STUDIES ON NEW DEHYDROPEPTIDASE INHIBITORS

IV. CHEMICAL TRANSFORMATION OF WS1358 COMPOUNDS AND AN IMPROVED SYNTHESIS OF WS1358A1

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The structures of compounds **3** (and **4**) and **5** (and **6**) derived from the natural products WS1358A1 (**1**) (and B1 (**2**)) have been determined by their spectroscopic evidence. By taking advantage of these transformations, an improved synthesis of A1 (**1**, racemate) has been achieved.

It has been shown that most carbapenem antibiotics are susceptible to deactivation by renal dehydropeptidase (DHP)[1]. Therefore, inhibitors of this enzyme suitable for combination with the antibiotics have been developed to protect them from inactivation in the kidney[2].

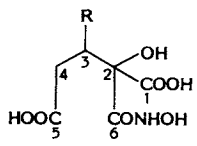
In the preceding papers[3,4], we reported the isolation and structural determination of new DHP inhibitors, WS1358A1 (**1**) and B1 (**2**) of microbial origin.

During the course of studies on these compounds, we found that A1 suffers chemical transformations to butyrolactone A2 (**3**) and *N*-hydroxyglutarimide A3 (**5**), which are readily reconverted to the original compound A1. As described in one of the preceding papers[3], these transformations were also found to expedite the purification of this series of natural products.

This paper describes the structures of these transformed products and a practical synthesis of WS1358A1 (racemate) by taking advantage of these transformations.

Results and Discussion

WS1358A1 (**1**, Na salt) was converted to the free acid by treatment with Dowex 50WX2 (H^+) and lyophilized to give a mixture of A1, A2 (**3**) and A3 (**5**) (see Fig. 1(A) for HPLC analysis). Separation by the preparative HPLC using YMC ODS-5 reverse-phase column (20 mM $NH_4H_2PO_4$-1 mM $Bu_4N^+OH^-$ (pH 4)), followed by acidification (Dowex 50WX2 (H^+)) and purification (Diaion SP-207 chromatography)



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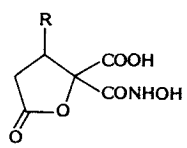
2 R=H



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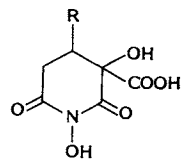

5 R=CH_3
6 R=H

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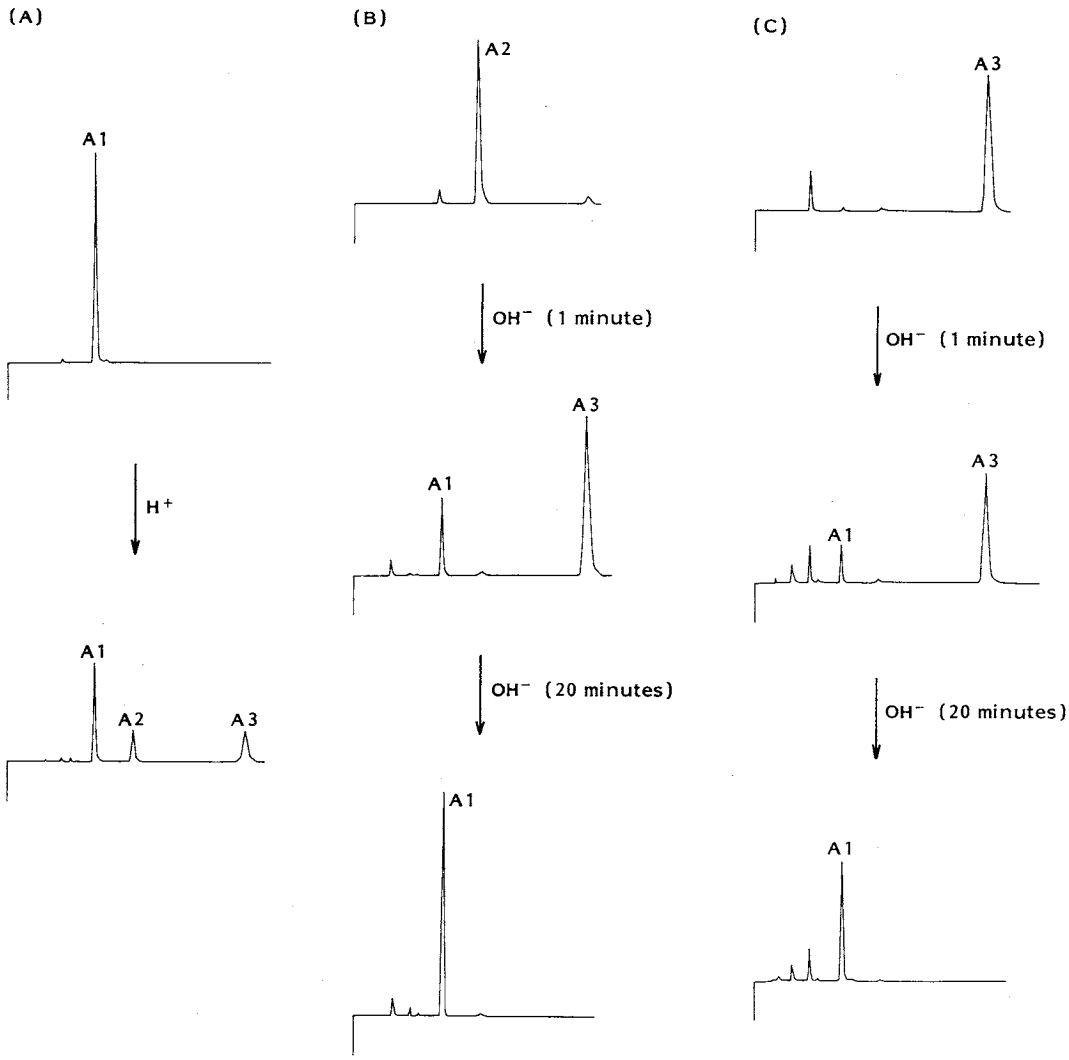
Fig. 1. Chemical transformation of natural WS1358A series: HPLC analysis.

