J. Braz. Chem. Soc., Vol. 7, No. 3, 187-191, 1996.
Printed in Brazil.

0103 – 5053 $6.00 + 0.00

Article

Chemical Constituents from *Pinus strobus* var. *Chiapensis*

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Received: January 2, 1996; May 24, 1996

O extrato hexânico da madeira de *P. strobus* var. *chiapensis* (Martinez), após fracionamento cromatográfico e cristalização, forneceu sitosterol; uma lignana, lariciresinol; cinco flavonóides: 5-hidroxi-7-metoxiflavanona(pinostrobina), 6-metilpinostrobina, 5-hidroxi-7-metoxiflavona(tectocrisina), 6-metiltectocrisina e 5,7-diidroxi-6-metilflavona (strobopinina); quatro estilbenos: 3-metoxi-5-hidroxiestilbeno(pinosilvina-monometileter), 3-metoxi-5-hidroxi-7,8-diidroestilbeno-(diidropinosilvina-monometileter), 3,5-dimetoxi-7,8-diidroestilbeno(di-idropinosilvina-dimetileter) e 3,5-dimetoxiestilbeno(pinosilvina-dimetileter) e três diterpenos: ácido isopimárico, ácido desidroabético e ácido copálico.

After chromatographic fractionation and recrystallization the hexanic extract of the wood of *P. strobus* var. *chiapensis* (Martinez) afforded sitosterol; a lignan, lariciresinol; five flavonoids: 5-hidroxy-7-methoxyflavanone(pinostrobin), 6-methylpinostrobin, 5-hidroxy-7-methoxyflavone(tectochrysin), 6-methyltectochrysin and 5,7-dihidroxy-6-methylflavone (strobopinin); four stilbenes: 3-methoxy-5-hidroxystibene (pinosylvin-monomethylether), 3-methoxy-5-hidroxy-7,8-dihydrostilbene(dihydropinosylvin-monomethylether), 3,5-dimethoxy-7,8-dihydrostilbene(dihydropinosylvin-dimethylether), and 3,5-dimethoxystilbene (pinosylvin-dimethylether), and three diterpenes: isopimaric acid, dehydroabietic acid, and copalic 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 *Amilosterum 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 *Pinus*[1,2,3]. The presence of flavonoids and pinosylvin methyl ether may explain the growth inhibition of this fungus in the hexane extract of this species[4].

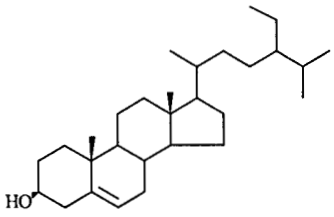
The chemical study of *Pinus* sp may make valuable contributions through its ecological and chemotaxonomic characterization[5].

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. *chiapenis* M. by chromatographic fractionation of the hexanic extract of the wood yielded sitosterol (**1**), lariciresinol (**2**), the flavonoids pinostrobin (**3**), 6-methylpinostrobin (**4**), 6-methylchrysin (**4b**), tectochrysin (**5**), and 6-methyltectochrysin (**6**), the stilbenes pinosylvin-dimethylther (**7**), dihydropinosylvin-dimethylether (**8**), pinosylvin- monomethyleter (**9**), dihydropinosylvin-monomethylether (**10**), and the diterpenes isopimaric acid



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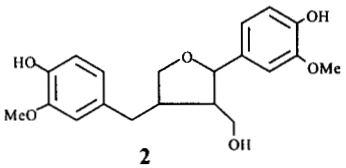

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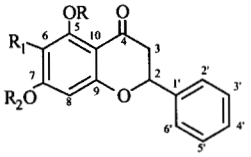
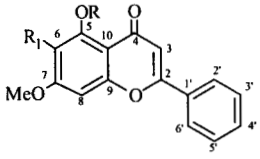
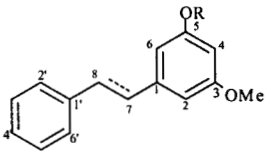


