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Cyclipostins, Novel Hormone-sensitive Lipase Inhibitors from *Streptomyces* sp. DSM 13381

II. Isolation, Structure Elucidation and Biological Properties

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Hormone-sensitive lipase (HSL) is a key enzyme of lipid metabolism and its control is therefore a target in the treatment of diabetes mellitus. Cultures of the *Streptomyces* species DSM 13381 have been shown to potently inhibit HSL. Ten inhibitors of HSL, termed cyclipostins, have been isolated from the mycelium of this microorganism and a further nine related compounds detected. Their structures were characterized by 2-D NMR experiments and by mass spectrometry and were found to comprise neutral cyclic enol phosphate esters with an additional γ-lactone ring. On account of their ester-bound fatty alcohol side chain, the cyclipostins have physicochemical properties similar to those of triglycerides. The outstanding characteristic of the cyclipostins is their strong anti-HSL activity, with IC_{50} values in the nanomolar range.

Hormone-sensitive lipase (HSL), the rate-limiting enzyme of intracellular triglyceride hydrolysis, is a major determinant of fatty acid mobilization in adipose tissue as well as in other tissues[1]. It plays a pivotal role in lipid metabolism, overall energy homeostasis, and cellular events involving fatty acid signaling. Fatty acids stored in the form of triglycerides are the main source of energy in the absence of dietary substrates. Hydrolysis of triglycerides involves a sequence of three reactions and is catalyzed by two enzymes: HSL and monoglyceride lipase. HSL on its own is sufficient to catalyze the hydrolysis of tri- and diglycerides, but the participation of monoglyceride lipase is required to achieve complete hydrolysis of monoglycerides[1,2]. Lipolysis is a regulated event; it is controlled mainly by the activity of the sympathetic nervous system and counteracted by plasma insulin levels[3].

In diabetes the normally balanced relationship between fats and glucose as metabolic fuels is disturbed. Fatty acids circulating in increased concentrations are metabolized in preference to glucose, while at the same time providing energy for excessive glucose production (gluconeogenesis), thereby resulting in raised glucose concentrations[4]. An elevated circulating free fatty acid concentration, a major feature of non-insulin-dependent or type II diabetes mellitus, is due to a dysregulation of adipose tissue function[5]. Because of its role in fatty acid mobilization, HSL is among the candidates for such dysregulation, and has been suggested as a target for the development of new antidiabetic drugs.

As a consequence, we have been investigating microbial cultures for the presence of possible inhibitors, and in fermentation solutions of *Streptomyces* sp. DSM 13381 we detected compounds that showed strong anti-HSL activity[6,7]. In this paper we describe these inhibitors, which we termed cyclipostins, their isolation from cultures of *Streptomyces* sp. DSM 13381, their structural characterization, and some biochemical properties.

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Experimental

General

Quantitative ultraviolet absorption spectra were recorded using a Perkin-Elmer 554 spectrometer (Perkin-Elmer Bodenseewerk, Überlingen, Germany); for all other purposes, including the performance of HPLC analyses, Hewlett-Packard series 1100 equipment fitted with diode array detectors was used. Preparative HPLC was performed using Pharmacia equipment (Uppsala, Sweden), Rainin Dynamax® pumps, and Büchi 684 fraction collectors (CH-9230 Flawil, Switzerland).

Isolation of Cyclipostins A and F

At the end of the fermentation[6,7] of *Streptomyces* species DSM 13381 (cultured on oat flake medium), 90 liters of fermentation broth was filtered with the addition of approx. 2% of filtration aid (*e.g.* Celite) and the cell mass (10 liters) extracted with 40 liters methanol. The methanolic solution containing the crude active substance mixture was filtered from the mycelium and concentrated under reduced pressure. The concentrate was then loaded onto a prepared 7 liters MCI gel, CHP20P (Mitsubishi Chemical Corporation, Tokyo) column, which was eluted with a gradient of water to 2-propanol. The eluate was collected in 10 liters fractions at a flow rate of 20 liters/hour and the fractions containing the cyclipostins (19 and 20) were each concentrated under reduced pressure. The fractions were investigated by HPLC. Fraction 19 comprised cyclipostins A to E and their isomers, fraction 20 cyclipostin F and isomers thereof. After concentration of fraction 19 under reduced pressure, 1 g of the concentrate was dissolved in water/methanol (1 : 1) and applied to a Nucleoprep 100-5 C_{18} AB® column (21×250 mm). The column was eluted with a gradient of 50~100% acetonitrile in 0.01% trifluoroacetic acid, at a flow rate of 50 ml/minute. The fractions containing cyclipostin A were concentrated under reduced pressure and passed repeatedly through an RP 250/10 Nucleosil 100-5 C18 HD (Macherey-Nagel, Düren, Germany) column. The column was eluted with a gradient of 50~66% acetonitrile in 0.01% trifluoroacetic acid adjusted to pH 3.5 by addition of a drop of ammonium hydroxide solution. The fractions which contained pure compound were combined and freeze-dried, affording 5.4 mg of pure cyclipostin A as a waxy solid. Repeated reversed-phase chromatography in analogous manner of the cyclipostin F containing fraction 20 gave 2.5 mg of pure inhibitor F.

Isolation of Cyclipostins N, P, P2, R, R2, S, T, and T2

To obtain the nonpolar cyclipostins N to T2, a 2000-liter batch of *Streptomyces* sp. DSM 13381 cultured in glycerol/soy meal nutrient medium was worked up. The cell mass (280 kg) was first separated from the culture filtrate and then extracted with two 800-liter volumes of 2-propanol. This alcoholic extract was concentrated under reduced pressure until the proportion of water to 2-propanol in the concentrate was approximately 1 : 1. The cyclipostin mixture was then subjected to solid-phase extraction with 100 liters of Amberlite XAD-7 (TosoHaas, Montgomeryville, USA). Elution of the loaded resin with a water/2-propanol mixture in which the 2-propanol content was increased stepwise yielded the HSL-inhibiting compounds in the fractions eluted with 60~85% 2-propanol (200 liters). The crude extract was concentrated under reduced pressure until the onset of turbidity and was then chromatographed in two portions on an MCI gel column (CHP20P, 75~150 μ, Mitsubishi Chem. Corp. Tokyo, Japan, column dimensions: 20 cm internal diameter×35 cm height, capacity 11 liters). The column was eluted with a gradient of 50 to 95% 2-propanol in water at a flow rate of 10 liters/hour, with the eluate collected in 10 liters fractions. Fractions 10 and 11 yielded 1.3 g cyclipostin P and 1 g cyclipostin P2 per run.

The subsequent fractions 13~16 contained mainly triglycerides. Further depletion of the neutral lipid content of fractions 10 and 11 was achieved by concentration of the inhibitor-containing fractions under reduced pressure and then cooling, which caused the triglycerides to crystallize out (f.p. 36~37°C; retention time in the described HPLC system: 18.1 minutes). The lipid composition of the latter (see Results) was determined by fatty acid analysis (GC Systems, HP 6890, Hewlett-Packard, using MIS software supplied by MIDI, Newark, Delaware, USA. GC-MS: InfraServ Wiesbaden, Germany).

