Phytochemistry, Vol. 24, No. 7, pp. 1545–1551, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

TERPENOIDS FROM THE SEED OF *CHAMAECYPARIS PISIFERA*: THE STRUCTURES OF SIX DITERPENOIDS

SHINICHI HASEGAWA, TOSHIMI KOJIMA and YOSHIYUKI HIROSE

Department of Applied Biological Science, Faculty of Science and Technology, Science University of Tokyo, Noda 278, Japan

(*Received* 9 *November* 1984)

Key Word Index—*Chamaecyparis pisifera*; Cupressaceae; chemotaxonomy; mono-, sesqui- and diterpenes; 20-*nor*-abietane; 9(10 → 20)-*abeo*-abietane.

Abstract—Six new diterpenes, 12-hydroxy-20-*nor*-abieta-1(10),2,8,11,13-pentaene, pisiferanol (10*S*,12-dihydroxy-9(10 → 20)-*abeo*-abieta-8,11,13-triene), pisiferadinol (20*S*-hydroxypisiferanol), 12-deoxypisiferanol, 1β-hydroxyisopisiferin (1*R*,12-dihydroxy-9(10 → 20)-*abeo*-abieta-8,10(20),11,13-tetraene), and the dimethylamine salt of O-methylpisiferic acid were isolated from the seed of *Chamaecyparis pisifera*. The structures of these diterpenes were established by spectral and chemical methods. In addition, 15 mono-, four sesqui- and nine diterpenes were identified.

INTRODUCTION

In a continuation of our recent studies [1, 2] on the chemical constituents of the seed of *Chamaecyparis obtusa*, we have investigated those of *Ch. pisifera* Endl. from the standpoint of chemotaxonomy.

In earlier studies on the terpenoid constituents of *Ch. pisifera*, several diterpenes, ferruginol (**1**), 15-hydroxyferruginol, pisiferol (**2**), pisiferal (**3**), pisiferic acid (**4**), O-methylpisiferic acid (**5**), pisiferin (**6**) and 6α-hydroxysandaracopimaric acid (**7**), were isolated from the leaves [3, 4], and chamaecynone, isochamaecynone and cadinane-type of sesquiterpenes from the heartwood [5–7].

In our preliminary paper [8] on the terpenes in the seed of this species we described the isolation of pisiferin (**8**) and isopisiferin (**9**), and revised the structure of the former from **6**, proposed by Yatagai *et al.* [2], to **8**. Further examination of the constituents led to the identification of a number of known mono-, sesqui- and diterpenes, and to the isolation of six new diterpenes. This paper describes the isolation and structural elucidation of these terpenes.

RESULTS AND DISCUSSION

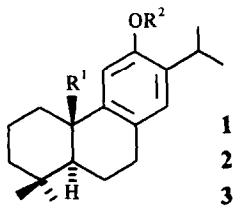
After removal of a crystalline material precipitated during storage in an ice-box, the ether extract of the seed was fractionated into neutral and acidic portions. The neutral portion was chromatographed on silica gel. The hexane eluate contained exclusively monoterpene hydrocarbons along with a small amount of sesqui- and diterpene hydrocarbons and paraffins ($C_{25}H_{52}$ to $C_{33}H_{68}$). The benzene eluate consisted mainly of triacylglycerols and bornyl and α-terpinyl acetates. The ether–hexane and ethyl acetate eluates consisted of oxygenated diterpenes and trace amounts of mono- and sesquiterpene alcohols. The compounds identified by GC/MS are shown in Table 1.

The acidic portion contained **4**, **5** and **7**, all of which had been isolated from the leaves. The precipitates obtained from the ether extract before treatment with an aqueous alkaline solution were recrystallized from ether to afford **5** and another crystalline compound, compound I, $C_{23}H_{37}O_3N$, mp 173–180°, which gave **5** on treatment with acid or acetic anhydride–pyridine. The IR and ^1H NMR spectra suggested I to be the dimethylamine salt of **5** (**10**), and this was confirmed by synthesis. The other diterpenes, compounds II–VI, isolated from the neutral portion together with **1**, **2**, **3**, **8** and **9**, were new compounds, and their structures were established as described below.

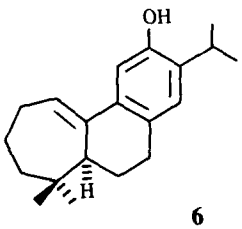
Table 1. Volatile components identified by GC/MS in the seed of *Chamaecyparis pisifera*

α-Pinine	Myrcenol
Camphene	*p*-Cymen-8-ol
Sabinene	Bornyl acetate
β-Myrcene	α-Terpinyl acetate
trans-β-Ocimene	Longifolene
Limonene	β-Caryophyllene
p-Cymene	Thujopsene
β-Phellandrene	γ-Cadinene
Terpinolene	Hibaene
p-Cymenene	Dolabradiene
Terpinen-4-ol	*ar*-Abietatriene

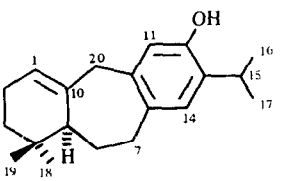
Compound II, $C_{19}H_{24}O$, amorphous solid, $[\alpha]_D$ −51.9°, was readily tinged with yellow on standing in the



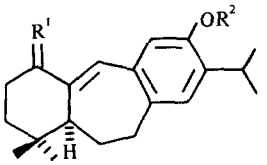
	R^1	R^2
1	Me	H
2	CH_2OH	H
3	CHO	H
4	COOH	H
5	COOH	Me
10	$COONH_2Me_2$	Me



