Structures of (–)-Mycousnine, (+)-Isomycousnine and (+)-Oxymycousnine, New Usnic Acid Derivatives from Phytopathogenic *Mycosphaerella nawae*

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The structures of three novel phytotoxins named (–)-mycousnine (II), (+)-isomycousnine (III) and (+)-oxymycousnine (IV), from the phytopathogenic fungus *Mycosphaerella nawae*, were elucidated on the basis of spectroscopic and chemical evidence. ¹H- and ¹³C-NMR signals of II, III and IV were assigned by ¹H-¹³C COSY, ¹³C-{¹H} LSPD, HMBC and NOESY. II and III were obtained directly from (+)-usnic acid (I) by heating with 15% hydrogen chloride in dry MeOH, and IV from II by H₂O₂ oxidation in pyridine. Methanolysis of IV gave a novel enonediol (V). II and III showed potent antimicrobial properties. These new metabolites are the first usnic acid derivatives isolated from fungi.

The isolation and structures of potent phytotoxins (–)-mycousnine (II) and (+)-isomycousnine (III), from *Mycosphaerella nawae*, which is a pathogen causing circular leaf spot disease of Japanese persimmon (*Diospyros kaki*), have been communicated in a previous report.^{1),*1} In subsequent studies, another phytotoxic usnic acid derivative, (+)-oxymycousnine (IV), was isolated and found to have an uncommon acylated enonediol partial structure. The present paper reports the structural determination of II, III and IV, including a full assignment of their NMR signals, and also the antimicrobial activities of these compounds. These metabolites are the first examples of compounds isolated from fungi that are directly related to biologically active²⁾ (+)-usnic acid (I).³⁾

Isolation of usnic acid derivatives

M. nawae was seed-cultured for 7 days at 24°C in constantly shaken Sakaguchi flasks with a medium containing maltose syrup, soybean flour, Pharmamedia and minor

nutrients. Numerous globular mycelia in this culture broth were inoculated into a 2% malt extract–2% commercial sugar–0.1% peptone medium, and were similarly cultured for 15–18 days. An EtOAc extract (240 mg) of the culture filtrate (4.0 l, pH 5.2) was separated on a BW-820MH silica gel column with *n*-hexane–EtOH mixtures containing AcOH or HCOOH. The eluent by 5:1 *n*-hexane–EtOH containing 0.5% AcOH gave II (mp 86–89°C, [α]_D –91°) and III (mp 78–79°C, [α]_D +146°).¹⁾ A fraction obtained by eluting with 5:2 *n*-hexane–EtOH containing 0.5% HCOOH was rechromatographed on a CQ-3 silica gel column (15:1 benzene–EtOAc containing 0.5% HCOOH) and gave colorless crystalline (+)-oxymycousnine (15 mg, mp 119–122°C, [α]_D +106°). Its dose-based phytotoxicity against leaves of Japanese persimmon was about one-tenth that of II and III.¹⁾ (+)-Oxymycousnine, however, was not detected in EtOAc extracts obtained from the potato-dextrose agar medium inoculated with this fungus.¹⁾

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*1 These compounds were designated as 'mycousnines' in ref. 1, but their names have been changed in this paper to 'mycousnines,' in accordance to the accepted name of usnic acid. The name 'usnic acid' (including this name in derivatives) was also used in the ref. 1 to designate 'usnic acid.' These misnomers have thus been corrected here.

Structures of (–)-mycousnine (II) and (+)-isomycousnine (III)

