *A. cunninghami* is rather different from that of the oleoresins of the other *Araucaria* species examined.^{1,7,8} Furthermore, it is peculiar that, although the acids (3a) and (3d) are present together with a roughly equivalent amount of the more common isomers with the exocyclic double bond [(2c) and (2d)], the $\Delta^{8(17)}$ isomers of the neutral compounds are completely absent.

EXPERIMENTAL

General experimental and procedures for the extraction of the resin and for the separation of acid and neutral fractions have already been described.¹ The oleoresin examined was collected from only one plant (Botanical Garden of the University in Naples, Italy).

Separation of the acid components. Acid fraction (13 g) was adsorbed on silica-gel (450 g, HCl washed). Elution with C_6H_6 containing increasing percentages of Et_2O gave 29 fractions then collected on the basis of TLC and spectral properties.

Esters (1) and (2b). The oily fractions 2–5 (3.8 g eluent C_6H_6) were adsorbed on silica-gel (100 g, HCl washed). Elution with petrol– Et_2O (98:2) gave a crude oily acid (380 mg) which by treatment with ethereal CH_2N_2 afforded the crystalline methyl commutate (1) m.p. 107–108° [$\alpha_D + 48^\circ$ (c 1.2) identical with an authentic sample. Further elution with petrol– Et_2O (19:1) then gave a second substance which treated with CH_2N_2 afforded the pure oily acetoxy ester (2b), [$\alpha_D + 51^\circ$ (c 0.9). Alkaline hydrolysis (KOH–MeOH, 10%) of (2b) afforded methyl isocupressate (2a) identical with authentic material. $LiAlH_4$ reduction of 2b gave agathadiol⁹ m.p. and m.m.p. 108–109°.

Acetoxy ester (3c). Fractions 6–14 (3.6 g eluent C_6H_6) readsorbed on silica-gel (100 g HCl washed; petrol– Et_2O , 9:1) gave an acetoxy acid (400 mg) whose treatment with CH_2N_2 afforded the pure oily acetoxy ester (3c), [$\alpha_D + 108.7^\circ$ (c 1) (Found: C, 73.40; H, 9.50. $C_{23}H_{36}O_4$ requires: C, 73.36; H, 9.64%). LAH reduction of 3c afforded the diol (3e) m.p. and m.m.p. 141–143°.

Methyl isocupressate (2a). Fractions 16–20 (0.9 g eluted with C_6H_6 – Et_2O , 19:1) treated with ethereal CH_2N_2 and chromatographed on alumina (petrol– Et_2O , 9:1) gave the oily methyl isocupressate (2a), [$\alpha_D + 50^\circ$ (c 0.9) identical with authentic material.

Hydroxy acid (3a). The crystalline fractions 22–24 (1.7 g, C_6H_6 – Et_2O , 9:1), after crystallization from C_6H_6 –hexane (7:3) gave the pure 3a m.p. 124–125°, [$\alpha_D + 128^\circ$ (c 1) (Found: C, 75.02; H, 10.51. $C_{30}H_{42}O_3$ requires: C, 74.96; H, 10.06%). Treatment of 3a with excess CH_2N_2 gave the corresponding oily ester (3b), [$\alpha_D + 124^\circ$ (c 0.9); ν_{max} 3550, 1725 cm^{-1} ; δ 5.46, 4.16, 1.68 (t, 1H, d, 2H and s, 3H, $-C(Me)=CH-CH_2O-$), 1.53 (s, 3H, $C_{17}-Me$), 1.07 (s, 3H, $C_{18}-Me$), 0.72 (s, 3H, $C_{20}-Me$). The $LiAlH_4$ reduction product of 3b was identical with the diol (3e).

7-Oxo-acetoxy ester (4). The oily fractions 25–29 (0.9 g eluent C_6H_6 – Et_2O , 4:1) were treated with excess CH_2N_2 and then directly acetylated (Ac_2O –Py over the night). The crude product was then adsorbed on alumina (grade III) and the elution with petrol– Et_2O (3:2) afforded the oily 4 which after further chromatographic purification had [$\alpha_D + 132^\circ$ (c 0.8) (Found: C, 70.68; H, 8.70. $C_{28}H_{34}O_5$ requires: C, 70.74; H, 8.78%). λ_{max}^{EtOH} 251 nm (ϵ 14000); ν_{max} 1740, 1725, 1670 cm^{-1} ; δ 3.05–2.70 (complex m, 2H, C_6 protons), 1.80 (s, 6H, vinylic Me's), 0.95 (s, 3H, $C_{20}-Me$).

