NOTE

A STUDY OF THE CONSTITUENTS OF THE BARK OF *TSUGA CHINENSIS PRITZ. VAR. FORMOSANA* (HAY.)

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Key Word Index—*Tsuga chinensis Pritz. var. formosana* (Hay.); pinaceae; barks; epimanool; larixol; (3,5-dihydroxy-2-methyl)phenyl 4′-hydroxybenzoate; kaempferol; epiafzelechin; epicatechin.

The bark of the Taiwan hemlock was found to contain long-chain alcohols, acids and esters, campesterol, β-sitosterol, 13-epimanool, larixol, dihydroconiferyl alcohol and the corresponding esters, *p*-hydroxybenzoic acid, kaempferol, epiafzelechin, epicatechin and a novel compound of (3,5-dihydroxy-2-methyl)phenyl 4′-hydroxybenzoate.

The Taiwan hemlock, *Tsuga chinensis Pritz. var. formosana* (Hay.), is one of the major forest trees indigenous to the high mountain area of Taiwan. We have recently reported[1,2] that the heartwood of this plant contains sterols, carboxylic acids, 13-epimanool, *o*-methoxyphenols, coniferaldehyde, cedrusin, α-conidendrin and several resinols. Since there is no previous investigation of the bark of this plant, we therefore carried out this work and herein present the results.

RESULTS AND DISCUSSION

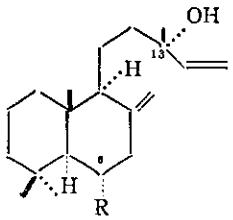
Fourteen compounds were isolated from the bark of *Tsuga chinensis Pritz. var. formosana* by means of consecutive solvent extraction and column chromatography. The long-chain alcohols (1) were found to be a mixture of do-, tetra- and hexacosanols (28:61:11) from their parent peaks at *m/z* 308, 336 and 364 in the mass spectrum. They were then compared with authentic samples in the GC analysis. Similarly, the long-chain acids (2) were determined to contain do-, tetra-, hexa- and octa-cosanoic acids as evidenced by the mass spectrum, which exhibited the pertinent parent peaks and a diagnostic peak at *m/z* 60 due to the McLafferty rearrangement. Structural elucidation of the long-chain esters (3) was based on the analysis of the saponification products, which consisted of alcohols **1** and acids **2**. Campesterol (**4**) and β-sitosterol (**5**) were identified by comparison with authentic samples through GC analysis.

The ^{1}H NMR spectrum of compound **6** showed the resonances of the four methyl groups and five olefinic protons. Since it had a parent peak at *m/z* 292, compound **6** was identified to be a diterpene, 13-epimanool, by comparison with an authentic sample[2]. The ^{1}H NMR of compound **7** also displayed four singlet methyl groups and five olefinic protons. The olefinic protons had patterns attributable to an exocyclic methylene and a mono-substituted double bond as those of 13-epimanool. In addition, compound **7** had a resonance at δ 3.79 (*ddd*, J=11, 11, 5 *Hz*) attributable to a methine proton geminal to the hydroxyl group. Thus, compound **7** was a diterpenoid diol, larixol[3,4], having one of the hydroxyl groups at the 6α-equatorial position.

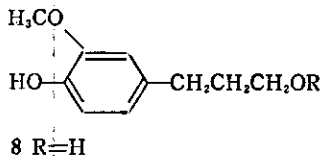
Compounds **8** and **9** were phenolic compounds as revealed by absorptions at 3600 (br, OH) and 1600 (aromatic) cm^{-1} in

