A STUDY OF THE CONSTITUENTS OF THE BARK OF *CHAMAECYPARIS FORMOSENSIS* MATSUM

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Key Word Index—*Chamaecyparis formosensis* Matsum; bark; dioxototarol; hinokiol; sugiol; abietane dione; secoabietane dial; sandaracopimaric acid.

Sixteen compounds were isolated from the bark of *Chamaecyparis formosensis* Matsum. These compounds include four categories: (a) long-chain alcohols, acids and esters, (b) sterols and their glucosides, (c) *o*-methoxyphenol and related compounds, (d) diterpenes of abietane, pimarane, secoabietane and totarane.

Chamaecyparis formosensis Matsum, known as Taiwan red cypress, is indigenous to the high mountain area of Taiwan. Some trees in A-Li Shan(阿里山) and La-La Shan(拉拉山) are thousands of years old and have very huge trunks. It is called red cypress since the bark appears to be a slightly reddish brown color. The bark is thin and the wood is frequently used in building and for expensive furniture. The chemical constitution of the root and wood of this plant has been investigated[1]. It is noteworthy, that a constituent, namely chamaecynone, is found to exhibit activity against termites[2]. Chamaecynone is an unusual nor-sesquiterpene containing an acetylenic group. We here report the constituents of the bark of *C. formosensis* Matsum. The constituents of the leaves will be reported in the accompanying article[3]. This study and related work thus provide a full description of the chemical constitution of the Taiwan red cypress.

RESULTS AND DISCUSSION

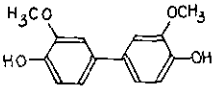
The methanolic extracts of the bark of *C. formosensis* Matsum were consecutively partitioned with hexane, chloroform and ethyl acetate. The components of these parts were then separated by column chromatography. Compounds **1-16** were isolated and classified into four categories: (a) long-chain alcohols, acids and esters, (b) sterols and their glucosides, (c) *o*-methoxyphenol and related compounds, (d) diterpenes.

The long-chain alcohols (**1**, 54 *mg*) were determined to be a mixture of do-, tetra- and hexacosanols (43:51:6) by comparison with the authentic samples through GC analyses (5% OV-17 column). The mass spectrum showed diagnostic fragments at *m/z* 308, 336 and 364 due to elimination of a water molecule. Sructural elucidation of the long-chain acids (**2**, 274 *mg*) was based on transformation into the corresponding methyl esters (CH_2N_2, Et_2O). The derivatives were determined to be methyl esters of do-, tetra- and hexacosanoic acids by comparison with the authentic samples through GC analyses. The structural elucidation of the long-chain esters (**3**, 95 *mg*) was based on analyses of the saponification products of the corresponding acids and alcohols. The esters **3** were thus determined to be *n*-docosyl do-, tetra- and hexacosanates.

From a mixture (278 *mg*) of β-sitosterol, stigmasterol and campesterol (**4**, **5** and **6**) the three compounds were isolated in a ratio of 89:7:4 as revealed by analysis with the authentic samples on a GC. Glycoside

7 (38 *mg*) was subjected to methanolysis to give the β-sitosterol and the glucose methyl acetals. Thus, the structure of **7** was assigned as β-sitosterol-3-*O*-D-glucoside.

The structures of *o*-methoxyphenol (**8**, 524 *mg*)[4] and the oxidative coupling dimer **9** (69 *mg*)[5] were determined by spectroscopic methods (MS, IR and ^{1}H NMR). Cinnamate esters **10** (42 *mg*) were hydrolyzed to give two alcohols and 4-hydroxy-3-methoxycinnamic acid[6]. The alcohol products were determined to be ei- and docosanols (1:2) by GC analysis.



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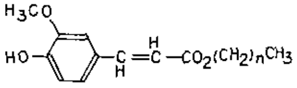


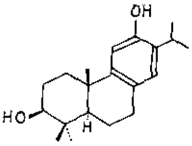
10 $n=19, 21$

Compound **11** (236 *mg*), M$^+$ 302, is an abietane-type diterpene[7] for it contains two aromatic protons, as revealed by two singlets at δ 6.69 and 6.73 in the ^{1}H NMR spectrum. Acetylation of **11** gave a product containing an alcoholic acetate group (1750 cm^{-1}, δ 2.00) and a phenolic acetate group (1725 cm^{-1}, δ 2.22). Diol **11** was identified as hinokiol by comparison (Mp, TLC, IR, MS and ^{1}H NMR) with an authentic sample previously obtained in this laboratory[8].

