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Stereospecific Synthesis of *trans*-Arachidonic Acids

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Abstract—An effective synthesis is described for the preparation of all four mono *trans* isomers of arachidonic acid via deoxidation of epoxide precursors with lithium diphenylphosphide and quaternization with methyl iodide. © 2001 Elsevier Science Ltd. All rights reserved.

Vascular inflammation, stroke, arteriosclerosis, and heart attack are associated with increased levels of lipid peroxidation products in the cardiovascular system. Evidence exists that these products originate from processes initiated by reactive oxygen free radicals.[1,2] Remarkable progress has been made recently to elucidate the structures of the oxidized lipids and the signaling role of lipids derived from peroxidation of arachidonic acid (AA), especially isoprostaglandins.[3,4] Inflammatory processes also lead to overproduction of NO and its oxidation product, NO_2.[5,6] However, very little is known about lipid peroxidation mechanisms that involve reactive nitrogen species such as NO_2.

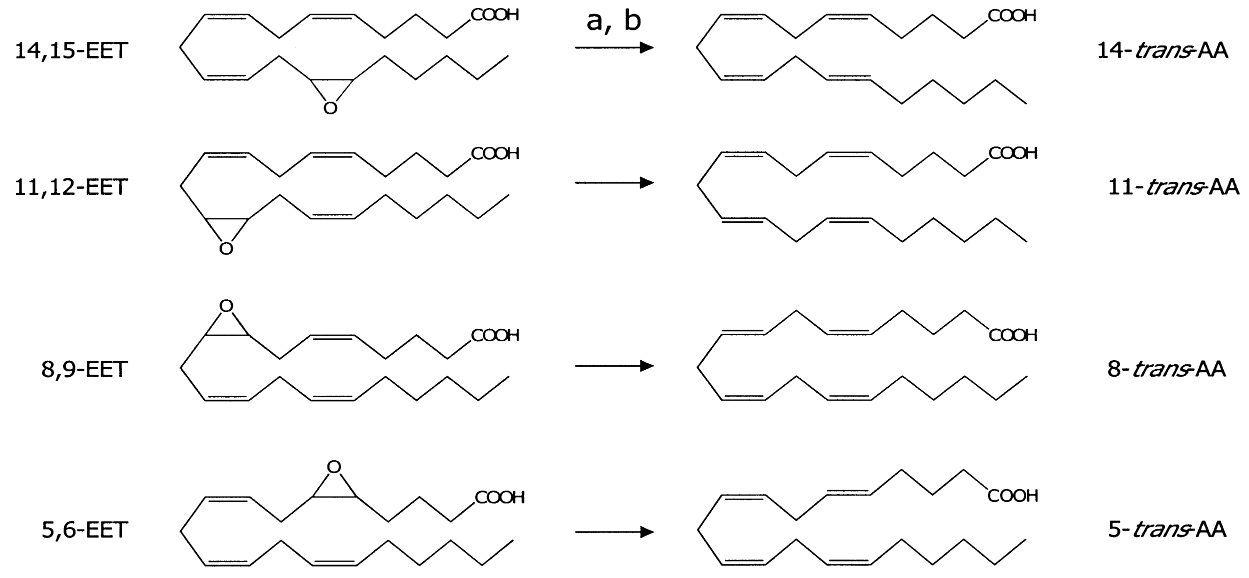

Figure 1. Synthesis of mono *trans*-arachidonic acid from corresponding *cis*-epoxyeicosatrienoic acids (EETs). (a) PPh_2Li in THF, 0 °C, 2 h at 23 °C; (b) CH_3I, 30 min.

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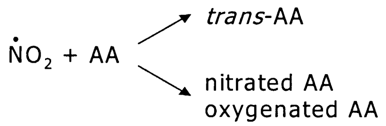

