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THE SYNTHESIS OF NORSURFACTIN,A HEMOLYTIC,ANTICOAGULANT CYCLODEPSIPEPTIDE

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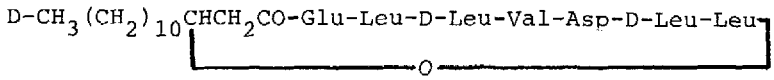
A large number of cyclic peptides having biological activity have been isolated from natural sources and many have been synthesized[1]. Syntheses of depsipeptides(compounds containing both amino and hydroxy residues linked through amide and ester bonds) and particularly cyclodepsipeptides are more rare. Most synthetic studies have involved the incorporation of α-hydroxyalkanoic acids having relatively short,branched side chains into the depsipeptide ring [2-5].

Relatively less attention has been given to cyclodepsipeptides containing β-hydroxyalkanoic acid units. Natural compounds of this kind generally have side chains associated with their β-hydroxy residues that are of moderate or substantial length. Compounds having these structural features include: isariin [6,7],the isarolides [8],peptidolipids NA [9,10],serratamolide [11],esperin[12] and surfactin [13](subtilysin [14]). Of this group,only isariin [15-17] and serratamolide [18] have been synthesized. The symmetrical,repeating sequence of serratamolide enables clever,but not generally applicable,synthetic methods to be employed. The techniques used to synthesize isariin do have some general applicability,but this synthesis was also more straightforward than it might have been since there are no side-chain functionalities. Peptidolipids NA, esperin and surfactin all contain amino acids with third functionality and this complicates the synthesis of these cyclodepsipeptides. One must manipulate protecting group and coupling strategies within the constraints imposed by the hydroxyl and carboxyl lactone-bond-forming functions and the glutamic and aspartic carboxylic acid side chains in addition to the usual amine and carboxyl functions which yield the amide bonds.

The development of a synthetic strategy for a molecule of the surfactin type proved more challenging than we had imagined. In later reports we will mention several approaches which,in principle,seem suitable but which,in practice,were not. This paper summarizes a successful total synthesis of a close analog of surfactin which we call norsurfactin. The only structural difference is that norsurfactin lacks a 13-methyl group in its β-hydroxy acid side chain, i.e.,the side chain is normal in norsurfactin and iso in surfactin.