(A) A1 ⟶ A2+A3, (B) A2 ⟶ (A3) ⟶ A1, (C) A3 ⟶ A1.



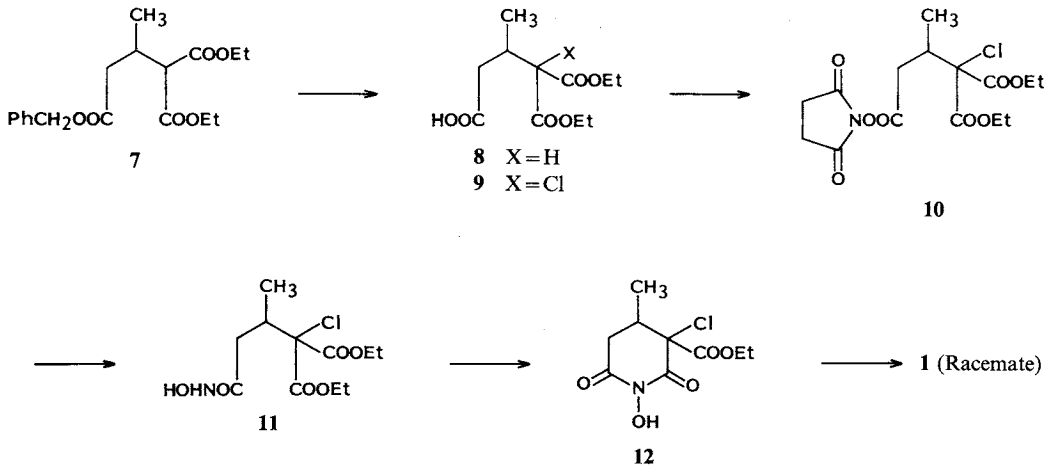
gave A2 as free acid and A3, after neutralization with 1 N NaOH, as Na salt. When treated with an excess of NaOH, A3 was reverted to A1 and A2 to A1 as well *via* A3 (see Figs. 1(B) and 1(C)).

WS1358A2 (**3**), $C_7H_9NO_6$ (FAB-MS, *m/z* 204 $(M+H)^+$), showed an absorption band at 1770 cm^{-1} in the IR spectrum suggesting the presence of a γ-lactone system in **3**. In the ^{13}C NMR spectrum, **3** exhibited a down-field shift of 5.4 ppm for C-2 as compared to the corresponding signal in **1** (acylation shift)[5]. These data suggest that WS1358A2 possesses the structure **3**.

WS1358A3 (**5**), $C_7H_8NO_6Na$ (elemental analysis); free acid, $C_7H_9NO_6$ (FAB-MS, *m/z* 204 $(M+H)^+$), showed carbonyl bands at 1730 and 1670 cm^{-1} in addition to 1630 cm^{-1} (carboxylate) in the IR spectrum of the Na salt, suggesting the presence of a *N*-hydroxyglutarimide function in **5**. Hence the structure of WS1358A3 was assigned to be **5**.

