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Siccanol: Sesterterpene Isolated from Pathogenic Fungus *Drechslera Siccans*

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Abstract : A bicyclic sesterterpene from *Drechslera siccans* was purified and structurally characterized. During the isolation procedure, the toxic component was monitored by the assay using Italian ryegrass (*Lolium multiflorum* Lam.), one of the host plants of the pathogen. Siccanol (**1**) named arbitrarily was shown to have a molecular formula of $C_{25}H_{38}O_4$. Siccanol completely inhibited the root growth of the Italian ryegrass seedlings at a level of 100 ppm.(Received February 13, 1996; accepted April 22, 1996)

Introduction

Drechslera siccans[1] is a pathogenic fungus which affects oats (*Avena sativa* L.) perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*Lolium multiflorum* Lam.). Fungal infections are characterized by dark brown irregular spots on the leaves of these hosts. The spots usually occur within one week after inoculation. Occasionally, these lesions have tan centers, which resemble an "eye-spot". These observations are consistent with the involvement of toxin(s) of some type, which are produced by the pathogenic fungus.[2]

In this paper, we report the isolation and the structure determination of a sesterterpene, named Siccanol, from *Drechslera siccans*, which shows toxic activity on Italian ryegrass. Although the fungus has already been reported to produce a phytotoxin, its chemical nature is markedly different from terpenes.[3-4]

Materials and Methods

Fungus and plant

A culture of *Drechslera siccans* was kindly given by Dr. M. Tsuda of Pesticide Research Institute at Kyoto University.

Italian ryegrass was used for the bioassay, the seeds of which were purchased from Takii seed Co (Kyoto, Japan).

Bioassay Methods

A specified amount of the test compound dissolved in MeOH was applied to a petri dish (35 mm in diameter) in which two layers of filter paper were laid. After the solvent was evaporated, 1 m*l* of water containing Tween 20 (Nakarai Chemicals Ltd., Kyoto, Japan) was added to this dish to dissolve the test compound. Nine germinated seedling (ca 5 mm) of plant were placed in the dish and grown in the dark at 26℃ for 72 hours. The inhibitory activity was evaluated by comparing the root length to that of the control.

Spectral Measurements

Optical rotations were measured on a JASCO model J-5 in MeOH solution at 22℃. The IR spectra were determined on a HORITA FT-200 ($CHCl_3$ solution). Electron ionization mass spectra (EI-MS) were obtained on a Hitachi M-80A. The UV spectra were measured on a Shimazu UV-3000. The ^1H and ^{13}C NMR spectra were determined on a Brucker AC 500 (500 MHz for ^1H and 125 MHz for ^{13}C) in $CDCl_3$, using the tetramethylsilane (TMS) signal as an internal standard. High perfomance liquid chromatography (HPLC) separation were perfomed on a Hitachi L-6200 apparatus equipped with L-4200H UV-Vis detector using a Cosmosil octadecyl silica (ODS) C18 column (20×250 mm i.d.).

Isolation of Siccanol

The fungus *D. siccans* was cultured on potato-sucrose agar plates (500 plates, 90 mm in diameter) in the dark at 26℃ for 14 days. The culture plates were then macerated with acetone, filtered and the filtrate condensed at 40℃ to give an aqueous residue, which was extracted

Key words : Sesterterpene, *Drechslera siccans*, Siccanol, Phytotoxins, Terpestacin, proliferin.
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Table 1. NMR(^{1}H, 500 MHz; ^{13}C 125 MHz) data for siccanol and its triacetyl derivative in $CDCl_3$

