ISOLATION AND CHARACTERIZATION OF THE FIRST HALOGEN CONTAINING POLYETHER ANTIBIOTIC X-14766A, A PRODUCT OF *STREPTOMYCES MALACHITOFUSCUS* SUBSP. *DOWNEYI*

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This report describes the isolation and chemical characterization of antibiotic X-14766A, the first halogen containing polyether antibiotic, from fermented cultures of *Streptomyces malachitofuscus* subsp. *downeyi*. The structure of this novel antibiotic was determined by X-ray analyses of the thallium and rubidium salts. In addition to the chlorine atom, which is attached to a salicylic acid chromophore, the molecule contains a tricyclic di-spiroketal ring system and an ethyl ester group.

In all sixty polyether antibiotics reported up to 1980, certain structural features are invariably found: A carboxyl group at one end of the molecule, a secondary or tertiary alcohol at the opposite end and in between, a multiplicity of cyclic ethers and C-methyl groups. These basic molecular requirements, involving many asymmetric centers, give the polyether antibiotics the ability to form lipid soluble salt-complexes with monovalent cations such as Na^+ and K^+, and in a minority of cases with divalent cations (Mg^{2+}, Ca^{2+}) also. This difference in cation selectivity plays an important role in the diverse biological activities of these naturally occurring ionophores[1], and has also been used together with glycosidation[2] as a method of classification for this antibiotic group.

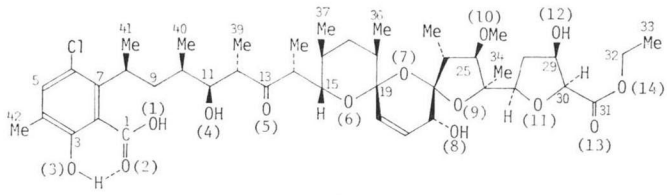
As this burgeoning group of antibiotics has grown, a number of distinguishing structural features have repeatedly appeared. These molecular characteristics can be used to further discriminate amongst members of the same valency class of polyether antibiotics. For instance, although there are now almost twenty polyether antibiotics which fall into the monovalent glycoside class, antibiotics K-41B[3] and A-130B[4] were recently reported as the first polyether *di*glycosides. A more common structural feature which occurs in half the known polyethers is the bicyclic spiro-ketal system first reported in the monovalent polyethers, monensin[5] and nigericin[6,7], but subsequently found in certain glycosides such as A204A[8] and carriomycin[9]. In addition, one group of the monovalent glycosides, dianemycin[10], lenoremycin[11], leuseramycin[12] and antibiotics A-130B and C[4], which all contain an α, β-unsaturated ketone, are also characterized by the presence of *two* spiroketals. In a further variation on this theme, the tricyclic di-spiroketal system first reported for salinomycin[13] has now been found in many other polyethers such as the noboritomycins[14] and antibiotic CP 44,161[15]. Other distinguishing structural features that occur with varying frequency amongst these ionophores are β-ketols, C-ethyls, methoxyls, cyclic hemiketals and aromatic chromophores, such as the pyrrole-2-carbonyl system found in the pyrrole-ether[2] antibiotics A23187[16] and X-14547A[17].

In this report, the isolation and characterization of the novel polyether antibiotic X-14766A is

reported. This antibiotic contains both a β-ketol and a tricyclic di-spiroketal ring system, but is distinguished from all other polyether antibiotics in having a halogen substituent, chlorine.

Isolation and Characterization

Antibiotic X-14766A (**1**) was isolated as part of a screen for novel ionophores by ethyl acetate extraction of whole fermentation broth from a culture of *Streptomyces malachitofuscus* subsp. *downeyi*.



