MYCOVERSILIN, A NEW ANTIFUNGAL ANTIBIOTIC

II. STRUCTURE ELUCIDATION

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The structure of a new antifungal antibiotic, mycoversilin, produced by *Aspergillus versicolor* $(N_5)_{17}$ was determined as **I** by various spectroscopic and chemical methods. Mycoversilin is a unique polynuclear aromatic compound having two methyl and six hydroxyl groups and two ether linkages. The acetyl derivative prepared was found to have no antifungal property.

In preceding papers[1,2] we described the fermentation, isolation and biological properties of a new antifungal antibiotic, mycoversilin. The producer organism was obtained from the mutagenic treatment[3] of an inactive parent, *Aspergillus versicolor* (N_5), and designated as $(N_5)_{17}$[4]. Mycoversilin has a narrow spectrum and is especially active against dermatophytes.

This paper describes the structure elucidation of the antibiotic as determined by UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectra as well as by chemical reactions.

Physico-chemical Properties

Mycoversilin is a colorless crystalline substance which melts at 242±1°C with decomposition. It is soluble in water, alcohol and acetone. The aqueous solution is acidic. It is highly soluble in dimethyl sulfoxide, but insoluble in benzene, chloroform and petroleum ether. It is optically inactive.

Color reactions are positive with neutral ferric chloride, potassium permanganate and bromine in carbon tetrachloride, indicating the presence of phenolic or enolic hydroxyl group and unsaturation respectively, while negative towards 2,4-dinitrophenylhydrazine, ninhydrin and Molisch tests, indicating the absence of carbonyl functions, peptides and sugars respectively.

Elemental analysis indicated the following composition:

Calcd for $C_{15}H_{16}O_8$:	C 60.00, H 4.48
Found:	C 60.17, H 4.46

The observation of a peak at *m/z* 360 in the mass spectrum and of eighteen carbon signals in the ^{13}C NMR spectrum supported the molecular formula, $C_{15}H_{16}O_8$.

Spectroscopic Study

The UV spectrum (Fig. 1) shows an absorption peak at λ_{max}^{EtOH} 269 nm (log ε 3.11). This indicates the presence of a phenolic moiety in the compound. In presence of alkali the peak suffers a bathochromic shift: $\lambda_{max}^{0.1N\ NaOH}$ 289 nm (log ε 3.09) characteristic of a phenol or enol function.

The IR spectrum (Nujol) of mycoversilin (Fig. 2) shows peaks at 3400~3100 cm^{-1} (broad band, hydroxyl), 1610 cm^{-1} (unsaturation), 1210 and 1025 cm^{-1} (ether linkage) and 950, 900 and 870 cm^{-1}

(skeletal vibration).

All these observations were further corroborated by the ^{1}H and ^{13}C NMR spectra of mycoversilin (Tables 1 and 2). Peak assignments for the respective spectra are given in Tables 1 and 2. The ^{1}H NMR spectrum showed signals for six methyl protons assignable to two methyl groups, two carbinyl protons, two olefinic protons and six hydroxyl protons.

The ^{13}C NMR spectrum displayed 16 signals assignable to 2 methyls, 2 olefines, 2 methines and 12 aromatic quaternary carbons.

Fig. 1. UV spectrum of mycoversilin.
—— EtOH, ---- EtOH+0.1 N NaOH.

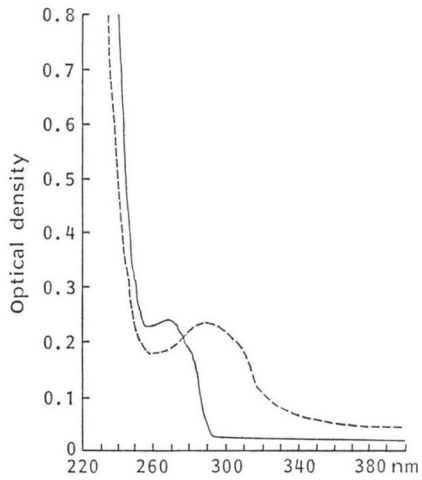


Fig. 2. IR spectrum of mycoversilin (Nujol).
The spectrum was recorded in a Perkin-Elmer Infrared spectrometer.

