VOL. 52, NO. 3, MAR. 1999 THE JOURNAL OF ANTIBIOTICS pp. 245 — 255

Glucolipsin A and B, Two New Glucokinase Activators Produced by *Streptomyces purpurogeniscleroticus* and *Nocardia vaccinii*

Jingfang Qian-Cutrone*, T. Ueki, Stella Huang, Kasim A. Mookhtiar[†],
Regina Ezekiel, Steven S. Kalinowski[†], Karen S. Brown[†], Jerzy Golik,
Susan Lowe, Dolores M. Pirnik, Robert Hugill, Judy A. Veitch,
Steven E. Klohr, Jeffrey L. Whitney and Susan P. Manly

Bristol-Myers Squibb Pharmaceutical Research Institute,
5 Research Parkway, Wallingford, Connecticut 06492, U.S.A.
[†]Bristol-Myers Squibb Pharmaceutical Research Institute,
Route 205 & Provinceline Road, Princeton, NJ 08543, U.S.A.

(Received for publication January 12, 1999)

During the screening of the natural products for their ability to increase the activity of glucokinase by relieving inhibition by long chain fatty acyl CoA esters (FAC), two novel compounds, glucolipsin A (**1**) and B (**2**) were isolated from the butanol extracts of *Streptomyces purpurogeniscleroticus* WC71634 and *Nocardia vaccinii* WC65712, respectively. The structures of these two compounds were established by spectroscopic methods and chemical degradation. Glucolipsin A (**1**) and B (**2**) relieved the inhibition of glucokinase by FAC with RC_{50} values of 5.4 and 4.6 μM.

One of the principal enzymes of glucose phosphorylation in glucose sensitive cells is glucokinase. Glucose phosphorylation influences circulating blood glucose levels, making glucokinase regulation one of the principle points for possible therapeutic intervention in diabetes. Long chain fatty acyl CoA esters (FAC) inhibit glucokinase activity allosterically and competitively with respect to glucose *in vitro*, while glucose itself activates it by a "mnemonic" mechanism[1]. The effect of FAC *in vivo* is as yet unknown. Compounds that ameliorate the inhibition of glucokinase by its negative allosteric effectors would "activate" the glucokinase activity in the cell. Such "activators" might bind to the fatty acyl CoA esters cofactor site or might sequester these negative effector molecules. We report here the discovery of novel compounds exhibiting such novel sequestering activity, thereby appearing to be glucokinase "activating" small molecules.

During the screening of natural products for their ability to relieve the inhibitory effects of stearoyl-CoA on glucokinase, the butanol extracts of *Streptomyces purpurogeniscleroticus* WC71634 and *Nocardia vaccinii* WC65712 were found to be active. When subjected to bioassay-guided fractionation, two novel, closely-related metabolites, designated as glucolipsin A (**1**) and B (**2**), were isolated as the major active components from the butanol extracts *Streptomyces purpurogeniscleroticus* and *Nocardia vaccinii*, respectively. The structures of glucolipsin A and B differ from one another only by one methylene group in the side chain. This report describes the fermentative production, isolation, structure elucidation and biological activities of these two compounds.

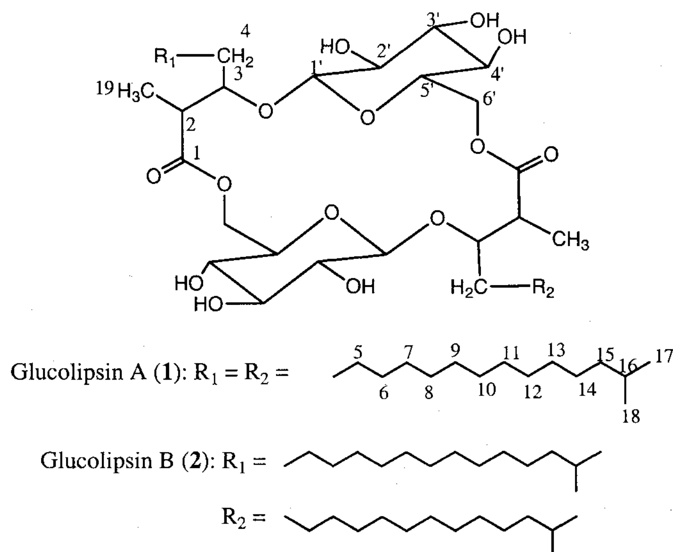
Materials and Methods

Taxonomy

Strain WC71634 and WC65712 are actinomycetes isolated by Mr. Yutaka Hoshino from soil collected in Bryce Canyon, Utah. Microscopic studies on strains WC71634 and WC65712 were carried out on ISP morphology media (ISP #2, ISP #3, ISP #4, ISP #5 and ISP #7) and observed at 7, 14 and 21 days incubation at 28°C according to the International Streptomyces Project Manual recommendations[2].

Spore chain morphology of WC71634 is spiral on

Fig. 1. Structures of glucolipsin A (**1**) and B (**2**).



