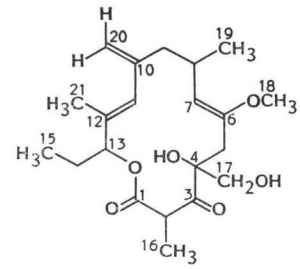
RUSTMICIN, A NEW MACROLIDE ANTIBIOTIC ACTIVE AGAINST WHEAT STEM RUST FUNGUS

Sir:

Stem rust (*Puccinia graminis* f. sp. *tritici*) is a serious wheat disease causing decrease of wheat production in many areas of the world[1] and has been partially controlled so far by the use of resistant races[2], eradication of the alternate host, *i.e.* barberry[3], or by synthetic fungicides[4~7]. We undertook the screening of new antibiotics active against wheat stem rust fungus using an inhibition assay of the germination of uredospores *in vitro* and pot test in green house. During the course of this screening, rustmicin (**I**) (formerly called P-59B1[8]) was obtained from the cultured broth of *Micromonospora narashinoensis* 980-MC$_1$. The present communication describes the isolation and structural elucidation of **I**.

Fermentation was carried out at 27°C with agitation of 400 rpm and aeration of 25 liters/minute for 4 days in two 50-liter jar fermentors each containing 25 liters of a medium consisting of soluble starch 2.5%, soybean meal 1.5%, dry yeast 0.2% and $CaCO_3$ 0.4% (pH 7.4). The activity against wheat stem rust fungus was monitored by an *in vitro* method, which assayed the germination of uredospores on the agar containing a sample 2 hours after inoculation at 20°C, throughout the purification. The active substance was isolated from the filtrate as described below. The cultured broth (*ca.* 47 liters) was harvested, adjusted to pH 7.0 with 2 N HCl and filtered. The filtrate was adsorbed on a Diaion HP20 column (5 liters) which was washed successively with H_2O and 50% aq MeOH (10 liters each) and finally the active metabolite was eluted with MeOH (10 liters). The MeOH concentrate (0.5 liter) was partitioned between H_2O and EtOAc three times. The combined organic layer (1 liter) was washed successively with 5% $NaHCO_3$, 0.01 N HCl and H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford a brownish oil (350 mg). This crude material was chromatographed over a Sephadex LH-20 column with MeOH and the active fractions were combined and evaporated *in vacuo* to dryness. The final purification of the antibiotic was achieved by reversed phase HPLC (Waters Radial PAK $_8C_{18}$) eluting with 70% aq MeOH to yield 7.5 mg of **I** in a pure form.

Fig. 1. Structure of rustmicin (**I**).



I is a neutral colorless oily material possessing the following physico-chemical properties: UV λ_{max}^{MeOH} nm (ε) 215 (9,500) and 240 (8,000); IR $\nu_{max}^{CHCl_3}$ 3600 (OH), 1730 and 1710 cm^{-1} (COO and C=O); EI-MS *m/z* 380 (M^+). Its molecular formula was established to be $C_{21}H_{32}O_6$ by high resolution mass spectral data (found 380.2196; calcd 380.2200). The signals in the ^{13}C NMR spectrum of **I** taken in $CDCl_3$ accounted for 21 carbons and 30 nonexchangeable protons (see Table 1). Two protons at δ_H 2.31 (1H, dd, J=11.0 and 2.0 Hz) and 3.46 (1H, br s) in the ^1H NMR spectrum of **I** revealed the presence of a primary and a tertiary alcohol, respectively.

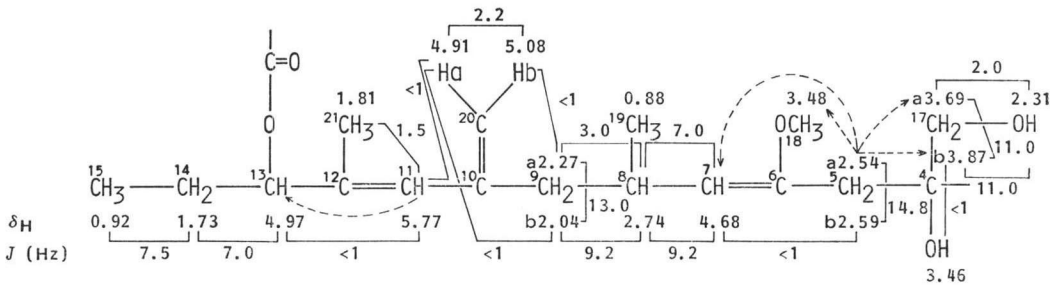
In the ^{13}C NMR spectrum, the resonance at δ_C 169.2 was assigned to an ester carbonyl due to an IR absorption band at 1730 cm^{-1}. The presence of a saturated ketone at δ_C 209.2 indicated that the UV absorption (λ_{max} 240 nm) is due to a conjugated diene system. Thus, the functional groups in **I** are summarized as follows: $CH_3\times4$, $CH_2\times3$, $CH\times2$, $CH_3O\times1$, $CH_2OH\times1$, $CH\text{–}O\times1$, $COH\times1$, $=CH_2\times1$, $=CH\times2$, $=C\times3$, $COO\text{–}\times1$ and $C=O\times1$. These data suggested the presence of one ring structure in **I**.