6



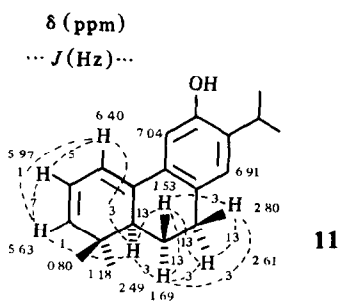
8



	R^1	R^2
9	H_2	H
24a	α-H,β-OH	H
24b	α-H,β-OAc	Ac
24c	α-H,β-OH	Me
25	O	Me
29	α-OH,β-H	H

air. The presence of a phenolic hydroxyl group was indicated by an IR absorption band at 3450 cm^{-1}, and a positive ferric chloride test. Catalytic hydrogenation of II over Pd–C gave a tetrahydro derivative, showing a UV absorption maximum at 282 nm (log ε 3.36), indicative of a simple phenolic ring. Thus, II was a tricarbocyclic compound having two double bonds and a phenolic ring. Since the UV spectrum of II showed absorption bands at 323 (log ε 4.31), 336 (4.39) and 351 (4.29) nm, the two double bonds appeared to be conjugated with the phenolic ring. The ^1H NMR spectrum (400 MHz) of II indicated the presence of two aromatic [δ 7.04 and 6.91 (each 1H, *s*)], three olefinic [δ 6.40, 5.97 and 5.63 (each 1H, *m*)], and a phenolic hydroxyl proton (δ 4.64), an isopropyl group attached to an aromatic ring [δ 3.17 (1H, septet, J = 6.8 Hz), 1.24 and 1.25 (each 3H, *d*, J = 6.8 Hz)], two tertiary methyls (δ 1.18, 0.80), an allylic methine [δ 2.49 (1H, *m*)], and two methylenes [δ 2.84–2.56 and 1.99–1.48 (each 2H)], one of which was benzylic. The ^{13}C NMR spectrum showed the occurrence of only one fully substituted sp^3 carbon atom (34.5 ppm) among nine sp^3 ones observed, suggesting the presence of two gem tertiary methyl groups. From the above spectral data, II was identified as a 20-*nor*-abietane type diterpene having two double bonds at C-1(10) and C-2. This was confirmed by a spin decoupling experiment, as shown in Fig. 1. Thus, II is 12-hydroxy-20-*nor*-abieta-1(10),2,8,11,13-pentaene (**11**).

Compound III, $C_{20}H_{30}O_2$, colourless prisms, mp 143–145°, $[\alpha]_D$ +24.9°, showed the presence of a phenolic hydroxyl (UV: 280 nm; IR: 3550, 1615, 1515, 1200 cm^{-1}; a positive ferric chloride test) and a tertiary hydroxyl groups [IR: 3310, 1100 cm^{-1}; ^{13}C NMR: 72.3 ppm (s)]. It gave a monoacetate (**12b**; mp 121–122°; IR: 3550, 1760, 1210 cm^{-1}) on treatment with acetic anhydride in pyridine. The ^1H NMR spectrum of III (Table 2) indicated the presence of two aromatic protons, an isopropyl group attached to an aromatic ring, two tertiary methyls and two benzylic methylenes; the one showing the signal of an AB quartet at δ 2.86, due to a geminal coupling and another multiplet at δ 2.70. Dehydration of III with thionyl chloride in pyridine afforded the same compounds, **8**, **9** and **13** [8] in a ratio of 5:3:2, that were previously derived from **2** on treatment with the same reagent. The above



11

Fig. 1

Table 2. ^1H NMR spectra of compounds III (**12a**), IV (**17a**), V (**23**) and VI (**24a**) (100 MHz, $CDCl_3$)

H	III	IV	V	VI
1				3.86 *m*
7	2.70 *m*	2.46 *m*	2.76 *m*	2.50 *m*
11	6.77 *s*	7.20 *s*	6.94 *s*	6.73 *s*
12			6.94 *s*	
14	6.87 *s*	6.81 *s*	6.96 *s*	6.76 *s*
15*	3.22 *sept*	3.23 *sept*	2.85 *sept*	3.24 *sept*
16*	1.19 *d*	1.19 *d*	1.23 *d*	1.20 *d*
17	1.20 *d*	1.19 *d*	1.23 *d*	1.22 *d*
18	0.91 *s*	0.86 *s*	0.88 *s*	0.88 *s*
19	0.93 *s*	0.89 *s*	0.92 *s*	0.54 *s*
20	2.86 *AB q*†	4.37 *br s*	2.77 *AB q*†	6.52 *s* (*br*)

*The J values between H-15 and H-16, 17 are 7 Hz
†J = 14 Hz and Δν = 42 Hz.

Table 3 ^{13}C NMR spectra of compounds III (**12a**), IV (**17a**) and V (**23**) (25.15 MHz, $CDCl_3$)

C	III	IV	V
1	41.6	34.2*	42.2*
2	18.7	18.3	18.7
3	42.3	42.5	42.5*
4	34.4	34.5	34.4
5	58.0	56.2	58.0
6	24.4	24.5	23.8
7	35.2	34.9*	36.3
8	134.9	133.7	143.7
9	133.5	135.9	133.2
10	72.3	76.4	70.6
11	118.6	113.0	131.9
12	152.3	152.5	124.0
13	132.6	132.3	147.8
14	126.6	126.2	126.5
15	26.5	26.5	33.7
16	22.6	22.4	24.0
17	22.9	23.1	24.0
18	32.3	32.5	32.2
19	21.7	21.8	21.6
20	51.1	78.8	50.9

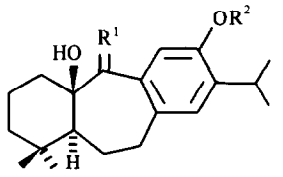
*May be interchanged.

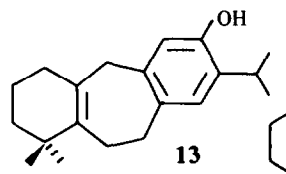
facts suggested the structure of III to be represented by the formula **12a** except for the absolute configuration at C-10. To determine the stereochemistry at C-10, the synthesis of **12a** from **9** *via* an epoxide was carried out. Epoxidation of **9** with *m*-chloroperbenzoic acid in the presence of potassium carbonate afforded two isomeric epoxides, **14** and **15**, in a ratio of 3:7. Examination of Dreiding models demonstrated that the β-face of the double bond at C-10(20) of **9** was fairly screened by the β-methyl group at C-4 and/or 7β-H, hence the α-epoxide was the predominant component formed, the structure being represented by **15**. Treatment of the two epoxides with lithium aluminium hydride yielded the corresponding tertiary alcohols, **12a** and **16**. The alcohol (**12a**) derived from the β-epoxide (**14**) was found to be identical with the natural product in all respects (mp, IR and ^{1}H NMR). Thus the structure of III was determined as **12a**, and named pisiferanol

