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A DEPSIPEPTIDE FUNGAL METABOLITE INHIBITOR OF CHOLESTERYL ESTER TRANSFER PROTEIN

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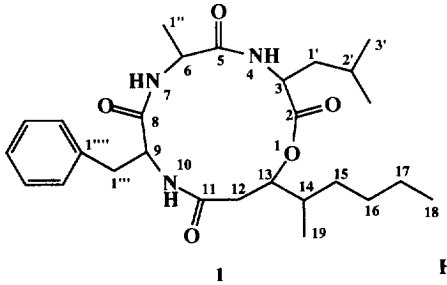
Abstract: The organic extract of the fermentation broth of a fungus was found to contain a depsipeptide SCH 58149 (**1**), containing three amino acids and a β-hydroxy acid, by spectroscopic studies. The amino acids were phenyl alanine, alanine and leucine and the β-hydroxy acid is 3-hydroxy-4-methyl octanoic acid. SCH 58149 exhibited weak activity against cholesterol ester transfer protein (CETP) with an IC_{50} of 50 μM.

In humans, monkeys, hamsters, rabbits and other species cholesteryl ester transfer protein (CETP) transfers cholesteryl esters from HDL to LDL, increasing concentration of the pro-atherogenic LDL while decreasing the anti-atherogenic HDL cholesterol. A low-LDL and high-HDL lipoprotein profile is strongly associated with decreased risk of cardiovascular diseases.[1-4] Genetic deficiency in CETP is associated with improved lipoprotein profiles and longevity, suggesting inhibition of CETP activity is beneficial to heart disease state. Although a slightly higher risk of cardiovascular disease has been found in humans deficient in CETP in a sub-population,[5,6] the growing body of evidence showing the profound beneficial effects of lower LDL and higher HDL suggests that specific and effective pharmaceutical inhibition of CETP's CE transfer activity may outweigh the risk and serve as an useful approach to improve dislipoproteinemia and heart diseases. As part of our continuing investigation of natural products as leads for cholesterol reducing agents, we screened ethyl acetate extracts of several fungal fermentation broths. A fungal broth belonging to *Acremonium* sp. was identified that displayed distinct activity in the CETP assay. Bioassay guided fractionation of this extract led to the isolation of **1**, a depsipeptide.



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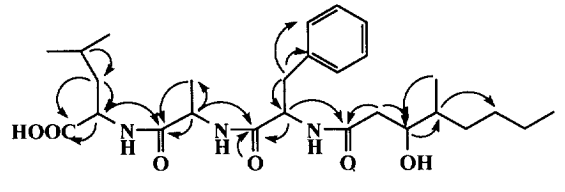

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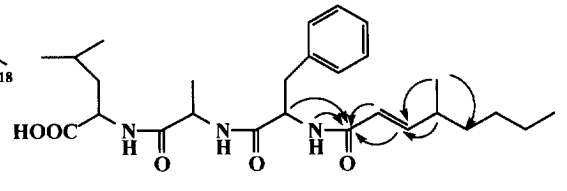

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A 1.5 L fermentation broth was extracted twice with 3 L of ethyl acetate. The organic layers were combined, dried over anhydrous Na_2SO_4 and the solvent removed. The solids from this oily extract were

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precipitated by dissolving the extract in dichloromethane and adding the solution to hexane. The resulting precipitate was filtered and dried to yield 1.5 g of solid containing all the CETP inhibitory activity. Six hundred mg of this precipitate was loaded on a Sephadex LH-20 column (5 × 40 cm) packed in methanol and eluted with the same solvent. The elution of active compound was monitored by their activity in CETP assay. The active fractions were collected and dried to yield 150 mg of enriched complex. Separation of the active compound was achieved by reverse phase preparative HPLC on a Water's Deltapak C-18 silica column (3 × 30 cm), eluting with a mixture of acetonitrile and water (45:55 v/v). Acetonitrile was removed from the active peak eluate, and the aqueous solution was freeze-dried to yield 12 mgs of **1**.