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The crude extract was concentrated under reduced pressure to a dark oil which was dissolved in *n*-hexane and the solution extracted twice with equal volumes of acetonitrile. The pooled extracts were evaporated under reduced pressure and the residue dissolved in diethyl ether. The ethereal solution was washed in turn with 1 N HCl, water, saturated aqueous Na_2CO_3 and water, then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Addition of *n*-hexane to the concentrate yielded crude sodium salt, which was recrystallized from methylene chloride and *n*-hexane to give antibiotic X-14766A, sodium salt, m.p. 219°C, $[\alpha]_D$ −4.7° (*c* 1, $CHCl_3$).

Calcd. for $C_{43}H_{62}ClO_{14}Na$ (861.4): C, 59.95; H, 7.26; Cl, 4.12; Na, 2.67.

Found: C, 60.30; H, 7.27; Cl, 4.25; Na, 2.15.

A solution of the sodium salt in ethyl acetate was washed with 1 N HCl and the solvent removed under reduced pressure. Crystallization from acetonitrile yielded the free acid form of antibiotic X-14766A as a monohydrate, m.p. 160°C, $[\alpha]_D$ −11.3° (*c* 1, $CHCl_3$).

Calcd. for $C_{43}H_{63}ClO_{14}\cdot H_2O$ (857.4): C, 60.24; H, 7.64; Cl, 4.13; H_2O, 2.10.

Found: C, 60.92; H, 7.88; Cl, 3.42; H_2O, 1.97.

The thallium salt of antibiotic X-14766A was prepared from the free acid by treating an ethyl acetate solution with saturated aqueous TlOH. The salt was isolated by evaporation of the organic phase under reduced pressure and crystallization from *n*-hexane - ethanol to yield the crystalline thallium salt, m.p. 194~196°C, $[\alpha]_D$ +29° (*c* 1, $CHCl_3$).

Calcd. for $C_{43}H_{62}ClO_{14}Tl$ (1042.8): C, 49.53; H, 5.99; Cl, 3.40; Tl, 19.58.

Found: C, 50.03; H, 6.02; Cl, 3.42; Tl, 17.87.

The rubidium salt was prepared from aqueous RbOH in an identical manner to that described for the thallium salt. Crystallization from *n*-hexane - ethanol yielded crystals containing one molecule of hexane for every two of the rubidium salt of X-14766A, m.p. 195°C.

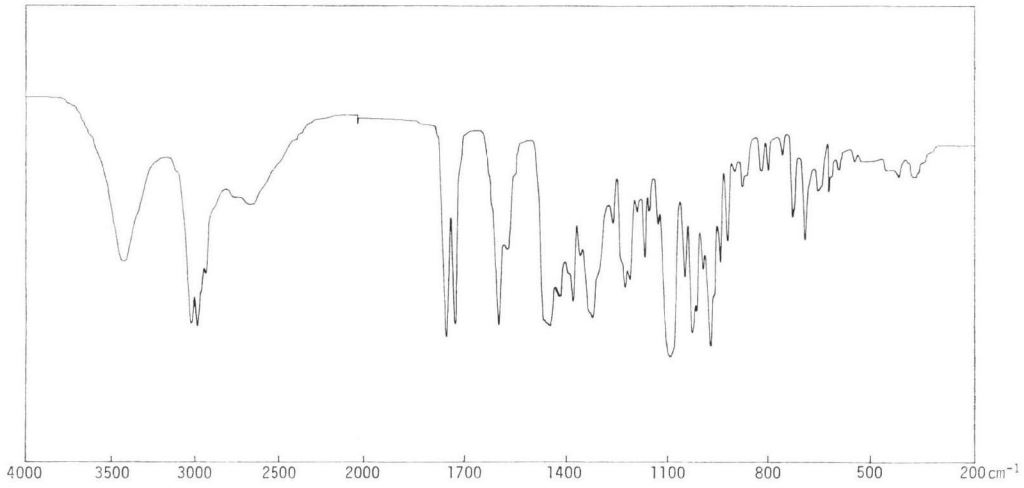
Calcd. for $C_{43}H_{62}ClO_{14}Rb\cdot C_3H_7$ (966.94): C, 57.13; H, 7.19; Cl, 3.67; Rb, 8.84.