Compound IV, $C_{20}H_{30}O_3$, colourless glassy solid, $[\alpha]_D$ +26.1°, gave a positive ferric chloride test and a UV absorption maximum at 281 nm. The IR spectrum showed hydroxyl absorption bands at 3550 and 3320 cm^{-1} but no carbonyl absorption. The presence of carbon signals at 152.5 (*s*), 78.8 (*d*) and 76.4 (*s*) ppm in the ^{13}C NMR spectrum suggested that IV contained a phenolic, a secondary and a tertiary hydroxyl groups, which was further supported by the formation of a diacetate (**17b**, mp 139–140°) on treatment with acetic anhydride in pyridine, and of a monomethyl ether (**17c**, mp 149–150°) by methylation with diazomethane The ^{1}H NMR spectrum of IV (Table 2) showed the presence of a trialkylated phenolic ring, an isopropyl group attached to an aromatic ring, two tertiary methyl groups, and a hydroxyl-bearing methine proton [δ4.37, *s* (*br*), sharpened by D_2O addition]. Oxidation of **17c** with manganese dioxide afforded a keto-arylaldehyde [**18**, $C_{21}H_{30}O_3$, UV: 226 (log ε4.36), 267 (4.06), 322 nm (3.59); IR: 2700, 1710, 1680 cm^{-1}; ^{1}H NMR: δ10.23 (1H, *s*, Ar–CHO)], suggesting that the secondary hydroxyl group was located at the benzylic position, adjacent to the tertiary hydroxyl group.

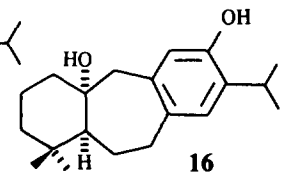
On treatment with mesylchloride in pyridine, IV gave a monochloride (**19**, $C_{20}H_{29}O_2Cl$, mp 153–156°), which was transformed into III with lithium aluminium hydride and into **14** with methanolic potassium hydroxide. The above spectral data and chemical transformations proved IV to be 20-hydroxypisiferanol. The stereochemistry of the hydroxyl group at C-20 was suggested to be *cis* to that at C-10 since, in the presence of *p*-toluenesulfonic acid, IV in acetone gave readily the corresponding acetonide (**20**) at room temperature. On the other hand, the epimer of IV at C-20 (**21**), prepared from IV *via* **22** by acetic anhydride–DMSO oxidation followed by sodium borohydride reduction, gave no acetonide under the same condition. Further confirmation of the configuration at C-20 was obtained from the CD spectrum of the bis(*p*-dimethylaminobenzoate) of IV (**17d**) according to the exciton chirality method [9]. The CD spectrum indicated the first negative and the second positive splitting-type of Cotton effects, λ_{ext} 322 ($\Delta\varepsilon$ −16.7) and 297 nm (+4.8). Hence, IV was established as 20*S*-hydroxypisiferanol (**17a**), and named pisiferadinol.

Compound V, $C_{20}H_{30}O$, colourless gum, $[\alpha]_D$ +50.8°, contained a simple alkylated benzene ring as indicated by UV absorption maxima at 265 (log ε 2.86) and 273 nm (2.87), IR absorption bands at 1610, 1505, 890 and 855 cm^{-1}, and a negative ferric chloride test. A hydroxyl group, shown by an IR stretching band at 3350 cm^{-1}, was shown to be tertiary by the presence of a signal (70.6 ppm) of a fully substituted carbon atom bearing an oxygen atom in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum of V (Table 2) showed the presence of three aromatic protons, an isopropyl group linked to a benzene ring, two tertiary methyl and two benzylic methylene groups. The above spectral features coupled with the close similarity of the chemical shifts for C-1 to C-7, C-10, C-18 and C-19 between the ^{13}C NMR spectra of III and V (Table 3) suggested V was the 12-deoxy derivative of III, and this was also supported by mass fragments, *m/z* 160 [a]$^+$, 145 [a − Me]$^+$, 126 [b]$^+$ and 111 [b − Me]$^+$, as shown in Fig. 2. Thus, V is 12-deoxypisiferanol (**23**).





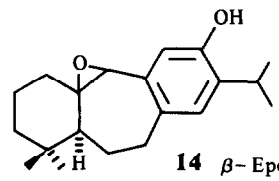
13



16

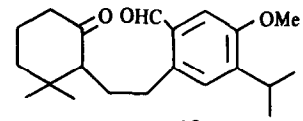
	R^1	R^2
12a	H_2	H
12b	H_2	Ac
17a	α-H,β-OH	H
17b	α-H,β-OAc	Ac
17c	α-H,β-OH	Me
17d	α-H,β-OCOAr	COAr
19	α-Cl,β-H	H
21	α-OH,β-H	H
22	O	H

Ar = p-dimethylaminophenyl

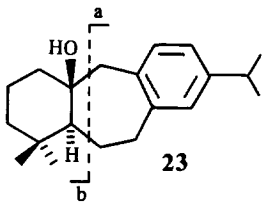


14 β-Epoxide

15 α-Epoxide



18



23

Fig 2.

Compound VI, $C_{20}H_{28}O_2$, colourless gum, $[\alpha]_D$ −122.9°, gave a positive ferric chloride test. The presence of phenolic and secondary hydroxyl groups was shown by a hydroxyl stretching band at 3300 cm^{-1} in the IR spectrum and by two carbon signals at 151.3 (*s*) and 75.0 (*d*) ppm in the ^{13}C NMR spectrum, and this was further supported by the formation of a diacetate (**24b**, IR: 1750, 1720 cm^{-1}) on treatment of VI with acetic anhydride in pyridine. The ^1H NMR spectrum of VI (Table 2) shows the presence of two aromatic protons and a trisubstituted olefinic proton, an isopropyl group attached to an aromatic ring, two tertiary methyl groups and a methine group bearing a hydroxyl. In addition, the close similarity between the UV spectra of VI [261 (log ε4.04) and 299 nm (3.54)] and **9** [8] suggested that VI was a hydroxyl derivative of **9**. Manganese dioxide oxidation of the monomethyl ether (**24c**), prepared from VI with diazomethane, gave an enone (**25**, IR: 1675 cm^{-1}; UV: 250, 303, 340 nm) and dehydration of VI with thionyl chloride in pyridine gave a conjugated diene (**26**, mp 107–108°, UV: 227, 288 nm), hence the secondary hydroxyl group was shown to be located at C-1. Further, the configuration of the hydroxyl group was presumed to be β-equatorial because the half-width value of the proton signal at C-1, in the ^1H NMR, was 16 Hz, suggesting the proton to be α-axial. In order to establish the structure, including the configuration of the hydroxyl group, VI and its epimer at C-1 were prepared from **8** as follows Epoxidation of **8** with *m*-chloroperbenzoic acid followed by isomerization of resulting epoxides, **27** and **28**, with potassium *t*-butoxide in DMSO afforded the corresponding allyl alcohols, **24a** and **29**, respectively. The alcohol, **24a**, derived from **27**, was identical with VI in all respects (TLC, IR and ^1H NMR). As the orientation of the epoxy ring of **27** was established to be β-oriented by the conversion of **27** into III with lithium aluminium hydride, VI was 1β-hydroxyisopisiferin (**24a**).

