Tetrahedron Vol 39, No 4 pp 599 to 605 1983
Printed in Great Britain

0040-4020/83/040599-07$03.00/0

STRUCTURE AND STEREOCHEMISTRY OF TOROMYCIN; STUDIES OF ITS ACID-CATALYZED REARRANGEMENT

TIKAM C. JAIN*, GERALD C. SIMOLIKE
Research & Development Division, Smith Kline & French Laboratories, Philadelphia, PA 19101, U.S.A

and

LLOYD M. JACKMAN
Department of Chemistry, Pennsylvania State University, University Park, PA 16802, U.S.A

(*Received in the USA 23 December 1981*)

Abstract—An antibiotic from *Streptomyces* has been shown to be identical with toromycin and gilvocarcin V. Toromycin has been shown by FT NOE difference spectroscopy to be a C-β-fucofuranoside and to undergo acid-catalyzed rearrangement to an approx. 1 : 1 : 2.5 mixture of toromycin and the corresponding C-α-fucofuranoside and C-β-fucopyranoside.

In 1971, Hatano, Horii et al.[1] reported the isolation of the antibiotic B21085, from *Streptomyces collinus* subsp. *albescens*. Subsequently, this group reported the details[2] of the isolation and biological activity of B21085, which they have named toromycin. The results of chemical and spectroscopic studies[3] leading to the structure **1**(R_2 = -CH=CH_2) in which R_1 is a 6-deoxyhexose, the ring size and stereochemistry of which was not established. Recently, Takahashi et al.,[4] have described an antibiotic, gilvocarcin V, isolated from *Streptomyces gilvotanareus*, for which they have unequivocally established the structure and stereochemistry **2**. Their proof of structure is based on the X-ray crystallographic structure of a congener, gilvocarcin M **3** and the close similarity between ^{1}H and ^{13}C NMR parameters of the sugar residues in gilvocarcins V and M. This latter group of workers noted the close similarity between gilvocarcin V and toromycin in terms of the presence of a common chromophore. However, they did not conclude that the two natural products have the same structure (the report of antibiotic DC-38-A identical with toromycin except the stereochemistry and ring size of the C-glycosyl moiety has also been cited in Ref. 3).

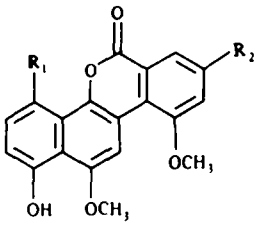
We have also isolated this antibiotic from a strain of *Streptomyces* species (designated as AAC-324). The antibiotic was obtained from the mycelium of the culture broth which is in agreement with the Takeda report[2] for toromycin. The antibiotic consistently analyzed for $C_{27}H_{36}O_9.1\frac{1}{2}H_2O$; neither the degree of hydration nor the biological activity was lost when the antibiotic was dried at 100° *in vacuo* over P_2O_5 for several hours. The melting point of our isolate is 234–235° compared with 255–260° of toromycin and 264–267° of gilvocarcin V. These differences in melting points may reflect differing degrees of hydration; the analysis reported for toromycin corresponds to the anhydrous antibiotic and no combustion data were reported for gilvocarcin V. Except for this anomaly, the physical and spectral characteristics of our isolate ($[\alpha]_D$ - 217.8°) are identical with those of toromycin ($[\alpha]_D$ - 217°) and gilvocarcin V ($[\alpha]_D$ - 216°).

The structure and stereochemistry of the antibiotic was deduced independently in this laboratory from proton and carbon NMR spectra of the parent molecule and its several reaction products, and this proved to be the same as gilvocarcin V. Furthermore, a comparison of the proton spectrum of the crystalline tetraacetate of our isolate with that reported for toromycin leaves no doubt that the antibiotics from all three sources are the same compound. It may also be pointed out that individual preparations of toromycin when converted to the corresponding tetraacetate revealed the presence of varying amounts (0–10%) of dihydrotoromycin **4** tetraacetate. Furthermore, separation of toromycin from dihydrotoromycin, **4** is exceedingly difficult.

Our spectroscopic studies leading to the elucidation of the structure of toromycin closely paralleled those of the two Japanese groups.[3,4] However, we have also obtained independent proof of configuration of the sugar residue. This led us to study the products of the acid catalyzed rearrangement of toromycin which, involving as it does the anomeric position of the C-fucosyl residue, is of considerable importance since it leads to closely related analogues of known stereochemistry, and of potentially different biological activity. We now present our findings in this area.

Because of the conformational mobility of the five membered ring, it is usually difficult to establish the configuration of furanose sugars from considerations of vicinal interproton coupling constants.[5] The sugar residue of toromycin is no exception in this regard. Data for toromycin and its tetraacetate are given in Table 1 in which it can be seen that there are considerable differences in two sets of coupling constants indicating that the conformations for the two systems are quite different. The relative configuration at C(2') and C(3') is, however, established through the observation that $^3J(2',3') < 1.0$ Hz for the tetraacetate. This requires a dihedral angle of 90° which is only possible with the *trans* configuration.[6] The configurations at the remaining positions of the furanose ring have been established by the determination of averaged interproton distances by a combination of quantitative nuclear Overhauser enhancement (NOE) FT difference spectroscopy[7] and measurements of proton spin lattice relaxation times, T_1.

