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NEW DITERPENES FROM *CHAMAECYPARIS PISIFERA*

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Key Word Index—*Chamaecyparis pisifera*; Cupressaceae; diterpenes; pisiferin; 15-hydroxyferruginol; *O*-methyl pisiferic acid; pisiferal.

Abstract—Three new diterpenes, pisiferin, 15-hydroxyferruginol, and *O*-methyl pisiferic acid have been isolated and the presence of 12-hydroxyabieta-8,11,13-trien-20-al has been confirmed in studies of the leaves of *Chamaecyparis pisifera*. The structure of pisiferin, a phenolic diterpene with a new carbon skeleton, has been determined by chemical degradation. The presence of diterpenes of the ferruginol type in the leaves of four varieties of *C. pisifera* has been examined.

INTRODUCTION

Previously we reported the isolation and structural determination of three new diterpenes of the ferruginol type, pisiferic acid (**1**) [1], pisiferol (**2**), and methyl pisiferate (**3**) [2], from the leaves of *Chamaecyparis pisifera* (Cupressaceae).

Recently, the antimicrobial activity of pisiferic acid (**1**) has been reported [3]. Since we are interested in antimicrobial substances in plants, we further examined the leaves of *C. pisifera*. We present here the isolation and structural determination of three new diterpenes, a phenolic diterpene with a new carbon skeleton, named pisiferin (**4**), 15-hydroxyferruginol (**5**) and *O*-methyl pisiferic acid (**6**). We also report the identification of 12-hydroxyabieta-8,11,13-trien-20-al (**7**) which we have named pisiferal.

Furthermore, we searched for diterpenes of the ferruginol type in the leaves of four varieties of *C. pisifera* Endl., *C. pisifera* Endl. var. *plumosa* Beissn., *C. pisifera* Endl. var. *plumosa* form. *aurea* Beissn., *C. pisifera* Endl. var. *squarrosa* Beissn. and *C. pisifera* Endl. var. *filifera* Beissn.

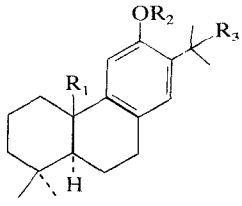
RESULTS AND DISCUSSION

The leaves of *C. pisifera* were extracted with methanol at room temperature. Successive CC of the extract on Si gel, and then PLC purification on Si gel yielded four compounds A, B, C and D.

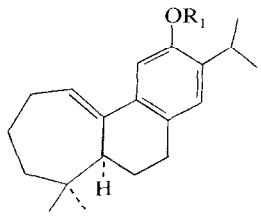
Compound A, named pisiferin, $C_{20}H_{28}O$ (M^+ *m/e* 284), colorless oil, contained two *para*-aromatic protons [^{1}H NMR δ 6.50 (1H, *s*), 6.87 (1H, *s*)], a trisubstituted double bond [ν_{max}^{oil} 810; δ 5.40 (1H, *t*, $J = 4$)], a phenolic OH [ν_{max} 3440, 1270 cm^{-1}; ^{13}C NMR δ 150.8 *s*; orange color with Pauly's reagent (diazotized sulfanilic acid) [5]], an isopropyl group on an aromatic ring [δ 1.22 (3H, *d*, $J = 7$), 1.23 (3H, *d*, $J = 7$)], and two tertiary methyl groups [δ 0.88 (3H, *s*), 0.91 (3H, *s*)]. The UV absorption maxima [λ_{max}^{MeOH} 267, 290, 310 nm] indicated that pisiferin was a styrene derivative. Hydroboration of pisiferin yielded two secondary alcohols [(**10**), colorless plates mp 77.9–79.8°, IR ν_{max}^{KBr} 3350 (OH), 1270 (phenolic OH, yellowish orange with Pauly's reagent), 1040 (secondary OH), 1606, 1500 cm^{-1} (phenyl); ^{1}H NMR δ 0.89 (3H, *s*), 1.09 (3H, *s*), 1.21, 1.24 (each 3H, *d*, $J = 7$, —CH(Me)$_2$), 3.19 (1H, *m*, —CH—OH), 6.64 (1H, *s*) and 6.88 (1H, *s*, aromatic protons); ^{13}C NMR δ 68.6 *d* (>CH—OH); (**11**), colorless plates mp 82.2–83.9°, IR ν_{max}^{KBr} 3500–3200 (OH), 1275 (phenolic OH, yellowish orange with Pauly's reagent), 1046 (secondary OH), 1610, 1500 cm^{-1} (phenyl); ^{1}H NMR δ 0.73, 0.93 (each 3H, *s*), 1.21, 1.23 (each 3H, *d*, $J = 7$, —CH(Me)$_2$), 3.11–3.31 (1H, *m*, —CH—OH), 6.65 (1H, *s*) and 6.87 (1H, *s*, aromatic protons); ^{13}C NMR δ 74.9 *d* (>CH—OH)].