We described *trans*-arachidonic acid isomers (*trans*-AA) as the major product of the NO_2 radical-mediated *cis–trans* isomerization of AA.[7,8] Structural analysis has revealed that NO_2 causes AA isomerization without rearrangement of the double bonds.[7] We have also reported that *trans*-AA isomers are present in normal human plasma in relatively high amounts (23–89 ng/mL).[7] Injection of bacterial endotoxin (LPS) into the rat has resulted in a more than 2-fold increase of the blood plasma levels of *trans*-AA.[9] Our studies suggest that *trans*-AA are formed endogenously by free radical processes initiated by NO_2 and could potentially function as novel mediators of inflammation and other pathophysiological processes. Several studies have suggested that dietary *trans* fatty acids originating from hydrogenated fat may play a role in development of cardiovascular disease.[10–12] We hypothesized that because *trans* fatty acids could be formed endogenously, they may also contribute to the cardiovascular and other complications resulting from NO overproduction. Although *trans*-AA as a group is well separated from AA by reversed-phase high performance liquid chromatography (RP-HPLC), our attempts to separate individual isomers by this technique were not successful. Thus, to further understand the biological role of these new fatty acids, their metabolism, and their impact on biological membrane fluidity and permeability, we developed a convenient synthesis for the preparation of pure *trans*-AA isomers from the individual regioisomeric *cis*-epoxyeicosatrienoic acids (EETs) (Fig. 1). The EET regioisomers (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET) were prepared as described[13–16] and dried azeotropically before being transformed stereospecifically to the corresponding *trans*-AA regioisomers[17] by modification of literature procedure.[18] Reactions were performed in dried glassware under argon atmosphere, using freshly distilled solvents. Tetrahydrofuran (THF) was distilled from benzophenone ketyl under nitrogen. Methyl iodide was freshly distilled under argon. All other reagents were commercially obtained and were used as received. *n*-BuLi in THF was added to a solution of diphenylphosphine in THF at 0 °C and stirred for 2 h at 23 °C. To this was added a solution of EET in THF over 5 min. After stirring for 2 h, freshly distilled CH_3I was added to the reaction mixture, which was then allowed to stand for 30 min. The reaction mixture was diluted with EtOAc, washed with H_2O, then with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by SiO_2 PTLC afforded *trans*-AA as a colorless oil (74%). TLC analysis (EtOAc/hexane 3:7) revealed one spot at $R_f \sim 0.31$. RP-HPLC analysis revealed a single peak at 28–32 min. The EETs had specific activity of 18–20 cpm/nmol (^{14}C label was at carbon 1). The radioactivity label was helpful to establish the concentration of products after purification. Thin-layer chromatography (TLC) was performed using E. Merck silica gel pre-coated plates

