Journal of Chromatography, 330 (1985) 177–179
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 785

Note

Thin-layer and gas–liquid chromatographic separation of trihydroxy pentacyclic triterpene alcohols from *Calendula officinalis* flowers

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(Received April 3rd, 1985)

Investigations on the content of triterpene alcohols in *Calendula officinalis* flowers have established that compounds with different numbers of hydroxy groups are present. The structures of monohydroxy[1], dihydroxy[1,2] and trihydroxy[3,4] alcohols have been determined.

Free monools and their acetates can be separated into individual components by thin-layer chromatography (TLC) on silver nitrate-impregnated silica gel[1], except for α-amyrine from β-amyrine, which can be achieved by gas–liquid chromatography (GLC)[5]. Separation of a mixture of free triterpene diols, their acetates and their oxidation products can be achieved by GLC[6].

This paper reports the conditions that permit the TLC separation of a mixture of triterpene triol triacetates from *Calendula officinalis* into individual compounds; their GLC separation is also described.

EXPERIMENTAL

Materials

Free triterpene triols were obtained from dry *Calendula officinalis* flowers by extraction with boiling diethyl ether for 3 days followed by alkaline hydrolysis. The ether extract obtained after hydrolysis was evaporated *in vacuo*. The crude lipid fraction was adsorbed on silica gel and applied to a silica gel column. Compounds were eluted with *n*-hexane–chloroform (0 → 100% chloroform). Crude triterpene polyols were obtained from the eluate containing 80–100% of chloroform.

Acetylation

Acetylation of the fraction of triterpene polyols was carried out in the usual manner with acetic anhydride and pyridine at room temperature giving, after preliminary TLC purification, a mixture of triol triacetates.

TLC and TLC on $AgNO_3$-impregnated plates of triterpene triols

Merck TLC sheets (20 × 20 cm) pre-coated with silica gel (Kieselgel 60, 0.2 mm) were used for the analysis of triterpene triol triacetates on a non-impregnated phase. The solvent system used was chloroform–methanol (75:1). For TLC on $AgNO_3$-impregnated plates Kieselgel G (Merck) was used, glass plates being coated

0021-9673/85/$03.30

with a layer of adsorbent containing 10% of $AgNO_3$. The solvent system was chloroform (freed from methanol)–diethyl ether (50:1). The chloroform freed from methanol was prepared by shaking commercial chloroform with water (four times), drying with $MgSO_4$ and distilling over anhydrous $MgSO_4$. In both TLC procedures the compounds were rendered visible by spraying with 50% sulphuric acid and heating.

GLC

GLC was carried out on a Chrom-4 instrument equipped with a flame ionization detector and a 120 cm × 4 mm I.D. column of SE-30 on Gas-Chrom Q (Applied Science Labs.); the column temperature was 270°C and the carrier gas (nitrogen) pressure was 0.74 kg/cm^2.

RESULTS AND DISCUSSION

Pentacyclic triterpene monools and diols cannot be separated using normal TLC, owing to their similar polarities. The difference in the position of the double bond allows their separation on $AgNO_3$-impregnated TLC plates. However, the introduction of a third hydroxy group changes the polarity and the separation of triol triacetates is possible even on normal TLC plates. R_F values of pentacyclic triol triacetates isolated from *Calendula officinalis* flowers are presented in Table I.

TABLE I

R_F VALUES OF TRITERPENE TRIOL TRIACETATES FROM *CALENDULA OFFICINALIS* FLOWERS

Compound	*R_F value*	
	Normal TLC	*TLC on $AgNO_3$-impregnated plate*
Olean-12-ene-3β,16β,28-triol triacetate	0.79	0.56
Lup-20(29)-ene-3β,16β,28-triol triacetate	0.70	0.48
Tarax-20-ene-3β,16β,30-triol triacetate	0.65	0.35
Tarax-20-ene-3β,16β,22α-triol triacetate	0.51	0.25
Urs-12-ene-3β,16β,21-triol triacetate	0.51	0.20

TABLE II

RELATIVE RETENTION TIMES (RRT) OF TRITERPENE TRIOL TRIACETATES FROM *CALENDULA OFFICINALIS* FLOWERS

Compound	*RRT**
Tarax-20-ene-3β,16β,22α-triol triacetate	1.66
Olean-12-ene-3β,16β,28-triol triacetate	1.91
Urs-12-ene-3β,16β,21-triol triacetate	2.25
Tarax-20-ene-3β,16β,30-triol triacetate	2.29
Lup-20(29)-ene-3β,16β,28-triol triacetate	3.03

* RRT of free erythrodiol = 1.00.

After TLC separation the purity of compounds was checked by GLC. Table II gives the relative retention times for isolated triol triacetates on an SE-30 column.

The results indicate that the separation of a mixture of marigold triols is possible using TLC followed by TLC on $AgNO_3$-impregnated plates. The spectroscopic analysis of these triols[4] showed that in all of them two hydroxy groups are in the 3β and 16β positions. Third hydroxy group in a ring E or between rings D and E confers a sufficiently different polarity to the compound to allow separation by the reported methods.

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