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DITERPENES FROM *PINUS TAEDA*

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Key Word Index—*Pinus taeda*; Pinaceae; diterpene acids.

Abstract—Solvent partition and chromatographic fractionation of the wood of *Pinus taeda* afforded a mixture of aliphatic ketones and esters, sitosterol, stilbene and five diterpenes namely, pimaric acid, isopimaric acid, dehydroabietic acid, 12α,13β-dihydroxyabiet-8(14)-en-18-oic acid and 7α,9α,11α-trihydroxyabiet-8(14)-en-18-oic acid. The structures of these compounds were elucidated by IR, 1D and 2D NMR techniques including NOE and also mass spectral data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Pinus taeda L. is a tree which has a high commercial value and is cultivated in the southern region of Brazil.

There are a number of papers describing the chemical constituents of *P. taeda*. Mixtures of triglycerides and fatty acids have been reported [1] along with condensed tannins [2, 3] and arabinogalactan proteins (β-lectin) [4]. Hydroxycoumarins have been obtained from fungus cultivated in *P. taeda* [5]. Although diterpenes from the pimarane and abietane series are commonly found in Pinaceae [6–11], there are no reports on the isolation of these compounds from *P. taeda* [6].

Industries that cultivate tropical Pinus in the south of Brazil have suffered great losses due to the infestation of *P. taeda* wood with the wood wasp *Sirex noctilio*. Consequently, a number of studies have been carried out concerning the growth of the fungus *Amilosterum areolatum* which is associated with the wood wasp in different tropical Pinus species [12, 13].

As the chemical composition of metabolites may influence the growth of the fungus associated with this pest, it is only fitting that a study of the chemical constituents of the wood and resin of this tropical *Pinus* should be undertaken.

Diterpenes are frequently found in Pinaceae. Some show some biological activity, e.g. ibosol, which is anti-microbial and anti-leukaemic [14]; dehydroabietanes which are antimicrobial [15, 16] and polyfolic acid which has been shown to reduce the action of larvae of herbivore insects [17].

RESULTS AND DISCUSSION

CC of the hexane fraction from the partition of the ethanolic extract afforded, besides a mixture of aliphatic acids, a mixture of aliphatic esters and ketones, sitosterol, pimaric acid, isopimaric acid and dehydroabietic acid, two new diterpenes: 12α,13β-dihydroxyabiet-8(14)-en-18-oic acid (**5**) and 7α,9α,11α-trihydroxyabiet-8(14)-en-18-oic acid (**6**). These substances were identified by their IR, ^{1}H and ^{13}C NMR and mass spectral data

The molecular formulas $C_{20}H_{30}O_2$ and $C_{20}H_{28}O_2$ were assigned to compounds **1** and **3**, respectively, by a combination of mass spectroscopy {**1**, $[M]^+ = m/z$ 302 (10%) and **3**, $[M]^+ = m/z$ 300 (33%)} and quantitation of the number of methyl methylene, methine and quaternary carbon atoms revealed in the ^{13}C NMR spectra (PND and DEPT).

The information afforded by the IR spectrum was in accordance with the presence of the functional groups mentioned below. Comparison of the spectral data for **1** and **3** with literature values [18, 19] established that **1** was pimaric acid and **3** was dehydroabietic acid [14].

Irradiation of H-20 (δ 0.75, *s*) showed enhancements for H-19 (10%) and H-6β (2%) and irradiation of H-17 afforded enhancements for H-14 (12%) and H-15 (20%) for compound **1**.

Irradiation of H-20 of compound **3** afforded enhancements for H-19 (5%), H-7β (3%) and H-6β (2%). Also, irradiation of H-19 showed a NOE at H-20 (5%). These data allowed the conclusion that **1** is pimara-8(14)-15-dien-18-oic acid (pimaric acid) [19] and that **3** is abieta-8,11,13-trien-18-oic acid (dehydroabietic acid) [12, 20]. This is the first report of these acids in *P. taeda*.

