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Molecules of Interest

# Oleanolic acid

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ABSTRACT

Oleanolic acid (3β-hydroxyolean-12-en-28-oic acid) is a pentacyclic triterpenoid compound with a widespread occurrence throughout the plant kingdom. In nature, the compound exists either as a free acid or as an aglycone precursor for triterpenoid saponins, in which it can be linked to one or more sugar chains. Oleanolic acid and its derivatives possess several promising pharmacological activities, such as hepatoprotective effects, and anti-inflammatory, antioxidant, or anticancer activities. With the recent elucidation of its biosynthesis and the imminent commercialization of the first oleanolic acid-derived drug, the compound promises to remain important for various studies. In this review, the recent progress in understanding the oleanolic acid biosynthesis and its pharmacology are discussed. Furthermore, the importance and potential application of synthetic oleanolic acid derivatives are highlighted, and research perspectives on oleanolic acid are given.



## 1. Introduction

The natural product oleanolic acid (3β-hydroxyolean-12-en-28-oic acid) (Fig. 1A) is a biologically active pentacyclic triterpenoid compound that has been isolated from more than 1620 plant species, including many food and medicinal plants (Fai and Tao, 2009; Fukushima et al., 2011; Liu, 1995). The compound is especially prevalent in plants belonging to the Oleaceae family, among which olive (*Olea europaea*), the plant species after which the compound was named (Simonsen and Ross, 1957), and that still serves as the main source of commercial oleanolic acid (Sporn et al., 2011). Often, oleanolic acid is present in combination with its isomer, ursolic acid (3β-hydroxyurs-12-en-28-oic acid) (Fig. 1B), with which it shares many pharmacological properties (Liu, 1995). Unconjugated triterpenoids, such as oleanolic acid, are often found in the epicuticular waxes of plants, preventing water loss and serving as a first defense barrier against pathogens (Heinzen et al., 1996). For instance, on olive leaves, oleanolic acid is present as almost pure crystals, that form a physical barrier against fungal attack (Kubo and Matsumoto, 1984; Kubo et al., 1985). Oleanolic acid not only occurs as a free acid, but also serves as an aglycone precursor for triterpenoid saponins, in which it is linked to one or more sugar chains (Liu, 1995; Szakiel et al., 2003, 2005). In plants, the various glycoconjugated oleanolic acid molecules may function as defense compounds against herbivores or pathogens, or as allelopathic agents (Szakiel et al., 2003, 2005).

## 2. Biosynthesis of oleanolic acid

Being a triterpenoid, oleanolic acid belongs to a large group of structurally diverse natural products that includes sterols, steroids, and triterpenoid saponins (Phillips et al., 2006; Xu et al., 2004). In plant cells, the precursor molecule of the primary sterol metabolism, 2,3-oxidosqualene, is synthesized in the cytosol from isopentenyl pyrophosphate derived from the mevalonate pathway (Chappell, 2002; Phillips et al., 2006; Xu et al., 2004). The subsequent cyclization of 2,3-oxidosqualene forms the branch-point between the primary sterol and the secondary triterpenoid metabolism leading to oleanolic acid. For the biosynthesis of phytosterols, 2,3-oxidosqualene is cyclized by cycloartenol synthase (CAS) to yield the tetracyclic plant sterol precursor cycloartenol (Fig. 2) (Abe, 2007; Corey et al., 1993). Predating the emergence of plants, CAS is the ancestral enzyme of all plant oxidosqualene cyclases (OSCs) involved in the secondary metabolism (Phillips et al., 2006). For oleanolic acid biosynthesis, 2,3-oxidosqualene is cyclized to the pentacyclic oleanane-type triterpenoid backbone β-amyrin by the OSC β-amyrin synthase (BAS) (Fig. 2) (Abe, 2007;

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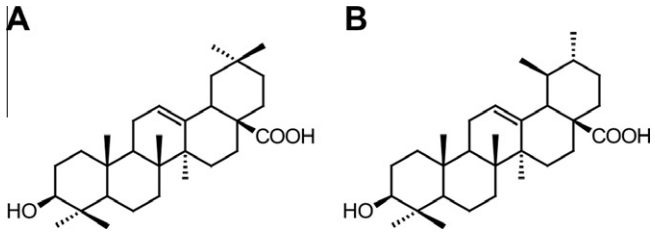


**Fig. 1.** Chemical structures of (A) oleanolic acid and (B) ursolic acid.

Kushiro et al., 1998). BAS was first cloned from the medicinal plant *Panax ginseng* (Kushiro et al., 1998), and later from a variety of other plant species, including olive (Saimaru et al., 2007). In the final step of the oleanolic acid biosynthesis, β-amyrin is oxidized in a sequential three-step oxidation at the C-28 position by a single cytochrome P450 enzyme to yield oleanolic acid through erythrodiol (Fig. 2). The cytochrome P450 enzyme involved in this multistep oxidation, CYP716A12, was first discovered in barrel medic (*Medicago truncatula*) (Carelli et al., 2011; Fukushima et al., 2011), and, more recently, the conserved functionality of the CYP716A subfamily towards oxidizing various triterpene backbones was shown using CYP716A homologs of grape (*Vitis vinifera*) and ginseng (*P. ginseng*) (Fukushima et al., 2011; Han et al., 2011).

## 3. Detection of oleanolic acid

Various analytical techniques, including thin layer chromatography (TLC) (Oleszek, 2002), liquid chromatography (LC) (Burnouf-Radosevich and Delfel, 1984; Claude et al., 2004; Liang et al., 2009; Oleszek, 2002), capillary electrophoresis (CE) (Qi et al., 2006; Unger, 2009) and gas chromatography (GC) with flame ionization (Janicsák et al., 2003; Schinor et al., 2004) or mass spectrometry (MS) detection (Budzikiewicz et al., 1963; Burnouf-Radosevich et al., 1985; Gu et al., 2006; Razboršek et al., 2008) are described for the separation and determination of pentacyclic triterpenoids. Among these, GC with electron impact MS detection (GC–EI/MS) is undoubtedly the most powerful method for the separation, quantification and structural determination of position isomers with very similar structures, such as, for instance, oleanolic acid (Fig. 1A) and ursolic acid (Fig. 1B). However, due to the high molecular weight and polarity of oleanolic acid, and, hence, its low volatility, derivatization by silylation (Burnouf-Radosevich et al., 1985; Janicsák et al., 2003; Razboršek et al., 2008; Sánchez Ávila et al., 2007), acetylation (Ghosh et al., 1985), or methylation (Assimopoulou and Papageorgiou, 2005; Gu et al., 2006) prior to GC analysis is mandatory.

