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TRITERPENE GLYCOSIDES ANALYZED BY TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

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Two-dimensional TLC with judiciously chosen solvent systems that differ in pH values is proposed for identification of triterpene glycosides in plant extracts (mono- and bisdesmosidic, acidic, sulfated, acylated).

Key words: triterpene glycosides, two-dimensional thin-layer chromatography.

Triterpene glycosides of many plant families are known to be complex mixtures of compounds with similar chromatographic mobilities. Preparative separation of such mixtures usually includes either production of narrow glycoside fractions using a single elution system with subsequent separation of these fractions in other chromatographic systems or on other adsrobents or a combination of chromatographic methods that differ in physicochemical properties for separation of glycoside fractions, e.g., gel-permeation, ion-exchange, extraction, reversed-phase, and ordinary adsorption chromatography.

Combinations of different chromatographic methods are practically impossible for analytical separation of natural mixtures of glycosides using TLC. The single method for substantially increasing the resolution is two-dimensional (2D) TLC with different solvent systems in two directions.

2D TLC is well known and widely applied for separation of complex mixtures of natural compounds, especially phenolic glycosides, amino acids, alkaloids, and several other compounds [1]. Thus, the principal problem usually consists of an empirical selection of the optimal solvent pair, often without a theoretical prediction of the resulting effect. Previous work on 2D TLC of triterpene glycosides includes 2D separation of *Panax trifolius* [2] and *Herniaria glabra* [3] glycosides in which, however, separation of glycoside groups was not addressed and a study in which only acidic chromatographic systems of different composition were used [3].

Triterpene plant glycosides are known to contain a wide variety of aglycones and hydrocarbons [4]. A single organ (leaves, stems, roots, etc.) sometimes contains several dozen glycosides. The most common glycoside groups can be defined in order to identify common structural features and to develop a strategy for preparative separation: neutral bisdesmoside glycosides (glycosides with two carbohydrate residues without glucuronic acid and free, i.e., unglycosylated, carboxylic groups in the aglycone); bisdesmoside glycosides with additional free carboxylic groups in the aglycone, usually on C-4 or C-20, or monodesmoside glycosides with a free carboxylic group on C-17 that forms an acylglycoside bond in the corresponding bisdesmoside glycosides; acidic glycosides with glucuronic acid residues; acidic glycosides with sulfate residues that esterify aglycone hydroxyls or carbohydrates; glycosides with acyl (usually acetyl) groups in the carbohydrates and, more rarely, aglycones.

We used 2D TLC with judiciously chosen solvent systems that differ in pH value, acidic, neutral, and alkaline, to detect these glycoside groups in plant extracts. Neutral systems are $CHCl_3$—CH_3OH mixtures saturated with water. The $CHCl_3$:CH_3OH ratio depends on the polarity of the glycosides to be separated. Acidic systems are obtained by adding formic acid (3-5%) to neutral ones. Alkaline chromatographic systems are prepared by saturation with aqueous ammonia (25%) instead of water.

The proposed principle of combining two chromatographic systems with different pH values is a modified version of the diagonal technique that is usually used in reactions on chromatographic plates [5].

Using combinations of neutral (direction I) and alkaline (direction II) solvent systems can differentiate the most frequently encountered groups of mono- and bisdesmosidic glycosides.

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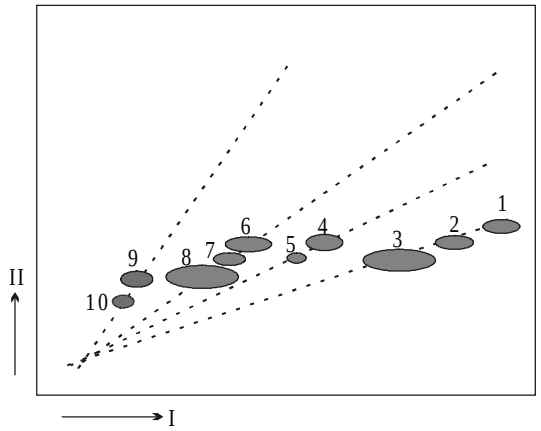

Fig. 1

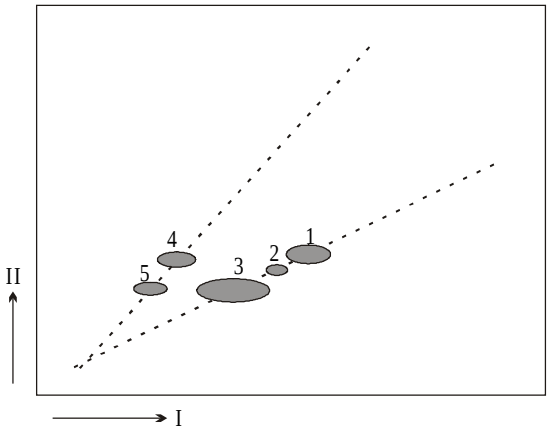

Fig. 2

Fig. 1. Two-dimensional chromatograph of extract of *Hedera taurica* leaves. Solvent systems: neutral (I) and alkaline (II); hederagenin 3-O-α-L-arabinopyranoside (1), 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosides of oleanolic acid (2) and hederagenin (3), 3-sulfates of oleanolic (4) and echinocystic (5) acids, 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters of 1-5, respectively (6-10).

