NOTES

A THIRD METABOLITE FROM *SPICARIA DIVARICATA* NRRL 5771

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The purification, structures and antimicrobial activities of two pharmacologically active metabolites from *Spicaria divaricata* NRRL 5771 (*Paecilomyces varioti* BAINIER), SC–28762, 3, 3′-di-(methoxycarbonylmethyl)-3,3′,4,4′-tetrahydro-9, 9′, 10, 10′-tetrahydroxy-7, 7′-dimethoxy-1, 1′-dioxo-8, 8′-bi-1H-naphtho-[2, 3-c] pyran (viriditoxin)[2] and SC–28763, 3-(2-oxopropyl)- 3′-methoxycarbonylmethyl-3, 3′, 4, 4′-tetrahydro-9, 9′, 10, 10′-tetrahydroxy-7, 7′-dimethoxy-1, 1′-dioxo-8, 8′-bi-1H-naphtho [2, 3-c] pyran were reported previously[1]. Since that publication, a third metabolite, SC–30532, 3, 3′-di-(2-oxopropyl)-3, 3′, 4, 4′-tetrahydro-9, 9′, 10, 10′-tetrahydroxy-7, 7′-dimethoxy-1, 1′-dioxo-8, 8′-bi-1H-naphtho [2, 3-c] pyran, with antianaerobic activity was discovered in the fermentation extract.

A fraction enriched with SC–30532 was obtained from the mother liquor of SC–28763. Purification was achieved using low pressure liquid chromatography (LPLC)[3]. The columns were eluted with solvent systems consisting of methylene chloride and ethyl acetate in a gradient mode at 100 psi. The fractions were monitored by thin-layer chromatography (TLC). The TLC plates were developed with chloroform - ethanol - formic acid (90: 4: 6, v/v). The developed plates were observed under short wavelength ultraviolet light at 254 nm. SC–30532, eluted with methylene chloride - ethyl acetate (80: 20), was crystallized from methylene chloride - ethyl acetate as greenish crystals with m. p. of 224~227°C (dec.).

Physicochemical Properties

The physicochemical properties of SC–30532

Table 1.

Physicochemical properties	SC–30532
Melting point (0°C)	224~227°C (dec)
Elementary analysis	Found / Calcd.
C	64.16 / 64.76
H	4.79 / 4.80
O	30.42 / 30.45
Mass spectral	
M+	630
M−44	586
M−44−18	568
M−44−18−18	550
M−44−44	542
M−44−44−18	524
M−44−44−32	510
M−44−44−18−18	506
Molecular formula	$C_{34}H_{30}O_{12}$
Molecular weight	630.58
Ultraviolet absorption (acetonitrile)	225 nm (ε31,500) 262 nm (ε70,800) 274 nm (ε56,800) 340 nm (ε14,100) 370 nm (ε20,500) 388 nm (ε18,900)
Infrared absorption (chloroform)	3420, 1730, 1660, 1640, 1590, 1390, 1320, 1265 cm^{-1}
Optical rotation $[\alpha]_D^{28}$ (chloroform)	−190.5°
Circular dichroism (chloroform)	$[\theta]_{305}$ 0, $[\theta]_{276}$ −745,000 $[\theta]_{265}$ 0, $[\theta]_{257}$ +169,000
Optical rotatory dispersion (chloroform)	a = −13,915

Fig. 1.

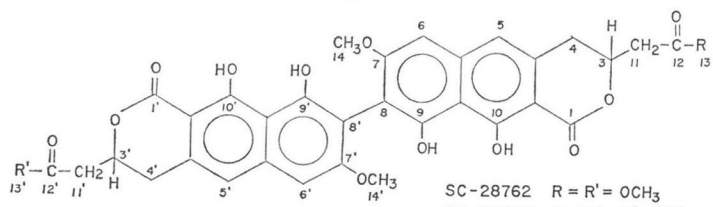

Table 2. ^{1}H NMR of SC-30532

Proton	Chemical shift (ppm) and multiplicity	Coupling constants (Hz)
3-H	4.98, ddt	$J_{3,4a}=6.5$, $J_{3,4b}=6.0$, $J_{3,11}=7.5$ (1)
3'-H	4.98, ddt	
4a-H	2.77, dd	$J_{4a,b}=17.0$, $J_{4a,3}=6.5$
4'a-H	2.77, dd	
4b-H	3.08, dd	$J_{4a,b}=17.0$, $J_{4b,3}=6.0$
4'b-H	3.08, dd	
5-H	6.27, broad, s	
5'-H	6.27, broad, s	
6-H	6.80, s	
6'-H	6.81, s	
9-OH	9.72, broad, s	
9'-OH	9.72, broad, s	
10-OH	13.73, s	
10'-OH	13.73, s	
11-H_2	2.85, d	$J_{11,3}=7.5$ (1)
11'-H_2	2.85, d	
13-H_3	2.21, s	
13'-H_3	2.21, s	
14-H_3	3.78, s	
14'-H_3	3.80, s	

Note: (1) 4a- and 11-protons are overlapped, J is estimated.

