Tetrahedron Letters,Vol.23,No.51,pp 5435-5438,1982 0040-4039/82/515435-04$03.00/0
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(STUDIES ON AI-77s, MICROBIAL PRODUCTS WITH PHARMACOLOGICAL ACTIVITY) STRUCTURES AND THE CHEMICAL NATURE OF AI-77s

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Summary: The structures of a novel gastroprotective substance AI-77-B and its analogues AI-77-C, D, F and G, which are produced by *Bacillus pumilus*, are described. Five of the asymmetric centers of AI-77-B have *S* absolute stereochemistry confirmed by X-ray in combination with chemical studies

AI-77-A, B, C, D, E, F and G have been isolated from the culture broth of *Bacillus pumilus AI-77* as structural analogues with characteristic fluorescence. AI-77-A and B, which were the major products in earlier and later fermentation stages respectively, exhibited potent gastroprotective activities against stress ulcers in rats, whereas the other AI-77s were minor products with less activities. Details of production, isolation, primary characterization and pharmacological activity of each compound have been reported[1]. AI-77-B appears to be part of a unique drug class because it has non-centralsuppresive, non-anticholinergic and non-antihistaminergic properties despite its potent antiulcerogenicity acting against stress ulcers. In order to clarify the relationship between the chemical structure and pharmacological activity we elucidate the structures of AI-77-B, C, D, F and G, here.

AI-77-B (1), mp 139.5-140 °C (dec.), FD mass M+1 425, showed UV λ_{max}^{MeOH} nm (ε) 246 (6250) and 314 (4450), CD (MeOH) $\Delta\varepsilon_{325}$=-0.42, $\Delta\varepsilon_{306}$=-0.67 and $\Delta\varepsilon_{257}$=-3.3, Fluorescence Excit. max 320 nm emmision max 470 nm and IR ν_{max}^{KBr} 1692 sh, 1680 sh, 1670 sh, 1662 and 1625 cm^{-1}. The UV spectrum of (1) agreed with those of mellein[2], λ_{max}^{EtOH} nm (ε) 246 (6500) and 314 (4100), and bacipfelacin[3], λ_{max}^{MeOH} nm (ε) 245 (6050) and 314 (4900). These findings suggest that AI-77-B contains a chromophore similar to 3,4-dihydro-8-hydroxyisocoumarin in its structure. The color reactions of (1) with ninhydrin and with $FeCl_3$ reagent indicate the presence of an amino acid moiety and a phenolic hydroxyl function, respectively.

γ-Lactone derivative (2), $C_{20}H_{26}N_2O_7 \cdot HCl$(M^+ m/e 406.1736, calcd. 406.1733) was obtained by evaporating an ethanol solution of (1) with HCl to dryness. In the IR spectrum of (2) a new absorption band appearing at 1790 cm^{-1} indicates the formation of a γ-lactone. In the ^{1}H-NMR spectrum (d_6-DMSO) of (2), the signal of a methine proton at δ3.64 in spectrum of (1) shifted downfield by 0.91 ppm.

Acetylation of (1) under different conditions afforded three kinds of acetate. The reaction

