Tetrahedron Letters,Vol.26,No.35,pp 4171-4174,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

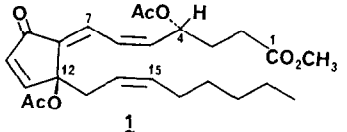
IDENTIFICATION OF A NEW EICOSANOID FROM IN VITRO BIOSYNTHETIC EXPERIMENTS WITH CLAVULARIA VIRIDIS. IMPLICATIONS FOR THE BIOSYNTHESIS OF CLAVULONES.

E. J. Corey and Peter T. Lansbury, Jr.
Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Yasuji Yamada
Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

Summary: A cell-free homogenate of Clavularia viridis converts tritiated arachidonic acid into a new eicosanoid identified as 4 (free acid) by chemical and chromatographic studies in comparison with synthetic substances. The isolation of 4 suggests a novel, non-prostanoid type pathway for the biosynthesis of the clavulones.

The clavulones, isolated from Clavularia viridis and exemplified by clavulone I (1), together with the more recently studied "punaglandins"[2] represent a new series of marine derived eicosanoids which are of considerable biological interest.[3] Although there are structural similarities between these eicosanoids and the prostaglandins, the pathways of biosynthesis are likely to differ significantly.[4] In this paper we report on the enzymatic transformation of arachidonic acid (the obvious predecessor of the clavulones)

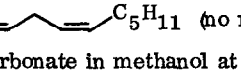


1

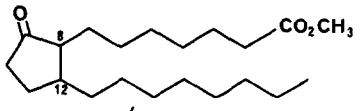
by a cell-free extract from the stolonifer Clavularia viridis to a new metabolite. The structure of this substance, obtained in tritiated form on sub-nanomolar scale, suggests that it is probably a precursor of the clavulones. From this structure an interesting new possibility can be deduced for the biosynthesis of the clavulones (and punaglandins). Structural identification of the metabolite, designated herein as pre-clavulone A (PC-A), was carried out by a radio tracer chemical study using high performance liquid chromatography (HPLC) analysis and comparison with synthetic compounds of known structure.

Clavularia viridis at -78°[5] was placed in pH 8, 0.05 M Tris buffer containing 1.0 M sodium chloride at 0° (ca. 1 g of coral in 10 ml of buffer) and homogenized in a blender. To the cold filtered homogenate (10 ml) was added 5,6,8,9,11,12,14,15-[^{3}H]-arachidonic acid (87 Ci/mMole) to give a concentration of 6 nM. Aerobic incubation was carried out at 23° for 50 min after which the mixture was extracted with hexane,[6] acidified with citric acid and extracted thoroughly with ethyl acetate. The ethyl acetate extracts contained a mixture of carboxylic acids as was shown by tlc analysis before and after treatment with diazomethane. The major component (ca. 5% yield) was isolated, after esterification of the mixture with

diazomethane, as the methyl ester (PC-A Me ester) by thin layer chromatography (tlc) followed by HPLC.[7] Some arachidonic acid (10%) was recovered unchanged; total recovery of radioactivity in the extracts was 50%. The amounts of metabolites obtainable were limited by the quantity of coral available in Cambridge and were insufficient for mass spectral identification.

