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(1R,6R,9S,10S)-9-CHLORO-10-HYDROXY-8-METHOXYCARBONYL-4-METHYLENE-2,5-DIOXABICYCLO[4.4.0]DEC-3-ONE-7-ENE , A FIRST CHLORINE-CONTAINING SHIKIMATE-RELATED METABOLITE FROM FUNGI

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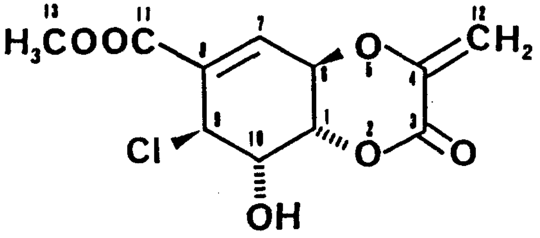
Summary: A new chlorine-containing metabolite has been isolated from two fungi, and its structure including absolute stereochemistry was established by spectroscopic and X-ray crystallographic studies.

We jointly[1] report the structure determination of (1R,6R,9S,10S)-9-chloro-10-hydroxy-8-methoxycarbonyl-4-methylene-2,5-dioxabicyclo[4.4.0]dec-3-one-7-ene (1), a first naturally occurring chlorine-containing shikimate-related metabolite, which possesses unique biological as well as chemical properties.

As a result of the screening, carried out in Yamagata group, for fungal metabolites inducing abnormal hyphal morphology of fungi[2], a new active metabolite was detected in the culture filtrate of an unidentified fungus. The fungus was cultured in malt-dextrose medium using a jar-fermenter for 10 days. The filtrate was extracted with ethyl acetate, and the extract was subjected to silica gel column chromatography. Elution with hexane-ethyl acetate system gave an active fraction, from which active colorless crystals were isolated upon careful concentration followed by adding hexane to the concentrated solution. The compound (1) did not give clear melting point, instead gradually decomposed on heating. The compound (1) induced irregular hyphal morphology of a phytopathogenic fungus Cochliobolus lunatus[2] at 10 μg/disc.

In Osaka group the same compound (1) and 3-methoxy-2,5-toluquinone (2)[3,4] were isolated from the culture filtrate of a fungus isolated from soil collected near Sakai city during the investigation[5] of looking for antimicrobial compounds having thiol reacting properties. This fungus was cultured in glucose-polypeptone medium for 5 days. Ethyl acetate extraction of the culture filtrate and successive purification of the extract by silica gel chromatography and crystallization from ethyl acetate in hexane afforded 1 as colorless crystals.



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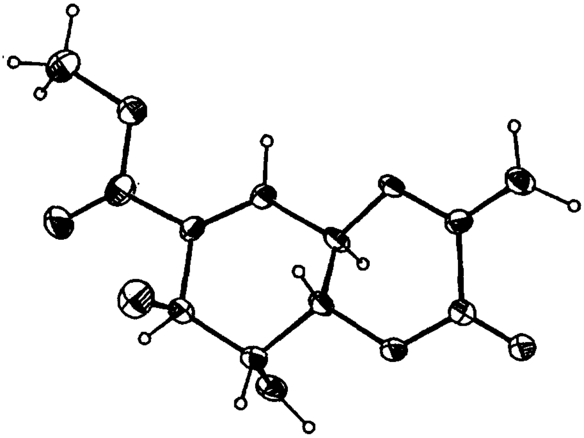
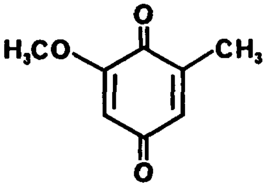


Figure. A perspective drawing of 1.



