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Review

S-Adenosyl-L-methionine: Beyond the universal methyl group donor

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Dedicated to Prof. Rodney Croteau at the occasion of his 60th birthday.

Abstract

S-Adenosyl-L-methionine (AdoMet or SAM) is a substrate in numerous enzyme-catalyzed reactions. It not only provides methyl groups in many biological methylations, but also acts as the precursor in the biosynthesis of the polyamines spermidine and spermine, of the metal ion chelating compounds nicotianamine and phytosiderophores, and of the gaseous plant hormone ethylene. AdoMet is also the source of catalytic 5′-deoxyadenosyl radicals, produced as reaction intermediates by the superfamily of radical AdoMet enzymes. This review aims to summarize the present knowledge of catalytic roles of AdoMet in plant metabolism.

Keywords: *S*-Adenosyl-L-methionine; Methyltransferases; Polyamines; Nicotianamine; Phytosiderophores; Ethylene; 5′-Deoxyadenosyl radicals

Contents

1. Introduction

The number of known enzymes that utilize *S*-adenosyl-L-methionine (**1**) (AdoMet or SAM, Fig. 1) has increased steadily in recent years. It is now clear that the transfer of methyl groups is only one role of this metabolite. Because of the vast number of methylated secondary products, methyltransferases are the most numerous among the AdoMet-utilizing enzymes in plants. Considering the richness of flora on Earth, and the fact that many identified or yet to be identified secondary products are produced only

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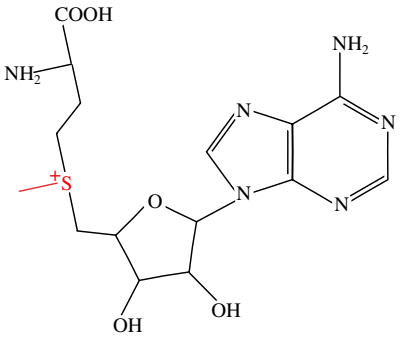


S-Adenosyl-L-Methionine, **1**

Fig. 1. Chemical structure of AdoMet. The sulfonium and the *S*-bound methyl group are highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by some plant species, many more AdoMet-utilizing methyltransferases will likely be discovered in the future. AdoMet (**1**) is also recognized as the substrate of a decarboxylase, of enzymes that catalyze transfer of aminopropyl or carboxypropyl groups, and of enzymes that catalyze generation of 5′-deoxyadenosyl radicals in plants. Reactions catalyzed by these enzymes lead to the biosynthesis of ethylene, polyamines, nicotianamine, phytosiderophores, and biotin. Finding enzymes that use AdoMet (**1**) as a substrate for entirely novel reactions in plants would not be surprising, as such enzymes are still being found in other organisms. For example, the recently discovered enzyme aclacinomycin-10-hydroxylase from *Streptomyces purpurascens* catalyzes an AdoMet-dependent hydroxylation reaction (Jansson et al., 2005).

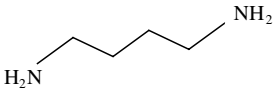
AdoMet (**1**) is synthesized from methionine and ATP in a reaction catalyzed by the enzyme AdoMet synthetase (Aarnes, 1977; Espartero et al., 1994; Izhaki et al., 1995; Konze and Kende, 1979; Schröder et al., 1997; Van Breusegem et al., 1994). The biosynthesis of methionine, and other members of the aspartate family of amino acids, is regulated by AdoMet (**1**) in plants. AdoMet (**1**) inhibits an isozyme of aspartate kinase in the presence of lysine (Azevedo et al., 1997), activates threonine synthase (Curien et al., 1998; Madison and Thompson, 1976), and affects stability of the mRNA for cystathionine γ-synthase (Chiba et al., 2003). Through these interactions, AdoMet (**1**) also regulates its own biosynthesis. This review focuses on AdoMet (**1**) as substrate in enzyme-catalyzed reactions in plants. Other related reviews have dealt with the biosynthesis of aspartate-derived amino acids and AdoMet (**1**) (Amir et al., 2002; Azevedo et al., 1997; Azevedo and Lea, 2001; Galili and Höfgen, 2002; Hesse et al., 2004; Hesse et al., 2001; Ravanel et al., 1998).

2. AdoMet as the precursor of polyamines

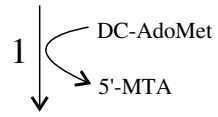
As the aminopropyl group donor in the biosynthesis of the polyamines spermidine (**3**) and spermine (**4**), AdoMet (**1**) is first decarboxylated to *S*-adenosyl-methioninamine in a reaction catalyzed by AdoMet decarboxylase (Dresselhaus et al., 1996; Hao et al., 2005; Mad Arif et al., 1994; Thu-Hang et al., 2002). Spermidine synthase (Yoon et al., 2000) then catalyzes transfer of the aminopropyl moiety of *S*-adenosyl-methioninamine to putrescine (**2**), yielding spermidine (**3**). Addition of another aminopropyl moiety to spermidine (**3**), catalyzed by spermine synthase (Hanzawa et al., 2000), yields spermine (**4**) (Fig. 2). Putrescine (**2**), spermidine (**3**), and spermine (**4**) are positively charged at cellular pH values, and are known to chemically interact with DNA, RNA, phospholipids, and some proteins. Abnormal phenotypes of plant mutants modified in polyamine metabolism suggest that these molecules are involved in the regulation of plant development (Clay and Nelson, 2005; Hanzawa et al., 2000; Imai et al., 2004a). Polyamines also appear to be involved in plant stress responses (Imai et al., 2004b; Kasukabe et al., 2004). Yet, their exact mode of action is unknown.

