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TWO SESQUILIGNANS FROM THE WOOD OF *ABIES MAROCANA*

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Key Word Index—*Abies marocana*; Pinaceae; wood; sesquilignans; sesquimarocanols A and B.

Abstract—From the chloroform extract of the wood of *Abies marocana*, consisting mainly of lignans, the acetoxyl derivatives of the new sesquilignans, 4,4″,7″,9,9″-pentahydroxy-3,3′,3″-trimethoxy-4′,8″:7,9′-bis-epoxy-8,8′-sesquineolignan and 4,4″,7″,9,9′,9″-hexahydroxy-3,3′,3″-trimethoxy-4′,8″-epoxy-8,8′-sesquineolignan were isolated; the trivial names, sesquimarocanols A and B, were subsequently assigned, respectively. Also, (9′*R*)-9′-hydroxylariciresinol tetraacetate, (7′*R*)-7′-hydroxylariciresinol tetraacetate and sesquipinsapol B pentaacetate, which had previously only been found in *A. pinsapo*, were isolated, together with some well known compounds. The new structures were established by spectroscopic methods. Certain cytotoxic activity assays were performed on the new compounds.

INTRODUCTION

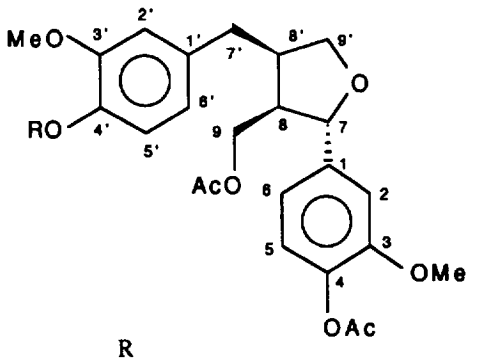
In the course of our investigations into the chemical composition of the hexane extract of *Abies marocana*, we have previously found endoperoxide diterpenoids [1] and cyclolanostanolides [2] as the main constituents, from both the needles and the wood [3]. In the present paper, the composition of the chloroform extract of the wood of this species is reported.

RESULTS AND DISCUSSION

The wood of *A. marocana* was extracted with hexane and then chloroform. The latter extract was defatted with methanol and after successive chromatographic assays on silica gel columns using hexane–ethyl acetate mixtures as eluent, the compounds matairesinol, veratric acid, vanillic acid, veratraldehyde and 3,4-dimethoxycinamaldehyde were isolated. The other fractions were acetylated and then chromatographed on silica gel columns resulting in the following well known substances: pinoresinol diacetate, 9-(*p*-coumaroyl)-lariciresinol triacetate, lariciresinol triacetate (**1a**), secoisolariciresinol tetraacetate (**2a**), (7′*S*)-7′-hydroxylariciresinol tetraacetate and dihydrodehydrodiconiferil alcohol triacetate. Other lignans produced, which had only previously been isolated by us from *A. pinsapo* were: (9′*R*)-9′-hydroxylariciresinol tetraacetate (**3a**) [4], (7′*R*)-7′-hydroxylariciresinol tetraacetate (**4a**) [4], sesquipinsapol B pentaacetate (**5a**) [5], together with the new sesquilignans, **6a** and **7a**. The ω-hydroxypropioguaiacone diacetate (**8a**), which has previously isolated as a natural product from *Bauhinia manca* [6], and whose biogenetic origin seems to be as an intermediary in lignan catabolism [7], was also found in *A. marocana*.

