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Analysis of sucrose esters – insecticides from the surface of tobacco plant leaves

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Abstract

Sucrose esters from the surface of leaves of *Nicotiana tabacum* L. have been shown to possess interesting biological activities. We developed a simple and effective method for their analysis using HPTLC silica gel plates, *n*-hexane–ethyl acetate (1:3, v/v) as developing solvent and aniline-diphenylamine as a detection reagent. Off-line TLC–MS was also used for the detection and identification of the compounds. Solutions containing sucrose esters upon alkaline hydrolysis give sucrose, which is used for indirect estimation by TLC of the sucrose ester content. The method is applicable for the screening for sucrose esters in plant extracts. The extract obtained from the surface of green leaves of oriental tobacco type Prilep P-23 contains sucrose esters and is effective against *Myzus persicae* (Sulzer) in laboratory and field experiments.

Keywords: Leaf surface compounds; *Nicotiana tabacum* L.; Sucrose esters of low fatty acids; Aphicid; HPTLC; Off-line TLC–MS

1. Introduction

The chemical composition of green, dried and fermented tobacco (*Nicotiana tabacum*) leaf and of cigarette smoke has been the subject of many reports in recent years and many components have been identified using modern analytical techniques [1,2]. Tobacco leaf surface compounds have also been studied [3–7] and shown to contain three main groups of compounds: aliphatic hydrocarbons (normal and branched chain, C_{25}–C_{36}), diterpenes of the duvane and/or labdane type, and sucrose esters (SEs) of saturated, normal and branched, short-chain (C_2–C_8) fatty acids. However, depending on their genetic background, tobaccos can exhibit large quantitative and qualitative differences in their leaf surface compounds and additional factors affecting levels of SEs are the stage of plant growth, soil, agronomic and climate conditions. Trichome gland biosynthesis of sucrose esters ends as the leaf matures and the SEs content retains practically unchanged during curing and fermentation.

SEs are recognized as precursors of important oriental tobacco smoke flavour components and have been positively correlated with tobacco quality. The most abundant tobacco leaf sucrose esters were identified as (6-*O*-acetyl-2,3,4-tri-*O*-acyl)-α-D-glucopyranosyl-β-D-fructofuranoside; with propionyl, butyryl, isobutyryl, valeryl, isovaleryl, 3-methylvaleryl, caproyl, methylcaproyl as the acyl group. As minor components glucose esters (GEs) and compounds with different esterification patterns of sucrose hydroxyl groups have been identified [4–6]. It was proved that SEs readily release free carboxylic acids on thermolysis in conditions similar to tobacco combustion [7].

Green tobacco leaf surface compounds can influence the resistance to pests and it has been shown that leaf surface extracts from different *Nicotiana* species can function as potent insecticides [8]. The content and composition of sucrose esters are important parameters in this regard. Compounds of this type have been synthesized in a search for a new class of bioinsecticides [9,10]. In contrast to toxic nicotine, SEs are non-toxic to humans and warm-blooded animals. While some products representing a new generation of bioinsecticides are already available, intensive investigation continues into the development of useful SEs [11].

Sucrose esters isolated from the leaf surface extracts of *N. tabacum* and *Nicotiana glutinosa* have been shown to possess also antibacterial and plant growth regulating activities [12]. Investigations showed that tobacco leaf surface GEs, SEs and some synthetic disaccharide esters could be potential cancer-preventive agents of a new type [13]. Studies have shown that

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some other related plants (potato, tomato, petunia) can also produce either glucose or sucrose esters (sometimes both) of short-chain fatty acids [14] as leaf surface compounds. Recently, bioactive (antiallergenic) sucrose esters containing *p*-coumaric acid and other acids were found in a traditional medicinal plant *Bidens parviflora* [15]. Sucrose esterified with isovaleric acid found in the stem of *Vernonia guinensis* (Asteraceae) presents probably the first report of SEs in this plant family [16] and opens the question how wide these interesting compounds are distributed in the plant kingdom.

Natural sugar fatty acid esters present structurally relatively simple compounds with numerous possible isomers. They belong to a large group of compounds that have been synthesized and studied as emulsifiers, stabilizers etc [17–22]. The degree of esterification of the sugar, its structure (mono-, di- or trisaccharide) and the fatty acid chain length are the main factors affecting the properties of these compounds as non-ionic surfactants. Beside classical methods of organic synthesis, enzymatic acylation is a challenging field for modification of sucrose and other saccharides into new products [23–25].