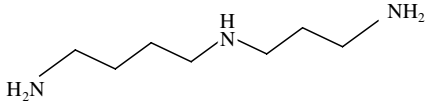
3. AdoMet as the precursor of nicotianamine and phytosiderophores

Nicotianamine (**5**) (Fig. 3), a strong chelator of iron and various transition metals, occurs widely in higher plants

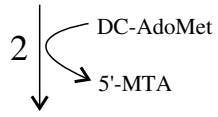


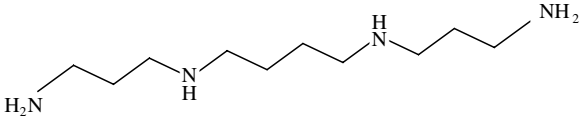
Putrescine, **2**





Spermidine, **3**





Spermine, **4**

Fig. 2. Biosynthesis of spermidine and spermine. Abbreviations: 1, spermidine synthase; 2, spermine synthase; DC-AdoMet, *S*-adenosyl-methioninamine; 5-MTA, 5-methylthioadenosine.

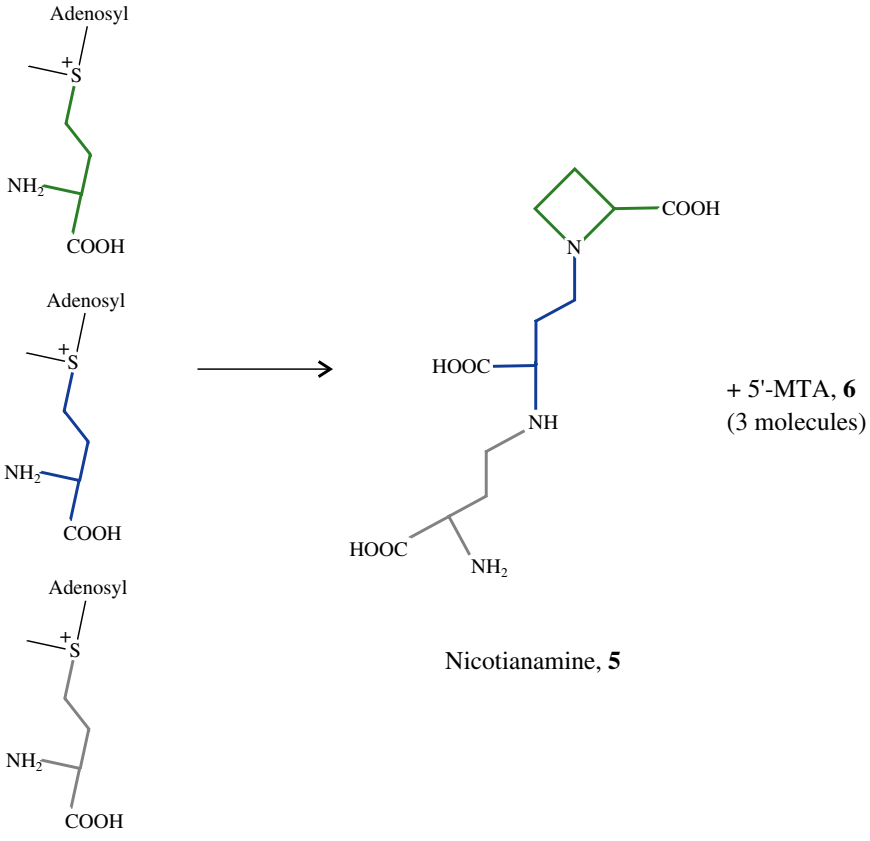


Fig. 3. Biosynthesis of nicotianamine. The AdoMet-derived moieties used in the synthesis of nicotianamine are highlighted in green, blue, and gray. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Mori, 1999; Takahashi et al., 2003). The synthesis of nicotianamine (**5**) from three molecules of AdoMet (**1**) includes two carboxypropyl group transfers and one azetidine ring formation, with three molecules of 5′-methylthioadenosine (5′-MTA) (**6**) released (Fig. 3). All three reactions are catalyzed by the enzyme nicotianamine synthase (Herbik et al., 1999; Higuchi et al., 1999; Ling et al., 1999). In non-graminaceous plants, nicotianamine (**5**) is thought to bind metal ions and to participate in their trafficking inside the plant (Higuchi et al., 2001; Takahashi et al., 2003). In graminaceous plants, nicotianamine (**5**) is also the precursor of phytosiderophores, which are essential in acquiring iron from soil (Mizuno et al., 2003). The roots of these plants secrete phytosiderophores into the soil, where they form complexes with poorly soluble Fe(III) ions. Phytosiderophore–Fe(III) complexes are then taken up by the roots via specific transporters, thus providing these plants with iron needed for various metabolic processes (Mori, 1999).

4. AdoMet as the precursor of ethylene

The biosynthesis of the plant hormone ethylene from AdoMet (**1**) proceeds in two steps. The enzyme 1-aminocyclopropane-1-carboxylate (ACC) synthase first catalyzes conversion of AdoMet to ACC and 5′-MTA (**6**). The enzyme ACC oxidase then catalyzes the conversion of ACC to ethylene (Adams and Yang, 1979; Bleecker and Kende, 2000; Boller et al., 1979; Hamilton et al., 1991; Kende, 1993; Spanu et al., 1991; Van der Straeten et al., 1990; Yu et al., 1979). Ethylene participates in regulation of growth, development, and responses to stress and pathogen attack in plants (Bleecker and Kende, 2000; Kende, 1993). The physiological roles of ethylene, regulation of ethylene biosynthesis, and ethylene signal transduction have recently been reviewed elsewhere (Chae and Kieber, 2005; Chang, 2003; Chen et al., 2005; Guo and Ecker, 2004; Klee, 2004; Wang et al., 2002).