II and III had the same molecular formula $C_{19}H_{20}O_8$ determined by HR-EIMS, and their spectral properties were closely similar to each other. II was presumed to be identical to (–)-usnic acid isomethoxide of unknown stereochemistry by a comparison of their molecular formula and spectral properties.⁴⁾ This was confirmed by its preparation from I¹⁾; I (177 mg) heated with 15% hydrogen chloride in dry MeOH afforded II, which was identifiable with (–)-usnic acid isomethoxide [74 mg, mp 88.5–89.5°C, $[\alpha]_D^{19} -103^\circ$ (CHCl₃); in lit.⁴⁾ mp 92–93°C, $[\alpha]_D^{25} -44^\circ$ (CHCl₃)]. Marked NOEs between C_{4a}-OMe [δ_H 3.54 (3H, s)] and both of the C₄-methylene protons [3.31 and 3.17 (ABq, $J=17$ Hz)] were observed in the ¹H-NMR spectrum of II.¹⁾ These observations indicated that the absolute configuration of the angular methyl on C_{9b} was the same as that of I¹⁾ and that the B/C ring junction was in a *cis* configuration. Thus, the stereostructure of II, (–)-usnic acid isomethoxide, was elucidated to be (4a*R*,9b*S*)-4a,9b-*cis*-2,6-diacetyl-1,4,4a,9b-tetrahydro-3,7,9-trihydroxy-4a-methoxy-8,9b-dimethyl-1-oxodibenzofuran (Fig. 1). An additional product (20 mg, mp 79.5–80.5°C, $[\alpha]_D +159^\circ$) was separated from the aforementioned reaction mixture of I and 15% hydrogen chloride in dry MeOH, and was identified as III by a comparison of mp, $[\alpha]_D$ and spectral data. Its new UV peak [ϵ 2,100] produced in an alkaline MeOH solution showed the presence of an isousnic acid skeleton in the molecule.¹⁾ The relative stereochemistry of a *cis* configuration was judged by an NOE observation between the C_{4a}-OMe [δ_H 3.50 (3H, s)] and both of the C₄-methylene protons [3.30 and 3.14 (ABq, $J=17$ Hz)]. The structure of III, therefore, was

elucidated to be that shown in Fig. 1. III was also derived from the methanolic hydrogen chloride-treated I, likely via a (4a*S*,9b*S*)-*trans*-isomer of II, by C_{4a}-O₅ bond cleavage of the cyclic ether ring, and its subsequent recyclization to the original ether ring with an isousnic acid skeleton.

These structures were further corroborated by an NMR study described later. ¹H- and ¹³C-NMR signals of II were assigned on the basis of their chemical shifts and its ¹H–¹³C COSY data, and by ¹³C-{¹H} long-range selective proton decoupling experiments (LSPD) using SPT difference spectrometry.⁵⁾ ¹H- and ¹³C-NMR data for II are shown in Table I. Two remaining olefinic carbon atoms appeared at 156.6 and 110.7 ppm in the ¹³C-NMR spectrum, and were respectively assigned from their chemical shifts to one (C_{5a}) bearing an oxygen atom and the other (C₂) bearing three carbon atoms. One (202.4 ppm) of the strongly hydrogen-bonded carbonyl carbon atoms (202.4 and 201.0 ppm) was assignable to the acetyl carbonyl carbon at 2 by a comparison

Table I. ¹³C-{¹H} LSPD AND ¹H–¹³C COSY DATA [δ (CDCl₃)] FOR (–)-MYCOUSNINE (II)

¹ H irradiated	¹³ C-{ ¹ H} LSPD data	¹ H– ¹³ C COSY data
	¹³ C with long-range coupling	
1.63	197.7, 109.6, 105.3, 59.8	1.73 ^a (1.63) ^b
2.04	163.4, 159.1, 107.2	7.2 (2.04)
2.59 and 2.56	202.4, 201.0	27.4 (2.56)
		31.2 (2.59)
3.31 and 3.17	194.6, 109.6	37.9 (3.31, 3.17)
3.54	109.6	51.3 (3.54)
9.40	159.1, 107.2	
13.36	163.4, 101.8	

^{a,b} Chemical shifts of ¹³C and ¹H, respectively.

Fig. 1. Structures of (+)-Usnic Acid (I), (–)-Mycousnine (II) and (+)-Isomycousnine (III).

Table II. ^1H - AND ^{13}C -NMR DATA (δ) FOR (–)-MYCOUSNINE (**II**, CDCl_3), (+)-ISOMYCOUSNINE (**III**, CDCl_3), (+)-OXYMYCOUSNINE (**IV**, CD_3CN) AND **V** (CD_3OD) ^1H -NMR data are in parenthesis.

