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Four New Metabolites Produced by Penicillium Citreo-viride B. on Addition of NaBr

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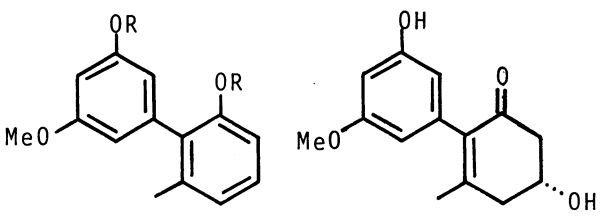
Four new metabolites (citreobiphenyl, precitreobiphenyl, citreoviranol, and demethylcitreoviranol) have been isolated from the mycelium of Penicillium citreo-viride B. (IFO 4692) incubated on the polished rice together with sodium bromide, and their structures have also been elucidated on the basis of their spectral data.

In connection with citreoviridin, a potent inhibitor of ATP-synthesis and ATP-hydrolysis catalyzed by mitochondrial enzyme system, a number of novel metabolites with a pyrone ring have been isolated from the mycelium of Penicillium citreo-viride B. (IFO 6200 and 6050).[1] Of some metabolites produced by another different strain of P. citreo-viride B. (IFO 4692), however, two phenolic compounds have been found.[2] From a biogenetic point of view, β-polyketo carboxylic acids are expected to be converted into the corresponding pyrones and/or phenolic compounds. In the present paper, we describe herein the structures of four metabolites [citreobiphenyl (1), precitreobiphenyl (2), citreoviranol (3), and demethylcitreoviranol (4)], which have been produced on addition of sodium bromide to the polished rice inoculated with P. citreo-viride B. (IFO 4692).

Polished rice (300 g) in deionized water (ca. 800 ml) was allowed to stand at room temperature for 30 min, then cooked using an electric rice cooker (99 °C, ca. 20 min) and transferred into an Erlenmyer flask (5 l), which was pasteurized



1 R = H
6 R = Ac

2

3 R = Me
4 R = H

5

at 120 °C for 20 min at 2 atm. After addition of sodium bromide (1 g), the polished rice so far treated was inoculated with a suspension of P. citreo-viride B. (IFO 4692) in sterilized water, incubated stationarily at 25 °C for 23 days 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 - 20%). After elution with 1% MeOH - $CHCl_3$ affording a mixture of hydrocarbons, further elution with the same solvent system gave rise to a colorless oil, which was separated by preparative TLC (Kieselgel PF_{254}) using $CHCl_3$ - acetone (10 : 1), $CHCl_3$ - acetone (5 : 1) and then hexane - acetone (2 : 1) to afford citreobiphenyl (1) in 0.064% yield.[3] The first fraction eluted with 2% MeOH - $CHCl_3$ was also separated by repeating preparative TLC (Kieselgel PF_{254}) with hexane - acetone (3 : 2) and then with $CHCl_3$ - acetone (2 : 1) to afford citreoviranol (3) in 0.23% yield.[3] On further elution with 2% MeOH - $CHCl_3$, the known metabolite, citreovirenone (5)[2] was also obtained in 0.42% yield.[3] The fraction eluted with 4% MeOH - $CHCl_3$ was separated by preparative TLC (Kieselgel PF_{254}) using AcOEt - MeOH (1 : 1) and then AcOEt to afford a trace amount of demethylcitreoviranol (4). Furthermore, the more polar fraction eluted with 4% MeOH - $CHCl_3$ was also separated by repeating preparative TLC (Kieselgel PF_{254}) [1) AcOEt - MeOH (2 : 1); 2) $CHCl_3$ - acetone (2 : 3)] to afford a trace amount of precitreobiphenyl (2). The physical data of these four newly isolated metabolites are shown below.