Fractions 10 and 11 were in each case pooled and rechromatographed on an MCI gel column (dimensions: 6×40 cm) in analogous manner to the above procedure, with a flow rate of 60 ml/minute. The fractions containing mostly cyclipostins N, R, R2, and P2 were pooled, as were the later fractions containing cyclipostins P, S, T, and T2. Preparative chromatographic separation of the individual constituents was in each case carried out by successive passage through 250/21 Nucleosil 100-5 C18 AB and 250/21 Nucleosil 100-7 C18 HD columns (both Macherey-Nagel, Düren, Germany), as the relative retention times on the solid supports were different. The columns were eluted with a gradient of 70 to 80% acetonitrile in 0.002% trifluoroacetic acid adjusted to pH 3.5 with dilute

ammonium hydroxide, at a flow rate of 50 ml/minute. Fractions containing pure cyclipostins were in each case freeze-dried, yielding 16 mg cyclipostin N (oil), 1.8 g cyclipostin P, approx. 1.5 g cyclipostin P2 (oil), 120 mg cyclipostin R, 80 mg cyclipostin R2 (oil), 7 mg cyclipostin S, 47 mg cyclipostin T, and 44 mg cyclipostin T2 (solid).

1.8 g of cyclipostin P was dissolved in 120 ml methanol, filtered to obtain a clear solution, and 20 ml water added. The mixture was then concentrated to about 100 ml under reduced pressure until slightly turbid, gently warmed to 45°C until clear again, and then gradually cooled to 1°C. Filtration and further crystallization gave 1.3 g of pure cyclipostin P, f.p. 57~59°C. $[\alpha]_D^{21}$ +3° (*c* 10, $CHCl_3$), CD (MeOH) $\Delta\varepsilon$ (λ_{max})+4.3 (232 nm). IR ν_{max} (KBr) cm^{-1} 2971, 2852, 1753, 1671, 1471, 1287, 1214, 996, 832. UV_{max}^{MeOH} nm (ε) 226 (11,000). NMR data are listed in Table 2. FAB-MS *m/z* 445.2717 (MH^+), calculated M.W. for $C_{23}H_{42}O_6P$: 445.2719.

HPLC Analysis of the Cyclipostins

HPLC analysis of the cyclipostins was carried out using an HP 1100® unit (Hewlett-Packard Co. USA) and YMC-Pack Pro C18® columns (AS-303, 250×4.6 mm, S-5 μm, 120 Å [YMC Co., Kyoto, Japan]). The columns were eluted with a flow rate of 1 ml/minute, a column temperature of 40°C, and a gradient of 0.05% trifluoroacetic acid to acetonitrile. The mobile phase reached 100% acetonitrile after 11 minutes, with elution thereafter isocratic with the same solvent. The compounds were detected through their UV absorbance at 210 nm. Under these conditions the cyclipostins have the retention times listed in Table 1.

NMR Spectroscopy

All NMR spectra were recorded on Bruker DRX 600 spectrometers operating at 600 MHz (^1H) and 150 MHz (^{13}C). Data were processed on an indigo2 station (Silicon Graphics) using Bruker XWINNMR software. DQF-COSY experiments were performed with a spectral width of 5 ppm. Spectra were recorded with 1024 increments in t_1 and 4096 complex data points in t_2, with 8 transients averaged for each t_1 value.

For the HMQC[8] spectra, 512 increments (16 scans) with 2048 complex data points in t_2 were collected using a sweep width of 5 ppm in the proton and 160 ppm in the carbon dimension. The HMBC[9] spectra were acquired with a sweep width of 5 ppm in the proton and 200 ppm in the carbon dimension. A total of 64 transients were averaged for each of 512 increments in t_1, and 2048 complex points in t_2 were recorded. A delay of 70 msec was used for the development of long range correlations.

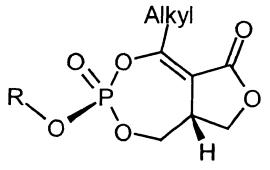
Mass Spectrometry

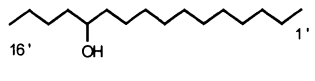
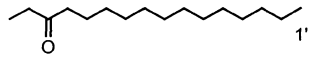
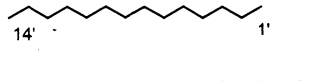
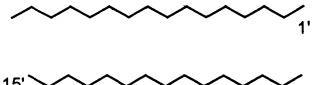
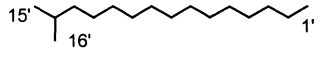
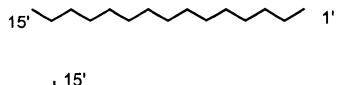
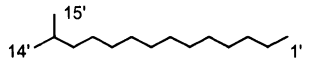
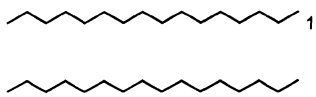
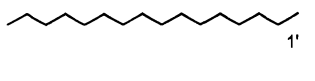
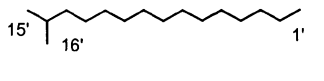
High resolution measurements were obtained on a VG ZAB 2SEQ sector field instrument using a FAB or EI source. MS/MS experiments were generally conducted on a VG Bio-Q triple quadrupole mass spectrometer by loop injection of the sample into the ESI source (solvent $MeOH/H_2O/HCO_2H$ 49.9 : 49.9 : 0.2, flow 5 μl/minute). Activation energies ranged from 20 to 40 eV. Additional MS^n (n=1~4) experiments were performed on a Bruker Apex III FTICR mass spectrometer with electrospray ionization to establish the elemental composition of the fragments.

Preparation of the Partially Purified Hormone-sensitive Lipase

Isolated rat adipocytes were obtained from epididymal fatty tissue of non-treated male rats (Wistar, 220~250 g) by collagenase treatment according to published procedures[10~12]. The adipocytes from 10 rats were washed by flotation with 3×50 ml of homogenization buffer (25 mM Tris/HCl, pH 7.4, containing 0.25 M sucrose, 1 mM EDTA, 1 mM DTT, 10 μg/ml leupeptin, 10 μg/ml antipain, 20 μg/ml pepstatin) and finally suspended in 10 ml of homogenization buffer. The adipocytes were homogenized in a Teflon-in-glass homogenizer (Braun-Melsungen) at 15°C (10 pulses at 1500 rpm) and the homogenate was centrifuged (Sorvall SM24 tubes, 5000 rpm, 10 minutes, 4°C). The lower layer between the supernatant fatty layer and the pellet was removed and the centrifugation repeated. The resulting lower layer was again centrifuged (Sorvall SM24 tubes, 20000 rpm, 45 minutes, 4°C) and the lower layer collected and treated with 1 g of heparin-Sepharose (Pharmacia-Biotech, CL-6B, washed 5 times with 25 mM Tris/HCl, pH 7.4, containing 150 mM NaCl). After incubation for 60 minutes at 4°C (shaking every 15 minutes), the batch was centrifuged (Sorvall SM24 tubes, 3000 rpm, 10 minutes, 4°C). The supernatant was adjusted to pH 5.2 by addition of acetic acid and incubated at 4°C for 30 minutes. The precipitates were collected by centrifugation (Sorvall SS34, 12000 rpm, 10 minutes, 4°C) and suspended in 2.5 ml of 20 mM Tris/HCl (pH 7.0, containing 1 mM EDTA, 65 mM NaCl, 13% of sucrose, 1 mM DTT, 10 μg/ml each of leupeptin/pepstatin/antipain). The suspension was dialyzed overnight at 4°C against 25 mM Tris/HCl (pH 7.4, containing 50% glycerol, 1 mM DTT, 10 μg/ml each of leupeptin, pepstatin, antipain) and then applied to a hydroxyapatite column (0.1 g per 1 ml of suspension, equilibrated with 10 mM potassium phosphate, pH 7.0, containing 30% glycerol, 1 mM DTT). The column was washed with four volumes of equilibration buffer at a

Table 1. Structural formulae and some physicochemical data of the isolated cyclipostins.