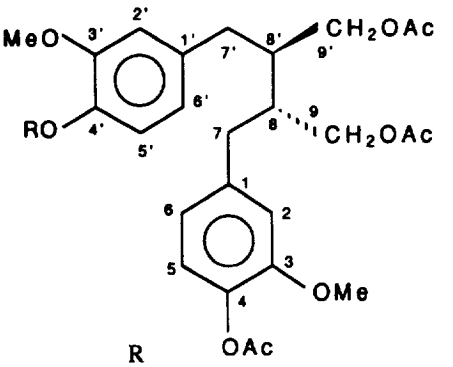
Compound **6a** was obtained as an oil, whose CI mass spectrum (*m/z* 707 $[M + 1 - OAc]^+$) and NMR spectral data agreed with a molecular formula of $C_{40}H_{46}O_{15}$. Its IR spectrum showed bands for aromatic rings (3014, 1605 and 1508 cm^{-1}) and acetoxyl groups (1742 cm^{-1}). The ^1H NMR spectrum (Table 1) indicated a sesquilignan structure related to **1a** (Table 1), their spectra being very much alike. The signals assigned to H-7, δ4.84 (J = 5.7 Hz) in **6a** and δ4.85 (J = 5.7 Hz) in **1a**, were particularly remarkable. However, in **6a** an additional C_6–C_3 unit appeared. The ^{13}C NMR spectrum (Table 2) confirmed the relationship between **6a** and **1a** [8] and a triacetyl-guaiacylglycerol ether was established as the structure for this unit, according specifically to the signals at δ74.51 (CH), 80.40 (CH) and 63.06 (CH_2) [9]. The relative configuration of the glycerol part was established as being *threo* by comparing the ^1H NMR data with the literature [9–12]. In **6a**, H-7″ resonated as a doublet (J = 6.2 Hz) at δ6.10. In compounds with an *erythro* configuration, $J_{7''-8''}$ fluctuated between 4.4 and 5.4 Hz, whereas that of the *threo* isomers did so between 6.0 and 6.4 Hz. The linkage between the third unit and the lariciresinol moiety was determined on the basis of the ^{13}C NMR spectral data. The common aliphatic portions of **1a** and **6a** showed identical signals. Hence, the linkage had to be on one of the aromatic rings. The signals of the unprimed ring were identical, whereas those of the primed one demonstrated variations due to the change from an acetoxyl group at 4′ in **1a** to an ether unit in **6a** with a downfield shift of C-4′ and upfield shifts of C-1′ and C-5′. Thus, **6a** was designated as 4,4″,7″,9,9″-pentahydroxy-3,3′,3″-trimethoxy-4′,8″:7,9′-bis-epoxy-8,8′-sesquineolignan pentaacetate and named sesquimarocanol A pentaacetate.

Compound **7a** was obtained as a syrupy liquid and showed the molecular formula $C_{42}H_{50}O_{16}$, in accordance with its CI mass spectrum (*m/z* 751 $[M + 1]^+$) and NMR spectral data. These spectra



