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Technical note

Purification of synthetic all-*E* lycophyll (ψ,ψ-carotene-16,16′-diol)

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Abstract

An efficient purification of synthetic all-*trans* (all-*E*) lycophyll is described. The synthetic preparation of the rare xanthophyll lycophyll produces a mixture of geometric isomers. Purification by HPLC using reverse-phase C30 silica affords milligram quantities of the desired all-*trans* isomer in >95% purity, as confirmed by ^{1}H NMR and LC/MS. Most recently, a facile work-up of the geometric mixture formed during total synthesis was found to provide multigrams of the targeted all-*E* geometric isomer of lycophyll. The acquisition of modest quantities of this specific lycopene analog allows its therapeutic potential to be explored.

Keywords: All-*trans* lycophyll; Carotenoids; HPLC analysis; Lycophyll; Lycophyll isomers; Semi-preparative chromatography

1. Introduction

Lycopene, the primary carotenoid found in tomato products, has been associated with a decreased risk of prostate cancer in epidemiological and observational studies [1–3]. It may also be linked to a lower incidence of myocardial infarction [4], and has been shown to be a more effective antioxidant than β-carotene in model system studies [5,6]. Unlike β-carotene, it demonstrates no pro-vitamin A activity in mammals [7]. This has raised interest in lycopene as a potential chemopreventive agent against various forms of chronic disease. Whether lycopene itself or some combination of phytochemicals found in tomato fruit are responsible for noted clinical benefits is currently unknown. Therefore, the search for efficacious antioxidant and/or therapeutic agents from tomato fruit continues.

With few exceptions, most carotenoids are hydrophobic and therefore lack appreciable water solubility. Problems with dose-proportionality in humans after oral administration of carotenoids can limit the absolute plasma and target tissue levels of carotenoids achievable by this route of administration [8,9]. Treatment of chronic disease may require sustained supraphysiologic levels of compound to achieve a desired effect. In such cases, modification of the parent carotenoid to increase its water dispersibility may facilitate parenteral administration, increasing the apparent biovailability to 100% [10–14]. As expected, lycopene and its natural dihydroxy analog lycophyll (ψ,ψ-Carotene-16,16′-diol; Fig. 1) share identical chromophores [15,16]. Lycophyll's allylic hydroxyls facilitate ongoing derivatization efforts using retrometabolic drug design [17].

Lycopene derived from processed tomato products can contain 79–90% of the all-*trans* (all-*E*) geometric isomer [3]. The predominance of the all-*E* isomer in consumed food products [18,19] suggests a benign safety profile. Total syntheses of lycophyll afford a mixture of geometric isomers, including the all-*trans* isomer [20,21]. Effective HPLC analyses of lycopene and various xanthophylls [3,16,22–24] have been reported, including the purification of lycopene geometric isomers by semi-preparative HPLC [25]. Recently, we were successful in accessing milligram quantities of all-*E* lycophyll using HPLC purification of the mixture of geometric isomers [21]. Additionally, an improved work-up of synthetic lycophyll provides the target compound at multigram scale.

2. Experimental

2.1. Crude sample preparation and characterization

Synthetic lycophyll was received directly after total synthesis at gram scale [21] and concentrated in vacuo. Microgram

Abbreviations: DCM, dichloromethane; HPLC, high-performance liquid chromatography; MTBE, methyl *tert*-butyl ether; THF, tetrahydrofuran; TFA, trifluoroacetic acid; DIBAL, diisobutylaluminum hydride

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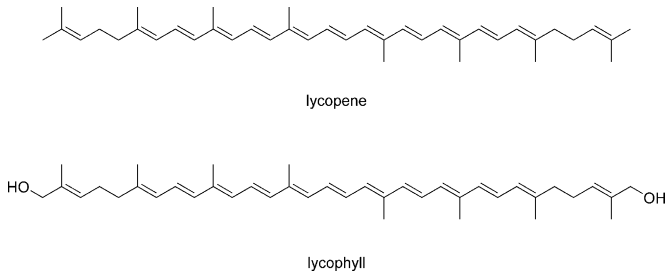


Fig. 1. Chemical structures of lycopene and lycophyll.