Citreobiphenyl (1) as a white powder: $C_{14}H_{14}O_3$ [m/z 230.0931 (M^+)]; IR (film) 3400 and 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 2.12(3H, s), 3.80(3H, s), 4.88(1H, s), 5.02(1H, s), 6.35(1H, dd, J = 2.2, 2.4 Hz), 6.41(1H, dd, J = 2.2, 2.4 Hz), 6.46 (1H, t, J = 2.2 Hz), 6.83 (2H, d, J = 7.8 Hz) and 7.16(1H, t, J = 7.8 Hz).

Precitreobiphenyl (2) as a solid: $C_{14}H_{16}O_4$ [m/z 248.1033 (M^+)]; IR (film) 3400, 1650, 1590 and 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.87(3H, s), 2.60(1H, dd, J = 7.8, 16.7 Hz), 2.62(1H, dd, J = 8.8, 15.1 Hz), 2.84(1H, dd, J = 5.0, 16.7 Hz), 2.85 (1H, dd, J = 3.4, 15.1 Hz), 3.76(3H, s), 4.38(1H, m), 6.12(1H, dd, J = 2.0, 2.4 Hz), 6.18(1H, dd, J = 2.0, 2.4 Hz) and 6.36(1H, t, J = 2.4 Hz).

Citreoviranol (3) as a white powder: $C_{15}H_{18}O_6$ [m/z 294.1082 (M^+)]; $[\alpha]_D^{30}$ -147° (c 0.196, $CHCl_3$); IR (film) 3420, 1670, 1625, 1580,and 1510 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.13(3H, d, J = 6.4 Hz), 1.27(1H, q, J = 11.5 Hz), 1.52(1H, dd, J = 11.0, 12.7 Hz), 2.05(1H, ddd, J = 2.0, 4.4, 11.5 Hz), 2.41(1H, ddd, J = 2.0, 4.9, 12.7 Hz), 3.00(1H, d, J = 16.3 Hz), 3.15(1H, d, J = 16.3 Hz), 3.83(3H, s), 4.10 (1H, m), 4.38(1H, m), 6.26(1H, br.s), 6.36(1H, d, J = 2.4 Hz) and 11.18(1H, s); ^{13}C NMR ($CDCl_3$) δ = 21.2(q), 38,1(t), 41.0(t), 42.0(t), 55.5(q), 63.6(d), 67.4 (d), 99.3(d), 100.9(s), 104.6(s), 107.1(d), 138.9(s), 164.4(s), 166.0(s) and 168.3(s).

Demethylcitreoviranol (4) as a solid: $C_{14}H_{16}O_6$ [m/z 280.0944 (M^+)]; IR (film) 3300, 1660, 1630, 1595,and 1515 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.14(3H, d, J = 6.2 Hz), 1.2 - 1.5(overlapped with signals of impure hydrocarbons), 2.05(1H, ddd, J = 1.8, 4.8, 12.5 Hz), 2.42(1H, ddd, J = 1.8, 4.8, 13.2 Hz), 3.00(1H, d, J = 16.3 Hz), 3.15(1H, d, J = 16.3 Hz), 4.11(1H, m), 4.37(1H, m), 6.21(1H, br.s), 6.30(1H, d,

J = 2.6 Hz) and 11.15(1H, s).

On the basis of ^{1}H NMR spectrum, the compound (1) with a molecular formula $C_{14}H_{14}O_3$ is regarded as a 2,6,3',5'-tetrasubstituted biphenyl having one Me, one MeO and two OH groups. When treated with Ac_2O - pyridine (room temp, overnight), 1 was readily converted into the corresponding diacetate (6),[4] whose ^{1}H NMR spectrum showed two doublets [δ 6.94(1H, d, J = 8 Hz); δ 7.16(1H, d, J = 8 Hz)] and one triplet [δ 7.28(1H, t, J = 8 Hz)] due to the three adjacent protons attached to one of the two aromatic rings. In the case of 1, the NMR signals assignable to them are observed at δ 6.83(2H, d, J = 7.8 Hz) and 7.16 (1H, t, J = 7.8 Hz). As judged from the NOE experiments, finally, the four substituents must be located as shown in Fig. 1.

