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Isolation and Structures of Citreovirenone and Citreovirone

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Two new metabolites (citreovirenone and citreovirone) have been isolated from the mycelium of Penicillium citreo-viride B. (IFO 4692) and their structures have also been elucidated on the basis of their spectral data coupled with some chemical evidence.

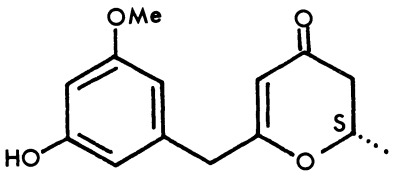
In connection with citreoviridin, a potent inhibitor of ATP-synthesis and ATP-hydrolysis catalyzed by mitochondrial enzyme system, we have isolated more than ten novel metabolites of Penicillium citreo-viride B.(IFO 6200 and 6050).[1] Further effort has been made on searching for physiologically active substances produced by another different strain of P. citreo-viride B. (IFO 4692) leading to the isolation of two new metabolites [citreovirenone (1) and citreovirone (2)], which have no pyrone ring, in addition to citreoviridin (3) and related mycotoxins.

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 mycelia of P. citreo-viride B. (IFO 4692) in sterilized water, was incubated stationarily at 25 °C for 3 weeks and extracted with acetone and then with AcOEt. The combined extracts were partitioned with AcOEt and water. The AcOEt extract was chromatographed on silica gel (Katayama Chemicals, Type 60) using a gradient solvent of MeOH - $CHCl_3$ (1 - 10%). After elution with 1% MeOH - $CHCl_3$ affording a mixture of hydrocarbons, the fractions eluted with 2 - 3% MeOH - $CHCl_3$ were further separated by repeating preparative TLC (Kieselgel PF_{254}) using hexane - acetone (1 : 1), hexane - AcOEt (1 : 10), and then hexane - acetone (1 : 1) or $CHCl_3$ - MeOH (20 : 1) to afford citreovirenone (1) and citreovirone (2) in 0.24 and 1.2% yields, respectively,[2,3] The physical data of these two newly isolated metabolites are shown below.

Citreovirenone (1) as a colorless oil: $[\alpha]_D^{30}$ -109.5° (c 0.66, $CHCl_3$); IR (film) 3300, 1660 (sh), 1640, 1590, and 1500 cm^{-1}; UV (MeOH) 209 (ε 20000), 222 (sh, 7000), and 267 nm (12000); ^1H NMR ($CDCl_3$ - D_2O) δ= 1.43(3H, d, J= 6.4Hz), 2.41 (1H, d, J= 6.0Hz), 2.42(1H, d, J= 10.9Hz), 3.41(2H, s), 3.75(3H, s), 4.50(1H, ddq, J= 6.0, 10.9, 6.4Hz), 5.32(1H, s), 6.31(2H, br.d, J= 1.9Hz), and 6.33 (1H, t, J= 1.9Hz);[4] ^{13}C NMR ($CDCl_3$)[4] δ= 20.29(q), 41.25(t), 42.35(t), 55.30 (q), 76.06(d), 100.2(d), 104.7(d), 107.3(d), 108.7(d), 137.5(s), 157.5(s),

161.0(s), 176.8(s), and 194.0(s); Found: m/z 248.1033. Calcd for $C_{14}H_{16}O_4$: M, 248.1047.

Citreovirone (2) as a colorless oil: $[\alpha]_D^{30}$ +21.8° (c 4.0, $CHCl_3$); IR (film) 3400, 1705, 1595, and 1500 cm^{-1}; UV (MeOH) 209 (ε 14000), 222 (sh, 7000), and 280 nm (1000); ^1H NMR ($CDCl_3$) δ= 2.92(1H, d, J= 6.8Hz), 2.92(1H, d, J= 5.2Hz), 3.66(2H, s), 3.76(3H, s), 4.37(1H, ddd, J= 4.0, 5.2, 6.8Hz), 5.77(1H, d, J= 4.0Hz), 6.27(1H, t, J= 1.6Hz), 6.32(1H, t, J= 1.6Hz), and 6.33(1H, t, J= 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ= 43.24(t), 50.81(t), 55.39(q), 72.44(d), 74.95(d), 100.6(d), 107.9(d), 108.9(d), 135.4(s), 157.2(s), 161.3(s), and 206.9(s); Found: m/z 292.0228. Calcd for $C_{12}H_{14}{}^{35}Cl_2O_4$: M, 292.0268.



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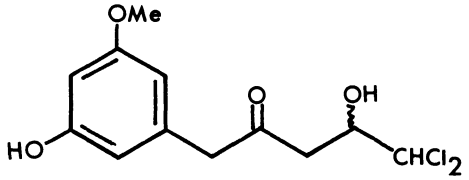

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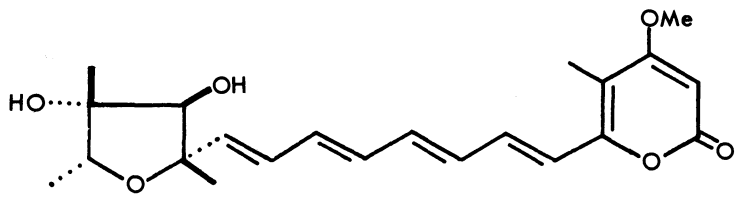

