

JUNOSMARIN, A NEW KHELLACTONE ESTER FROM CITRUS JUNOS TANAKA¹Motoharu Ju-ichi^{*}, Mami Inoue, Rika Tsuda, and Naomi Shibukawa

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Abstract ——— A new (-)-trans-khellactone ester, junosmarin, was isolated from the root bark of Citrus junos (Rutaceae) and the structure was assigned as 1 on the basis of spectral data and synthesis of its acetate 5.

Many angular type dihydropyranocoumarins having two chiral centers at C-3' and C-4' are known and the great majority of these compounds are derivatives of (+)-cis-khellactone (2) or (-)-cis-khellactone (3).² Though epimeric (-)-trans-khellactone (4) was isolated from a few plants,^{3,4} natural esters of this diol were unknown. In continuing our studies on the constituents of Citrus plants, we isolated a new (-)-trans-khellactone ester, junosmarin (1), from the root bark of Citrus junos Tanaka (Rutaceae). In this paper we wish to report the isolation and structure elucidation of this new compound.

Junosmarin (1) was obtained as an oily compound, $[\alpha]_D -16.4^\circ$ (CHCl₃), from the acetone extract of the plant by repeated column, centrifugal chromatography and PTLC on silica gel. The mass spectrum of 1 showed the molecular ion peak at m/z 346, and the ¹³C-NMR spectrum⁵ indicated the presence of nineteen carbons. From these results the molecular formula of 1 was established to C₁₉H₂₂O₆. The IR spectrum (CHCl₃) showed absorption at 3400, 1720, 1610 cm⁻¹ and the UV spectrum [$\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 221(sh, 3.73), 260(3.66), 278(3.71), 328(3.61)] indicated typical absorption of 7-oxygenated coumarin moiety.⁶ The ¹H-NMR spectrum (CDCl₃) revealed the signals at δ 7.66, 6.26 (each 1H, d, J=9.53), 7.35, 6.82 (each 1H, d, J=8.55), 5.21, 5.02 (each 1H, d, J=3.66), 1.48, 1.38 (each 3H, s), 3.50 (1H, br s), 2.22 (2H, d, J=6.34), 2.1 (1H, m) and 0.94 (6H, d, J=6.59) suggesting that 1 was a

isovalerylkhellactone. The isovaleryloxy group was assumed to locate at C-3' on the basis of the chemical shifts of C_{3'}-H (δ 5.21) and C_{4'}-H (δ 5.02).⁴ Acetylation of **1** afforded monoacetate (**5**), C₂₁H₂₄O₇, m/z 388(M⁺). The ¹H-NMR spectrum (CDCl₃) of **5** showed signals at δ 7.61, 6.25 (each 1H, d, J=9.52), 7.36, 6.81 (each 1H, d, J=8.54), 6.23, 5.31 (each 1H, d, J=4.39), 2.24 (2H, d, J=6.10), 2.13 (3H, s), 2.1 (1H, m), 1.44, 1.36 (each 3H, s) and 0.96 (6H, d, J=6.59). These data were not in accord with those of dihydrosamidin (**6**)⁷ which has cis-khellactone skeleton. It is well established that the relative configuration of C-3' and C-4' of khellactone derivatives could be assigned by ¹H-NMR spectrum.⁸ The coupling constant of J_{3',4'} appears in the range of 3.0-6.9 Hz for the trans compounds and about 5.0 Hz for the cis compounds. The methyl signals of 1,1-dimethylchromane ring generally resonate as discrete singlets by a difference 0.08-0.20 ppm in trans, and as a broad singlet or two singlets with little difference in cis compounds. On the basis of these spectral data, the structure of junosmarin was proposed as trans-3'-isovalerylkhellactone. Hydrolysis of **1** with 1N KOH was undertaken for obtaining information on the stereochemistry. Two isomeric diols (**2**) and (**4**) were formed in the ratio 2:7. The minor diol (**2**), C₁₄H₁₄O₅, [α]_D +53.1° (CHCl₃), ¹H-NMR (CDCl₃, δ): 5.22, 3.85 (each 1H, d, J=4.88, C_{4'}-H and C_{3'}-H), 1.47, 1.40 (each 3H, s) and the major diol (**4**), C₁₄H₁₄O₅, mp 191-193°C, [α]_D -23.26° (CHCl₃), ¹H-NMR (CDCl₃, δ): 5.00, 3.85 (each 1H, d, J=6.84, C_{4'}-H and C_{3'}-H), 1.53, 1.31 (each 3H, s) corresponded to (+)-cis-khellactone and (-)-trans-khellactone, respectively.⁹ Formation of mixture of diols was considered as a result of epimerization at the benzylic C-4' position during the alkaline hydrolysis and this epimerization is usually observed on khellactone esters.¹⁰ From these results, the structure of junosmarin was established as (**1**). Assignment of the trans configuration was further confirmed by the synthesis of junosmarin acetate (**5**). Reaction of trans-4'-acetylkhellactone (**7**)¹¹ with isovaleryl chloride in benzene afforded trans-3'-isovaleryl-4'-acetylkhellactone which was found to be identical with (**5**) by comparisons of ¹H-NMR, IR and mass spectra and TLC behaviors. Thus the structure of new coumarin was concluded as (-)-trans-3'-isovaleryl-khellactone. To the authors' knowledge, junosmarin is the first example of trans-khellactone ester from natural sources. Isolation of trans-khellactone is usually considered to the result of epimerization at C-4' during the isolation process or the basic treatment because cis-khellactone ester have been isolated together. In our experiments, however, we could not detect any cis-khellactone derivatives,

so we consider that junosmarin exists as a natural product.

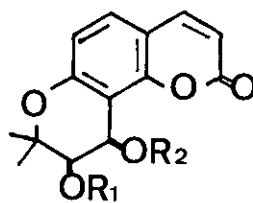


(1) $R_1 = \text{COCH}_2\text{CH}(\text{CH}_3)_2$, $R_2 = \text{H}$

(4) $R_1 = R_2 = \text{H}$

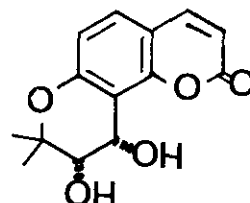
(5) $R_1 = \text{COCH}_2\text{CH}(\text{CH}_3)_2$, $R_2 = \text{COCH}_3$

(7) $R_1 = \text{H}$, $R_2 = \text{COCH}_3$



(2) $R_1 = R_2 = \text{H}$

(6) $R_1 = \text{COCH}_2\text{CH}(\text{CH}_3)_2$
 $R_2 = \text{COCH}_3$



(3)

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5. ^{13}C -NMR spectrum of junosmarin (1): δ 22.34 (q), 22.40 (q), 23.56 (q), 24.00 (q), 25.67 (d), 43.30 (t), 63.25 (d), 73.90 (d), 77.29 (s), 110.58 (s), 112.45 (s), 112.59 (d), 114.75 (d), 128.53 (d), 143.92 (d), 154.52 (s), 155.95 (s), 160.60 (s), 171.98 (s).
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