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Citreospirosteroid, a New Metabolite of a Hybrid Strain KO 0011 Derived from *Penicillium citreo-viride* B. IFO 6200 and 4692

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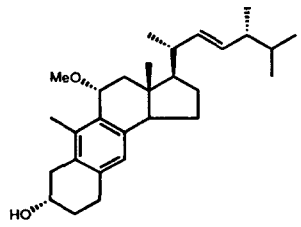
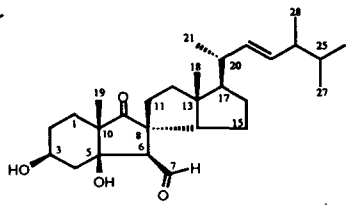
Abstract: A sterol with a spiro ring system of B/C rings, citreospirosteroid, has been isolated from the mycelium of a hybrid strain KO 0011 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Its stereostructure has also been elucidated on the basis of its spectral data. It inhibited the growth of hypocotyls of lettuce seedlings, but promoted the growth of roots of its seedlings. © 1997 Elsevier Science Ltd.

In a series of experiments, we have achieved more than ten hybrid strains by means of a cell fusion technique using two different strains *Penicillium citreo-viride* B. IFO 4692 and 6200.[1] Some of these hybrid strains produced a number of new interesting metabolites which have not been previously detected in mycelium of either parent strain. Recently, we could isolate an anthracene-type sterol with an aromatized B ring, citreoanthrasteroid (**1**).[2] Further investigation of other metabolites in the same mycelium of the hybrid strain KO 0011 resulted in the isolation of a novel spirosterol named citreospirosteroid (**2**).

According to essentially the same procedure as described in the previous papers,[3] the EtOAc extract (142 g) was chromatographed on silica gel using a gradient solvent of MeOH-$CHCl_3$ (1~50%). Elution with $CHCl_3$-MeOH (20:1) afforded a pale yellow powder, which was further separated by preparative TLC using hexane-EtOAc (2:1) to afford citreospirosteroid **2** in 0.0025% yield. The inhibitory activity of citreospirosteroid (**2**) towards the germination of lettuce seedlings was not observed. However, the growth of hypocotyls of lettuce seedlings was inhibited 63.7% and the growth of roots of its seedlings was promoted 122.2%, relative to the control, by this compound at 37.5 μg/cm^2.



citreoanthrasteroid (1)



citreospirosteroid (2)

Citreospirosteroid (**2**)[4] was obtained as a colorless powder; $[\alpha]_D^{24}$ +29.3° (*c* 0.3, $CHCl_3$) and analyzed for $C_{28}H_{44}O_4$ by HR-EIMS [M$^+$ 444.3236, Δ -0.1 mmu]. The IR absorptions at 3350 and 1735 and 1700 cm^{-1} suggested the presence of hydroxy group(s), five membered ketone (δc

227.7) and aldehyde (δc 208.7), respectively. The ^{1}H NMR spectrum of **2** showed the presence of an aldehyde proton (δ 10.1), one *trans* olefin, and six methyl groups of which four were secondary (δ 0.93, 0.92, 0.84, and 0.82), two were tertiary (δ 1.09 and 0.60). The ^{13}C NMR spectrum showed the presence of 28 carbons including two oxygenated carbons [a methine (δc 68.0, C3) and a quaternary (δc 83.3, C5)]. The gross structure of **2** was determined by detailed analyses of one and two dimentional NMR spectra. Especially, the spiro ring system of B/C rings was confirmed by observing HMBC correlations as follow: 6-H to C7, C8, C11 and C14; 7-H to C6; 11-Hβ to C6, C8, C9 and C12; 14-H to C6, C8, C9, C13, C15 and C18; 19-H_3 to C1, C5, C9 and C10. The relative stereochemistry of **2** was clarified by the NOE difference spectra (Figure 1). These NOE results suggested that A/B ring junction should be fused *cis*, because of NOE enhancements between 3-H and 6-H. Furthermore, as can be seen in Figure 1, NOE enhancements between 7-H and 11-Hβ, between 18-CH_3 and 7-H, and between 18-CH_3 and 6-H were observed. Thus, the relative structure of citreospirosteroid (**2**) including the side chain is clearly established as depicted in the formula.

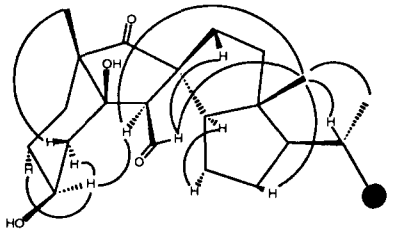


Figure 1. NOE experiments in CD_3OD at room temp. for **2**

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4. Physical data for citreospirosteroid (**2**): $[\alpha]_D^{24}$ +29.3° (c 0.3, $CHCl_3$); IR (film) υ_{max} 3350, 1735 and 1700 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 10.1 (1H, d, J= 4.8 Hz, H7), 5.25 (1H, dd, J=15.4, 8.1 Hz, H23), 5.13 (1H, dd, J= 15.4, 8.1 Hz, H22), 3.73 (1H, m, H3), 3.00 (1H, ddd, J= 14.8, 9.9, 7.7 Hz, H11β), 2.78 (1H, d, J= 4.8 Hz, H6), 2.47 (1H, dd, J= 13.2, 7.3 Hz, H14), 2.23 (1H, ddd, J= 14.8, 8.8, 1.8 Hz, H11α), 2.06 (1H, ddd, J= 13.2, 5.1, 1.8 Hz, H4α), 2.02 (1H, m, H16α), 1.88 (1H, m, H20), 1.84 (1H, m, H24), 1.78 (1H, m, H2α), 1.63 (1H, m, H16β), 1.55 - 1.15(9H, complex, H1α and β, H2β, H12α and β, H15α and β, H17α, H25), 1.42 (1H, dd, J= 13.2, 11.2 Hz, H4β), 1.09 (3H, s, H19), 0.93 (3H, d, J= 6.6 Hz, H21), 0.92 (3H, d, J= 6.6 Hz, H28), 0.84 (3H, d, J= 7.1 Hz, H26 or 27), 0.82 (3H, d, J= 7.1 Hz, H26 or 27), and 0.60 (3H, s, H18); ^{13}C NMR (100 MHz, CD_3OD): δ 227.7 (s, C9), 208.7 (d, C7), 135.9 (d, C22), 134.0 (d, C23), 83.3 (s, C5), 68.0 (d, C3), 67.7 (d, C14), 56.7 (d, C6), 54.5 (s, C13), 53.9 (s, C8 or C10), 53.7(s, C8 or C10), 53.6 (d, C17), 44.3 (d, C24), 41.0 (d, C20), 40.9 (t, C4), 40.5 (t, C11), 38.0 (t, C12), 34.9 (t, C16), 34.3(d, C25), 30.6 (t, C2), 30.1 (t, C1), 20.5 (q, C26 or C27), 20.4 (q, C26 or C27), 20.1 (q, C21), 19.6 (t, C15), 18.2 (q, C28), 14.5 (q, C18), and 14.2 (q, C19); HREI-MS $C_{28}H_{44}O_4$, found 444.3236 (M^+) (Δ -0.1 mmu).

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