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LIGNANS FROM THE WOOD OF *ABIES PINSAPO*

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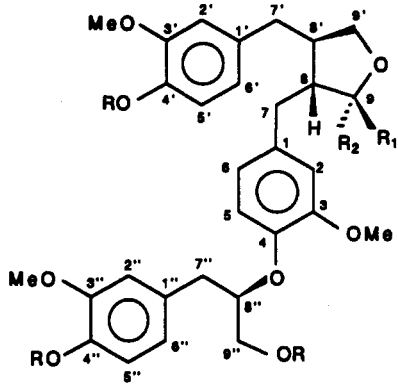
ABSTRACT.—Several lignans have been isolated from the $CHCl_3$ extract of the wood of *Abies pinsapo*. These include the new compounds 4,4′,9-trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan [**2**], (9′*R*)-9′-hydroxylariciresinol [**5**], and (7′*R*)-7′-hydroxylariciresinol [**7**], as well as a novel sesquilignan with the structure (8*R*,8′*R*,8″*R*,9*R*)-4′,4″,9,9″-tetrahydroxy-3,3′,3″-trimethoxy-4,8″:9,9′-*bis*-epoxy-8,8′-sesquineolignan [**8**], to which we have assigned the trivial name sesquipinsapol C. Compound **2** has been identified as a mixture of epimers, while compounds **5**, **7**, and **8** were obtained their acetoxyl derivatives. The new structures were established by spectroscopic methods and chemical transformations. Certain antimicrobial and cytotoxic activity assays have been carried out and applied to the isolates from *A. pinsapo*.

From the hexane extract of the wood of *Abies pinsapo* Boiss. (Pinaceae), we have found sesquiterpenoids related to juvabione in the acid fraction (1), as well as diterpenoids, triterpene lactones, and sterols (2) in the neutral fraction; and from the $CHCl_3$ extract two sesquilignans, sesquipinsapols A [**1**] and B have been reported (3). In this paper, lignans from the $CHCl_3$ extract are described.

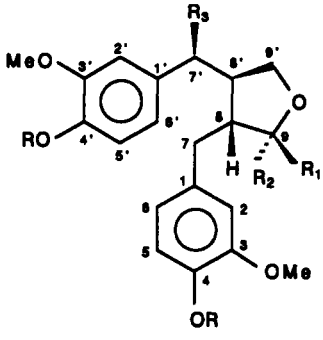
RESULTS AND DISCUSSION

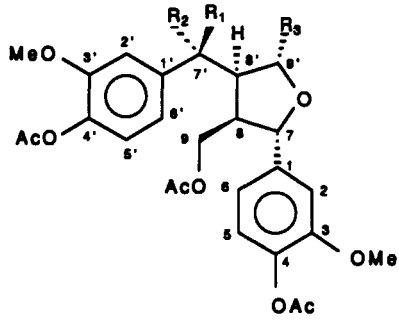
The wood of *A. pinsapo* was extracted with hexane and then $CHCl_3$. The latter extract was chromatographed on a Si gel column eluting with hexane/EtOAc mixtures of increasing polarity. Some fractions were rechromatographed to obtain compound **2** and lariciresinol [**3**] (4) as the major products. The other fractions were acetylated and then chromatographed on a Si gel column and subjected to prep. tlc. Compounds **5**, **7**, and **8** were isolated as acetates **5a**, **7a**, and **8a**, respectively, in addition to sesquipinsapol A triacetate (3), sesquipinsapol B pentaacetate (3), 9-(*p*-coumaroyl)-lariciresinol triacetate (5), (7′*S*)-todolactol A tetraacetate [**4a**] (6), (7′*S*)-7′-hydroxylariciresinol [**6a**], and isolariciresinol tetraacetate (4).