While we were investigating the structure of pisiferin, it was proved to be the same compound to that obtained from pisiferol (**2**) by treatment with thionyl chloride in C_6H_6 or with tosyl chloride in pyridine. Therefore, three structures, **4**, **8**, and **9** could be considered as the possible structure of pisiferin. However, by the following facts, the structure of pisiferin was elucidated to be **4**.

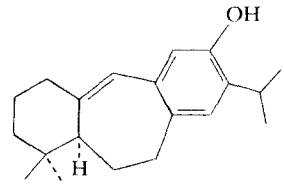
The dehydrogenation product of pisiferin showed UV absorption maxima (λ_{max}^{MeOH} 232, 286 nm) of a naphthalene skeleton. On the other hand, in the ^{1}H NMR spectrum of pisiferin, an olefinic proton on a trisubstituted double bond appeared as a triplet and its coupling constant ($J = 4$ Hz) suggested the coupling was with protons at the α carbon, but not long range couplings. Thus, the structure of pisiferin was considered to be **4**. Furthermore, in the ^{1}H NMR spectra of the hydroboration products **10** and **11**, a proton at the carbon with an attached hydroxyl group appeared as a multiplet, indicating that there were more than two protons at the α,α' carbons and that pisiferin had structure **4**. Thus, pisiferin (**4**) is a phenolic diterpene having a new carbon skeleton.



1 $R_1 = CO_2H$, $R_2 = R_3 = H$
2 $R_1 = CH_2OH$, $R_2 = R_3 = H$
3 $R_1 = CO_2Me$, $R_2 = R_3 = H$
5 $R_1 = Me$, $R_2 = H$, $R_3 = OH$
6 $R_1 = CO_2H$, $R_2 = Me$, $R_3 = H$
7 $R_1 = CHO$, $R_2 = R_3 = H$
13 $R_1 = Me$, $R_2 = R_3 = H$
14 $R_1 = CO_2Me$, $R_2 = Me$, $R_3 = H$



4 $R_1 = H$
15 $R_1 = Ts$



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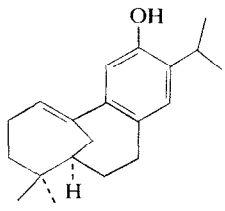

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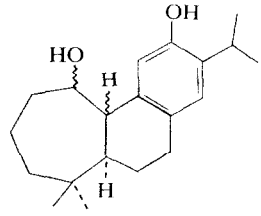

10 and **11**

Compound B, colorless needles, mp 62.4–64.5°, contained two *para*-aromatic protons [δ 6.98 (1H, *s*), 7.00 (1H, *s*)], a phenolic OH [ν_{max}^{KBr} 3400–3200, 1200 cm^{-1}; ^{13}C NMR δ 147.8 *s*; yellowish orange color with Pauly's reagent], three tertiary methyl groups [δ 0.88 (3H, *s*), 0.93 (3H, *s*), 1.20 (3H, *s*)] and an isopropyl OH group on an aromatic ring [ν_{max}^{KBr} 3570, 1120 cm^{-1}; ^{13}C NMR δ 70.6 *s* (tertiary OH); ^1H NMR δ 1.26 (3H, *s*), 1.27 (3H, *s*), 1.27 (3H, *s*) (two tertiary methyls)]. Compound B was treated with 10% HCl–MeOH to give a dehydration product (**12**), followed by hydrogenation over 10% Pd–C to yield the dihydro-compound (**13**). The NMR and IR spectra of **13** were identical with those of ferruginol [4]. Therefore, the structure of compound B was deduced to be 15-hydroxyferruginol (**5**).

