The Structure of Ikarugamycin, an Acyltetramic Acid Antibiotic Possessing a Unique *as*-Hydrindacene Skeleton[1)]

Shosuke Ito and Yoshimasa Hirata

Department of Chemistry, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464

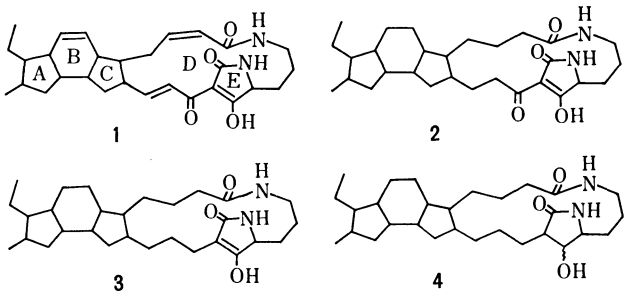
(Received December 25, 1976)

The structure and configuration of an antibiotic, ikarugamycin, have been established to be **1e** on the basis of chemical reactions, especially of oxidative degradation. It is suggested that the *as*-hydrindacene skeleton arises biogenetically *via* an intramolecular Diels-Alder reaction.

A new antibiotic, ikarugamycin ($C_{29}H_{38}N_2O_4$), possessing specific antiprotozoal activity has been isolated from the culture broth of *Streptomyces phaeochromogenes var. ikaruganensis* Sakai by Jomom *et al.*[2)] In this paper, we wish to describe evidence for assigning structure **1e** to ikarugamycin. A biogenesis of this antibiotic is also proposed.

Chromophoric Part of Ikarugamycin. On catalytic hydrogenation over PtO_2 for 1 h, ikarugamycin (**1**) absorbed 3 mol of hydrogen giving hexahydroikarugamycin (**2**), $C_{29}H_{44}N_2O_4$. The presence of three disubstituted double bonds in **1** was revealed by PMR spectrometry (Fig. 1). The double bond bearing strongly deshielded H_a and H_b protons (δ 6.96 and 6.64, respectively, in DMSO-d_6) must be trans substituted (J= 15.6 Hz). The other two pairs of olefinic protons (δ 5.6—6.2 in DMSO-d_6) should be attached to cis double bonds; although the H_d proton signal is obscured by those of the H_e and H_f protons, its counterpart, the H_c proton signal appears as a broad doublet with J= 12 Hz,[3)] and the H_e and H_f protons are observed as a broad AB quartet with J=10 Hz. The PMR spectrum of the hexahydro derivative (**2**) exhibited no signals for the olefinic protons.

The presence of an enolized β-tricarbonyl grouping in both **1** and **2** was suggested by an orange-red coloration with ferric chloride and the formation of greenish



copper complexes, as well as by the formation of an *N*-methylpyrazole derivative upon heating with methylhydrazine. Further catalytic hydrogenation of **2** over PtO_2 for 24 h resulted in conversion of one of the carbonyl groups into a methylene group[4)] giving deoxooctahydroikarugamycin (**3**), $C_{29}H_{46}N_2O_3$. The UV maximum for the compound displayed a bathochromic shift of 33 nm upon the addition of an alkali (Table 1). The PMR spectrum showed a signal at δ 10.32 attributable to an enol proton. These data, coupled with a pK_a value of 7.8 and the formation of an enol ether, as well as of an enol acetate, indicates that an enolized β-dicarbonyl group is present in **3**.

Upon reduction with $LiBH_4$, **3** was readily converted to deoxodecahydroikarugamycin (**4**), $C_{29}H_{48}N_2O_3$ (probably a mixture of epimers) which could also be formed

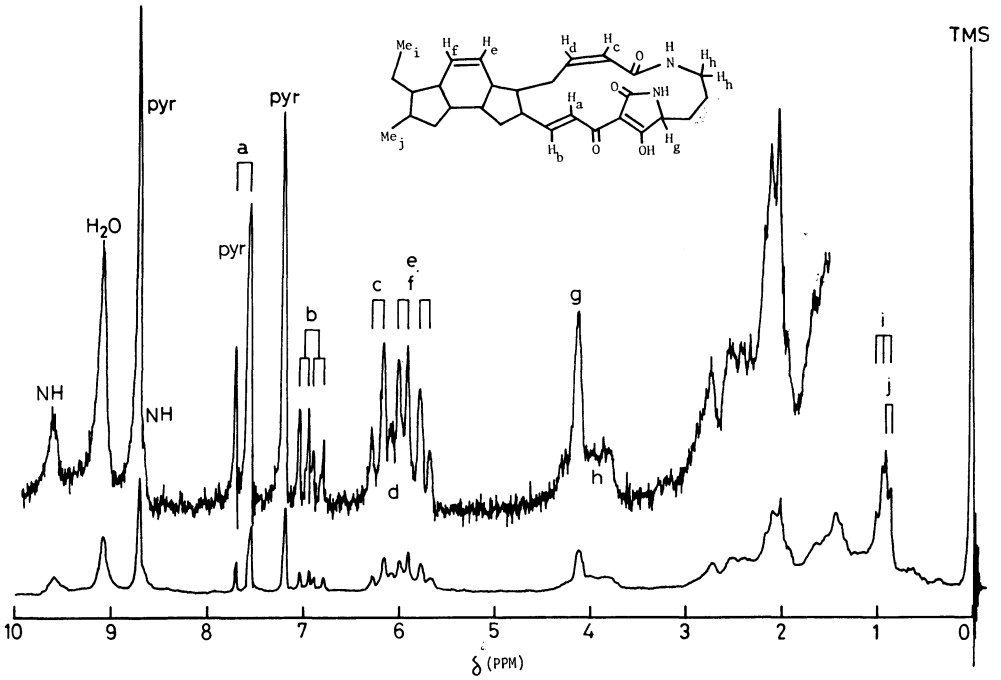


Fig. 1. The 100 MHz PMR spectrum of ikarugamycin (**1**) in pyr-d_5.

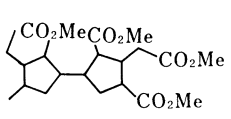
Table 1. UV spectra of ikarugamycin, its derivatives and related compounds

	λ_{max} (ε)	Solvent
Ikarugamycin (**1**)	227 (20700), 327 (17300)	MeOH
	243 (21400), 321 (13300)	0.1 M NaOH-MeOH
Hexahydroikarugamycin (**2**)	220 (5000), 280 (12400)	MeOH
	243 (10300), 279 (13600)	0.1 M NaOH-MeOH
Deoxooctahydroikarugamycin (**3**)	220 (6100), 240^{sh} (4600)	MeOH
	222 (3200), 273 (9300)	0.1 M NaOH-MeOH
Deoxodecahydroikarugamycin (**4**)	211 (910)	MeOH
Tenuazoic acid (**10**)[8]	217 (5100), 277 (12900)	EtOH
	239 (9600), 279 (12000)	0.1 M NaOH
Decahydroerythroskyrine[9]	225 (7200), 284 (12900)	EtOH
	246 (14100), 288 (14100)	0.1 M NaOH

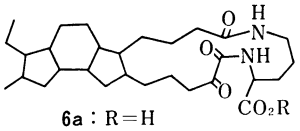
directly from **2** under more drastic conditions. That **4** no longer contains the β-dicarbonyl group is evident from its UV spectrum (Table 1) and the formation of an acetate.