SCH 58149 (**1**) showed molecular ion *m/z* 488 (M+H) in FAB mass spectrum suggesting the molecular weight 487 daltons. and thereby indicating the presence of nitrogens. The molecular formula of **1** was established as $C_{27}H_{42}N_3O_5$ by HRMS (high-resolution mass spectrum).[7] The UV spectrum (MeOH) displayed maxima at 210 and 280 nm and the IR spectrum in KBr showed peaks at 3315, 1675, 1645, and 1540 cm^{-1}, suggesting the presence of amide and ester functionalities. ^1H and ^{13}C NMR chemical shifts of **1** and its base hydrolysis products **2** and **3** are tabulated in Table 1. The ^1H NMR indicated the presence of five methyls, several methylenes and the presence of an aromatic ring. The ^{13}C NMR also showed 27 carbon signals in agreement with the number of carbons revealed by HRMS. APT ^{13}C NMR identified them as four >C=O, five aromatic =CH-, one aromatic =C<, six >CH-, six $>CH_2$ and five $-CH_3$. Four methyls were secondary and the fifth was a primary methyl as revealed by ^1H NMR. This information led us to believe the compound to be a peptide. Two mg of **1** in 2.0 ml 6N HCl was heated at 110 °C under vacuum overnight. The reaction mixture was diluted with water and freezedried and then redissolved in 0.5 mL water. The solution was analyzed for amino acids by TLC[8] and CIMS and showed the presence of alanine, leucine and phenylalanine. They accounted for seven of the nine degrees of unsaturation. The fourth carbonyl is an ester as indicated by the IR which accounted for an additional degree of unsaturation. This information in conjunction with the ^1H NMR data suggested a ring and that **1** may be a depsipeptide. ^1H, and ^{13}C NMR spectra were analyzed and indicated the presence of a β-hydroxy acid with an aliphatic chain.

The amino acids alanine, leucine and phenylalanine accounted for four methines, two methylenes, three secondary methyls thus suggesting the hydroxy acid should contain two methines, four methylenes, one secondary and one primary methyls. ^1H NMR of **1** showed methines at δ 4.43, 3.94, 4.17, 4.90, 2.10 and 1.05. The first three were due to protons attached to α carbon atoms of amino acids leucine, alanine, and phenylalanine, the methine at δ 4.90 was due to the proton attached to the oxygenated carbon on the hydroxy acid. The proton at δ 1.05 was due to γ- methine of leucine. ^1H NMR also showed methylene proton signals at δ 2.93, 1.40, 2.3–2.5, 2.10, 1.13, 1.30. The first two were due to the methylene on phenyl alanine and leucine and the others were on hydroxy acids. The methylene peak at δ 2.3–2.5 is split and showed coupling with proton linked to oxygenated sp^3 carbon (δ 4.90), which in turn showed correlation with another methylene at δ 2.10. This suggested probably the hydroxy acid is a β- hydroxy acid. The ^{13}C NMR ($CDCl_3$ + CD_3OH)showed carbon signals at 47.6, 51.7, 56.2, and 75.2 ppm, of which the first three were due to α- carbon atoms of amino acids alanine, phenylalanine and leucine respectively and the fourth was due to the oxygenated sp^3 carbon.

Further anlaysis of 2D(^{1}H-^{1}H), HETCOR and HMBC (heteronuclear multiple bond correlation) spectra established the hydroxy acid to be 3-hydroxy-4-methyl octanoic acid.

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts for **1–3** (DMSO-d_6)

		1		2		3	
Amino acid	Carbon #	^{1}H	^{13}C NMR	^{1}H	^{13}C	^{1}H	^{13}C
Leucine	2		168.2 (s)		170.6 (d)		171.0(s)
	3	4.43 (t, 6.5Hz)	55.3 (d)	4.52 (m)	53.6 (d)	4.42 (t)	54.2 (d)
	1'	1.40 (m)	27.5 (t)	1.50 (m)	28.9 (t)	1.55 (m)	28.8 (t)
	2'	1.05 (m)	33.5 (d)	1.15 (m)	37.3 (d)	1.2 (m)	36.6 (d)
	3'a	0.86 (d, 6.0Hz)	20.6 (q)	0.82 (d, 6.4Hz)	21.0 (q)	0.96 (d, 6.5 Hz)	18.8 (q)
	3'b	0.87 (d, 6.1Hz)	20.9 (q)	0.89 (d, 6.4Hz)	22.7 (q)	0.88 (d, 6.4Hz)	19.5 (q)
	-NH	7.12 (d, 8Hz)		8.08 (d, 8.3Hz)		7.97 (d, 8Hz)	
Alanine	5		169.3 (s)		171.2 (s)		172.3 (s)
	6	3.94 (dq, 6.5Hz)	47.1 (d)	4.23 (m)	47.9 (d)	4.6 (m)	48.0 (d)
	1"	1.18 (d, 7Hz)	14.6 (q)	1.20 (d, 7.1Hz)	13.9 (q)	1.25 (d, 7Hz)	14.6 (q)
	-NH	8.17 (d, 8.2Hz)		8.02 (d, 7.4Hz)		8.17 (d, 8Hz)	
Phenylalanine	8		169.5 (s)		171.8 (s)		173.1 (s)
	9	4.17 (t, 6.3Hz)	50.7 (d)	4.33 (d, 7.4Hz)	49.9 (d)	4.35 (dd, 6.5Hz)	53.8 (d)
	3	2.93 (dd, 10Hz)	39.5 (t)	3.02 (dd, 7, 2Hz)	40.4 (t)	3.05 (dd, 16, 4Hz)	37.4 (t)
	1'''		136.0 (s)		137.8 (s)		138.0 (s)
	2'''	7.25 (d)	127.6 (d)	7.26 (d)	129.0 (d)	7.28 (d)	129.0 (d)
	3'''	7.20 (d)	126.7 (d)	7.25 (d)	127.8 (d)	7.27 (d)	128.0 (d)
	4'''	7.25 (dd)	124.5 (d)	7.17 (dd)	126.0 (d)	7.17 (dd)	126.0 (d)
	5'''	7.20 (d)	126.7(d)	7.25 (d)	127.8 (d)	7.27 (d)	128.0 (d)
	6'''	7.25 (d)	127.6 (d)	7.26 (d)	129.0 (d)	7.28 (d)	129.0 (d)
	-NH	8.30 (d, 7.5Hz)		8.02 (d, 7.4Hz)		8.27 (d, 7Hz)	
3-hydroxy-4-methyl octanoic acid	1		168.8 (s)		173.8 (s)		164.8 (s)
	12	2.3–2.5 (m)	34.3 (t)	2.75 (dd, 14, 4Hz)	37.0 (t)	5.90 (d, 16Hz)	122.3 (d)
	13	4.90 (dt, 6.5Hz)	74.2 (d)	3.67 (m)	70.12 (d)	6.45 (dd, 16, 8 Hz)	148.2 (d)
	14	1.30 (m)	23.0 (d)	2.14 (m)	24.7 (d)	2.78 (dd, 2 Hz)	35.3 (d)
	15	2.10 (m)	34.2 (t)	2.14 (m)	32.3 (t)	2.2 (m)	37.4 (t)
	16	1.13 (m)	29.4 (t)	1.20 (m)	28.9 (t)	1.35 (m)	25.7 (t)
	17	1.30 (m)	21.1 (t)	1.30 (m)	22.3 (t)	1.40 (m)	22.1 (t)
	18	0.82 (t, 6.4Hz)	12.5 (q)	0.85 (t, 6.3Hz)	13.7 (q)	0.86 (t, 6.8 Hz)	11.5 (q)
	19	0.85 (d, 6.7Hz)	13.0 (q)	0.75 (d, 6.6Hz)	18.5 (q)	0.85 (d, 6.4 Hz)	13.8 (q)