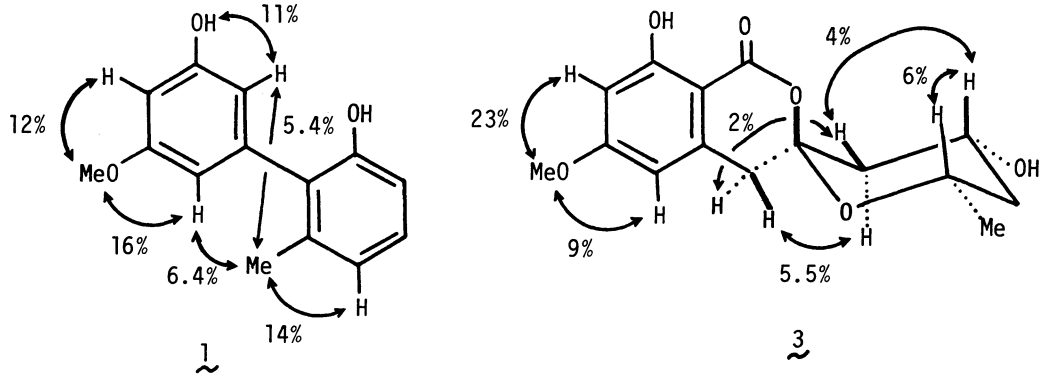
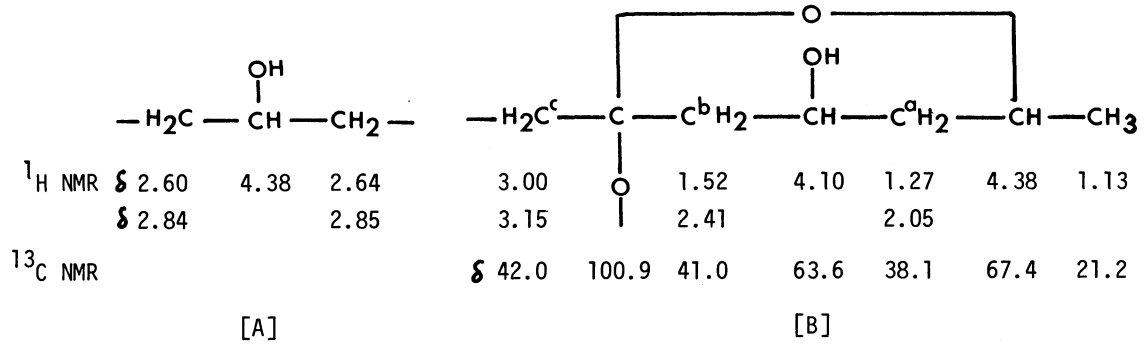


Fig. 1. NOE experiments of citreobiphenyl (1) and citreoviranol (3).

Precitreobiphenyl (2) with a molecular formula $C_{14}H_{16}O_4$ has a conjugated CO group (IR 1650 cm^{-1}) and one Me group attached to a double bond (δ 1.87) in addition to a 1,3,5-trisubstituted benzene (δ 6.12, 6.18 and 6.36). Furthermore, the decoupling experiments indicate the presence of a partial structure [A] in 2. From these data, the structure of precitreobiphenyl must be represented by 2. In fact, 2 was treated with p-TsOH in benzene (room temp., 3 h) to afford citreobiphenyl (1). From a biogenetic point of view, the stereochemistry of the sec.OH group in 2 may be the same as that in citreoviranol (3), as discussed later.

Citreoviranol (3) has a 1,2,3,5-tetrasubstituted benzene ring (δ 6.26 and 6.36) with a conjugated ester group (IR 1670 cm^{-1}; δ 168.3). In addition, ^{1}H and ^{13}C NMR spectra indicate the presence of a tetrahydropyrane moiety [B] with aid of decoupling and NOE experiments. Particularly, coupling constants of the



signals at δ 1.27, 1.52, 2.05 and 2.41 (C^a and C^b) indicate that both sec. Me and OH groups are in an equatorial configuration. As seen in Fig. 1, the NOE experiments strongly suggest that the isolated methylene group (C^c) is located as depicted in [B]. In the light of co-occurrence of citreovirenone (5),[2] furthermore, the stereostructure of citreoviranol is represented by 3, which is supported by NOE experiments, as seen in Fig. 1. Clearly, the phenolic OH group (δ 11.18) is adjacent to the lactone CO group (IR 1670 cm^{-1}) so that a hydrogen bond is formed between them.

