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Note

Gas–liquid chromatographic separation of triterpene monohydroxyalcohol esters

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Studies of the triterpene alcohols present in *Calendula officinalis* flowers have demonstrated that 90% of the monohydroxyalcohols are unesterified, the remaining 10% being esterified[1]. It has been shown that the alcoholic component of both fractions comprises the previously identified triterpene monools ψ-taraxasterol[2], α-amyrin, β-amyrin, taraxasteol and lupeol[3], whereas the acidic component includes acetic, lauric, myristic and palmitic acids[4].

Free monools can be separated from their acetates and esters with higher fatty acids by thin-layer chromatography (TLC) on silica gel. Mixtures of free triterpene monools and their acetates have been readily separated into individual components by TLC on silver nitrate-impregnated silica gel[3]. In contrast, attempts at the separation (with respect to both the acidic and alcoholic components) of monool esters with higher fatty acids have been unsuccessful[4].

The present study was designed to develop conditions permitting the gas–liquid chromatographic (GLC) separation of esters of triterpene alcohols with various fatty acids. Only few papers dealing with the separation of triterpene pentacyclic alcohols and their esters have so far been published. Ikekawa *et al.*[5] succeeded in separating α-amyrin from taraxasterol and Ikan and Gottlieb[6] investigated triterpene alcohols of ursane and lupane. The separation of β-amyrin from α-amyrin has been achieved[7].

EXPERIMENTAL

Materials

Free triterpene monools obtained from *Calendula officinalis* flowers were esterified with the use of commercial acetic and butyric anhydrides and also lauryl, myristyl and palmityl chlorides prepared by the reaction of phosphorus pentachloride with the corresponding fatty acids[8].

Esterification

Triterpene monool acetates and butyrates were prepared by reactions with the corresponding acid anhydrides at 80° for 90 min, and esters with higher fatty acids by reaction with the corresponding acid chlorides at 80° for 16 h.

Purification of esters

Acetates and butyrates. The mixture obtained by the reaction was evaporated to dryness with an air stream. The residue was dissolved in diethyl ether and purified by TLC on silica gel plates with *n*-hexane–chloroform–methanol (40:20:1).

Esters of higher fatty acids. For preliminary purification, the mixture obtained by the reaction was applied to a small column packed with silica gel (Koch-Light, Colnbrook, Great Britain) and eluted with the solvent mixture *n*-hexane–benzene (4:1). Subsequently, monool esters with higher fatty acids were purified by TLC on silica gel plates, followed by development with carbon tetrachloride.

Preparation of trimethylsilyl ethers

To 0.1 mg of free monool, 0.1 ml of a mixture obtained by combining 5.2 ml of hexamethyldisilazane, 3.2 ml of trimethylsilane and 4 ml of pyridine was added. The reaction mixture was allowed to stand for 1 h and then analyzed for the ether.

Gas–liquid chromatography

GLC was carried out on a Pye-Unicam instrument, using QF-1, OV-17 and OV-101 columns (5 ft.).

RESULTS AND DISCUSSION

Esters of triterpene pentacyclic alcohols with lower fatty acids (acetic and butyric acids) were prepared by reaction with the corresponding acid anhydrides, and esters with higher fatty acids (lauric, myristic and palmitic acids) by reaction with the corresponding acid chlorides, in analogy with the procedure used by Kuksis and Beveridge[9] for the preparation of sterol esters. These methods permitted esters of triterpene pentacyclic alcohols to be obtained in relatively high yield and with low contaminant content.

Esters of monools with higher fatty acids were separated on an OV-101 column. Good separation, with respect to the acid component, was attained, *i.e.*, laurates were separated from myristates and palmitates. Fractions of laurates, myristates and palmitates tended to resolve into two peaks, and consequently the separation of a mixture of monool esters with higher fatty acids resulted in six peaks. The retention times of esters of triterpene monools with higher fatty acids, measured against ψ-taraxasterol acetate and cholesterol added as internal standards, are presented in Table I.

In order to determine the alcoholic components corresponding to different peaks, esters of the individual alcohols with lauric acid were synthesized and separated on an OV-101 column. The relative retention times of monool laurates, measured against ψ-taraxasterol acetate and cholesterol, are given in Table II. The first peak corresponded to laurates of β-amyrin, α-amyrin and lupeol and the second peak to laurates of taraxasterol and ψ-taraxasterol.

Monool esters with higher fatty acids showed very long retention times. They exhibited wide peaks, resulting in a slight disagreement between the relative retention times of the esters determined singly and in admixture.

