

## The Chemistry of the Order Araucariales

### Part 4.\* The Bled Resins of *Agathis australis*

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The bled resin of the New Zealand kauri, *Agathis australis* (Lamb. ex D Don) Steud., contains as principal components agathic, *cis*-communic, *trans*-communic, sandaracopimaric and abietic acids, sandaracopimaradienol, *cis*-communol, *trans*-communol and agathic acid monomethyl ester.

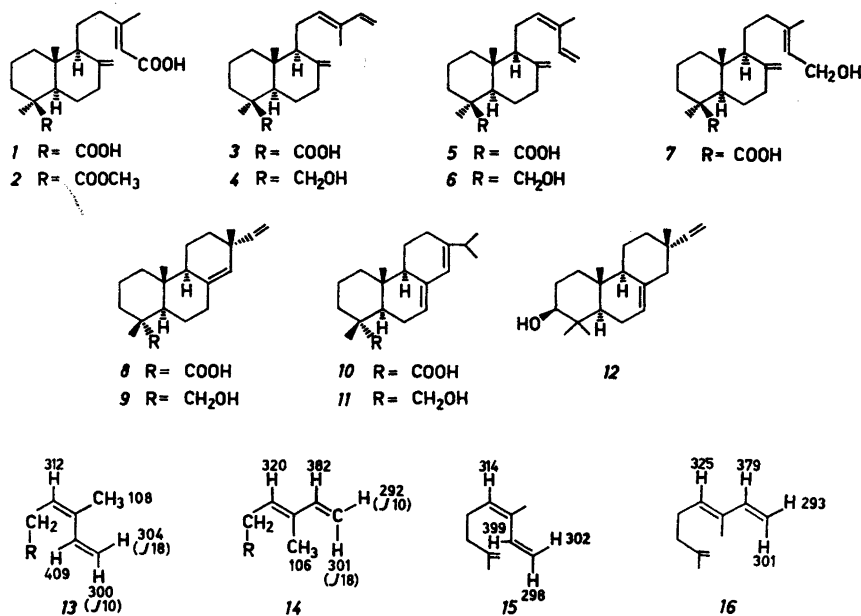
The bled resins of the New Zealand kauri ("kauri gum"), because of their economic importance in the past, have been the subject of a number of investigations but these have been somewhat inconclusive. Apart from the small amount of essential oil in the resin, the only recognisable products obtained have been very small yields of agathic acid (*I*) (0.5–1%)<sup>1</sup> and, very recently, of abietic acid (*10*) (1%) and sandaracopimaric acid (*8*) (1%).<sup>2</sup> The results of earlier work on resins of the genus *Agathis* have been summarised in a previous paper in this series.<sup>3</sup>

A detailed examination of kauri wood resin, described in the same paper, has now shown<sup>4</sup> that it contains isopimara-7,15-dien-3 $\beta$ -ol and the related ketols, araucarol, araucarone, araucarolone, (which is the main constituent) and araucarenolone. Kauri bled resin which forms the subject of the present paper is however quite different from the wood resin and contains none of these compounds.

The bled resin sold commercially has been mainly the "fossil" resin that is found in the soil in areas where kauri forests have formerly grown but also includes resin collected from natural accumulations on living trees. Thus the age of a sample of kauri bled resin may vary from a few weeks to many thousands of years. An excellent survey of the different types of resin, their occurrence and their physical properties has been given by Hosking.<sup>1</sup>

The present investigation has concerned almost entirely the acetone-soluble fraction of the crude resin. Very fresh bled resin is almost completely

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soluble in acetone, but on ageing the resin undergoes partial polymerisation (*cf.* Refs. 1 and 2) giving a fraction that is insoluble in acetone but soluble in benzene/acetone or benzene/alcohol mixtures. This material varies in amount from about 0 % in very fresh resin to about 50 % in a sample a few years old, it gives no distinct spots on thin layer chromatograms and on drying gives a hard brittle resin. It was not further investigated. The constituents involved in the formation of the acetone-insoluble resin are shown by the composition of resins of different ages given in Table 1.

