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NORLIGNAN FROM THE KNOT RESIN OF *ARAUCARIA ANGUSTIFOLIA*

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Key Word Index—*Araucaria angustifolia*; Araucariaceae; Parana pine; knot; norlignan; 2,3-bis-(*p*-hydroxyphenyl)-2-cyclopentene-1-one; 4,4′-dihydroxychalcone; cryptoresinol; lignan.

Abstract—A new norlignan, 2,3-bis-(*p*-hydroxyphenyl)-2-cyclopentene-1-one, was isolated from the knot resin powder of *Araucaria angustifolia*. The structure was elucidated by spectroscopic chemical analyses. In addition 4,4′-dihydroxychalcone, cryptoresinol (norlignan), four known lignans and one known norlignan, were isolated for the first time from the resin. A qualitative and quantitative comparison of these compounds in knot, heartwood and sapwood of *A. angustifolia* was performed from the viewpoint of plant chemistry. Detectable amounts of the new compound were present in both knot and heartwood.

INTRODUCTION

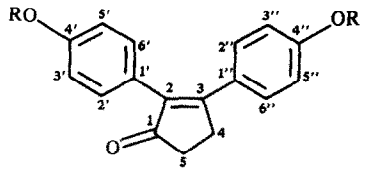
It is well established that *Araucaria angustifolia* (Parana pine in Brazil) and *Araucaria* species contain various lignans such as (+)-pinoresinol, (+)-pinoresinol monomethyl ether, (+)-pinoresinol dimethyl ether, (+)-isolariciresinol, (+)-isolariciresinol monomethyl ether, (−)-secoisolariciresinol and many other compounds [1, 2]. We have reinvestigated the phenolic constituents in knot resin, and in knot, heartwood and sapwood parts of Parana pine, and found three new phenolic compounds, in addition to the five known phenolic compounds found in this species. One of the new compounds is a new type of norlignan in the plant kingdom.

RESULTS AND DISCUSSION

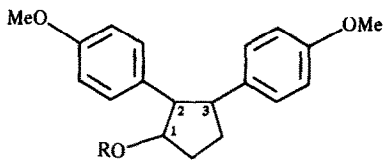
In Brazil the knot powder of *A. angustifolia* is used in flexographic inks, plastic laminates, furniture varnishes, and as a partial substitute for phenolic resins [3]. Eight phenolic compounds (**1**–**8**) were isolated from the powder.

On TLC analysis, compound **1** gave one spot which was positive to diazotized sulphanilic acid and 2,4-dinitrophenylhydrazine reagents. The IR spectrum of **1** suggested the presence of a carbonyl group, an olefinic double bond and an aromatic ring which might be conjugated to each other. This suggestion was supported by the absorption maximum in the UV spectrum (325 nm) and the yellow colour of compound **1**. Compound **1** gave one acetate (**1a**) and one methylate (**1b**), respectively. The latter was formed easily on usual diazomethane treatment. The mass spectra of **1**, **1a** and **1b** indicated molecular ions of *m/z* 266, 350 and 294, respectively. Thus, it was concluded that **1** has two aromatic hydroxyl groups. The molecular formula of the original compound is $C_{17}H_{14}O_3$.

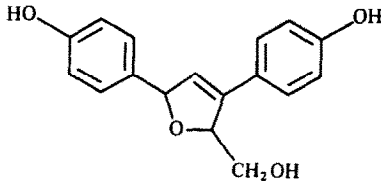
On ^1H and ^{13}C NMR analysis of the parent compound and its derivatives, it was possible to assign their signals as follows. The chemical shifts of the two methoxyl groups of compound **1b** [$C_{17}H_{12}O$-$(OMe)_2$] were located at δ3.81 and 3.82 in the ^1H NMR spectrum and at δ55.2 and 55.3 in ^{13}C NMR spectrum, respectively. This meant that they must have been formed from phenolic hydroxyl groups as mentioned above. Two pairs of A_2B_2



