STRUCTURES OF NEORUSTMICINS B, C AND D NEW CONGENERS OF RUSTMICIN AND NEORUSTMICIN A

Sir:

In the previous papers[1,2], we described the isolation and structures of rustmicin and neorustmicin A, 14-membered macrolide antibiotics produced by *Micromonospora narashinoensis* 980-MC_1 and *Micromonospora chalcea* 1302-AV_2, respectively. They inhibited elongation of the germ tube of the wheat stem rust fungus (*Puccinia graminis* f. sp. *tritici*) *in vitro* and prevented occurrence of the disease caused by this pathogen at very low concentrations (0.2~4 μg/ml). As a result of the continuing screening for new antibiotics active against this pathogen, new congeners were obtained from the cultured broth of another strain, *M. chalcea* 1304-AV_3. This communication describes the isolation and structural elucidation of these congeners named neorustmicins B, C and D (**I**, **II** and **III**, respectively).

M. chalcea 1304-AV_3 was cultured for 72 hours at 27°C in a production medium KG (100 ml in a 500-ml Erlenmeyer flask) consisting of glucose 2.5%, soybean meal 2.0%, dry yeast 0.2% and $CaCO_3$ 0.4% (pH 7.2). This culture was used as an inoculum for a 50-liter fermentor containing 25 liters of the KG medium and after 48 hours of cultivation, the fermentation broth was transferred to two 300-liter fermentors each containing 150 liters of the KG medium. Maximum production was reached after about 96 hours.

The extraction and purification procedures are outlined in Fig. 1. Acetone extract of the mycelial cake was concentrated *in vacuo* to afford an aqueous solution, which, after being combined with broth filtrate (pH 7.0), was adsorbed on a Diaion HP-20 column. Active substances were eluted with MeOH after washing the column with H_2O and 50% aq MeOH. The eluate was concentrated *in vacuo* and then partitioned between H_2O and EtOAc. The combined organic layer was washed successively with 0.1 N HCl, 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to dryness. The crude material thus obtained (29.2 g) was chromatographed on a silica gel column. The fraction containing neorustmicin

Fig. 1. Isolation of neorustmicins B (**I**), C (**II**) and D (**III**).

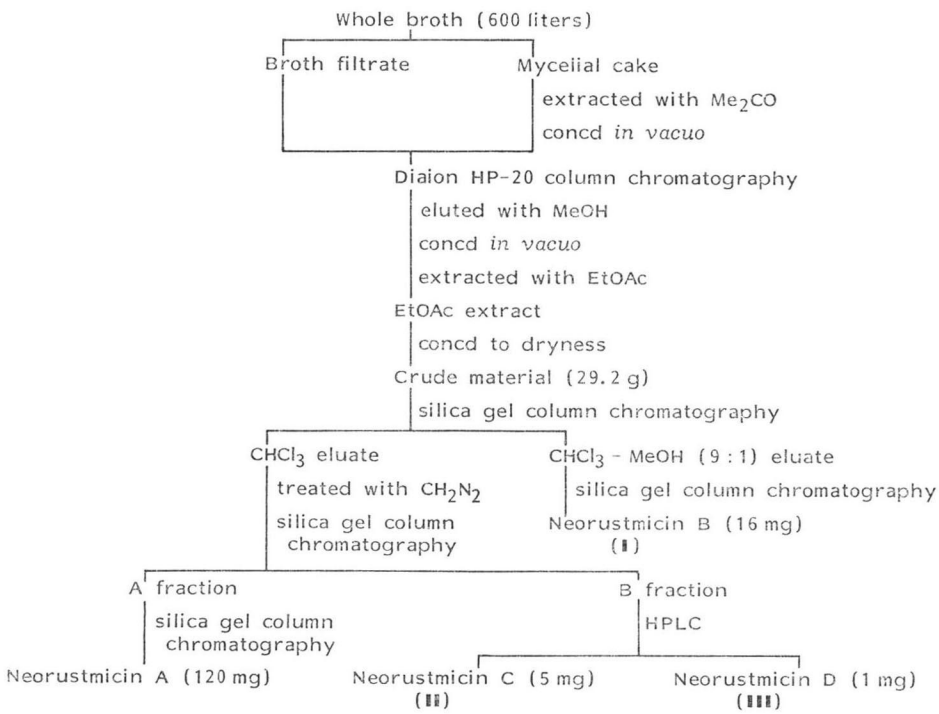
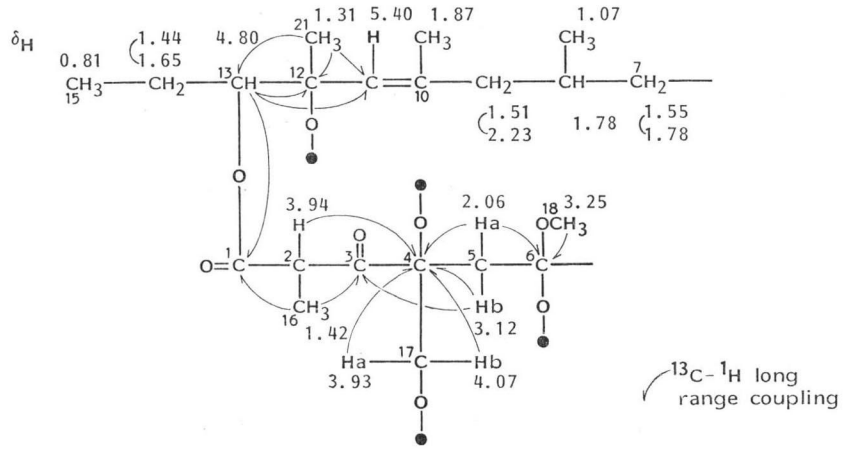


