[Chem. Pharm. Bull. 28(12)3601—3611(1980)]

Chemistry of Toromycin

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(Received June 27, 1980)

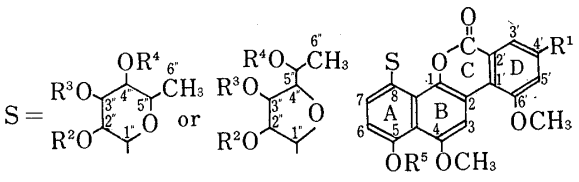
Chemical degradation and spectral studies on toromycin, an antibiotic isolated from *Streptomyces collinus* subsp. *albescens*, showed that toromycin is a polycyclic aromatic hydrocarbon having a C-glycosyl group at C-4, a vinyl group at C-8, two methoxyl groups at C-10 and C-12, and a phenolic hydroxyl group at C-1 of 6-oxo-6*H*-benzo[*d*]naphtho-[1,2-*b*]pyran (**1**).

Keywords——polycyclic aromatic; C-glycoside; 6-oxo-6*H*-benzo[*d*]naphtho[1,2-*b*]-pyran; (2-phenylnaphthalene)carbolactone; naphtho[1,2-*c*]isocoumarin; antibacterial; anti-PPLO; antiviral

Toromycin (**1**) is an antibiotic produced by *Streptomyces collinus* subsp. *albescens* and shows activities against pleuropneumonia-like organisms, DNA-type viruses, trichomonases, and gram-positive bacteria, including mycobacteria. Its isolation and physico-chemical properties have been reported.[2]

The present report proposes the structure of **1**, except for the stereochemistry and ring size (furano or pyrano) of the C-glycosyl moiety of **1**, and discusses the evidence supporting the proposed structure as well as the chemistry of **1**. Some structure-activity relationships between **1** and its derivatives will be presented.

Toromycin is a yellow crystalline substance having a molecular formula of $C_{27}H_{26}O_9$; it shows a blue color upon reaction with ferric chloride-potassium ferricyanide and dissolves in dilute alkali (2 N NaOH) to give a red wine-colored solution.[2]



Compound	R^1	R^2	R^3	R^4	R^5
1	$-CH=CH_2$	H	H	H	H
1b	$-CH=CH_2$	Ac	Ac	Ac	Ac
2	$-CH_2CH_3$	H	H	H	H
1′	$-CH=CH_2$	H	H	H	H
1′b	$-CH=CH_2$	Ac	Ac	Ac	Ac
1′c	$-CH=CH_2$	H	$H_3C>C<CH_3$		H
1′d	$-CH=CH_2$	Ac	$H_3C>C<CH_3$		Ac

Chart 1

The proton nuclear magnetic resonance (PMR) spectrum of **1** indicated the presence of one secondary C-methyl, two methoxyls, one phenolic hydroxyl, one vinyl, five aromatic protons, and five methine protons,[2] and the carbon-13 nuclear magnetic resonance (CMR) spectrum indicated the presence of sixteen aromatic carbons (C<u>H</u>×5, C<u></u>×7, C<u></u>–O–×4), two olefinic carbons (C<u>H</u>$_2$×1, C<u>H</u>×1), one carbonyl carbon (C<u></u>=O), and eight aliphatic carbons (C-C<u>H</u>$_3$×1, O–C<u>H</u>$_3$×2, C<u>H</u>×5).

The catalytic hydrogenation (platinum oxide in dioxane) of **1** gave dihydrotoromycin (**2**), $C_{27}H_{28}O_9$, [*m/e* 496.17 (M^+)]. The ultraviolet (UV) spectra showed that the peak at 288 nm in **1** was shifted to 277 nm in **2**, and the PMR and CMR spectra indicated that the vinyl group

1) Location: *Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.*
2) K. Hatano, E. Higashide, M. Shibata, Y. Kameda, S. Horii, and K. Mizuno, *Agric. Biol. Chem.* (Tokyo), **44**, 1157 (1980).

Table I. CMR Chemical Shifts of **1**, **2**, and **4** in DMSO-d_6

	1	**2**	**4**
$\underline{C}H_2$=$\underline{C}$H–	116.5(t), 135.1(d)	—	—
$\underline{C}H_3\underline{C}H_2$–	—	14.8(q), 28.0(t)	15.6(q), 28.7(t)
$\underline{C}H_3$O–	55.9(q), 56.3(q)	56.0(q), 56.3(q)	55.6(q), 56.9(q)
Aromatic $\underline{C}$–H	101.0(d), 111.7(d), 113.9(d), 118.7(d), 128.8(d)	101.3(d), 111.5(d), 117.3(d), 119.5(d), 128.8(d)	108.1(d), 109.6(d), 109.9(d), 119.0(d), 127.8(d)
Aromatic $\underline{C}$	112.6(s), 114.6(s), 121.6(s), 122.5(s), 123.4(s), 125.8(s), 138.2(s)	112.8(s), 114.5(s), 121.0(s), 121.5(s), 123.5(s), 125.7(s), 145.8(s)	115.5(s), 119.4(s), 122.3(s), 124.9(s), 126.6(s), 144.3(s), 145.1(s)
Aromatic $\underline{C}$–O	141.9(s), 151.3(s), 152.5(s), 156.9(s)	141.6(s), 151.4(s), 152.5(s), 156.7(s)	141.6(s), 149.0(s), 153.0(s), 156.9(s)
C-Glycosyl $\underline{C}H_3$	20.3(q)	20.3(q)	20.2(q)
C-Glycosyl O–$\underline{C}$–H	66.6(d), 78.9(d×2), 80.9(d), 86.0(d)	66.5(d), 78.9(d×2), 80.9(d), 86.0(d)	67.0(d), 78.4(d), 79.7(d), 81.3(d), 88.3(d)
Lactone $\underline{C}$=O	159.3(s)	159.5(s)	—
–$\underline{C}H_2$OH	—	—	61.4(t)

in **1** had been changed to an ethyl group [CMR: δ 14.8 (q), 28.0 (t); PMR: δ 1.26 (3H, t, J=7), 2.72 (2H, q, J=7)] in **2**. These findings suggested the presence of a vinylbenzene partial structure.

Acetylation (Ac_2O–pyridine) of **1** gave the acetate (**1b**) which did not show the phenol color reaction with ferric chloride-potassium ferricyanide. The PMR spectrum of **1b** showed the presence of four acetyl groups (δ 1.47, 2.10, 2.27, and 2.31). The high field signal at δ 1.47 was assigned to 2″-O-acetyl in the C-glycosyl moiety (magnetic anisotropy of the aromatic ring).

When **1** was treated with acid (reflux for 2 hr in 50% aqueous AcOH) or alkali (reflux for 30 min in dioxane–1 N NaOH 1: 1), followed by silica gel chromatography, an isomerized toromycin (**1′**) was isolated. The isomer **1′** has the same molecular formula, $C_{27}H_{26}O_9$ as **1**, and the UV spectrum of **1′** (λ_{max}^{MeOH} (nm): 247, 277, 288, and 396) is similar to that of **1**. However, the infrared (IR) absorption of the C=O group shifted from 1690 cm^{-1} (in **1**) to 1710 cm^{-1} (in **1′**) and the optical rotation changed from $[\alpha]_D$ −204° (in **1**) to $[\alpha]_D$ −40° (in **1′**). Some changes in the PMR spectra were also observed in the signals of the C-1″ proton and C-methyl protons of the C-glycosyl moiety, and one of the *ortho* aromatic protons, as will be discussed later (Table V).

The isomer **1′** gave a tetraacetyl derivative (**1′b**; Ac_2O–pyridine), monoisopropylidene derivative (**1′c**; 2,2-dimethoxypropane-*p*-toluenesulfonic acid), and monoisopropylidene diacetyl derivative (**1′d**; Ac_2O–pyridine). In the PMR spectrum of **1′d**, the chemical shifts of one methyl and five methine protons [δ 1.46 (d, J=6.5, C-6″), 4.40 (m, C-5″), 4.20 (dd, J=2.5, J=6, C-4″), 4.44 (dd, J=6, J=8, C-3″), 5.16 (dd, J=8, J=10, C-2″), 6.12 (d, J=10, C-1″)] were dispersed enough to support the partial structure I upon spin decoupling studies.