Position	II	III	IV	V
1	197.7	198.0	189	— ^a
2	110.7	110.3	126.0	130.6
3	194.6	194.7	167.9	164.8
4	37.9 (3.31, 3.17) ^b	37.7 (3.30, 3.14) ^b	35.0 (3.40, 3.21) ^b	35.3 (3.37, 3.09) ^b
4a	109.6	108.7	111.5	111.8
5a	156.6	160.2	158.5	158.7
6	101.8	100.4	102.8	102.7
7	163.4	165.6	163.8	163.6
8	107.2	106.7	107.3	107.5
9	159.1	156.0	159.9	160.7
9a	105.3	104.7	108.1	109.5
9b	59.8	60.2	58.5	57.5
Methyl groups				
2-COMe	27.4 (2.56)	27.4 (2.57)		
2-OCOMe			20.6 (2.18)	
4a-OMe	51.3 (3.54)	50.6 (3.50)	51.3 (3.47)	50.0 (3.50)
6-Me		7.2 (1.99)		
6-COMe	31.2 (2.59)		31.5 (2.60)	31.2 (2.64)
8-Me	7.2 (2.04)		7.6 (1.96)	7.4 (2.00)
8-COMe		32.6 (2.71)		
9b-Me	17.3 (1.63)	16.9 (1.64)	16.6 (1.63)	16.6 (1.67)
Carbonyl groups				
2-COMe	202.4	202.3		
2-OCOMe			168.9	
6-COMe	201.0		203.0	202.9
8-COMe		203.5		
Hydroxyl groups				
2-OH				(<i>c</i> 6.0) ^c
3-OH	(18.15)	(18.22)	(<i>c</i> 7.4) ^c	(<i>c</i> 6.0) ^c
7-OH	(13.36)	(9.63)	(13.45)	(13.55) ^c
9-OH	(9.40)	(14.31)	(<i>c</i> 7.4) ^c	(<i>c</i> 6.0) ^c

^a The signal corresponding to the 1-position of **V** did not appear in ^{13}C -NMR (COM) at the low concentration employed.^b ABq ($J=18$ – 17 Hz).^c Chemical shifts in CD_3COCD_3 .

of chemical shifts of the corresponding carbonyl carbons in **II** and **III** (Table II). In addition, NOESY spectra of **II** showed clear cross peaks at δ_{H} 2.59 (3H, s, C_6 -COMe) $\rightleftharpoons$ 3.54 (3H, s, C_{4a} -OMe), 3.54 (C_{4a} -OMe) $\rightleftharpoons$ 3.31 and 3.17 (ABq, C_4 - H_2), and 9.40 (1H, s, C_9 -OH) $\rightleftharpoons$ 1.63 (3H, s, C_{9b} -Me). From these observations, the ^1H - and ^{13}C -NMR signals

of **II** were reasonably assigned as shown in Table II. The NMR signals of **III** were also assigned in a similar manner (Table II); its ^{13}C - $\{^1\text{H}\}$ LSPD revealed long-range couplings between the methyl protons at δ_{H} 1.99 (C_6 -Me) and the carbons at δ_{C} 165.6 (C_7), 160.2 (C_{5a}) and 100.4 (C_6). Chemical shift differences for the carbons at 5a and 9 in **II** and **III** are

explainable from the substituent effects of the acetyl and methyl groups on their benzene rings. Thus, NMR-signal assignments of fungal usnic acid derivatives **II** and **III** were established. The previous assignments of ^1H - and ^{13}C -NMR signals of **I** [δ_{H} 1.79 ($\text{C}_2\text{-COMe}$), 2.12 ($\text{C}_{9\text{b}}\text{-Me}$) and 2.67 ($\text{C}_8\text{-Me}$); δ_{C} 163.8 ($\text{C}_{5\text{a}}$), 157.4 (C_7) and 155.1 (C_9)]⁶ need partial revision.

(-)-Pseudoplacodiolic acid has been isolated from lichen *Rhizoplaca chryssoleuca*, and its stereostructure, the (4a*R*,9b*R*)-4a,9b-*trans*-stereoisomer of **II**, has been established by an X-ray crystal analysis.³⁾

Structure of (+)-oxymycousnine (**IV**)

The molecular formula of (+)-oxymycousnine was determined to be $\text{C}_{19}\text{H}_{20}\text{O}_9$ by HR-EIMS. Its IR spectrum showed two carbonyl absorptions at 1753 and 1624 (br.) cm^{-1} , the latter of which was closely similar to the characteristic carbonyl band of **II**. The UV absorptions [λ_{max} nm (ϵ): 340 (sh. 2,300), 280 (25,000) and 225 (14,000) in MeOH, and 333 (8,400) and 281 (25,000) in alkaline MeOH] were respectively similar to those of **II**, suggesting the presence of a 2,4-dihydroxyacetophenone moiety in the molecule.¹⁾ In addition, characteristic mass peaks at m/z 250 ($\text{C}_{13}\text{H}_{14}\text{O}_5$) and 235 ($\text{C}_{12}\text{H}_{11}\text{O}_5$) suggested the presence of the same dihydrobenzofuran moiety as that of **II**.⁴⁾ ^1H - and ^{13}C -NMR also gave numbers of signals similar to those for **II**. The ^1H -NMR spectrum, however, was too simple and hardly informative of the structure. Thus, HMBC (heteronuclear multiple-bond connectivity) NMR spectra were taken to reveal the connectivities between protons and carbon atoms separated by one to three bonds (Table III).⁷⁾ Only one among the carbon

