Chemotaxonomy of the Genus *Euchresta*. III. Three New Flavonoids in the Roots of *Euchresta japonica*

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Three new flavonoids isolated from the roots of *Euchresta japonica*, designated as euchrenones a_4, b_4 and b_5, were identified as 5,7-dihydroxy-6,8-di(γ,γ-dimethylallyl)-[6‴,6‴-dimethylpyrano(2‴,3‴:4′,3′)]flavanone, 5,7-dihydroxy-2′-methoxy-4′,5′-methylenedioxy-6,8-di(γ,γ-dimethylallyl)isoflavone and 5,7,2′-trihydroxy-4′,5′-methylenedioxy-6,8-di(γ,γ-dimethylallyl)isoflavone by means of spectral analysis.

Keywords *Euchresta japonica*; Leguminosae; prenylated flavanone; prenylated isoflavone; euchrenone a_4; euchrenone b_4; euchrenone b_5

The roots of *Euchresta japonica* (Leguminosae) have been used in Japan as an antiinflammatory, antiarrhythmic, anticancer and antiulcer agent in place of Chinese crude drug derived from *Sophora tonkinensis*.[1,2] Many flavonoids with relevant biological activities have been isolated by Komatsu and his co-workers.[3] The chemical constituents of *E. japonica* have been further investigated by us not only to search for substances with medicinal potency but also to characterize chemotaxonomically the genus *Euchresta* which consists of four or five species; *E. japonica* (western Japan), *E. formosana* (Taiwan), *E. horsfieldii* (Java, Thailand and southern China), *E. tubulosa* (China), and *E. trifoliolata* (China).[4] The last species has been suggested to be the same as *E. japonica*.[5] The flavonoids of the roots so far isolated by us are prenylated flavanones (euchrenones a_1—a_3),[6] isoflavones (euchrenones b_1—b_3)[7] and coumaronochromone (euchretin A).[8] In a continuation of our studies on the constituents, three new minor flavonoids, designated as euchrenone a_4 (flavanone) and euchrenones b_4 and b_5 (isoflavones), were isolated from the roots. We described the structure elucidation of the new flavonoids in this paper.

Euchrenone a_4 (**1**), $C_{30}H_{34}O_5$ (M^+ at *m/z* 474) was isolated as a colorless oil from a fraction eluted with benzene on silica gel column chromatography of a methanol extract of *Euchresta japonica* HOOK. f. ex REGEL, and gave positive results with both the ferric chloride and the magnesium-hydrochloride tests. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, two double doublets (1H each, $J=17.1$ and 3.1 Hz, and $J=17.1$ and 12.9 Hz) at 2.77 and 3.03 ppm, and a double doublet (1H, $J=12.9$ and 3.1 Hz) at 5.26 ppm were assignable to the protons at C-3 and C-2 of the flavanone skeleton, respectively. Furthermore the ^{1}H-NMR spectrum showed the presence of a dimethylpyran ring [1.60 (2×Me), 5.64 and 6.33 (1H each, $J=10$ Hz, CH=CH–)], two γ, γ-dimethylally groups [1.72 (2×Me), 1.75 and 1.82 (Me), 3.33 and 3.45 ppm (2H each, d, $J=6.7$ Hz, $2\times CH_2$), 5.19, 5.22 (1H each, d, $J=6.7$ Hz, CH=C<)] and two hydroxy groups (6.32 and 12.32 ppm). One singlet at 12.32 ppm was assigned to a chelated hydroxy group at C-5. A typical ABX system at 6.78 (1H, d, $J=8.4$ Hz), 7.03 (1H, d, $J=2.2$ Hz) and 7.15 ppm (1H, dd, $J=8.4$ and 2.2 Hz) ppm indicated the presence of the C-3′,4′ disubstituted B ring moiety. No signals corresponding to any aromatic hydrogen on the A ring moiety was observed in the ^{1}H-NMR spectrum. As shown in Fig. 1, in the mass spectrum (MS), the prominent fragments of *m/z* 288