Compound	Empirical formula	M.W.	R (fatty alcohol)	Alkyl	HPLC* RT
Cyclipostin A:	$C_{23}H_{41}O_7P$	460.55		methyl	12.7 min
Cyclipostin F:	$C_{23}H_{39}O_7P$	458.54		methyl	13.2 min
Cyclipostin N:	$C_{21}H_{37}O_6P$	416.50		methyl	15.9 min
Cyclipostin P:	$C_{23}H_{41}O_6P$	444.55		methyl	17.7 min
Cyclipostin P2:	$C_{23}H_{41}O_6P$	444.55		methyl	17.3 min
Cyclipostin R:	$C_{22}H_{39}O_6P$	430.53		methyl	16.7 min
Cyclipostin R2:	$C_{22}H_{39}O_6P$	430.53		methyl	16.4 min
Cyclipostin S:	$C_{24}H_{43}O_6P$	458.58		ethyl	18.5 min
Cyclipostin T:	$C_{25}H_{45}O_6P$	472.61		propyl	19.1 min
Cyclipostin T2:	$C_{25}H_{45}O_6P$	472.61		propyl	18.7 min

* The HPLC procedure is described in the Experimental section.
The fatty alcohol side chain is attached at $C^{1'}$ to the ester oxygen atom of the phosphoric acid.

flow rate of 20~30 ml/hour. The HSL was then eluted with a volume of equilibration buffer containing 0.5 M potassium phosphate, dialyzed (see above), and concentrated by ultrafiltration (Amicon Diaflo PM 10 filter) 5~10 times at 4°C. The partially purified HSL can be stored at −70°C for 4~6 weeks. HSL activity in this fraction was not reduced significantly by 1 M NaCl or antiserum to bovine lipoprotein lipase. Furthermore, the presence of 100 mM NaF, which completely blocks purified rat adipose HSL[2], reduced the release of fatty acids from trioleylglycerol by the partially purified HSL preparation by 69~78%. These data indicate that the major portion of the lipolytic activity in the preparation is based on HSL and only minor portions (less than 3 and 30%, respectively) are due to contaminating lipoprotein lipase (sensitive toward NaCl) and monoacylglycerol hydrolase (resistant toward NaF).

Assay for HSL Activity

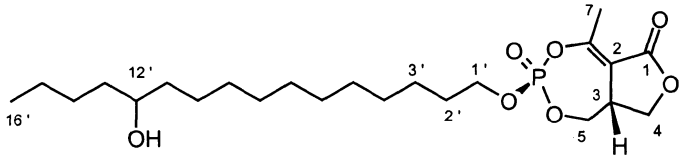
For the preparation of the substrates, a mixture of 25~50 μCi of [^{3}H]glyceryl trioleate (in toluene) and 6.8 μmol of unlabeled glyceryl trioleate or a mixture of 12~25 μCi of [^{3}H]cholesteryl oleate (in toluene),

10.5 μmol of unlabeled cholesteryl[13] oleate, and 0.6 mg of phospholipid (phosphatidylcholine/phosphatidylinositol 3 : 1 w/v) was dried in a jet of N_2 and taken up in 2 ml of 0.1 M potassium phosphate (KP_i, pH 7.0) by ultrasound treatment (Branson 250, microtips, setting 1~2, 2×1 minute, with an interval of 1 minute between pulses). After addition of 1 ml of KP_i and further sonication (4×30 sec, in ice at 30 sec intervals), 1 ml of 20% BSA (bovine serum albumin in KP_i) was added (final concentration of glyceryl trioleate/cholesteryl oleate: 1.7/0.9 mM). For the reaction, 100 μl of substrate solution was pipetted into 100 μl of HSL solution (HSL prepared as above, diluted in 20 mM KP_i, containing 1 mM EDTA, 1 mM DTT, 0.02% BSA, 20 μg/ml pepstatin, 10 μg/ml leupeptin) and incubated at 37°C for 30 minutes. After addition of 3.25 ml of methanol/chloroform/heptane (10 : 9 : 7) and 1.05 ml of 0.1 M K_2CO_3/0.1 M boric acid (pH 10.5), the batch was mixed well and finally centrifuged (800 g, 20 minutes). After phase separation, one equivalent of the upper phase (1 ml) was removed and the radioactivity (released fatty acids) determined by liquid scintillation measurement[14]. For the evaluation of inhibitory compounds, 2 μl of compound at the 100-fold final assay concentration (in 100% DMSO) was added to the 100 μl of substrate solution and incubated at 37°C for 15 minutes, followed by addition of 100 μl of HSL solution and further incubation. The DMSO concentration in the final assay was thus a constant 1% at each concentration tested, which did not interfere with the HSL activity. Substances were routinely tested in four independent reactions with the phase separations each performed in triplicate. Inhibition of the enzymatic activity of the HSL by the test substance was quantified by comparison with a noninhibited control reaction, with the IC_{50} value calculated from an inhibition curve generated from at least 10 different concentrations of the test substance. Data were analyzed using the Graphit software package (Elsevier/Biosoft).

Lipolysis Assay

For the preparation of isolated rat adipocytes, epididymal fat pads were obtained from male Sprague-Dawley rats (150~180 g) killed by decapitation. The main fat pad blood vessel was removed with forceps and the pads were cut into small pieces. Digestion was carried out at 37°C in a shaking water bath by treating 1 g of sliced pad for 20 minutes with 3 ml of 1 mg/ml collagenase (type I, CLS, Worthington) in 1% BSA, fraction V, in KRH (Krebs-Ringer solution buffered with 25 mM HEPES/KOH, pH 7.4, containing 2.5 mM $CaCl_2$, 2 mM glucose, 1 mM sodium pyruvate, 0.5 units/ml adenosin deaminase and 200 nM phenylisopropyl adenosine). The adipocytes were filtered through nylon mesh of sufficient pore size that no pressure other than gentle teasing with a plastic spatula was necessary for the cells to pass through. After three cycles of centrifugation (500 g, 1 minute), aspiration of the infranatant, and resuspension in KRH containing 1% BSA, the cells were suspended in KRH containing 4% defatted BSA. Small aliquots of the final suspension were aspirated into capillary hematocrit tubes and centrifuged for 1 minute in a Microhematocrit centrifuge in order to determine what fraction of the suspension consisted of fat cells[15]. Results expressed as activities per unit volume of packed adipocytes (corresponding to equivalent numbers of cells) are based on this centrifugation procedure. Lipolysis was initiated by addition of a 100 μl aliquot of the adipocyte suspension to 700 μl of incubation medium [prewarmed to 37°C and consisting of KRH, 4% defatted BSA and 1 U/ml adenosine deaminase (type I, Sigma) and/or 1 μM isoproterenol, as indicated], with or without addition of 8 μl of a solution of the appropriate test substance in 100% DMSO (at a concentration corresponding to 100 times its concentration in the final mixture). Thus, the final DMSO concentration in the incubation mixture was no greater than 1% and did not affect cell viability (as determined by the sensitivity of the adipocytes in respect of isoproterenol-induced lipolysis and its inhibition by insulin). Incubations with or without addition of 1 μM isoproterenol were performed in 5 ml polypropylene vials agitated in a shaking water bath (140 cycles/minute, amplitude 4.5 cm) at 37°C for 20, 40, and 60 minutes. The incubation was terminated by addition of 200 μl of 50 mM EDTA buffered to pH 7.4 with Tris. For the glycerol determination, the whole mixture was mixed rapidly and homogenized with a ground glass pestle in a 1 ml ground glass homogenizing tube (10 pulses). The homogenate was transferred immediately to a 1.5 ml Eppendorf cup precooled to 4°C, after which the extracts were maintained at 4°C. The homogenate was then centrifuged (10,000 g, 15 minutes) and the infranatant below the fat cake removed with a glass Pasteur pipette, taking care not to aspirate the membrane pellet, and transferred to another Eppendorf cup. 300 μl aliquots of the infranatant were added to 300 μl volumes of 10% (w/v) $HClO_4$. The precipitate was in each case removed by centrifugation (10,000 g, 2 minutes) and the supernatant neutralized with 20% (w/v) KOH, followed by addition of 50 μl of 1 M Tris/HCl, pH 7.4. A 50 μl sample was then incubated (5 minutes, 37°C) with 1 ml of 0.1 M HEPES/KOH, pH 7.5, containing 2 mM ATP, 0.5 mM 4-aminoantipyrine, 1 mM EDTA, 0.5 U glycerol kinase, 4 U glycerol-3-phosphate oxidase, 2 U peroxidase, 2.7 mM