Compound C, colorless needles, mp 80–82°, contained an aldehyde group [ν_{max}^{KBr} 2720, 1690.cm^{-1}; ^1H NMR δ 9.79 (1H, *s*)], two *para*-aromatic protons [δ 6.60 (1H, *s*), 6.81 (1H, *s*)], a phenolic OH [ν_{max}^{KBr} 3400, 1225 (OH), 1605, 1500, 1450 cm^{-1} (phenyl), orange color with Pauly's reagent], an isopropyl group on an aromatic ring [δ 1.21 (6H, *d* $J = 7$)] and two tertiary methyl groups [δ 0.84 (3H, *s*), 1.10 (3H, *s*)]. These data showed that compound C was 12-hydroxyabieta-8,11,13-trien-20-al (**7**), which we have named pisiferal, and which has been prepared previously from pisiferol (**2**) [1] by Jones oxidation. Pisiferal (**7**) was isolated from natural sources for the first time.

Compound D, colorless plates, mp 63.6–64.8°, contained aromatic protons [^1H NMR δ 6.74 (1H, *s*), 6.90 (1H, *s*); ^{13}C NMR 107.4 *d*, 127.0 *d*], an isopropyl group on an aromatic ring [δ 1.17 (6H, *d* $J = 7$)], a methoxyl group [ν_{max}^{KBr} 1240, 1050 cm^{-1}; δ 3.73 (3H, *s*)], two tertiary methyl groups [δ 0.83 (3H, *s*), 0.96 (3H, *s*)] and a carboxyl group [ν_{max}^{KBr} 2800–2400, 1680 cm^{-1}; ^{13}C NMR δ 180.8 *s*]. Except for the presence of a methoxyl group in compound D, the above spectral data were similar to those of pisiferic acid (**1**). Hence, compound D was treated with diazomethane to yield a methyl ester. The NMR, IR spectra and TLC of the ester were identical with those of methyl *O*-methyl pisiferate (**14**) [1] made by methylation of pisiferic acid (**1**). Thus, compound D was concluded to be *O*-methyl pisiferic acid (**6**). Compound **6** is a genuine natural product and not an artifact produced during extraction, because it could also be obtained when the use of methanol was avoided during extraction and isolation.

The presence of the diterpenes of the ferruginol type including pisiferin (**4**) in the leaves of four varieties of *C. pisifera* Endl. was examined by TLC. The results are summarized in Table 1. Four varieties

Table 1. Relative amounts of diterpenes of the ferruginol type contained in the leaves of *C. pisifera* and its varieties

Compound	Ferruginol (**13**)	Pisiferin (**4**)	Methyl pisiferate (**3**)	Pisiferal (**7**)	Pisiferol (**2**)	Pisiferic acid (**1**)
R_f*	0.83	0.80	0.57	0.53	0.47	0.43
C. pisifera Endl.	t†	t	m	s	s	l
C. pisifera Endl. var. *plumosa* Beissn.	t	t	m	s	s	m
C. pisifera Endl. var. *plumosa* form. *aurea* Beissn.	s	t	m	s	m	m
C. pisifera Endl. var. *aquarrosa* Beissn.	s	s	l	s	m	m
C. pisifera Endl. var. *filifera* Beissn.	m	s	l	t	m	m

* TLC on Si gel, eluent $CHCl_3$–MeCOOH, 60:1.