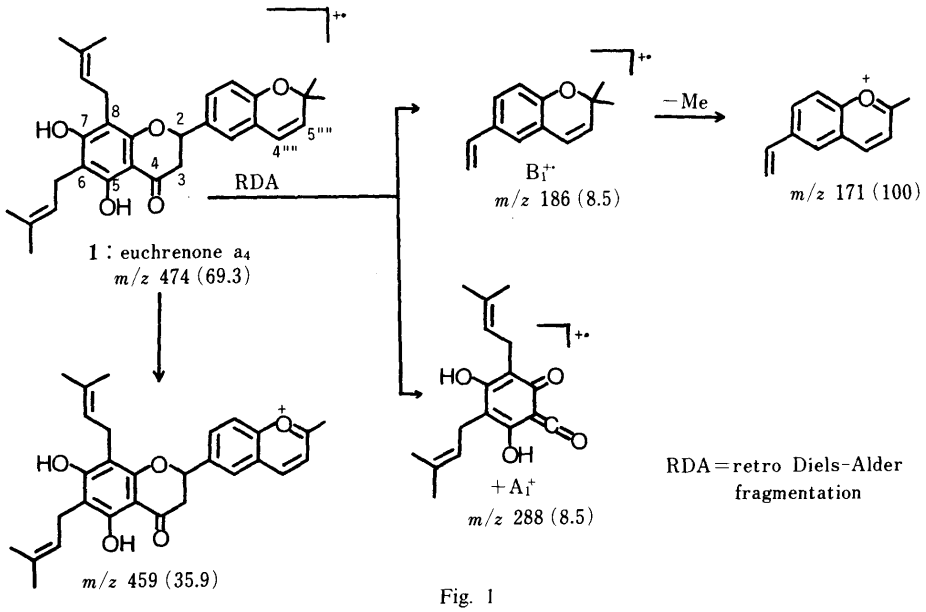

Fig. 1

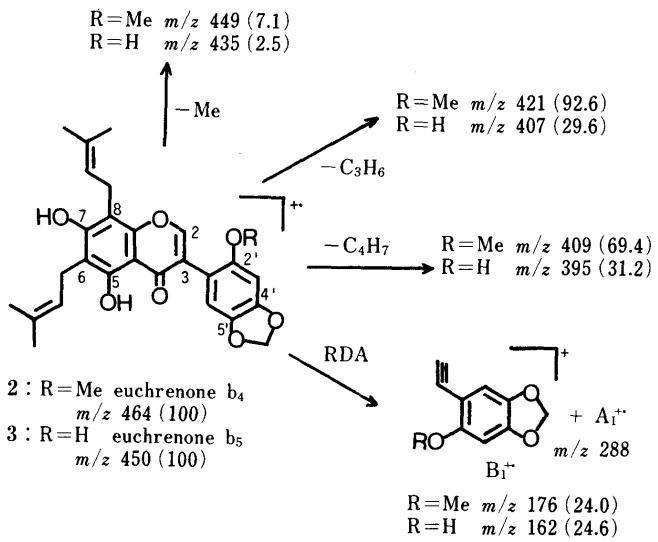

Fig. 2

based on the A ring and *m/z* 186 based on the B ring giving the base peak *m/z* 171 by successive demethylation, indicated the presence of a dimethylpyran moiety on the B ring. Consequently, the structure of euchrenone a_4 was established to be 5,7-dihydroxy-6,8-di- (γ,γ-dimehylallyl)-[6′′′′,6′′′′-dimethylpyrano (2′′′′,3′′′′:4′,3′)]flavanone[2,3-dihydro-5,7-dihydroxy-6,8(γ,γ-dimethylallyl)-2-(2,2-dimethylchromene-6-yl)-4-(4*H*)chromenone]. Euchrenone a_4 is considered to be a derivative of euchrenone a_3.[6]

Euchrenone b_4 (**2**), $C_{27}H_{28}O_7$ (M^+ at *m/z* 464), was isolated from a fraction eluted with C_6H_6–EtOAc as a pale yellow oil. In the ^{1}H-NMR spectrum, a sharp singlet due to the proton at C-2 of the isoflavone skeleton was observed at 7.86 ppm. Furthermore, the ^{1}H-NMR spectrum showed the presence of two γ,γ-dimethylallyls [1.74, 1.77, 1.83, 1.85 (Me), 3.46—3.48 (4H, m, $2\times CH_2$), 5.22, 5.25 ppm (1H each, br t, J=7.0 Hz, –CH=C<), one methoxy (3.74 ppm), one methylenedioxy (5.96 ppm) and two hydroxy groups (6.29 and 13.14 ppm). One singlet could be assigned to a chelated hydroxy group at C-5. Two aromatic proton signals at 6.62 and 6.97 ppm (singlets) were assigned to the protons at C-3′ and C-6′, which indicated that **2** had a 2′,4′,5′-trioxygenated B ring moiety. In the MS, the prominent fragment of *m/z* 176 (Fig. 2) supported the presence of a methoxy group and a methylenedioxygroup on the B ring. Consequently, the structure of euchrenone b_4 was concluded to be 5,7-dihydroxy-2′-methoxy-4′,5′-methylenedioxy-6,8-di(γ,γ-dimethylallyl)isoflavone [5,7-dihydroxy-6,8-di(γ,γ-dimethylallyl)-3-(2-methoxy-4,5-methylenedioxyphenyl)-4-(4*H*)-chromenone].