Fig. 1. Structural assignment proposed for cyclipostin A isolated from *Streptomyces* sp. DSM 13381.



p-chlorophenol, 0.04% Triton X-100 and 2 mM $MgSO_4 \cdot 7H_2O$, and the glycerol content determined from the absorbance at 505 nm[16]. For the determination of free fatty acids, 300 μl aliquots of the incubation mixture were added to 3 ml of chloroform/heptane (1 : 1 v/v) containing 2% (v/v) methanol, and the liberated free fatty acids determined with copper reagent and bathocuproine[17]. The inhibitory activities of the cyclipostins were calculated from the combined results (glycerol and free fatty acid release) by plotting the log of the dose against the percent response (with the isoproterenol-induced lipolysis set at 100%). Data in the linear portion of the log(dose)-percent response curves were analyzed using parallel-line bioassay techniques.

Results

Fermentation[6,7] of *Streptomyces* sp. DSM 13381 in nutrient solutions containing 2% oat flakes and trace elements resulted in the formation of the polar cyclipostins A~F (Table 1), but in only low yield. Higher anti-HSL activities were achieved with a richer medium containing 5 g/liter glucose, 20 g/liter glycerol, 20 g/liter soy meal, 5 g/liter yeast extract, 3 g/liter NaCl, and trace elements, though the product spectrum no longer corresponded to the original distribution of the HSL inhibitors. The isolated HSL inhibitors were the more oxygen-poor components cyclipostins N to T2 (Table 1).

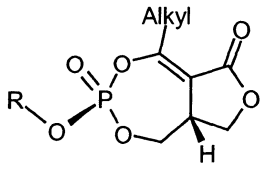
Isolation of the active principle from cultures of *Streptomyces* sp. DSM 13381 was hindered by the instability of the active, nonpolar compounds on silica gel and on reversed-phase columns under TFA-acidified conditions, which meant they could not be isolated in pure form. In neutral media, on the other hand, separation on reversed-phase solid supports was unsatisfactory. Isolation in pure form was ultimately achieved by the observation that the compounds were sufficiently stable to allow separation on reversed-phase supports between pH 3 and pH 4. Repeated chromatographic purification steps on various reversed-phase solid supports with different properties ultimately afforded the pure cyclipostins. Cultures of *Streptomyces* sp. DSM 13381 grown on oat flake medium yielded cyclipostins A~F, some of which were isolated. Thus, 5.4 mg of cyclipostin A and 2.5 mg of cyclipostin F were each obtained in pure form from the mycelium of 90 liters of fermentation solution.

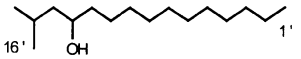
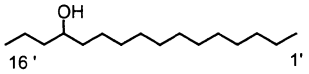
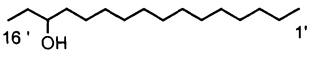
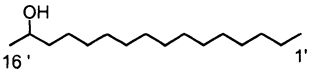
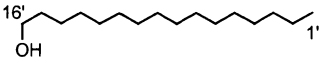
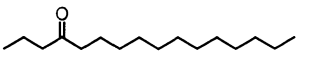
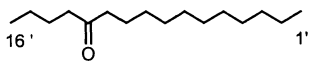
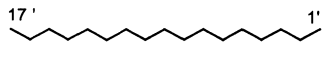
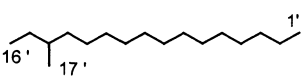
Culturing *Streptomyces* sp. DSM 13381 on glycerol/soy meal nutrient media gave the nonpolar cyclipostins N to T2 in comparatively higher yields of up to 2 mg/liter of cyclipostin P as the principal constituent, or 4 g in 2000 liters. However, under these fermentation conditions, the more highly oxidized cyclipostins A~F (Fig. 1) are absent. Isolated from 2 m^3 of culture solution were 16 mg cyclipostin N (oil), 1.3 g of crystalline cyclipostin P, approx. 3 g cyclipostin P2 (oil), 120 mg of crystallized cyclipostin R, 80 mg cyclipostin R2 (oil), 7 mg cyclipostin S, 47 mg cyclipostin T, and 44 mg cyclipostin T2 (solid).

The more oxygen-poor cyclipostins N to T2 are of similar polarity to triglycerides, which meant they could be separated from the inhibitor-containing fractions only with difficulty. The content of these neutral lipids was reduced by crystallization at 1°C in aqueous acetonitrile solution. Recrystallizations of the separated lipids afforded triglycerides with constant composition, f.p. 35~36°C. Both NMR spectra and GC determinations revealed approx. 10% 14:0 *iso*-, approx. 5% 14:0-, approx. 10% 15:0 *iso*, approx. 12% 15:0 *anteiso*, approx. 35% 16:0 *iso*, approx. 24% 16:0- and approx. 3.5% 17:0 *anteiso* fatty acids, a distribution that has been reported for the cell wall lipids of[17] of streptomycetes.

The more carbon-rich and the straight-chain cyclipostins are solids that can be crystallized, the hydroxylated and branched-chain HSL-inhibitors are oils. The solidification

Table 2. Structural formulae and some physicochemical data of the detected, but not preparatively isolated cyclipostins.



Compound	Empirical formula	M.W.	R (fatty alcohol)	Alkyl	HPLC* RT
Cyclipostin A2:	$C_{23}H_{41}O_7P$	460.55		methyl	12.5 min
Cyclipostin B	$C_{23}H_{41}O_7P$	460.55		methyl	12.6 min
Cyclipostin C	$C_{23}H_{41}O_7P$	460.55		methyl	12.6 min
Cyclipostin D	$C_{23}H_{41}O_7P$	460.55		methyl	12.6 min
Cyclipostin E	$C_{23}H_{41}O_7P$	460.55		methyl	12.6 min
Cyclipostin G	$C_{23}H_{39}O_7P$	458.54		methyl	13.3 min
Cyclipostin H	$C_{23}H_{39}O_7P$	458.54		methyl	13.3 min
Cyclipostin Q	$C_{24}H_{43}O_6P$	458.58		methyl	18.6 min
Cyclipostin Q3	$C_{24}H_{43}O_6P$	458.58		methyl	18.3 min

* The HPLC procedure is described in the Experimental section.
The fatty alcohol side chain is attached at $C^{1'}$ to the ester oxygen atom of the phosphoric acid. The structures of cyclipostins A2 and Q3 were determined by NMR, those of B~E by GC-MS, and those of cyclipostins G, H, and Q by ESI-MS.

point of the crystalline cyclipostin P is 58~59°C. Although the compound possesses two asymmetric centers it has a low optical rotation only. Cyclipostin P was shown by chiral HPLC to be a single component. The inhibitors are colorless and absorb UV light with a maximum at λ_{max} 226 nm (ε 11,000). The UV spectrum indicates the presence of an α,β-unsaturated carbonyl group; the principal features of the IR spectrum are absorbances at 1753 cm^{-1} (γ-lactone) and 1671 cm^{-1} (enol ether).