Acid isomerization of 2c. Pure 2c (180 mg) was dissolved in anhyd- $CHCl_3$ (40 ml) and dry gaseous HCl was bubbled into the soln at 0° for 1 hr. The soln was then washed with 2 N Na_2CO_3 aq. and the organic layer evaporated to give the crude 3a (160 mg) which after purification had m.p. 125°, [$\alpha_D + 128^\circ$ and was identical with the natural material.

Chromic oxidation of 3c. To 3c (270 mg) dissolved in AcOH (2 ml) and Ac_2O (0.5 ml), solid CrO_3 (55 mg) was added with stirring and kept at r.t. for 10 min. The crude product, after chromatography (alumina III; petrol– Et_2O , 3:2), afforded 4 (120 mg) identical with the natural product.

Separation of the neutral compounds. Neutral fraction (3 g) was adsorbed on alumina (90 g; grade III). Elution with petrol containing increasing percentages of Et_2O afforded 35 fractions collected in three groups: (A) unhydroxylated fractions 2–19 (1.37 g eluted with petrol– Et_2O , 19:1); (B) oily hydroxylated fractions 21–32 (1 g eluted with petrol– Et_2O , 4:1); (C) crystalline hydroxylated fractions 33–35 (0.3 g with Et_2O).

Acetoxy aldehyde (3h) and diacetate (3i). Fraction A (1 g) was readsorbed on silica-gel (30 g). Elution with petrol– Et_2O , 19:1 (600 ml) gave the crude 3h (350 mg). PLC (hexane– Et_2O , 9:1, 4 ×) of the crude material then afforded the oily rather unstable 3h, [$\alpha_D + 67^\circ$ (c 1). Treatment of 3h with semicarbazide acetate yielded the corresponding crystalline semicarbazone m.p. 126–128°, [$\alpha_D + 58^\circ$ (c 0.9) (Found: C, 68.20; H, 9.36; N, 10.41. $C_{23}H_{37}O_3N_3$ requires: C, 68.45; H, 9.24; N, 10.41%). $LiAlH_4$ reduction of 3h gave the diol 3e, m.p. and m.m.p. 141–143°. Further elution with the same solvent (800 ml) then gave the oily diacetate 3i, [$\alpha_D + 49^\circ$ (c 1) (Found:

⁷ BRUNS, K. (1968) *Tetrahedron* **24**, 3417.

⁸ CAPUTO, R., MANGONI, L. and MONACO, P. (1972) *Phytochemistry* **11**, 839.

⁹ ENZELL, C. (1961) *Acta Chem. Scand.* **15**, 1303.

C, 73.72; H, 9.84. $C_{24}H_{38}O_4$ requires: C, 73.85; H, 9.74%. Alkaline hydrolysis (KOH-MeOH, 10%) of **3i** quantitatively afforded **3e**.

Monoacetate (3g) and hydroxy aldehyde (3f). Fraction B by PLC (C_6H_6 -Me₂CO, 4:1) gave a first fraction with higher R_f enriched in **3g** and a second fraction with lower R_f enriched in **3f**. Further chromatographic purification of the first fraction then gave pure oily monoacetate (**3g**), $[\alpha]_D^{25} + 51^\circ$ (c 1.1) (Found: C, 75.70; H, 10.70. $C_{22}H_{36}O_3$ requires: C, 75.81; H, 10.41%). Alkaline hydrolysis of **3g** gave the diol **3e**, m.p. and m.m.p. 142°. Chromatographic purification of the second fraction afforded the pure oily hydroxy aldehyde (**3f**), $[\alpha]_D^{25} + 68^\circ$ (c 1) (Found: C, 78.90; H, 10.71. $C_{20}H_{32}O_2$ requires: C, 78.89; H, 10.59%). ν_{max} 3580, 2700, 1725 cm^{-1} ; δ 9.79 (s, 1H, -CHO), 5.48 and 1.73 (t, 1H and s, 3H, -C(Me)=CH-), 1.64 (s, 3H, C_{17} -Me). NaBH₄ reduction of **3f** quantitatively afforded **3e** identical with authentic material.

Diol (3e). Fraction C after chromatographic purification on alumina (grade III; petrol-Et₂O, 7:3) was crystallized from C_6H_6 -hexane (7:3) and afforded the pure diol (**3e**) m.p. 141-143°, $[\alpha]_D^{25} + 54^\circ$ (c 1.2) (Found: C, 78.31; H, 11.49. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%). ν_{max} 3500, 3350 cm^{-1} ; δ 5.48, 1.71 and 4.17 (t, 1H, s, 3H and d, 2H, -C(Me)=CH-CH₂-O-), 3.62 (ABq, J 11 Hz, 2H, -C-CH₂-O-), 2.58 (s, 3H, C_{17} -Me). Pure **3e** showed undepressed m.p. when mixed with the LiAlH₄ reduction product of **3b**.

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