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STRUCTURE AND SYNTHESIS OF WB-3559 A, B, C AND D, NEW FIBRINOLYTIC AGENTS ISOLATED FROM FLAVOBACTERIUM sp.

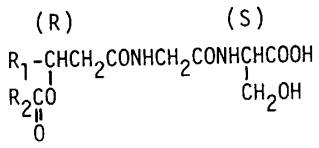
Itsuo Uchida,* Keizo Yoshida, Yoshio Kawai, Shigehiro Takase, Yoshikuni Itoh, Hirokazu Tanaka, Masanobu Kohsaka, and Hiroshi Imanaka
Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 1-6, 2-chome, Kashima, Yodogawaku, Osaka 532, Japan

WB-3559 A, B, C, and D are new fibrinolytic agents isolated from Flavobacterium sp. No. 3559. The structure of these four compounds and the total synthesis of WB-3559 D are described.

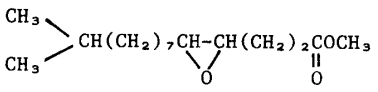
KEYWORDS———fibrinolytic agent; Flavobacterium sp.; WB-3559; acyl peptide; structure elucidation; synthesis

In the course of our screening program for biologically active principles from fermentation sources, several new fibrinolytic agents were isolated from Flavobacterium sp. No. 3559.[1] This communication deals with the structure elucidation of these four compounds and total synthesis of one of the components: WB-3559 D. WB-3559 was designated as a mixture of the four components (A, B, C, and D) all of which have almost the same Rf value on TLC (MeOH-H_2O (7:3), Rf 0.73). They were finally separated by liquid chromatography in the ratio of 1:4:1:4.[1] Each isolated component is an entirely colorless wax and they have the following molecular formulae based on elemental analysis:[2] WB-3559 A, $C_{36}H_{66}N_2O_7$; WB-3559 B, $C_{37}H_{68}N_2O_7$; WB-3559 C, $C_{36}H_{68}N_2O_7$; WB-3559 D, $C_{37}H_{70}N_2O_7$. As supplies of the isolated components were limited, a mixture of the four components (WB-3559) was used for the structural study.

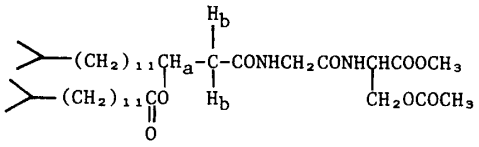
LC analysis of the hydrogenation (10% Pd-C) products of WB-3559 showed that the original four peaks due to WB-3559 A, B, C, and D changed to two peaks corresponding to WB-3559 C and D in the ratio of 1:4. WB-3559 was treated with



	R_1	R_2
WB 3559 A	$-(CH_2)_{10}-$	$-(CH_2)_7CH{=}CHCH_2CH_2$
WB 3559 B	$-(CH_2)_{11}-$	$-(CH_2)_7CH{=}CHCH_2CH_2$
WB 3559 C	$-(CH_2)_{10}-$	$-(CH_2)_{11}$
WB 3559 D	$-(CH_2)_{11}-$	$-(CH_2)_{11}$



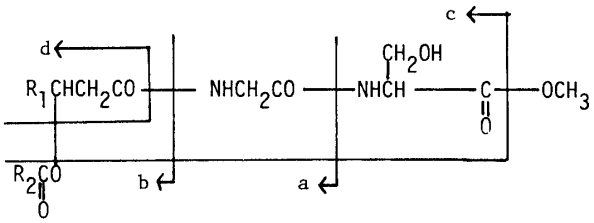
(1)



