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ISOLATION AND IDENTIFICATION OF A STILBENE DERIVATIVE FROM THE ANTARCTIC SPONGE *KIRKPATRICKIA VARIOLOSA*

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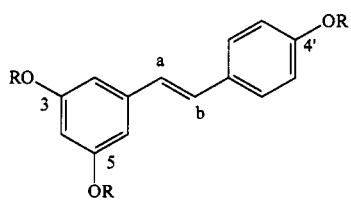
ABSTRACT.—The Antarctic red sponge *Kirkpatrickia variolosa* yielded a new stilbene derivative, resveratrol triacetate (3,4′,5-triacetoxystilbene, **1**), in addition to a known variolin alkaloid. The isolation, characterization, and unambiguous assignments of the ^{1}H- and ^{13}C-nmr spectra of **1** are reported.

Recent studies have shown that Antarctic marine invertebrates, despite their lack of predation by fish (1), are capable of producing substances with a variety of biological activities. For example, the Antarctic sponges *Latrunculia apicalis* and *Dendrilla membranosa* produce compounds that have been shown to exhibit cytotoxicity, antimicrobial activity, and deterrence toward the major Antarctic sponge predator, the sea star *Perknaster fuscus* (2–6), while the pelagic pteropod *Clione antarctica* produces the fish-feeding deterrent pteroenone (7,8).

In our continuing evaluation of the chemical ecology of the Antarctic benthos, we have examined the conspicuous red sponge *Kirkpatrickia variolosa* Kirkpatrick (family Myxillidae, order Poecilosclerida), which has been reported recently to elaborate several pyridopyrrolopyrimidine alkaloids, the variolins (9,10). The variolins display cytotoxic, antiviral, and antifungal properties (9,10) and are deterrent toward the spongivorous *P. fuscus* (6). During these investigations of the pigments from *K. variolosa*, we have found a new natural product stilbene derivative, resveratrol triacetate (3,4′,5-triacetoxystilbene) [**1**], which we wish to report in this communication, along with accurate ^{1}H- and ^{13}C-nmr chemical shift assignments.

Compound **1** was isolated as an amorphous solid and displayed a mol wt of 354 by lsims ([M^{+}+H] *m/z* 355). The ^{1}H-nmr spectrum of **1** (Table 1) suggested the presence of a trans-disubstituted double bond flanked by quaternary carbons based on the presence of two doublet olefinic proton resonances at δ 7.22 and δ 7.32 (J=16.4 Hz). Further analysis of the ^{1}H-nmr data showed a three-proton spin-system consisting of two doublets at δ 7.25 and one triplet at δ 6.86, indicative of a 1,3,5-trisubstituted aromatic ring. Two additional doublets, at δ 7.14 and δ 7.65, each integrating for two protons, indicated the presence of a second 1,4-disubstituted, aromatic moiety. Compound **1** also displayed a singlet at δ 2.27, integrating for six hydrogens, as well as a second 3-hydrogen singlet at δ 2.26, suggesting the presence of three acetoxyl methyl groups. Thus, two aromatic rings, a trans-olefin, and three acetoxy groups accounted for the observed molecular formula.

The ^{13}C-nmr spectrum of **1** (Table 1) confirmed the presence of 20 carbon atoms including three acetoxy groups (δ 21.0, 169.5, and 169.6). ^{1}H-^{1}H COSY



1 R=Ac
2 R=H

TABLE 1. ^{1}H- and ^{13}C-Nmr Assignments [δ, multiplicity, *J* (Hz)] of Resveratrol Triacetate [**1**].[a]

Position	^{1}H	^{13}C
1		140.6
2	7.25 (d, 1.98)	117.9
3		152.7
4	6.86 (t, 1.98)	115.8
5		152.7
6	7.25 (d, 1.98)	117.9
1′		135.5
2′	7.65 (d, 8.5)	128.5
3′	7.14 (d, 8.5)	123.1
4′		151.8
5′	7.14 (d, 8.5)	123.1
6′	7.65 (d, 8.5)	128.5
a	7.22 (d, 16.4)	127.9
b	7.32 (d, 16.4)	130.5
3x-CH_3CO ..	2.26 (s), 2.27 (s)	21.0
3x-CH_3CO ..		169.5, 169.6

