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CONSTITUENTS OF *TRITONIA CROCOSMAEFLORA*, II. TRICROZARIN B, AN ANTITUMOR NAPHTHAZARIN DERIVATIVE[1]

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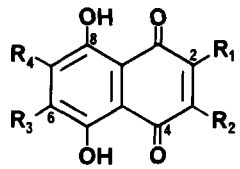
Tritonia crocosmaeflora Lemoine (Iridaceae) is a hybrid between *Tritonia aurea* Pappe. and *Tritonia pottsii* Benth., which was introduced into Japan in the late 19th century as a garden plant and is now naturalized in several areas of this country. In connection with our interest in naturally occurring biologically active compounds, it was found that the MeOH extract of the fresh bulbs of *T. crocosmaeflora* exhibited antimicrobial activity against Gram-positive bacteria, fungi, and yeast (1), and a novel, antimicrobial naphthazarin derivative, tricrozarin A [**1**], was isolated from the extract. Later, it was found that the extract showed also strong cytocidal activity against HeLa S_3 cells in vitro. So, we investigated the active component responsible for this activity, and a cytocidal naphthazarin derivative designated as tricrozarin B [**2**] was isolated. This paper deals with the isolation and physicochemical and biological characterization of **2**.



	R_1	R_2	R_3	R_4
1	OMe	OMe	OCH_2O	
2	OMe	OMe	OMe	H
3	OMe	H	OMe	OMe
4	OH	OH	OH	H
5	$CH(CH_3)OH$	OMe	OMe	OMe

Tricrozarin B ($C_{13}H_{12}O_7$, 0.00016% yield) was crystallized from MeOH as deep red needles and has a uv-vis and nmr spectra suggesting a naphthazarin nucleus (1-3).

Because $C_{10}H_2O_4$ out of $C_{13}H_{12}O_7$ has been assigned to the naphthazarin skeleton, the remainder is $C_3H_{10}O_3$ and consists of an aromatic hydrogen and three methoxyls. These three methoxyl groups can be attached to the naphthazarin skeleton as in structure **2** or **3**.

In the ^{13}C-nmr spectrum of tricrozarin B, signals corresponding to 13 carbons were observed. Among these signals, the signal at δ_C 107.4 assigned to the sp^2 methine was further split into a doublet ($^3J_{CH}$=5 Hz) clearly. This indicated that this carbon is next to the carbon bearing a hydroxyl group, and the structure of tricrozarin B was established as **2** (2,3,6-trimethoxynaphthazarin), which corresponds to the tri-*O*-methylated derivative of spinochrome D [**4**] (4) and physicochemical data of **2** are in accordance with those reported for the latter compound. Whereas it was demonstrated that **2** did not exist as a mixture of various tautomers between **2** and **3** as reported previously (4) but only existed as indicated in Figure 1 through various nmr experiments.

On the other hand, in the LSPD experiments of tricrozarin B [**2**], when signals at δ_H 12.910 (C_5-O*H*) and δ_H 13.017 (C_8-O*H* were irradiated, signals at δ_C 146.7 (C_3) and δ_C 149.4 (C_2) were simplified, respectively. Observation of these couplings are quite interesting because these couplings must have occurred via strong hydrogen bonds as in-

[1]For part 1 in this series see Masuda *et al.* (1).

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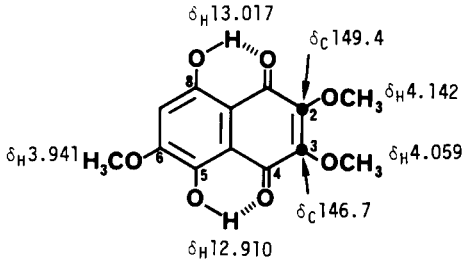

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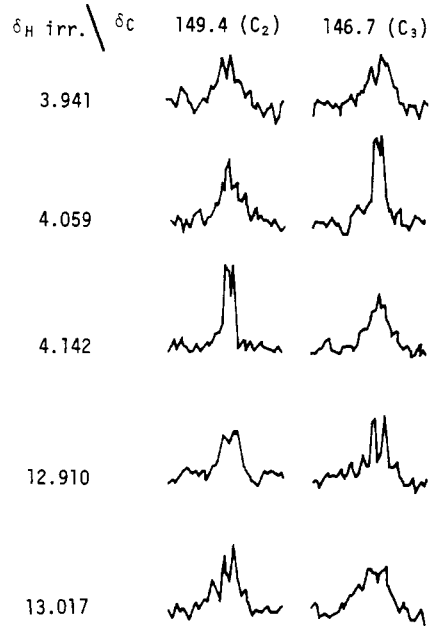

FIGURE 1. LSPD experiments of tricrozarin B [**2**].

dicated in Figure 1. This is the first report of this type of ^{1}H-^{13}C coupling.