R
1 H
1a Ac
1b Me



R
1c H
1d Ac



3 (cryptoresinol)

system signals in the ^{1}H NMR spectrum (δ7.06 and 7.16) indicated the presence of two 1,4-disubstituted aromatic nuclei whose carbons were assigned to the signals at δ113.8, 114.1, 125.0, 128.1, 129.8, 130.7, 159.1 and 160.9 in the ^{13}C NMR spectrum. This was also supported by the mass spectrum of **1b**, which contained the characteristic ion $[MeO\text{–}\phi\text{–}CH_2]^+$, at *m/z* 121 [2, 4]. The remaining four protons (δ_H 2.67 and 3.02) were regarded as two methylene signals (δ_C 29.2 and 34.6 for the two carbons), and they were coupled with each other with a symmetrical multiple pattern. This established that the two methylene groups are located in the same aliphatic ring, and one of them (δ3.02) is situated adjacent to the carbonyl group.

The ^{13}C NMR chemical shifts of the olefinic carbons without protons, C-2 and C-3 (δ138.0 and 166.8) suggested the presence of a cyclic pentene structure, in which a carbonyl group was present (δ208.1) [5]. It was considered that the structure of 1-(*p*-hydroxyphenyl)-2-(*p*-hydroxybenzoyl)-1-cyclobutene was improbable for **1**, because one characteristic fragment ion $[MeO\text{–}\phi\text{–}C{\equiv}O]^+$ (*m/z* 105) was not recognized in the mass spectrum of **1b** [5]. The most appropriate structure of compound **1** was deduced to be that of 2,3-bis-(*p*-hydroxyphenyl)-2-cyclopentene-1-one. This structure was confirmed by instrumental analysis of the derivatives, 1-hydroxy-2,3-bis-(*p*-methoxyphenyl)-cyclopentane (**1c**) and 1-acetoxyl-2,3-bis-(*p*-methoxyphenyl)-cyclopentane (**1d**). The former (**1c**) was derived from the methylate [2,3-bis-(*p*-methoxyphenyl)-2-cyclopentene-1-one] (**1b**) by hydrogenation of Pd-C, and the latter (**1d**) was prepared by acetylation of the former (**1c**). In the ^{1}H NMR spectrum of **1d**, the newly derived proton from the carbonyl group [C(1)H(OAc)] was recorded at δ5.43 as a complex multiplet, and also the assignment of other protons in the cyclic pentane was consistent with the proposed structure.

This is the first report of the isolation of 2,3-bis-(*p*-hydroxyphenyl)-2-cyclopentene-1-one (**1**) in nature. Compound **1** is a new type of norlignan, but there was a question as to whether or not this compound was an artifact of the isolation procedure used or a degradation product formed by wood decaying fungi. This question is now irrelevant, because the new norlignan has been isolated from the heartwood of Japanese cypress (*Chamaecyparis obtusa*, Cupressaceae), in addition to the detection in separately imported heartwood and knot of *A. angustifolia* [H. Ohashi, unpublished work]. The skeletal structure of **1** (HO–ϕ–C=C–ϕ–OH) is related to that (HO–ϕ–C–C–ϕ–OH) of the norlignan, sequirin D (2-hydroxy-5-(*p*-hydroxybenzyl)-5,6-dihydronaphtalene) from *Sequia sempervirens* (Taxodiaceae) [6].

4,4'-Dihydroxychalcone (**2**) and cryptoresinol (**3**) were newly isolated from Parana pine knot resin and their structures were determined by direct comparison with authentic compounds [7–9]. The remarkable chalcone has been isolated from the heartwood of Japanese cypress, and the latter norlignan has been isolated from the heartwoods of Japanese cedar (*Cryptomeria japonica*, Taxodeaceae) and Japanese cypress.

Four known lignans (**4–7**) and one norlignan (**8**) were also isolated from *A. angustifolia* knot resin and identified as (−)-secoisolariciresinol (**4**), (+)-isolariciresinol (**5**), (+)-isolariciresinol monomethyl ether (**6**), (+)-pinoresinol monomethyl ether (**7**) and hinokiresinol (**8**), by direct comparison with authentic specimens or comparison of analytical data [1, 3, 10, 11]. Nevertheless, in the present experiment, the known lignans of (+)-pinoresinol and (+)-pinoresinol dimethyl ether could not be isolated from the resin powder.

