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# The Chemistry of the Order Araucariales

## 2.* The Wood Resin of *Agathis australis*

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The wood of the New Zealand kauri, *Agathis australis* (Lamd. ex D. Don) Steud. (= *A. australis* Salisbury), contains the four closely related ketols, araucarolone (11), araucarone (12), araucarol (13) and araucarenolone (15), together with isopimaradien-3$\beta$-ol (14), $\beta$-sitosterol and a number of minor constituents.

The resins derived from various species of the genus *Agathis* have in the past found considerable industrial use in the production of varnishes, lacquers and floor coverings. Manila copal from *Agathis dammara* (Lamb.) L. C. Rich. (= *A. alba* (Lamb.) Foxw.) has still some importance but the resin from the New Zealand kauri (mostly "fossil" resin), of which more than 500 000 tons have been produced over the past hundred years, is now no longer significant.

In spite of the former economic importance of kauri resin, little has been known of its chemical constituents. The earliest recorded investigation was made in 1843 at about the time when export of the resin from New Zealand first began.[1] A sample of the resin was found by Tschirch and Niederstadt[2] in 1901 to consist of: acidic material 75 %, neutral 12 %, oil 13 %. They obtained agathic acid (1) in 1.5 % yield from the acid fraction. The structure of agathic acid was elucidated, by Hosking and Ruzicka[3] and the oil was shown by Hosking[4] to be a mixture of *d*-$\alpha$-pinene and dipentene. After the completion of the present work Gough[5] has isolated about 1 % each of sardarcopimaric (8) and abietic (9) acids from the fossil resin of *A. australis* and suggested that the petroleum insoluble bulk of the resin consists predominantly of a copolymer of communic acid (3) and communol (4).

Agathic acid has been isolated from a number of other *Agathis* resins[6,7] including Manila copal from *A. dammara* (up to 15 %), *A. vitiensis* (Seem.)

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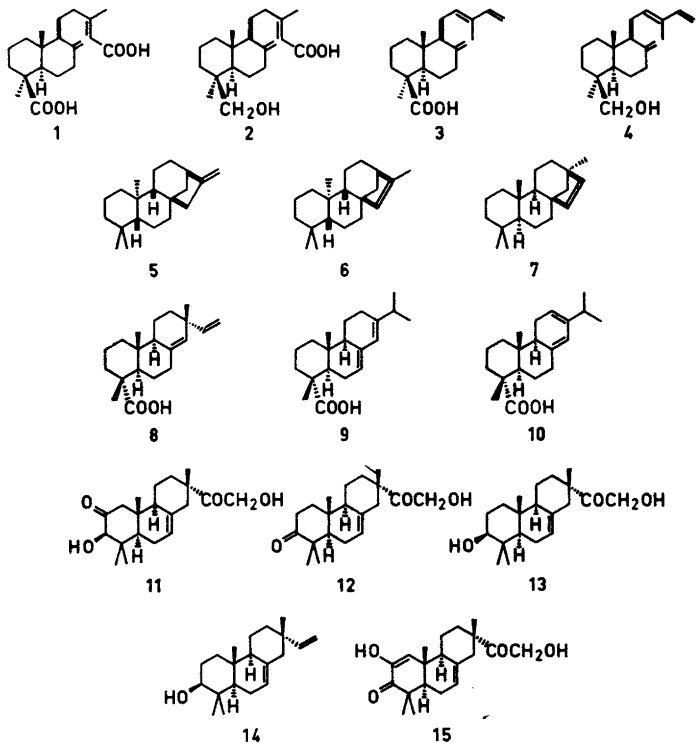



Warb. resin (5 %), *A. palmerstonii* F. Meull. resin (8 %), *A. robusta* (Moore) F. Muell. (= *A. Brownii* (Lemaire) L. H. Bailey) resin (1 %) and quite recently by Carman [8] from *A. microstachya* Bailey and White oleoresin (45 %). Carman and Dennis [9] have also isolated communic acid (3) and laevopimaric acid (10) from *A. robusta* oleoresin but were unable to find any agathic acid or the dundathic and dundatholic acids isolated by Baker and Smith [10] from this resin. In view of the rather drastic conditions used in obtaining them, dundathic and dundatholic acids are very probably artifacts. In addition to agathic acid, Manila copal also contains the very closely related agatholic acid (2).[11]

Much more is known of the leaf oil of *Agathis australis*. In addition to several mono- and sesquiterpenes it has been found to contain the diterpenes, (−)-kaurene (5), (−)-isokaurene (6) and cupressene (7, = hibaene).[12–14]

Although most of the previous work on kauri resin has been done on either fresh or fossil bled resins, it seemed that the wood resin should have undergone less change since its formation and should be easier to investigate. The present paper describes the isolation of the main constituents of the wood resin. Structural work on the compounds obtained is described elsewhere.[15]

Extraction of a fresh sample of kauri heartwood with acetone gave a brown resin (7.5 %) which was taken up in ether/acetone and separated into ether-insoluble (1 %), neutral (4.8 %) and acidic fractions (1.7 %).

