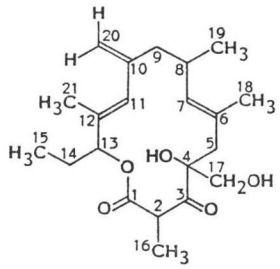
NEORUSTMICIN A, A NEW MACROLIDE ANTIBIOTIC ACTIVE AGAINST WHEAT STEM RUST FUNGUS

Sir:

Stem rust caused by the infection of *Puccinia graminis* f. sp. *tritici* is known as one of the several limiting factors of wheat production. We have previously reported the isolation and structural elucidation of rustmicin active against this pathogen with the screening systems using an inhibition assay of spore germination *in vitro* and plant inoculation test in green house[1]. During the course of further screening, a new antibiotic neorustmicin A (**I**) was found in the cultured broth of *Micromonospora chalcea* 1302-AV_2. This communication reports the isolation, structural elucidation and some biological activities of **I**.

Fermentation was carried out at 27°C for 4 days in 500-ml Erlenmeyer flasks each containing 100 ml of the same medium used for fermentation of rustmicin. The active substance was isolated from the mycelium. The mycelial cake was obtained by centrifugation of the whole broth (*ca.* 10 liters) and extracted with acetone (10 liters). The extract was evaporated *in vacuo* to give an aqueous solution which was extracted with three 1.5-liter portions of EtOAc. The combined organic layer was washed successively with 5% $NaHCO_3$, 0.01 N HCl and H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The oily residue (850 mg) was chromatographed on a silica gel column developing with EtOAc-benzene (1: 3). The active fractions were combined and concentrated *in vacuo* to give a brownish oil (20 mg). Further purification of the oily material by preparative TLC (EtOAc-benzene, 1: 3, Rf 0.35) gave 5.3 mg of **I** as a yellowish oily material.

Fig. 1. Structure of neorustmicin A (**I**).



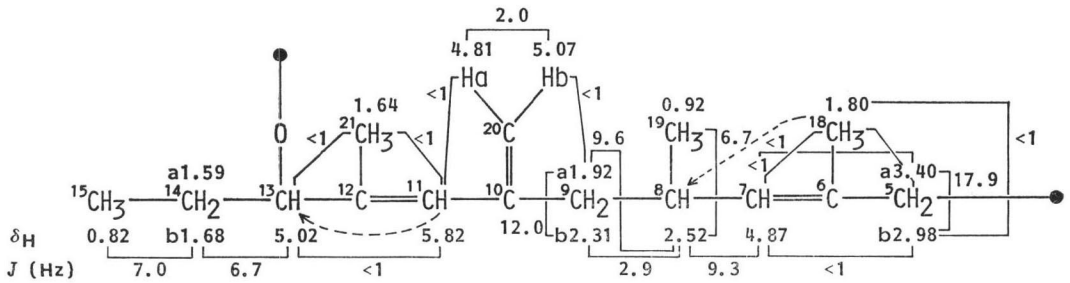
Physico-chemical properties of neorustmicin A are as follows: UV λ_{max}^{MeOH} 212 (ε 13,700) and 233 nm (11,000); IR $\nu_{max}^{CHCl_3}$ 3540, 1740 (sh) and 1720 cm^{-1}; EI-MS *m/z* 364 (M^+); $[\alpha]_D^{31}$ −83.1° (*c* 0.25, MeOH). The molecular formula of **I** was established to be $C_{21}H_{32}O_5$ by high resolution mass spectral data (obsd 364.2288; calcd 364.2251). Its molecular formula is one less oxygen than that of rustmicin. In addition to the UV and IR spectra, ^1H and ^{13}C NMR spectral data of **I** are very close to those of rustmicin (^1H NMR data will be given below and ^{13}C NMR data were shown in Table 1).

Comparison of ^1H and ^{13}C NMR spectral data between rustmicin and **I** indicated that a methoxy signal at δ_H 3.48 and δ_C 57.0 of rustmicin was lost and a methyl signal at δ_H 1.64 and δ_C 17.5 was newly observed in **I**. This finding suggested that the methoxy group at C-6 of rustmicin has been replaced by a methyl group at C-6 in **I**. At the same time, a quaternary carbon signal attached to the methyl group and the adjacent carbon signals in ^{13}C NMR spectrum of **I** must

Table 1. ^{13}C NMR signals of **I** taken in $CDCl_3$.