Compound **2** was obtained as an amorphous powder with the molecular formula $C_{20}H_{24}O_6$, established according to its mass spectrum (*m/z* 360, $[M]^+$) and ^1H- and ^{13}C-nmr data. In its ir spectrum, absorption bands representing aromatic rings (1604, 1511 cm^{-1}) and hydroxyl groups (3413 cm^{-1}) were observed. The ^1H- (Table 1) and ^{13}C-nmr spectra (Table 2) of **2** indicated that the compound belonged to the dibenzylbutyrolactol class (6–8) and was a mixture of two isomers occurring in approximately a 2:1 ratio. The main differences lay in the signals assignable to carbons 9 and 7, which appeared at δ 103.46 and 39.21 ppm, respectively, for the major isomer, and at δ 98.92 and 33.46 for the minor one. The singlets in the ^1H-nmr spectrum at δ 3.76, 3.82, and 3.83 (all integrating for 6 protons) were assigned to two aromatic methoxyl groups. The four benzylic and two methine protons gave rise to two complex multiplets between δ 2.37 and 2.80 ppm and between δ 1.94 and 2.18, respectively, and the hemiacetal proton appeared as a multiplet at δ 5.23, integrating in total for one proton. Several lignans with lactol rings have been reported as mixtures of epimers in solution (7,8). To establish the relative stereochemistry of the chiral centers of the molecule, **2** was oxidized with Ag_2CO_3 to obtain the dibenzylbutyrolactone matairesinol [**9**] (8), which is a known



1 R=H, R_1R_2:=O
1a R=Ac, R_1R_2:=O
8a R=Ac, R_1=OAc, R_2=H





2α R=H, R_1=H, R_2=OH, R_3=H
2β R=H, R_1=OH, R_2=H, R_3=H
2a R=Ac, R_1=OAc, R_2=H, R_3=H
4a R=Ac, R_1=OAc, R_2=H, R_3=OAc
9 R=H, R_1R_2:=O, R_3=H

3a R_1=H, R_2=H, R_3=H
5a R_1=H, R_2=H, R_3=OAc
6a R_1=OAc, R_2=H, R_3=H
7a R_1=H, R_2=OAc, R_3=H

constituent of *A. pinsapo* (3). Compound **9** was reduced with DIBAH at −78° (7) to yield **2** as a mixture of epimers. Compound **2** was then determined to be epimers of (8*R*,8′*R*)-4,4′,9-trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan. The configuration at C-9 of each epimer could be established according to the ^{13}C-nmr data for C-7 and C-9. The upfield shift of C-7 in the minor isomer (−5.75 ppm) could be explained only for a synperiplanar relative disposition between C-7 and the hydroxyl group (9), which corresponded to an α-configuration of this OH on C-9; its upfield shift at C-9 (−4.54 ppm) also indicated a pseudoaxial disposition for the OH (10). When the mixture of epimers was acetylated, only one product was obtained [**2a**], in whose ^{1}H-nmr spectrum H-9 resonated as a doublet at δ 6.05 with a small coupling constant (J=1.6 Hz). This indicated that H-8 was oriented at nearly 90° to H-9, in agreement with a β-configuration of the acetoxyl group at C-9.

Compound **5a** was obtained as a colorless syrupy liquid with a positive optical rotation, whose cims (*m/z* 573 for $[M+29]^+$ and *m/z* 544 for $[M]^+$) and ^{13}C-nmr spectra agreed with a molecular formula of $C_{28}H_{32}O_{11}$. In the ^{1}H-nmr spectrum (Table 1), the typical signals of two guaiacyl rings, two aromatic acetoxyl groups, two aliphatic acetates (1.92 and 2.02 ppm), a singlet signal for a proton attached to an acetylated hemiacetal carbon at δ 6.14, a doublet signal for an oxygenated benzylic proton at δ 4.96 (J=8.8 Hz), and two double doublets for a methylene attached to an acetoxyl group at δ 4.20 (J=7.6 and 11.2 Hz) and δ 4.33 (J=6.5 and 11.2 Hz) were apparent. The ^{13}C-nmr

TABLE 1. ^{1}H-Nmr Data of Compounds **2**, **2a**, **5a**, **6a**, and **7a**.[a]

