Information on biosynthesis of ephedrine or pseudoephedrine by ephedra:

Biosynthesis of ephedrine and pseudoephedrine by ephedra begins with L-phenylalanine (Phe). Phe is the initial precursor of ephedrine and pseudoephedrine and catalyzed by phenylalanine ammonia lyase (PAL) [S1]. Recent studies suggest that there are at least two possible pathways of Phe side chain- shortening in ephedra benzoic acid biosynthesis: β-oxidative CoA-dependent and non-β-oxidative CoA-independent routes [S2, S3]. A proposed biosynthesis pathway for the biosynthesis of ephedrine and pseudoephedrinein in ephedra is described in Figure S1 [S4-S8]. Figure and references for details can be found in supporting information.

In the β-oxidative CoA-dependent pathway, the first step is the formation of cinnamoyl-CoA, which is catalyzed by cinnamate: CoA ligase. Subsequently, a hydratase converts cinnamoyl-CoA to 3-hydroxy-3-phenylpropanoyl-CoA, and a dehydrogenase catalyzes the 3-hydroxy-3-phenylpropanoyl-CoA into 3-oxo-3-phenylpropanoyl-CoA. In the end, 3-ketoacyl-CoA thiolase catalyzes the formation of benzoyl-CoA (Figure S1) [S6-S8].

In the non-β-oxidative CoA-independent routes, cinnamic acid was converted to 3-hydroxy-3-phenylpropionic-aicd under the catalysis of a hydratase. After retro-aldol cleavaging, 3-hydroxy-3-phenylpropionic-aicd was converted to benzaldehyde. Subsequently, two different dehydrogenase (benzaldehyde dehydrogenase and aldehyde oxidases 4) catalyze the formation of benzoic acid from benzaldehyde. After undergoing condensation with pyruvate, benzaldehyde, benzoyl-CoA and/or benzoic acid was catalyzed by a ThDP-dependent carboligase to form 1-phenylpropane-1,2-dione. Transamination of 1-Phenylpropane-1,2-dione yields (S)-cathinone, which is reduced to (1S,2S)-pseudonorephedrine and (1R,2S)-norephedrine. In the end, N-Methylation of these two ephedrines completes the pathway in ephedra (Figure S1) [S4, S5, S7, S8].

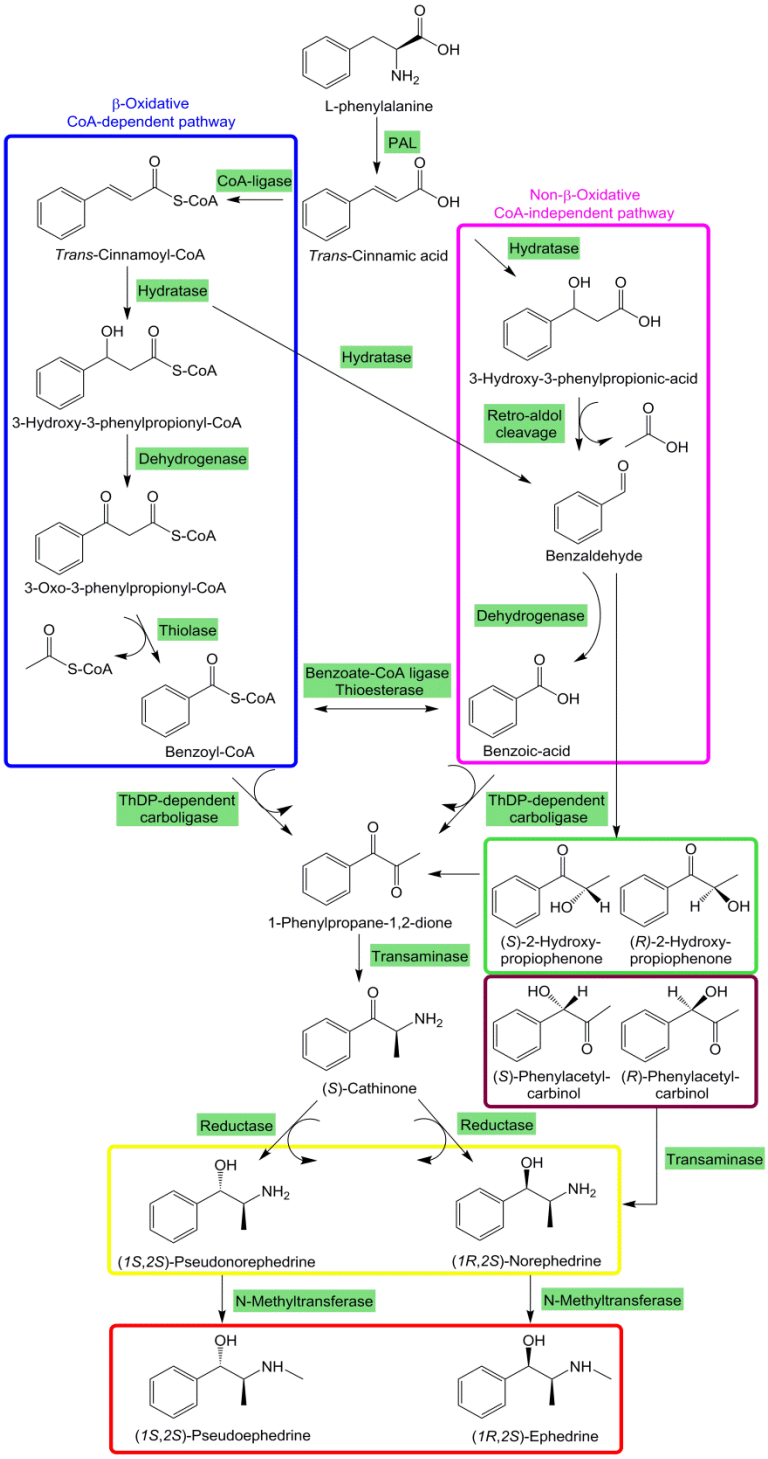

FIGURE S1. Proposed pathways for the biosynthesis of ephedrine and pseudoephedrinein in ephedra. A β-oxidative CoA-dependent pathway of L-phenylalanine side chain-shortening is shown in blue boxes, however a non-β-oxidative CoA-independent pathway is shown in pink boxes. Abbreviations: PAL, phenylalanine ammonia lyase; CoA, Coenzyme A; ThDP, thiamine diphosphate.

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