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Molecules of Interest

Noscapine comes of age

Xue Chen [1], Thu-Thuy T. Dang [1], Peter J. Facchini *

Department of Biological Sciences, University of Calgary, Alberta, Canada

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ABSTRACT

Noscapine is a phthalideisoquinoline alkaloid, which represents a class of plant specialized metabolites within the large and structurally diverse group of benzylisoquinoline alkaloids. Along with the narcotic analgesic morphine, noscapine is a major alkaloid in the latex of opium poppy (*Papaver somniferum*) that has long been used as a cough suppressant and has undergone extensive investigation as a potential anticancer drug. Cultivated opium poppy plants remain the only commercial source of noscapine. Despite its isolation from opium more than two centuries ago, the almost complete biosynthesis of noscapine has only recently been established based on an impressive combination of molecular genetics, functional genomics, and metabolic biochemistry. In this review, we provide a historical account of noscapine from its discovery through to initial investigations of its formation in opium poppy. We also describe recent breakthroughs that have led to an elucidation of the noscapine biosynthetic pathway, and we discuss the pharmacological properties that have prompted intensive evaluation of the potential pharmaceutical applications of noscapine and several semi-synthetic derivatives. Finally, we speculate on the future potential for the production of noscapine using metabolic engineering and synthetic biology in plants and microbes.

1. Introduction

Phthalideisoquinoline alkaloids are a class of benzylisoquinoline alkaloids (BIAs) that can be divided into two subgroups depending on the configuration of the isoquinoline moiety. Classic phthalideisoquinolines possess an intact tetracyclic skeleton, whereas secophthalideisoquinolines display a cleavage on the B ring of the isoquinoline group resulting in the formation of a dimethylaminoethyl side chain (Blasko et al., 1982; MacLean, 1985). Examples of several phthalideisoquinoline and secophthalideisoquinoline alkaloids are shown in Fig. 1. Phthalideisoquinolines exhibit a broader taxonomic distribution occurring in the plant families Papaveraceae, Berberidaceae, and Ranunculaceae. In contrast, secophthalideisoquinolines are found exclusively in the Papaveraceae.

The phthalideisoquinoline noscapine was one of the first isolated alkaloids and continues to draw considerable attention owing to its important pharmacological properties. Noscapine has long been used as a cough suppressant (La Barre and Plisner, 1959; Put et al., 1974) and, more recently, the inhibitory effects of noscapine on the growth of various tumors have raised awareness of its potential as an anticancer drug (Ye et al., 1998; Mahmoudian and Rahimi-Moghaddam, 2009; Fang et al., 2012). Opium poppy (*Papaver somniferum*) remains the only commercial source for noscapine, in addition to the narcotic analgesics morphine, codeine, and semi-synthetic derivatives of thebaine. The *de novo* chemical synthesis of noscapine is hindered by the occurrence of two chiral centers in the molecule. However, despite the passage of more than two centuries since its discovery and extensive investigation of its pharmaceutical potential, the chemistry and molecular biochemistry of noscapine biosynthesis have only recently been established. In this review, we provide a historical account of the discovery, structural elucidation, and pharmacological investigation of noscapine and other phthalideisoquinoline alkaloids. The rapid advances in biochemical genomics that led to an elucidation of noscapine biosynthesis in opium poppy are discussed. Finally, we speculate on the biotechnological potential for the production of noscapine-based pharmaceuticals in plants and microbes using metabolic engineering and synthetic biology.

2. Discovery, structural elucidation, and chemical synthesis of noscapine

2.1. Discovery

Noscapine was first isolated from opium in 1803 by the French manufacturing chemist Charles Derosne, who named the compound "sel narcotique de Derosne" (Derosne's salt). Pierre Jean Robiquet later demonstrated that Derosne's salt was not a morphine

* Corresponding author. Tel.: +1 403 220 7651; fax: +1 403 298 9311.
E-mail address: pfacchin@ucalgary.ca (P.J. Facchini).
[1] Authors contributed equally to this work.