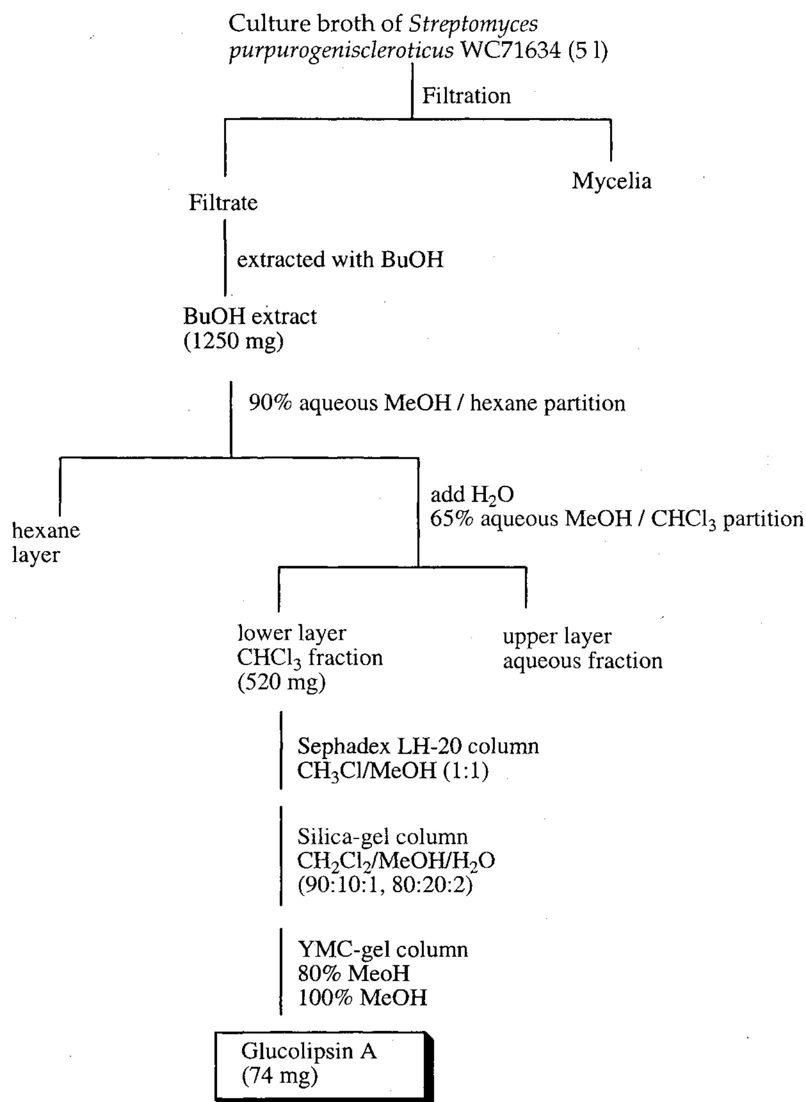
salts - starch agar (ISP #4), and yeast - malt agar (ISP #2). Sporulation is thin on these media and absent on Oatmeal agar (ISP #3). The spore surface is smooth. Sclerotia are present in salts - starch agar and yeast - malt agar after 14 days incubation. Aerial mass color is in the White Series (CHM#13ba, Alabaster Tint; ISCC-NBS #231, Purplish white)[3]. Colony reverse is yellow/brown on salts - starch agar; reddish/brown on yeast - malt agar and glycerol - asparagine agar. Melanoid pigments are not produced on tyrosine agar and the modified Arai and Mikami melanin formation test was also negative[4]. The amino acid components of the cell wall are L-diaminopimelic acid (DAP) and alanine and the sugar components are galactose, arabinose and ribose. Carbon utilization studies showed that glucose, mannitol, arabinose, fructose, inositol, lactose, sucrose, rhamnose, galactose, ribose, xylose, raffinose, salicin and melibiose were all utilized for growth when incorporated into ISP #9 as the sole carbon source. Based on the above characteristics, this organism was identified as *Streptomyces purpurogenisscleroticus*.

Strain WC65712 forms yellow cream colored colonies with white aerial mycelium which fragments into bacillary and coccoid elements. Colony reverse is yellow/brown on yeast - malt agar (ISP #2), asparagine agar (ISP #5) and tyrosine agar (ISP #7). Diffusible pigments form purple on salts - starch agar (ISP #4) and brown on tyrosine agar (ISP #7). Melanoid pigments are not formed on tyrosine agar and are not detected by the modified Arai and Mikami melanin formation test[3]. The amino acid component of the cell wall is *meso*-DAP plus alanine and aspartic acid. The sugar components are galactose, arabinose and ribose. Carbon utilization studies showed that glucose, mannitol, glycerol, arabinose, fructose, innositol, lactose, rhamnose, galactose, xylose and melibiose were utilized for growth when incorporated into inorganic salts agar (ISP #9) as sole carbon sources. Based on the above characteristics, this organism has been identified as a strain of *Nocardia vaccinii*[5].

Fermentation

Both cultures, WC71634, *Streptomyces purpurogenisscleroticus*, and WC65712, *Nocardia vaccinii*, were grown in test tubes on agar slants which consisted of the following per liter of distilled water: Japanese soluble starch, 5 g; glucose, 5 g; fish meat extract, 1 g; yeast extract, 1 g; N-Z case, 2 g; NaCl, 2 g; $CaCO_3$, 1 g; agar, 15 g. Both cultures were incubated at 32°C for 14 days and then the surfaces were swabbed into 50 ml of vegetative medium in a 250 ml flask, which contained the following per liter of distilled water: Japanese potato starch, 20 g; dextrose, 5 g; N-Z case, 3 g; yeast extract, 2 g; fish meat extract, 5 g; $CaCO_3$, 3 g. The flasks were incubated at 32°C at 250 rpm on a gyrotary shaker. Frozen vegetative preparations were prepared by mixing a culture grown for 3 days in the vegetative medium with

Fig. 2. Isolation and purification procedures of glucolipsin A (1).



an equal volume of 20% (w/v) glycerol/10% (w/v) sucrose, and aliquots frozen in a dry ice-acetone bath, and stored at −80°C. From the frozen stock, 4 ml was used as an inoculum into 100 ml of the vegetative medium described above in a 500 ml flask. Both cultures were grown for 3 days at 32°C at 250 rpm and then 4 ml was used to inoculate 100 ml of the production medium in a 500 ml flask. The production medium for WC71634, *Streptomyces purpurogenisscleroticus*, contained the following per liter of distilled water: Japanese potato starch, 15 g; fructose, 10 g; distillers solubles, 5 g; fish meat extract, 2 g; $MgSO_4 \cdot 7H_2O$, 3 g; $CaCO_3$, 5 g. The production medium of WC65712, *Nocardia vaccinii* contained the following per liter of distilled water: glycerol, 20 g; beet molasses, 10 g; Pharmamedia, 10 g; $CaCO_3$, 3 g. In both cases, the pH was adjusted to 6.5 prior to autoclaving. Both cultures were incubated for 6 days at 32°C at 250 rpm.