We found in the conversion of A3 to A1 that the hydrolysis was regiospecific to yield A1 almost as a single product and, therefore, we anticipated that a stereoselective synthesis of A3 could be entirely

Fig. 2. Improved synthesis of WS1358A1 racemate.



corresponding to a regio- and stereoselective synthesis of A1. Thus, we undertook the synthesis of A1 according to the synthetic route outlined in Fig. 2. We envisioned that, in this synthesis, the cyclization of **11** to **12** would proceed thermodynamically, resulting in a stereoselective synthesis of **12**. Hydrolysis of **12** would then stereoselectively afford the final product **1** (racemate) as described above, although is was ambiguous at that time whether the desired configuration would be obtained.

The starting material **7**, prepared by Michael condensation[6] of benzyl crotonate and diethyl malonate, was hydrogenolysed and the resulting carboxylic acid **8** was chlorinated with NaOCl to give chloride **9**. Activation of the carboxylic acid in **9** with *N*-hydroxysuccinimide and dicyclohexylcarbodiimide, followed by treatment with hydroxylamine produced a mixture of hydroxamic acid **11** and *N*-hydroxyglutarimide **12** together with other minor products. It was clear that, in the above reactions, **12** was formed by cyclization of **11** (see below for a corroborative evidence). The separation of **11**, **12**, and the minor products at this stage was found to be difficult, because **11** was partially cyclized to **12** during the period of purification by chromatography on silica gel. Therefore, the mixture was directly subjected to the next reaction and treated with 1 N NaOH. The reaction mixture was acidified using Dowex 50WX2 (H^+) and lyophilized to give a crude mixture, which was applied to a Diaion SP-207 column chromatography to give pure A3 (**5**, racemate) as the first eluent. Later fractions containing A2 and the minor products were further purified by another SP-207 chromatography, followed by preparative HPLC (YMC ODS-5) to give a partially purified A2 (**3**) and one of the minor products. The product A2 was treated with Dowex 50WX2 (H^+) and followed by further purification by Diaion SP-207 chromatography and lyophilization to afford A2 (**3**, racemate). The minor product isolated as above was also purified in a similar manner and, after neutralization to pH 6 with 1 N NaOH, lyophilized to give pure product A3′ (see Fig. 3). The spectral data of A3′ was quite similar to those of A3 (**5**), revealing that A3′ is a diasteroisomer of A3. The ratio of A3 and A3′ just after the hydrolysis of **11** and **12** was about 5 : 1 on HPLC.

Transformation of the A series of compounds is summarized in Fig. 3. On treatment with 0.5 N NaOH (1 minute), A2 (**3**) was cyclized to A3 (**5**), which in turn was exposed to a prolonged treatment with the same alkaline medium (*e.g.* 20 minutes) to give A1 (**1**). A similar treatment of A3′ (0.5 N NaOH, 20 minutes) gave A1′ as well. As shown in Fig. 3, A3′ might be converted into A1′ *via* A2′ (corresponding to the

Fig. 3. Proposed pathway of chemical transformation of WS1358A series.

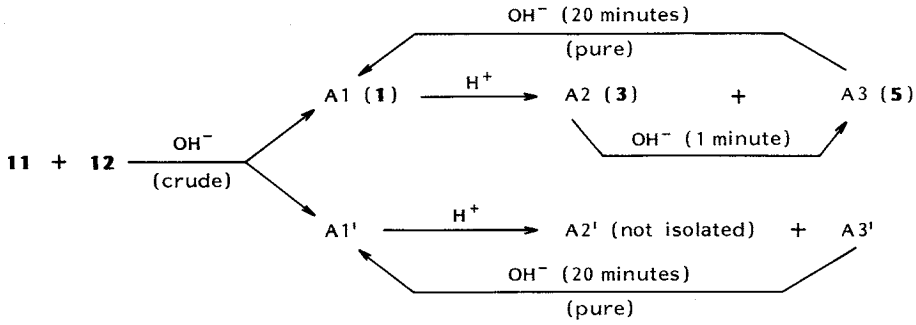


Fig. 4. Chemical transformation of natural WS1358B series: HPLC analysis.