(2)

sodium methoxide in methanol (r.t. 3h) to give two kinds of products: fatty acid methyl ester and acyl peptide. Analysis by gas chromatography showed that the fatty acid methyl ester was a mixture of two compounds in the ratio of ca. 1:1, and GC-MS demonstrated the molecular weights of these compounds to be 256 and 254, respectively. The mixture was subjected to catalytic hydrogenation over 10% Pd-C and converged to the former, which was identical with methyl 13-methyltetradecanoate.[3] The presence and position of the double bond in the latter compound were deduced as follows. Treatment of the mixture with m-chloroperbenzoic acid gave an epoxide (1), and with osmium tetroxide a γ-lactone ($C_{15}H_{28}O_3$ (M^+ 256); ν_{max} ($CHCl_3$) 1765cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ:4.45 (1H,m), 3.9 (1H, m)). The assignment of the fragment peaks[4] in the EIMS of the epoxide (1) (m/z: 270 (M^+), 183, 129, 117, 85) indicated that the latter fatty acid methyl ester is methyl-13-methyl-4-tetradecenoate. The acyl peptide produced by methanolysis of WB-3559 was deduced from its FDMS to be a mixture of two compounds (m/z: 453 ($M+Na)^+$, 439 $(M+Na)^+$), which was hydrolized with 6N HCl at 100°C for 20 h to leave two known amino acids, i.e. glycine and serine (1:1) characterized by conventional amino acid analysis. The hydrolized products were treated with carbobenzoxy chloride and subsequently with diazomethane to furnish carbobenzyloxy-L-serine

Table. EI Mass Fragmentation Peaks of (3), (4), (5), and (6)

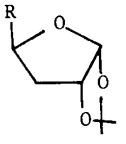


	WB 3559 A methyl ester (3)	WB 3559 B methyl ester (4)	WB 3559 C methyl ester (5)	WB 3559 D methyl ester (6)
	R_1= $\rangle$—$(CH_2)_{10}$ R_2= $\rangle$—$(CH_2)_7CH{=}CHCH_2CH_2$	R_1= $\rangle$—$(CH_2)_{11}$ R_2= $\rangle$—$(CH_2)_7CH{=}CHCH_2CH_2$	R_1= $\rangle$—$(CH_2)_{10}$ R_2= $\rangle$—$(CH_2)_{11}$	R_1= $\rangle$—$(CH_2)_{11}$ R_2= $\rangle$—$(CH_2)_{11}$
M^+	m/z 652	666	654	668
a	534	548	536	550
b	477	491	479	493
c	382	396	382	396
d	237	251	237	251

methyl ester ($[\alpha]_D^{20}$ +7.42 (c=0.7, $CHCl_3$); synthetic sample from L-serine: $[\alpha]_D^{20}$ + 7.48 (c=1.0, $CHCl_3$)). Therefore, all serine molecules contained in WB-3559 apparently have the L-configuration.

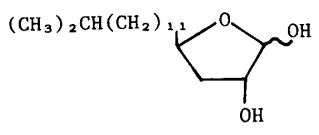
The unknown structure of the acyl part in WB-3559 was characterized by ^{1}H-NMR spectral study as follows. The isolated WB-3559 D was transformed into the monoacetate methyl ester (2) by treatment with diazomethane and then with acetic anhydride in pyridine. In the 100 MHz ^{1}H-NMR spectrum of 2 ($CDCl_3$, δ:7.12 (1H, d, 8.0 Hz), 6.70 (1H, t, 4.5 Hz), 5.20 (1H, tt, 6.0 and 6.0 Hz), 4.82 (1H, td, 4.0 and 8.0 Hz), 4.40 (2H, d, 4.0 Hz), 4.00 (2H, d, 4.5 Hz), 3.80 (3H, s), 2.54 (2H, d, 6.0 Hz), 2.06 (3H, s), 0.85 (6H, d, 6.0 Hz)), a doublet (Hb) at δ2.54 was changed to a singlet on irradiation of a triplet triplet signal (Ha) at δ5.20. This result enabled us to identify a β-acyloxy acyl unit in its molecule. The sequence of the β-acyloxy acyl unit, glycine and L-serine in the structure of WB-3559 D was elucidated by analysis of the EIMS of its methyl ester (6) obtained by treatment with diazomethane. The structure of the other three components: WB-3559 A, B, and C were also clarified by analyzing the mass fragment peaks in the EIMS of their respective methyl esters (3), (4), (5). The table shows the assignment of each fragment peak, which agrees with the corresponding chemical structures. The absolute configuration of the acyl part in WB-3559 D was determined by total synthesis starting from D-glucose as the chiral origin.