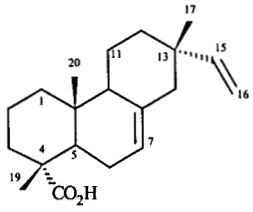
3: R=R_1=H, R_2=Me
3a: R=Ac, R_1=H,R_2=Me
4: R=H,R_1=R_2=Me
4a: R=R_1=R_2=Me
4b: R=R_2=H,R_1=Me
4c: R=Ac,R_1=R_2=Me



5: R=R_1=H
5a: R=Ac, R_1=H
6: R=H,R_1=Me
6a: R=R_1=Me
6b: R=Ac,R_1=Me



7: R=Me
8: R=Me; 7,8-dihydro
9: R=H
10: R=H; 7,8-dihydro



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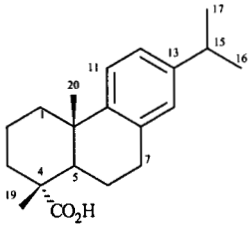

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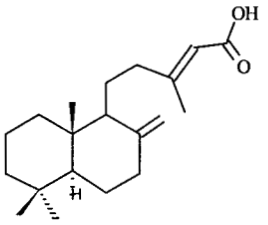

13

Scheme 1.

(**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, EIMS and ^{1}H- and ^{13}C-NMR spectral data, and by comparison with the literature data[10,11,12].

The molecular formula $(OCH_3)_2(CH_2)_3(CH)_9C_6$ 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 lariciresinol (**2**)[13,14].

The structures of known flavonoids **3-6**[6,7,15] were determined by IR, EI mass and NMR spectra, including NOE and 2D[^{1}H,^{1}H-COSY and ^{1}H,^{13}C-COSY(^{n}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, EI 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 (θ = 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 ^{n}J$_{CH}$ (n = 1,2 and 3 with ^{1}H,^{13}C-COSY), the ^{1}H,^{1}H-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 EI mass spectra of **7 + 8** showed peaks at m/z 242 ($M^{+\cdot}$ of **8**, 30%) and 240 ($M^{+\cdot}$ 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-dimetylether. The IR and EI mass spectra of **9 + 10** and the usual NMR experiments were done with this mixture of stylbenes 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.

	3a		**4c**		**5a**		**6b**	
C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	79.5	5.44 (dd,13.3;2.6)	79.6	5.46 (dd,13.3;2.8)	163.4	-	162.0	-
3	45.0	3.01 (dd,13.3;17.7)	45.2	3.03 (dd,13.6;16.6)	108.3	6.60(s)	108.3	6.74(s)
		2.71 (dd,17.7;2.6)	-	2.73 (dd,16.6;2.8)	-	-	-	-
4	188.7	-	189.0	-	176.4	-	176.6	-
5	164.2	-	164.0	-	150.5	-	158.3	-
6	99.5	6.27(d,2.0)	107.3	-	98.9	6.60(d,2,0)	105.9	-
7	165.5	-	166.5	-	150.4		158.3	-
8	104.7	6.42(d,2.0)	97.1	6.42(s)	108.2	6.87(d,2,0)	108.3	6.77(s)
9	151.8	-	162.3	-	150.4	-	150.4	-
10	107.9	-	105.0	-	111.1	-	105.9	-
11	138.3	-	138.6	-	131.4	-	131.4	-
2',6'	126.1	7.42(s)	126.1	7.40(m)	126.2	7.84(m)	126.1	7.87(m)
3',5'	128.8	7.42(s)	128.8	7.40(m)	128.9	7.47(m)	128.9	7.51(m)
4'	128.7	7.42(s)	128.8	7.40(m)	131.2	7.47(m)	131.3	7.51(m)
OCH_3	55.8	3.82(s,3H)	56.0	3.82(s)	55.9	3.89(s)	55.9	3.87(s)
CH_3	-	-	8.1	1.99(s)	-	-	7.5	2.01(s)
OCO	169.5	-	171.0	-	169.7	-	169.7	-
$OCCH_3$	21.1	2.36(s,3H)	21.4	2.43(s)	21.1	2.42(s)	21.1	2.43(s)