Upon EI, pentacyclic triterpenoids that contain a C-12–C-13 double bond undergo a retro-Diels-Alder (rDA) cleavage of the C-ring, leading to fragments consisting of the ABC*-rings (dienophile) and the C*DE-rings (diene), thereby retaining the charge on the diene portion (Fig. 3, pathway 1, C* indicates a partial C-ring) (Budzikiewicz et al., 1963; Burnouf-Radosevich et al., 1985). As such, fragmentation of trimethylsilylated oleanolic acid leads to an ion at *m*/*z* 320 (Fig. 3, pathway 1). This ion is subject to further fragmentation, thereby losing its TMSi-carboxy group, which leads to an ion at *m*/*z* 203 (Fig. 3). An additional characteristic (although low abundant) ion is observed at *m*/*z* 279 (Fig. 3, pathway 2), and is the result of the transfer of a hydrogen atom during cleavage of the C-ring, leading to a charged ABC* portion of the molecule (Budzikiewicz et al., 1963; Burnouf-Radosevich et al., 1985; Razboršek et al., 2008). Additional loss of TMSiOH from this ion leads to the fragment ion observed at *m*/*z* 189. Next to the fragment ions derived from the rDA cleavage, abundant ion signals are present at *m*/*z* 600 (molecular ion), *m*/*z* 585 (loss of methyl group), *m*/*z* 482 (loss of TMSi-carboxy group), *m*/*z* 393 (loss of TMSi-carboxy and TMSiOH groups), and *m*/*z* 73 (TMSi-peak) (Fig. 3) (Burnouf-Radosevich et al., 1985; Razboršek et al., 2008).

## 4. Pharmacology

One of the important pharmacological properties attributed to oleanolic acid is its hepatoprotective effect. It has been shown that oleanolic acid is not only effective in protecting the liver from acute chemically induced liver injury, but also protects the liver from fibrosis and cirrhosis caused by chronic liver diseases. The hepatoprotective effects of oleanolic acid allow its use in China as an over-the-counter oral drug to treat humans for liver disorders such as viral hepatitis (Liu, 1995, 2005; Reisman et al., 2009; Wang et al., 2010). Despite its use as a hepatoprotective drug, the mechanism of action of oleanolic acid remains to be fully elucidated (Wang et al., 2010). It has been shown that oleanolic acid increases nuclear accumulation of Nrf2, a key transcriptional regulator of antioxidant and detoxifying enzymes, thereby leading to the induction of Nrf2-dependent genes that play a role in the protection of the liver (Klaassen and Reisman, 2010; Liu et al., 2008; Reisman et al., 2009). In later studies, it has been shown that the increased nuclear accumulation of Nrf2 by oleanolic acid is due to the activation of intracellular signaling cascades mediated through the PI3K/Akt, JNK, and ERK pathways (Feng et al., 2011; Wang et al., 2010). However, hepatoprotective effects were also observed in Nrf2-null mice, indicating that oleanolic acid also

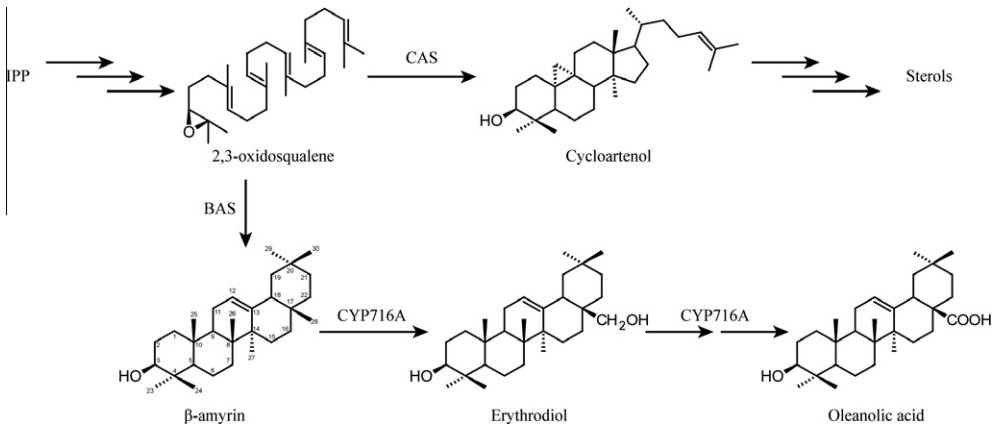


**Fig. 2.** Oleanolic acid biosynthetic pathway. BAS = β-amyrin synthase, CAS = cycloartenol synthase, IPP = isopentenyl pyrophosphate.