† Relative amounts were determined by the spot sizes (diameters) observed after charring with 10% H_2SO_4. 15-Hydroxyferruginol (**5**) and *O*-methylpisiferic acid (**6**) were not examined because insufficient amounts of these compounds were isolated. t: trace (<1 mm), s: small (1–2 mm), m: medium (3–6 mm), l: large (7 mm<).

contained all six diterpenes of the ferruginol type examined. It seems that these diterpenes are contained in the leaves of *C. pisifera* and its varieties. However these compounds were not contained in the leaves of *C. obtusa* Endl., the near species of *C. pisifera* in the genus *Chamaecyparis*.

EXPERIMENTAL

Mps were determined on a Mettler FP5 melting point apparatus and were uncorr. NMR spectra were measured in $CDCl_3$ with TMS as an internal standard. CC was on Si gel (Merck, Kieselgel 60, 70–230 mesh), and PLC on Si gel (Merck, Kieselgel $GF_{254+366}$).

Separation of pisiferin (**4**), *15-hydroxyferruginol* (**5**), *pisiferal* (**7**), *and O-methyl pisiferic acid* (**6**). The fresh leaves of *Chamaecyparis pisifera* (300 g), collected in Tokyo in January 1978, were extracted with MeOH at room temp. for 1 week. The extract was concd to give a dark brown sticky solid (7.2 g). The solid (2.0 g) was chromatographed on Si gel to yield three fractions (A, C_6H_6 0–500 ml, B, 501–800 ml, C, 801–1200 ml). Fraction A was subjected to PLC on Si gel (eluent C_6H_6) to give pisiferin (**4**) (85 mg) $[\alpha]_D^{2.5}+20.8°$ (*c* 0.48, MeOH). (Calc. for $C_{20}H_{28}O$: C, 84.45; H, 9.92. Found: C, 84.13; H, 9.79%). Fraction B was chromatographed on Si gel repeatedly and an enriched fraction of 15-hydroxyferruginol (**5**) obtained by elution with C_6H_6 was crystallized from hexane–C_6H_6 to give colorless needles of 15-hydroxyferruginol (9 mg) $[\alpha]_D^{2.5}=-8.2°$ (*c* 0.73, MeOH). Fraction C was rechromatographed on Si gel; elution with C_6H_6 yielded an enriched pisiferal (**7**) fraction and a crude *O*-methyl pisiferic acid (**6**). Each fraction was subjected to PLC on Si gel to yield pure pisiferal (**7**) (180 mg, eluent C_6H_6–Et_2O, 2:1, R_f 0.32 $[\alpha]_D^{2.5}$ 164.1° (*c* 0.61, MeOH)) and *O*-methyl pisiferic acid (**6**) (25 mg, eluent C_6H_6–Et_2O, 2:1, R_f 0.21 $[\alpha]_D^{2.5}+127.2°$ (*c* 0.27, MeOH)) respectively.

Hydroboration of pisiferin (**4**). A soln of BF_3-etherate (0.3 ml) in Et_2O (5 ml) was added dropwise to a soln of pisiferin ((81 mg) and $LiAlH_4$ (70 mg) in Et_2O (14 ml) with stirring for 5 min at room temp. under N_2. The soln was then allowed to stand for 3 hr at room temp. and finally refluxed for 4 hr. After a drop of Me_2CO was added to the soln, it was extracted with Et_2O. Evapn yielded a white solid. The mixture of the solid, 3% NaOH–EtOH (13 ml) and 30% H_2O_2 (3 ml) was stirred for 1 hr at room temp., followed by reflux for 5 min. The reaction mixture was extracted with $CHCl_3$, followed by evapn to yield a mixture of alcohol **10** and **11**. PLC of the mixture on Si gel (eluent $CHCl_3$–MeCOOH, 20:1) yielded alcohol **10** (32 mg, R_f 0.50) and **11** (21 mg, R_f 0.68).

Reaction of pisiferol (**2**) *with thionyl chloride.* A soln of pisiferol (**2**) (284 mg) and thionyl chloride (0.3 ml) in C_6H_6 (40 ml) was heated at reflux for 5 hr. The soln was diluted with H_2O, dried (dry Na_2SO_4) and evapd to give a yellow oil (203 mg). PLC of the oil on Si gel (eluent C_6H_6) yielded pisiferin (**4**) (163 mg).

