A NOVEL COUMARONOCHROMONE FROM THE STEMS OF EUCHRESTA JAPONICA AND ITS ANTIBACTERIAL ACTIVITY

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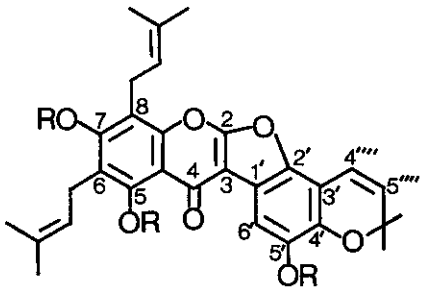
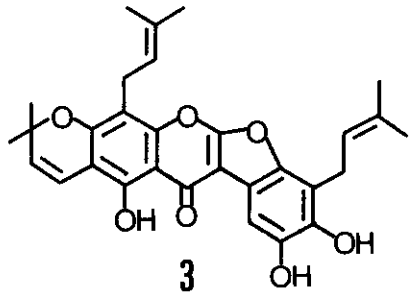
Abstract —— The structure of a novel highly substituted coumaronochromone, designated as euchretin A, from the stems of Euchresta japonica was established by spectral evidences. Its antibacterial activity is also described.

In earlier papers we reported the isolation and structure elucidation of new prenylated flavanones (euchrenone a_1-a_3)[1] and isoflavones (euchrenone b_1-b_3)[2] from the roots of Euchresta japonica Hooker fil. ex Regel (Leguminosae). In the course of our continuing chemotaxonomical search on the genus Euchresta, a new highly substituted coumaronochromone (1)[3], designated as euchretin A, mp 208-209°C, was isolated as pale yellow needles from the C_6H_6 extract of the stems by column chromatography on silica gel when eluted with C_6H_6-EtOAc (10 : 1). In the high resolution mass spectrum (M^+: m/z 502.1992), its empirical formula was confirmed as $C_{30}H_{30}O_7$ (Calcd. 502.1991). In the ^1H nmr spectrum, two two-proton doublets (J= 7.0 Hz) at 3.45 and 3.59, two-proton multiplet at 5.23-5.25, and two one-proton doublets (J= 9.9 Hz) at 5.99 and 6.75 ppm indicated that 1 had two γ,γ-dimethylallyl groups and a chromene ring. Further, three one-proton singlets at 7.83, 8.31 and 13.26 ppm showed the presence of three hydroxy groups. Each absorption band in its ir (3450 and 1680 cm^{-1}; chelated OH and C=O) and uv (237, 264, 290sh, and 349 nm) spectra suggested a basic skeleton for 1 to be one of isoflavone derivatives. Among them, a coumaronochromone structure was proposed because of the absence of a characteristic signal to be assignable for a proton at C-2 (ca. 8 ppm) in ^1H nmr spectrum and a carbon at C-2 (ca. > 150 ppm) in ^{13}C nmr spectrum (isoflavone numbering). The proposal was supported by the evidence that the uv spectrum of 1 resembled closely



1: R = H (euchretin A)

2: R = CH_3



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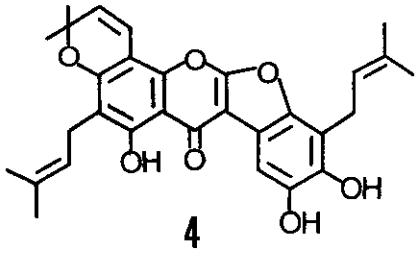