Thin layer chromatograms of the acetone-soluble resin with hexane/acetone (80:20) on silica gel showed eight significant spots corresponding to fractions: 1 hydrocarbons, 2 esters and compounds of similar  $R_F$ , diterpene alcohols with 3 equatorial C(4)-CH<sub>2</sub>OH and 4 axial C(4)CH<sub>2</sub>OH, diterpene acids with, 5 axial C(4)COOH and 6 equatorial C(4)COOH, 7 diterpene ester acids, and 8 diterpene di-acids. The resin was separated into corresponding fractions by column chromatography on silica gel with hexane/acetone mixtures and in some cases further separated by chromatography on silver nitrate/silica gel. The separations were followed by thin layer chromatography and gas chromatography. The possibility of isomerisation or loss of any significant component was minimised by comparing NMR spectra of the original acetone-soluble resins and of some of the crude fractions.

The compounds identified in the mixture are listed in Table 1.

The fractions of the acetone-soluble resin corresponding to the first two spots of the thin layer chromatogram gave quite complex gas chromatograms. These components are still under investigation and will be described in a later communication.

Table 1. Approximate percentages of the main constituents of the acetone-soluble bled resin of *Agathis australis*.

	Fresh resin	Resin 2-4 years old	"Fossil" resin
Sandaracopimaric acid (8)	4	7	15
Sandaracopimaradienol (9)	5	15	10
Abietic acid (10)	20	17	30
<i>trans</i> -Communic acid (3)	7	7	—
<i>trans</i> -Communol (4)	4	7	—
<i>cis</i> -Communic acid (5)	7	—	—
<i>cis</i> -Communol (6)	6	—	—
Agathic acid (1)	10	5	}15
Agathic acid monomethyl ester (2)	20	10	
Hydrocarbons, esters etc. (fractions 1 and 2)	10-15	10-20	7
Percentage of acetone-soluble resin in crude resin	95	50	35

The fraction containing diterpene alcohols with an equatorial C(4)CH<sub>2</sub>OH group (fraction 3) was almost entirely sandaracopimaradienol (9).<sup>5</sup>

Fraction 4, containing diterpene alcohols with an axial C(4)CH<sub>2</sub>OH group, was a mixture of *trans*-communol (4)<sup>6,7</sup> and the previously unknown *cis*-communol (6) (see below). The components of the mixture were identified by retention index (as defined by Kovats<sup>8</sup>) on a neopentylglycol succinate column and by comparison of the NMR spectrum of the mixture and the spectra of *trans*-communol and *cis*-communol prepared by lithium hydrido-aluminate reduction of *trans*-communic acid and *cis*-communic acid.

Fraction 5 containing diterpene acids with an axial C(4) carboxyl group was a mixture of *trans*-communic acid (3)<sup>6,7,13</sup> and *cis*-communic acid (5) (see below). These were separated by chromatography of their methyl esters on silver nitrate/silica gel. *trans*-Communol and *trans*-communic acid are also found together in the oleoresin of *Pinus elliottii*.<sup>9</sup> In *Agathis*, *trans*-communic acid has also been found in *A. palmerstonii* and *A. robusta*.<sup>10</sup>

Fraction 6 containing diterpene acids with an equatorial C(4) carboxyl group was a mixture of abietic acid (10) and sandaracopimaric acid (8)<sup>11</sup> which was methylated and separated by chromatography on silver nitrate/silica gel. Abietic acid and sandaracopimaric acid have also been isolated by Gough from kauri "fossil" bled resin.<sup>2</sup> *A. palmerstonii* and *A. robusta* oleoresins have recently been shown by Carman<sup>12</sup> to contain neo-abietic and laevopimaric acids in addition to abietic acid. Ultraviolet spectra show that laevopimaric acid is not a significant component of the present *A. australis* samples.

The fractions corresponding to the two spots of lowest  $R_F$  on the thin layer chromatogram contained agathic acid monomethyl ester (2) and agathic acid (1), respectively. The monomethyl ester must have structure 2 since on methylation with diazomethane it gives agathic acid dimethyl ester and since the C(4) methyl band is shifted from 37 cps in agathic acid to 32 cps in the monomethyl ester. In accordance with this structure it was very resistant to alkaline hydrolysis while esters of the C(15) allyl carboxyl group are readily

hydrolysed (*cf.* Ref. 1, p. 15). Although agathic acid is a common constituent of *Agathis* bled resins, this monomethyl ester does not appear to have been isolated previously.