Structural Elucidation by NMR

The structural elucidation of all compounds is based on the analyses of various 2D-NMR experiments including DQF-COSY[18], HMQC[8], and HMBC[9] spectra. The unusual coupling patterns in the ^1H spectra and the splitting

Table 3. ^{1}H chemical shifts of isolated cyclipostins.

	A	F	N	P	P2	R	R2	S	T	T2
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.85	3.87	3.87
4	4.46/3.86	4.46/3.85	4.45/3.84	4.47/3.85	4.46/3.85	4.45/3.85	4.46/3.85	4.45/3.85	4.46/3.85	4.46/3.85
5	4.31/4.25	4.30/4.25	4.30/4.25	4.30/4.25	4.30/4.25	4.30/4.25	4.30/4.25	4.29/4.24	4.29/4.26	4.29/4.25
6	-	-	-	-	-	-	-	-	-	-
7	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.98/2.82	2.89/2.83	2.90/2.83
8	-	-	-	-	-	-	-	1.16	1.65	1.65
9	-	-	-	-	-	-	-	-	0.98	0.98
1'	4.25	4.25	4.24	4.24	4.24	4.24	4.24	4.25	4.25	4.24
2'	1.73	1.73	1.73	1.73	1.73	1.73	1.73	1.74	1.74	1.74
3'	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.42	1.42	1.42
4'-10'	1.37-1.26	1.37-1.27	1.35-1.26	1.34-1.29	1.34-1.29	1.37-1.25	1.37-1.25	1.34-1.29	1.34-1.29	1.37-1.25
11'	1.46-1.33	1.37-1.27	1.35-1.26	1.34-1.29		1.37-1.25	1.29	1.34-1.29	1.34-1.29	1.37-1.25
12'	3.49	1.53	1.35-1.26	1.34-1.29	1.29	1.37-1.25	1.16	1.34-1.29	1.34-1.29	1.29
13'	1.46-1.33	2.43	1.31	1.34-1.29	1.17	1.17	1.51	1.34-1.29	1.34-1.29	1.17
14'	1.37-1.26	-	0.89	1.34-1.29	1.52	1.30	0.87	1.34-1.29	1.34-1.29	1.52
15'	1.34	2.45		1.31	0.87	0.89	0.87	1.31	1.31	0.87
16'	0.91	1.00		0.89	0.87			0.89	0.89	0.87

of several signals in the ^{13}C spectra indicated the presence in the molecule of a phosphorus center. The correlations in the DQF-COSY and HMBC spectra led us to deduce a bicyclic core structure, as was previously reported for the acetylcholinesterase inhibitor cyclophostin[19], a major difference being that the *O*-methyl group of cyclophostin is replaced by various long-chain alkyl substituents. The chemical structure of the different side chains was deduced from correlations in the DQF-COSY and HMBC spectra, with the overall chain length (exact number of CH_2 groups) derived from the molecular weight. In the case of cyclipostin A, the position of the hydroxyl group was determined by MS fragmentation studies (see below). For cyclipostins S, T and T2, the signal of the methyl group C7 was missing; in these compounds the methyl substituent is replaced by an ethyl (S) or propyl group (T, T2), as demonstrated by correlations in the HMBC spectra and by the $^{13}C/^{31}P$ couplings (in all the cyclipostins C7 shows a $^{13}C/^{31}P$ coupling constant of between 4.5 and 5.0 Hz).

Mass Spectrometric Characterization of the Cyclipostins

Cyclipostins are easily ionized by ESI or FAB in the positive mode and their elemental compositions were determined using a high-resolution sector-field instrument (Table 5). For their structure elucidation, the protonated ions were subjected to collision-induced dissociations carried out using FTICR and/or triple quadrupole mass analyzers. The general fragmentation pattern of all the cyclipostins is similar (Scheme 1). By far the lowest energy path involves elimination of the alkyl side chain, yielding the bicyclic core **1**. As expected for cyclic phosphate-containing ions, the fragmentation of **1** is accompanied by extensive rearrangements, which renders further structural assignments impossible. However, it is possible to distinguish alkyl=Me from alkyl=Et, Pr on the basis of the relative fragment intensities (Table 2). For alkyl=Et, Pr, elimination of H_3PO_4 dominates, whereas for alkyl=Me, elimination of C_2H_2O, HPO_3, H_3PO_4 and $C_7H_6O_2$ occurs

Table 4. ^{13}C chemical shifts of isolated cyclipostins.

	A	F	N	P	P2	R	R2	S	T	T2
1	171.08 (1.7Hz)[a)]	171.07 (1.7 Hz)[a)]	171.07 (1.7 Hz)[a)]	171.08 (1.7 Hz)[a)]	171.05 (1.7 Hz)[a)]	171.06 (1.7 Hz)[a)]	171.06 (1.7 Hz)[a)]	170.87 (1.7 Hz)[a)]	170.98 (1.7 Hz)[a)]	170.98 (1.7 Hz)[a)]
2	114.61 (3.4 Hz)[a)]	114.58 (3.2 Hz)[a)]	114.60 (3.1 Hz)[a)]	114.60 (3.0 Hz)[a)]	114.60 (3.2 Hz)[a)]	114.58 (3.2 Hz)[a)]	114.58 (3.1 Hz)[a)]	113.66 (3.1 Hz)[a)]	114.39 (3.1 Hz)[a)]	114.40 (3.1 Hz)[a)]
3	40.75	40.76	40.74	40.74	40.74	40.75	40.75	40.77	40.78	40.78
4	66.04	66.04	66.03	66.05	66.02	66.04	66.03	66.04	66.02	66.02
5	69.39 (6.0 Hz)[a)]	69.40 (6.0 Hz)[a)]	69.39 (5.9 Hz)[a)]	69.40 (6.0 Hz)[a)]	69.38 (6.0 Hz)[a)]	69.40 (6.0 Hz)[a)]	69.39 (6.0 Hz)[a)]	69.17 (6.0 Hz)[a)]	69.23 (5.9 Hz)[a)]	69.23 (5.9 Hz)[a)]
6	161.47 (8.0 Hz)[a)]	161.48 (8.0 Hz)[a)]	161.47 (8.0 Hz)[a)]	161.47 (8.0 Hz)[a)]	161.46 (8.0 Hz)[a)]	161.48 (8.0 Hz)[a)]	161.47 (8.0 Hz)[a)]	165.80 (8.3 Hz)[a)]	164.69 (8.7 Hz)[a)]	164.69 (8.7 Hz)[a)]
7	17.89 (4.6 Hz)[a)]	17.90 (4.6 Hz)[a)]	17.90 (4.9 Hz)[a)]	17.90 (4.6 Hz)[a)]	17.90 (4.6 Hz)[a)]	17.90 (5.0 Hz)[a)]	17.90 (4.9 Hz)[a)]	25.05 (4.6 Hz)[a)]	33.35 (4.5 Hz)[a)]	33.35 (4.5 Hz)[a)]
8	-	-	-	-	-	-	-	10.86	20.63	20.63
9	-	-	-	-	-	-	-	-	13.84	13.84
1'	71.61 (6.6 Hz)[a)]	71.60 (6.6 Hz)[a)]	71.60 (6.6 Hz)[a)]	71.62 (6.9 Hz)[a)]	71.60 (6.9 Hz)[a)]	71.61 (7.0 Hz)[a)]	71.60 (6.9 Hz)[a)]	71.57 (6.9 Hz)[a)]	71.57 (6.6 Hz)[a)]	71.57 (6.9 Hz)[a)]
2'	31.16 (6.6 Hz)[a)]	31.15 (6.6 Hz)[a)]	31.16 (6.2 Hz)[a)]	31.16 (6.3 Hz)[a)]	31.16 (6.3 Hz)[a)]	31.16 (6.2 Hz)[a)]	31.16 (6.6 Hz)[a)]	31.19 (6.3 Hz)[a)]	31.18 (6.2 Hz)[a)]	31.18 (6.2 Hz)[a)]
3'	26.39	26.37	26.38	26.38	26.39	26.38	26.38	26.41	26.42	26.42
4'-10'	30.85-30.58	31.17-30.08	30.76-30.11	30.76-30.11	31.04-30.11	30.74-30.10	31.02-30.10	30.76-30.11	30.78-30.11	31.03-30.11
11'	38.44[b)]	31.17-30.08	30.76-30.11	30.76-30.11	31.04-30.11	30.74-30.10	28.51	30.76-30.11	30.78-30.11	31.03-30.11
12'	72.45	24.98	33.06	30.76-30.11	28.53	30.74-30.10	40.24	30.76-30.11	30.78-30.11	28.52
13'	38.15[b)]	43.09	23.72	30.76-30.11	40.25	33.06	29.15	30.76-30.11	30.78-30.11	40.25
14'	30.85-30.58	214.69	14.41	33.07	29.15	23.71	23.02	33.07	33.06	29.15
15'	23.84	36.54		23.72	23.04	14.40	23.02	23.73	23.72	23.03
16'	14.43	8.09		14.42	23.04			14.43	14.42	23.03