scale amounts were dissolved in THF and filtered through a 0.45 micron PVDF membrane filter. Characterization of synthetic lycophyll was performed using high-performance liquid chromatography/mass spectrometry (LC/MS) on an Agilent VL1100 Series LC/MS equipped with an HP autosampler, a UV–vis photodiode array detector (PDA) and an APCI positive source. An Agilent Zorbax Eclipse 3.5 μm XDB-C18 column was used with a 0.025% TFA/H_2O (A) and 0.025% TFA/MeCN (B) mobile phase system with the following gradient: increase from 30 to 50% B over 5 min; increase from 50 to 98% B over 3.3 min; hold at 98% B for 16.9 min; return to 30% B over 0.2 min and re-equilibrate for 5 min; or a YMC Carotenoid C30 S-5 reverse-phase column (spherical 5 μm particle size, Waters) with a YMC guard column was used with a mobile phase system of MTBE (A) and MeOH (B) run isocratic at 40:60 A:B. Proton nuclear magnetic resonance (NMR) spectra (in $CDCl_3$) were obtained on a Varian Unity INOVA 500 spectrometer operating at 500.111 MHz (megahertz).

2.2. HPLC purification

Synthetic lycophyll purification was performed using HPLC on a Waters system with a Millipore 600E System Controller, a 996 PDA, and a 717 Autosampler. Removal of non-carotenoid impurities and isolation of individual lycophyll geometric isomers were performed using a YMC Carotenoid C30 S-5 reverse-phase column (spherical 5 μm particle size, Waters) with a YMC guard column, mobile phase system of MTBE (A) and MeOH (B) run isocratic at 40:60 A:B.

2.3. Precipitation method

Synthetic lycophyll was dissolved in a solvent mixture consisting of dichloromethane/alcohol/triethylamine (80:19:1). The resulting solution was slowly evaporated to dryness. The residue was then slurried with minimal DCM, filtered, washed with alcohol, then with acetone, and dried under vacuum to yield all-*trans* lycophyll at >90% purity (AUC).

3. Results and discussion

Recently we reported the total synthesis of the rare xanthophyll lycophyll, a di-hydroxylated member of the lycopene family of C40 carotenoids [21]. As seen in Scheme 1, the synthesis utilizes an endgame Wittig condensation using two equivalents of C10 ylide headgroup per equivalent of C20 dialdehyde. Preparation of lycophyll results in a mixture of lycopene *cis*- and *trans*-geometric isomers.

Initially, attempted thermal isomerization of the synthetic geometric mixture was unsuccessful. Refluxing in several solvents including acetone, heptane, toluene, and THF/petroleum ether resulted in significant decomposition of the lycopene chromophore with some desired all-*E* geometric enrichment. As previous authors have noted, total synthesis of C40 carotenoids

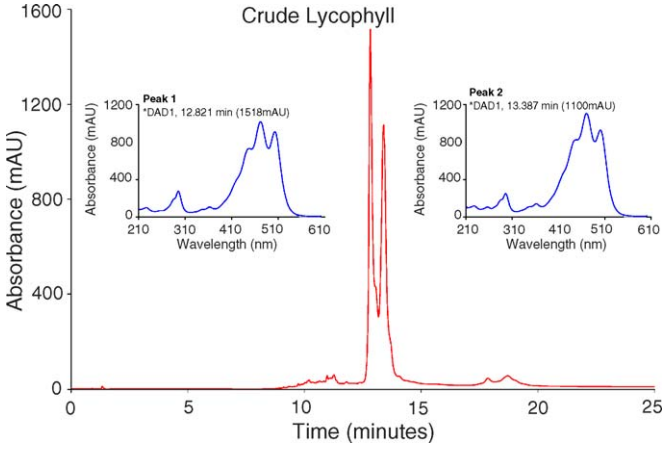
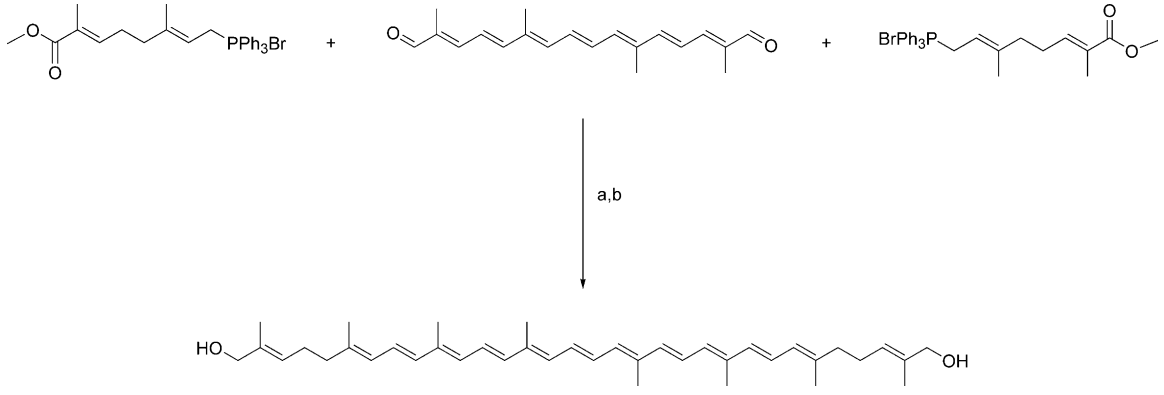


Fig. 2. HPLC analysis of synthetic lycophyll using C18 column.