The structure of **I** was determined mainly based on spin decoupling and NOE experiments. The olefinic proton resonating at δ_H 5.77 (H-11; 1H, br s) showed long range couplings with a methyl at δ_H 1.81 and two protons at δ_H 4.91 and 4.97; irradiation of H-11 sharpened these three signals. The proton at δ_H 4.91 was coupled to the signal at δ_H 5.08 by a small coupling constant (J=2.2 Hz). These two resonances were ascribed to an exomethylene whose ^{13}C resonance was observed at δ_C 116.9. The conjugated diene system in **I** proved to be located

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

No.	Functional group	δ_C	No.	Functional group	δ_C
1	-COO-	169.2	12	=C	134.8
2	-CH	51.1	13	-CH-O	81.4
3	-C=O	209.2	14	$-CH_2$	25.8
4	-COH	81.8	15	$-CH_3$	9.9
5	$-CH_2$	33.9	16	$-CH_3$	13.9
6	=C	148.2	17	$-CH_2OH$	67.4
7	=CH	124.1	18	$-OCH_3$	57.0
8	-CH	30.7	19	$-CH_3$	21.0
9	$-CH_2$	46.0	20	$=CH_2$	116.9
10	=C	143.4	21	$-CH_3$	15.0
11	=CH	129.3			

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

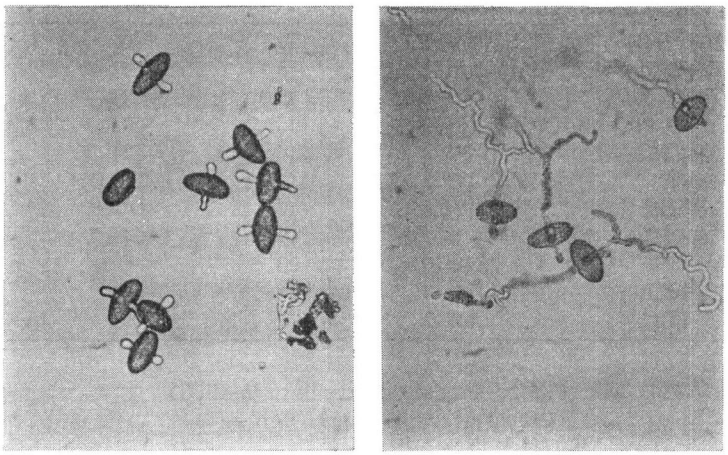


in the partial structure as shown in Fig. 2. Then, the ^1H chemical shift of the only one oxymethine (δ_H 4.97, δ_C 81.4) mentioned above is responsible for the formation of an ester function. The connectivity from C-13 to C-15 and from C-7 to C-20 were straightforwardly revealed by the consecutive spin decoupling. The methine proton at δ_H 4.68 (H-7) was assigned to an olefinic proton based on a selective proton decoupling (δ_C 124.1). The allylic couplings between H-7 and methylene protons (H-5a and -5b) proved the connectivity from C-5 to C-7.

The connectivity from C-5 to C-15 was further extended by an NOE experiment; in addition to H-7, the irradiation of H-5a showed enhancements of the signals due to the methoxy group at δ_H 3.48 and the primary alcohol group at δ_H 3.69 and 3.87. This finding indicated either one of the methoxy (δ_H 3.48) or isolated hydroxymethyl was directly attached to C-6 and the other is combined to C-5 across the non-protonated carbon (C-4), *i.e.* the isolated carbonyl or *tert*-alcohol. The long range coupling observed between the hydroxymethyl proton at δ_H 3.87 and a broad singlet hydroxy proton at δ_H 3.46 enabled to determine the partial structure from C-5 to C-17. The double bond between C-6 and -7 proved to be in *Z* configuration by NOE enhancement observed with H-7 on the irradiation of H-5a as described above. Similarly, the geometry between C-11 and -12 was determined to *E* configuration by NOE enhancement with H-13 on the irradiation of H-11.

At this point, there remained two other partial structures, CH_3CH (δ_H 1.44, d and 3.73, q) and C=O, which can be accommodated in α-ketolactone $-C(OH)CH(CH_3)COCOO-$ or β-ketolactone $-C(OH)COCH(CH_3)COO-$. Calculation of this methine attached to the ketone (δ_H 3.73) by the rule reported by CURPHEY[9] indicated that the β-ketolactone (δ_H 3.65) was in good agreement with the observed value than the α-ketolactone (δ_H 2.60). The methine substituted by two carbonyl and a methyl in pikromycin[10] resonated at δ_H 4.30. This data supported the β-ketolactone system in I. Accordingly, the structure of rustmicin was elucidated as shown in Fig. 1.

Fig. 3. Inhibition of elongation of germ tube of *Puccinia graminis* by rustmicin (I): On the left is rustmicin (I) at 1 μg/ml and, right, control.



The gross structure of rustmicin is similar to those of the 14-membered β-ketolactone antibiotics such as pikromycin[10~12] and narbomycin[13], but different from nonglycosylated macrolides such as albocycline[14,15].

Rustmicin showed strong activity against wheat stem rust fungus *in vitro* and pot test in green house, MIC being 1 and 0.8 μg/ml, respectively. Rustmicin inhibits the elongation of the germ tube accompanied with the swelling of tube tops *in vitro* (see Fig. 3). This unique antifungal effect is interesting as compared with the biological activities of the other macrolide antibiotics. Rustmicin exhibits antifungal but not siginificant antibacterial activity.

Acknowledgment

We wish to thank Dr. AKITOSHI TAJIMI, National Grassland Research Institute, for his generous gift of wheat stem rust fungus.

This investigation was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

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(Received July 24, 1985)

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