Short Communications

A proposal for the biosynthesis of the clavulones, a new family of eicosanoids

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Summary. The biosynthesis of the newly discovered clavulones is suggested to proceed from arachidonic acid by a novel pathway, outlined in chart I, involving an unbroken series of 10 free radical intermediates and an uptake of 3 O_2 molecules.

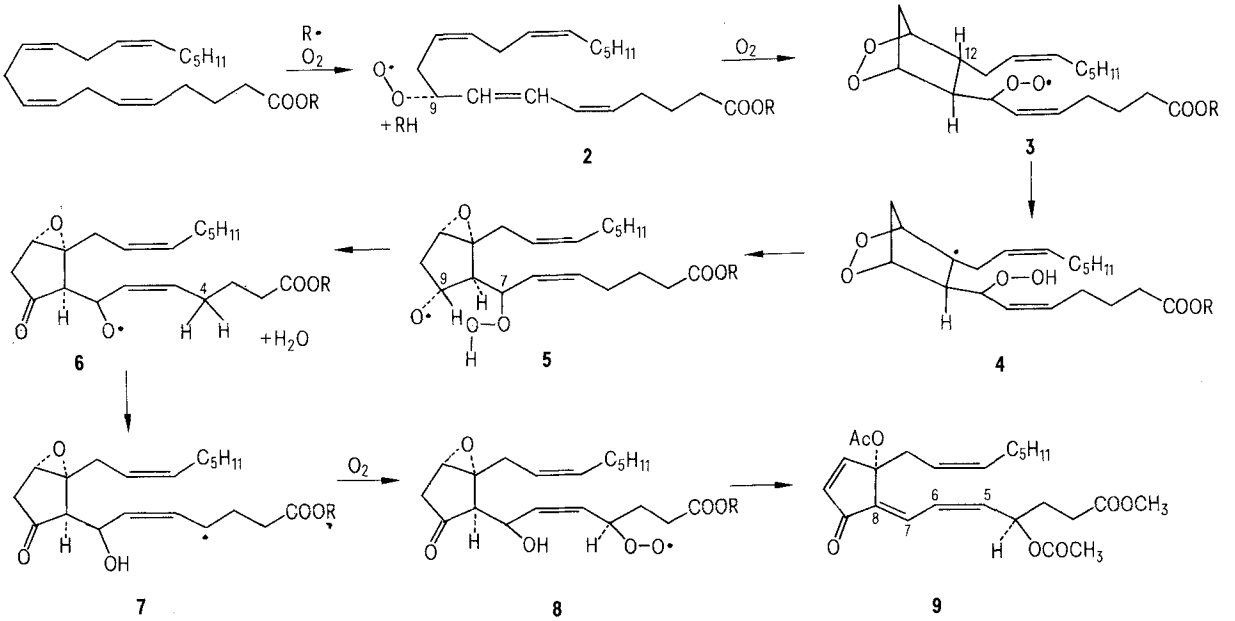
In recent papers[2,3] the structures of 3 new eicosanoids[4], clavulones I, II and III, have been disclosed. Calvulone I is represented by formula **1**, clavulone II is the 5,6-*E*-isomer of **1**, and clavulone III is the 5,6-*E*, 7,8-*Z*-isomer of **1**. Although it seems probable that these prostaglandin (PG)-like structures[3] are derived from arachidonic acid, the pathway of biosynthesis, which has not been discussed, constitutes a fascinating problem. We suggest herein a novel biogenetic route from arachidonic acid to the clavulones. Despite the fact that each step in the proposed scheme finds good synthetic or mechanistic analogy, the overall process is unusual both for the number of consecutive internal free radical reactions involved and the level of molecular complexity which is generated from an initially formed free radical through a series of transient species and the incorporation of *three* molecules of O_2. Because the suggested pathway entails a continuous succession of metastable intermediates, it is in this respect reminiscent of the 'non-stop' conversion of 2,3-oxidosqualene to sterols or triterpenoids which proceeds via a multistep cationic sequence. In both cases a noteworthy economy is evident in terms of the number of enzymes which are likely to be required.