Position	Siccanol(**1**)		Triacetyl derivative(**2**)
	δC	δH	δH
1	49.6d[a]	2.72(dd, 11.4, 2.1)[b]	2.83(dd, 11.4, 2.1)
2	28.8t	2.08(m), 2.44(d, 9.0)	2.08(m)c, 2.40(d, 9.0)
3	128.9d	5.38(dd, 5.9, 3.5)	5.48(dd, 5.9, 3.5)
4	136.5s	–	–
5	76.5d	4.07(dd, 9.7, 3.6)	5.16[c]
6	29.8t	1.70(m), 1.75(m)	1.70, 1.84
7	34.9t	1.78(m), 2.18(m)	1.82
8	132.9s	–	–
9	124.3d	5.13(m)	5.13[c]
10	23.8t	2.11(m), 2.26(m)[c]	2.11, 2.20
11	40.3t	2.01(m), 2.24(m)[c]	2.01, 2.20
12	138.0s	–	–
13	121.5d	5.25(dd, 10.4, 5.4)	5.25(dd, 10.4, 5.4)
14	39.9t	1.75(M)[c]	1.75
		2.36(dd, 13.7, 10.6)	2.36(dd, 13.7, 10.6)
15	49.0s	–	–
16	207.8s	–	–
17	148.8s	–	–
18	146.6s	–	–
19	37.1d	2.66(m)	2.89(m)
20	66.1t	3.80(dd, 10.4, 5.5)	4.15
		3.85(dd, 10.4, 7.0)	
21	10.4q	1.56(s)	1.57(s)
22	15.3q	1.64(s)	1.63(s)
23	15.6q	1.65(s)	1.64(s)
24	16.2q	0.99(s)	1.00(s)
25	14.4q	1.29(d, 7.3)	1.23(d, 7.3)
$COCH_3$			2.01(s)
$COCH_3$			2.02(s)
$COCH_3$			2.26(s)

[a]All assignments are based on the results of ^{13}C-^{1}H COSY and INEPT., [b]Proton signal multiplicity and coupling constant(J=Hz) are in parentheses., [c]Overlapping multiplet signal.

with a mixture of hexane, ethyl acetate and benzene (1 : 1 : 1, v/v/v). After evaporating the solvent *in vacuo*, the concentrated organic component (4.6 g) was subjected to silica gel (230 g) column chromatography, using a solvent gradient system from n-hexane to EtOAc and then to MeOH. During the isolation procedure, the toxic component was monitored by the assay using Italian ryegrass seedlings. The active fractions eluted with the 100% EtOAc and MeOH were further purified by with a Cosmosil ODS column and solvent system of 80% aq. MeOH, to give an active compound (18 mg).

Physicochemical data for Siccanol (1).

UV (MeOH) λmax(ε) nm: 233 (950), 263 (1380); $[\alpha]_D^{15}$-23°(c 0.6, MeOH); IR ν($CHCl_3$): 3500, 3020, 1699, 1670, 1220, 1211 cm^{-1}; EI-MS(m/z) 402(M^+, 40), 384(39), 353 (5), 257(4), 248(7), 215(13), 187(16), 147(42), 137(68), 93 (58), 81(100), 55(68); ^{1}H- and ^{13}C-NMR: see Table 1.

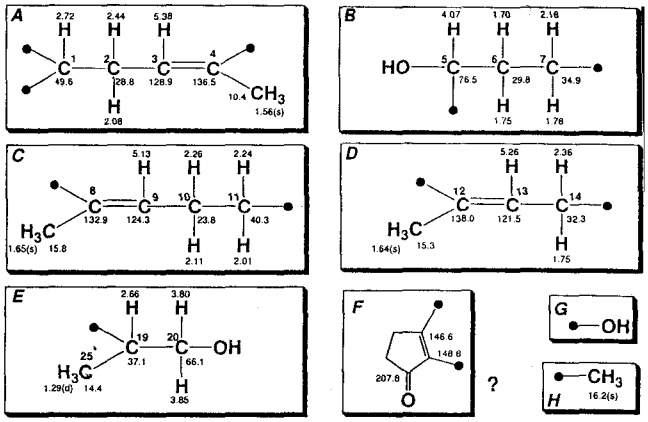


Fig. 1. Partial structures of siccanol. The sequences were confirmed by ^{1}H-^{1}H and ^{13}C-^{1}H COSY NMR spectra.

Acetylation of Siccanol.