In the PMR spectra of **1** to **4**, two 1H broad singlets were always observed in the range of δ 6—9, which disappeared upon the addition of deuterium oxide. This indicates the presence of two secondary amide groups in **1** to **4**, which is further supported by the IR spectrum of **4** showing, in addition to amide NH (3430 cm^{-1}) and amide II (1520 cm^{-1}) bands, two amide carbonyl bands at 1695 and 1655 cm^{-1}. All of the oxygen and nitrogen atoms present in **1** can thus be assigned to a β-tricarbonyl system in which one of the carbonyl groups is involved as an amide carbonyl group, and to an additional amide group.

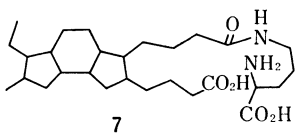
The relative position of the two amide nitrogen atoms was then established by ozonolysis of **1**. Thus, ether extraction of the oxidation mixture yielded a carboxylic acid which was isolated as bicyclic tetramethyl ester **5**, $C_{14}H_{22}(CO_2Me)_4$, and the water-soluble part gave, after acid hydrolysis, L-ornithine and oxalic acid. These results led to the formulation of the chromophoric part of ikarugamycin as **1a**; in order to account for the formation of **5**, as well as the shift of a UV absorption maximum from 327 nm for **1** to 280 nm for **2** (Table 1), two double bonds should be located at the positions shown in **1a**.



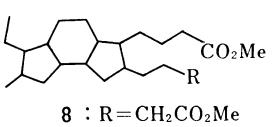
5



6a : R=H
6b : R=Me

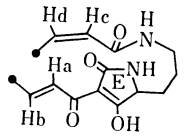


7

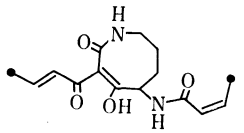


8 : R=CH_2CO_2Me
11 : R=CO_2Me

In principle, an alternative structure **1b** is also consistent with these chemical data. However, such a possibility can be ruled out by the formation of α-DNP-ornithine[5] from **3** *via* **6** and **7**. Chromic acid oxidation of **3** resulted in cleavage of the enolic double bond to give the keto acid **6a**, $C_{29}H_{46}N_2O_5$ and similarly, permanganate oxidation of the enol ether of **3** afforded the corresponding keto ester **6b**, $C_{30}H_{48}N_2O_5$. Treatment of **6a** or **6b** with alkaline hydrogen peroxide cleaved the α-keto amide group, giving an amino acid, **7**, which was characterized as its *N*-acetyl dimethyl ester. Acid hydrolysis of the DNP derivative of **7** gave α-DNP-ornithine and a carboxylic acid isolated as trimethyl dimethyl ester **8**, $C_{21}H_{36}(CO_2Me)_2$. Thus, the partial structure **1a** was established for ikarugamycin. The configurations of the two double bonds were assigned on the following basis. The occurrence of the H_a proton



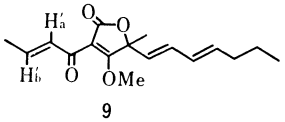
1a



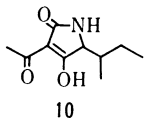
1b

at a lower field than the H_b proton is unusual for olefinic protons in a trans α,β-unsaturated carbonyl system;[6] an example of such a case is found in aspertetronin A (**9**) which gives a PMR spectrum exhibiting H_a' and H_b' protons at δ 7.32 and 7.06, respectively.[7]

Natural products having the acyltetramic acid chromophore are already known and examples include tenuazoic acid (**10**),[8] erythroskyrine,[9] *etc.*[10,11] As expected, the peculiar UV spectral behavior of hexahydroikarugamycin (**2**) closely parallels that of other acyltetramic acids (Table 1). Further chemical evidence for **1a** was provided by alkaline hydrolysis of **2**. The reaction led to cleavage of the tricarbonyl system and the amide bonds to give the carbocyclic part of **2**, a carboxylic acid isolated as dimethyl ester **11**, $C_{20}H_{34}(CO_2Me)_2$.



9



10

Drastic Oxidation of Ikarugamycin, Hexahydroikarugamycin, and Deoxooctahydroikarugamycin. From the molecular formula and the functional groups (three double bonds, a diketo amide, and an amide), ikarugamycin (**1**) is a pentacyclic compound. The formation of the bicyclic tetramethyl ester **5** by ozonolysis of **1** and of the tricyclic dimethyl ester **11** by hydrolysis of **2** indicates that i) **1** possesses three carbocyclic rings, one of which contains the cis double bond not shown in **1a** and ii) two

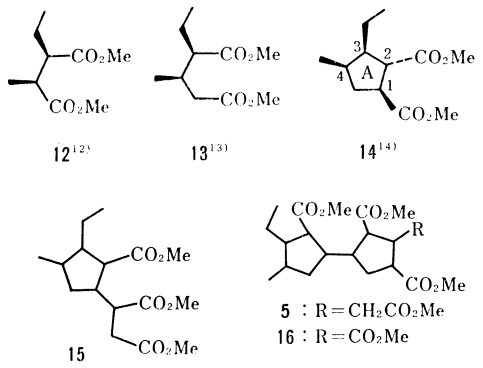


Fig. 2. Permanganate oxidation products of ikarugamycin (**1**).

side chains in the partial structure **1a** are connected with the carbocyclic part, thereby forming a large-membered lactam ring. Although there are 16 carbon atoms in **1** which are not included in **1a**, little information has so far been obtained beyond that revealed by the PMR spectrum of **1** (Fig. 1): the presence of a primary methyl (δ 0.93) and a secondary methyl (δ 0.88) groups. Since the carbon skeleton of **1** appeared to be unique, an attempt was made to obtain information on the structure of the carbocyclic part by oxidative degradations which were vigorous enough to cleave carbon–carbon single bonds. This approach was remarkably successful, as the following results show.

(a) Oxidation of Ikarugamycin (1): Prolonged oxidation of **1** with $KMnO_4$ in pyridine–water at 60 °C yielded, after esterification with diazomethane, a series of esters which was effectively isolated by preparative GLC. The products, including **5** as the major one, are listed in Fig 2 in the order of their GLC retention times. The PMR sepctrum of **14** shows a doublet (J= 7.5 Hz) at δ 0.85 due to a secondary methyl group, a triplet (J=6.7 Hz) at δ 0.89 due to a primary methyl group, a complex multiplet between δ 1.2—2.4 (6H), a triplet (J=8.0 Hz) at δ 2.79 and a multiplet (ddd, J= 8.5, 8.0, and 7.5 Hz) at δ 3.09 attributable to two methine protons on carbons bearing ester groups, and two 3H singlets at δ 3.63 and 3.64 due to two methoxyl groups. Irradiation at δ 3.09 caused the triplet at δ 2.79 to collapse to a doublet (J=8.0 Hz), indicating a vicinal arrangement of the two methoxycarbonyl groups. On the basis of these data, coupled with the concomitant formation of **13**, the diester was formulated to be **14** (not considering stereochemistry). The *r*-1, *t*-2, *c*-3, *c*-4-configuration has been unambiguously established by the synthesis of the four possible 3,4-*cis* diastereomers.[14] The trimethyl ester **15** produced a mass spectrum containing three characteristic peaks at *m/e* 169 due to the $M^+-MeO_2CCH_2\dot{C}HCO_2Me$ ion, at *m/e* 146 due to the $(MeO_2CCH_2CH_2CO_2Me)^+$ ion formed by MacLafferty rearrangement,[15] and at *m/e* 109 due to the $C_8H_{13}^+$ ion from the cyclopentane ring. This, together with the formation of **22** from **2** (see below), suggests the structure **16** for the ester.