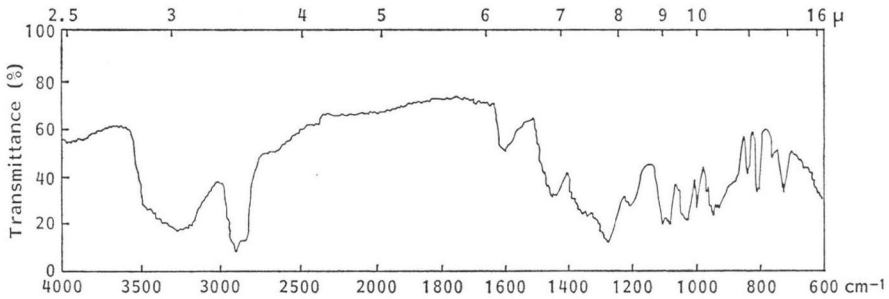


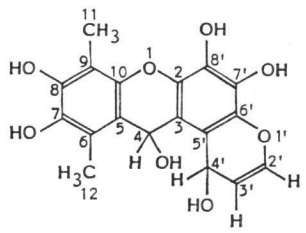
Table 1. ^{1}H NMR spectrum of mycoversilin in DMSO.

Chemical shift (ppm)	No. of protons	Multiplicity	Assignment
1.84	3	s	$HO\rangle C=C(6)\langle CH_3$
2.06	3	s	$HO\rangle C=C(9)\langle CH_3$
4.84	2	brs	$\rangle C(4 \text{ or } 4')\langle {}^{H}_{OH}$
5.51	1	m	$\rangle C(3')$–H
5.64	1	d	$O\rangle C(2')$–H
6.67	1	s	$\rangle$C–OH Partially D_2O exchangeable
8.04	5	m	$\rangle$C–OH D_2O exchangeable

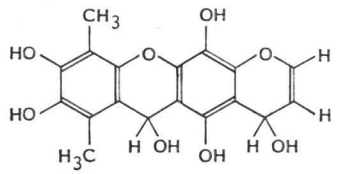
Spectrum was recorded in DMSO at 5°C using TMS as an internal standard.

Table 2. ^{13}C NMR spectrum of mycoversilin in DMSO.

Chemical shift (ppm)	Corresponding No. of carbons	Multiplicity	Assignment
144.07	1	s	
143.43	1	s	
137.91	1	s	
134.80	2	s	
134.39	1	s	>C(2,3,5~10 and 5'~8')
128.22	1	s	
126.81	1	s	
119.18	2	s	
111.43	1	s	
110.90	1	s	
104.15	1	d	O–C(2')<H
89.41	1	d	=C(3')<H, C
79.43	1	d	>C(4 or 4')<H, OH
73.33	1	d	>C(4 or 4')<H, OH
12.27	1	q	=C<C(11 or 12)H$_3$
11.33	1	q	=C<C(11 or 12)H$_3$



I



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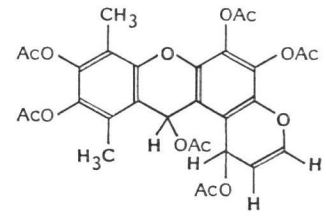

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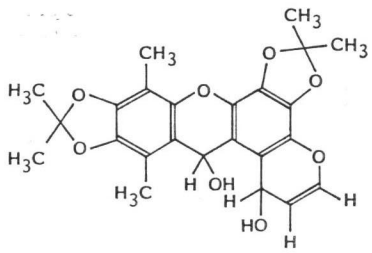
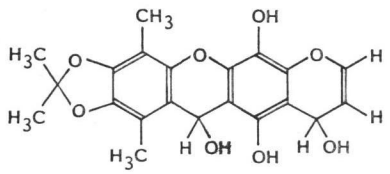


