Pergamon

Tetrahedron Letters 39 (1998) 4497–4500

TETRAHEDRON LETTERS

Revised Structure of Flavolipin and Synthesis of Its Isomers

Masao Shiozaki* and Noriko Deguchi
Exploratory Chemistry Research Laboratories, Sankyo Company, Limited
Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140-8710, Japan

Tomio Ishikawa and Hideyuki Haruyama
Analytical and Metabolic Research Laboratories, Sankyo Company, Limited
Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140-8710, Japan

Yohko Kawai and Masahiro Nishijima
Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases
Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan

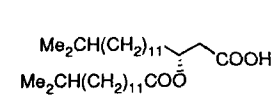
Received 13 March 1998; revised 8 April 1998; accepted 10 April 1998

Abstract: *The proposed structure of natural flavolipin was revised as N-[N-[(3R)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine as a result of synthetic study and biological activity test.*

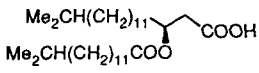
A serine-containing lipid, flavolipin,[1] which was isolated by Kawai et al. from an opportunistic pathogen, *Flavobacterium meningosepticum*, exhibits definite hemagglutinating activity[2] and strongly stimulates macrophages to generate immunoregulatory substances. However, it exhibits none of the lethal toxicity in mice which is exhibited by lipopolysaccharide.[3] This fact suggests that it is a nontoxic immunoactivator. Therefore, we tried to synthesize the proposed flavolipin to determine the configuration of the natural flavolipin and to investigate the biological activities of its stereoisomers. However, the proposed structure (**2**) was negated by our synthetic study.[4] Judging from the FAB MS and ^{1}H NMR analyses of natural flavolipin, the proposed flavolipin is lacking a glycine moiety. Moreover, the natural flavolipin yielded negative results in the ninhydrin test, characteristic of amino acids. Therefore, we estimated the structure of flavolipin as *N*-[*N*-[15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]seryl]glycine (**6**) or *N*-[*N*-[15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]serine (**8**) rather than the amino acids **3**, **4** and **5**. In particular, compounds **4** and **5** may not exist in these forms. In this paper, we describe the synthesis of all stereoisomers of estimated flavolipin to confirm the structure.