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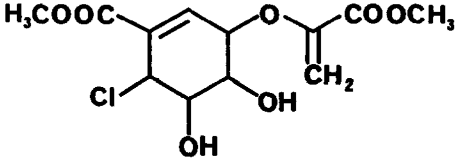

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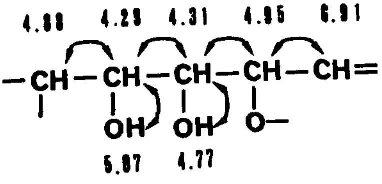

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The compound (1) showed positive Beilstein test and the following physicochemical properties: $[\alpha]_D$ +94.5°(c 0.86, EtOH); $C_{11}H_{11}O_6Cl$ (M^+ m/z 274.0188 and 276.0241; calcd. 274.0244 (^{35}Cl) and 276.0214 (^{37}Cl), respectively); UV (EtOH) 230 nm (sh, ε=6600); IR ($CHCl_3$) 3360, 1720, 1630, 1295 cm^{-1}; ^{1}H-NMR (500 MHz, d_6-acetone) δ 6.96 (1H, d, J=2 Hz, H-7), 5.57 (1H, d, J=4 Hz, -OH), 5.52 (1H, d, J=1.5 Hz, H-12), 5.07 (1H, d, J=1.5 Hz, H-12), 5.05 (1H, dd, J=9 and 2 Hz, H-6), 4.94 (1H, d, J=2.5 Hz, H-9), 4.92 (1H, dd, J=9 and 2 Hz, H-1), 4.52 (1H, m, H-10), 3.82 (3H, s, H-13); ^{13}C-NMR (125 MHz, d_6-acetone) δ164.8 (C-11), 160.1 (C-3), 148.5 (C-4), 135.7 (C-7), 131.9 (C-8), 103.6 (C-12), 75.6 (C-1), 71.6 (C-10), 71.1 (C-6), 54.6 (C-9), 52.7 (C-13). Peak assignments were based on the results of 2D NMR spectral measurements including H-H COSY, H-C COSY and HMBC spectra. In the HMBC spectrum the hydroxyl proton signal appeared at δ 5.57 showed cross peaks which were correlated to the carbon signals of δ 54.6 (C-9) and δ 75.6 (C-1), indicating the connectivity of C-10 to C-9 and to C-1, respectively. The olefinic proton (H-7) signal appeared at δ 6.96 showed correlated peaks to the carbon signals of δ 164.8 (C-11), δ 75.6 (C-1) and δ 54.6 (C-9), indicating the connectivity though these carbons to the proton (H-7). The methoxyl protons signal appeared at δ 3.82 showed correlation peaks to the carbons C-11 and C-8, indicating the connectivity of the methoxycarbonyl group to C-8. Correlation peaks were also observed in the enolpyruvyl moiety[6]. Treatment of 1 with sodium methoxide afforded a diester derivative (3); colorless oil; CI-MS m/z 307 $[M+1]^+$; UV (EtOH) 218 nm (sh, ε=8160); IR ($CHCl_3$) 3450, 3000, 2950, 1720, 1620, 1440, 1250, 1200, 1170, 1090 cm-1; ^{1}H-NMR (270 MHz, d_6-acetone) δ 6.91 (1H, d, J=2.1 Hz, H-2), 5.48 (1H, d, J=2.7 Hz, H-10), 5.07 (1H, d, J=4.0, 5-OH), 5.04 (1H, d, J=3.1 Hz, H-10), 4.95 (1H, dd, J=1.8 and 7.9 Hz, H-3), 4.88 (1H, d, J=2.7 Hz, H-6), 4.77 (1H, dd, J=1.5 and 5.5 Hz, 4-OH), 4.31 (1H, overlapped, H-4), 4.29 (1H, overlapped, H-5), 3.79 and 3.77 (each 3H, $-OCH_3$); ^{13}C-NMR (67.5 MHz, d_6-acetone) δ 165.9 and 164.5 (2 x -COO-), 151.7 (C-8), 138.2 (C-2), 131.6 (C-1), 98.2 (C-10), 77.5, 75.5, and 69.1 (3 x -O-CH-), 55.9 (C-6), 53.1 and 53.0 (2 x $-OCH_3$). The signals on the cyclohexene ring revealed partial structure (4) by the irradiation method. Thus, this diester formation indicated the location of the enolpyruvyl moiety.

