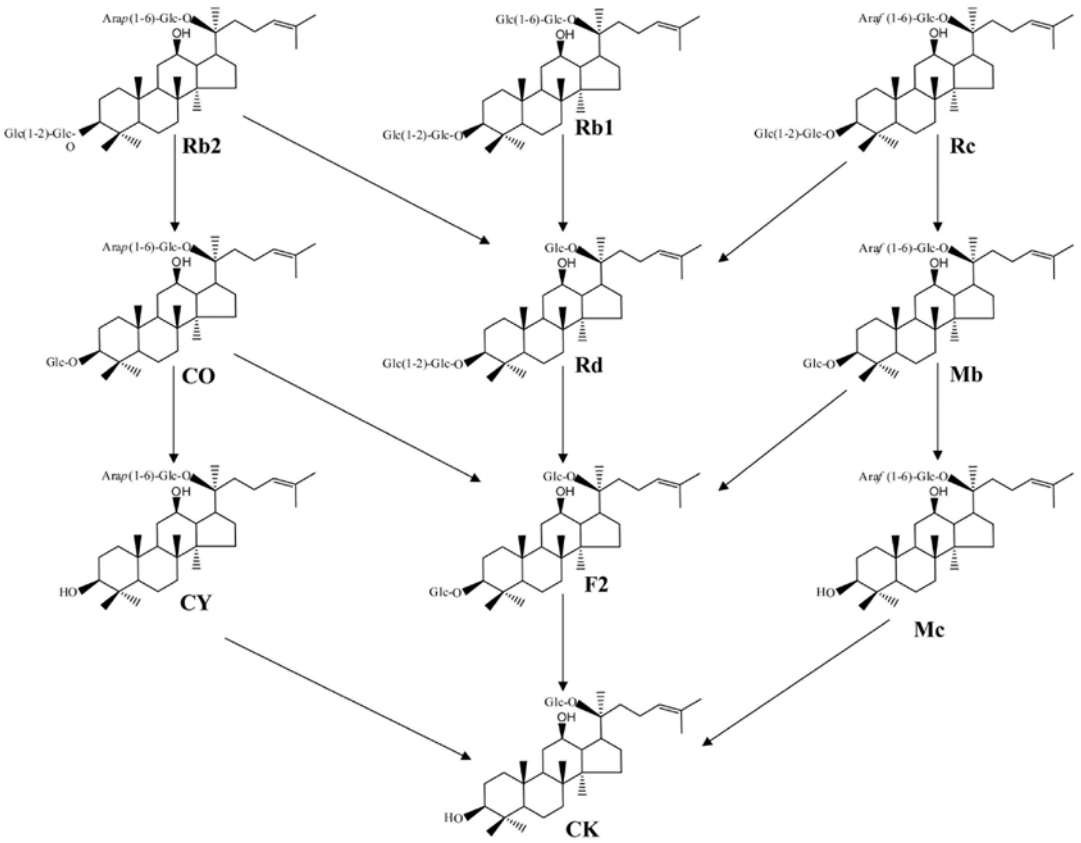
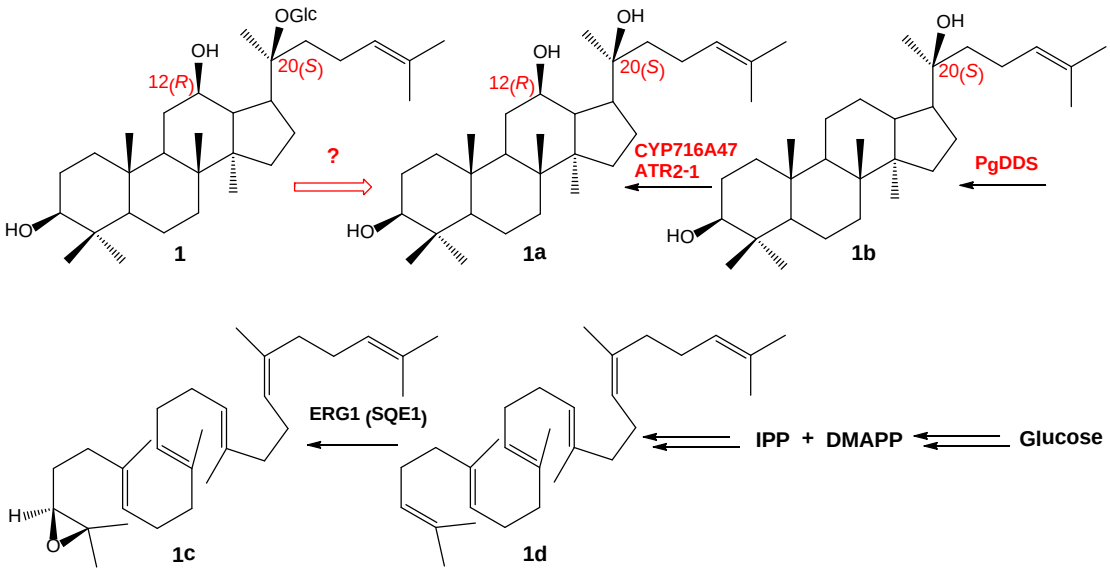
A