The structure of the *cis*-communic acid isolated from fraction 5 was shown by its NMR and ultraviolet spectra and its chromatographic behaviour. Like *trans*-communic acid it is rather labile and was difficult to purify. It clearly contains an axial carboxyl group at C(4) (thin layer  $R_F$ ; methyl peak at 40 cps; AB multiplet at 214 cps in the related alcohol), a C(8) exocyclic methylene group (peaks at 290 and 268 cps) and a conjugated diene grouping  $\lambda_{\max}$  235  $m\mu$  ( $\epsilon$  20 000); methyl peak at 106 cps, vinyl region with 6 protons). The very close similarity, apart from the vinyl region, of its NMR spectrum to that of *trans*-communic acid shows that, given a normal isoprenoid skeleton, it can differ only in the configuration of the C(12):C(13) double bond. That it has the same carbon skeleton as *trans*-communic acid was shown by catalytic hydrogenation of the methyl ester to the hexahydro compound (*cf.* Ref. 7), identical with that prepared from the *trans* isomer (infrared spectrum, GLC retention index).

The main difference in the NMR spectrum of the two isomers is in the position of the quartet due to the C(14) proton. In the new isomer this appears as expected for the *cis* compound (13) well downfield (409 cps,  $J$  10 and 18 cps) of its position in the *trans*-compound (14) where it appears at 382 cps. The configuration of the C(12):C(13) double bond in *trans*-communic acid has very recently been assigned by Norin<sup>13</sup> on the basis of the similarity of its NMR spectrum with that of *trans*- $\alpha$ -ocimene. Comparison of the NMR data for *cis*-communic acid (13) and *trans*-communic acid (14) ( $\text{CDCl}_3$ ) and for *cis*- $\alpha$ -ocimene (15) and *trans*- $\alpha$ -ocimene (16) ( $\text{CCl}_4$ )<sup>14</sup> shows excellent agreement between the two pairs of isomers and provides confirmation of the *cis* configuration for the new communic acid isomer. The ultraviolet absorption spectra maxima of the two communic acid isomers (*cis* 235  $m\mu$ ; *trans* 232  $m\mu$ ) are in accord with those of *cis*- and *trans*- $\alpha$ -ocimene<sup>14</sup> (*cis* 234.5  $m\mu$ ; *trans* 231  $m\mu$ ) and other similar *cis* and *trans* pairs.

The values given in Table 1 for the percentages of the different components in the bled resin are approximate estimates based on column chromatographic weights and GLC relative peak area. No attempt was made to allow for small amounts of water and volatile lower terpenes in the resin. Further refinement is at present unjustified in view of the variations possible in the original source and the changes proceeding in the resin with age. However the results obtained appear to account for all significant peaks in the NMR spectrum and the gas chromatogram and for all the spots on the thin layer chromatogram (phosphomolybdic acid spray) apart from the material present in the less polar fractions 1 and 2.

The fresh bled resin that exudes from the bark of *Agathis australis* is thus a mixture of agathic acid, communic acid, abietic acid, sandaracopimaric acid, and related compounds. As the resin ages, *cis*-communol and *cis*-communic acid are lost first and in the sample of resin which was one or two years old they were practically absent and *trans*-communol and *trans*-communic acid were very much reduced. In the "fossil" resin sample which contained 60–70 % of acetone-insoluble material the communic acid type of compound is

insignificant, sandaracopimaric acid is proportionately increased and a thin layer chromatogram shows minor spots that are not evident in chromatograms of fresh resin. The composition of the "fossil" resin varies also with the environment in which it has lain since it was formed. Thus in "swamp resin" the small amount of  $\alpha$ -pinene usually found is replaced by fenchyl alcohol.<sup>1</sup>

The compounds found in kauri bled resin in this investigation are all in accord with the biogenetic pattern discussed in a previous paper and with the correlation in the *Agathis* resin diterpenes between the four different types of ring A oxygenation (3, 18, 19 or none) and the structures of ring C or the corresponding side chain.<sup>3</sup> A careful examination was made of the NMR spectra of samples of fractions 3, 4, 5, and 6 but no indication was found of the presence of any compound with equatorial oxygenation at C(4) and a communic acid or agathic acid side-chain or with axial oxygenation at C(4) and a sandaracopimaric or abietic acid type ring C.

