Asymmetric Synthesis of a 3-Acyltetronic Acid Derivative, RK-682, and Formation of Its Calcium Salt during Silica Gel Column Chromatography

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RK-682 was reported to be a potent protein tyrosine phosphatase inhibitor. We found that (*R*)-3-hexadecanoyl-5-hydroxymethyltetronic acid (1) was easily converted to its calcium salt during column chromatography on Silica gel 60, and this calcium salt was identical to RK-682 originally isolated from a natural source. Here we report details of the asymmetric synthesis of (*R*)-1 and its conversion to the calcium salt. Fast atom bombardment mass spectrometric (FAB-MS) analysis of the free and calcium salt forms of RK-682 is also reported.

Key words RK-682; tetronic acid derivative; silica gel; calcium salt; FAB-MS

RK-682 was isolated by a RIKEN group from *Streptomyces* sp. 88-682 and found to have interesting biological activities.[1)] These activities may be due to blocking of protein phosphorylation, since RK-682 inhibits protein tyrosine phosphatases (CD45, VHR). The chemical structure of RK-682 was assigned as 3-hexadecanoyl-5-hydroxymethyltetronic acid (**1**) at that stage.[1a,b)] The same structure had already been assigned by a Takeda group to a compound isolated from cultures of *Streptomyces* sp. AL-462 that inhibits the activity of phospholipase A_2.[2)] The Takeda group also reported a synthesis of (+)-**1** from D-ribose, and the structure of their compound, including the absolute stereochemistry, was confirmed to be (*R*)-**1**. However, the reported spectral data, including optical rotation and melting point, for RK-682 were not identical with those of Takeda's compound. To clarify this structural problem of RK-682, we have developed a versatile synthetic route to optically active **1**, and in our preliminary communication, we briefly reported the asymmetric synthesis of (*R*)- and (*S*)-**1**.[3,4)]

Since 3-acyltetronic acid is known to exist in two sets of tautomeric forms (**i/ii** and **iii/iv** in Fig. 2),[5)] we first suspected that such tautomeric properties may be the origin of the difference of the spectral data between RK-682 and (*R*)-**1**. However, this was not the case, and we found that RK-682 was distinct from (*R*)-**1**. Acid treatment of RK-682 gave (*R*)-**1**, and, interestingly, Silica gel column chromatography of (*R*)-**1** afforded an unknown less-soluble compound whose spectral data were identical to those reported for RK-682. In this paper, we report structural analysis of this less-soluble compound, as well as details of the asymmetric synthesis of (*R*)-**1**.

Results and Discussion

Asymmetric Synthesis of (*R*)-1 For efficient synthesis of optically pure (*R*)-**1**, the commercially available optically active glyceric acid derivative (*R*)-**2** was chosen as a starting material,[6)] and synthesis was achieved as shown in Fig. 3. Specifically, (*R*)-**2** was first converted to the hydroxyester (*R*)-**3** *via* deprotection of the acetonide group and selective protection of the primary alcohol with a trityl group. The β-ketoester group was introduced into (*R*)-**3** by silver salt-promoted condensation with the thioester **6** to give (*R*)-**7** in 74%

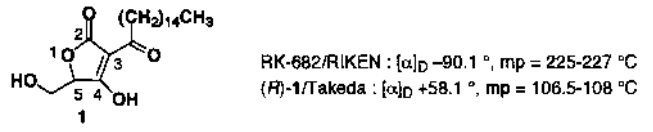


Fig. 1

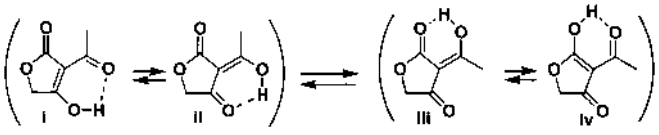
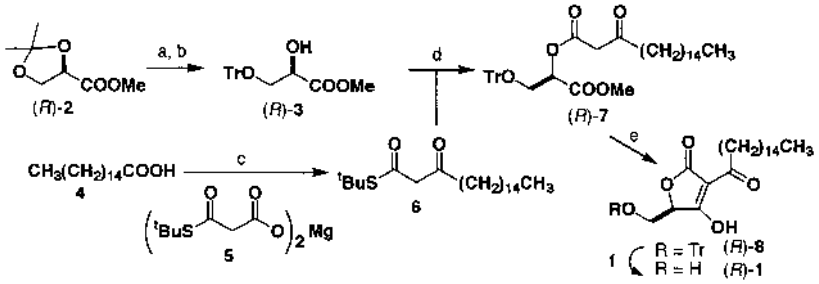


Fig. 2



(a) 1 N aq. HCl, MeOH, 23 °C; (b) Ph_3CCl, NEt_3, cat. DMAP, 23 °C, 69% (2 steps); (c) CDI, THF, 23 °C, then **5**, 93%; (d) CF_3COOAg, **6**, THF, 23 °C, 74%; (e) Bu_4NF, THF, 23 °C, 95%; (f) 1 N aq. HCl, MeOH, 75%.