The proton NOE difference spectra for the sugar protons are shown in Fig 1. It is immediately obvious from the observed, mutual enhancements of the signals of H(1') and H(4') that these protons must be *cis* to each



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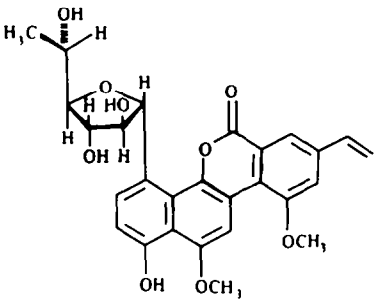

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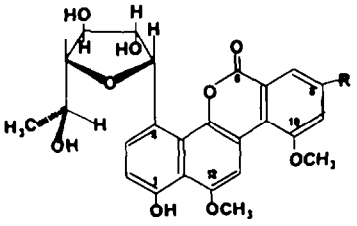
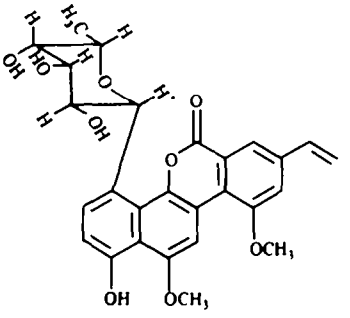


2. R = $-CH=CH_2$
3. R = $-CH_3$
4. R = $-CH_2-CH_3$



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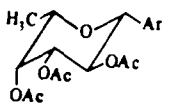

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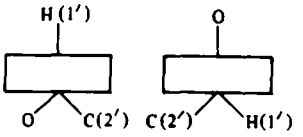

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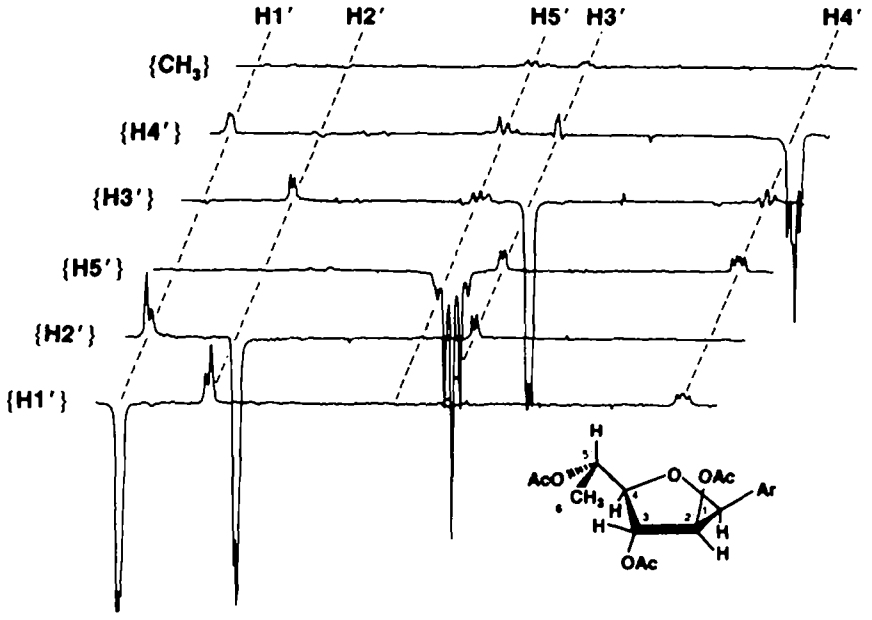

Fig. 1. FT NOE difference spectra of toromycin tetraacetate in $CDCl_3$ at 200 MHz. Large negative signals correspond to resonances of protons being irradiated.

Table 1. Chemical shifts and coupling constants for the sugar protons of toromycin and some deratives in $CDCl_3$

	δ (ppm)						^3J (Hz)				
COMPOUND	1'	2'	3'	4'	5'	6'	1',2'	2',3'	3',4'	4',5'	5',6'
TOROMYCIN (a)	6.147	4.625	3.93	3.627	3.93	1.281	4.6	2.5	4.1	4.7	6.4
TOROMYCIN TETRAACETATE	6.535	6.170	5.185	4.202	5.389	1.434	3.2	0.9	3.8	6.4	6.4
TOROMYCIN ACETONIDE	5.880	4.297	4.550	4.267	4.518	1.425	4.0	4.9	6.8	2.1	6.7
TOROMYCIN ACETONIDE DIACETATE	6.220	5.307	4.539	4.289	4.581	1.491	9.6	8.0	5.2	2.3	6.6
TOROMYCIN ISOMER A TETRAACETATE	6.284	5.809	5.432	5.478	4.684	1.223	10.2	9.5	3.4	1.1	6.6
TOROMYCIN ISOMER B TETRAACETATE	6.762	5.438	4.899	4.386	5.309	1.425	0.8	0.7	1.8	6.7	6.5