Although the coupling constants ($J_{1,6}$=9 Hz and $J_{1,10}$=2.1 Hz) observed in the ^{1}H NMR spectrum of 1 indicated the trans and cis configurations at C-1 to C-6 and C-1 to C-10, respectively, the stereochemistry at C-9 could not be assigned owing to the small coupling constant ($J_{9,10}$=2.5 Hz) observed between H-9 and H-10 and conformational ambiguity of the cyclohexene part in 1.

The complete structure and stereochemistry of 1 were established by single-crystal X-ray analysis. Crystals were monoclinic, space group $P2_1$, a=14.128(3), b=6.323(1), c=6.725(1) Å, β=98.35(2)°, D_c=1.53 g cm^{-3}, Z=2. All unique diffraction intensities with $2\theta \leq 130°$ were collected in the variable speed $2\theta/\omega$ scan mode on a Rigaku AFC-5UD four-circle diffractometer with graphite-monochromated Cu-K_α (1.5418 Å). A total of 1111 reflections were judged to be observed after correction for Lorentz, polarization, and background effects. The structure was solved by direct methods using MULTAN 84[7]. Block-diagonal least squares refinements with anisotropic thermal parameters for the non-hydrogen atoms converged to a current R factor of 0.090. At last stage, the anomalous scattering factor corrections for the chlorine atom were introduced into structure-factor calculations to establish the absolute configuration. For the configuration of 1, R was 0.084 whereas for the inverted configuration it was 0.085[8].

Figure shows a perspective drawing of X-ray structure, and the absolute configuration of this compound should thus be represented by formula 1. The atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

The compound (1) is interesting in considering biogenetic relationship with other chlorinated shikimate-derived natural products such as methyl 4-chloroindoleacetate[9].

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References and Notes.

(1) At the annual meeting of The Agricultural Chemical Society of Japan held on April in 1989 at Niigata, we recognized that both groups handled the same compound. Both samples were found to be identical each other by direct comparisons carried out in both laboratories.

(2) M. Nukina, Y. Sato, M. Ikeda, and T. Sassa, Agric. Biol. Chem., 45, 789 (1981).

(3) R. S. Sood, K. Roy, G. C. S. Reddy, J. Reden, and B. N. Ganguli,J. Antibiot.,35, 985 (1982).

(4) Yamagata group found that the addition of p-benzoquinone to the ethyl acetate extract solution greatly reduced polymerization of the extract. The latter, without such additives, tended to produce organic solvent insoluble materials during rotary evaporation of the ethyl acetate extract solution.

(5) M. Nakagawa, A. Hirota, H. Sakai, and A. Isogai, J. Antibiot., 35, 778 (1982); B. K. Park, M. Nakagawa, A. Hirota, and M. Nakayama, Agric. Biol. Chem., 51, 2861 (1987); Y. H. Hsu, A. Hirota, S. Shima, M. Nakagawa, H. Nozaki, T. Tada, and M. Nakayama, ibid., 51, 3455 (1987).

(6) The remaining correlation peaks in the HMBC spectrum of 1 were as follows; $\delta_H - \delta_C$: 5.52 (H-12) - 160.1 (C-3), 5.52 (H-12) - 148.5 (C-4), 5.07 (H-12) - 160.1 (C-3), 5.07 (H-12) - 148.5 (C-4), 4.94 (H-9) - 71.6 (C-10), 4.94 (H-9) - 75.6 (C-1), 4.94 (H-9) - 131.9 (C-8), 4.94 (H-9) - 135.7 (C-7), 4.92 (H-1) - 71.1 (C-6), 4.92 (H-1) - 71.6 (C-10).

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