Fig. 3

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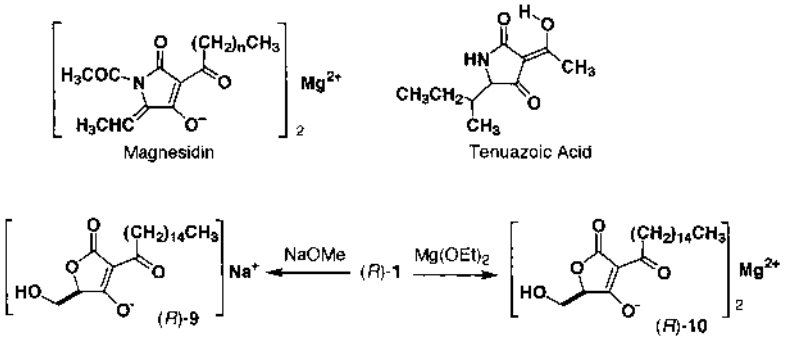

Fig. 4

yield. The thioester **6** was synthesized from hexadecanoic acid (**4**) by using the magnesium salt **5**.[7] Construction of the key 3-acyltetronic acid skeleton without racemization was achieved under the conditions reported by Ley and co-workers.[8] Cyclization of the β-ketoester (*R*)-**7** proceeded smoothly with tetrabutylammonium fluoride to give the desired 3-acyltetronic acid (*R*)-**8** in 95% yield. Deprotection of the trityl group using 1 N HCl afforded (*R*)-**1** in 75% yield. The overall yield of the last three steps was improved to 75% by omitting the chromatographic purification of **7** and **8**. All spectral data, including ^{1}H-NMR in dimethyl sulfoxide (DMSO)-d_6, the optical rotation, and the melting point of synthetic (*R*)-**1**, were identical to those reported by the Takeda group.[2]

Structural Analysis of RK-682 As shown in Fig. 2, 3-acyltetronic acid is known to exist in two sets of tautomeric forms (**i**/**ii**, **iii**/**iv**).[5] In fact, the ^{1}H-NMR spectrum of 3-acetyltetronic acid in $CDCl_3$ was reported to show two sets of peaks corresponding to the two tautomers (**i**/**ii**, **iii**/**iv**). Saito and Yamaguchi have suggested that 3-acetyltetronic acid exists as the **i**/**ii** form in methanol-d_4, but as the **iii**/**iv** form in DMSO-d_6.[5b] Since the ^{1}H-NMR spectrum reported by the Takeda group was measured in DMSO-d_6, and that reported by the RIKEN group was measured in methanol-d_4, we first measured the ^{1}H-NMR spectrum of the synthetic (*R*)-**1** in methanol-d_4 for comparison. But the ^{1}H-NMR spectrum of this synthetic (*R*)-**1** in methanol-d_4 was not identical to that previously reported for RK-682,[1a] indicating that the two samples are distinct compounds.

Next, we found that treatment of the original RIKEN sample of RK-682[9] with 0.5 N aqueous hydrochloric acid afforded a compound whose spectral data, including optical rotation, were identical with those of synthetic (*R*)-**1**. This suggested that RK-682 was a metal salt. Indeed, 3-acyltetronic acids are known to be extremely acidic (pK_a values of 3-acetyltetronic acid and 3-acetyl-5-methyltetronic acid were reported to be 0.8 and 0.5, respectively),[5a] and a Ciba-Geigy group reported isolation of the sodium salt of **1** from *Actinomycete* strain DSM7357.[10] It is also known that the antibiotic magnesidin, a structurally related tetramic acid derivative, is a magnesium salt.[11] Another tetramic acid derivative, tenuazoic acid, was reported to form complexes with several metal ions.[12] Therefore, to examine the possibility that **1** could be isolated as a salt, the sodium salt (*R*)-**9** and magnesium salt (*R*)-**10** were prepared by the treatment of (*R*)-**1** with 1 eq of sodium methoxide in methanol or with 1/2 mol eq of magnesium ethoxide in tetrahydrofuran (THF). The ^{1}H-NMR spectrum, IR spectrum and melting point of (*R*)-**9** were similar to those of RK-682, but those of (*R*)-**10** were distinct. Secondary ion mass spectrometry (SI-MS) of (*R*)-**9**, however, clearly eliminated the possibility of RK-682 being a sodium salt. SI-MS of (*R*)-**9** showed molecular-related ion peaks at *m*/*z* 391 ($M+H^+$) and 413 ($M+Na^+$), but RK-682 showed only a low intensity peak at *m*/*z* 377. This clearly indicated that RK-682 is not a Na salt. It appeared that RK-682 might be some other metal salt.

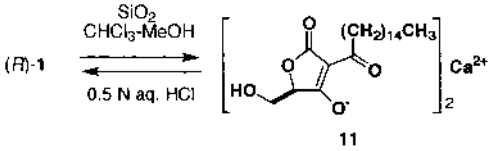

Fig. 5

Conversion of (*R*)-1 to Its Calcium Salt 11 by Silica Gel Chromatography In the course of these studies, we found that (*R*)-**1** was converted to a new compound **11**, which is less soluble in organic solvent, during column chromatography on Silica gel 60 (Merck) (MeOH–$CHCl_3$). The spectral data and melting point of this less-soluble compound **11** were found to be identical to those of RK-682. In addition, (*R*)-**1** was recovered by the treatment of **11** with 0.5 N aqueous hydrochloric acid as well as the case of RK-682. Since Silica gel column chromatography (MeOH–$CHCl_3$) was employed in the isolation of RK-682, we concluded that RK-682 was identical to this less-soluble compound **11**.[13]

To identify the unknown element in **11**, we next carried out X-ray fluorescence analysis of **11**. Since **11** was obtained upon Silica gel column chromatography, we first suspected that RK-682 might be a silicon complex; however, the content of silicon was less than 0.5 wt%. The data indicated that a significant amount of calcium (*ca.* 3.8 wt%) was contained in **11**, in addition to carbon (*ca.* 65 wt%) and oxygen (*ca.* 29 wt%). No potassium was detected, and the contents of other atoms (Na, Mg, Al, S, Cl) were less than 1%. Quantitative analysis of **11** by ICP-AES (inductively coupled plasma atomic emission spectroscopy) gave a calcium content of 3.4 wt%. Thus, we presumed that **11** is mainly a calcium salt of (*R*)-**1**. Analysis data supplied by Merck suggested that Silica gel 60 contains 0.11 wt% of calcium. It is surprising that **1** can react efficiently with such a small amount of calcium contained in the Silica gel 60 to give **11**.[14] The contents of ions are variable from sample to sample, depending on the amount and lot of the Silica gel used, and no salt formation