Proton	**2**		**2a**	**5a**	**6a**	**7a**
	α	β				
H-2	6.42–6.81 m		6.66 d (1.8)	6.90 d (2.0)	6.99 d (1.9)	7.00 d (1.8)
H-5	6.42–6.81 m		6.92 d (7.9)	7.00 d (8.0)	7.00 d (8.0)	6.99 d (8.2)
H-6	6.42–6.81 m		6.63 dd (1.8, 7.9)	6.85 dd (2.0, 8.0)	6.89 dd (1.8, 8.0)	6.87 dd (1.8, 8.2)
H-7	2.37–2.80 m		2.67 d (7.7)	4.96 d (8.8)	4.56 d (8.0)	4.58 d (7.2)
H-8	1.94–2.18 m		2.28 m	2.58–2.92 m	2.38 m	2.67 m
H-9a	5.23 m		6.05 d (1.6)	4.20 dd (7.6, 11.2)	4.11 dd (6.7, 11.3)	3.96 dd (7.0, 9.4)
H-9b	—		—	4.33 dd (6.5, 11.2)	4.34 dd (4.6, 11.3)	4.12 dd (4.2, 9.4)
H-2′	6.42–6.81 m		6.57 d (1.9)	6.81 d (2.0)	6.69 br s	6.79 d (1.8)
H-5′	6.42–6.81 m		6.90 d (8.5)	6.97 d (8.0)	7.02 d (8.0)	6.97 d (8.1)
H-6′	6.42–6.81 m		6.59 dd (1.9, 8.5)	6.79 dd (2.0, 8.0)	6.94 dd (1.9, 8.0)	6.82 dd (1.8, 8.1)
H-7′a	2.37–2.80 m		2.54 dd (7.9, 13.8)	2.58–2.92 m	5.77 d (9.8)	—
H-7′b	2.37–2.80 m		2.82 dd (7.5, 13.8)	2.58–2.92 m	—	5.70 d (8.8)
H-8′	1.94–2.18 m		2.28 m	2.58–2.92 m	2.66 m	2.89 m
H-9′a	4.09 t (8.0)	4.00 dd (7.0, 8.5)	3.77 m	6.14 s	3.65–4.00 m	3.70–3.85 m
H-9′b	3.57 t (8.0)	3.47 m	4.11 dd (6.9, 8.8)	—	3.65–4.00 m	3.70–3.85 m
OMe	3.76 s		3.75 s	3.80 s	3.84 s	3.77 s
	3.82 s	3.83 s	3.76 s	3.81 s	3.85 s	3.84 s
OAc	—		2.03 s	1.92 s	1.96 s	1.94 s
	—		2.08 s	2.02 s	2.04 s	2.04 s
	—		—	2.28 s	2.30 s	2.27 s
	—		—	2.29 s	2.30 s	2.29 s
OH	5.76, 5.77, 5.78 s					

[a]Coupling constants in Hz are given in parentheses.

TABLE 2. ^{13}C-Nmr Chemical Shifts (in ppm) of Compounds **2**, **2a**, **5a**, **6a** and **7a**.[a]