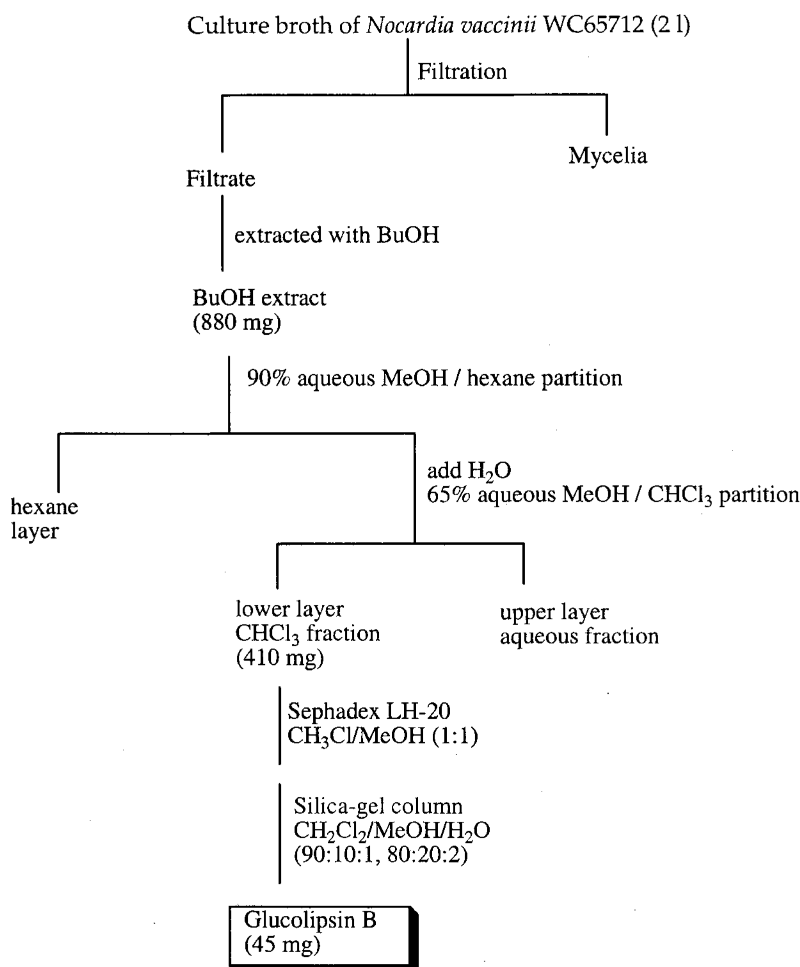
Extraction and Isolation

The isolation and purification procedures of glucolipsin A (**1**) and B (**2**) are illustrated in Fig. 2 and Fig. 3, respectively.

Alkaline Hydrolysis of 1

A 3 ml 2% NaOH solution was added to a solution of 15 mg of **1** dissolved in 3 ml methanol. The solution was stirred at room temperature for 6 hours. The reaction

Fig. 3. Isolation and purification procedures of glucolipsin B (**2**).



was monitored by TLC (Si-gel, CH_2Cl_2-MeOH-H_2O 90:10:1). The reaction mixture was diluted with water, neutralized to pH 7 with 0.1 N HCl and evaporated. The aqueous solution was then extracted with *n*-butanol. The extract was then concentrated *in vacuo* to dryness and purified by preparative TLC (silica, CH_2Cl_2-MeOH-H_2O 90:10:1) to give white hygroscopic powder **3** (8 mg).

3: ^{1}H-NMR (500 Hz, methanol-d_4) δ 4.39 (1H, d, J=7.7 Hz), 3.88 (1H, m), 3.81 (1H, dd, J=11.7 and 2.3 Hz), 3.69 (1H, dd, J=11.7 and 5.0 Hz), 3.38 (3H, s), 3.36 (1H, t, 9.8 Hz), 3.32 (1H, dd, J=9.1 and 7.8 Hz), 3.29 (1H, m), 3.18 (1H, t, J=9.7 Hz), 2.54 (1H, m), 1.50~1.59 (5H, m), 1.25~1.39 (18H, m), 1.16 (2H, m), 1.09 (3H, d, J=7.0), 0.85 (6H, d, J=6.7 Hz). ^{13}C-NMR: see Table 2.

Acid Methanolysis of **3**

A solution of **3** (7 mg) in 1 N HCl-MeOH (3 ml) was stirred overnight at room temperature. The reaction was monitored by TLC (Si-gel, CH_2Cl_2-MeOH-H_2O 90:10:1). The reaction mixture was diluted with water and then extracted with ether. Evaporation of the ethereal extract gave a white amorphous powder of **4** (2 mg). ^{13}C-NMR of **4**: see Table 2.

Instrumental Analyses

The UV spectrum was taken on a Shimadzu UV2100 spectrophotometer; the IR spectrum was recorded on a Perkin Elmer FT-IR 1800 spectrometer; Electrospray mass spectra (MS) were taken on a Finnigan TSQ7000 triple quadruple mass spectrometer, the high resolution FAB-MS analysis was performed with a Kratos MS50 mass spectrometer, and all ^{1}H- ^{13}C-NMR spectra

including COSY, HETCOR, COLOC and HMBC were taken on a Bruker AM-500 spectrometer (1H, 500 MHz; ^{13}C, 125 MHz).