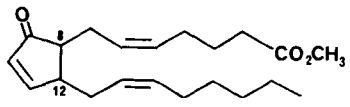
Initial characterization of PC-A Me ester was made by testing its reactivity toward various functional-group selective reagents using tlc and HPLC to analyze for the occurrence of reaction. The probable absence of the following structural units was deduced: (1) hydroperoxide, peroxide (no reaction with triphenylphosphine in methanol at 23° for 40 min), epoxide (no reaction with trimethylsilyl chloride - sodium iodide in CH_3CN at 23° for 6 hr),  (no reaction with soybean lipoxygenase), 1° or 2° acetate (no reaction with potassium carbonate in methanol at 23° for 2 hr). The presence of a carbonyl function (probably ketonic) was indicated by semicarbazone formation (semicarbazide-EtOH-H_2O-NaOAc, 23° for 24 hr), oxime formation (hydroxylamine hydrochloride, MeOH-NaOAc, 23° for 27 hr) and reaction with sodium borohydride (MeOH, 30 min at -20°), all of which gave more polar products. Significantly, the sodium borohydride reduction resulted in concurrent formation of a slightly less polar product along with the more polar product. The less polar product was converted to a more polar compound at a relatively slower rate. These results suggested that PC-A Me ester could be an α,β-enone which undergoes conjugate addition of hydride to yield less polar non-conjugated ketone as well as alcohol from carbonyl reduction. This idea was further supported by the finding that PC-A Me ester undergoes hydrogenation (H_2, Pd-C, ethanol 23° for 12 hr) to form a single product (HPLC system A[7]) which was significantly less polar (system A,[7] 15 : 1) and the fact that this hydrogenation product was reduced slowly by sodium borohydride as compared to PC-A Me ester. The catalytic hydrogenation product (PC-A-H_6 Me ester) was chromatographically indistinguishable from methyl 8,12-trans-9-oxoprostanoate ($\underset{\sim}{2}$) (prepared from prostaglandin A_2 methyl ester acetate, H_2, Pd-C in EtOH at 23°).[8] However, reduction of $\underset{\sim}{2}$ with sodium borohydride (EtOH, 1.5 hr at 0°) afforded two diastereomeric alcohols, neither of which co-eluted (solvent A[7]) with the product from sodium borohydride reduction of PC-A-H_6 Me ester. Although treatment of PC-A-H_6 Me ester with 1% methanolic sodium methoxide did not lead to a change in chromatographic mobility, it was evident that isomerization had occurred since reduction by sodium borohydride gave two products that co-eluted with the two borohydride reduction products from $\underset{\sim}{2}$. These results suggested that PC-A-H_6 Me ester is the 8,12-cis-isomer of $\underset{\sim}{2}$ ($\underset{\sim}{3}$). Support for this assignment was obtained from the finding that sodium borohydride reduction of synthetic cis cyclopentanone $\underset{\sim}{3}$[9] afforded one major alcohol which co-eluted with the borohydride product from PC-A-H_6 Me ester. Further confirmation for the formulation of PC-A-H_6 Me ester as $\underset{\sim}{3}$ was obtained by isomerizing it to $\underset{\sim}{2}$ (NaOMe - MeOH as above) and converting it to the following derivatives which co-eluted with the corresponding derivatives made from $\underset{\sim}{2}$: (1) N,N-dimethylhydrazone (system A), O-methyl oxime (system A), (3) benzyl ester (systems A, B, C, D), (4) benzyl ester 9-alcohols (produced in a ratio 3 : 2 by sodium borohydride reduction, analysis by solvent C, 7 : 1).

Since hydrogenation of PC-A Me ester using Rh-C catalyst afforded PC-A-H_6 Me ester cleanly whereas clavulones under these conditions gave mixtures of 12-acetate-containing and 12-acetate-cleavage



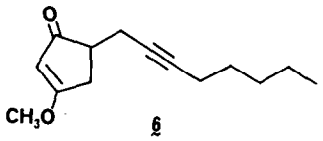
2 H-8/H-12 trans

3 H-8/H-12 cis

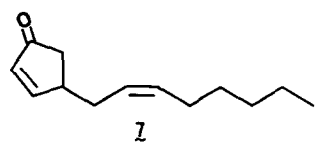


4 H-8/H-12 cis

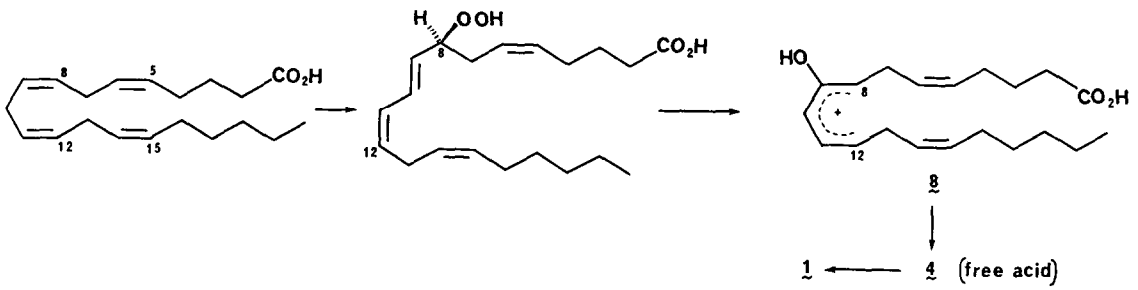
5 H-8/H-12 trans



6



7



8

1 ← 4 (free acid)

products, it appeared that PC-A Me ester possessed a hydrogen at C(12) rather than an acetoxy substituent as in the clavulones. In this case, PC-A Me ester could reasonably be formulated as the cis cyclopentenone 4. This was confirmed as follows. The trans-cyclopentenone 5 was synthesized and shown to be chromatographically different from PC-A Me ester. However, treatment of PC-A Me ester with 1,8-diazabicyclo[5.4.0]undec-7-ene in THF (23° for 11 hr) transformed it into a slightly less polar isomer which co-eluted with synthetic 5 (in systems A, B, C and D).

