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PRENYLISOFLAVONES FROM *EUCHRESTA JAPONICA*

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Key Word Index—*Euchresta japonica*; Leguminosae; euchrenone b_1; euchchrenone b_2, euchrenone b_3; prenylisoflavone

Abstract—Three new prenylisoflavones, designated euchrenones b_1, b_2 and b_3, were isolated from the roots of *Euchresta japonica*. The structures were established on the basis of spectroscopic methods and chemical evidence.

INTRODUCTION

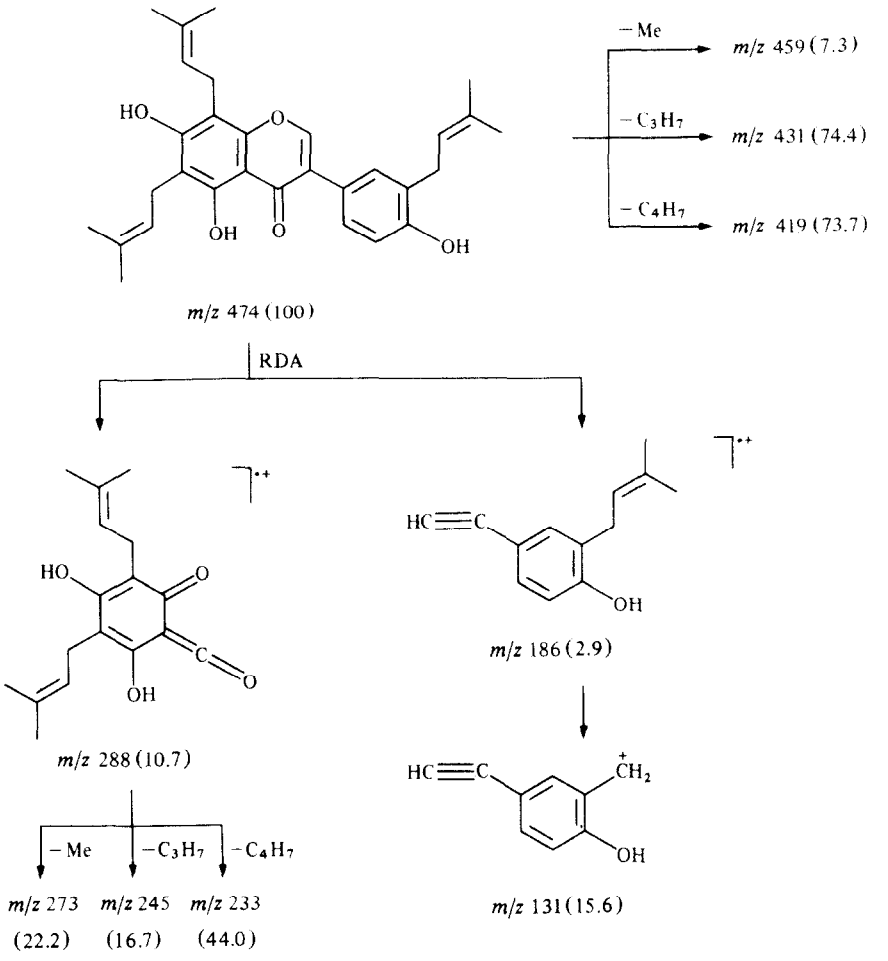
Isolation and characterization of three new flavanones, euchrenone a_1, a_2 and a_3 was described in our previous paper [1]. Up to the present, several new flavanones in *E. japonica* have been reported [1–4], but isoflavones in the plants have not been presicely investigated. In continuation of our studies on flavonoids in the roots of *E. japonica*, three new isoflavones, designated euchrenones b_1, b_2 and b_3, were isolated. In this paper, we describe the structure elucidation of the compounds. To distinguish the basic structures of the compounds in the genus *Euchresta*, the name euchrenone a_x is used for the flavanones, while the name euchrenone b_x is used to designate the isoflavones.