R

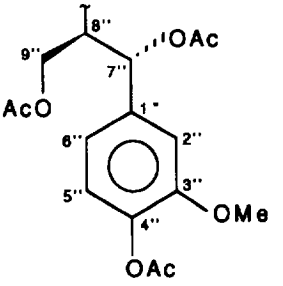
1a Ac



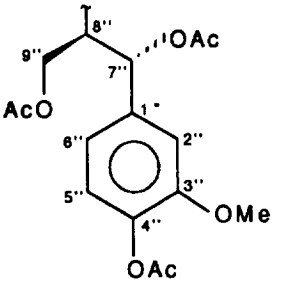
R

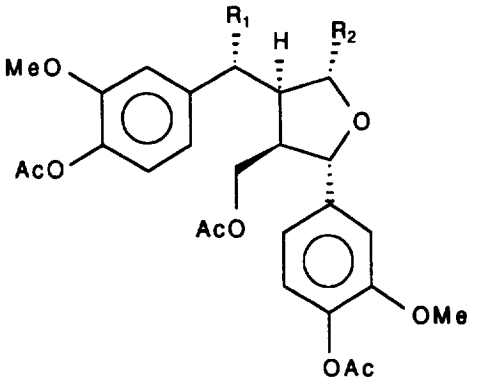
2a Ac

6a

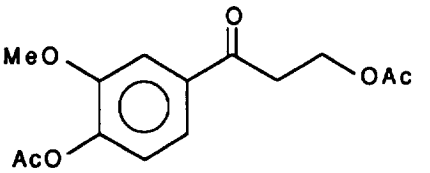


7a

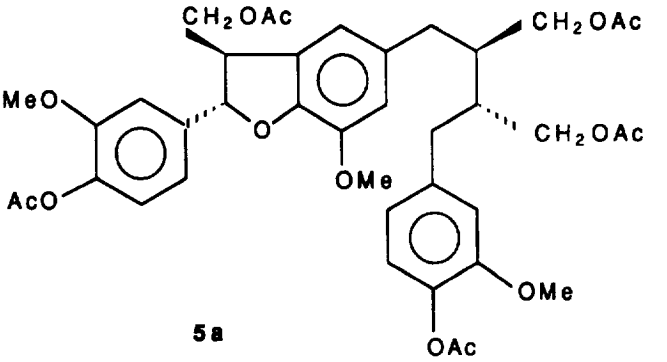




	R_1	R_2
3a	H	OAc
4a	OAc	H



8a



5a

indicated that **7a** was also a sesquilignan, in this case, related to **2a**. Comparison of the ^{13}C NMR spectra (Table 2) revealed that the signals of their C-1 to C-9 carbons were similar, whereas the other aromatic ring was modified in **2a**. At the same time, the third C_6–C_3 unit was similar to that in **6a**, with signals at δ74.54 (C-7″), 80.47 (C-8″) and 63.02 (C-9″). The relative configuration of the glycerol part of the molecule was also established as being *threo*, because H-7″ appeared in the ^1H NMR (Table 1) as a doublet at δ6.10, with a coupling

Table 1. ^{1}H spectral data for compounds **1a**, **6a**, **2a** and **7a** (300 MHz, $CDCl_3$, TMS)

H	1a	6a	2a	7a
2	6.95 *d* (1.9)*	6.95 *br s*	6.63 *d* (1.9)	6.64 *d* (1.1)
5	6.99 *d* (8.1)	6.99 *d* (7.5)	6.90 *d* (7.9)	6.89 *d* (8.0)
6	6.86 *dd* (1.9, 8.1)	6.86 *dd* (1.2, 7.5)	6.60 *dd* (1.9, 7.9)	6.60 *dd* (1.1, 8.0)
7a	4.85 *d* (5.7)	4.84 *d* (5.7)	2.66 *dd* (7.3, 14.1)	2.60 *m*
7b	—	—	2.69 *dd* (7.6, 14.1)	2.60 *m*
8	2.59 *m*	2.59 *m*	2.12 *m*	2.12 *m*
9a	4.20 *dd* (7.6, 11.2)	4.20 *dd* (7.7, 11.1)	4.02 *dd* (5.8, 11.4)	4.00 *dd* (5.5, 11.9)
9b	4.37 *dd* (6.5, 11.2)	4.37 *dd* (6.8, 11.1)	4.23 *dd* (5.6, 11.4)	4.19 *dd* (4.9, 11.9)
2′	6.75 *br s*	6.65 *br s*	6.63 *d* (1.9)	6.57 *br s*
5′	6.94 *d* (7.9)	6.78 *d* (7.9)	6.90 *d* (7.9)	6.73 *d* (6.8)
6′	6.73 *dd* (2.0, 7.9)	6.68 *dd* (2.0, 7.9)	6.60 *dd* (1.9, 7.9)	6.54 *br d* (6.8)
7′a	2.56 *dd* (2.0, 13.0)	2.53 *dd* (6.4, 12.7)	2.66 *dd* (7.3, 14.1)	2.60 *m*
7′b	2.86 *dd* (4.8, 13.0)	2.88 *dd* (7.1, 12.7)	2.69 *dd* (7.6, 14.1)	2.60 *m*
8′	2.72 *m*	2.72 *m*	2.12 *m*	2.12 *m*
9′a	3.75 *m*	3.75 *m*	4.02 *dd* (5.8, 11.4)	4.00 *dd* (5.5, 11.9)
9′b	4.09 *dd* (6.5, 8.7)	4.06 *dd* (6.8, 10.2)	4.23 *dd* (5.6, 11.4)	4.19 *dd* (4.9, 11.9)
2″	—	6.95 *br s*	—	6.95 *br s*
5″	—	6.83 *d* (8.2)	—	6.83 *d* (6.8)
6″	—	6.75 *dd* (2.0, 8.2)		6.75 *br d* (6.8)
7″	—	6.10 *d* (6.2)	—	6.11 *d* (6.3)
8″	—	4.57 *pq*† (6.2)	—	4.52 *pq*† (6.3)
9″a	—	4.03 *dd* (5.6, 11.7)	—	4.00 *m*
9″b	—	4.30 *dd* (4.4, 11.7)	—	4.31 *dd* (4.4, 11.2)
OMe	3.81 *s*, 3.83 *s*	3.80 *s*, 3.83 *s*, 3.84 *s*	3.74 *s*	3.71 *s*, 3.72 *s*, 3.80 *s*
OAc	2.02 *s*	1.98 *s*, 2.01 *s*, 2.04 *s*	2.05 *s*	2.01 *s*, 2.02 *s*, 2.04 *s*
	2.29 *s*	2.27 *s*, 2.28 *s*	2.29 *s*	2.21 *s*

