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ISOLATION AND STEREOSTRUCTURES OF CITREOVIRAL, CITREODIOL, AND EPICITREODIOL

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Summary: Three new metabolites (citreoviral, citreodiol, and epicitreodiol) have been isolated from the mycelium of Penicillium citreo-viride B. (IFO 6050) and their stereostructures have been elucidated on the basis of their spectral data coupled with some chemical evidence. Furthermore, the absolute configurations of citreodiol and epicitreodiol have been determined by syntheses of the corresponding antipodes starting from L-rhamnose.

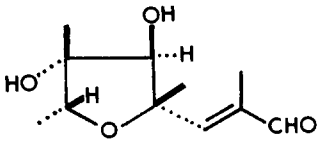
In connection with citreoviridin, a potent inhibitor of ATP-synthesis and ATP-hydrolysis catalyzed by mitochondrial enzyme system, we have isolated several novel metabolites of Penicillium citreo-viride B. (IFO 6200).[1] Further effort has been made on searching for physiologically active substances produced by another strain of P. citreo-viride B. (IFO 6050), resulting in the isolation of three new metabolites, as described herein.

According to essentially the same procedure as described in the previous papers,[1] the polished rice (250 g), which was inoculated with a suspension of mycelium of P. citreo-viride B. (IFO 6050) in a sterilized water, was incubated stationarily at 25 °C for 21 days and extracted with acetone and then with AcOEt. The combined extracts were partitioned between AcOEt and water. The AcOEt extract was chromatographed on silica gel (Wakogel C-100) using a gradient solvent of MeOH - $CHCl_3$ (1 - 20%). After elution with 2% MeOH - $CHCl_3$, the first fraction eluted with 4% MeOH - $CHCl_3$ was further separated by repeating preparative TLC (Kieselgel PF_{254}) using AcOEt and then $CHCl_3$ - MeOH (19 : 1) to afford citreoviral (1) as a colorless oil, in 0.1% yield[2]: $C_{11}H_{18}O_4$ [m/z 214.1212(M^+)]; $[\alpha]_D^{27}$ +2.7° (c 7.0, $CHCl_3$); IR (film) 1680 and 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ1.20(3H, d, J= 6Hz), 1.27(3H, s), 1.41(3H, s), 1.87(3H, br.s), 3.86(1H, q, J= 6Hz), 3.97(1H, br.s), 6.69(1H, br.s), and 9.38(1H, s).

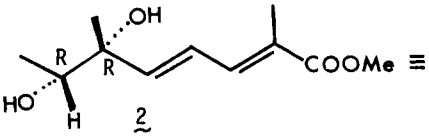
Further elution with 4% MeOH - $CHCl_3$ afforded an oil, which was separated by repeating preparative TLC (Kieselgel PF_{254}) using AcOEt - acetone (2 : 1) and then $CHCl_3$ - acetone (2 : 1) to give an inseparable mixture of citreodiol (2) and epicitreodiol (3) in 0.04% yield (relative ratio: 2/3 = 2/3).[2,3] These two metabolites were characterized as the corresponding carbonates (4 and 5), which were quantitatively obtained on treatment of the mixture with excess carbonyldiimidazole in benzene (30 °C, 15 h) [4: mp 89 - 90 °C (from hexane - AcOEt); $C_{12}H_{16}O_5$ [m/z 240.0997(M^+)]; $[\alpha]_{300}^{26}$ +70° (c 0.1, MeOH); IR (film) 1805, 1710, 1645, and 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ1.41(3H, d, J= 7Hz), 1.53(3H, s), 2.00(3H, d, J= 1Hz), 3.81(3H, s),

4.55(1H, q, J= 7Hz), 6.01(1H, d, J= 15Hz), 6.70(1H, dd, J= 15, 11Hz), and 7.16(1H, dq, J= 11, 1Hz). 5: mp 98 - 100 °C (from hexane - AcOEt); $C_{12}H_{16}O_5$ [m/z 240.1007(M^+)]; $[\alpha]_{300}^{26}$ +233° (c 0.2, MeOH); IR (film) 1805, 1710, 1645, and 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ1.33(3H, d, J= 7Hz), 1.67(3H, s), 2.02(3H, d, J= 1Hz), 3.83(3H, s), 4.55(1H, q, J= 7Hz), 5.92(1H, d, J= 15Hz), 6.72(1H, dd, J= 15, 11Hz), and 7.19(1H, dq, J= 11, 1Hz)].