So far, in the family of Labiatae, icetexone [10], romulogarzone [11], nilgherron [12] and barbatusol [13] have been known as diterpenes having the 9(10 → 20)-*abeo*-abietane skeleton and a series of tanshinones [14] as 20-*nor*-abietane diterpenes, but this is the first time that both types of diterpenes have been found in one species of a family other than Labiatae.

EXPERIMENTAL

General. Mps: uncorr; UV: EtOH; ^1H (60 and 100 MHz) and ^{13}C (25.15 MHz) NMR: $CDCl_3$ (unless noted otherwise, TMS; MS: 70 eV, direct insertion; GC/MS: 5% OV-17 (2 m × 3 mm), temp. programmed 60–330° at 5°/min, He 60 ml/min), 20 eV, TLC: silica gel 60 F-254 (Merck) and RP-8 F-254 (Merck).

Extraction. Seeds (400 g), collected in Chiba Prefecture, Japan, in 1982, were ground and extracted with Et_2O (2 l. × 2). The Et_2O extract was concd to *ca* 500 ml and left to stand at 4°, giving a ppt. of a crystalline material. After removal of the crystals (*ca* 1 g) by filtration, the filtrate was treated successively with aq. $NaHCO_3$, Na_2CO_3 and NaOH (each 5%, 500 ml × 2) to yield strongly acidic (142 mg), less strongly acidic (1.2 g), weakly acidic (5.7 g) and neutral (23.4 g) portions.

Isolation of O-methylpisiferic acid (**5**) *and its dimethylamine salt* (**10**). The crude crystals obtained from the Et_2O extract before alkaline treatment were recrystallized from Et_2O to give **5** (127 mg), mp 149–150° (needles), $[\alpha]_D^{26}$ +166.7° (*c* 0.78, MeOH), and **10** (877 mg)

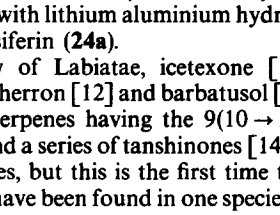
Compound **10**, mp 173–180° (fine needles), $[\alpha]_D^{26}$ +129.6° (*c* 0.79, MeOH), UV λ_{max} nm (log ε): 282 (3.44), 288 (3.43), IR ν_{max}^{KBr} cm^{-1}: 3400, 1550, 1510, 1390, 1255, 1060; ^1H NMR (60 MHz, Me_2CO-d_6): δ6.83, 6.70 (each 1H, *s*), 3.63 (3H, *s*), 2.97, 0.88 (each 6H, *s*), 1.14 (6H, *d*, *J* = 7 Hz); MS *m/z* (rel. int.): 375 $[M]^+$ (100), 330 (30), 285 (72), 284 (33), 269 (32), 215 (27), 213 (27), 203 (26), 189 (60), 69 (46).

Preparation of **10** *from* **5**. Into an ice-cold soln of **5** was passed gaseous Me_2NH evolved from its aq. soln with the aid of a stream of N_2 The crystals deposited, mp 165–175°, were identical with **10** (IR and ^1H NMR)

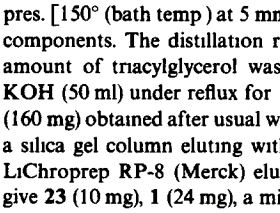
Isolation of 6α-hydroxysandaracopimaric acid (**7**) *and pisiferic acid* (**4**). The concentrated Et_2O solns of the less strongly and weakly acidic portions gave a powdery ppt. on standing, which was filtered and recrystallized from MeOH to give **7** (628 mg) as colourless fine needles, mp 268–270°, $[\alpha]_D^{26}$ −8.0° (*c* 1.25, EtOH) Upon recrystallization from aq. MeOH, the filtrates gave **4** (*ca* 4 g) as colourless prisms, mp 158–160°, $[\alpha]_D^{26}$ +159.6° (*c* 1.1, MeOH).

Fractionation of the neutral portion. The neutral portion of the Et_2O extract (19 g) was chromatographed on a silica gel column (26 × 4 cm) eluting with 500 ml each of hexane, C_6H_6, Et_2O–hexane (1:2) and EtOAc to yield 3.3, 6.4, 4.9 and 3 6 g of the eluates, respectively, which were analysed by GC/MS in order to identify volatile components

Isolation of 12-*deoxypisiferanol* (**23**), *ferruginol* (**1**), *pisiferin* (**8**), *isopisiferin* (**9**) *and* 12-*hydroxy*-20-nor-*abieta*-1(10),2,8,11,13-*pentaene* (**11**) The C_6H_6 eluate (5.2 g) was distilled under reduced pres. [150° (bath temp) at 5 mm Hg] in order to remove volatile components. The distillation residue (4 5 g) containing a large amount of triacylglycerol was saponified with 1 M ethanolic KOH (50 ml) under reflux for 15 hr The unsaponifiable matter (160 mg) obtained after usual work-up was chromatographed on a silica gel column eluting with Et_2O–hexane (1·20) and on a LiChroprep RP-8 (Merck) eluting with H_2O–MeOH (1.9) to give **23** (10 mg), **1** (24 mg), a mixture of **8** and **9** (70 mg), and **11** (32 mg) as well as nonacosan-10-ol (15 mg). After acetylation



26



27 β – Epoxide

28 α – Epoxide

(Ac_2O–pyridine) of the mixture of **8** and **9**, the acetate (80 mg) was subjected to chromatography on $AgNO_3$–silica gel (1:9, 20 g) eluted with $CHCl_3$–hexane (1:2) followed by deacetylation with $LiAlH_4$ to afford pure **8** (44 mg) and **9** (16 mg), both of which had been reported in the preliminary paper [8].