atoms, which gave a signal at 158.5 ppm, showed the absence of connectivity with the protons and/or carbon atoms, and this carbon atom was reasonably assigned to that of 5a-position by a comparison of its chemical shift with that of the 5a-carbon of **II**. The disclosed connectivities between the protons and carbon atoms, and their chemical shifts (δ_{C} 126.0 for the 2-carbon⁸⁾) clearly elucidated the structure of (+)-oxymycousnine as **IV** shown in Fig. 2.

The presence of an acetoxy group at the 2-position in **IV** was then confirmed. The IR ($\nu_{\text{C=O}}$ 1753 cm^{-1}) and EIMS [m/z 350 ($\text{M}^+ - \text{CH}_2\text{CO}$)] data of **IV** suggested the presence of an acetylated enol moiety. Upon methanolysis at 60°C for 6 hr, **IV** gave methyl acetate and a labile enonediol (**V**, $\text{C}_{17}\text{H}_{18}\text{O}_8$). ^{13}C -NMR signals of **V** were assigned on the basis of their chemical shifts and its $^{13}\text{C}\{-^1\text{H}\}$ LSPD data (Table II). Changes in the carbon chemical shifts of the 2- and 3-positions [**IV**→**V**: δ 126.0→130.6 and 167.9→164.8, respectively] induced by de-*O*-acetylation clearly indicated the presence of the acetoxy group at 2. The close similarity of the ^1H -NMR chemical shifts of $\text{C}_{4\text{a}}\text{-OMe}$ and $\text{C}_{9\text{b}}\text{-Me}$

Table III. HMBC NMR DATA [δ (CD_3CN)]
FOR (+)-OXYMYCOUSNINE (**IV**)

^1H	^{13}C with $^1J_{\text{CH}}$	^{13}C with $^2J_{\text{CH}}$ or $^3J_{\text{CH}}$
1.63	16.6	189, 111.5, 108.1, 58.5
1.96	7.6	163.8, 159.9, 107.3
2.18	20.6	168.9
2.60	31.5	203.0, 102.8
3.21	(35.0) ^a	167.9, 126.0, 111.5, 58.5
3.40	(35.0) ^a	167.9, 126.0, 111.5
3.47	51.3	111.5
13.45		163.8, 107.3, 102.8

^a Weak cross peaks.

Fig. 2. Structures of (+)-Oxymycousnine (**IV**), **V** and **VI**.

Table IV. ANTIMICROBIAL SPECTRUM (MIC $\mu\text{g/mL}$) OF (–)-MYCOUSNINE (II), (+)-ISOMYCOUSNINE (III) AND (+)-OXYMYCOUSNINE (IV)

Test organism	II	III	IV
<i>Staphylococcus aureus</i>	6.2	6.2	> 100
<i>Bacillus subtilis</i>	6.2	6.2	> 100
<i>Escherichia coli</i>	> 100	> 100	> 100
<i>Candida albicans</i>	100	100	> 100
<i>Trichophyton asteroides</i>	25	6.2	> 100
<i>T. rubrum</i>	25	25	> 100

between II and IV showed that these groups of IV were in a *cis* configuration.¹⁾ CD spectra of II and IV showed the first Cotton effect with the same sign respectively at 347 and 360 nm, suggesting that the absolute stereochemistry of IV was the same as that of II. This was confirmed by a direct derivation of IV from II in a fair yield by H_2O_2 oxidation in pyridine. From the biosynthetic point of view, IV seems to be derived from II by a Baeyer-Villiger type of oxidation previously mentioned.

