

# NEW LABDANE DITERPENES FROM *ARAUCARIA COOKI*\*

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**Key Word Index**—*Araucaria cooki*; Araucariaceae; conifer oleoresin; labdane diterpenes.

**Abstract**—Eight new and seven known labdane diterpenes were identified in the oleoresin of *Araucaria cooki*.

## INTRODUCTION

IN PURSUING our chemical study of oleoresins produced by conifers belonging to the *Araucaria* genus,<sup>1-3</sup> we examined the oleoresin exuded from the trunk of *A. cooki*. This paper describes the isolation from this resin of a number of bi- and tri-cyclic diterpenes with labdane, abietane and isopimarane skeletons. Some of them have been found in nature for the first time.

## RESULTS

Fresh oleoresin, collected at the end of summer, was treated with cold ether. A rather large ether-insoluble polymeric fraction was removed by filtration. Evaporation of the ethereal solution then gave a viscous clear yellow oil which was separated into an acid fraction (66%) and a neutral fraction (34%).

TABLE 1. PROPERTIES OF ACIDIC DITERPENES

Compounds*		m.p.	$[\alpha]_D$	Ref.
7,13-Abietadien-18-oic acid (abietic acid)	(1a)	170–172°	–109°	4
Methyl 15-hydroxy-8(17), E-13-labdadien-19-oate (methyl isocupressate)	(2a)	oil	+50°	5
Methyl 13 $\beta$ -hydroxy-8(17), 14-labdadien-19-oate	(3a)	oil	+57°	—
Methyl 13 $\beta$ -hydroxy-8, 14-labdadien-19-oate	(4a)	oil	+140°	—
15-Acetoxy-8(17), E-13-labdadien-19-oic acid (acetyl-isocupressic acid)	(2b)	oil	+49°	2
13 $\beta$ -Acetoxy-8(17), 14-labdadien-19-oic acid	(3b)	oil	+57°	—

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

Chromatographic separation of the acid components led to the compounds listed in Table 1.

Abietic acid (1a) and the already known isocupressic acid (characterized as methyl ester

\* Part IV in the series "Diterpenoids of the Araucariaceae". For Part III see Ref. 3. After the completion of this work, the compounds (3a) and (4a) were described by Carman *et al.*<sup>1,3</sup> in a study of the oleoresin from *Agathis robusta*.

<sup>1</sup> CAPUTO, R. and MANGONI, L. (1974) *Phytochemistry* **13**, 467.

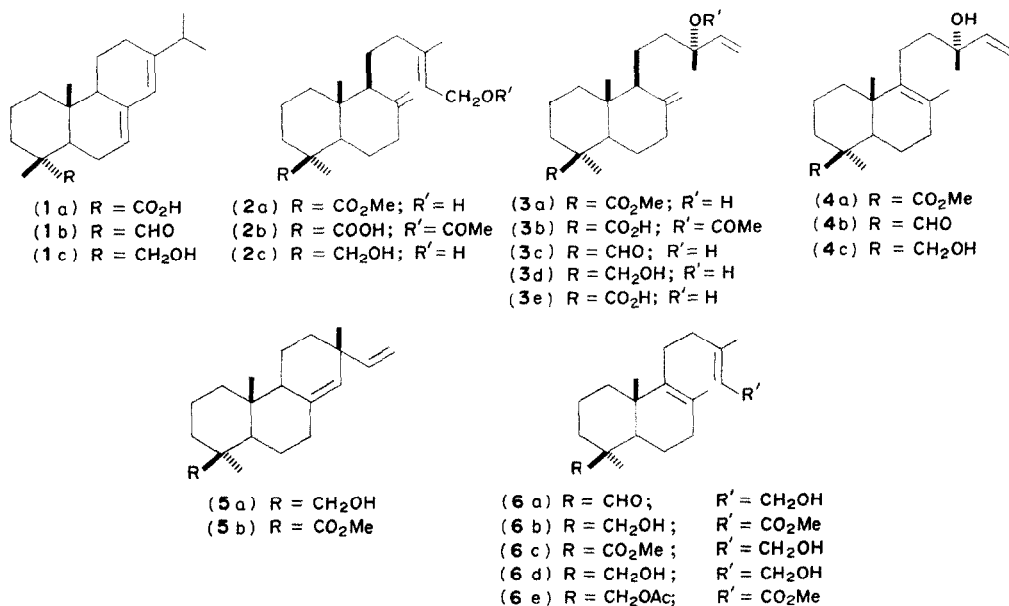
<sup>2</sup> CAPUTO, R., DOVINOLA, V. and MANGONI, L. (1974) *Phytochemistry* **13**, 475.

