Methoxybenzaldehydes in plants: insight to the natural resources, isolation, application and biosynthesis

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Methoxybenzaldehydes in plants are one of the important groups of benzoate derivatives. Some of them, exhibiting refreshing fragrance can be used as flavouring ingredients in food and cosmetics. Therefore, they have important roles in food and cosmetic industries. Methoxybenzaldehydes also exhibit significant medicinal properties and thus have certain prospects in pharmaceutical industry. Biosynthesis of benzoic acid in plants has been explored in the last decade. There has been focus on benzaldehyde and methoxybenzaldehyde biosynthesis as well. There have been several studies regarding the biosynthetic route and mechanism of formation of benzaldehyde via benzoic acid from cinnamate and further addition of 'methoxy' group to it in the last few years. Still there are many ambiguities regarding the medicinal properties and biosynthesis of methoxybenzaldehydes. This review highlights the latest advances in fragrant methoxybenzaldehyde research and the knowledge gaps till date. The review also discusses the occurrence of methoxybenzaldehydes in plants, their separation methods, medicinal properties and biosynthesis.

Keywords: Biosynthesis, plants, medicinal properties, methoxybenzaldehyde, vanillin.

BIOSYNTHESIS of benzoic acid and its derivatives has been in focus in the last few years, but methoxybenzaldehyde biosynthesis remains a partially explored area[1]. Methoxybenzaldehydes generally have a characteristic fragrance and contain a single 'methoxy' group in the *ortho*, *para* or *meta* position of the benzene ring of benzaldehyde (Figure 1). They are regarded as natural products of plants with medicinal and industrial importance[2,3]. For example, vanillin, a popular and widely used flavouring methoxybenzaldehyde was an area of interest for plant biologists in the last decade due to its direct usage in food and beverage industries. Its specific use as flavouring agent as well as preservative makes it industrially important. On the other hand, ambiguities of its biosynthetic route have not yet been fully explored. Vanillin is extracted mainly from the orchid *Vanilla planifolia* and, to a lesser extent, from *Vanilla tahitienis* and *Vanilla pompona*[4]. Another interesting methoxybenzaldehyde, 2-hydroxy-4-methoxybenzaldehyde (MBALD), is found mainly in the roots of plants belonging to the family Apocynaceae. The root extracts are also used as flavouring agent, mostly in the southern part of India, specially as food and drink ingredient[5,6]. MBALD was reported to exhibit medicinal properties such as anti-acetylecholinesterase, antityrosinase and antileukemic activities[2,3,7,8]. There are many other plants which produce and accumulate different homologous methoxybenzaldehydes in different organs (Figure 1) and these have to be considered for future research in this area. In brief, the importance of methoxybenzaldehydes in natural products research cannot be overlooked as they have functional and potential roles in medicine, agriculture and industry. Therefore, up-to-date knowledge is required for further progress in methoxybenzaldehyde research. In this article, we discuss the available scientific literature regarding the occurrence of methoxybenzaldehydes in plants as natural products, methods of separation, medicinal properties and biosynthesis. Thus, we aim to compile information on the research in this field and review the latest advances in the fragrant methoxybenzaldehyde research.

Occurrence of methoxybenzaldehydes in plants

Accumulation of fragrant methoxybenzaldehydes in plant is reported mainly in roots, bark and pod, although there are examples of plants producing methoxybenzaldehydes in other organs like leaves and seeds. It has been reported that MBALD is the major chemical constituent (80%) found in the essential oils from root extract of *Hemidesmus indicus*[5]. Apart from *H. indicus*, *Decalepis hamiltonii* has also been reported to accumulate MBALD in roots as a chief chemical constituent (96%)[9,10] (Table 1). Accumulation of MBALD was found to be enhanced after chitosan and yeast extract treatment in *H. indicus* field roots[1,11]. Enhanced accumulation of MBALD was

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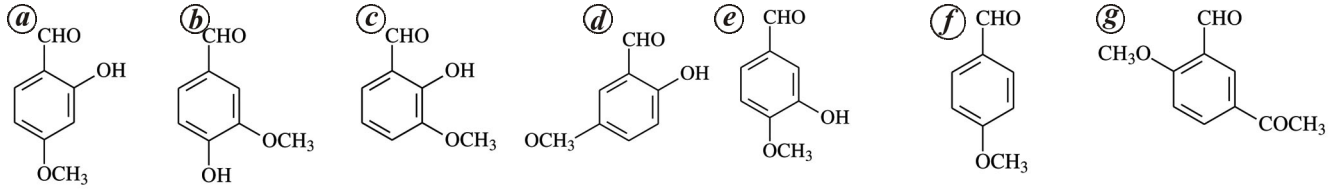

Figure 1. Different methoxybenzaldehydes found in plants. (***a***) 2-Hydroxy-4-methoxybenzaldehyde (MBALD); (***b***) 4-Hydroxy-3-methoxybenzaldehyde (vanillin); (***c***) 2-Hydroxy-3-methoxybenzaldehyde; (***d***) 2-Hydroxy-5-methoxybenzaldehyde; (***e***) 3-Hydroxy-4-methoxybenzaldehyde; (***f***) 4-Methoxybenzaldehyde (anisaldehyde) and (***g***) 3-Acetyl-6-methoxybenzaldehyde.