However, attempts to isolate a sugar residue by the hydrolysis of **1** or **1′** were unsuccessful, even by vigorous acid hydrolysis. These results showed the presence of a C-glycosidic linkage, instead of an O-glycosidic linkage. The monoisopropylidene derivative **1′c** showed a blue color on reaction with ferric chloride-potassium ferricyanide, but the monoisopropylidene diacetyl derivative **1′d** gave no color with ferric chloride-potassium ferricyanide and showed the signal (δ 1.53) of the acetyl group influenced by the magnetic anisotropy of the aromatic ring in the PMR spectrum . These results indicated that these monoisopropylidene derivatives (**1′c** and **1′d**) are 3″, 4″ (or 3″, 5″)-monoisopropylidene derivatives and that **1** and **1′** have three secondary alcoholic groups and one phenolic hydroxyl group.

The nature of the carbon skeleton was established on the basis of the following spectral and degradative data. The PMR spectra of **1** showed the presence of one pair of aromatic protons in the *meta* position [δ 7.52 (d, J=1) and 7.82 (d, J=1)], one pair of aromatic protons in the *ortho* position [δ 6.86 (d, J=8.5) and 8.02 (d, J=8.5)], and one uncoupled proton at δ 8.24 (s). Zinc-dust distillation of **1** gave a product whose UV spectrum is very similar to that of 1,2-benzofluorene or 2,3-benzofluorene. These data suggested that **1** might contain a 2-phenylnaphthalene system, and this conjecture was confirmed by the following experiments.

The oxidative degradation of **1** with alkaline permanganate gave the benzenecarboxylic acid (**6**) as colorless needles, $C_{10}H_8O_7$. The PMR spectrum of **6** showed the presence of one pair of *meta* aromatic protons and one methoxyl group, and **6** gave the trimethyl ester (**6b**), $C_{13}H_{14}O_7$ [*m/e*: 282 (M^+)] upon treatment with diazomethane. The methoxybenzenetricarboxylic acid **6** and its trimethyl ester **6b** were respectively identical with authentic 6-methoxy-1,2,4-benzenetricarboxylic acid and its trimethyl ester, which were prepared by permanganate oxidation of 2,3,5-trimethylanisole.[3)]

Treatment of **1** with alkaline hydrogen peroxide gave pale yellow leaflets (**5**), $C_{11}H_{10}O_5$ [*m/e*: 222 (M^+)]. The PMR of **5** showed the presence of one methoxyl, one AMX-type vinyl system, and one pair of *meta* aromatic protons. The alkaline permanganate oxidation of **5** gave **6**, and **5** was converted to the anhydride **5b** (IR: ν C=O 1840 and 1875 cm^{-1}) by heating. These data showed the compound **5** to be 3-methoxy-5-vinyl-1,2-benzenedicarboxylic acid and demonstrated the presence of the partial structure II in **1**.

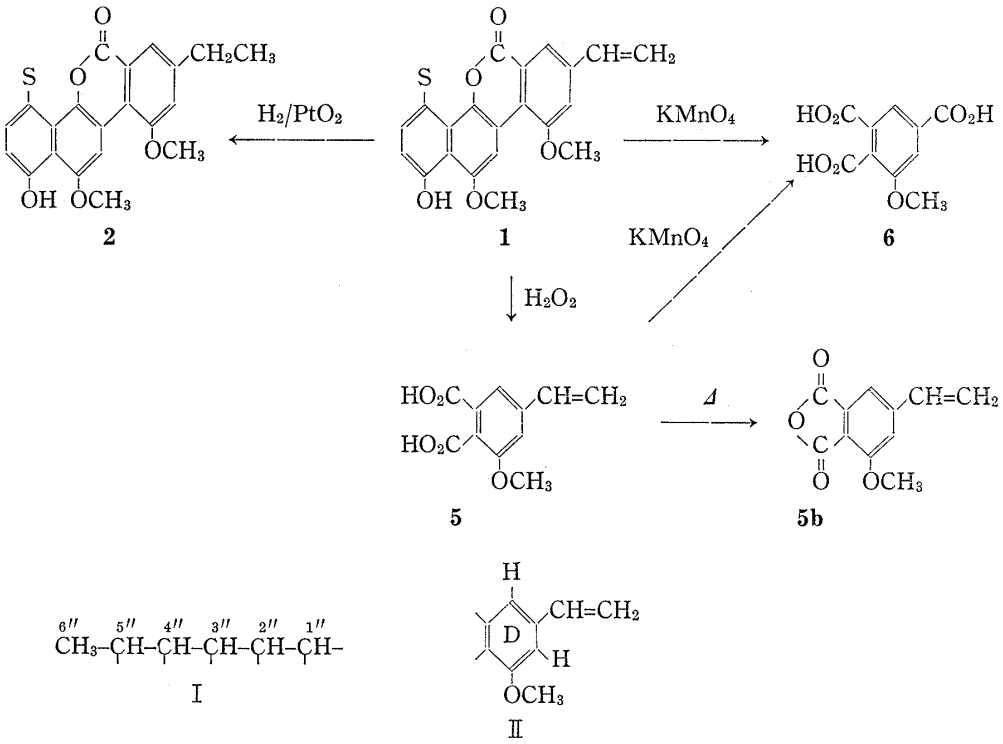


Chart 2

Reduction of **1** with sodium borohydride gave tetrahydrotoromycin **3**. The IR spectrum of **3** did not show any characterisitc absorption peak in the C=O region. The catalytic hydrogenation of **3** gave hexahydrotoromycin **4**, $C_{27}H_{32}O_9$ [*m/e* 500 (M^+)]. The PMR spectrum of **4** showed the presence of one ethyl (δ: 1.27, 3H, t, J=7; δ: 2.68, 2H, q, J=7) and two phenolic hydroxyls (δ 9.67 and 7.86 in DMSO-d_6; disappeared upon addition of D_2O) and the

3) C.A. Buehler, R.B. Spees, and P.A. Sanguinetti, *J. Am. Chem. Soc.*, **71**, 11 (1949).

CMR spectrum of **4** indicated that the C=O group in **2** had changed to a CH_2 group. Furthermore, reduction of **1b** with sodium borohydride gave a compound which showed a blue color upon reaction with ferric chloride-potassium ferricyanide and gave the hexaacetate upon further acetylation (Ac_2O–pyridine). These data suggested the cleavage of the lactone bridge and the appearance of a new phenolic hydroxyl group upon reduction with sodium borohydride. The new phenolic hydroxyl group (at δ 7.86 in **4**) was assigned to the *peri*-position with respect to the C-glycosyl group as described below, and its chemical shift (higher field signal than the original phenolic hydroxyl) may be explained by hindrance of the solvation of the phenolic hydroxyl group with hydrogen-bonding solvent (DMSO-d_6) by the C-glycosyl group.

The sodium borohydride reduction product **3** was methylated with dimethyl sulfate-potassium hydroxide to give the permethylated derivative (**3b**). Alkaline potassium permanganate oxidation of **3b** gave two benzenecarboxylic acids (**6** and **8**) and one benzil derivative, **7**. One of the two benzenecarboxylic acids was identified as 6-methoxy-1,2,4-benzenetricarboxylic acid (**6**), which is the same as the compound obtained by permanganate oxidation of **1**. The other benzenecarboxylic acid **8**, $C_{10}H_8O_7$ gave the trimethyl ester, $C_{13}H_{14}O_7$ [*m/e* 282 (M^+)] upon treatment with diazomethane. The PMR spectrum of the trimethyl ester of **8** showed the presence of one pair of *ortho* aromatic protons and four methoxyl groups, and **8** was identified as 4-methoxy-1,2,3-benzenetricarboxylic acid by comparison with an authentic sample.[4] The compound **7**, $C_{20}H_{14}O_{12}\cdot 2H_2O$ showed a characterisitc C=O IR absorption at 1760 cm^{-1}. The PMR spectrum of **7** showed the presence of four aromatic protons (one pair each of *meta* and *ortho* aromatic protons) and two methoxyl groups. Esterification of **7** with diazomethane gave the tetramethyl ester (**7b**), $C_{24}H_{22}O_{12}$ [*m/e* 502 (M^+)], and hydrogen

