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Evaluation of the antioxidant activity and capacity of some natural N^6-substituted adenine derivatives (cytokinins) by fluorimetric and spectrophotometric assays

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ABSTRACT

Four natural N^6-substituted adenine derivatives (cytokinins) were evaluated for the first time in vitro for they antioxidant capacity by using fluorimetric and spectrophotometric assays, i.e., the oxygen radical absorbance capacity (ORAC), trolox equivalence antioxidant capacity (TEAC) and the 2-deoxyribose degradation (2-DRA) assays. The results from the TEAC assay show that only N^6-(4-hydroxybenzyl)adenine (*p*-topolin) shows an electron transfer capacity due to the presence of a phenolic moiety in the N^6-position. The results from the ORAC test show that the antioxidant activity of N^6-furfuryladenine (kinetin, K) is the highest up to a concentration of 1 μM, whereas at concentrations higher than 1 μM *p*-topolin is the most efficient antioxidant. Analysis of the kinetic data suggests that, compared to the other cytokinins, more sites of the molecular structure of *p*-topolin are available for the quenching of peroxyl radicals. The hydroxyl radical scavenger ability, as measured by the 2-DRA assay, showed that all tested cytokinins react in this test and that N^6-(Δ^2-isopentenyl)adenine is slightly more potent, probably because of the allylic methylene group present in the N^6-isopentenyl moiety. Our data suggest that a part of the biological activity of the evaluated cytokinins is likely to be related to an intrinsic antioxidant capacity.

1. Introduction

Cytokinins (CKs) constitute a class of natural products that are structurally related to adenine and exert a hormonal activity in plants promoting cell division and growth, retardation of senescence or protection against abiotic oxidative stress [1]. Scheme 1 shows the general structure CKs (1) and the structure of the most representative natural CKs, i.e., N^6-(Δ^2-isopentenyl) adenine (iPA), N^6-furfuryladenine (kinetin, K), N^6-benzyladenine (BA), and the three isomeric hydroxylated derivatives of BA (topolins, T) [1].

Many biological activities of CKs in plants or in mammals can be explained by their effect on various biochemical parameters involved in cellular oxidative stress [2–7]. In this paper, we present the results of a study designed to check the intrinsic antioxidant capacity of the CKs shown in Scheme 1; among topolins, we have selected the para-isomer *p*-topolin. Among different assays currently available to evaluate the antioxidant activity, we have selected hydrogen atom transfer or electron transfer assays [8], specifically the oxygen radical absorbance capacity (ORAC) assay and the trolox equivalence antioxidant capacity (TEAC) assay, respectively. The ORAC assay (Scheme 2) is based on the capability of an antioxidant (AH) of inhibiting the loss of fluorescence of 2,7-dichlorofluorescein. Fluorescence loss is induced by the reaction of 2,7-dichlorofluorescein with peroxyl radicals generated from 2,2′-azo-bis(2-methylpropionamidine) (ABAP). AH reacts faster than 2,7-dichlorofluorescein with peroxyl radicals [8]. The TEAC assay (Scheme 3) is a spectrophotometric assay that relies on the antioxidant (AH) capability of quenching the colored radical cation formed by the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) treated with persulfate ions. The antioxidant capability of the selected CKs was compared with that of trolox which was used as reference [8].

In addition to these assays, we evaluated the scavenging capability of the CKs against the hydroxyl radical •OH by means of the 2-deoxyribose degradation assay (2-DRA) (Scheme 4). This assay

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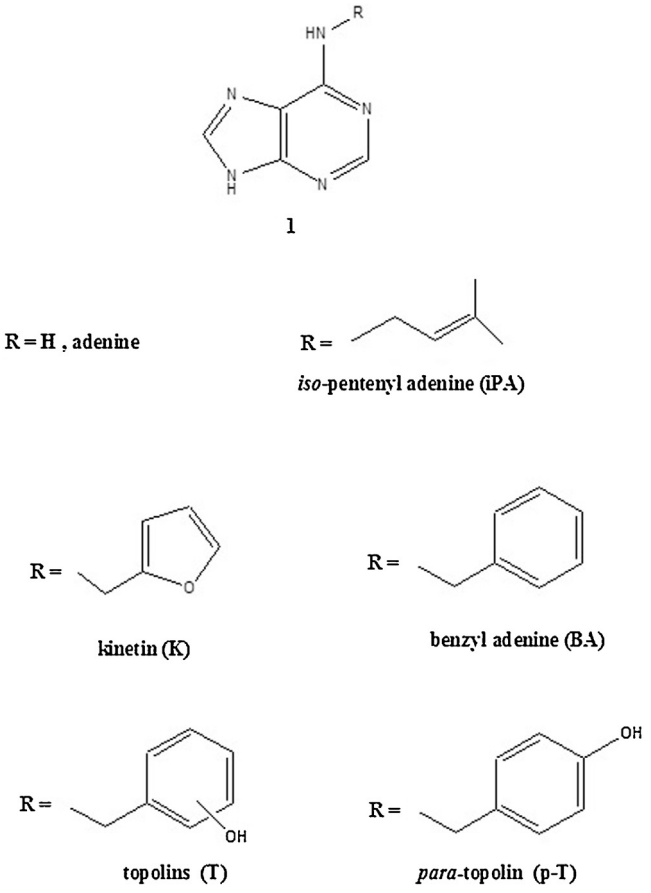


