PROPIOXATINS A AND B, NEW ENKEPHALINASE B INHIBITORS

III. TOTAL SYNTHESIS OF PROPIOXATIN A

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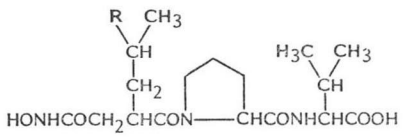
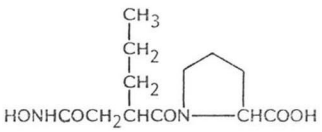
Propioxatin A, a potent enkephalinase B inhibitor produced by *Kitasatosporia setae* SANK 60684, was synthesized. The synthetic route involved a regio-selective synthesis of *O*-benzyl-α-propylsuccinic acid monohydroxamic acid *via* the acid chloride of α-propylsuccinic acid. The stereoisomer of the *N*-acyl moiety of natural propioxatin A was analyzed by X-ray crystallography in the form of the di-*O*-benzyl ester and was determined as *S*. Devalyl propioxatin A synthesized by the same method showed a higher *Ki* value for enkephalinase B than propioxatin A.

In preceding papers[1,2], we reported on the structural determination of propioxatins A and B, which were isolated from the fermentation broth of *Kitasatosporia setae* SANK 60684, as inhibitors of enkephalinase B. Propioxatins A (**1a**) and B (**1b**) were found to be dipeptides with *N*-acyl containing hydroxamic acid. In the present paper we report a total synthesis of propioxatin A and its devalyl derivative (**2**). This synthetic route involved a position specific sequence for the β-monohydroxamic acid of α-alkyl-succinic acid.

α-Propylsuccinic acid (**3**) was synthesized by the condensation of α-bromo-*n*-valeric acid methyl ester with malonic acid diethyl ester in the presence of sodium ethoxide in absolute ethanol and followed by hydrolysis with sodium hydroxide solution and decarboxylation with hydrochloric acid at reflux temperature. On treatment of **3** with oxalyl chloride, the acid chloride was obtained after removal of excess oxalyl chloride. The crude acid chloride was allowed to react with *O*-benzyl hydroxylamine in chloroform to give a monohydroxamic acid derivative (**4**, approximately 90%) and a small amount of the α-isomer. This position selective reaction of acid chloride with hydroxylamine can be attributed to an attack on the less hindered side of α-alkyl-succinic anhydride. The structure was confirmed by the mass spectrum of the methyl ester **4**: *m/z* 280 $(M+H)^+$. Subsequent reaction of **4** with L-prolyl-L-valine benzyl ester, in the presence of dicyclohexylcarbodiimide (DCC) in chloroform followed by hydrogenolysis of **5** in the presence of PtO_2 furnished the desired product having the L,L-configuration. On HPLC, this material was found to be a mixture of isomers (1 : 1) at C-2″ of the



1a R = H
1b R = CH_3



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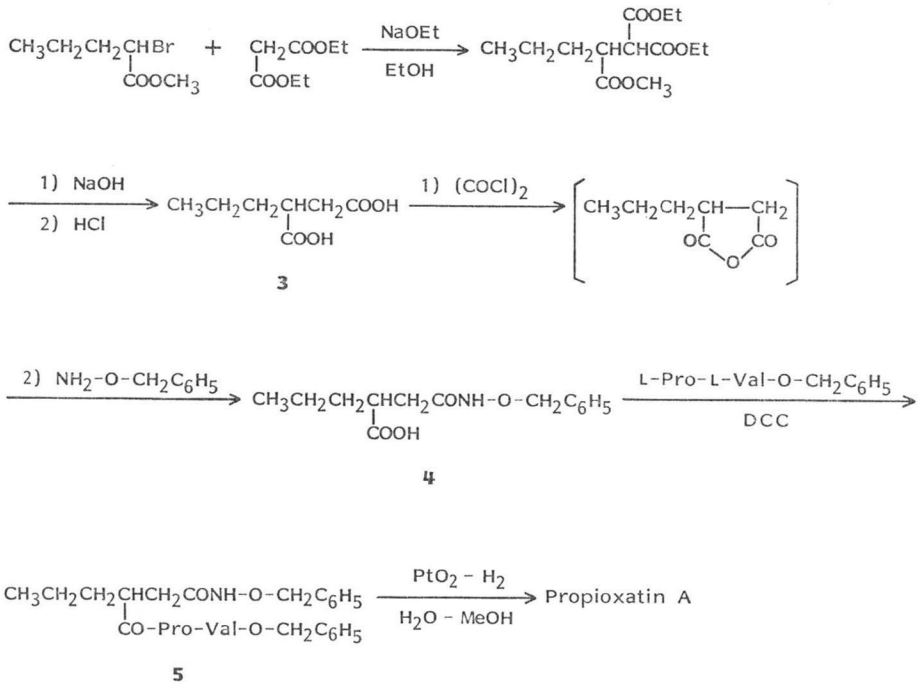
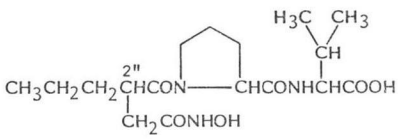
Scheme 1. Total synthesis of propioxatin A.





