

in the nonpolar fractions.¹¹ Column chromatography (ethyl acetate) or HPLC (8:92 isopropyl alcohol:hexane) of the polar fraction afforded the most active component ajoene (**4**) as a colorless, odorless oil of formula $C_9H_{14}S_3O$ (elemental analysis¹² and CI-MS using methane and ammonia): IR 1050 (s, C—S(O)—C), 1650 cm^{-1} (s, C=C); UV λ_{max} 240 nm; 1H NMR (250 MHz) δ 6.38 (dt, $J = 14.8, 1$ Hz, 1 H, =CHSS), 5.9 (m, 3 H, =CHCH₂), 5.4 (m, 2 H, CH₂=CHCH₂S(O)), 5.2 (m, 2 H, CH₂=CHCH₂S), 3.5 (m, 4 H, CH₂S(O)CH₂), 3.36 (d, $J = 7.2$ Hz, 2 H, SSCH₂); ^{13}C NMR δ 134.7, 132.6, 125.7, 123.7, 119.3, 116.9, 54.5, 53.1, 41.4. The spectroscopic data are consistent with the structure (*E*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, (*E*)-CH₂=CHCH₂S(O)CH₂CH=CHSSCH₂CH=CH₂^{6,8} (**4-E**). An isomeric compound with 1H NMR (250 MHz) δ 6.56 (dt, $J = 9.5, 1$ Hz, 1 H, =CHSS), 5.8 (m, 3 H, =CHCH₂), 5.4 (m, 2 H, CH₂=CHCH₂S(O)), 5.2 (m, 2 H, CH₂=CHCH₂S), 3.5 (m, 4 H, CH₂S(O)CH₂), 3.38 (d, $J = 7.2$ Hz, 2 H, SSCH₂) and ^{13}C NMR δ 138.5, 132.7, 125.7, 123.8, 119.3, 118.2, 55.1, 49.7, 42.2 was identified as the *Z* isomer of **4**. The three compounds **3**, **4**, and **7** account for more than 75% of the platelet aggregation inhibitory activity of garlic extract.

We suggest (Scheme I) that compounds **3–8** are formed by decomposition of alliin (**1**), itself formed from a stable precursor by action of the allinase enzyme followed by dehydrative coupling of 2-propenesulfenic acid (steps a and b).¹³ S-Thioallylation of **1** followed by Cope-type elimination and readdition of 2-propenesulfenic acid^{14a} should give (*E,Z*)-ajoene (**4**) (steps c and d) while unimolecular decomposition of **1** (steps e and f) should afford thioacrolein (**9**) which would dimerize, following mechanisms previously advanced by one of us.^{1d,14b} In accord with this proposal, (*E,Z*)-ajoene (**4**) could be obtained by refluxing a solution of **1**¹⁵ (10% in 3:2 Me₂CO:H₂O)¹⁶ for 4 h, centrifuging, and extracting (CH₂Cl₂) the upper layer, which had been diluted with methanol and repeatedly extracted with pentane to remove nonpolar materials. Flash chromatography of the methylene chloride concentrate (0.34 g from 1 g of **1**; ca. 34% yield of slightly impure **4**) gave **4** (4:1 *E:Z*) in 17% yield. Synthetic **4** was identical in all respects with the natural material. GC analysis of the pentane-soluble fraction (0.52 g from 1 g of **1**) including the methanol-water-washed centrifugate indicated a 21:17:50:12 mixture of **5/3/7/8**, respectively. This ratio changed to 4:4:75:17 when **1** was decomposed in the same solvent mixture at 37 °C for 2 days or 25 °C for 7 days, reflecting partial decomposition of some compounds at the higher temperature and/or different temperature dependence of the reactions of Scheme I. The near identity of the 4.4:1 ratio of **7** to **8** observed in the 37 or 25 °C decomposition of **1** and the 4.5:1 ratio of **7** to **8** found from dimerization at -180 °C of thioacrolein (**9**) from flash vacuum pyrolysis of diallyl sulfide⁵ provides support for steps e and f.¹⁷ Steps g and i are supported by formation of propene on refluxing **1** in water and occurrence¹⁰ of sulfur dioxide and allyl alcohol in

garlic extracts; steps h and j employ previously proposed mechanisms^{1d,18} to rationalize formation of **3** and **5**. The mechanisms of Scheme I are also supported by studies on the decomposition of *S*-methyl 2-propenethiosulfinate.¹⁹