While oxygenation of the C(4) methyl groups might be expected to follow cyclisation of rings A and B, the specificity of the oxygenation of the equatorial and axial methyl groups at C(4) in the different groups of compounds found in *Agathis* gives some indication that the orientation at C(4) may be implicit in the cyclising configuration of the acyclic precursor and is possibly determined by two alternative ways of closing ring A.

Isopimara-7,15-dien-3 $\beta$ -ol (12) and its oxidation products araucarol, araucarone, araucarolone and araucarenolone which are present in very large amount in the wood resin of *Agathis australis*<sup>3</sup> are apparently absent from the bled resin. Compounds such as isocupressic acid (7, R = COOH) which might be expected to form a precursor of both agathic acid and the communic acids cannot be present in more than small amount and have not yet been identified in the present material though isocupressic acid itself occurs in *Cupressus sempervirens*.<sup>15</sup> No evidence was found of the presence of compounds that could have been formed by an allylic rearrangement of a precursor of this type, and manool and manool oxide could not be detected on thin layer chromatograms.

The function of conifer resins is often regarded as uncertain. However the bled resin of *Agathis australis* appears to be excellently suited as a protective material for repairing damage to the outer layers of the tree. It is exuded as a latex from canals that are present only in the bark and hardens on exposure to air and light, initially by loss of water and a monoterpene solvent and then more slowly by polymerisation. The wood resin probably also has protective functions since kauri timber is very durable. A preservative adapted for the interior of the trunk would not of course be expected to have the same properties as a preservative for the exterior surface.

Particularly for a long-lived tree like the kauri, both types of resin would confer a very definite genetic advantage as protective agents. Isoprenoid products are required by plants for a variety of physiological purposes and provide a readily available base from which suitable coating and plugging materials could evolve.

#### EXPERIMENTAL

NMR spectra (CDCl<sub>3</sub>; cps from TMS) were recorded on a Varian DP 60 spectrometer. Analyses are by Dr. A. P. Campbell, University of Otago.

**Resin samples.** Fresh kauri resin samples were collected from the stumps and butts of newly felled trees. These samples contained small amounts of acetone-insoluble material none of which was soluble in benzene/alcohol. The 2–4 year resin sample was a single lump collected from a living tree; its age could only be estimated. The "fossil" resin sample was of unknown age but of a type which could be hundreds of years old or older.

**Extraction.** The crude resins were broken into small pieces and extracted twice for 24 h with 5–10 times their weight of acetone. Since the material was somewhat labile it was extracted only as required.

The acetone-insoluble resins were rubbery gelatinous substances giving a hard brittle resin on drying and were soluble in benzene/acetone, benzene/alcohol and similar mixtures. Thin layer chromatograms of this material showed only indefinite streaking.

**Chromatography.** Column chromatograms were run on silica gel (Merck) using permanent columns regenerated for each run by washing first with acetone and then with hexane. The silver nitrate/silica gel column was prepared from silica gel containing 5 % silver nitrate and was regenerated with ether/hexane (70:30) after each run.

Gas chromatograms were run at 180°C on 3 % neopentylglycol succinate on Gas Chrom P treated with hexamethyldisilazane; all gas chromatographic data are given as retention indexes ( $I_{180}^{\text{NPGS}}$ ). Relative peak areas were estimated as the product of peak height and retention time.

**Separation.** Thin layer chromatograms (silica gel G, (Merck)) of the resin samples in hexane/acetone (80:20) showed significant spots (phosphomolybdic acid) at 0.99 (Fraction 1), 0.92 (2), 0.84 (3), 0.77 (4), 0.40 (5), 0.31 (6), 0.22 (7), 0.11 (8), ( $\beta$ -sitosterol reference spot 0.55).

The resin was separated into fractions corresponding to the thin layer spots by column chromatography with hexane/acetone mixtures, usually after separation into acid and neutral fractions.