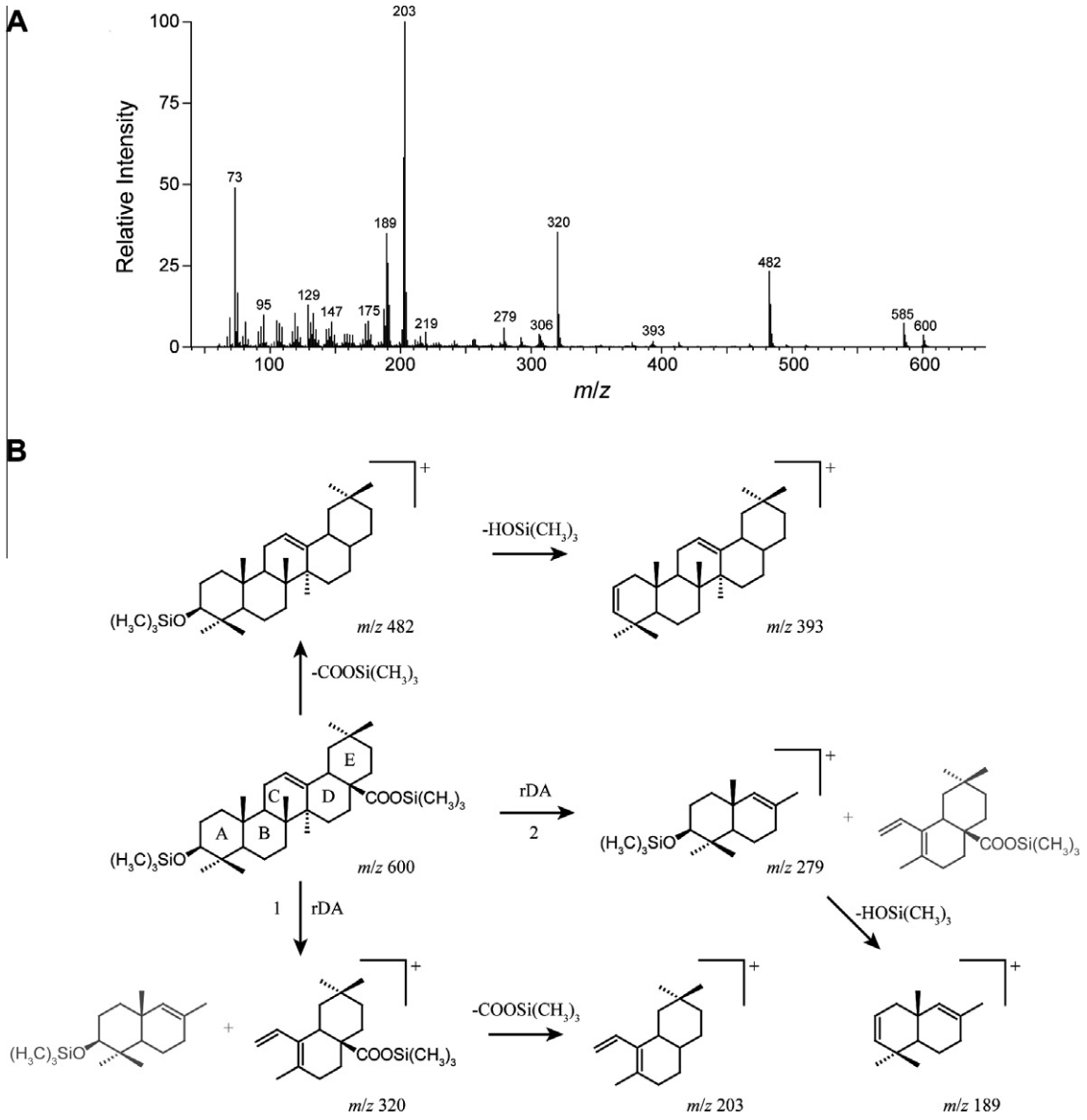


**Fig. 3.** Fragmentation of trimethylsilylated oleanolic acid. (A) EI-MS spectrum. (B) Fragmentation mechanism leading to the characteristic fragment ions observed in the EI-MS spectrum.

activates Nrf2-independent cytoprotective mechanisms (Reisman et al., 2009).

One such Nrf2-independent hepatoprotective mechanism could be the inhibition of bile acid synthesis in the liver. The accumulation of abnormally high levels of bile acids in the liver leads to cytotoxic effects that may cause liver injury, fibrosis, or cirrhosis (Hofmann, 1999). A key player in the maintenance of bile acid homeostasis is the farnesoid X receptor (FXR), a ligand-regulated transcription factor that regulates bile acid biosynthesis and its excretion from liver cells. Activation of FXR occurs through binding of bile acids to the ligand binding domain (LBD) of the protein, thereby altering the conformation of the transcription factor and allowing the binding of co-activators that activate the transcription of the FXR target genes (Ananthanarayanan et al., 2001; Chiang, 2004; Claudel et al., 2005; Parks et al., 1999; Sinal et al., 2000). In a recent study, it has been shown that oleanolic acid binds to the LBD of the FXR *in vitro*, and that treatment of HepG2 hepatocarcinoma cells with oleanolic acid modulates the expression of several FXR target genes, such as the suppression of CYP7A1, a rate-limiting bile acid biosynthetic enzyme (Liu and Wong, 2010). Hence, part of the Nrf2-independent hepatoprotective effects of oleanolic acid may be explained by its functioning as an FXR modulator.

Since the translocation of Nrf2 to the nucleus leads to the onset of the expression of a whole set of cytoprotective genes (Kensler et al., 2007; Kobayashi and Yamamoto, 2005; Li and Kong, 2009), other effects attributed to oleanolic acid might be (partially) explained. For instance, several studies have reported on the antioxidant activities of oleanolic acid (Ovesná et al., 2006; Somova et al., 2003a,b; Sultana and Ata, 2008), but only recently it has been shown that oleanolic acid is not only a free radical-scavenger acting through direct chemical reactions with reactive oxygen species, but also that the main antioxidant activity of the molecule is due to the Nrf2-mediated increased expression of antioxidant enzymes such as catalase and thioredoxin peroxidase, and the enhanced biosynthesis of the antioxidant glutathione (Wang et al., 2010).

Next to hepatoprotective and antioxidant effects, oleanolic acid is also reported to exert anticancer and anti-inflammatory activities (Dzubak et al., 2006; Petronelli et al., 2009). Oleanolic acid and

related triterpenoids target cancer cells by inducing apoptosis and modulating the tumor environment by, among others, their anti-inflammatory activities (Laszczyk, 2009). Inflammation plays an important role in the development and progression of cancer (Mantovani et al., 2008), and NF-κB, a key transcription factor involved in inflammation, is commonly overexpressed in cancer cells, thereby suppressing apoptosis of the tumor cells and maintaining a chronically inflamed microenvironment beneficial for cancer proliferation (Laszczyk, 2009; Van Waes, 2007). Several studies have shown the anti-inflammatory and anticancer potential of oleanolic acid, most likely by targeting NF-κB, however, its exact mode of action remains to be discovered (Laszczyk, 2009).