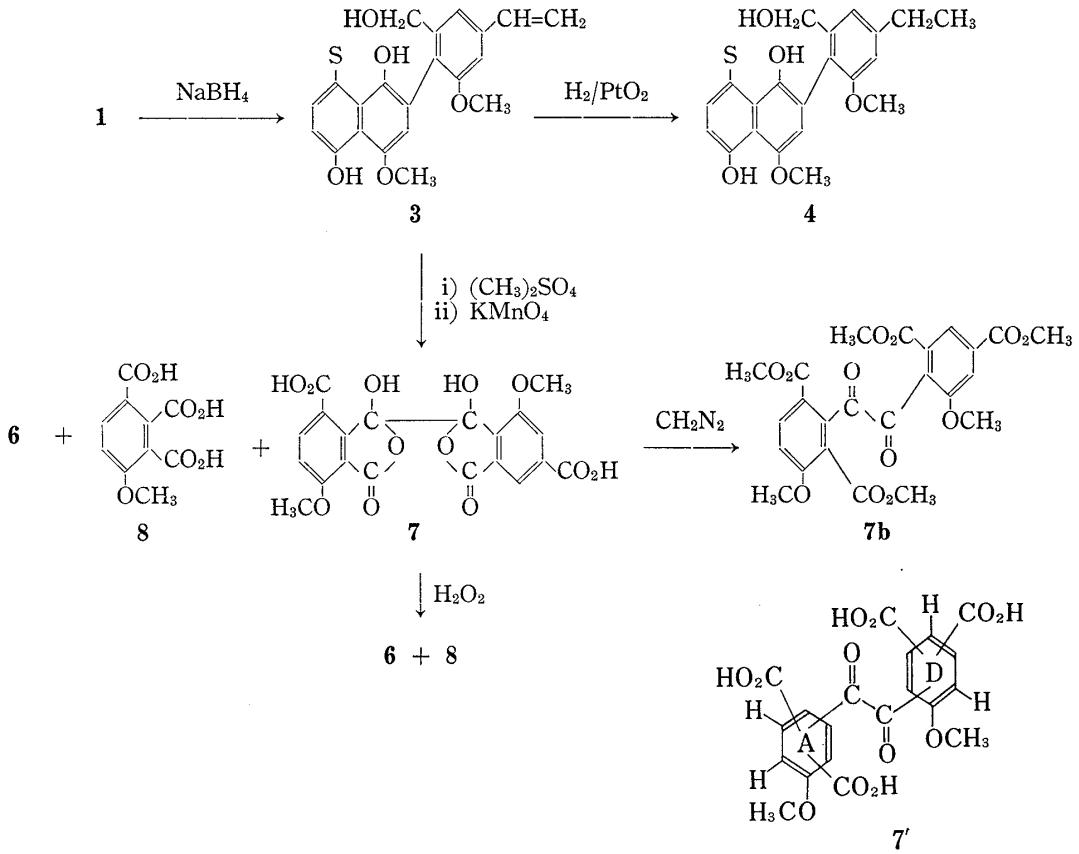


Chart 3

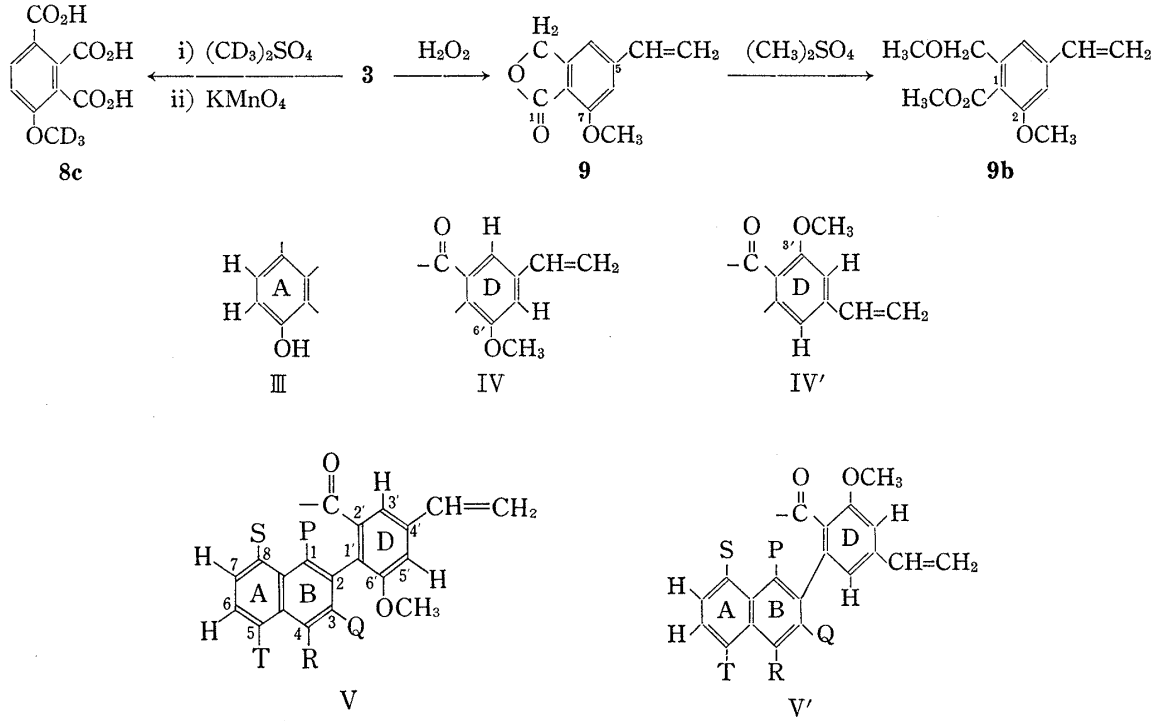
4) D. Gardner, J.F. Grove, and D. Ismay, *J. Chem. Soc.*, **1954**, 1817.

peroxide oxidation of **7** in acetic acid gave **6** and **8**. These data suggested the presence of the partial structure **7'**.

Similarly, the sodium borohydride reduction product **3** was methylated with dimethyl sulfate-d_6-potassium hydroxide to give the perdeuteriomethylated derivative **3c**. Alkaline permanganate oxidation of **3c** gave 4-deuteriomethoxy-1,2,3-benzenetricarboxylic acid (**8c**). In view of these data, the partial structure III can be suggested for the Ring A moiety of **1**.

Alkaline hydrogen peroxide oxidation of **3** gave colorless needles (**9**), $C_{11}H_{10}O_3$ [*m/e* 190 (M^+), IR: 1745 cm^{-1}]. The PMR spectrum of **9** showed the presence of one pair of *meta* aromatic protons, as well as $-CH=CH_2$, $-OCH_3$, and $-CH_2-$ groups. Treatment of **9** with dimethyl sulfate and potassium hydroxide gave a colorless oil (**9b**), $C_{13}H_{16}O_4$ [*m/e* 236 (M^+)]. The PMR spectrum of **9b** showed the presence of one pair of *meta* aromatic protons at δ 6.88 (1H, d, $J=1$) and 7.02 (1H, d, $J=1$), one vinyl system at 5.30 (1H, d, $J=11$), 5.76 (1H, d, $J=18$), and 6.80 (1H, dd, $J=11$ and 18), $-CH_2OCH_3$ at 4.43 (2H, s) 3.30 (3H, s), and two methoxyls at 3.83 (3H, s) and 3.86 (3H, s). These data showed that **9** is 7-methoxy-5-vinylphthalide or 4-methoxy-6-vinylphthalide and that **9b** is methyl 2-methoxy-6-methoxymethyl-4-vinylbenzencarboxylate or methyl 3-methoxy-2-methoxymethyl-5-vinylbenzenecarboxylate; the $-OCH_2-$ group of these compounds was derived from the lactone carbonyl of **1**.

On the basis of these results, the structure IV or IV' was assigned to the Ring D moiety of **1**, and the structure V or V' can be proposed as a partial structure of **1**.