Screen for Compounds Relieving Inhibition of Glucokinase by Stearoyl CoA

The screen for glucokinase activity involves spectrophotometric analysis of an NAD^+ analogue (thionicotinamide adenine dinucleotide; thio-NAD) in a coupled enzyme system[6]. This system employs the use of glucose 6-phosphate dehydrogenase from Leuconostoc mesenteroides as the coupling enzyme. The glucose-6 phosphate formed from glucose and ATP by glucokinase is oxidized to glucose-6-phosphate-lactone by glucose 6-phosphate dehydrogenase using NAD^+ analogue as the cofactor. This analogue, when reduced, yields a compound that absorbs maximally at 405 nm. The enzyme is diluted in 25 mM HEPES, pH 7.1 to yield an increase at 0.3 absorbance units at 405 nm for 30 minutes at 37°C. Standard reaction conditions were as follows: 40 μl 62 mM HEPES buffer, pH 7.1, 7.5 mM $MgCl_2$, 10 mM DTT, 10 μl 50 mM ATP, 10 μl glucose 6-phosphate degydrogenase (2 units/ml), 10 μl of glucokinase solution, 10 μl of 20 μM stearoyl-CoA, 10 μl of sample and 10 μl 5 mM thio-NAD solution including 50 mM glucose, was incubated for 30 minutes at 37°C. The amount of NADH converted from NAD^+ was measured by visible spectrophotometry at 405 nm in THERMO max microplate reader at 0 minute and 30 minutes.

Every 96-well microtiter plate contained eighty test sample, eight control samples (no stearoyl-CoA and no test samples) and eight blanks (no test samples). Any samples that were activated 80% or more relative to the control value (GK plus stearoyl-CoA) were re-tested and activation % values confirmed.

$$\text{Activation } \% = \frac{\text{Test sample } \Delta T\ (30\ \text{min} \sim 0\ \text{min}) - \text{Blank } \Delta T\ (30\ \text{min} \sim 0\ \text{min})}{\text{Control } \Delta/T\ (30\ \text{min} \sim 0\ \text{min}) - \text{Blank } \Delta T\ (30\ \text{min} \sim 0\ \text{min})}.$$

Secondary Assay for Fatty Acyl Coenzyme A

Since fatty acyl CoA esters are reactive compounds by virtue of the reactive thioester group, it is possible that compounds react with FACs and deplete free FAC concentrations. Furthermore, FAC could also be sequestered by compounds in a tight complex such as that produced by the sequesterants, cyclodextrins. In order to determine whether these mechanisms of action of compounds were in play in the glucokinase assays, FAC concentrations were measured after incubation with the compounds under glucokinase activity assay conditions. The assay used was a coupled assay using FAC oxidase and horse radish peroxidase. Fatty acyl-coenzyme A oxidase (FACO) oxidizes fatty acid CoA esters at the beta position, generating hydrogen peroxide. Hydrogen peroxide production is monitored by measuring the horse-radish peroxidase (HRP)-dependent generation of dichlorofluorescein from dihydrodichlorofluorescein (Molecular Probes, OR), using an absorbance change at 502 nm. HRP and FAC oxidase from *Arthrobacter* sp. were from Sigma Chemical Co. (St. Louis, MO). A 2 mM stock of dihydrodichlorofluorescein diacetate (DCFDA) was prepared in 95% ethanol and stored at −20°C wrapped in foil. DCFDA is pretreated with weak base to hydrolyze the acetate groups and generate the actual substrate (which is very sensitive in its susceptibility to autooxidation). One volume of the 2 mM DCFDA is treated with four volumes (combined volumes above) 25 mM $NaPOP_4$, pH 7.4. HRP stock (3750 U/ml) was prepared in 25 mM $NaPO_4$, pH 7.4 and stored at 4°C (stable for eight months). FAC oxidase stock (200 U/ml) was prepared in 25 mM $NaPO_4$, pH 7.4 and stored at −20°C in aliquots. FACO samples were thawed just prior to use. The 10× assay buffer was composed of 250 mM HEPES, pH 7.1～7.3, 50 mM $MgCl_2$, 50 mM D-glucose, and 25 mM ATP.

Two protocols were used for these assays that differed only in the amount of FAC oxidase and HRP used. When the total amount of FAC was to be estimated, *i.e.* to check if any FAC had reacted with the compound, the final concentration of FAC oxidase was 0.2 units/ml and HRP was 7.5 units/ml. In this protocol, called the end point assay, the reaction was run by adding water, 10× assay buffer, compound or vehicle and stearoyl-CoA to reaction tubes in the order given. A standard curve was run for stearoyl-CoA (0, 0.5, 1, 2, 3 and 5 μM final concentrations). All compounds were tested at 5 and 25 μM against 2 μM stearoyl-CoA. Tubes were incubated at 37°C for 15 minute "preincubation". DCFDA was added with a multipipettor (final concentration 16 μM). Enzyme mix (10 μl) to give final concentration given above was added to tubes at 15 second intervals. Tubes were sealed and vortexed and placed at 37°C at 15 second intervals so that the incubation times would be constant. After the 15 minutes incubation, tubes were removed, contents placed in cuvettes and absorbance at 502 nm was measured using a Varian Cary 3 spectrophotometer Since the blanks change with time, the precise incubation period is critical. Compounds were tested by the same assay for the inhibition of HRP by using added hydrogen peroxide.

Table 1. Phisico-chemical properties of glucolipsin A (**1**) and B (**2**).