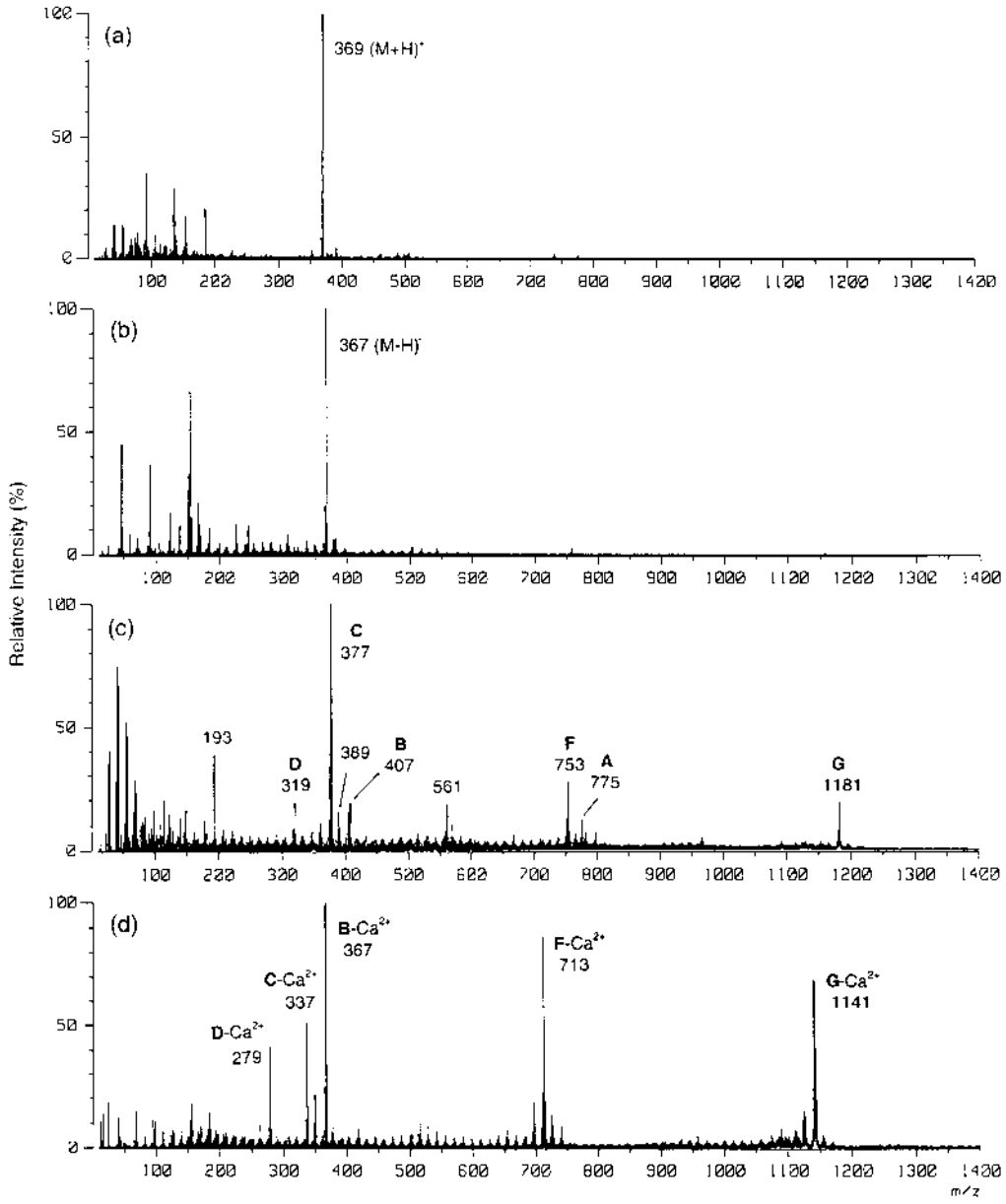

Fig. 6. a, b Positive and Negative Ion FAB-MS of **1** [Matrix: Glycerol, *m*-Nitrobenzyl Alcohol (2 : 1)]
c, d Positive and Negative Ion MS of **11** [Matrix: (c) Dithiothreitol, α-Thioglycerol (1 : 1),[15] (d) Triethanolamine].

was observed when spherical Silica gel 60 was used.