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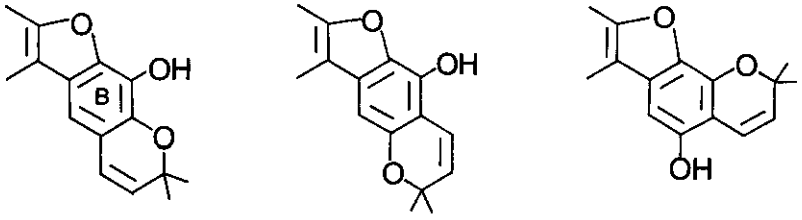
Chart 1

to those of lisetin[4], millettin[5] and a relative[6]. A proton appearing as a small coupled doublet at 7.34 ppm, which was shielded diamagnetically by a carbonyl group, was assigned to a proton at C-6' in the fixed B ring moiety of isoflavone like a coumarono-chromone or a rotenoid[4]. Hence three possible structures as shown in 1, 3 and 4 in Chart 1 can be permitted considering that euchretin A was a coumaronochromone compound possessing three hydroxy-, two γ,γ-dimethylallyl groups and one chromene ring. However, other possible partial structures due to the oxygenation pattern in B ring as shown in A (2',3',4'-), B and C (2',3',5'-) in Chart 2 have to be considered in addition to 1, 3 and 4 (2',4',5'-trioxygenation). By the following experiments and their results, the plausible structure of euchretin A was achieved as 1. By a usual methylation (MeI, K_2CO_3 in acetone), euchretin A gave a trimethyl ether (2)[7] as colorless nee-



A B C

Chart 2

dles, mp 163-165°C. Its empirical formula $C_{33}H_{36}O_7$ (Calcd. 544.2461) was confirmed by high resolution mass spectrometry (M^+: m/z 544.2460). In the mass spectrum of 2, a fragment based on $[A_1^+ + H]$ was observed at m/z 317 ($C_{19}H_{25}O_4$), which exhibited that the A ring of 2 bears two methoxy and two γ,γ-dimethylallyl groups, and the B ring was deduced to possess a chromene ring in the above result. Hence two possible structures (3 and 4) were eliminated in the result. The signal area of a proton at C-6' (7.52 ppm)[8] increased by 34% in monitering a methoxy signal at 3.96 ppm, which indicated that the methoxy group was adjacent to the proton at C-6', and made it clear one of hydroxy groups to be present at C-5' in 1. On the other hand, the proton at C-6' was observed it to couple with a proton at C-5''' by 0.4 Hz in the case of 1 and 0.3 Hz in 2 by a long range coupling ($^7J_{6',5'''}$). The result brought a partial structure due to the B ring moiety as shown in Chart 3. Therefore, the structure of a new coumaronochromone in Euchresta japonica was concluded to be 5,7,5'-trihydroxy-6,8-di(3,3-dimethylallyl)-[6'',6''-dimethylpryrano (2'',3'':4',3')]coumaronochromone.

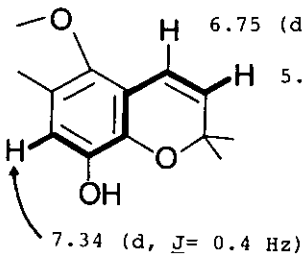


Chart 3

The efficiency of euchretin A against antibacteria was found by a cylinder plate method according to Metzner[9]. The results are listed in Table I.

Table I. Results of the Antibacterial Activity of Euchretin A

test organism	inhibition zone in mm		
	euchretin A[a)]	parabene[a)b)]	EtOH
Escherichia coli	9.1	9.3	9.1
Staphylococcus aureus	8.7	8.7	8.0
Candida tropicalis	9.4	10.3	8.0

