SYNTHESSES AND ACTIVITIES OF N-SUBSTITUTED DERIVATIVES OF SIASTATIN B†

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N-Substituted derivatives of siastatin B have been obtained by a chemical modification. Some derivatives showed potent inhibitory activities against Streptococcus sp. and Clostridium perfringens neuraminidases.

Most of the naturally occurring poly- or multifunctional piperidines are specific and potent inhibitors of glycosidases. They have many potential applications not only as molecular tools to investigate important biological processes but also as therapeutic agents such as antimetastatic, antitumor-poliferation, antiviral agents, etc.¹)

Such a multifunctional piperidine, siastatin B (1), was isolated as an inhibitor of neuraminidase by UMEZAWA et al.²) from a Streptomyces culture. It inhibits neuraminidases isolated from microorganisms and animal tissues as well as β-glucuronidase and N-acetyl-β-D-glucosaminidase, and somewhat resembles structurally sialic acid (N-acetylneuraminic acid, 2) (Fig. 1). After achievement of the total synthesis of 1³⁻⁵) and its analogues,⁶⁻⁹) some N-(1,2-dihydroxypropyl) derivatives of 1 have been synthesized, and

Fig. 1.

1 Dedicated to the late Professor HAMAO UMEZAWA on the occasion of the 30th anniversary of the Institute of Microbial Chemistry.
N-[(2S)-1,2-dihydroxypropyl]siastatin B (3), N-[(2R)-1,2-dihydroxypropyl]siastatin B (4), N-[(2S)-1,2-dihydroxypropyl]-4-deoxysiastatin B (5) and N-[(2R)-1,2-dihydroxypropyl]-4-deoxysiastatin B (6), inhibitors of *Streptococcus* sp. and *Clostridium perfringens* neuraminidases, have been obtained. In this paper, the syntheses and the biological activities of N-substituted derivatives of siastain B, 4-deoxysiastatin B and 3,4-didehydro-4-deoxysiastatin B (7~21) are presented.

Synthesis

In the course of our molecular graphics study\(^8,9\) of the relationship between structure and biological activity among such inhibitors, we became interested in the substitution at the imino group of the piperidine ring which we believe interacts with the glycopyranosyl binding site to inhibit the enzymatic process. Thus, compounds 7~21 were prepared from 1, its methyl ester (22), 4-deoxysiastatin B methyl ester\(^8\) (23), 3,4-didehydro-4-deoxysiastatin B\(^8\) (24) or its methyl ester\(^8\) (25), by N-benzylation or reductive N-alkylation. Treatment of 1, for example, with benzyl chloride and triethylamine in an aqueous N,N-dimethylformamide (DMF) solution gave 7 in 73% yield (Scheme 1). Compounds 20 and 21 were similarly prepared from 24.

Scheme 1.
and 25. Reductive N-alkylation of 23 with benzaldehyde by sodium cyanoborohydride (NaBH$_3$CN) in methanol afforded 8 which was converted into 9 by alkaline hydrolysis in a good yield. Compounds 10~17 were also efficiently obtained from 22 and the corresponding aldehydes by the similar reaction sequences. On the other hand, compounds 18 and 19 were prepared by reductive N-alkylation from 1 with 2-methylpropanal and 2,2-dimethylpropanal, respectively, in good yields.

**Biological Activities**

As shown in Table 1, compounds 7, 9, 11, 13, 15, 17, 18, 19 and 20 showed inhibitory activity against *Streptococcus* sp. and *Clostridium perfringens* neuraminidases, whereas their methyl esters (8, 10, 12, 14, 16 and 21) did not inhibit these enzymes. Remarkably, compounds 9, 13 and 17 strongly affected *Streptococcus* sp. neuraminidase more effectively than the well-known inhibitor, 2,3-didehydro-2-deoxy-N-acetylneuraminic acid$^{10)}$ (DDNA, 26). Compound 7 also inhibited yeast $\alpha$-glucosidase and sweet potato $\beta$-amylase at IC$_{50}$ values of 13.0 and 18.0 $\mu$g/ml, respectively. No other analogues showed inhibitory activity against glycosidases ($\alpha$-glucosidase from yeast, $\beta$-glucosidase from almond, $\alpha$-mannosidase from soybean, $\beta$-glucuronidase from bovine liver, $\alpha$-amylase from porcine pancreas, $\beta$-amylase from sweet potato, $\alpha$- and $\beta$-galactosidase from *Escherichia coli*, $\beta$-galactosidase from bovine liver, N-acetyl-$\alpha$-galactosidase from chicken liver and N-acetyl-$\beta$-glucosaminidase from bovine liver). Further evaluation of the biological activities of these analogues are in progress.

