Identification of Difenylenedioxide-2,3-quinones Obtained from an
Alternative Pathway for Catechol Metabolism in Tobacco

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Derivados de difenilenodióxido-2,3-quinona produzidos pela incubação de proteínas, parcialmente purificadas, obtidas das folhas de \textit{Nicotiana tabacum} var. \textit{Xanthi}, \textit{Campsis radicans}, \textit{Tecoma stans} e \textit{Populus deltoides} com cateriais foram identificados com base em seus dados de RMN \textsuperscript{1}H, \textsuperscript{13}C e EM. Os produtos de oxidação dos cateriais foram similares àqueles previamente isolados pela incubação de cateriais com extratos de folhas de espinafre.

Diphenylenedioxide-2,3-quinone derivatives produced by incubating partially purified proteins from the leaves of \textit{Nicotiana tabacum} var. \textit{Xanthi}, \textit{Campsis radicans}, \textit{Tecoma stans} and \textit{Populus deltoides} with catechols were identified based on their \textsuperscript{1}H- and \textsuperscript{13}C-NMR and MS data. The catechol oxidation products were similar to those previously isolated from the incubation of catechols with spinach leaf extracts.

Keywords: \textit{Nicotiana tabacum} var. \textit{Xanthi}, \textit{Campsis radicans}, \textit{Tecoma stans}, \textit{Populus deltoides}, catechol oxidation

Introduction

Catechol oxidase is an intracellular enzyme widely distributed in the plant kingdom. It contains copper, with a molecular weight between 116 and 128 kDa\textsuperscript{1,2,3}, and is frequently classified as polyphenol oxidase. In general it catalyses two distinct reactions: the insertion of oxygen in an \textit{ortho} position into an existing hydroxyl group, followed by the oxidation of the diphenol to the corresponding quinone, often referred to as cresolase activity. It can also catalyze only the oxidation step referred to above, and in this case its activity is referred to as catecholase. Since it is only one of several phenol oxidizing enzymes known to occur in plant tissues, cases of incorrect identification cannot be ruled out\textsuperscript{4}, particularly when crude preparations have been used. Furthermore, catechol oxidase is considered a difficult enzyme to purify, especially from plant tissues. Tanning reactions and binding to cell membranes in many tissues occur during the isolation of the enzyme, resulting in changes in its properties as well as in its oligomerization state. Such reactions can be partially prevented by isolation under N\textsubscript{2} or by the presence of reducing agents\textsuperscript{1}.

This paper presents two derivative products of the incubation of catechol and 4-chlorocatechol with cellular leaf extracts from \textit{Nicotiana tabacum} var. \textit{Xanthi}. Diphenylenedioxide-2,3-quinone derivatives were produced in small amounts and were identified on the basis of their \textsuperscript{1}H- and \textsuperscript{13}C-NMR and MS data. Cellular leaf extracts from \textit{Campsis radicans}, \textit{Tecoma stans} and \textit{Populus deltoides} also presented similar products through incubation with catechol and 4-chlorocatechol.

The oxidation of catechol has been reported to occur in several plant species, leading different authors to classify them as catechol oxidizing plants\textsuperscript{4,5,6}.

Experimental

Leaves from \textit{Nicotiana tabacum} var. \textit{Xanthi} (25 g fresh weight) were frozen in liquid nitrogen, crushed with a mortar and pestle, and suspended in water (25 mL). The
mixture was filtered through cheese cloth and centrifuged (5,000 rpm) at 4 °C. The supernatant was treated with 3 volumes of cold acetone (-20 °C), and the precipitate was collected and thoroughly dried in vacuo.

Either catechol, or 4-chlorocatechol or 4-methylcatechol (250 mg) in water (125 mL) was added to 250 mL of 0.1 M phosphate buffer, pH 7.4. Tobacco leaf acetone powder (0.5 g) dispersed in water (20 mL) was stirred in, and the mixture was incubated for 5 min at 25 °C. Since the product was unstable in an alkaline medium, the pH was immediately adjusted to 5.0 with HCl 0.1 M. The reaction mixture was extracted 3 times with chloroform (150 mL). After drying this mixture over anhydrous sodium sulfate, it was evaporated to a small volume in vacuo, and the concentrated solution was applied to a column (1.8 x 12 cm) of silica gel. The main orange-red zone was eluted rapidly with chloroform. It was concentrated in vacuo, and the product was precipitated by adding an excess of petroleum ether. After recrystallization from acetone, the product obtained as red prisms was used in spectroscopic analysis.

