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Chemical structures, biosynthesis, bioactivities, biocatalysis and semisynthesis of tobacco cembranoids: An overview

Ning Yan, Yongmei Du, Xinmin Liu, Hongbo Zhang, Yanhua Liu, Peng Zhang, Daping Gong, Zhongfeng Zhang*

Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao 266101, China

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ABSTRACT

Cembranoids, 14-membered carbocyclic diterpenes composed of four isoprene units, are mainly found in plants of the *Nicotiana* and *Pinus* genera, as well as in soft coral and other marine organisms. We summarise the chemical structures of the eighty-seven naturally occurring tobacco cembranoids (**1–52**), nor-cembranoids (**53–74**), seco-cembranoids (**75–82**) and cyclised cembranoids (**83–87**) reported to date. The most prevalent cembranoids in tobacco are (1S,2E,4S,7E,11E)-cembra-2,7,11-triene-4,6-diol (**1**) and (1S,2E,4R,7E,11E)-cembra-2,7,11-triene-4,6-diol (**2**), which are biosynthesised in the plastid from the reactions of geranylgeranyl diphosphate with cembrantrien-ol synthase and cytochrome P450 hydroxylase via the 2-C-methyl-D-erythritol-4-phosphate metabolic pathway. The accumulation of cembranoids in tobacco is affected by genetic and environmental factors, including fertiliser, water and light exposure, soil-type and temperature. Tobacco-derived cembranoids are known to display antifungal, antibacterial, anti-human immunodeficiency virus, anti-tumour and neuroprotective properties. A large number of biologically active (primarily anti-invasive, anti-proliferative, anti-migratory and neuroprotective) compounds have been prepared from the semisynthesis of tobacco cembranoids, many via biocatalytic methods. The documented bioactivities of tobacco cembranoids make them excellent templates for the future development of drugs to treat cancer, AIDS, Alzheimer's disease, Parkinson's disease, stroke and other neurodegenerative diseases. These findings indicated that although tobacco has long been associated with negative effects on human health, it may ultimately be able to play a therapeutic role.

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Abbreviations: 8-OH-CPP, 8-α-hydroxy-copalyl-pyrophosphate; AChRs, acetylcholine receptors; AIDS, acquired immunodeficiency syndrome; CBT-diol, cembratrien-diol; CBT-ol, cembratrien-ol; CBTS, cembrantrien-ol synthase; CDK, cyclin-dependent protein kinase; CYP450, cytochrome P450 hydroxylase; DMAPP, dimethylallyl diphosphate; DFP, diisopropylfluorophosphate; EA, early antigen; EBV, Epstein–Barr virus; EC_{50}, concentration for 50% of maximal effect; ERK, extracellular signal regulated kinase-1,2; GGPP, geranylgeranyl diphosphate; HIV, human immunodeficiency virus; IC_{50}, 50% inhibitory concentration; IPP, isopentenyl diphosphate; MDR, multidrug resistance; MEP, 2-C-methyl-D-erythritol-4-phosphate; nAChR, nicotinic acetylcholine receptor; NMDA, *N*-methyl-D-aspartate; OP, organophosphate; PI3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; SAR, structure–activity relationship; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

* Corresponding author at: 11th Keyuan Jingsi Rd., Laoshan Dist. of Qingdao, Shandong Prov. 266071, China. Fax: +86 532 88702239.
E-mail address: zhangzhongfeng@caas.cn (Z. Zhang).

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1. Introduction

Cembranoids are a type of macrocyclic diterpene consisting of four isoprene units bonded 'head to tail'. These natural products feature a 14-carbon macrocyclic skeleton, three symmetrically distributed methyl groups, a lone isopropyl group and a plane of symmetry along the C1–C8 axis of the core (Fig. 1; Sun and Li, 2011). Cembranoids are primarily distributed in plants of the *Nicotiana* and *Pinus* genera, as well as in marine organisms (e.g. soft coral), where they are thought to play a role in survival (El Sayed and Sylvester, 2007; Liu et al., 2015; Sun and Li, 2011). The first cembranoid-type compound reported was found in the oleoresin secreted from the trunk of a pine tree (Dauben et al., 1962). Shortly thereafter came the identification of cembratrien-diol (CBT-diol) in tobacco, which existed as α-CBT-diol (**1**) and β-CBT-diol (**2**) (Roberts and Rowland, 1962). In plants of the *Nicotiana* genus, cembranoids are most prevalent in the secretions on the surface of the leaves and flowers (Severson et al., 1985). Since the identification of α- and β-CBT-ol in 1962 (Roberts and Rowland, 1962), tremendous advances have been made regarding the chemical structures, biosynthesis, bioactivities, biocatalysis and semisynthesis of tobacco cembranoids. For example, El Sayed and Sylvester (2007) were one of the first to summarise the anti-tumour activity of these compounds. In recent years, significant progress has been made in studies of the antimicrobial (Aqil et al., 2011; Duan et al., 2015; Ferchmin et al., 2014b; Rodriguez et al., 2010, 2011), anti-tumour (El Sayed et al., 2011; Nacoulma et al., 2013; Zubair et al., 2014) and neuroprotective activities (Eterović et al., 2011, 2013; Ferchmin et al., 2014a, 2015; Martins et al., 2015; Vélez-Carrasco et al., 2015) of tobacco cembranoids. In addition, a range of studies have investigated their biocatalysis (Baraka et al., 2011; El Sayed et al., 2008a,b; Le-Huu et al., 2015), semisynthesis (Baraka et al., 2011; El Sayed et al., 2008a,b; Eterović et al., 2013) and the factors affecting their accumulation (Li et al., 2011; Wang et al., 2014a,b; Yang et al., 2014; Zhu et al., 2011). In addition, Wang et al. (2014c) summarised the biosynthetic pathways and regulatory factors central to tobacco cembranoids. Therefore, this review summarises the chemical structures and key properties/activities of tobacco cembranoids in order to provide an overview of current understanding of their development and utilisation.