(a) Solvent was DMSO-d_6

other. In order to extract the relative configurations at C(1') and C(2'), and hence at all four positions of the furanose rings, it is necessary to compare the relative contributions, $T_1(i, j)$, which H(1') amd H(3') make to the dipole–dipole relaxation of H(2'). The spin lattice relaxation times, $T_1^{(i)}$, for all the sugar protons as well as the quantitative NOE's, $\eta^{(i)}$ were determined for toromycin tetraacetate. The contribution of the jth proton to the relaxation of the ith proton is given by a simple equation which suffices for the system under investigation since indirect NOE's are not observed:

$$T_1^{(i,j)} = 0.5T_1^{(i)}/\eta^{(i)}$$

These contributions are then related to the internuclear distances, r, through the expression

$$T_1^{(i,j)}/T_1^{(m,n)} = \gamma_{ij}^6/\gamma_{m,n}^6$$

The results for toromycin tetraacetate are given in Table 2.

Although the computation of the internuclear distances includes the assumption of isotropy of rotational diffusion, as well as considerable experimental error in the determination of $\eta^{(i)}$, the sixth power dependence of $T_1^{(i)}$ on internuclear distance greatly reduces the uncertainties and the results in Table 2 leave no doubt that H(2') is considerably closer to H(1') than to H(3') and hence H(1') is *cis* to H(2'). Table 2 also includes a comparison with approximate internuclear distances taken from a Dreiding model in which the dihedral angle H(1')–C(1')–C(2')–H(2') is held at 90°.

The configuration at C(5') is not readily established from these experiments although the observation of small but significant enhancements of H(3'), H(4'), and H(5') on irradiation of the C-methyl protons suggest the fucose configuration.

In order to establish more firmly the configuration at C(5') we attempted to prepare the acetonide of toromycin with the expectation that this would be the 3',5'-isopropylidene derivative in which the six membered ring should have a well-defined conformation. The NMR data for the resulting product, together with those for the corresponding diacetate, are included in Table 1. The coupling constants for the acetonide diacetate, in particular, were difficult to reconcile with the relative configuration established for the furanose ring and we concluded that an acid catalyzed isomerization at C(1')

Table 2. Proton spin lattice relaxation times, NOE's and internuclear distances for the sugar residue of toromycin tetraacetate

i	j	$T_1(i)$ sec	$T_1(i)$ sec$^{(j)}$	$T_1(j)$ sec$^{(i)}$	$\eta^{i\{j\}}$ %	$\eta^{\{i\}j}$ %	rij (observed) Å	rij (calculated)[a] Å
1'	2'	0.725	1.8	1.9	20	20	2.3	2.3
1'	4'	-	4.0	3.6	9	9	2.6	2.5
2'	3'	0.77	5.1	3.9	7.5	9	2.7[b]	2.7
3'	4'	0.71	18	9.3	2	3.5	3.0	2.8
3'	5'	-	8.9[c]	4.4	4	8	2.6	2.5
4'	5'	0 65	4.3	3.9	7.5	9	2.6	2.9

(a) From Dreiding model

(b) This is an assumed value

(c) Too long because of proximity of the 3' to the 5' and cis-8 resonances

Table 3. Chemical shifts (ppm) and coupling constants (Hz; in parentheses) for the protons of the aromatic nuclei of toromycin isomer B tetraacetate and its dihydro derivative in $CDCl_3$

POSITION	TOROMYCIN ISOMER A TETRAACETATE	DIHYDROTOROMYCIN ISOMER A TETRAACETATE	TOROMYCIN ISOMER B TETRAACETATE	DIHYDROTOROMYCIN ISOMER B TETRAACETATE
2	7.201 (8.3)	7.188 (8.3)	7.171 (8.3)	7.159 (8.3)
3	7.853 (8.3; 1.2) [a]	7.844 (8.3; 1.2) [a]	7.876 (8.3; 1.2) [a]	7.867 (8.3; 1.2) [a]
α [d]	6.822 (17.6; 10.8)	2.832 (q, 7.5) [b]	6.819 (17.6; 10.8)	2.798 (q, 7.5) [b]
β-trans [d]	5.983 (17.6)	1.362 (t, 7.5) [c]	5.942 (17.6)	1.341 (t, 7.5) [c]
β-cis [d]	5.486 (10.8)	-	5.456 (10.8)	-
7	8.159 (1.7)	7.983	8.056 (1.7)	7.891
9	7.396 (1.7)	7.232	7.365 (1.7)	7.195
10 (CH_3O)	4.110	4.091	4.106	4.077
11	8.587	8.603	8.558	8.575
12 (CH_3O)	3.997	3.992	4.011	4.007