Next, chromic acid oxidation in 3 M H_2SO_4–acetic acid at 80 °C was examined. Compared with the permanganate oxidation, the reaction led to more extensive cleavage of carbon bonds as indicated by the products (Fig. 3). The structure for **19** was suggested by the empirical formula $C_6H_8(CO_2Me)_4$ and by the formation of **17** and **18**. The relative configuration of **18** has been established by synthesis.[18]

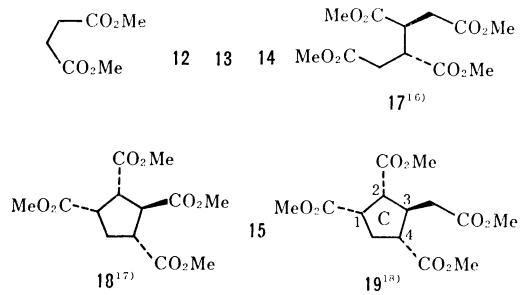


Fig. 3. Chromic acid oxidation products of ikarugamycin (**1**).

(b) Oxidation of Hexahydroikarugamycin (2) and Deoxooctahydroikarugamycin (3): As expected, chromic acid oxidation of **2** under conditions similar to those for **1** gave a completely different series of products (Fig. 4). The structures of **21** and **22** were deduced from their mass and PMR spectra (see Experimental) and were then confirmed by synthesis (of mixtures of diastreomers).[20] The mass spectrum of **23** shows two characteristic peaks at *m/e* 174 and 160 due to the $(MeO_2CCH_2CH_2CH_2CH_2CO_2Me)^+$ and $(MeO_2CCH_2CH_2CH_2CO_2Me)^+$ ions, respectively, thus indicating the structure. Its *threo* configuration was established by synthesis.[20] Structure of **24** was suggested by its mass spectrum and the formation of **20** and **23**, and was eventually confirmed by synthesis (of a mixture of diastereomers).[20]

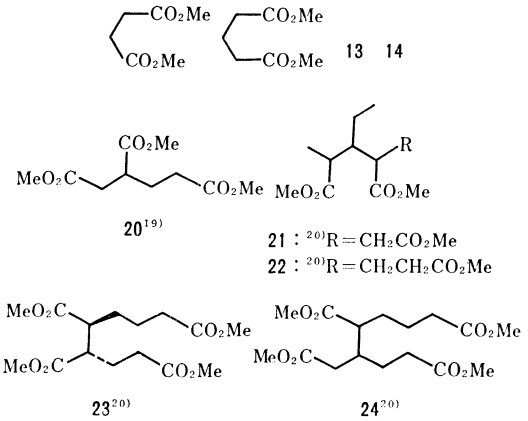


Fig. 4. Chromic acid oxidation products of hexahydroikarugamycin (**2**).

Finally, chromic acid oxidation of **3** was examined, since it should give information concerning the position of the methylene group in **3** which was derived from a carbonyl group in the β-tricarbonyl system of **2**. The oxidation afforded, in addition to all the products from **2**, the trimethyl ester **25** and the tetramethyl esters **26** and **27** (Fig. 5) which are the higher homologs of **20**, **23**, and **24**, respectively. Structure of **27** was suggested by its mass spectrum and the formation of **25** and **26**, and was then confirmed by synthesis.[20]

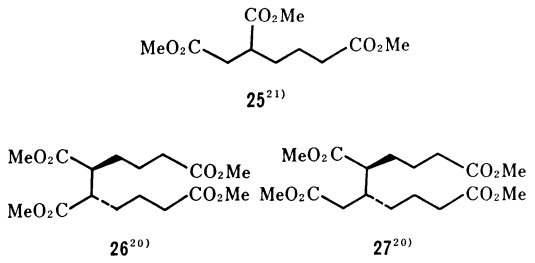
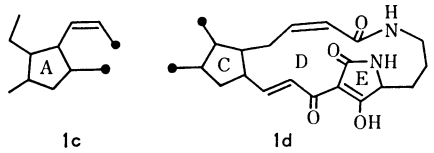


Fig. 5. Chromic acid oxidation products of deoxooctahydroikarugamycin (**3**) (in addition to the compounds shown in Fig. 4).

Structure of Ikarugamycin. The formation of **14** from **1** and of **22** from **2** and **3** but not from **1** can be accounted for by the partial structure **1c** for ikarugamycin. From the structural relationships among **17**, **24**, and **27**, coupled with the formation of **19** from **1**, partial structure **1a** can now be extended to **1d**. The

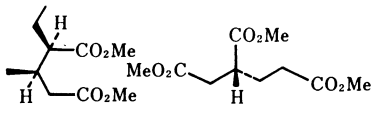


1c 1d

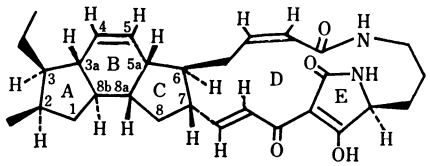
coupling patterns of the H_a–H_d protons in the PMR spectrum of **1** (Fig. 1) are fully consistent with this structure. There then remain two possibilities to explain how these two parts, **1c** and **1d**, could be connected to make up the remaining B ring. This problem was settled by the following experiment. Triester **15** was prepared by the permanganate oxidation of **1** in pyridine–deuterium oxide and was examined by mass spectrometry which showed that the succinyl residue in **15** contained only *ca.* 0.3 deuterium. This indicates that **15** was formed without decarboxylation and therefore, that the C-1 position in **14** should be connected to C-1 (not to C-2) in **19**. Consequently, ikarugamycin can now be represented by structure **1**.

Stereostructure and Biogenesis of Ikarugamycin. In ikarugamycin there are eight consecutive asymmetric centers at the C-2, C-3, C-3a, C-8b, C-5a, C-6, and C-7 positions on rings A, B, and C, and one at the junction of rings D and E. The configuration of the latter is evident from L-ornithine. The relative configurations on rings A and C are also apparent from those of **14** and **19**, respectively. That the latter were formed without epimerization was proved by the oxidation of **1** in deuterium-containing solvents. Thus, the permanganate oxidation in pyridine–deuterium oxide yielded **14** which according to the mass spectrum consisted of d_0 (65%), d_1 (30%), and d_2 (5%) derivatives. Also, the chromic acid oxidation in 3M D_2SO_4–D_2O gave **19** consisting of d_0 (85%) and d_1 (15%) derivatives.