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meconate, but rather a new compound that he called narcotine (Robiquet, 1817) and suggested the elemental formula $C_{23}H_{25}O_7N$, which was later corrected to $C_{22}H_{23}O_7N$ (Matthiessen and Foster, 1863). The hydrolysis products of noscapine, hydrocotarnine and opianic acid, were later used to construct a structural formula for the alkaloid (Roser, 1888), which was later confirmed via the synthesis of noscapine from meconine and cotarnine (Perkin and Robinson, 1911; Robinson, 1917) or the synthesis of isonoscapine from opianic acid and hydrocortanine (Jones et al., 1912). The related compound narcotoline (Fig. 1) was also isolated from opium (Pfeifer and Weiss, 1955; Pfeifer, 1957) and opium poppy seed capsules (Baumgarten and Christ, 1950; Bognár et al., 1967). Noscapine and narcotoline are classified as classic phthalideisoquinolines owing to their intact tetracyclic scaffolds, and are confined to members of the genus *Papaver* (Papaveraceae). Noscapine is the second most abundant alkaloid in opium poppy latex after morphine, with the highest content found in Russian and Indian opium at 12 and 7.7%, respectively (Bernath, 2006).

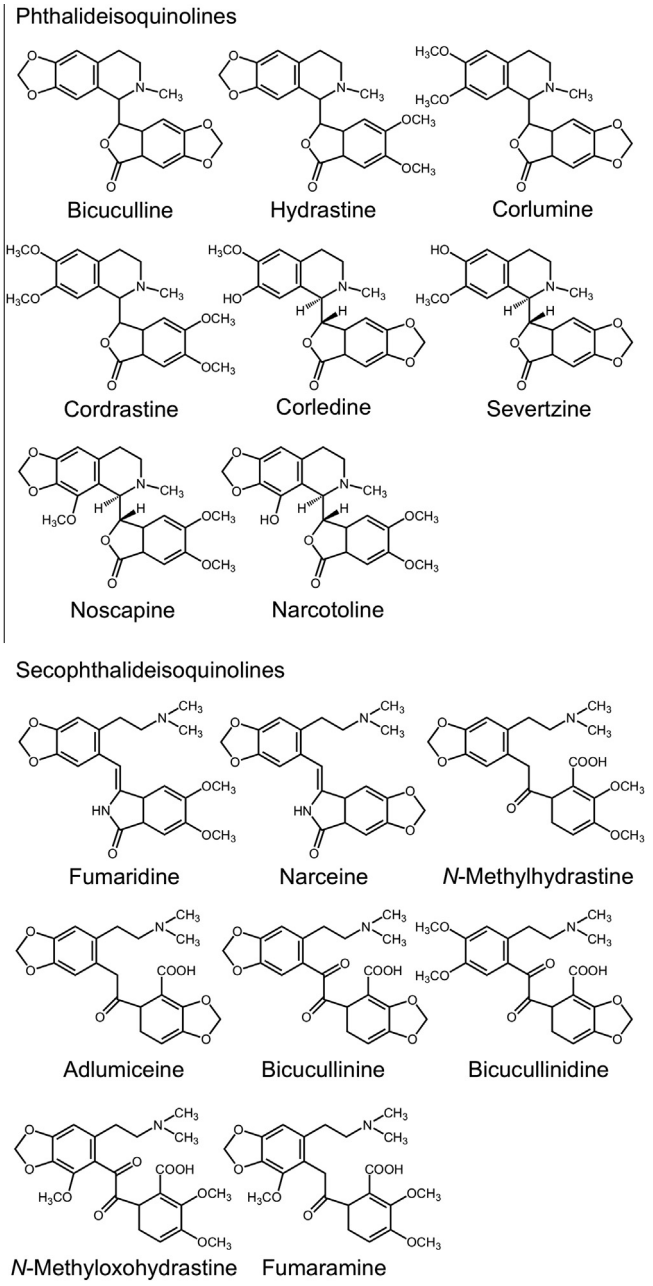

Fig. 1. Several phthalideisoquinoline and secoisoquinoline alkaloids found in plants.

