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ISOLATION AND STRUCTURE OF WALLICHOSIDE, A NOVEL PTEROSIDE FROM *PTERIS WALLICHIANA*

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Key Word Index—*Pteris wallichiana*; Pteridaceae; sesquiterpene; pteroside; wallichoside; structure and absolute configuration.

Abstract—Wallichoside has been isolated from the rhizomes of *Pteris wallichiana* and its structure and absolute configuration has been established as the 3-β-D-glucoside of 2*S*, 3*S*-pterosin C on the basis of UV, IR, NMR and MS data of wallichoside and its derivatives.

INTRODUCTION

Pteris wallichiana, an Indian fern of the genus Pteridaceae grows in the Himalayan region. The finding [1] that extracts of the plant show considerable antibiotic activity led us to undertake a thorough examination of the rhizomes of the plant.

RESULTS AND DISCUSSION

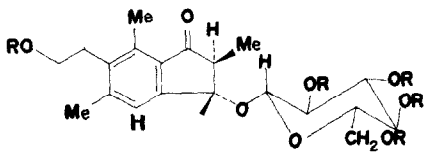
The rhizomes of *P. wallichiana* were extracted with ethanol and the residue from the alcoholic extract was treated in the manner described in the Experimental. The *n*-butanol extract showed appreciable antibiotic activity against *Staphylococcus aureus*. Si gel chromatography of this fraction led to the isolation of a crystalline glucoside that we designated as wallichoside, $C_{20}H_{28}O_8$, mp 216–218°, $[\alpha]_D$ + 22.2°, which was active against *S. aureus*. The present work establishes the structure and absolute configuration of wallichoside as (**1**). Thus it is related to the general class of sesquiterpenoids that are 1-indanone derivatives, called pterosins, of which more than thirty members have been isolated from different species of ferns in recent years [2–14]. Some of these pterosins have been isolated in the free state and some as glucosides called pterosides. The glucoside residue occurs mostly on the oxygen atom in the ethanol side chain and rarely on the oxygen function at C-3. The only other member besides wallichoside having the glucoside residue at C-3 is pteroside Q reported recently by Murakami *et al.* [12].

The UV absorbance of wallichoside (**1**) λ_{max}^{EtOH} nm (log ε): λ_{max} 219 (38,200), 258 (16,300) and 300 (2000) 219 (4.58), 258 (4.21) and 300 (3.30) is characteristic of 1-indanone derivatives. The IR spectra showed bands at 3100–3400 (OH), 1700 (carbonyl) and 1600 cm^{-1} (aromatic).

Wallichoside pentaacetate (**2**), $C_{30}H_{38}O_{13}$, mp 172–175°, $[\alpha]_D$ + 41.1°, M^+ at *m/e* 606 showed similar UV and IR spectra. The 270 MHz NMR spectrum showed signals at 1.34 (3H, *d*, *J* 7 Hz, Me at C-2); 2.015 (3H, *s*), 2.04 (6H, *s*), 2.081 (3H, *s*) and 2.104 (3H, *s*) due to the five acetate methyls; 2.45 (3H, *s*, Me at C-5), 2.67 (3H, *s*, Me at C-7), 2.75 (1H, *dd*, *J* 4 and 7 Hz, H at C-2); 3.05 (2H, *t*, *J* 7 Hz) and 4.14 (2H, *t*, *J* 7 Hz) due to the protons of the ethanol side chain; 4.65 (1H, *d*, *J* 4 Hz, H at C-3) and 7.155 (1H, *s*, aromatic H at C-4). The β-glucoside linkage was established by the signal of the anomeric H at 4.87 (1H, *d*, *J* 8 Hz) [7, 15–17]. The MS of **2** showed besides the molecular ion, peaks at *m/e* 259 and 185 corresponding to the ions **3** and **4** respectively.

The dihedral angle between the H at C-2 and that at C-3 in both **1** and **2** is close to 120° as is clear from the coupling constant (4 Hz) [6,18]. Such an arrangement places the H at C-2 and the oxygen function at C-3 in *cis*-orientation (dihedral angle close to 0°) meeting the stereoelectronic requirement for the elimination of a molecule of glucose. Indeed when wallichoside **1** was boiled with water containing one drop of aq KOH a crystalline orange yellow solid (**5**), $C_{14}H_{16}O_2$, mp 97–99°, M^+ 216 was obtained. D-Glucose was identified in the aqueous solution. Compound (**5**) showed UV absorption at λ_{max}^{EtOH} nm (log ε): λ_{max} 216 (3.94), 252 (4.61) 334 (3.40) and 411 (2.70) 216 (8,800), 252 (40,600), 334 (2500) and 411 (500) characteristic of substituted indenones. In IR the carbonyl band now shifted to 1680 cm^{-1} due to further conjugation. The 60 MHz NMR spectrum of **5** showed signals at 1.80 (3H, *d*, *J* 1.7 Hz, Me at C-2), 2.28 (3H, *s*, Me at C-5), 2.48 (3H, *s*, Me at C-7); 2.89 (2H, *t*, *J* 7.5 Hz) and 3.70 (2H, *t*, *J* 7.5 Hz) protons on the ethanol side chain; 6.57 (1H, *s*, H at C-4) and 6.90 (1H, *q*, *J* 1.7 Hz, H at C-3). The MS of **5** showed the base peak at *m/e* 185 corresponding to the ion **4**.