It has already been established that (−)-*erythro*-2-ethyl-3-methylglutarate, $[\alpha]_D$ −14.9°, has the 2*R* : 3*S* (**13a**) configuration.[13b] The natural **13** obtained by the oxidation of **1** has $[\alpha]_D$ −8.6° and, therefore, must have the absolute configuration of **13a**. The absolute configuration of trimethyl (+)-1,2,4-butanetricarboxylate, $[\alpha]_D$ +16.2°, has also been reported to be *R* (**20a**).[19b] Therefore, the absolute configuration of the natural **20** ($[\alpha]_D$ +12.6°) obtained by the oxidation of **2** and hence that of C-7 in ring C was established as shown below. From the combination of these absolute configurations, ikarugamycin can now be assigned the stereostructure **1e**.

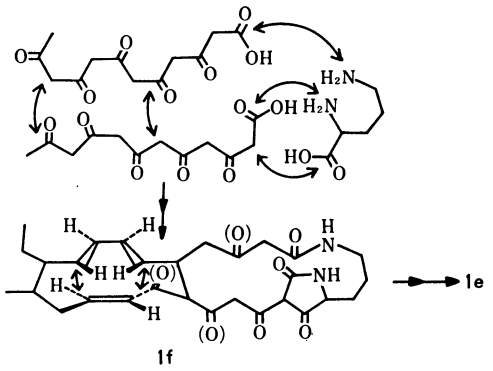


13a 20a



1e

Since, to the best of our knowledge, ikarugamycin is the first example of a natural product with an *as*-hydrindacene skeleton, its biogenesis appears to be of considerable interest. It has been proved that other natural acyltetramic acids, such as tenuazoic acid (**10**), can be biosynthesized from an amino acid (L-isoleucine in case of **10**) and a polyacetate (diacetate in case of **10**).[8b,9b,10b] It appears likely, therefore, that ikarugamycin can also be derived from L-ornithine and two hexaacetate chains as outlined in Scheme **1**. Although exactly how the unique carbon skeleton is formed remains a matter of conjecture, it is suggested that an intramolecular Diels-Alder reaction between the butadiene part and the double bond in a hypothetical intermediate such as **1f** could lead to the formation of the decahydro-*as*-indacene skeleton of ikarugamycin.



1f

Scheme 1. A biogenesis of ikarugamycin (**1**).

Experimental

The mp values are uncorrected. The IR spectra were recorded with JASCO model IR-S and DS-402G spectrophotometers and for the UV spectra a Perkin-Elmer model 202 spectrophotometer was used. The PMR spectra were recorded on JEOL models JNM-C60H and 4H-100 spectrometers; the chemical shifts are given in ppm relative to internal TMS. The mass spectra were obtained with a Hitachi model RMU-6C mass spectrometer. Optical rotations were determined with a JASCO model ORD/UV-5 spectrophotometer

or a JASCO model DIP-SL polarimeter (for **13** and **20**) and pK_a values were determined from titration curves taken with a Radiometer model TTT1 titrater. For TLC, Merch GF_{254} or PF_{254} silica gel was used and for column chromatography, Mallinckrodt silicic acid. Gas-liguid chromatography (GLC) was carried out with a Varian Aerograph model 1828-4 chromatograph. The column used for preparative GLC was composed of 5% SE-30 on celite 545 (3 m×0.95 cm).

*Properties of Ikarugamycin (**1**).* Repeated recrystallization of crude **1** from MeOH gave colorless needles, mp 228—229 °C (dec), $[\alpha]_D^{25}+390°$ (*c* 0.19, DMF), slightly soluble in DMF, DMSO, THF, EtOH, MeOH, and pyridine. The antibiotic showed pK_a 5.6 (67% EtOH); IR ($CHCl_3$): 3450, 1700, 1665, 1642, 1580, 1510 cm^{-1}; (KBr): 3380, 3220, 1700, 1678, 1645, 1583, 1519 cm^{-1}; PMR (pyr-d_5): δ 0.88 (3H, d, J=7 Hz), 0.93 (3H, t, J=7), 3.6—4.4 (2H, m), 4.1 (1H, narrow m), 5.72 and 5.95 (each 1H, br ABq, J=10), 6.0 (1H, m), 6.20 (1H, br d, J=12), 6.94 (1H, dd, J=15.6 and 9.5), 7.62 (1H, d, J=15.6), 8.7 and 9.6 (each 1H, br s, D_2O exchangeable); (DMSO-d_6): δ 5.6—6.2 (4H, m), 6.64 (1H, dd, J=15.0 and 9.5 Hz), 6.96 (1H, J=15.0), 7.79 and 8.62 (each 1H, br s, D_2O exchangeable). Found: C, 72.67; H, 8.20; N, 5.90%; *m/e* 478.2771. Calcd for $C_{29}H_{33}N_2O_4$: C, 72.77; H, 8.00 N, 5.85%; M, 478.2731.

*Copper Complex of **1**.* A soln of **1** (50 mg) and cupric acetate (12 mg) in EtOH (30 ml) was heated under reflux for 2 h. Crystals deposited after cooling, which were recrystallized from EtOH to give yellow-green needles (21 mg), mp>280 °C. Found: C, 63.50; H, 7.65; N, 5.13%. Calcd for $(C_{29}H_{37}N_2O_4)_2Cu\cdot 4H_2O$: C, 63.86; H, 7.58; N, 5.14%.

*Hexahydroikarugamycin (**2**).* A soln of **1** (600 mg) in EtOH (200 ml) was hydrogenated in the presence of PtO_2 (100 mg) for 1 h at room temp and atmospheric pressure. It was filtered and evaporated to a small volume under reduced pressure. The solid that precipitated was recrystallized from $CHCl_3$ to give 350 mg of **2** as needles, mp 243—244 °C (dec), $[\alpha]_D^{25}+110°$ (*c* 0.44, $CHCl_3$), pK_a 5.1 (67% EtOH), IR ($CHCl_3$): 3450, 1710, 1662, 1610, 1520 cm^{-1}; PMR ($CDCl_3$): δ 5.86 and 6.0 (each 1H, br s, exchangeable with D_2O), no other signals above δ 4.0. Found: C, 71.51; H, 9.27; N, 5.82%; *m/e* 484.3303. Calcd for $C_{29}H_{44}N_2O_4$: C, 71.86; H, 9.15; N, 5.78; M, 484.3301.

*Copper Complex of **2**.* Green crystals, mp>280 °C (from EtOH). Found: C, 65.01; H, 8.74; N, 4.99. Calcd for $(C_{29}H_{43}N_2O_4)_2Cu\cdot 2H_2O$: C, 65.21; H, 8.50; N, 5.25%.

*N-Methylpyrazole Derivative of **2**.* A soln of **2** (48 mg) and *N*-methylhydrazine sulfate (20 mg) in 90% EtOH (5 ml) was heated under reflux for 30 h. The product was subjected to preparative TLC ($CHCl_3$–MeOH, 4 : 1), giving 19 mg of an oil, mass: *m/e* 494 (M^+); IR ($CHCl_3$): 3360, 1680, 1654, 1540 cm^{-1}; UV (MeOH): λ_{max} 226 nm (ε 4900); PMR ($CDCl_3$): δ 3.85 (3H, s), 4.1 (1H, m), 6.8 (1H, br s,) 8.2 (1H, br s).

