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BIOMIMETIC SYNTHESIS OF CITREOVIRIDIN-TYPE COMPOUNDS AND ISOLATION OF EPICITREOVIRIDINOL, A NEW METABOLITE OF PENICILLIUM PEDEMONTANUM IFO 9583

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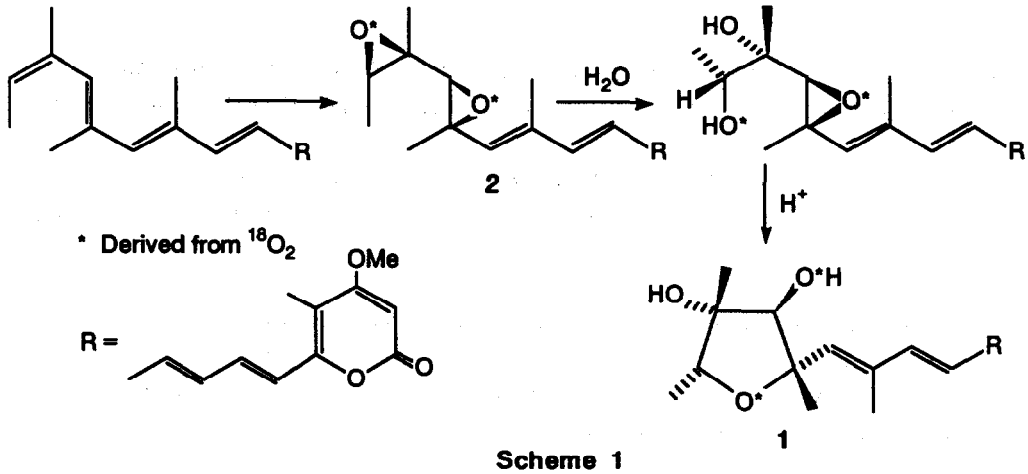
Summary On epoxidation using *m*-chloroperbenzoic acid, citreoviridin and its acetate have been converted to isocitreoviridinol, epiisocitreoviridinol and epicitreoviridinol and their acetates, respectively. Particularly, epicitreoviridinol has been newly isolated from the mycelium of *Penicillium pedemontanum* IFO 9583.

Citreoviridin and related metabolites[1] are quite attractive because of their novel structures as well as physiological properties inhibiting ATP-synthesis and ATP-hydrolysis catalyzed by mitochondrial enzyme systems.[2] Among them, the synthesis of citreoviridin (1) was first accomplished by us[3] and quite recently by other two groups[4]. In addition, several citreoviridinol-type compounds have also been synthesized including citreoviridinol,[5] neocitreoviridinol,[5] aurovertin B,[6] asteltoxin,[7] and verrucosidin.[8] Furthermore, ^{13}C- and ^{18}O-isotope incorporation studies of citreoviridin (1) by Vleggaar et al.[9] clarified the origin of five oxygen atoms included in its structure, and they postulated a biosynthetic pathway for citreoviridin, as shown in Scheme 1, wherein a bis-epoxide (2) is regarded as a key intermediate.