**Experimental**

**General Methods**

Melting points were determined with a Yanagimoto apparatus and are uncorrected. IR spectra were determined on a Hitachi Model 260-10 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. $^1$H NMR spectra were recorded with a JEOL JNM-GX400 spectrometer. Chemical shifts are expressed in $\delta$ values (ppm) with tetramethylsilane as an internal standard. Mass spectra were taken by a JEOL JMS-SX102 in the FAB mode.

**N-Benzylsiastatin B (7)**

To a solution of siastatin B (1, 50 mg) in a mixture of DMF (3 ml) and H$_2$O (1 ml) were added triethylamine (0.5 ml) and benzyl chloride (0.2 ml), and the mixture was stirred at room temperature overnight. Evaporation of the solvent gave an oil. The oil was subjected to preparative TLC on silica gel developed with a mixture of CHCl$_3$-MeOH-conc aq ammonia (20:10:3) to give a colorless amorphous solid of 7 (51.6 mg, 73%): $[\alpha]^5 +3.2^o$ (c 0.48, H$_2$O); IR (KBr) cm$^{-1}$ 3400 (sh), 3150, 3050, 2825, 2600 (sh), 1730, 1690, 1550, 1410, 1290, 1230, 1170, 1140, 1110, 1040, 990, 960, 920, 885; $^1$H NMR (400 MHz, D$_2$O) $\delta$ 2.05 (3H, s, NCOCH$_3$), 2.54 (1H, ddd, $\gamma$= 2.5, 4 and 12Hz, 3-H), 2.75 (1H, t, $\gamma$=12Hz, 2-H$ax$), 2.94 (1H, dd, $J= 4$ and 12 Hz, 2-H$eq$), 3.85 and 4.05 (2H, ABq, $J= 13$ Hz, CH$_2$Ph), 4.37 (1H, t, $J= 2.5$ Hz, 4-H), 4.51 (1H, d, $J= 9.4$ Hz, 6-H), 7.35~7.45 (5H, m, Ph); MS (FAB, positive) $m/z$ 309.2 (M + H)$^+$, 250.1, 207.1, 115.0, 75.0, 57.0.

**N-Benzyl-4-deoxyksiastatin B Methyl Ester (8)**

To a solution of 23 (50 mg) in MeOH (1.3 ml) were added benzaldehyde (0.18 ml) and NaBH$_3$CN in methanol afforded 8 which was converted into 9 by alkaline hydrolysis in a good yield. Compounds 10~17 were also efficiently obtained from 22 and the corresponding aldehydes by the similar reaction sequences. On the other hand, compounds 18 and 19 were prepared by reductive N-alkylation from 1 with 2-methylpropanal and 2,2-dimethylpropanal, respectively, in good yields.

