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CONSTITUENTS OF *TRITONIA CROCOSMAEFLORA*, I. TRICROZARIN A, A NOVEL ANTIMICROBIAL NAPHTHAZARIN DERIVATIVE

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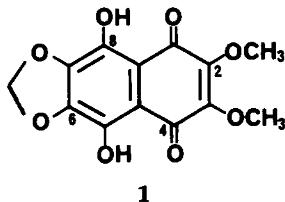
ABSTRACT.—A novel naphthazarin derivative, tricrozarin A, has been isolated from the fresh bulbs of *Tritonia crocosmaeflora* and was characterized as 5,8-dihydroxy-2,3-dimethoxy-6,7-methylenedioxy-1,4-naphthoquinone (2,3-dimethoxy-6,7-methylenedioxynaphthazarin). Tricrozarin A exhibits antimicrobial activity against gram-positive bacteria, fungi, and yeast in vitro and is the first tetra-oxygenated naphthazarin derivative isolated from higher plants.

Tritonia crocosmaeflora Lemoine (Iridaceae) is a hybrid between *Tritonia aurea* Pappe. and *Tritonia pottsii* Benth. that was introduced into Japan in the late-19th century as a garden plant and is now naturalized in several areas of Japan. As part of our continuing study on naturally occurring biologically active compounds, it was found that the MeOH extract of the fresh bulbs of *T. crocosmaeflora* exhibited antimicrobial activity against some gram-positive bacteria, fungi, and yeast. This paper describes the isolation and characterization of a novel, antimicrobial naphthazarin derivative designated as tricrozarin A (2,3-dimethoxy-6,7-methylenedioxynaphthazarin) [**1**].



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Fresh bulbs of *T. crocosmaeflora* were mixed with dry ice and crushed, followed by extraction with MeOH at room temperature. The combined MeOH extracts were concentrated in vacuo to give a brown syrup and subjected to antimicrobial activity tests against various kinds of microorganisms. As a result, it was found that the MeOH extract and a C_6H_6 extract of the MeOH extract showed antimicrobial activities against *Bacillus subtilis*, *Micrococcus luteus*, *Aspergillus niger*, *Mucor racemosus*, *Candida albicans*, and *Saccharomyces sake*. The extract was fractionated, and the fractions were monitored for antimicrobial activity. The C_6H_6 extract was chromatographed on Si gel and a Sephadex LH-20 column in succession. By a combination of the column chromatography and biological activity tests described above, a new naphthazarin derivative, tricrozarin A was isolated.

Tricrozarin A (0.0001% yield) was crystallized from MeOH as deep red needles, mp 187°, and the molecular formula ($C_{13}H_{10}O_8$) was established by hrms (found, *m/z* 294.034; calcd for $C_{13}H_{10}O_8$, 294.038). The uv spectrum [λ max (MeOH) 236, 264, 341, 481, 529 (sh), and 582 (sh) nm] attested to the presence of a naphthoquinone moiety (1,2). The bathochromic shift [λ max 242, 346, 506 (sh), 547, and 585 nm] in the

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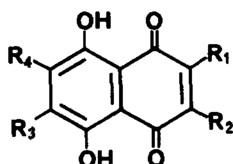
uv spectrum on the addition of base and the broad absorption in the ir spectrum at ν max 3445 cm^{-1} indicated the presence of at least one phenolic OH group. The ^1H-nmr spectrum (400 MHz, $CDCl_3$) was quite simple, displaying a six-proton singlet at δ 4.109 attributed to two methoxyls, a two-proton singlet at δ 6.239 attributed to a methylenedioxy moiety, and a two-proton singlet at δ 12.419 attributed to two phenolic OH groups.

The ^1H-nmr data suggested a naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) [**2**] nucleus, since the last characteristic lower field singlet (2H) corresponded to the signals *peri* to the carbonyl function (3). The bathochromic shift of the uv spectrum of this compound compared with 1,4-naphthoquinone [λ max ($CHCl_3$) 245, 251, 257 (sh), and 335] and 5-hydroxy-1,4-naphthoquinone (juglone) [λ max (MeOH) 248, 340 (sh), 407 (sh), and 422 nm], and a strong carbonyl absorption at ν max 1605 cm^{-1} (4) also supported this hypothesis.