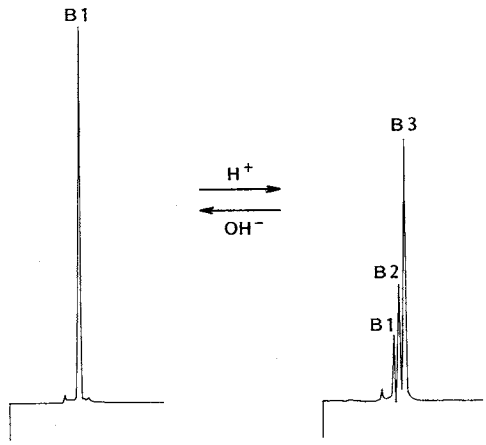


Table 1. Inhibitory activity (IC_{50}) of synthetic and natural WS1358 compounds against porcine DHP.

Compound	IC_{50}(ng/ml)	
	Synthetic	Natural
A1 (**1**)	1.5	0.83
A1′	230	nd
A2 (**3**)	16	10
A3 (**5**)	78	45
A3′	4,300	nd
B1 (**2**)	340	150
B2 (**4**)	nd	2,000
B3 (**6**)	nd	>10,000

nd: Not determined.

diasteroisomer of A2) which could not be isolated in the synthesis described above.

The minor product B1 (**2**) from the natural source was found to show a similar behavior. Thus, after acidification with Dowex 50WX2 (H^+), B1 was lyophilized to give B2 (**4**) and B3 (**6**) together with B1 unchanged (see Fig. 4). Isolation of B2 and B3 was performed by a procedure similar to that used for the A series of compounds.

WS1358B2 showed an IR absorption band at 1780 cm^{-1}, indicating the γ-lactone structure **4** for B2, while the IR spectrum of B3 showed, in addition to 1680 cm^{-1} (carboxylic acid), carbonyl bands at 1730 and 1640 cm^{-1} which were assignable to the carbonyls of the *N*-hydroxyglutarimide structure, leading to the structure **6** for B3. The other physical data of B2 and B3 are recorded in Experimental. On treatment with 0.5 N NaOH, B2 and B3 showed a behavior similar to those of A2 and A3, respectively, and finally the two compounds reverted to B1 (**2**).

Each product of both A and B series was thus chemically characterized and, fortunately, the synthetic route described above was amenable for obtaining stereoselectively the major natural product A1 although its stereochemistry remained equivocal.

The biological activities of the product described above are summarized in comparison with those of the natural A1 (**1**) and B1 (**2**) in Table 1. The activities of the natural A2 (**3**) and A3 (**5**) were considerably less than that of A1 and this was the case of the B series of products. The synthetic compounds A1, A2, A3 and B1 (all racemates) were approximately 2-fold less active than the corresponding natural products,

respectively. The diasteroisomer A1′ and A3′ were considerably less active even when compared with the synthetic A1 and A3, respectively.

Experimental

General

IR spectra were recorded on a Jasco A-120 spectrometer. ^1H and ^{13}C NMR spectra were measured on a Bruker AM400 spectrometer. Mass spectra were determined with a VG ZAB-SE spectrometer. Analytical HPLC was performed on a Hitachi 655 liquid chromatography using a YMC ODS-5 column (4.6 × 250 mm) and preparative HPLC was carried out using a YMC ODS-5 column (20 × 250 mm).

WS1358A2 (3) and A3 (5)

WS1358A1 (**1**, Na salt, 100 mg) was dissolved in water (2 ml) and passed through a column of Dowex 50WX2 (H^+, 3 ml) and eluted with water (10 ml). The eluate was adjusted to pH 1.8 with 6 N HCl and lyophilized to give a powder (76 mg, see Fig. 1(A) for HPLC analysis). Preparative HPLC of this mixture was carried out on YMC ODS-5 using a buffer of 20 mM $NH_4H_2PO_4$ - 1 mM $Bu_4N^+OH^-$ (pH 4) as eluant. The fraction containing A2 was purified by chromatography on Diaion SP-207 (7.5 ml) eluting with water and the eluate was passed through a column of Dowex 50WX2 (H^+, 7 ml) and eluted with water. The eluate was lyophilized to give A2 (**3**, 24 mg) as the free acid: FAB-MS, *m/z* 204 $(M+H)^+$; IR (KBr) cm^{-1} 1780~1640 (br); ^1H NMR (D_2O) δ 3.16 (1H, m), 2.87 (1H, dd, J=18 and 8.5 Hz), 2.49 (1H, dd, J=18 and 8 Hz), 1.23 (3H, d, J=7 Hz); ^{13}C NMR (D_2O) δ 180.6 (s), 171.8 (s), 168.6 (s), 91.2 (s), 39.6 (d), 37.7 (t), 17.9 (q).