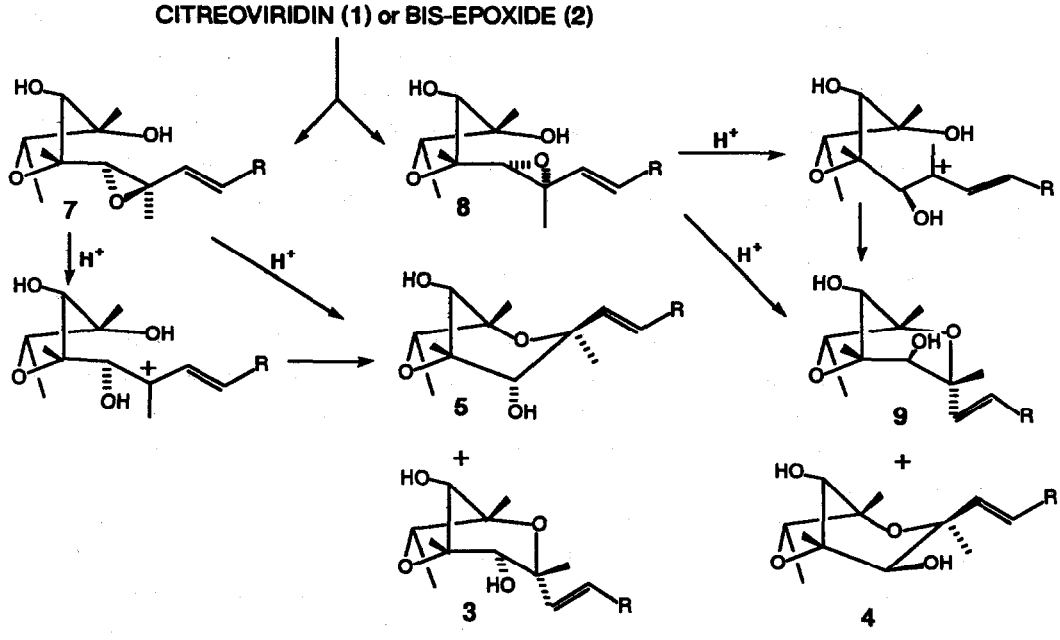
Recently, we could isolate three citreoviridin-type metabolites (3, 4 and 5),[1] having a 2,6-dioxabicyclo[3.2.1]octane ring similar to that of aurovertin B (6). As judged from their stereostructures, further epoxidation of 1 or its precursor (2) does not take place in a stereospecific manner, but the terminal double bond is attacked by monooxygenase from both sides to afford two unstable epoxides (7 and 8), which are spontaneously converted to four possible stereoisomers (3, 4, 5 and 9), as shown in Scheme 2. Of them citreoviridinol (3), isocitreoviridinol (4) and epiisocitreoviridinol (5) have been found in nature,[1] and the remaining stereoisomer (9) named epicitreoviridinol, will be discussed later.

From a biogenetic point of view, epoxidation of citreoviridin (1) was carried out under various conditions, particularly using *m*-chloroperbenzoic acid (1.2 equiv) and $NaHCO_3$ (2.9 equiv) in CH_2Cl_2 (-20 °C - room temp., 10 h, under Ar) to afford 4,[1] 5[1] and epicitreoviridinol (9)[10] in 3.6, 4.4 and 3.4% yields, respectively.[11] Monoacetylcitreoviridin (10) was also subjected to epoxidation using *m*-chloroperbenzoic acid (1.04 equiv) in CH_2Cl_2 (room temp., overnight, under Ar) to give monoacetates (11, 12 and 13)[12] corresponding to 4, 5 and 9 in 19, 2.1 and 4.4% yields, respectively. The compound (9), named epicitreoviridinol, is quite similar to citreoviridinol (3)[1] in spectral data. Finally, the stereostructure of epicitreoviridinol was unambiguously determined by chemical transformation of the monoacetate (13)[13] to citreoviridinol (3), as follows.

The monoacetate (13) was subjected to Moffatt oxidation [DMSO / DCC / pyridine / TFA in benzene (0 °C - room temp., overnight)] to afford the corresponding ketone (14),[13] in almost quantitative yield, which was reduced with $NaBH_4$ in EtOH (0 °C, 1 h) to give an alcohol (15)[1] in 63% yield. Finally, 15 was treated with



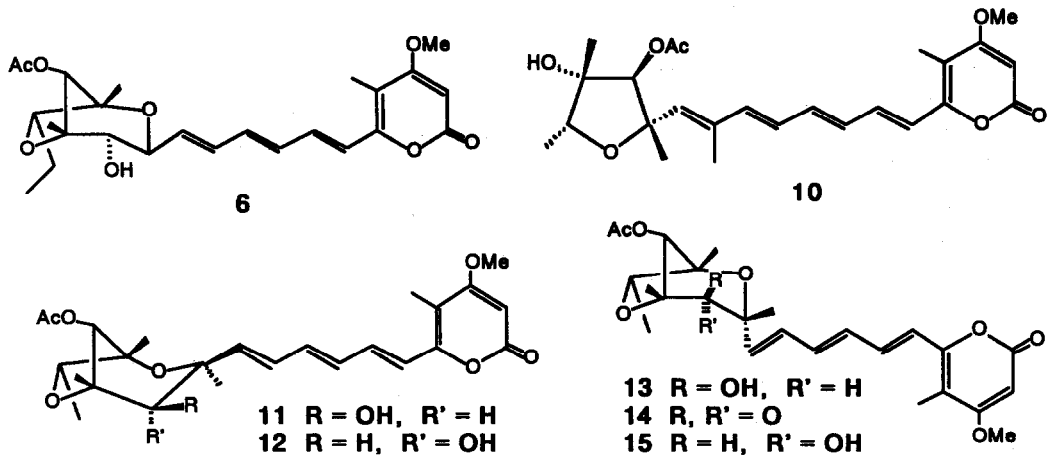
Scheme 1



Scheme 2

K_2CO_3 in MeOH (room temp., 1 h) to give rise to citreoviridinol (3)[1] in ca. 100% yield. The newly formed compound (9), epicitreoviridinol, was also isolated from the mycelium of *P. pedemontanum* IFO 9583.