5. AdoMet as the source of 5′-deoxyadenosyl radicals

Radical AdoMet enzymes catalyze reductive cleavage of AdoMet (**1**) to yield methionine (**7**) and a catalytic, highly oxidizing 5′-deoxyadenosyl radical intermediate (Fig. 4A) (Jarrett, 2003; Layer et al., 2004). This reactive radical abstracts hydrogen from a carbon atom of a substrate molecule to yield 5′-deoxyadenosine (**8**) and a substrate radical (Fig. 4B). The substrate radical is then converted into the

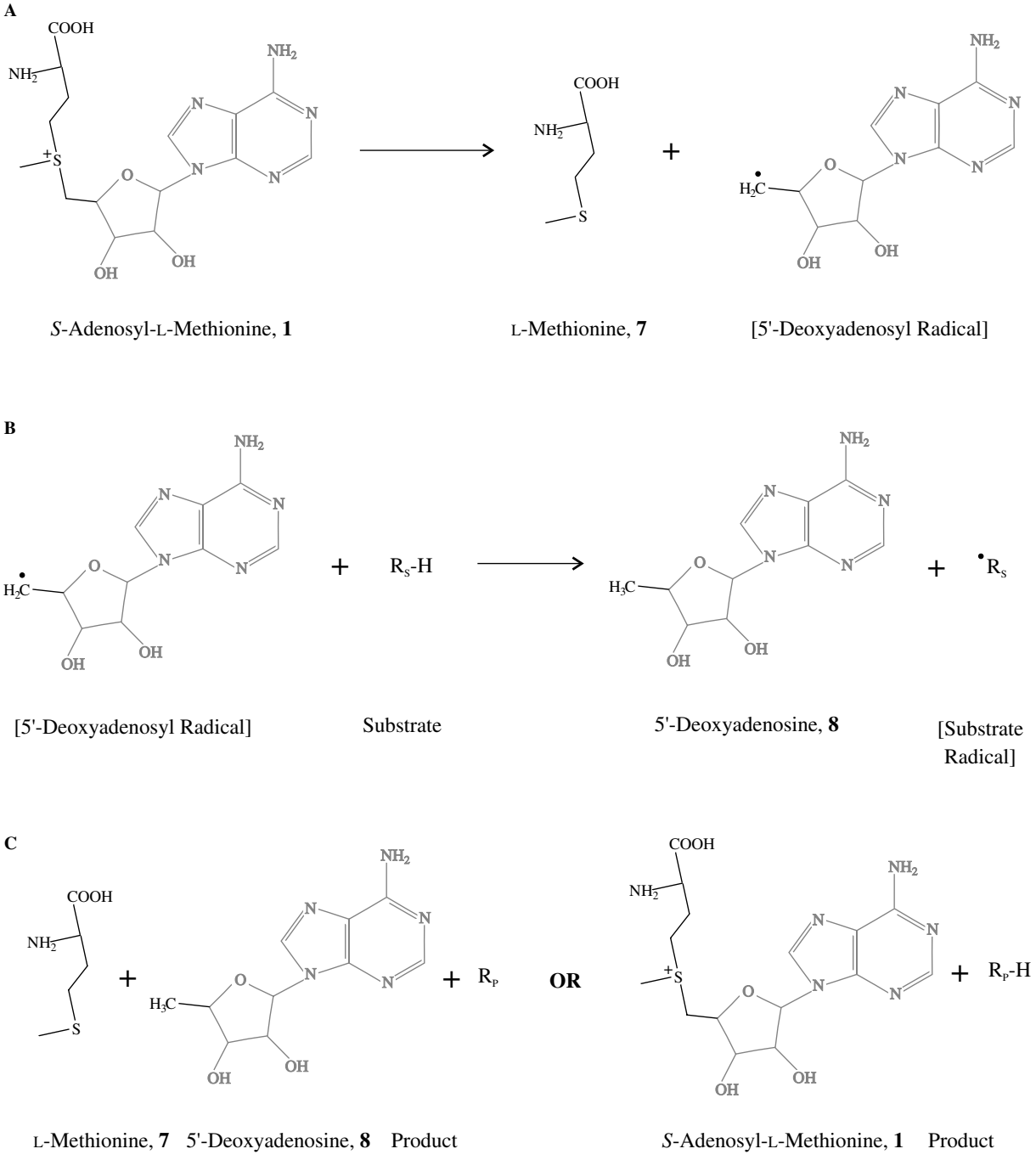


Fig. 4. AdoMet as the source of 5′-deoxyadenosyl radicals. (A) Reductive cleavage of AdoMet; (B) generation of a substrate radical; (C) the final reaction products.

final reaction product(s). In most cases, methionine (**7**) and 5′-deoxyadenosine (**8**) are released with the final reaction product (Fig. 4C). In few cases, however, AdoMet (**1**) is released instead, after being restored when the product radical re-abstracts a hydrogen from 5′-deoxyadenosine (**8**) (Fig. 4C) (Jarrett, 2003; Layer et al., 2004).

The substrate of a radical AdoMet enzyme can be a small molecule or a catalytic glycine residue of another protein. All radical AdoMet enzymes contain a catalytically essential iron–sulfur (4Fe–4S) cluster, which initiates cleavage of AdoMet (**1**) by transfer of an electron to its sulfonium ion; and a signature sequence motif, which binds the iron–sulfur cluster (Sofia et al., 2001).

Biotin synthase catalyzes conversion of dethiobiotin (**9**) to biotin (**10**) (Fig. 5); it is the only radical AdoMet enzyme that has been cloned and characterized in plants (Baldet et al., 1997; Baldet and Ruffet, 1996; Patton et al., 1996; Picciocchi et al., 2001; Picciocchi et al., 2003; Weaver et al., 1996). Considering that the superfamily of radical AdoMet enzymes is predicted to contain more than 600

