A Diepoxide Cyclization Cascade Initiated through the Action of Pig Liver Esterase

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An enantiomerically pure, functionalised diepoxide is converted, upon treatment with pig liver esterase under buffered aqueous conditions, into a cyclized product *via* a stepwise, stereospecific diepoxide cyclization; the crystal structure of the bis-lactone (**5**) has been determined.

The Cane–Westley model of polyether ionophore antibiotic biosynthesis invokes a polyepoxide cyclization cascade to account for the stereocontrolled formation of the tetrahydrofuranoid segments of these antibiotics from polyunsaturated fatty acid precursors.[1] According to this model the biosynthesis of monensin-A proceeds *via* a triepoxide which rearranges by the route shown in Scheme 1. The chemical feasibility of closely related cascades, under acidic conditions, has been demonstrated recently by the groups of Still,[2] and Schreiber,[3] who independently achieved stereocontrolled cyclizations from diastereoisomerically pure di- and tri-epoxide precursors. In this communication we report the synthesis of enantiomerically pure diepoxides which, notably, undergo a diepoxide cyclization initiated by the action of pig liver esterase in buffered aqueous solution.

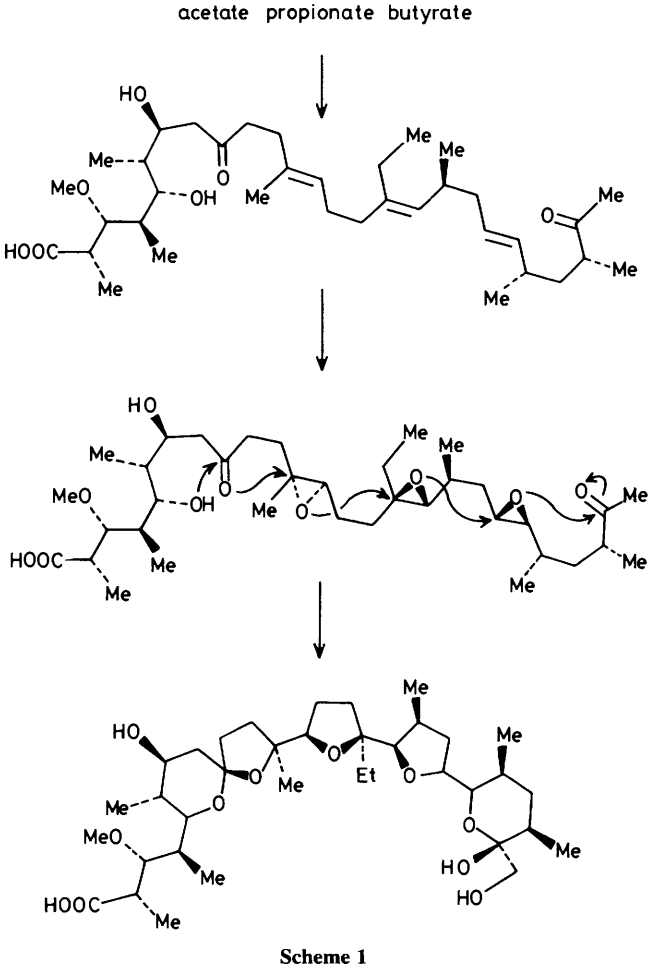
The synthesis of a model diepoxide is outlined in Scheme 2. Thus geranyl acetate provides the carbon backbone upon which two enantioselective epoxidations may be elaborated in excellent overall chemical yield, and with a high degree of stereospecificity [90% enantiomeric excess (e.e.) at each epoxidation].† All new compounds gave spectroscopic and analytical data entirely in accord with the structures shown, whereas the relative and absolute configurations of diepoxides (**1**) and (**2**) are those expected based on the known steric course of the titanium catalysed epoxidation.[4]

Of special interest are the conditions under which diepoxides (**1**) and (**2**) have been transformed *via* a stereospecific bis-cyclization into the lactones (**3**) and (**4**), respectively (Scheme 3). In initial experiments, the treatment of either (**1**) or (**2**) sequentially with base (1 M aqueous NaOH, 1 equiv.) and acid (aq. AcOH) gave in each case a mixture of products, including in low yields the expected lactones (**3**) and (**4**). We were pleased to discover, however, that when either starting ester was incubated with commercial pig liver esterase in buffered aqueous solution, ester hydrolysis occurred, and from the acidified aqueous solution the lactones (**3**) and (**4**) could be extracted in high yield. The structures of these products shown are supported in particular by ^{1}H n.m.r., 2-D COSY, and ^{13}C n.m.r. spectroscopy, and in the case of (**4**) was proven unambiguously by single crystal *X*-ray diffraction analysis‡ of the bis-lactone (**5**) (Figure 1) obtained after Jones' oxidation of (**4**).