*Deoxooctahydroikarugamycin (**3**).* A soln of **2** (500 mg) in EtOH (170 ml) was hydrogenated in the presence of PtO_2 (150 mg) for 24 h. It was filtered and evaporated to give an oily residue which was crystallized from MeOH, affording 350 mg of **3** as prisms, mp 155—157.5 °C, pK_a 7.8 (67% EtOH), mass: *m/e* 470 (M^+); IR ($CHCl_3$): 3470, 3250, 1775, 1705, 1665, 1510 cm^{-1}; PMR (DMSO-d_6): δ 7.09, 7.85, and 10.32 (each 1H, br s, D_2O exchangeable). Found: C, 74.12; H, 9.99; N, 6.04%. Calcd for $C_{29}H_{46}N_2O_3$: C, 74.00; H, 9.85: N, 5.95%.

*Enol Ether of **3**.* Treatment of **3** (140 mg) with diazomethane yielded, after preparative TLC, 97 mg of an oil mass: *m/e* 484 (M^+); IR ($CHCl_3$): 3450, 3300, 1677, 1652, 1515 cm^{-1}; UV (MeOH): λ_{max} 224 (ε 5300) and 242^{sh} nm (4600): PMR ($CDCl_3$): δ 3.98 (3H, s).

*Enol Acetate of **3**.* Acetylation of **3** with acetic anhydride–pyridine gave an oily enol acetate, mass: *m/e* 512 (M^+), 470, 452; IR ($CHCl_3$): 3450, 3340, 1781, 1690, 1664, 1515, 1185 cm^{-1}; UV (MeOH): λ_{max} 222 nm (ε 7000); PMR ($CDCl_3$): δ 2.29 (3H, s).

*Deoxodecahydroikarugamycin (**4**).* *(a) From **3**:* A mixture of **3** (100 mg) and $LiBH_4$ (100 mg) in THF (20 ml) was stirred for 24 h at room temp. Water and then 2M HCl were added to the ice-cooled mixture until it became a clear, neutral soln which was then extracted with $CHCl_3$. The extract was washed with water, dried, and evaporated. The residue was crystallized from MeOH to give 68 mg of **4** as needles, mp 220—222 °C, mass: *m/e* 472 (M^+), 454; IR ($CHCl_3$): 3430, 3500—3200, 1695, 1655, 1520 cm^{-1}; PMR (DMSO-d_6): δ 4.4 (1H, m), 5.0, 7.65, 7.88 (each 1H, br, exchangeable with D_2O). Found: C, 71.38; H, 10.18; N, 5.62%. Calcd for $C_{29}H_{48}N_2O_3\cdot H_2O$: C, 70.98: H, 10.27; N, 5.71%.

*(b) From **2**:* A suspension of **2** (150 mg) and $LiBH_4$ (150 mg) in dimethoxyethane (45 ml) was heated under reflux for 6 h with stirring. The work-up described in *(a)* afforded 68 mg of **4**.

*Acetate of **4**.* Acetylation of **4** gave an oily acetate, mass: *m/e* 514 (M^+), 454; IR ($CHCl_3$): 3460, 3400—3200, 1741, 1699, 1662, 1521, 1240 cm^{-1}; PMR ($CDCl_3$): δ 2.18 (3H, s), 5.53 (1H, m).

*Ozonolysis of **1**: Formation of **5**, Oxalic Acid, and* L-*Ornithine.* Through a soln of **1** (100 mg) in MeOH (70 ml) at—70 °C ozonized oxygen was passed until the soln turned pale purple. After the excess of ozone had been expelled by a nitrogen stream, the solvent was removed at room temp and to the residue 98% formic acid (10 ml) and 35% hydrogen peroxide (1 ml) were added. The mixture was stirred for 1 h at 0 °C and then 14 h at room temp. After decomposition of the excess oxidant with $NaHSO_3$ (1 g), the mixture was evaporated to dryness and the residue was dissolved in 0.5 M H_2SO_4 (10 ml) and extracted with ether; the aqueous layer was saved for further experiment (see below). The ether extract was washed with water, dried, and evaporated to leave an oil which was then treated with diazomethane. The crude ester thus obtained was purified by preparative TLC (hexane–ether, 1 : 1), giving 40 mg of the tetramethyl ester **5**, mass: *m/e* 426 (M^+), 366, 334 (base peak), 306, 246; IR (CCl_4): 1740 cm^{-1}; PMR (CCl_4): δ 0.88 (3H, t, J=6.2 Hz), 0.88 (3H, d, J=6.5), 3.57 (3H, s), 3.59 (6H, s), 3.64 (3H, s), Found: *m/e* 426.2254. Calcd for $C_{22}H_{34}O_8$: M, 426.2253.

The aqueous layer (see above) was adjusted to 1 M with respect to H_2SO_4 by the addition of concd H_2SO_4 and heated under reflux for 4 h. Continuous extraction with ether afforded a crude acid which was recrystallized from ether-CCl_4, giving 16 mg of oxalic acid dihydrate (identified by IR) as prisms. The dimethyl ester derivative, mp 51—54 °C, also had an IR spectrum identical with that of dimethyl oxalate. The aqueous layer remaining after the continuous extraction was neutralized with 0.2 M $Ba(OH)_2$ and the $BaSO_4$ precipitated was removed by filtration. The filtrate was concentrated to a small volume under reduced pressure and applied to a column (1×6 cm) of Dowex 50W-X8 (H^+ form). Elution with 1 M NH_4OH (60 ml) afforded an amino acid which was crystallized from dil HCl (pH *ca.* 4)-EtOH to give 15 mg of L-ornithine monohydrochloride (identified by IR) as prisms, mp 224—229 °C (dec), $[\alpha]_D^{25}+40°$ (*c* 0.42, 5 M HCl), lit,[22] $[\alpha]_D^{25}+37.5°$ (*c* 2, 5 M HCl). Found: C, 35.29; H, 7.88; N, 16.60%. Calcd for $C_5H_{12}N_2O_2\cdot HCl$: C, 35.61; H, 7.77; N, 16.61%.

Keto Acid ***6a***. A suspension of **3** (94 mg) and CrO_3 (80 mg) in 3 M H_2SO_4 (10 ml) was heated at 80 °C for 2 h with stirring. Cooling of the resulting solution caused precipitation of crystals which were filtered, washed well with water, and recrystallized from MeOH–water, yielding 68 mg of microcrystals, mp 222—223 °C, IR (KBr): 3300, 2800—2300, 1745, 1725, 1665, 1617, 1545 cm^{-1}. Found: C, 67.56: H, 9.59; N, 5.32%. Calcd for $C_{29}H_{46}N_2O_5 \cdot MeOH$: C, 67.38; H, 9.43; N, 5.24%.

Keto Ester ***6b***. To a soln of the enol ether of **3** (145 mg) in a mixture of acetone (20 ml) and water (30 ml) were added KH_2PO_4 (75 mg), $MgSO_4$ (150 mg), and $KMnO_4$ (75 mg). The mixture was stirred for 24 h at room temp and then made clear and neutral by the addition of $NaHSO_3$ powder and 2 M HCl. The soln was extracted with $CHCl_3$ and the extract washed with water, dried, and evaporated to dryness. Crystallization of the residue from MeOH–water afforded 100 mg of **6b**, mp 173—174 °C; mass: *m/e* 516 (M^+), 488; IR (KBr): 3430, 3310, 1760, 1739, 1660, 1639, 1550 cm^{-1}; PMR (pyr-d_5): δ 3.59 (3H, s), 3.63 (3H, s), 4.90 (1H, m), 8.5 and 9.7 (each 1H, br, exchangeable with D_2O). Found: C, 67.51; H, 9.25; N, 5.15%. Calcd for $C_{30}H_{48}N_2O_5 \cdot MeOH$: C, 67.85; H, 9.55; N, 5.11%.