P, Q, R=–H, –OCH_3, or –O–
S, T=–OH or C-glycosyl

Chart 4

The IR spectrum of **9** showed a characteristic C=O absorption at 1745 cm^{-1}, which suggested that **9** is a 7-methoxyphthalide (1748 cm^{-1}) rather than a 4-methoxyphthalide (1765 cm^{-1}),[5] and the structure of the Ring D moiety of **1** was determined to be the 6'-methoxy structure IV by the following nuclear Overhauser effect (NOE) experiments.

5) L.A. Duncanson, J.F. Grove, and J. Zealley, *J. Chem. Soc.*, **1953**, 1331.

The NOE technique was applied for assignment of the positions of the substituent groups on the 2-phenylnaphthalene skeleton of the structure V (or V'). In the NOE experiment on dihydrotoromycin **2** (Table II), saturation of the two methoxyl signals which showed the same chemical shifts at δ 3.98 resulted in enhancements (7%) of the integrated area of the phenol signal at δ 9.60, and this result suggests that the phenolic hydroxyl on Ring A and the methoxyl on Ring B are in a *peri* position with respect to each other.

Table II. NOE of **2** in DMSO-d_6

Proton signal observed (δ, ppm)	Proton signal saturated (δ, ppm)	Percent increase in integrated area of signal
9.60(5-phenolic OH)	3.98(4-OCH_3 and 6'-OCH_3)	7
8.24(3-H)	3.98(4-OCH_3 and 6'-OCH_3)	30
7.29(5'-H)	3.98(4-OCH_3 and 6'-OCH_3)	13
7.64(3'-H)	3.98(4-OCH_3 and 6'-OCH_3)	0

Furthermore, the very strong NOE (30% increase) on the uncoupled aromatic proton on Ring B at δ 8.24 upon irradiation of two methoxyls at δ 3.98 [*cf.* 13% increase between δ 7.29 (obs.) and 3.98 (sat.) and no increase between δ 7.64 (obs.) and 3.98 (sat.)] suggested that the uncoupled aromatic proton is spatially close to both methoxyls. In view of these results, the 4,6'-dimethoxy structure (Q=–H, R=–OCH_3, T=–OH in V) can be proposed as a partial structure of **1**. The 4,6'-dimethoxy structure was further confirmed by NOE experiments with hexahydrotoromycin **4** and tetra-O-acetyl isomerized toromycin **1'b**, as shown in Tables III and IV.

Table III. NOE of **4** in DMSO-d_6

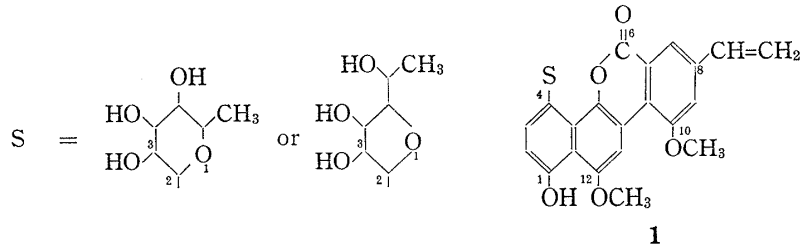
Proton signal observed (δ, ppm)	Proton signal saturated (δ, ppm)	Percent increase in integrated area of signal
6.54(3-H)	3.93(4-OCH_3)	20
6.85(5'-H)	3.93(4-OCH_3)	0
6.54(3-H)	3.68(6'-OCH_3)	0
6.85(5'-H)	3.68(6'-OCH_3)	19
3.68(6'-OCH_3)	6.85(5'-H)	16
3.93(4-OCH_3)	6.85(5'-H)	0
3.93(4-OCH_3)	6.54(3-H)	13
3.68(6'-OCH_3)	6.54(3-H)	0

Table IV. NOE of **1'b** in $CDCl_3$

Proton signal observed (δ, ppm)	Proton signal saturated (δ, ppm)	Percent increase in integrated area of signal
3.96(4-OCH_3)	8.48(3-H)	11
4.00(6'-OCH_3)	8.48(3-H)	4
4.00(6'-OCH_3)	7.30(5'-H)	11
3.96(4-OCH_3)	7.30(5'-H)	0
8.48(3-H)	4.00(6'-OCH_3 and partially 4-OCH_3)	24
7.30(5'-H)	4.00(6'-OCH_3 and partially 4-OCH_3)	17
8.48(3-H)	3.96(4-OCH_3 and partially 6'-OCH_3)	35
7.30(5'-H)	3.96(4-OCH_3 and partially 6'-OCH_3)	10
2.38(5-OAc)	3.96(4-OCH_3 and partially 6'-OCH_3)	5

In the PMR spectra of **4** and **1'b**, the two methoxyl groups on Rings B and D showed different chemical shifts, and the NOE experiments with **4** and **1'b** showed that the uncoupled aromatic proton and the methoxyl group on Ring B were *ortho* with respect to each other (C-3 and C-4 positions) and also that one of the aromatic *meta* protons and the methoxyl on Ring D were *ortho* with respect to each other (C-5' and C-6' positions). In the NOE experiment with **1'b**, it was also confirmed that the methoxyl group on Ring D and the uncoupled aromatic proton on Ring B were spatially close. Therefore, the methoxyls at δ 3.93 in **4** and δ 3.96 in **1'b** were assigned to the C-4 position on Ring B, and the methoxyls at δ 3.68 in **4** and δ 4.00 in **1'b** were assigned to the C-6' position on Ring D. The placement of the two methoxyl groups at C-4 and C-6' positions also reasonably explains the findings that no NOE between δ 3.68 (obs.) and δ 6.54 (sat.) in **4** was observed (because of the free rotation about the 2-1' bond in **4**) and that a considerable chemical shift difference was observed between the C-3 protons of **2** (δ 8.24) and **4** (δ 6.54) as well as **1** (δ 8.24) and **3** (δ 6.46) (the anisotropic effect of Ring D).

Consequently, the lactone ring (Ring C) in **1** must be formed between C-1 and C-2' of the 2-phenylnaphthalene skeleton, and the C-glycosyl group must be attached at C-8 of the 2-phenylnaphthalene skeleton on Ring A; these data meant that the structure of **1** could be assigned as 1-hydroxy-4-(3,4,5-trihydroxy-6-methyl-2*H*-tetrahydropyran-2-yl)-10,12-dimethoxy-6-oxo-8-vinyl-6*H*-benzo[*d*]naphtho[1,2-*b*]pyran or 1-hydroxy-4-[3,4-dihydroxy-5-(1-hydroxymethyl)tetrahydrofur-2-yl]-10,12-dimethoxy-6-oxo-8-vinyl-6*H*-benzo[*d*]naphtho[1,2-*b*]pyran[6] (Chart 5), and the structure of the benzil derivative **7** was determined to be 3,6'-dimethoxy-2,6,2',4'-benziltetracarboxylic acid.



1

Chart 5

The frequencies of the IR carbonyl bands of **1** (1690 cm^{-1}) and its isomerized compound **1'** (1710 cm^{-1}) are compatible with the observed range of carbonyl bands of 2',1-(2-phenylnaphthalene)carbolactones, and a shift in the frequency from 1690 to 1710 cm^{-1} can be rationalized in terms of the release of intramolecular hydrogen bonding between the lactone moiety and the C-glycosyl moiety. The structural difference between **1** and **1'** as well as that between **2** and **2'** was attributed to the configurational change of C-1'' or the conformational change of the C-glycosyl moiety in view of the changes of the PMR spectra (Table V) as well as the change of the optical rotations.

Table V

Compound	C-1''	$-CH_3$	Aromatic *ortho* protons
1	δ 6.13 (d, J=5)	δ 1.28 (d, J=6)	δ 8.02 and 6.86 (each d, J=8.5)
2	δ 6.11 (d, J=5)	δ 1.27 (d, J=6)	δ 8.00 and 6.84 (each d, J=8.5)
1'	δ 5.62 (d, J=8.5)	δ 1.08 (d, J=6.5)	δ 7.72 and 6.85 (each d, J=8.5)
2'	δ 5.60 (d, J=8.5)	δ 1.06 (d, J=6.5)	δ 7.68 and 6.85 (each d, J=8.5)

6) During the preparation of this paper, the structure of antibiotic DC-38-A, which is identical with that of toromycin except that the stereochemistry and ring size of the C-glycosyl moiety remain unsolved, was disclosed in a Japanese patent. [Kyowa Hakko Kogyo Co., Ltd., Japan Patent Provisional Publication 55-57586 (1980)].