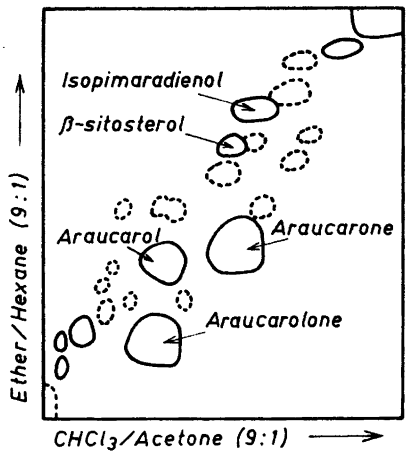


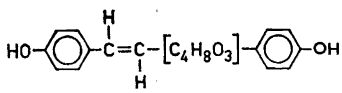

*Fig. 1.* Constituents of the neutral fraction of *Agathis australis* wood resin.

Two-dimensional thin layer chromatography on silica gel (Fig. 1) showed that the neutral fraction contains three main components. These were separated by column chromatography on silica gel and were shown to be the three closely related ketols, araucarolone (11), araucarone (12) and araucarol (13).[15] These three compounds represent approximately 45, 20, and 5 %, respectively, of the neutral resin. The neutral fraction also contains isopimaradien-3$\beta$-ol (14) and $\beta$-sitosterol as minor constituents but no terpene hydrocarbon could be detected on thin layer chromatography. According to its NMR spectrum the fastest moving spot was a mixture of fatty acid esters.

The acidic fraction of the wood resin was separated into bicarbonate-soluble, carbonate-soluble and sodium hydroxide-soluble fractions by successive extractions of an ether/acetone solution.

The sodium hydroxide-soluble fraction contains as principal components the diosphenol 15,[15] closely related to the ketols obtained from the neutral fraction, and four other components of lower $R_F$ on a thin layer plate ($R_F$:s 0.04, 0.14, 0.32 and 0.39 in hexane/acetone 7:3). These four components are apparently a set of related phenolic compounds: they are non-crystalline and somewhat unstable. The substance of $R_F$ 0.39 and $R_F$ 0.04 were characterised by ultraviolet and NMR spectra.

The substance of $R_F$ 0.04 also forms the major part of the bicarbonate-soluble fraction and is thus the most important of these phenolic substances (it is about 5 % of the total resin). We have accordingly given it the name agatharesinol. Present evidence on its structure can be explained by the partial formula 16. The substance of $R_F$ 0.39 is probably, from its NMR spectrum, an acetonide of agatharesinol and may have been formed during

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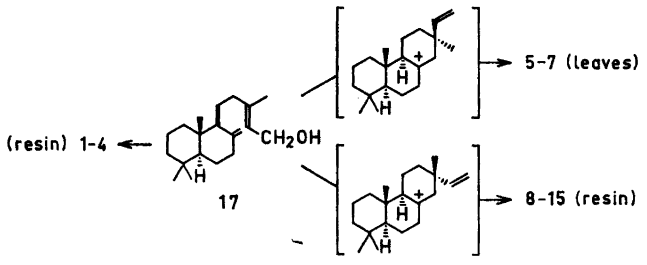
the acetone extraction of the resin. It is readily obtained by treatment of agatharesinol in acetone with dimethyl sulfate and sodium bicarbonate. The evidence for the structure of agatharesinol and the results of investigations at present in progress on the structures and relationships of these phenolic compounds will be discussed in a separate communication.

The carbonate-soluble fraction according to thin layer chromatography contains four main components. The fastest moving spot, from its NMR spectrum contains principally a linoleic-type acid or acids (triplet at 325 cps). The other three components were mixtures of diterpene acids which give NMR spectra that are clearly related to those of the four main neutral compounds. Thin layer chromatography and an NMR spectrum of the crude acid fraction showed that agathic acid was not a significant component of this sample of resin.

The composition of the wood resin from *Agathis australis* is thus quite different from that of the bled resins of *Agathis* species that have been investigated so far, including the bled resin of *Agathis australis* which in a current investigation[16] in this laboratory is shown to contain the principal components sandarcopimaric (8) and communic (3) acids, the corresponding alcohols, agathic acid (1) and its monomethyl ester. Apart from the phenolic substances the bulk of the wood resin is made up of derivatives of isopimar-7-ene. It contains little or none of the compounds with a labdane skeleton that are present in the *Agathis* bled resins and none of the diterpene hydrocarbons present in the leaves. As yet it is only from the leaf oil that the compounds of abnormal configuration have been obtained.