a) concentration: 0.1% in EtOH, b) p-hydroxybenzoyl methyl ester

REFERENCES AND FOOTNOTES

1. M. Mizuno, K. Tamura, T. Tanaka, and M. Iinuma, Phytochemistry, in press.

2. M. Mizuno, K. Tamura, T. Tanaka, and M. Iinuma, Phytochemistry, in press.

3. 1 (euchretin A); MS m/z (rel. int.): 502 (100), 487 (33.6), 459 (53.9), 447

(45.9), 431 (17.3), 403 (20.0), 391 (56.0), 243 (5.7), 231 (2.7), 216 (2.9), 215 (10.0), 208 (28.3), 188 (28.0). ^{1}H nmr (acetone-d_6, 300 MHz)δ : 1.50 (6H, br s, 2 x CH_3), 1.67, 1.69, 1.79, 1.88 (3H, each s, CH_3), 3.45, 3.59 (2H, each d, J= 7.0 Hz, Ar-CH_2-CH=C<), 5.23-5.25 (2H, m, Ar-CH_2-CH=C<), 5.99 (1H, dd, J= 9.9, 0.4 Hz, H-5""), 6.75 (1H, d, J= 9.9 Hz, H-4""), 7.34 (1H, d, J= 0.4 Hz, H-6'), 7.83, 8.31, 13.26 (1H, each s, OH-C_7, C_5, and C_5). uv λ_{max}^{MeOH} nm (log ε): 237 (4.59), 264 (4.72), 290sh (4.32), 349 (4.24); +NaOMe: 248, 276, 366; +$AlCl_3$: 237, 265, 291sh, 355; +NaOAc: 237, 265, 291, 356. ir ν_{max}^{KBr} cm^{-1}: 3450 (chelated OH), 3300 (OH), 1680 (chelated C=O), 1380. ^{13}C nmr (acetone-d_6, 75 MHz) δ : (CH_3) 18.02, 25.87, 27.52, (CH_2) 22.56, 22.67, (CH) 109.98, 115.64, 122.77, 133.77, (quar.) 77.95, 98.22, 104.46, 108.01, 108.06, 113.14, 115.70, 132.76, 132.97, 139.31, 145.24, 151.48, 158.88, 159.23, 165.85, 180.19.

4. C.P. Falshaw, W.D. Ollis, J.A. Moore, and K. Magnus, Tetrahedron Suppl., 1966, 7, 333.
5. K.V.S. Raju, G. Srimannarayana, B. Ternai, R. Stanley, and K.R. Markham, Tetrahedron, 1981, 37, 957.
6. S. Tahara, J.L. Ingham, and J. Mizutani, Agric. Biol. Chem., 1985, 49, 1775.
7. 2 (euchretin A trimethyl ether); MS m/z (rel. int.): 544 (95.5), 529 (100), 513 (9.3), 501 (5.9), 487 (17.1), 475 (100), 317 (7.3), 272 (4.8), 257 (14.7), 229 (14.1), 213 (16.0). ^{1}H nmr (acetone-d_6, 300 MHz) δ : 1.58, 1.62, 1.82, 1.92 (3H, each s, CH_3), 3.37, 3.65 (2H, each d, J= 7.0 Hz, Ar-CH_2-CH=C<), 3.89, 3.93, 3.95 (3H, each s, OCH_3), 5.24, 5.32 (1H, each t, J= 7.0 Hz, Ar-CH_2-CH=C<), 5.97 (1H, dd, J= 9.9, 0.3 Hz, H-5""), 6.80 (1H, d, J= 9.9 Hz, H-4""), 7.49 (1H, d, J= 0.3 Hz, H-6'). ^{13}C nmr (acetone-d_6, 75 MHz) δ : (CH_3) 18.02, 18.13, 25.83, 27.71, 56.99, 62.54, 62.86, (CH_2) 23.98, 24.02, (CH) 104.81, 115.70, 122.84, 124.32, 133.52, (quar.) 77.44, 100.34, 108.26, 115.98, 116.13, 121.05, 128.36, 132.05, 133.19, 140.40, 148.25, 153.36, 158.47, 161.55, 164.41, 173.30.
8. NOE of 2 was measured in $CDCl_3$ (300 MHz); δ 3.83, 3.95, 3.96 (3H, each s, OCH_3), 7.52 (1H, d, J= 0.4 Hz, H-6').
9. J. Metzner, H. Bekemeier, M. Paintz, and E. Schneidewind, Pharmazie, 1979, 34, 97.

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