**Table 1. IC$_{50}$ ($\mu$g/ml) of siastatin B (I) and its analogues against N-acetylneuraminidases.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>N-Acetylneuraminidase</th>
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<tr>
<td></td>
<td>C. perfringens</td>
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<td>1</td>
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<td>50</td>
</tr>
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<td>12</td>
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</table>
(69 mg), and the mixture was stirred at room temperature overnight. Another portion of benzaldehyde (0.09 ml) and NaBH₃CN (33 mg) were added to the mixture, and then the reaction mixture was further stirred at room temperature for 4 hours. Addition of water and evaporation of the solvent gave a solid, which was taken up in chloroform. Evaporation of the solvent gave a foam. The foam was subjected to preparative TLC developed with a mixture of CHCl₃-MeOH-conc aq ammonia (20:10:3) to give a colorless amorphous solid of 8 (58 mg, 95.7%): [α]D –29° (c 0.14, MeOH); IR (KBr) cm⁻¹ 3425, 3275, 3100 (sh), 3050, 2970, 2900, 1740, 1650, 1580, 1505, 1465, 1450, 1400, 1385, 1350, 1340 (sh), 1310, 1270, 1250, 1205, 1185, 1165, 1140, 1100, 1090, 1070, 1030, 990, 970, 940; ¹H NMR (400 MHz, CD₃OD) δ 1.58 (1H, q, J=12Hz, 4-Hax), 1.98 (3H, s, NCOCH₃), 2.16 (1H, t, J=12Hz, 2-Hax), 2.27 (1H, dtd, J=2, 4 and 12Hz, 4-Heq), 2.57 (1H, tt, J=4 and 12Hz, 3-H), 2.97 (1H, tt, J=4 and 12Hz, 3-H), 2.97 (1H, ddd, J=2, 4 and 12 Hz, 2-Heq), 3.16 and 3.95 (2H, ABq, J=13.6Hz, CH₂Ph), 3.45 (1H, ddd, J=4, 8 and 12Hz, 5-H), 4.11 (1H, d, J=8 Hz, 6-H), 7.18 ~ 7.32 (5H, m, Ph); MS (FAB, positive) m/z 307.3 (M+H)+, 248.2, 75, 57.

N-Benzyl-4-deoxysiastatin B (9)
To a solution of 8 (45 mg) in MeOH (2.3 ml) was added 1 m NaOH (0.59 ml), and the mixture was stirred at room temperature for 5 hours. Evaporation of the solvent gave a solid. The solid was subjected to preparative TLC on silica gel developed with a mixture of CHCl₃-MeOH-conc aq ammonia (20:10:3) to give a colorless amorphous solid of 9 (43 mg, 100%): [α]D –3.5° (c 0.79, MeOH); IR (KBr) cm⁻¹ 3450, 3250, 1680, 1600, 1520, 1480, 1420, 1320, 1230, 1180, 1120, 1090 (sh), 1050 (sh), 980, 940, 910; ¹H NMR (400MHz, CD₃OD) δ 1.64 (1H, dt, J=10.4 and 12.8Hz, 4-Hax), 1.97 (3H, s, NCOCH₃), 2.23 (1H, dtd, J=-2, 4 and 12.8Hz, 4-Heq), 2.27 (1H, dd, J=10 and 11.6Hz, 2-Hax), 2.42 (1H, tt, J=4 and 10 Hz, 3-H), 2.97 (1H, ddd, J=2, 4 and 11.6 Hz, 2-Heq), 3.22 and 3.89 (2H, ABq, J=13.6Hz, CH₂Ph), 3.45 (1H, ddd, J=4, 8 and 10.4Hz, 5-H), 4.19 (1H, d, J=8 Hz, 6-H), 7.15 ~ 7.34 (5H, m, Ph); MS (FAB, positive) m/z 293.2 (M+H)+, 234.2, 75, 57.

N-(2-Fluorophenyl)methylsiastatin B Methyl Ester (10)
Compound 10 was obtained from 22 and 2-fluorobenzaldehyde by a similar procedure as was used for the preparation of 8 (yield 92.2%): [α]D –22° (c 0.16, MeOH); IR (KBr) cm⁻¹ 3340, 3070, 2975, 2930, 2840, 1950, 1660, 1595, 1550, 1500, 1470, 1440, 1420, 1400, 1390, 1360, 1345, 1325, 1300, 1280, 1260, 1230, 1220, 1180, 1150, 1120, 1110, 1090, 1080, 1030, 970, 950, 920, 900; ¹H NMR (400 MHz, CD₃OD) δ 1.99 (3H, s, NCOCH₃), 2.6 ~ 2.9 (3H, m, 2-H₂ and 3-H), 3.32 and 3.94 (2H, ABq, J=14Hz, CH₂Ph), 3.39 (1H, dd, J=3 and 9Hz, 5-H), 3.64 (3H, s, CO₂CH₃), 4.33 (1H, broad, d, J=3Hz, 4-H), 4.47 (1H, d, J=9 Hz, 6-H), 7.0 ~ 7.5 (4H, m, Ph); MS (FAB, positive) m/z 341.2 (M+H)+, 282.1, 180.1, 75, 57.