Diterpene **2** was identified as a third component of

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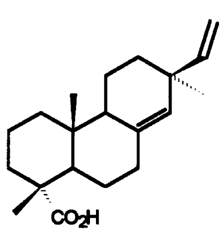

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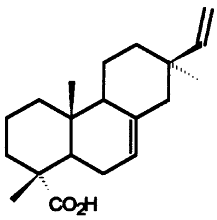

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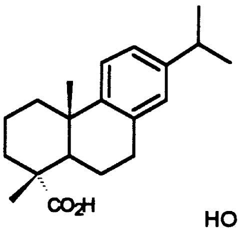

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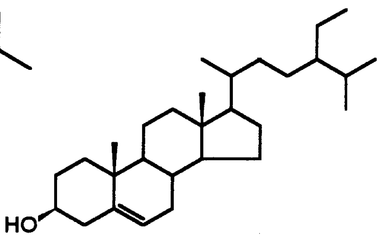

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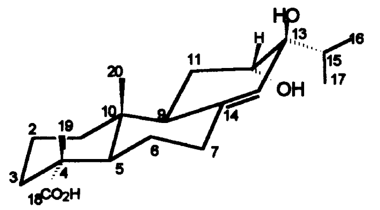

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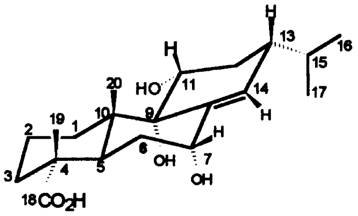

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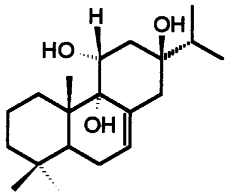

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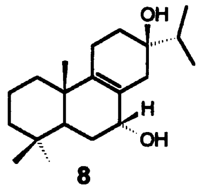

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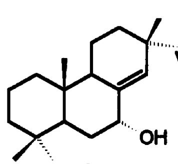

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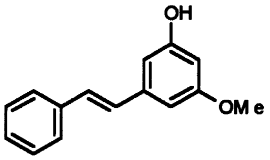

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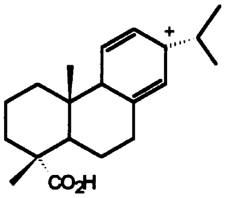
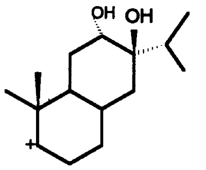
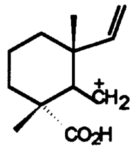


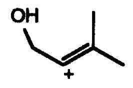
5a
m/z 301(19)



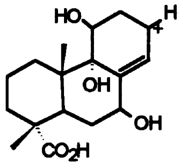
5b
m/z 237(25)



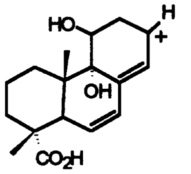
5c
m/z 195(23)



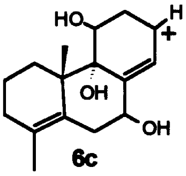
5d
m/z 85(75)



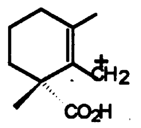
6a
m/z 309(41)



6b
m/z 291(41)



6c
m/z 263(69)



6d
m/z 167(15)

a fraction containing **1**–**3**. An additional signal at δ 5.36 in the ^{1}H NMR spectrum corresponded to H-7 of isopimaric acid, previously identified in another *Pinus* species [7]. The chemical shifts for C-7 (δ 122.0) and C-8 (δ 136.0) were in accordance with literature values [7].

β-Sitosterol (**4**) was identified by comparison of its mp and ^{1}H and ^{13}C NMR spectral data with literature values [21–23].

The IR spectra of **5** and **6** showed carboxylic carbonyl (1700 cm^{-1}) and alcohol (3500 and 1100 cm^{-1}) absorptions. Comparison of the ^{13}C NMR data allowed the quantitation of the number of methyl methylene, methine and quaternary carbon atoms. These data established that **5** and **6** were unsaturated acid diterpenes.

Olefinic proton signals at δ 5.20 (**5**) and 5.54 (**6**), doublets at δ 0.86 (**5**) and 0.88 (**6**) and also the methyl signals at δ 0.68 and 1.03 (**5**) and 0.71 and 1.06 (**6**) were in agreement with tricyclic diterpenes of the abietane series. Unsaturated carbon signals at δ 137.4 and 125.2 (**5**) and δ 142.0 and 126.3 (**6**) were compatible with a double bond at C-8/C-14.

The doubling of the methyl signals was attributed to their interaction with other multiplets at δ 1.60 (**5**) and 1.73 (**6**) which can be seen in their ^{1}H-^{1}H COSY spectra.