According to essentially the same procedure as described in the previous paper,[1] the polished rice (450 g), which was inoculated with a suspension of mycelium of *P. pedemontanum* IFO 9583 in a sterilized water, was incubated stationarily at 20 - 23 °C for 32 days and then extracted with EtOAc. The EtOAc extract (14.9 g) was chromatographed on silica gel (Wako gel C-60) using a gradient solvent of MeOH - $CHCl_3$ (0/100 - 50/100 v/v). After elution with MeOH - $CHCl_3$ (1 : 20) giving a mixture of hydrocarbons, elution with a gradient solvent (5/100



- 6/100 v/v) afforded a brown oil which was rechromatographed on silica gel using $CHCl_3$ - acetone (20 : 1) to give citreoviridin (1)[1] in 4.7% yield.[14] Further elution with MeOH - $CHCl_3$ (7 : 100) provided a reddish brown oil which was separated by repeated preparative TLC (Kieselgel PF_{254}) using $CHCl_3$ - MeOH (10 : 1) and then hexane - EtOAc - acetone (1 : 1 : 1) to give epineocitreoviridinol [1] in 0.17% yield.[14] The fractions eluted with MeOH - $CHCl_3$ (7/100 - 10/100 v/v) were combined and then subjected to repeated preparative TLC (Kieselgel PF_{254}) using hexane - EtOAc - acetone (1 : 1 : 1) and then $CHCl_3$ - MeOH (10 : 1) to afford isocitreoviridinol (4) and a new metabolite (9), named epicitreoviridinol,[10] which was completely identical with the synthetic one derived from citreoviridin (1).

The present study means biomimetic synthesis of citreoviridin-type metabolites (4, 5 and 9) and strongly suggests a plausible mechanism of the formation of citreoviridinol and related compounds with a 2,6-dioxabicyclo[3.2.1]octane ring, as shown in Scheme 2, wherein the third epoxidation does not occur in a stereospecific manner in contrast to that of aurovertin B (6).[15] In addition, epicitreoviridinol (9) was found in nature, as expected.[16]

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10. Epicitreoviridinol as yellow powder: $[\alpha]_D^{22}$ -46° (c 0.20, $CHCl_3$); $C_{23}H_{30}O_7$ [m/z 418.2000 (M^+)]; IR (film) 3430, 1690, 1620 and 1540 cm^{-1}; δ ($CDCl_3$) 1.19 (3H, d, J = 6.5 Hz), 1.22 (3H, s), 1.37 (3H, s), 1.39 (3H, s), 1.96 (3H, s), 3.83 (3H, s), 3.91 (1H, s), 4.05 (1H, q, J = 6.5 Hz), 4.11 (1H, s), 5.49 (1H, s), 6.2 - 6.5 (5H, complex) and 7.18 (1H, dd, J = 11, 15 Hz).
11. Citreoviridinol (**3**) has not been obtained.
12. On hydrolysis with excess K_2CO_3 in MeOH (room temp., 1 h), these acetates (**11**, **12** and **13**) were readily converted to **4**, **5** and **9** respectively, in quantitative yields.
13. The spectral data for the new compounds were in accord with the structures assigned, and only selected data are cited: **13**: $C_{25}H_{32}O_8$ [m/z 460.2116 (M^+)]; IR (film) 3425 and 1730 cm^{-1}; δ ($CDCl_3$) 2.14 (3H, s), 3.92 (1H, br.s) and 5.44 (1H, s). **14**: $C_{25}H_{30}O_8$ [m/z 458 (M^+)]; IR (film) 1755 and 1736 cm^{-1}; δ ($CDCl_3$) 2.18 (3H, s) and 5.24 (1H, s).
14. Based on the weight of the EtOAc extract.
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16. Any amount of citreomontanin has not been isolated (see S. Rebuffat, D. Davoust, L. Molho, and D. Molho, Phytochemistry, **19**, 427 (1980); *ibid.*, **20**, 1279 (1981).

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