Scheme 1. The structure of natural N^6-substituted adenine derivatives (CKs).

is based on the formation of malondialdehyde (MDA) and other β-dicarbonyl compounds (MDA-like compounds) that result from the degradation of 2-deoxyribose after its reaction with hydroxyl radicals in situ generated by the Fenton reaction. The formation of MDA and MDA-like compounds and their inhibition by CKs is spectrophotometrically monitored following their reaction with thiobarbituric acid (TBA) [9–11].

2. Materials and methods

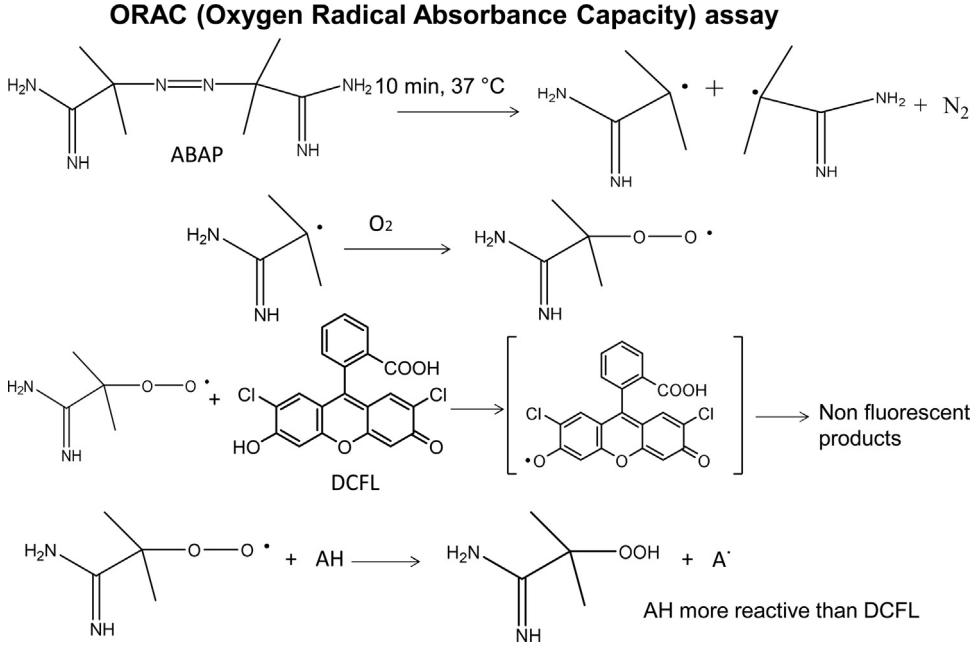
2.1. Materials

CKs were obtained from OlChemIm Ltd., (Olomouc, Czech Republic) and their purity was checked by HPLC with UV absorbance detection (270 nm), using a Symmetry Shield RP18 3.5 μm, 4.6 × 150 mm column from Waters (Milan, Italy), methanol–water mixtures as mobile phases, and a flow rate of 0.7 ml/min. A gradient elution from 40:60 to 90:10 (v/v) methanol–water within 7 min was used. The retention times of BA, *p*-topolin, kinetin, and iPA were 3.95, 3.97, 4.40 and 6.25 min, respectively. Their chemical purity resulted in 95%, 96%, 95% and 96%, respectively. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, iron(III) chloride, 2-deoxyribose, 30% hydrogen peroxide, EDTA disodium salt dihydrate, ascorbic acid, thiobarbituric acid, 2,7-dichlorofluorescein, trolox, 2,2′-azo-bis(2-methylpropionamidine) dihydrochloride (ABAP) and all other inorganic and organic reagents and solvents were purchased by Sigma–Aldrich Co. (Milan, Italy).

2.2. The ORAC assay

2.2.1. Reagent preparation

Stock solutions of 2,7-dichlorofluorescein (1 mM) and ABAP (200 mM) were prepared in 75 mM phosphate buffer (pH 7.0) and distilled water, respectively, and kept at −30 °C. For the assay, the 2,7-dichlorofluorescein solution was daily diluted with phosphate buffer to obtain a 500 nM solution. Trolox solution was prepared daily in a water–ethanol (50:50, v/v) to obtain a 100 μM solution. Fresh solutions of the tested compounds were prepared daily by dissolving each cytokinin in water–ethanol (50:50, v/v). The concentration range of the cytokinins was from 5 to 50 μM, with the



Scheme 2. Mechanism of ORAC assay. The hydrogen transfer from 2,7-dichlorofluorescein to peroxyl radicals yields different products finally not fluorescent. AH is a generic antioxidant which reacts with peroxyl radicals faster than 2,7-dichlorofluorescein.