N-(2-Fluorophenyl)methylsiastatin B (11)
Compound 11 was obtained from 10 by a similar procedure to that used for the preparation of 9 (yield 91.1%): [α]D –7° (c 0.97, MeOH); IR (KBr) cm⁻¹ 3400, 1640, 1610, 1580, 1560, 1500, 1470, 1410, 1315, 1290, 1270, 1250, 1235, 1220, 1200, 1175, 1150, 1120, 1100, 1070, 960, 950, 930; ¹H NMR (400 MHz, CD₃OD) δ 1.99 (3H, s, NCOCH₃), 2.48 (1H, ddd, J=11, 4.5 and 3 Hz, 3-H), 2.72 (1H, t, J=11 Hz, 2-Hax), 2.83 (1H, dd, J=11 and 4.5 Hz, 2-Heq), 3.35 and 3.90 (2H, ABq, J=14 Hz, CH₂Ph), 3.38 (1H, dd, J=8 and 3 Hz, 5-H), 4.21 (1H, t, J=3 Hz, 4-H), 4.59 (1H, d, J=8 Hz, 6-H), 6.95 ~ 7.5 (4H, m, Ph); MS (FAB, positive) m/z 327 (M+H)+, 268, 207, 109, 75, 57.

N-(2-Methylphenyl)methylsiastatin B Methyl Ester (12)
Compound 12 was obtained from 22 and 2-methylbenzaldehyde by a similar procedure to that used for the preparation of 8 (yield 85.6%): [α]D⁻ –28° (c 0.31, MeOH); IR (KBr) cm⁻¹ 3340, 2960, 2920, 2830, 1740, 1660, 1540, 1500, 1465, 1440, 1390, 1340, 1320, 1300, 1250, 1220, 1170, 1145, 1110, 1095, 1070, 1055, 1020, 965, 950, 940, 910; ¹H NMR (400 MHz, CD₃OD) δ 1.98 (3H, s, NCOCH₃), 2.32 (3H, s, CH₃), 2.56 ~ 2.78 (3H, m, 2-H₂ and 3-H), 3.13 and 3.98 (2H, ABq, J=13 Hz, CH₂Ph), 3.42 (1H, dd, J=3 and 9 Hz, 5-H), 4.32 (1H, broad t, J=3 Hz, 4-H), 4.45 (1H, d, J=9 Hz, 6-H), 7.04 ~ 7.30 (4H, m, Ph); MS (FAB, positive) m/z 337.2 (M+H)+, 318.2, 105, 75, 57.
N-(2-Methylphenyl)methylsiastatin B (13)

Compound 13 was obtained from 12 by a similar procedure to that used for the preparation of 9 (yield 97.4%): $\alpha$ 15° (c 1.95, MeOH); IR (KBr) cm⁻¹ 3400, 3250, 1690, 1635, 1610, 1590 (sh), 1570 (sh), 1540, 1470, 1410, 1380 (sh), 1340, 1310, 1290, 1210, 1170, 1150, 1110, 1090, 1050, 960, 925; ¹H NMR (400 MHz, CD₃OD) δ 1.99 (3H, s, NCOCH₃), 2.31 (3H, s, CH₃), 2.46 (1H, ddd, J = 3, 4 and 11 Hz, 3-H), 2.68 (1H, t, J = 11 Hz, 2-H₇₄), 2.79 (1H, dd, J = 4 and 11 Hz, 2-H₆₄), 3.20 and 3.90 (2H, ABq, J = 13 Hz, CH₂Ph), 3.40 (1H, dd, J = 3 and 8 Hz, 5-H), 4.20 (1H, t, J = 3 Hz, 4-H), 4.59 (1H, d, J = 8 Hz, 6-H), 7.04 ~ 7.30 (4H, m, Ph); MS (FAB, positive) m/z 323.2 (M + H)⁺, 264.1, 105.1, 75, 57.