[a] $J_{1',3}$

[b] Methylene of the 8-ethyl group

[c] Methyl of the 8-ethyl group

[d] Protons of the vinyl group; cis- and trans- refer to the relationship between hydrogen atoms

Table 4. ε max values for toromycin acetonide, toromycin isomers A, B and their corresponding acetates

COMPOUND	ε max at nm							
	245	265	275	285	320	335	380	390
TOROMYCIN ACETONIDE	26,700	-	20,030	21,140	-	-	-	12,760
TOROMYCIN ACETONIDE DIACETATE	25,720	-	20,080	20,780	12,680	-	14,660	-
TOROMYCIN ISOMER A	23,670	12,870	18,010	11,840	-	-	-	9,980
TOROMYCIN ISOMER A TETRAACETATE	31,030	18,619 (268nm)	29,376 (277nm)	-	14,560	14,560	14,900	-
TOROMYCIN ISOMER B	33,450	18,530	23,160	-	-	-	-	12,140 (395nm)
TOROMYCIN ISOMER B TETRAACETATE	36,960	21,520	32,550 (276nm)	-	14,790	14,560	14,790 (385nm)	-

may have occurred. Proof of this premise was provided by the treatment of toromycin with toluene-p-sulfonic acid in acetonitrile whereupon an isomer of toromycin (isomer A) was isolated from the crude reaction product. This compound was converted to the acetonide and its corresponding diacetate which were identical with the acetonide and acetonide diacetate reported by Horii et al.[4b] who, however, did not establish either the ring size or configuration of the sugar residue.

Under the acid conditions (H^+/CH_3CN) we have employed, (see Experimental) toromycin gives a mixture of several products, the resolution of which is complicated in those preparations of toromycin which were contaminated with the dihydro derivative. A major product, isomer A was isolated in pure form and was characterized as its tetraacetate. It appears to be identical with the tetraacetate described by Horii *et al.*[4b] (see Experimental). The chemical shift of H(5') indicates that this position does not carry an acetoxyl group so that the sugar must have the pyranose ring system. The configuration of isomer A tetraacetate is readily established by the noe difference spectra shown in Fig. 2. Particularly striking features in these spectra are the large mutual enhancements of the signals arising from H(1'), H(3'), and H(5'), which clearly requires them to bear a 1',3',5'-triaxial relation to each other. In contrast, irradiation of H(2') has little or no effect on the intensities of the absorptions of the other protons of the sugar moiety. Irradiation of H(5') enhances the resonance of H(4'), as well as those of H(1') and H(3'), and therefore establishes the *cis* relation between H(4') and H(5'). These results can only be accommodated by assigning the sugar moiety in isomer A the β-fucopyranosyl configuration, **5**, a configuration which clearly is in agreement with the magnitudes of the vicinal coupling constants reported in Table 1. Toromycin isomer A is therefore 4-β-fucopyranosyl-1-hydroxy-10,12-dimethoxy-8-vinyl-6H-benzo[*d*]-naphtho[1,2-*b*] pyran-6-one **6**.

In addition to isomer A, a second isomer (referred to as isomer B) was detected by thin layer chromatography and bioautography against *B. subtilis*. Although we have not obtained this isomer completely free from its dihydro derivative, the 360 MHz NMR spectrum of the mixture of the corresponding tetraacetates was sufficiently well resolved to allow the total assignments of the two components. The ^1H NMR data for aromatic nucleus of isomer B and its corresponding dihydro derivative are presented in Table 3 and those for the sugar protons of the former have been included in Table 1. The chemical shift of H(4') establishes the presence of the furanose ring in isomer B. The vicinal coupling constants for the ring protons are in accord with the presence of the C-α-fucofuranosyl residue. Thus $^3J_{1',2'}$ as well as $^3J_{2',3'}$ are less than 1 Hz and $^2J_{3',4'}$ is only 1.8 Hz. This demonstrates the all-*trans* substitution of the ring, which evidently adopts the envelope (V3)[6] conformation. Toromycin isomer B is therefore 4-α-fucofuranosyl-1-hydroxy-10,12-dimethoxy-8-vinyl-6H-benzo[d]naphtho [1,2-b] pyran-6-one **7**.

Both the α and β *C*-furanosides exhibit coupling between the H(1') and the 3 proton of the aromatic ring (1.2 and 0.9 Hz, respectively) indicating that the conformation **8a** is preferred. In contrast, this coupling constant is approximately zero in the C-β-pyranoside suggesting that now the sugar residue adopts the conformation **8b**.