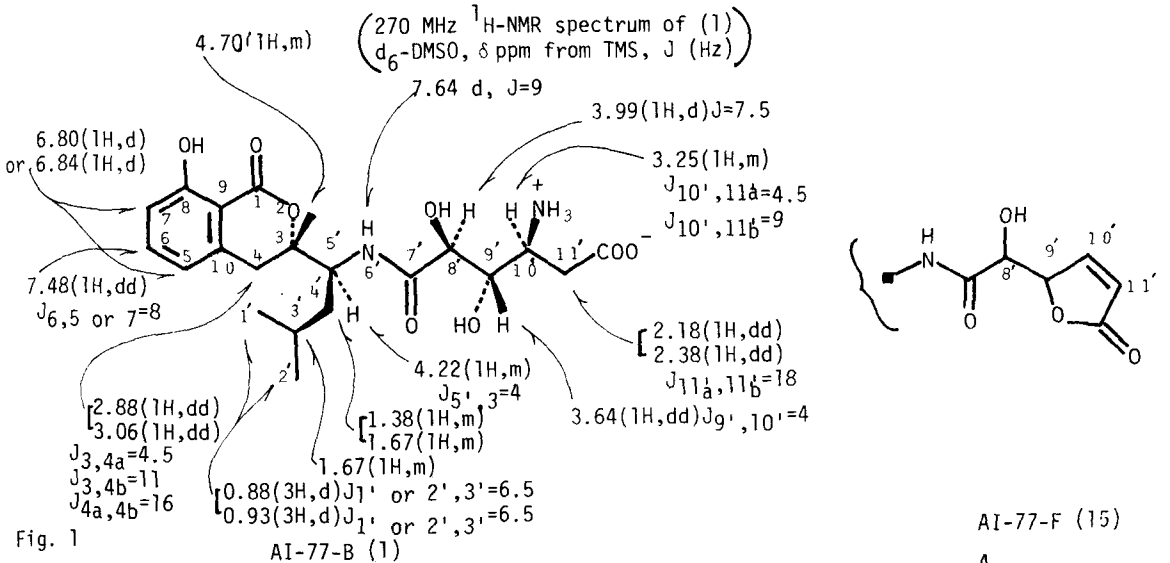

Fig. 1

of (1) with acetic anhydride in pyridine at 0 °C gave exclusively monoacetate (3)[4]. Treatment of (1) with $ZnCl_2$ in acetic anhydride at 50 °C afforded diacetate (4)[4]. Extensive acetylation of (1) with acetic anhydride in pyridine at 50 °C gave triacetate (5)[4]. The UV spectrum of (5) revealed blue shifts [236 (ε, 7150) and 288 nm (ε,1805) in MeOH] with accompanying disappearance of the fluorescence.

Acid hydrolysis of (1) with 2 N HCl at 105 °C for two days afforded chromophore moiety (6) and pyrrolidonecarboxylic acid[5] (7) predominately. Both compound (6) and (7) were separated from the hydrolysate by column chromatography (Amberlite XAD-2). Chromophore moiety (6) was obtained as white needles from a methanol eluate of the column and identified as the chromophore moiety of baciphelacine[3]. The fraction eluated with water gave pyrrolidonecarboxylic acid (7). Compound (8) was obtained as the monosodium salt by treating compound (7) with 1 N NaOH (pH 10) at room temperature overnight, followed by adjusting the pH to 6.5 with Dowex 50 (H^+) and lyophilizing (yield 87 %). The spectral data[6] give reasonable grounds to conclude compound (8) to be 2,3--dihydroxy-4-amino-hexanedioic acid. Leucine (11) was obtained by the oxidative degradation[7] of diacetyl derivative (9) of chromophore moiety (6) with excess potassium permanganate in neutral aqueous solution, followed by deprotection of the amine group with acid hydrolysis (yield 23.3 % by amino acid autoanalysis). After purification by column chromatography with Dowex 50 (H^+), leucine was crystallized from hot water and then identified optically as L-leucine within experimental error; $[\alpha]_D^{23}$ +15.4 (c 1.57, 6 N HCl), authentic L-leucine $[\alpha]_D^{23}$ +15.2 (c 1.58, 6 N HCl).

Based on the above studies and detailed spin decoupling experiments on the ^{1}H-NMR spectrum in addition to the ^{13}C-NMR spectrum, the structure of AI-77-B (1) was proposed and confirmed by the following single crystal X-ray analysis (Fig. 1).

Crystals grown in aqueous solutions were used for X-ray diffraction study using CuKα radiation. The crystal data are : AI-77-B tetrahydrate, $C_{20}H_{28}N_2O_8 \cdot 4H_2O$, FW=496.5, monoclinic, space group $P2_1$, Z=2, a=15.902(7), b=10.161(5), c=7.571(4) Å, β=95.28(5)°, V=1218 Å^3, D_m=1.356 Mgm^{-3}, D_c=1.354 Mgm^{-3}. The structure shown in Fig. 3 was determined and refined on the basis of 2655 observed structure factors (90 % of those involved within 2θ<165) to an R value of 0.054 inclu-