Plant chemistry was used to find the main phenolic compounds (**1–5**, **7** and **8**) in knot, heartwood and sapwood of Parana pine. They were identified by co-TLC or HPLC with authentic compounds isolated from knot resin. Their amounts were determined to be as shown in Table 1. The sapwood contained almost all of the main compounds though in only small amounts. The knot contained more of them than the heartwood. It was considered that the differences between the parts of Parana pine were due to differences in their biosynthetic and rate of translocation to the respective tissues. The results are very suggestive as to the site of synthesis of such secondary metabolites.

EXPERIMENTAL

General. Mp: uncorr; TLC: silica gel using the following solvent systems; (1) C_6H_6–EtOAc–HOAc (40:10:1) and (2) C_6H_6–EtOAc–HOAc (40:20:1). After development, compounds were located by spraying with diazotized sulphanilic acid reagent and/or exposure to UV light (254 and 365 nm). IR: KBr; GC: 2% Silicone SE-30 on Chromosorb W (80–100 mesh), stainless 0.3 (ϕ) × 200 cm; column temp., 270°; carrier gas and flow rate, N_2 at 30 ml min^{-1}; detector, FID; HPLC: Unisil-Q 60-5, 0.4 (ϕ) × 25 cm; eluting solvent, *n*-hexane–EtOAc (1:2); flow rate, 1 ml min^{-1}, monitor, UV (275 nm); MS: EIMS, direct

Table 1. Amounts of main phenolic compounds in different parts of *A. angustifolia*

Compound	Heartwood	Sapwood	Knot	Knot resin*
2,3-Bis-(*p*-hydroxyphenyl)-2-cyclopentene-1-one (**1**)	0.1		t	0.1
4,4'-Dihydroxychalcone (**2**)	t	0.01	0.4	0.7
Cryptoresinol (**3**)	t		t	t
Secoisolariciresinol (**4**)	7.9	0.13	6.4	21.0
Isolariciresinol (**5**)	0.9	0.06	3.8	15.7
Pinoresinol monomethyl ether (**7**)	1.1	0.04	3.1	2.7
Hinokiresinol (**8**)	0.2	0.03	3.7	0.7
Total	10.2	0.27	17.0	41.3

Calculated in percentages for one gram of the dried resin or wood powder.
*: Commercial; t: trace.

insertion probe, 70 eV; NMR: 270 or 60 MHz, shifts recorded relative to an int. TMS standard.

Origin of samples. The knot resin and knot of *A. angustifolia* were provided by Gifu Shellac Seizousho Co., Ltd (Gifu) and the timber by Wanibe Shoukai Co., Ltd (Nagoya). Their identities were guaranteed by companies in Brazil, from where the samples had been imported.

Extraction and isolation. Knot resin powder was applied to silica gel (Wako gel C-200) and eluted with a mixture of $CHCl_3$ and MeOH (9:1), followed by further separation and purification by repeated CC, prep. TLC and fractional recrystallizations. In addition to the 5 known phenolic compounds (**4–8**), 3 phenolic compounds (**1–3**) were isolated and purified.