2. Chemical structures of tobacco cembranoids

We have provided the chemical structures of the eighty-seven naturally occurring tobacco cembranoids (**1**–**52**), nor-cembranoids (**53**–**74**), seco-cembranoids (**75**–**82**) and cyclised cembranoids (**83**–**87**) that have been reported to date in Figs. 1–5.

2.1. Cembranoids

The first cembranoid-type compound reported in tobacco was CBT-diol, which existed as [(1S,2E,4S,7E,11E)-cembra-2,7,11-triene-4,6-diol (α-CBT-diol, **1**) and (1S,2E,4R,7E,11E)-cembra-2,7,11-triene-4,6-diol (β-CBT-diol, **2**)] (Fig. 1; Roberts and Rowland, 1962). Cembratrien-ol (CBT-ol) also exists as two isomers, (1S,2E,4S,7E,11E)-cembra-2,7,11-triene-4-ol (α-CBT-ol, **3**) and (1S,2E,4R,7E,11E)-cembra-2,7,11-triene-4-ol (β-CBT-ol, **4**) (Fig. 1). Among cultivated tobacco varieties, the CBT-diol content is typically at least 100 times higher than that of CBT-ol, thus α-CBT-diol (**1**) and β-CBT-diol (**2**) are the predominant cembranoid compounds in these plants (Guo and Wagner, 1995a). α-CBT-diol (**1**) and β-CBT-diol (**2**) are key metabolites in the biosynthesis of the other cembranic compounds (Wahlberg and Enzell, 1984). Wahlberg and Enzell (1984) reported these biogenetic reactions and provided the chemical structures of eighteen tobacco cembranoids (**1–18**) (Fig. 1). Most of these cembranoids had a hydroxyl substituent at C-4 and were commonly divided into two series: those having a 4R- and those having a 4S-configuration (Wahlberg and Enzell, 1984).

The hydroxyl groups (**7**–**25**) were identified at C-8, C-10, C-11, C-12, C-13 or C-20 (Fig. 1). Among these, cembranoids **7–9** were identified as (1S,2E,4R,6R,7E,11S)-, (1S,2E,4S,6R,7E,11S)-, and (1S,2E,4S,6R,7E,11R)-2,7,12(20)-cembratriene-4,6,11-triols (Wahlberg and Enzell, 1984). The 4,6,12-triols (**10–13**) all had 1S,2E,6R,7E,10E-configurations and were diastereoisomers with respect to the configurations of C-4 and C-12 (Wahlberg and Enzell, 1984). Olsson et al. (1991) isolated five new cembranoids from Greek tobacco flowers: (1S,2E,4S,6E,8S,11S)-2,6,12(20)-cembratriene-4,8,11-triol (**14**), the 12S- and 12R-epimers of (1S,2E,4S,6E,8S,10E)-2,6,10-cenbratriene-4,8,12-triol (**15** and **16**) and the 12S- and 12R-epimers of (1S,2E,4R,6E,8S,10E)-2,6,10-cenbratriene-4,8,12-triol (**17** and **18**). Forsblom et al. (1993) isolated two new cembranoids from Greek tobacco flowers: (1S,2E,4R,6R,7E,11E,13R)-2,7,11-cembratriene-4,6,20-triol (**19**) and (1S,2E,4S,6R,7E,11Z)-2,7,11-cembratriene-4,6,20-triol (**20**). Five new cembranoids were isolated from Greek tobacco flowers and shown to be (1S,2E,4S,6R,7E,10R,11E)-2,7,11-cembratriene-4,6,10-triol (**21**), the corresponding (10S)-, (4R*)- and (4R*,10S)-diastereoisomers (**22**–**24**) and (1S*,2E,4R*,6R*,7E,10S,11Z)-2,7,11-cembratriene-4,6,10-triol (**25**) (Olsson et al., 1993).

The hydroperoxyl groups (**26–30**) were identified at C-11 or C-12 (Fig. 2). These cembranoids were identified as the (1S,2E,4R,6R,7E,11S)- and (1S,2E,4S,6R,7E,11S)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diols (**26** and **27**) and the (1S,2E,4R,6R,7E,10E,12S)-, (1S,2E,4S,6R,7E,10E,12S)- and (1S,2E,4S,6R,7E,10E,12R)-12-hydroperoxy-2,7,10-cembratriene-4,6-diols (**28–30**) (Wahlberg and Enzell, 1984).

The methyl ethers (**31–38**) were identified at C-4, C-6 or C-8 (Fig. 2). Among these, 4-*O*-methyl-(1S,2E,4R,7E,11E)-2,7,11-cembratriene-4,6-diol (**32**), 4-*O*, 6-*O*-methyl-(1S,2E,4R,7E,11E)-2,7,11-cembratriene-4,6-diol (**36**) and 4-*O*, 8-*O*-dimethyl-(1S,2E,4R,6E,8S,11E)-2,6,11-cembratriene-4,8-diol (**37**) were identified as new natural products from tobacco in 1983 (Bylov et al., 1983). Baraka et al. (2011) isolated