RESULTS AND DISCUSSION

Euchrenone b_1 (**1**), $C_{30}H_{34}O_5$ [M^+ at *m/z* 474] was isolated from a fraction eluted with benzene during silica gel column chromatography of a methanolic extract of *Euchersta japonica* (roots). In the ^{1}H NMR spectrum, a one proton singlet at δ 7.88 was chracteristic of an isoflavone and assignable to H-2. This skeleton was also supported by the following colour tests; positive to ferric chloride (greenish-brown) and negative to Mg–HCl. The presence of three isoprenyl groups was shown in the ^{1}H NMR spectrum by six methyl signals (δ 1.74, 1.77, 1.78, 1.79, 1.83 and 1.85), three two-proton doublets (δ 3.39, 3.43 and 3.46) (Ar–CH_2–CH=C<), and three one proton doublets at δ 5.2–5.4 (Ar–CH_2–CH=C<). Three hydroxy groups were revealed by resonances observed at δ 5.27, 6.35 and 13.18 Furthermore, a typical ABX system at δ 6.86 (*d*, J = 8.43 Hz), 7.16 (*d*, J = 2.20 Hz) and 7.20 (*dd*, J = 8.43, 2.20 Hz) showed the presence of three aromatic protons in the B ring. In the EI mass spectrum, the molecular ion was detected at *m/z* 474 and other prominent fragments are shown in Fig. 1. The fragment ion peaks at *m/z* 288 and 186 caused by a usual RDA cleavage revealed information about the structure of **1**. The former ion resulted from the A ring moiety and showed that the ring possessed two isoprenyl groups at C-6 and C-8 in addition to two hydroxyl at C-5 and C-7. On the other hand, the latter ion arose from the B ring moiety and showed that the B ring had an isoprenyl and a hydroxyl group which were at positions C-3′ and C-4′, respectively, as shown by the ^{1}H NMR spectrum [5]. Therefore the structure of euchrenone b_1 was concluded to be 5,7,4′-trihydroxy-6,8,3′-tri-(γ,γ-dimethylallyl) isoflavone (**1**).

Euchrenone b_2 (**2**), $C_{30}H_{34}H_6$ [M^+ at *m/z* 490] was isolated from the benzene extract of the stems of *E. japonica*. The ^{1}H NMR spectrum together with the UV spectrum (272, 310 sh nm) and the colour tests (Mg–HCl and $FeCl_3$) suggested that **2** was also an isoflavone derivative. As in the case of euchrenone b_1, compound **2** was shown by ^{1}H NMR to possess three isoprenyl groups and no aromatic proton in the A ring. The differences of **2** from **1** were that **2** exhibited four hydroxy groups (δ 5.48, 6.48, 8.40 and 12.61), and two *ortho*-coupled doublets (6.48 and 6.89) each J = 8.43 Hz. The spectral data indicated a hydroxy group located at C-2′ compared with euchrenone b_1, and made the B ring substitution 2′,3′,4′ [6]. In the EI mass spectrum, this deduction was supported by the presence of a fragment (*m/z* 202) based on the B ring, which differed from the corresponding ion of **1** by 16 mass units. From the spectral data described above, the structure of euchrenone b_2 was established as 5,7,2′,4′-tetrahydroxy-6,8,3′-tri-(γ,γ-dimethylallyl)isoflavone (**2**).

Euchrenone b_3 (**3**), $C_{27}H_{26}O_7$ [M^+ at *m/z* 462] was isolated from the benzene fraction eluted before euchrenone b_1. It was suggested that **3** was an isoflavone derivative from its UV, ^{1}H NMR spectra and the colour tests. The ^{1}H NMR spectrum showed the presence of a 2,2-dimethylpyran unit as two-proton doublets (δ 5.75 and 6.74 J = 10.0 Hz), a two-proton doublet (3.40, J = 6.7 Hz) and a one-proton triplet (5.22, J = 6.7 Hz), but two methyls on the pyran ring were indistinguishable from those of an isoprenyl group. A methoxy group at δ 3.75, a methylendioxy group at 6.00, and a chelated hydroxy group at 13.19 were also observed as sharp singlets. In the EI mass spectrum, significant ion-peaks were obseved at *m/z* 286 $[A_1]^+$ and *m/z* 176 $[B_1]^+$ by RDA cleavage in addition to the molecular ion-peak (*m/z* 462). Considering $[B_1]^+$ to be 176, the B ring possessed both methoxy and methylendioxy groups, the position of which were considered to be C-2′ and C-4′,5′ by the chemical shifts of two aromatic protons at δ 6.77 (H-3′) and 6.84 (H-6′) [7]. The ion-peak based on the A ring



yielded a further two fragments at *m/z* 271 $[A_1-Me]^+$ and 215 $[A_1-Me-C_4H_7]^+$, which indicated that the A ring fused with 2, 2-dimethylallylpyran and had an isoprenyl group. These spectral data indicated that the alternative structures **3** and **4** should be represented by differences in the positions of the 2, 2-dimethylpyran unit and the isoprenyl group. To clarify the structure of euchrenone b_3, a chroman compound was prepared by cyclization of the isoprenyl moiety with an acid medium [8]. The chroman **5** which gave a negative $FeCl_3$ test was different from the original compound R_f on TLC, but it exhibited a close similarity to **3** in its mass spectral fragmentations. Consequently, the structure of euchrenone b_3 was shown to be 5-hydroxy-6-isoprenyl-[6″,6″-dimethylpyrano (2″,3″:7,8)]-2′-methoxy-4′,5′-methylenedioxyisoflavone (**3**).