Finally, the structure of **11** was confirmed by detailed analysis of fast atom bombardment mass spectra (FAB-MS). Figure 6 shows positive and negative ion FAB-MS of **1** and **11**. A strong $(M+H)^+$ peak at *m/z* 369 and $(M-H)^-$ peak at 367 were observed in the spectra of **1** (Figs. 6a, b). In contrast, no significant peak at *m/z* 369 was observed in the spectrum of **11** (Fig. 6c). FAB-mass spectral patterns of **11** (Figs. 6c, d) were completely different from those of **1**. Peaks observed in the FAB-MS of **11** were assigned on the basis of HR-FAB-MS data and collisionally activated dissociation (CAD) spectra as shown in Fig. 7.[16] Dissociation of protonated **11** (ion **A**, Calcd for $C_{42}H_{71}O_{10}Ca$: 775.4673, Found: 775.4681) would give the cation **B** at *m/z* 407. Association of cation **B** to **11** affords the cation **G** (Calcd for $C_{63}H_{105}O_{15}Ca_2$: 1181.6705, Found: 1181.6709). It is likely that the cation **C** (Calcd for $C_{20}H_{33}O_4Ca$: 377.2005, Found: 377.2029) is formed from the cation **B** by elimination of formaldehyde. Since no significant peak at *m/z* 339, corresponding to the elimination of formaldehyde from protonated **1**, was observed in the spectrum of **1** (Fig. 6a), this elimination is presumably enhanced by the calcium salt formation. The structure of the cation **D** (Calcd for $C_{18}H_{31}O_2Ca$: 319.1950, Found: 319.1960) is unclear, but a possible candidate is expected to be formed by elimination of the $C_2H_2O_2$ fragment from **C**. The cation **F** (Calcd for $C_{40}H_{65}O_8Ca_2$: 753.3931, Found: 753.3991) would be formed by double elimination of formaldehyde from **A** and further association of Ca^{2+}. Figure 8 shows CAD spectra of the ions **A** (*m/z* 775) and **C** (*m/z* 377). The cation **A** gave the ions **E**, **B** and **C**, and the cation **C** gave the ion **D**. Fragmentation of the ion **B** to **C** was shown by the metastable ion at *m/z* 349.2 in the spectrum of Fig. 6c. Anions at *m/z* 367, 337, 279, 713 and 1141 are formally corresponding to the cations **B**, **C**, **D**, **F** and **G** minus

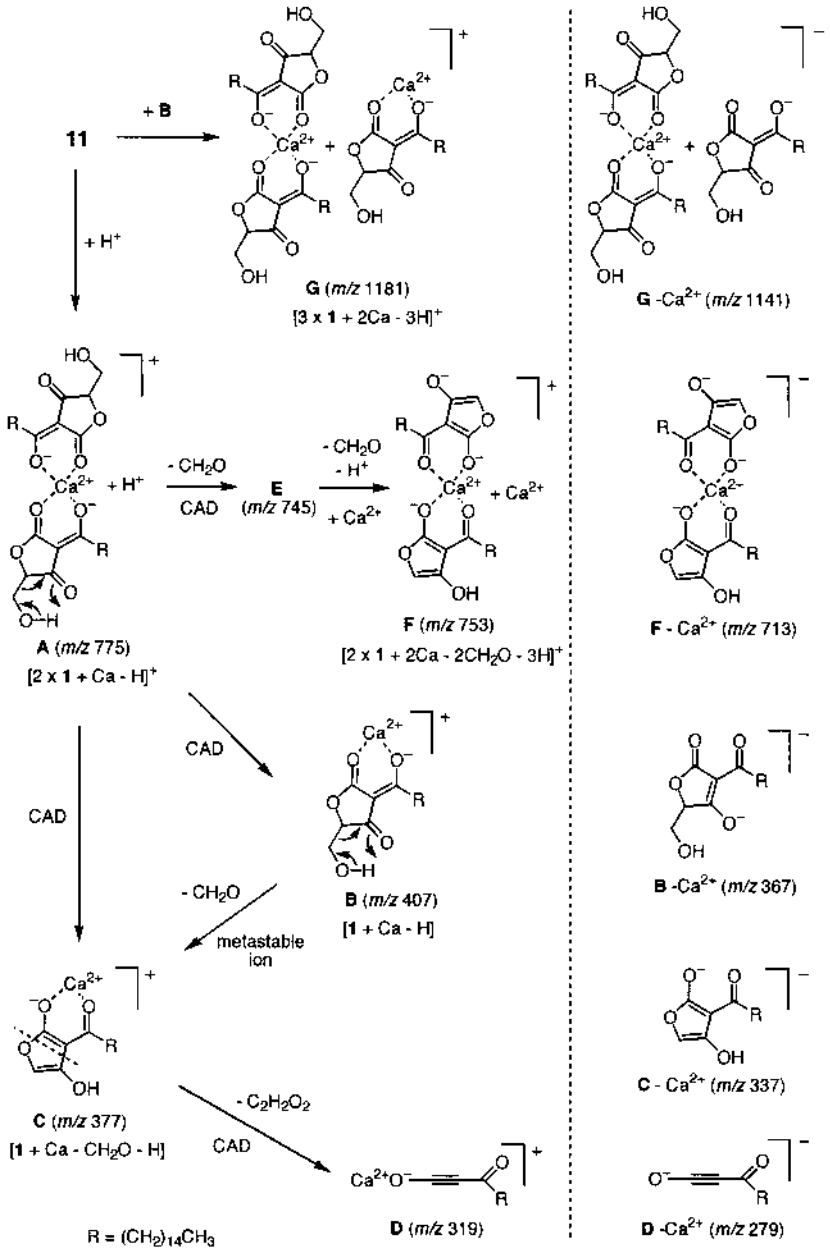

Fig. 7. Assignment of the Peaks Observed in FAB-MS

Only one structure is shown for each ion, although several tautomeric forms are possible.

Ca^{2+}, respectively. Although no large molecular-related ion peak, which would be helpful to identify the counter cation, was observed in the FAB-MS of **11**, the characteristic 40 atom mass unit difference observed in the positive and negative mass spectra (*m*/*z* 1181 *vs*. 1141, 753 *vs*. 713, 407 *vs*. 367, 377 *vs*. 337, and 319 *vs*. 279) suggested involvement of Ca^{2+} in the structure. Thus, comparison of the positive and negative ion mass spectra is useful for analyzing the structure of divalent cation salts.

The ^{1}H-NMR spectra of **1** and **11** in methanol-d_4 show only one set of peaks. The proton at the C5 position of **11** (4.40 ppm) shows an upfield shift compared to that of **1** (4.73 ppm). The ^{1}H-NMR spectrum of a mixture of **1** and **11** shows only one set of peaks with intermediate chemical shift, suggesting a rapid equilibrium between **1** and **11** in methanol. Table 1 shows the ^{13}C-NMR chemical shift values of **1** and **11**. Peaks were assigned using the heteronuclear multipe quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) techniques. Two types of chelate, **11a** and **11b**, are expected to exist for the calcium salt. Since large chemical shift changes were observed for the carbonyl carbons at positions 2 and 7, **11a** might be the predominant form in methanol-d_4.