0040-4039/98/$19.00
PII: S0040-4039(98)00798-9

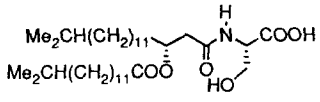
Figure 1



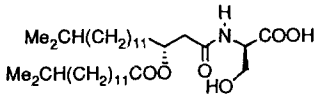
1*R*



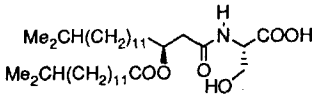
1*S*



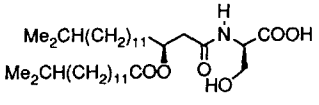
2a



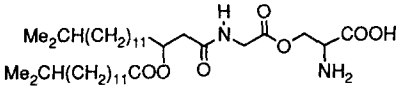
2b



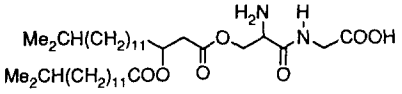
2c



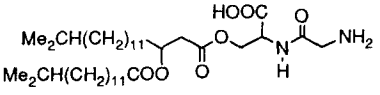
2d



3

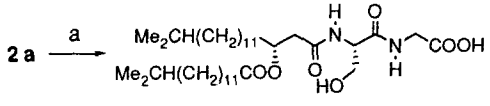


4

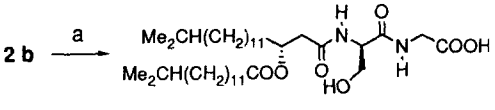


5

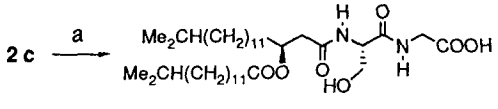
Scheme 1



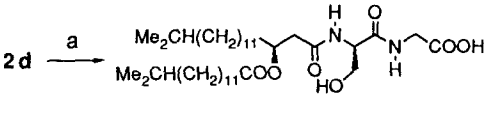
6a



6b



6c

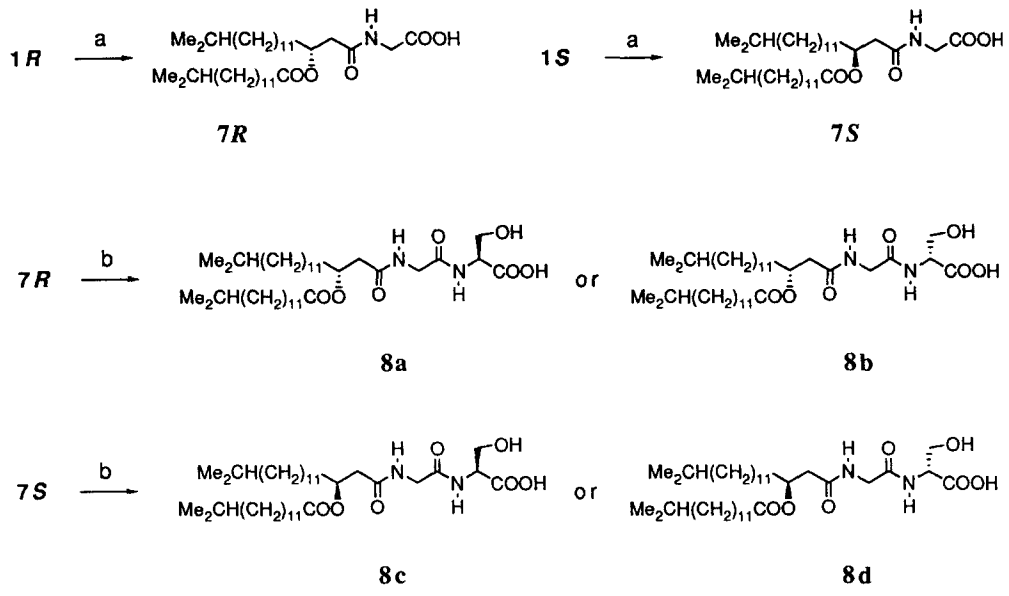


6d

Reagents and conditions: a) (i) glycine benzyl ester, DCC, CH_2Cl_2, 24 °C, 16 h, 70-91%; (ii) H_2, Pd/C, EtOAc, 2.5 h, 71-96%.

At first, we tried to synthesize lipid-serylglycine compounds **6**. The four stereoisomers **2a** (3*R*-L-Ser) **2b** (3*R*-D-Ser), **2c** (3*S*-L-Ser) and **2d** (3*S*-D-Ser), obtained from 15-methyl-3(*R*)-(13-methyltetradecanoyl-

Scheme 2



Reagents and conditions: a) (i) $(COCl)_2$, CH_2Cl_2, 24 °C, 1 h, then glycine benzyl ester hydrochloride, Et_3N, CH_2Cl_2, 24 °C, 1 h, 57-69%; (ii) H_2, Pd/C, EtOAc, 2 h, 94-98%; b) (i) L or D-serine benzyl ester, DCC, CH_2Cl_2, 24 °C, 2 h, 72-82%; (ii) H_2, Pd/C, EtOAc, 2.5 h, 50-73%.

oxy)hexadecanoic acid (**1*R***) and 15-methyl-3(*S*)-(13-methyltetradecanoyloxy)hexadecanoic acid (**1*S***) according to our reported method,[4] were reacted with glycine benzyl ester using dicyclohexylcarbodiimide (DCC) as a condensing reagent to give the corresponding amides. Next, hydrogenolytic deprotection of each benzyl ester gave the corresponding acids **6a** (3*R*-L-Ser-Gly-OH, $[\alpha]_D^{24}$ -7.8° (c 0.50, $CHCl_3$)), **6b** (3*R*-D-Ser-Gly-OH, $[\alpha]_D^{24}$ +7.6° (c 0.75, $CHCl_3$)), **6c** (3*S*-L-Ser-Gly-OH, $[\alpha]_D^{24}$ -8.0° (c 0.23, $CHCl_3$)) and **6d** (3*S*-D-Ser-Gly-OH, $[\alpha]_D^{24}$ +7.1° (c 0.63, $CHCl_3$)). Disappointingly, none of these compounds was identical to the natural flavolipin.