Compound **5** was converted to the acetate (**6**), $C_{16}H_{18}O_3$, mp 86–88°, M^+ 258, which was previously



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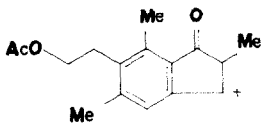
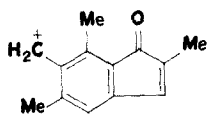


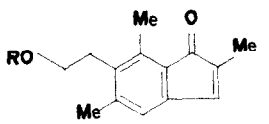
m/e 259

(3)



m/e 185

(4)



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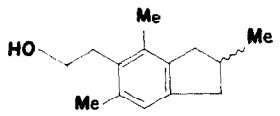
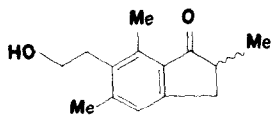
(6) R = Ac



(7)



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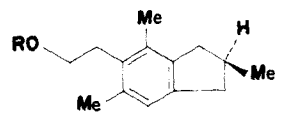
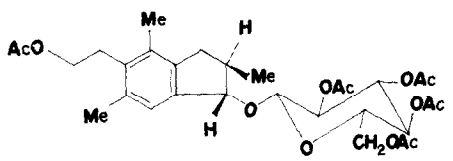


(9) R = H

(11) R = Ac



(10)

prepared by Natori *et al.* [6] but the mp reported was 71–72°. However a direct comparison* of the two acetates showed them to be identical. Thus the gross structure of wallichoside (**1**) was settled. In order to determine the absolute configuration of **1** it was necessary to reduce the carbonyl group without affecting the asymmetric centre at C-2. Natori *et al.* [7] reduced the carbonyl group of pterosins by the Clemmensen procedure. We decided to effect the reduction catalystically under mild conditions. To find out the most suitable condition we first carried out preliminary experiments on the indenone (**5**). Thus, when **5** was hydrogenated in ethyl acetate with 10% Pd–C catalyst the recemic desoxodihydro derivative **7** was obtained, $C_{14}H_{20}O$, mp 73–77°, M^+ 204. The UV, IR, NMR and MS data were consistent with this structure. On the other hand, hydrogenation of **5** with neutral 5% Pd–C [19] catalyst in ethyl acetate did not reduce the carbonyl group and racemic pterosin B (**8**), was obtained, $C_{14}H_{18}O_2$, mp 89–91°, M^+ 218.

We now directed our attention to the determination of the absolute configuration at C-2 in wallichoside (**1**). Since the relationship between the substituents at C-2 and C-3 has already been established, the determination of the configuration at C-2 would settle the absolute configuration at C-3 also. With this object we planned to convert wallichoside pentaacetate (**2**) into the desoxo-compound (**9**) whose absolute configuration was known [7]. Thus when **2** was hydrogenated in ethyl acetate with 10% Pd–C catalyst only the carbonyl group was reduced to yield desoxowallichoside pentaacetate (**10**), mp 149–151°, $[\alpha]_D$-70°, M^+ 592 whose UV, IR, NMR and MS data were consistent with this structure. However, when the same hydrogenation was carried out in the presence of a drop of perchloric acid, compound **11** was obtained as a gum, (M^+ 246) which on hydrolysis with alkali yielded compound **9**, $C_{14}H_{20}O$, M^+ 204, having $[\alpha]_D$ + 2.0°, which agreed well with the value (+3°) reported by Natori *et al.* [7] for the 2*S* isomer (**9**). Hence wallichoside (**1**) is the 3-glucoside of 2*S*, 3*S*-pterosin C [6, 7].

*We are indebted to Prof. S. Natori for this comparison.

EXPERIMENTAL

All mps are uncorrected. The UV absorption spectra were taken in 95% EtoH. Unless otherwise stated optical rotations were taken in $CHCl_3$. The MS were recorded at 80 eV using a direct inlet system. We are indebted to Prof. W. Herz, Florida State University, U.S.A. for the 270 MHz NMR spectra.

Extraction of the rhizomes of P. wallichiana: *Wallichoside* (**1**). Dried and pulverised rhizomes of *P. wallichiana* (2.7 kg) were extracted in a Soxhlet apparatus with EtOH for 20 hr.