The Na salt of A2 was prepared by neutralization of a solution of the free acid in water to pH 7 with 1 N NaOH and subsequent lyophilization: IR (KBr) cm^{-1} 1770, 1650, 1600; ^1H NMR (D_2O) δ 3.08 (1H, m), 2.82 (1H, dd, J=18 and 8 Hz), 2.40 (1H, dd, J=18 and 7 Hz), 1.15 (3H, d, J=7 Hz); ^{13}C NMR (D_2O) δ 181.9 (s), 174.4 (s), 170.4 (s), 93.4 (s), 38.9 (d), 38.5 (t), 18.4 (q).

The fraction containing A3 on the preparative HPLC was similarly purified. The eluate obtained by a Dowex 50WX2 column chromatography was neutralized to pH 7 with 1 N NaOH and lyophilized to give A3 (**5**, 32 mg) as the Na salt: IR (KBr) cm^{-1} 1730, 1670, 1630; ^1H NMR (D_2O) δ 2.86 (1H, dd, J=17.5 and 5 Hz), 2.76 (1H, dd, J=17.5 and 9.5 Hz), 2.62 (1H, m), 0.97 (3H, d, J=6.5 Hz); ^{13}C NMR (D_2O) δ 177.4 (s), 174.2 (s), 173.9 (s), 83.0 (s), 38.6 (t), 34.5 (d), 15.9 (q).

The free acid was prepared by treatment of a solution of the Na salt in water with Dowex 50WX2 (H^+) and subsequent lyophilization: FAB-MS, *m/z* 204 $(M+H)^+$; IR (KBr) cm^{-1} 1720~1620 (br); ^1H NMR (D_2O) δ 2.83 (1H, dd, J=17 and 5 Hz), 2.83~2.65 (2H, m), 0.96 (3H, d, J=7 Hz); ^{13}C NMR (D_2O) δ 175.5 (s), 173.4 (s), 171.9 (s), 82.3 (s), 38.0 (t), 34.4 (d), 15.6 (q).

WS1358B2 (4) and B3 (6)

Compounds B2 and B3 were obtained in a manner similar to that for A2 and A3 as the free acids. For HPLC analysis, see Fig. 4. B2 (**4**): FAB-MS, *m/z* 190 $(M+H)^+$; IR (KBr) cm^{-1} 1780, 1730, 1680; ^1H NMR (D_2O) δ 2.80~2.71 (4H, m); ^{13}C NMR (D_2O) δ 181.1 (s), 173.2 (s), 168.9 (s), 88.6 (s), 31.9 (t), 30.1 (t). B3 (**6**): FAB-MS, *m/z* 190 $(M+H)^+$; ^1H NMR (D_2O) δ 3.04~2.88 (2H, m), 2.45 (1H, m), 2.26 (1H, m); ^{13}C NMR (D_2O) δ 175.1 (s), 173.8 (s), 172.3 (s), 79.3 (s), 31.0 (t), 30.0 (t).

Conversion of A2 (3) to A1 (1) *via* A3 (5)

A sample (5 mg) of **3** (free acid) was dissolved in 0.5 N NaOH (5 ml) and, after 1 minute, the solution was analyzed using HPLC. After being left at room temperature for 20 minutes, the solution was again subjected to HPLC analysis. The data were shown in Fig. 1(B).

Conversion of A3 (5) to A1 (1)

A sample (5 mg) of **5** (free acid) was dissolved in 0.5 N NaOH (5 ml) and, after being left at room temperature for 1 and 20 minutes subjected to HPLC analysis. The data were shown in Fig. 1(C).

