Chemical Constituents from Pinus strobus var. Chiapensis

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O extração hexânica da madeira de P. strobus var. chiapensis (Martinez), após fracionamento
cromatográfico e cristalização, foi obtido sitosterol; uma liganina, larciresinol; cinco flavonóides:
5-hidroxi-7-metoxiflavanona(pinoestrobina), 6-metilpinostrobina, 5-hidroxi-7-metoxiflavona(tecto-
crisina), 6-metiltectocrisina e 5,7-didroxi-6-metilflavona (strobrinina); quatro estilbenos: 3-meto-
xi-5-hidroxiestilbeno (pinoestilbeno-monometileter), 3-metoxi-5-hidroxi-7,8-didroxiestilbeno-
(diidropinoestilbeno-monometileter), 3,5-dimetoxi-7,8-didroxiestilbeno (diidropinoestilbeno-di-
metileter) e 3,5-dimetoxiesticilbeno (pinoestilbeno-dimetiileter) e três diterpenos: ácido isopimarico,
ácido desidroabético e ácido copárico.

After chromatographic fractionation and recrystallization the hexanic extract of the wood of P.
strobus var. chiapensis (Martinez) afforded sitosterol; a lignan, larciresinol; five flavonoids:
5-hydroxy-7-methoxyflavone(pinoestrobina), 6-methylpinostrobina, 5-hydroxy-7-methoxyfla-
vote(pectochrysin), 6-methylectochrysin and 5,7-dihydroxy-6-methylflavone (strobopinina); four
stilbenes: 3-methoxy-5-hidroxicristena (pinosylvin-monomethyl ether), 3-methoxy-5-hidroxi-7,8-
dihidrostilbeno (dihidropinosylvin-monomethyl ether), 3,5-dimethoxy-7,8-dihidrostilbeno (dihy-
dropinosylvin-dimethyl ether), and 3,5-dimethoxystilbeno (pinosylvin-dimethyl ether), and three
diterpenes: isopimarinic acid, dehydroabietic acid, and copallic acid.

Keywords: Pinus strobus var. chiapensis, Pinaceae, flavonoids, stilbenes, diterpenes

Introduction

The relationship of different extracts from pine wood species with the growth of the fungus "Amilostereum areolatum" which is associated with Sirex noctilio ("Wood wasp"), a pest in pine cultivation in the south of Brazil, has motivated us to study different specimens of Pinus1,2,3. The presence of flavonoids and pinosylvin methyl ether may explain the growth inhibition of this fungus in the hexane extract of this species4.

The chemical study of Pinus sp may make valuable contributions through its ecological and chemotaxonomic characterization5.

The chemical study of plants is frequently used to identify special metabolites that have biological activity. However, it is even more interesting to do so in cultivated plants.

Some reports have been made on the chemical study of P. strobus L.6-9 The purpose of this paper is the chemical study of P. strobus var. chiapensis Martinez which has not been previously reported.

Results and Discussion

Our phytochemical investigation of P. strobus var. chiapensis M. by chromatographic fractionation of the hexanic extract of the wood yielded sitosterol (1), larciresinol (2), the flavonoids pinoestrobina (3), 6-methylpinostrobina (4), 6-methylectochrysin (4b), tectochrysin (5), and 6-
methylectochrysin (6), the stilbenes pinosylvin-dimethyl ether (7), dihydropinosylvin-dimethyl ether (8), pinosylvin-monomethyl ether (9), dihydropinosylvin-monomethyl ether (10), and the diterpenes isopimaric acid...
(11), dehydroabietic acid (12) and copalic acid (13). The substances 1, 2, 6, 7, 8, 11 and 12 have not been previously detected in P. strobus L.

The identification of β-sitosterol(1) was based on the analysis of IR, ElMS and $^1$H- and $^{13}$C-NMR spectral data, and by comparison with the literature data$^{10,11,12}$. The molecular formula (OCH$_3$)$_2$(CH$_2$)$_3$(CH)$_3$C$_6$ of 2 was determined on the basis of PND and DEPT $^{13}$C-NMR spectra, which can be represented by C$_{18}$H$_{15}$(OCH$_3$)$_2$, corresponding to a C$_{18}$ lignan skeleton. The $^1$H- and $^{13}$C-NMR data are identical to those registered in the literature for larciresinol (2)$^{13,14}$.