## 5. Chemical derivatives of oleanolic acid

Because of its inherent pharmacological activities, availability, and low production costs, oleanolic acid was considered to be a good starter molecule for further synthetic modifications (Sporn et al., 2011). Chemical modification of oleanolic acid on its three "active" portions, the C-3 hydroxy, the C-12–C-13 double bond, and the C-28 carboxylic acid, has led to a series of new synthetic oleanane triterpenoids (Honda et al., 1997, 2002, 1998; Sporn et al., 2011). Compared to oleanolic acid, several of these compounds showed an increased ability to block the synthesis of inducible nitric oxide synthase (iNOS), an enzyme that plays a key role in the inflammation process (Suh et al., 1998, 1999). Compounds belonging to the most potent molecules of this sort are 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) and its C-28 methyl ester, CDDO-Me (Fig. 4A). Both compounds have enone functions in their A- and C-rings, and an electron-withdrawing nitrile group at the C-2 position in the A-ring (Honda et al., 1998), features that were shown to be essential for the activity of the synthetic triterpenoids (Couch et al., 2005; Dinkova-Kostova et al., 2005; Yates et al., 2007). The electron-withdrawing nitrile group activates the A-ring enone, thereby allowing it to serve as an acceptor of a Michael addition to covalently, but reversibly, bind activated sulfhydryl groups of cysteine residues in protein targets (Fig. 4B) (Dinkova-Kostova et al., 2005; Liby et al., 2007; Sporn et al., 2011). One of the direct molecular targets of CDDO is Keap1 (Dinkova-Kostova et al., 2005; Liby et al., 2005, 2007), a protein that interacts with Nrf2, thereby sequestrating Nrf2 in the cytoplasm, which facilitates its ubiquitination and subsequent proteasomal degradation (Itoh et al., 1999; Kensler et al., 2007; Kobayashi et al., 2004; Li and Kong, 2009). Binding of CDDO to Keap1 disrupts critical cysteine residues in Keap1, leading to the release of Nrf2, which hinders its ubiquitination and finally leads to stabilization and nuclear translocation of the transcription factor (Liby et al., 2007; Sporn et al., 2011). In the nucleus, Nrf2 activates the transcription of phase 2 response genes, leading to a coordinated antioxidant and anti-inflammatory response (Kobayashi and Yamamoto, 2005). Next to Keap1, there are several other protein targets of synthetic triterpenoids, making them candidate drugs for the prevention and treatment of many diseases where oxidative and inflammatory stress play a key role (Liby et al., 2007; Sporn et al., 2011; Yore et al., 2011). The most advanced pharmaceutical application of synthetic triterpenoids is in the treatment of chronic kidney disease (CKD), which is characterized by a progressive and irreversible loss of kidney function that finally leads to kidney failure. The progress of the disease is correlated with an increased inflammation of the kidneys, and treatment of CKD patients with CDDO-Me (generic name: bardoxolone methyl) leads to improved inflammation measures and kidney function (Pergola et al., 2011). To date, bardoxolone methyl is in a late-stage clinical development for the treatment of CKD (Pergola et al., 2011; Pollier et al., 2011; Sporn et al., 2011; Yore et al., 2011).

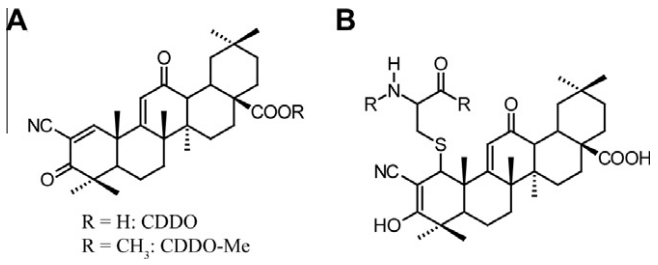


**Fig. 4.** (A) Chemical structures of CDDO and CDDO-Me. (B) Michael addition of an activated cysteine residue.

## 6. Research perspectives

The success-story of bardoxolone methyl and related synthetic oleanane triterpenoids shows the potential of a semi-synthetic chemistry approach using oleanolic acid as starter molecule. However, these successes came with only three "active" portions of the molecule (see above). Several plant species accumulate oleanolic acid derivatives, with additional enzymatically attached functionalities on the triterpenoid backbone. These compounds possess various biological activities, and may thus be useful starter molecules for semi-synthetic chemistry programs, in which also the extra functionalities might be targeted for modification. Maslinic acid (Fig. 5), for instance, a natural triterpenoid that is also derived from olive, possesses multiple biological activities and was shown to act as an inhibitor of glycogen phosphorylase, making it an interesting compound for the treatment of diseases caused by abnormalities in glycogen metabolism, such as type 2 diabetes (Wen et al., 2005, 2006). Likely, the compound is derived from oleanolic acid with the C-2 hydroxy generated in a single enzymatic step (Fig. 5). Like oleanolic acid, the compound is used as starter molecule for further synthetic modifications, resulting in compounds with improved biological activities compared to maslinic acid (Qiu et al., 2009; Wen et al., 2006).