V functions as an enediol and behaves as a reductone; its exposure to atmospheric oxygen at room temperature gave a vicinal tricarbonyl compound (VI, $\text{C}_{17}\text{H}_{16}\text{O}_8$).⁹⁾

Biological activities of II, III and IV

As shown in Table IV, II and III showed marked antimicrobial activity against Gram-positive bacteria and *Trichophyton* spp., while IV showed no antimicrobial activity. II with dosages of 25–50 $\mu\text{g}/\text{disc}$ also showed growth self-inhibiting activity against *M. nawae* on an agar nutrient plate. Interestingly, IV showed significant selective antiviral activity in a plaque reduction test using an influenza B virus; 70% plaque inhibition was obtained at 30 $\mu\text{g}/\text{ml}$, whereas the 50% cytotoxic dose against the carrier cells was 190 $\mu\text{g}/\text{ml}$. In this connection, it has been suggested that cyclic vicinal triketones have potent antiviral properties.¹⁰⁾ II and III were too highly cytotoxic against the carrier cells (50% cytotoxic doses: 4.6–3.6 $\mu\text{g}/\text{ml}$) to assay their actual antiviral activity.

Experimental

Melting points (mp) were determined with a Yanagimoto micro-apparatus and are uncorrected. EIMS spectra and HR-EIMS data were obtained by using a JEOL JMS-D300 mass spectrometer. UV spectra were recorded on Hitachi 124 and JASCO UVIDEK-510 spectrophotometers, and IR spectra on a JASCO A-202 spectrophotometer. CD spectra were obtained on a JASCO J-600 spectropolarimeter. ^1H -NMR spectra were taken on a JEOL FX-90Q (90 MHz) spectrometer or a Bruker AM-400 (400 MHz) spectrometer, and ^{13}C -NMR spectra on JEOL FX-90Q (22.5 MHz) and Bruker AM-400 (100 MHz) instruments, using tetramethylsilane as an internal standard. ^1H - ^{13}C COSY, NOESY and HMBC NMR data were obtained on a Bruker AM-400 NMR spectrometer. LSPD, using SPT difference spectrometry, was performed on a JEOL FX-90Q instrument equipped with an irradiation power controlling unit; ^{13}C -signals with long-range couplings were observed as ones with a differential sign. GC analysis was performed on a Shimadzu GC-7A gas chromatograph, and BW-820MH and CQ-3 silica gel (Fuji Davison Chemical Ltd.) were used for column chromatography. (+)-Usnic acid was purchased from Wako Pure Chemical Industries Ltd.

Isolation of (–)-mycousnine (II), (+)-isomycousnine (III) and (+)-oxymycousnine (IV). A mycelial suspension of *M. nawae* in 1 ml of sterilized water was inoculated into a 100 ml culture medium containing 4% maltose syrup, 2% soybean flour, 1% Pharmamedia, 0.3% soybean oil, 0.3% CaCO_3 , 0.001% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.0001% $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$ in a 500-ml Sakaguchi flask, and then the flasks were incubated at 24°C for 7 days on a reciprocal shaker. Numerous globular, greenish mycelia in 6 ml of this culture broth were successively inoculated into a 100 ml culture medium containing 2% malt extract, 2% commercial sugar and 0.1% peptone in a 500-ml Sakaguchi flask, before the flasks were incubated at 25°C for 15–18 days on a reciprocal shaker. The culture filtrate obtained (4 l, pH 5.4–5.0) was saturated with NaCl and partitioned against EtOAc. The residue (240 mg) obtained by evaporation of combined EtOAc extracts was chromatographed by stepwise elution over BW-820MH silica gel with a 5:1 *n*-hexane–EtOH mixture (0.5% AcOH) to give the fraction (61 mg) containing II and III. This fraction was further separated on a CQ-3 silica gel column with a 40:1 *n*-hexane–EtOAc mixture (0.5% HCOOH) to give II (25 mg) and III (7 mg) as pale yellow crystals.¹⁾ Subsequent elution with a 5:2 *n*-hexane–EtOH mixture (0.5% HCOOH) gave the fraction (26 mg) containing IV. This was further purified on a CQ-3 silica gel column with a 15:1 benzene–EtOAc mixture (0.5% HCOOH) to yield IV (15 mg) as colorless crystals.