Compound **5** has a carbinolic C and a C—H (δ 72.0 and 67.3, respectively). Comparison of these chemical shifts with those of **1** and model compounds **7**–**9** [16, 24, 25] allowed the placement of the hydroxyl groups at C-12 and C-13 and justified the absence of an interaction, in the 2D spectrum, of a proton at this position with H-15 which was responsible for the doubling of the CH_3-16 and CH_3-17 signals mentioned above. Also, the similarities of the chemical shifts for C-13, 15, 16 and 17 of **5** and ibosol (**7**) were in accordance with the hydroxyls at these positions. The γ-effect of the oxygen atom of the alcohol function at C-12 on C-9 justified the fact that this carbon, which in **1** absorbs at δ 50.6, is shielded in **5** (δ 47.0) (Table 1). Other information that corroborates the proposed structure are the values of the fragment ions **5a**, **5b**, **5c** and **5d**. These data led to the structure of **5** as 12α,13β-dihydroxyabiet-8(14)-en-18-oic acid which is a new compound.

Compound **6** was found to contain three carbinol carbons at δ 71.0 and 69.3 (CHs), and 76.8 (C). The ^{1}H-^{1}H COSY spectrum showed a coupling between H-14 [5.54, *s* (*br*)], H-13 (2.34, *m*) and H-7 [3.68, *s* (*br*)]. Comparison of the chemical shifts for **6** with those of **1** and model compounds **7**–**9** [16, 24, 25] showed the absence of some signals due to the position of the hydroxyls in this structure (Table 1).

The intense signal at δ 41.9 (CH) was attributed to C-5 and C-13. The γ-effects of the hydroxy groups at C-7 and C-9 accounted for the difference in chemical shift between C-5 of **6** and of **1** (Table 1). Another reason for positioning a hydroxy group at C-7 was the chemical shift of δ 142.0 for C-8, which is subject to β-effect from the oxygen atom.

The mass spectrum contained a signal compatible with the $[M]^+$ of **6** (m/z 352) and other signals which corresponded to fragment ions such as **6a**, **6b**, **6c** and **6d** which were in accordance with the structure of 7α,9α,11α-trihydroxyabiet-8(14)-en-18-oic acid which has, until now, not been described in the literature.

GC/MS analysis of a fraction containing aromatic material, revealed a main component (R_t 1.307 min) whose mass spectrum showed m/z peaks (rel. int.) at: 226 $[M]^+$ (54) 149 (56), 123 (10) and 77 (20). Analysis of the ^{1}H and ^{13}C NMR spectra and comparison with literature data [7] allowed this component to be identified as pinossilvin monomethyl ether (**10**).

Table 1. ^{1}H NMR (200 MHz) and ^{13}C NMR (50.3 MHz) data for compounds, **1**, **5** and **6** and model compounds **7**, **8** and **9**

	1*,†	**5***		**6***		**7**	**8**	**9**
C	δ_C‡	δ_H (*J*, Hz) [^{1}H-^{1}H COSY]	δ_C‡	δ_H (*J*, Hz) [^{1}H-^{1}H COSY]	δ_C‡	δ_C	δ_C	δ_C
1	38.5		37.1		38.6	37.7	37.1	38.7
2	17.7	1.2–1.3 (*m*)	17.6	1.2–1.7 (*m*)	17.8	18.7	48.9	18.4
3	37.1		36.4		37.8	41.1	41.4	35.2
4	45.9		46.1		45.9	33.0	33.0	37.2
5	48.2	1.5–1.7 (*m*)	48.5	1.75 (*m*)	41.9	43.4	49.7	39.6
6	24.0	1.2–1.3 (*m*)	24.4	1.49 (*m*)	24.9	24.3	30.1	28.7
7	34.6	2.0–2.2 (*m*)	34.9	3.68 (*d*(1)) [H-13, 14]	69.2	130.6	72.1	73.3
8	137.4		137.4		142.0	134.0	126.8	139.4
9	50.6	2.0–2.2 (*m*)	47.0		70.4	76.3	141.8	46.3
10	37.9		37.5		37.3	42.1	38.4	38.3
11	18.2	2.0–2.2 (*m*) [12]	25.4	3.91 (*s*(1)) [H-12]	71.0	68.8	21.0	18.4
12	36.2	3.73 (*dd*, 12.0; 4.0)	67.3	1.49 (*m*) [11, 13, 14]	38.6	39.0	31.8	34.4
13	37.7		72.0	2.24 (*m*) [12, 14, 15]	41.9	73.9	72.2	37.6
14	127.5	5.2 (*s*(1))	125.2	5.54 (*s*(1)) [7, 13, 12]	126.3	41.7	36.5	134.1
15	146.6	1.5–1.7 (*m*) [16, 17]	34.3	1.73 (*m*) [13, 16, 17]	31.9	34.6	34.4	148.4
16	112.6	0.86 (*d*, 6.0) [15]	16.6	0.88 (*d*, 6.0) [15]	16.1	16.8	16.7	110.7
17	29.4	0.86 (*d*, 6.0) [15]	17.3	0.88 (*d*, 6.0) [15]	16.3	16.7	16.8	25.8
18	179.1		178.5		179.3	33.8	33.0	70.9
19	16.7	1.1 (*s*)	16.4	1.04 (*s*)	16.9	22.6	21.6	18.1
20	14.4	0.75 (*s*)	14.6	0.68 (*s*)	14.1	17.2	20.2	14.9
OH		3.78 (*s* (b))		4.23/3.50 (*s*, *b*)				

* DMSO-d_6.