a) $^{13}C/^{31}P$ coupling constant
b) cannot be distinguished

with almost equal frequency. Loss of H_3PO_4 presumably involves abstraction of a proton; this process is more difficult from a primary carbon than from a secondary carbon center, which explains the relatively low degree of elimination from alkyl=Me.

The lack of basic sites in the cyclipostin molecules means that spectra in the ESI^- mode are weak and characterized by adducts such as $[M+HCO_2]^-$ and $[M+F_3CCO_2]^-$ rather than by simple deprotonated ions. MS/MS spectra in the negative mode are best studied with cyclipostins A~E, which form the most intense anions on account of their hydroxyl group. The anion [cyclipostin $A+HCO_2]^-$ breaks down *via* loss of the adduct ($-HCO_2H$), the side chain ($-C_{16}H_{34}O$, $-HCO_2H$), and the rearranged core ($-HCO_2H$, $-CO_2$), ($-HCO_2H$, $-C_7H_6O_2$), ($-C_7H_6O_2$, $-H_2O$). The anionic bicyclic core $[\mathbf{1}-2H]^-$ gives exclusively PO_3^- upon fragmentation. The anionic fragmentation processes are thus very similar to those obtained in the positive mode.

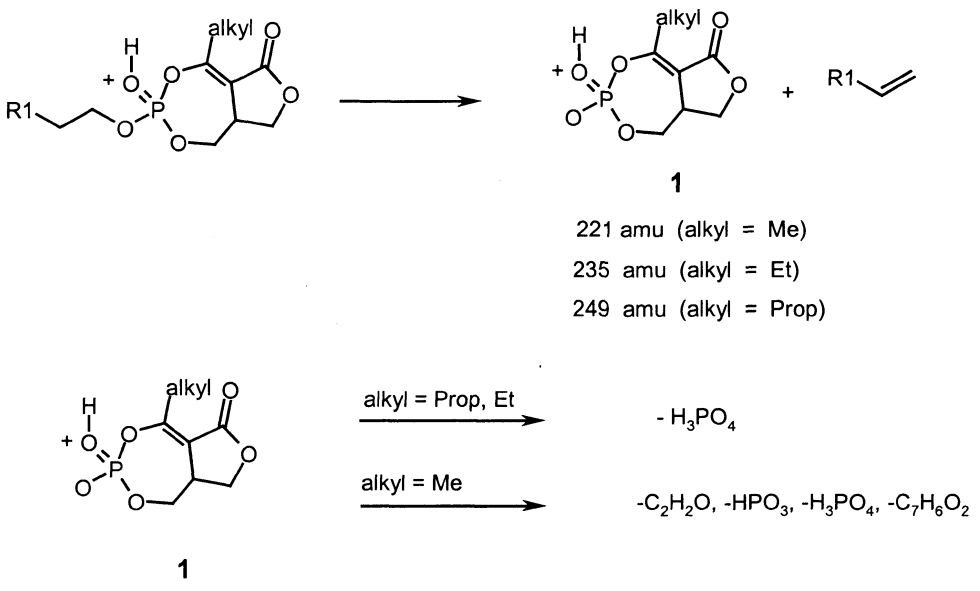
In all the oxidized cyclipostin derivatives, the oxidation

Table 5. Theoretical and experimental monoisotopic masses of the quasimolecular ions, and characteristic ESI+ fragment masses of cyclipostins.

Compound	Theor. mass in amu	Exp. mass in amu	Characteristic ESI^+ fragment masses
Cyclipostins A-E	$m(C_{23}H_{41}O_7PLi) = 467.2750$	467.2757	443, 221, 179, 141, 123, 99
Cyclipostins F-H	$m(C_{23}H_{40}O_7P) = 459.2512$	459.2515	239, 221, 179, 141, 123, 99
Cyclipostin N	$m(C_{21}H_{38}O_6P) = 417.2406$	417.2405	221, 179, 141, 123, 99
Cyclipostin P	$m(C_{23}H_{42}O_6P) = 445.2719$	445.2717	221, 179, 141, 123, 99
Cyclipostin P2	$m(C_{23}H_{42}O_6P) = 445.2719$	445.2721	221, 179, 141, 123, 99
Cyclipostin Q	$m(C_{24}H_{44}O_6P) = 459.2875$	459[a)]	221[a)]
Cyclipostin R	$m(C_{22}H_{40}O_6P) = 431.2563$	431.2561	221, 179, 141, 123, 99
Cyclipostin R2	$m(C_{22}H_{40}O_6P) = 431.2563$	431.2564	221, 179, 141, 123, 99
Cyclipostin S	$m(C_{24}H_{44}O_6P) = 459.2875$	459.2883	235, 137, 119, 109
Cyclipostin T	$m(C_{25}H_{46}O_6P) = 473.3032$	473.3030	249, 151, 133, 109
Cyclipostin T2	$m(C_{25}H_{46}O_6P) = 473.3032$	473.3035	249, 151, 133, 109

[a)] Only normal ESI-MS measured.

Scheme 1. General fragmentation pattern of cyclipostins.