A major challenge in drug development with plant-derived natural products is the availability of sufficient amounts of the candidate compounds (Carter, 2011; Pollier et al., 2011). For oleanolic acid, which can be extracted from by-products of the olive industry (Sporn et al., 2011), this may not be a major issue (yet), but for oleanolic acid-derived triterpenoids that accumulate at very low concentrations *in planta*, or only in rare plant species, obtaining sufficient amounts of the compound may become a serious bottleneck. Alternatively, a constant and cheap supply of the compounds could be obtained by expressing the plant biosynthesis genes leading to oleanolic acid and its derivatives in heterologous hosts, such as *Escherichia coli* or yeast (*Saccharomyces cerevisiae*), a technique referred to as heterologous biosynthesis or synthetic biology (Pollier et al., 2011; Zhang et al., 2011). Heterologous biosynthesis of oleanolic acid has already been achieved in *S. cerevisiae*, by expressing *M. truncatula* CYP716A12 in yeast cells that also express *Lotus japonicus* BAS and *L. japonicus* cytochrome P450 reductase, an enzyme required for efficient electron transfer to plant cytochrome P450 enzymes in recombinant yeast (Fig. 5) (Fukushima et al., 2011). To increase the production of oleanolic acid in yeast, heterologous biosynthesis could be combined with metabolic engineering of the sterol pathway in *S. cerevisiae*. For instance, a 50% increase in β-amyrin production has been achieved in yeast by combining the expression of an N-terminal truncated 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) with the restriction of the expression of lanosterol synthase (LAS) (Kirby et al., 2008) (Fig. 5). The activity and abundance of HMGR, the main control point of sterol biosynthesis in yeast, is regulated through degradation mediated by the N-terminal transmembrane regions of the

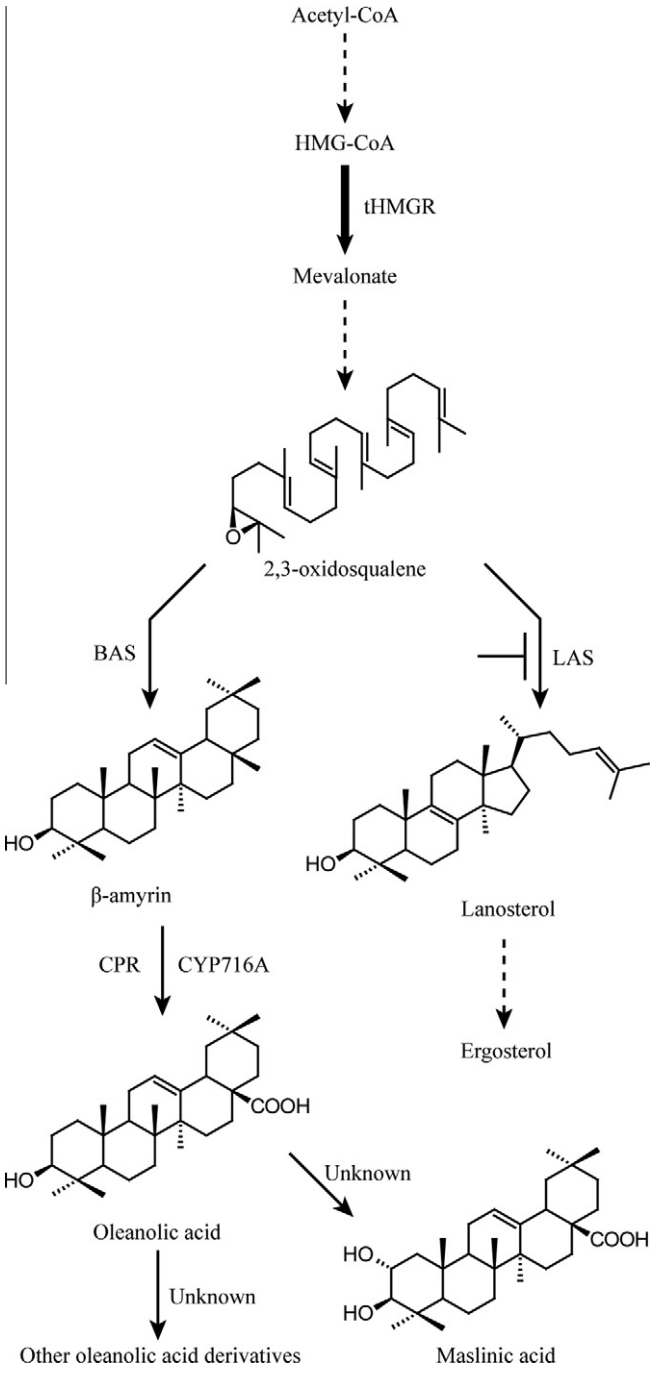


**Fig. 5.** Metabolic engineering of the yeast sterol pathway and heterologous biosynthesis of oleanolic acid and its derivatives. Dashed lines indicate multiple biosynthetic steps. tHMGR = N-terminal truncated 3-hydroxy-3-methylglutaryl-CoA reductase, BAS = β-amyrin synthase, LAS = lanosterol synthase, CPR = cytochrome P450 reductase.

enzyme. Expression of an N-terminal truncated form of HMGR overcomes this regulation and leads to an increased production of 2,3-oxidosqualene. LAS is an OSC that converts 2,3-oxidosqualene into lanosterol, the sterol precursor in yeast, and hence, down-regulation of LAS provides more 2,3-oxidosqualene substrate for BAS (Kirby et al., 2008). By expressing additional biosynthesis genes in the engineered oleanolic acid producing yeast strain, oleanolic acid-derived triterpenoids could be produced in sufficient amounts to make them candidate compounds for drug development or starter molecules for semi-synthetic chemistry programs. To date, these enzymes remain to be discovered, however, large-scale gene discovery programs running worldwide will soon deliver the necessary resources for such programs.

