Bioorganic & Medicinal Chemistry Letters, Vol.1, No.10, pp. 535-538, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

THE ENZYMATIC BAEYER-VILLIGER OXIDATION: SYNTHESIS OF THE C_{11}-C_{16} SUBUNIT OF IONOMYCIN

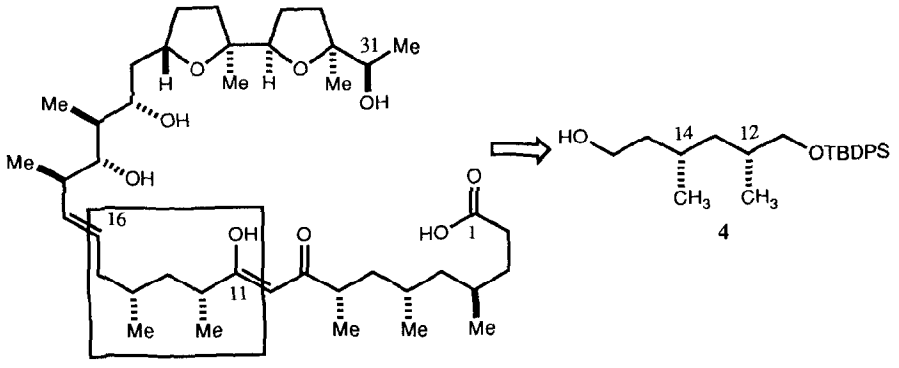
Michael J. Taschner* and Quin-Zene Chen

Department of Chemistry, The University of Akron,
Akron, Ohio 44325-3601

(*Received* 22 *August* 1991)

Abstract: An efficient synthesis of the C_{11}-C_{16} subunit of ionomycin from *cis*-3,5-dimethyl cyclohexanone using as the enzymatic Baeyer-Villiger oxidation to establish the correct absolute stereochemistry at C_{12} and C_{14} is reported.

Ionomycin (**1**) is a member of a large class of natural products called polyether antibiotics. It, like the others in this group, has been obtained from one of the numerous Streptomyces bacteria.[1] The polyether antibiotics have been shown to elicit a range of biological activity largely due to their ability of complex various inorganic cations which aids in the transport of these cations across membrane barriers.[2] It is singular among the ionophores in that it is doubly charged and thus has the ability to form 1:1 neutral complexes with various divalent cations. The isolation of the calcium complex of ionomycin from *Streptomyces conglobatus* was reported by Meyers, *et. al.* in 1978.[3] The X-ray structure and the absolute configurations for 2 ionomycin complexes were delineated in 1979.[4] Ionomycin has attracted attention from the synthetic community and in 1990 two independent syntheses from the laboratories of Hanessian[5] and Evans[6] were reported.



Ionomycin **1**

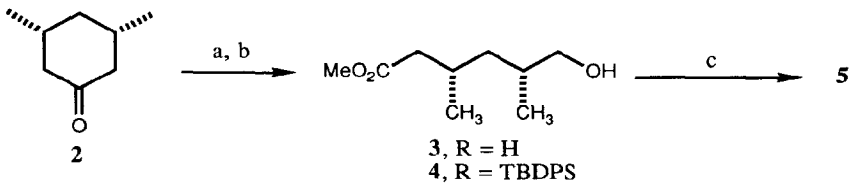
As in the previous syntheses, one of the key synthons was a segment which would ultimately become the C_{11}-C_{16} subunit. Evans prepared **5** in 8 steps employing asymmetric bond construction. The alkylation of a propionimide derived from (*S*)-valine controlled the C_{14} stereochemistry. The C_{12} stereocenter was installed via the alkylation of a prolinol amide enolate.[6] The "chiral pool" approach utilized by Hanessian prepared a related intermediate in 25 steps from L-glutamic acid.[7] The following communication describes a 3 step synthesis of **5** utilizing the enzymatic Baeyer-Villiger oxidation[8] of *cis*-3,5-dimethyl cyclohexanone (**2**).

The relative stereochemistry between the two methyls at C_{14} and C_{16} would be controlled by virtue of their cis relationship on the cyclohexane ring. The absolute stereochemistry would be controlled by discriminating between which carbon atom α to the carbonyl migrates during the oxidative rearrangement. The enzymatic Baeyer-Villiger oxidation using the enzyme cyclohexanone oxygenase (E.C. 1.14.13.-), isolated from the bacteria *Acinetobacter* NCIB 9871, has been shown to be capable of discerning between the two enantiotopic carbons flanking the carbonyl of mesomeric cyclohexanones.[9] In this initial report, it was found that the ε-lactone produced from *cis*-3,5-dimethyl cyclohexanone possessed the 3*S*, 5*R* absolute configuration at what will ultimately be C_{14} and C_{12}, respectively. This intermediate with the correct absolute configuration at the two stereogenic centers and the two ends functionally differentiated was exactly what was required for the efficient preparation of the C_{11}-C_{16} subunit.

The reactions during the initial phase of the study with the cyclohexanone oxygenase were performed using purified enzyme[10] and the $NADP^+$/NADPH recycling technique.[11] Furstoss has since shown that reactions can be performed using a whole-cell process.[12] However, with *Acinetobacter* NCIB 9871 the use of tetraethyl pyrophosphate was found to be necessary to isolate reasonable quantities of Baeyer-Villiger derived products from the substrates being employed. During the course of this and other investigations, it has been found that the whole-cell process with this strain of *Acinetobacter* affords good yields of the Baeyer-Villiger products, usually in the form of their hydroxy esters after acidic workup and CH_2N_2 treatment, without the use of the pyrophosphate.

Thus, subjecting *cis*-3,5-dimethyl cyclohexanone to the whole-cell process as essentially described by Furstoss, provided the hydroxy ester **3** ($[\alpha]_D$ = -1.01° (c 3.26, $CHCl_3$) in 82% isolated yield. This hydroxy ester compared favorably with the one obtained from the $NaOCH_3/CH_3OH$ reaction of the ε-lactone prepared with purified enzyme. The differences in the whole-cell process used here were some minor reductions in the times for incubation, the elimination of the tetraethyl pyrophosphate, periodic additions of 50% NaOH to control the pH,

and periodic additions of cyclohexanol during the initial incubations. Reaction of the hydroxy ester with t-$BuPh_2SiCl$ employing the Hernandez conditions[13] produced the silyl ether **4** ($[\alpha]_D$ = +2.83° (c 3.2, $CHCl_3$). Reduction of the ester with $LiAlH_4$ afforded the C_{11}-C_{16} subunit **5** ($[\alpha]_D$ = +3.29° (c 1.58, CH_2Cl_2),[14] which was identical to that reported in the literature.[6]



a) i. *Acinetobacter* NCIB 9871, ii. CH_2N_2, 82%; b) t-$BuPh_2SiCl$, Et_3N, DMAP, 97%; c) $LiAlH_4$, Et_2O, 95%.

The use of enzymes has become increasingly popular within the synthetic community.[15] The short, efficient synthesis of this ionomycin subunit illustrates the advantage enzymes sometimes have over conventional methodology. In addition, it demonstrates a useful application of the enzymatic Baeyer-Villiger reaction in the context of organic synthesis.[16] Further applications of this interesting and useful transformation are currently in progress. These developments will be the subject of future communications.

Acknowledgment. The assistance and information provided by Professor Chris Walsh and Dr. Jack Chen is gratefully acknowledged.

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