Review

Synthesis of aromatic cytokinins for plant biotechnology

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Cytokinins represent an important group of plant growth regulators that can modulate several biotechnological processes owing to their ability to influence almost all stages of plant development and growth. In addition, the use of purine based cytokinins with aromatic substituent in C6 position of the purine moiety in tissue culture techniques is currently experiencing a surge in interest, made possible by the ongoing systematic synthesis and study of these compounds. This review article outlines progress in the synthesis of aromatic cytokinins, the *in vitro* and *in vivo* effects of these substances and insights gleaned from their synthesis. As the purine moiety in these compounds can be substituted at several positions, we examine each of the substitution possibilities in relation to the derivatives prepared so far. The discussion highlights the gradual simplification of their preparation in relation to their application in practice and summarizes the relevant organic chemistry literature and published patents.

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Introduction

Purine based cytokinins (CKs) are naturally occurring plant growth hormones. Their homeostasis *in planta* influence almost all stages of plant development and directly affect processes of plant growth, often in concert with other plant hormones [1]. Although the first discovered naturally occurring CK was the isoprenoid derivative *trans*-zeatin isolated from *Zea mays* in 1963 [2], the first aromatic cytokinin (ARCK) was synthesized and tested as a specific cell-division factor much earlier. A postdoctoral fellow, Carlos Miller, experimented with coconut milk and used UV spectrum analysis in combination with other techniques to show that the prepared concentrates contained purines. In 1955, Miller tested coconut milk concentrates and discovered that they were highly active in tobacco pith tissue culture. After several experiments, Miller *et al.* isolated a white crystalline solid from aged DNA by chromatographic procedures and identified its structure as N^6-furfuryladenine. The authors suggested that the compound promoted cell division (cytokinesis) and named it kinetin (KIN) [3,4]. In fact, discovery of this compound was largely driven by the need to enhance cytokinesis in *in vitro* plant regeneration [5].

The first attempt to use CKs in plant tissue culture appeared immediately after the discovery of KIN [6,7]. In 1956, the term 'kinin' was coined by Miller for all substances able to promote cytokinesis and *permit continuous growth of various plant tissues in vitro* [5]. In the same laboratory, another aromatic purine derivative, 6-benzylaminopurine (BAP), was synthesized and shown to stimulate cell division in cultured tissues [5]. The above mentioned compounds formed the cornerstone of the first generation of synthetic ARCKs able to elicit plant growth and stimulate cell division. Since that time, micropropagation techniques have been used in conjunction with ARCKs in culture media to provide a rapidly developing biotechnological strategy for the commercialization of many important plant species. The main achievements so far are in the micropropagation of bananas [8,9], strawberries [10], apples [11], roses [12], melons [13] and many other plants, especially ornamental and/or medicinal [14,15]. For traditional medicinal plants, tissue culture is often the best way to reduce the cost of seedling/plant production whilst enhancing their chance of survival in nature [16]. One of the most widely used and reasonably cheap CK 6-benzylaminopurine (BAP), also known as N^6-benzyladenine (BA), unfortunately causes side effects, such as shoot-tip necrosis [17], problematic acclimatization in the greenhouse [18] and inhibition of rooting [19], that complicate micropropagation processes, especially in rare and susceptible medicinal plants [16,20].

Therefore, several CK derivatives have been prepared to avoid the unfavorable properties of BAP. Although the most obvious solution was to use KIN or coconut water containing KIN and other CK derivatives in the media [21], many laboratories attempted to design more sophisticated and effective ARCKs. Notably, the adenine moiety can be substituted at several positions, such as N1, C2, N3, N6, N7, C8 and N9, as shown in Fig. 1. This opens up possibilities for preparing a wide range of derivatives with interesting biological properties thanks to modern combinatorial chemistry. In addition, if the N6-position is substituted with phenyl or benzyl, further substitutions can be made on the phenyl/benzyl ring, extending the range of interesting derivatives available. Further, hydroxylated and methoxylated BAP derivatives were discovered in plants as natural phytohormones [22–24].

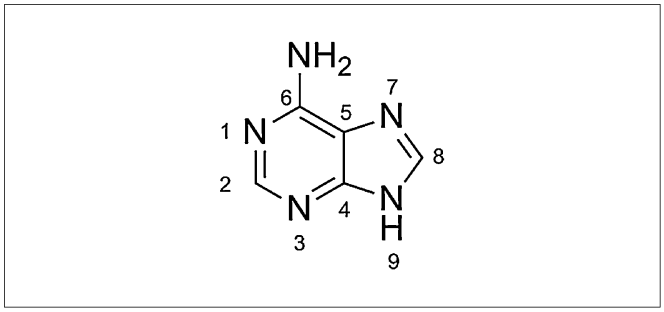

FIGURE 1
Structure of adenine with numbered atom positions.

The earliest syntheses usually started from substituted pyrimidine or imidazole precursors and generated the purine by sophisticated building-block synthesis. This type of method also allowed the preparation of N1-, N3-, N7-derivatives of purine [25]. Over the past 15 years, an extensive library has been constructed containing thousands of compounds derived from naturally occurring ARCKs, mostly obtained by the reaction of 6-halogenopurines with the appropriate aromatic amines. The first artificial representatives were prepared in the laboratory by substitution of the benzyl ring, for example, 6-(3-methoxybenzylamino)purine and 6-(3-hydroxybenzylamino)purine [26], currently called topolins [22]. Substitution of one or more hydrogen atoms by -hydroxy, -methoxy, -halogen, -mercapto or -alkyl groups or their mutual combinations has been proposed as an effective strategy for CK effect improvement [27]. New CK derivatives have been prepared not only by substitution of the benzyl ring but also by various substitutions in the purine moiety, especially at the C2, C8 and N9 atoms. These compounds are often called second generation CKs because they exhibit CK properties but do not usually exist in nature [28–36]. ARCKs of the second generation, successfully tested for biotechnological applications, usually possess a combination of these features: they contain substitution at the C2 and/or N9, eventually C8-positions of the purine moiety and concurrently substitution of the benzyl ring attached to the N6-atom of adenine moiety. In this review, we summarize developments in the synthetic procedures of ARCKs currently attracting interest for their possible use in biotechnologies, especially plant tissue culture. Our main aim was to map the continual process of searching for new, more effective structural motifs that are straightforward to synthesize for widespread biotechnological use.

Monosubstituted ARCKs: N6 adenine atom substitutions

Kinetin discovery and synthesis

Kinetin was the first known ARCK discovered in 1955 by Miller and his collaborators [3,5]. The synthesis of KIN was reported in the same paper [5]. Although the first attempt at the condensation of adenine with furfural was unsuccessful, the authors managed to perform furfuryl chloride and adenine condensation under alkaline conditions established with sodium bicarbonate [5]. The improved synthesis was based on 6-methylmercaptopurine and freshly redistilled furfurylamine as the starting materials. KIN was obtained after heating the abovementioned mixture at 115–120°C for nine hours [5,37]. This method with minor or major modifications is still used