Table 1. Methoxybenzaldehydes in plants and their medicinal properties

Plant methoxybenzaldehydes	Plants	Medicinal properties	Reference
2-Hydroxy-4-methoxybenzaldehyde	*Hemidesmus indicus*, *Decalepis hamiltonii*, *Mondia whitei*, *Rhus vulgaris*, *Sclerocarya caffra*	Antibacterial, antifungal, anti-acetylcholinesterase, antityrosinase, antileukemic, larvicidal	2–5, 7–9, 21, 37
4-Hydroxy-3-methoxybenzaldehyde	*Vanilla planifolia* *Vanilla tahitienis* *Vanilla pompona*	Antifungal, antibacterial	30–34
2-Hydroxy-3-methoxybenzaldehyde	*Hemidesmus indicus*, *Mondia whitei*	NA	12
2-Hydroxy-5-methoxybenzaldehyde	*Periploca sepium*	NA	13
3-Hydroxy-4-methoxybenzaldehyde	*Mondia whitei*	Antileukemic	8
4-Methoxybenzaldehyde	*Pipinella anisum* L.	Antityrosinase, anticancerous, antifungal	13, 36
3-Acetyl-6-methoxybenzaldehyde	*Encelia farinosa*	NA	14

NA, not available.

also found in micro-propagated *Decalepis hamiltonii* upon treatment with triacontanol (TRIA)[10]. Some plants of families Asclepiaceae (*Mondia whitei*), Anacardiaceae (*Rhus vulgaris*) and Anacardiaceae (*Sclerocarya caffra*) of African origin were also found to accumulate MBALD[7]. In *M. whitei*, another aromatic methoxybenzaldehyde can be found which is called isovanillin (3-hydroxy-4-methoxybenzaldehyde)[12]. In *H. indicus* along with MBALD, isovanillin was also found in trace amount[7]. *Periploca sepium*, a traditionally used Chinese medicinal plant, accumulates 2-hydroxy-5-methoxybenzaldehyde and *o*-vanillin (2-hydroxy-3-methoxybenzaldehyde) in its root burk[13]. Anise (*Pipinella anisum* L., Apiaceae), a Mediterranean and South Asian (including India) annual herb is reported to accumulate *p*-anisaldehyde (4-methoxybenzaldehyde) in its seed oil[14] (Table 1). *Encelia farinosa*, a desert shrub in northwestern Mexico and southeastern United States was found to produce 3-acetyl-6-methoxybenzaldehyde in its leaves[15]. Dry beans of vanilla orchid, *V. planifolia* were found to accumulate 4-hydroxy-3-methoxybenzaldehyde (vanillin) as the major flavour compound, which is one of the world's most popular flavours[16]. Though the green beans of *V. planifolia* are flavourless, curing of the beans for a long time helps in the accumulation of this flavouring methoxybenzaldehyde[17]. The abundance of MBALD mostly in plant extracts suggests that this compound is present as soluble form in plant cells, whereas vanillin could also be found in cell wall-bound pool of phenolics as glucovanillin[18]. In most of the plants vanillin can be found in trace amounts, including tobacco[19].

Isolation of fragrant methoxybenzaldehydes from plants

Previously separation of fragrant methoxybenzaldehydes was based on thin layer chromatography (TLC) and gas chromatography[6,20]. MBALD from petroleum ether extract of culture was separated previously by TLC using petroleum ether and ethyl acetate (19 : 1; v/v and 95 : 5; v/v) as mobile phase[20,21]. MBALD was also isolated from root bark of *Periploca sepium* by silica gel column chromatography and TLC-based methods, where extraction was carried out by hydrodistillation and MBALD was separated from the essential oils[22]. In each case GC-MS, NMR or FT-IR was preferred for compound identification. As the TLC method was found to be problematic for preparative separation due to chances of impurities during collection of the separated compounds