Found: C, 57.47; H, 7.15; Cl, 4.02; Rb, 8.40.

The sodium salt of antibiotic X-14766A is insoluble in water, less than 1% (w/v) soluble in methanol, ethanol or DMSO, but quite soluble in THF (20%) and chloroform (30%).

The infrared absorption spectrum of the sodium salt (Fig. 1) contains three carbonyl absorption at 1593 (aromatic CO_2^-), 1719 (ketone) and 1740 cm^{-1} (ester), which shift to 1655, 1710 and 1730 cm^{-1} respectively in the free acid form of the antibiotic. Hydroxyl (3300 cm^{-1}) and ether (1100 cm^{-1})

Fig. 1. IR Spectrum of the sodium salt of antibiotic X-14766A (KBr).



functions were also indicated.

The ultraviolet spectrum in ethanol of the sodium salt exhibited maxima at 210 nm (ε 30400) and 307 nm (ε 3410). Potentiometric titration in 50% aqueous ethanol gave two pKa′ values for antibiotic X-14766A at 4.03 (corresponding to approximately 3.0 in water) for the carboxyl group and 12.82 (11.8) for the phenol.

Mass spectrometry of the sodium salt yielded a molecular ion consistent with the molecular formula of $C_{43}H_{62}ClO_{14}Na$ (861). Chlorine containing peaks at *m/z* 222 and 207 suggest that the antibiotic probably undergoes the same thermolytic retroaldol cleavage and decarboxylation reactions in the mass spectrometer as observed previously for lasalocid[18]. Evidence for the ketonic fragment from the retroaldol cleavage was found in mass spectral peaks at *m/z* 395, 252, 159 and 143, which are accounted for in Scheme 1.

The proton nmr spectrum of antibiotic X-14766A sodium salt in $CDCl_3$ revealed the presence of *ten* methyl groups in the molecule at δ 0.72 (d, J=8 Hz), 0.87 (d, J=6.5 Hz), 0.91 (d, J=6.5 Hz), 1.00 (d, J=7 Hz), 1.08 (d, J=7 Hz), 1.11 (t, J=7.5 Hz), 1.15 (d, J=7 Hz), 1.35 (d, J=7 Hz), 2.00 (s, methyl C-34 in **1**) and 2.75 (s, aromatic methyl C-42), a methoxyl as a singlet at δ 3.44, olefinic protons at δ 5.95 (–C<u>H</u>=CH–CHOH, J=11.5 Hz) and 6.09 (–CH=C<u>H</u>–CHOH, J=11.5, 2.5 Hz), an aromatic singlet at 6.99 and hydroxyl protons at δ 5.07, 5.72, 10.39 and 11.60.

The ^{13}C nmr spectrum of antibiotic X-14766A sodium salt (Fig. 2) in $CDCl_3$ was consistent with a molecular formula of $C_{43}H_{62}ClO_{14}Na$. The spectra of the salt and free acid form of antibiotic X-14766A (**1**) were provisionally assigned as shown in Table 1 by comparison with the published assignments of lasalocid A[19], salinomycin[20] and noboritomycin A[14]. Carbons (and oxygens) are numbered for **1** in accordance with the proposals[21] made earlier for a universal method of notation for the polyether antibiotics.

The complete structure of antibiotic X-14766A was determined from the X-ray crystallographic analyses of the rubidium and thallium salts. Crystals of the rubidium salt of **1** are triclinic, whereas those of the thallium salt are monoclinic. Both salts are unusual in that both crystallize with *two* independent molecules in the unit cell (primed and unprimed), but differ in that the rubidium salt

Scheme 1. Mass spectral fragmentation of X-14766A.