Next, we tried to synthesize lipid-glycylserine compounds **8**. Compounds **1*R*** and **1*S*** were converted with oxalyl chloride to their corresponding acid chlorides, which were treated with glycine benzyl ester hydrochloride and Et3N to give the corresponding amides, and then hydrogenolytic deprotection of each benzyl ester of the amides gave the corresponding acids **7*R*** (3*R*-Gly-OH)[5] and **7*S*** (3*S*-Gly-OH).[6] The compounds **7*R*** and **7*S*** were treated with L- and D-serine benzyl esters using DCC as a condensing reagent to give the corresponding amides, and then hydrogenolytic deprotection of each benzyl ester gave the corresponding acids **8a** (3*R*-Gly-L-Ser-OH, $[\alpha]_D^{24}$ +18.9° (c 0.39, $CHCl_3$))[5,7], **8b** (3*R*-Gly-D-Ser-OH, $[\alpha]_D^{24}$ -26.2° (c 0.58, $CHCl_3$)), **8c** (3*S*-Gly-L-Ser-OH, $[\alpha]_D^{24}$ +25.6° (c 0.38, $CHCl_3$)) and **8d** (3*S*-Gly-D-Ser-OH, $[\alpha]_D^{24}$ -18.8° (c 0.52, $CHCl_3$)).

Fortunately, compounds **8a** and **8d** were identical to natural flavolipin with respect to ^{1}H NMR[8] and FAB MS spectra.

The macrophage stimulation activity of **8a** was almost the same as natural flavolipin, and that of **8d** was practically inactive. Therefore, from this synthetic study we clearly determine the structure of natural flavolipin to be *N-[N-[(3R)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine*.[9]

Thus we could synthesize all eight isomers of flavolipin in a stereocontrolled manner, and determine the correct structure of flavolipin. We are now investigating the biological activities of all compounds of **6** and **8**, and the results will be reported in due course.

References

1. (a) Y. Kawai and I. Yano, *Eur. J. Biochem.*, **136**, 531-538 (1983). (b) Y. Kawai, I. Yano, and K. Kaneda, *Eur. J. Biochem.*, **171**, 73-80 (1988). (c) Y. Kawai, I. Yano, K. Kaneda, and E. Yabuuchi, *Eur. J. Biochem.*, **175**, 633-641 (1988).
2. (a) Y. Kawai and K. Akagawa, *Infection and Immunity*, **57**, 2086-2091 (1989). (b) Y. Kawai, K. Kaneda, Y. Morisawa, and K. Akagawa, *Infection and Immunity*, **59**, 2560-2566 (1991).
3. D. H. Murray and J. Prokop, *J. Pharm. Sci.*, **54**, 1468-1473 (1965).
4. M. Shiozaki, N. Deguchi, T. Mochizuki, and M. Nishijima, *Tetrahedron Lett.* **37**, 3875-3876 (1996).
5. Compound (**7*R***) was already reported in the following articles: (a) I. Uchida, K. Yoshida, Y. Kawai, S. Takasa, Y. Itoh, H. Tanaka, M. Kohsaka, and H. Imanaka, *J. Antibiotics*, **38**, 1476-1486 (1985), and (b) I. Uchida, K. Yoshida, Y. Kawai, S. Takase, Y. Itoh, H. Tanaka, M. Kosaka, and H. Imanaka, *Chem. Pharm. Bull.*, **33**, 424-427 (1985).
6. Compound (**7*S***) has been reported as belonging to the N-type calcium channel blockers from a marine bacterium, *Cytophaga* sp. SANK 71996. T. Morishita, A. Sato, M. Hisamoto, M. Oda, K. Matsuda, A. Ishii, and K. Kodama, *J. Antibiotics*, **50**, 457-468 (1997).
7. K. Yoshida, M. Iwami, Y. Umehara, M. Nishikawa, I. Uchida, M. Kohsaka, H. Aoki, and H. Imanaka, *J. Antibiotics*, **38**, 1469-1475 (1985).
8. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.86 (12H, d, *J*=6.7 Hz), 1.12-1.20 (4H, m), 1.25 (34H, bs), 1.46-1.64 (6H, m), 2.30 (2H, t, *J*=7.5 Hz), 2.45-2.58 (2H, m), 3.88-4.12 (4H, m), 4.62 (1H, d, *J*=6.9 Hz), 5.18 (1H, quintet, *J*=5.7-6.7 Hz), 5.3-5.8 (2H, broad, COOH, OH), 7.15 (1H, bs, NH), 7.62 (1H, d, *J*=6.9 Hz, NH).
9. This compound was reported as WB-3559 D, isolated from *Flavobacterium* sp. No. 3559 (reference 7), and it was synthesized by Uchida et al (reference 5). Therefore, it becomes clear that flavolipin is the same compound as WB-3559 D.