the IR spectra. The ^1H NMR spectrum of phenol **8** showed an aromatic methoxy group at δ 3.86 (s), a signal for primary alcohol ($CH_2C\underline{H}_2OH$) at δ 3.65 (*t*, J=7 *Hz*) and three aromatic protons at δ 6.63 (*d*, J=8 *Hz*), 6.67 (br, s) and 6.81 (*dd*, J=8, 1.5 *Hz*). Since compound **8** had MW of 182, it was inferred to be 3-(4′-hydroxy-3′-methoxy)phenyl propanol, the dihydroconiferyl alcohol. Phenol **9**, ν_{max} 1735 cm^{-1}, was found to be a mixture of esters (2:6:1) with parent peaks at *m/z* 504, 532 and 560. Saponification of **9** gave the products dihydroconferyl alcohol (**8**) and do-, tetra- and hexa-cosanoic acids, thus indicating the structure of esters **9** (*n*=20, 22 and 24). Compound **10**, M^+ *m/z* 138, was recrystallized from EtOAc, mp. 212-214°. Since there were only aromatic resonances of the A_2X_2 pattern occurring at δ 6.90 (*d*, J=10 *Hz*) and δ 7.91 (*d*, J=10 *Hz*) in the ^1H NMR spectrum, compound **10** was readily determined to be *p*-hydroxybenzoic acid[5]. A novel phenolic compound (**11**), 3300, 1600 cm^{-1}, was isolated in a small quantity from the acetone-soluble portion of the bark. Although phenol **11** exhibited an absorption at 1680 cm^{-1} attributable to the conjugated carbonyl group, it gave no hydrazone product on treatment with 2,4-dinitrophenylhydrazine. Thus, this absorption (1680 cm^{-1}) was interpreted to be a functionality of an unsaturated ester. Besides four A_2X_2 type aromatic protons (at δ 6.87 and 8.00), the ^1H NMR spectrum of **11** also revealed an aromatic methyl (at δ 1.90) and two *meta*-oriented aromatic protons (H-4, at δ 6.24 and H-6 at δ 6.12). Thus, ester **11** was inferred to be (3,5-dihydroxy-2-methyl)phenyl 4′-hydroxybenzoate. The chemical shifts of H-4 and H-6 were consistent with those calculated using a standard empirical rule[6]. The occurrence of the methyl group at relatively high field was presumably due to the shielding effects of the hydroxyl and ester groups. The assigned structure of **11** was supported by its mass spectrum, which displayed a parent peak of *m/z* 260 and a base peak of *m/z* 121 due to the fragmentation at the ester linkage. Methylation of phenol **11** with diazomethane afforded a crystalline product, of which ^1H NMR spectrum showed resonances of three aromatic methoxy groups at δ 3.76, 3.83 and 3.90.

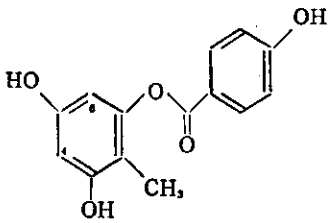
A yellow crystalline compound (**12**), M^+ *m/z* 286, was found to be optically inactive. The IR spectrum revealed the absorptions of hydroxyl (3500 cm^{-1}), conjugated carbonyl



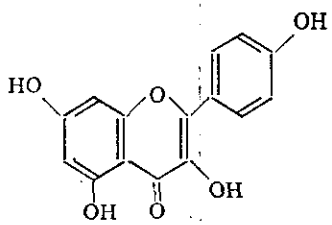
6 R=H
7 R=OH



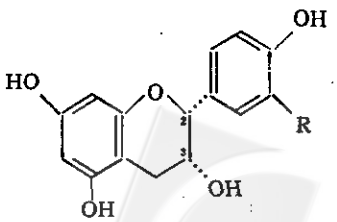
8 R=H
9 R=C(CH2)nCH3
O



11



12



13 R=H
14 R=OH

(1650 cm^{-1}) and aryl (1600 cm^{-1}) groups, thus indicating **12** to be a flavonol compound. The ^{1}H NMR of **12** showed only six aromatic protons at δ 6.29 (1H, *d*, J=1 *Hz*), 6.59 (1H, *d*, J=1 *Hz*), 7.01 (2H, *d*, J=8 *Hz*) and 8.15 (2H, *d*, J=8 *Hz*). Based on above properties, flavonol **12**, mp. 276-279°, was determined to be kaempferol (lit.[7] mp. 279-280°). Compound **13**, M^+ *m/z* 274, has a ^{1}H NMR spectrum similar to that of kaempferol, showing four aromatic protons of the A_2X_2 pattern and two others in the *meta*-orientation. In addition, flavan **13** displayed resonances of the aliphatic hydroxyl group at δ 3.54 (*d*, J=5 *Hz*) and the geminal methine proton at δ 4.20 (m). Thus, compound **13** was assigned as epiafzelechin, $[\alpha]_D$−53° (lit.[7] −59°). The protons at C-2 and C-3 were in the *cis* configuration as indicated by the relatively sharp signal ($W_{1/2}$=3 *Hz*) of H-2 at δ 4.92[6]. Compound **14**, M^+ *m/z* 290, contained five aromatic protons as revealed by the ^{1}H NMR spectrum. The acetylation product of **14** showed five acetyl methyl groups at δ 1.89 (*s*, 3H, CH_3CO_2R) and δ 2.26 (*s*, 12H, four CH_3CO_2Ar) in the ^{1}H NMR spectrum. Thus, compound **14** contained one aliphatic hydroxyl and four aromatic hydroxyl groups. Based on the above information, compound **14** was assigned to be epicatechin[7], of which H-2 occurred at δ 4.87 (br, *s*, $W_{1/2}$=3 *Hz*) in the ^{1}H NMR spectrum[7].

