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DEVELOPERS FOR TLC OF TRITERPENE GLYCOSIDES

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Color reactions are used to detect triterpene glycosides on TLC plates because pure triterpene glycosides are colorless compounds. The reactions involve aromatic aldehydes in strong mineral acids [1-3], a mixture of acetic anhydride and conc. H_2SO_4 (Liebermann—Burchard reagent) [1, 3, 4], $Ce(SO_4)_2$ or Fe(III) salts in inorganic acids [1], $SbCl_3$ [1, 5], and phosphomolybdic acid [1, 4]. Triterpene glycosides are also detected in chromatograms using H_2SO_4 or phosphotungstic acids of various concentrations, a mixture of chlorosulfonic and acetic acids, or hemolysis of erythrocytes [1, 5].

Table 1 contains a list of reagents that we propose for detecting glycosides of oleanic acid and hederagenin, the most commonly encountered aglycons among triterpene glycosides [1]. Model compounds can be both mono- and bisdesmoside glycosides: oleanic acid 3-O-α-L-arabinopyranoside, 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside, and 3-O-sulfato-28-O-α-L-rahmnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside; hederagenin 3-O-α-L-arabinopyranoside, 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside, and 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-glucopyranoside; oleanic acid 3-O-sulfate, and nonglycosylated aglycons. TLC was performed on Sorbfil PTSKh-P-A-UV-254 plates (Russian Federation). Chromatograms were heated to 100°C. The solvent systems $CHCl_3$:CH_3OH:NH_4OH (25%) (100:20:3 and 100:30:5) were used.

The initial color of the glycoside spots changes with time. Thus, the lilac-violet spots corresponding to hederagenin and its glycosides that are obtained by treatment with reagents **1**-**9** become blue after 15-20 min. The pink spots of oleanic acid and its glycosides acquire a slightly lilac tint. This makes it possible to differentiate the analyzed aglycons and their glycosides by the color of the spots on the TLC plate after treatment with the corresponding reagents. The color of the spots fades over 1-2 h. After a day, it is practically gone, which is indicative of its relative stability.

TABLE 1. Composition of Mixtures for Triterpene Glycoside Detection

Reagent	Composition
$AlCl_3$-H_2SO_4	1 g $AlCl_3 \cdot 6H_2O$ in 50 mL 2 N H_2SO_4
$CdSO_4$-H_2SO_4	1g $CdSO_4$ in 50 mL 2 N H_2SO_4
$CoSO_4$-H_2SO_4	1g $CoSO_4 \cdot 7H_2O$ in 50 mL 2 N H_2SO_4
$Cr_2(SO_4)_3$-H_2SO_4	1 g $Cr_2(SO_4)_3 \cdot 6H_2O$ in 50 mL 2 N H_2SO_4
$CuSO_4$-H_2SO_4	1 g $CuSO_4 \cdot 5H_2O$ in 50 mL 2 N H_2SO_4
$KMnO_4$-H_2SO_4	0.05 g $KMnO_4$ in 50 mL 2 N H_2SO_4
$MnCl_2$-H_2SO_4	1g $MnCl_2 \cdot 4H_2O$ in 50 mL 2 N H_2SO_4
$NiSO_4$-H_2SO_4	1 g $NiSO_4 \cdot 7H_2O$ in 50 mL 2 N H_2SO_4
$SnCl_2$-H_2SO_4	1 g $SnCl_2 \cdot 2H_2O$ in 50 mL 2 N H_2SO_4
$ZnCl_2$-H_2SO_4	1 g $ZnCl_2$ in 50 mL 2 N H_2SO_4
Benzaldehyde-H_2SO_4	0.5 mL benzaldehyde in 50 mL 2 N H_2SO_4
p-Hydroxybenzaldehyde-H_2SO_4	0.1 g *p*-hydroxybenzaldehyde in 50 mL 2 N H_2SO_4

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Certain researchers detected glycosides using $SnCl_2$ [5]. However, its aqueous and ethanolic solutions did not give satisfactory results in our hands. Therefore, we used reagent **9** containing H_2SO_4.

The spots from reagent **10** are unstable (hederagenin glycosides appear first as a brown band; oleanic acid, pink, the 3-O-sulfate, pale lilac), fade after 10-15 min, and acquire a gray tint. However, after this the bands of triterpene glycosides and pure aglycons are observed in UV light as light blue or light green spots.

We modified Komarovskii reagent, which is used to detect both steroids [3] and triterpene glycosides [1] (reagent **12**, Table 1). Reagents **11** and **12** give with triterpene glycosides and unglycosylated alglycons brightly colored spots. For hederagenin glycosides and the aglycon itself, they acquire a lilac color that changes to blue after 5-10 min. Oleanic acid and its glycosides give a pink color with these reagents that lasts for about one day.

Furthermore, spots of oleanic acid 3-O-sulfate and its 28-O-glycoside that are produced using reagents **1**-**9**, **11**, and **12** quickly become pale with a gray-blue tint. This makes it possible to differentiate triterpene glycosides and sulfates by observing their spots on TLC plates.

Thus, the ability to use reagents **1**-**12** to detect triterpene glycosides and their aglycons on TLC plates has been demonstrated.

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