We have repeated the isomerization of toromycin in aqueous acetic acid[3]. The crude reaction product was exhaustively acetylated and the resulting crude acetate was rigorously analyzed by 360 MHz ^1H NMR spectroscopy. This revealed the presence of four sugar C-methyl signals in the ratio of 1 : 1 : 2.5 : 1. The first three are due to toromycin, isomers B and A, respectively. We have, as yet, been unable to isolate and characterize the fourth component. Also present are the corresponding dihydro derivatives which constitute about 10% of the mixture.

In summary, the identity of toromycin with gilvocarcin

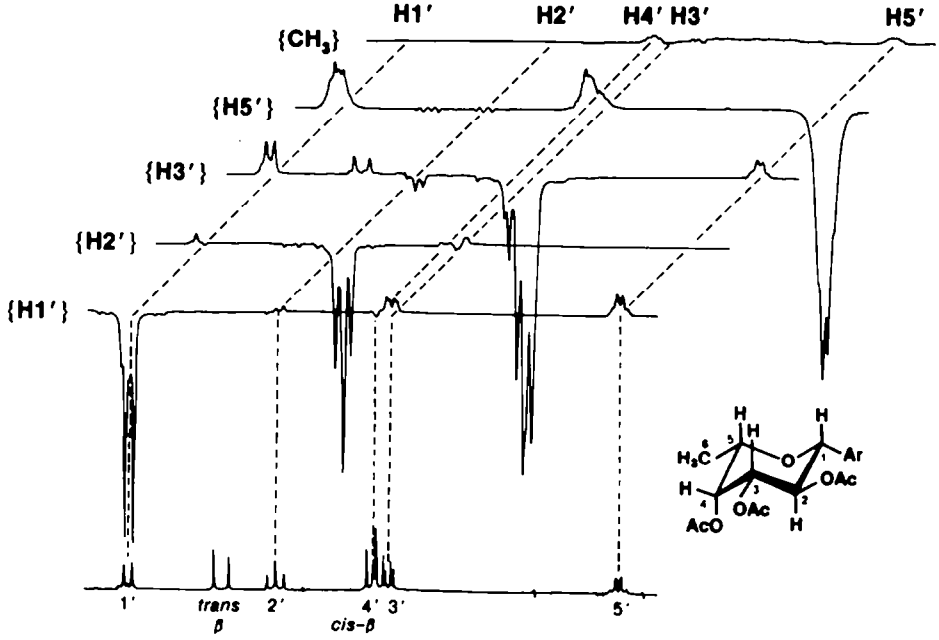

Fig. 2. FT NOE difference spectra of toromycin isomer A tetraacetate in $CDCl_3$ at 200 MHz. Large negative signals correspond to resonances of protons being irradiated.

V has been unambiguously established and the nature of the acid catalyzed rearrangement of toromycin has been elucidated. Toromycin 1 is unusual in that, as far as we are aware, it is the first example of a C-glycoside antibiotic and in our opinion represents a new class of antibiotics. This structural feature is biogenetically significant in the sense that the chromophore present in toromycin has also been found in chartreusin[8] which, however, has the ubiquitous O-glycosidic linkage. It is possible that the presence of a C-glycosidic rather than O-glycosidic linkage might confer hydrolytic stability while retaining important hydrophilic character.

EXPERIMENTAL

Mps were determined on a Kofler hot-stage microscope and are uncorrected. IR spectra were recorded in KBr pellets on a Perkin–Elmer Model 299B Spectrophotometer. UV spectra were obtained with Cary 15 Spectrophotometer. Electron-impact and FD mass spectra were measured with Varian CH-5 and Hitachi-Perkin–Elmer RMU - 6E mass spectrometers. Specific rotations were determined at 25° with a Perkin–Elmer Model 241 MC Polarimeter. ^1H and ^{13}C NMR spectra were obtained with Bruker IUP200 and WP360 spectrometers. The NOE and spin lattice relaxation data were determined for solutions which had been rigorously degassed by repeated cycles of freezing and thawing. The quantitative NOE measurements were made using the alternating phase (PAPS) mode. Waiting times of $10 \times T_1$ were used to ensure complete equilibration of the spin systems. The T_1 values were determined using the 180-τ-90 sequence and three parameter fitting of the recovery curves. HPLC was performed with an Altex Model 152 chromatograph with UV detector at 254 nm.