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## References

Abe, I., 2007. Enzymatic synthesis of cyclic triterpenes. Nat. Prod. Rep. 24, 1311–1331.
Ananthanarayanan, M., Balasubramanian, N., Makishima, M., Mangelsdorf, D.J., Suchy, F.J., 2001. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. J. Biol. Chem. 276, 28857–28865.
Assimopoulou, A.N., Papageorgiou, V.P., 2005. GC–MS analysis of penta- and tetracyclic triterpenes from resins of *Pistacia* species. Part I. *Pistacia lentiscus* var. Chia.. Biomed. Chromatogr. 19, 285–311.
Budzikiewicz, H., Wilson, J.M., Djerassi, C., 1963. Mass spectrometry in structural and stereochemical problems. XXXII. Pentacyclic triterpenes. J. Am. Chem. Soc. 85, 3688–3699.
Burnouf-Radosevich, M., Delfel, N.E., 1984. High-performance liquid chromatography of oleanane-type triterpenes. J. Chromatogr. A 292, 403–409.
Burnouf-Radosevich, M., Delfel, N.E., England, R., 1985. Gas chromatography–mass spectrometry of oleanane- and ursane-type triterpenes – application to *Chenopodium quinoa* triterpenes. Phytochemistry 24, 2063–2066.
Carelli, M., Biazzi, E., Panara, F., Tava, A., Scaramelli, L., Porceddu, A., Graham, N., Odoardi, M., Piano, E., Arcioni, S., May, S., Scotti, C., Calderini, O., 2011. *Medicago truncatula* CYP716A12 is a multifunctional oxidase involved in the biosynthesis of hemolytic saponins. Plant Cell 23, 3070–3081.
Carter, G.T., 2011. Natural products and Pharma 2011: strategic changes spur new opportunities. Nat. Prod. Rep. 28, 1783–1789.
Chappell, J., 2002. The genetics and molecular genetics of terpene and sterol origami. Curr. Opin. Plant Biol. 5, 151–157.
Chiang, J.Y.L., 2004. Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. J. Hepatol. 40, 539–551.
Claude, B., Morin, P., Lafosse, M., Andre, P., 2004. Evaluation of apparent formation constants of pentacyclic triterpene acids complexes with derivatized β- and γ-cyclodextrins by reversed phase liquid chromatography. J. Chromatogr. A 1049, 37–42.
Claudel, T., Staels, B., Kuipers, F., 2005. The Farnesoid X receptor: a molecular link between bile acid and lipid and glucose metabolism. Arterioscler., Thromb., Vasc. Biol. 25, 2020–2031.
Corey, E.J., Matsuda, S.P.T., Bartel, B., 1993. Isolation of an *Arabidopsis thaliana* gene encoding cycloartenol synthase by functional expression in a yeast mutant lacking lanosterol synthase by the use of a chromatographic screen. Proc. Nat. Acad. Sci. U.S.A. 90, 11628–11632.
Couch, R.D., Browning, R.G., Honda, T., Gribble, G.W., Wright, D.L., Sporn, M.B., Anderson, A.C., 2005. Studies on the reactivity of CDDO, a promising new chemopreventive and chemotherapeutic agent: implications for a molecular mechanism of action. Bioorg. Med. Chem. Lett. 15, 2215–2219.
Dinkova-Kostova, A.T., Liby, K.T., Stephenson, K.K., Holtzclaw, W.D., Gao, X., Suh, N., Williams, C., Risingsong, R., Honda, T., Gribble, G.W., Sporn, M.B., Talalay, P., 2005. Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. Proc. Nat. Acad. Sci. U.S.A. 102, 4584–4589.
Dzubak, P., Hajduch, M., Vydra, D., Hustova, A., Kvasnica, M., Biedermann, D., Markova, L., Urban, M., Sarek, J., 2006. Pharmacological activities of natural triterpenoids and their therapeutic implications. Nat. Prod. Rep. 23, 394–411.
Fai, Y.M., Tao, C.C., 2009. A review of presence of oleanolic acid in natural products. Nat. Proda Med. 2, 77–290.
Feng, J., Zhang, P., Chen, X., He, G., 2011. PI3K and ERK/Nrf2 pathways are involved in oleanolic acid-induced heme oxygenase-1 expression in rat vascular smooth muscle cells. J. Cell. Biochem. 112, 1524–1531.
Fukushima, E.O., Seki, H., Ohyama, K., Ono, E., Umemoto, N., Mizutani, M., Saito, K., Muranaka, T., 2011. CYP716A subfamily members are multifunctional oxidases in triterpenoid biosynthesis. Plant Cell Physiol. 52, 2050–2061.
Ghosh, A., Misra, S., Dutta, A.K., Choudhury, A., 1985. Pentacyclic triterpenoids and sterols from seven species of mangrove. Phytochemistry 24, 1725–1727.
Gu, J.-Q., Wang, Y., Franzblau, S.G., Montenegro, G., Timmermann, B.N., 2006. Dereplication of pentacyclic triterpenoids in plants by GC–EI/MS. Phytochem. Anal. 17, 102–106.
Han, J.-Y., Kim, H.-J., Kwon, Y.-S., Choi, Y.-E., 2011. The cytochrome P450 enzyme CYP716A47 catalyzes the formation of protopanaxadiol from dammarenediol-II during ginsenoside biosynthesis in*Panax ginseng*. Plant Cell Physiol. 52, 2062–2073.
Heinzen, H., de Vries, J.X., Moyna, P., Remberg, G., Martinez, R., Tietze, L.F., 1996. Mass spectrometry of labelled triterpenoids: thermospray and electron impact ionization analysis. Phytochem. Anal. 7, 237–244.