2,3-*Bis*-(p-*hydroxyphenyl*)-2-*cyclopentene*-1-*one* (**1**). Yellow amorphous (Et_2O), mp 280–283°. TLC (solvent system 1): R_f 0.19 (colouration: diazotized sulphanilic acid reagent, orange; 2,4-dinitrophenylhydrazine reagent, reddish orange); GC: R_t (min): 6.9; HPLC R_t (min): 5.8; UV λ_{max}^{MeOH} nm: 238, 325; IR ν_{max}^{KBr} cm^{-1}: 3270 (OH), 1645 (conjugated C=O), 1615 (conjugated C=C), 1600 (Ar. C=C), 1565, 1510, 1360, 1295, 1180, 825 (1,4-disubstituted phenyl); MS *m/z* (rel. int.): 266 $[M]^+$ ($C_{17}H_{14}O_3$) (100), 249 $[M-17]^+$ (16), 237 $[M-29]^+$ (9), 223 $[M-43]^+$ (51), 210 (31), 207 (11), 181 (14), 165 (10), 152 (12), 133 [HO–ϕ–CH–CH–CH_2]$^+$ (9), 107 [OH–ϕ–CH_2]$^+$ (9) [2, 5]; ^{1}H NMR (Me_2CO-d_6) δ: 2.56 (2H, *m*, H-4), 2.87 (2H, *m*, H-5), 7.30 (4H, *dd*, A_2B_2 system, J=8.4 Hz, H-2″, H-6″, H-3″, H-5″), 7.40 (4H, *dd*, A_2B_2 system, J=8.4 Hz, H-2′, H-6′, H-3′, H-5′); ^{13}C NMR (pyridine-d_5) δ: 29.0 (C-4), 34.0 (C-5), 116.2 (C-3″, C-5″), 116.5 (C-3′, C-5′), 120.4 (C-1″), 127.4 (C-1′), 130.7 (C-2″, C-6″), 132.7 (C-2′, C-6′), 137.8 (C-3), 160.7 (C-4″), 161.7 (C-4′), 166.8 (C-2), 207.3 (C-1 C=O).

2,3-*Bis*-(p-*acetoxyphenyl*)-2-*cyclopentene*-1-*one* (*diacetate*) (**1a**). Compound **1** was acetylated with Ac_2O in pyridine. Needles (**1a**) (MeOH), mp 240–242°. MS *m/z* (rel. int.): 350 $[M]^+$ [$C_{17}H_{12}O_3(C_2H_3O)_2$] (19.0), 308 $[M-42]^+$ (64), 266 $[M-84]^+$ (100), 249 (21), 237 (10), 223 (32), 210 (16), 207 (14), 195 (9), 181 (10), 165 (10), 152 (13), 133 (12), 107 (13), 77 (7), 43 (52); ^{1}H NMR ($CDCl_3$) δ: 2.28 (6H, *s*, Ac-4′, Ac-4″), 2.70 (2H, *m*, H-4), 3.00 (2H, *m*, H-5), 7.06 (4H, *dd*, A_2B_2 system, J=8.8 Hz, H-2″, H-6″, H-3″, H-5″), 7.16 (4H, *dd*, A_2B_2 system, J=8.8 Hz, H-2′, H-6′, H-3′, H-5′).

2,3-*Bis*-(p-*methoxyphenyl*)-2-*cyclopentene*-1-*one* (*dimethylate*) (**1b**). Compound **1** was methylated with CH_2N_2 in Et_2O. Needles (**1b**) (MeOH), mp 109–110°. MS *m/z* (rel. int.): 294 $[M]^+$ [$C_{17}H_{12}O_3(Me)_2$] (100), 279 $[M-15]^+$ (5), 263 (5), 251 (24), 223 (13), 221 (15), 152 (13), 147 (15), 121 [MeO–ϕ–CH_2]$^+$ (29); ^{1}H NMR ($CDCl_3$) δ: 2.67 (2H, *m*, H-4), 3.02 (2H, *m*, H-5), 3.81, 3.82 (6H, each *s*, MeO-4′; MeO-4″), 6.99 (4H, *dd*, A_2B_2 system, J =8.8 Hz, H-2″, H-6″, H-3″, H-5″), 7.13 (4H, *dd*, A_2B_2 system, J =8.8 Hz, H-2′, H-6′, H-3′, H-5′); ^{13}C NMR ($CDCl_3$) δ: 29.2 (C-4), 34.6 (C-5), 55.2, 55.3 (MeO-4″, MeO-4′), 113.8 (C-3″, C-5″), 114.1 (C-3′, C-5′), 125.0 (C-1″), 128.1 (C-1′),129.8 (C-2″, C-6″), 130.7 (C-2′, C-6′), 138.0 (C-3), 159.1 (C-4″), 160.9 (C-4′), 166.8 (C-2), 208.1 (C-1 C=O).