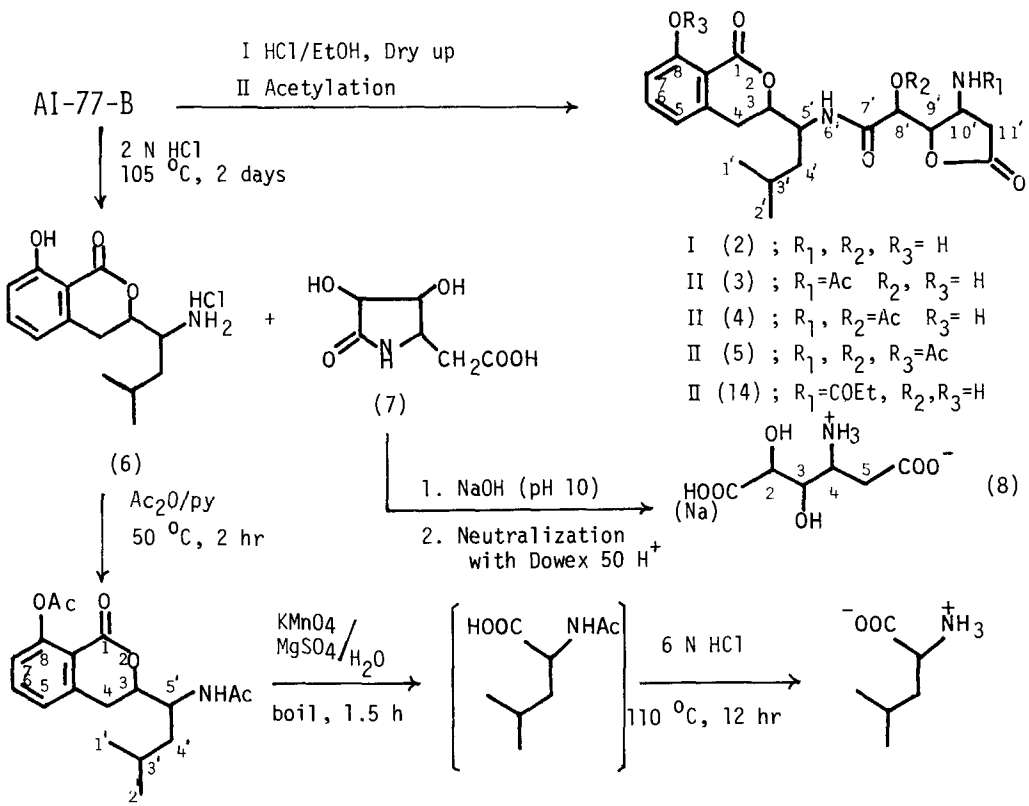

Fig. 2

ding 27 hydrogen atoms. Nine of those bonded to the oxygen atoms of crystal water and hydroxyl groups were not included. To determine the absolute configuration, the values of $r = |F_c(hkl)| / |F_c(h\bar{k}\bar{l})|$ were calculated by taking into account the anomalous dispersion effect of C, N and O atoms for CuKα radiation. Comparison with the observed values for all the 11 Friedel pairs giving the largest or smallest r values, indicated the configuration presented in Fig. 3. It is then confirmed that all the five asymmetric centers of (1) have the same absolute configuration and they were indicated to be S by the X-ray anomalous dispersion method. The configuration at C-5' has been related to L-leucine which was obtained from chromophore moiety (6) in the above chemical studies. For additional crystallographic details consult reference 9. Perhaps chromophore moiety (6) is

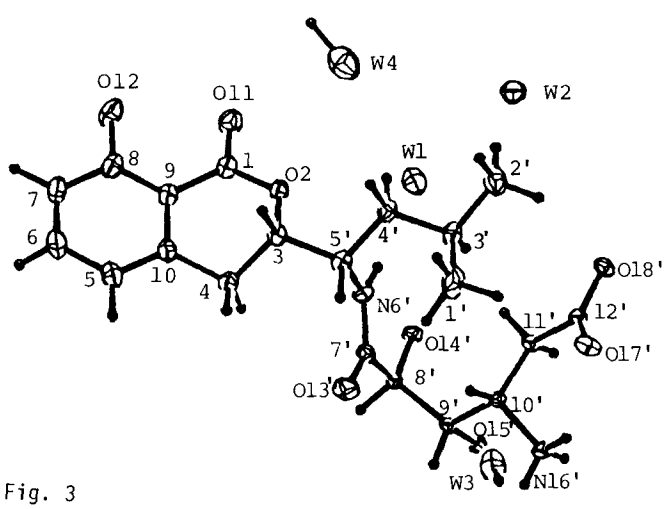

Fig. 3

derived biogenetically from four molecules of acetic acid and one molecule of L-leucine.

