Gualamycin, a Novel Acaricide Produced by *Streptomyces* sp. NK11687

II. Structural Elucidation†

KOUICHI TSUCHIYA, SHINICHI KOBAYASHI, TAKASHI KUROKAWA, TAIZO NAKAGAWA and NOBUYOSHI SHIMADA

Applied Microbiology Research Center, Nippon Kayaku Co. Ltd., 225-1 Horigome, Koshikiya, Ageo, Saitama 362, Japan

HIKARU NAKAMURA

Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawaku, Tokyo 141, Japan

YOICHI IITAKA

The Department of Biological Sciences, The Nishi Tokyo University, 2525 Yatsuzawa, Uenohara, Kitatsuru, Yamanashi 409-01, Japan

MASAYUKI KITAGAWA and KUNIAKI TATSUTA

Waseda University, Graduate School of Science and Engineering, Okubo, Shinjuku, Tokyo 169, Japan

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A novel acaricide, gualamycin was isolated from the culture broth of *Streptomyces* sp. NK11687. The structure of gualamycin was determined to be (2*R*,3*S*,4*S*)-2-*O*-[4-*O*-(2-amino-2-deoxy-β-D-gulopyranosyl)-α-D-galactopyranosyl]-2,3,4-trihydroxy-4-[(2*S*,3*S*,4*S*,5*S*)-3,4-dihydroxy-5-hydroxymethylpyrrolidin-2-yl] butanoic acid by FAB-MS, ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, IR, X-ray crystallographic analyses and synthetic studies.

Gualamycin (Fig. 1) is a new microbial secondary metabolite having the acaricidal activity. The taxonomy, production, isolation and preliminary characterization of gualamycin were reported in the preceding paper[1]. In this paper we describe the structural elucidation.

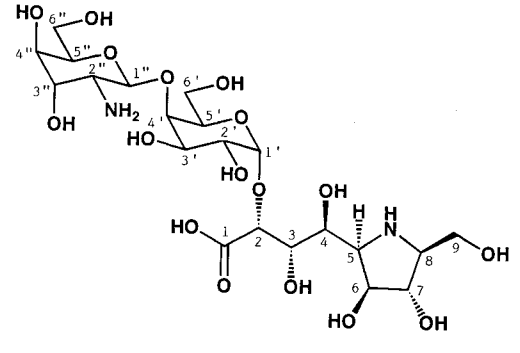
Results and Discussion

The IR spectrum of gualamycin (Fig. 2) showed major absorptions at 3412, 2942, 1607 cm^{-1} indicating polyhydroxy groups, C-H bonds and carboxyl group. The high resolution positive ion FAB-MS of gualamycin was recorded as 591.2288, which corresponded to $C_{21}H_{39}N_2O_{17}$ (Calcd 591.2249; M+H).

The ^{1}H NMR and ^{13}C NMR spectra of gualamycin in deuterium oxide are shown in Figs. 3 and 4, respectively. Chemical shifts and DEPT data in the NMR spectra are in Table 1. The ^{13}C NMR spectrum of gualamycin revealed the presence of twenty-one carbons, which were assigned to one carbonyl, two anomeric methines, three amino methines, twelve oxy methines and three oxy methylenes by the heteronuclear multiple-quantum coherency (HMQC) spectrum and DEPT experiments.

In addition, the ^{1}H-^{1}H COSY data showed the couplings among 2-H and 3-H, 3-H and 4-H, 4-H and 5-H, 5-H and 6-H, 6-H and 7-H, 7-H and 8-H, 8-H and 9-H, 1′-H and 2′-H, 2′-H and 3′-H, 3′-H and 4′-H, 4′-H

Fig. 1. Structure of gualamycin.



† Dedicated to Prof. S. ŌMURA on his 60th birthday.

Fig. 2. IR spectrum of gualamycin (KBr).