Compound **12** (68 *mg*), M$^+$ 300, has a conjugated carbonyl group (1650 cm^{-1}), and its ^{1}H NMR spectrum showed the resonances of two aromatic protons as two singlets at δ 6.98 and 7.99. The phenolic moiety of **12** was revealed by its acetate derivative, of which the acetate methyl group appeared at δ 2.30(s) in the ^{1}H NMR spectrum. Compound **12** was identified as sugiol, a diterpenoidal ketophenol, by comparison with an authentic sample[9]. The resonance of H-14 at a low field of δ 7.99 is due to the deshielding effect of the adjacent carbonyl group at C-7.

Compound **13** (213 *mg*), M$^+$ 314, was identified as 12-hydroxyabieta-8, 11, 13-triene-3, 7-dione by comparison with an authentic sample previously isolated in this laboratory[10]. Diketone **13** has two IR absorptions at 1690 and 1660 cm^{-1}. Two *para* aromatic protons occurred as singlets at δ 6.75 and 7.93 in the ^{1}H NMR spectrum. Two C-4 methyl groups, occurring at δ 1.20 and 1.40, were apparently deshielded by the C-3 carbonyl group.

Compound **14** (32 *mg*), M$^+$ 316, showed resonances of two *para* aromatic protons at δ 6.93(s) and 7.78(s) as well as resonances of two aldehyde protons at δ 9.82 (*d*, J=4 *Hz*) and 10.37(s) in the ^{1}H NMR spectrum. The corresponding IR absorptions of the carbonyl groups appeared at 1705 (RCHO) and 1658 cm^{-1} (ArCHO). Dial **14** was recognized as a secoabietane-type diterpene, and its structure was inferred to be 12-hydroxy-6, 7-secoabieta-8, 11, 13-triene-6, 7-dial[11]. The absolute configuration of C-5 and C-10 in (+)-dial **14** was rigorously determined by a chemical correlation with the structure of (+)-sugiol[12].



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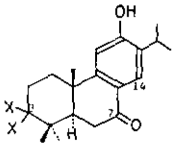
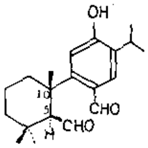
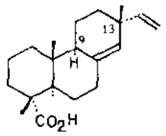


12 X=H
13 X, X=O



14

Compound **15** (16 *mg*), M$^+$ 302, was recrystallized from acetone/hexane, mp. 168-169°C. The carboxylic acid moiety was revealed by absorption at 3300—2500 cm^{-1} in the IR spectrum. The terminal olefinic moiety ($RCH=CH_2$) was revealed by the resonances at δ 4.78 (*dd*, J=10, 1.5 *Hz*), 4.92 (*dd*, J=17, 1.5 *Hz*), and 5.72 (*dd*, J=17, 10 *Hz*) in the ^{1}H NMR spectrum. Thus, compound **15** was inferred to be a pimarane-type diterpenoid acid[7]. Since the vinyl proton at C-14 exhibited as a broad singlet (at δ 5.21) with a narrow half width of 5 *Hz*, the H-9 was assigned to α-orientation (orthogonal to H-14)[13]. By comparison



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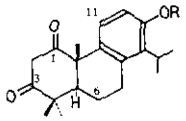

16 R=H
17 R=CH_3

with the ^1H NMR spectrum reported by E. Wenkert *et al.*, compound **15** was assigned as sandaracopimaric acid (mp. 169°C)[13,14], but not the C-13 epimer (pimaric acid, mp. 212°C)[15].

Compound **16** (323 *mg*), M^+ 314, showed two IR absorptions of carbonyl groups at 1715 and 1690 cm^{-1}. In the ^1H NMR spectrum, two *ortho* aromatic protons occurred at δ 6.60 (*d*, J=8 *Hz*) and 7.23 (*d*, J=8 *Hz*). Compound **16** was inferred to be a novel totarane-type diterpene, namely 1, 3-dioxototarol. The IR and ^1H NMR spectra of **16** exhibited compatible characteristics with those of a methyl ether analog **17**[16]. Two carbonyl groups in **16** were assigned at C-1 and C-3 positions instead at C-3 and C-6. In the ^1H NMR spectra, the C-11 protons of compounds **11-14** usually appeared at the fields of δ 6.75-6.98, however, the corresponding aromatic proton of **16** was significantly deshielded to δ 7.23 by the carbonyl group at C-1. Two geminal protons at C-2 of **16** exhibited the resonances at δ 3.35 (*d*, J=18 *Hz*) and 3.80 (*d*, J=18 *Hz*) as the AB pattern. The different fragmentation in the mass spectra was also observed. While compounds **11-14** and most of tricyclic diterpenes yielded intense fragments of M^+-15 and/or M^+-57, diterpene **16** showed very minor signals at the corresponding positions.