After the removal of EtOH the extract was thoroughly digested with Et_2O and filtered. The Et_2O insol residue was stirred with H_2O at room temp. and filtered. The aq. filtrate was first shaken with EtOAc and then with *n*-BuOH. The *n*-BuOH extract was conc under red. pres. at 65–70° to nearly 200 ml. The residual syrup was poured into *ca* 2 l. of Et_2O when an amorphous solid precipitated out. The amorphous solid was chromatographed on Si gel (230 g). Elution with EtOAc–MeOH (9:1) yielded a solid (13.3 g), mp 195–200°, which was rechromatographed on Si gel (150 g). Elution with the same solvent mixture furnished a solid (3.5 g), mp 206–210°, which on crystallisation from MeOH–Me_2CO yielded pure wallichoside (**1**), mp 216–218°, $[\alpha]_D$ + 22.2° (MeOH). (Found: C, 58.95; H, 7.24. $C_{20}H_{28}O_8$. CH_3OH requires: C, 58.86; H, 7.53%). MS *m/e*: 216, 185 (100%), 141, 128 and 115. (Molecular ion not observed).

Wallichoside pentaacetate (**2**). Wallichoside (**1**; 0.15 g) was acetylated on heating with C_5H_5N (1.5 ml) and Ac_2O (1.5 ml) for 3 hr on a water bath. Working up in the usual manner gave a crude acetate which was chromatographed over alumina (6 g, deactivated with 0.18 ml of 10% aq. AcOH). Elution with C_6H_6 furnished a solid, which on crystallisation from MeOH gave colourless crystals of wallichoside pentaacetate (**2**), mp 172–175°, $[\alpha]_D$ + 41.1°. (Found: C, 59.68; H, 6.61. $C_{30}H_{38}O_{13}$ requires: C, 59.40; H, 6.27%). UV: λ_{max}^{EtOH} nm (log ϵ): 220 (4.56), 256 (4.19) and 298 (3.34) IR ν_{max} cm^{-1}: 1760, 1748, 1600 and 1240. MS *m/e*: 606 (M^+), 546, 486, 426, 367, 366, 347, 331, 271, 259, 229, 216, 211, 199, 185, 169, 141, 128, 115 and 109.

Alkali treatment of wallichoside (**1**): *indenone derivative* (**5**). A soln of wallichoside (**1**; 0.55 g) in warm H_2O (60 ml) and one drop of 1% aq. KOH was boiled for 0.5 hr when the soln turned yellow. The soln was allowed to stand overnight when orange yellow crystals of the indenone derivative (**5**) were obtained mp 97–99°. (Found: C, 77.59; H, 7.42. $C_{14}H_{16}O_2$ requires: C, 77.75; H, 7.46%). *IR* ν_{max} cm^{-1} 3330, 1680, and 1595. MS *m/e*: 216 (M^+) 185 (100%), 170, 155, 141, 128 and 115.

Identification of D-*glucose*. The above alkaline filtrate after the separation of compound **5** was neutralised and thoroughly shaken with Et_2O. The aq. soln was conc to a small vol. and passed through Dowex-1 and Dowex-50 ion-exchange resins. The deionised solution on paper chromatography showed the presence of D-glucose. We are indebted to Prof. S. P. Sen, University of Kalyani, who performed this analysis.

Acetate of the indenone derivative (**6**). The indenone derivative (**5**; 0.1 g) was acetylated in the usual manner with C_5H_5N (1 ml) and Ac_2O (1 ml). The crude acetate on crystallisation from aq. MeOH furnished yellow crystals of the acetate (**6**), mp 86–88° (Lit. [6] mp 71–72°). (Found: C, 74.42; H, 6.97. Calc. for $C_{16}H_{18}O_3$: C, 74.39; H, 7.02%). UV λ_{max}^{EtOH} nm (log ϵ): 216 (3.92), 251 (4.67), 333 (3.37) and 406 (2.72). IR ν_{max} cm^{-1}: 1740 and 1235 (acetate). NMR (60 MHz): δ 1.84 (3H, *d*, *J* 1.7 Hz, Me at C-2), 2.05 (3H, *s*, $C\underline{H}_3$–COO–), 2.32 (3H, *s*, Me at C-5), 2.53 (3H, *s*, Me at C-7), 2.96 (2H, *t*, *J* 7.5 Hz, Ar–$C\underline{H}_2$–CH_2–O), 4.15 (2H, *t*, *J* 7.5 Hz, –CH_2–$C\underline{H}_2$–OAc), 6.60 (1H, *s*, H at C-4) and 6.96 (1H, *q*, *J* 1.7 Hz, H at C-3). MS *m/e*: 258 (M^+), 198 (100%, M^+ – AcOH), 185, 170, 155, 141, 128 and 115.