Hofmann, A.F., 1999. The continuing importance of bile acids in liver and intestinal disease. Arch. Intern. Med. 159, 2647–2658.
Honda, T., Finlay, H.J., Gribble, G.W., Suh, N., Sporn, M.B., 1997. New enone derivatives of oleanolic acid and ursolic acid as inhibitors of nitric oxide production in mouse macrophages. Bioorg. Med. Chem. Lett. 7, 1623–1628.
Honda, T., Honda, Y., Favaloro Jr., F.G., Gribble, G.W., Suh, N., Place, A.E., Rendi, M.H., Sporn, M.B., 2002. A novel dicyanotriterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile, active at picomolar concentrations for inhibition of nitric oxide production. Bioorg. Med. Chem. Lett. 12, 1027–1030.
Honda, T., Rounds, B.V., Gribble, G.W., Suh, N., Wang, Y., Sporn, M.B., 1998. Design and synthesis of 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid, a novel and highly active inhibitor of nitric oxide production in mouse macrophages. Bioorg. Med. Chem. Lett. 8, 2711–2714.
Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D., Yamamoto, M., 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 13, 76–86.
Janicsák, G., Veres, K., Kállai, M., Máthé, I., 2003. Gas chromatographic method for routine determination of oleanolic and ursolic acids in medicinal plants. Chromatographia 58, 295–299.
Kensler, T.W., Wakabayash, N., Biswal, S., 2007. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu. Rev. Pharmacol. Toxicol. 47, 89–116.
Kirby, J., Romanini, D.W., Paradise, E.M., Keasling, J.D., 2008. Engineering triterpene production in *Saccharomyces cerevisiae* - β-amyrin synthase from *Artemisia annua*. FEBS J. 275, 1852–1859.
Klaassen, C.D., Reisman, S.A., 2010. Nrf2 the rescue: effects of the antioxidative/electrophilic response on the liver. Toxicol. Appl. Pharmacol. 244, 57–65.
Kobayashi, A., Kang, M.-I., Okawa, H., Ohtsuji, M., Zenke, Y., Chiba, T., Igarashi, K., Yamamoto, M., 2004. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol. Cell. Biol. 24, 7130–7139.
Kobayashi, A., Yamamoto, M., 2005. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. Antioxid. Redox Signaling 7, 385–394.
Kubo, I., Matsumoto, A., 1984. Secreted oleanolic acid on the cuticle *Olea europaea* (Oleaceae); a chemical barrier to fungal attack. Cell. Mol. Life Sci. 40, 937–938.
Kubo, I., Matsumoto, A., Takase, I., 1985. A multichemical defense mechanism of bitter olive *Olea europaea* (oleaceae) – is oleuropein a phytoalexin precursor? J. Chem. Ecol. 11, 251–263.
Kushiro, T., Shibuya, M., Ebizuka, Y., 1998. *β*-amyrin synthase – cloning of oxidosqualene cyclase that catalyzes the formation of the most popular triterpene among higher plants. Eur. J. Biochem. 256, 238–244.
Laszczyk, M.N., 2009. Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy. Planta Med. 75, 1549–1560.
Li, W., Kong, A.-N., 2009. Molecular mechanisms of Nrf2-mediated antioxidant response. Mol. Carcinog. 48, 91–104.
Liang, Z., Jiang, Z., Fong, D.W., Zhao, Z., 2009. Determination of oleanolic acid and ursolic acid in *Oldenlandia diffusa* and its substitute using high performance liquid chromatography. J. Food Drug Anal. 17, 69–77.
Liby, K., Hock, T., Yore, M.M., Suh, N., Place, A.E., Risingsong, R., Williams, C.R., Royce, D.B., Honda, T., Honda, Y., Gribble, G.W., Hill-Kapturczak, N., Agarwal, A., Sporn, M.B., 2005. The synthetic triterpenoids, CDDO and CDDO-imidazolide, are potent inducers of heme oxygenase-1 and Nrf2/ARE signaling. Cancer Res. 65, 4789–4798.
Liby, K.T., Yore, M.M., Sporn, M.B., 2007. Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. Nat. Rev. Cancer 7, 357–369.
Liu, J., 1995. Pharmacology of oleanolic acid and ursolic acid. J. Ethnopharmacol. 49, 57–68.
Liu, J., 2005. Oleanolic acid and ursolic acid: research perspectives. J. Ethnopharmacol. 100, 92–94.
Liu, J., Wu, Q., Lu, Y.-F., Pi, J., 2008. New insights into generalized hepatoprotective effects of oleanolic acid: key roles of metallothionein and Nrf2 induction. Biochem. Pharmacol. 76, 922–928.
Liu, W., Wong, C., 2010. Oleanolic acid is a selective farnesoid X receptor modulator. Phytother. Res. 24, 369–373.
Mantovani, A., Allavena, P., Sica, A., Balkwill, F., 2008. Cancer-related inflammation. Nature 454, 436–444.
Oleszek, W.A., 2002. Chromatographic determination of plant saponins. J. Chromatogr. A 967, 147–162.
Ovesná, Z., Kozics, K., Slameňová, D., 2006. Protective effects of ursolic acid and oleanolic acid in leukemic cells. Mutat. Res. 600, 131–137.
Parks, D.J., Blanchard, S.G., Bledsoe, R.K., Chandra, G., Consler, T.G., Kliewer, S.A., Stimmel, J.B., Willson, T.M., Zavacki, A.M., Moore, D.D., Lehmann, J.M., 1999. Bile acids: natural ligands for an orphan nuclear receptor. Science 284, 1365–1368.
Pergola, P.E., Krauth, M., Huff, J.W., Ferguson, D.A., Ruiz, S., Meyer, C.J., Warnock, D.G., 2011. Effect of bardoxolone methyl on kidney function in patients with T2D and Stage 3b-4 CKD. Am. J. Nephrol. 33, 469–476.
Petronelli, A., Pannitteri, G., Testa, U., 2009. Triterpenoids as new promising anticancer drugs. Anti-Cancer Drugs 20, 880–892.
Phillips, D.R., Rasbery, J.M., Bartel, B., Matsuda, S.P.T., 2006. Biosynthetic diversity in plant triterpene cyclization. Curr. Opin. Plant Biol. 9, 305–314.
Pollier, J., Moses, T., Goossens, A., 2011. Combinatorial biosynthesis in plants: a (p)review on its potential and future exploitation. Nat. Prod. Rep. 28, 1897–1916.