Siccanol (5 mg) was treated with Ac_2O (500μl) and pyridine (500μl) at 40℃ for 10 hours. The product was purified by chromatography on Cosmosil ODS-18 column eluted with 80% aq. MeOH to give a pure triacetyl derivative (**2**. 8.6 mg, Pale-yellow oil). mp:$[\alpha]_D^{22}$-53°(c 0.68, MeOH); EI-MS (m/z): 528(M^+, 15) 486(6), 468(55), 426 (65), 408(12), 384(6), 366(22), 257(7), 217(35), 175(18), 147 (52), 133(48), 93(52), 81(75), 43(100); ^{1}H-NMR(500 MHz, $CDCl^3$): see Table 1.

Results

The molecular formula of Siccanol was determined as $C_{25}H_{38}O_4$ on the basis of HR-EI-MS (obsd. m/z of 402.2776, calcd. 402.2771). The IR spectrum indicated the presence of OH (3500 cm^{-1}) and carbonyl (1699 cm^{-1}) groups. The ^{1}H-NMR spectrum of siccanol gave signals assignable to 35 protons, and ^{13}C-NMR spectrum with an aid of INEPT experiment showed the presence of 5 CH_3 groups, 7 CH_2 groups, 6 CH groups and 7 non proton-bearing carbon atoms. A peak at δC 207.8 ppm suggested the carbonyl group of siccanol to be attributed to ketone form. Also, total 8 signals of sp^2 carbons were observed, suggesting the presence of four pairs of C=C units. The relationship between the proton and the carbon signals were determined based on the analysis of the correlation signals in ^{13}C-^{1}H COSY spectrum.

Acetylation of siccanol afforded a triacetate derivative, suggesting the presence of 3 alcoholic hydroxy groups in siccanol.

The molecular formula indicated the degree of unsaturation of siccanol to be 7. Since one C=O group and four C=C double bonds are involved, as described above, siccanol was considered to have two ring struc-

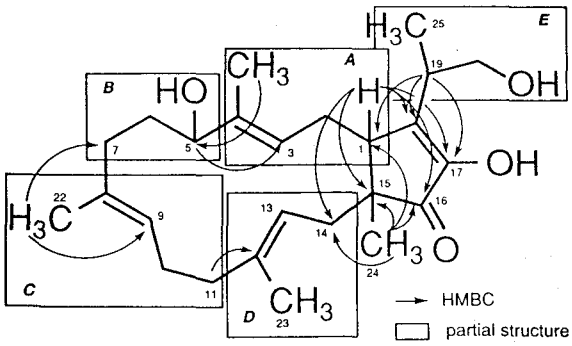


Fig. 2. Observed signal correlations ^{1}H-^{1}H COSY and HMBC experiments for siccanol. The partial structures were confirmed by ^{1}H-^{1}H and ^{13}C-^{1}H COSY NMR spectra.

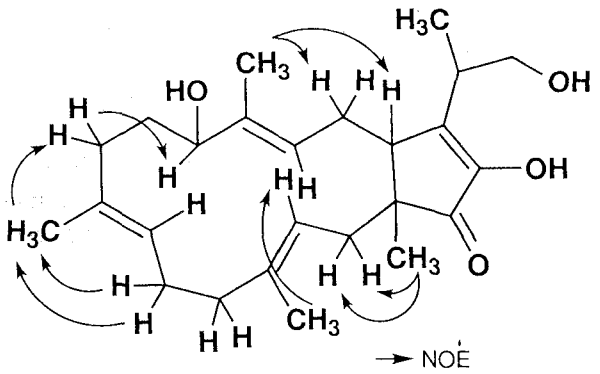
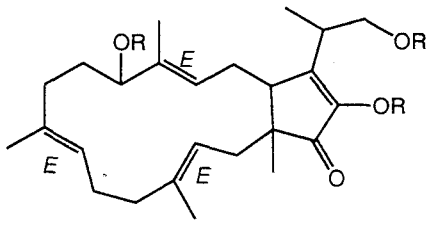


Fig. 3. Significant NOE correlations in siccanol.



1: R = H
2: R = Ac

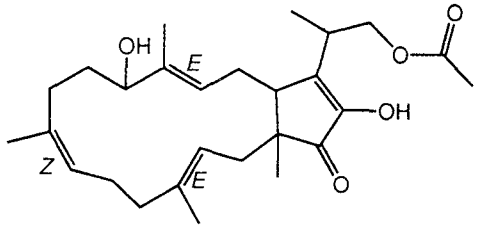


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Fig. 4. Structures of siccanol(1), triacetate siccanol(2) and proliferin(3).

ture.