Ten mg of **1** was stirred with 5 mL of 0.25 N sodium hydroxide for 24 h. The solution was acidified and freezedried. The solids obtained were further separated by HPLC on a Water's Deltapak C-18 column (3.0 × 30

cm) eluting with acetonitrile and water (ACN:0.05%, TFA 4:6) afforded 3.6 and 1.4 mgs of **2** and **3**, respectively. Compound **2** on Cs^+ ion liquid secondary ionization mass spectrum (SIMS) displayed an intense molecular ion at *m/z* 506 $(M+H)^+$ and sodiated peak at *m/z* 528 $[M+Na]^+$ and **3** on SIMS showed *m/z* 488 $[M+H].^+$ This mass ion difference along with the presence of two sp^2 carbon signal in ^{13}C NMR suggested the presence of a double bond formed due to dehydration of the β-hydroxy acid. The other fragment ions at 375 [M-Leu], 304 [M-Leu-Ala], 276 [M-Leu-Ala-CO], 203 [Leu+Ala-CO] in SIMS of **2** and 357 [M-Leu], 286 [M-Leu-Ala], 258 [M-CO-Leu-Ala], 203 [Leu+Ala-H_2O] in SIMS of **3** suggested the amino acid sequence in the molecule. Analysis of significant ^1H and ^{13}C long range correlations confirmed the structure of **1**. The absolute stereochemistry of amino acids or β- hydroxy acid is not established.

Compound **1** showed IC_{50} value of 50 μM in the CETP- SPA[9] assay but compound **2** and **3** were inactive. The effects of **1** on the integrity of CETP substrates HDL and LDL were tested by incubation with lipoprotein followed by agarose electrophorosis analysis. No changes of the lipoprotein migration was observed for either HDL or LDL. Recently two CETP inhibitors have been reported from our group.[9,10] Compound **1** has comparable IC_{50} to that reported from a fungus (40 μM)[10] but significantly less active than that from a marine sponge(0.3 μM)[9].

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   Spectral data for SCH 58149 (**1**): FABMS *m/z* 488(M+H); HRFABMS obsd. for $C_{27}H_{42}N_3O_5$ (obsd. 488.3128, calcd 488.3124). (**2**): SIMS *m/z* 528 [M+Na], 506 [M+H], 375 [M-Leu], 304 [M-Leu-Ala], 276 [M-Leu-Ala-CO], 203 [Leu+Ala-CO]; IR (Neat): 3306, 2960, 1715, 1636, and 1540 cm^{-1}. (**3**): SIMS *m/z* 488 [M+H], 357 [M-Leu], 286 [M-Leu-Ala], 258 [M-CO-Leu-Ala], 203 [Leu+Ala-H_2O]; IR (Neat): 3290, 2960, 1715, 1665, 1623, and 1540 cm^{-1}.
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