In summary we have efficiently synthesized (*R*)-**1**, and found that it is easily converted to its calcium salt **11** during Silica gel column chromatography. RK-682 originally isolated from a natural source was found to be identical to this calcium salt **11**. This unusual property of **1** suggests that care must be taken in handling a wide variety of 3-acyltetronic acid derivatives, which are frequently found as components of biologically active natural products.[17] It is noteworthy that the acid-treated form, (*R*)-**1**, is now supplied as "RK-682"

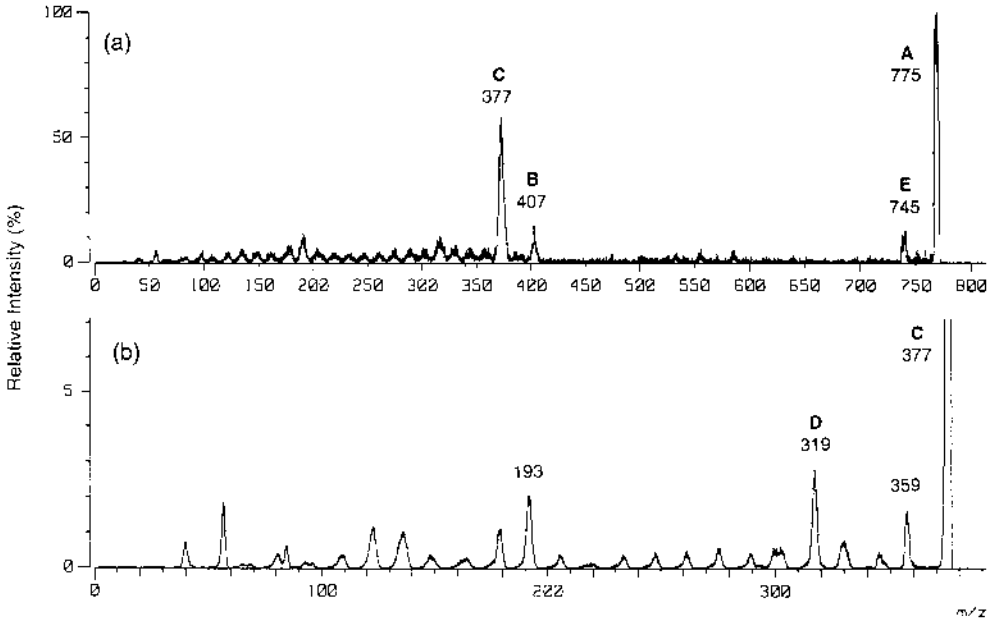

Fig. 8. CAD Spectra of a) Ion A at *m/z* 775 and b) Ion C at *m/z* 377 from the Spectrum of **11** in Fig. 3c

Table 1. ^{13}C-NMR Chemical Shifts of **1** and **11**

	1	**11**
2-C	174.59	178.63
3-C	100.79	99.19
4-C	196.10	196.69
5-C	83.69	83.18
6-C	61.63	62.65
7-C	196.48	198.96

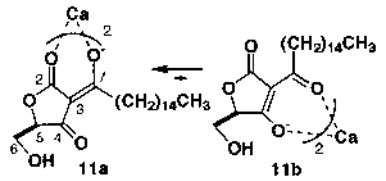

Fig. 9

for biological research use.[13)]

Experimental

General Methods Infrared (IR) spectra were measured on an FT/IR-5300 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Brucker AM-400, AC-200P, or AVANCE 500 NMR spectrometer with tetramethylsilane used as an internal standard. Electron ionization mass spectra (EI-MS) and chemical ionization mass spectra (CI-MS) were obtained with a Hitachi M-80B mass spectrometer. SI-MS were obtained with a Hitachi M-80A mass spectrometer. FAB-MS were measured on a JEOL JMS-HX110 double-focusing mass spectrometer of EBE arrangement. The ion acceleration voltage was 10 kV, and xenon gas was accelerated at a voltage of 6 kV. CAD spectra were obtained with helium as the collision gas. Ultraviolet (UV) spectra were measured on a Hitachi U-3210 spectrophotometer. Optical rotation was measured on a Horiba SEPA-200 polarimeter. In general, reactions carried out under anhydrous conditions utilized dry solvents under an argon atmosphere.

Methyl (*R*)-2-Hydroxy-3-triphenylmethyloxypropionate (3) To a solution of methyl (*R*)-2,2-dimethyl-1,3-dioxolane-4-carboxylate **2** (2.57 g, 16.0 mmol) in methanol (40 ml) was added 1 N aqueous HCl (20 ml), and the mixture was stirred at 23 °C for 4 h. After the evaporation of methanol, the residue was neutralized with saturated aqueous $NaHCO_3$. The mixture was successively extracted with AcOEt containing 10% iso-PrOH. The organic layers were combined, dried over Na_2SO_4, and concentrated to give crude methyl (*R*)-2,3-dihydroxypropionate (1.39 g, 72%) as a colorless oil. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 2.67 (2H, br s), 3.85 (3H, s), 3.87 (2H, m), 4.30 (1H, t, 3.5 Hz).