2.2. Chemical and structural characterization

Like most other phthalideisoquinolines (Fig. 1), noscapine is oxygenated at C-6, C-7, C-4′ and C-5′, and contains asymmetric centers at C-1 and C-9. However, noscapine and narcotoline bear an extra oxygen at C-8 compared with other phthalideisoquinolines (Manske, 1944; Blasko et al., 1982). Noscapine is a water insoluble, weak tertiary base that forms unstable salts in dilute acids (pH ~3) and, thus, can be extracted in solvents such as chloroform (Annett, 1923; Annett and Bose, 1923; Barbier, 1950; Ugai, 1960). Several methods have been reported for the isolation and extraction of noscapine from opium (Ramanathan and Chandra, 1981), opium poppy straw (Bayer, 1961), or unripe opium poppy seed capsules using the Kabay method (Biniecki and Rylski, 1956; Peyroux et al., 1972) or chromatography (Ayyangar and Bhide, 1988).

The stereochemistry of noscapine was determined by optical rotatory dispersion (Ohta et al., 1963, 1964) based on the absolute stereochemistry of hydrastine (Battersby and Spencer, 1964, 1965). The structural properties of noscapine, including ^1H and ^{13}C NMR chemical shift assignments (Uhrín and Proksa, 1989; Janssen et al., 1989), mass spectra (Ohashi et al., 1963; Tatematsu et al., 1967), and UV absorption spectra (Piniazhko, 1964; Sangster and Stuart, 1965), have been reported.

2.3. Chemical synthesis of noscapine and derivatives

Noscapine can be chemically synthesized from the treatment of an appropriately substituted *N*-methyl-3,4-dihydroisoquinolinium salt and a bromophthalide with zinc in acetonitrile (Shono et al., 1983), or from a benz[d]indeno[l,2-b]azepine skeleton (Kametani et al., 1975). An analogous pathway leads to the biosynthesis of noscapine in opium poppy, involving oxidation of the protoberberine scoulerine at N-7 and C-8 while maintaining the integrity of the C-14 asymmetric center (Kametani et al., 1977). Noscapine analogs such as nornarcotine have been prepared via the reaction of narcotine *N*-oxide with ferric citrate (Allen et al., 1984). (+)-β-Narcotine was prepared by specifically inverting noscapine at the chiral C-1 by hydrolysis of the product obtained from the reaction of noscapine with cyanogen bromide (Gaál et al., 1971). Noscapine can also be obtained by treating narcotoline with diazomethane (Pfeifer, 1957). Alternatively, synthesis of several phthalideisoquinolines, including hydrastine (Fig. 1), and spirobenzylisoquinolines has been reported by condensation of 3,4-methylenedioxyphthalide-α-carboxylic acid with phenethylamine, followed by cyclization and reduction (McLean and Dime, 1977; Falck and Manna, 1981). Recently, an efficient route for the synthesis of several benzylisoquinoline alkaloids, including noscapine and bicuculline (Fig. 1) was achieved using diastereoselective addition of 1-siloxy-isobenzofurans to an iminium ion (Soriano et al., 2010).

3. Biosynthesis

3.1. Origin of the carbon scaffold

The foundational hypothesis established by pioneering chemical investigations on BIA biosynthesis (Winterstein and Trier, 1910; Robinson, 1917, 1955) suggested that the condensation of tyrosine derivatives yielded norlaudanosoline as the central intermediate to structural variants derived via methylation, aromatization, and intra- and intermolecular oxidative coupling (Robinson, 1955). An alternative theory suggested that phthalideisoquinoline alkaloid biosynthesis began with hydrated