Carbon	2		2a	5a	6a	7a
	α	β				
C-1	132.67	132.33	137.86	138.56	139.51[b]	139.26[b]
C-2	111.62	111.29	113.03	110.55	110.37	109.84
C-3	146.58[b]		151.08[b]	151.21	151.27	151.23[c]
C-4	144.00[c]	143.95[c]	138.40	139.73	139.89[b]	140.13[b]
C-5	114.41	114.24	122.78	122.75	122.77	122.84
C-6	121.42	121.58	120.99	118.58	118.91	118.03
C-7	33.46	39.21	39.22	84.10	84.89	83.31
C-8	51.82	52.85	51.55	48.14	47.94	48.12
C-9	98.92	103.46	103.05	61.33	64.51	63.57
C-1′	132.01	131.49	138.26	137.55	138.00[b]	137.30[b]
C-2′	111.29	111.12	112.75	113.07	111.60	111.39
C-3′	146.45[b]		151.05[b]	151.21	151.27	151.29[c]
C-4′	143.95[c]	143.80[c]	138.78	139.55	139.39[b]	139.76[b]
C-5′	114.34	114.16	122.78	122.96	123.09	123.03
C-6′	121.23		120.70	120.87	119.49	119.28
C-7′	38.76	38.33	38.08	31.78	77.27	75.95
C-8′	42.87	45.74	44.83	47.34	50.35	49.24
C-9′	72.62	72.32	73.61	101.28	69.65	69.88
OMe	55.73		55.84	55.80	56.05	56.01
	55.89		56.90	56.00	56.05	56.01
OCO*Me*	—		20.72	20.64	20.73	20.71
	—		21.36	21.24	21.21	21.15
OC*O*Me	—		169.12	169.90	168.91	168.81
	—		170.36	170.71	170.93	170.79

[a]The number of directly attached protons to each carbon was verified with the DEPT pulse sequence.
[b]Interchangeable values.
[c]Interchangeable values.

spectrum of compound **5a** (Table 2) was similar to that of lariciresinol triacetate [**3a**] (4) except for an additional acetoxyl group. This function was located at C-9′, since the signal of a methylene group at δ 72.7 in **3a** was substituted in **5a** for that of a methine at δ 101.28. The configuration at C-9′ was established considering that in the ^{1}H-nmr spectrum, H-9′ resonated as a singlet, because H-8′ was oriented at 90° to H-9′. This observation is possible only if the OAc group has an α- disposition. Thus, **5a** was identified as (9′*R*)-9′-hydroxylariciresinol tetraacetate, which has not previously been isolated from a natural source.

Compound **6a** was obtained in this investigation mixed with **7a** (20%), while, in turn, **7a** contained **6a** (25%) as an impurity. Their molecular formula, $C_{28}H_{32}O_{11}$, was determined on the basis of their ^{13}C-nmr and mass spectra, in which both molecular ions appeared at *m/z* 544. Their ir spectra, which exhibited absorption bands characteristic of acetoxyl groups and aromatic rings were almost identical. Their ^{1}H-nmr (Table 1) and ^{13}C-nmr (Table 2) spectra indicated that **6a** and **7a** were epimeric. The ^{1}H-nmr spectrum of **6a** exhibited signals that suggested this substance had the structure, 7′-hydroxylariciresinol tetraacetate (5,11). Andersson *et al.* (11) have determined that the stereochemistry of carbons 7, 8, and 8′ is identical to that of lariciresinol by chemical correlations, but did not establish the stereochemistry of C-7′. Moreover, Ozawa *et al.* (5) did not define the stereochemistry of any chiral center in this compound. In comparing the ^{1}H-nmr spectrum of **6a** with that of the 7′*S* epimer of todolactol A tetraacetate [**4a**], the same configuration was apparent at C-8′ (6). In compounds **4a** and

6a, H-7′ resonated as a doublet at δ 5.79 with J=9.9 Hz, and at δ 5.77 with J=9.8 Hz, respectively; C-7′ in **4a** and in **6a** resonated at δ 77.0 and δ 77.27, respectively. Thus, **6a** had the structure (7′*S*)-7′-hydroxylariciresinol tetraacetate. In their ^{1}H-nmr spectra, the coupling constant (J=8.8 Hz) of H-7′, resonating as a doublet at δ 5.70 for **7a**, was smaller than that observed for **6a** (δ 5.77, J=9.8 Hz) (6,12). Also, in their ^{13}C-nmr spectra, C-7′ of **7a** appeared at δ 75.95, and at δ 77.27 for **6a**. This information suggested that **7a** had the 7′*R* configuration. Therefore, **7a** was assigned the structure (7′*R*)-7′-hydroxylariciresinol tetraacetate, and has been obtained for the first time as a natural product.

