Chemistry of Natural Compounds, Vol. 42, No. 2, 2006

USE OF HETEROPOLYACIDS FOR TLC ANALYSIS OF TRITERPENE GLYCOSIDES

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UDC 543.544:547.918

We reported earlier on reagents for detecting triterpene glycosides on TLC plates that included various salts or aromatic aldehydes and H_2SO_4 [1]. In this communication we describe the use for these purposes of mixtures containing the heteropolyacids phosphotungstic $H_7[P(W_2O_7)_6]$ (**1**), phosphomolybdic $H_7[P(Mo_2O_7)_6]$ (**2**), and silicotungstic $H_8[Si(W_2O_7)_6]$ (**3**), which also give color reactions with triterpene glycosides.

Acid **1** is used as an alcoholic solution (25%) to detect triterpene glycosides on TLC plates because it gives distinct and well preserved spots [2]. Acid **2** is a general reagent and is used to identify not only saponins but also lipids, antioxidants, and other compounds [3]. Acid **3** has been used to detect vitamin D on TLC plates and paper [4] and as a qualitative reagent for alkaloids [5]. The use of the last to detect triterpene glycosides has not been reported.

We used solutions of **1**-**3** in $CHCl_3$:CH_3OH and with added *p*-hydroxybenzaldehyde or H_2SO_4. Oleanane-type glycosides with hederagenin and oleanolic and echinocystic acids as the aglycons were detected. It has been found that treatment of the chromatograms with $CHCl_3$:CH_3OH solutions of **1** and **3** with subsequent heating causes spots of hederagenin and its glycosides to acquire a bluish-violet color; oleanolic and echinocystic acids and their glycosides, reddish-pink. On the other hand, **2** gives in all instances blue spots on a yellowish-green background. Thus, this reagent cannot initially differentiate glycosides by the type of aglycon.

A solution with a high concentration of **1** (25%) [2] gives a rather strong background if the chromatograms are heated. Therefore, we used a lower concentration of **1**. After the chromatograms were treated with this reagent and heated, the spots of the compounds appeared on a light pinkish-brown background. On the other hand, there was practically no visible background if **3** was used in this instance.

If chromatograms were treated with reagents with added *p*-hydroxybenzaldehyde or H_2SO_4, the same color effects were noted for **1** and **3** as when the $CHCl_3$:CH_3OH solutions were used. However, in this instance the spots were more distinct, more strongly colored, and more stable. Spots of glycosides persisted longer than any if treated with reagents containing H_2SO_4. Furthermore, adding *p*-hydroxybenzaldehyde and H_2SO_4 increased the sensitivity of the detecting reagents and enabled the concentration of heteropolyacid to be decreased. Detection of glycosides and pure aglycons using **2** with added *p*-hydroxybenzaldehyde or H_2SO_4 also did not enable the type of aglycon to be established initially because all spots were dark blue on a blue background.

Triterpene glycosides of known structure that were isolated by us from various organs of Crimea ivy *Hedera taurica* Carr. and *H. canariensis* Willd. were used for TLC. These were oleanolic acid 3-*O*-α-L-arabinopyranoside, 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranoside, and 3-*O*-sulfate-28-*O*-α-L-rhamnopyranosyl-(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, 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabionopyarnosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside, 3-*O*-β-D-glucopyranoside, and 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside; and echinocystic acid 3-*O*-α-L-arabinopyranoside and 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranoside, in addition to nonglycosylated hederagenin and oleanolic and echinocystic acids.

We used the following solutions: **1**-**3** (3 g) as the crystalline hydrates ($H_7[P(W_2O_7)_6]\cdot nH_2O$, $H_7[P(Mo_2O_7)_6]\cdot nH_2O$, and $H_8[Si(W_2O_7)_6]\cdot nH_2O$) in $CHCl_3$:CH_3OH (1:1, v/v, 50 mL); **1**-**3** (2 g) and *p*-hydroxybenzaldehyde (0.5 g) in $CHCl_3$:CH_3OH (1:1, v/v, 50 mL); and **1**-**3** (2 g) in H_2SO_4 (2 N, 50 mL). TLC was performed on Sorbfil plates (RF) grade PTSKh-P-A-UF-254

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 0009-3130/06/4202-0238

of silica-gel particle size 5-7 μm (STKh-1A type sorbent). Chromatograms were heated to 100°C. Elution used $CHCl_3$:CH_3OH:NH_4OH (25%) (100:20:3 and 100:30:5).

Thus, the ability to use reagents containing **1**-**3** to detect triterpene glycosides and their aglycons on TLC plates was demonstrated. Reagents with **1** and **3** can differentiate glycosides according to the aglycon. Heteropolyacid **3** was used for the first time to detect triterpene glycosides by TLC analysis.

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