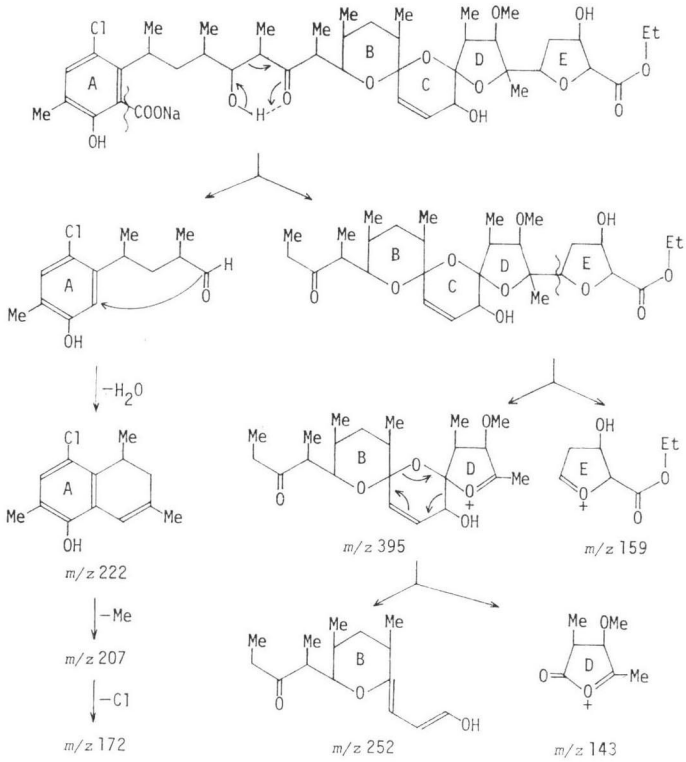
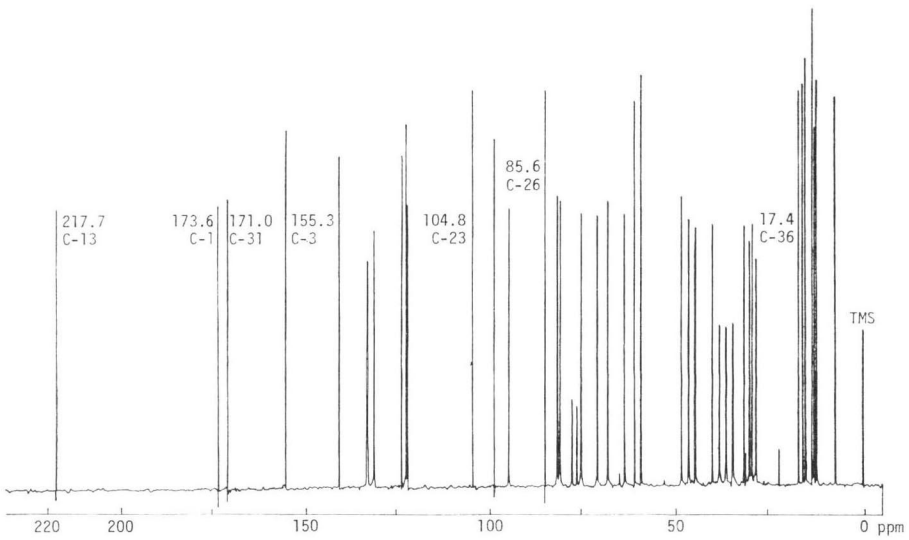


Fig. 2. ^{13}C NMR spectrum of the sodium salt of antibiotic X-14766A in $CDCl_3$.



contains solvent.

The Rb—O and Tl—O distances in the two salts are listed in Table 2 and the conformation of the primed molecule of the thallium and rubidium salts is shown in Fig. 3. The same seven oxygen atoms, O-1, 4, 5, 9, 11, 12 and 13 are involved in all four salt modifications, but in the rubidium salt the Rb—O

Table 1. Assignments of the ^{13}C NMR spectra for the sodium salt and free acid form of antibiotic X-14766A in $CDCl_3$.