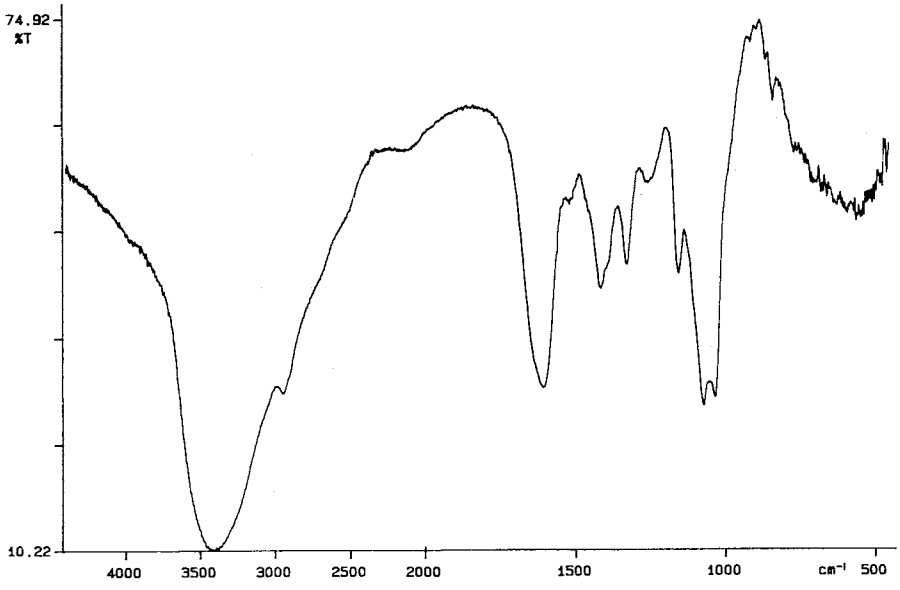


Fig. 3. ^{1}H NMR spectrum of gualamycin (400 MHz in D_2O).

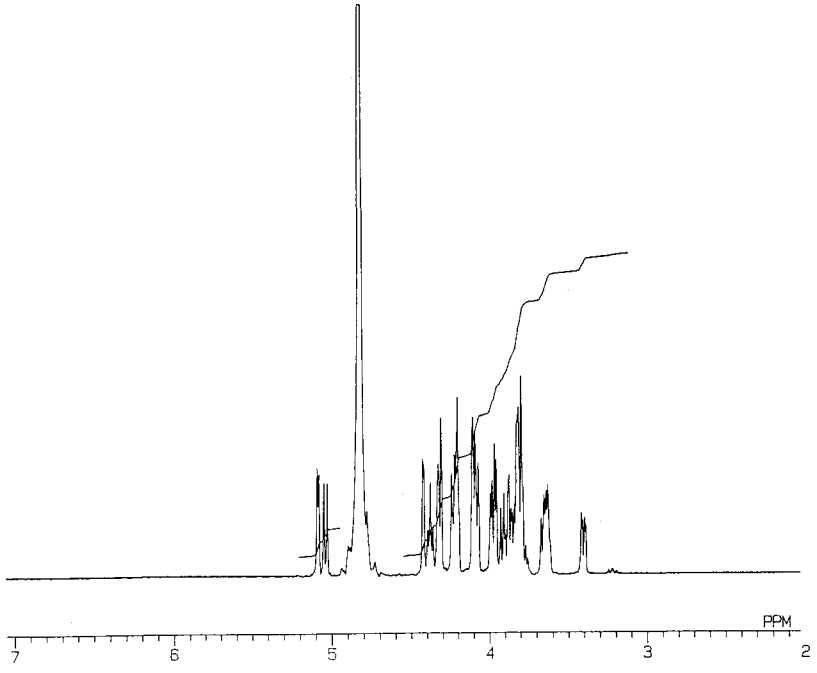
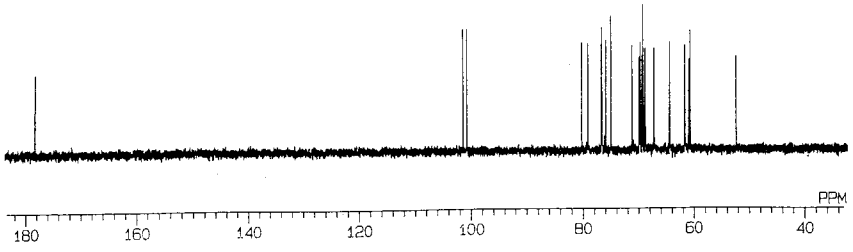


Fig. 4. ^{13}C NMR spectrum of gualamycin (100 MHz in D_2O).



and 5′-H, 5′-H and 6′-H, 1″-H and 2″-H, 2″-H and 3″-H, 3″-H and 4″-H, 4″-H and 5″-H, 5″-H and 6″-H, respectively. Partial structures A, B, C and D (Fig. 5) can be constructed from above NMR data. The partial structure (A) from C-1″ to C-6″ suggested an existence of β-amino sugar and the partial structure (B) from C-1′ to C-6′ suggested an existence of α-sugar by the coupling constants. The HMBC data between 1-C and 2-H indicated the connection of the partial structures C and D. The HMBC data between 2-C and 1′-H, 2-H and 1′-C, 1′-C and 5′-H, 1′-H and 5′-C and 4′- C and 1″-H, 4′-H and 1″-C indicated the connections of the partial structures B and C, A and B, respectively.