IV



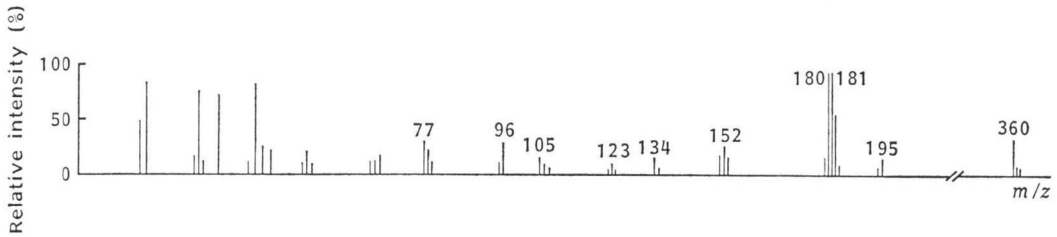
V

Discussion

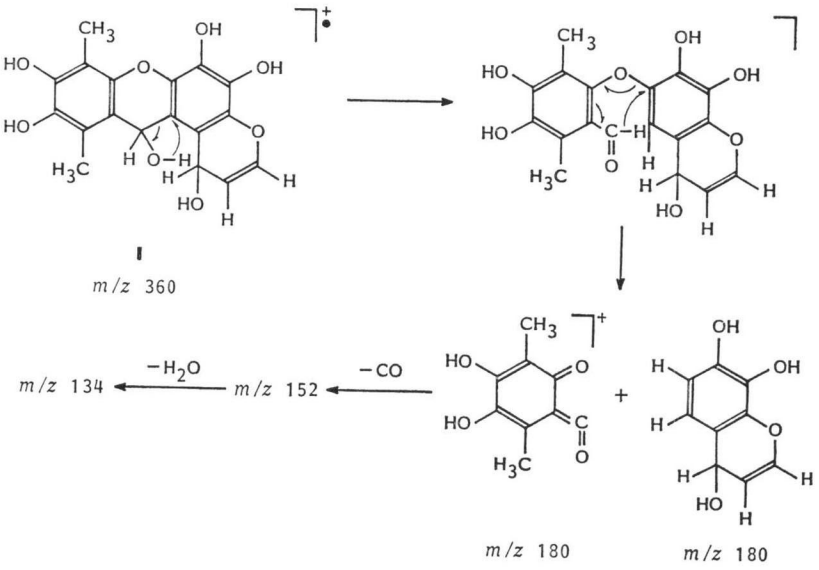
From the elemental and mass spectral analysis mycoversilin (**I**) was found to have the molecular formula, $C_{18}H_{18}O_8$. The carbon and hydrogen ratio indicated that it may be a polynuclear aromatic compound. The UV spectrum showed the presence of a phenolic moiety which was further confirmed by IR spectrum. The IR spectrum also showed the presence of ether function.

The presence of six hydroxyl groups was demonstrated by preparation of its hexaacetate (**III**) (M^+,

Fig. 3. Mass spectrum of mycoversilin.



Scheme 1.



m/z 612). The antibiotic does not contain any carbonyl function (>C=O) as was evident from its IR and ^{13}C NMR spectra. Consequently, it results that the other two oxygen atoms in mycoversilin (**I**) are present as ether functions. The ^{1}H NMR spectrum of **I** did not show the presence of any aromatic proton. It exhibited two olefinic protons, two carbinyl protons and six *C*-methyl protons.

Considering the 18 carbon atoms, functional groups (–OH, –C–O–C–, polynuclear aromatic system and unsaturation), the distribution of hydrogen atoms as arrived at by proton signals of the ^{1}H NMR spectrum and the environment of carbon atoms as indicated by ^{13}C NMR spectrum of mycoversilin, the antibiotic might be imagined as having the structure **I** or **II**. Both these structures agreed with the molecular formula, color reactions and most of the physico-chemical properties.