† Values in $CDCl_3$ are very similar to those in DMSO-d_6.

‡ Multiplicity determined using PND and DEPT ($\theta = 90^\circ$ and $\theta = 135^\circ$) spectra.

EXPERIMENTAL

General

Mp: uncorr.; IR: KBr pellets or NaCl film; NMR: Bruker AC-200 (200 MHz for ^1H and 50.3 MHz for ^{13}C) with $CDCl_3$ or DMSO-d_6 as solvents and TMS as int. standard; GC/MS (70 eV): Hewlett-Packard 5890/5988-A GC/MS spectrometer; CC and TLC: Merck or Aldrich silica gel with the suitable mesh size.

Plant material

The trunk of an adult tree was collected at the José Velho farm in São Francisco de Paula/RS by the forest engineer Acácio Geraldo de Carvalho. This material was used in the study of the development of the wood wasp (*Sirex noctilio*) in *Pinus taeda*.

Extraction and isolation of chemical constituents

A portion of the trunk (1.2 kg) was ground and extracted via maceration with EtOH. This extract was submitted to solvent partition affording 32.5 g of residue from the hexane fr. and 2.0 g from the MeOH fr.

The residue from the hexane fr. was submitted to CC on silica gel using hexane as initial eluent followed by EtOAc and MeOH. 430 frs of 150 ml were collected and each evaporated under reduced pressure. The residues were analysed by TLC and the frs pooled accordingly.

The first frs were composed mainly of a mixture of aliphatic esters and ketones, the major constituent being dodecyl tetradecanoate. Frs 32–60 were crystallized from MeOH giving a mixture of two compounds which, after prep. TLC using hexane–EtOAc (9:1), were identified as compounds **1** (mp 170–172°, 60 mg) and **3** (mp 183–184°, 50 mg). Group 80–82 was analysed by TLC revealing the presence of three compounds which by ^1H and ^{13}C NMR analysis were found to be **1**–**3** (30 mg). Frs 110–132 were crystallized from MeOH and gave 300 mg β-sitosterol (**4**, mp 136–138°); group 135–183 is an oily material whose GC/MS analysis revealed a major component identified as **10**; pool 190–237 was submitted to CC and crystallization from MeOH giving a mixture of **3**+**4**+**10**; frs 286–301 afforded, after addition of MeOH and filtration on silica gel, 20 mg of **5** (oil) and the mother liquor was shown to have a mixture of **1**–**3** (30 mg, oil). Finally, addition of EtOAc and MeOH to groups 302–350 followed by silica gel filtration and prep. TLC, afforded **6** (oil, 26 mg). The more polar fractions were shown to be a mixture of aliphatic acids.

12α,13β-Dihydroxyabiet-8(14)-en-18-oic acid (**5**). Oil; IR ν_{max}^{film} cm^{-1}: 3500, 2950, 1700, 1385, 1370, 1270, 1080, 950; ^1H NMR (200 MHz, DMSO-d_6 and ^{13}C NMR (50.3 MHz, DMSO-d_6): Table 1; MS, *m/z* (rel. int.): 336 $[M]^+$ (absent), 318 $[M-H_2O]^+$ (1), 301 [318–OH]$^+$ (19), 274(2), 273 [318–propane]$^+$ (5), 237 (25), 195 (22), 162 (18), 149 (11), 123 (18), 85 (75), 83 (100).

7α,9α,11α-Trihydroxyabiet-8(14)-en-18-oic acid (**6**). Oil, IR ν_{max}^{film} in cm^{-1}: similar to **5**; ^1H NMR (200.13 MHz, DMSO-d_6) and ^{13}C NMR (50.3 MHz, DMSO-d_6): Table 1; MS, *m/z* (rel. int.): 352 $[M]^+$ (52), 334 (12), 316 (6), 309 (49), 306 (9), 292 (9), 291 (41), 275 (25), 263 (69), 235 (28), 199 (6), 185 (14), 180 (8), 167 (14), 154 (10), 149 (22), 43 (100).

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