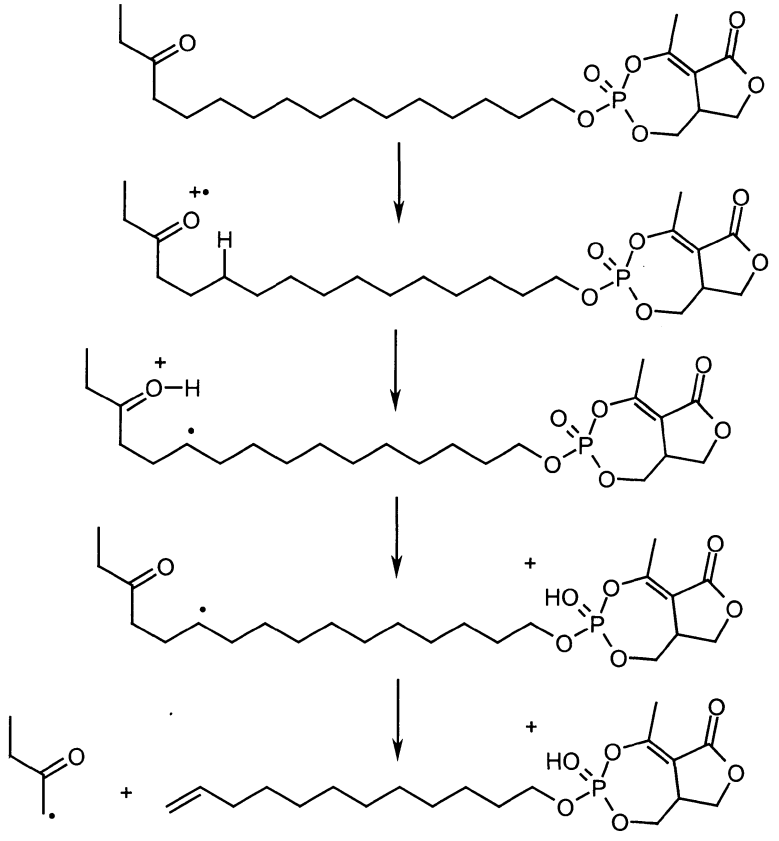
center is located in the side chain, as demonstrated by ESI-MS/MS experiments. However, ESI-MS/MS failed to establish the exact position of oxidation, therefore EI experiments were used to induce odd-electron fragmentations.

In the case of ketone derivatives, cleavage in the β-position to the keto group indicates its location in the chain. This process is similar to a McLafferty rearrangement, except that a hydrogen atom is transferred to the neighboring basic phosphate group prior to cleavage (Scheme 2). On the basis of the transformations outlined in Scheme 2, a mixture of three components with oxo groups

Table 6. Relative ion intensities in MS^3 spectra of selected fragments in cyclipostins P, S, and T.

Cyclipostin	Alkyl	MS^3 precursor mass		Neutral loss					
			Precursor ion	C_2H_2O	C_3H_6O	C_4H_8O	HPO_3	H_3PO_4	$C_7H_6O_2$
P	Me	221	100	25			25	30	20
S	Et	235	65		3			100	
T	Pr	249	90			1		100	

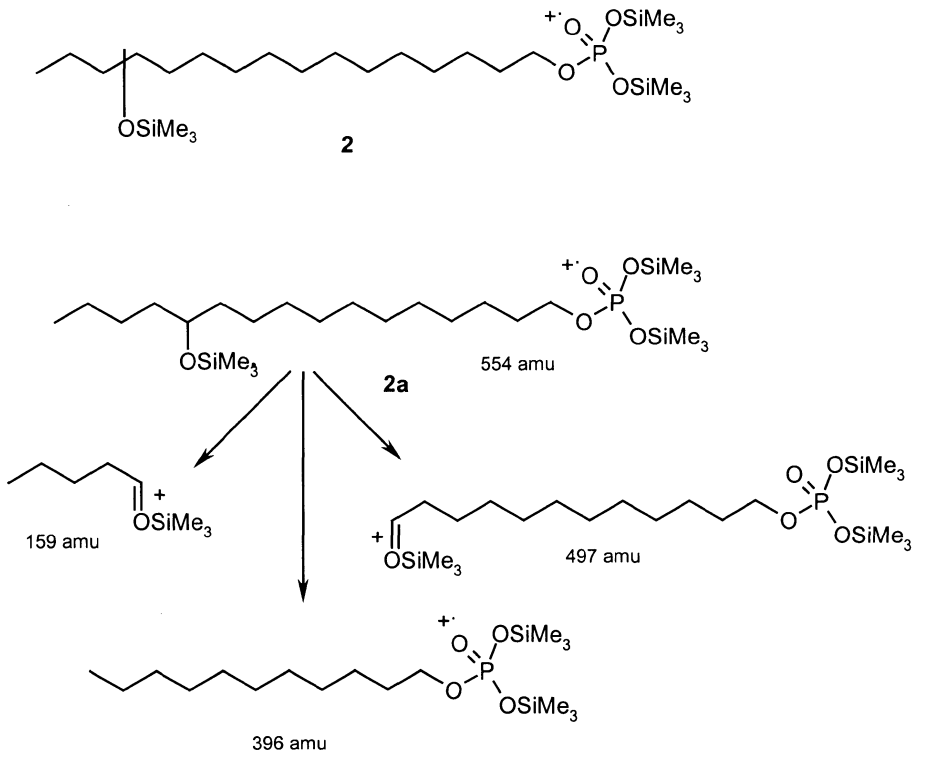
Scheme 2. Proposed mechanism for EI fragmentation of ketones.



at C14 (cyclipostin F, 63%), C13 (cyclipostin G, 10%), and C12 (cyclipostin H, 27%) was identified.

Alcohols were derivatized to improve their volatility prior to analysis. Silylation using *N*-methyl-*N*-(trimethylsilyl)trifluoracetamide (MSTFA) followed by GCMS was accompanied by breakdown of the bicyclic structure, with formation of the silylated alcohols **2**. However, we were able to establish unambiguously the position of the hydroxy group in **2** from the α-cleavage reactions that occur either side of the hydroxy group with loss of an enol ether moiety. For example, fragmentation of **2a** (554 amu) on electron ionization gave fragments at m/z=497, 159, and 396 amu, indicating that the oxidation site is C12 (Scheme 3). The GCMS chromatogram contained four species corresponding to the structure **2**, with relative intensities of 83.1% (cyclipostins A and A2,

Scheme 3. Proposed mechanism for EI fragmentation of derivatized alcohols.



hydroxy group at C12), 10.3% (cyclipostin B, C13), 6.3% (cyclipostin C, C14), and 0.4% (cyclipostin E, C16).

In summary, mass spectrometric high-resolution and fragmentation experiments give easy access to mainly four kinds of information on cyclipostins: (i) the molecular formula, (ii) the length of the alkyl side chain, (iii) the position of hydroxy and oxo groups in the side chain, and (iv) differentiation between R_2=Me and R_2=Et, Pr.

Biological Properties

As can be seen from the data shown in Table 7, cyclipostins inhibit hormone-sensitive lipase at nanomolar concentrations. We observed reasonable coincidence of the IC_{50} values for inhibition of HSL (determined using both glyceryl trioleate and cholesteryl oleate as the substrate) in the cell-free assay system (Table 7) and for inhibition of HSL in intact isolated rat adipocytes, measured as release of either glycerol or fatty acids, the lipolysis products: the cyclipostins A, P, P2, and R inhibit the lipolysis in rat adipocytes with $IC_{50} \leq 0.2\,\mu M$ (Table 8). In the cellular assay system, cyclipostins do not interfere with stimulation of lipolysis by adenosine deaminase and isoproterenol (Fig. 2). Furthermore, cyclipostins are able to completely block lipolysis (total glycerol and fatty acid release) from adipocytes in the basal (data not shown) and stimulated state (Fig. 2). These data are strong evidence for direct inhibition of HSL rather than interruption of the signaling cascade leading to activation of HSL. The similar activity of the various cyclipostin components between inhibition of HSL in the cell-free and cellular systems and only moderate loss of potency in intact rat adipocytes compared with the partially purified rat HSL suggest that cyclipostins cross the plasma membrane with high efficiency. Efficient membrane transfer of cyclopostins would also explain the observation that inhibition of lipolysis in rat adipocytes by cyclipostins, as measured by the apparent IC_{50} values, was not enhanced by increasing the total incubation and assay time, as reflected in the approximately linear increments of glycerol release with time at each inhibitor concentration (Fig. 2) and the constancy in the calculated IC_{50} values for the different incubation times. Neither did the duration of preincubation of glyceryl trioleate with the cyclipostins (>5 minutes) prior to the start of the cleavage reaction have any significant effect on the cell-free assay system (data not given). Furthermore, inhibition of lipolysis by

Table 7. IC_{50} values for inhibition of rat HSL by various cyclipostins.