	1	**2**
Appearance	White powder	White powder
Molecular formula	$C_{50}H_{92}O_{14}$	$C_{49}H_{90}O_{14}$
HRFAB-MS (*m/z*) $(M+Na)^+$	939.6366	925.6196
$[\alpha]_D$ (25°C)	+11.39 (*c* 0.05, CH_3OH)	
IR (KBr) (cm^{-1})	3350, 2923, 2853, 1734, 1652, 1464, 1361, 1310, 1278, 1185, 1076, 1046, 1016	3364, 2924, 2854, 1734, 1646, 1464, 1364, 1311, 1279, 1186, 1076, 1047, 1016

When simple sequestration was to be tested a kinetic assay measuring the initial rate of stearoyl CoA oxidation was used. In this assay, called the kinetic assay, the FAC oxidase was reduced to 0.002 ~ 0.005 units/ml, while HRP was increased to ~11 units/ml. These amounts resulted in measurable, reproducible rates. The assay is run exactly as the earlier assay up until the preincubation step. After this step, the reaction mix is transferred to a cuvette, HRP and DCFDA added, and the mix incubated in the spectrophotometer at 37°C to obtain a stable blank rate. FAC oxidase is finally added and the rate of change of absorbance at 502 nm monitored for 3 minutes. Rates were calculated after subtraction of the blank rate. The percent ratio of the rate in presence of compound to the rate in the absence of compound was used as % inhibition.

Results and Discussion

Isolation

The fractionation of the fermentation broths of *Streptomyces purpurogenisscleroticus* WC71634 and *Nocardia vaccinii* WC65712 were monitored by the glucokinase activation assay, and the detailed procedures are illustrated in Fig. 2 and Fig. 3. Briefly, the fermentation broths were divided into filtrates and mycelia by filtration. The filtrates were extracted with butanol. The butanol extracts were processed by sequential solvent partition against hexane and prequibrated chloroform. In both cases, the chloroform fractions were active. From chloroform fraction of *Streptomyces purpurogenisscleroticus* WC71634, glucolipsin A was isolated by column chromatography on Sephadex LH-20, silica gel and finally YMC gel, while glucolipsin B was purified from the chloroform fraction of *Nocardia vaccinii* WC65712 by column chromatography on Sephadex LH-20, and subsequently on silica gel.

Physico-chemical Properties

Glucolipsin A and B were obtained as amorphous white solids. Both substances were soluble in methanol, chloroform, methylene chloride, and insoluble in water. Some important physico-chemical properties of these two compounds are summarized in Table 1. The ^{1}H- and ^{13}C-NMR spectra of glucolipsin A, which are almost identical to those of glucolipsin B, are reproduced in Fig. 4, 5.

Structure Elucidation

Glucolipsin A: (**1**)

High resolution FABMS analysis revealed that **1** has a molecular formula of $C_{50}H_{92}O_{14}$, with 5 degrees of unsaturation. The IR spectrum showed absorption bands at 3364 and 1734 cm^{-1}, which are characteristic of hydroxyl and ester groups. The presence of an ester carbonyl group was confirmed by the ^{13}C-signal at δ 175.7 in the ^{13}C-NMR spectrum. The ^{1}H- and ^{13}C-NMR spectra further revealed the presence of a sugar moiety and a fatty acid fragment. Furthermore, three methyl groups, one oxymethine, two methines and a number of methylene groups (around twelve) in the fatty acid portion were readily indicated. The signals arising from these methylene groups were heavily overlapped in both ^{1}H-, and ^{13}C-NMR spectra. The above mentioned structural fragments were accounted for only about half of its molecular weight, thus, the compound should be

Fig. 4. ^{1}H-NMR spectrum of glucolipsin A (**1**).

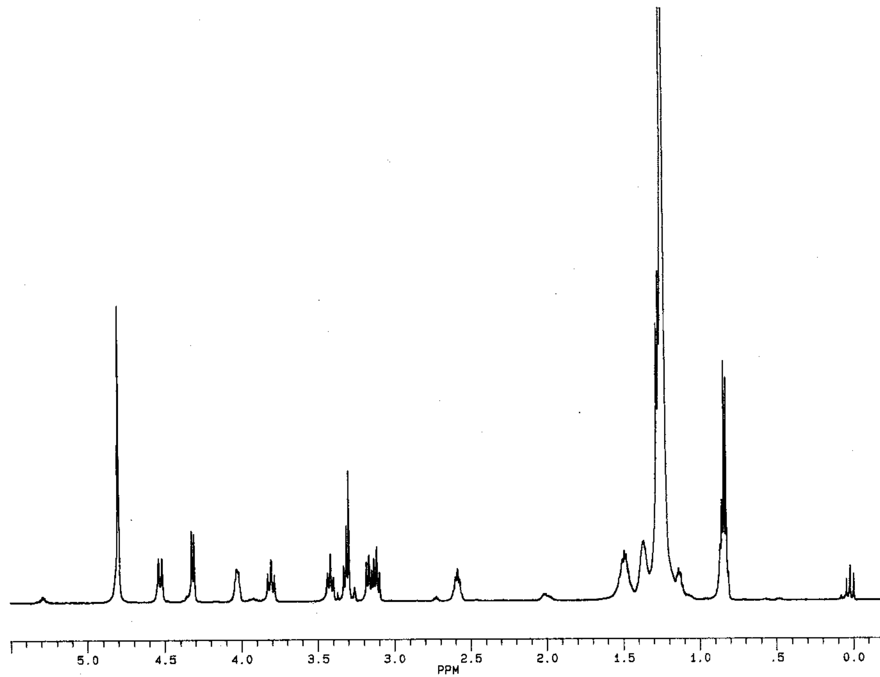


Fig. 5. ^{13}C-NMR spectrum of glucolipsin A (**1**).