Based on 1H-1H COSY experiment eight partial structures (A-H, Fig. 1) were constructed, where two hydroxyl groups were allocated in the partial structures B and E, taking each of the carbon chemical shifts (δc 76.5 at C-5 and δc 66.1 ppm at C-20) into account.

These partial structures were combined together by analyzing the HMBC spectrum. As shown in Fig. 2, siccanol was proposed to be a rather unusual bicyclic sesterterpene, which was constituted from 5 and 15-membered rings.

In the 15-membered ring of siccanol, there are three carbon-carbon double bonds, C(3)=C(4), C(8)=C(9) and C(12)=C(13). All three configuration were presumed to be *E* by ROESY experiments, since no correlation was observed between the methyl proton and the olefinic proton for each of C=C double bond moiety. It is generally well known that a methyl group of trisubstituted olefins located in *trans* position of a olefinic proton is more shielded to give a chemical shift of δc 15~20 ppm, than that in *cis* position (23~26 ppm)[5]. Therefore, rather shielded carbon chemical shifts of the methyl groups, C-21(δ 10.4), C-22(δ 15.3) and C-23(δ 15.6), also supported this presumption. The stereochemistry relative to the ring fusion was also presumed to be *trans*, since no correlation was observed between the protons H-1 and H-24. The absolute structure of siccanol in terms of C-1, C-5, C-15, and C-19 were not determined in this study.

Discussion

Phytotoxin from *D. siccans*, arbitrarily named siccanol (**1**), was determined to be a bicyclic sesterterpene. The deduced planar structure was identical with that of terpestacin[6-8], a syncytium formation inhibitor that might act as an AIDS-controlling agent, isolated from the culture of a fungi *Arthrinium* sp. The NMR spectroscopic data of siccanol were in good agreement with those of terpestacin. However, the stereochemistry of these compounds could be different, because their optical rotations were opposite: $[\alpha]_D^{15}$ (c 0.6 MeOH) for siccanol was −23°, while for terpestacin it was reported to be $[\alpha]_D^{22}$ +26°(c 0.5 $CHCl_3$). Further investigation is required to determine the absolute configurations for siccanol. The structure of siccanol is also closely related to proliferin (**3**)[9], which was isolated from *Fusarium proliferatum* as a cytotoxic compound to brine shrimp(*Artemia salina*). The differences between siccanol and proliferin are the substituents at C-20 and the configurations of the C(8)=C(9) double bond. However, the triacetate of siccanol had spectral data in agreement with that reported for the diacetate of proliferin, suggesting that siccanol has the same absolute structure as proliferin. In addtion, the chemical shift of C-22 of proliferin is almost the same as that of siccanol. Reviewing the report of proliferin carefully, the conclusion drawn for the configuration of the C(8)=C(9) appears to be based on the noise signal

observed in the NOESY experiment. Therefore, the correction in terms of the configuration of this double bond of proliferin is required. No information is available about the stereochemistry of proliferin.

Siccanol completely inhibited the root growth of Italian ryegrass seedlings, one of the host plants of the fungus, at the level of 100 ppm. It was considered that siccanol played some roles in the expression of disease symptoms by this fungus.

Acknowledgments

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식물 병원균 *Drechslera siccans*로 부터 분리한 세스터테르펜류 Siccanol의 구조

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초록 : *Drechslera siccans*의 배양 추출물로부터 이탈리안 라이그라스 (*Lolium multiflorum* Lam.) 뿌리의 생육저해 활성을 나타내는 물질을 분리정제한 다음에 2차원 NMR을 포함한 각종 기기분석을 이용하여 구조를 결정하였다. Siccanol (**1**)이라고 명명한 이 화합물의 분자식은 $C_{25}H_{38}O_4$이었으며 100 ppm에서 숙주 식물중의 하나인 이탈리안 라이그라스에 대하여 100%의 저해 활성을 나타내었다.

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