Table 2. Bioactive doses of different methoxybenzaldehydes

Methoxybenzaldehydes	Bioactivity	Bioactive dosage	Reference
2-Hydroxy-4-methoxybenzaldehyde	Antibacterial, antifungal, antiacetylcholin-esterase, antityrosinase, antileukemic, larvicidal	*A. tumefaciens* (IC_{50}: 63.49 μg ml^{-1})	22
		E. coli (IC_{50}: 101.88 μg ml^{-1})	
		P. lachrymans (IC_{50}: 141.74 μg ml^{-1})	
		S. typhimurium (IC_{50}: 95.05 μg ml^{-1})	
		X. vesicatoria (IC_{50}: 131.86 μg ml^{-1})	
		B. subtilis (IC_{50}: 111.49 μg ml^{-1})	
		S. aurius (IC_{50}: 63.29 μg ml^{-1})	
		S. haemolyticus (IC_{50}: 161.9 μg ml^{-1})	
		C. albicans (IC_{50}: 99.99 μg ml^{-1})	22
		M. oryzae (IC_{50}: 161.90 μg ml^{-1})	
		IC_{50}: 0.047 mmol	2
		IC_{50}: 0.03 mmol	3, 7
		Effective at 0.54 μg ml^{-1} against Jurkat cells[a]	8
		LD_{50}: 22 μg ml^{-1}	38
4-Hydroxy-3-methoxybenzaldehyde	Antifungal, antibacterial, antiacetylcholin-esterase	MIC (mean): 1.69 μg ml^{-1} against mould	32, 33
		MIC (mean): 2.99 μg ml^{-1} against yeast	
		MIC: 15–75 mmol	34
		IC_{50}: 0.037 mmol	2
		IC_{50}: 0.037 μg ml^{-1}	
2-Hydroxy-3-methoxybenzaldehyde	NA	NA	NA
2-Hydroxy-5-methoxybenzaldehyde	NA	NA	NA
3-Hydroxy-4-methoxybenzaldehyde	Antileukemic, antityrosinase	Effective at 0.6 μg ml^{-1} against Jurkat cells[b]	8
		IC_{50}:0.32 mmol	37
4-Methoxybenzaldehyde	Anticancerous, antifungal	IC_{50}: 400–800 μg ml^{-1}	37
		MIC: 250-600 μg ml^{-1} against different Candida strains	35
3-Acetyl-6-methoxybenzaldehyde	NA	NA	NA

NA, Not available. a and b, Effective amounts of 2-hydroxy-4-methoxybenzaldehyde and 3-hydroxy-4-methoxybenzaldehyde were calculated according to the content present in *Hemidesmus indicus* root extract reported by Fimognari *et al.*[8].

and GC displayed a number of difficulties due to instability of the derivatized compounds and being destructive process by itself, both are not suitable for preparative work. Therefore, high performance liquid chromatography method was preferred for separation and preparative analysis of MBALD using Waters symmetry® reverse phase column and aqueous methanol as mobile phase (68 : 32). Methanolic extract (50%) of *H. indicus* roots was used for analysis and validation, and compound identified by electron spray ionization mass spectrophotometry (ESI-MS). Along with MBALD, 2-hydroxy-4-methoxybenzoic acid was also successfully separated by this method[6,10]. On the other hand, separation of vanillin has remained an area of interest in food and cosmetic industries for decades. Though numerous quality methods are available for separation and quantification of compounds from vanilla extracts, including TLC, HPTLC (high performance thin layer chromatography), isotope ratio MS and HPLC, the search continues for a simple and rapid HPLC-based method for isolation of this fragrant methoxybenzaldehyde from ethanolic vanilla pod extract[23–28]. A RP-HPLC-based method was developed for efficient separation of compounds using which maximum number of compounds, including vanillin has been efficiently separated in a single run using RP Purospher ®-Star RP-18e column and a photodiode array detector[29]. In this method, ACN/methanol (solvent A, 1 : 1 v/v) and water/acetic acid (solvent B, 99.8 : 0.2 v/v, pH 2.88) were used as mobile phase with a gradient elution[29]. Further, an ultra-high performance liquid chromatography (UHPLC) method was developed for rapid and better separation of vanillin from vanilla bean extract using a C_{18} RP column (ACQUITY UPLC BEH C_{18}, 50 mm × 2.1 mm, 1.7 μm particle size)[30]. Another rapid HPLC method for separation of vanillin was established, where Synergi Hydro-RP reverse phase column (4 μ, 250 mm × 4.60 mm) was used as stationary phase and water/methanol (68 : 32, v/v) as a mobile phase with an isocratic elution[2].

Medicinal properties

Antimicrobial activities

MBALD in the essential oil from the root bark of *P. sepium* showed activities against both bacteria and fungi. MIC values ranged from 80 to 250 μg ml^{-1}, and IC_{50} values from 63.29 to 161.90 μg ml^{-1}. On the other hand, MFC values ranged from 125 to 250 μg ml^{-1}. Amphotericin-

resistant strain of *Candida albicans* was found to be sensitive to MBALD (IC_{50} = 99.99 μg ml^{-1}), which showed its potential as an antimicrobial against antibiotic-resistant *C. albicans*. MBALD also exhibited potential activity against *Staphylococcus aureus* and *Staphylococcus haemolyticus* with IC_{50} values 63.29 and 78.74 μg ml^{-1} respectively[22]. Excluding these, MBALD showed antimicrobial activity against many other microorganisms (Table 2). Vanillin was found to exhibit antifungal activities[31,32]. Vanillin inhibited the growth of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Debaryomyces hansenii* and *Zygosaccharomyces rouxii* in culture medium and apple puree for 40 days when used at a concentration of ~13 mmol. However, it was less effective in banana puree, in which ~20 mmol was adequate to inhibit the growth of *Z. bailii*[32]. Vanillin (3–7 mmol) showed inhibition against the growth of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus parasiticus* for 2 months when applied to fruit-based agar (apple, banana, mango, papaya and pineapple)[31]. In addition, 10–20 mmol vanillin exhibited complete inhibition of growth of *Candida parapsilosis* and *S. cerevisiae* when applied in soft drinks over an 8-week storage period at 25°C (ref. 33). Study on the mode of action of vanillin against *E. coli*, *Lactobacillus plantarum* and *Listeria innocua* revealed vanillin as a primarily membrane-active compound[34]. Antifungal activity was shown by 4-methoxybenzaldehyde against different mould strains (mean mould MIC = 1.69 mmol) and yeast strains (mean yeast MIC = 2.99 mmol).