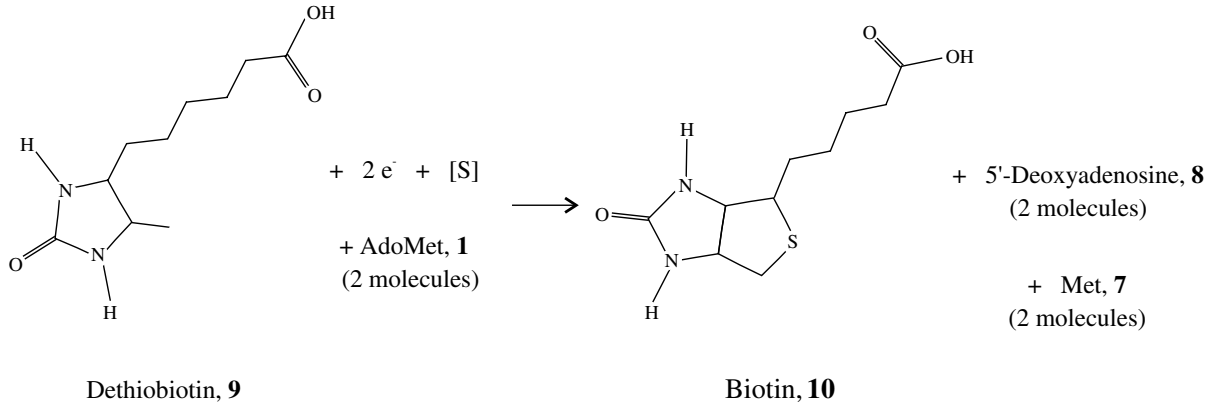


Fig. 5. The reaction catalyzed by biotin synthase.

enzymes (Sofia et al., 2001), the discovery of new members of this superfamily in plants is likely.

6. AdoMet as the universal methyl group donor

AdoMet (**1**) is the methyl group donor in a wide variety of enzyme-catalyzed reactions in plants. *O*-, *N*-, and *C*-methyltransferases are the three major families of methyltransferases based on the chemical nature of the substrate. Enzymes that act on halide ions, and that catalyze the addition of a methylene group to the *cis*-double bond of fatty acids, also exist in plants.

The substrates of AdoMet-dependent methyltransferases form a chemically diverse group of compounds that participate in both primary and secondary metabolism. Examples include lipids, pectin, alkaloids, phytosterols, osmoprotectants, the precursors of lignins, lignans, suberins, hydroxycinnamic acids, flavonoids, stilbenes and other aromatics, as well as various volatile fragrance and aroma compounds. Some methyltransferases exhibit strict specificity for a single substrate; many others accept a broad range of substrates. All share three highly conserved motifs that have been implied in AdoMet (**1**) binding (Kagan and Clarke, 1994).

6.1. *O*-methyltransferases

Most known plant *O*-methyltransferases (OMTs) act on hydroxyl and carboxyl moieties of small molecules. A classification of plant OMTs that act on small molecules has been proposed, based on the chemical nature of the methyl acceptor molecule (Ibrahim et al., 1998). According to this classification, plant OMTs are assigned to Class "A" if they act on the hydroxyl groups of phenylpropanoids, Class "B" if they act on flavonoids, Class "C" if they act on alkaloids, and Class "D" if they act on aliphatic substrates. Since this classification was proposed, new OMTs that do not fall into any of the four classes above have been identified in plants (see below).

Plants also contain an OMT that does not act on a small molecule. This enzyme is protein-L-isoaspartate *O*-methyltransferase (Amaral et al., 2001; Mudgett and Clarke, 1993; Mudgett and Clarke, 1994; Mudgett and Clarke, 1996; Thapar and Clarke, 2000), which catalyzes the transfer of methyl groups to protein-bound L-isoaspartyl (**12**) and D-aspartyl residues that result from spontaneous protein damage. Methylation of these abnormal residues into an α-methyl ester (**13**) initiates their repair to L-aspartyl residues (**11**) (Fig. 6).

Class A OMTs act on phenylpropanoids and include caffeoyl coenzyme A 3-*O*-methyltransferase (CCOMT) and caffeic acid 3-*O*-methyltransferase (COMT). These two enzymes are essential to the biosynthesis of coniferyl and sinapyl alcohols, the precursors of lignins, lignans, and other phenylpropanoids. CCOMTs catalyze methylation of caffeoyl CoA to feruloyl CoA, and of 5-hydroxyferuloyl CoA to sinapoyl CoA, in vitro and probably in vivo (Anterola and Lewis, 2002; Boerjan et al., 2003; Dixon et al., 2001; Lewis et al., 1999; Ye et al., 2001). Since the discovery (Pakusch et al., 1989) and subsequent cloning (Schmitt et al., 1991) of the first plant CCOMT from parsley cell suspension cultures, these enzymes, and their roles in tissue lignification, have been studied extensively. Advances in the understanding of their biochemical and physiological roles have been comprehensively covered in recent reviews (Anterola and Lewis, 2002; Boerjan et al., 2003; Dixon et al., 2001; Lewis et al., 1999; Ye et al., 2001).

Although *O*-methylation of caffeic acid by plant extracts was discovered more than 40 years ago (Finkle and Nelson, 1963), the in vivo substrates of COMTs are still being debated (Anterola and Lewis, 2002; Boerjan et al., 2003; Dixon et al., 2001; Gauthier et al., 1998; Kota et al., 2004; Lewis et al., 1999; Maury et al., 1999). The in vitro substrates of these enzymes reportedly include caffeic acid, caffeoyl CoA, caffeyl aldehyde, caffeyl alcohol, 5-hydroxyferulic acid, 5-hydroxyferuloyl CoA, 5-hydroxyconiferyl aldehyde, 5-hydroxyconiferyl alcohol, protocatechuic aldehyde, protocatechuic acid, 3,4-dihydroxy, 5-methoxybenzaldehyde, isovanillin, luteolin, and quercetin. Each COMT can *O*-methylate caffeic acid and 5-hydroxyferulic