To a solution of the crude methyl (*R*)-2,3-dihydroxypropionate (1.39 g, 11.6 mmol) in CH_2Cl_2 (60 ml) were added triphenylchloromethane (4.20 g, 15.1 mmol), triethylamine (2.3 ml, 16.5 mmol), and *N*,*N*-dimethylaminopyridine (DMAP) (99.4 mg, 0.814 mmol), and the mixture was stirred at 23 °C for 24 h. The reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by Silica gel column chromatography (CH_2Cl_2) to give (*R*)-**3** (3.99 g, 95%) as a colorless solid.

^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.16 (1H, d, J=7.7 Hz), 3.35 (1H, dd, J=9.5, 3.4 Hz), 3.48 (1H, dd, J=9.5, 3.0 Hz), 3.77 (3H, s), 4.25 (1H, ddd, J=7.7, 3.4, 3.0 Hz), 7.10—7.50 (15H, m). IR (neat) cm^{-1}: 3520, 1745, 1450, 1230, 1125. EI-MS *m/z*: 362 (M^+), 285 (M^+-OH, $COOCH_3$), 243 (Ph_3C^+). HR-MS (M^+) Calcd for $C_{23}H_{22}O_4$: 362.1516, Found 362.1515. $[\alpha]_D^{20}$ −6.93° (c=1.36, CH_3OH : $CHCl_3$=1 : 4); mp 88—90 °C.

***S*-*tert*-Butyl 3-Oxo-octadecanethioate (6)** To a solution of [(*tert*-butylthio)carbonyl]acetic acid[18)] (2.76 g, 15.6 mmol) in THF (45 ml) was added magnesium ethoxide (897 mg, 7.84 mmol), and the mixture was stirred at 23 °C for 24 h. Evaporation of the solvent afforded its magnesium salt **5** (3.16 g, quant.) as a colorless solid. To a solution of palmitic acid (**4**) (100 mg, 0.39 mmol) in THF (4 ml) was added 1,1′-carbonyldiimidazole (CDI) (103 mg, 0.78 mmol), and the mixture was stirred at 23 °C for 6 h. After addition of the magnesium salt (267 mg, 0.78 mmol), the mixture was stirred at 23 °C for 1.5 h, then the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl and extracted with AcOEt. The organic layer was washed with saturated aqueous NH_4Cl and brine, dried (Na_2SO_4), and concentrated. The residue was purified by Silica gel column chromatography (hexane : AcOEt, 50 : 1) to give **6** (134 mg, 93%) as a colorless oil

^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 0.88 (3H, m), 1.25 (24H, m), 1.48 (9H, s), 1.61 (2H, m), 2.53 (2H, t, J=7.2 Hz), 3.56 (2H, s). IR (neat) cm^{-1}: 2930, 1725, 1680, 1620. EIMS *m/z*: 314 (M^+-*tert* Bu+H), 281 (M^+-S^tBu), 239 (M^+-CH_2COS *tert* Bu). CI-MS (isobutane) *m/z*: 371 (M^++H). HRMS (M^+) Calcd for $C_{22}H_{42}O_2S$: 370.2902, Found 370.2879.

Methyl (*R*)-2-(3-Oxo-octadecanoyl)oxy-3-triphenylmethyloxypropionate (7) To a solution of **6** (570 mg, 1.54 mmol) and (*R*)-**3** (557 mg, 1.54 mmol) in THF (12 ml) was added silver trifluoroacetate (0.41 g, 1.84 mmol), and the mixture was stirred overnight at 23 °C while shielded from light. The mixture was diluted with ether, passed through a short Silica gel column, and concentrated. The residue was purified by Silica gel column chromatography (hexane : AcOEt, 10 : 1) to give **7** (728 mg, 74%, a 6 : 1 mixture of keto and enol forms) as a colorless oil.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.88 (3H, t, J=6.7 Hz), 1.25 (24H, m), 1.52 (2H, m), 2.24 (2/7H, t, J=7.5 Hz), 2.62 (6/7H, dt, J=17.5, 7.3 Hz), 2.63 (6/7H, dt, J=17.5, 7.3 Hz), 3.46—3.56 (2H, m), 3.55 (6/7H, d, J=15.4 Hz), 3.59 (6/7H, d, J=15.4 Hz), 3.74 (18/7H, s), 3.75 (3/7H, s), 5.20 (1/7H, s), 5.26 (6/7H, dd, J=4.6, 3.5 Hz), 5.30 (1/7H, t, J=4.1 Hz), 7.22—7.33 (9H, m), 7.38—7.44 (6H, m), 11.76 (1/7H, s). IR (neat) cm^{-1}: 2930, 1750, 1720, 1220, 1100. EI-MS m/z: 362 ($M^+-COCH_2CO(CH_2)_{14}CH_3+H$), 285, 259 ($Ph_3CO^+$), 243 ($Ph_3C^+$). CI-MS (isobutane) m/z: 642 (M^+), 643 (M^++H). HR-MS ($M^+-C_{18}H_{32}O_2$) Calcd for $C_{23}H_{22}O_4$: 362.1516, Found 362.1543.

(*R*)-3-Hexadecanoyl-5-(triphenylmethyloxymethyl)tetronic Acid (8) To a solution of **7** (436 mg, 0.68 mmol) in THF (2.2 ml) was added tetrabutylammonium fluoride (1 M THF solution, 0.88 ml, 0.88 mmol), and the mixture was stirred at 23 °C for 2 h. After the addition of tetrabutylammonium fluoride (0.14 ml, 0.14 mmol), the mixture was further stirred overnight at 23 °C. The reaction was quenched by the addition of 6 N aqueous HCl (0.17 ml, 1.03 mmol) and the mixture was poured into ice-water; the whole was then extracted with ether. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by Silica gel column chromatography ($CHCl_3$: MeOH, 1 : 0—20 : 1). The combined and concentrated fractions containing the desired product were redissolved in AcOEt (50 ml), and the solution was washed with 0.5 N aqueous HCl and water, dried (Na_2SO_4), and concentrated to give **8** (395 mg, 95%) as a pale yellow oil.