monomethylether (**9**) and dihydropinosylvin-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, dehydroabietic 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 (δ = 1.25) resulted in 8% NOE at Me-20 (δ = 0.90), consistent with a 1,3-diaxial orientation of these methyl groups in **11** and **12**. The absence of NOE at H-7 (δ = 5.36, brd) was used to confirm the Me-17 in β 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-^{13}C-COSY($^{n}J_{CH}$, 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 *chiapensis* 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 as average height of 5.5 m x10-15 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**

(60 mg), **9** + **10** (340 mg), **11** (23 mg), **11** + **12** (20 mg), **4b** (10 mg) and **11** + **12** + **13** (30 mg). Some fractions had a mixture of the compounds with another that was purified from other fractions. The chloroform extract yielded a mixture of stilbenes (**7-10**) and aliphatic esters.

Methylation of **4**

A mixture of **4** (9.0 mg), Me_2SO_4 (1 mL), dry K_2CO_3 (0.2 g) and dry Me_2CO (5 mL) was heated under reflux for 2 h. After removal of the inorganic salts with H_2O, the solution was dried over Na_2SO_4 and concentrated to dryness. The crude residue was subjected to silica gel C.C. Elution with $CHCl_3$ gave 6-methyl-pinostrobin-5-methyl ether (**4a**, 8.5 mg) as an amorphous powder: ^{1}H-NMR ($CDCl_3$, TMS, δ): 7.43 (m, 5H, Ar-H), 6.33 (s, 1H, H-8), 5.42 (dd, 1H, J = 2.5 and 12.5 Hz, H-2), 3.84 (s, 3H, OMe-7), 3.82 (s, 3H, OMe-5), 3.03 (dd, 1H, J = 13.0 and 17.0 Hz, H-3ax), 2.75 (dd, 1H, J = 2.5 and 17.0 Hz, H-3eq), 2.07 (s, 3H, Me-6).

Methylation of **6**

A mixture of **6** (10.0 mg), Me_2SO_4 (1 mL), dry K_2CO_3 (0.2 g) and dry Me_2CO (5 mL) was submitted to the same work-up as above, yielding **6a** (9.0 mg) as an amorphous powder: ^{1}H-NMR ($CDCl_3$, TMS, δ): 7.8 (m, 2H, H-2',6'), 7.5 (m, 3H, H-3',5',4'), 6.77 (s, 1H, H-3), 6.7 (bs, 1H, H-8), 3.95 (s, 3H, OMe-5), 3.88 (s, 3H, OMe-7), 2.2 (s, 3H, Me-6).

*Catalytic reduction of methylpinosylvin (***9***)*

The mixture of **9** + **10** (200 mg) in ethanol (5.0 mL) under an atmosphere of H_2 (50 psi) in the presence of 10% Pd/C (0.5 g) was stirred for 5 h at room temp. The mixture was filtered through silica gel and the solvent was removed under reduced pressure to yield **10** (150 mg) as an oil, ^{1}H-NMR ($CDCl_3$, TMS, δ): 7.2 (m, 5H, Ar-H), 6.26 (d, 1H, J = 2.0 Hz), 6.25 (d, 2H, J = 2.0 Hz,), 5.0 (sl, H-O), 3.74 (s, 3H, OMe), 2.85 (m, 4H, H-7.8); ^{13}C-NMR (PND,DEPT, θ:90 and 135, $^nJ_{CH}$, n = 1,2,3): 161.7 (C-3), 156 (C-5), 144.5 (C-1), 141.6 (C-1'), 128.4 (C-2',6'), 128.3 (C-3',5'), 126.0 (C-4'), 107.9 (C-6), 106.7 (C-2), 55.2 (OMe), 37.9 (C-8), 37.7 (C-7).