The acid-isomerized toromycin (**1'**) retains substantial biological activities, though it is not as potent as the parent compound **1**. However, hydrogenation of the vinyl group to an ethyl group markedly reduced the antimicrobial activities (about 50—100 times less active than **1** against *Bacillus subtilis*), and the antimicrobial activities of the monoisopropylidene derivative (**1'd**) and tetra-O-acetyl derivative (**1b**) were also markedly reduced.

Experimental

Dihydrotoromycin (2) (Catalytic Hydrogenation of Toromycin (1))——A solution of **1** (112 mg) in dioxane (500 ml) was hydrogenated over PtO_2 (100 mg) at room temperature and atmospheric pressure. After 3 hr, the catalyst was removed by filtration. The filtrate was concentrated, and MeOH was added to the concentrated solution. The resulting crystalline compound was recrystallized from dioxane–MeOH to give **2** as pale yellow needles (93 mg). mp 218—220° (dec.), $[\alpha]_D^{22}$ −200.0° (c=0.5, DMSO), IR ν_{max}^{KBr} cm^{-1}: 1690 (C=O), UV λ_{max}^{MeOH} nm ($\varepsilon \times 10^{-3}$): 244.5 (42.9), 267 (28.0), 276 (38.0), 307.5 (11.7), 330 (9.8), 345.5 (7.8), 389 (10.8), PMR (DMSO-d_6) δ: 1.26 (3H, t, J=7, C<u>H</u>$_3$–CH$_2$–), 1.27 (3H, d, J=6, C<u>H</u>$_3$–CH–), 2.72 (2H, q, J=7, –C<u>H</u>$_2$–CH$_3$), 3.51 (1H, t, J=5, –O–CH–<u>H</u>), *ca.* 3.85 (2H, 2×–O–CH–<u>H</u>, overlapped), 3.98 (6H, s, –OC<u>H</u>$_3$×2), 4.57 (1H, t, J=5, –O–CH–<u>H</u>), 6.11 (1H, d, J=5, –O–CH–<u>H</u> at C-1″), 6.84 and 8.00 (each 1H, d, J=8.5, aromatic *ortho*), 7.29 and 7.64 (each 1H, d, J=1, aromatic *meta*), 8.24 (1H, s, aromatic 5-substituted), 9.60 (1H, s, phenolic OH), MS *m/e*: 496.1704 (M$^+$, Calcd 496.1733), *Anal.* Calcd for $C_{27}H_{28}O_9$: C, 65.31; H, 5.68. Found: C, 64.83; H, 5.58.

Zinc Dust Distillation of Toromycin (1)——Zinc dust (30 g), an intimate mixture of **1** (5.0 g) and zinc dust (100 g), and zinc dust (30 g) were packed in that order in a round-bottomed flask (200 ml) with a neck. The mixture was heated in a furnace (100° for 2 hr, 100—200° for 2 hr, 200—300° for 3 hr, and then the temperature in the furnace was raised by 50° an hour up to 550°). The oily substances which were distilled in the range of about 300 to 500° were chromatographed on an alumina column with petroleum ether–benzene (10: 1) as a developing solvent to give colorless needles (4 mg). UV λ_{max}^{hexane} nm ($E_{1cm}^{1\%}$): 220 (1300), 255 (1890), 264 (2500), 287 (629), 303 (618), 315 (640), 342 (152).

Tetrahydrotoromycin (3) (Reductive Cleavage of the Lactone Moiety of Toromycin (1) with $NaBH_4$)——A solution of $NaBH_4$ (0.61 g) in MeOH (20 ml) was added to a suspension of **1** (2.0 g) in dioxane (100 ml) with stirring. The reaction mixture was stirred for 1 hr at room temperature, then dioxane (50 ml) and a solution of $NaBH_4$ (0.3 g) in MeOH (10 ml) were added successively. The mixture was further stirred until it became a clear solution. After removal of solvents *in vacuo*, EtOAc (200 ml) and water (100 ml) were added to the residue. After adjustment to pH 2 with 1 N HCl with stirring, the EtOAc layer was separated. The aqueous layer was again extracted with EtOAc (100 ml×2). The combined EtOAc extracts were washed with water, dried over Na_2SO_4, and concentrated *in vacuo* to give a yellowish-brown foam (1.9 g). This foam was used as the starting material for the following O-methylation reaction without further purification. For further purification, the foam was chromatographed on silica gel. The column was eluted with EtOAc to give **3**. PMR (DMSO-d_6) δ: 1.23 (3H, d, J=6, –CH–C<u>H</u>$_3$), 3.72 (1H, s, –OC<u>H</u>$_3$), 3.90 (1H, s, –OC<u>H</u>$_3$), 4.23 (2H, s, –C<u>H</u>$_2$–O–), 5.25, 5.80, and 6.70 (each 1H, dd×3, J=1 and 11; J=1 and 17; J=11 and 17, respectively, AMX type vinyl), 6.10 (1H, d, J=3, –O–CH–<u>H</u> at C-1″), 6.46 (1H, s, aromatic 5-substituted), 6.69 and 7.78 (each 1H, d×2, J=8.5, aromatic *ortho*), 7.02 and 7.24 (each 1H, d×2, J=1, aromatic *meta*), 8.00 (1H, s, phenolic OH).

Hexahydrotoromycin (4) (Catalytic Hydrogenation of 3 to 4)——A solution of **3** (600 mg) in MeOH (150 ml) was hydrogenated over PtO_2 at room temperature and atmospheric pressure. After stirring for 1 hr, the catalyst was removed by filtration. The filtrate was concentrated to dryness *in vacuo*. The residue was chromatographed on silica gel. The column was eluted with EtOAc to give **4** (260 mg) as a pale yellow amorphous powder. UV λ_{max}^{MeOH} nm ($\varepsilon \times 10^{-3}$): 229 (55.2), 287 (9.3), 321.5 (10.4), 339 (11.2), 354 (12.3). IR ν_{max}^{KBr} cm^{-1}: 1630, 1580, 1520, but no peak corresponding to the lactone carbonyl at 1710—1690 cm^{-1}. PMR (DMSO-d_6) δ: 1.22 (3H, d, J=6, –CH–C<u>H</u>$_3$), 1.27 (3H, t, J=7, –CH$_2$–C<u>H</u>$_3$), 2.68 (2H, q, J=7, –C<u>H</u>$_2$–CH$_3$), 3.68 (3H, s, –OC<u>H</u>$_3$ at C-6′), 3.93 (3H, s, –OC<u>H</u>$_3$ at C-4), 4.23 (2H, –C<u>H</u>$_2$–O–), 6.16 (1H, d, J=3, –O–CH–<u>H</u> at C-1″), 6.54 (1H, s, aromatic 5-substituted), 6.74 and 7.82 (each 1H, d, J=8, aromatic *ortho*), 6.85 and 7.06 (each 1H, d, J=1, aromatic *meta*), 7.93 (1H, s, phenolic OH at C-1), 9.73 (1H, s, phenolic OH at C-5). MS *m/e*: 500 (M$^+$), 482 (M$^+$−18).

Alkaline Permanganate Oxidation of Toromycin (1)——$KMnO_4$ (10 g) was added to a solution of **1** (5.0 g) in 2% KOH (400 ml) in portions with stirring, and then three additional 5.0 g portions of $KMnO_4$ were added at intervals of one hour. The reaction mixture was allowed to stand overnight, and the insoluble material was removed by filtration. The filtrate was passed through a column of Amberlite IR-120 (H$^+$, 150 ml) and the combined effluent and washings were evaporated to dryness *in vacuo*. The residue was dissolved in a small amount of water and the solution was neutralized with 1 N NaOH. The solution was passed through a column of Dowex 1×2 (formate form, 80 ml). The column was washed with water and then eluted with

5 M formic acid. The eluate was concentrated to give crystals, and recrystallization from water gave 6-methoxy-1,2,4-benzenetricarboxylic acid (**6**, 800 mg) as colorless needles. mp 253—255°. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 217 (29800), 314 (4270), $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 218 (24300), 250 (sh), 301 (3700). PMR (DMSO-d_6) δ: 3.84 (3H, s, $-OC\underline{H}_3$), 7.70 and 8.00 (each 1H, d×2, J=1—2, aromatic *meta*). *Anal.* Calcd for $C_{10}H_8O_7$: C, 50.01; H, 3.36. Found: C, 49.83; H, 3.32.