Anticancer activities

Some methoxybenzaldehydes showed anticancer activities. Methoxybenzaldehydes have shown promising inhibitory activity against cancerous cells. Anisaldehyde exhibited cytotoxic effect against MCF 7, Hep G2 and ME 180. IC_{50} values for these three cell lines were found to be 400, 600 and 800 μg ml^{-1} (ref. 35). Decocted extract of *H. indicus* root containing significant amount of MBALD and 3-hydroxy-4-methoxybenzaldehyde showed antileukemic effect in Jurkat cell lines[7]. Two synthetic methoxybenzaldehydes, 2-(benzyloxy)-4-methoxybenzaldehyde and 2-(benzyloxy)-5-methoxybenzaldehyde, exhibited potential anti-carcinogenic activity against HL-60 cell line[36].

Antityrosinase and antiacetylcholinesterase activities

MBALD exhibited potential inhibitory effect on tyrosinase. Both the monophenolase and diphenolase activities of tyrosinase were inhibited by MBALD. Monophenolase activity was inhibited at 0.5 mmol concentration, whereas ID_{50} value for inhibiting diphenolase activity was found to be 0.03 mM (refs 2, 7). In another report, anisaldehyde from *Pimpinella anisum* L. inhibited diphenolase activity of tyrosinase[37]. Both MBALD and vanillin showed mixed type of inhibition against acetylcholinesterase. But vanillin was found to be more potent (IC_{50} = 0.037 mmol) than MBALD (IC_{50} = 0.047 mmol) as inhibitory agent[2].

Larvicidal activities

MBALD isolated from *M. whitei* showed prospective larvicidal activity against *Anopheles gambiae* (LD_{50} = 22 μg ml^{-1}). Two other closely related congeners, 2-benzoyloxy-4-methoxybenzaldehyde and 2-benzyloxy-4-methoxybenzaldehyde, also showed larvicidal activity (LD_{50} = 22 and 10 μg ml^{-1} respectively)[38].

Biosynthesis of fragrant methoxybenzaldehydes

Biosynthesis of benzoic acid and its derivatives is an area of interest among plant biochemists because of their importance in defence and metabolism in plants. Despite this, biosynthesis of benzoic acid is not well-understood, and further advances in benzoic acid research revealed that its biosynthesis pathway provides a well-balanced control over synthesis and channelling intermediates to particular benzoic acid derivatives[39–41]. Benzoic acid and its derivatives were reported to originate from either phenylalanine or directly from shikimate-derived products such as isochorismate[42,43]. Thereafter, benzoic acid pathway at the transcript level was reported in root tissues of *Petunia hybrida*[44]. The β-oxidative pathway has also been explored[45].

Methoxybenzoates, being benzoic acid derivatives, fall under the area of interest in the context of benzoic acid metabolomics because some of them have fragrant and flavouring properties. Biosynthesis of methoxybenzaldehyde is directly related to benzoic acid biosynthesis as both follow similar central phenylpropanoid pathway. But the works reported on methoxybenzaldehyde biosynthesis do not complete the picture. Specifically, methoxybenzaldehydes, like MBALD and vanillin, which are accumulated in plants in significantly high amount, were found to be synthesized via phenylpropanoid pathway using cinnamate and hydroxycinnamate as their intermediates; although most of the enzymes involved in the pathway are not well characterized and some of them remain indeterminate[11,46]. Therefore, a proper and organized compilation of previous reports is needed for further research on elucidating methoxybenzaldehyde biosynthesis in plants.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) biosynthesis

The biosynthetic pathway of vanillin is considered first because of two reasons. The first one concerns with the