B



Supplementary Information, Figure S1. The potential approaches for compound K manufacture.

A, The current bio-deglycosylation pathway to produce compound K based on Gao *et al*. [1] with some modification: the best yield of CK production reported *via* deglycosylating PPD-type ginsenosides reached more than 80% (*Mol* conversion)

under the optimized fermentation conditions, which means that about 1.225 g/L CK could be obtained with 5 g/L total ginsenosides (containing 3.4% Rb1, 20.0% Rb2 and 29.9% Rb3) as substrate after 144 h fermentation [2]. **B**, the potential CK biosynthetic pathway designed in chassis yeast. CYP716A47: cytochrome P450 from *Panax ginseng* [3]; ATR2-1: NADPH-cytochrome P450 reductase from *Arabidopsis thaliana* [4]; PgDDS: Dammarenediol-II synthase from *P. ginseng* [5]; Squalene epoxidase and other enzymes involved in the biosynthesis of isoprenoide precursors are all originated from *Saccharomyces cerevisiae* [6]. The empty arrow indicates the potential pathway from PPD to CK based on retrobiosynthetic analysis.

References

1 Gao J, Xu W, Fang Q *et al.* Efficient biotransformation for preparation of pharmaceutically active ginsenoside Compound K by *Penicillium oxalicum* sp. 68. *Ann Microbiol* 2013; **63**:139-149.

2 Zhou W, Yan Q, Li JY, Zhang XC, Zhou P. Biotransformation of *Panax notoginseng* saponins into ginsenoside compound K production by *Paecilomyces bainier* sp. 229. *J Appl Microbiol* 2008; **104**:699-706.

3 Han J-Y, Kim H-J, Kwon Y-S, Choi Y-E. The Cyt P450 enzyme CYP716A47 catalyzes the formation of protopanaxadiol from dammarenediol-II during ginsenoside biosynthesis in *Panax ginseng*. *Plant Cell Physiol* 2011; **52**:2062-2073.

4 Urban P, Mignotte C, Kazmaier M, Delorme F, Pompon D. Cloning, yeast expression, and characterization of the coupling of two distantly related *Arabidopsis thaliana* NADPH-cytochrome P450 reductases with P450 CYP73A5. *J Biol Chem* 1997; **272**:19176-19186.

5 Tansakul P, Shibuya M, Kushiro T, Ebizuka Y. Dammarenediol-II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in *Panax ginseng*. *FEBS Lett* 2006; **580**:5143-5149.

6 Jandrositz A, Turnowsky F, Högenauer G. The gene encoding squalene epoxidase from *Saccharomyces cerevisiae*: cloning and characterization. *Gene* 1991; **107**:155-160.