EXPERIMENTAL

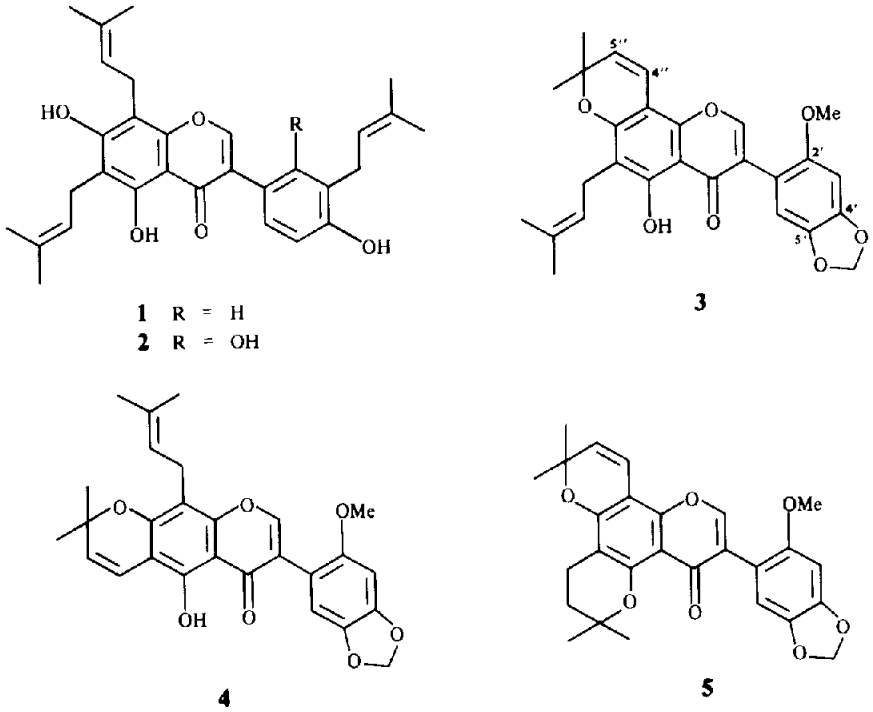
^{1}H NMR spectra were obtained at 270 MHz using TMS as int. standard. MS were record at 70 eV with a direct inlet system.

Plant material. The roots of *Euchresta japonica* were collected at Mt. Taradake, Nagasaki Prefecture (Kyusyu), Japan in Oct. 1985. The stems of *E. Japonica* were collected at Mt. Goji, Miyazaki Pref. (Kyushu), Japan in Dec. 1985. Vouchers are deposited at the Herbarium of Gifu Pharmaceutical University.

Extraction and isolation of compounds **1**–**3**. Dried roots (1.0 kg) of *E. japonica* were crushed into pieces and extracted with MeOH (2.5 l) under reflux (12 hr × 8). The MeOH soln was concd *in vacuo* and the combined extract (380 g) was subjected to silica gel CC using C_6H_6 as a solvent to give crude compounds **1** and **3**, which were rechromatographed on silica gel eluted C_6H_{14}–EtOAc (10:1). The fractions containing **1** and **3** were purified by PLC to give euchrenones b_1 (**1**) (15 mg) and b_3 (**3**) (8 mg). Dried stems (1.2 kg) of *E. japonica* were crushed into pieces and extracted with C_6H_6 (3 l) under reflux (12 hr × 10). The C_6H_6 soln was concentrated *in vacuo* and the combined extract (50 g) was subjected to silica gel CC using C_6H_6 as a solvent to give crude compound **2**, which was purified by gel filtration to give euchrenone b_2 (**2**) (20 mg).