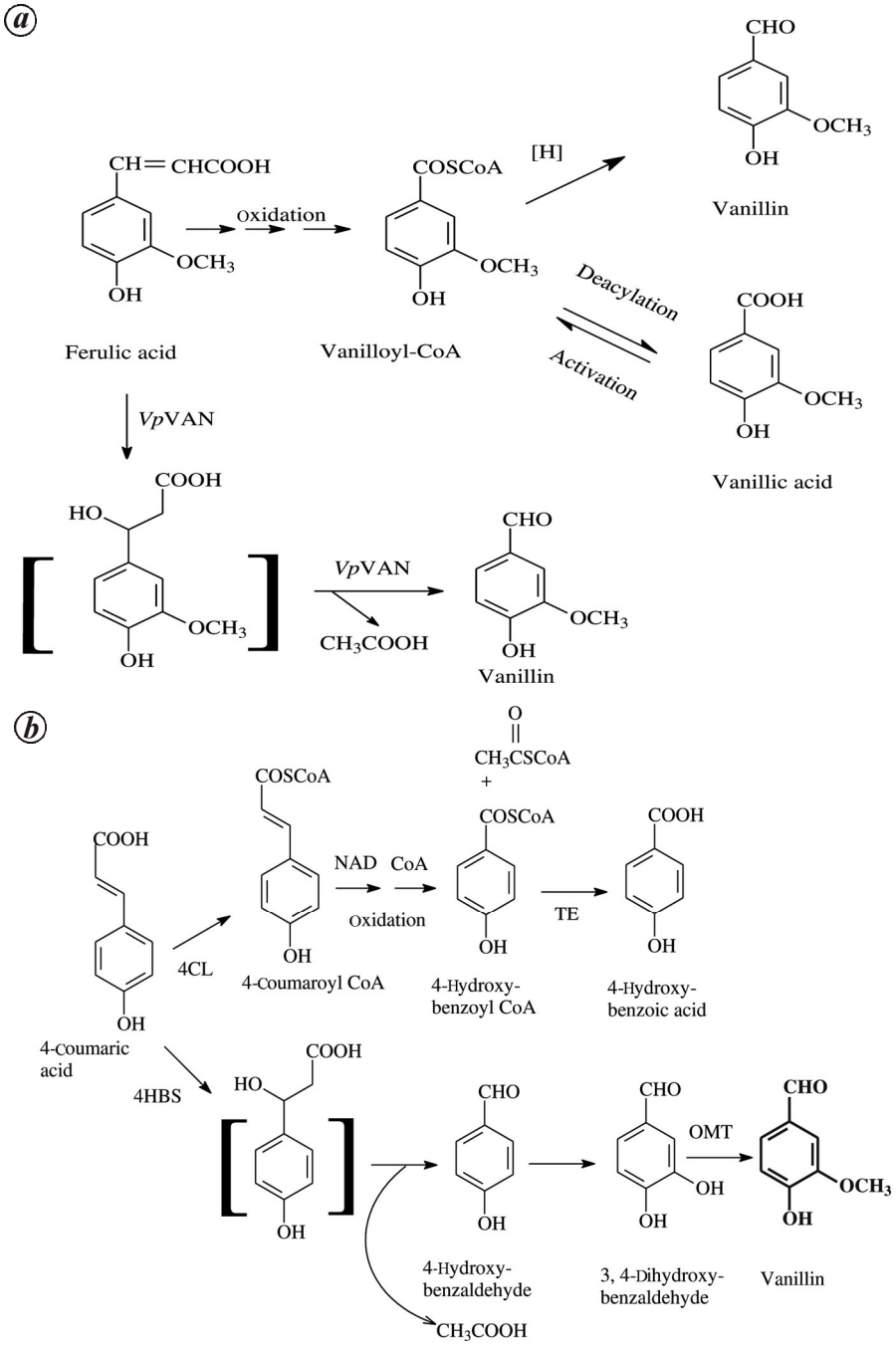

Figure 2. Proposed biosynthetic pathway of vanillin by (***a***) Zenk[49] and Gallage *et al.*[63]; (***b***) Podstolski *et al.*[16] and Pak *et al.*[64].

relationship of vanillin with the central phenylpropanoid pathway and benzoic acid biosynthesis, including 4-hydroxybenzoic acid and salicylic acid (2-hydroxybenzoic acid). The second is the possibility of biotechnological approaches for production of this commercially important flavouring compound to fulfil its worldwide demands.

The first part of vanillin biosynthetic pathway leads to the synthesis of phenylalanine via shikimate pathway, which was generally accepted by different groups of investigators and they executed the experiments in either green beans or in cell cultures[16]. Vanillin is present in the green beans exclusively in conjugated form with β-D-glucoside. This conjugated form does not show any flavouring characteristics. The vanilla flavour is developed during the 'curing' process after harvesting the beans approximately 6–8 months after pollination. During the curing process, interaction of vanillin-β-D-glucoside with