Monool esters with lower fatty acids (acetic and butyric acids) and trimethylsilyl ethers were separated on an OV-17 column, while butyrates were additionally separated on an OV-101 column. Both columns permitted good separations with

TABLE I

RELATIVE RETENTION TIMES OF TRITERPENE MONOOL ESTERS WITH HIGHER FATTY ACIDS

Esters	*OV-101 column (300°)**	
	B	*A*
(1) Laurates	6.58	15.80
	7.83	18.80
(2) Myristates	9.25	22.20
	11.83	28.40
(3) Palmitates	14.67	35.20
	18.25	43.80
Mixture of 1–3		
Peak 1	6.58	15.80
Peak 2	7.83	18.80
Peak 3	9.35	22.24
Peak 4	11.40	27.36
Peak 5	14.30	34.32
Peak 6	17.60	42.24

* Internal standard: A, cholesterol (R_t = 1.00); B, ψ-taraxasterol acetate (R_t = 1.00).

respect to the acidic component and a partial separation with respect to the alcoholic component. The results of the separation of monool esters with lower fatty acids and of trimethylsilyl ethers are presented in Table III. Mixtures of monool acetates and butyrates and of monool trimethylsilyl ethers separated into three peaks corresponding to the derivatives of the same compounds: 1, β-amyrin; 2, α-amyrin and lupeol; 3, ψ-taraxasterol and taraxasterol.

Free monools were separated on OV-17 and QF-1 columns. There was a slight difference in comparison with the separation of esters with lower fatty acids and trimethylsilyl ethers, namely, the three peaks obtained corresponded to: 1, β-amyrin;

TABLE II

RELATIVE RETENTION TIMES OF TRITERPENE MONOOL LAURATES

Laurate	*OV-101 column (300°)**	
	B	*A*
(1) β-Amyrin	6.15	14.76
(2) α-Amyrin	6.61	15.86
(3) Lupeol	6.80	16.32
(4) Taraxasterol	7.70	18.48
(5) ψ-Taraxasterol	8.05	19.32
(6) Mixture of 1–5		
Peak 1	6.58	15.80
Peak 2	7.83	18.80

* Internal standard: A, cholesterol (R_t = 1.00); B, ψ-taraxasterol acetate (R_t = 1.00).

TABLE III

RELATIVE RETENTION TIMES OF TRITERPENE MONOOL ACETATES, BUTYRATES AND TRIMETHYLSILYL ETHERS

Derivative of	*Column*			
	*OV-17 (285°), acetates**	*OV-17 (290°), butyrates**	*OV-101 (290°), butyrates**	*OV-17 (265°), TMS ethers***
(1) β-Amyrin	1.99	3.45	2.85	1.65
(2) α-Amyrin	2.23	3.75	3.15	1.83
(3) Lupeol	2.28	3.68	3.15	1.88
(4) ψ-Taraxasterol	2.82	4.65	3.69	2.28
(5) Taraxasterol	2.94	4.68	3.69	2.30
(6) Mixture of 1–5				
Peak 1	1.99	3.08	2.85	1.64
Peak 2	2.30	3.50	3.15	1.85
Peak 3	2.82	4.38	3.69	2.27

* Internal standard: cholesterol (R_t = 1.00).
** Internal standard: cholesterol TMS ether (R_t = 1.00).

2, α-amyrin, lupeol and taraxasterol; 3, ψ-taraxasterol. The results of the separation of free monools are presented in Table IV.

Combination of the GLC separation of free triterpene alcohols and their esters with lower fatty acids or trimethylsilyl ethers allowed the quantitative determination of most of the triterpene monools. Analysis of one of monool acetates, butyrates or trimethylsilyl ethers permits the quantitative determination of β-amyrin and also of the sum of lupeol and α-amyrin and the sum of taraxasterol and ψ-taraxasterol. Analysis of free monools permits the quantitative determination of β-amyrin and ψ-taraxasterol and of the sum of α-amyrin, lupeol and taraxasterol. Therefore, only α-amyrin and lupeol could not be separated and determined individually.

The present study indicates that, with the use of GLC, good separations of esters of triterpene alcohols with higher fatty acids can be obtained. However, we did

TABLE IV

RELATIVE RETENTION TIMES OF FREE TRITERPENE MONOOLS

Compound	*Column**			
	QF-1 (235°)		*OV-17 (280°)*	
	A	*D*	*A*	*D*
(1) β-Amyrin	1.76	1.00	1.45	1.00
(2) α-Amyrin	1.95	1.12	1.65	1.14
(3) Lupeol	1.88	1.08	1.67	1.15
(4) Taraxasterol	1.98	1.14	1.71	1.18
(5) ψ-Taraxasterol	2.40	1.30	2.00	1.41
(6) Mixture of 1–5				
Peak 1	1.75	1.00	1.45	1.00
Peak 2	1.95	1.12	1.67	1.15
Peak 3	2.39	1.30	2.00	1.41

* Internal standard: A, cholesterol (R_t = 1.00); D, β-amyrin (R_t = 1.00).

not succeed in establishing conditions that would permit the separation of all individual monools.

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