<sup>3</sup> CAPUTO, R., MANGONI, L. and MONACO, P. (1974) *Gazz. Chim. Ital.*

<sup>4</sup> RUZICKA, L., STERNBACH, L. and JEGER, O. (1941) *Helv. Chim. Acta* **24**, 504.

<sup>5</sup> MANGONI, L. and BELARDINI, M. (1964) *Gazz. Chim. Ital.* **94**, 1108.

(2a)) and acetyl-isocupressic acid (2b) were identified on the basis of the spectral properties and were then shown to be identical with authentic samples. Two further hydroxy-acids, obtained as a mixture, were esterified with ethereal  $\text{CH}_2\text{N}_2$  and then isolated as methyl esters (3a) and (4a) by  $\text{AgNO}_3$  silica chromatography.



The hydroxy ester (3a) showed IR and NMR spectra superimposable to those of methyl cupressate<sup>5</sup> but had a higher optical rotation. Reduction of 3a with  $\text{LiAlH}_4$  led to the crystalline 13-epiturulosol (3d) m.p.  $114-115^\circ$ ,  $[\alpha]_D + 42.3$  thus proving that the hydroxyester (3a) is the epimer of methyl cupressate with the  $\text{C}_{13}\text{S}$  configuration.

The NMR spectrum of the hydroxyester (4a) was identical with that of 3a in the vinyl region, but no further olefinic protons were present. Furthermore, it showed a 3-proton singlet of a vinyl methyl, thus suggesting that it was the  $\Delta^8$  isomer of 3a. Acid rearrangement of 4a led to the known<sup>2</sup> hydroxyester (6c),  $[\alpha]_D + 124^\circ$  thus proving the assigned structure. In this way, chirality at  $\text{C}_{13}$  was destroyed; since biogenetic considerations suggested 4a having the same  $\text{C}_{13}\text{S}$  configuration as 3a, this latter was submitted to isomerization with lithium in diaminoethane.<sup>6</sup> In fact, a reasonable yield of the isomer (4a) was obtained. Finally, the acetoxyacid (3b) by alkaline hydrolysis gave the acid 3e and by  $\text{LiAlH}_4$  reduction 13-epiturulosol (3d).

Our attention was now turned to the neutral fraction of the resin, which after chromatography, gave the compounds listed in Table 2.

All the structures were assigned on the basis of the spectral characteristics and all the known substances were compared with authentic samples either directly or through their derivatives. The crystalline diol (4c) turned out to be identical in every respect with the  $\text{LiAlH}_4$  reduction product of the above hydroxyester (4a). The hydroxy aldehyde (3c) which

<sup>5</sup> CAMBIE, R. C. and FRANICH, R. A. (1970) *Australian J. Chem.* **23**, 93.

TABLE 2. PROPERTIES OF NEUTRAL DITERPENES

Compounds		m.p.	$[\alpha]_D$	Ref.
7,13-Abietadien-18-al (abietinal)	(1b)	oil	-116°	7
8(14), 15-Isopimaradien-18-ol (sandaracopimaradienol)	(5a)	oil	-7°	8
13 $\beta$ -Hydroxy-8(17), 14-labdadien-19-al	(3c)	oil	+36°	—
8(17), 14-Labdadiene-13 $\beta$ , 19-diol (13-epitorulosol)	(3d)	114–115°	+42°	9
13 $\beta$ -Hydroxy-8, 14-labdadien-19-al	(4b)	oil	+95°	—
8,14-Labdadien-13 $\beta$ , 19-diol	(4c)	89–91°	+76°	—
15-Hydroxy-8, E-13-labdadien-19-al	(6a)	oil	+68°	—
Methyl 19-hydroxy-8, E-13-labdadien-15-oate	(6b)	oil	+56°	—
8(17), E-13-Labdadiene-15, 19-diol (agathadiol)	(2c)	109–110°	+33°	10

was spectrally almost identical with torulosal<sup>10</sup> was reduced by  $\text{LiAlH}_4$  to give the crystalline 13-epitorulosol (3d). Furthermore, structures of hydroxy-aldehydes 4b and 6a were assigned on the basis of their conversion, by  $\text{LiAlH}_4$  reduction, into the diol (4c) and into the known<sup>2</sup> diol (6d) respectively. Finally, the hydroxy ester (6b) which was characterized as the crystalline acetyl derivative (6e) m.p. 58–60°,  $[\alpha]_D + 58.4^\circ$  was transformed by  $\text{LiAlH}_4$  reduction into the diol (6d).