The percentages of the different components present in the resin (Table 1) were estimated from gas chromatograms of the neutral fraction and of the esters obtained by methylation (diazomethane) of the crude acid fraction and fractions 5 and 6. (A mixture of methyl sandaracopimarate, methyl *cis*-communol and methyl *trans*-communol was not resolved by gas chromatography alone under these conditions and it was necessary to make a preliminary separation by column or thin layer chromatography).

**Fractions 1 and 2.** Fresh resin contained about 10 % of these two fractions. Gas chromatograms showed them to contain a complex mixture of substances; these are still under investigation.

**Sandaracopimaradienol (9).** Fraction 3 was an oil containing almost entirely a single component (tlc in hexane/acetone 70/30; glc; NMR) which was identified as sandaracopimaradienol,<sup>5</sup>  $[\alpha]_D -7^\circ$  ( $\text{CHCl}_3$ ,  $c$  2.1),  $I_{180}^{\text{NPGS}}$  2750, NMR 360–285 (m, 3H,  $\text{CH}_2=\text{CH}-$ ), 311 (s, C(14)H), 196 (AB, 2H,  $-\text{CH}_2\text{OH}$ ), 62, 50, 48 (3 s, 9H,  $\text{CH}_3-$ ). The NMR and infra-red spectra were identical with those of a sample obtained by reduction of sandaracopimaric acid with lithium hydrido-aluminate.

**trans-Communol and cis-communol.** Fraction 4 from fresh resin showed two glc peaks at  $I_{180}^{\text{NPGS}}$  2830 and 2785. The compound giving the peak at 2830 was identified as *trans*-communol<sup>6,7</sup> by comparison with a sample prepared by reduction of *trans*-communol acid with lithium hydrido-aluminate. The NMR spectrum of *trans*-communol (382 (q,  $J$  17 and 11 cps, C(14)H), 326 (t, C(12)H), 320–291 (m, 2H, C=CH<sub>2</sub>), 291 and 270 (2 s, C(8)=CH<sub>2</sub>), 214 (AB,  $J$  10 cps,  $-\text{CH}_2\text{OH}$ ), 107 (s, vinylic CH<sub>3</sub>-), 58, 42 (2 s, 6H, CH<sub>3</sub>-)) was identical with that of fraction 4 except for the vinyl peaks due to *cis*-communol.

The compound giving the peak at 2785 was identified as *cis*-communol by comparison with a sample prepared by reduction of *cis*-communol acid (see below) with lithium hydrido-aluminate. The reduction product was an oil and could not be obtained completely pure but its NMR spectrum (409 (q,  $J$  17 and 11 cps, C(14)H), 326–291 (m, 3H, C(12)H and C(15)H<sub>2</sub>), 291 and 270 (2 s, C(8)=CH<sub>2</sub>)) agreed with that of fraction 4 except for the vinyl peaks due to *trans*-communol.

**trans-Communol acid.** Fraction 5 from resin 1 gave an NMR spectrum with vinyl peaks at 420–270 cps which were identical with those of fraction 4 and methyl peaks at 107, 58, and 40 cps (3 s, 9H). Methylation with diazomethane gave an oil which showed two glc peaks of about equal area at  $I_{180}^{\text{NPGS}}$  2570 and 2530 and a much smaller peak at 2515.

Chromatography of the methyl esters (160 mg) on a silver nitrate/silica gel column gave three fractions. The third fraction (50 mg) on crystallisation from methanol followed by sublimation gave methyl *trans*-communate, m.p. 105–106°,  $\lambda_{\max}$  232  $\mu$  ( $\epsilon$  25 000),  $I_{180}^{\text{NPGS}}$  2570, nmr: 382 (q,  $J$  18 and 10 cps, C(14)H), 320 (t, C(12)H), 292 and 268 (2 s, C(8)=CH<sub>2</sub>), 217 (3H, CH<sub>3</sub>O-), 106, 72, 34 (3 s, CH<sub>3</sub>-),  $\nu_{\max}$  (nujol) 1725, 1640, 1605, 990, 980, 960, 890, 840, 820  $\text{cm}^{-1}$ .