Euchrenone b_1 (**1**). ^{1}H NMR ($CDCl_3$): δ 1.78, 1.79, 1.83 1.85 (each 3H, *s*, Me × 6), 3.38, 3.43, 3.45 (each 2H, *d*, J = 6.7 Hz, Ar–C<u>H</u>$_2$–CH=C< × 3), 5.20–5.40 (3H, *m*, Ar–CH$_2$–C<u>H</u>=C< × 3), 6.34 (1H, *s*, OH), 6.86 (1H, *d*, J = 8.43 Hz, H-5′), 7.16 (1H, *d*, J = 2.20 Hz, H-2′), 7.20 (1H, *dd*, J = 8.43, 2.20 Hz, H-6′), 7.88 (1H, *s*, H-2), 13.18 (1H, *s*, C-5–OH). MS *m/z* (rel. int.): 474 (100), 459 (7.3), 431 (74.4), 419 (73.7), 363 (3.3), 288 (10.7, 273 (22.2), 245 (16.7), 133 (44.0), 218 (6.7), 186 (2.9), 131 (15.6). R_f 0.58 (C_6H_{14}–EtOAc, 9:4).

Euchrenone b_2 (**2**). UV λ_{max}^{MeOH} nm: 273, 310 sh; λ + NaOMe: 279; λ + $AlCl_3$: 240, 272 sh, 315, 363; λ + $AlCl_3$/HCl: 240, 270 sh, 313, 363; λ + NaOAc: 277, 315 sh; λ + NaOAc/H_3BO_3: 272, 315



Scheme 1.

sh. ^{1}H NMR ($CDCl_3$): δ 1.25, 1.83, 1.85 (each 6H, *br s*, $CH_3 \times 6$), 3.47, 3.50, 3.54 (each 2H, *d*, $J = 6.70$ Hz, Ar–C$\underline{H}_2$–CH=C< x 3), 5.20–5.33 (3H, *m*, Ar–CH_2–C$\underline{H}$=C< × 3), 5.48 (1H, *s*, OH), 6.48 (1H, *s*, OH), 6.48 (1H, *d*, $J = 8.43$ Hz, H-5'), 6.89 (1H, *d*, $J = 8.43$ Hz, H-6'), 7.99 (1H, *s*, H-2), 8.40 (1H, *s*, OH), 12.61 (1H, *s*, C-5–OH). MS *m/z* (rel. int.): 490 (100), 337 (33.6), 435 (49.1), 391 (40.0), 379 (35.6), 363 (9.3), 335 (22.1), 323 (29.9), 288 (1.2), 245 (1.6), 233 (8.0), 217 (14.4), 203 (6.6), 202 (5.5), 189 (32.5), 177 (17.7), 147 (8.0), 217 (15.5). R_f 0.48 (C_6H_{14}–Me_2CO, 2:1).

Euchrenone b_3 (**3**). UV λ_{max}^{MeOH} nm (log ε): 238 (4.38), 272 (4.59), 305 sh (4.25). ^{1}H NMR ($CDCl_3$): δ 1.50 (6H, br *s*, Me × 2), 1.66, 1.80 (each 3H, *s*, Me × 2), 3.40 (2H, *d*, $J = 6.70$ Hz, Ar–C$\underline{H}_2$–CH=C<), 3.75 (3H, *s*, OMe), 5.22 (1H, *t*, $J = 6.70$ Hz, Ar–CH_2–C$\underline{H}$=C<), 5.75 (1H, *d*, $J = 10.0$ Hz, H-5''), 6.00 (2H, *s*, –OCH_2O–), 6.74 (1H, *d*, $J = 10.0$ Hz, H-4''), 6.77 (1H, *s*, H-3'), 6.84 (1H, *s*, H-6'), 8.11 (1H, *s*, H-2), 13.19 (1H, *s*, C-5–OH). MS *m/z* (rel. int.): 462 (70.0), 447 (100), 419 (50.0), 407 (47.1), 286 (1.2), 269 (8.9), 227 (12.9), 215 (13.3), 195 (13.1), 176 (2.2). R_f 0.31 (C_6H_{14}–EtOAc, 6:1).

Cyclization of euchrenone b_3 *to chroman* (**5**). Euchrenone b_3 (2 mg) was taken up in a soln of HCO_2H (0.2 ml) and conc. H_2SO_4 (2 drops). The mixture was allowed to stand at room temp. for 24 hr. The reaction mixture was poured into H_2O and extracted with C_6H_6. By the usual work-up of the C_6H_6 extract, compound **5** was obtained as an oil. Compound **5**: $FeCl_3$ negative, R_f 0.52 (C_6H_6–EtOAc, 6:1).

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