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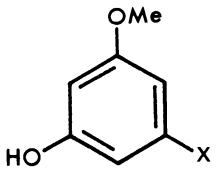
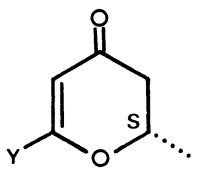
Citreovirenone (1) with a molecular formula $C_{14}H_{16}O_4$ has a trisubstituted aromatic ring [A] on the basis of its spectral data, particularly the chemical shifts and/or coupling constants of ^1H and ^{13}C NMR spectra (^1H NMR δ= 3.75, 6.31, and 6.33; ^{13}C NMR = 55.30, 100.2, 107.3, 108.7, 137.5, 157.5, and 161.0.[5] In addition, the dihydropyrone moiety [B] must be present in the structure of citreovirenone, as judged from the ^1H and ^{13}C NMR spectra (^1H NMR δ= 1.43, 2.41, 2.42, 4.50, and 5.32; ^{13}C NMR δ= 20.29, 41.25, 76.06, 104.7, 176.8, and 194.0), which include the signals corresponding to those of hepialone (4),[6] except for both ethyl and methyl ones. Thus, the two moieties [A] and [B] are connected to each other through the remaining one methylene group (^1H NMR δ= 3.41; ^{13}C NMR δ= 42.35).

As depicted in 1, finally, the absolute configuration of citreovirenone was unambiguously determined by comparing its CD spectrum with that of hepialone (4)[6] [1: $[\alpha]_D^{30}$-109.5°; CD (c 0.938, EtOH, 24 °C) $\Delta\varepsilon$ -1.02 at 316 nm. 4: $[\alpha]_D^{20}$ +106.4° (c 1.09, EtOH); CD (EtOH) $\Delta\varepsilon$ +0.89 at 312 nm].

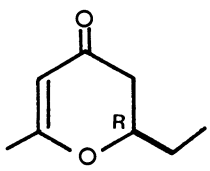
Citreovirone (2) with a molecular formula $C_{12}H_{14}Cl_2O_4$ also has a trisubstituted aromatic ring [A] (^1H NMR δ= 3.76, 6.27, 6.32, and 6.33; ^{13}C NMR δ= 55.39, 100.6, 107.9, 108.9, 135.4, 157.2, and 161.3). Furthermore, a partial structure



[A]



[B]



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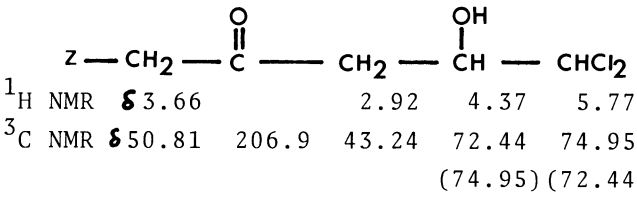
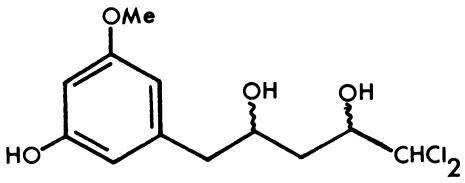


[C]



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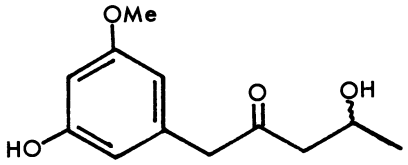

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[C] must be included in the structure of citreovirone (2) on the basis of its spectral data (see [C]) together with some chemical evidence, as follows.

On irradiation at δ4.37, each signal at δ2.92 and 5.77 became singlet. Furthermore, on $NaBH_4$ reduction of 2 in dioxane - MeOH (9 : 1) (0 °C - room temp , 25 min) giving rise to the corresponding dihydro compound (5) [$C_{12}H_{16}{}^{35}Cl^{37}ClO_4$ (m/z 296.0401(M^+)); IR (film) 3350 (br) cm^{-1} and no CO absorption band; ^1H NMR ($CDCl_3$) δ= 4.19(2H, m, 2 x (-CH(OH)-))], the signals at δ2.92 and 3.66 in 2 were shifted to δ1.97 and 2.68 as multiplets, respectively. When treated with Bu^n_3SnH (12 equiv.) in anhydrous toluene containing catalytic amounts of AIBN (room temp , 1 h and then refluxing temp , 2 h), citreovirone (2) was readily converted into a dechloro compound (6) [$C_{12}H_{16}O_4$ (m/z 224.1047(M^+)); IR (film) 3400 and 1705 cm^{-1}], in the ^1H NMR spectrum of which the newly formed methyl doublet was observed at δ1.15(3H, d, J= 6.5 Hz). Thus, the structure of citreovirone must be represented by 2, including these two moieties [A] and [C]. The absolute configuration of citreovirone (2) remains unsettled.

Clearly, citreovirenone (1) and citreovirone (2) both are hepta- and hexaketides in origin, respectively. From a biogenetic point of view, it is noted that these two phenolic compounds (1 and 2) co-occur with citreoviridin (3) and related pyrones in the case of the strain of P. citreo-viride B. (IFO 4692) different from the other ones (IFO 6200 and 6050).

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References

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2) Based on the weight of the AcOEt extract.

3) After elution of citreovirenone (1) and citreovirone (2), both citreoviridin (3) and citreoviridinol have been obtained, in 3.2 and 0.24% yields respectively, from the fractions eluted with 3 - 4% MeOH - $CHCl_3$.

4) ^1H and ^{13}C NMR spectra were taken on a JNM-GX 400 FT-NMR spectrometer using tetramethylsilane as an internal standard.

5) ^1H and ^{13}C NMR signals are tentatively assigned.

6) T. Matsumoto, I. Kubo, and D. L. Wagner, 50th National Meeting of the Chemical Society of Japan, Tokyo, March 1985, Abstr., No 1M07; K. Uchino, Y. Yamagiwa, T. Kamikawa, and I. Kubo, Tetrahedron Lett., 26, 1319 (1985).

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