From cims, the $[M+1]^+$ at *m/z* 709 and the ^{1}H- and ^{13}C-nmr data of tetraacetate **8a**, with a negative optical rotation, allowed the deduction of a molecular formula of $C_{38}H_{44}O_{13}$. The molecule possessed four acetoxyl and three methoxyl groups and the remaining 27 carbons were assigned to a sesquilignan skeleton. The ^{1}H-^{1}H COSY nmr spectrum showed signals corresponding to three 1,3,4-trisubstituted aromatic rings (δ 6.52–6.93), three methoxyl groups (δ 3.73, 3.75, and 3.79), two aliphatic acetoxyl groups (δ 2.02 and 2.03), and two aromatic acetates (δ 2.27 and 2.28). Comparison of the ^{1}H-nmr spectra of **8a** and **2a** revealed that their protons for H-7 through H-9 and H-2′ through H-9′ were similar, and indicated that **8a** represents the structure of **2a** with an additional C_6-C_3 unit through one of the aromatic rings, as in compound **1** (3). The ^{13}C-nmr spectrum, assigned according to the ^{1}H-^{13}C HETCOR spectrum, also indicated the similarities between **8a** and **2a** for C-7 through C-9 and C-1′ through C-9′, and between **8a** and **1a** for C-1 through C-6 and C-1″ through C-9″ (3), so that the configurations of the chiral centers should correspond to **2a** for C-8, 8′ and 9, and to **1a** for C-8″. Furthermore, the difference in optical rotation between **2a** and **8a** (+12.1°) was similar to the difference between the alcohols **1** and **9** (+26.7°) which corroborated the assignment of the same configurations at C-8, 8′, 8″, and 9. Therefore, **8a** was designated as (8*R*,8′*R*,8″*R*,9*R*)-4′,4″,9,9″-tetrahydroxy-3,3′,3″-trimethoxy-4,8″:9,9′-*bis*-epoxy-8,8′-sesquineolignan tetraacetate, and was named sesquipinsapol C.

The activity of the $CHCl_3$ extract and alcohols **1**, **2**, **3**, and **9** towards several Gram-positive bacteria (*Bacillus megaterium, B. subtilis, B. licheniformis* CECT 20, *B. licheniformis* CECT 491, *B. circulans* CCM 2048, *Staphylococcus aureus* ATCC 8, *Micrococcus luteus*), Gram-negative bacteria (*Pseudomonas* sp. and *Alcaligenes faecalis*), and fungi (*Aspergillus niger* CECT 2089 and *Penicillium A*) was studied. None of the assayed samples proved to be active against the selected fungi and minimal activity was observed against the Gram-positive and Gram-negative bacteria. Cytotoxicity against the cancer cell lines P-388, A-549, and HT-29, cytotoxicity against CV-1 (monkey kidney fibroblast cells), and antiviral activity against HSV-1 (herpes simplex virus-1) and VSV (vesicular stomatitis virus) were studied for compounds **2**, **3**, **4a–8a**, and **9**. All the compounds were inactive against the assayed virus at the level of 40 μg/ml. The cytotoxic activities were moderate (see Experimental).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-Nmr, ^{13}C-nmr, DEPT, and 2D nmr spectra were recorded on a Bruker AM 300 spectrometer in $CDCl_3$ using TMS as internal standard. Uv spectra in MeOH were obtained on a uv-vis Bausch and Lomb model Spectronic 2000 spectrophotometer, and ir spectra on a Perkin-Elmer 983G instrument. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Low-resolution eims (70 eV) were determined on a Hewlett-Packard 5988A mass spectrometer, with cims recorded using CH_4 as ionizing gas. Cc was carried out using Si gel Merck 60 (70–230 mesh) eluted with mixtures of hexane/EtOAc of increasing polarity. Anal. tlc was performed on layers of Si gel 60 G (Merck) of 0.25-mm thickness, using a 7% phosphomolybdic acid solution in EtOH to visualize the spots. Prep. tlc was carried out on layers of Si Merck 60 PF_{254} of 1-mm thickness with hexane-EtOAc (1:3) as eluent.