Table 3. ^{13}C NMR of SC-30532

Carbon number	Chemical shift (ppm)
1	170.7
3	75.4
4	33.0
4a	139.1 (c)
5	114.0
5a	107.9 (a)
6	98.1
7	158.9
8	98.6 (8'=98.7)
9	160.8 (b) (9'=160.7)
9a	109.9 (a)
10	163.0 (b)
10a	132.0 (c) (10a'=132.1)
11	47.8
12	203.7
13	30.7
14	56.1

Notes: a, b, c-assignments may be reversed.
Solvent, deuterated chloroform.
Trimethyl silane as standard reference.

are summarized in Tables 1, 2 and 3. The elementary analysis and mass spectral data are consistent for a compound of $C_{34}H_{30}O_{12}$.

Both ultraviolet (UV) and infrared (IR) spectra of SC-30532 are quite similar to those of SC-28763. In the IR spectrum, the absence of the 1743 cm^{-1} band in SC-30532 indicated the absence of a methoxycarbonyl group and the enhancement of the 1730 cm^{-1} band indicated the presence of an additional acetyl group.

The proton nuclear magnetic resonance (NMR) of SC-30532 exhibits a chemical shift at 2.21 ppm of twice the integrated value of SC-28763, whereas the chemical shift at 3.72 ppm is absent. This suggests that SC-30532 contains two acetyl groups and no methoxycarbonyl group (Fig. 1). This is corroborated by ^{13}C NMR in which the intensity of the chemical shift at 203.7 ppm of the carbonyl carbon of the acetyl groups in enhanced, whereas the chemical shift at 170.5 ppm of the carbonyl carbon of the methoxycarbonyl is absent. All the data are consistent for the structure assigned to SC-30532.

Microbiological Properties

The antimicrobial properties of SC-30532 are reported in Table 4. Tests were conducted by two-fold serial dilution in liquid media, the highest concentration of the compound being 1,000 μg/ml. The antimicrobial activities against the aerobic and facultative anaerobic bacteria *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella paratyphi* ATCC 11737 were determined in Typticase Soy Broth (Bioquest, Cockeysville, Md.); against the anaerobic bacterium *Clostridium perfringens*

Table 4. Minimal inhibitory concentration of SC-30532

	μg/ml
Staphylococcus aureus ATCC 6538	125
Salmonella paratyphi A ATCC 11737	>1,000
Pseudomonas aeruginosa ATCC 9027	>1,000
Clostridium perfringens ATCC 13124	16
Candida albicans ATCC 10231	>1,000
Candida albicans M41-106	>1,000
Trichophyton mentagrophytes ATCC 10270	>1,000
Microsporum gypseum M41-10	>1,000
Verticillium albo-atrum M64-2	125
Trichomonas vaginalis ATCC 30021	1

ATCC 13124 in Fluid Thioglycollate Broth (Bioquest); against the fungi *Microsporum gypseum* M41–10, *Trichophyton mentagrophytes* ATCC 10270, *Verticillium albo-atrum* M64–2, *Candida albicans* ATCC 10231 and *Candida albicans* M41–106 in SABOURAUD Dextrose Broth (Difco Laboratories, Detroit, Mi.) and against the anaerobic protozoan *Trichomonas vaginalis* ATCC 30001 in Diamond Medium[4] containing 5% horse serum.

SC–30532 inhibited the anaerobic organisms, *Cl. perfringens* and *T. vaginalis*, at low concentrations but demonstrated little or no activity against the aerobic and facultative anaerobic bacteria and the fungi. This activity was similar to that shown by SC–28763.

In comparing the structures, it is of interest that when methoxycarbonyl groups are present as in SC–28762, there is little or no antianaerobic activity against the microorganisms tested. When one or two methylcarbonyl groups are present in place of the methoxycarbonyl groups as in SC–28763 and SC–30532, there is a good antianaerobic activity.

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