Compound **23**, gum, $[\alpha]_D^{26}$ +50.8° (*c* 2.04, $CHCl_3$); UV λ_{max} nm (log ε): 266 (2.86), 274 (2.87); IR ν_{max}^{KBr} cm^{-1}: 3350, 1610, 1505, 1470, 1160, 890, 855, 720; ^1H NMR and ^{13}C NMR: see Tables 2 and 3; HRMS *m/z* (rel. int.): 286.2296 $[M]^+$ (Calc. for $C_{20}H_{30}O$: 286.2298) (9), 160 $[C_{12}H_{16}]^+$ (96), 145 $[C_{11}H_{13}]^+$ (43), 126 $[C_8H_{14}O]^+$ (43), 111 $[C_7H_{11}O]^+$ (100).

Compound **11**, amorphous solid, $[\alpha]_D^{26}$ −51.9° (*c* 1.17, $CHCl_3$); UV λ_{max} nm (log ε): 323 (4.31), 336 (4 39), 351 (4.29); IR ν_{max}^{KBr} cm^{-1}. 3450, 1500, 1165, 903, 858, 782, 752, 738; ^1H NMR: see Fig. 1; ^{13}C NMR: 151 2 (*s*), 140.3 (*d*), 134.3 (*s*), 133.8 (*s*), 132 8 (*s*), 130.8 (*s*), 126.5 (*d*), 122.3 (*d*), 115 8 (*d*), 109 4 (*d*), 46 5 (*d*), 34 5 (*s*), 30.5 (*t*), 28.1 (*q*), 26 9 (*d*), 23.3 (*t*), 22.7 (*q*), 22.5 (*q*), 18 6 (*q*) ppm; HRMS *m/z* (rel. int.): 268.1823 $[M]^+$ (Calc. for $C_{19}H_{24}O$: 268.1828) (76), 253 $[C_{18}H_{21}O]^+$ (63), 211 $[C_{15}H_{15}O]^+$ (100)

Catalytic hydrogenation of **11**. Compound **11** (10 mg) was hydrogenated over 10% Pd–C in EtOAc for 5 hr. The product obtained as a solid was an almost pure tetrahydro derivative. UV λ_{max} nm (log ε): 282 (3.36); IR ν_{max}^{KBr} cm^{-1}. 3250, 1610, 1230, 1170, 1050, 1010, 885, 850, 760; ^1H NMR (60 MHz): δ6 81, 6 43 (each 1H, *s*), 4.46 (1H, *s*, OH), 3.13 (1H, *sept*, *J* = 7 Hz), 1 24 (6H, *d*, *J* = 7 Hz), 1.08, 0.97 (each 3H, *s*); MS *m/z* (rel. int.): 272 $[M]^+$ (100), 257 (56), 229 (11), 173 (9), 147 (16), 133 (9), 69 (8)

Isolation of pisiferal (**3**) *and pisiferanol* (**12a**). The Et_2O–hexane eluate (4 g) was chromatographed on LiChroprep Si 60 (Merck) eluting with increasing amounts of Et_2O in hexane (1·5 → 1:2) to give **3** (346 mg), mp 132–133° (needles from hexane), $[\alpha]_D^{26}$ +418.7° (*c* 0.68, MeOH), **5** (1.9 g) and **12a** (1.1 g).

Compound **12a**, mp 143–145° (prisms from hexane), $[\alpha]_D^{26}$ +24.9° (*c* 2.11, MeOH); UV λ_{max} nm (log ε): 280 (3.46); IR ν_{max}^{KBr} cm^{-1}· 3550, 3310, 1615, 1515, 1270, 1200, 1100, 980, 950, 890, 820, 800; ^1H NMR and ^{13}C NMR: see Tables 2 and 3; HRMS *m/z* (rel. int.): 302.2236 $[M]^+$ (Calc. for $C_{20}H_{30}O_2$. 302.2247) (8), 284 $[M-H_2O]^+$ (1), 269 $[M-H_2O-Me]^+$ (2), 176 $[C_{12}H_{16}O]^+$ (100), 163 $[C_{11}H_{15}O]^+$ (19), 161 $[C_{11}H_{13}O]^+$ (25); the monoacetate (**12b**): mp 121–122°; IR ν_{max}^{KBr} cm^{-1}: 3550, 1760, 1210; ^1H NMR (100 MHz): δ2.25 (3H, *s*, OAc).

Dehydration of **12a**. To a soln of **12a** (490 mg) and pyridine (0.7 ml) in dry C_6H_6 (20 ml) was added dropwise $SOCl_2$ (0.3 ml) at room temp After being stirred for 30 min, the reaction mixture was poured into H_2O and extracted with C_6H_6. The extract was washed with 5% HCl and brine, dried over $MgSO_4$ and the solvent removed. The residue was chromatographed on silica gel (15 g) eluting with EtOAc–hexane (1·10) to yield a mixture of **8**, **9** and **13** (268 mg), which were separated from one another in a ratio of 5.3:2 by chromatography on $AgNO_3$–silica gel (1·9)

Epoxidation of **9**. A mixture of **9** (83 mg), *m*-chloroperbenzoic acid (86 mg), K_2CO_3 (50 mg) and CH_2Cl_2 (10 ml) was stirred for 2 hr at room temp. After dilution with Et_2O, the soln was washed successively with 5% $NaHSO_3$, 5% $NaHCO_3$ and brine, and evaporated. The product was purified by prep. TLC on silica gel developing with Et_2O–$CHCl_3$ (1:7) to yield **14** (R_f 0 41, 14 mg) and **15** (R_f 0.35, 33 mg)

Compound **14**, mp 136–138° (prisms from hexane); UV λ_{max} nm (log ε): 289 (3.44); IR ν_{max}^{KBr} cm^{-1}: 3200, 1245, 875, 845, 790; ^1H NMR (60 MHz): δ6.90, 6.72, 3.53 (each 1H, *s*), 6.10 [1H, *s* (*br*), OH], 3.17 (1H, *sept*, *J* = 7 Hz), 1 20, 1.17 (each 3H, *d*, *J* = 7 Hz), 1 05, 0.94 (each 3H, *s*); MS *m/z* (rel. int): 300 $[M]^+$ (100), 271 (17), 257 (14), 215 (23), 189 (14), 178 (38), 177 (43), 174 (18), 162 (23), 159 (14)

Compound **15**, mp 93–95° (prisms from pentane); UV λ_{max} nm (log ε): 285 (3.48); IR ν_{max}^{KBr} cm^{-1}· 3280, 1280, 910, 900, 850, 800, 760; ^1H NMR (60 MHz): δ6.97, 6.82, 3.70 (each 1H, *s*), 6.10 [1H, *s* (*br*), OH], 3.23 (1H, *sept*, *J* = 7 Hz), 1.25 (6H, *d*, *J* = 7 Hz), 1.04, 0.79 (each 3H, *s*); MS *m/z* (rel. int.): 300 $[M]^+$ (100), 215 (20), 178 (33), 177 (36), 176 (25), 163 (17), 162 (21).