AI-77-C (12) was identified as a monoacetate (3) and AI-77-D (13) was identified as the monopropionate (14) obtained by acylation of (1) with propionic anhydride in pyridine at 0 °C. Spectral data of AI-77-F (15)[10] were characteristic of a butenolide [IR 1790 and 1755 cm^{-1}, ^{1}H-NMR δ 5.35(1H,dd), 6.24 (1H,dd) and 7.55 (1H,d) -CH-CH=CH-CO-]. Detailed spin decoupring experiments on the ^{1}H-NMR spectrum led to elucidation of the structure of AI-77-F (15) as shown in Fig.1 The UV spectrum of AI-77-G (16) agreed with that of (1) determined after standing overnight in 0.1 N NaOH solution. By evaporating an ethanol solution of (16) with HCl to dryness, γ-lactone derivative (2) was obtained in excelent yield (98 %). From these findings and spectral data the structure of (16) was determined to be ring opening form of δ-lactone of AI-77-B (1).

Although AI-77-C, D, F and G have been isolated from the culture broth of *Bacillus pumilus AI-77* , two of the latter cannot be proved to be either chemically degraded products or biogenetic products.

Acknowledgments : The authors are indebted to prof. T. Goto (Department of Agricultural Chemistry, Faculty of Agriculture, Nagoya University) and Prof. Y. Yamada (Tokyo College of Pharmacy) for valuable suggestions. We thank Mr. I. Nagoya (NMR spectra) and Mr. K. Tamura (Mass Spectra) in our laboratry.

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4.

Comp.	Formula	mass M^+	mp °C(dec.)	-NHCOCH_3	-OCOCH_3	-NHCOCH_3	ϕ-OH	H-8'(d_6-DMSO)
(3)	$C_{22}H_{28}N_2O_8$	448.1830	210-211	1.69 s		7.88 d	10.85 s	4.32 d δ ppm
(4)	$C_{24}H_{30}N_2O_9$	490.1939	216-217	1.59 s	2.08 s	8.38 d	10.80 s	5.18 d
(5)	$C_{26}H_{32}N_2O_{10}$	532.2028	170	1.59 s	2.09 s,2.27 s	8.42 d		5.19 d

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6. ^{1}H-NMR spectrum (200 MHz in D_2O) δ ppm from TMS: H-2 4.25(1H,d J=4), H-3 4.15(1H,dd $J_{3,4}$=2.5), H-4 3.92(1H,m $J_{4,5a}$=2, $J_{4,5b}$=4), H-5 2.79(1H,dd) and 2.96(1H,dd $J_{5a,5b}$=17). IR ν_{max}^{KBr} 1710, 1600 and 1570 sh cm^{-1}. FD mass M+1 194 and M+Na 216.

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8. The ^{13}C-NMR assignments were based on chemical shifts and substituent effects as well as correlation of the ^{1}H-NMR and ^{13}C-NMR spectra by selective decoupling methods: (270 MHz in d_6-DMSO) δ ppm from TMS, C-3 80.9, C-4 28.9, C-5 118.4, C-6 136.2, C-7 115.2, C-8 160.9, C-9 108.3, C-10 140.6, C-1' or C-2' 21.5 or 23.3, C-3' 38.6 (40.1 in CD_3OD), C-4' 24.0, C-5' 48.1, C-8' or C-9' 71.4 or 71.6, C-10' 50.4, C-11' 33.4, C-1, C-7', C-12' 168.9, 172.7, 174.6

9. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre.

10. $C_{20}H_{23}NO_7$ (M^+ m/e 389.1474, calcd. 389.1473); white needles, mp 182-183 °C; UV λ_{max}^{MeOH} nm(ε) 245(6100), 312(4200); CD (dioxane) $\Delta\varepsilon_{327}$=-0.31, $\Delta\varepsilon_{303}$=-0.47, $\Delta\varepsilon_{258}$=-4.5; ^{13}C-NMR (d_6-DMSO, δppm from TMS)21.2, 23.2, 24.0, 29.1, 38.8, 48.5, 70.8, 80.9, 84.2, 108.4, 115.6, 118.8, 121.9, 136.7, 140.3, 154.7, 161.1, 169.1, 170.1, 173.2.

(Received in Japan 14 September 1982)