Qi, S.D., Ding, L., Tian, K., Chen, X.G., Hu, Z.D., 2006. Novel and simple nonaqueous capillary electrophoresis separation and determination bioactive triterpenes in Chinese herbs. J. Pharm. Biomed. Anal. 40, 35–41.
Qiu, W.-W., Shen, Q., Yang, F., Wang, B., Zou, H., Li, J.-Y., Li, J., Tang, J., 2009. Synthesis and biological evaluation of heterocyclic ring-substituted maslinic acid derivatives as novel inhibitors of protein tyrosine phosphatase 1B. Bioorg. Med. Chem. Lett. 19, 6618–6622.
Razboršek, M.I., Vončina, D.B., Doleček, V., Vončina, E., 2008. Determination of oleanolic, betulinic and ursolic acid in *Lamiaceae* and mass spectral fragmentation of their trimethylsilylated derivatives. Chromatographia 67, 433–440.
Reisman, S.A., Aleksunes, L.M., Klaassen, C.D., 2009. Oleanolic acid activates Nrf2 and protects from acetaminophen hepatotoxicity via Nrf2-dependent and Nrf2-independent processes. Biochem. Pharmacol. 77, 1273–1282.
Saimaru, H., Orihara, Y., Tansakul, P., Kang, Y.-H., Shibuya, M., Ebizuka, Y., 2007. Production of triterpene acids by cell suspension cultures of *Olea europaea*. Chem. Pharm. Bull. 55, 784–788.
Sánchez Ávila, N., Priego Capote, F., Luque de Castro, M.D., 2007. Ultrasound-assisted extraction and silylation prior to gas chromatography-mass spectrometry for the characterization of the triterpenic fraction in olive leaves. J. Chromatogr. A 1165, 158–165.
Schinor, E.C., Salvador, M.J., Turatti, I.C.C., Zucchi, O.L.A.D., Dias, D.A., 2004. Comparison of classical and ultrasound-assisted extractions of steroids and triterpenoids from three *Chresta* spp. Ultrason. Sonochem. 11, 415–421.
Simonsen, J.L., Ross, W.C.J., 1957. The terpenes: The Triterpenes and Their Derivatives, Vol. 5: Hydroxy acids, Hydroxy lactones, Hydroxyaldehydo Acids, Hydroxyketo Acids and the Stereochemistry of the Triterpenes. Cambridge University Press, Cambridge.
Sinal, C.J., Tohkin, M., Miyata, M., Ward, J.M., Lambert, G., Gonzalez, F.J., 2000. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 102, 731–744.
Somova, L.I., Shode, F.O., Ramnanan, P., Nadar, A., 2003a. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies *africana* leaves. J. Ethnopharmacol. 84, 299–305.
Somova, L.O., Nadar, A., Rammanan, P., Shode, F.O., 2003b. Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. Phytomedicine 10, 115–121.
Sporn, M.B., Liby, K.T., Yore, M.M., Fu, L., Lopchuk, J.M., Gribble, G.W., 2011. New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress. J. Nat. Prod. 74, 537–545.
Suh, N., Honda, T., Finlay, H.J., Barchowsky, A., Williams, C., Benoit, N.E., Xie, Q.-W., Nathan, C., Gribble, G.W., Sporn, M.B., 1998. Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. Cancer Res. 58, 717–723.
Suh, N., Wang, Y., Honda, T., Gribble, G.W., Dmitrovsky, E., Hickey, W.F., Maue, R.A., Place, A.E., Porter, D.M., Spinella, M.J., Williams, C.R., Wu, G., Dannenberg, A.J., Flanders, K.C., Letterio, J.J., Mangelsdorf, D.J., Nathan, C.F., Nguyen, L., Porter, W.W., Ren, R.F., Roberts, A.B., Roche, N.S., Subbaramaiah, K., Sporn, M.B., 1999. A novel synthetic oleanane triterpenoid, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid, with potent differentiating, antiproliferative, and anti-inflammatory activity. Cancer Res. 59, 336–341.
Sultana, N., Ata, A., 2008. Oleanolic acid and related derivatives as medicinally important compounds. J. Enzyme Inhib. Med. Chem. 23, 739–756.
Szakiel, A., Grzelak, A., Dudek, P., Janiszowska, W., 2003. Biosynthesis of oleanolic acid and its glycosides in *Calendula officinalis* suspension culture. Plant Physiol. Biochem. 41, 271–275.
Szakiel, A., Ruszkowski, D., Janiszowska, W., 2005. Saponins in *Calendula officinalis* L. – structure, biosynthesis, transport and biological activity. Phytochem. Rev. 4, 151–158.
Unger, M., 2009. Capillary electrophoresis of natural products: current applications and recent advances. Planta Med. 75, 735–745.
Van Waes, C., 2007. Nuclear factor-κB in development, prevention, and therapy of cancer. Clin. Cancer Res. 13, 1076–1082.
Wang, X., Ye, X.-l., Liu, R., Chen, H.-L., Bai, H., Liang, X., Zhang, X.-D., Wang, Z., Li, W.-l., Hai, C.-X., 2010. Antioxidant activities of oleanolic acid in vitro: possible role of Nrf2 and MAP kinases. Chem.-Biol. Interact. 184, 328–337.
Wen, X., Sun, H., Liu, J., Wu, G., Zhang, L., Wu, X., Ni, P., 2005. Pentacyclic triterpenes. Part 1: The first examples of naturally occurring pentacyclic triterpenes as a new class of inhibitors of glycogen phosphorylases. Bioorg. Med. Chem. Lett. 15, 4944–4948.
Wen, X., Zhang, P., Liu, J., Zhang, L., Wu, X., Ni, P., Sun, H., 2006. Pentacyclic triterpenes. Part 2: Synthesis and biological evaluation of maslinic acid derivatives as glycogen phosphorylase inhibitors. Bioorg. Med. Chem. Lett. 16, 722–726.
Xu, R., Fazio, G.C., Matsuda, S.P.T., 2004. On the origins of triterpenoid skeletal diversity. Phytochemistry 65, 261–291.
Yates, M.S., Tauchi, M., Katsuoka, F., Flanders, K.C., Liby, K.T., Honda, T., Gribble, G.W., Johnson, D.A., Johnson, J.A., Burton, N.C., Guilarte, T.R., Yamamoto, M., Sporn, M.B., Kensler, T.W., 2007. Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes. Mol. Cancer Ther. 6, 154–162.
Yore, M.M., Kettenbach, A.N., Sporn, M.B., Gerber, S.A., Liby, K.T., 2011. Proteomic analysis shows synthetic oleanane triterpenoid binds to mTOR. PLoS ONE 6, e22862.
Zhang, H., Boghigian, B.A., Armando, J., Pfeifer, B.A., 2011. Methods and options for the heterologous production of complex natural products. Nat. Prod. Rep. 28, 125–151.