LiAlH$_4$ reduction of **15** *and* **14**. A soln of **15** (20 mg) in dry Et_2O (10 ml) containing $LiAlH_4$ (10 mg) was refluxed for 18 hr. EtOAc and then H_2O-satd Et_2O were added to the soln to destroy the reagent. Filtration followed by evaporation left a solid, which was recrystallized from hexane to afford **16** (16 mg) as colourless prisms

Compound **16**, mp 154–156°, UV λ_{max} nm (log ε): 282 (3.55); IR ν_{max}^{KBr} cm^{-1}· 3250, 1615, 1510, 1100, 915, 890; ^1H NMR (60 MHz); δ6.85, 6.62 (each 1H, *s*), 6.15 [1H, *s* (*br*), OH], 3 20 (1H, *sept*, *J* = 7 Hz), 3.10–2.60 (4H, *m*), 1.23 (6H, *d*, *J* = 7 Hz), 1 19, 0 95 (each 3H, *s*); MS *m/z* (rel. int.): 302 $[M]^+$ (24), 284 (13), 177 (35), 176 (100), 163 (30), 161 (38), 147 (12)

On a similar treatment as above, **14** gave a product identical with **12a** in all respects.

Isolation of pisiferol (**2**), *1β-hydroxyisopisiferin* (**24a**) *and pisiferadinol* (**17a**). The EtOAc eluate (3.5 g) was chromatographed on silica gel (150 g) eluting with increasing amounts of Et_2O in $CHCl_3$ (1 5 → 1.3) to afford in order of elution **2** (490 mg), mp 95–97° (fine needles from Et_2O–hexane), $[\alpha]_D^{26}$ +80.6° (*c* 0.85, MeOH), **4** (910 mg), **24a** (150 mg) and **17a** (1.6 g)

Compound **24a**, gum, $[\alpha]_D^{26}$ −122.9° (*c* 1.34, MeOH); UV λ_{max} nm (log ε): 261 (4.04), 299 (3 54); IR ν_{max}^{neat} cm^{-1}: 3300, 1615, 1515, 900, 785; ^1H NMR see Table 2; ^{13}C NMR: 151 3 (*s*), 144.7 (*s*), 134.5 (*s*), 133 6 (*s*), 133.1 (*s*), 125.5 (*d*), 120.7 (*d*), 117.3 (*d*), 75.0 (*d*), 53.0 (*d*), 40.1 (*t*), 36.5 (*s*), 32 4 (*t* × 2), 31.2 (*t*), 29.6 (*q*), 26 7 (*d*), 22 8 (*q*), 22.6 (*q*), 19.9 (*q*) ppm; HRMS *m/z* (rel. int.): 300 2079 $[M]^+$ (Calc. for $C_{20}H_{28}O_2$: 300 2090) (42), 282 $[M-H_2O]^+$ (100), 267 $[M-H_2O-Me]^+$ (34), 239 $[C_{17}H_{19}O]^+$ (20), 231 $[C_{15}H_{19}O_2]^+$ (19), 197 $[C_{14}H_{13}O]^+$ (19), the diacetate: IR ν_{max}^{neat} cm^{-1}. 1750, 1720, 1200, ^1H NMR (60 MHz). δ5 20 (1H, *m*, C<u>H</u>–OAc), 2.30, 2.14 (each 3H, *s*, OAc × 2).

Compound **17a**, glassy solid, $[\alpha]_D^{26}$ +26.1° (*c* 1 27, MeOH); UV λ_{max} nm (log ε). 281 (3 33), IR ν_{max}^{KBr} cm^{-1} 3550, 3320, 1618, 1512, 1425, 1270, 1195, 1070, 1035, 955, 890, 800, ^1H and ^{13}C NMR: see Tables 2 and 3; HRMS *m/z* (rel. int.). 318 2184 $[M]^+$ (Calc for $C_{20}H_{30}O_3$: 318.2196) (9), 300 $[M-H_2O]^+$ (100), 285 $[M-H_2O-Me]^+$ (30), 192 $[C_{12}H_{16}O_2]^+$ (82), 162 $[C_{11}H_{14}O]^+$ (61), 111 $[C_7H_{11}O]^+$ (66); the diacetate (**17b**): mp 139–140°; IR ν_{max}^{KBr} cm^{-1}: 3470, 1760, 1720, 1250, 1230; ^1H NMR (100 MHz): δ5.97 (1H, *s*, C<u>H</u>–OAc), 2 30, 2 20 (each 3H, *s*, OAc × 2); the dibenzoate: mp 171–172°; the monomethyl ether (**17c**) mp 149–150°

MnO_2 oxidation of **24c**. To a soln of **24c** (56 mg) in Et_2O (10 ml), prepared from **24a** by treatment with Et_2O soln of CH_2N_2 (room temp., 3 days), was added activated MnO_2 (500 mg), and the mixture was stirred at room temp. for 2 hr After filtration followed by evaporation, the residue was purified on silica gel (1 g) eluting with EtOAc–hexane (1:10) to yield **25** (37 mg)

Compound **25**, gum; UV λ_{max} nm (log ε): 250 (sh), 303 (4.02), 340 (sh); IR ν_{max}^{neat} cm^{-1}: 1675, 895, 750; ^1H NMR (60 MHz): δ7 50, 6.90, 6.74 (each 1H, *s*), 3 36 (1H, *sept*, *J* = 7 Hz), 1.26 (6H, *d*, *J* = 7 Hz), 3 80, 1 07, 0.92 (each 3H, *s*); MS *m/z* (rel. int): 312 $[M]^+$ (100), 297 (19), 286 (30), 271 (25), 269 (39), 243 (33), 199 (27), 41 (23).

Dehydration of **24a** To a soln of **24a** (20 mg) and pyridine (0 1 ml) in Et_2O (10 ml) was added a drop of $SOCl_2$ at 0° Upon work-up the product was recrystallized from pentane to yield **26** as colourless prisms.