Methyl 6-Methoxy-1,2,4-benzenetricarboxylate (6b) from 6——A large excess of diazomethane in Et_2O was added to a solution of **6** (100 mg) in MeOH (5 ml). The reaction mixture was concentrated to dryness *in vacuo*. The residue was recrystallized from EtOAc to give the methyl ester **6b** (87 mg). mp 143—145°. PMR ($CDCl_3$) δ: 3.87 (3H, s), 3.88 (3H, s), 3.92 (6H, s) (total 12H, $-CO_2C\underline{H}_3\times3$ and $-OC\underline{H}_3$), 7.72 and 8.20 (each 1H, d×2, J=1—2, aromatic *meta*). MS *m/e*: 282 (M^+). *Anal.* Calcd for $C_{13}H_{14}O_7$: C, 55.32; H, 5.00. Found: C, 55.36; H, 5.07.

Compounds **6** and **6b** were identical with 6-methoxy-1,2,4-benzenetricarboxylic acid and its trimethyl ester obtained by potassium permanganate oxidation of 2,3,5-trimethylanisole, respectively.

Alkaline Hydrogen Peroxide Oxidation of Toromycin (1)——3% H_2O_2 (50 ml) was added to a solution of **1** (5.0 g) in 5% KOH (200 ml) with stirring and the reaction mixture was kept at approximately 80°. Three additional 50 ml portions of 5% H_2O_2 were added at intervals of one hour at the same temperature. The resulting clear pale yellow reaction mixture was passed through a column of Amberlite IR-120 (H^+, 300 ml), and the effluent was extracted with EtOAc (500 ml). The EtOAc extract was shaken with 1% $NaHCO_3$. The aqueous layer was acidified with 5% HCl and shaken with EtOAc. The EtOAc layer was washed with water and evaporated to dryness *in vacuo*. The residue was crystallized from water to give 3-methoxy-5-vinyl-1,2-benzenedicarboxylic acid (**5**, 132 mg) as pale yellow leaflets. mp 180—182°. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 225 (25550), 255 (sh) 317 (7000); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 223 (24400), 260 (sh), 309 (3600). PMR (DMSO-d_6) δ: 3.84 (3H, s, $-OC\underline{H}_3$), 5.36, 5.96, and 6.78 (each 1H, dd×3, J=1 and 11; J=1 and 18; J=11 and 18, respectively, AMX type vinyl), 7.38 and 7.52 (each 1H, d, J=1—2, aromatic *meta*). MS *m/e*: 222 (M^+), *Anal.* Calcd for $C_{11}H_{10}O_5$: C, 59.64; H, 4.54. Found: C, 59.21; H, 4.57.

The benzenedicarboxylic acid **5** was melted for 10 min at 180—185° to give 3-methoxy-5-vinyl-1,2-benzenedicarboxylic anhydride (**5b**). IR ν_{max}^{KBr} cm^{-1}: 1875 and 1840 (C=O).

Per-O-methylation of Tetrahydrotoromycin (3)——Me_2SO_4 (15 ml) was added dropwise to a solution of crude **3** (5.0 g) in 10% KOH (15 ml) with stirring and the reaction mixture was stirred for 3 hr at room temperature. The resulting precipitate was collected by filtration, then washed with 10% KOH and water. The precipitate was vacuum-dried in a desiccator to give a yellowish-brown amorphous powder (5.0 g). This crude per-O-methyl derivative (**3b**) was used for the following reaction without further purification.

Alkaline Permanganate Oxidation of Per-O-methyltetrahydrotoromycin (3b)——Crude **3b** (5.0 g) was suspended in 2% KOH (200 ml) and $KMnO_4$ (10 g) was added to the suspension with stirring. The reaction mixture was kept at approximately 80°, and five 5.0 g portions of $KMnO_4$ were added to the reaction mixture at intervals of 30 min. The reaction mixture was stirred for a further 3 hr, then MeOH (50 ml) was added and the mixture was worked up. After filtration, the filtrate was passed through a column of Amberlite IR 120 (H^+, 150 ml). The combined effluent and washings were evaporated to dryness *in vacuo*. The residue was dissolved in water and neutralized with 1 N NaOH. The solution was passed through a column of Dowex 1×2 (formate form, 100 ml). After being washed with water, the column was eluted with 5 M formic acid (1 l) and 10 M formic acid (1 l). The fractions which were eluted with 5 M formic acid were evaporated to dryness *in vacuo*. The residue was dissolved in water and neutralized with 1 N NaOH. The solution was chromatographed on a column of Dowex 50W×2 (H^+, 100 ml) and eluted with water. Compound **8** was contained in the early fractions, and **6** was contained in the late fractions. These fractions were concentrated to dryness *in vacuo* and crystallized from water to give **8** (colorless prisms, mp 219—222°, *Anal.* Calcd for $C_{10}H_8O_7$: C, 50.01; H, 3.36. Found: C, 50.26; H, 3.47) and **6** (colorless needles, mp 253—255°). Compound **8** was dissolved in a small quantity of MeOH and treated with a solution of diazomethane in Et_2O in the usual way to give **8b**, as colorless needles (from MeOH), mp 90—92°. PMR ($CDCl_3$) δ: 3.82, 3.83, 3.84 and 3.87 (each 3H, s×4, $-CO_2C\underline{H}_3\times3$ and $-OC\underline{H}_3$), 6.67 and 7.97 (each 1H, d×2, J=8.5, aromatic *ortho*). The fractions which were eluted with 10 M formic acid were concentrated to dryness *in vacuo*. The residue was crystallized from water to give **7** as white needles. mp 284—286°. IR ν_{max}^{KBr} cm^{-1}: 1760. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 221 (57400), 311 (7480). $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 275—280 (sh), 333 (3860). PMR (DMSO-d_6) δ: 3.98 (6H, s, $-OCH_3\times2$), 7.34 and 8.22 (each 1H, d×2, J=8, aromatic *ortho*), 7.64 and 7.82 (each 1H, d×2, J=1, aromatic *meta*). *Anal.* Calcd for $C_{20}H_{14}O_{12}\cdot2H_2O$: C, 50.13; H, 3.55. Found: C, 49.80; H, 3.76. Compound **7** was treated with diazomethane in the usual way to give the tetramethyl ester (**7b**). mp 203—205°. IR ν_{max}^{KBr} cm^{-1}: 1750. PMR ($CDCl_3$) δ: 3.88, 3.90, 3.92 and 3.96 (each 3H, s×4, $-CO_2C\underline{H}_3$), 3.80 (6H, s, $-OC\underline{H}_3\times2$), 7.07 and 8.06 (each 1H, d×2, J=8, aromatic *ortho*), 7.81 and 8.21 (each 1H, d×2, J=1, aromatic *meta*). MS *m/e*: 502 (M^+). *Anal.* Calcd for $C_{24}H_{22}O_{12}$: C, 57.37; H, 4.41. Found: C, 57.26; H, 4.43.

Hydrogen Peroxide Oxidation of the Benzil Derivative 7——The benzil derivative **7** (100 mg) was dissolved in AcOH (5 ml), and then 3% H_2O_2 (10 ml) was added to the solution. The mixture was warmed at approximately 80° for 4 hr. After removal of the solvent *in vacuo*, the residue was dissolved in water and neutralized with 1 N NaOH and passed through a column of Dowex 1×2 (formate form). After being washed with water, the column was eluted with 5 M formic acid. The benzenetricarboxylic acid **8** and **6** were eluted in this order.