Fig. 2. Two-dimensional chromatograph of extract of *Hedera taurica* stems. Solvent systems: neutral (I) and alkaline (II); 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters of hederagenin 3-O-α-L-arabinopyranoside (1) and hederagenin 3-O-β-D-glucopyranoside (2), hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside (3), 3-O-β-D-glucuronopyranosides of oleanolic acid (4) and hederagenin (5).

Thus, glycosides of each group are shifted along lines starting at the origin but with different slopes. Monodesmosidic glycosides are always located on lines with smaller slope (Fig. 1).

This occurs because increasing the pH of the chromatographic system ionizes the free carboxylic group on C-17 of the aglycone, which considerably increases the polarity of such glycosides and decreases their chromatographic mobility compared with a neutral chromatographic system. Bisdesmosidic glycosides with an additional free carboxylic group in the aglycone on C-4, C-14, or C-20 should behave analogously.

Using the combination of solvent systems described above should enable the isolation of yet another group of glycosides that have a carbohydrate chain on the aglycone carboxylic group and are sulfated at the hydroxyl on C-3 of the aglycone or the carbohydrate. These sulfates are located on lines with larger slopes than neutral bisdesmosidic glycosides (Fig. 1). Such behavior can be explained by the fact that the sulfate group is completely ionized even in neutral solution. Increasing the pH value does not affect its polarity. However, changing from a neutral to an alkaline chromatographic system causes a small overall increase of polarity for neutral glycosides owing to partial ionization of sugar hydroxyls. For the same chromatographic mobility in a neutral system, neutral glycosides usually have a larger number of monosaccharide residues than glycosides with sulfates, the presence of which is equal in polarity to about two carbohydrates. Therefore, representatives of these glycoside groups differ in chromatographic mobility in the second direction.

Using a combination of neutral and alkaline solvent systems for acidic bisdesmosidic glycosides with a glucuronic acid causes no significant difference in their behavior compared with neutral bisdesmosidic glycosides. Obviously, this is due to the fact that the carboxylic group in uronic acids is much more acidic than in the aglycone because the α-position has an oxygen substituent so that it is significantly ionized even in neutral solution. Therefore, changing to an alkaline system does not substantially increase the polarity of such an acidic glycoside and noticeably decrease the chromatographic mobility. As a result, glycosides with uronic acids lie on practically the same lines as glycosides without uronic acids. However, using a combination of neutral and acidic solvents does place glycosides with uronic acids on a separate line with a larger slope than neutral glycosides (Fig. 2). This occurs because ionization of uronic carboxylic groups is substantially suppressed in the acidic system, which decreases the polarity of the glycoside and increases its chromatographic mobility.

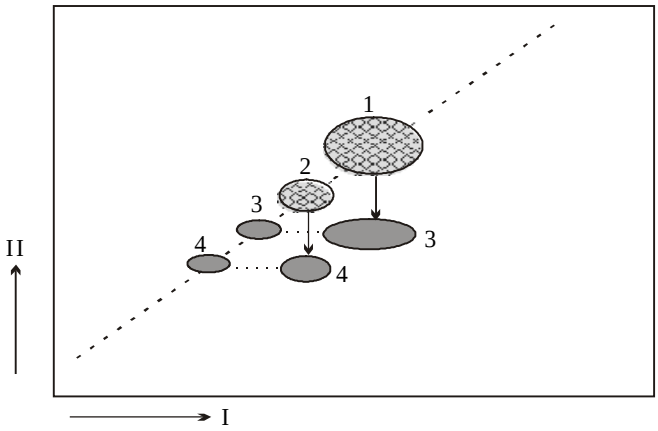

Fig. 3. Two-dimensional chromatograph of extract of *Tetrapanax papyriferum* stems. Solvent systems: both neutral and acidic; 28-O-α-L-rhamnopyranosyl-(1→4)-O-(6-O-acetyl-β-D-glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl esters of 3-O-[β-D-galactopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)-O-α-L-arabinopyranosides of oleanolic (1) and echinocystic acids (2), 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters of 3-O-[β-D-galactopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-O-α-L-arabinopyranosides of oleanolic (3) and echinocystic acids (4).

Detection of glycosides with acyl residues is most successful if the diagonal technique with movement in one direction, exposure of the chromatograph to ammonia vapor at room temperature for 2-3 h, and subsequent movement in a different direction in the same solvent system is used. The presence in the extract of acylated glycosides is easily detected by the appearance of deacylated derivatives that are formed after treatment with ammonia, which lie on a line of smaller slope compared with the usual glycosides. The position of the starting acylated glycosides can be easily determined by comparing the 2D chromatographs with complete (exposure to ammonia vapor for more than 2 h) and partial (exposure for ~1 h) removal of acyls.

Thus, the starting acyl derivatives are located further along the diagonal of the 2D chromatograph than their deacylated counterparts (Fig. 3) whereas the corresponding native glycosides without acyls, which are always present in the plant, are situated on a single horizontal.

EXPERIMENTAL

We used Silufol TLC plates and the following solvent systems: neutral, $CHCl_3$—CH_3OH—H_2O (100:30:5 for glycosides with 1→4 sugar residues and 100:40:7 for glycosides with 4→6 sugar residues); acidic, $CHCl_3$—CH_3OH—H_2O (100:30:5 or 100:40:7) with addition of formic acid (3-5%) immediately before chromatography; alkaline, $CHCl_3$—CH_3OH—NH_3 (aqueous, 25%) (100:30:6 or 100:40:10).

Extracts containing triterpene glycosides of various groups were obtained from leaves of *Hedera taurica* [6], stems of *Hedera taurica* [7], and stems of *Tetrapanax papyriferum*.

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