Table 1. Physico-chemical properties of **I**, **II** and **III**.

	I	II	III
Appearance	Neutral colorless oil	Neutral colorless oil	Neutral colorless oil
Molecular formula	$C_{21}H_{34}O_7$	$C_{20}H_{32}O_5$	$C_{20}H_{32}O_5$
FAB-MS (*m/z*)	421 $(M+Na)^+$, 437 $(M+K)^+$		
EIHR-MS (*m/z*): found	367.2091 $(M-OCH_3)^+$	352.2197 (M^+)	352.2203 (M^+)
calcd	367.2120	352.2249	352.2249
UV λ_{max}^{MeOH} nm (ε)	216 (1,900)	245 (4,500)	220 (4,000)
IR ν cm^{-1} ($CHCl_3$)	3500, 1745, 1710	3450, 1720, 1675	3450, 1720
$[\alpha]_D^{22}$ ($CHCl_3$)	$-7.1°$ (*c* 0.68)	$+12.0°$ (*c* 0.2)	

Fig. 2. Partial structure of **I**.
400 MHz ^1H NMR spectral data of **I** taken in $CDCl_3$ was measured.



A, **II** and **III** obtained by elution with $CHCl_3$ was treated with CH_2N_2 to enable easier separation from contaminated fatty acids and rechromatographed on a silica gel column (benzene - EtOAc, 9: 1) to give two active fractions, one containing a major component (120 mg) identified as neorustmicin A, and the other containing **II** and **III**. Final separation of **II** and **III** was achieved by reversed phase HPLC (Waters Radial PAK $_8C_{18}$; H_2O - MeOH, 6: 4).

The fraction eluted with $CHCl_3$ - MeOH (9: 1) from the first silica gel column contained **I** and was further purified by rechromatography with silica gel (benzene - EtOAc, 8: 3) to give 16 mg of **I** in a pure form.

Physico-chemical properties of **I**, **II** and **III** are as shown in Table 1.

The molecular formula of **I** was established to be $C_{21}H_{34}O_7$ by EI high resolution mass spectral (EIHR-MS) data based on the fragment ion $(M-OCH_3)^+$ (found *m/z* 367.2091; calcd for $C_{20}H_{31}O_6$ *m/z* 367.2120) and by the ion peaks in the FAB mass spectrum (FAB-MS): *m/z* 421 $(M+Na)^+$ and *m/z* 437 $(M+K)^+$.