The oleoresins of the *Araucaria* species so far examined<sup>1–3,11</sup> show a rather varied chemical composition. Diterpenes with labdane (or rearranged labdane)<sup>1</sup> skeleton, however, are more frequent than those with pimarane or abietane skeletons. Furthermore, labdane resins show a marked tendency to polymerize. Such polymers are elsewhere known<sup>12</sup> to consist of large quantities of diterpenes like communic acid and communol, thus increasing the actual amounts of labdane materials present in the oleoresin. Thus the oleoresins of *Araucaria* strongly resemble those of *Agathis* (also *Araucariaceae*) and at the same time show marked affinities with the resins from most genera of the *Cupressaceae*.

## EXPERIMENTAL

General experimental procedures have already been described.<sup>1</sup> The oleoresin examined was collected from only one plant (Botanical garden of the University in Palermo, Italy).

*Separation of the acid components.* The acid extract (22 g) was adsorbed on silica-gel (700 g, HCl washed). Elution with petrol containing increasing percentages of  $\text{Et}_2\text{O}$  yielded 21 fractions then collected in four groups on the basis of TLC and spectral evidences.

*Abietic acid (1a).* The crystalline fractions 3–8 (8.6 g, petrol- $\text{Et}_2\text{O}$ , 19:1) after repeated crystallizations from hexane yielded abietic acid (1a) m.p. 170–172°,  $[\alpha]_D - 109^\circ$  (c 1.2) identical with authentic material.

*Acetoxy acids (2b) and (3b).* The oily fractions 9–16 (8.75 g, petrol- $\text{Et}_2\text{O}$ , 9:1) were rechromatographed on silica-gel (250 g, HCl washed). Elution with petrol- $\text{Et}_2\text{O}$  (19:1) gave the pure oily 2b (3.2 g),  $[\alpha]_D + 49^\circ$  (c 1:1) compared with an authentic sample. Further elution with petrol- $\text{Et}_2\text{O}$  (93:7) then gave the oily acetoxy acid (3b) (5.4 g),  $[\alpha]_D + 56.7^\circ$  (c 1.7); MW 362 (MS). Alkaline hydrolysis (KOH-MeOH 10%) of 3b gave the hydroxy-acid 3e.  $\text{LiAlH}_4$  reduction of 3b gave the crystalline 13-epitorulosol (3d) m.p. and m.m.p. 114–115°.

*Hydroxy esters (4a) and (3a).* The fractions 17–19 (3 g, petrol- $\text{Et}_2\text{O}$ , 7:3) were treated with excess ethereal  $\text{CH}_2\text{N}_2$  and then adsorbed on silica-gel impregnated with  $\text{AgNO}_3$  (95 g). Elution with petrol- $\text{Et}_2\text{O}$  (17:3) yielded

<sup>7</sup> CHIRKOVA, M. A. (1966) *Izv. Sib. Otd. Akad. Nauk. SSSR* **2**, 99.

<sup>8</sup> IRELAND, R. E. and SCHIESS, P. (1963) *J. Org. Chem.* **28**, 6.

<sup>9</sup> ROWE, J. W. and SHAFTE, J. W. (1967) *Tetrahedron Letters* 2528.

<sup>10</sup> ENZELL, C. (1961) *Acta Chem. Scand.* **15**, 1303.

<sup>11</sup> BRUNS, K. (1968) *Tetrahedron* **24**, 3417.

<sup>12</sup> GOUGH, L. J. and MILLS, J. S. (1972) *Nature* **239**, 527.

<sup>13</sup> CARMAN, R. M., CRAIG, W. J. and SHAW, I. M. (1973) *Australian J. Chem.* **26**, 209.

the pure oily hydroxy ester (**4a**) (2.4 g),  $[\alpha]_D + 139.8^\circ$  (c 1.8); MW 334 (MS) (Found: C, 75.27; H, 10.32.  $C_{21}H_{34}O_3$  requires: C, 75.40; H, 10.25%).  $\nu_{\max}$  3530, 1725  $\text{cm}^{-1}$ ;  $\delta$  4.9–6.1 (3H, ABX system, vinyl protons), 1.57 (3H, s, vinyl-Me), 1.29 (3H, s,  $C_{16}$ -Me), 1.19 (3H, s,  $C_{20}$ -Me), 0.76 (3H, s,  $C_{18}$ -Me). LAH reduction of **4a** gave the crystalline diol (**4c**) m.p. 89–91°.  $[\alpha]_D + 76^\circ$  (c 1.2). Further elution with petrol-Et<sub>2</sub>O (3:1) then gave the second hydroxy ester (**3a**) (0.5 g);  $[\alpha]_D + 5.7^\circ$  (c 1.7); MW 334 (MS) (Found: C, 75.60; H, 10.16.  $C_{21}H_{34}O_3$  requires: C, 75.40; H, 10.25%).  $\nu_{\max}$  3540, 1725  $\text{cm}^{-1}$ ; NMR spectrum identical with methyl cupressate.<sup>6</sup> A pure sample of (**3a**) reduced by LiAlH<sub>4</sub> yielded the crystalline 13-epitorulosol (**3d**) m.p. and m.m.p. 114–115°.