The gross structure of the diterpenes so far isolated from *Agathis* is readily accounted for by currently accepted biogenetic concepts,[17] the cyclisation of the initial acyclic precursor here probably involves an intermediate such as 17 (or its antipode). These compounds also show a relationship between the ring A oxygenation and the cyclisation of ring C in that the dicyclic compounds are oxygenated at the axial C(4) methyl group, the tricyclic at C(3) or at the equatorial C(4) methyl group and the tetracyclic lack oxygen. Since sandarcopimaric acid, but not isopimaric acid, has been isolated from *Agathis* species the former type of compound here represents a more likely precursor than the latter, thus indicating that in the tricyclic diterpenes of *Agathis* oxygenation at C(3) may more specifically be associated with a C(7):C(8) double bond and oxygenation at the equatorial C(4) methyl group with a C(8):C(14) double bond. However, lack of evidence regarding the stage at which oxygenation



occurs in any of the above groups of compounds makes it at present impossible to say if there in *Agathis* is a direct relationship between the two processes.

Further investigations of the distribution of the compounds described here and of the minor constituents of kauri and other *Agathis* species are in progress.

## EXPERIMENTAL

NMR spectra were recorded on a Varian DP 60 spectrometer (deuterochloroform solutions except where otherwise specified; scaled in cps from TMS (60 Mc)).

*Chromatography.* Column chromatograms were run almost entirely on silica gel (Merck). For large scale chromatography the silica gel columns were used repeatedly and were regenerated by washing with approximately a half column volume of acetone and then a half column volume of the solvent to be used in the next separation. When strongly adsorbed materials were left on the column it was stripped with acetone/acetic acid or ethanol/water before washing with acetone.

Thin layer chromatograms were run on Merck silica gel G or GF. They were developed by spraying with phosphomolybdic acid and heating at 110° for 10 min.

*Extraction.* A block of fresh kauri heartwood (approx. 10 kg), collected in North Auckland, New Zealand, was milled and extracted with acetone (twice for 24 h at room temperature). The extract was concentrated to small volume under reduced pressure and the dark brown resin, still containing some acetone, was diluted with ether. The insoluble material that separated from the solution was again dissolved in a small amount of acetone and reprecipitated by adding ether. The material still insoluble, amounting to 1 % of the weight of dry wood, was discarded. The material soluble in ether/acetone was separated into neutral (4.8 %) and acidic (1.7 %) fractions by extraction of the combined ether/acetone solutions with 5 % sodium hydroxide solution. Acetone was added as required during this separation to keep the resin in solution.

*Neutral fraction.* The neutral resin was precipitated twice from acetone solution by adding hexane. This removed much of the compounds giving spots with an $R_F$ greater than that of araucarone. The remainder when dissolved in a small amount of ethanol and left in the refrigerator for several days deposited a crystalline mixture of araucarolone and araucarone (1.3 %). Column chromatography gave the approximate percentages of the main components of the total neutral resin as: forerun 10, araucarone 20—25, araucarol 5—10, araucarolone 45, final fraction 10.

*Araucarolone* (11) was most conveniently obtained by recrystallisation of the crystalline part of the neutral fraction from acetone/hexane and then from ethanol, m.p. 157—159°, $[\alpha]_D$ −42° ($CHCl_3$, *c* 5.4). Further amounts were obtained by chromatography of the main neutral fraction on silica gel with ether/hexane mixtures.

*Araucarone* (12) was obtained by chromatography of the crystalline neutral fraction with ether/hexane mixtures or by chromatography of the main neutral fraction, first with ether/hexane mixtures and then with chloroform/acetone (4:1). It was recrystallised from ethanol, m.p. 115—116°, $[\alpha]_D$ −51° ($CHCl_3$, *c* 2.2).

*Araucarol* (13) was obtained from the main neutral fraction by chromatography in hexane/ether (1:4) to remove araucarolone and then with chloroform/acetone (4:1) to remove araucarone. When pure it crystallised readily from ethanol, m.p. 124—125°, $[\alpha]_D$ −24° ($CHCl_3$, *c* 1.7).

*Isopimara-7,15-dien-3β-ol* (14). The neutral material soluble in hexane and the forerun fractions available from chromatography of the main neutral fraction were combined (24 g) and chromatographed with ether/hexane mixtures. Ether/hexane (7:3) eluted a fraction (2.6 g) containing largely isopimaradien-3β-ol. It was recrystallised several times from ethanol, m.p. and mixed m.p. 146—147°, $[\alpha]_D$ −36° ($CHCl_3$, *c* 1.8), NMR spectrum identical with that of authentic material.