The keto ester was also obtained by esterification (MeOH–HCl) of keto acid **6a**.

Amino Acid ***7***. To a soln of **6a** (68 mg) in MeOH (10 ml) were added 1 M NaOH (10 ml) and 35% hydrogen peroxide (0.9 ml). After stirring for 64 h at room temp, the mixture was acidified with 6 M HCl, which caused precipitation of an amorphous powder. It weighed 64 mg, was homogeneous (R_f 0.7) on TLC in 1-butanol–acetic acid–water, 4 : 1 : 2, and became pink with ninhydrin.

N-*Acetyl Dimethyl Ester of* ***7***. To a stirred soln of **7** (18 mg) in 1 M NaOH (1 ml) was added acetic anhydride (0.1 ml) at room temp. After a time, an additional 1 M NaOH (1 ml) was added and the mixture was extracted with $CHCl_3$. The aqueous layer was acidified with 2 M HCl, extracted with $CHCl_3$, and the extract washed with a sat NaCl soln, dried, and evaporated. The residue was then treated with diazomethane and the product was purified by preparative TLC ($CHCl_3$–MeOH, 9 : 1) to give 5 mg of an oil, mass: *m/e* 562 (M^+) ; IR ($CHCl_3$): 3460, 3300, 1739, 1673, 1513 cm^{-1}; PMR ($CDCl_3$): δ 2.04 (3H, s), 3.67 (3H, s), 3.75 (3H, s), 4.5 (1H, m), 6.0 (1H, br s), 6.50 (1H, br d, J=8 Hz).

Hydrolysis of the DNP Derivative of ***7***: *Formation of* ***8*** *and 2,4-Dintrophenylornithine*. To a suspension of **7** (40 mg) in EtOH (2 ml) and water (2 ml) were added $NaHCO_3$ (0.2 g) and 2,4-dinitrofluorobenzene (0.1 ml). After stirring at 40 °C for 6 h, the EtOH was removed under reduced pressure and the aqueous residue was extracted with ether. The ether layer was washed with water, and the aqueous layers were combined, acidified with 6 M HCl, and extracted with EtOAc. The extract was washed with water and a sat NaCl aq soln. and dried. Evaporation of the solvent left an oil which was subjected to preparative TLC ($CHCl_3$–MeOH–acetic acid, 90 : 9 : 1), giving 30 mg of the DNP derivative as an amorphous powder. This was dissolved in concd HCl (3 ml) and acetic acid (3 ml) and heated to 110 °C. After 24 h, additional concd HCl (2 ml) was added and heating was continued for another 12 h. The hydrolysate was concentrated to dryness under reduced pressure and the residue, taken up in 50% MeOH, was placed on a column (1.2×2 cm) of Dowex 50W-X8 (H^+ form). Elution with 50% MeOH yielded a crude carboxylic acid which was esterified with diazomethane. Preparative TLC (hexane–ether, 2 : 1) of the crude ester yielded 3 mg of oily **8**, mass *m/e* 406 (M^+), 388 (base peak), 374, 356; IR (CCl_4): 1743 cm^{-1}; PMR (CCl_4): δ 3.61 (6H, s). Found: *m/e* 406.3074. Calcd for $C_{25}H_{42}O_4$: M, 406.3083.

The Dowex column was then eluted with 0.5 M NH_4OH (40 ml) and 1 M NH_4OH (20 ml) to afford an oil which was crystallized from dil HCl. Orange crystals (3 mg) of α-DNP-ornithine were obtained (identified by IR and paper chromatography in comparison with an authenic sample[5]). The R_f values of the α- and δ-DNP-ornithines on paper chromatography in 1-butanol saturated with water were 0.48 and 0.52, respectively.

Alkaline Hydrolysis of ***2***: *Formation of* ***11***. A soln of **2** (25 mg) in ethylene glycol (1 ml) was prepared by heating at 150 °C. To this soln, 40% NaOH (0.3 ml) was added and the mixture heated at 100 °C for 24 h. The ice-cooled mixture was acidified with dil HCl, extracted with EtOAc, and the extract was treated with diazomethanne. After decomposition of the excess diazomethane with acetic acid, the EtOAc soln was washed with water, an aq $NaHCO_3$ soln, and again with water, and then dried. Evaporation of the solvent left an oil which was purified by preparative TLC (hexane–ether, 4 : 1), giving 6 mg of dimethyl ester **11** as an oil, mass: *m/e* 392 (M^+), 374, 361, 360, 343, 319 (base peak); IR (CCl_4): 1743 cm^{-1}; PMR (CCl_4): δ 0.89 (3H, t, J=7 Hz), 0.93 (3H, d, J=7), 3.62 (6H, s). Found: *m/e* 392.2951. Calcd for $C_{24}H_{40}O_4$: M, 392.2926.

Oxidation of ***1*** *with $KMnO_4$ in Pyridine–Water*. A mixture of **1** (478 mg) and $KMnO_4$ (3.5 g) in pyridine (40 ml) and water (40 ml) was stirred at room temp overnight and then at 60 °C for 5 h. The solvents were evaporated under reduced pressure, water was added, and the evaporation repeated. To the residue were added $NaHSO_3$ powder and concd HCl until the mixture became a clear, acidic soln. The latter was continuously extracted with ether for 8 h and the extract was dried and evaporated giving a mixture of carboxylic acids which was then esterified with diazomethane. The esters (260 mg) thus obtained were partially separated by column chromatography on silica gel (5 g); elution with hexane–ether, 4 : 1 and then 1 : 1 gave 35 and 64 mg of oil, respectively. Preparative GLC of the former (column temp: 130—270 °C, 20 °C/min) afforded **12** (1.5 mg), **13** (1.0 mg), **14** (4.2 mg), and **15** (0.3 mg), and that of the latter (column temp: 270 °C) **16** (1.1 mg) and **5** (17 mg).

Dimethyl erythro-*2-Ethyl-3-methylsuccinate* (***12***): $[\alpha]_D^{25}$ +2.2° (*c* 0.44, $CHCl_3$); mass: *m/e* 157 (M^+−OMe), 102, 101 (base peak), 88, 87; PMR (CCl_4): δ 0.89 (3H, t, J=7.2 Hz), 1.10 (3H, d, J=7.0), 1.2—1.8 (2H, m), 2.4—2.7 (2H, m), and 3.66 (6H, s). This compound was identified by means of IR, PMR, mass spectrometry, and GLC with an authentic sample of the *erythro* isomer.[12]

Dimethyl erythro-*2-Ethyl-3-methylglutarate* (***13***): $[\alpha_D^{25}]$ −8.6° (*c* 0.64, $CHCl_3$), lit[13b] $[\alpha]_D^{25}$ −14.9° (*c* 1.19, $CHCl_3$); mass: *m/e* 171 (M^+−OMe), 142, 129, 102, 101 (base peak), 87, 74; PMR (CCl_4): δ 0.89 (3H, t, J=6.8 Hz), 0.93 (3H, d, J=6.3), 1.1—2.7 (6H, m), 3.61 (3H, s), and 3.62 (3H, s). This ester was identical with an authenic sample of the *erythro* isomer (IR, PMR, mass, and GLC).[13]

Dimethyl c-*3-Ethyl*-c-*4-methyl*-r-*1*,t-*2-cyclopentanedicarboxylate* (***14***):[14] $[\alpha]_D^{25}$ +23° (*c* 0.60, $CHCl_3$); mass: *m/e* 228 (M^+), 199, 197, 168, 109 (base peak).