Among naturally occurring tri- or tetra-oxygenated naphthazarin derivatives, lomazarin [**5**] (5) and tricrozarin A [**1**] (1) are the only previous examples isolated from the plant kingdom, and most of these kinds of compounds have been isolated from marine invertebrates such as the sea urchin (4, 6-11), sea cucumber (12,13), and starfish (14). As described above, **2** had been obtained previously through semisynthesis by the methylation of spinochrome D [**4**] (4) which was isolated from the sea urchin (8); this is the first report of its occurrence in nature.

Tricrozarin B [**2**] showed no antimicrobial activity, whereas it showed potent cytocidal activity against HeLa S_3 cells (IC_{50} 0.007 μg/ml) in vitro and antitumor activity against sarcoma 180 murine tumor in vivo (T/C 146%, 10×5.0 mg/kg). We are now investigating the antitumor activities of this compound against various kinds of murine tumors in vivo, and the results will be published elsewhere.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Yanagimoto MP-3 hot stage microscope and are uncorrected. Uv spectra were recorded on a Shimadzu model UV-200S spectrophotometer and ir spectra on a Jasco model A-102 interferometer. Mass spectra were obtained with a Jasco model DX-300 mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian XL-400 instrument. Kieselgel 60 (Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for column chromatography and DC-Fertigplatten Kieselgel 60 (Merck) was used for tlc analysis and for preparative tlc.

PLANT MATERIAL.—The plant material used in this study was collected in October 1984 in Kagoshima prefecture, Japan, and a voucher specimen was placed on file at the Kitasato Institute, Tokyo, Japan.

EXTRACTION AND FRACTIONATION.—Fresh bulbs (5.0 kg) of *T. crocosmaeflora* were treated as described in the previous paper (1). The C_6H_6 layer was chromatographed over Si gel (100 g) using $CHCl_3$ and $CHCl_3$/MeOH as solvent to give fraction A (yellow solid, 0.82 g), fraction B (red solid, 0.62 g), and fraction C (yellow solid, 0.82 g).

ISOLATION OF TRICROZARIN B [**2**].—Fraction B (0.62 g) was chromatographed over Sephadex LH-20 (ϕ2.0×50 cm) using MeOH as solvent, and the fractions containing tricrozarin B [**2**] were collected and further purified by preparative tlc using $CHCl_3$ as solvent. Tricrozarin B [**2**] was recrystallized from MeOH to give deep red needles (7.8 mg); mp 176-177°; ir ν max (KBr) 3450, 2935, 1590, 1484, 1418, 1280, 1205, 1175, 1111, 1077, 1000, 990 cm^{-1}; uv λ max (MeOH) 232, 318, 468 (sh), 492, 520 (sh) nm; uv λ max (MeOH-HCl) 232, 317, 471 (sh), 492, 522 (sh) nm; uv λ max (MeOH-NaOH) 232, 322, 500 (sh), 532, 567 nm; ms *m/z* 280 (M^+), 265, 261, 250, 249, 246, 236, 234, 218, 209, 207, 191, 166, 138; hrms *m/z* 280.059 (M^+, calculated for $C_{13}H_{12}O_7$, 280.058); ^{1}H

nmr (400 MHz, $CDCl_3$) δ 3.941 (s, 3H, C_6-OCH_3), 4.059 (s, 3H, C_3-OCH_3), 4.142 (s, 3H, C_2-OCH_3), 6.385 (s, 1H, C_7-H), 12.910 (s, 1H, C_5-*OH*), 13.017 (s, 1H, C_8-*OH*); ^{13}C nmr (100 MHz, $CDCl_3$) δ 56.7 (C_6-OCH_3), 61.6 (C_3-OCH_3), 61.7 (C_2-OCH_3), 104.9 (C_{8a}), 107.4 (C_7), 109.3 (C_{4a}), 146.7 (C_3), 149.4 (C_2), 159.1 (C_6), 161.1 (C_5), 170.0 (C_8), 172.3 (C_1), 174.8 (C_4).

ANTIMICROBIAL ACTIVITY OF TRICROZARIN B [**2**].—The antimicrobial spectrum of **2** was determined as described previously (1).

ANTI-HELA S_3 ACTIVITY OF TRICROZARIN B[**2**].—The anti-HeLa S_3 activity of **2** was determined as described previously (15).

ANTITUMOR ACTIVITY OF TRICROZARIN B [**2**].—Sarcoma 180 cells (1×10^6) were inoculated ip into ICR mice (5-week old females). Mice were given ip injections of the test compound on days 1-10. Antitumor activity was evaluated by $T/C\times100$ (%), where T was the median survival days (MSD) of the treated group and C the MSD of the control group.

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