N-(2-Trifluoromethylphenyl)methylsiastatin B Methyl Ester (14)

Compound 14 was obtained from 22 and 2-trifluorobenzaldehyde by a similar procedure to that used for the preparation of 8 (yield 89.8%): $\alpha$ 12° (c 0.47, MeOH); IR (KBr) cm⁻¹ 3370, 2980, 2950, 2860, 1750, 1670, 1550, 1450, 1390, 1325, 1300, 1255, 1230, 1180, 1170, 1150, 1130, 1120, 1080, 1050, 1030, 980, 970, 915; ¹H NMR (400MHz, CD₃OD) S 1.95 (3H, s, NCOCH₃), 2.61-2.76 (3H, m, 2-H₂ and 3-H), 3.46 (1H, dd, J = 3 and 8 Hz, 5-H), 3.46 and 4.06 (2H, ABq, J = 15 Hz, CH₂Ph), 3.64 (3H, s, COOCH₃), 4.36 (1H, t, J = 3Hz, 4-H), 4.52 (1H, d, J = 8 Hz, 6-H), 7.34 ~ 7.88 (4H, m, Ph); MS (FAB, positive) m/z 389.1 (M + H)⁺, 332.1, 230.1, 159.1.

N-(2-Trifluoromethylphenyl)methylsiastatin B (15)

Compound 15 was obtained from 14 by a similar procedure to that used for the preparation of 9 (yield 100%): $\alpha$ 2.2° (c 1.96, MeOH); IR (KBr) cm⁻¹ 3420 (sh), 3350, 2950, 2860, 1750, 1670, 1550, 1450, 1390, 1325, 1300, 1255, 1230, 1180, 1170, 1150 (sh), 1145 (sh), 1130, 1090, 1070, 1050, 960, 930, 910; ¹H NMR (400MHz, CD₃OD) 5 1.95 (3H, s, NCOCH₃), 2.56 (1H, ddd, J = 3, 6 and 11.5Hz, 3-H), 2.65-2.80 (2H, m, 2-H₂ and 3-H), 3.46 (1H, dd, J = 3 and 8 Hz, 5-H), 3.50 and 4.00 (2H, ABq, J = 15 Hz, CH₂Ph), 4.27 (1H, t, J = 3 Hz, 4-H), 4.62 (1H, d, J = 8 Hz, 6-H), 7.3 ~ 8.0 (4H, m, Ph); MS (FAB, positive) m/z 377.2 (M + H)⁺, 318.2, 159.1, 75, 57.

N-Propylsiastatin B Methyl Ester (16)

Compound 16 was obtained from 22 and propanal by a similar procedure to that used for the preparation of 8 (yield 64.3%): $\alpha$ 22° (c 0.16, MeOH); IR (KBr) cm⁻¹ 3500 (sh), 3430, 3300, 2975, 2880, 2850, 1750, 1740, 1710, 1660, 1560, 1440, 1380, 1340, 1320, 1300 (sh), 1290, 1275, 1225, 1210, 1185, 1170, 1130, 1110, 1060, 1010, 980, 950, 930, 920, 910; ¹H NMR (400MHz, D₂O) S 0.85 (3H, t, J = 7Hz, CH₃), 2.09 (3H, s, NCOCH₃), 1.3-1.7 (2H, m, CH₂), 2.31 and 2.65 (each 1H, ddd, J = 5, 11 and 13Hz, NCH₂), 2.72 (1H, t, J = 13 Hz, 2-H₇₄), 2.89 (1H, ddd, J = 3, 4 and 13 Hz, 3-H), 3.10 (1H, dd, J = 4 and 13 Hz, 2-H₆₄), 3.49 (1H, dd, J = 3 and 10 Hz, 5-H), 4.35 (1H, d, J = 10 Hz, 6-H), 4.45 (1H, t, J = 3 Hz, 4-H); MS (FAB, positive) m/z 275.2 (M + H)⁺, 216.2, 114.1, 72.1.