II. mp 86–89°C (MeOH); $[\alpha]_D^{20}$ -91° ($c=0.18$, CHCl_3); EIMS m/z : 376 [M^+ , 22% (relative abundance)], 344 (10), 260 (12), 250 (25), 235 (100) and 217 (18); HR-EIMS m/z :

376.1118 [M^+ , $C_{19}H_{20}O_8$ (376.1158)], 250.0819 [$C_{13}H_{14}O_5$ (250.0841)] and 235.0601 [$C_{12}H_{11}O_5$ (235.0606)]⁴⁾; UV λ_{max} (MeOH) nm (ϵ): 340 (sh, 3,200), 281 (28,000) and 223 (20,000), and λ_{max} (MeOH-0.5N NaOH) nm (ϵ): 332 (10,000) and 270 (30,000); IR ν_{max} (KBr) cm^{-1} : 3400 and 3130 (br.), 3000–2500, 1622 (br.), 1450, 1359, 1317, 1290, 1204, 1112, 1026, 989, 931, 840, 817, 780 and 764; CD λ_{ext} (EtOH) nm ($\Delta\epsilon$): 347 (–6.0) and 317 (–8.9) [306 (0)]; ¹H- and ¹³C-NMR data: Tables I and II. **III**. mp 78–79°C (MeOH); [α]_D²⁰ +146° (c =0.15, $CHCl_3$); EIMS m/z : 376 [M^+ , 29% (relative abundance)], 344 (12), 260 (15), 250 (76), 235 (100) and 217 (22); HR-EIMS m/z : 376.1192 [M^+ , $C_{19}H_{20}O_8$ (376.1158)], 250.0839 [$C_{13}H_{14}O_5$ (250.0841)] and 235.0631 [$C_{12}H_{11}O_5$ (235.0606)]⁴⁾; UV λ_{max} (MeOH) nm (ϵ): 335 (sh, 2,900), 280 (25,000) and 228 (17,000), and λ_{max} (MeOH-0.5N NaOH) nm (ϵ): 375 (2,100) and 275 (26,000); IR ν_{max} (KBr) cm^{-1} : 3160 (br.), 3000–2500, 1616 (br.), 1418, 1362, 1316, 1147, 1110, 998, 934, 892, 851 and 810; CD λ_{ext} (EtOH) nm ($\Delta\epsilon$): 320 (+2.7) and 287 (+5.4) [274 (0)]; ¹H- and ¹³C-NMR data: Table II. ¹³C-¹H LSPD data: δ_H ($CDCl_3$) 1.64 (3H, s; ¹H irradiated) $\Rightarrow \delta_C$ 198.0, 108.7, 104.7 and 60.2 (each ¹³C-signal with long-range coupling); 1.99 (3H, s) $\Rightarrow$ 165.6, 160.2 and 100.4; 2.57 (3H, s) $\Rightarrow$ 202.3; 2.71 (3H, s) $\Rightarrow$ 203.5; 9.63 (OH, s) $\Rightarrow$ 165.6, 106.7 and 100.4; 14.31 (OH, s) $\Rightarrow$ 156.0 and 106.7. **IV** gave a greenish color distinctive from the other related metabolites on silica gel TLC plates visualized with a vanillin-conc. H_2SO_4 reagent. **IV**. mp 119–122°C (n -hexane- $CHCl_3$); [α]_D¹⁹ +106° (c =0.3, MeCN); EIMS m/z : 392 [M^+ , 19% (relative abundance)], 364 (6), 350 (16), 250 (72), 235 (100) and 217 (17); HR-EIMS m/z : 392.1073 [M^+ , $C_{19}H_{20}O_9$ (392.1107)], 350.0985 [$C_{17}H_{18}O_8$ (350.1001)], 250.0827 [$C_{13}H_{14}O_5$ (250.0841)] and 235.0601 [$C_{12}H_{11}O_5$ (235.0606)]; UV: see the text; IR ν_{max} (KBr) cm^{-1} : 3580, 3510, 3440 (br.), 3000–2300 (br.), 1753, 1624 (br.), 1572, 1368, 1318, 1203, 1112, 1077, 995, 878 and 822; CD λ_{ext} (EtOH) nm ($\Delta\epsilon$): 360 (–3.7) and 314 (+9.3) [306 (0)]; ¹H- and ¹³C-NMR data: Tables II and III.