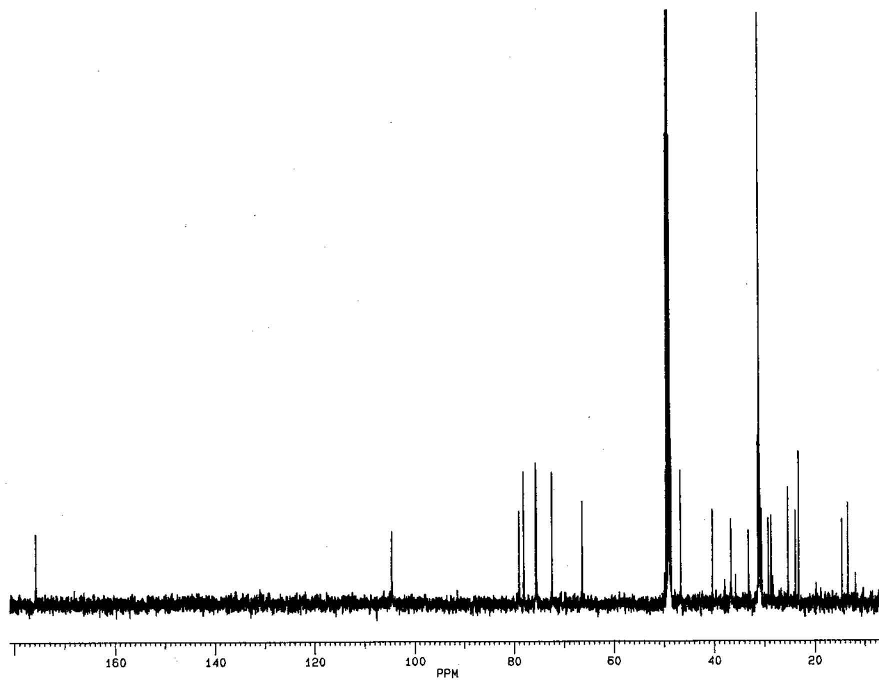


Table 2. ^{13}C-NMR data of glucolipsin A (1), B (2) and the degradation products 3 and 4 (methanol-d_4).

Carbon position	1	2	3	4
1	175.7	175.7	175.2	175.3
2	46.6	46.6	47.1	43.0
3	78.9	78.9	84.1	70.5
4	36.6	36.6	33.5	32.0
5	25.2	25.2	26.9	25.6
6	28.6[a]	28.6[b]	30.8[c]	28.0[d]
7	30.5[a]	31.5[b]	30.8[c]	27.4[d]
8	30.9[a]	30.9[b]	30.8[c]	30.0[d]
9	30.9[a]	30.9[b]	30.8[c]	30.0[d]
10	30.9[a]	30.9[b]	30.8[c]	30.0[d]
11	30.9[a]		30.8[c]	30.0[d]
12	31.1[a]	31.1[b]	30.8[c]	30.0[d]
13	31.2[a]	31.2[b]	30.8[c]	30.0[d]
14	33.1[a]	33.1[b]	33.1[c]	30.0[d]
15	40.2	40.2	40.3	39.0
16	29.2	29.2	29.2	29.3
17	23.1	23.1	23.1	22.7
18	14.5	14.5	14.6	22.7
19	13.4	13.4	13.3	14.1
1′	104.5	104.4	104.5	
2′	75.6	75.6	75.5	
3′	78.0	78.0	78.1	
4′	72.4	72.3	71.6	
5′	75.4	75.4	77.8	
6′	66.3	66.3	62.8	
OCH_3			49.9	51.4

[a,b,c,d] Interchangable methylene carbons.

Fig. 6. Important long range correlations (HMBC) of glucolipsin A (1).

Glucolipsin A (1): $R_1 = R_2 =$

a dimer of two symmetric units with two glucosyl moieties and two identical aliphatic fragments. The dimer of above functional group and structural fragments accounted for only two degrees of unsaturation, the remaining degree of unsaturation must be attributed to a ring, which should link the two monomeric units to each other.

Detailed 1D and 2D-NMR studies (Table 2, 3) including COSY, HETCOR and HMBC led to the establishment of the connectivity of the core structure of the molecule. Fig. 6 showed the important HMBC cor-

Table 3. ^{1}H-NMR data of glucolipsin A (1) and B (2) (methanol-d_4).

δ in ppm (# of protons, mult. J in Hz)			
1		2	
δ 4.57	(1H, d, 10.5)	δ 4.57	(1H, d, 10.6)
4.36	(1H, d, 7.7)	4.35	(1H, d, 7.7)
4.05	(1H, m)	4.06	(1H, m)
3.85	(1H, t, 9.9)	3.85	(1H, t, 10.1)
3.46	(1H, t, 9.9)	3.46	(1H, t, 9.8)
3.35	(1H, dd, 9.1, 9.8)	3.35	(1H, dd, 9.1, 9.9)
3.20	(1H, dd, 7.8, 9.0)	3.20	(1H, dd, 7.8)
3.15	(1H, t, 9.8)	3.16	(1H, t, 9.9)
2.63	(1H, m)	2.63	(1H, m)
1.49∼1.55	(3H, m)	1.50∼1.55	(3H, m)
1.40	(2H, m)	1.41	(2H, m)
1.31	(3H, d, 7.0)	1.31	(3H, d, 7.0)
1.25∼1.29	(18 H, m)	1.25∼1.29	(18H, m)
1.17	(2H, m)	1.17	(2H, m)
0.87	(6H, d, 6.6)	0.87	(6H, d, 6.6)

relations of this compound. The proton coupling constants and the patterns of chemical shift of sugar moiety were characteristic of a glucose. The doublet at 4.38, $J = 10.7$ Hz, which was assigned as the anomeric proton indicated the β configuration of the glucosyl moiety. Furthermore, the anomeric carbon must be linked to C-3 of the aliphatic portion, because of the long range correlation between C-1′ (δ 104.5) and H-3 (δ 4.05). The C-6′ of the glucosyl must be connected to the C-1 of aliphatic chain *via* an ester linkage, due to the long range correlation between C-1 (δ 175.7) and 6-Ha′ (δ 4.57) and 6-Hb′ (δ 3.85). This ester linkage between C-1 and C-6′ was further supported by the significant downshifts of the chemical shifts of C-6 at δ 66.3 (about 4 ppm) and H-6a′ (1 ppm), compared with the corresponding signals of unsubstituted glucose. Furthermore, the signals H-6a′ appeared as a doublet instead of a double doublet, which suggested also that hydroxyl at

Fig. 7. Degradation schema of glucolipsin A (**1**).