The synthesis of 5 was accomplished by the sequence: (1) deprotonation of 3-methoxy-2-cyclopentenone (lithium diisopropylamide, THF, -78°) and alkylation with 1-iodo-2-octyne to afford 6 (87%); (2) Lindlar hydrogenation of acetylene 6 to the corresponding Z-olefin in 9 : 1 toluene-triethylamine as solvent; (3) ketone reduction (diisobutylaluminum hydride, -78°), and hydrolysis with 1N hydrochloric acid to give enone 7 (71%); (4) deprotonation of 7 (LDA, THF, -78°) followed by treatment with methyl (Z)-7-iodo-5-heptenoate (-78° to 23°).

If PC-A is an intermediate in the biosynthesis of clavulones, the remainder of the process can be formulated in a straightforward way involving enzymatic oxygenation at carbons 4, 7 and 12. The mechanism of formation of PC-A (4, acid) is intriguing. A radical endoperoxide pathway[4] seems unlikely. On the other hand, the structural similarity of PC-A to cis-jasmonic acid suggests analogous biosynthesis[10] via the hydroperoxide cyclase pathway as an attractive possibility. In this pathway the cis-4,5-disubstituted 2-cyclopentenone structure is formed starting from a hydroperoxide, possibly by pericyclic closure of a 2-hydroxy-pentadienyl cation. This analysis suggests that 4 may be formed from 8-HPETE via the cationic

intermediate 8. Experiments to test this hypothesis using 8-HPETE in the cell-free Clavularia viridis system and also to mimic chemically the cyclization 8 ⟶ 4 are planned.[11]

References and Notes

1. (a) H. Kikuchi, Y. Tsukitani, K. Iguchi, and Y. Yamada, Tetrahedron Letters, 23, 5171 (1982); (b) ibid., 24, 1549 (1983).

2. B. J. Baker, R. K. Okuda, P. T. K. Yu, and P. J. Scheuer, J. Am. Chem. Soc., 107, 2976 (1985).

3. M. Fukushima, T. Kato, Y. Yamada, I. Kitagawa, S. Kurozumi, and P. J. Scheuer, Proc. Amer. Assoc. Cancer Res., 26, 249 (1985).

4. See E. J. Corey, Experientia, 39, 1084 (1983) for a biosynthetic proposal via radical intermediates.

5. Clavularia viridis was collected by one of us (Y. Y.) off Ishigaki Island and immediately cooled to -78° (dry ice) and maintained at that temperature. Biosynthetic activity was maintained at that temperature for approximately one year.

6. Native clavulones (mainly clavulone I) were extracted in this way and analyzed by PMR and HPLC. No appreciable tritiation was found in this clavulone fraction.

7. HPLC analysis and separation was performed with a DuPont Zorbax silica column (25 cm x 6 mm). UV-active standards were detected by UV absorption; tritiated materials were detected by scintillation counting of collected column fractions (usually 2 ml). Solvent systems used for HPLC were:

   A, hexane-tetrahydrofuran (THF), ca. 15 : 1

   B, hexane-isopropyl alcohol

   C, hexane-t-butyl methyl ether

   D, methylene chloride-THF

   Solvent ratios were adjusted to obtain optimal separation of peaks. Retention volumes were in the range 13-37 ml.

8. HPLC co-elution was demonstrated as follows. Standard and radiolabelled substance were co-injected into the HPLC column with benzene. Fractions of equal volume were collected and assayed for tritium by scintillation counting. Collected peaks were reinjected in a second solvent system, collected and assayed for tritium. Substances differing in retention volume by 3% were routinely separated and distinguished.

9. The cis-cyclopentanone 3 was obtained by hydrogenation (H_2, Pd-C, EtOH, 23° for 14 hr) of 4-desacetoxy-1 which was synthesized by aldol condensation using the method of E. J. Corey and M. M. Mehrotra, J. Am. Chem. Soc., 106, 3384 (1984) with methyl Z-6-formyl-5-hexenoate as the aldehyde component.

10. B. A. Vick and D. C. Zimmerman, Biochem. Biophys. Res. Commun., 111, 470 (1983) and references therein.

11. This research was assisted financially by grants from the National Institutes of Health and the National Science Foundation.

(Received in USA 6 June 1985)