The ready availability of ajoene (**4**) permits study of the nature of its antithrombotic activity. Preliminary results indicate that when rabbits are fed 20 mg/kg of body weight of (*E,Z*)-**4** 100% inhibition of collagen-induced platelet aggregation is seen for a 24-h period after feeding. In vitro tests provide other interesting information on inhibition by **4**: the effect of **4** increases with time of incubation with platelets; its effect cannot be reversed by washing platelets; aggregation induced by all known inducers is inhibited; rabbit granulocyte aggregation is also inhibited.²⁰ These physiological observations suggest that the age-old belief in the therapeutic effect of garlic on the circulatory system may indeed have some basis.

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Registry No. **4**, 92285-01-3; (*E*)-**4**, 92284-99-6; (*Z*)-**4**, 92285-00-2; alliin, 539-86-6.

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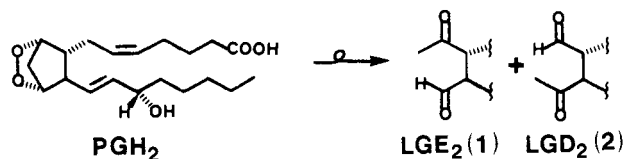
Asymmetric Total Synthesis of Levuglandin E₂¹

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It is fascinating that the prostaglandin (PG) endoperoxide PGH₂ is extraordinarily unstable in the aqueous environment of its biosynthesis.² Recently we discovered that this solvent-induced decomposition yields ($\approx 20\%$) two levulinialdehyde derivatives,³ levuglandin E₂ (LGE₂) (**1**) and LGD₂ (**2**), in addition to the



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(3) Zagorski, M. G.; Salomon, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 3498.

(9) Heterocycles **7** and **8** (incorrectly identified as 3-vinyl-6*H*-1,2-dithiin) were formed on preparative GC of **1** but were said not to be present in garlic extracts.¹⁰

(10) Brodnitz, M. H.; Pascale, J. V.; Van Derslice, L. *J. Agric. Food Chem.* **1971**, *19*, 273-275.

(11) HPLC analysis of commercial garlic oil or pearls showed that **4**, **7**, and **8** were absent; however, the latter three compounds would be unlikely to survive the steam distillation used to produce the commercial essential oil of garlic.

(12) Anal. Calcd for $C_9H_{14}S_3O$: C, 46.2; H, 5.98; S, 41.0. Found: C, 45.8; H, 5.90; S, 40.8.

(13) Stoll, A.; Seebeck, E. *Adv. Enzymol. Relat. Subj. Biochem.* **1951**, *11*, 377-400.

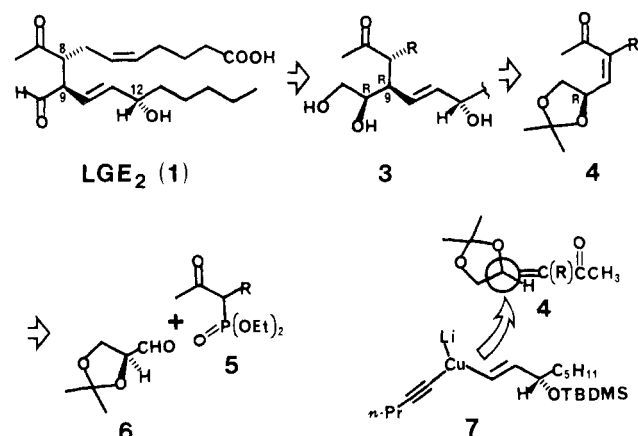
(14) (a) For a related process involving γ -chlorination of allylic sulfides, see: Mura, A. J., Jr.; Bennett, D. A.; Cohen, T. *Tetrahedron Lett.* **1975**, 4433-4436. (b) For thioaldehyde formation from thiosulfonates, see: Baldwin, J. E.; Lopez, R. G. *Tetrahedron* **1983**, *39*, 1487-1498.