Each fraction was concentrated to dryness *in vacuo* and treated with diazomethane to give the trimethyl ester **8b** and **6b**. For further purification, **8b** and **6b** were chromatographed on a column of silica gel (solvent: $CHCl_3$–EtOAc 20: 1).

4-Trideuteriomethoxy-1,2,3-benzenetricarboxylic Acid (8c)——Dimethyl sulfate-d_6, $(CD_3)_2SO_4$ (0.3 ml) was added dropwise to a solution of **3** (1.0 g) in 10% KOH (3 ml) with stirring at room temperature for 3 hr. The resulting precipitate was collected by filtration and washed with 10% KOH and then with water to give a yellowish-brown powder (850 mg). Oxidation of the powder with $KMnO_4$ was carried out by a method similar to that described above for the oxidation of the per-O-methyl derivative **3b**. The crude oxidation product was purified by a Dowex 1×2 (formate form) column chromatography with 5 M formic acid as a developing solvent to give **8c** as colorless prisms. mp 219—222°. **8c** was treated with diazomethane in the usual manner to give the trimethyl ester **8d** as colorless prisms mp 89—91°. MS: *m/e* 285 (M^+, $C_{13}H_{11}D_3O_7$).

Alkaline Hydrogen Peroxide Oxidation of Tetrahydrotoromycin (3)——3% H_2O_2 (50 ml) was added to a solution of **3** (5.0 g) in 5% KOH (200 ml) with stirring. The reaction mixture was warmed at approximately 80°, and three 50 ml portions of 3% H_2O_2 were added at intervals of 30 min. The solution was cooled and passed through a column of Amberlite IR 120 (H^+, 300 ml). The effluent and washings were combined and extracted with EtOAc (300 ml×2). The EtOAc extract was concentrated *in vacuo*, and petroleum ether was added to the concentrate to give a yellow precipitate. The precipitate was dissolved in $CHCl_3$, and chromatographed on a column of silica gel. The column was eluted with EtOAc–$CHCl_3$ 1: 1, and the eluate was concentrated to give crystals. Recrystallizations from EtOAc gave **9** (220 mg) as colorless needles. mp 111—113°. IR ν_{max}^{KBr} cm^{-1}: 1745 (C=O). UV λ_{max}^{MeOH} nm (ε): 216 (23800), 268 (19600), 313 (4750). PMR (DMSO-d_6) δ: 3.96 (3H, s, $-OC\underline{H}_3$), 5.16 (2H, s, $-C\underline{H}_2O-$), 5.43, 5.85, and 6.74 (each 1H, dd×3, J=<1 and 11, J=<1 and 18, J=11 and 18, respectively, AMX type vinyl), 6.80 and 7.00 (each 1H, d×2, J=<1, aromatic *meta*). MS *m/e*: 190 (M^+). *Anal.* Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.32; H, 5.20.

Methyl 2-Methoxy-6-methoxymethyl-4-vinyl-benzenecarboxylate (9b)——The methoxy-vinyl-phthalide **9** (100 mg) was dissolved in 10% KOH (3 ml) by warming in a water bath (80°). The solution was cooled to room temperature, then Me_2SO_4 (0.2 ml) was added. The reaction mixture was stirred for 3 hr and then extracted with Et_2O. The extract was washed with water and the solvent was evaporated off. The residue was dissolved in $CHCl_3$ and chromatographed on a column of silica gel. The column was eluted with $CHCl_3$ and the eluate was concentrated to give **9b** as a colorless oil (65 mg). PMR ($CDCl_3$) δ: 3.30 (3H, s, $-CH_2OC\underline{H}_3$), 3.83 (3H, s, $-OC\underline{H}_3$), 3.86 (3H, s, $-OC\underline{H}_3$), 4.43 (2H, s, $-C\underline{H}_2OCH_3$), 5.30, 5.76 and 6.80 (each 1H, dd×3, J=1 and 11, J=1 and 18, J=11 and 18, respectively, AMX type vinyl), 6.88 and 7.02 (each 1H, d×2, J=1, aromatic *meta*). MS: *m/e* 236 (M^+).

Tetra-O-acetyltoromycin (1b)——Ac_2O (7 ml) was added dropwise to a solution of **1** (1.0 g) in pyridine (50 ml) with stirring at ice-water bath temperature. The reaction mixture was stirred for 3 hr at room temperature and allowed to stand overnight, then concentrated *in vacuo*. The residue was partitioned between $CHCl_3$ and water at ice-water bath temperature. The $CHCl_3$ extract was washed with water and then concentrated *in vacuo*. The residue was triturated with MeOH to give a pale yellow powder (1.24 g). The powder was chromatographed on a column of silica gel with $CHCl_3$ to give **1b**. UV λ_{max}^{MeOH} nm (ε): 246 (34000), 289 (39000), 323 (14000), 334.5 (13000), 348 (12000), 388 (15000). PMR (DMSO-d_6) δ: 1.39 (3H, d, J=6.5, $-\underset{|}{C}H-C\underline{H}_3$); 1.47, 2.10, 2.27, and 2.31 (each 3H, s×4, acetyl×4), 3.83 (6H, s, $-OC\underline{H}_3\times2$); 4.12 (1H, dd, J=4 and 6), 5.09 (1H), 5.32 (1H, m), 6.04 (1H), and 6.38 (1H, d, J=3) (C-glycosyl methine); 5.32 (1H, dd, J=<1 and 11), 5.79 (1H, dd, J=<1 and 17.5), and 6.64 (1H, dd, J=11 and 17.5) (AMX type vinyl), 7.05 and 7.96 (each 1H, d, J=8.5, aromatic *ortho*), 7.14 and 7.90 (each 1H, d, J=1—2, aromatic *meta*), 8.28 (1H, s, aromatic 5-substituted).

Tetra-O-acetyl-dihydrotoromycin (2b)——A solution of tetra-O-acetyltoromycin (**1b**, 600 mg) in dioxane (60 ml) was hydrogenated over PtO_2 (30 mg) at room temperature and atmospheric pressure. After stirring for 3 hr, the catalyst was removed and dioxane was evaporated off *in vacuo*. The residue was triturated with MeOH to give **2b** (530 mg) as a pale yellow amorphous powder. mp 210—212° (dec.). MS *m/e*: 664 (M^+), 649 (M^+-CH_3), 621 (M^+-CH_3CO). UV λ_{max}^{MeOH} nm ($\varepsilon\times10^{-3}$): 244.5 (37.0), 268 (25.0), 277 (38.0), 302 (9.5), 325 (10.4), 337 (12.4), 377 (10.0). PMR (DMSO-d_6) δ: 1.28 (1H, t, J=7.5, $-CH_2-C\underline{H}_3$), 1.41 (3H, d, J=6.5, $-\underset{|}{C}H-C\underline{H}_3$); 1.48, 2.10, 2.28, and 2.32 (each 3H, s×4, acetyl×4); 2.69 (2H, q, J=7.5, $-C\underline{H}_2-CH_3$), 3.86 (6H, s, $-OC\underline{H}_3\times2$); 4.13 (1H, dd, J=3.5 and 6), 5.11 (1H, dd, J=<1 and 3.5), 5.33 (1H, quin., J=6), 6.09 (1H, d, J=3), 6.46 (1H, d, J=3) (C-glycosyl methine); 7.03 and 7.81 (each 1H, d×2, J=<1, atomic *meta*), 7.07 and 7.98 (each 1H, d×2, J=8, aromatic *ortho*), 8.36 (1H, s, aromatic 5-substituted).