The aldehyde (7)[5] transformed from D-glucose was subjected to the Wittig reaction with 11-methyldodecyl triphenylphosphorane[6] to lead to the olefine (8).[8] After hydrogenation of 8 over PtO_2 (quant.), the acetonide was cleaved by acid treatment (0.1N HCl, 70°C, 2h) to furnish the diol (9) in 90% yield. The crucial conversion of the diol (9) into the -hydroxycarboxylic acid (10) was completed in four steps (46% overall yield): (1) diol cleavage with $NaIO_4$, dioxane-H_2O; (2) protection of the alcohol with dihydropyran, p-TsOH; (3) oxidation with Ag_2O, EtOH-H_2O, 25°C, 4 h; (4) treatment with 5% citric acid. From the key intermediate (10),[8] the synthesis was accomplished by stepwise elongation of the molecules. First, the N-hydroxysuccinimide ester of 10 was coupled with H-GlyOBzl to give 11 in 95% yield. Then, acylation of 11 with 13-methyl-tetradecanoyl chloride[7] in pyridine followed by hydrogenation over Pd-black in AcOH, furnished the N-acyl glycin (12) in 71% yield. Finally, coupling with L-serine was carried out by active ester procedure. Thus, N-hydroxysuccinimide ester prepared from 12 with



(7) R= CHO

(8) R= $-CH=CH(CH_2)_9CH(CH_3)_2$



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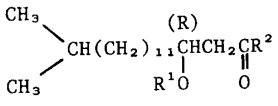

(10) R^1= H, R^2= OH

(11) R^1= H, R^2= $NHCH_2COOCH_2C_6H_5$

(12) R^1= $CO(CH_2)_{11}CH(CH_3)_2$, R^2= $NHCH_2COOH$

(13) R^1= $CO(CH_2)_{11}CH(CH_3)_2$, R^2= $NHCH_2CONH\overset{(S)}{C}HCOOH$ (CH_2OH)

DCC was condenced with L-serine in the presence of triethylamine to afford the N-acyl peptide (13), $[\alpha]_D^{22}$ +19.5° (c=0.3, $CHCl_3$), which was identical with natural WB-3559 D in all respects. Therefore, the absolute configuration of the acyl part in WB-3559 D was established to be the R configuration. The analogy of $[\alpha]_D$ values among WB-3559 A, B, C and D allowed assignment of the absolute configurations in the acyl part of WB-3559 A, B and C to be all R configuration.