Table 1. ^{13}C and ^{1}H NMR chemical shifts of gualamycin in D_2O.

Carbon number	Carbon type[a]	δ_C[b]	δ_H[c]
1	C=O	178.1	
2	CH–O	80.1	4.31 (1H, m)
3	CH–O	75.9	4.08 (1H, d, J=7.1 Hz)
4	CH–O	67.1	4.22 (1H, t, J=8.3 Hz)
5	CH–N	64.3	3.87 (1H, dd, J=2.54, 7.62 Hz)
6	CH–O	76.5	4.42 (1H, d, J=2.94 Hz)
7	CH–O	76.7	4.10 (1H, br d)
8	CH–N	69.1	3.64 (1H, m)
9	CH_2–O	60.6	3.90 (2H, m)
1′	O–CH–O	101.3	5.08 (1H, d, J=4.2 Hz)
2′	CH–O	69.6	3.97 (1H, dd, J=4.2, 10.9 Hz)
3′	CH–O	69.8	4.23 (1H, br d, J=8.3 Hz)
4′	CH–O	79.0	4.32 (1H, m)
5′	CH–O	71.1	4.38 (1H, t, J=6.6 Hz)
6′	CH_2–O	60.9	3.64 (1H, dd, J=6.6, 15.6 Hz)
			3.80 (1H, dd, J=6.6, 15.6 Hz)
1″	O–CH–O	100.6	5.04 (1H, d, J=8.8 Hz)
2″	CH–N	52.3	3.40 (1H, dd, J=2.6, 8.8 Hz)
3″	CH–O	69.3	4.20 (1H, t, J=2.6 Hz)
4″	CH–O	68.7	3.83 (1H, m)
5″	CH–O	74.9	4.09 (1H, t, J=5.9 Hz)
6″	CH_2–O	61.6	3.80 (2H, m)

[a] Based on ^{13}C DEPT NMR experiments.
[b] 100 MHz in D_2O (ppm).
[c] 400 MHz in D_2O (ppm).

Acidic Methanolysis of Gualamycin (I) and Isolation of II and III

By refluxing in 5% methanolic hydrogen chloride solution for 10 hours, gualamycin was split into methyl α- and β-glycosides and an aglycone methyl ester (III), the latter of which was a new pyrrolidine derivative. The resulting precipitates were recrystallized from aqueous methanol to give colorless needles (III).

The filtrates were evaporated to give the mixture of methyl α- and β-glycosides which was treated with acetic anhydride in pyridine at room temperature to yield colorless oil of a hepta-acetyl glycosides. The acetate (II) was purified by silica gel colum chromatography and HPLC. This was determined to be methyl 2,3,6-tri-*O*-acetyl-4-*O*-(3,4,5-tri-*O*-acetyl-2-deoxy-2-acetamido-β-

Fig. 5. Partial structures, ^{1}H-^{1}H COSY and HMBC coupling patterns of gualamycin.

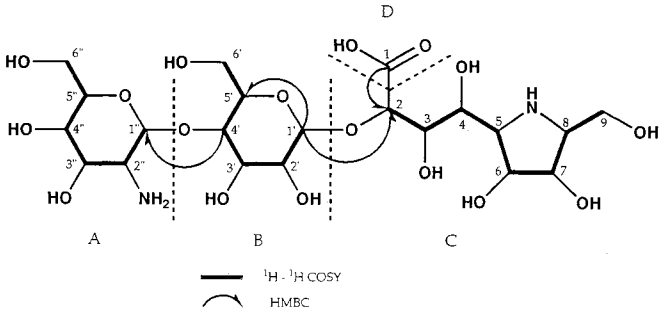


Fig. 6. Acidic methanolysis of gualamycin.