EXPERIMENTAL

General

Melting points were obtained on a Yanagimoto micromelting point apparatus, and are uncorrected. Infrared spectra were taken on a Jasco IRA-1 infrared spectrophotometer. ^1H NMR spectra were recorded on a Varian EM-390 or a Jeol JNM-FX-100 spectrometer using TMS as internal standard. Mass spectra were recorded on a Jeol JMS-300 mass spectrometer operating at an ionizing voltage of 70 *eV*. Specific rotations were obtained on a Jasco Dip-180 digital polarimeter. Gas chromatography was carried out on a Hewlett Packard 5710A gas chromatograph. The silica gel used for column and thin layer chromatographies was purchased from the Merck Co..

Plant material

The bark of *C. formosensis* Matsum was collected in May 1981 in the mountains (2-3 *km*) of Nan-Tou County (南投縣望鄉). The bark was sliced, air-dried (830 *g*) and extracted exhaustively with methanol (4×9 *L*). The methanolic extracts were concentrated *in vacuo* to about 2 *L*, and subsequently partitioned with hexane, chloroform and ethyl acetate. The contents of the hexane-, chloroform-, ethyl acetate-, and methanol-soluble portions weighed 1.9 *g*, 3.3 *g*, 2.6 *g* and 26 *g*, respectively.

Column chromatography

The components of the hexane-, chloroform-, and ethyl acetate-soluble portions were separated on the columns using 60 *g*, 90 *g* and 60 *g* of silica gel respectively. Compounds **1-6** and **14** were obtained from the first column by eluting with gradients of hexane-ethyl acetate. Compounds **1**, **2**, **4-13**, **15** and **16** were obtained from the second column by eluting with gradients of hexane-ethyl acetate and ethyl acetate-methanol. Compounds **13** and **16** were obtained from the third column by eluting with gradients of chloroform-ethyl acetate and ethyl acetate-methanol.

Hinokiol (11)

Colorless crystals from ethanol, mp. 239-241°C (lit[8] 240-242°C), $[\alpha]_D^{25}$+67° (MeOH, lit[9] +66.2°). IR (KBr) 3540, 3280, 1600 cm^{-1}. ^1H NMR (acetone-d_6) δ 0.85 (3H, *s*, Me-4), 1.03 (3H, *s*, Me-4), 1.14 (3H, *s*, Me-10), 1.16 (6H, *d*, J=6.5 *Hz*), 3.00 (1H, *s*, ROH), 3.08

(1H, *m*), 3.96 (1H, *m*, H-3), 6.69 (1H, *s*, H-11), 6.73 (1H, *s*, H-14), 8.38 (1H, *s*, ArOH). MS *m/z* (rel. intensity) 302 (85, M$^+$), 287 (42), 269 (100), 227 (53), 199 (51). Acetylation of **11** gave colorless crystals of a diacetate derivative, mp. 142-144°*C* (from EtOH), $[\alpha]_D^{25}$+68° (MeOH). IR (KBr) 2950, 2850, 1750 (C=O, alcoholic acetate), 1725 (C=O, phenolic acetate) *cm*$^{-1}$. ^{1}H NMR (CDCl$_3$) δ 0.94 (3H, *s*), 0.96 (3H, *s*), 1.20 (6H, *d*, J=7 *Hz*), 1.22 (3H, *s*), 2.01 (3H, *s*, CH$_3$CO$_2$R), 2.38 (3H, *s*, CH$_3$CO$_2$Ar), 4.61 (1H, *dd*, J=9, 6 *Hz*), 6.78 (1H, *s*), 6.98 (1H, *s*).

Sugiol (12)

Colorless crystals from methanol, mp. 291-293°*C* (lit$^{9)}$ 292-294°*C*), $[\alpha]_D^{25}$+25° (MeOH, lit$^{9)}$ +26°). IR (KBr) 3150 (OH), 1650 (C=O, conjugated), 1575, 1500 *cm*$^{-1}$. ^{1}H NMR (pyridine-*d*$_5$) δ 0.83 (3H, *s*), 0.86 (3H, *s*), 1.12 (3H, *s*), 1.30 (6H, *d*, J=7 *Hz*), 3.52 (1H, *m*, ArCHMe$_2$), 6.86 (1H, *s*), 7.78 (1H, *s*). MS *m/z* (rel. intensity) 300 (68, M$^+$), 285(100), 243(34), 217(45), 215(44), 203(42). Acetylation of **12** resulted in a monoacetate derivative, mp. 163-165°*C* (from MeOH). IR (KBr) 1750, 1660, 1200 *cm*$^{-1}$. ^{1}H NMR (CDCl$_3$) δ 0.94 (3H, *s*), 0.99 (3H, *s*), 1.10-1.38 (9H, *m*), 1.38-1.90 (7H, *m*), 2.30 (3H, *s*, CH$_3$CO$_2$Ar), 2.65-2.80 (2H, *m*), 6.98 (1H, *s*, H-11), 7.99 (1H, *s*, H-14).