Benzyl 4,4-Diethoxycarbonyl-3-methylbutyrate (7)

A solution of diethyl malonate (8.16 g, 0.051 mol) in benzene (30 ml) was added to a suspension of sodium hydride (60% dispersion in mineral oil, 0.2 g, 5 mmol) in benzene (30 ml) under nitrogen atmosphere. After the evolution of hydrogen ceased, a solution of benzyl crotonate (9 g, 0.051 mol) in benzene (30 ml) was added to above mixture. The mixture was refluxed for 15 hours and, after cooling to room temperature, the reaction mixture was poured into ice water (200 ml). The organic layer was separated and the aqueous layer was extracted with ether. The benzene and ether layers were combined, washed with water, and dried over magnesium sulfate. Filtration and evaporation gave a crude product (17 g) which was further evaporated under reduced pressure (2 mmHg) to give **7** as the residue: EI-MS, *m/z* 336 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.35 (5H, m), 5.12 (2H, s), 4.19 (4H, q, J=7 Hz), 3.41 (1H, d, J=7 Hz), 2.75 (1H, m), 2.61 (1H, dd, J=16 and 5 Hz), 2.37 (1H, dd, J=16 and 8.5 Hz), 1.26 (6H, t, J=7 Hz), 1.08 (3H, d, J=7 Hz).

4,4-Diethoxycarbonyl-3-methylbutyric Acid (8)

A solution of **7** (3.36 g, 0.01 mol) in dioxane (50 ml) was hydrogenated over 10% Pd-C (0.5 g) under 5 atm pressure of hydrogen. After removal of the catalyst by filtration, the filtrate was concentrated to give an oily residue (2.43 g), which was purified by chromatography on silica gel (60 g) eluting with 5% methanol in chloroform to give **8** as an oil (2.11 g): EI-MS, *m/z* 247 $(M+H)^+$; ^{1}H NMR ($CDCl_3$) δ 4.21 (4H, q, J=7 Hz), 3.42 (1H, d, J=7 Hz), 2.76 (1H, m), 2.64 (1H, dd, J=16 and 5 Hz), 2.38 (1H, dd, J=16 and 8.5 Hz), 1.28 (6H, t, J=7 Hz), 1.11 (3H, d, J=7 Hz).

4-Chloro-4,4-diethoxycarbonyl-3-methylbutyric Acid (9)

Sodium hypochlorite (available chlorine 5% minimum, 25 ml, 0.017 mol) was added dropwise (30 minutes) to a solution of **8** (3.69 g, 0.015 mol) in water (50 ml) with stirring under room temperature. The pH of the mixture was reached to 7.0. After stirring for an additional 30 minutes, the reaction mixture was acidified to pH 2.0 with 1 N HCl and extracted with ether. The extract was washed with water, dried over magnesium sulfate and evaporated to give a crude oil, which was purified by chromatography on silica gel (150 g) eluting with 5% methanol in chloroform to give **9** as an oil (3.56 g): FAB-MS, *m/z* 281, 283 $(M+H)^+$; IR $(CHCl_3)$ cm^{-1} 1740, 1710; ^{1}H NMR ($CDCl_3$) δ 4.30 (2H, q, J=7 Hz), 4.29 (2H, q, J=7 Hz), 3.14 (1H, m), 2.87 (1H, dd, J=16.5 and 2.5 Hz), 2.33 (1H, dd, J=16.5 and 10 Hz), 1.31 (3H, t, J=7 Hz), 1.29 (3H, t, J=7 Hz), 1.12 (3H, d, J=7 Hz).

4-Chloro-4,4-diethoxycarbonyl-3-methylbutyric Acid Succinimide Ester (10)

To a mixture of **9** (2.81 g, 10 mmol) and *N*-hydroxysuccinimide (1.15 g, 10 mmol) in methylene chloride (50 ml) was added dicyclohexylcarbodiimide (2.06 g, 10 mmol) and the mixture was stirred at room temperature for 15 hours. After removal of the precipitate by filtration, the filtrate was concentrated to give **10** as a crude oil (2.96 g), which was used for the next reaction without purification: ^{1}H NMR ($CDCl_3$) δ 4.30 (2H, q, J=7 Hz), 4.29 (2H, q, J=7 Hz), 3.13 (1H, m), 3.10 (1H, dd, J=16.5 and 2.5 Hz), 2.85 (4H, s), 2.59 (1H, dd, J=16.5 and 10 Hz), 1.30 (3H, t, J=7 Hz), 1.29 (3H, t, J=7 Hz), 1.15 (3H, d, J=7 Hz).