The structures of known flavonoids 3-6$^{5,7,15}$ were determined by IR, El mass and NMR spectra, including NOE and 2D[1H,1H-COSY and 1H,13C-COSY($^3$J$_{CH}$, n = 1.2 and 3)], in addition to comparison with the literature data$^{16,17}$. Irradiation at δ = 3.82 (OMe-7) of 4a and 6a resulted in 8% NOE at δ = 6.1 (H-8), and irradiation at δ = 3.89 (OMe-7) of 6a resulted in 9% NOE at δ = 6.3 (H-8). The irradiation at δ = 3.82 (OMe-5) did not produce NOE at Ar-H. This data, along with long-range couplings detected between 6-methyl proton and C-6 (δ = 106.0 in 4 and δ = 105.09 in 6) by $^2$J$_{CH}$, and C-5(4: δ = 106.3, 6: δ = 162.1) and C-7(4: δ = 165.7, 6: δ = 165.5) by $^3$J$_{CH}$ in 2D $^1$H,$^{13}$C-COSY NMR spectra were used to establish the methyl position in 4, 4b and 6. The $^1$H and $^{13}$C-NMR data of the acetyl derivatives 3a, 4c, 5a and 6b (Table 1) were used to confirm the structure of the natural flavonoids 3, 4, 5 and 6.

The stilbene mixtures of 7 + 8 and 9 + 10 had their structures determined by IR, El mass and $^{1}$H- and $^{13}$C-NMR spectral analyses and comparison with the literature$^{18,19,20}$. The $^{13}$C-NMR[PND and DEPT (ω = 135 and 90°)] spectra were used to distinguish the CH$_3$, CH$_2$, CH and C signals of 7 and 8. The 2D carbon-proton correlation experiments modulated with $^3$J$_{CH}$ (n = 1.2 and 3 with 1H,$^{13}$C-COSY), the 1H,1H-COSY, in addition to the analysis of NOE difference spectra, were used to establish the chemical shift correlations of protons and carbons in 7 and 8. The El mass spectra of 7 + 8 showed peaks at m/z 242 (M$^+$ of 8, 30%) and 240 (M$^+$ of 7, 100%), and others at m/z(%): 179(10), 178(10), 165(45), 151(55), 91(35) and 77(16), which is compatible with pinosylvin-dimethylether and dihydropinosylvin-dimethylether. The IR and El mass spectra of 9 + 10 and the usual NMR experiments were done with this mixture of stilbenes to identify pinosylvin-
Table 1. $^1$H- (200 MHz) and $^{13}$C- (50.3 MHz) spectral data for acetyl derivatives 3a, 4c, 5a and 6b in CDCl$_3$ with TMS as the internal standard.

<table>
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<th>3a</th>
<th>4c</th>
<th>5a</th>
<th>6b</th>
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<tr>
<td>C</td>
<td>$\delta_C$</td>
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<td>2</td>
<td>79.5</td>
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<tr>
<td>3</td>
<td>45.0</td>
<td>3.01 (dd, 13.3; 17.7)</td>
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<td></td>
<td>2.71 (dd, 17.2; 2.6)</td>
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<td>2.73 (dd, 16.6; 2.8)</td>
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<tr>
<td>4</td>
<td>188.7</td>
<td>-</td>
<td>189.0</td>
<td>-</td>
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<tr>
<td>5</td>
<td>164.2</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>6</td>
<td>99.5</td>
<td>6.27(d, 2.0)</td>
<td>107.3</td>
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<tr>
<td>7</td>
<td>165.5</td>
<td>-</td>
<td>166.5</td>
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</tr>
<tr>
<td>8</td>
<td>104.7</td>
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<tr>
<td>9</td>
<td>151.8</td>
<td>-</td>
<td>162.3</td>
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<td>10</td>
<td>107.9</td>
<td>-</td>
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<td>11</td>
<td>138.3</td>
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<td>21.1</td>
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<td>21.4</td>
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monomethylether (9) and dihydrobisylvinline
monomethylether (10). The hydrogenation product of 9+10 gave the $^1$H and $^{13}$C(PND and DEPT) and $^1$H,$^1$H-COSY NMR spectra of 10 (see Experimental).