Scheme 1. (a) LiOMe in MeOH, toluene, reflux and (b) DIBAL, THF, 0 °C.

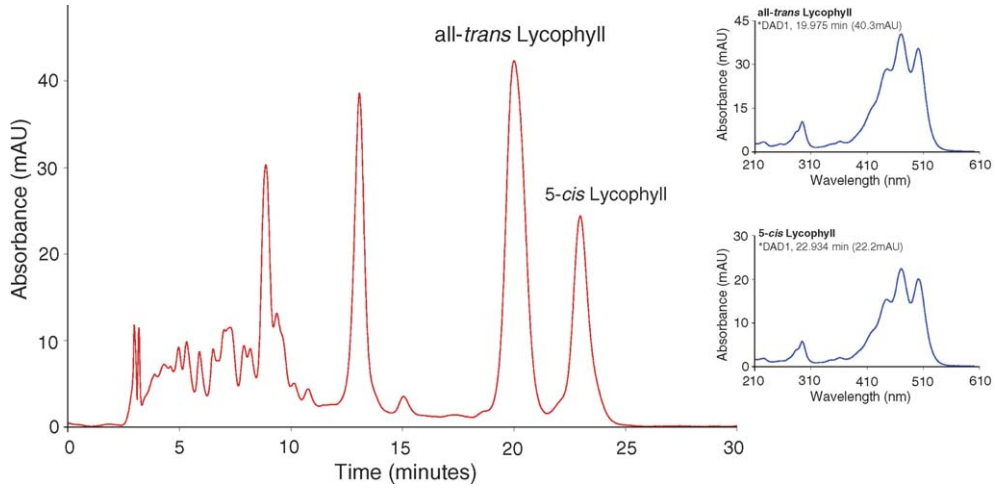


Fig. 3. HPLC analysis of synthetic lycophyll using C30 column.

generates a mixture of geometric isomers, some of which are particularly resistant to thermodynamic isomerization, such as the 5-*cis* isomer [26–28].

HPLC analysis of synthetic lycophyll using a reverse-phase C18 column confirmed the formation of the desired lycopene chromophore (Fig. 2). Inspection of the electronic absorption spectra of chromatographic peaks suggested a significant number of geometric isomers. Employment of a reverse-phase C30 column further deconvoluted the synthetic mixture of lycophyll geometric isomers, as seen in Fig. 3. Analysis of the electronic absorption spectra of early-running chromatographic peaks showed significant blue-shifting of the lycopene chromophore, suggesting a large degree of *cis*-isomer content. Electronic absorption spectra of the two latest-running chromatographic peaks lacked evidence of either blue-shifting or of appreciable absorption in the "*cis*-peak" region. It was therefore speculated that these late-running peaks could be the all-*E* and 5-*Z* isomers of lycophyll, as similarly evidenced in characterization studies of lycopene geometric isomers [25]. Attempts to chromatographically resolve lycophyll geometric isomers using normal-phase silica or alumina, with or without 1% triethylamine buffer, were not successful.

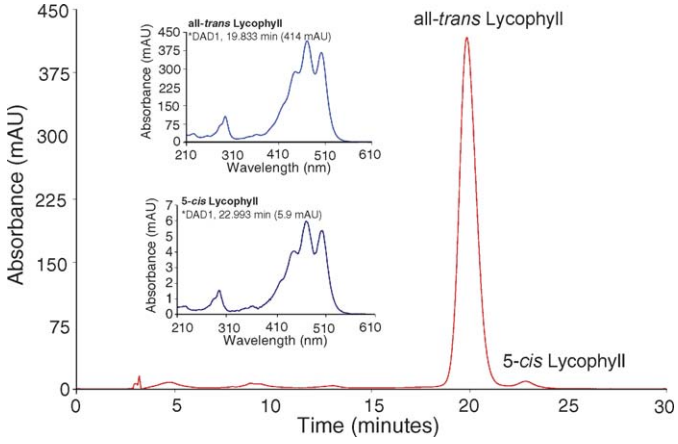


Fig. 4. HPLC analysis of chromatographically purified lycophyll using C30 column.