Trimethyl Ester ***15***: Mass: *m/e* 314 (M^+), 169*, 146 (base peak), 109. Found: *m/e* 314.1724. Calcd for $C_{16}H_{26}O_6$: M, 314.1729.

Tetramethyl Ester ***16***: PMR (CCl_4): δ 3.58, 3.59, 3.65,

* The compositions of these peaks were established by high-resolution mass spectrometry.

and 3.67 (each 3H, s). Found: *m/e* 412.2075. Calcd for $C_{21}H_{28}O_8$: M, 412.2097.

Oxidation of ***1*** *with CrO_3 in H_2SO_4–Acetic Acid.* A suspension of **1** (500 mg) and CrO_3 (2.5 g) in 3 M H_2SO_4 (25 ml) and acetic acid (25 ml) was heated at 80 °C with stirring. After 16 h, the oxidation mixture was concentrated under reduced pressure, water was added, and the concentration repeated. The residue was taken up in concd NH_4OH (8 ml) and the still strongly acidic soln was continuously extracted with ether for 30 h. The crude acids (108 mg) thus obtained were first esterified with diazomethane and the resultant esters were heated under reflux in MeOH (10 ml) containing concd H_2SO_4 (1 drop) for 1 h to convert some ethyl esters formed during the ether extraction into methyl esters. The MeOH was evaporated, the residue diluted with water, and extracted with ether. The extract was washed with an aq $NaHCO_3$ soln, water, and a sat NaCl soln, and then dried. Evaporation of the solvent left a mixture of methyl esters (110 mg) which was passed through a silica gel (3 g) column. Elution with ether gave 85 mg of an oil which was subjected to preparative GLC (column temp: 200—280 °C, 4 °C/min). The products were (in the order of increasing retention time): dimethyl succinate (4.7 mg), **12** (0.5 mg), **13** (3.8 mg), **14** (1.0 mg), **17** (7.8 mg), **18** (0.2 mg), **15** (0.5 mg), and **19** (1.6 mg).

Tetramethyl meso-*1,2,3,4-Butanetetracarboxylate (**17**)*: Mp 74—74.5 °C (from ether hexane), lit,[16] mp 75 °C; mass: *m/e* 259 (M^+–OMe), 139 (base peak); PMR (CCl_4): δ 2.1—3.4 (6H, m), 3.66 (6H, s), and 3.69 (6H, s). Found: C, 49.65; H, 6.25%. Calcd for $C_{12}H_{18}O_8$: C, 49.48; H, 6.43%. This compound was identical with an authentic sample of the *meso* isomer (IR, PMR, mass, and GCL).[16]

Tetramethyl r-*1*, c-*2*, t-*3*, c-*4-Cyclopentanetetracarboxylate (**18**)*: Found: *m/e* 302.0976. Calcd for $C_{13}H_{18}O_8$: M, 302.1001. This ester was identified with an authenic sample having the configuration depicted in **18** (IR, mass, and GLC).[17]

Trimethyl t-*3-(Methoxycarbonylmethyl)*-r-*1*,c-*2*,c-*4-cyclopentanetricarboxylate (**19**)*:[18] Mass: *m/e* 316 (M^+), 285, 224, 165 (base peak); PMR (CCl_4): δ 2.0—3.4 (8H, m), 3.58 (3H, s), 3.60 (6H, s), and 3.63 (3H. s). Found: *m/e* 316.1160. Calcd for $C_{14}H_{20}O_8$: M, 316.1158.

Oxidation of ***2*** *with CrO_3 in H_2SO_4.* A mixture of **2** (330 mg) and CrO_3 (1.2 g) in 3 M H_2SO_4 (35 ml) was stirred at 80 °C for 8 h. The work-up described for the CrO_3 oxidation of **1** gave 93 mg of methyl esters which were separated by preparative GLC (column temp: 120—280 °C, 10 °C/min). The products were: dimethyl succinate (13.8 mg), dimethyl glutarate (3.2 mg), **13** (1.4 mg), **14** (0.3 mg), **20** (2.2 mg), **21** (0.4 mg), **22** (0.9 mg), **23** (2.9 mg), and **24** (4.2 mg).

*Trimethyl 1,2,4-Butanetricarboxylate (**20**)*: $[\alpha]_D^{25}+12.6°$ (*c* 0.68, acetone), lit[19b] $[\alpha]_D^{25}+16.2°$ (*c* 13.7, acetone); mass: *m/e* 201 (M^+–OMe); PMR (CCl_4): δ 1.6—2.4 (7H, m), 3.61, 3.62, and 3.64 (each 3H, s). This compound was identified with an authetic sample.[19]

*Trimethyl 3-Ethyl-1,2,4-pentanetricarboxylate (**21**)*: Mass: *m/e* 243 (M^+–OMe)*, 214 (M^+–HCO_2Me), 187 (fragmentation between C-3 and 4)*, 155 (base peak), 146 [$(MeO_2CCH_2CH_2CO_2Me)^+$]*; PMR (CCl_4): δ 0.88 (3H, t, J=7.0 Hz), 1.20 (3H, d, J=7.0), 3.69, 3.71, and 3.73 (each 3H, s). This ester exhibited a mass spectrum and GLC behavior identical to a synthetic sample (of a mixture of diastereomers).[20]

*Trimethyl 4-Ethyl-1,3,5-hexanetricarboxylate (**22**)*: Mass: *m/e* 257 (M^+–OMe)*, 228 (M^+–HCO_2Me)*, 201 (fragmentation between C-4 and 5)*, 169 (base peak), 160 [$(MeO_2CCH_2CH_2CH_2CO_2Me)^+$]*, 129; PMR ($CCl_4$): δ 0.89 (3H, t, J=7.6 Hz), 1.18 (3H, d, J=7.2), 3.68 (6H, s), 3.70 (3H, s). This ester also exhibited a mass spectrum and GLC behavior identical to a synthetic sample (of a mixture of diastereomers).[20]

Tetramethyl threo-*1,3,4,7-Heptanetetracarboxylate (**23**)*: Mass: *m/e* 301 (M^+–OMe)*, 174*, 160*, 59 (base peak); PMR (CCl_4): δ 1.2—2.9 (12H, m), 3.63 (6H, s), and 3.67 (6H, s). This compound was identical with a synthetic sample of the *threo* isomer (IR, PMR, mass, and GLC).[20]

*Trimethyl 3-(Methoxycarbonylmethyl)-1,4,7-heptanetricarboxylate (**24**)*: Mass: *m/e* 315 (M^+–OMe)*, 174 [$(MeO_2CCH_2CH_2CH_2CH_2CO_2Me)^+$]*, 59 (base peak); PMR (CCl_4): δ 1.1—2.7 (14H, m), 3.62 (9H, s), and 3.66 (3H, s). This ester exhibited a mass spectrum and GLC behavior identical to a synthetic sample (of a mixture of the *threo* and *erythro* isomers).[20]

Oxidation of ***3*** *with CrO_3 in H_2SO_4–Acetic Acid.* A mixture of **3** (100 mg) and CrO_3 (600 mg) in 3M H_2SO_4 (5 ml) and acetic acid (5 ml) was stirred for 9 h at 80 °C. Work-up of the oxidation mixture gave 50 mg of a mixture of methyl esters which was separated by preparative GLC (column temp: 140—290 °C, 10 °C/min). The products were: dimethyl succinate (10 mg), dimethyl glutarate (3.2 mg), **13** (0.2 mg), **20** (1.0 mg), **25** (0.3 mg), **21** (0.1 mg), **22** (0.2 mg), **23** (0.9 mg), **24** plus **26** (2.3 mg), and **27** (0.9 mg).