REFERENCES AND NOTES

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2) WB-3559 A, B, C and D have the following physical properties. WB-3559 A : mp 144-145°C ; $[\alpha]_D^{25}$+15.3° (c=0.3, $CHCl_3$) ; Anal. Found: C, 67.37, H, 10.42, N, 4.14, Calcd. for $C_{36}H_{66}N_2O_7$: C, 67.67, H, 10.41, N, 4.39 ; ν_{max} ($CHCl_3$) 3350, 2950, 2860, 1725, 1660 cm^{-1} ; ^{1}H-NMR ($CDCl_3$-CD_3OD) δ:5.35 (2H, m), 5.20 (1H,m), 4.53 (1H, m), 3.92 (4H,m), 2.55 (2H, d, 6 Hz), 2.36 (4H, m), 2.04 (2H, m), 1.60 (4H, m), 1.10-1.50 (30H, m), 0.85 (12H, d, 6 Hz). WB-3559 B : mp 126-127°C ; $[\alpha]_D^{22}$ +16.7° (c=0.33, $CHCl_3$) ; Anal. Found: C, 67.95, H, 10.43, N, 4.10, Calcd. for $C_{37}H_{68}N_2O_7$: C, 68.06, H, 10.50, N, 4.29 ; ν_{max} ($CHCl_3$) 3350, 2950, 2860, 1730, 1715, 1660 cm^{-1} ; ^{1}H-NMR ($CDCl_3$-CD_3OD) δ:5.35 (2H,m), 5.20 (1H, m), 4.53 (1H, m), 3.92 (4H,m), 2.55 (2H, d, 6 Hz), 2.36 (4H, m), 2.04 (2H, m), 1.60 (4H, m), 1.10-1.50 (32H, m), 0.85 (12H, d, 6 Hz). WB-3559 C : mp 154-155°C ; $[\alpha]_D^{25}$ +15.9° (c=0.37, $CHCl_3$) ; Anal. Found: C, 67.28, H, 10.48, N, 4.15, Calcd. for $C_{36}H_{68}N_2O_7$: C, 67.46, H, 10.69, N, 4.37 ; ν_{max} ($CHCl_3$) 3350, 2950, 2860, 1725, 1660 cm^{-1} ; ^{1}H-NMR ($CDCl_3$-CD_3OD) δ:5.20 (1H, m), 4.53 (1H,m), 3.92 (4H,m), 2.54 (2H, d, 6 Hz), 2.32 (2H, t, 7 Hz), 1.60 (4H, m), 1.10-1.50 (38H, m), 0.85 (12H, d, 6 Hz). WB-3559 D : mp 137-138°C ; $[\alpha]_D^{22}$ +19.75° (c=0.8, $CHCl_3$) ; Anal. Found: C, 67.74, H, 10.55, N, 4.19, Calcd. for $C_{37}H_{70}N_2O_7$: C, 67.85, H, 10.77, N, 4.28 ; ν_{max} ($CHCl_3$) 3350, 2950, 2870, 1725, 1660 cm^{-1} ; ^{1}H-NMR ($CDCl_3$-CD_3OD) δ:5.20 (1H,m), 4.53 (1H, m), 3.92 (4H, m), 2.54 (2H, d, 6 Hz), 2.32 (2H, t, 7Hz), 1.60 (4H,m), 1.10-1.50 (40H, m), 0.85 (12H, d, 6 Hz).

3) The authentic sample was synthesized as follows. (i) Wittig condensation of isobutyraldehyde and 10-carboxydecyl triphenylphosphorane (ii) hydrogenation (iii) CH_2N_2 treatment.

4) R. T. Aplin and L. Coles, J. Chem. Soc., Chem. Commun., 1967, 858.

5) D.H. Murray and J. Prokop, J. Pharm. Sci., 54, 1468 (1965).

6) The Wittig reagent was prepared in the following sequences.
$Cl(CH_2)_{10}Cl \xrightarrow{a,\ b} (CH_3)_2CH(CH_2)_{10}Cl \xrightarrow{c,\ d} (CH_3)_2CH(CH_2)_9CH{=}P(Ph)_3$
(a) NaI, CH_3COCH_3, (b) $(CH_3)_2CHMgCl$, $CuCl_2$, LiCl, (c) $(C_6H_5)_3P$, (d) n-BuLi (1eq), THF, r.t., 1 h.

7) This compound was prepared by treatment of 13-methyltetradecanoic acid with thionyl chloride; see ref. 3.

8) The structure of the intermediates was confirmed by the following physical evidence. 8 : EIMS, m/z 338 (M^+) ; ^{1}H-NMR ($CDCl_3$) δ:5.80 (1H, d, 4 Hz), 5.67-4.83 (3H, m), 4.70 (1H, t, 4 Hz), 2.28-1.97 (2H, m), 1.73-1.17 (25H, m), 0.87 (6H, d, 7 Hz). 10 : FDMS, m/z 287 (M^+ +1) ; ^{1}H-NMR ($CDCl_3$)δ:7.00-6.77 (2H, br), 4.00 (1H, m), 2.50 (2H, d, 6 Hz), 1.60-1.17 (23H, m), 0.87 (6H, d, 7 Hz).

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