[a]Recorded in Me_2CO-d_6 at 360 MHz (^{1}H) or 90 MHz (^{13}C); chemical shift values reported as δ (ppm) relative to solvent (δ_H=2.04, δ_C=29.9).

relationships permitted the assignment of aromatic proton chemical shifts and also supported the substitution pattern. ^{1}H-^{13}C one-bond (HMQC) data were used to assign carbon resonances (Table) to their attached protons. ^{1}H-^{13}C three-bond (HMBC) correlations from the H-2 and H-6 proton resonances at δ 7.25 to the olefinic carbon resonance at δ 127.9 identified the latter as C_a. This further confirmed the chemical shift assignment of the two olefinic protons H_a and H_b of resveratrol [**2**] carried out by nOe experiments (11). Further two-bond HMBC correlations to the carbon resonance at δ 152.7 from the two-proton resonance (δ 7.25) fixed the C-3 and C-5 assignments. The third oxygen-bearing carbon (δ 151.8) showed long-range correlation from H-2′ and H-6′ (δ 7.65) while C-1′, resonating at δ 135.5, correlated to a proton signal at δ 7.14 (H-3′ and H-5′).

The identity of **1** was further confirmed by acetylation (Ac_2O/pyridine) of an authentic sample of resveratrol [**2**], yielding a triacetate identical in all respects to the natural product.

To the best of our knowledge this is the first report of the occurrence of a stilbene in a marine organism, although similar structural types include a chalcone, found in the scleractinian coral *Tubastrea micrantha* (12), and a nor-isoflavone isolated from marine snail, *Nerita albiella* (13). A literature search revealed that compound **1** is known previously as a semi-synthetic compound; this report contained errors in nmr chemical shift assignments (14). Stilbenes are found in many plant genera, and some are considered to be phytoalexins (15,16). They exhibit a variety of biological and pharmacological activities including protein tyrosine kinase (PTK) and protein kinase C (PKC) inhibitory effects (11). The reports of biological studies conducted with a number of stilbene and flavonoid compounds, and of kinase inhibition in particular, indicate the importance of free phenolic functions for the observed biological effects (11,17). Resveratrol triacetate [**1**] is therefore expected to be inactive as a kinase inhibitor.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—High-field nmr spectra were recorded at 360 MHz for ^{1}H and 90 MHz for ^{13}C on a Bruker AMX 360 instrument. Lsims was measured on a Finnigan MAT 95Q spectrophotometer at the University of Florida. The uv spectrum was obtained on a Hewlett-Packard 8452A diode-array spectrometer. Si gel 60 (230–400 mesh) was used for cc. A Waters 401 hplc system with a Waters 490E uv detector and Alltech normal-phase (10×250 mm, 5 μm) Si gel column were used for hplc.

SPONGE MATERIAL.—*Kirkpatrickia variolosa* samples were collected using scuba at depths of between 30 and 40 m from Hut Point and Danger Slopes on Ross Island, Antarctica. A voucher specimen of *K. variolosa* is on hand in the Biology Department, University of Alabama at Birmingham.

EXTRACTION AND ISOLATION.—The freeze-dried sponge material (100 g) was extracted sequentially with hexane, $CHCl_3$, MeOH, and MeOH-H_2O (7:3). The concentrated $CHCl_3$ extract (45 mg) was chromatographed by Si gel flash cc. Gradient elution from 0–15% MeOH in CH_2Cl_2 afforded four fractions. The fraction eluted with CH_2Cl_2 was further purified twice by hplc employing a normal-phase silica column eluting with 2% MeOH in CH_2Cl_2 to yield **1** as white solid (1.6 mg). A yellow pigment in the aqueous MeOH extract was isolated by LH-20 cc (MeOH) and

repeated reversed phase hplc (50% aqueous MeOH), and was identified as variolin A by comparison of its ^{1}H- and ^{13}C-nmr data with those previously reported (10).

Resveratrol triacetate [**1**].—Amorphous white solid (MeOH): mp 115–118° [lit. (8) 118.5–119.5°]; uv λ max (EtOH) (log ε) 300 (4.40), 312 (4.39), 326 (4.16) nm; ^{1}H- and ^{13}C-nmr data, see Table 1; lsims *m/z* [M^{+}+H] 355.

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