*Trimethyl 1,2,5-Pentanetricarboxylate (**25**)*: Mass: *m/e* 215 (M^+–OMe), 146; PMR (CCl_4): δ 1.4—1.8 (4H, m), 2.1—2.9 (5H, m), 3.65, 3.66, and 3.69 (each 3H, s), This ester was identical with an authentic sample (IR, PMR, mass, and GLC).[21]

Tetramethyl threo-*1,4,5,8-Octanetetracarboxylate (**26**)*: The PMR spectrum(CCl_4) of an inseparable mixture of **24** and **26** in the presence of Eu(DPM)[23] gave rise to six methoxyl signals, two of which came from **26** and the others from **24**; the intensities of the former increased upon the addition of a synthetic sample of **26**.[20]

Trimethyl threo-*5-(Methoxycarbonylmethyl)-1,4,8-octanetricarboxylate (**27**)*: Mass: *m/e* 329 (M^+–OMe)*, 174 [$(MeO_2CCH_2CH_2CH_2CH_2CO_2Me)^+$]*; PMR ($CCl_4$): δ 1.1—1.9 (8H, m), 1.9—2.6 (8H, m), 3.64 (9H, s), and 3.67 (3H, s). This compound was identical with a synthetic sample of the *threo* isomer (IR, PMR, mass, and GLC).[20]

$KMnO_4$ Oxidation of ***1*** *in Pyridine–D_2O.* A mixture of **1** (100 mg) and $KMnO_4$ (700 mg) in pyridine (10 ml) and D_2O (10 ml) was heated at 60 °C with stirring. After 5 h, concd HCl and $NaHSO_3$ powder were added to the ice-cooled mixture until it became an acidic, clear soln. This was extracted with ether and the extract was washed with water and a sat NaCl aq soln, and dried. Evaporation of the ether left an oil which was treated with diazomethane. The esters were separated by preparative GLC (column temp: 140—290 °C, 10 °C/min). Esters **14** (0.7 mg) and **15** (0.2 mg) were obtained.

CrO_3 Oxidation of ***1*** *in 3 M D_2SO_4.* A mixture of **1** (100 mg) and CrO_3 (500 mg) in 3 M D_2SO_4–D_2O (10 ml) was heated at 80 °C for 12 h with stirring. The reaction mixture was then continuously extracted with ether for 10 h and a subsequent work-up gave esters which were separated by preparative GLC giving 1.1 mg of **19**.

The authers are grateful to the Fujisawa Phamaceutical Co., Ltd., for the generous gift of ikarugamycin and for the high-resolution mass spectrometry. They are also indebted to Dr. Tadao Kondo (Nagoya University) for the 100 MHz PMR measurements.

References

1) A preliminary account of this work appeared in: S.

Ito and Y. Hirata, *Tetrahedron Lett.*, **1972**, 1181, 1185, 2557.

2) K. Jomon, Y. Kuroda, M. Ajisaka, and H. Sasaki, *J. Antibiot.*, **25**, 271 (1972).

3) The olefinic protons of *N*-methyl-*cis*-cinnamanilide have $J=12.5$ Hz, while those of the *trans* isomer, 15.5 Hz: R. M. Coates and E. F. Johnson, *J. Am. Chem. Soc.*, **93**, 4016 (1961).

4) For an example of a similar reaction, see H. Smith, *J. Chem. Soc.*, **1953**, 803.

5) F. Sanger, *Biochem. J.*, **40**, 261 (1949).

6) C. Pascual, J. Meiler, and W. Simon. *Helv. Chem. Acta*, **49**, 164 (1966).

7) J. A. Ballantine, V. Ferrito, C. H. Hassal, and V. I. P. Jones, *J. Chem. Soc.*, **1969**, 56. See also Ref. 11 for an example of the occurrence of an α-proton at an unusually low field (δ 7.13) in a dienoyltetramic acid system.

8) a) C. E. Stikings, *Biochem. J.*, **72**, 332 (1959); b) C. E. Stickings and R. J. Townsend, *ibid.*, **78**, 412 (1961).

9) a) J. Shoji, S. Shibata, U. Sankawa, H. Taguchi, and Y. Shibanuma, *Chem. Pharm. Bull.*, **13**, 1240 (1965); b) S. Shibata, "Chemistry of Microbial Products, Preprint of Symposium held on April 1964 at Tokyo," University of Tokyo, Tokyo (1964), pp. 225—227.

10) a) C. W. Holzapfel, *Tetrahedron*, **24**, 2101 (1968); b) C. W. Holzapfel and D. C. Wilkins, *Phytochemistry*, **10**, 351 (1971).

11) F. A. Mackellar, M. F. Grostic, E. C. Olson, R. J. Wnuk, A. R. Branfman, and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **93**. 4943 (1971).

12) J. H. Golden and R. P. Linstead, *J. Chem. Soc.*, **1958**, 1732.

13) a) H. R. Snyder and R. E. Putnan, *J. Am. Chem. Soc.*, **76**, 33 (1954); b) S. Ito and Y. Hirata, *Bull. Chem. Soc. Jpn.*, **46**, 672 (1973).

14) S. Ito and Y. Hirata, *Bull. Chem. Soc. Jpn.*, **50**, 227 (1977).

15) H. Budzikiewicz, C. Djerassi, D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Cal. (1967), p 155.

16) K. Alder and M. Schumacher, *Justus Liebigs Ann. Chem.*, **564**, 96 (1949).

17) K. Alder, H.-H. Mölls, and R. Reeber, *Justus Liebigs Ann. Chem.*, **611**, 7 (1958).

18) S. Ito and Y. Hirata, *Bull. Chem. Soc. Jpn.*, **46**, 603 (1973).

19) a) F. W. Kay and W. H. Perkin, Jr., *J. Chem. Soc.*, **1906**, 1640; b) K. Freudenberg and J. Geiger, *Justus Liebigs Ann. Chem.*, **575**, 145 (1952).

20) These new compounds were synthesized in unambiguous manners. The syntheses will be reported elsewhere.

21) M. E. Dobson, J. Ferns, and W. H. Perkin, Jr., *J. Chem. Soc.*, **1909**, 2012.

22) S.-C. J. Fu, K. R. Rao. S, M. Birnbaum, and J. P. Greenstein, *J. Biol. Chem.*, **199**, 207 (1952).

23) J. K. M. Sanders and D. H. Williams, *J. Am. Chem. Soc.*, **93**, 641 (1971).