† The enantiospecificity at each Sharpless epoxidation[4] was assayed by 360 MHz ^{1}H n.m.r. spectroscopy, after conversion of the product epoxy-alcohol into the corresponding Mosher derivatives[5] by reaction with (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride.

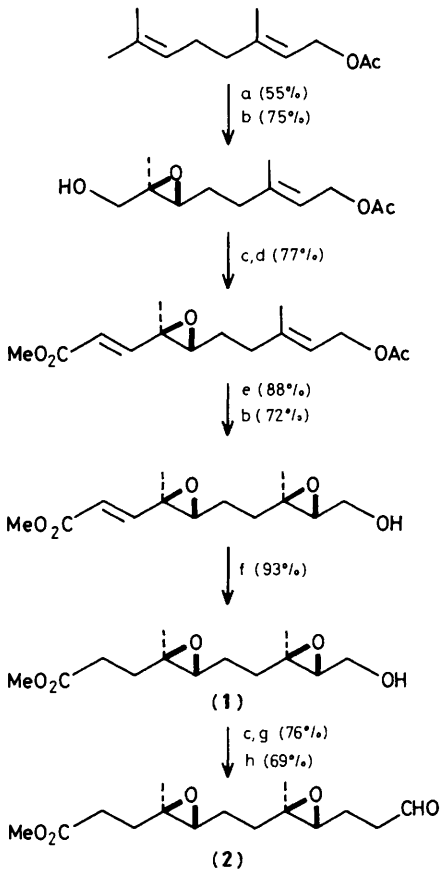
‡ *Crystal data* for dilactone (**5**): $C_{14}H_{20}O_5$, M = 268.3, orthorhombic, a = 8.401(2), b = 9.651(3), c = 16.989(3) Å, U = 1377 Å^3, space group $P2_12_12_1$, Z = 4, D_c = 1.29 g cm^{-3}, μ(Cu-K_α) = 8 cm^{-1}. Data were measured on a Nicolet R3m diffractometer with Cu-K_α radiation (graphite monochromator) using ω-scans. The structure was solved by direct methods and refined anisotropically to give R = 0.043, R_w = 0.055 for 1083 independent observed reflections [$|F_o| > 3\sigma(|F_o|)$, $\theta < 58°$]. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

It appears therefore that in each case a bis-cyclization cascade has occurred, proceeding through the stereospecific opening of each epoxide in a 5-*exo-tet* manner. The esterase catalysed transformation of bis-epoxide (**1**), however, was investigated in more detail by conducting the reaction in an n.m.r. tube. In this way the rate of esterase catalysed release of methanol was seen by n.m.r. spectroscopy to match exactly the rate of appearance of an intermediate whose structure was established by ^{1}H 1-D, 2D-COSY, and ^{13}C n.m.r. spectroscopy, as being the monocyclic lactone (**6**). The conversion of (**1**) into (**6**) was complete within 1 h under the conditions of this reaction.§ Thereafter, upon standing for a further 24 h,