Chart I summarizes the biosynthetic pathway which is envisaged. The initial step is a 9-lipoxygenation, considered to be analogous to that in PG biosynthesis, to give peroxy radical **2**. This is followed by formation from **2** and O_2 of a PGG_2-like endoperoxide-peroxy radical (**3**). 1,5-Hydrogen atom migration from C (12) to oxygen converts **3** to the β-peroxy radical **4**. The next step, **4** → **5**, is a rearrangement of a β-peroxy radical with O–O bond cleavage to an oxirane-alkoxy radical. This kind of reaction has already been observed in chemical systems by Bloodworth[5], and in a separate article we describe additional examples[6], so there can be no doubt of its feasibility. 1,5-Hydrogen transfer from C(9) of **5** to the nearby terminal oxygen of the C(7) hydroperoxide forms the 9-keto 7-alkoxy radical **6** and a water molecule. 1,5-Hydrogen migration in **6** from C(4) to the 7-oxy radical affords **7** which by capture of O_2 leads to peroxy radical **8**. At this point all of the oxygens of the clavulones are in place. Conversion of **8** to clavulone I (or clavulones II and III) then involves a collection of routine steps which include (ordering arbitrary):

(1) 4-peroxy radical → 4-hydroperoxide → 4-hydroxyl → 4-acetate; (2) β,γ-epoxy ketone → γ-hydroxy-α,β-cyclopentenone; (3) β-elimination of the C(7) hydroxy group.

As indicated above, in this proposed biosynthesis of **8** all intermediates are free radical and 3 O_2 molecules are fixed. All intramolecular radical transfer processes beyond the well precedented endoperoxide **3** involve sterically favorable 6-membered cyclic transition states (chart I: **3** → **4**; **5** → **6** and **6** → **7**)[7]. The chirality of the initial lipoxygenation which is required to generate the correct absolute configuration of the clavulones (arachidonic acid → **2**) corresponds to that for PG biosynthesis.

The mechanism outlined in chart I leads to a number of predictions which are subject to experimental test. The feasibility of a simple biomimetic synthesis of clavulones is implied by this proposal as is the possibility that the PG and clavulone synthetase enzymes may bear an unusually interesting relationship to one another, possibly differing in only a small number of amino acid units.



1 This research was assisted by a grant from the National Science Foundation.
2 Kikuchi, H., Tsukitani, Y., Iguchi, K., and Yamada, Y., Tetrahedron Lett. *23* (1982) 5171.
3 Kikuchi, H., Tsukitani, Y., Iguchi, K., and Yamada, Y., Tetrahedron Lett. *24* (1983) 1549.
4 The term 'eicosanoid' (Corey, E.J., Adv. Prostaglandin Thromboxane Res. *6* (1980) 19) is more appropriate to the clavulones than 'prostanoid', which has been used previously[2,3], because the pathways for the biosynthesis of prostaglandins and clavulones differ so markedly.
5 Bloodworth, A.J., and Bylina, G.S., J. chem. Soc. Perkin I *1972*, 2433.
6 Corey, E.J., Schmidt, G.E., and Shimoji, K., Tetrahedron Lett. *24* (1983) in press.
7 For a review of such intramolecular radical reactions see Kalvoda, J., and Heusler, K., Synthesis (1971) 501.

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Stability of ceftizoxime in aqueous solution

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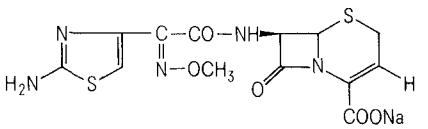
Summary. Ceftizoxime, when assayed using the disc agar-diffusion method with *Bacillus subtilis* ATCC 6633 as the indicator microorganism in buffered (pH 6) medium, retains its potency in aqueous solution at 4 °C for about 3 months. Even after 4 months, some residual activity can be found. However, when this solution was assayed on the non-buffered medium, a seemingly quicker but misleading loss of potency was observed. This resulted from inadequate sensitivity of the assay. The sensitivity of the ceftizoxime assay was increased by lowering the incubation temperature from 37 °C to 30 °C. The pH- and incubation temperature-dependent potency must be kept in mind when assaying ceftizoxime.