Compound **26**, mp 107–108°; UV λ_{max} nm (log ε): 227 (4 30), 288 (4.48), IR ν_{max}^{KBr} cm^{-1} 3520, 1610, 1500, 1165, 910, 785, 765,

745; ^{1}H NMR (60 MHz). δ6 88, 6.50 (each 1H, *s*), 6.27 [1H, *s* (*br*)], 6 18 [1H, *d* (*br*), J = 10 Hz], 5.67 (1H, *dt*, J = 10 and 4 Hz), 4 60 (1H, *s*, OH), 3.17 (1H, *sept*, J = 7 Hz), 1.25 (6H, *d*, J = 7 Hz), 0.97, 0.79 (each 3H, *s*); MS *m/z* (rel int.): 282 $[M]^+$ (100), 267 (33), 239 (16), 225 (15), 197 (13).

Epoxidation of **8**. A mixture of **8** (130 mg), K_2CO_3 (100 mg), *m*-chloroperbenzoic acid (135 mg), and CH_2Cl_2 (20 ml) was stirred at room temp. for 5 hr. Upon work-up the product was purified by prep. TLC on silica gel developing with Et_2O–$CHCl_3$ (1:7) to yield **27** (R_f 0.45, 74 mg) and **28** (R_f 0.33, 28 mg).

Compound **27**, mp 156–158° (needles from hexane); IR ν_{max}^{KBr} cm^{-1}: 3250, 1610, 1235, 900, 880, 780; ^{1}H NMR (60 MHz): δ6.86, 6.38 (each 1H, *s*), 5.00 (1H, *s*, OH), 3.63–2.34 (6H, *m*), 1.24, 1 22 (each 3H, *d*, J = 7 Hz), 0.88, 0.76 (each 3H, *s*); MS *m/z* (rel int.) 300 $[M]^+$ (100), 285 (55), 213 (12), 201 (14), 163 (23), 159 (13), 43 (13).

Compound **28**, gum; IR ν_{max}^{neat} cm^{-1} 3300, 1610, 1230, 900, 880, 750; ^{1}H NMR (60 MHz): δ6.80, 6.29 (each 1H, *s*), 5.13 [1H, *s* (*br*) OH], 3.85–2.90 (6H, *m*), 1.20 (6H, *d*, J = 7 Hz), 0.92, 0.78 (each 3H, *s*); MS *m/z* (rel. int.); 300 $[M]^+$ (100), 285 (66), 213 (22), 201 (17), 163 (36), 145 (19), 43 (44).

Isomerization of **28** *and* **27** *to allyl alcohols*. A soln of **28** (32 mg) and potassium *t*-butoxide (50 mg) in dry DMSO (1 ml) was stirred under N_2 at 90° for 15 hr. After neutralization by addition of 5% HCl under cooling, the soln was extracted with Et_2O. Upon work-up the product was purified by prep. TLC on silica gel developing with Et_2O–$CHCl_3$ (1·3) to yield **29** (R_f 0.36, 23 mg).

Compound **29**, gum; UV λ_{max} nm (log ε): 263 (4.02), 302 (3.56), IR ν_{max}^{neat} cm^{-1}: 3250, 1610, 1500, 1035, 960, 900; ^{1}H NMR (60 MHz): δ6.78, 6.47 (each 1H, *s*), 6 30 [1H, *s* (*br*)], 5.50 [1H, *s* (*br*), OH], 4 30 (1H, *m*, $W_{1/2}$ = 6 Hz), 3.17 (1H, *sept*, J = 7 Hz), 1.23 (6H, *d*, J = 7 Hz), 1.00, 0.70 (each 3H, *s*); MS *m/z* (rel. int.): 300 $[M]^+$ (99), 282 (100), 267 (45), 244 (44), 239 (33), 231 (27), 197 (23), 163 (28), 147 (27), 81 (23).

On a similar treatment as above, **27** gave a product (R_f 0 28) identical with **24a** in all respects.

MnO_2 *oxidation of* **17c**. To a soln of **17c** (100 mg) in Et_2O (20 ml) was added activated MnO_2 (300 mg) and the mixture was stirred at room temp. for 30 min Filtration followed by evaporation left pure **18** (80 mg) as an unstable colourless oil.

Compound **18**, UV λ_{max} nm (log ε): 226 (4.36), 267 (4.06), 322 (3.59); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2700, 1710, 1680, 1600, 1500, 1265, 1185, 1065, 890; ^{1}H NMR (60 MHz) δ10 23, 7.27, 7 01 (each 1H, *s*), 3.30 (1H, *sept*, J = 7 Hz), 1.24 (6H, *d*, J = 7 Hz), 3.83, 1.01, 0 75 (each 3H, *s*), MS *m/z* (rel. int.): 330 $[M]^+$ (9), 312 (14), 204 (100), 192 (31), 176 (45), 111 (45)

Conversion of **17a** *into* **12a** *and* **14** To a soln of **17a** (100 mg) in pyridine (3 ml) was added dropwise mesyl chloride (0 1 ml) at 0°, and the mixture was stirred for 30 min Upon work-up the product was recrystallized from hexane to yield **19** (68 mg) as colourless prisms

Compound **19**, mp 153–156°; IR ν_{max}^{KBr} cm^{-1}: 3400, 1610, 1510, 1220, 1165, 950, 845, 780, 770, 675; ^{1}H NMR (60 MHz) δ6.90, 6.61, 4.46 (each 1H, *s*), 3.15 (1H, *sept*, J = 7 Hz), 1.21 (6H, *d*, J = 7 Hz), 1.10, 0.91 (each 3H, *s*); MS *m/z* (rel. int): 338 $[M+2]^+$ (4), 336 $[M]^+$ (11), 212 (56), 210 (100), 198 (23), 195 (14), 162 (18), 139 (15), 111 (20).

On treatment of **19** with $LiAlH_4$ (reflux in Et_2O for 3 hr) and with 1 M methanolic KOH (room temp., 30 min) **19** afforded **12a** and **14**, respectively.

Preparation of acetonide **20** *from* **17a**. A soln of **17a** (41 mg) and *p*-TsOH (5 mg) in dry Me_2CO (10 ml) was stirred at room temp for 3 hr After concn the residue was dissolved in Et_2O, and the soln was washed with 5% $NaHCO_3$ and brine, dried and evaporated. The product was chromatographed on silica gel eluting with increasing amount of Et_2O in $CHCl_3$ to afford **20** (10 mg) and unreacted **17a** (28 mg).