Carbon[a]	Antibiotic X-14766A sodium salt		Multiplicity	Antibiotic X-14766A (1) free acid	
	Function	Chemical shift[b]		Chemical shift	Difference in shift (δ Na-δ H)
1	CO_2H	173.6[c]	s	168.4	+5.2
2	$=C-CO_2H$	124.1[d]	s	117.4[d]	+6.7
3	$=C-OH$	155.3	s	155.7	−0.4
4	$=C-CH_3$	122.5[d]	s	124.4[d]	−1.9
5	$=CH$	133.2	d	135.7	−2.5
6	$=C-Cl$	122.9[d]	s	125.2[d]	−2.3
7	$=C-CH_2$	141.0	s	141.1	−0.1
8	$CH(CH_3)$	30.0	d	34.1	−4.1
9	CH_2	36.8	t	38.0	−1.2
10	$CH(CH_3)$	30.6	d	31.5	−0.9
11	CH(OH)	75.9	d	77.3	−1.4
12	$CH(CH_3)$	48.9	d	47.6	+1.3
13	$C=O$	217.7	s	213.3	+4.4
14	$CH(CH_3)$	46.9	d	45.9	+1.0
15	$CH-O$	81.5	d	81.8	−0.3
16	$CH(CH_3)$	32.0	d	32.2	−0.2
17	CH_2	38.6	t	38.0	+0.6
18	$CH(CH_3)$	40.5	d	40.4	+0.1
19	$OC-O$	99.1	s	98.1	+1.0
20	$=CH$	122.9	d	121.8	+1.1
21	$=CH$	131.4	d	130.8	+0.6
22	CH(OH)	71.6	d	73.3[e]	−1.7
23	$OC-O$	104.8	s	103.2	+1.6
24	$CH(CH_3)$	45.0	d	45.6	−0.6
25	$CH(OCH_3)$	95.1	d	94.3	+0.8
26	$C(CH_3)-O$	85.6	s	83.7	+1.9
27	$CH-O$	68.8	d	73.2[e]	−4.4
28	CH_2	35.0	t	35.3	−0.3
29	CH(OH)	64.3	d	64.0	+0.3
30	$CH-O$	82.2	d	82.3	−0.1
31	$C=O$	171.0[c]	s	172.1	−1.1
32	CH_2CH_3	61.6	t	61.1	+0.5
33	CH_2CH_3	13.9[c]	q	14.2	−0.3
34	CH_3-C-O	16.4	q	15.8	+0.6
35	CH_3-CH	13.3	q	14.1	−0.8
36	CH_3-CH	17.4	q	17.1	+0.3
37	CH_3-CH	15.6	q	15.6	0
38	CH_3-CH	12.6[c]	q	11.9	+0.7
39	CH_3-CH	12.7	q	12.2	+0.5
40	CH_3-CH	7.6	q	7.6	0
41	CH_3-CH	28.9	q	27.3	+1.6
42	$CH_3-C=$	15.7	q	15.6	+0.1
OMe	CH_3-O	59.7	q	60.3	−0.6

[a] As indicated for X-14766A (see **1**).

[b] Downfield from internal Me_4Si.

[c] Incorrectly assigned in the noboritomycin paper (Ref. 14).

[d],[e] In each case, these signals could be interchanged.

distances are on average 0.10 A greater in the primed than in the unprimed molecule; the average difference in the thallium salt is only 0.04 A.

The same intramolecular hydrogen bonds involving O–3 to O–2, O–4 to O–1 and O–8 to O–12 exist in both salts, but the typical head-to-tail O–12 to O–2 bond which is observed in both forms of the thallium salt and one of the rubidium salts is absent in the primed rubidium salt, which accounts for the longer Rb—O distances in that form.

In all four molecules, the carboxylate group is twisted significantly out of the plane of its phenyl

Table 2. Coordination distances in the two salts of antibiotic X-14766A.