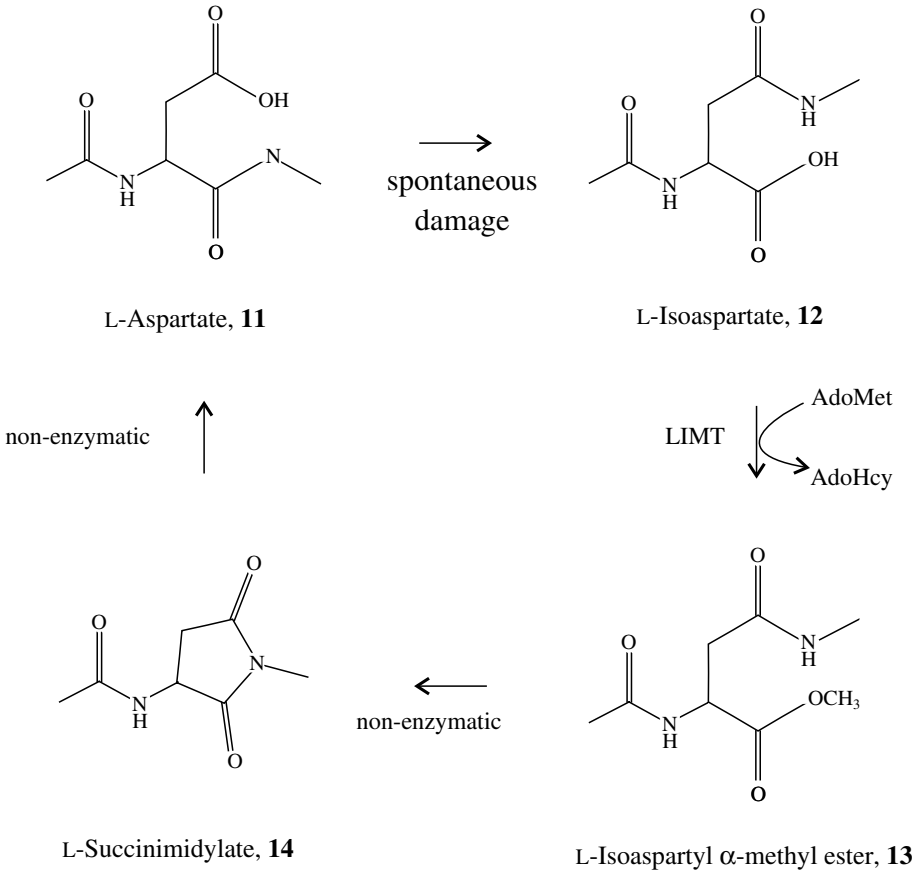


Fig. 6. Repair of protein-bound L-isoaspartyl residues. LIMT, protein-L-isoaspartate *O*-methyltransferase.

acid, as well as one or more of the other compounds listed above (Anterola and Lewis, 2002; Boerjan et al., 2003; Dixon et al., 2001; Gauthier et al., 1998; Kota et al., 2004; Lewis et al., 1999; Maury et al., 1999). Advances in the understanding of the biochemical and physiological roles of COMTs have been reviewed recently (Anterola and Lewis, 2002; Boerjan et al., 2003; Dixon et al., 2001; Lewis et al., 1999; Ye et al., 2001).

Another Class A OMT, β-peltatin 6-*O*-methyltransferase, catalyzes a step in the biosynthesis of 6-methoxypodophyllotoxin, a cytotoxic lignan from *Linum nodiflorum* (Kranz and Petersen, 2003). Class A OMTs also participate in the biosynthesis of many fruit flavor and floral scent components in various plant species (Gang, 2005). For example, chavicol and eugenol *O*-methyltransferases catalyze, respectively, the final step in the biosynthesis of methylchavicol and methyleugenol in sweet basil (Gang et al., 2002); orcinol *O*-methyltransferase (Lavid et al., 2002b; Scalliet et al., 2002) and phloroglucinol *O*-methyltransferase catalyze the three methylation steps in the biosynthesis of 1,3,5-trimethoxybenzene in chinese rose (Wu et al., 2004); and furaneol *O*-methyltransferase catalyzes the methylation of furaneol to methoxyfuraneol in strawberry (Lavid et al., 2002a; Wein et al., 2002).

Class B OMTs catalyze methylation of the hydroxyl groups of flavonoids and include chalcone and isoflavone *O*-methyltransferases. Chalcone *O*-methyltransferase (also known as isoliquiritigenin 2′-*O*-methyltransferase) catalyzes methylation of 4,2′,4′-trihydroxychalcone to 4,4′-dihydroxy-2′-methoxychalcone, an inducer of nodulation genes (Maxwell et al., 1992; Maxwell et al., 1993). Isoflavone 7-*O*-methyltransferase in vitro catalyzes methylation of the 7-hydroxyl-group of the isoflavonoids daidzein, genistein, and 6,7,4′-trihydroxyisoflavone; and of the pterocarpans 6a-hydroxymaackiain and maackiain (He and Dixon, 1996; He et al., 1998). The proposed role of this enzyme in vivo is methylation of 2,4′,7-trihydroxyisoflavanone, an unstable product of isoflavone synthase, into 2,7-dihydroxy-4′-methoxyisoflavanone (Liu and Dixon, 2001). This reaction is a step in the biosynthesis of medicarpin, a phytoalexin present in alfalfa. A related class B OMT, 6a-hydroxymaackiain 3-*O*-methyltransferase, catalyzes the final step in pisatin synthesis in pea (Wu et al., 1997). Chalcone *O*-methyltransferase and isoflavone 7-*O*-methyltransferase from alfalfa are the first plant methyltransferases to be structurally characterized (Zubieta et al., 2001). Besides the AdoMet-binding catalytic domain, which is structurally conserved among all family members investigated since the X-ray crystal structure of an OMT was first elucidated (Vidgren et al., 1994), plant OMTs contain a second domain involved in enzyme dimerization and substrate binding (Zubieta et al., 2001).

Class C OMTs catalyze methylation of alkaloids and include reticuline 7-*O*-methyltransferase and norcoclaurine 6-*O*-methyltransferase from opium poppy (Ounaroon et al., 2003), as well as norcoclaurine 6-*O*-methyltransferase, 3′-hydroxy-*N*-methylcoclaurine 4′-*O*-methyltransferase, and scoulerine 9-*O*-methyltransferase, all three catalyzing steps in benzylisoquinoline alkaloid biosynthesis in *Coptis* (*Ranunculaceae*) (Choi et al., 2002). A class D representative, *myo*-inositol *O*-methyltransferase, catalyzes the biosynthesis of the osmoprotectant D-ononitol in *Mesembryanthemum crystallinum* (Rammesmayer et al., 1995).