Diethyl 2-Chloro-2-((2-hydroxyaminocarbonyl-1-methyl)ethyl)malonate (11) and Ethyl 3-Chloro-1-hydroxy-4-methyl-2,6-dioxo-3-piperidinecarboxylate (12)

A solution of hydroxylamine hydrochloride (834 mg, 12 mmol) in water (10 ml) was, after neutralization by adding 1 N NaOH (15 ml), added to a solution of **10** (2.96 g, 10 mmol) in ethanol (30 ml) with stirring at 0°C. The mixture was stirred at room temperature for 5 hours and at 80°C for 2 hours. The reaction mixture was acidified to pH 2 with 1 N HCl, diluted with water (150 ml), and extracted with ethyl acetate. The extract was washed with water, dried over magnesium sulfate, and evaporated to give a crude mixture (2.7 g), which was purified by chromatography on silica gel (90 g) eluting with 5% methanol in chloroform to give a mixture of **11** and **12** (1.78 g). A 100-mg sample of this mixture was subjected to a preparative TLC (silica gel plate, 0.5 mm) developing with 10% 2-propanol in benzene to give **11** (44 mg, oil) and **12** (45 mg, oil). **11** (major diastereomer): FAB-MS, *m/z* 296, 298 $(M+H)^+$ and 318, 320 $(M+Na)^+$; IR $(CHCl_3)$ cm^{-1} 1740, 1665; ^{1}H NMR (CD_3OD) δ 4.28 (4H, q, J=7 Hz), 3.10 (1H, m), 2.47 (1H, dd, J=14 and 2.5 Hz), 2.03 (1H, dd, J=14 and 11 Hz), 1.29 (6H, t, J=7 Hz), 1.04 (3H, d, J=7 Hz). **12** (major

diastereomer): FAB-MS, m/z 250, 252 $(M+H)^+$ and 272, 274 $(M+Na)^+$; IR $(CHCl_3)$ cm^{-1} 1750, 1695; ^1H NMR (CD_3OD) δ 4.35 (2H, q, $J=7$ Hz), 3.11 (1H, m), 2.82 (2H, m), 1.31 (3H, t, $J=7$ Hz), 1.04 (3H, d, $J=7$ Hz); ^{13}C NMR (CD_3OD) δ 168.2, 166.3, 165.3, 75.2, 64.9, 37.1, 34.0, 14.7, 14.2.

Alkaline Treatment of **11** and **12**

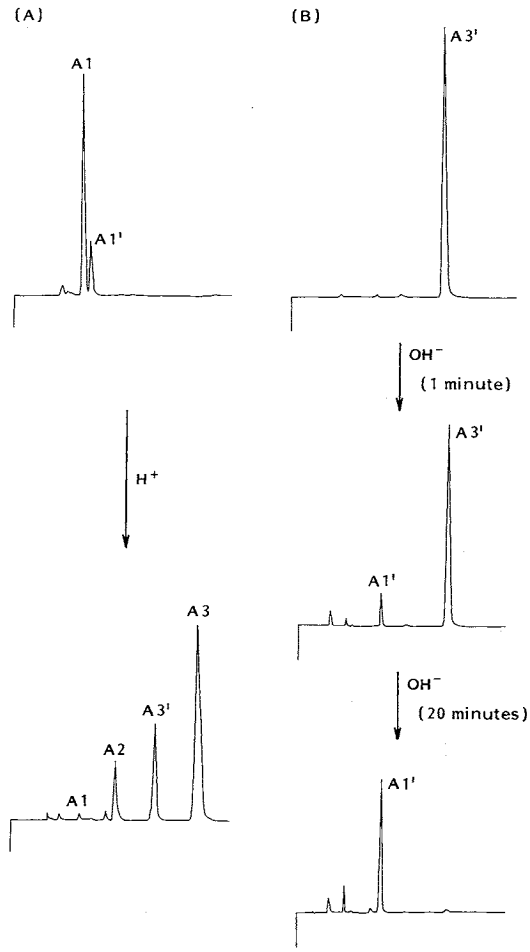
A mixture of **11** and **12** (400 mg) was dissolved in 1 N NaOH (8 ml) and stirred at room temperature for 3 hours. After neutralization to pH 7 with 1 N HCl, the mixture was analyzed by HPLC using a column of YMC ODS-5. The data were shown in Fig. 5(A). The ratio of A1 (**1**) and its diastereomer was about 5 : 1.

The mixture obtained above was acidified by passing through a column of Dowex 50WX2 (H^+, 6 ml) eluting with water and the eluate was lyophilized to give a crude product containing A1, A2, A3, and their diastereoisomer (324 mg). This mixture was applied to a column of Diaion SP-207 (60 ml) eluting with water. The fractions containing A3 only were lyophilized to give pure A3 (**5**, racemate, 97 mg): The IR, ^1H and ^{13}C NMR spectra and the retention time on HPLC were all identical with those of the natural product.