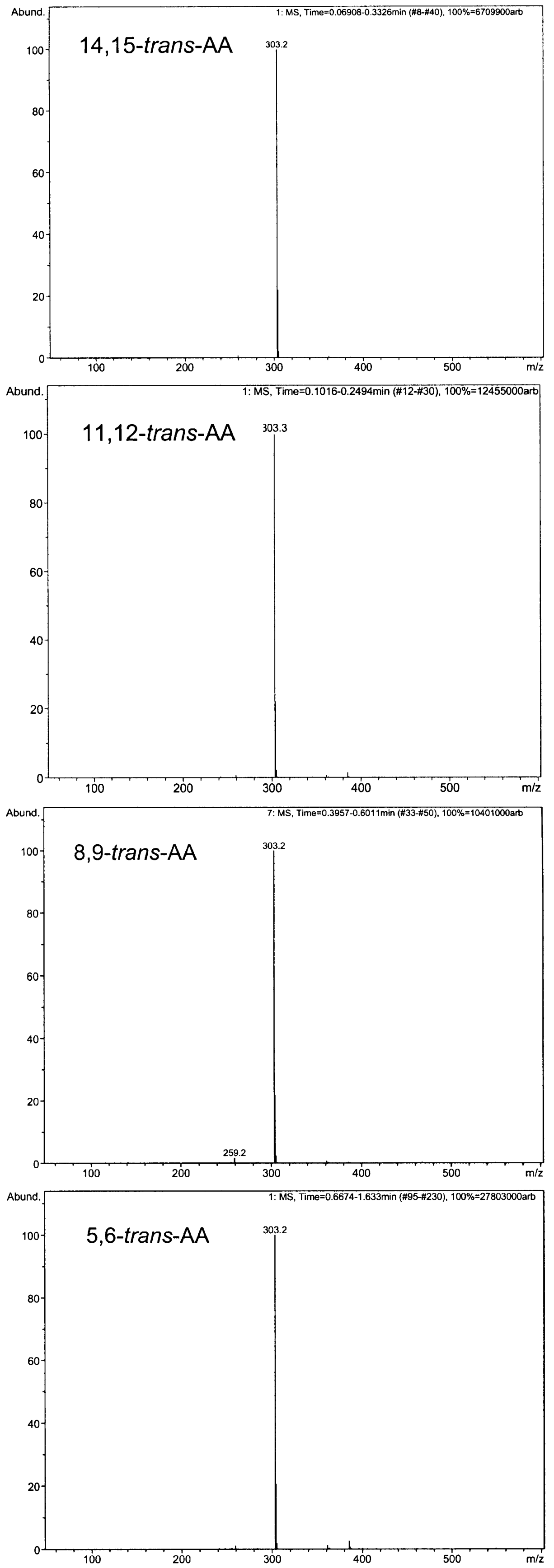

Figure 2. Electrospray mass spectra (negative ions) of 14,15-*trans*-AA, 11,12-*trans*-AA, 8,9-*trans*-AA, and 5,6-*trans*-AA.

(0.25 mm). ^{1}H NMR was performed on a 400 MHz instrument in $CDCl_3$.[19] Mass spectral analyses were performed at The New York Medical College Mass Spectrometry Center using electron ionization (EI), chemical ionization (CI) or electrospray (ESI) (Fig. 2). Methyl esters were prepared with fresh diazomethane in diethyl ether. Pentafluorobenzyl esters were prepared from pentafluorobenzyl bromide and diisopropylethylamine in acetonitrile. ESI mass spectrometry (negative ions) was performed on a Brucker Daltonics Esquire ion trap mass spectrometer using a syringe pump for sample introduction (200 μL/h). The following parameters were used for mass spectra acquisition: capillary: +4 kV, end plate offset: −0.5 kV, skimmer 1: −35 V, capillary exit offset: −60 V, accumulation time: 30 ms, dry gas (nitrogen) temperature: 300 °C, nebulizer pressure: 5 psi, nebulizer flow: 5 L/min. RP-HPLC was performed on an octadecylsilyl column (250×4.6 mm) and *trans*-AA isomers were eluted with a gradient of acetonitrile in water (62.5–100% in 50 min) at 1 mL/min. The effluent from the column was analyzed by an ultraviolet detector, which monitored the wavelength at 205 nm (Fig. 3).