PLANT MATERIAL.—The wood of *A. pinsapo* was collected in Sierra Bermeja, Málaga, Spain, in the

month of June 1989, and was identified by Prof. F. Valle, Departamento de Biología Vegetal, Universidad de Granada. A voucher specimen (No. MGC 9040) is deposited in the Herbarium of the University of Málaga.

EXTRACTION AND FRACTIONATION.—The crushed wood (4.6 kg) was extracted and processed as described in ref. (3). From 20 g of the $CHCl_3$ extract, in addition to the other products, after successive Si gel cc, **2** (750 mg) and lariciresinol [**3**] (800 mg) were isolated. After acetylation of some fractions, successive Si gel cc, and, finally, prep. tlc, the following compounds were isolated in order of increasing polarity: 9-(*p*-coumaroyl)-lariciresinol triacetate (60 mg), (7′*S*)-todolactol A triacetate [**4a**] (250 mg), **5a** (200 mg), (7′*S*)-7′-hydroxylariciresinol tetraacetate [**6a**] (38 mg) including **7a** (20%), **7a** (114 mg) mixed with **6a** (25%), isolariciresinol tetraacetate (70 mg), and **8a** (99 mg).

(8R,8′R)-4,4′,9-trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan [**2**].—Amorphous powder. $[\alpha]_D^{25}$ −40.9° (*c*=1.05, $CHCl_3$); ir ν max (film) 3413, 2937, 1604, 1511, 1450, 1429, 1365, 1270, 1236, 1153, 1123, 1033, 931, 859 cm^{-1}; eims *m/z* 360 $[M]^+$ (2), 342 (23), 205 (24), 204 (23), 188 (10), 138 (34), 137 (100), 122 (12), 85 (10), 83 (15), 81 (38); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

ACETYLATION OF **2**.—Compound **2**, representing a mixture of **2α** and **2β** (28 mg), was treated with Ac_2O-pyridine (1:1) (2 ml) at room temperature overnight. The usual workup yielded the triacetate **2a** (30 mg) as a colorless syrupy liquid: $[\alpha]_D^{25}$ −33.9° (*c*=1.22, $CHCl_3$); uv λ max (MeOH) (ε) 208.1 (19800), 271.5 (6000), 278.0 (5600) nm; ir ν max (film) 3012, 2938, 1761, 1602, 1507, 1458, 1418, 1368, 1269, 1198, 1152, 1034, 941, 827 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

OXIDATION OF **2** TO MATAIRESINOL [**9**].—To a solution of **2** (19 mg) in toluene (10 ml), Ag_2CO_3 (100 mg) was added, and the mixture was heated slowly under N_2 with stirring until reflux. After 5 h at this temperature, the reaction mixture was cooled, filtered, and the solvent removed *in vacuo* to obtain a viscous mass (19 mg), which was purified by prep. tlc. The product (16 mg) was identical to **9** (3).

REDUCTION OF **9** TO **2**.—To a solution of **9** (132 mg, 0.37 mmol) in 1.3 ml of dry CH_2Cl_2 and 4.5 ml of dry toluene under N_2 at −78°, a solution of DIBAH (0.93 ml of 20% solution in toluene, 1.3 mmol) was added dropwise. After 2 h of stirring at the same temperature, 1 ml of MeOH was added and the reaction was warmed to room temperature. Then Et_2O (3 ml), brine (3 ml), H_2O (3 ml) containing 0.3 ml of 6N HCl, and, finally, Et_2O (4 ml) were added. The mixture was decanted and the aqueous phase was extracted with Et_2O (3×15 ml). The organic phases were dried over anhydrous Na_2SO_4, filtered, and evaporated, yielding 105 mg of a crude product, which, after prep. tlc, gave 30 mg of starting material and 51 mg of **2α** and **2β** in the ratio 1:2, respectively.