As judged from the IR (1680 and 1630 cm^{-1}) and ^1H NMR (δ1.87, 6.69, and 9.38) spectra, citreoviral (1) has a partial structure [-$\overset{|}{C}$-CH=C(Me)-CHO]. In addition, the signals due to the remaining moieties in 1 are observed in the ^1H NMR spectrum of citreoviridin (6),[4] indicating that citreoviral has the same substituted tetrahydrofuran moiety as that of 6. Finally, the stereostructure of citreoviral (1) was established in connection with citreoviridin (6), as follows: citreoviridin in MeOH was subjected to ozonolysis (-40∼-50 °C,

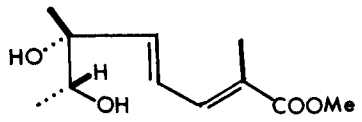


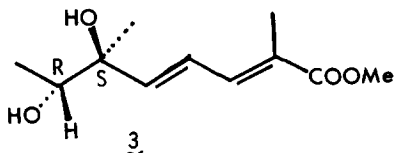
1



2

13 (antipode of 2)





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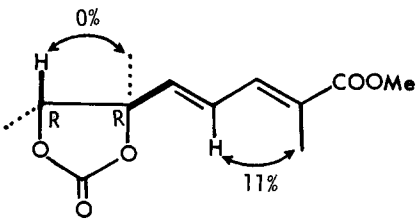
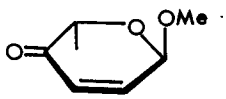
15 (antipode of 3)



4

14 (antipode of 4)



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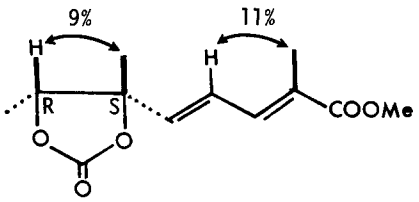
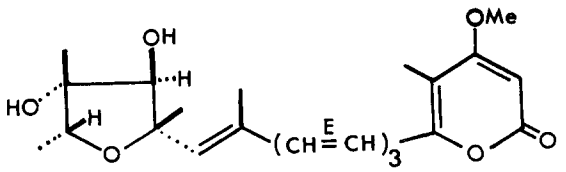


5

16 (antipode of 5)



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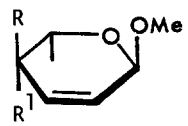
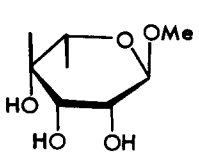


8 R = Me, R^1= OH

9 R = OH, R^1= Me



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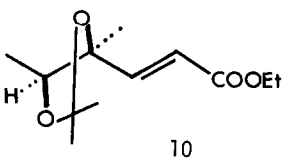

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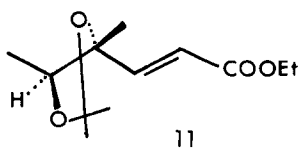

11

40 min) followed by decomposition with excess Me_2S to give several oxidation products, which were carefully separated by preparative TLC [Kieselgel PF_{254}; $CHCl_3$ - MeOH (95 : 5)] to give citreoviral (1) in 26% yield.

The two carbonates (4 and 5) are quite similar to each other in their spectral data. Particularly, on the basis of the ^1H NMR spectra coupled with some NOE experiments (see 4 and 5), their stereostructures may be depicted in 4 and 5, respectively. Finally, the absolute stereostructures of citreodiol and epicitreodiol were unambiguously determined by syntheses of the corresponding antipodes starting from L-rhamnose, as follows.

The known enone (7)[5] derived from L-rhamnose was treated with MeLi in ether under argon atmosphere (-68 °C, 40 min) to afford a mixture of two olefins (8 and 9)[6] in 82% yield, which was readily converted into the corresponding ethyl esters (10 and 11)[7] in 11 and 14% overall yields, respectively, in 5 steps [1) O_3 in MeOH (0 °C, 1 h); 2) excess Me_2S in MeOH (0 °C ~ room temp., 30 min); 3) Ph_3P=CHCOOEt in benzene (refluxing temp., 2 days); 4) p-TsOH in MeOH (room temp., 2.5 h); 5) 2,2-dimethoxypropane in acetone containing p-TsOH (room temp., overnight)]. The former (10) has been also synthesized from the known compound (12),[8] which was stereoselectively derived from L-rhamnose, in 4 steps [1) $Pb(OAc)_4$ (1 equiv.) in benzene (room temp., overnight); 2) Ph_3P=CHCOOEt in benzene (70 °C, 12 h); 3) p-TsOH in MeOH (room temp., 4 h); 4) 2,2-dimethoxypropane in acetone containing p-TsOH (room temp., overnight)].[9]

