Studies towards the total synthesis of an epoxy isoprostane phospholipid, a potent activator of endothelial cells

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We report studies toward the total synthesis of an epoxy isoprostane, namely the preparation of compound 9 which is an analogue of the elimination product 7 of the naturally occurring epoxy isoprostane 4 by a straightforward route using a three-component coupling, and have shown by several spectroscopic criteria that it closely resembles the natural material.

We report herein the total synthesis of the epoxy isoprostane analogues 8 and 9. Isoprostanes,1 isomers of the well-known prostaglandins, were discovered in 1990,2 and several substitution patterns in the cyclopentane ring are known (D, E, and F, 1–3). They are formed in both biological systems and in vitro, via a free-radical induced oxidation process, from arachidonic acid in a pathway independent of cyclooxygenases.4,5 Since the formation of isoprostanes is not an enzymatic process, a large number of stereo- and regiosomers are formed with the relative ratio of the different isomers being dependent on the exact conditions under which the isoprostanes were formed. We recently reported the isolation and biological activity of an in vitro oxidation product of arachidonyl phosphocholine 4, an oxidized phospholipid with the same biological properties as several substitution patterns in the cyclopentane ring are known (D, E, and F, 1–3). They are formed in both biological systems and in vitro, via a free-radical induced oxidation process, from arachidonic acid in a pathway independent of cyclooxygenases.4,5 Since the formation of isoprostanes is not an enzymatic process, a large number of stereo- and regiosomers are formed with the relative ratio of the different isomers being dependent on the exact conditions under which the isoprostanes were formed. We recently reported the isolation and biological activity of an in vitro oxidation product of arachidonyl phosphocholine 4, an oxidized phospholipid with the same biological properties as

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The epoxy aldehyde ester 22 was prepared in five steps from the ester aldehyde 17 (prepared in one step by ozonolysis of cyclopentene).10 Wittig reaction using (triphenylphosphoranylidene)acetaldelyde 18 gave an EZ mixture of the aldehyde 19.11 Selective reduction at low temperature gave the alcohol 20. Sharpless asymmetric epoxidation12 of this substrate was earlier reported to be problematic.13 Using a mild work-up allowed the desired compound 21 to be isolated in good yield and ee.14 Although oxidation of this epoxy alcohol 21 with tetrapropyl perunethane TPAP/NMO gave low yields and Dess-Martin periodinane oxidation gave non-reproducible yields, Swern oxidation afforded the desired epoxyaldehyde 22.

Before attempting the required 3-component coupling15 we first studied a simpler model, namely the addition of allylcopropane 23 in the presence of trimethylsilyl chloride (TMSCl)17 to give the crude silyl enol ether 24. Regeneration of the enolate with methylthium18 and trapping with the optically active aldehyde 22 afforded in 90% yield a mixture of four diastereomers 25 (two major and two minor). Elimination of the mesylate19 and chromatographic separation gave a 1:1 mixture of two diastereomers of the E-enoone 26E in 19% yield and predominantly one diastereomer of the Z-enoone 26Z in 6% yield. Analogous trapping of the zinc enolate (made by the addition of 1 eq of ZnCl2 to the enolate) afforded the hydroxy ketone 25 as four isomers (in an approximate 1:1:1:1 ratio) in lower yield (33%). Elimination of this mixture gave 26E and 26Z in 22% and 6% yield, respectively. The proton NMR spectrum of 26E matched generally that of the isolated acids 6 and 7 but the differences in the structures are so significant that a close comparison was not expected. However, the UV and mass spectra were more informative. Compound 26E had a λmax of 253 nm while the λmax of both 6 and 7 were 252 nm. In the ESI-MS (and the APCI-MS), compound 26E showed an exact mass of 278.1518 and peaks at m/z of 131 and 159 which correspond to the breakdown of the epoxide and match the peaks of m/z of 115 and 143 seen in the mass spectra of 6 and 7.

Of the several good methods developed for the preparation of prostaglandin-like molecules, we chose a modification of Noyori’s 3-component coupling.26 The enantiomerically enriched 4:4-tert-butylmethyislyoxy-2-cyclopenten-1-one 16 was prepared from the prochiral diacetate 10 (made from cyclopentadiene), using deacetylation with electric cell acetylcholine esterase.9 Replacement of the acetyl group by a silyl group was performed in four steps via 12-14 to give the monoalcohol 15 which on PCC oxidation gave 16.10

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Having established that the 3-component coupling and elimination worked even with the sensitive epoxide functionality, we then carried out the process on the chiral cyclopentene-1,4-Addition of allylcopper to 16 in the presence of TMSCl afforded the crude silyl enol ether 27. Regeneration of the enolate and trapping with the aldehyde 28 gave the desired product 29 in 27% yield together with the enone 30 in 23% yield, in which elimination of the silyloxy group had occurred. Elimination of the hydroxy group was carried out as before with the silyl ether 28 giving the desired analogue of 6, the enone 8, in 44.5% yield along with the Z isomer 30 in 16.5% yield. Similar treatment of the enone 29 afforded the analogue of 7, the diene 9 in 15% yield along with the Z isomer 31 in 32% yield. The structural assignment of 8 and 9 was based largely on NOE data, e.g., NOESY spectra for both compounds showed interactions between H5 and H7 and, more importantly, between H6 and H5. As shown in Table 1, the peaks for H11, H10, H9, H12, H8, and H6 were at nearly identical chemical shift and had virtually identical coupling constants in the two compounds. The only significant differences were observed in the olefinic protons of the allyl group and the cis-2-ocetyl unit, as would be expected. This adds compelling evidence to the assignment of the structure of 7 to the compound derived from the natural material. By contrast, the proton NMR spectrum of the enone 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the proton NMR spectrum of 7. As shown in Table 1, the peaks for H11, H10, H9, H12, H8, and H6 were at nearly identical chemical shift and had virtually identical coupling constants in the two compounds. The only significant differences were observed in the olefinic protons of the allyl group and the cis-2-ocetyl unit, as would be expected. This adds compelling evidence to the assignment of the structure of 7 to the compound derived from the natural material. By contrast, the proton NMR spectrum of the enone 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials 6 and 7. In particular, the proton NMR spectrum of 9 matched very closely the relevant regions of the spectra of the analogues 8 and 9 with the naturally derived materials

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Thus our studies strongly support the unambiguous structural assignment of the naturally occurring epoxy isoprostane formed by free radical-induced oxidation of arachidonyl phosphatidylcholine. Furthermore the strategy and methods reported herein should pave the way for a total synthesis of the epoxy isoprostane phospholipid 7. We are currently carrying out biological investigations of the analogues 8, 9, 26(EZ), 30, and 31 and the synthesis of the epoxy isoprostanes 6 and 7 and their derived phospholipids 4 and 5 to further study the endothelial activation by this novel class of compounds.

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Notes and references