Because assignment of the naphthazarin skeleton ($C_{10}H_2O_4$) has been accomplished, the remaining atoms consisted of two methoxyls ($2\times$-OCH_3) and a methylenedioxy (-OCH_2O-) moieties. These three functional groups can be attached to the naphthazarin skeleton only as **1** or **3**.



	R_1	R_2	R_3	R_4
2	H	H	H	H
3	OCH_2O		OCH_3	OCH_3
4	$COCH_3$	OH	OH	H
5	OH	H	$COCH_3$	OH
6	OH	OH	OH	OH
7	OCH_3	OH	OH	OH
8	OH	OCH_3	OH	OCH_3
9	OH	OCH_3	OCH_3	OH

In the ^{13}C-nmr spectrum of tricrozarin A, signals corresponded to 13 carbons were observed. Among these signals, a methoxyl signal at δ 61.6 ($2\times$C), a methylenedioxy signal at δ 104.4, a signal attributed to C_{4a} and C_{8a} at δ 108.0 ($2\times$C), and a carbonyl signal at δ 182.8 ($2\times$C) were readily assigned, and three quarternary carbon signals at δ 142.6, 146.4, and 147.3 (each $2\times$C) and the positions of two methoxyls and methylenedioxy moieties remained to be assigned.

In the fully coupled ^{13}C-nmr spectrum of tricrozarin A, a signal at δ 147.3 ($2\times$C, q, $^3J_{CH}=3$ Hz) was assigned to the carbon bearing a methoxyl group. A signal at δ 142.6 ($2\times$C, dt, $^3J_{CH}=7$ and 2 Hz) was coupled to a methylenedioxy moiety and hydroxyl groups and the last signal at δ 146.4 ($2\times$C, $^2J_{CH}=5$ Hz) was assigned to the carbon bearing a hydroxyl group, respectively. So, the positions of two methoxyls and methylenedioxy groups were elucidated to be C_2 and C_3 (methoxyls) and C_6 and C_7 (methylenedioxy), respectively.

The above assignments were confirmed by long range selective decoupling experiments. When a signal at δ 6.239 (-OCH_2O-) was irradiated, the signal at δ 142.6 ($2\times$C, dt, $^3J_{CH}=7$ and 2 Hz) was changed into a doublet ($^3J_{CH}=7$ Hz), whereas no change occurred in signals at δ 146.4 and 147.3. On the other hand, when a signal at δ 12.419 (-O*H*) was irradiated, signals at δ 142.6, 146.4, and 108.0 (C_{4a} and C_{8a}, $2\times$C, d, $^3J_{CH}=6$ Hz) were changed into a triplet (δ 142.6, $^3J_{CH}=2$ Hz) and two singlets, respectively, although no change was observed in the signal at δ 147.3.

From all of the observations described above, the structure of tricrozarin A was concluded to be **1** (2,3-dimethoxy-6,7-methylenedioxynaphthazarin).

In the ^{13}C nmr of tricrozarin A [**1**], chemical shift of the carbonyl signal (δ 182.8) was similar to that of 1,4-naphthoquinone (δ 184.9) rather than that of naphthazarin [**2**] (δ 172.9) (5). So, it is obvious that **1** does not exist as tautomers between **1** and **3** but only exists as **1**, though it was reported that **2** and some of the naphthazarin derivatives cannot be accurately represented by any one species such as **4** (in the case of spinochrome A) but must be considered as a mixture of several rapidly interconverting tautomers between **4** and **5** (5-7).