Isomerized Toromycin (1′)——Toromycin (**1**, 2.0 g) was suspended in 50% aqueous acetic acid (200 ml) and refluxed for 2 hr. The resulting solution was concentrated *in vacuo*, and water was added to precipitate a crude powder. The precipitate was washed with water and dissolved in MeOH (about 200 ml). The MeOH solution was concentrated *in vacuo*, and Et_2O was added to the concentrate to give a yellow powder (1.8 g). The powder (1.2 g) was dissolved in acetone (about 200 ml) and absorbed on a column of alumina (Woelm acid, 3×25 cm). The column was washed with acetone and then eluted with acetone–MeOH (1: 1) to give isomerized toromycin (**1′**, 0.5 g). IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O). λ_{max}^{MeOH} nm: 247, 277, 288 and 396. TLC (silica gel, EtOAc: MeOH 9: 1): *Rf* 0.40. $[\alpha]_D^{21}$ −40.6 (c=1, DMSO). PMR (DMSO-d_6) δ: 1.08 (3H, d, J=

6.5, –CH–CH$_3$), 3.4—4.7 (4H, C-glycosyl methine×4), 3.94 and 3.99 (each 3H, s×2, –OCH$_3$), 5.62 (1H, d, J=8.5, C-glycosyl C-1″); 5.38 (1H, dd, J=<1 and 11), 5.97 (1H, dd, J=<1 and 17.5), and 6.78 (1H, dd, J=11 and 17.5) (AMX type vinyl), 6.85 and 7.72 (each 1H, d, J=8.5, aromatic *ortho*), 7.43 and 7.83 (each 1H, d, J=1, aromatic *meta*), 8.22 (1H, s, aromatic 5-substituted), 9.74 (1H, s, phenolic OH). *Anal.* Calcd for $C_{27}H_{26}O_9$: C, 65.58; H, 5.30. Found: C, 65.13; H, 5.26. MIC (μg/ml): *Staphylococcus aureus* (FDA 209P) 0.5, *Bacillus subtilis* (PCI 219) 1.0, *B. cereus* (IFO 3514) 1.0, *Sarcina lutea* (IFO 3232) 0.5, *Micrococcus flavus* (IFO 3242) 0.5, *Mycobacterium sp.* 607 5.0, *M. phlei* (IFO 3142) 2.0, *M. smegmatis* (IFO 3083) 1.0, *Trichomonas vaginalis* (No. 4F) 12.5, *Mycoplasma gallisepticum* (S6) 0.5—1.

Tetra-O-acetyl Isomerized Toromycin (1′b)——Ac_2O (1.0 ml) was added dropwise to a solution of **1′** (0.18 g) with stirring at ice-water bath temperature. The reaction mixture was stirred for 1 hr and allowed to stand overnight at room temperature, then concentrated *in vacuo*. The concentrate was partitioned between $CHCl_3$ and water at ice-water bath temperature. The $CHCl_3$ extract was washed with water and then concentrated *in vacuo*. EtOAc was added to the residue to give light yellow crystals (0.18 g). PMR ($CDCl_3$) δ: 1.23 (3H, d, J=6.5, –CH–CH$_3$); 1.66, 2.02, 2.24, and 2.38 (each 3H, s×4, acetyl×4); 3.96 and 4.00 (each 3H, s×2, –OCH$_3$×2); 4.67 (1H, q, J=6), *ca.* 5.5 (2H), 5.79 (1H, t, J=9), 6.31 (1H, d, J=9.5) (C-glycosyl methines); 5.46 (1H, dd, J=<1 and 10), 5.94 (1H, dd, J=<1 and 17.5), and 6.79 (1H, dd, J=10 and 17.5) (AMX type vinyl), 7.19 and 7.87 (each 1H, d, J=8, aromatic *ortho*), 7.30 and 8.09 (each 1H, d, J=1—2, aromatic *meta*), 8.48 (1H, s, aromatic 5-substituted). TLC (silica gel; $CHCl_3$–EtOAc 2:1): *Rf* 0.55.

Isomerized Dihydrotoromycin (2′)——A solution of **1′** (600 mg) in MeOH (600 ml) was hydrogenated over PtO_2 (100 mg) in the same manner as **2**. After removal of the catalyst and solvent, the residue was dissolved in a large quantity of EtOAc and passed through a column of silica gel (Merck, acid-washed) and then eluted with EtOAc. The effluent was concentrated *in vacuo* and acetone was added to the concentrate to give **2′** as pale yellow needles (102 mg). UV λ_{max}^{MeOH} nm (ε): 255 (53800), 267 (30600), 276 (39700), 382 (11200). IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O). PMR (DMSO-d_6) δ: 1.06 (3H, d, J=6.5, –CH–CH$_3$), 1.25 (3H, t, J=7.5, –CH$_2$–CH$_3$), 2.59 (2H, q, J=7.5, –CH$_2$–CH$_3$), 3.4—4.7 (4H, C-glycosyl methine×4), 3.94 (6H, s, –OCH$_3$), 5.60 (1H, d, J=8.5, C-glycosyl C-1″), 6.85 and 7.68 (each 1H, d×2, J=8.5, aromatic *ortho*), 7.24 and 7.65 (each 1H, d×2, J=1, aromatic *meta*), 8.25 (1H, s, aromatic 5-substituted), 9.74 (1H, s, phenolic OH).

Isopropylidene Derivative (1′c) of 1′——**1′** (0.50 g) was stirred in 2,2-dimethoxypropane (10 ml) with *p*-toluenesulfonic acid (10 mg) at room temperature. After stirring for 2 hr, pyridine (1 ml) was added to the resulting solution and the whole was further stirred for 30 min. The reaction mixture was evaporated to a syrup *in vacuo*, and petroleum ether was added to the syrup to give a brownish-yellow powder (0.52 g). The crude powder was chromatographed on a column of aluminum oxide (Woelm neutral) with $CHCl_3$. The eluate was concentrated *in vacuo*, and petroleum ether was added to give **1′c** (0.30 g) as a yellow powder. PMR (DMSO-d_6) δ: 1.20 (3H, d, J=6.5, –CH–CH$_3$), 1.32 and 1.48 (each 3H, s×2, isopropylidene), 3.94 (6H, s, –OCH$_3$×2), 3.0—4.7 (4H, C-glycosyl, methine×4), 5.58 (1H, d, J=9, C-glycosyl C-1″); 5.38 (1H, dd, J=<1 and 11), 5.96 (1H, dd, J=<1 and 17.5), and 6.78 (1H, dd, J=11 and 17.5) (AMX type vinyl); 6.86 and 7.70 (each 1H, d×2, J=8.5, aromatic *ortho*), 7.59 and 7.82 (each 1H, d×2, J=1, aromatic *meta*), 8.24 (1H, s, aromatic 5-substituted), 9.74 (1H, s, phenolic OH).

Mono-isopropylidene-di-acetyl Derivative (1′d) of 1′——Ac_2O (1 ml) was added dropwise to a solution of the isopropylidene derivative **1′c** (100 mg) in pyridine (5 ml) with swirling at ice-water bath temperature. After standing overnight, the reaction mixture was concentrated *in vacuo* and the residue was partitioned between $CHCl_3$ and water. The $CHCl_3$ extract was washed with water, and then evaporated to dryness *in vacuo*. The residue was chromatographed on a column of silica gel with $CHCl_3$ to give **1′d**. TLC (silica gel, EtOAc–$CHCl_3$ 1:1 v/v); *Rf* 0.56 (*cf.* *Rf* 0.43 (**1′c**), 0.09 (**1′**)). PMR (DMSO-d_6) δ: 1.30 and 1.32 (each 3H, s×2, isopropylidene), 1.46 (3H, d, J=6.5, –CH–CH$_3$), 1.53 (3H, s, acetyl), 2.27 (3H, s, acetyl), 3.82 and 3.84 (each 3H, s×2, –OCH$_3$), 4.20 (1H, dd, J=2.5 and 6, C-4″), 4.40 (1H, m, C-5″), 4.44 (1H, dd, J=6 and 8, C-3″), 5.16 (1H, dd, J=8 and 10, C-2″), 6.12 (1H, d, J=10, C-1″); 5.30 (1H, dd, J=<1 and 11), 5.79 (1H, dd, J=<1 and 17.5), and 6.65 (1H, dd, J=11 and 17.5) (AMX type vinyl, respectively); 7.07 and 7.92 (each 1H, d×2, J=8.5, aromatic *ortho*), 7.15 and 7.95 (each 1H, d×2, J=2, aromatic *meta*), 8.29 (1H, s, aromatic 5-substituted).

Acknowledgement The authors are grateful to Dr. E. Ohmura of this Division for his encouragement throughout this study. The authors are also grateful to Dr. Y. Kameda and Mr. H. Kawashima for their skillful assistance in a part of this study.