Toromycin. Our isolation procedure closely parallels that of the Takeda group[2] except that the antibiotic was crystallized and recrystallized (8 times) from CH_2Cl_2 containing 5% MeOH.† Toromycin was obtained as yellow needles, m.p. 234–235°; $[\alpha]_{589}$ - 217.8°, $[\alpha]_{578}$ - 226.2°, $[\alpha]_{546}$ - 277.0° (c 0.25, DMSO); IR 3380, 1700 (sh), 1670, 1370, 1240, 1130, 1000, 840, 780 cm^{-1} (*cf* gilvocarcin V: 3380, 1690 cm^{-1}; toromycin: 1680 cm^{-1}; $UV^{EtOH}_{\lambda max}$ 247, 277 (shoulder), 287, 400 nm (*cf* gilvocarcin V:[4] 248, 287, 398 nm; toromycin[3]: 247, 277,‡ 288, 398 nm); MS (EI, 70 e) m/e (% rel. int.) 494 (7.3, molecular ion); MS (FD, 15 mA) *m/e* (% rel int) 494 (19.35, M+), 989 (0.83, dimer + 1);¶ exact mass measurement: found 494.1608 ($C_{27}H_{26}O_9$), calc 494.1577; ^1H NMR (δ) 8.302 (1H, s), 8.046, 6.890 (1H each, d × 2, J = 8.4Hz), 7.863, 7.563 (1H each, d × 2, J = 1.55 Hz) (5 aromatic protons); 6.843 (q, J = 17.7 and 11.0 Hz), 6.044 (d, J = 17.7 Hz), 5.443 (d, J = 11.0 Hz) (vinylic protons 3); 4.190 and 4.137 (3H each, s × 2, two methoxyls); 6.172 (1H, d, J = 4.7 Hz, C1'H), 4.655 (1H, q, J = 4.7 and 2.9 Hz, C2'H), 3.89 (1H, q, J = 3.9 and 2.9 Hz, C3'H), 3.562 (1H, q, J = 3.9 and 5.2 Hz, C4'H), 3.89 (1H, q, J = 5.2 and 6.5 Hz, C5'H), 1.276 (3H, d, J = 6.5 Hz, C6'H) (sugar protons;§) 9.600, 5.044, 4.498, 4.879 (four hydroxyls, exchanged with D_2O and confirmed as tetraacetate: 2.355, 2.296, 2.121, 1.494); ^{13}C NMR (DMSO) δ 159.5, 157.2 ($J_{C,OMe}$ = 3.7 Hz), 152.6 ($J_{C,OH}$ = 3.4 Hz), 151.6 ($J_{C,OMe}$ = 3.7Hz), 142.2, 138.4, 135.3, 128.9, 126.0 ($J_{C,H\&6,89}$ = 7.2 Hz, $J_{C\ anomeric\ H}$ = 3.5 Hz), 123.6, 122.9, 122.0, 119.0 (exhibits two *meta* splittings of ~ 5.5 Hz), 116.9, 114.8, 114.2 (exhibits two *meta* splittings of ~ 5.5 Hz), 112.7, 111.9 ($J_{C,OH}$ = 6.7 Hz), 101.2 (all sp^2 carbons), 85.7, 80.8, 78.9, 78.7, 66.5, 56.5, 56.0, 20.2 (sugar carbons and two methoxyls), anomeric carbon absorbs at 80.8 ppm with $J_{C,H}$ = 153 Hz. The Japanese groups[3,4] have reported identical chemical shifts without the coupling constants. Found: C, 62.38, 62.08; H, 5.36, 5.32. $C_{27}H_{26}O_9.1\frac{1}{2}H_2O$ requires: C, 62.18; H, 5.56%.

Toromycin acetonide. Toromycin (50 mg) in dry acetone (300 ml) was stirred with toluene-p-sulfonic acid (500 mg) at room temp in the dark for three days. The solution was concentrated to ~ 5 ml and precipitated with ether; silica gel column chromatography of the solid using 5% methanol in CH_2Cl_2 as eluant gave several fractions: TLC-pure fractions were pooled and crystallized from acetone to give mustard colored crystals (35 mg), mp 171–174°; silica gel TLC Rf 0.80 (Et_2O–CH_2Cl_2, 70 : 30); IR 3400, 1710 (sh), 1700, 1366, 1330, 1288, 1240, 1128, 870, 780 cm^{-1}; $UV^{MeOH}_{\lambda max}$ 245, 265 (sh), 275, 285, 390 nm; the ε max values for toromycin acetonide and other compounds not reported in the literature have been compiled in Table 4. MS (FD, 20mA) *m/e* (%rel. int.) 534 (10.38, molecular ion). (Found: C, 66.36; H, 5.41.$C_{30}H_{30}O_9.1/2H_2O$ requires: C, 66.29; H, 5.70%). The crystalline acetonide (6mg) was converted to the corresponding diacetate by the pyridine-acetic anhydride method. The product was crystallized from ether as light yellow needles, m.p. 182–187°; IR 3390, 1739, 1724 (sh), 1379, 1325, 1299, 1220 cm^{-1}; $UV^{CH_2Cl_2}_{\lambda max}$ 245, 265 (sh), 275, 285, 320, 330 (broad), 380 nm; ^1H NMR (Table 1), high-resolution mass measurement: found 618.210 ($C_{34}H_{34}O_{11}$), calc 618.210.