N-Propylsiastatin B (17)

Compound 17 was obtained from 16 by a similar procedure to that used for the preparation of 9 (yield 82%): $\alpha$ 9° (c 0.44, H₂O); IR (KBr) cm⁻¹ 3450, 3220, 2970, 2880, 2730, 1690, 1670, 1610, 1550, 1390, 1340, 1310, 1270, 1240, 1215, 1160, 1140, 1110, 1060, 1010, 970, 920; ¹H NMR (400 MHz, D₂O with a few drops of pyridine-d₅) S 0.76 and 0.78 (each 3H, d, J = 7 Hz, CH₃), 1.67 (1H, t, J = 12 Hz, 2-H₇₄), 2.92 (1H, dd, J = 4 and 12 Hz, 2-H₆₄), 3.45 (1H, dd, J = 3 and 9.5 Hz, 5-H), 4.33 (1H, t, J = 9.5 Hz, 4-H), 4.34 (1H, d, J = 9.5 Hz, 6-H); MS (FAB, positive) m/z 261.2 (M + H)⁺, 202.1, 75, 72.1.

N-2-Methylpropylsiastatin B (18)

Compound 18 was obtained from 1 and 2-methylpropanal by a similar procedure to that used for the preparation of 9 (yield 77.2%): $\alpha$ 11° (c 0.4, H₂O); IR (KBr) cm⁻¹ 3400, 3350, 3320, 3260, 3225, 2975, 1700, 1620, 1550, 1470, 1410, 1390, 1350, 1310, 1270, 1250, 1220, 1170, 1140, 1115, 1065, 1010, 980, 955, 930; ¹H NMR (400 MHz, D₂O with a few drops of pyridine-d₅) δ 0.76 and 0.78 (each 3H, d, J = 7 Hz, CH₃ x 2), 1.67 (1H, t, J = 7 Hz, CH₃ x 2), 2.00 (3H, s, NCOCH₃), 2.13 and 2.30 (2H, dd, J = 5 and 13,
\( J = 9 \) and \( 13 \) Hz, \( \text{CH}_2 \), 2.48 \( \sim \) 2.62 (2H, m, \( 2\text{-H} \) and \( 3\text{-H} \)), 2.88 \( \sim \) 3.00 (1H, m, \( 2\text{-H} \)), 3.47 (1H, dd, \( J = 3 \)\ and \( 9 \) Hz, \( 5\text{-H} \)), 4.28 (1H, d, \( J = 9 \) Hz, \( 6\text{-H} \)), 4.31 (1H, t, \( J = 3 \) Hz, \( 4\text{-H} \)); MS (FAB, positive) \( m/z \) 275.2 (M + H)\(^+\), 216.2, 143.1, 128.1, 86.1, 57.1.

**N-(2,2-Dimethylpropyl)siasstatin B (19)**

Compound 19 was obtained from 1 and 2,2-dimethylpropanal by a similar procedure to that used for the preparation of 8 (yield 49.4\%): \( [\alpha]^25_0 + 13^\circ \) (c 0.61, H\(_2\)O); IR (KBr) cm\(^{-1}\) 3420, 3200, 1680, 1640, 1600, 1490, 1410, 1170, 1140, 1110, 1040, 1000, 980, 940, 920; \(^1\)H NMR (400 MHz, D\(_2\)O with a few drops of pyridine-d\(_5\) ) \( \delta \) 0.84 (9H, s, \( \text{CH}_3 \times 3 \)), 2.07 (3H, s, NCOCH\(_3\)), 2.11 and 2.30 (2H, ABq, \( J = 15 \) Hz, \( \text{CH}_2 \)), 2.70 (1H, dt, \( J = 3.5 \) and 11 Hz, 3-H), 2.85 (1H, t, \( J = 13 \) Hz, 2-H\(_{ax}\)), 3.04 (1H, br s, 6-H), 4.34 (1H, brs, 4-H), 4.47 (1H, d, \( J = 8 \) Hz, 6-H); MS (FAB, positive) \( m/z \) 289.2 (M + H)\(^+\), 230.2, 142.2, 100.1, 71.1, 57.