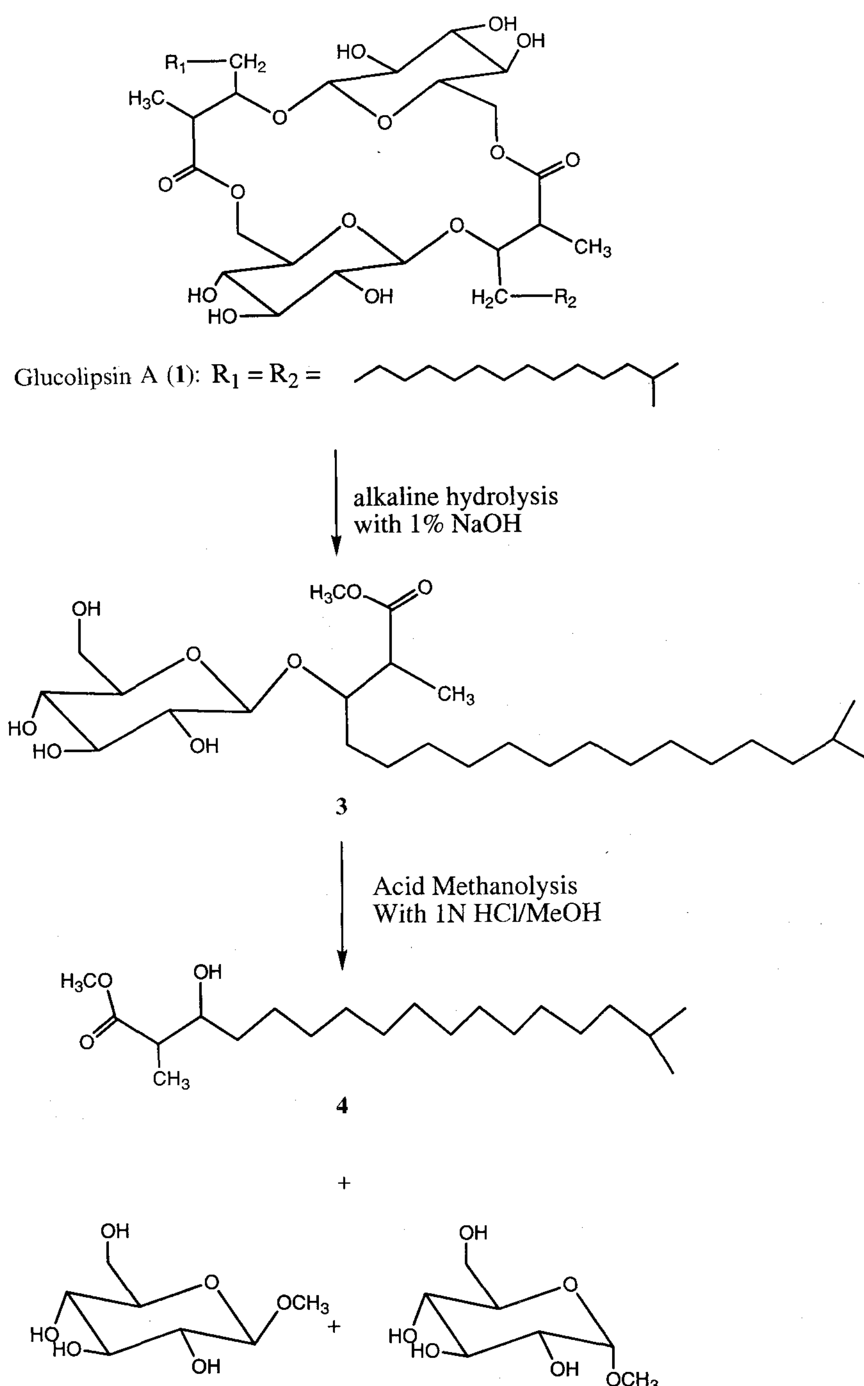
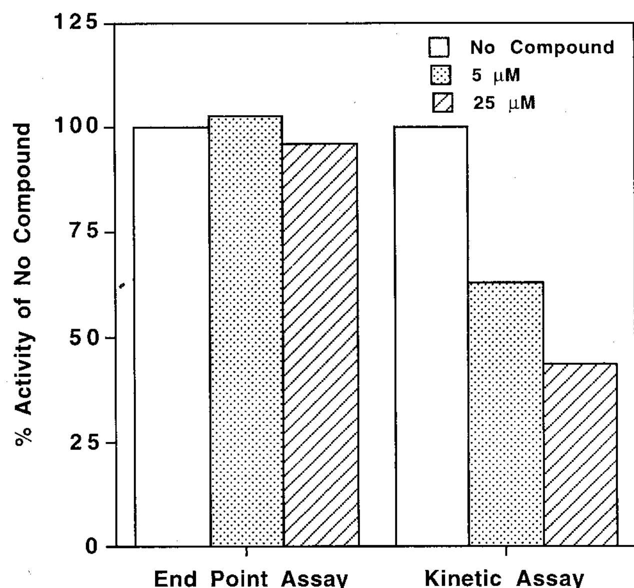


Fig. 8. Effect of incubation of glucolipsin A (**1**) on stearoyl CoA on stearoyl CoA chemical integrity as measured by the end point assay and free stearoyl CoA concentration as measured by the kinetic assay.



Details of the assay and rationale are described under Methods.

C-6′ was substituted.