Isomerization of Toromycin in acetonitrile. A solution of toromycin (40 mg) in acetonitrile (500 ml) and toluene-p-sulfonic acid (40 mg) was refluxed for 10 h. The solution was concentrated and the residual solid was washed with a small volume of cold water, filtered and dried to give light greenish yellow solid $[\alpha]_D$ - 29.6° (c 1.0, $CHCl_3$). Found: C, 64.64; H, 5.33.$C_{27}H_{26}O_9$ $\frac{1}{2}H_2O$ requires: C, 64.41; H, 5.37%. TLC of the crude reaction product on silica plate in 5% MeOH in CH_2Cl_2 showed three distinct spots (detected by UV and bioautography against *B. subtilis*). The more polar spot (Rf 0.27) referred to as isomer A and the less polar isomer B (Rf 0.58) were distinguishable from toromycin (Rf 0.40) in 5% MeOH in CH_2Cl_2.

Toromycin isomer A. Silica gel column chromatography of the crude product and gradient elution with 1 to 5% MeOH in CH_2Cl_2 yielded several fractions, which on the basis of TLC-bioautography were pooled into two major cuts containing the two isomers. The isomer A isolated from the pool was purified by preparative TLC (three times in 5% MeOH in CH_2Cl_2) and crystallized from CH_2Cl_2–CH_3OH: yield (15 mg), mp 172–74°, $[\alpha]_{589}$ - 51.10°, $[\alpha]_{578}$ - 54.6°, $[\alpha]_{546}$ - 66.7° (c 0.5, DMSO), toromycin under identical conditions exhibited $[\alpha]_{589}$ - 232.4°, $[\alpha]_{578}$ - 247.8°, $[\alpha]_{546}$ - 300°; IR 3380, 1710, 1700 (sh), 1620, 1590, 1450, 1370, 1300, 1250, 1130, 1060, 1000, 900, 850, 790 cm^{-1}; $UV^{MeOH\text{-}CH_2Cl_2}_{\lambda max}$ 245, 265, 275, 285, 390 nm; MS (FD, 25 mA) *m/e* (%rel. int.) 494 (100, molecular ion), high-resolution mass measurement found 494.155 ($C_{27}H_{26}O_9$), calc 494.158. Found: C, 63.59; H, 5.48. $C_{27}H_{26}O_9.\frac{1}{4}H_2O$ requires: C, 63.84; H, 5.42%). The acetonide, m.p. 270–72°, prepared from the polar isomer A was identical with the above acetonide (TLC, IR, UV, ^1H NMR).

Toromycin isomer A tetraacetate. Toromycin isomer A (7 mg) was converted to its tetraacetate by the pyridine-acetic anhydride method. It was crystallized from EtOAc–Et_2O, m.p. 279–282°; $[\alpha]_{589}$ + 100.5°, $[\alpha]_{578}$ + 108.0°, $[\alpha]_{546}$ + 128.3° (c 0.3, $CHCl_3$); IR 1750–1730, 1710, 1250, 1220, 890 cm^{-1}; $UV^{CH_2Cl_2}_{\lambda max}$ 245, 268, 277, 288 (sh), 320, 335, 380 nm; high-resolution mass measurement: found 662.198 ($C_{35}H_{34}O_{13}$), calc 662.200; ^1H NMR (Tables 1 and 3). Found: C, 63.19; H, 5.51. $C_{35}H_{34}O_{13}$ requires C, 63.44; H, 5.14%. Some preparations were contaminated with up to 10% dihydro analog, the NMR data for both compounds are given in Table 3. The melting points of these contaminated samples were virtually identical as for the pure isomer A tetraacetate. Surprisingly, the contamination with dihydro-analog does not exhibit significant effect on the melting point of the parent isomer. This observation is consistent with our experience with toromycin tetraacetate whose melting point of 193–195° was not depressed when mixed with dihydrotoromycin tetra-acetate (mp 211–214°). Toromycin tetraacetate ($[\alpha]_D$ - 143.2°; c 0.3, $CHCl_3$) was found to be identical (mp, IR, UV, MS, ^1H NMR) with the tetra-acetate reported by Horii[3] and Takahashi.[4]

†Not surprisingly, in view of the presence of the vinyl group on an aromatic ring, solutions of toromycin displayed a tendency to polymerize over prolonged periods yielding a gelatinous insoluble polymer.

‡The maximum at 277 nm in the published spectrum of toromycin suggests possible contamination by either gilvocarcin M and dihydrotoromycin or both. The latter compound in our hand shows a distinct maximum at 275 nm.

¶The dimerization invariably occurs in the mass spectrometer.

§Identical chemical shifts and multiplicities have been reported for toromycin and gilvocarcin V.