*β-Sitosterol.* The fractions immediately following those containing isopimaradien-3β-ol in the chromatogram described above gave a mixture (2.4 g) which on crystallisation from methanol gave β-sitosterol as main component, m.p. and mixed m.p. 156—158°, NMR spectrum identical with that of authentic material.

*Acidic fraction.* A solution of the acidic material in a small amount of acetone was diluted with ether and then extracted successively with bicarbonate solution, carbonate solution and sodium hydroxide solution.

The sodium hydroxide-soluble fraction (approx. 0.5 % of the wood) showed five main spots on a thin layer chromatogram on silica gel GF (Merck) run in hexane/acetone (7:3) (approx. $R_F$:s 0.04, 0.14, 0.32, 0.39, and 0.57). These spots could all be detected by ultraviolet light or with phosphomolybdic acid.

The carbonate-soluble fraction (approx. 0.9 % of the wood) was taken up in chloroform and decanted from a small amount of insoluble material containing the compounds present in the sodium hydroxide-soluble fraction. The product, according to its behaviour on thin layer chromatograms in chloroform/acetone/acetic acid and ether/hexane/methanol mixtures was a mixture of carboxylic acids. Comparison with reference spots on thin layer chromatograms and the NMR spectrum of the total carbonate-soluble fraction indicated that isopimaric acid and agathic acid were not present in significant amount in the resin from this sample of wood. The fastest moving component appears from its NMR spectrum to contain mainly a linoleic type unsaturated fatty acid or acids, NMR (cps): 325, t ($-CH=CH-$); 167, t ($=CH-CH_2-CH=$); 53, distorted triplet ($CH_3-CH_2CH_2CH_2-$).

The bicarbonate-soluble fraction (approx. 0.3 % of the wood) contains one major component. This is identical with the substance giving the $R_F$ 0.04 spot on the thin layer chromatogram of the sodium hydroxide-soluble fraction.

*Araucarenolone* (15). The sodium hydroxide-soluble fraction was chromatographed with ether/hexane (9:1) and the material corresponding to the spot at $R_F$ 0.57 was crystallised from acetone and then ethanol giving araucarenolone, m.p. 143–144°, $[\alpha]_D$ −58° ($CHCl_3$, *c* 2.0). Further amounts of araucarenolone were obtained by chromatography of the material remaining in the ether solution after extraction of the sodium hydroxide-soluble fraction.

*Agatharesinol.* The bicarbonate-soluble fraction chromatographed on silica gel with chloroform/ethanol/acetone (7:1:2) gave agatharesinol as a pale amber resin, $\lambda_{max}^{EtOH}$ 266 mμ (ε 26 000), $[\alpha]_D$ −26° (acetone, *c* 2.3). (Found: C 64.6; H 6.6. $C_{18}H_{20}O_5 \cdot H_2O$ requires C 64.7; H 6.6). NMR (cps, acetone): 526, s, 2H (O*H*); 430–394, m, 8H (aromatic protons); 376, m, 2H (vinyl protons); 245–197, b, $\nu_{max}^{film}$ 1610, 1515, 1450, 1240, 1175, 1020, 970, 830 $cm^{-1}$. Acetylation with acetic anhydride/pyridine at 20° gave a colourless acetate as a resin, $\lambda_{max}^{EtOH}$ 256 mμ (ε 24 000) $[\alpha]_D$ −19° (acetone, *c* 1.0). NMR (cps): 438–412, m, 8H (aromatic protons); 376, AB, 2H (vinyl protons); 327, m, 1H (C*H*OAc); 267–225, m, 4H; 133, 133, 120, 110, 4 s, 12H ($CH_3CO$).

The substance of $R_F$ 0.39 (hexane/acetone 7:3) after purification by chromatography on silica gel with chloroform/acetone (9:1) was a colourless resin, $\lambda_{max}^{EtOH}$ 263 mμ (ε 28 000), NMR (cps, $CDCl_3$/10 % deuteroacetone): 438–405, m, 8H; 378, AB, 2H (vinyl protons); 279–203, m, 4H; 84, 81, 2 s, 6H ($CH_3C$). (Found: C 69.2; H 7.3. $C_{21}H_{24}O_5 \cdot C_3H_6O$ requires C 69.5; H 7.3). Treatment of agatharesinol (50 mg) in acetone (5 ml) with dimethyl sulfate (0.5 ml) and sodium bicarbonate (1.0 g) on a steam bath for 2 h followed by chromatographic purification with chloroform/acetone (9:1) gave the substance of $R_F$ 0.39 which was shown to be identical with material from the sodium hydroxide-soluble fraction by its NMR spectrum and by thin layer chromatography (identical $R_F$:s in chloroform/acetone (9:1) and in hexane/acetone (65:35)).

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