There appeared to be more than one possible connection between the two identical glucosyl moieties and the two identical fatty acid portions. However, taking into account the fact that there was only one remaining degree of unsaturation, the connection between sugar and fatty acid moieties in this structure can be only proposed as shown in Fig. 1. The connectivities among C-1 ester carbonyl (δ_C 175.7), C-2 methine (δ_C 46.6, δ_H 2.63), C-3 oxymethine (δ_C 78.9, δ_H 4.07) and C-19 methyl (δ_C 13.4, δ_H 1.31) of each hydroxyl fatty acid fragments were clearly suggested by their proton spin systems and confirmed by the HMBC correlations (Fig. 6) between the related carbons and protons. The attachment of the long fatty chain to C-3 oxymethine was indicated by the proton coupling between H-3 and the methylene protons arising from the fatty chain. Each of the two fatty chains should be composed of the remaining functional groups: two methyl groups, one methine and 12 methylene groups, which could be easily deduced from its molecular weight or formula. The two methyl groups (C-17, δ_C 14.5, δ_H 0.87; C-18, δ_C 23.1 δ_H 0.87) should be linked to the methine (C-16, δ_C 29.16), since the signals of both methyl groups appeared as doublets. In addition, there are HMBC correlations between C-16 and the protons of the methyl groups as well as between C-18 and 17-H, also between C-17 and 18-H.

In order to confirm the proposed structure, chemical degradation studies (saponification and acid methanolysis) (Fig. 7) were carried out. The spectral data of the degradation products were analysed. Upon saponification, **1** afforded the monomer **3**. Acidic methanolysis of **3** yielded **4**. The structure of compound **3** was confirmed by their spectral data. The ^{1}H-, ^{13}C-NMR spectra of **3** were similar to those of **1** except the signals of C-6′, H-6a′ and H-6b′. Compared to the corresponding signals of compound **1**, the signals of C-6′ was shifted to upfield to 62.8, and the signals of H-6a′ and H-6b′ were shifted also upfield and appeared as two double doublets at 3.82 and 3.70, respectively. These changes suggested that the C-6 of the glucosyl moiety should bear a free hydroxyl group. The structure of compound **4** was confirmed by the ^{1}H-NMR spectrum and well supported by the diagnostic EI-MS fragments at *m/z* 329, 297, 311, 85, 71, 57 and 43. Thus, the structure of compound **1** was established as shown in Fig. 1.

Glucolipsin: (**2**)

Compound **2** showed the same physical appearance and chemical properties as compound **1**. Its IR, ^{13}C-NMR and ^{1}H-NMR spectral data were almost identical to those of **1**, as well. The only difference between these two compounds was observed in the molecular formula and weight. High resolution FAB mass spectrometry showed that compound **2** has a molecular weight of 925.6196 $(M+Na)^+$ and a chemical composition of $C_{49}H_{90}O_{14}$. Thus, this compound should be composed of one less methylene group than compound **1** as shown in Fig. 1.

Biological Activity

Glucolipsin A and B relieved the inhibition of glucokinase by long chain fatty acyl CoA esters (FAC) with RC_{50} values of 5.4 and 4.6 μM, respectively. Glucolipsin A was further tested in the secondary assay. glucolipsin A did not modify stearoyl CoA, since preincubation of the compound did not result in any change in stearoyl CoA levels as measured by an enzymatic assay. However, the compound did appear to deplete the free concentration of stearoyl CoA since the rate of stearoyl

CoA oxidation was dose dependently inhibited by the compound (Fig. 10). The sequestration of stearoyl CoA by glucolipsin A and consequent lowering of its free concentration would then result in de-inhibition of glucokinase by stearoyl CoA.

Compound **1** was mainly produced by *Streptomyces purpuro-genisclerotics*, while compound **2** was a major product of *Nocardia vaccinii*. However, both *Streptomyces purpurogenisclerotics* and *Nocardia vaccinii* produce compound **1** and **2** based on FAB-MS analysis. In addition, a series of analogs, which differ from **1** and **2** only in the length of the fatty chain with molecular ions $(M+Na)^+$ at m/z 883, 897, 911, 953, were also detected in both cultures by FAB-MS. The structures of compound **1** and **2** are related to the antiviral antibiotics, cycloviracin B_1 and B_2[7,8].

Acknowledgments

The authors wish to acknowledge Dr. S. MAMBER for his advise and help with the programming on the ThermoMax UV/Vis platereader; Dr. D. VYAS and S. FORENZA for helpful discussion.

References

1) STORER, A. C. & A. CORNISH-BOWDEN: Kinetic evidence for a "Mnemonic" mechanism for rat liver glucokinase. Biochem. J. 165: 61∼69, 1977
2) SHERLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313∼340, 1968
3) American Can. Co., Color Harmony Manual. Chicago, 1958
4) MIKAMI, Y; K. YOKOYAMA & T. A. ARAI: Modified Arai and Mikami melanin formation test of Streptomycetes. Int. J. Syst. Bacteriol. 27: 290, 1977
5) NONOMURA, H.: Key for Classification and Identification of 458 Species of the Streptomycetes Included in ISP, 1974
6) Florini, J. R.: Assay of creatine kinase in microtiter dishes using thio-NAD to allow monitoring at 405 nm. Analytical Biochem. 182: 399∼404, 1989
7) TSUNAKAWA, M.; N. KOMIYAMA, O. TENMYO, K. TOMITA, K. KAWANO, C. KOTAKE, M. KONISH & T. OKI: New antiviral antibiotics, cycloviracin B1 and B2. I. Production, isolation, physico-chemical properties and biological activity. J. Antibiotics 45: 1467∼1471, 1992
8) TSUNAKAWA, M.; C. KOTAKE, T. YAMASAKI, T. MORIYAMA, M. KONISH & T. OKI: New antiviral antibiotics, cycloviracin B1 and B2. II. Structure determination. J. Antibiotics 45: 1472∼1480, 1992