(15) Prepared by oxidation of **5** (CH₃CO₂H).

(16) This solvent mixture was chosen because it maximizes the yield and *E/Z* ratio of **4** and gave an initially homogeneous solution with **1**. The *Z* isomer is the major product on decomposition of neat **1** or a solution of **1** in acetone or benzene/water.

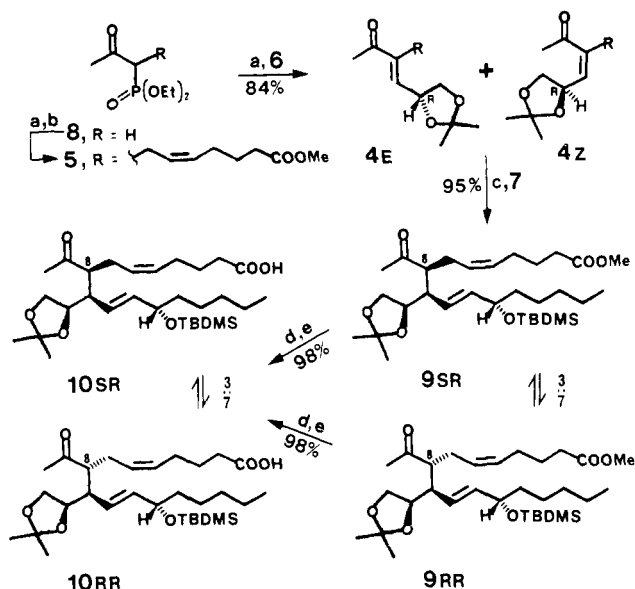
(17) Direct observation of deep blue thioacrolein (**9**) could be achieved by distilling **1** into a liquid-nitrogen-cooled trap; 2-propenesulfenic acid could also be trapped with an alkyne as reported previously.^{7b}

Scheme I



previously recognized products PGE_2 and PGD_2 .⁴ Separation of levuglandins from this mixture is hampered by their proclivity toward dehydration. Although the more stable of these levuglandins was isolated by HPLC, neither mass spectral nor nuclear magnetic resonance data⁵ were deemed adequate for a choice between structures 1 or 2 for this ketoaldehyde. Owing to the limited availability of PGH_2 , we turned to total synthesis as a practical alternative source of levuglandins to facilitate thorough chemical characterization and biological evaluation. We now report an efficient asymmetric synthesis of LGE_2 [8(*R*)-acetyl-9(*R*)-formyl-12(*S*)-hydroxy-5(*Z*),10(*E*)-heptadecadienoic acid].

LGE_2 is a challenging target for total synthesis. Besides a sensitive vinylogous β -hydroxy carbonyl array, this acyclic compound incorporates three asymmetric centers, two of which are epimerizable. A key feature of our synthetic strategy (Scheme I) is generation of the sensitive vinylogous β -hydroxy aldehyde functional array late in the synthesis employing a mild periodate cleavage of a vicinal diol 3.⁶ In analogy with a strategy for synthesis of prostaglandins,⁷ a convergent construction of the levuglandin skeleton is achieved by stereospecific Michael addition of a vinyl nucleophile to chiral enone 4. The asymmetry of 4 is provided by *L*-glyceraldehyde acetonide (6),⁸ which affords the enone upon olefination with phosphonate 5. Recent precedent⁹ indicates that 1,4-addition of the vinyl cuprate 7 will proceed by a Felkin-type transition state¹⁰ in which carbon-carbon bond formation occurs anti to the polar allylic alkoxy. Therefore, an *R* configuration at the alkoxy-substituted allylic center in 4 will foster generation of the *R* configuration required at position 9 in LGE_2 (1). Precedents extant at the inception of this project indicated that chiral allylic alkoxy-substituted centers direct stereospecific addition of nucleophiles to Michael acceptors, but the sense of asymmetric induction varies with the nature of the nucleophile,¹¹ and the outcome for additions of vinyl cuprates was

Scheme II^a

^a (a) NaH, (b) RBr, (c) MgBr_2 , (d) $\text{NaOH}/\text{H}_2\text{O}$, (e) HCl at pH 3.