(9R)-9′-Hydroxylariciresinol tetraacetate [**5a**].—Colorless oil. $[\alpha]_D^{25}$ +2.0° (*c*=1.70, $CHCl_3$); uv λ max (MeOH) (ε) 217.5 (13100), 271.0 (4800), 277.5 (4600) nm; ir ν max (film) 3018, 2939, 1763, 1739, 1604, 1509, 1464, 1420, 1369, 1267, 1220, 1199, 1126, 1035, 906, 860 cm^{-1}; cims *m/z* 573 $[M+29]^+$ (2), 544 $[M]^+$ (1), 486 (6), 485 (20), 426 (12), 425 (46), 61 (100); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

(7′S)-7′-Hydroxylariciresinol tetraacetate [**6a**].—Obtained impure with 20% of **7a**, in accordance with ^1H-nmr data. Colorless oil; ir ν max (film) 2938, 1762, 1740, 1605, 1507, 1464, 1421, 1368, 1219, 1198, 1157, 1033, 906, 858 cm^{-1}; eims *m/z* 544 $[M]^+$ (1), 167 (6), 153 (8), 151 (15), 137 (12), 43 (100); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

(7′R)-7′-Hydroxylariciresinol tetraacetate [**7a**].—Obtained impure with 25% of **6a**, in accordance with ^1H-nmr data. Colorless oil; ir ν max (film) 2938, 1762, 1740, 1605, 1507, 1464, 1421, 1368, 1219, 1198, 1157, 1033, 906, 858 cm^{-1}; eims *m/z* 544 $[M]^+$ (1), 205 (42), 178 (22), 167 (2), 151 (13), 137 (7), 43 (100); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

(8R,8′R,9R,8″R)-4′,4″,9,9″-Tetrahydroxy-3,3′,3″-trimethoxy-4,8″:9,9′-bis-epoxy-8,8′-sesquineolignan (sesquipinsapol C tetraacetate) [**8a**].—Colorless oil; $[\alpha]_D^{25}$ −21.8° (*c*=1.21, $CHCl_3$); ir ν max (film) 3010, 2938, 2850, 1762, 1739, 1603, 1508, 1464, 1418, 1368, 1268, 1220, 1199, 1153, 1123, 1035, 1011, 942, 824 cm^{-1}; cims *m/z* 709 $[M+1]^+$ (1), 691 (4), 677 (11), 650 (16), 649 (42), 648 (13), 631 (25), 389 (26), 265 (100), 261 (16), 223 (10), 61 (32); ^1H nmr (300 MHz, $CDCl_3$) δ 2.02 (3H, s, OAc), 2.03 (3H, s, OAc), 2.27 (3H, s, OAc), 2.28 (3H, s, OAc), 2.28 (2H, m, H-8, -8′), 2.49 (1H, dd, *J*=7.8 and 13.8 Hz, H-7′a), 2.65 (2H, dd, *J*=2.7 and 7.3 Hz, H-7), 2.76 (1H, dd, *J*=7.3 and 13.8 Hz, H-7′b), 2.96 (1H, dd, *J*=6.2 and 14.1 Hz, H-7″), 3.09 (1H, dd, *J*=6.5 and 14.1 Hz, H-7″b), 3.73 (3H, s, OMe), 3.75 (3H, s, OMe), 3.77 (3H, s, OMe), 3.77 (1H, m, H-9′a), 4.09 (1H, dd, *J*=6.9 and 8.8 Hz, H-9′b), 4.21 (2H, dd, *J*=1.4 and 6.2 Hz, H-9″), 4.51 (1H, dqui, *J*=1.4 and 6.2 Hz, H-8″), 6.03 (1H, d, H-9), 6.52 (1H, dd, *J*=2.0 and 8.1 Hz, H-6), 6.57 (1H, d, *J*=1.9 Hz, H-2′), 6.58 (1H, m, H-6′), 6.59 (1H, d, *J*=2.0 Hz, H-2), 6.77 (1H, d, *J*=8.1 Hz, H-5), 6.81 (1H, dd, *J*=1.9 and 8.0 Hz, H-6″), 6.88 (1H, d, *J*=8.6 Hz, H-5′), 6.90 (1H, d, *J*=1.9 Hz, H-2″), 6.93 (1H, d, *J*=8.0 Hz, H-5″); ^{13}C nmr (75 MHz, $CDCl_3$) δ 20.67 (OAc), 20.84 (OAc), 21.35