The locations of the hydroxyl groups were revealed by the preparation of the acetonide (**IV**) which did not show any color change with ferric chloride solution, and this finding might be taken to mean that the aromatic hydroxyls were present in vicinal positions as in **I**; structure **II** would have two free phenolic hydroxyl groups even after monoacetonide (**V**) formation.

Moreover, the ^{13}C NMR data of two methyl carbons indicated that both the methyl groups are located adjacent to hydroxyl groups.

Structure **I** also received further support from a detailed analysis of the mass spectrum (Fig. 3). The mass spectrum displayed, in addition to the molecular ion peak at *m/z* 360, other significant peaks at 195, 180, 152, 134, 123, 96 and 77. The base peak at *m/z* 180 was crucial in the determination of the structure of mycoversilin which could consists of two symmetrical parts each with a molecular weight of 180 in the molecule. The genesis of base peak at *m/z* 180 might be rationalised as shown in Scheme 1.

From all the foregoing physical and chemical evidences, the structure of mycoversilin might be **I**. The ^{13}C NMR data (Table 2) are compatible with this structure.

Experimental

General

All melting points were uncorrected. The UV spectrum was recorded on a Cary ID spectrometer. The IR spectrum was measured on a Perkin-Elmer Infrared spectrometer. The ^{1}H NMR spectrum was recorded on a Jeol FX-100 NMR spectrometer (100 MHz) and ^{13}C NMR spectrum on a Jeol FX-100 NMR spectrometer (25.05 MHz) using TMS as internal standard. The mass spectrum was recorded with a Hitachi spectrometer model RMU-6L.

Isolation of Mycoversilin (I)

The isolation of pure mycoversilin was reported elsewhere[2].

Acetylation of I

To a solution of mycoversilin (50 mg) in dry pyridine (2 ml) was added acetic anhydride (5 ml). The mixture was kept overnight at room temperature, then poured into crushed ice with stirring, filtered, washed with ice-cold water and dried. This was then recrystallized from $CHCl_3$ - petroleum ether to give colorless crystalline derivative (**III**) (45 mg), mp 121°C (Found: C 58.92, H 4.56; Calcd for $C_{30}H_{28}O_{14}$: C 58.83, H 4.61); IR ν_{max}^{Nujol} 1740 (acetate CO), 1243, 980, 920, 900 and 868 cm^{-1} (no absorption above 3000 cm^{-1}), MS *m/z* 612 (M^+), 570 ($M^+-C_2H_2O$), 528 ($570-C_2H_2O$), 486, 444, 402, 386, 342, 306, 264 and 180 (100%). It is interesting to note that the hexaacetate had no antifungal activity.

Acetonide of I

To a solution of mycoversilin (50 mg) in acetone (2 ml) were added two drops of conc H_2SO_4. The product was extracted with $CHCl_3$ and then worked up. The crystalline product was recrystallized from $CHCl_3$. The yield was 45 mg, mp 182°C. This compound does not respond to ferric chloride color reaction, thereby indicating that there is no free phenolic or enolic hydroxyl group.

Acknowledgment

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References

1) SAMANTA, A. K.; H. K. KOLE, S. K. GOSWAMI & S. K. BOSE: Mycoversilin, a new antifungal antibiotic from a mutant derivative of *Aspergillus versicolor*. Ind. J. Exptl. Biol. 21: 577~578, 1983
2) SAMANTA, A. K. & S. K. BOSE: Mycoversilin, a new antifungal antibiotic. I. Fermentation, isolation and biological properties. J. Antibiotics 37: 728~732, 1984
3) KOLE, H. K. & S. K. BOSE: A note on the development of a technique for regeneration of a degenerated culture. J. Appl. Bacteriol. 48: 433~436, 1980
4) KOLE, H. K.; A. K. SAMANTA, S. K. GOSWAMI & S. K. BOSE: Attempt at gene reversion of a desired phenotype as a rescue against strain degeneration. J. Appl. Bacteriol. 53: 163~167, 1982