Preparation of (–)-mycousnine (II) and (+)-isomycousnine (III) from (+)-usnic acid (I). Pulverized **I** (300 mg) was heated in 15% hydrogen chloride in dry MeOH (115 ml) under reflux for 6 hr. To the resulting mixture was added brine, before extracting with EtOAc. The EtOAc extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The MeOH-soluble part (177 mg) of the residue was chromatographed on a CQ-3 silica gel column with a 10:1 n -hexane-EtOAc mixture (0.5% HCOOH) to give **II** [74 mg, mp 88.5–89.5°C (MeOH), [α]_D¹⁹ –103° (c =0.16, $CHCl_3$)] and **III** [20 mg, mp 79.5–80.5°C (MeOH), [α]_D¹⁹ +159° (c =0.17, $CHCl_3$)] as pale yellow crystals. The unchanged **I** (124 mg) was recovered from the MeOH-insoluble part. These synthetic **II** and **III** were respectively identical to natural **II** and **III** in all respects (EIMS, IR, UV and ¹H-NMR already described). The IR and ¹H-NMR data of **II** were also in fair agreement with those of (–)-usnic acid isomethoxide.⁴⁾

II has previously been identified as (–)-usnic acid isomethoxide, which was prepared from **I** according to Takahashi's procedure.¹⁾

Methanolysis of (+)-oxymycousnine (IV). A CD_3OD solution of **IV** (10 mg in 0.2 ml) in an NMR tube was warmed to 60°C and stood for 6 hr while monitoring with an NMR spectrometer. $AcOCD_3$ in the resulting mixture was directly identified against an authentic sample prepared from $AcCl$ and CD_3OD by GC [t_R : 3.0 min (column, 2m PEG-20M (3%, Gaschrom Q); column temp., 30°C; carrier gas, He; detector, FID)]. Removal of the solvents from the foregoing reaction mixture mixed with MeOH gave **V** as a colorless solid. **V**. [α]_D¹⁹ +165° (c =0.4, MeCN); EIMS m/z : 350 [M^+ , 100% (relative abundance)], 319 (7), 250 (36), 235 (80), 220 (25) and 217 (20); HR-EIMS m/z : 350.1042 [M^+ , $C_{17}H_{18}O_8$ (350.1002)]; UV λ_{max} (MeOH) nm (ϵ): 340 (sh, 11,000), 280 (14,000) and 224 (9,100), and λ_{max} (MeOH-0.5N NaOH) nm (ϵ): 333 (13,000) and 281 (10,000); IR ν_{max} (KBr) cm^{-1} : 3400 (br.), 1625 (br.), 1319, 1289, 1203, 1104, 1072, 997, 967, 823 and 768; ¹H- and ¹³C-NMR: Table II. ¹³C-¹H LSPD data: δ_H (CD_3OD) 1.67 (3H, s; ¹H irradiated) $\Rightarrow \delta_C$ 111.8, 109.5 and 57.5 (each ¹³C-signal with long-range coupling); 2.00 (3H, s) $\Rightarrow$ 163.6, 160.7 and 107.5; 2.64 (3H, s) $\Rightarrow$ 202.9. An EtOAc solution of **V** (1 mg in 2 ml) in a 30-ml round-bottomed flask was evaporated to dryness in a rotary vacuum evaporator. When **V** spread in a thin film was stood overnight in contact with air, a significant part was converted into vicinal triketone **VI**. The EIMS spectrum of the air-exposed **V** showed additional intense peaks originating from **VI** at m/z 348 (M^+), 320 (M –CO) and 288 [M –(CO–MeOH)]; HR-EIMS m/z : 348.0883 [M^+ , $C_{17}H_{16}O_8$ (348.0845)] and 320.0939 [$C_{16}H_{16}O_7$ (320.0896)].

H_2O_2 oxidation of (–)-mycousnine (II). **II** (15 mg) was dissolved in pyridine (0.25 ml) and treated with 30% H_2O_2 (0.013 ml) at 30°C, after which the reaction mixture was stirred for 1 hr. The resulting orange solution was cooled to 0°C, acidified with 1N HCl, and extracted 3 times with EtOAc. The combined EtOAc extracts were worked up in the usual way to give a crystalline solid. This was further purified by CQ-3 silica gel column chromatography with a 10:1 benzene-EtOAc mixture (0.5% HCOOH) and recrystallized from $CHCl_3$ - n -hexane to yield colorless **IV** [10 mg, mp 116–120°C, [α]_D¹⁴ +100° (c =0.6, MeCN)] whose spectral data [IR, EIMS and ¹H-NMR (60 MHz)] were completely identical with those of an authentic sample of **IV**.

Biological activities. Antibacterial and antifungal activities (MICs) were determined by an agar dilution method. The antiviral property was determined by a plaque reduction method using an influenza B virus (Singapore/222/79)-MDCK (NBL-2) cell system,¹¹⁾ and the cytotoxicity against MDCK cells was examined by an MTT assay.¹²⁾

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