A novel family of *O*-methyltransferases was discovered in plants after the classification above was proposed. This family includes the enzymes that catalyze methylation of the carboxyl group of benzoic (**16**), salicylic (**18**), or jasmonic (**20**) acid to produce the corresponding volatile methyl esters (Fig. 7) (Dudareva et al., 2000; Effmert et al., 2005; Murfitt et al., 2000; Negre et al., 2003; Ross et al., 1999; Seo et al., 2001; Zubieta et al., 2003). All three methyl esters occur in fruit flavor and floral scent in various plant species (Effmert et al., 2005; Negre et al., 2003; Ross et al., 1999). Methyl salicylate (**19**) and methyl jasmonate (**21**) participate in plant responses to pathogen attack (Farmer and Ryan, 1990; Shulaev et al., 1997). Methyl jasmonate (**21**) is also involved in the regulation of various developmental processes in plants (Creelman and Mullet, 1995; Creelman and Mullet, 1997). The X-ray crystal structure of salicylic acid carboxyl methyltransferase from *Clarkia breweri* (Zubieta et al., 2003) revealed a dimer structure broadly similar to those of chalcone *O*-methyltransferase and isoflavone 7-*O*-methyltransferase from alfalfa (Zubieta et al., 2001).

6.2. *N*-methyltransferases

Plant *N*-methyltransferases act on proteins, DNA, phosphoethanolamine, and secondary metabolites. *N*-methylation of histones and DNA plays a crucial role in the organization of chromatin structure, and thus transcriptional regulation, in plants and animals. Histones can be methylated in ε-amino groups of selected lysine residues by histone lysine methyltransferases (HKMTs) (Cheng et al., 2005). All the investigated plant HKMTs carry a signature SET domain, which contains the active site of the enzyme. The first two SET domain HKMTs have been characterized from mammalian sources (Rea et al., 2000). Characterization of plant SET domain HKMTs and investigation of their physiological roles are currently active research areas (Jackson et al., 2002; Naumann et al., 2005; Springer et al., 2003).

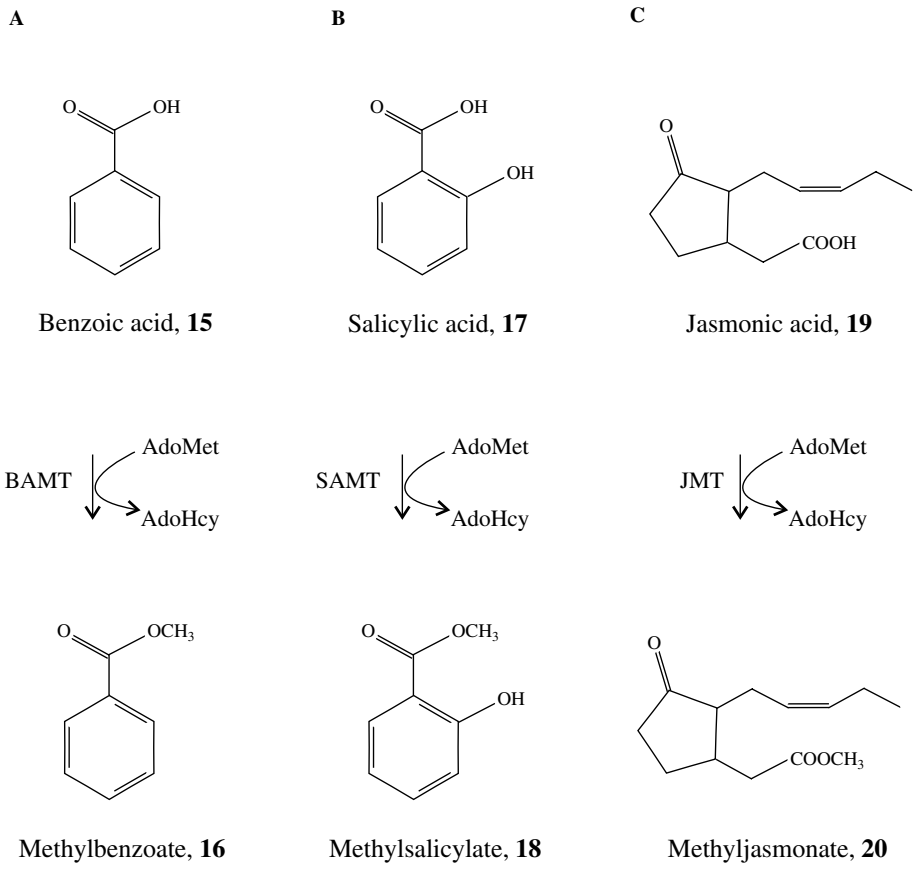


Fig. 7. Biosynthesis of volatile methyl esters. (A) methylbenzoate; (B) methylsalicylate; (C) methyljasmonate. BAMT, benzoic acid carboxyl methyltransferase; SAMT, salicylic acid carboxyl methyltransferase; JAMT, jasmonic acid carboxyl methyltransferase.

Histones can also be methylated in the guanidino group of selected arginine residues by protein arginine methyltransferases (PRMTs) (Cheng et al., 2005). Histone PRMT activity has been detected in plants (Disa et al., 1986; Gupta et al., 1982), and multiple putative PRMT genes have been found in plant genomes (Cheng et al., 2005). However, no plant histone PRMTs have yet been cloned and characterized.

Three types of cytosine DNA methyltransferases exist in plants (Tariq and Paszkowski, 2004). The first type maintains CpG methylation and is represented by MET1 from *Arabidopsis* (Ronemus et al., 1996). The second type maintains CpNpG methylation and is represented by chromomethylase (CMT) from *Arabidopsis* (Lindroth et al., 2001). The third type methylates DNA de novo (Cao and Jacobsen, 2002) and is represented by the domains rearranged methylase (DRM) from *Arabidopsis*. Studies of DNA methylation and histone *N*-methylation suggest that these two processes act in concert to regulate transcription in plants (Lindroth et al., 2004; Naumann et al., 2005; Tariq and Paszkowski, 2004).

Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) is another substrate of protein *N*-methyltransferases in plants. This enzyme consists of eight large and eight small subunits; it is methylated at a lysine residue of the large subunit, and at the α-amino group of the *N*-terminal methionine of the mature form of the small subunit (Ying et al., 1999). Methylation of the large subunit is mediated by a SET domain protein methyltransferase (Trievel et al., 2002; Trievel et al., 2003). The physiological roles of Rubisco methylation are unknown. Targeting of proteins that interact with Rubisco is one role proposed for the large subunit methylation (Trievel et al., 2002).

In addition to protein and DNA methyltransferases, which serve regulatory roles, *N*-methyltransferases involved in the biosynthesis of various plant metabolites have been characterized. For example, phosphoethanolamine *N*-methyltransferase catalyzes the three sequential methyl group transfers that convert phosphoethanolamine (**22**) to phosphocholine (**23**) (Fig. 8A), the precursor of choline (Nuccio et al., 2000). Choline is used in the biosynthesis of the phospholipid phosphatidylcholine, which makes up 40–60% of lipids in non-plastid membranes (Bolognese and McGraw, 2000; Moore, 1990). Choline is also used in the biosynthesis of the osmoprotectant glycine betaine in some plants (Rathinasabapathi et al., 1997). Another *N*-methyltransferase, β-alanine *N*-methyltransferase from the plant *Limonium latifolium*, is also involved in the biosynthesis of an osmoprotectant. This enzyme catalyzes the three sequential methyl group transfers that convert β-alanine (**24**) to the osmoprotectant β-alanine betaine (**25**) (Fig. 8B) (Raman and Rathinasabapathi, 2003).

Plant *N*-methyltransferases also participate in the biosynthesis of many alkaloids, some of which are pharmaceutically important. Methylation of putrescine to *N*-methylputrescine by putrescine *N*-methyltransferase is the first committed step in the biosynthesis of tropane, nicotine, and probably calystegine alkaloids in *Solanaceae* and *Convolvulaceae* (Sato et al., 2001; Stenzel et al., 2005; Zhang et al., 2004). Methylation of coclaurine to *N*-methylcoclaurine by coclaurine *N*-methyltransferase, along with the three *O*-methylations (see above), is a step in benzylisoquinoline alkaloid biosynthesis in *Coptis* (Choi et al., 2002). Three *N*-methylation steps are required for the biosynthesis of caffeine in tea and coffee (Fig. 9) (Ashihara et al., 1996; Kato et al., 2000; Kato et al., 1999; Mösli Waldhauser et al., 1997; Ogawa et al., 2001; Uefuji et al., 2003). The first step is methylation of xanthosine (**26**) by xanthosine methyltransferase, yielding 7-methylxanthosine (**27**). Ribose removal from this product is then catalyzed by 7-methylxathosine nucleosidase, yielding 7-methylxathine (**28**). Methylation of 7-methylxanthine (**28**) to 3,7-dimethylxanthine (theobromine) (**29**) is catalyzed by 7-methylxanthine methyltransferase (also known as theobromine synthase). Theobromine (**29**) is then methylated by 3,7-dimethylxanthine methyltransferase to yield 1,3,7-trimethylxanthine (caffeine) (**30**). Plants contain multiple enzymes, which differ in biochemical properties and substrate specificities, capable of catalyzing these methylation steps in vitro and possibly in vivo (Kato et al., 1999; Ogawa et al., 2001; Uefuji et al., 2003).

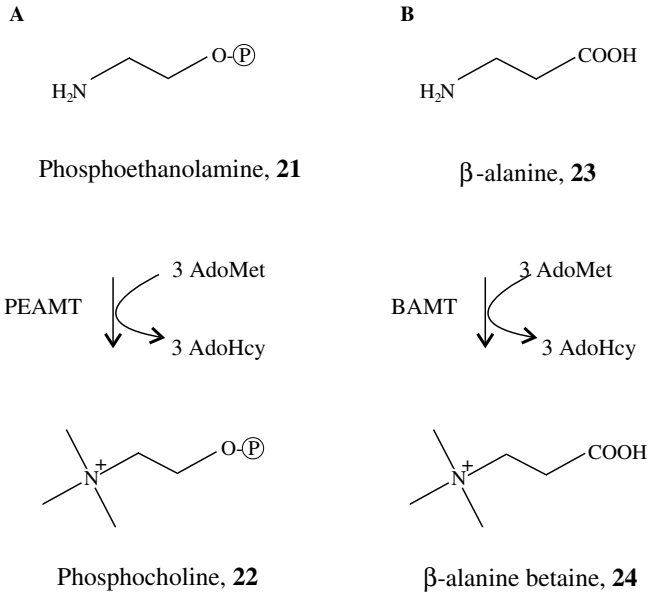


Fig. 8. Biosynthesis of the N-methylated products of phosphoethanolamine and β-alanine. (A) phosphocholine; (B) β-alanine betaine. PEAMT, phosphoethanolamine *N*-methyltransferase; BAMT, β-alanine *N*-methyltransferase.

6.3. *C*-methyltransferases

Only four types of *C*-methyltransferases have so far been identified in plants: two act on lipids (Bao et al., 2003; Nes, 2003), one catalyzes a step in the biosynthesis of the cofactor siroheme (Leustek et al., 1997), and one a step in the biosynthesis of vitamin E (Cho et al., 2005).