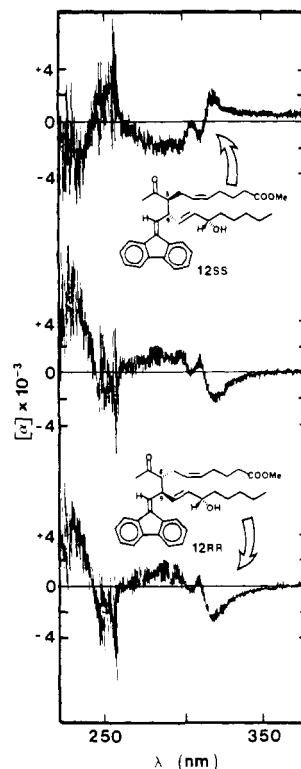


Figure 1. ORD spectra of flurenlydene derivatives: top, 12-SS; middle, flurenlydene derivative of LGE_2 methyl ester obtained from PGH_2 ; bottom, 12-RR.

not yet known. Therefore, since acetonides of both D- and L-glyceraldehyde are readily available,⁸ syntheses were executed by using 4 and its enantiomer and the final products were correlated with LGE_2 .

(4) At pH 8, products which were isolated from decomposition of [1-¹⁴C] PGH_2 by extraction of the acidified solution with diethyl ether, separated and identified by TLC, and quantified by liquid scintillation counting included PGE_2 (71%) and PGD_2 (20%).^{2c} It is important to note that these are distributions of radioactive products isolated, and not absolute yields.

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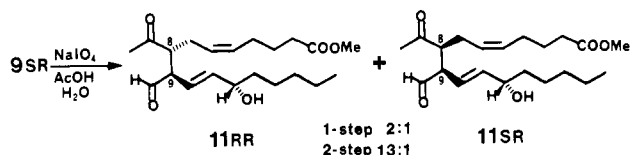
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The LGE₂ skeleton was assembled as outlined in Scheme II. Alkylation of (diethylphosphono)acetone (8) with methyl 7-bromohept-5(Z)-enoate¹² affords 5 whose sodium salt reacts with isopropylidene-L-glyceraldehyde (6) to produce the isomeric enones 4-Z and 4-E (1:2.3). Reaction of either isomeric enone with vinyl cuprate 7,¹³ prepared from 1-iodo-3(S)-[(*tert*-butyldimethylsilyl)oxy]-1(E)-octene¹⁴ afforded identical mixtures of 9-RR¹⁵ (70%), which has the C-8 configuration of LGE₂, and the C-8 epimer 9-SR¹⁵ (30%). Interestingly, Michael addition occurred only in the presence of MgBr₂, which presumably serves as a Lewis acid catalyst.¹⁶ That 9-RR and 9-SR are epimeric at position 8 is evident from the observation that saponification of either ester afforded identical mixtures of epimeric acids 10-RR (70%) and 10-SR (30%).

Unexpectedly, treatment of either 9-RR or 9-SR with aqueous acetic acid followed by sodium periodate afforded the same 13:1 mixture of LGE₂ methyl ester (11-RR) and the 8-epi isomer (11-SR). This remarkably high preference for the required 8R configuration contrasts with the 7:3 equilibrium ratio observed for 10-RR and 10-SR. The 1,2-dihydroxyethyl substituent in the intermediate vicinal diol 3 apparently plays a role in the stereoselective epimerization since efficient interception of this intermediate allows conversion of 9-SR to mixtures richer in the 11-SR epimer. Thus, treatment of 9-SR with aqueous acetic acid in the presence of sodium periodate yielded a 2:1 mixture of 11-RR and 11-SR. Further discussion of this remarkable stereoselection is