*cis*-Communate acid. The second fraction from silver nitrate/silica gel chromatography of the methyl esters of fraction 5 was an oil (40 mg) which on sublimation gave methyl *cis*-communate, m.p. 41–42°,  $[\alpha]_{\text{D}} + 45^\circ$  (CHCl<sub>3</sub>,  $c$  2.3),  $\lambda_{\max}^{\text{(EtOH)}}$  235  $\mu$  ( $\epsilon$  20 000),  $I_{180}^{\text{NPGS}}$  2530, NMR: 408 (q,  $J$  10 and 18 cps, C(14)H), 327–302 (m, 3H, C(12)H and C(15)H<sub>2</sub>), 293 and 271 (2H, C(8)=CH<sub>2</sub>), 217 (3H, CH<sub>3</sub>-), 108, 72, 33 (3s, CH<sub>3</sub>-). (Found: C 79.5; H 10.2. C<sub>21</sub>H<sub>32</sub>O<sub>2</sub> requires C 79.7; H 10.2).

*Methyl hexahydrocommunate*. Methyl *cis*-communate (20 mg) was hydrogenated (PtO<sub>2</sub>/H<sub>2</sub>/EtOH) for 3 h and the product was filtered through alumina in hexane/ether solution and distilled giving methyl hexahydro-communate as an oil (15 mg),  $I_{180}^{\text{NPGS}}$  2375,  $\nu_{\max}$  (film) 1727, 1230, 1195, 1150, 1095, 990, 805, 770  $\text{cm}^{-1}$ . The infrared spectrum and retention index were identical with those of a specimen prepared in the same way from methyl *trans*-communate.

*Sandaracopimaric acid*. Fraction 6 according to its NMR spectrum and a gas chromatogram of the methyl ester (diazomethane) contained two components. Crystallisation of the crude fraction from ethanol gave sandaracopimaric acid, m.p. 171–172°,  $[\alpha]_{\text{D}} - 13^\circ$  (CHCl<sub>3</sub>,  $c$  2.0); NMR: 361–285 (m, 3H, -CH=CH<sub>2</sub>) 313 (s, C(14)H), 72, 62, 50 (3) s, CH<sub>3</sub>-). The methyl ester gave  $I_{180}^{\text{NPGS}}$  2530.

*Abietic acid*. Fraction 6, after methylation with diazomethane was chromatographed on silver nitrate/silica gel. Elution with ether/hexane mixtures gave as first fraction, methyl sandaracopimarate, and then on further elution, methyl abietate as an oil,  $\lambda_{\max}^{\text{(EtOH)}}$  241  $\mu$ ; NMR: 348 (C(14)H), 322 (C(7)H), 219 (3H, CH<sub>3</sub>O-), 76 (s, CH<sub>3</sub>-), 60 (d, CH<sub>3</sub>-), 50 (s, CH<sub>3</sub>-),  $I_{180}^{\text{NPGS}}$  2715. The identity of the methyl abietate was confirmed by comparison of retention index and NMR spectrum with those of a commercial sample.

*Agathic acid*. Fraction 8, on crystallisation from acetone and then methanol gave agathic acid, m.p. 202–203°,  $[\alpha]_{\text{D}} + 54^\circ$  (CHCl<sub>3</sub>,  $c$  2.1); NMR: 337, 290 268 (3 s, 3H, C=CH-), 131, 75, 37 (3 s, CH<sub>3</sub>-). The dimethyl ester (diazomethane) gave  $I_{180}^{\text{NPGS}}$  3005; NMR: 131, 71, 30 (3 s, CH<sub>3</sub>-).

*Agathic acid monomethyl ester*. Fraction 7 was an oil which gradually solidified on standing to give almost pure agathic acid monomethyl ester, m.p. 81–86°,  $[\alpha]_{\text{D}} + 57^\circ$  (CHCl<sub>3</sub>,  $c$  2.7); NMR: 337, 290, 268 (3 s, 3H, C=CH-), 218 (s, CH<sub>3</sub>O-), 131, 71, 31 (3 s, CH<sub>3</sub>-). (Found: C 72.5; H 9.5. Calc for C<sub>21</sub>H<sub>32</sub>O<sub>4</sub>: C 72.4; H 9.3). The dimethyl ester (diazomethane) was identical (NMR, infrared spectrum, retention index) with a sample prepared from agathic acid.

Agathic acid and its monomethyl ester gave only faint spots with phosphomolybdic acid after normal heating (10 min at 110°) but these spots gradually intensified on standing.

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