Different chromatographic techniques have been used for the analysis of sugar fatty acid esters including glass capillary gas chromatography (as trimethylsilyl derivatives) with FID and MS detection at studying of tobacco leaf surface constituents [3–6]. A TLC method was developed for detection of different types of tobacco leaf surface compounds [3]. TLC and RP-HPLC with evaporative light scattering (ELSD) and mass spectrometric (MS) detector were used for the determination of some commercial sucrose fatty acid esters [26]. Spectrophotometric estimation of the total SEs content by rhodamine B has potential in studies of natural and synthetic SEs [27].

Sucrose esters, having no chromophore, are unsuitable for UV–vis detection in HPLC. Because of toxicity of the reagent, silylation is not useful for the screening stage of SEs and TLC remains an attractive choice. Because tobacco types are not all able to produce SEs at the usual levels (0–100 μg/cm^2) [4,6], we sought to develop a TLC method for an indirect estimation of SEs on the surface of tobacco leaf via the sucrose produced by alkaline hydrolysis. A secondary goal was a simple TLC screening method for the direct qualitative analysis of SEs in green and fermented tobacco leaves. These compounds affect the quality and pest resistance of tobacco and development of a rapid, high-throughput screening procedure will benefit entomologists and tobacco breeders who require rapid results on large numbers of phenotypes. It can be used also for screening of leaf surface SEs of other plants, for analysis of fractions from chromatographic separations and/or isolations on preparative level (e.g. for off-line MS identification) and for screening purposes of synthetic products with similar chemical composition as natural SEs.

2. Experimental

2.1. Chemicals

Reagents and solvents were of analytical grade. Dichloromethane, methanol, *n*-hexane, acetonitrile and anhydrous sodium sulphate were purchased from Fluka (Buchs, Switzerland); potassium hydroxide pellets, perchloric acid 70%, orthophosphoric acid 85%, perchloric acid 70%, aniline and 2-aminoethyl diphenylborinate from Merck (Darmstadt, Germany); and diphenylamine from Sigma (Steinheim, Germany).

2.2. Sampling of plant material

Leaf surface extracts were prepared from different tobacco varieties and types: oriental (Prilep P-23, Prilep NS 72, Prilep 156/1), semi-oriental (Jaka JB 125/3), flue-cured (Virginia MV-1) and burley (B-2/93), grown at Tobacco Institute, Prilep, Republic of Macedonia, under conditions normally used for their production. Technologically mature, undamaged leaves were collected in August. Cured and naturally fermented leaf samples from the same tobacco varieties were also collected for analysis one year later. Some wild types of tobacco (*N. glutinosa*, *Nicotiana forgetiana*, *Nicotiana rustica*, *Nicotiana paniculata*) were included for the SEs TLC screening test.

2.3. Extraction of tobacco leaf surface compounds

Six whole tobacco leaves were individually dipped into 200 mL of dichloromethane for 30 s. The extract was filtered through anhydrous Na_2SO_4 [5,6]. The bulk of the solvent was removed on a rotary evaporator and the last few millilitres in a stream of nitrogen. The dry residue was weighed and stored at −20 °C pending analysis.

For the routine TLC screening test of SEs, the leaf surface extract was prepared as follows: a 20 cm^2 segment of a green plant leaf (area on both sides of the leaf) was cut and dipped into 10 mL of dichloromethane. The solvent was removed in a stream of nitrogen. For the TLC examination the rest was dissolved in 100 μL of methanol, vortexed and centrifuged and 15 μL of this test solution was applied to the plate.

2.4. Alkaline hydrolysis

On the residue in the tube obtained after the washing of six whole leaves, 1 mL of 80% methanol in water and 1 mL of *n*-hexane was poured, vortexed and centrifuged. The upper hexane layer was removed. From the methanol extract 0.5 mL was pipetted into a vial, 0.5 mL of 0.2 M KOH was added, mixed well and left covered at room temperature for 24 h. The reaction mixture was neutralized by conc. $HClO_4$. The presence of sucrose in the test solutions was established by TLC.

2.5. TLC determination of sucrose

Standard solution containing glucose (0.2 mg/mL), fructose (0.2 mg/mL) and sucrose (0.15 mg/mL) in 80% methanol in water was prepared. Different volumes of standard (2–12 μL) and sample test solutions obtained after alkaline hydrolysis were applied by Linomat IV (Camag, Muttenz, Switzerland). HPTLC silica gel 60 F_{254}, 20 cm × 10 cm, thickness 0.2 mm (Merck) plates were developed in a twin trough saturated chamber for 20 cm × 10 cm plates (Camag, Muttenz, Switzerland), using acetonitrile–water (17:3, v/v), containing 0.05% of 2-