1-*Hydroxy*-2,3-*bis*-(p-*methoxyphenyl*)-*cyclopentane* (**1c**). The Methylate (**1b**) was reduced with 5% Pd-C in EtOH. The product (**1c**) was purified by prep. TLC (*n*-hexane–EtOAc, 4:1). The oily product was used in the next procedure after MS analysis. MS *m/z* (rel. int.): 298 $[M]^+$ [$C_{17}H_{16}O_3(Me)_2$] (33), 280 (5), 256 (5), 177 (13), 165 (8), 164 (6), 163 (18), 152 (6), 151 (17), 150 (100), 149 (15), 148 (38), 147 (25), 137 (25), 135 (64), 134 (71), 121 (70), 91 (24).

1-*Acetoxy*-2,3-*bis*-(p-*methoxyphenyl*)-*cyclopentane* (**1d**). Compound **1c** was acetylated with Ac_2O in pyridine. The product (**1d**) was purified by prep. TLC (developing solvent system: see above). The oily product was submitted to MS and NMR analyses. MS *m/z* (rel. int.): 340 $[M]^+$ [$C_{17}H_{15}O_3(Me)_2(Ac)$] (41), 280 (100), 250 (17), 249 (17), 174 (37), 161 (35), 149 (33), 135 (30), 122 (61), 116 (13), 81 (24); ^{1}H NMR ($CDCl_3$) δ: 2.08 (2H, *m*, H-4), 2.17 (3H, *s*, Ac-1), 2.30 (2H, *m*, H-5), 3.50 (1H, *m*, H-3), 3.73 (6H, *s*, MeO-4′), 3.78 (1H, *m*, H-2), 5.43 (1H, *m*, OAc-1), 6.70 (4H, *dd*, A_2B_2 system, J=8.8 Hz, H-2″, H-6″, H-3″, H-5″), 6.74 (4H, *dd*, A_2B_2 system, J=8.8 Hz, H-2′, H-6′, H-3′, H-5′).

4,4′-*Dihydroxychalcone* (**2**) *and cryptoresinol* (**3**). Compound **2** and **3** were identified as 4,4′-dihydroxychalcone and cryptoresinol (norlignan), respectively by direct comparison with authentic specimens [7–9].

4,4′-*Dihydroxychalcone* (**2**). Yellow needles (aq. MeOH), mp, mmp 203–205° [7]. TLC (solvent system 1) R_f 0.62; GC: R_t (min): 7.2; HPLC: R_t (min): 3.7; ^{1}H NMR (Me_2CO-d_6) δ: 7.24 (4H, *dd*, A_2B_2 system, J=8.4 Hz, H-2, H-6, H-3, H-5), 7.46 (4H, *dd*, A_2B_2 system, J=8.8 Hz, H-2′, H-6′, H-3′, H-5′), 7.70 (2H, *dd*, AB system, J=15.8 Hz, –CH=CH– *trans*).

Cryptoresinol (**3**). Pale pink needles (aq. MeOH), mp, mmp 230–232° [8, 9]. TLC (solvent system 2) R_f 0.27; GC: R_t (min): 13.5; IR ν_{max}^{KBr} cm^{-1}: 3460, 3250, 1640, 1605, 1510, 1443, 1360, 1271, 1252, 1217, 1168, 1105, 1082, 1050, 1008, 975, 918, 838, 785, 724, 690; MS *m/z* (rel. int.): 284 $[M]^+$ ($C_{17}H_{16}O_4$) (14), 266 (38), 253 (25), 240 (28), 225 (35), 149 (12), 146 (15), 136 (50), 131 (57), 121 (100), 107 (70).

Quantitative determination of lignans and norlignans in heartwood, sapwood and knot of A. angustifolia. The knot, heartwood and sapwood powders of Parana pine (each 5 g) were extracted with hot MeOH and the concd extracts fractionated with *n*-hexane and EtOAc, respectively. Each EtOAc-soluble fraction was analysed by co-GLC or HPLC, using authentic specimens isolated from knot resin (Table 1). The quantitative analysis was undertaken by GC. Each peak area was determined on the basis of a calibration curve prepared for secoisolariciresinol, and further calculated in percentages for one gram of dried wood powder or resin powder. For GC analysis, the sample fr. was derivatized with TMSi prior to analysis

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