EXPERIMENTAL

General:

Melting points were obtained on Yanagimoto Micromelting Point Apparatus, and are uncorrected. Infrared spectra were taken on the Jasco IRA 1 spectrophotometer using either a KBr film or the neat oil. ^{1}H NMR spectra were recorded on the Varian EM-390 spectrometer using TMS as internal standard. Mass spectra were recorded on the Jeol JMS-300 spectrometer operating at an ionizing voltage of 70 *eV*. Specific rotations were obtained on the Jasco Dip-180 digital polarimeter. Gas chromatography was carried out on the Hewlett Packard 5710 A gas chromatograph. The silica gels used for column and thin layer chromatographies were purchased from the Merck Co.

Plant material:

The wood of *Tsuga chinensis Pritz. var. formosana* (Hay.) was collected in May 1981 in the high mountain areas (2-3 *km*) of Nan-Tou County, Taiwan (南投縣望鄉). The bark of the plants was sliced, air dried (1533 *g*) and extracted exhaustively with acetone.

Method:

The acetone extract of the bark was consecutively partitioned with hexane and chloroform. The hexane-soluble portion was concentrated *in vacuo* to give 31.8 *g* of oil, while the chloroform-soluble portion gave 9.1 *g* of oil. The residue of acetone-soluble portion was 111.3 *g* of oil. The three portions were subjected to column chromatography, and eluted exhaustively with gradients of hexane, ethyl acetate and methanol. From the hexane portion, compounds **1** (9.17 *g*), **2** (2.78 *g*), **3** (6.78 *g*), **4** (2.60 *g*), **5** (7.80 *g*), **6** (0.80 *g*), **7** (2.39 *g*), and **9** (5.85 *g*) were isolated. From the chloroform portion, compounds **4** (2.50 *g*), **5** (7.49 *g*), **6** (0.60 *g*), **7** (1.60 *g*), **8** (2.36 *g*), **9** (0.61 *g*), **10** (0.30 *g*) and **12** (0.30 *g*) were isolated. From the acetone portion, compounds **8** (1.62 *g*), **11** (0.19 *g*), **12** (1.10 *g*), **13** (0.71 *g*) and **14** (2.72 *g*) were isolated.

Larixol (7):

Colorless needle crystals from EtOAc/C_6H_{14}=1:1, mp. 99-101° (lit.[3] 101°). $[\alpha]_D^{25}$ +51.7° (c 1.0, $CHCl_3$, lit.[3] +57°). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 3120 (=CH), 1643 (C=C), 994, 907 ($CH=CH_2$), 889 ($CR=CH_2$). EIMS *m/z* (rel. int.): 288 (1, M^+ −H_2O), 273 (4), 270 (2), 255 (7), 69 (100). ^{1}H NMR ($CDCl_3$) δ 0.67 (3H, *s*, Me-10), 0.97 (3H, *s*, Me-4), 1.14 (3H, *s*, Me-4), 1.25 (3H, *s*, Me-13), 1.10-2.20 (13H), 2.63 (1H, *dd*, J=12, 5 *Hz*, H-7), 3.79 (1H, *ddd*, J=11, 11, 5 *Hz*, H-6), 4.57 (1H, br, *s*, H-17), 4.84 (1H, br, *s*, H-17), 5.01 (1H, *dd*, J=10.5, 1.5 *Hz*, H-15), 5.15 (1H, *dd*, J=18,

1.5 *Hz*, H-15), 5.90 (1H, *dd*, J=18, 10.5 *Hz*, H-14).

(3, 5-Dihydroxy-2-methyl) phenyl 4′-hydroxybenzoate (11):