**Methyl isocupressate (2a).** The oily fractions 20–21 (1.1 g, petrol-Et<sub>2</sub>O, 3:2) were treated with excess ethereal CH<sub>2</sub>N<sub>2</sub> and then chromatographed on silica-gel (32 g). Elution with petrol-Et<sub>2</sub>O (7:3) gave the pure oily methyl isocupressate (**2a**) (0.95 g),  $[\alpha]_D + 50^\circ$  (c 1.4) compared with an authentic sample. LiAlH<sub>4</sub> reduction of **2a** gave the crystalline agathadiol (**2c**) m.p. and m.m.p. 109–110°.

**Acid rearrangement of 4a.** Pure hydroxy ester (**4a**) (200 mg) was dissolved in CHCl<sub>3</sub> (50 ml) and dry gaseous HCl was bubbled into the soln for 3 hr. The soln was then washed with 2N Na<sub>2</sub>CO<sub>3</sub> aq. and the organic layer evaporated to give the crude **6c** (190 mg) which after purification had  $[\alpha]_D + 124^\circ$  and was identical with authentic material.

**Isomerization of 3a.** Pure hydroxy ester (**3a**) (200 mg) was added to diaminoethane (10 ml) containing Li (300 mg) and refluxed for 20 hr. Workup of the reaction mixture gave a crude product which after chromatography (silica-gel-AgNO<sub>3</sub>; C<sub>6</sub>H<sub>6</sub>-Et<sub>2</sub>O, 9:1) afforded the pure oily (**4a**) (110 mg; 55% Y).  $[\alpha]_D + 140^\circ$  identical with authentic material.

**Separation of the neutral components.** The neutral fraction (12.6 g) was adsorbed on alumina (380 g; grade III). C<sub>6</sub>H<sub>6</sub> eluted a less polar fraction (3.5 g) consisting of hydrocarbons (IR, NMR) and whose composition has not been yet investigated. Benzene with increasing percentages of ether gave 24 fractions then collected in five groups according to TLC and spectral evidences.

**Abietinal (1b) and sandaracopimaradienol (5a).** The oily fractions 1–5 (3.5 g, C<sub>6</sub>H<sub>6</sub>-Et<sub>2</sub>O, 19:1) were rechromatographed on silica-gel (105 g, petrol-Et<sub>2</sub>O, 19:1). The first 1400 ml eluted abietinal (**1b**) (150 mg),  $[\alpha]_D - 115.8^\circ$  (c 1.3) whose LAH reduction yielded the corresponding alcohol (**1c**)  $[\alpha]_D - 73.4^\circ$ . Further 2500 ml then eluted 2 not yet identified isomeric alcohols of lower MW [222 (MS)]. Further elution with 1200 ml more of the same eluent gave the crude (**5a**) (1750 mg) which was directly acetylated (Ac<sub>2</sub>O-Py). The resulting acetate, after chromatographic purification, was hydrolyzed (KOH-MeOH, 10%) to give the pure oily (**5a**) (1400 mg),  $[\alpha]_D - 7^\circ$  (c 2) identical with a sample specially prepared by LAH reduction of (**5b**).