Acetylation of **3, 4, 5** *and* **6**

The flavonoids **3** (15.0 mg), **4** (15.0 mg), **5** (10.0 mg) and **6** (15.0 mg) were dissolved in a mixture of pyridine (1.0 mL) and Ac_2O (1.0 mL), and the solution was allowed to stand for 4 h at room temperature. The usual work up yielded **3a** (14.0 mg), **4c** (14.5 mg), **5a** (9.0 mg), and **6b** (15.0 mg), respectively.

Acknowledgments

The authors are grateful to CNPq for research fellowships, and for grants from Conselho National de Desenvolvimento Cientifico e Tecnológico (CNPq), Fundação e Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), and Financiadora de Estudos e Projetos (FINEP-PADCT).

References

1. Carvalho, A.G. de *Bioecologia de Sirex Noctilio F. (Hymenoptera, Siricidae) em Povoamento de P. taeda L.; Doctoral Thesis*, Universidade Federal do Paraná, Curitiba-PR-Brazil, 1992.
2. Equipe da DURAFLORA S/A *Susceptibilidade de toras de pinheiros tropicais ao ataque da vespa da madeira-Sirex noctilio*(Hymenoptera, Siricidae); Diretoria Florestal; 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.
4. Kemp, M.S.; Burden, R.S. *Phytochemistry* **1986**, *25*(6), 1261.
5. Zinkel, D.F. *Pine Resin Acids as Chemataxonomic and Genetic Indicators,* TAPPS Conf.Papers, For. Boil. Wood Chem. Conf.; Madison, WI **1977**, 53.
6. Strack, D.; Heilemann, J.; Mömken, M.; Wray, V. *Phytochemistry* **1988**, *27*, 3517.
7. Lindstedt, G.; Misiorny, A. *Acta Chem. Scand.* **1951**, *5*, 121.
8. Olusegum, E. *Z. Pflanzenphysiol* **1980**, *99(3),* 235.
9. Zinkel, D.F.; Magee, T.V. *Phytochemistry* **1987**, *26,* 769.
10. Dutra, N.N.; Alves, H. de M.; Carvalho, M.G. de; Braz-Filho, R. *Química Nova* **1992**, *15,*10.
11. Rubinstein, I.; Goad, L.J.; Clague, A.D.H.; Mulhesirn, L.J. *Phytochemistry* **1976**, *15,* 195.
12. Itoh, T.; Yoshida, K.; Tamura,T.; Matsumoto, T. *Phytochemistry* **1982**, *21,* 727.
13. Chun-Nanlin, Whey Pim Tome *J. Nat. Prod.* **1991**, *54,* 998.
14. Subba Raju, G.V.; Pillai, K.R. *Indian J. Chem.* **1989**, *28B,* 558.
15. Lebreton, P.; Sartre, J. *Can. J. For. Res.* **1983**, *13,* 145.
16. Agrawal, P.K. *Carbon-13 NMR of flavonoids, Central Inst. of Medicinal and Aromatic plants*; P.K. Agrawal, ed., Elsevier; Amsterdam, 1989, p 101, 127.

17. Marbry, T.J.; Markham, K.R.; Thomas M. *The systematic identification of flavonoids*; Springer: New York, 1970.
18. Rowe, J. W.; Bower, C.L.; Wagner, E.R. *Phytochemistry* **1969**, *8,* 235.
19. Strock, D.; Heilmann, J.; Monkein, M.; Wrey, V. *Phytochemistry* **1983**, *22,* 3517.
20. Geydon, O.H.; Bianchin, J.P. *Bul. Soc. Chim.* (France) **1978**, *Part II*, 43.
21. Wenkert, E.; Buckwalter, B.L. *J. Am. Chem. Soc.* **1972**, *94,* 4367.
22. Chamy, M.C.; Piovano, M.; Gambaro, V.; Garbarino, J.A.; Nicoletti, M. *Phytochemistry* **1987**, *26,* 1763.
23. Atta-ur-Rahman; Ahmad, V.U.; *^{13}C-NMR of Natural Products, Diterpenes*; Plenum Press: New York and London, 1992, *93*, 264, 297.