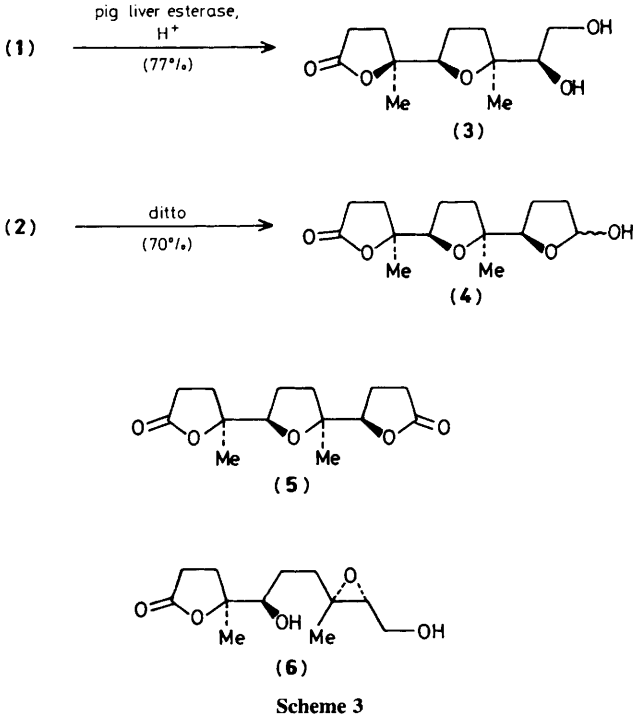


Scheme 1

§ A typical incubation contained 50 mM phosphate buffer (2.5 ml, pH 8.0), substrate (24 mg), and esterase (320 U) at *ca.* 21 °C. The reaction was complete within 1 h under these conditions, and the pH was maintained in the range 7.5—8.0 throughout the reaction. For n.m.r. experiments the incubation was conducted in deuteriated buffer under the same conditions.



Scheme 2. *Reagents:* a, SeO_2, Bu^tO_2H, salicylic acid, CH_2Cl_2; b, $Ti(OPr^i)_4$, CH_2Cl_2, (−)-di-isopropyl tartrate, Bu^tO_2H, 4 Å sieve; c, pyridine–SO_3, Me_2SO, Et_3N, CH_2Cl_2, room temp.; d, $Ph_3P{=}CHCO_2Me$, CH_2Cl_2, −40 °C; e, K_2CO_3, MeOH; f, $(K^+{}^-O_2CN)_2$, AcOH, dioxane; g, $Ph_3P{=}CHCHO$, CH_2Cl_2, −40 °C; h, H_2, Rh/Al_2O_3, EtOAc.



Scheme 3

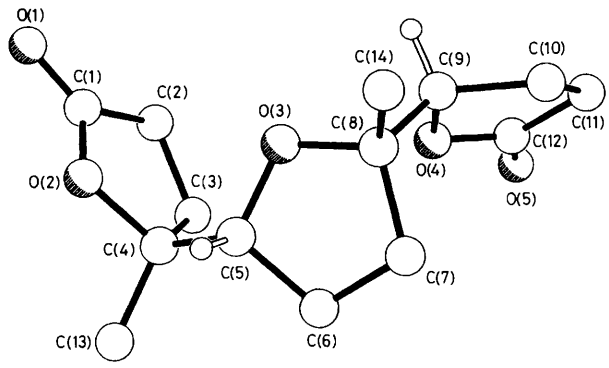


Figure 1. Crystal structure of the bis-epoxide (**5**).

the intermediate (**6**) had rearranged to afford the bis-cyclized material (**3**), which could be recovered from the aqueous solution in excellent yield. Similar observations were made of a stepwise conversion of (**2**) into (**4**), *via* a related intermediate, under the same conditions.

Thus our observations show that a bis-cyclization, which occurs apparently as a cascade process in aqueous solution at low pH, becomes under neutral or slightly basic conditions a stepwise process, with as expected, the carboxylate anion proving to be a more effective nucleophile during the conversion of (**1**) into (**6**) than is a secondary hydroxy group during the second stage from (**6**) to (**3**), as evidenced by the vast difference in rate for each step.

These observations raise interesting questions concerning the mechanism of any putative enzyme catalysed cascade, as implicated in the monensin biosynthetic pathway, shown in Scheme 1. A rapid cyclization could be initiated by nucleophilic attack from the hemiacetal in the triepoxide intermediate, and be catalysed by an array of acid and base groups on an enzyme surface. On the other hand, an alternative mechanism may be considered involving electrophilic catalysis, initiated by epoxide opening and proceeding at each stage through a tertiary carbocation, and quenched finally by the hemiacetal hydroxy group. In this way a smoothly orchestrated triepoxide cyclization cascade may be envisaged that is controlled by the ability of the enzyme to bind the substrate in a suitable conformation. Models of such an electrophilically driven polyepoxide cyclization cascade remain an interesting objective for future research.

The authors thank the S.E.R.C. for a studentship.

Received, 24th October 1986; Com. 1524

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