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Isolation of lycopene from crude tomato extract via selective inclusion in deoxycholic acid

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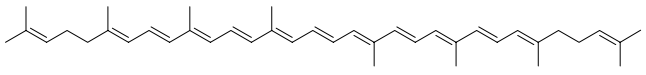
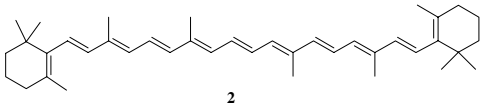
Abstract—Deoxycholic acid has been used as host in the separation of C-40 carotenoid isomers. The methodology was successfully applied to recover almost pure lycopene from commercial tomato paste.

The isomer separation via selective enclathration processes has been recently studied in a variety of host–guest systems and bile acids have shown a particular ability in the inclusion of organic guest molecules such as aliphatic and aromatic hydrocarbons, alcohols, ketones, esters, nitriles, epoxides, and amides[1] In recent papers, we have demonstrated the inclusion ability of some bile acid derivatives for the resolution of organic racemates[2] including the precise definition of the structures involving the host–guest assemblies.[3–5] In this frame, we have studied various bile acid hosts for the separation of close C-40 carotenoid isomers: lycopene[6] (**1**), β-carotene (**2**).



1



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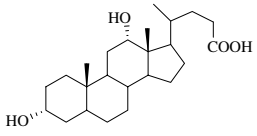

3

We found that deoxycholic acid, (3α,12α-dihydroxy-5β-colan-24-oic-acid, DCA), (**3**), in solid state, selectively included lycopene from an equimolar mixture of lycopene and β-carotene. In fact, co-grinding 50 mg of DCA and 3 mg each of lycopene and β-carotene for 20 min in a vial, only lycopene was enclathrated in the DCA lattice (see photos in Fig. 1).

Thus, after the addition of dichloromethane to the solid mixture, the DCA·lycopene inclusion compound was recovered by filtration. The TLC analysis of inclusion adduct showed the unique presence of the lycopene carotenoid.[7] This selectivity is probably due to the DCA crystalline assembly having channels suitable for accommodating relatively large linear guest molecules.[8,9] With these results in hand, we decided to apply this methodology to separate lycopene from crude tomato extract (tomato oleoresin).

Lycopene, the red pigment present in some common vegetables,[10] is one of the important content of human dietary foods because of its nutraceutical, epidemiological and pharmaceutical value.[11,12] It is also a natural coloring substance used in food industry as food dye. The most important source of lycopene is tomato '*Lycopersicon esculentum*' and its processed food products, in which lycopene constitutes more than 60% of the carotenoids present. Conventional methods for the extraction of carotenoid from many sources use pure solvents such

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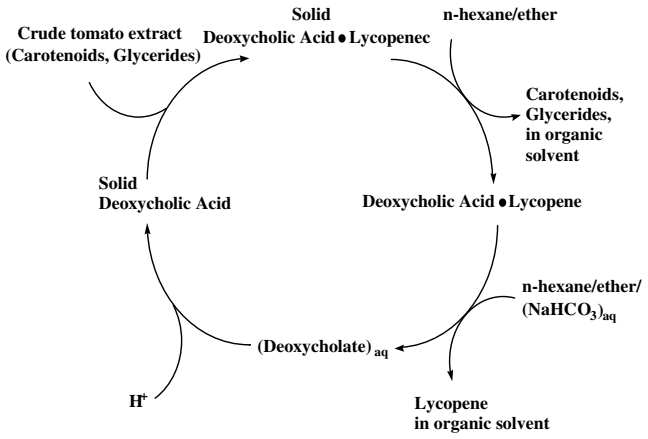
Figure 1. Dichloromethane solution containing an equimolar mixture of lycopene (red dye) and β-carotene (yellow dye): left, as extant; right, after inclusion of lycopene in DCA.

as dichloromethane or the mixture of polar–non-polar solvents (e.g., hexane–acetone–ethanol), while the supercritical fluid extraction (SFE) with CO_2 has been recently proposed.[13] The final isolation of pure lycopene from the crude carotenoids mixture is generally achieved by chromatographic methods (HPLC, TLC, column chromatography).[10]

Solid commercially available DCA (1 g) is added to a solution of dichloromethane (5 ml) containing crude tomato extract (1.1 g) coming from commercial tomato paste.[14] The heterogeneous mixture was left to stand, at room temperature, for 48 h and a solution of ether/*n*-hexane (20 ml) was added. The reddish crystals, filtered off and washed, were analyzed by ^{1}H NMR[15] which confirmed the presence of lycopene in a host/guest ratio of about 28/1. The lycopene guest was recovered by dissolving the inclusion compound in a mixture of aqueous $NaHCO_3$ and ether/*n*-hexane; the organic layer, separated and evaporated under reduced pressure, gave 70 mg (70% yield) of lycopene which does not need further purification (see UV, ^{1}H NMR in Supplementary data).



Scheme 1.

As expected, the bile acid host was able to selectively enclathrate only lycopene leaving the other molecules (carotenoid and glycerides) in solution. The host may be recovered and used for further cycles upon the treatment of the aqueous basic ($NaHCO_3$) layer with dilute mineral acid. Our separation process is shown in Scheme 1.

In conclusion, a new methodology for the isolation of lycopene from a crude mixture has been established based on the selective inclusion in DCA. The major advantages of this methodology are its efficiency and simplicity, the mild conditions employed, the quantitative recovery of both host (DCA) and guest (lycopene) compounds.

We are currently extending this readily accessible and low cost methodology to the separation of other classes of natural organic compounds.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.10.127.

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6. 90–95% all-*trans* lycopene, from Sigma.
7. TLC was performed on precoated 0.25 mm Silica gel plate (Merck) with developing solution of cyclohexane: methylene chloride (9:1); R_f lycopene 0.21, R_f β-carotene 0.49.
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14. The crude tomato extract was obtained as following: to about 180 g of tomato paste was added 200 ml of 95% ethanol. The mixture was stirred with a spatula for 15 min and then filtered through a small piece of glass wool pressing as much liquid as possible from the paste. The 'dehydrated' material was then extracted two times with 150 ml of dichloromethane and the extract concentrated under reduced pressure giving 1.1 g of crude extract. This mixture, containing mainly triglycerides, β-carotene and lycopene, was analyzed by UV and ^{1}H NMR (see Supplementary data) being the lycopene 0.1 g (weight after preparative chromatographic column: silica gel, *n*-hexane/dichloromethane 80:20).
15. ^{1}H NMR ($CDCl_3/CD_3OD$), selected δ (ppm from residue $CHCl_3$ at 7.27 ppm): 0.65 (s, 3H, 18-CH_3 DCA), 0.88 (s, 3H, 19-CH_3 DCA), 0.95 (d, 3H, $J = 6.5$ Hz, 21-CH_3 DCA), 1.97 (s, 3H, lycopene), 3.55 (m, 1H, 3β DCA), 3.95 (br s, 1H, 12β DCA), 5.09 (br, 2H lycopene), 5.90–6.08 (m, 16H lycopene).