β-D-glucosidase releases free vanillin[16,46]. In another report, it was proposed that there is a pathway of glucovanillin formation from the glucoside of the 4-hydroxybenzyl alcohol after analysis of glucosides in green vanilla pods[47]. The mechanism of vanillin-β-D-glucoside formation is not clear because of ambiguities in the chain-shortening mechanism and other reactions that result in the conversion of *trans*-cinnamate to vanillin-β-D-glucoside via vanillin. An early report of vanillin and vanillic acid production from ferulic acid suggested a CoA-dependent β-oxidative cleavage of side chain of feruloyl-CoA leading the formation of vanilloyl-CoA that is further reduced to vanillin; simultaneously, an alternative deacylation leads to formation of vanillic acid (Figure 3 *a*)[48]. The route of this pathway is supported by radiolabelling studies[49]. In a recent study, ferulic acid was reported as a good precursor of vanillin, where it was proposed that the methyl group is donated by methionine to phenylpropanoids via *S*-adenosylmethionine[50]. The authors also depicted that vanillin can be directly synthesized via ferulic acid and immediately glycosilated as glucovanillin. If levels become high, 4-coumaric acid, ferulic acid, 4-hydroxybenzaldehyde (4-HBALD), 4-hydroxybenzyl alcohol, and vanillin are immediately glucosylated to form the respective glucose esters of C_6–C_3 compounds and glucosides of C_6–C_1 compounds due to their toxicity in free condition. Reports have also shown that addition of ferulic acid to *V. planifolia* cell culture or tissue culture increased the levels of vanillin production[51,52]. A more complex pathway was proposed for *V. planifolia* cell culture on the basis of the results of feeding radiolabelled compounds to its tissue cultures[53,54]. According to this proposal, caffeic acid (3,4-dihydroxycinnamic acid) produced via phynylpropanoid pathway is methylated at 4′ position to produce isoferulic acid (3-hydroxy-4-methoxycinnamic acid), which is further methylated at 3′ position to produce 3,4-dimethoxycinnamic acid followed by 4′ demethylation, glucosilation and a late-stage side-chain shortening (Figure 4). This late-stage side-chain shortening results in the production of vanillic acid (or its β-D-glucoside), which is further reduced to vanillin. Another non-oxidative route for vanillin biosynthesis was proposed for conversion of 4-coumaric acid to 4-HBALD, a possible precursor of vanillin biosynthesis. This type of reaction was proposed to occur during biosynthesis of 4-hydroxybenzoic acid in potato tubers, elicited carrot cell cultures and in elicited carrot hairy root cultures and in an alternative pathway to shikonin in *Lithospermum* erythrorhizon[55–58]. It was reported that vanillin is produced from feruloyl-CoA through a non-oxidative process by a catalytic enzyme enoyl-SCoA hydratase/isomerase family[59,60]. *V. planifolia* cell culture was shown to synthesize 4-HBALD with other benzoic acid derivatives, including 4-coumaric acid, 4-hydroxybenzyl alcohol, 3,4-dihydroxybenzaldehyde (3,4-DHBALD), 4-hydroxy-3-methoxybenzyl alcohol and vanillin with a consistency of pattern and levels of these metabolites[61]. Further, a thiol-dependent but cofactor-independent hydroxybenzaldehyde synthase (HBS) was evident and purified from *V. planifolia* cell culture that was shown to be responsible for side-chain shortening mechanism and produced 4-HBALD directly from 4-coumaric acid which indicated 4-HBALD as an intermediate in vanillin biosynthesis. This enzyme hydrolyses isolated-double bond at alpha position of the carboxylic group of 4-coumaric acid with subsequent cleavage of the side chain, thus yielding acetate and 4-HBALD (Figure 2 *b*). When the substrate

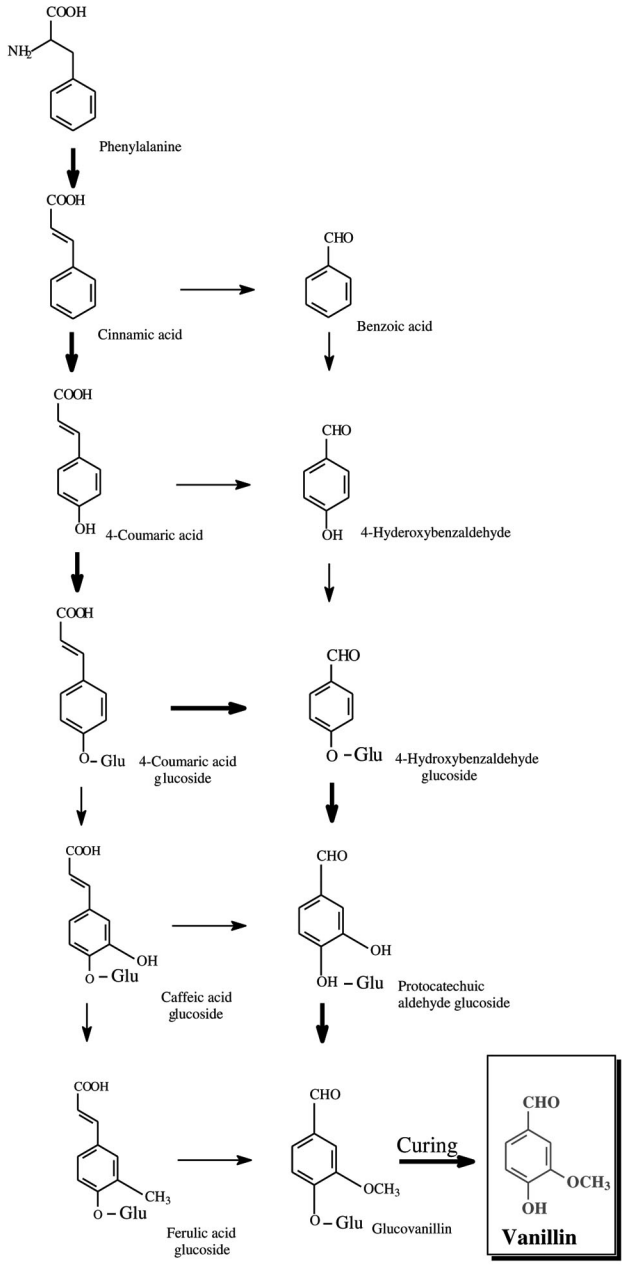

Figure 3. Pathway of vanillin biosynthesis proposed by Dignum *et al.*[17].