Catalytic hydrogenation of the indenone derivative (**5**). (a) *Indane derivative* (**7**). The indenone derivative (**5**; 0.2 g) in EtOAc (10 ml) was shaken in an atmosphere of H_2 with 10% Pd–C catalyst. Usual work up furnished a colourless solid which was chromatographed over Si gel (10 g). Elution with petrol–C_6H_6 (3:2) yielded the indane derivative (**7**), mp 73–77°. (Found: C, 82.22; H, 9.75. $C_{14}H_{20}O$ requires: C, 82.30; H, 9.87%). UV λ_{max}^{EtOH} nm (log ϵ): 220 (4.12) and 272 (3.23) nm. IR ν_{max} cm^{-1}: 3150 (OH) and 1600 (aromatic). NMR (60 MHz): δ 1.17 (3H, *d*, *J* 6 Hz, Me at C-2), 2.27 (3H, *s*, Me at C-5), 2.36 (3H, *s*, Me at C-7), 2.95 (2H, *t*, *J* 7.5 Hz, Ar–$C\underline{H}_2$–CH_2O–), 3.76 (2H, *t*, *J* 7.5 Hz, –CH_2–$C\underline{H}_2$–OH) and 6.90 (1H, *s*, H at C-4). MS *m/e*: 204 (M^+), 189, 186, 174, 173 (100%), 158, 143, 128 and 115.

(b) (±)-*Pterosin B* (**8**). The indenone derivative (**5**; 0.13 g) in EtOAc (10 ml) was similarly shaken in H_2 with 5% neutral Pd–C catalyst [19] and worked up in the usual manner to furnish a crystalline solid, which on crystallisation from petrol (60–80°) yielded colourless (±)-pterosin B (**8**), mp 89–91°. (Found: C, 76.87; HH, 8.31. $C_{14}H_{18}O_2$ requires: C, 77.03; H, 8.31%). UV λ_{max}^{EtOH} nm (log ϵ): 218 (4.50), 260 (4.17) and 305 (3.36). IR ν_{max} cm^{-1}: 3330 (OH), 1670 (ketone) and 1590 (aromatic). NMR (60 MHz): δ 1.27 (3H, *d*, *J* 7 Hz, Me at C-2), 2.43 (3H, *s*, Me at C-5), 2.68 (3H, *s*, Me at C-7), 3.02 (2H, *t*, *J* 7.5 Hz, Ar–$C\underline{H}_2$–CH_2–O), 3.77 (2H, *t*, *J* 7.5 Hz, CH_2–$C\underline{H}_2$–OH) and 7.09 (1H, *s*, H at C-4). MS *m/e*: 218 (M^+), 203, 187 (100%), 175, 159, 144, 141, 129, 128 and 115.

Catalytic hydrogenation of wallichoside pentaacetate (**2**). (a) *Desoxowallichoside pentaacetate* (**10**). Wallichoside pentaacetate (**2**; 0.2 g) and 10% Pd–C in EtOAc (15 ml) was shaken in an atmosphere of H_2. Usual work up furnished a solid which on crystallisation from Me_2CO–petrol gave colourless crystals of the desoxoacetate (**10**), mp 149–151°, $[\alpha]_D$ −70°. (Found: C, 60.57; H, 6.62. $C_{30}H_{40}O_{12}$ requires: C, 60.80; H, 6.80%). UV λ_{max}^{EtOH} nm (log ϵ): 220 (4.06) and 270 (2.97). IR ν_{max} cm^{-1}: 1740 and 1240 (acetate). NMR (60 MHz): δ 1.03 (3H, *d*, *J* 7.5 Hz, Me at C-2); 1.81 (3H), 1.98 (3H), 2.05 (3H), 2.08 (3H) and 2.13 (3H) due to 5 $C\underline{H}_3COO$ groups; 2.43 and 2.47 (3H each, *s*) due to aromatic Me at C-5 and C-7; 3.05 (2H, *t*, *J* 7.5 Hz, Ar–$C\underline{H}_2$–CH_2–O) and 7.04 (1H, *s*, H at C-4). MS *m/e*: 592 (M^+); 533, 488, 472, 331, 245, 201, 185, 171, 169, 157, 142, 141, 128, 127, 115 and 109.

(b) *Indane derivative* (**9**). Wallichoside pentaacetate (**2**; 0.15 g) and 10% Pd–C (0.15 g) in EtOAc (15 ml) and a drop of perchloric acid was shaken in an atmosphere of H_2. Usual work up furnished **11** as a gum (0.07 g), M^+ 246 which was hydrolysed by refluxing with 2 ml of 1N NaOH in EtOH. Usual work up yielded **9** as a low melting solid, $[\alpha]_D$ + 2.0° having identical IR, MS and TLC with the racemic indane (**7**).

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