*Coupling constants (J in Hz) given in parentheses.
†*pq*: pseudoquadruplet.

constant of 6.3 Hz. Therefore, the structure 4,4″,7″,9,9′,9″-hexahydroxy-3,3′,3″-trimethoxy-4′,8″-epoxy-8,8′-sesquineolignan hexaacetate, was assigned to **7a**, together with the trivial name sesquimarocanol B hexaacetate.

Cytotoxicity against the cancer lines P-388, A-549, HT-29 and MEL-28 (human melanoma) were studied for **6a** and **7a**. The cytotoxic activities were only moderate.

Studies carried out on the hexane extracts of woods from *A. marocana* and *A. pinsapo* [3, 13, 14], revealed that the major products were essentially the same and that the structural skeletons were identical, *viz.* sesquiterpenoids related to juvabione, abietane and labdane diterpenoids, and cycloartane triterpenoids. The chloroform extracts of *A. marocana* and *A. pinsapo* woods [4, 5] were made up almost exclusively of lignans. The major products were the same from both trees (matairesinol, lariciresinol and secoisolariciresinol) and some of the minor ones (**3a**, **4a** and **5a**) have been found only in these species. This similarity may corroborate the possibility that they are two varieties of the same species, rather than two different species, as their botanical features indicate [15].

EXPERIMENTAL

Extraction and isolation. Wood of *A. marocana* Trabut was collected on Mont Talasmtane (calcareous chain of Yebala, Western Rif, Morocco) in March 1987 and was identified by Prof. F. Valle (Departamento de Biologia Vegetal, Universidad de Granada). A voucher specimen is deposited in the Herbarium of this University. Crushed wood (16 kg) was extracted with $CHCl_3$ in a Soxhlet apparatus for 8 hr, after being extracted with hexane. The soln was evapd *in vacuo*, giving 23 g of extract (0.14% of wood wt) which was defatted with MeOH to yield 12.5 g of a lignan-enriched fr. After successive silica gel CC, matairesinol (900 mg), veratric acid (6 mg), vanillic acid (9 mg), veratraldehyde (55 mg) and 3,4-dimethoxycinamaldehyde (25 mg) were isolated. After acetylation of some frs and successive silica gel CC, the following compounds were isolated in order of increasing polarity: pinoresinol diacetate (33 mg), 9-(*p*-coumaroyl)-lariciresinol triacetate (42 mg), **1a** (357 mg), **8a** (40 mg), **3a** (30 mg), **2a** (2.11 g), (7′*S*)-7′-hydroxylariciresinol tetraacetate (162 mg), **4a** (78 mg), isolariciresinol tetraacetate (10 mg), **6a** (12 mg), **5a** (25 mg), dihydrodehydrodiconiferyl alcohol triacetate (11 mg) and **7a** (12 mg).

4′,4″,7″,9,9″-Pentahydroxy-3,3′,3″-trimethoxy-4,8″:7,9′-bis-epoxy-8,8′-sesquineolignan pentaacetate (*sesquimarocanol A pentaacetate*) (**6a**). Eluted with hexane–EtOAc (1:1). Oil. $[\alpha]_D^{25}$ + 2° ($CHCl_3$; *c* 0.85). IR ν_{max}^{film} cm^{-1}: 3014, 2938, 1742, 1605, 1508, 1463, 1421, 1369, 1222, 1157, 1123, 1035, 905, 801, 756. CIMS *m/z* (rel. int.) 707 $[M + 1 - OAc]^+$ (1), 487 (1), 385 (1), 367 (1), 261 (5), 163 (21), 61 (100). ^{1}H NMR, see Table 1. ^{13}C NMR, see Table 2.