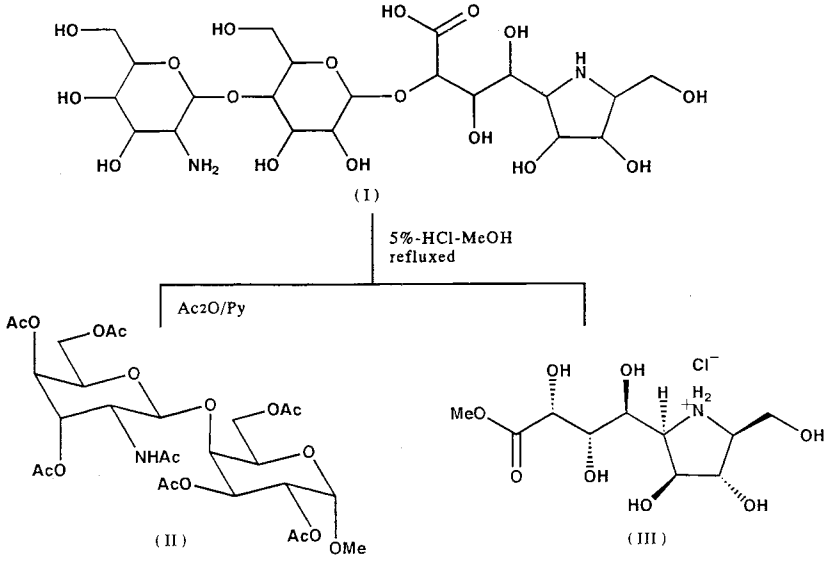
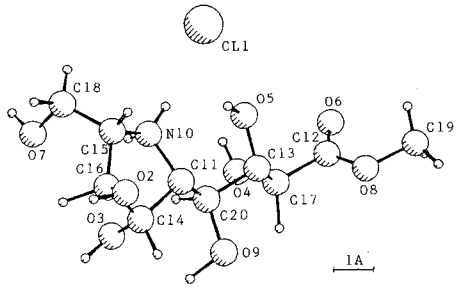


Fig. 7. X-ray molecular structure of aglycone (III)·HCl drawn by PLUTO program[4].



gulopyranosyl)-α-galactopyranoside by ^1H and ^{13}C NMR spectra. The absolute structure of this acetyl glycoside was determined to be the β-D-gulopyranosyl-α-D-galactopyranoside by comparing the optical rotation ($[\alpha]_D^{20}$ +42.6°) with that of the synthetic product[2].

The aglycone (III) was determined to be methyl 2,3,4-trihydroxy-4-(3,5-dihydroxy-5-hydroxymethylpyrroridin-2-yl) butanate·HCl by ^1H and ^{13}C NMR spectrum in DMSO-d_6. The crystals of aglycone (III) were found to be optimum, which formed in the monoclinic space group C2 with a=15.4304(11) Å, b=5.1337(8) Å, c=17.1172(10) Å, β=95.470(5)°; V=1349.8 Å^3; Z=4; Dx 1.563 g/cm^3. The structure was determined by the direct method using SHELXS86[3]. Parameters were refined by using anisotropic temperature factors to R=0.033 for 1545 reflections. The absolute configuration was determined using anomalous dispersion of Cl, O, N and C atoms for CuK_α radiation†. A perspective view of aglycone (III)·HCl drawn by PLUTO program[4] is illustrated in Fig. 7. This configuration was further confirmed by using the hydrobromide crystal of aglycone (III) and the whole structure, (2*R*,3*S*,4*S*)-2-*O*-[4-*O*-(2-amino-2-deoxy-β-D-gulopyranosyl)-α-D-galactopyranosyl]-2,3,4-trihydroxy-4-[(2*S*,3*S*,4*S*,5*S*)-3,4-dihydroxy-5-hydroxymethylpyrrolidin-2-yl] butanoic acid, was also confirmed by the enantiospecific synthesis[2]. As reported in the preceding paper[1], it showed 100% acaricidal activity at 250 μg/ml against mites. The synthetic studies on gualamycin will be reported separately[2].

Experimental

IR, FAB-MS and NMR

IR spectrum was recorded on a Perkin Elmer model 1600 FT-IR. FAB-MS spectrum was obtained on JEOL JMS-AX505HA mass spectrometer. NMR spectrum were recorded on JEOL GX-400 NMR spectrometer with ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz.