The synthetic intermediate (10) was treated successively with diisobutylaluminum hydride in THF (-76 °C, 80 min), PDC in DMF (0 °C, 80 min), (α-carbomethoxyethylidene)-triphenylphosphorane in benzene (refluxing temp., 1 h), and then p-TsOH in MeOH (room temp., 5 h) to afford (+)-citreodiol (13) (63% overall yield from 10) as a colorless oil: $C_{11}H_{18}O_4$ [m/z 215.1320(M^+ + 1)]; $[\alpha]_D^{22}$ +4.3° (c 1, $CHCl_3$); IR (film) 3450, 1690br., 1630, and 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ1.18(3H, d, J= 7Hz), 1.31(3H, s), 1.97(3H, d, J= 1Hz), 3.70(1H, q, J= 7Hz), 3.80(3H, s), 6.10(1H, d, J= 15Hz), 6.70(1H, dd, J= 15, 11Hz), and 7.19(1H, dq, J= 11, 1Hz). According to the same procedure as described in 2 and 3, this diol (13) was almost quantitatively converted into the corresponding carbonate (14), whose spectral data were completely identical with those of the natural one (4) except for optical rotation ($[\alpha]_{300}^{26}$ -73° (c 0.3, MeOH)). Thus, citreodiol (2) is regarded as [6R, 7R]-6,7-dihydroxy-2,6-dimethyloct-2,4-dienoic acid methyl ester.

According to the same procedure as described in 10, the epimer (11) was also converted easily into (-)-epicitreodiol (15) (62% overall yield for 11) as a colorless oil: $C_{11}H_{18}O_4$ [m/z 215.1303(M^+ + 1)]; $[\alpha]_D^{21}$ -7.1° (c 2.3, $CHCl_3$); IR (film) 3450, 1700br., 1635, and 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ1.17(3H, d, J= 7Hz), 1.35(3H, s), 1.97(3H, d, J= 1Hz), 3.78(3H, s), 3.78(1H, overlapped with MeO signal), 6.10(1H, d, J= 15Hz), 6.67(1H, dd, J= 15, 11Hz), and 7.19(1H, dq, J= 11, 1Hz). Furthermore, (-)-epicitreodiol (15) was also converted into the corresponding carbonate (16), which showed the same spectral data as those of the natural one except for optical rotation ($[\alpha]_{300}^{26}$ -280° (c 0.2, MeOH)), indicating that epicitreodiol (3) is [6S, 7R]-6,7-dihydroxy-2,6-dimethyloct-2,4-dienoic acid methyl ester.

From a biogenetic point of view, these newly isolated metabolites (1, 2, and 3) are quite interesting because of close similarity in their structures, although there a possibility that citreoviral (1) is formed on catabolic oxidation of citreoviridin (6). Presumably, both citreodiol (2) and epicitreodiol (3) are biosynthesized from the enzyme-bound 3,5,7-tri-

oxooctanethioate and two molecules of methionine (C_2- and C_6-methyls), although their carbon skeleton is compatible with "isoprene rule". Biosynthetic study on this point is further in progress. Synthetic studies on citreoviral (1) as well as on citreoviridin (6), the absolute configuration of which remains still unsettled, are also in progress.

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References and Notes

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2. Based on the weight of the AcOEt extract.
3. The high resolution mass spectrum of the mixture is almost identical with those of synthetic samples of (+)-citreodiol (13) and (-)-epicitreodiol (15). In addition, the ^{1}H NMR spectrum has all of the signals observed in both 13 and 15.
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6. This mixture was directly used for the next experiment.
7. 10 as a colorless oil: $C_{12}H_{20}O_4$ [m/z 213.1149(M^+- 15)]; $[\alpha]_D^{22}$ +45° (c 1.4, $CHCl_3$); IR (film) 1725 and 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ1.23(3H, s), 1.26(3H, d, J= 7Hz), 1.30(3H, t, J= 6.5Hz), 1.40(3H, s), 1.48(3H, s), 4.03(1H, q, J= 7Hz), 4.20(2H, q, J= 6.5Hz), 6.08(1H, d, J= 15Hz), and 6.88(1H, d, J= 15Hz).
   11 as a colorless oil: $C_{12}H_{20}O_4$ [m/z 213.1130(M^+- 15)]; $[\alpha]_D^{23}$ -4.6° (c 4.3, $CHCl_3$); IR (film) 1725 and 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ1.23(3H, d, J= 7Hz), 1.32(3H, t, J= 6.5Hz), 1.42 (3H, s), 1.45(3H, s), 1.53(3H, s), 4.07(1H, q, J= 7Hz), 4.22(2H, q, J= 6.5Hz), 6.07(1H, d, J= 15Hz), and 6.90(1H, d, J= 15Hz).
8. R. D. King and W. G. Overrend, Carbohydr. Res., 9, 423 (1969).
9. In this case, only (+)-citreodiol (13) has been able to be synthesized from L-rhamnose.

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