Cyclipostin	IC_{50}
Cyclipostin A	20 nM
Cyclipostin P	30 nM
Cyclipostin P2	40 nM
Cyclipostin S	20 nM

Various cyclipostin concentrations (2 nM~10 μM) were preincubated with the substrate glyceryl trioleate or cholesteryl oleate prior to initiation of the cleavage reaction by addition of partially purified rat HSL as described in the Experimental section. IC_{50} values were calculated from the fitted inhibition curves as the mean (n=4).

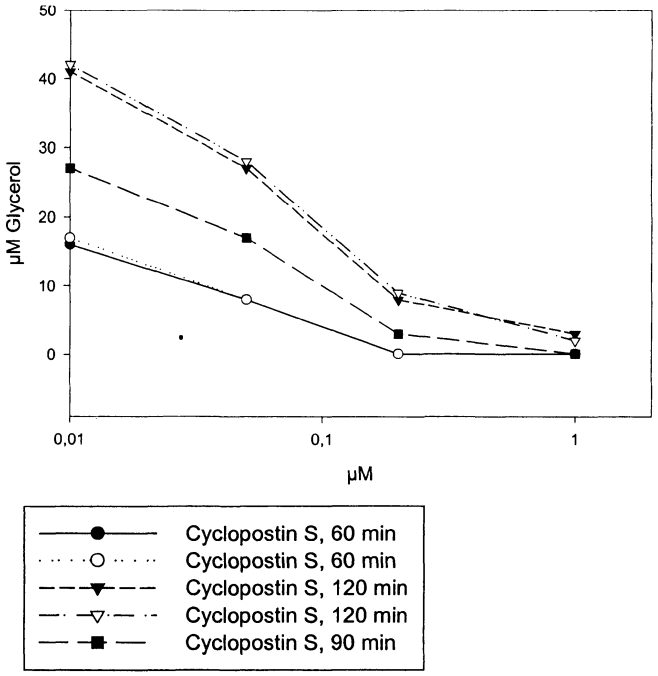
Table 8. IC_{50} values for inhibition of lipolysis by various cyclipostins in isolated rat adipocytes.

Cyclipostin	IC_{50}
Cyclipostin A	190 nM
Cyclipostin P	85 nM
Cyclipostin P2	150 nM
Cyclipostin S	40 nM

Various cyclipostin concentrations (10 nM~10 μM) were preincubated with isolated rat adipocytes in the presence of adenosine deaminase prior to stimulation of lipolysis with 1 μM isoproterenol. After incubation, the amounts of glycerol and fatty acids released from the cells were determined as described in the Experimental section. IC_{50} values were calculated from the fitted inhibition curves as the mean (n=3).

cyclipostin P in intact rat adipocytes was not significantly reduced after several cycles of washing (which lowers the active substance concentration in the incubation medium by a factor of about 1000) and subsequent challenge of the cells with isoproterenol. Taken together, these findings make cyclipostins attractive candidates as lead compounds for the development of drugs for the treatment of metabolic disturbances associated with increased peripheral lipolysis, such as metabolic syndrome and type II diabetes.

Fig. 2. Effect of incubation time on the inhibitory potency of cyclipostins in lipolysis.



Isolated rat adipocytes were incubated in the presence of adenosine deaminase (open symbol) or isoproterenol (filled symbol) with various concentrations of cyclipostin S for the periods indicated, followed by determination of glycerol.

Discussion

A number of lipase inhibitors have previously been reported in the literature: esterastin[20], ebelactone[21], lipstatin[22], valilactone[23], and the panclicins[24] are β-lactones with long-chain alkyl substituents. They act as esterase inhibitors and-on account of their strongly inhibitory action on pancreatic lipase-a derivative, tetrahydrolipstatin[25], has now risen to prominence in the treatment of obesity.

The cyclipostins differ from previously described lipase inhibitors in their basic chemical framework. The new inhibitors of hormone sensitive lipase are neutral cyclic enol phosphate esters with a fused γ-lactone ring, and show no structural similarity to the known compounds. The bicyclic phosphorus-containing ring has up to now been described only for the compound cyclophostin[19] isolated from *Streptomyces lavendulae*. Cyclophostin which harbors a phosphoric acid methyl ester and exhibits strong anti-acetylcholinesterase activity. In place of this methoxy

substituent, the cyclipostins have a long-chain fatty alcohol residue, which gives the new compounds their high anti-HSL activity.

Likewise little known is the phosphorus-free carbon skeleton of the cyclipostins. The phosphorus-free hydrolysis products of the cyclipostins, 2-acetyl- and 2-*n*-butyryl-3-hydroxymethyl-butyrolactone, natural products given the names 1718-E and 1718-E/1[26], were isolated from *Streptomyces antibioticus* ssp. *antibioticus* Tü 1718 and evaluated as A-factors. These butanolides, the syntheses of which were reported in 1988[27], were found to act as weak inductors of virginiamycin production during fermentation of *Streptomyces virginiae*. Aside from this, there have been no other literature reports of this simple chemical structure type.

A point to note here is the striking similarity of the cyclipostins to the neutral lipids isolated from the same culture of *Streptomyces* sp. DSM 13381. The solubility properties of the cyclipostins, their polarity, and their melting points are so similar to those of the strain-specific triglycerides, it is tempting to suggest that some form of molecular mimicry must be in operation.

The outstanding property of the cyclipostins is their anti-HSL activity at the level of both the partially purified enzyme and isolated adipocytes. They inhibit rat HSL and adipocyte lipolysis in sub-micromolar concentrations. Preliminary data demonstrate an anti-HSL and anti-lipolytic activity of the cyclipostins with regard to the partially purified human enzyme and primary human adipocytes of similar potency as for the rat counterparts. Thus, cyclipostins may therefore be suitable as agents for control of the physiologically important lipolysis process and thus for the treatment of diabetes mellitus.

The extraordinary activity of the recently discovered cyclipostins raises the question of how secondary metabolites of streptomycetes are able to cause strong and selective inhibition of enzymes of phylogenetically far-removed species such as rats and humans. The genome structure of human hormone-sensitive lipase[28] has been characterized and shows sequence homologies with certain bacterial proteins[29]. Similarities exist with respect not only to *Moraxella* and *E. coli* lipases, but also to *N*-acetylhydrolases from *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*, a notable common feature being the catalytic center -Gly-Asp-Ser-Ala-Gly-. Together with certain other proteins, the abovementioned enzymes comprise a special group of esterases and lipases that differ markedly from other hydrolases. The regulatory function in respect of the metabolism of the production organism that is attributed to microbial secondary metabolites[30] may explain the presence in cultures of *Streptomyces* sp. DSM 13381 of the HSL-inhibiting cyclipostins.

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