No.	Functional group	δ_C	No.	Functional group	δ_C
1	–COO–	171.7	12	=C	133.0
2	–CH	45.1	13	–CH–O	82.4
3	–C=O	207.5	14	$-CH_2$	25.3
4	–COH	81.3	15	$-CH_3$	10.1
5	$-CH_2$	46.2	16	$-CH_3$	12.9
6	=C	125.8	17	$-CH_2OH$	65.9
7	=CH	135.2	18	$-CH_3$	17.5
8	–CH	34.9	19	$-CH_3$	21.6
9	$-CH_2$	45.7	20	$=CH_2$	115.4
10	=C	144.4	21	$-CH_3$	15.2
11	=CH	133.6			

Fig. 2. Partial structure of neorustmicin A (I).
Dotted arrows indicate NOE observed.



be shifted corresponding to the change from the methoxy of rustmicin to the methyl of **I**. The structure of **I**, however, was determined by the analysis of 2D COSY[2], ^{13}C-^{1}H shift correlation[2] and long range selective proton decoupled (LSPD) spectra[3], because ^{13}C NMR signals of rustmicin were tentatively assigned.

Based on INEPT experiments the functionality of carbon atoms in **I** is as follows: $CH_3\times5$, $CH_2\times3$, $CH\times2$, CH_2–$O\times1$, CH–$O\times1$, C–$O\times1$, $=CH_2\times1$, $=CH\times2$, $=C\times3$, COO–$\times1$ and $C=O\times1$. The signal at δ_C 171.7 and 207.5 were assigned to an ester and a saturated ketone carbonyl due to IR absorption bands at 1740 and 1720 cm^{-1}, respectively. These data revealed **I** had one ring system and two hydroxyl groups. By the analysis of 2D COSY, two connectivities of the protonated carbons were clarified; one was CH_3CH (δ_H 1.35, d and 2.94, q) and the other shown in Fig. 2. The oxymethine signal (H-13) at δ_H 5.02 was distinguished clearly from olefinic protons by ^{13}C-^{1}H shift correlation spectrum (δ_C 82.4). A newly generated olefinic methyl (H-18) was assigned to δ_H 1.80, not 1.64 and the olefinic methyl (H-21) resonating at δ_H 1.81 in rustmicin was assigned to the signal at δ_H 1.64. This partial structure has provided an evidence in confirmation of the structure of **I**. These two partial structures were connected by LSPD experiments. The signal of the ester carbonyl at δ_C 171.7 was collapsed on irradiation of the oxymethine proton H-13 at δ_H 5.02. Similarly, irradiation of the methyl protons at δ_H 1.35 attached to the methine in the former partial structure collapsed an ester carbonyl and a ketone carbonyl signals, δ_C 171.7 and 207.5, respectively. This result proved the connectivity from C-5 to C-3 *via* the ester function.

Table 2. Antimicrobial spectrum of neorustmicin A against phytopathogenic microorganisms.

Test organism	Growth (100 μg/ml)
Alternaria kikuchiana	+
Botrytis cinerea	+
Cochliobolus miyabeanus	−
Colletotrichum lagenarium	−
Diaporthe citri	−
Fusarium oxysporum f. *lycopersici*	+
Gibberella fujikuroi	+
Glomerella cingulata	+
Pellicularia sasakii	+
Piricularia oryzae	++
Corynebacterium michiganense	+++
Erwinia aroideae	+++
Pseudomonas lachrymans	+++
Pseudomonas tabaci	+++
Xanthomonas campestris pv. *citri*	+++
Xanthomonas campestris pv. *oryzae* H-5809	+++

At this point, there remained a primary and a tertiary alcohol and one ring system must be present in **I**. Thus, the structure of neorustmicin A was determined to be **I**.

The double bond between C-11 and -12 proved to be ***E*** configuration by NOE enhancement observed with H-13 on irradiation of H-11. Similarly, the geometry between C-6 and -7 was determined to be ***E*** configuration by NOE enhancement observed with H-8 on irradiation of the methyl protons (δ_H 1.80).

Neorustmicin A is a new macrolide antibiotic consisting of a 14-membered lactone and, like rustmicin, it has an exomethylene group in the molecule and is not glycosylated.

Neorustmicin A showed strong activity against wheat stem rust fungus as much as in rustmicin; it inhibited the germ tube elongation at 0.2 μg/ml

in vitro, and protected wheat leaves from rust infection at concentration of 4 μg/ml. An antimicrobial spectrum of neorustmicin A against phytopathogenic microorganisms is shown in Table 2. Neorustmicin A showed inhibitory activity against *Diaporthe citri, Cochliobolus miyabeanus* and *Colletotrichum lagenarium* at concentration of 100 μg/ml. Thus, the following became clear; neorustmicin A is specifically active against wheat stem rust fungus.

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