12-Hydroxyabieta-8,11,13-triene-3,7-dione (13)

Colorless crystals from ethanol, mp. 213-215°*C*. IR (KBr) 3390 (OH), 1690 (C=O), 1660 (C=O), 1595 (C=C) *cm*$^{-1}$. ^{1}H NMR (CDCl$_3$) δ 1.12 (3H, *s*), 1.20 (3H, *s*), 1.26 (6H, *d*, J=7 *Hz*), 1.40 (3*H*, *s*), 1.80-2.80 (7H, *m*), 6.75 (1H, *s*, H-11), 7.15 (1H, *br. s*, ArOH), 7.93 (1H, *s*, H-14). MS *m/z* (rel. intensity) 314 (92, M$^+$), 299(69), 257(36), 217(46), 125(100).

12-Hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (14)

Colorless crystals from chloroform/hexane, mp. 191-192°*C*, $[\alpha]_D^{25}$+22° (c 1.2, MeOH). IR (KBr) 3350, 1705 (RCHO), 1658 (ArCHO) *cm*$^{-1}$. ^{1}H NMR (CDCl$_3$) δ 0.70 (3H, *s*), 0.99 (3H, *s*), 1.22 (6H, *d*, J=6 *Hz*, ArCH(C$\underline{H}_3$)$_2$), 1.45 (3H, *s*, Me-10), 3.14 (1H, *d*, J=4 *Hz*, C$\underline{H}$CHO), 3.05-3.28 (1H, *m*, ArCHMe$_2$), 6.93 (1H, *s*, H-11), 7.02 (1H, *br. s*, ArOH), 7.78 (1H, *s*, H-14), 9.82 (1H, *d*, J=4 *Hz*, RCHO), 10.37 (1H, *s*, ArCHO). MS *m/z* (rel. intensity) 316 (45, M$^+$), 301(65), 287(63), 273(51), 255(47), 231(70), 203(100).

Sandaracopimaric Acid (15)

Colorless crystals from acetone/hexane, mp. 168-169°*C* (lit$^{7)}$ 169°*C*). IR (KBr) 3300-2500 (acid), 1685, 1630, 990, 900 (CH=CH$_2$) *cm*$^{-1}$. ^{1}H NMR (CDCl$_3$) δ 0.84 (3H, *s*, Me-10), 1.04 (3H, *s*, Me-13), 1.21 (3H, *s*, Me-4), 1.10-2.10 (15H, *m*), 2.16 (1H, *m*, H-9), 4.78 (1H, *dd*, J=10, 1.5 *Hz*), 4.92 (1H, *dd*, J=17, 1.5 *Hz*), 5.21 (1H, *br. s*, $W_{1/2}$=5 *Hz*, H-14), 5.72 (1H, *dd*, J=17, 10 *Hz*). MS *m/z* (rel. intensity) 302(50, M$^+$), 287(65), 167(39), 121(100).

1,3-Dioxototarol (16)

Colorless crystals from acetone/hexane, mp. 237-239°*C*, $[\alpha]_D^{25}$+135° (c 0.2, CHCl$_3$). IR (KBr) 3460, 1715, 1690, 1580, 800 *cm*$^{-1}$. ^{1}H NMR (CDCl$_3$) δ 1.23 (3H, *s*), 1.28 (6H, *d*, J=7.5 *Hz*), 1.30 (3H, *s*), 1.36 (3H, *s*), 1.40-3.10 (5H, *m*), 3.20 (1H, *m*, ArCHMe$_2$), 3.35 (1H, *d*, J=18 *Hz*, H-2), 3.80 (1H, *d*, J=18 *Hz*, H-2), 6.60 (1H, *d*, J=8 *Hz*, H-12), 6.65 (1H, *br. s*, ArOH), 7.23 (1H, *d*, *J*=8 *Hz*, H-11). MS *m/z* (rel. intensity) 314(44, M$^+$), 299(7), 271(84), 243(47), 229(100).

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