**Hydroxy-aldehydes (3c) and (4b) and hydroxy-ester (6b).** Fractions 6–9 (1.1 g, C<sub>6</sub>H<sub>6</sub>-Et<sub>2</sub>O, 9:1) were rechromatographed on silica-gel (32 g). Elution with petrol-Et<sub>2</sub>O (17:3; 390 ml) gave the oily hydroxy-aldehyde (**3c**) (0.4 g),  $[\alpha]_D + 36.3^\circ$  (c 1.4); MW 304 (MS) (Found: C, 78.95; H, 10.50.  $C_{20}H_{32}O_2$  requires: C, 78.89; H, 10.59%).  $\nu_{\max}$  3500, 2700, 1710  $\text{cm}^{-1}$ ;  $\delta$  9.79 (1H, s, -CHO). LiAlH<sub>4</sub> reduction of **3c** yielded 13-epitorulosol (**3d**) m.p. and m.m.p. 114–115°. Elution with petrol-Et<sub>2</sub>O (41:9 400 ml) then gave the second hydroxy-aldehyde (**4b**) (0.32 g),  $[\alpha]_D + 95^\circ$  (c 1.1); MW 304 (MS) (Found: C, 78.76; H, 10.72.  $C_{20}H_{32}O_2$  requires: C, 78.89; H, 10.59%).  $\nu_{\max}$  3510, 2705, 1710  $\text{cm}^{-1}$ ;  $\delta$  9.77 (1H, s, -CHO), 1.02 (3H, s,  $C_{20}$ -Me), 0.82 (3H, s,  $C_{18}$ -Me). LiAlH<sub>4</sub> reduction of **4b** gave the crystalline diol (**4c**) m.p. 89–91°.  $[\alpha]_D + 76^\circ$  (c 1.2) identical with the LiAlH<sub>4</sub> reduction product of **4a**. Finally, elution with petrol-Et<sub>2</sub>O (4:1; 350 ml) gave the crude hydroxy-ester (**6b**) (0.21 g) which was directly acetylated. The crude acetate (**6c**), after chromatographic purification, was crystalline, m.p. 58–60° (from MeOH),  $[\alpha]_D + 58.4^\circ$  (c 1.9); MW 376 (MS) (Found: C, 73.32; H 9.61.  $C_{23}H_{36}O_4$  requires: C, 73.36; H, 9.64%).  $\nu_{\max}$  1740, 1725  $\text{cm}^{-1}$ ;  $\delta$  5.71 (1H, br, vinyl proton), 4.09 (2H, q,  $J$  11.4 Hz, -CH<sub>2</sub>OAc), 3.69 (3H, s, -COOMe), 1.57 (6H, s, vinyl-Me's). Alkaline hydrolysis (KOH-MeOH, 10%) of (**6c**) and subsequent treatment of the crude product with ethereal CH<sub>2</sub>N<sub>2</sub> gave the pure oily hydroxyester (**6b**).  $[\alpha]_D + 56.2^\circ$ . LiAlH<sub>4</sub> reduction of **6b** yielded the crystalline diol (**6d**) m.p. 141–143°.  $[\alpha]_D + 54^\circ$  identical with authentic material.

**Hydroxy-aldehyde (6a).** Fractions 10–15 (0.3 g eluted with C<sub>6</sub>H<sub>6</sub>-Et<sub>2</sub>O, 4:1) rechromatographed on silica-gel (10 g petrol-Et<sub>2</sub>O, 8:2) gave the oily hydroxy-aldehyde (**6a**) (0.25 g),  $[\alpha]_D + 68^\circ$  (c 1.4). LiAlH<sub>4</sub> reduction of **6a** gave the crystalline diol (**6d**) m.p. and m.m.p. 141–143°.

**Diols (4c) and (3d).** The crystalline fractions 16–21 (0.6 g, C<sub>6</sub>H<sub>6</sub>-Et<sub>2</sub>O, 3:2) after several crystallizations from hexane-C<sub>6</sub>H<sub>6</sub> (7:3) gave the pure diol (**4c**) (0.4 g) m.p. 89–91°.  $[\alpha]_D + 76^\circ$  (c 1.2); MW 306 (MS) (Found: C, 78.22; H, 11.36.  $C_{20}H_{34}O_2$  requires: C, 78.38; H, 11.18%).  $\nu_{\max}$  3550  $\text{cm}^{-1}$ ;  $\delta$  1.54 (3H, s,  $C_{17}$ -Me), 0.98 (3H, s,  $C_{18}$ -Me), 0.91 (3H, s,  $C_{20}$ -Me) identical (m.m.p. 89°) with the LiAlH<sub>4</sub> reduction product of the hydroxy-ester (**4a**). The mother liquors of crystallization of (**4c**) were rechromatographed on silica-gel (6 g; eluent petrol-Et<sub>2</sub>O, 7:3) and after several crystallizations from hexane-C<sub>6</sub>H<sub>6</sub> (7:3) yielded 13-epitorulosol (**3d**) m.p. 114–115°.  $[\alpha]_D + 42.3^\circ$  (c 1.3) identical with authentic material.

**Agathadiol (2c).** The crystalline fractions 22–24 (100 mg, C<sub>6</sub>H<sub>6</sub>-Et<sub>2</sub>O, 1:1) after repeated crystallizations from MeOH gave the pure agathadiol (**2c**) m.p. 109–110°.  $[\alpha]_D + 33^\circ$  (c 1.4) identical with authentic material.

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