**Ar-Benzyl-3,4-didehydro-4-deoxysiastatin B (20) and its Methyl Ester (21)**

Compounds 20 and 21 were obtained in yields of 65 and 78\%, respectively from 24 and 25 by the similar procedures to those used for the preparation of 7 from 1.

**20**: \( [\alpha]^26_0 + 94^\circ \) (c 0.24, H\(_2\)O); \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) 2.02 (3H, s, NCOCH\(_3\)), 3.37 (1H, dt, \( J = 1.5 \) and 18 Hz, 2-H), 3.54 (1H, dt, \( J = 1.5 \) and 18 Hz, 2-H), 3.78 and 3.90 (2H, ABq, \( J = 13 \) Hz, \( \text{CH}_2 \)), 4.14 (1H, r,b, \( J = 1.5 \) and 3 Hz, 5-H), 4.80 (1H, br s, 6-H), 6.55 (1H, dt, \( J = 1.5 \) and 4 Hz, 4-H), 7.3 \( \sim \) 7.7 (5H, m, Ph).

**21**: \( [\alpha]^5_0 + 131^\circ \) (c 0.55, H\(_2\)O); \(^1\)H NMR (400 MHz, CD\(_3\)OD) \( \delta \) 1.97 (3H, s, NCOCH\(_3\)), 3.17 (1H, dt, \( J = 2 \) and 18 Hz, 2-H), 3.37 (1H, dt, \( J = 1.5 \) and 18 Hz, 2-H), 3.65 and 3.78 (2H, ABq, \( J = 14 \) Hz, \( \text{CH}_2 \)), 3.72 (3H, s, COOCH\(_3\)), 3.97 (1H, m, 5-H), 4.91 (1H, d, \( J = 3 \) Hz, 6-H), 6.88 (1H, dt, \( J = 2 \) and 5 Hz, 4-H), 7.2 \( \sim \) 7.4 (5H, m, Ph).

**Siastatin B Methyl Ester (22)**

A solution of 1 (487 mg) in dry MeOH (15 ml) was stirred with Amberlist 15 (H\(^+\)) (500 mg) at room temperature for 1 day. After addition of conc aq ammonia (pH \( \sim \) 9), the resin was filtered off. Evaporation of the filtrate gave a solid, which was subjected to the column chromatography on silica gel. Elution with a mixture of CH\(_2\)Cl\(_2\)-MeOH-conc aq ammonia (20: 10: 3) gave a colorless solid of 22 (434 mg, 83.7\%). The solid was crystallized from MeOH to give colorless crystals: MP 177 \( \sim \) 178°C; \( [\alpha]^25_0 + 18^\circ \) (c 0.59, H\(_2\)O); IR (KBr) cm\(^{-1}\) 3530, 3380, 3300, 3130, 3000, 2960, 2930, 1770, 1750, 1670, 1585, 1465, 1450, 1400, 1365, 1345, 1320, 1295, 1285, 1250, 1220, 1185, 1155, 1145, 1110, 1095, 1065, 1030, 1000, 980, 960, 930, 910; \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) 2.44 (3H, s, NCOCH\(_3\)), 3.85 (1H, ddd, \( J = 2, 6 \) and 11 Hz, 3-H), 3.0 \( \sim \) 3.15 (2H, m, 2-H\(_2\)), 3.55 (1H, dd, \( J = 2 \) and 10 Hz, 5-H), 3.75 (3H, s, COOCH\(_3\)), 4.49 (1H, t, \( J = 2 \) Hz, 4-H) and 4.61 (1H, d, \( J = 10 \) Hz, 6-H); MS (FAB, positive) \( m/z \) 233.2 (M + H)\(^+\), 174.1, 75, 57.

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**References**