^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.84 (3H, t, J=6.7 Hz), 1.21 (24H, m), 1.54 (2H, br tt, J=7.3, 7.3 Hz), 2.78 (2H, m), 3.20 (1H, dd, J=10.3, 3.7 Hz), 3.34 (1H, dd, J=10.3, 2.4 Hz), 4.73 (1H, m), 7.24—7.34 (15H, m). IR (neat) cm^{-1}: 3450, 2925, 2860, 1775, 1700, 1610, 710. EI-MS m/z: 350 (M^+-Ph_3COH), 243 (Ph_3C^+). CI-MS (isobutane) m/z: 610 (M^+), 611 (M^++H). HR-MS ($M^+-C_{19}H_{16}O$) Calcd for $C_{21}H_{34}O_4$: 350.2455, Found 350.2454. $[\alpha]_D^{20}$ +48.27° (c=1.02, $CHCl_3$).

(*R*)-3-Hexadecanoyl-5-hydroxymethyltetronic Acid (1) To a solution of **8** (315 mg, 0.52 mmol) in methanol (30 ml) was added 1 N aqueous HCl (0.52 ml, 0.52 mmol), and the mixture was stirred at 23 °C for 48 h. After removal of the solvent *in vacuo*, the residue was purified by Silica gel column chromatography ($CHCl_3$: MeOH, 1 : 0—20 : 1—10 : 1). The combined and concentrated fractions containing the desired product were redissolved in AcOEt (100 ml), washed with 0.5 N aqueous HCl and water, dried (Na_2SO_4), and concentrated to give **1** (143 mg, 75%) as a colorless solid.

^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.85 (3H, t, J=6.8 Hz), 1.23 (24H, m), 1.49 (2H, m), 2.73 (2H, t, J=7.4 Hz), 3.66 (1H, dd, J=12.3, 3.6 Hz), 3.74 (1H, dd, J=12.3, 2.6 Hz), 4.66 (1H, m). ^{1}H-NMR (methanol-d_4, 500 MHz) δ: 0.90 (3H, t, J=6.9 Hz), 1.22—1.46 (24H, m), 1.65 (2H, br tt, J=7.5, 7.5 Hz), 2.85 (2H, t, J=7.5 Hz), 3.86 (1H, dd, J=12.6, 3.5 Hz), 3.94 (1H, dd, J=12.6, 2.7 Hz), 4.68 (1H, br t, J=2.9 Hz). ^{13}C-NMR (methanol-d_4, 125 MHz, 33 °C) δ: 14.45 (22-CH_3), 23.74 (21-CH_2), 26.49 (9-CH_2), 30.46, 30.60, 30.73, 30.76, 30.78 (10—19-CH_2), 33.09 (20-CH_2), 37.51 (8-CH_2), 61.63 (6-CH_2O), 83.69 (5-CH), 100.79 (3-C), 174.59 (2-C=O), 196.10 (4-C=O), 196.48 (7-C=O); IR (KBr) cm^{-1}: 3350, 2925, 2850, 1750, 1665, 1610, 1470, 1050. EI-MS m/z: 368 (M^+), 350 (M^+-H_2O), 337 (M^+-CH_2OH), 319, 185, 172, 154. HR-MS (M^+) Calcd for $C_{21}H_{36}O_5$: 368.2560, Found 368.2557. UV λ_{max} (MeOH) nm (ε): 232 (12319), 267 (15083). $[\alpha]_D^{20}$ +58.06° (c=0.47, $CHCl_3$); mp 105—108 °C.

Improved Synthesis of (*R*)-1 from (*R*)-3 To a solution of **6** (1.32 g, 3.50 mmol) and (*R*)-**3** (1.27 g, 3.51 mmol) in THF (25 ml) was added silver trifluoroacetate (930 mg, 4.21 mmol), and the mixture was stirred for 23 h at 23 °C while shielded from light. Silver trifluoroacetate (231 mg after 5 h and 387 mg after 22 h) was further added to complete the reaction. The mixture was diluted with ether, passed through a short Silica gel column, and concentrated to give crude **7**.

To a solution of this crude **7** in THF (12 ml) was added tetrabutylammonium fluoride (1 M THF solution, 4.6 ml, 4.6 mmol), and the mixture was stirred at 23 °C for 41 h. Tetrabutylammonium fluoride (0.7 ml after 2 h, 1.75 ml after 17 h, and 1.75 ml after 25 h) was further added to complete the reaction. The reaction was quenched by the addition of 1 N aqueous HCl (2 ml) and poured into ice-water; the mixture was then extracted with ether. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated to give crude **8** (2.916 g).

To a solution of the crude **8** (2.907 g) in methanol (70 ml) was added 1 N aqueous HCl (4 ml), and the mixture was stirred at 23 °C for 66 h. After removal of the solvent *in vacuo*, the residue was purified by Silica gel column chromatography ($CHCl_3$: MeOH, 1 : 0—10 : 1—3 : 1). The combined and concentrated fractions containing the desired product were redissolved in AcOEt, washed with 0.5 N aqueous HCl and water, dried (Na_2SO_4), and concentrated to give (*R*)-**1** (967 mg, 75% in 3 steps) as a colorless solid.

(*R*)-3-Hexadecanoyl-5-hydroxymethyltetronic Acid Sodium Salt (9) To a solution of (*R*)-**1** (26.3 mg, 0.071 mmol) in methanol (2.5 ml) was added sodium methoxide (1 M MeOH solution, 71.3 μl, 0.071 mmol) at 0 °C. The mixture was stirred at 23 °C for 1 h, and the solvent was removed *in vacuo* to give the sodium salt (*R*)-**9** (27.5 mg, quant) as a colorless solid.