6 (2"-*R*)
7 (2"-*S*)

N-acyl moiety. The isomers were separated by HPLC. The shorter retention time compound (**6**) was identical with natural propioxatin A with respects to spectral data. The less mobile compound (**7**) seemed to be an isomer of **6** at the C-2″ of the succinic acid portion.

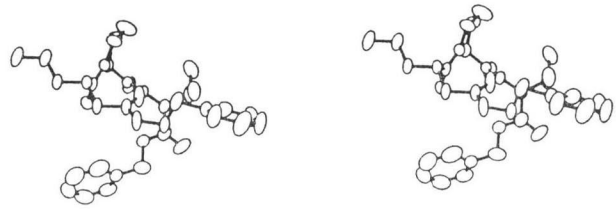
Condensation of **4** with L-proline benzyl ester and hydrogenolysis with PtO_2 as described above afforded an isomeric mixture at C-2″ of the devalyl derivative (**2**).

The stereochemistry was elucidated by means of X-ray crystallography of compound **5′** which gave a stereoisomer at C-2″ of natural propioxatin A by hydrogenolysis of **5** with PtO_2 as mentioned above. The X-ray crystallographical data of **5′** are as follows: $C_{31}H_{41}N_3O_6$, MW 551.7, orthorhombic, $P2_12_12_1$, $a = 17.058(3)$, $b = 15.059(2)$, $c = 11.906(2)$ Å, $U = 3062.4$ Å^3, $Z = 4$, $Dx = 1.20$ g·cm^{-3}, $\mu(CuK_\alpha) = 0.6$ mm^{-1}. Intensities were recorded on a Rigaku AFC-5R apparatus using graphite-monochromatized CuK_α radiation. A total of 2,545 independent reflections were corrected for Lorentz and polarization factors but not absorption. The structure was solved by MULTAN[3] and refined by block-diagonal least-squares methods. The positions of the hydrogen atoms were estimated from standard geometry. A final least-squares refinement with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms lowered the R-value by 0.072 (Rw=0.079, w=1.0; 2,451 observed reflections with $Fo \geq 2\sigma(Fo)$).

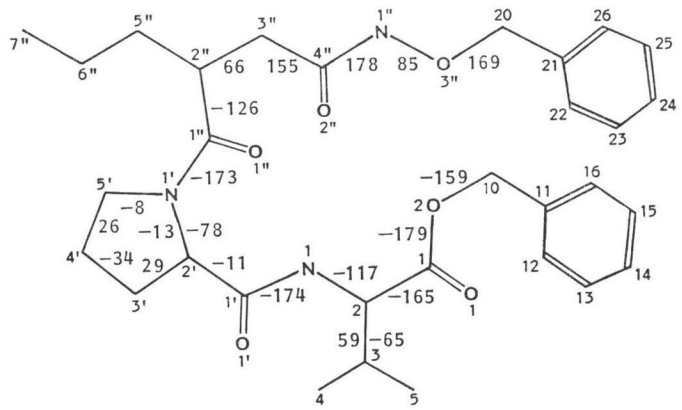
A stereo-drawing by ORTEP[4] and the conformational angles are shown in Fig. 1, which also shows the atom labels used. Since the prolyl and valyl residues have been confirmed to be L-amino acid as

Fig. 1. (a) A stereo-drawing by ORTEP and (b) conformational angles of **5'**.