Table 2. ^{13}C NMR spectral data for compounds **1a**, **6a**, **2a** and **7a*** ($CDCl_3$, TMS)

Carbon	1a	6a	2a	7a
C-1 ...	138.7	138.90	137.9	138.94†
C-2 ...	109.5	109.80	112.7	113.06
C-3 ...	150.9	150.86	150.8	151.00
C-4 ...	141.4	141.60	138.4	138.74†
C-5 ...	122.5†	122.19	122.4	122.68
C-6 ...	117.6	117.85	120.8	121.03
C-7 ...	82.7	82.95	35.2	35.26
C-8 ...	49.0	49.07	39.5	39.82
C-9 ...	62.6	62.76	64.1	64.24
C-1′ ...	138.0	135.20	137.9	134.85
C-2′ ...	112.6	112.98	112.7	113.06
C-3′ ...	150.9	150.86	150.8	150.78
C-4′ ...	138.7	146.00	138.4	146.31
C-5′ ...	122.6†	118.94	122.4	118.82
C-6′ ...	120.4	120.75	120.8	121.21
C-7′ ...	33.4	33.26	35.2	34.92
C-8′ ...	42.1	42.26	39.5	39.82
C-9′ ...	72.7	72.88	64.1	64.24
C-1″ ...	—	135.40	—	135.39
C-2″ ...	—	111.88	—	111.76
C-3″ ...	—	151.17	—	151.17
C-4″ ...	—	140.00	—	139.94
C-5″ ...	—	122.89	—	122.88
C-6″ ...	—	119.64	—	119.63
C-7″ ...	—	74.51	—	74.54
C-8″ ...	—	80.40	—	80.47
C-9″ ...	—	63.06	—	63.02
OMe ...	55.8	55.90	55.7	55.84, 56.00
OCO*Me*	20.6	20.69, 20.86, 20.91	20.6, 20.8	20.74, 21.05
OCOMe	168.9, 170.7	169.01, 169.20 170.90, 171.07	168.8, 170.7	168.87, 169.17, 169.77 170.65, 170.68, 171.03

*6a and 7a, 75 MHz, 1a and 2a data from ref. [8], 25.2 MHz.
†Interchangeable values.
Number of protons directly attached to each carbon verified using DEPT pulse sequence.

4′,4″,7″,9,9′,9″-*Hexahydroxy*-3,3′,3″-*trimethoxy*-4,8″-*epoxy*-8,8′-*sesquineolignan hexaacetate* (*sesquimarocanol B hexaacetate*) (**7a**). Eluted with hexane–EtOAc (9:11). Oil $[\alpha]_D^{25}$ + 13° ($CHCl_3$, *c* 0.46). IR ν_{max}^{film} cm^{-1}, 3013, 2960, 1740, 1605, 1508, 1463, 1419, 1369, 1226, 1199, 1156, 1123, 1035, 944, 905, 800, 756, 664. CIMS *m/z* (rel. int.) 751 $[M + 1]^+$ (3), 691 (2), 533 (35), 501 (5), 369 (5), 331 (6), 246 (13), 245 (100), 61 (14). ^{1}H NMR, see Table 1. ^{13}C NMR, see Table 2.

Cytotoxic activity. *In vitro* cytotoxic activity of **6a** and **7a** against cell lines P-388 (murine lymphocytic leukemia), A-549 (human lung carcinoma), HT-29 (human colon carcinoma) and MEL-28 (human melanoma) were determined by methods described in ref. [16]. Compounds **6a** and **7a** gave IC_{50} values of 2.5 $\mu g\,ml^{-1}$ against P-388 and A-549. The IC_{50} of **6a** and **7a** against MEL-28 was 10 and 5 $\mu g\,ml^{-1}$, respectively, and against HT-29, the IC_{50} was greater than 5 $\mu g\,ml^{-1}$.

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