The fourth new compound (4) is regarded as demethylcitreoviranol, whose ^1H NMR spectrum is almost identical with that of citreoviranol (3) except for the following points: the former has no MeO group, while the methyl singlet (δ 3.83) due to MeO group is observed in 3.

From a biogenetic point of view, these metabolites (1 - 5) so far obtained are derived from a common precursor (7), as demonstrated in Fig. 2. Particularly, it is noted that these different types of metabolites co-occur on addition of sodium bromide to the polished rice inoculated with P. citreo-viride B. (IFO 4692). However, we have not yet found any metabolite containing Br atoms. Further study on this point is in progress.

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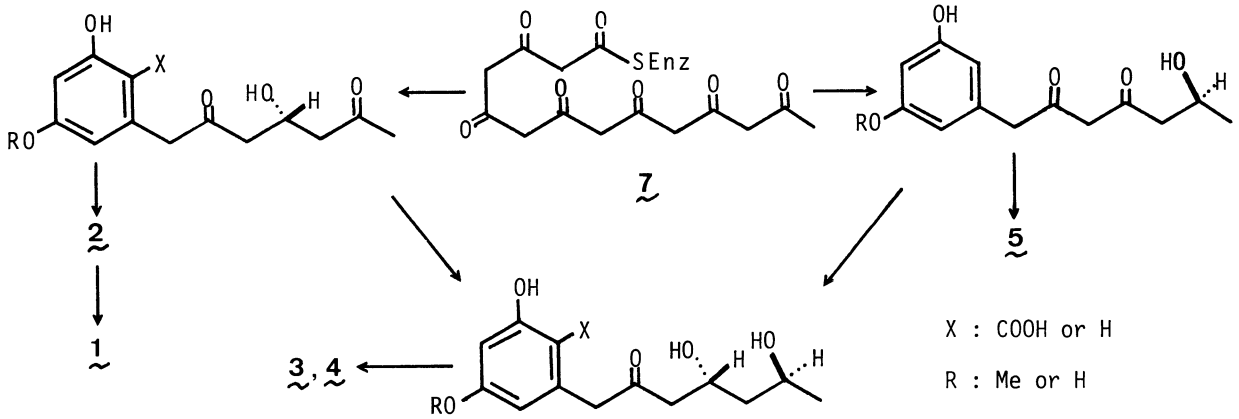

Fig. 2. Biogenesis of citreobiphenyl, citreoviranol and related compounds.

References

1) S. Nishiyama, Y. Shizuri, S. Yamamura, Y. Terada, K. Kawai, and H. Furukawa, Tetrahedron Lett., 26, 6239 (1985) and many references cited therein.
2) Y. Shizuri, M. Nagahama, S. Yamamura, K. Kawai, N. Kawai, and H. Furukawa, Chem Lett., 1986, 1129.
3) Based on the total weight of the acetone and AcOEt extracts.
4) 5 as a pale yellow powder: $C_{15}H_{18}O_5$ [m/z 314.1131 (M^+)]; IR (film) 1765, 1615 sh., and 1580 cm^{-1}; ^1H NMR ($CDCl_3$) = 1.98(3H, s), 2.16(3H, s), 2.28(3H, s), 3.79(3H, s), 6.51(1H, br.s), 6.61(1H, br.s), 6.63(1H, t, J = 2 Hz), 6.94(1H, d, J = 8 Hz), 7.16(1H, d, J = 8 Hz) and 7.28(1H, t, J = 8 Hz).

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