deferred to a full account of this work. The aldehydic ¹H NMR resonance for 11-RR in CDCl₃ occurs at δ 9.47 as found for the methyl ester of LGE₂ derived from PGH₂ while the corresponding resonance for 11-SR occurs at δ 9.56. For comparison with LGE₂ methyl ester derived from PGH₂, 8-*epi*-9-*epi*-LGE₂ methyl ester 11-SS was obtained from 9-SS by substituting isopropylidene-D-glyceraldehyde for the L isomer 6 in Scheme II. The ¹H and ¹³C NMR spectra of 11-RR, 11-SS, and LGE₂ methyl ester obtained from PGH₂ are almost identical. To facilitate correlation, these keto aldehydes were converted to fluorenylidene derivatives 12 by chemoselective Wittig reaction with 9-fluorenylidene-*n*-butylphosphorane.¹⁷ The adducts 12 show intense UV absorptions (ε_{max} ≈ 40000; hexane) at both 258 and 229 nm. Furthermore, in contrast with LGE₂ (1), analytically pure fluorenylidene derivatives were readily isolated quantitatively by HPLC on partisil. As expected, 12-RR and the fluorenylidene derivative of LGE₂ methyl ester derived from PGH₂ exhibit identical ORD curves while the curve for 12-SS is virtually a mirror image (Figure 1).

Levuglandin E₂ was prepared in good yield from the ketal 10-RR (or 10-SR) by treatment with aqueous acetic acid followed by sodium periodate. The ¹H NMR spectrum of synthetic LGE₂ is identical with that of the more stable levuglandin obtained from PGH₂ except for tiny absorptions owing to minor impurities in the latter sample which were absent in the synthetic product. The total synthesis now makes LGE₂ readily available. The present results also demonstrate that epimerization of LGE₂ at positions 8 or 9 is not extensive under the conditions of its solvent-induced formation from PGH₂.

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Supplementary Material Available: Physical data and purification of compounds 4-6 and 8-12 (12 pages). Ordering information is given on any current masthead page.

The Nature of Restrictions in the Binding Site of Rhodopsin. A Model Study

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Continuing the effort of early workers,¹ the research teams at Hawaii and elsewhere have recently completed the synthesis of all 16 possible geometric isomers of vitamin A.² Among the 14 stable retinal isomers, 10 have been shown to form visual pigment analogues: 11-cis, 9-cis,¹ 9-cis,13-cis,^{1,3} 7-cis, 7-cis,9-cis, 7-cis,13-cis, 7-cis,9-cis,13-cis,⁴ 7-cis,11-cis,⁵ 9-cis,11-cis,⁶ and 7-cis,9-cis,11-cis.^{7,8} The notable exceptions are the all-trans and the 13-cis isomers, which do not yield stable analogues. However, it is known that after introduced into the binding site, such geometries can be generated as transient forms, i.e., in lumirhodopsin (or Meta-I)⁹ and photo-Meta-II-465.¹⁰

The failure of these two isomers to form stable pigments has been rationalized on the ground of a longitudinal restriction of the binding site.^{11,12} This explanation is quite satisfactory for the all-trans isomer, which has the longest distance between the center of the cyclohexenyl ring and the carbonyl carbon.¹¹ Since the 13-cis isomer is not uniquely longer than several other isomers, conformational rigidity was also considered important.¹²

An analysis of longitudinal restrictions based on the length of the chromophore overlooks the tetherlike function of the *n*-butyl group of Lys-296.¹³ Also, the model implies freedom of in-plane motion of the imino carbon relative to a rigid cyclohexenyl ring. This is only partially correct because within the binding site the relative distance of the primary (the protonated Schiff base) and the secondary binding sites (the hydrophobic pocket) is likely to vary only within the limits allowed by conformational changes of the butyl group and to a smaller extent the chromophore itself. We now present a different approach in analyzing the shape of the binding site on the basis of its behavior toward retinal isomers.

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