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SYNTHETIC CONFIRMATION OF THE STRUCTURE OF ISARIIN, A CYCLODEPSIPEPTIDE METABOLITE OF ISARIA CRETACEA

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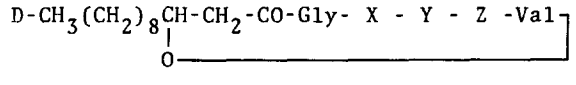
Isariin, a peptide metabolite of Isaria cretacea, is a representative of its several congeners isolated from several Isaria species.[1,2] Vining and coworkers[1,3] have first proposed the structure (1) for isariin from the degradative and high-resolution mass spectral informations, but Kiryushkin et. al.[4] have independently presented a different structure (2), thus its structure being remained to be settled.



(1) X = Val, Y = D-Leu, Z = Ala

(2) X = Ala, Y = Val, Z = D-Leu

The present study[5] has been undertaken to make a choice between the above alternative structures and obtain the synthetic confirmation of the structure. There has been very little synthetic work of a naturally occurring depsipeptide with a long chain 3-hydroxy fatty acid except only one example, serratamolide[6] which was synthesized by using rather peculiar hydroxyacyl insertion reaction.

By a simpler route as outlined in Chart, we have now synthesized the cyclohexadepsipeptide (1) which was completly identical with natural isariin. Our syntheses were designed to minimize racemisation. The substituted linear hexadepsipeptide BOC-Val-D-Leu-Ala-Val-D-Hyd-Gly-OBzl (5)[7] required

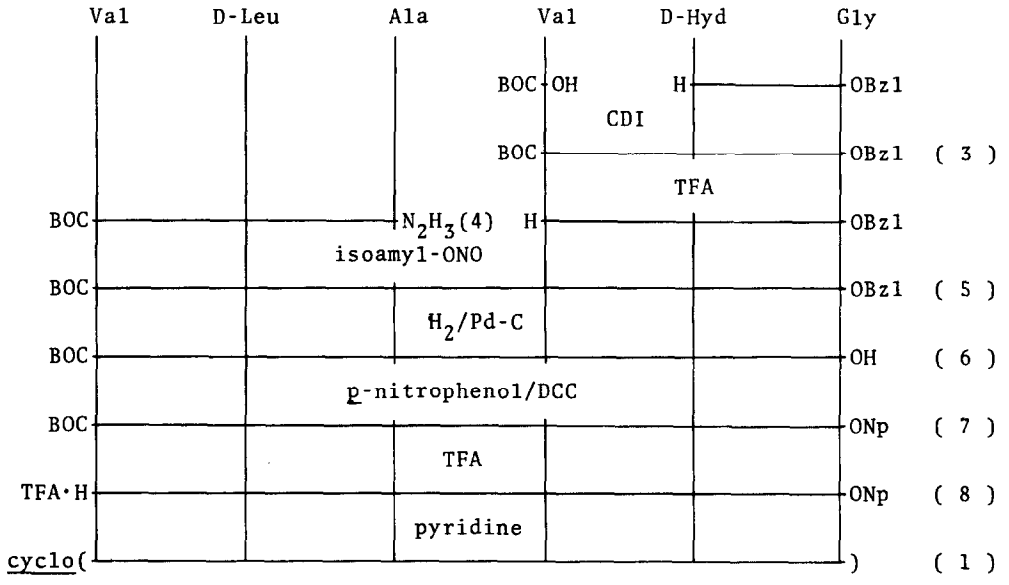


Chart. Synthetic Route to Isariin

BOC = t-butyloxycarbonyl, OBzl = benzyl ester,
ONp = p-nitrophenyl ester, DCC = N,N'-dicyclohexylcarbodiimide
CDI = N,N'-carbonyldiimidazole, Hyd = $CH_3(CH_2)_8$-CH(O-)-CH_2-CO-,
TFA = trifluoroacetic acid

as an intermediate was obtained in 21% overall yield starting from D-3-hydroxydodecanoic acid. Ester bond formation between BOC-Val-OH and H-D-Hyd-Gly-OBzl[8] by the use of CDI[9] yielded the tridepsipeptide BOC-Val-D-Hyd-Gly-OBzl (3) (amorphous powder, Rf^1 0.77), which was coupled, after removal of the BOC-group by acidolysis with trifluoroacetic acid, with BOC-Val-D-Leu-Ala-$NHNH_2$ (4) [mp 199 - 202°, $[\alpha]_D^{27}$-17° (c=1, EtOH), Rf^2 0.39] to give the required hexadepsipeptide (5) [mp 110 - 115°, $[\alpha]_D^{18}$-12° (c=1, MeOH), Rf^1 0.23, Rf^2 0.60]. The benzyl group was removed by catalytic hydrogenation and the resulting (6) (85%, powder, Rf^3 0.82) was converted to the activated ester BOC-Val-D-Leu-Ala-Val-D-Hyd-Gly-ONp (7) (60%, powder, Rf^1 0.29) by p-nitrophenol. Then the BOC-group was eliminated by the acidolysis to give TFA·H-Val-D-Leu-Ala-Val-D-

Hyd-Gly-ONp (8) (85%, powder, Rf^1 0.33), which was subjected to cyclization by the high dilution technique in pyridine at 55°. The desired cyclodepsipeptide cyclo(Val-D-Leu-Ala-Val-D-Hyd-Gly) (1) was purified by successive column chromatographies on Dowex 50W x4 (MeOH-dioxane-H_2O = 25:25:10 v/v) and Sephadex LH-20 (MeOH) and then preparative t.l.c. over silica gel ($CHCl_3$-MeOH-pyridine = 95:5:3 v/v), and finally crystallized from EtOH-H_2O to furnish as colorless needles in 23% yield, [mp 249 - 251°, $[\alpha]_D^{20}$-2.7° (c=0.5, $CHCl_3$), ORD^{10} (c=0.075, EtOH) $[\alpha]^{20}$ (mμ): 0° (224), -2060° (230), -3030° (240) (trough), -1280° (260), -105° (400). $IR\nu_{max}^{KBr}cm^{-1}$: 1735, 1635, 1535. m/e 637 (M^+). Anal. Calcd. for $C_{33}H_{59}O_7N_5$: C, 62.11; H, 9.32; N, 10.98. Found : C, 61.84; H, 9.20; N, 10.73. Amino acid ratios in acid hydrolysate : Gly 1.00, Ala 1.00, Val 2.01, Leu 0.98]. Synthetic (1) gave a single spot (ninhydrin negative, pale yellow with the iodine-toluidine reagent) on t.l.c. in different solvent systems (Rf^1 0.35, Rf^2 0.44, Rf^3 0.93) and was shown to be identical with a sample of natural isariin in mp, mixed mp, $[\alpha]_D$, ORD, infrared and mass spectra, and chromatographic behaviours, thus presenting unambiguous evidence that the structure of isariin is (1).

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References and Notes

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5) Amino acid symbols except D-Leu and Gly denote the L-configuration. The following solvent systems are used for thin-layer chromatography (Kieselgel G, Merck): Rf^1; $CHCl_3$-MeOH-AcOH (95:5:3 v/v), Rf^2; $CHCl_3$-MeOH-pyridine (95:5:3 v/v), Rf^3; n-BuOH-AcOH-H_2O (4:1:5 v/v).

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