Isolation of II and III from the Acidic Methanolysis of Gualamycin (I)

A solution of gualamycin (I, 655 mg) in 12 ml of 5% methanolic hydrogen chloride was refluxed for 10 hours. The solution was then concentrated to 1 ml *in vacuo* and poured into 20 ml of methanol. The white precipitate was collected by filtration and thoroughly dried *in vacuo*. The precipitate was recrystallized from aqueous methanol to give 224 mg of colorless needles (III). mp 181~182°C (dec.), $[\alpha]_D^{20}$ +51.3° (*c* 0.5, H_2O) TLC: Rf 0.31 (BuOH - AcOH - H_2O, 4:1:1).

^1H NMR (400 MHz DMSO-d_6) δ 9.39 (1H, br), 7.99 (1H, br), 5.80 (1H, br), 5.64 (1H, d, *J*=2.93 Hz), 5.49 (1H, d, *J*=7.7 Hz), 5.42 (1H, d, *J*=5.9 Hz), 5.27 (1H, t, *J*=4.7 Hz), 4.73 (1H, d, *J*=9.5 Hz), 4.42 (1H, d, *J*=5.5 Hz), 4.13 (1H, br), 4.03 (1H, dd, *J*=8.6, 7.7 Hz), 3.80 (2H, m), 3.66 (3H, s), 3.64 (2H, m), 3.50 (1H, br), 3.38 (1H, m).

^{13}C NMR (100 MHz DMSO-d_6) δ 173.5, 75.3, 74.8, 74.7, 69.9, 69.3, 64.7, 64.4, 59.6, 51.5.

The filtrate of the above methanolyzate was concentrated *in vacuo* to yield 548 mg of crude oil. The oil was stirred with 2 ml of acetic anhydride and 4 ml of pyridine for 12 hours at room temperature, and the solution was added to water (50 ml). The resulting solution was extracted with ethyl acetate (3×60 ml). The organic phase was washed with 1 N hydrochloric acid (2×50 ml), saturated aqueous $NaHCO_3$ (50 ml) and water (2×50 ml), dried with anhydr $MgSO_4$ and then evaporated. The residue was chromatographed on a silica gel (BW-700, 2 i.d. × 15 cm) using $CHCl_3$ - MeOH to afford 403 mg of colorless oil. The colorless oil was purified by using a reversed-phase HPLC column (CAPCELL PAK C18 15 i.d. × 250 mm, flow rate 9 ml/minute) with a mobile phase of H_2O - CH_3CN (70:30). The fractions of peak were concentrated *in vacuo* to yield 43.4 mg of colorless oil (II). $[\alpha]_D^{20}$ +42.6° (*c* 0.5, MeOH) TLC: Rf 0.33 ($CHCl_3$ - MeOH, 40:1).

^1H NMR (400 MHz $CDCl_3$) δ 5.50 (1H, d, *J*=3.3 Hz), 5.39 (1H, dd, *J*=10.6, 2.2 Hz), 5.35 (1H, dd, *J*=10.6,

† The following Tables have been submitted to the editorial office of the Journal of Antibiotics.

Table 2. Crystal and experimental data of aglycone (III)·HCl.

Table 3. The final atomic coordinates and equivalent isotropic temperature factors Beq of the crystal of aglycone (III)·HCl.

Table 4. Comparison of the calculated and observed intensity ratio, |F(hkl)+|2/|F(hkl)−|2 with the estimated standard deviations for the crystal of aglycone (III)·HCl. The ratios were calculated only for such reflections which satisfy ||Fobs+|−|Fobs−||>2σ[Fobs].

Table 5. Estimation of the effect of anomalous dispersion of the crystal of aglycone (III)·HCl by Engel's method[5]. The DELA values were calculated for hkl reflection using the data for h'k'l' in order to reduce the absorption effect of the diffraction intensities; where, h'k'l' was chosen such that |h−h'|≦2, |k−k'|≦2, |l−l'|≦2, excluding h=h', k=k', l=l'.

2.9 Hz), 4.98 (1H, d, $J=3.3$ Hz), 4.85 (1H, dd, $J=10.6$, 2.9 Hz), 4.70 (1H, d, $J=8.8$ Hz), 4.2 (6H, m), 4.1 (2H, m), 3.42 (3H, s), 2.18 (3H, s), 2.12 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.08 (3H, s), 2.06 (3H, s), 2.04 (3H, s).

^{13}C NMR (100 MHz $CDCl_3$) δ 170.7, 170.7, 170.1, 169.4, 168.8, 168.2, 99.6, 97.5, 75.1, 71.1, 71.0, 68.9, 67.8, 67.6, 66.1, 63.6, 61.9, 55.3, 50.1, 23.0, 20.8, 20.7.

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