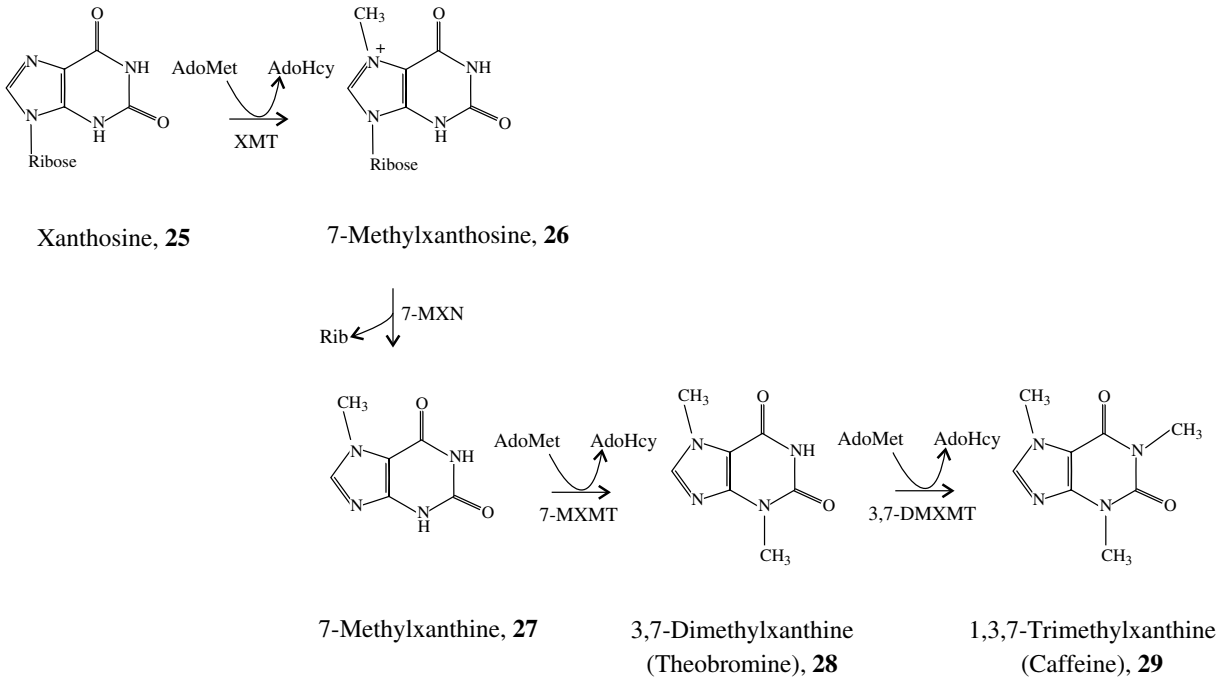


Fig. 9. Biosynthesis of caffeine. XMT, xanthosine methyltransferase; 7-MXN, 7-methylxanthosine nucleosidase; Rib, ribose; 7-MXMT, 7-methylxantine methyltransferase; 3,7-DMXMT, 3,7-dimethylxanthine methyltransferase.

Sterol methyltransferases catalyze addition of a methyl group to the carbon-24 of the side chain of C_{28} and C_{29} steroids. This addition creates a chiral center in the sterol side chain. The generated phytosterols become components of plant membranes, or are further metabolized to brassinosteroid hormones. *C*-methylation of phytosterols is one origin of their structural and functional diversity (Bouvier-Navé et al., 1998; Diener et al., 2000; Nes, 2003; Nes et al., 2003).

Cyclopropane fatty-acid synthases catalyze the addition of a methyl group across a double bond of unsaturated fatty acids to yield products containing a three-member carbocyclic ring (Fig. 10) (Bao et al., 2002; Bao et al., 2003). Carbocyclic fatty acids occur in several plant orders (Bao et al., 2002; Bohannon and Kleiman, 1978). Their functions may include carbon and energy storage in seeds (Kleiman et al., 1969), and resistance to fungal attack (Schmid and Patterson, 1988).

Uroporphyrinogen III methyltransferase acts on uroporphyrinogen III to yield precorrin-2. Precorrin-2 is a precursor of siroheme, the cofactor for sulfate and nitrate reductases. Uroporphyrinogen III methyltransferase from *Arabidopsis* has been cloned, and shown to be localized in plastids (Leustek et al., 1997). Another enzyme that participates in the cofactor metabolism is γ-tocopherol methyltransferase. This enzyme catalyzes methylation of γ-tocopherol (one chemical form of vitamin E) into the biologically more potent α-tocopherol (Cho et al., 2005).

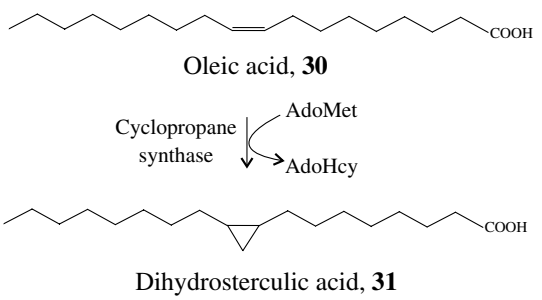


Fig. 10. The reaction catalyzed by cyclopropane fatty-acid synthase.

6.4. Thiol and halide ion methyltransferases

Thiol methyltransferases, found in *Brassicaceae*, act on various organic thiols such as thiophenol, thiocyanate, thiosalicylic acid, and 4,4′-thiobisbenzenethiol (Attieh et al., 2002; Attieh et al., 2000). The proposed role of these enzymes in vivo is methylation of the hydrolysis products of glucosinolates to volatile sulfur compounds, which are thought to participate in the defense against insects and pathogens (Attieh et al., 2002). These thiol methyltransferases also accept halide ions (chloride, bromide, and iodide) as substrates to produce methyl halides; these activities, however, are probably of little or no relevance in vivo (Attieh et al., 2000; Attieh et al., 1995).

Halide methyltransferases are present in the salt-marsh plant *Batis maritima* (Ni and Hager, 1998; Ni and Hager, 1999), and in the marine microalga *Pavlova pinguis* (Ohsawa et al., 2001). Thiols are not the preferred substrates of the enzyme from *B. maritima*. It is therefore improbable that this enzyme is involved in the metabolism of sulfur compounds (Ni and Hager, 1998). One view is that it maintains the concentration of chloride ions in the cytosol (Ni and Hager, 1999).

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