Ceftizoxime is a member of the third generation parenteral 2-amino-4-thiazolyl-α-methoxyimino-acetyl cephalosporins. It is unique since it contains only 1 H-atom at the 3-position of the β-lactam-dihydro-thiazine nucleus as shown in the figure 1.



Chemical structure of ceftizoxime.

Ceftizoxime possesses a wide spectrum of antibacterial activity with an unusually great potency and a high degree of β-lactamase stability[1]. The compound has an excellent therapeutic application and safety record. Therefore, it is important to make its bio-assay at optimal conditions. In this report, the results of the study of its stability in aqueous solutions employing such optimal conditions is communicated.

Materials and methods. A solution of ceftizoxime containing 10 µg/ml was prepared by dissolving 2.5 mg in 250 ml of deionized water. This stock-solution was kept at 4 °C throughout the study. Initially and later at selected times between August 15 and December 12, 2-fold dilutions were made from the stock-solution to obtain concentrations of 5, 2.5, 1.25, 0.6 and 0.3 µg/ml. These were assayed using the disc (6.35 mm diameter, Schleicher-Schuell Inc.) agar-diffusion method. Both commercial 'Penseed' agar and 'Penseed' agar buffered to pH 6.0 with phosphate buffer were seeded with the appropriate dilutions of *B. subtilis* ATCC 6633 spores. Prior to assay, discs saturated with the appropriate dilutions of ceftizoxime were placed on the surface of the seeded agar plates as described earlier[2]. The plates were incubated overnight at 37 °C, but on 2 occasions were additionally incubated at 30 °C. The diameters of the inhibition zones were measured and recorded. In all assays and for each dilution, 3 discs were used and the data given in the tables represent the average values of the inhibition zone diameters of the 3 discs.

Results and conclusion. The table represents the average diameters (mm) of the inhibition zones for each concentration used (5, 2.5, 1.25, 0.6, and 0.3 µg/ml). Over the study period of 2 months, the inhibition zone sizes (a reflection of activity) did not change markedly, thus reflecting maintained stability. After 2 months, inhibition zone sizes diminished slowly, and after 4 months, diminished at a faster rate. For assay purposes (*B. subtilis* ATCC 6633), the buf-

Time related stability of ceftizoxime (µg/ml) in aqueous solution under various assay conditions

Date of assay	Diameter of inhibition zones in mm																			
	pH 6, 37 °C					Non-buffered 37 °C					pH 6, 30 °C					Non-buffered, 30 °C				
	5*	2.5*	1.25*	0.6*	0.3*	5*	2.5*	1.25*	0.6*	0.3*	5*	2.5*	1.25*	0.6*	0.3*	5*	2.5*	1.75*	0.6*	0.3*
8/15	23	20	16.5	13.4	10	21.5	15.5	11.5	9	0										
8/16	23	19.5	16.5	14	9	20.5	16.5	13	9	0	28.5	25	20	17.5	14	29.5	25.5	21.5	17.5	11.5
8/27	23.4	22	18.5	15.5	9	20.5	18	14.5	10.5	0										
9/4	24.5	22	18.5	15.5	11	22	18.5	14	11	0										
9/13	24.5	22	18	14	10.5	19	16	12.5	9	0										
9/17	24.5	21.5	18	14.5	11.5	19	16.5	11.5	7.5	0										
10/15						20	16.5	13.5	7.5	0										
10/18	22.5	20	16.5	12.5	7.5	21	18	12.5	7.5	0										
10/30	24.5	21.5	18	12.5	7	20.5	17	12.5	6.5	0										
11/8	22.5	20	18	13	9	15.5	12	9	0	0	27	23	21	18	12	18	13	9	0	0
12/12	19	15.5	11	0	0	18	13	10	0	0										

* Ceftizoxime, µg/ml.