	Rb—O distance (A)			Tl—O distance (A)	
	unprimed	primed		unprimed	primed
O(9)	2.791	2.901	O(9)	2.757	2.652
O(1)	2.825	2.878	O(1)	2.756	2.822
O(5)	2.799	2.925	O(13)	2.846	2.748
O(13)	2.939	2.960	O(5)	2.953	2.882
O(12)	2.972	3.102	O(11)	2.910	2.944
O(11)	3.010	3.150	O(4)	3.001	2.980
O(4)	2.994	3.221	O(12)	3.049	2.952

The average estimated standard deviation for Rb—O and Tl—O is 0.010A.

Table 3. X-Ray crystallographic data and details of the analyses of the two salts of antibiotic X-14766A.

Space group	Rubidium P1	Thallium $P2_1$
a (A)	11.575(3)	17.488(6)
b (A)	13.582(4)	17.531(6)
c (A)	15.970(5)	15.497(5)
α (°)	77.19(2)	
β (°)	83.24(2)	102.42(2)
γ (°)	88.08(2)	
Z	2	4
d_{calcd} (g cm^{-3})	1.324	1.492
μ(Cu Kα) (cm^{-1})	26.0	78.0
Crystal size (mm)	0.12×0.30×0.60	0.20×0.40×0.8
Maximum θ (°)	57	57
Number of reflections	6,495	7,942
Number of observed reflections	5,322	7,098
Absorption correction	yes	yes
Least squares refinement	block diagonal (two blocks)	block diagonal (two blocks)
Rb/Tl, Cl atoms	anisotropic	anisotropic
O and C atoms	isotropic	isotropic
Hydrogen atoms	iso (fixed)	iso (fixed)
Final R	0.095	0.055
Final wR	0.101	0.065
Final difference map—largest peak (eA^{-3})	$<\pm 1.0$	$<\pm 1.4$