The structure of **I** was determined by proton spin decoupling and long range ^{13}C-^1H *J* modulated difference (JMD) experiments[3]. Spin decoupling experiments showed three partial structures: $CHCH_3$ (C-2 and C-16), CH_2CH-$(CH_3)CH_2C(CH_3)=CH$ (C-7 to C-11), and $CH(O-)CH_2CH_3$ (C-13 to C-15). They were extended to the structure shown in Fig. 2 by JMD experiments. Irradiation of 13-H (δ_H 4.80) proved long range couplings of this proton with an oxyquaternary carbon (C-12) at δ_C 75.3, an olefinic carbon (C-11) at δ_C 123.8 and an ester carbonyl carbon (C-1) at δ_C 168.3. Accordingly, the oxymethine carbon of the third partial structure (C-13) was attached to the olefinic carbon of the second partial structure (C-11) *via* the oxyquaternary carbon (C-12) and the ester carbonyl (C-1) across an oxygen atom. Irradiation of a

Fig. 3. Partial structure of **II**.

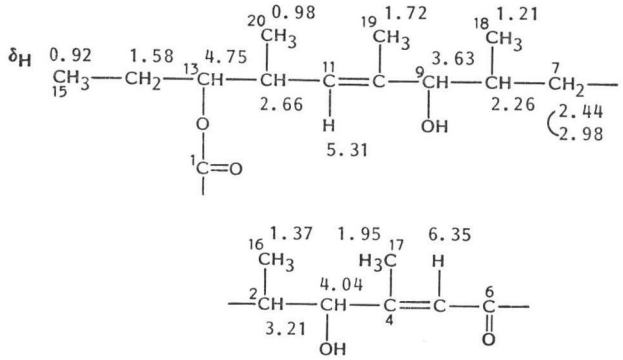
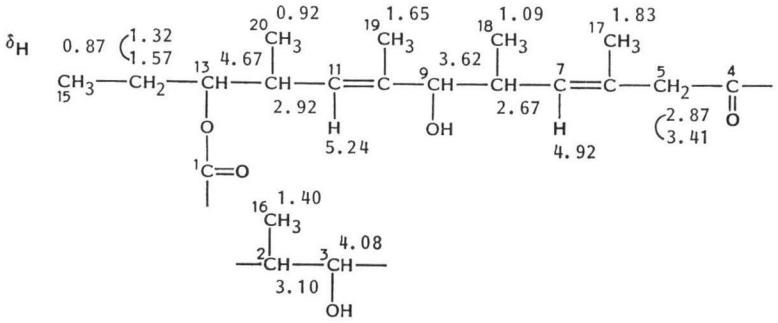


Fig. 4. Partial structure of **III**.



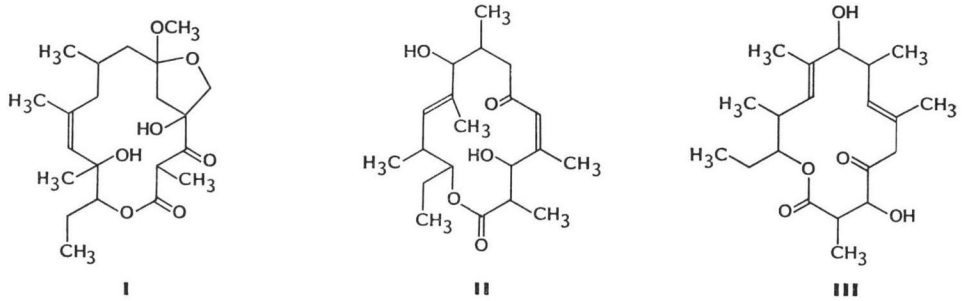
singlet methyl proton at δ_H 1.31 (21-H) indicated that this methyl was attached to the oxyquaternary carbon (C-12). Thus, the alcoholic portion of the ester was extended from C-7 to C-15.