So far, spinochrome E [**6**] [isolated from the spines of sea urchins (*Paracentroutus lividus* and *Psammechinus miliares*)] (8-10), namakochrome [**7**] [isolated from sea cucumber (*Polycheira rufescens*)] (11,12), 2,6-dihydroxy-3,7-dimethoxynaphthazarin [**8**], and 2,7-dihydroxy-3,6-dimethoxy naphthazarin [**9**] [isolated from star fish (*Acanthaster planci*)] (13) have been isolated as naturally occurring tetra-oxygenated naphthazarin derivatives from the above marine invertebrates, respectively. It is noteworthy that tricrozarin A [**1**] isolated and characterized herein is not only a novel naphthazarin derivative but also the first example of a tetra-oxygenated naphthazarin derivative isolated from higher plants.

Tricrozarin A [**1**] showed antimicrobial activity against *Bacillus subtilis* PCI 219 (MIC 5.1 μg/ml), *Micrococcus luteus* ATCC 9341 (MIC 112.0 μg/ml), *Aspergillus niger* ATCC 6275 (MIC 81.3 μg/ml), *Mucor racemosus* IFO 5403 (MIC 10.2 μg/ml), *Candida albicans* KF 1 (MIC 81.3 μg/ml), and *Saccharomyces sake* KF 26 (MIC 81.3 μg/ml).

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. ^1H- 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 the Kagoshima prefecture, Japan, and voucher specimens are preserved in our laboratory.

EXTRACTION AND FRACTIONATION.—Fresh bulbs (5.0 kg) of *T. crocosmaeflora* were mixed with dry ice (20 kg) and crushed using a Hosokawa FM-1 Feather mill (Hosokawa Iron Works, Osaka, Japan). The crushed bulbs were extracted with MeOH (3×74.0 liters) at room temperature, and the combined MeOH extracts were concentrated in vacuo to ca. 4 liters. To this extract C_6H_6 (2×4.0 liters) was added, and the combined C_6H_6 layers were dried over Na_2SO_4 (anhydrous) and concentrated in vacuo to give a reddish brown solid (3.7 g). The C_6H_6 layer was chromatographed over Si gel (100 g) using $CHCl_3$/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), respectively.

ISOLATION OF TRICROZARIN A [**1**].—Fraction B (0.62 g) was chromatographed over Sephadex LH-20 (ϕ2.0×50 cm) using MeOH as solvent, and the fractions containing tricrozarin A [**1**] were collected and further purified by preparative tlc using $CHCl_3$ as solvent. Tricrozarin A [**1**] was recrystallized from MeOH to give deep red needles (5.7 mg), mp 187°; ir ν max (KBr) 3445, 2960, 2930, 2860, 1605, 1449, 1364, 1284, 1245, 1215, 1169, 1130, 1069, 1016, 928, 767, 743 cm^{-1}; uv λ max (MeOH) 236, 264, 341, 481, 529 (sh), 582 (sh) nm; uv λ max (MeOH-HCl) 235, 266, 342, 473, 526 (sh) nm; uv λ max (MeOH-NaOH) 242, 346, 506 (sh), 547, 585 nm; hrms *m/z* 294.034 (294.038 calcd. for $C_{13}H_{10}O_8$), 279.010 (279.007 calcd. for $C_{12}H_7O_8$), and 264.025 (264.028 calcd. for $C_{12}H_8O_7$); ^1H nmr (400 MHz, $CDCl_3$) δ 4.109 (s, 6H, C_2- and C_3-OCH_3), 6.239 (s, 2H, -OCH_2O-), 12.419 (s, 2H, C_5- and C_8 -*OH*); ^{13}C nmr (100 MHz, $CDCl_3$) δ 61.6 (2×C, C_2- and C_3-OCH_3), 104.4 (-OCH_2O-), 108.0 (2×C, C_{4a} and C_{8a}), 142.6 (2×C, C_6 and C_7), 146.4 (2×C, C_5 and C_8), 147.3 (2×C, C_2 and C_3), 182.8 (2×C, C_1 and C_4).

ANTIMICROBIAL TESTS OF TRICROZARIN A [**1**].—The antimicrobial spectrum of tricrozarin A [**1**]

was determined using 8-mm paper discs and Mueller-Hinton agar medium (Difco) for bacteria and potato broth agar medium for fungi or yeasts. Antimicrobial activity was observed after 24 h at 37° for bacteria and longer incubation at 27° for fungi and yeasts.

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