Semi-preparative reverse-phase HPLC of synthetic lycophyll provided milligram quantities of >95% all-*E* lycophyll (Fig. 4), as confirmed by LC/MS (Fig. 5) and ^{1}H NMR (Fig. 6) analyses [21].

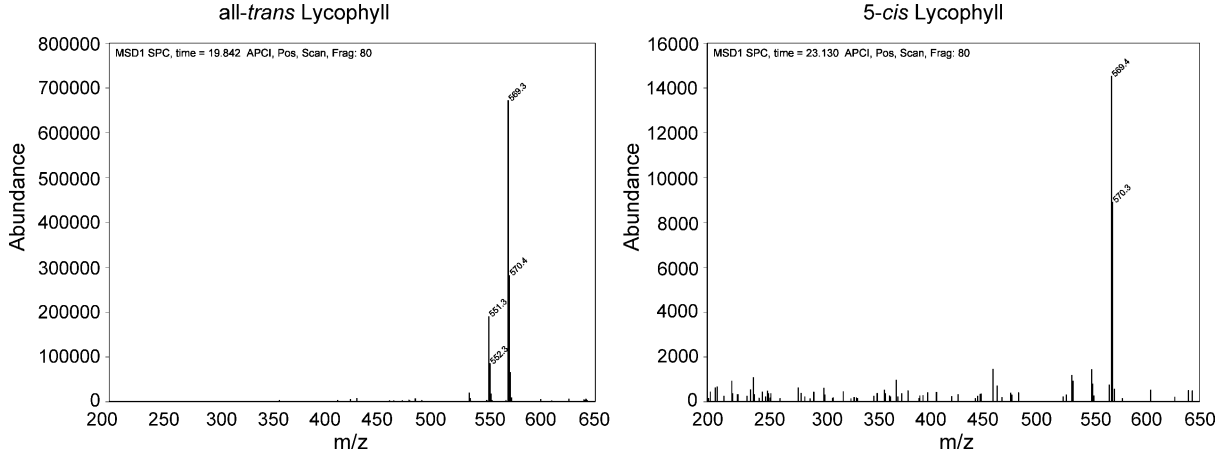


Fig. 5. MS data of chromatographically purified lycophyll.

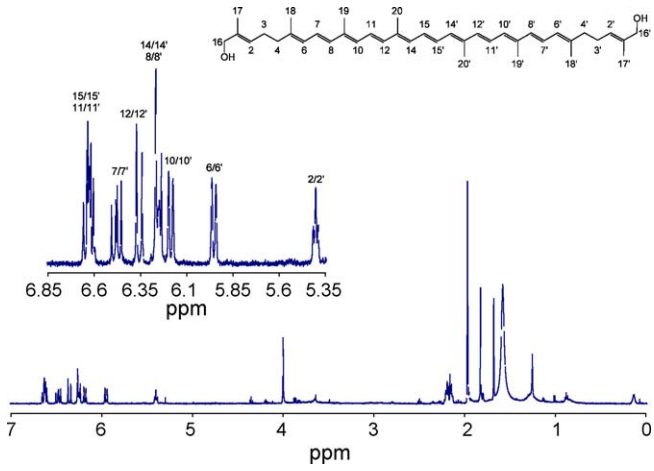


Fig. 6. ^{1}H NMR data of chromatographically purified lycophyll.

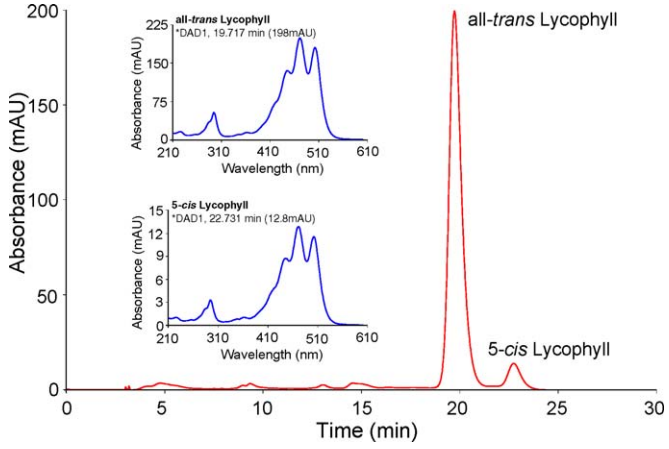


Fig. 7. HPLC analysis of precipitated lycophyll using C30 column.