Subsequently, the ester carbonyl carbon and a ketone (δ_C 206.9) proved to show long range couplings with the methyl of the first partial structure (16-H) by irradiating this methyl proton (δ_H 1.42). Similarly, an oxyquaternary carbon (δ_C 85.2, C-4) proved to be long-range-coupled with the methine of the first partial structure (2-H). These observations revealed the connectivity of the non-protonated carbons with C-2, *i.e.* the connectivity from C-1 to C-4. Moreover, the oxyquaternary carbon C-4 was elucidated to be coupled with isolated methylene protons 5-Ha and 5-Hb (δ_H 2.06 and 3.12, respectively) and isolated hydroxymethyl protons 17-Ha and 17-Hb (δ_H 3.93 and 4.07, respectively) in addition to C-2 by irradiations of these protons. The partial structure thus obtained was extended to C-5. Irradiation of one of the isolated methylene proton (5-Ha) showed a long range coupling with a ketal carbon (δ_C 110.6, C-6) in addition to C-4. This ketal carbon was also revealed to be coupled with a methoxy proton (18-H) by a long range coupling. Hence, the partial structure from C-4 to C-6 was determined.

At this point, C-6 was obviously connected to C-7 due to the chemical shifts of 7-H (δ_H 1.55 and 1.78). The chemical shift of C-6 (δ_C 110.6) indicated formation of a five-membered ketal ring[4], *i.e.* C-6 must be attached to C-17 across an oxygen atom. The oxyquaternary carbons C-4 and C-12 were assigned to *tert*-alcohols. The geometry of the double bond between C-10 and C-11 proved to be *Z* configuration due to the chemical shift of the attached methyl (C-20, δ_C 21.7)[5]. Accordingly, the structure of neorustmicin B was established as shown in Fig. 5.

The EI-HRMS data of **II** and **III** as shown in Table 1 exhibited that they had the same molecular formula ($C_{20}H_{32}O_5$). The separated signals in the ^1H NMR spectra of **II** and **III** taken in $CDCl_3$ accounted for 30 non-exchangeable protons ($CH_3\times6$, $CH_2\times2$, $CH\times3$, $CH\text{-}O\times3$ and $C{=}H\times2$) and suggested the presence of two

Fig. 5. Structure of neorustmicins B (I), C (II) and D (III).



I II III

alcohols.

The structures of **II** and **III** were determined by spin decoupling experiments. Two partial structures of **II** and **III** as shown in Figs. 3 and 4, respectively, were easily confirmed. A ketone C-6 of **II** was attached to an olefinic carbon C-5 because this ketone (δ_C 199.0) was assigned to an α,β-unsaturated ketone. The chemical shifts of isolated methylene protons 5-Ha and 5-Hb of **III** (δ_H 2.87 and 3.41) indicated that the methylene carbon C-5 was attached to a ketone C-4. Lower chemical shift of 13-H (δ_H 4.75) of **II** as compared with those of 3-H and 9-H of **II** (δ_H 4.04 and 3.63) was ascribed to the formation of an ester linkage. The structure of **II** was established as shown in Fig. 5 because of no coupling between 2-H and 7-H. The geometry of the double bond between C-4 and C-5 was determined to be *Z* configuration by nuclear Overhauser effect (NOE) enhancement observed with 5-H on irradiation of 17-H while that between C-10 and C-11 determined to be *E* configuration by NOE enhancement with 12-H on the irradiation of 19-H.

Similarly, chemical shift of 13-H of **III** (δ_H 4.67) was attributable to the formation of an ester linkage. There were no direct information on the connectivity between the partial structures of **III** shown in Fig. 4. Taking account of the biosynthesis of known macrolide antibiotics[6~8], rustmicin and neorustmicins are assumed to belong to polyketide substances formed by the condensation of acetates and propionates. Thus, it is reasonable to connect between C-2 and the ester carbonyl of **III** to give the structure of **III** as shown in Fig. 5. The geometries of the double bonds were determined to be *E* configuration by NOE enhancement with 8-H and 12-H on the irradiation of 17-H and 19-H, respectively.

Neorustmicin B inhibited elongation of the germ tube of wheat stem rust fungus at 1.0 μg/ml being accompanied with swelling of the tube tops *in vitro* as did rustmicin and neorustmicin A. Similarly, neorustmicins C and D inhibited elongation of the germ tube at 4 and 5 μg/ml, respectively.

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