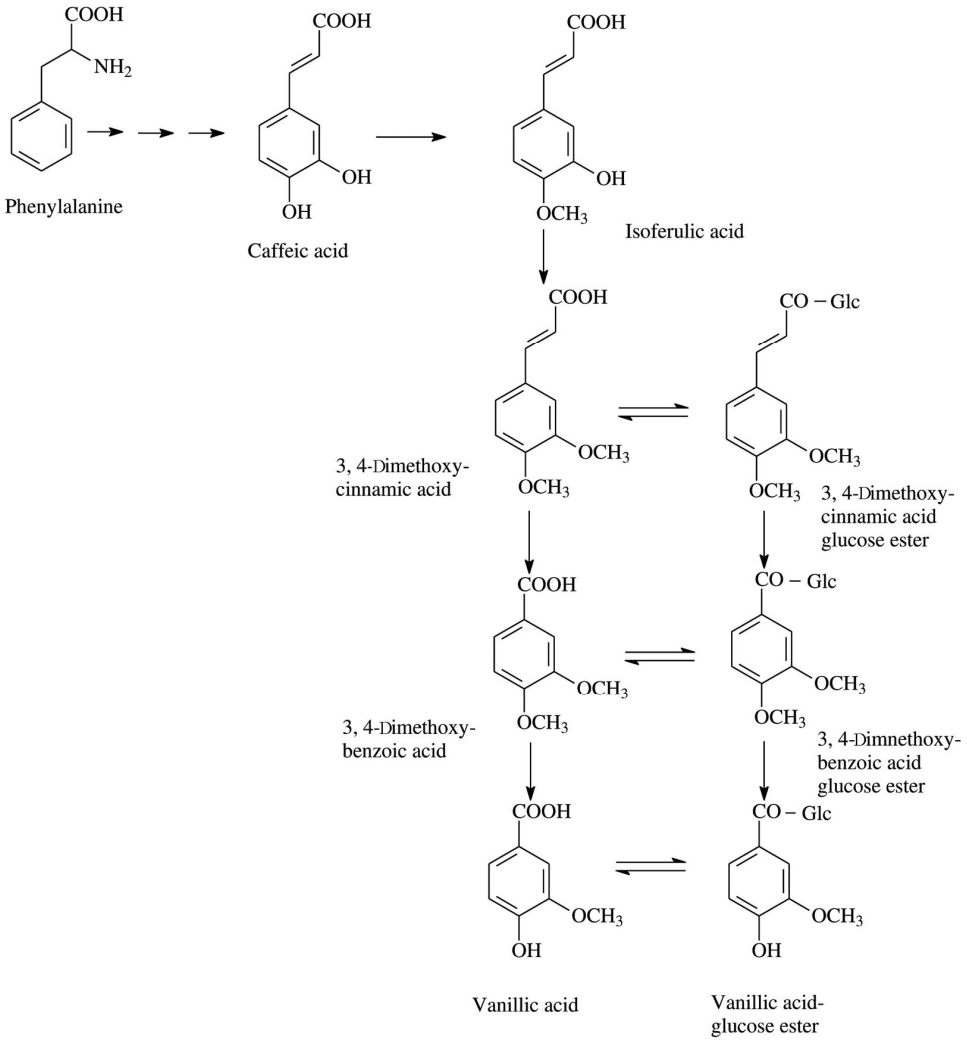

Figure 4. Proposed biosynthetic pathway of vanillic acid by Funk and Brodelius[54,55].

specificity was tested, it showed maximum activity with 4-coumaric acid rather than *o*-coumaric acid, caffeic acid, cinnamic acid and sinapic acid. Ferulic acid also showed only 2% of activity with 4-coumaric acid, which corroborated the previous proposal (Figure 3) that 4-coumaric acid is the major precursor of vanillin biosynthesis rather than ferulic acid[15,16]. This interpretation about involvement of HBS was supported by the results of feeding experiments in *V. planifolia* cultures, in which 4-HBALD accumulated after feeding 4-coumaric acid[62]. On the contrary, according to Gallage *et al.*[63], side-chain shortening occurred in ferulic acid, which produced vanillin by direct conversion. Recently, this group identified a gene *VpVAN* in *V. planifolia* that encoded an enzyme having C_2 side-chain shortening activity (Figure 3 *a*). The next step after 4-HBALD biosynthesis is hydroxylation at position 3′ on the ring, which results in 3,4-DHBALD or protocatechuic aldehyde. This 3′ hydroxyl group is then methylated, producing vanillin. Excluding HBS, none of the proposed enzymes was characterized until evidence of a multifunctional *O*-methyltransferase (OMT) was described in *V. planifolia*[64]. The authors purified and characterized this enzyme by cloning and functional expression directly from *V. planifolia* tissue that revealed it to be a 40.66 kDa protein after affinity purification. This enzyme was proved to methylate the 3′ hydroxyl group of a broad range of substrates, including 3,4-DHBALD. Preference of caffoeoyl aldehyde and 5-OH-coniferaldehyde over 5-OH-ferulic acid, 3,4-DHBALD and caffeic acid suggested the involvement of this OMT in lignin biosynthesis. Though multiple plausible outlines have been suggested for biosynthesis of vanillin, ambiguities still remain about the precursor of vanillin, intermediate compounds and the enzymes involved. The identification and characterization of HBS, HCHL and VpVAN enzymes proposed that they are either encoded by the same gene or different isoforms. They are involved in similar chain-shortening mechanism on either 4-coumaric acid or ferulic acid,