Recently, selective precipitation of all-*E* lycophyll from the synthetic mixture of geometric isomers was developed. This simple purification method allows access to multigram quantities of the target lycophyll isomer (Figs. 7 and 8). It was found

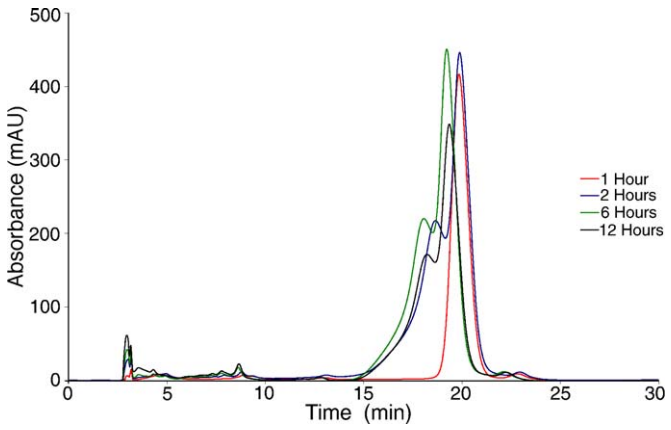


Fig. 9. HPLC analysis of chromatographically purified lycophyll in THF solution using C30 column.

that if synthetic lycophyll is dissolved in a minimum amount of DCM and then diluted with alcohol, mainly the all-*trans* geometric isomer readily precipitates out of solution; the majority of *cis*-containing geometric isomers remain in solution. As DCM is removed in vacuo, more of the *trans*-enriched isomers precipitate out of solution. Filtration and subsequent washing of the precipitate removes remaining *cis*-enriched isomers, yielding the desired all-*E* isomer (91%), along with a minor amount of 5-*Z* isomer (6%).

As seen in Fig. 9, a solution of all-*E* lycophyll (>95%) was observed to appreciably isomerize within a few hours as evidenced by an increase in the *cis*-isomer shoulder peak versus the all-*trans* chromatographic peak. As lycophyll remains in solution, a decrease in chromatographic retention time correlates to an increase in *Z*-isomer content, accompanied by blue-shifting of electronic absorption spectra. The observed propensity for lycopene's chromophore to undergo facile geometric isomerization reveals insight into the dynamic quality of *trans*-enriched lycophyll in solution.

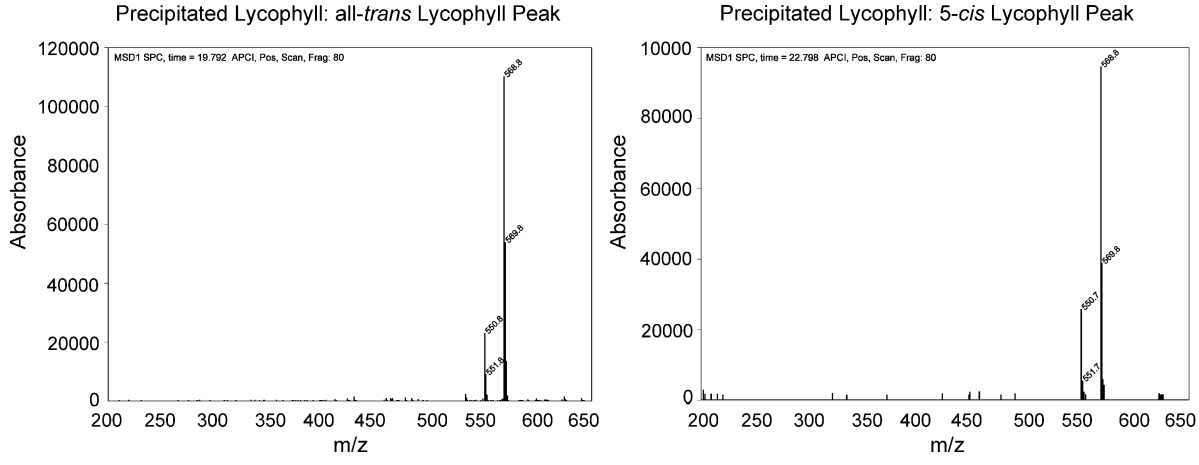


Fig. 8. MS data of precipitated lycophyll.

4. Conclusion

HPLC purification of synthetic lycophyll yields the desired all-*trans* geometric isomer in excellent purity at milligram scale. An alternative, recently developed precipitation of synthetic lycophyll allows access to multigram quantities of the all-*E* isomer. This facile process promotes elucidation of the therapeutic profile of this specific lycopene analog.

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