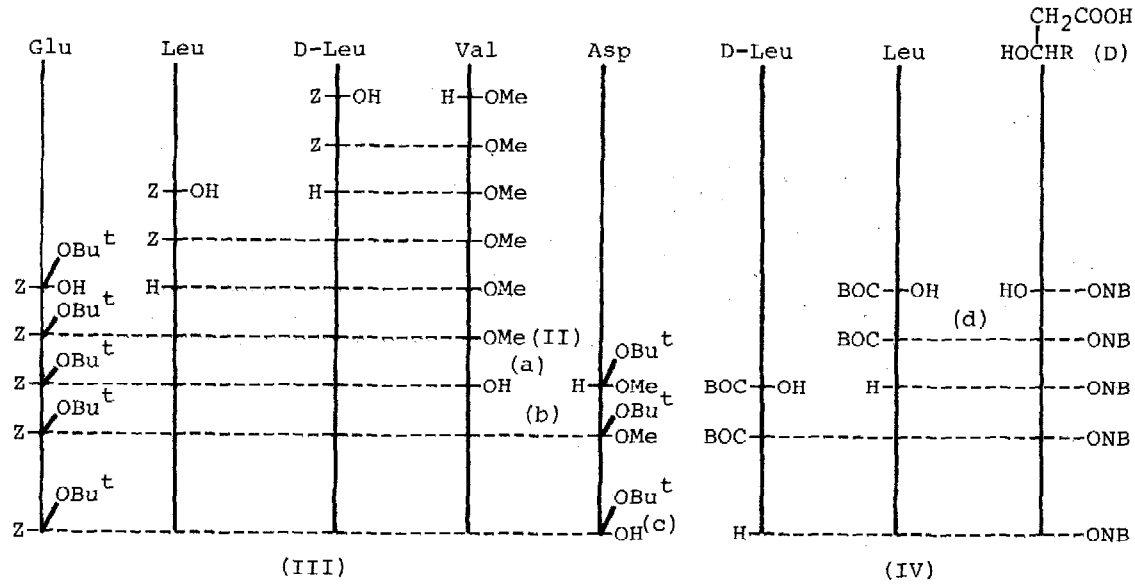
Fragments III and IV were built up as shown in Scheme I using rather conventional procedures. The somewhat unorthodox approach to III was dictated in part by the fact that quantities of II were available from other synthetic approaches. This general route,which could also be accomplished in a more racemization-free stepwise manner,avoids repeated exposure of OBu^t ester groups to acidic conditions. The fragment coupling between pentapeptide III and depsi tripeptide IV was accomplished in 75% yield using the N,N-dicyclohexylcarbodiimide/hydroxybenztriazole (DCC-HOBT) technique. Terminal deprotection by hydrogenolysis gave V(94%). Depsipeptide V was cyclized(three days)under high dilution conditions (1.8×10^{-3}M peptide) in a 34:1 (v:v) methylene chloride/ N,N-dimethylformamide solvent using DCC(2.7 equivalents) and N-hydroxysuccinimide(HOSu,4.1 equivalents).The yield of protected cyclodepsipeptide was 41% after purification by column chromatography (silica gel) followed by high-pressure gel permeation chromatography. Norsurfactin was prepared by removal of the side chain OBu^t ester groups(anhydrous trifluoroacetic acid);the yield was 65% from VI after purification by preparative layer chromatography on silica gel followed by HPLC on C-18/Porasil B. The infrared spectrum was indistinguishable from that of natural surfactin; $[\alpha]_D^{25} = +27.1^\circ$ (c 1,$CHCl_3$) and -35.2° (c 1,CH_3OH). Found: C,60.77; H,9.03; N,9.30. Calculated: C,61.09; H,8.97; N,9.59 for $C_{52}H_{91}N_7O_{13}$. Amino acid analysis: Asp,0.99; Glu,0.98; Leu,4.05; Val,0.94.

Under the conditions reported by Bernheimer[14] norsurfactin showed hemolytic activity comparable to that of a sample of natural surfactin that had been similarly purified. The anticoagulant activities[19] of synthetic norsurfactin and natural surfactin were likewise comparable.

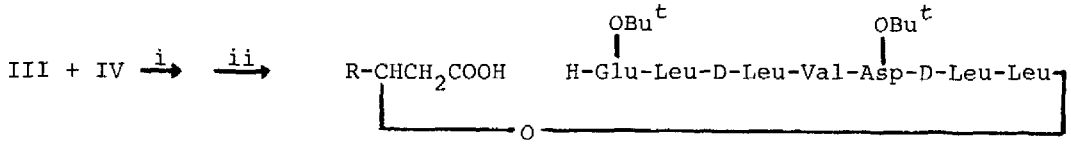


NORSURFACTIN (I)

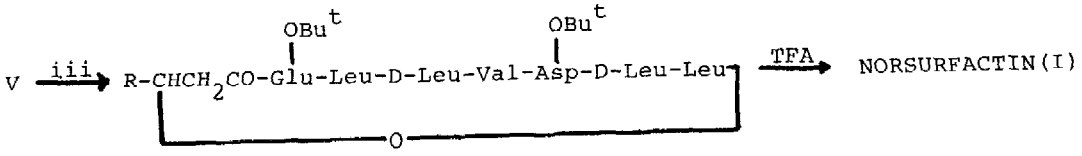
SCHEME I. Synthesis of Norsurfactin.



(a) 71%, NaOH-75%dioxane; (b) 65%, DCC-HOBT; (c) 82%, as in (a); (d) 97%, CDI-Na/Imid.
R=$CH_3(CH_2)_{10}$-; NB=p-nitrobenzyl; Z=benzyloxycarbonyl; BOC=t-butyloxycarbonyl



i=DCC-HOBT, 5 days
ii=H_2/Pd-C, MeOH



VI

iii=DCC-HOSu, CH_2Cl_2-DMF, 3 days

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