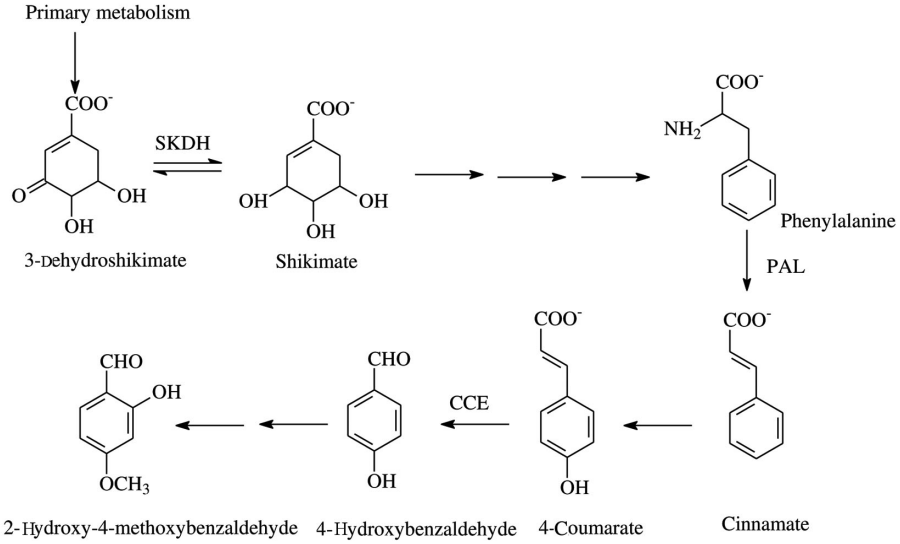

Figure 5. Proposed biosynthetic pathway 2-hydroxy-4-methoxybenzaldehyde by Kundu *et al.*[11].

which leads to vanillin production. Thus, due to divergence in the reports, this is clearly a fertile area for future research.

MBALD (2-hydroxy-4-methoxybenzaldehyde) biosynthesis

After discovering the production of MBALD in different plant systems, it was obvious to conduct research to know its biosynthetic pathway[9,10,12,65]. In recent years, the biosynthetic route of another fragrant methoxybenzaldehyde, MBALD has been explored mainly in a well-known Indian medicinal plant, *H. indicus*, commonly known as 'Indian Sarsaparilla'. The fragrant mature root of this plant contains significant amount of MBALD (about 3.2 ± 0.2 mg/g dry wt)[6]. Phenylalanine ammonia-lyase (PAL), the first enzyme of phenylpropanoid pathway was found to be directly involved in MBALD biosynthesis. Inhibition of PAL by aminooxyacetic acid reduced the amount of MBALD in elicited roots of *H. indicus*. Elicitation is a standard method to enhance the phenolic metabolism in excised plant tissue, organs and cell cultures[66]. In chitosan-elicited root, both PAL activity and MBALD content increased significantly, which clearly indicates biosynthesis of MBALD is PAL-mediated and it follows the central phenylpropanoid pathway[1,11]. Being a homologue of vanillin, formation of MBALD was further investigated by following the hydroxybenzoic acid biosynthetic pathway. In another report, correlation of shikimate pathway with MBALD biosynthesis has been established considering the possible biosynthetic path in yeast extract-elicited *H. indicus* roots[11]. Inhibition of shikimate pathway with glyphosate, a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitor, was found to result in reduction of MBALD in the root along with the increment of shikimic acid. Glyphosate treatment also reduced the PAL activity. An evidence of C_2 side-chain cleavage activity was also found in *H. indicus* roots that catalysed the C_2 side-chain cleavage of the C_6–C_3 compound (4-coumaric acid) leading to the formation of C_6–C_1 compound (4-HBALD; Figure 5). This phenomenon supports the proposed pathway of vanillin biosynthesis through the formation of hydroxybenzaldehyde by a chain-cleaving mechanism. Inhibition of shikimate pathway decreased this C_2 chain-cleaving enzyme, which also emphasizes that shikimate pathway modulates the downstream enzymes involved in MBALD biosynthesis. There are no reports available on the downstream enzymes of MBALD biosynthesis after C_2 side-chain shortening. Therefore, there is scope in this area to conduct research in future.

Conclusion and perspectives

Methoxybenzaldehydes are one of the families of major hydroxycinnamate derivatives. They have industrial importance for their flavouring properties as well as medicinal values. In the last few years, different bioactive characteristics of methoxybenzaldehydes from different plants have been explored. For example, antityrosinase activity of MBALD, inducing effect of decocted extract of *H. indicus* root containing significant amount of MBALD and 3-hydroxy-4-methoxybenzaldehyde showing antileukemic effect, acetylcholinesterase inhibitory potential of both the MBALD and vanillin. This information establishes methoxybenzaldehydes as a promising group of compounds with simple, small structure from the pharmacological and industrial perspective. Therefore, it is important to study the biosynthetic routes of methoxybenzaldehydes including characterization of the

involved enzymes. It will be useful for metabolic engineering of the methoxybenzaldehydes producing plants to increase the methoxybenzaldehyde contents in plants for industrial purposes.

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