Euchrenone b_5 (**3**), $C_{26}H_{26}O_7$ (M^+ 450), was isolated from a more polar fraction than **2**. The ^{1}H-NMR spectrum of **3** was very similar to that of **2**. But no signal due to a methoxy group was observed. In the MS, the fragments based on the B ring were smaller than those of **2** by 14 mass units (Fig. 2). These data indicated that the substituent at C-2′ in the B ring was a hydroxy group. Therefore the structure of euchrenone b_5 was characterized as 5,7,2′-trihydroxy-4′,5′-methylenedioxy-6,8-di(γ,γ-dimethylallyl)isoflavone [5,7-dihydroxy-6,8-di(γ,γ-dimethylallyl)-3-(2-hydroxy-4,5-methylenedioxyphenyl)-4(4*H*)-chromenone].

The structures of other minor flavonoids in *E. japonica* are being investigated in relation with those of *E. formosana* and *E. horsfieldii*.

Experimental

The dried roots (1.3 kg) of *E. japonica*, collected in Miyazaki prefecture, were extracted successively with *n*-C_6H_{12}, C_6H_6, $CHCl_3$, EtOAc and MeOH. The C_6H_6 extract was concentrated *in vacuo*, and then eluted with benzene and benzene–AcOEt (10 : 1) from a silica gel column. A fraction eluted with benzene was repeatedly purified by prepatative thin layer chromatography (TLC) using *n*-hexane–EtOAc (8 : 1) as the solvent to give **1** (10 mg). A benzene–EtOAc (10 : 1) fraction was also purified by a similar method to give **2** (8 mg) and **3** (5 mg).

Euchrenone a_4 (1) $C_{30}H_{34}O_5$, MW 474, a colorless oil. EIMS *m/z* (rel. int.): 474 (69.3), 459 (35.9), 419 (16.5), 288 (8.5), 273 (32.5), 233 (32.0), 186 (8.5), 177 (27.5), 171 (100). ^{1}H-NMR ($CDCl_3$) δ: 1.60 (6H, br s, 2 × Me), 1.72 (6H, br s, 2 × Me), 1.75, 1.82 (3H each, s, Me), 2.77 (1H, dd, J=17.1, 3.1 Hz, H-3), 3.03 (1H, dd, J=17.1, 12.9 Hz, H-3), 3.33, 3.45 (2H each, br d, J=6.7 Hz, Ar-C<u>H</u>$_2$–CH=C<), 5.19, 5.22 (1H each, br t, J=6.7 Hz, –CH$_2$–C<u>H</u>=C<), 5.26 (1H, dd, J=12.9, 3.1 Hz, H-2), 5.64 (1H, d, J=10 Hz, H-5′′′′), 6.32 (1H, s, C_7-OH), 6.33 (1H, d, J=10 Hz, H-4′′′′), 6.78 (1H, d, J=8.4 Hz, H-5′), 7.03 (1H, d, J=2.2 Hz, H-2′), 7.15 (1H, dd, J=8.4, 2.2 Hz, H-6′), 12.32 (1H, s, C_5-OH).

Euchrenone b_4(2) $C_{27}H_{28}O_7$, MW 464, a pale yellow oil. EIMS *m/z* (rel.int.): 464 (100), 449 (7.1), 421 (92.6), 409 (52.4), 393 (32.5), 365 (52.9), 353 (93.6), 288 (2.6), 189 (25.1), 176 (20.4), 162 (27.0). ^{1}H-NMR ($CDCl_3$) δ: 1.74, 1.77, 1.83, 1.85 (3H each, br s, Me), 3.46—3.48 (4H, m, 2 × –C<u>H</u>$_2$–CH=C<), 3.74 (3H, s, OMe), 5.22, 5.25 (1H each, br t, J=7.0 Hz, –CH$_2$C<u>H</u>=C<), 5.96 (2H, s, –OCH$_2$O–), 6.29 (1H, s, C_7-OH), 6.62 (1H, s, H-3′), 6.69 (1H, s, H-6′), 7.86 (1H, s, H-2), 13.14 (1H, s, C_5-OH).

Euchrenone b_5 (3) $C_{26}H_{26}O_7$, MW 450, a pale yellow oil. EIMS *m/z* (rel. int.): 450 (100), 435 (2.5), 407 (29.6), 395 (31.2), 379 (21.7), 351 (23.0), 339 (55.8), 288 (7.9), 189 (57.1), 162 (24.6). ^{1}H-NMR ($CDCl_3$) δ: 1.73, 1.77, 1.83, 1.86 (3H each, br s, Me), 3.45—3.48 (4H, m, 2 × –C<u>H</u>$_2$CH=C<), 5.24—5.26 (2H, m, 2 × –CH$_2$C<u>H</u>=C<), 5.96 (2H, s, –OCH$_2$O–), 6.26 (1H, s, OH), 6.56 (1H, s, H-3′), 6.87 (1H, s, H-6′), 8.02 (1H, s, H-2), 13.10 (1H, s, C_5-OH).

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