The same procedure was used with Campsis radicans, Tecoma stans and Populus deltoides leaf extracts, and the oxidation products presented similar characteristics.

**Diphenylenedioxide-2,3-quinone (1).** Red solid; m.p. 260-261 °C dec., UV λ max (CHCl3): 240, 275, 390 nm (log ε 4.30, 4.26, 3.87); IR ν max (KBr): 3060, 2350, 1653, 1583, 1490, 1388, 1258, 1197, 1098, 859, 677 cm⁻¹, MS m/z (rel.int.): Scheme 1. ¹H-NMR (200 MHz, CDCl3): δ 6.45 (s, H-1 and 4), 6.97 (d, J = 8Hz, H-6 and 7), 7.24 (d, J = 8Hz, H-5 and 8).

**6-Chlorodiphenylenedioxide-2,3-quinone (2).** Red solid, m.p. undet., UV λ max (CHCl3): 240, 283, 390 nm (log ε 4.32, 4.28, 3.88); IR ν max (KBr): 3060, 2350, 1653, 1583, 1490, 1388, 1258, 1197, 1098, 859, 781, 677 cm⁻¹, MS m/z (rel.int.): Scheme 1. ¹H-NMR (200 MHz, CDCl3): δ 6.32 (s, H-4 or 1), 6.33 (s, H-4 or 1), 7.29 (dd, J = 8.8, 2.3, H-7), 7.42 (d, J = 8.8, H-8), 7.58 (d, J = 2.3, H-5). ¹³C-NMR (50.32 MHz, CDCl3): δ 108.6 (C-1 or C-4), 108.8 (C-4 or C-1), 117.5 (C-5), 118.1 (C-8), 128.8 (C-7).

**Results and Discussion**

The dried acetone extract, prepared from frozen Nicotiana tabacum var. Xanthi leaves, contains enzymes (polyphenol oxidase) which oxidize catechol and chlorocatechol to the quinone (diphenylenedioxide-2,3-quinone, 1) and Cl-quinone (6-chlorodiphenylenedioxide-2,3-quinone, 2), respectively. These substances were characterized by their spectroscopic data. The UV absorptions of 1 and 2 are characteristic of ortho-quinone (240, 289 ± 4 and 390 nm), and the IR data for both are similar, with the exception of compound 2 which has a characteristic chloride absorption at 781 cm⁻¹. The fragmentation of com-

![Scheme 1](image-url)
pounds 1 and 2 in the MS (Scheme 1) confirmed the
proposed structures. Structure 1 was confirmed by the
$^1$H-NMR data and Structure 2 by the $^1$H- and partial $^{13}$C-
NMR data. As compound 1 is a symmetrical molecule, its
$^1$H-NMR spectrum only contains three signals, the attribu-
tions of which were determined on the basis of their cou-
pling constants. In 6-chlorodiphenylene-dioxide-2,3-
quinone (2) the signals attributed to H-4 and H-1 are not
equivalent, appearing at δ 6.32 and 6.33. The multiplicity
of the signals of H-5 (7.58 δ) and H-8 (7.42 δ) appear as
doublets, while H-7 (7.29 δ) appears as a double doublet,
as expected.

The enzyme (polyphenol oxidase) presents activity
with catechol, 4-chlorocatechol and 4-methylcatechol in
the pH range of 7 to 8. Its temperature optimum was 30 °C.
The enzyme is insensitive to DIECA, but its activity is
increased by the addition of CuSO$_4$. It is inhibited by
cyanide, benzoic acid and ascorbic acid.

We have checked the ability of other plants to oxidize
catechol and 4-chlorocatechol to quinones (Campsis, Tec-
coma, Nicotiana and Populus). In these studies the same
products (1 and 2) were obtained as when using N. tabacum
extracts. This property could be a general feature observed
in several plants which could be exploited in the recovery
of waste sites. Additionally, it is first necessary to establish
whether these plants have endogenous hydroxylases with
phenol oxidase activity which can convert chlorinated phe-
nols to catechol, and then to carry out the subsequent
oxidation.

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