Reaction of pisiferol (**2**) *with tosyl chloride.* A mixture of pisiferol (265 mg), tosyl chloride (191 mg) and dried Py (5 ml) was allowed to stand at room temp. for 12 hr. After dilution with H_2O, the reaction mixture was extracted with $CHCl_3$. Evapn of the $CHCl_3$ followed by PLC of the residue on Si gel (eluent C_6H_6) yielded pisiferin (**4**) (181 mg) and 13 mg of tosylate (**15**), pale yellow needles, mp 75.4–77.1°, IR ν_{max}^{KBr} cm^{-1}: 1600, 1480 (phenyl), 1365, 1190–1170 (SO_2), 830–800 (trisubst. double bond); ^1H NMR: δ 0.87 (3H, *s*), 0.90 (3H, *s*), 1.02 (3H, *d*, $J=7$), 1.07 (3H, *d* $J=7$), 2.44 (3H, *s*), 5.34 (1H, *t* $J=4$), 6.67 (1H, *s*), 6.92 (1H, *s*), 7.30 (2H, *d*, $J=8$), 7.76 (2H, *d*, $J=8$).

Reaction of pisiferol (**2**) *with oxalic acid.* A soln of pisiferol (**2**) (210 mg) and oxalic acid (180 mg) in EtOH (30 ml) and H_2O (2 ml) was refluxed for 5 hr. In this case, pisiferol (**2**) was recovered unchanged.

Dehydrogenation of pisiferin (**4**) *with* 10% Pd–C. Pisiferin (10 mg) was heated at 310° for 6 hr with 10% Pd–C (10 mg) on a salt bath ($NaNO_3$–KNO_3 1:1) and then extracted with MeOH, followed by concn to give a dehydrogenation product (2 mg).

Ferruginol (**13**) *from 15-hydroxyferruginol* (**5**). A soln of 15-hydroxyferruginol (**5**) (8 mg) in 10% HCl–MeOH (1.5 ml) was refluxed for 6 hr. The soln was extracted with $CHCl_3$, followed by evapn to yield a dehydration product (4 mg). A soln of the dehydration product (4 mg) in MeOH (2 ml) was hydrogenated over 10% Pd–C for 15 hr. After filtration, the soln was concd to give ferruginol (**13**) (3 mg).

Methyl O-methyl pisiferate (**14**) *from O-methyl pisiferic acid* (**6**). An Et_2O soln (2 ml) of CH_2N_2 freshly prepared from *N*-nitroso, *N*-methyl urethane [6] was mixed with a soln of *O*-methyl pisiferic acid (**6**) (10 mg) in Et_2O (2 ml) and the mixture was allowed to stand at room temp. for 12 hr. After a drop of glacial HOAc was added, the soln was extracted with Et_2O. Evapn yielded methyl *O*-methyl pisiferate (**14**) (7 mg).

Extraction of C. pisifera *Endl., its varieties and* C. obtusa *Endl.* The fresh leaves of *C. pisifera*, its four varieties, *C. pisifera* Endl. var. *plumosa* Beissn., *C. pisifera* Endl. var. *plumosa* form. *aurea* Beissn., *C. pisifera* Endl. var. *squarrosa* Beissn. and *C. pisifera* Endl. var. *filifera* Beissn., and *C. obtusa* Endl. (each 5 g), collected in Ibaraki, Japan in January 1979, were each extracted separately with MeOH at room temp. for 1 week. Evapn. of the MeOH yielded extracts which were subjected to TLC on Si gel.

Isolation of O-methyl pisiferic acid (**6**) *from* C_6H_6 *extract of the leaves of* C. pisifera. The fresh leaves of *C. pisifera* (50 g), collected in Tokyo in January 1978, were extracted with C_6H_6 at room temp. for 1 week. The extract was concd to give a dark green solid (1.0 g). CC of the solid (820 mg) on Si gel (100 g) gave *O*-methyl pisiferic acid (12 mg) by elution with C_6H_6.

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