Toromycin isomer B. The pool containing isomer B plus some toromycin was repeatedly purified by preparative TLC in 5% MeOH in CH_2Cl_2. Finally, the preparation was crystallized from methanol: yield 7 mg, mp 214–220° (dec); $[\alpha]_{589}$ + 64.3°, $[\alpha]_{578}$ + 70.3°, $[\alpha]_{546}$ + 91.3° (c 0.6, DMSO); IR 3380, 1700 (sh), 1690, 1610, 1582, 1445, 1370, 1300, 1245, 1130, 1010, 910, 870, 840, 780 cm^{-1}; $UV_{\lambda max}^{MeOH-CH_2Cl_2}$ 245, 265, 275, 285(sh), 395 nm; MS (FD, 25 mA) *m/e* (% rel. int.) 495 (74.62, M + 1), 496 (28.19, M + 2); exact mass measurement: found 494.156 ($C_{27}H_{26}O_9$), calc 494.158. Found: C, 64.60; H, 5.40. $C_{27}H_{26}O_9 \cdot 1/2\ H_2O$ requires: C, 64.41; H, 5.37%. By means of the pyridine and acetic anhydride method, isomer B was converted to the corresponding tetraacetate, yellow precipitate from EtOAc–hexane: TLC Rf 0.83 (EtOAc); IR 1735, 1210, 1230 cm^{-1}; $UV_{\lambda max}^{CH_2Cl_2}$ 245, 265, 276, 285 (sh), 320, 335 (broad), 385 nm; exact mass measurement: found 662.198 ($C_{35}H_{34}O_{13}$), calc 662.200; found: 664.215 ($C_{35}H_{36}O_{13}$), calc 664.216. For ^1H NMR of isomer B and the corresponding dihydro analog, see Table 3. Because of the apparent similarity in the Rf values of these two compounds, we could not resolve them by preparative thin layer chromatography.

Isomerization of toromycin in aq. HOAc. A solution of toromycin (150 mg) in aq. HOAc (100 ml) was refluxed for 2 hr and filtered to remove some gelatinous polymer. The filtrate was lyophilized and washed with ether to give light greenish yellow solid. TLC of the crude solid in 5% MeOH in CH_2Cl_2 showed three distinct spots (UV and bioautography against *B. subtilis*) which were identical with our two isomers and toromycin (mixed TLC). Reversed phase HPLC of the crude product on LiChrosorb RP-8 (10μ, 4.6 × 250 mm; mobile phase 0.015 M heptafluorobutyric acid/CH_3CN, 6 : 4; flow rate 1 ml/min) showed four distinct peaks with retention times 7.5, 8.4, 8.6 and 9.1 min due to an unidentified compound, isomers A, B and toromycin, respectively.

Treatment of the crude product with pyridine and large excess of Ac_2O at room temp (3 days) yielded the corresponding tetraacetates which were examined by 360 MHz ^1H NMR spectroscopy. The sugar C-methyl region of the spectrum showed the presence of toromycin (δ, 1.432), isomer B (δ, 1.427), isomer A (δ, 1.225) and an unknown compound (δ, 1.219) in the ratio of 1 : 1 : 2.5 : 1, respectively. In addition, the spectrum showed the presence of approximate 10% of the corresponding dihydro compounds. The ratio of these isomeric products did not change when refluxing was continued for 7 h. Horii *et al.*[3] essentially used the same conditions to isomerize toromycin and reported the formation of only one solid product ($[\alpha]_D$ − 40.6°, DMSO) which appears to be identical with our crystalline toromycin isomer A ($[\alpha]_D$ − 51.1°, DMSO). These workers did not report the concomitant formation of dihydro analogs.

The crude reaction product was passed on neutral alumina (grade I) column in acetone. Unlike the TLC profile of the crude product, fractions showing less polar behaviour on TLC plate were obtained and processed further to get bright yellow needles, (35 mg), mp 274–278°; $[\alpha]_{589}$ + 22.4°, $[\alpha]_{578}$ + 23.1, $[\alpha]_{546}$ + 27.6 (c 0.4, $CHCl_3$). Found: C, 67.21; H, 5.81. $C_{30}H_{30}O_9$ requires: C, 67.41; H, 5.66%. UV, IR and ^1H NMR spectra of this crystalline product were identical with those of the acetonide prepared by direct treatment of toromycin and acetone in the presence of toluene-p-sulfonic acid. Again, the difference in the melting points of the two crystalline products reflects the degree of hydration in the molecule. It may be pointed out that the Takeda group did not report this secondary reaction on alumina surface. Further gradient elution of the column with 10–50% MeOH in acetone gave several fractions which on the basis of TLC-bioautography against *B. subtilis* were combined into two pools. Preparative TLC of the two pools followed by several crystallizations yielded isomers A and B, respectively, further characterized by their corresponding tetraacetates. Isomers A, B and the corresponding tetraacetates were identical in all respects with the products obtained *via* the acetonitrile toluene-p-sulfonic acid method, apart from differences in the degree of contaminations by the corresponding dihydro compounds.

Acknowledgements—We are grateful to the members of our Microbiology and Analytical and Physical Chemistry Departments for their help in this work. We are especially thankful to Gerry Roberts for mass spectral data reported herein. Finally, it is a pleasure to thank G. Udowenko for his technical skill.

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