Compound **20**, gum; IR ν_{max}^{neat} cm^{-1}: 3350, 1610, 1515, 1170, 1040, 950, 905, 850; ^{1}H NMR (60 MHz): δ6.83, 6.48, 4.50 (each 1H, *s*), 5.67 (1H, *s*, OH), 3.14 (1H, *sept*, J = 7 Hz), 1 58, 1.50, 0.95, 0.77 (each 3H, *s*), 1 20, 1.10 (each 3H, *d*, J = 7 Hz), MS *m/z* (rel int): 358 $[M]^+$ (19), 343 (28), 300 (100), 282 (30), 271 (37), 205 (29), 163 (27), 43 (37), 41 (37).

Preparation of bis (p-*dimethylaminobenzoate*) (**17d**) A soln of **17a** (100 mg) and *p*-dimethylaminobenzoyl chloride (280 mg) in pyridine (5 ml) was heated at 70° for 1 hr. The reaction mixture was poured into ice-H_2O and extracted with Et_2O. Upon work-up the product was chromatographed on silica gel (5 g) eluting with $CHCl_3$ to yield **17d** (154 mg) as a colourless glassy solid.

Compound **17d**, UV λ_{max} nm (log ε): 230 (sh), 316 (4.84); IR ν_{max}^{KBr} cm^{-1}: 3505, 1705, 1605, 1535, 1270, 1180; ^{1}H NMR (60 MHz): δ8.01 [4H, *d* (*br*), J = 8 Hz], 7.22, 7 09, 6.29 (each 1H, *s*), 6.62 (4H, *m*), 2.98, 2 94 (each 6H, *s*), 1.20, 1.18 (each 3H, *d*, J = 7 Hz), 0.95, 0.91 (each 3H, *s*); CD (*c* 0.005 g/l, EtOH): $\Delta\varepsilon_{340}$ −1.3, $\Delta\varepsilon_{322}$ −16.7, $\Delta\varepsilon_{307}$ 0 0, $\Delta\varepsilon_{297}$ +4.8, $\Delta\varepsilon_{270}$ +2.2, $\Delta\varepsilon_{233}$ 0.0.

Oxidation of **17a** *to* **22**. A mixture of **17a** (250 mg), Ac_2O (1 ml) and DMSO (1 ml) was stirred at room temp. for 23 hr. After addition of H_2O (10 ml) the soln was stirred for an additional hr and extracted with Et_2O. The extract was washed with 5% $NaHCO_3$ and brine, dried and evaporated. The product was purified by prep. TLC on silica gel developing with EtOAc–hexane (1:6) to yield **22** (R_f 0.21, 81 mg) and its 12-O-acetyl derivative (R_f 0.44, 147 mg)

Compound **22**, gum; UV λ_{max} nm (log ε). 221 (4.00), 263 (3.39), 306 (3.27); IR ν_{max}^{neat} cm^{-1}: 3350, 1660, 1605, 1195, 1060, 995; ^{1}H NMR (60 MHz): δ6.86, 6.62 (each 1H, *s*), 5 82, 2.88 (each 1H, *s*, OH × 2), 3.23 (1H, *sept*, J = 7 Hz), 1.25, 1.20 (each 3H, *d*, J = 7 Hz), 1.06, 0.91 (each 3H, *s*); MS *m/z* (rel. int.): 316 $[M]^+$ (23), 191 (28), 190 (31), 178 (100), 163 (30), 111 (30).

$NaBH_4$ *reduction of* **22**. To a soln of **22** (56 mg) in MeOH (6 ml) was added an excess of $NaBH_4$ at 0° After being stirred for 50 min, the soln was concd, diluted with H_2O and extracted with Et_2O. Upon work-up the product was purified by prep. TLC on silica gel developing with $CHCl_3$–Et_2O (2:3) to yield **21** (R_f 0.50, 54 mg) and **17a** (R_f 0.33, 4 mg).

Compound **21**, solid; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3300, 1090, 960, 890; ^{1}H NMR (60 MHz). δ6.83, 6.54, 4.10 (each 1H, *s*), 3.17 (1H, *sept*, J = 7 Hz), 1.20 (6H, *d*, J = 7 Hz), 0.99, 0 92 (each 3H, *s*); MS *m/z* (rel. int.): 318 $[M]^+$ (10), 300 (100), 285 (24), 192 (71), 162 (30), 111 (32).

Acknowledgements—We wish to thank Dr. Keiji Kanamitsu, Tokyo University Forest, Chiba, for supplying us the seed, and Dr Mitsuyoshi Yatagai, Forestry and Forest Products Research Institute, for sending the IR and ^{1}H NMR spectra of **2**, **3**, **4** and **5**. We are also indebted to Dr. Yasuo Fujimoto, Institute of Physical and Chemical Research, for the measurement of the ^{1}H NMR (400 MHz) spectrum of **11**, and Prof. Takashi Handa of this university for the measurement of the CD spectrum of **17a**.

REFERENCES

1. Hirose, Y., Hasegawa, S, Ozaki, N. and Iitaka, Y (1983) *Tetrahedron Letters* **24**, 1535.
2 Ozaki, N., Hasegawa, S and Hirose, Y (1983) *Phytochemistry* **22**, 1771.
3 Yatagai, M. and Takahashi, T. (1980) *Phytochemistry* **19**, 1149
4. Fukui, H., Koshimizu, K. and Egawa, H (1978) *Agric Biol Chem.* **42**, 1419.

5. Nakatsuka, T. and Hirose, Y. (1955) *J Japan Forestry Soc.* **37**, 452.
6 Erdtman, H and Vorbrüggen, H. (1960) *Acta Chem. Scand* **14**, 2161
7 Saeki, I, Summoto, M and Kondo, T (1973) *Holzforschung* **27**, 93
8 Hasegawa, S and Hirose, Y (1984) *Chem. Letters* 1837
9. Harada, N. and Nakanishi, K. (1970) *Chem. Commun* 310
10 Watson, W H, Taira, Z., Dominguez, X A, Gonzales, H., Guiterrez, M. and Aragon, R (1976) *Tetrahedron Letters* 2501.
11. Dominguez, X. A., González, F. H., Aragon, R., Gutierrez, M, Marroguin, J. S. and Watson, W. (1976) *Planta Med.* **30**, 237.
12. Miyase, T., Ruedi, P. and Eugster, C. H. (1977) *Helv. Chim Acta* **60**, 2789.
13. Kelecom, A. (1983) *Tetrahedron* **39**, 3603.
14 Kakisawa, H., Hayashi, T, Okazaki, I. and Ohashi, M (1968) *Tetrahedron Letters* 3231.