(a)



(b)



mentioned above, the atomic configuration at the C-2″ atom of the *N*-acyl moiety is determined as *S*. The backbone of the molecule is folded, in which proline and *N*-acyl moieties are at the corner and an intramolecular hydrogen bond, N(1)...O(2″) = 2.997(8) Å, is formed between the NH of valine and the CO of the hydroxamyl moiety. If the side groups are disregarded, this conformation feature is almost identical with the β-turn of type II′ found in cyclic-Gly-L-Pro-L-Ser-D-Ala-L-Pro[5]. Although β-turn structures have been reported for many oligopeptides so far, type II′ β-turns have been observed only rarely, at least in crystals of relatively small and linear peptides. The pyrrolidine ring of proline exists in the conformation between C_S-C^β-exo and C_2-C^γ-endo[6] with C^β and C^γ deviating from the $NC^\alpha C^\delta$ plane by −0.345(5) and 0.191(5) Å, respectively. The valine side chain takes a gauche II conformation, and is compatible with *N*-(*tert*-butoxycarbonyl)-L-Val-L-Pro-Gly-L-Val[7]. There is one intermolecular hydrogen bond, N(1″)H...O(6″) = 2.756(8) Å, which connects the molecules related by a 2-fold screw axis parallel to the *c* axis. All the other intermolecular contacts correspond to van der Waals interactions.

Ki values for enkephalinase B indicated that natural propioxatin A, the synthetic one (**6**), the isomer (**7**), and the isomeric mixture of devalyl derivative (**2**) were 1.3×10^{-8}, 5.0×10^{-8}, 1.0×10^{-6} and 2.0×10^{-6} M, respectively.

Experimental

Synthesis of α-Propylsuccinic Acid (**3**)

Synthesis of the title compound was described in a previous paper[2].

Synthesis of α-Propylsuccinic Acid β-*O*-Benzyl Monohydroxamic Acid (**4**)

α-Propylsuccinic acid (**3**, 1 g) was dissolved in 5 ml of oxalyl chloride and then allowed to stand at room temp overnight. After removal of the oxalyl chloride under reduced pressure, the residue was dissolved in 75 ml of $CHCl_3$ and a solution of 3.4 g *O*-benzyl hydroxylamine HCl salt in 75 ml of $CHCl_3$ and 3 ml of triethylamine was added. The reaction mixture was allowed to stand at room temp overnight. After addition of H_2O, the mixture was extracted with $CHCl_3$, the $CHCl_3$ layer concd to a small volume, charged on a Sephadex LH-20 column (200 ml) and developed with $CHCl_3$ - EtOAc (1 : 1), to yield 4.4 g of the title compound (**4**), mp 71~74°C.

Condensation of L-Prolyl-L-valine Benzyl Ester with **4**

To a stirred solution of 2 g of **4** in 20 ml of $CHCl_3$, a solution of 2.6 g of L-prolyl-L-valine benzyl ester in 20 ml of $CHCl_3$, and 1 ml of triethylamine were added in the presence of 1.6 g of DCC. After stirring at room temp overnight, the reaction mixture was filtered and extracted with H_2O. The $CHCl_3$ layer was concd and chromatographed by HPLC (TSK column, ODS, 50% CH_3CN, 1 ml/minute, UV 230 nm) to yield 2.9 g of **5**. Peaks 1 and 2 were isomers at C-2'' of the succinic acid moiety and the configuration of the more mobile compound (peak 1) was the same as of the modified natural product.

Synthesis of (±)-Propioxatin A

A solution of 150 mg of condensation products (isomeric mixtures, **5**) in 7 ml of MeOH - H_2O (5 : 2) was hydrogenated over 20 mg of PtO_2 for 4 hours. The solution was filtered and concd under reduced pressure. The residue was separated by HPLC (TSK column, ODS, 15% CH_3CN - 0.1% TFA) to give 20 mg of (±)-propioxatin A.

Condensation of L-Proline Benzyl Ester with **4**, and Hydrogenation with PtO_2

To a stirred solution of 100 mg of **4** in 5 ml of $CHCl_3$, a solution of 81 mg of L-proline benzyl ester in 5 ml of $CHCl_3$ and 50 μl of triethylamine were added in the presence of 74 mg of DCC. The reaction mixture was worked up as above, the condensation product was dissolved in 7 ml of MeOH - H_2O (5 : 2) and hydrogenated over 20 mg of PtO_2 for 3 hours. Isolation by HPLC as described gave 20 mg of (±)-devalyl propioxatin A (**2**).

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