Compounds 11, 12 and 13 were identified as isopimaric acid, dehydropinacol acid and copalic acid, respectively, by $^1$H and $^{13}$C(PND and DEPT) NMR analysis of 11, 11+12, and the 11 + 12 + 13 mixture, in addition to comparison with the literature data 21,22,23 and the NOE difference spectral analysis. Irradiation at Me-19 ($\delta = 1.25$) resulted in 8% NOE at Me-20 ($\delta = 0.90$), consistent with a 1,3-diaxial orientation of these methyl groups in 11 and 12. The absence of NOE at H-7 ($\delta = 5.36$, brd) was used to confirm the Me-17 in $\beta$ orientation. The mixture of the diterpenes 11+12+13 was analyzed by spectral data, mainly $^1$H- and $^{13}$C-NMR(PND and DEPT) and 2D$^1$H-$^1$H-COSY($^{13}$C$_n$, n = 1,2,3) and $^1$H-$^1$H-COSY, and also by comparison with the values from the corresponding compounds reported in the literature 21-23.

**Experimental**

**General procedure**

Melting points were determined using a Kofler hot stage instrument and are uncorrected; NMR spectra were measured in CDCl$_3$, using TMS as the internal standard, employing a Bruker AC-200 ($^1$H: 200 MHz; $^{13}$C: 50.3 MHz). Mass spectra were obtained with CG-MS HP-5988A (EI, 70 eV). Infrared spectra were recorded as KBr discs on a Perkin-Elmer 1420 spectrophotometer.; C.C. Silica Gel S (Riedel, 0.032-0.063 mm); TLC: silica gel G (Merck), and the spots were visualized by UV (254 nm) and exposure to iodine vapor.

**Plant material**

P. strobus var chiaepensis Martinez was collected in November 1990, at the Fazenda Monte Alegre, Agudo - SP, Brazil, with collaboration from the technicians of DURAFLORA S/A. The specimen was 16 years old with an average height of 5.5 m x 10-15 cm diameter in the D.P.A

**Extraction and isolation of the constituents**

The powdered wood (430 g) was extracted with hexane and chloroform in a Soxhlet apparatus. The solvent was removed under vacuum to yield 4.7 g of hexanic and 2.5 g of chloroformic residues. The hexanic residue was fractionated by C.C. of silica gel, and the solvent was hexane gradually enriched with EtOAc and MeOH, to afford 450 fractions of 20 mL. The resulting fractions were separated by flash column chromatography, preparative TLC (Si gel), and recrystallization of the products, yielding 1 (18 mg), 2 (80 mg), 3 (50 mg), 4 (25 mg), 5 (230 mg), 6 (80 mg), 7 + 8.
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References


2. Equipe da DURAFLORA S/A Susceptibilidade de toras de pinheiros tropicais ao ataque da vespa da madeira-Sirex noctilio(Hymenoptera, Siricidae); Diretor José Flórestal; Botucatu-SP, Brazil, 1992.

3. a: Carvalho, M.G. de; Lopes, J. de J.S.; Runjaneck, V.M.; Carvalho, A.G. de Resumos-SBQ 1991, PN-048; b: Carvalho, M.G. de; Cranchi, D.C.; Meleiro, L.A. da C.; Carvalho, A.G. de Resumos-SBQ 1993, PN-081; c: Carvalho, M.G. de; Cranchi, D.C.; Filho, A.de O.; Carvalho, A.G. de Resumos-SBQ 1994, PN-086; d: Carvalho, M.G. de; Velloso, C.R.X.; Carvalho, A.G. de; Castellano, C. Resumos-SBQ 1995, PN-65.


10. Dutra, N.N.; Alves, H. de M.; Carvalho, M.G. de; Braz-Filho, R. Química Nova 1992, 15, 10.


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