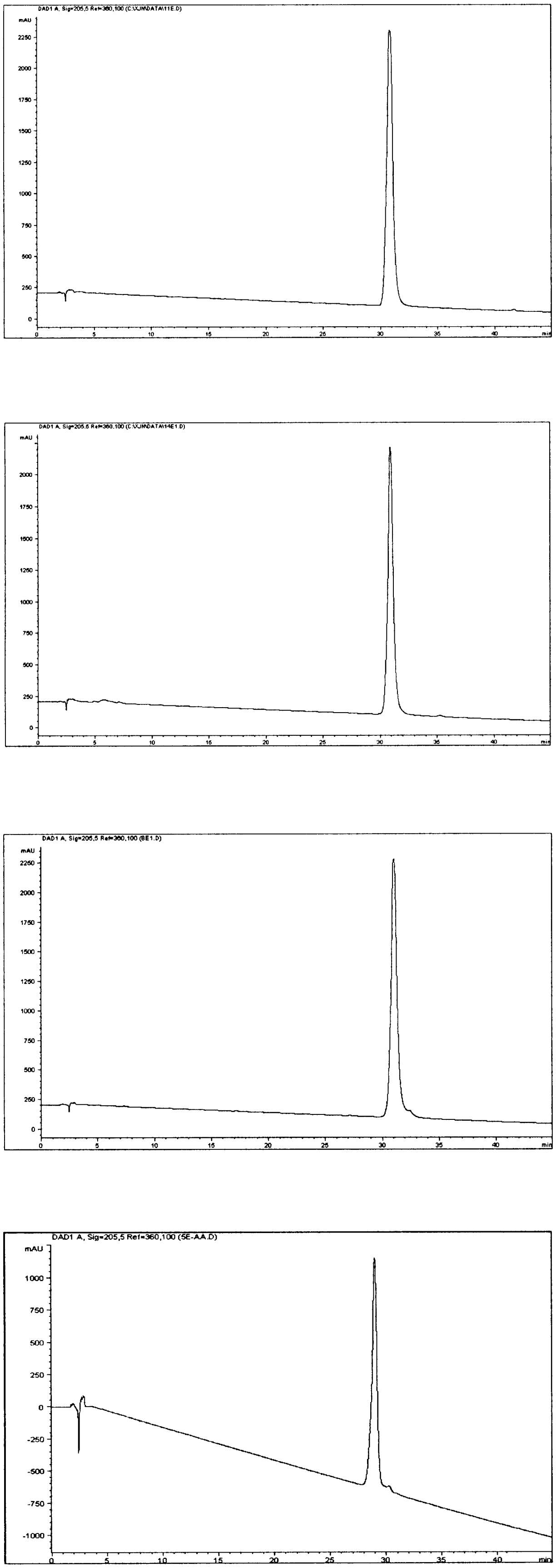

Figure 3. RP-HPLC purification of 14,15-, 11,12-, 8,9-, and 5,6-*trans*-AA (from top to bottom) using UV absorbance detection at 205 nm.

Synthetic *trans*-AAs are thus conveniently prepared in multimilligram amounts by a straightforward process described above which should expedite biological research in endogenous formation of *trans*-fatty acids. The individual isomers could be tested separately or as a mixture. This protocol also allows preparation of isotopically-labeled analogues of *trans*-AA that will be useful for metabolic and quantitative mass spectrometric studies. A similar approach can be used to prepare *trans* isomers of other fatty acids.

Acknowledgements

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17. *n*-BuLi (0.1 mL, 2.5 M solution in THF) was added to a solution of diphenylphosphine (46 mg, 0.25mmol) in THF (3 mL) at 0 °C and stirred for 2 h at 23 °C. To this solution was added dropwise a solution of EET (20 mg, 0.0625 mmol) in THF (3 mL) over 5 min. After stirring for 2 h at room temperature, freshly distilled CH_3I (35 mg, 0.25 mmol) was added dropwise to the reaction mixture, which was then allowed to stand for 30 min (the color of the reaction turns from red to white). The reaction mixture was diluted with EtOAc (40 mL), washed with H_2O (2×20 mL), then with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by SiO_2 PTLC afforded *trans*-arachidonic acid as a colorless oil (14 mg, 74%). TLC: EtOAc/hexane (3:7), $R_f \sim 0.31$.

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19. **^{1}H NMR (400 MHz, $CDCl_3$): 14,15-(*trans*)-AA**: δ 0.88 (t, $J = 6.4$ Hz, 3H), 1.22–1.38 (m, 6H), 1.71 (quintet, $J = 7.4$ Hz, 2H), 1.98 (dd, $J = 7.0$, 7.0 Hz, 2H), 2.13 (dd, $J = 7.0$, 7.0 Hz, 2H), 2.36 (apparent t, $J = 7.4$ Hz, 2H), 2.74–2.82 (m, 6H), 5.32–5.44 (m, 8H).

11,12-(*trans*)-AA: δ 0.89 (t, $J = 6.4$ Hz, 3H), 1.24–1.38 (m, 6H), 1.71 (quintet, $J = 7.4$ Hz, 2H), 2.03 (dd, $J = 7.0$, 7.0 Hz, 2H), 2.14 (dd, $J = 7.0$, 7.0 Hz, 2H), 2.37 (apparent t, $J = 7.4$ Hz, 2H), 2.74–2.82 (m, 6H), 5.32–5.44 (m, 8H).

8,9-(*trans*)-AA: δ 0.89 (t, $J = 6.4$ Hz, 3H), 1.24–1.38 (m, 6H), 1.71 (quintet, $J = 7.4$ Hz, 2H), 2.05 (dd, $J = 7.0$, 7.0 Hz, 2H), 2.14 (dd, $J = 7.0$, 7.0 Hz, 2H), 2.37 (apparent t, $J = 7.4$ Hz, 2H), 2.74–2.82 (m, 6H), 5.32–5.44 (m, 8H).

5,6-(*trans*)-AA: δ 0.89 (t, $J = 6.4$ Hz, 3H), 1.24–1.38 (m, 6H), 1.71 (quintet, $J = 7.4$ Hz, 2H), 2.07 (dd, $J = 7.0$, 7.0 Hz, 4H), 2.37 (apparent t, $J = 7.6$ Hz, 2H), 2.74–2.82 (m, 6H), 5.32–5.44 (m, 8H).