(OAc), 37.80 (C-7′), 37.91 (C-7″), 39.17 (C-7), 44.78 (C-8′), 51.57 (C-8), 55.78 (OMe), 55.84 (OMe), 64.86 (C-9″), 73.76 (C-9′), 78.93 (C-8″), 103.07 (C-9), 112.68 (C-2′), 113.09 (C-2), 113.81 (C-2″), 118.20 (C-5), 120.62 (C-6′), 121.07 (C-6), 121.56 (C-6″), 122.57 (C-5″), 122.68 (C-5′), 133.61 (C-1), 136.34 (C-1″), 138.19 (C-1′), 138.43 (C-4″), 138.83 (C-4′), 145.77 (C-4), 150.77 (C-3), 150.87 (C-3″), 150.97 (C-3′), 169.08 (OAc), 170.29 (OAc), 170.82 (OAc). Assignments were made according to ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR nmr spectra obtained for this isolate.

ANTIMICROBIAL SCREENING.—Products were assayed for antimicrobial activity by the disk diffusion method. Brain-heart agar (BHA) plates (Pronadisa, Hispanagar) were inoculated with 0.1 ml of 8 h-old cultures of the microbial strains, and 3MM Whatman paper disks (5-mm diameter), previously impregnated with 1 mg of extract, **1**, **2**, **3**, and **9**, were deposited onto the plates. The plates were kept at 4° for 2 h to allow diffusion and then incubated at 28°. The results were read 18 h after incubation and measured as a function of the growth inhibition halo (mm) that appeared around the disks.

All the fungi and bacteria employed belong to the collection of the Departamento de Microbiología de la Universidad de Granada. The Gram-positive bacteria *Bacillus megaterium, B. subtilis, B. licheniformis* CECT 20, *B. licheniformis* CECT 491, *B. circulans* CCM 2048, *Staphylococcus aureus* ATCC 8, *Micrococcus luteus* and the Gram-negative bacteria *Pseudomonas* sp. and *Alcaligenes faecalis* were grown in BHI (brain-heart infusion). The fungi, *Aspergillus niger* CECT 2089 and *Penicillium A,* were grown in CM medium (1% glucose, 0.5% yeast extract and 0.5% malt extract). No inhibition was detected in the fungi. In the case of the bacteria, a small inhibition halo was observed (0–3 mm) when the concentration was 1 mg/disk. The best results were against *Bacillus licheniformis* CECT 491. At 200 μg/disk of extract an inhibition halo of 7 mm was observed. Compounds **2** and **9** yielded halos of 4 and 10 mm, respectively, at 350 μg/disk, whereas **1** showed no inhibition.

CYTOTOXIC AND ANTIVIRAL ACTIVITIES.—In vitro cytotoxic activity of compounds **2**, **3**, **4a–8a**, and **9** against the cell lines P-388 (murine lymphocytic leukemia), A-549 (human lung carcinoma), and HT-29 (human colon carcinoma), cytotoxicity against CV-1 (monkey kidney fibroblast), and antiviral activity against HSV-1 (herpes simplex virus) and VSV (vesicular stomatitis virus) were determined by methods described in ref. (13). Compounds **2**, **3**, **6a**, **7a**, and **9** exhibited IC_{50} values of >5 μg/ml against all the cell lines assayed. An IC_{50} =5 μg/ml was determined for **4a** against A-549, HT-29 and CV-1, for **5a** against HT-29, and for **8a** against P-388. The IC_{50} of **5a** against P-388 was 2.5 μg/ml.

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