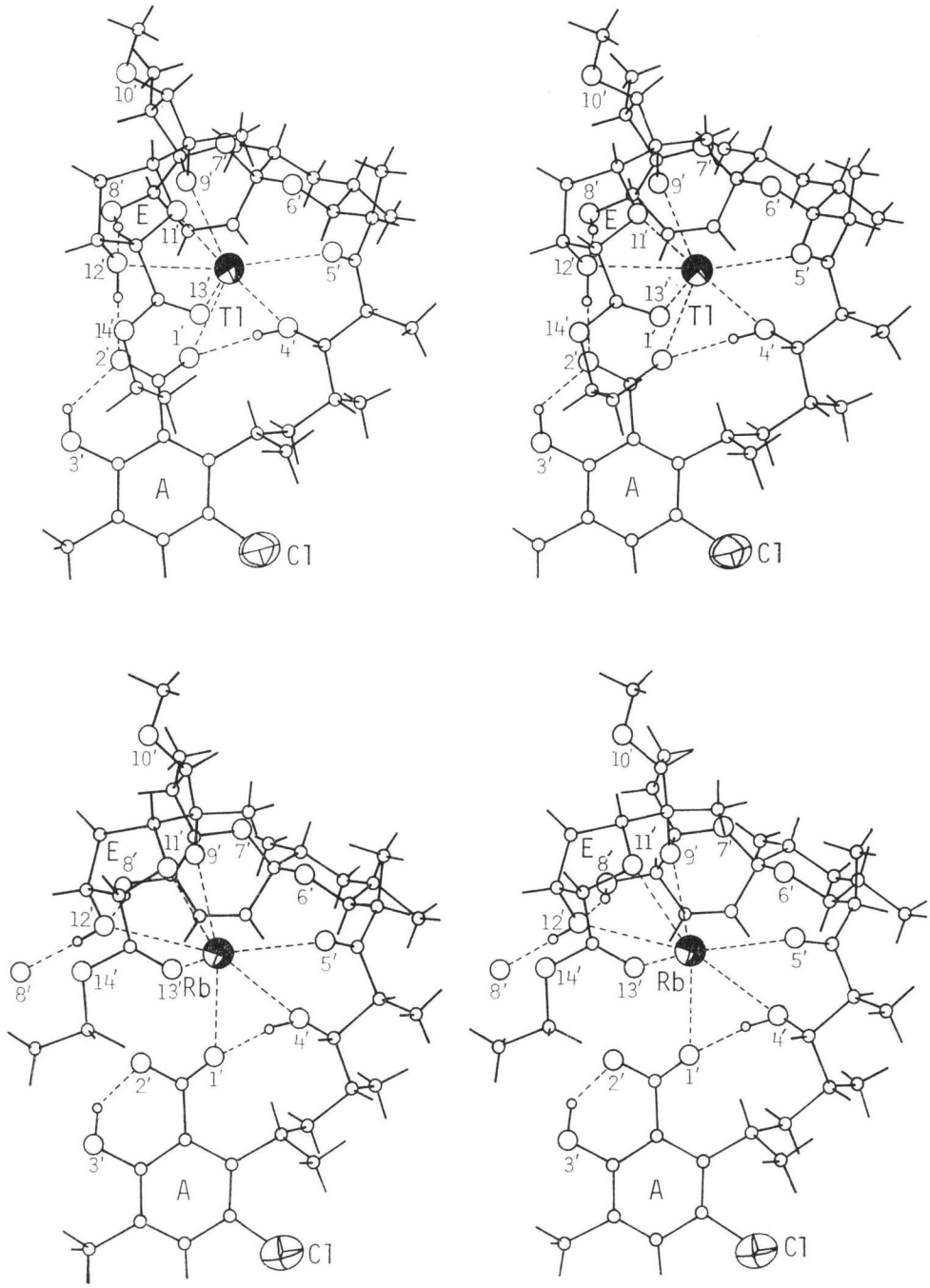
ring to an extent of 22° in the rubidium salt and 33° in the thallium salt due to a combination of the O–1 to O–4 hydrogen bond and cation coordination.

The absolute configuration of antibiotic X-14766A was determined from the thallium salt. A different data set (Zr filtered Mo Kα radiation, ω scans) was used. Both enantiomers were refined taking into account the anomalous dispersion of the thallium and chlorine atoms. The final weighted R values for 5,135 observed reflections (thallium and chlorine atoms anisotropic, other atoms isotropic, hydrogens not refined) were 0.0455 for the correct configuration as shown for **1** and 0.0597 for its antipode. The details of the X-ray crystallographic analyses are summarized in Table 3. The intensity data were measured on a Hilger-Watts diffractometer (Ni filtered Cu Kα radiation, θ–2θ scans, pulse height discrimination) and the crystal structures solved by the heavy atom method.

A comparison of the structure of antibiotic X-14766A with the corrected structures[22] of the noboritomycins shows that the chlorine atom is the only difference between this novel polyether antibiotic and noboritomycin A.

The most intriguing question regarding the biosynthesis of antibiotic X-14766A (and the noboritomycins) is the origin of carbons C-31, 32 and 33. One possibility is that all three originally constituted a single propionate unit which was present in a precursor as a propionyl (or equivalent) substituent at C-30. This substrate could then be oxidized *via* a Bayer-Villiger type reaction[23] to **1** by an oxygenase (+NADPH) analogous to those

Fig. 3. A stereoscopic drawing of the primed molecule of the thallium and rubidium salts of antibiotic X-14766A.



which catalyze the conversion of *cyclo*pentanone to 5-valerolactone[24] and *cyclo*hexanone to the ε-lactone of 6-hydroxyhexanoic acid[25].

The taxonomy and microbiological characteristics of *Streptomyces malachitofuscus* subsp. *downeyi* and the antimicrobial and other biological activities of antibiotic X-14766A are presented in the accompanying paper[26].

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