The fractions containing A2 and A3′ mainly were lyophilized to give a mixture of A2 and A3′ (105 mg), which was subjected to preparative HPLC on YMC ODS-5 eluting with a buffer of 20 mM $NH_4H_2PO_4$-1 mM $Bu_4N^+OH^-$ (pH 4) at a flow rate of 5.0 ml/minute. The fraction containing A2 was concentrated to give a crude product of A2 (42 mg), which was acidified by a Dowex 50WX8 (H^+) column (10 ml) eluting with water and the eluate was concentrated to about 2 ml. This concentrate was purified by chromatography on Diaion SP-207 (7 ml) eluting with water and the eluate was lyophilized to give A2 (**3**, racemate, 32 mg): The IR, ^1H and ^{13}C NMR and the retention time on HPLC were all identical with those of the natural product.

Fig. 5. Chemical transformation of synthetic WS-1358A series: HPLC analysis.

(A) A1 (A1′) ⟶ A2 + A3 (A3′), (B) A3′ ⟶ A1′.



The fraction containing A3′ on the preparative HPLC described above was concentrated to give a crude product of A3′ (20 mg), which was purified in a similar manner using Dowex 50WX8 and Diaion SP-207. The fraction of the final Diaion SP-207 chromatography was adjusted to pH 6.2 (1 N NaOH) and lyophilized to give A3′ (the diastereoisomer of A3, racemate, 16 mg): IR (KBr) cm^{-1} 1680, 1640; ^1H NMR (D_2O) δ 2.87 (2H, d, $J=9$ Hz), 2.39 (1H, m), 1.00 (3H, d, $J=6.5$ Hz); ^{13}C NMR (D_2O) δ 175.5 (s), 174.5 (s), 174.4 (s), 83.5 (s), 39.3 (t), 35.4 (d), 16.8 (q).

WS1358A1 (**1**, Racemate)

A sample (500 mg) of the synthetic compound A3 (**5**, racemate) was dissolved in 0.5 N NaOH (10 ml) and allowed to stand for 3 hours at room temperature, the solution was then neutralized by passing through a column of Dowex 50WX2 (H^+, 6 ml) eluting with water. The eluate was subjected to chromatography on DEAE-Toyopearl (Cl^-, 45 ml) eluting with 0.02 M NaCl solution and the eluate was concentrated and adjusted to pH 2.0 with 6 N HCl. The concentrate was further purified by chromatography on Diaion SP-207 (60 ml) eluting with water and the eluate was neutralized to pH 7 with 1 N NaOH, then lyophilized

to give A1 (**1**, racemate, Na salt, 213 mg): The IR, ^1H and ^{13}C NMR spectra and the retention time on HPLC were all identical with those of the natural product.

Diastereoisomer A1′ (Racemate) of WS1358A1

A sample (20 mg) of A3′ (racemate) described above was treated with 0.5 N NaOH in a manner similar to that for the conversion of A3 to A1, giving pure A1′ (racemate, Na salt, 9 mg): IR (KBr) cm^{-1} 1650, 1620; ^1H NMR (D_2O) δ 2.76 (1H, m), 2.08 (1H, dd, $J=14$ and 3 Hz), 1.99 (1H, dd, $J=14$ and 11 Hz), 0.87 (3H, d, $J=6.5$ Hz); ^{13}C NMR (D_2O) δ 184.5 (s), 177.6 (s), 173.2 (s), 86.0 (s), 41.9 (t), 38.9 (d), 16.0 (q). For HPLC analysis, see Fig. 5(B).

HPLC Analysis

A Model 635 Hitachi liquid chromatograph was used in this study. A 15-cm TSK gel ODS 80TM column was used with a mobile phase consisting of 0.2 M $NH_4H_2PO_4$ (pH 4) containing 1 mM $(n\text{-}C_4H_9)_4$-NOH for WS1358A compounds. A 25-cm YMC ODS-5 column was used with a mobile phase of 0.1% TFA for WS1358B compounds. The flow rate was 1 ml/minute and solutes were detected by UV (210 nm). The retention times of A1, A2, A3, A1′ and A3′ were 7.15, 10.35, 19.55, 8.13 and 14.75 minutes, respectively. Those of B1, B2 and B3 were 3.73, 3.98 and 4.29 minutes, respectively.

Biological Evaluation

Inhibitory activity (IC_{50}) of the compounds described above against porcine DHP was measured by the method described in the preceding paper[3].

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