Colorless viscous oil, $[\alpha]_D^{25}$ ±0° (c 0.2, EtOH). IR ν_{max}^{neat} cm^{-1}: 3300 (OH), 1680 (C=O, conjugated), 1600, 1450, 1240, 1100. EIMS *m/z* (rel. int.): 260 $[M]^+$ (16), 140 (27), 139 (21), 121 (100), 93 (20). ^1H NMR (CD_3OD) δ 1.90 (3H, *s*, $ArCH_3$), 6.12 (1H, *d*, J=1.5 *Hz*, H-6), 6.24 (1H, *d*, J=1.5 *Hz*, H-4), 6.87 (2H, *d*, J=10 *Hz*, H-3′, H-5′), 8.00 (2H, *d*, J=10 *Hz*, H-2′, H-6′). Methylation of the methanolic solution of compound **11** with diazomethane gave a tetramethyl derivative, mp. 108-110°. ^1H NMR (CD_3OD) δ 1.92 (3H, *s*, $ArCH_3$), 3.76 (3H, *s*, $ArOCH_3$), 3.83 (3H, *s*, $ArOCH_3$), 3.90 (3H, *s*, $ArOCH_3$), 6.30 (1H, *d*, J=1.5 *Hz*), 6.43 (1H, *d*, J=1.5 *Hz*), 7.04 (2H, *d*, J=10 *Hz*), 8.12 (2H, *d*, J=10 *Hz*).

Kaempferol (12):

Yellow crystals from EtOAc, mp. 276-279° (lit.[7] 279-280°, dec.). $[\alpha]_D^{25}$ ±0° (c 0.1, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3350, 1650, 1600, 1560, 1490, 1160. EIMS *m/z* (rel. int.): 286 $[M]^+$ (100), 285 (33), 258 (13), 153 (7), 134 (11), 121 (12). ^1H NMR (acetone-d_6) δ 6.29 (1H, *d*, J=1 *Hz*, H-6), 6.59 (1H, *d*, J=1 *Hz*, H-8), 7.01 (2H, *d*, J=8 *Hz*, H-3′, H-5′), 8.15 (2H, *d*, J=8 *Hz*, H-2′, H-6′).

Epiafzelechin (13):

Colorless crystals from $CHCl_3$/MeOH= 4:1, mp. 247-249° (lit.[7] 240-243°). $[\alpha]_D^{25}$ −53° (c 0.5, EtOH, lit.[7] −59°). IR ν_{max}^{KBr} cm^{-1}: 3350, 1610, 1510, 1470, 1205, 1130. EIMS *m/z* (rel. int.) 274 $[M]^+$ (40), 139 (100), 136 (50), 108 (26), 107 (43). ^1H NMR (acetone-d_6) δ 2.80 (2H, *m*, H-4), 3.54 (1H, *d*, J=5 *Hz*, OH-3), 4.20 (1H, *m*, H-3), 4.92 (1H, br, *s*, $W_{1/2}$=3 *Hz*, H-2), 5.90 (1H, *d*, J=1.5 *Hz*, H-6), 6.00 (1H, *d*, J=1.5 *Hz*, H-8), 6.75 (2H, *d*, J=9 *Hz*, H-3′, H-5′), 7.33 (2H, *d*, J=9 *Hz*, H-2′, H-6′), 7.86 (1H, *s*, ArOH), 8.02 (1H, *s*, ArOH), 8.16 (1H, *s*, ArOH).

Epicatechin (14):

Colorless crystals from EtOAc/MeOH= 4:1, mp. 245-247° (dec., lit.[7] 237-239°). $[\alpha]_D^{25}$ −54° (c 0.3, EtOH, lit.[7] −69°, mercury light). IR ν_{max}^{KBr} cm^{-1}: 3500, 3200, 1620, 1520, 1465, 1440, 1150. EIMS *m/z* (rel. int.) 290 $[M]^+$ (48), 272 (8), 152 (45), 139 (100), 123 (44). ^1H NMR (acetone-d_6) δ 2.70 (2H, *m*, H-4), 4.10-4.30 (1H, *m*, H-3), 4.87 (1H, br, *s*, $W_{1/2}$=3 *Hz*, H-2), 5.90 (1H, *d*, J=1.5 *Hz*), 6.01 (1H, *d*, J=1.5 *Hz*), 6.80 (2H, br, *s*, H-2′, H-5′), 7.03 (1H, br, *s*, H-6′). Acetylation of epicatechin (Ac_2O, pyr) gave pentaacetate derivative as product. ^1H NMR ($CDCl_3$) δ 1.89 (3H, *s*, CH_3CO_2R), 2.26 (12H, *s*, three CH_3CO_2Ar), 2.92 (2H, br, *s*, H-4), 5.09 (1H, br, *s*, H-2), 5.38 (1H, *m*, H-3), 6.53 (1H, *d*, J=1.5 *Hz*, H-6), 6.65 (1H, *d*, J=1.5 *Hz*, H-8), 7.21 (2H, br, *s*, H-2′, H-5′), 7.32 (1H, br, *s*, H-4′).

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