^{1}H-NMR (methanol-d_4, 400 MHz) δ: 0.90 (3H, t, J=6.9 Hz), 1.28 (24H, m), 1.56 (2H, m), 2.74 (2H, m), 3.73 (1H, dd, J=12.3, 5.0 Hz), 3.88 (1H, dd, J=12.3, 2.8 Hz), 4.30 (1H, dd, J=5.0, 2.8 Hz). IR (neat) cm^{-1}: 3400, 2920, 2850, 1720, 1645, 1570, 1460, 1030. SI-MS (*m*-nitrobenzyl alcohol) m/z: 413 ($M+Na^+$), 391 ($M+H^+$), 176, 136, 73, 43, 41 23 (Na^+, bp). UV λ_{max} (MeOH) nm (ε): 233 (13821), 265 (17343). $[\alpha]_D^{20}$ +55.7° (c=0.42, $CHCl_3$); mp 220—230 °C (dec.).

(*R*)-3-Hexadecanoyl-5-hydroxymethyltetronic Acid Magnesium Salt (10) To a solution of (*R*)-**1** (17.4 mg, 0.047 mmol) in THF (2.0 ml) was added magnesium ethoxide (2.7 mg 0.024 mmol) at 0 °C. The mixture was stirred at 23°C for 4 h, and the solvent was removed *in vacuo* to give the magnesium salt (*R*)-**10** (19.9 mg, quant) as a colorless solid. ^{1}H-NMR (methanol-d_4, 400 MHz) δ: 0.90 (6H, t, J=6.9 Hz), 1.29 (48H, m), 1.64 (4H, m), 2.86 (4H, m), 3.87 (2H, dd, J=12.6, 3.3 Hz), 3.94 (2H, dd, J=12.6, 2.7 Hz), 4.72 (2H, m). IR (neat) cm^{-1}: 3395, 2925, 2855, 1735, 1635, 1565, 1530, 1500, 1475, 1340, 1080, 1040. SI-MS (*m*-nitrobenzyl alcohol+NaCl) m/z: 781 ($M+Na^+$), 759 ($M+H^+$). $[\alpha]_D^{20}$ +161.3° (c=0.19, $CHCl_3$); mp 245—260 °C (dec.).

(*R*)-3-Hexadecanoyl-5-hydroxymethyltetronic Acid Calcium Salt (11) A solution of (*R*)-**1** (50.4 mg, 0.137 mmol) in $CHCl_3$: MeOH (5 : 1) was loaded onto a Silica gel column (Merck, Silica gel 60, particle size 0.040—0.063 mm, cat. # 109385, 5.0 g), and eluted with the same solvent. Removal of the solvent of the combined fractions afforded the calcium salt **11** (52.8 mg) as a colorless solid. No salt formation was observed when spherical Silica gel 60 (Kanto Chemical, particle size 0.04—0.050 mm, cat. # 37562) was used.

^{1}H-NMR (methanol-d_4, 400 MHz) δ: 0.90 (3H, t, J=6.9 Hz), 1.25—1.50 (24H, m), 1.60 (2H, br tt, J=7.6, 7.6 Hz), 2.79 (2H, t, J=7.6 Hz), 3.81 (1H, dd, J=12.3, 4.2 Hz), 3.90 (1H, dd, J=12.3, 2.7 Hz), 4.40 (1H, dd, J=4.2, 2.7 Hz). ^{13}C-NMR (methanol-d_4, 125 MHz, 30 °C) δ: 14.46 (22-CH_3), 23.75 (21-CH_2), 26.25 (9-CH_2), 30.50, 30.77, 30.80, 30.84, 30.86 (10—19-CH_2), 33.10 (20-CH_2), 40.68 (8-CH_2), 62.65 (6-CH_2O), 83.18 (5-CH), 99.19 (3-C), 178.63 (2-C=O), 196.69 (4-C=O), 198.96 (7-C=O). IR (neat) cm^{-1}: 3400, 2925, 2855, 1730, 1635, 1560, 1470, 1040—1100 (broad). Elemental Analysis Found C 62.51, H 9.32 (residual material 7.61%).[19] mp 180—200 °C (dec). The $[\alpha]_D$ value was unstable due to the low solubility of **11**.

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13) (*R*)-**1** and **11** showed similar inhibitory activity to a dual-specificity phosphatase, VHR, suggesting that **11** is hydrolyzed in the assay buffer (pH 6.0).[3] RK-682 (acid-treated free form isolated from a natural source=(*R*)-**1**) is commercially available from Wako Pure Chemicals Industries Inc.
14) According to the ICP-AES analysis data supplied by Merck, the following elements were present in Silica gel 60: Al (0.035%), Ca (0.11%), Fe (0.01%), Mg (0.018%), Na (0.06%), S as SO_4 (0.1%), Si (+++), Ti (0.02%), Zr (0.006%). We used *ca.* 50—100 fold excess (by weight) of silica gel for the conversion of **1** to **11**. This means that the compound **1** extracted calcium from silica gel quite efficiently. It is also noteworthy that the Mg content in the same sample of **11** quantified by ICP-AES was 0.7 wt%, which was significantly higher than those of other metals such as sodium (sodium content of **11** quantified by atomic absorption spectrum was 1600 ppm) indicating that **1** prefers divalent cations.
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19) Quantification by ICP-AES and atomic absorption suggested that the molar ratio of Ca : Mg : Na is about 72 : 25 : 6. The elemental analysis data are in good agreement with the calculated values for $(C_{21}H_{35}O_5)_2Ca_{0.72}Mg_{0.25}Na_{0.06}\cdot 2H_2O$: C 62.48, H 9.24.