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SHORT COMMUNICATION

STEROL AND TRITERPENE ALCOHOL ESTERS FROM *CALENDULA OFFICINALIS*

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Abstract—Sterol esters as well triterpene monol and diol esters isolated from the flowers of *Calendula officinalis* contain as alcohol components all the types of sterols and triterpenic alcohols present in this plant. Sterols and triterpene monols are esterified with acetic, lauric, myristic and palmitic acids. Triterpene diols are esterified with lauric, myristic and palmitic acids. The main diol esters are 3-monoesters; diesters are present only in very small amount.

INTRODUCTION

IT APPEARS from the available data that the acidic components of the sterol and triterpene esters in plants are mostly long-chain fatty acids (mainly C_{14}–C_{20});[1–4] less common are lower fatty acids, e.g. acetic acid,[2,5,6] and aromatic acids such as dihydrocaffeic acid[7] and cinnamic acid.[8]

We have previously shown[9–11] that the flowers of *Calendula officinalis* (Compositae) contain a number of sterols and mono- and dihydroxy pentacyclic triterpene alcohols, both free and esterified.[12,13] The esters represent about 20% of total sterols occurring in flowers of *C. officinalis*, 10% of monols and 98% of triterpene diols.

Preliminary studies[12] indicated that a fraction of sterols and trierpene monols is esterified with acetic acid. The experiments described in this paper were aimed at a more complete qualitative and quantitative analysis of these triterpenoid esters.

RESULTS

A light petroleum extract (57 g) of 500 g of dry calendula flowers was chromatographed on aluminium oxide. The eluate was examined before and after hydrolysis by TLC. Four fractions of different compostion containing triterpenoid esters were obtained. The fractions

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[12] Z. KASPRZYK, G. TUROWSKA and E. BARANOWSKA, *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **17**, 399 (1969).
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(designated I–IV according to their increasing polarity) were further purified by preparative TLC. Chromatographically homogenous fractions were lightly coloured oils. From fractions I and III mixtures of sterols and triterpene monols were obtained by hydrolysis, from fractions II and IV mixtures of triterpenic diols.

Chromatographic comparison of the ester fractions with synthetic[14] acetates, butyrates myristates and stearates of sitosterol, ψ-taraxasterol and the mono- and diesters of faradiol indicated that fraction I contains esters of sterols and monols and higher fatty acids, fraction II diesters of diols and higher fatty acids, fraction III acetates of monols and sterols and fraction IV monoesters of diols and higher fatty acids or diacetates of diols (Fig. 1). Components of the fraction IV could be acetylated, yielding products of higher chromatographic mobility. This confirms that they are monoesters of higher fatty acids and excludes the possibility that fraction IV contains diacetates. GLC analysis of the acidic components of this fraction showed that it contained fatty acids C_{12}–C_{16}. In order to elucidate whether the monoesters of diols are 3-monoesters or if the acyl moieties are bound to a second OH group at C-12 or C-16, a sample of fraction IV was oxidized with CrO_3, hydrolysed and then acetylated. The resulting mixture of monoacetylmonoketo-diols was examined by ORD and CD. The product showed a negative Cotton effect of the optical rotatory dispersion ($[\Phi]_{250} = +2145$, $_{270} = +2850$, $_{285} = +1739$, $_{294} = 0$, $_{300} = -1043$, $_{308} = -2145$, $_{316} = -1913$, $_{330} = -850$, $_{350} = -435$) and a negative circular dichroism curve ($\Delta\epsilon_{250} = -0{\cdot}037$, $_{270} = -0{\cdot}372$, $_{293} = -1{\cdot}059$, $_{310} = -0{\cdot}416$, $_{320} = -0{\cdot}058$). Previous observations of Sliwowski and Kasprzyk[15] showed that synthetic 3-acetyl monoketones of all diols found in calendula show a negative Cotton effect and a negative circular dichroism curve. On the other hand, for all 3-keto (12 or 16)-monoacetyl derivatives of the same diols positive Cotton effects are observed and positive circular dichroism curves. This finally proves that the fatty acid moieties are attached to the 3-OH group in natural monoesters of diols of calendula.

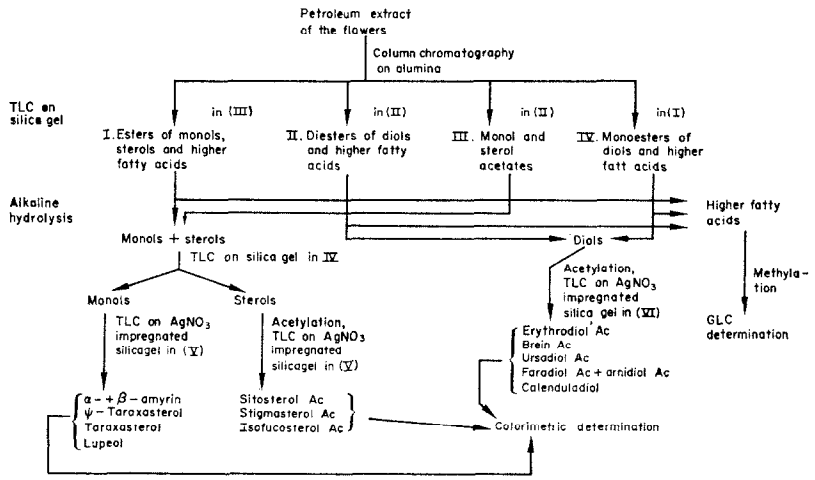

FIG. 1. FRACTIONATION AND EXAMINATION OF TRITERPENOID ESTERS FROM THE FLOWERS OF *C. officinalis*.

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TABLE 1. COMPOSITION OF THE TRITERPENOID ESTER FRACTION FROM THE FLOWERS OF *Calendula officinalis*

Ester fraction	Content (% of dry wt. of flowers)	Alcohol components %		Acidic components % C_2	C_{12}	C_{14}	C_{16}
I. Esters of monols and sterols	0·344	Sitosterol	15·1	—	54	14	32
		Stigmasterol	22·7				
		Isofucosterol	—				
		α- and β-amyrin	21·9				
		ψ-Taraxasterol	15·3				
		Taraxasterol	—				
		Lupeol	25·0				
II. Diesters of diols	0·020	Erythrodiol	trace	—	3	42	55
		Brein	10·8				
		Ursadiol*	13·3				
		Faradiol and arnidiol	50·9				
		Calenduladiol	25·0				
III. Esters of monols and sterols	0·066	Sitosterol	9·0	100	—	—	—
		Stigmasterol	14·4				
		Isofucosterol	2·6				
		α- and β-amyrin	—				
		ψ-Taraxasterol	19·0				
		Taraxasterol	25·6				
		Lupeol	29·4				
IV. 3-Monoesters of diols	4·070	Erythrodiol	—	—	5	42	53
		Brein	8·6				
		Ursadiol*	6·1				
		Faradiol and arnidiol	75·5				
		Calenduladiol	9·8				

* A new triterpene diol.[16]

The isolated ester fractions I–IV were hydrolysed and the triterpenoid alcohols were then quantitatively determined by colorimetry[18] and the fatty acids by GLC. Figure 1 schematically presents the operations applied to the fractions studied. Results of the quantitative determinations are given in Table 1. The data indicate that only about 16% of the esterified monols and sterols is bound to acetic acid. No diol acetates were found. The higher fatty acids bound to triterpenoids are lauric, myristic and palmitic acids. Comparison of the quantity of acid components of monol, sterol and diol esters clearly indicates a higher content of lauric acid in monol and sterol esters and of palmitic acid in diol esters. The composition of the fraction of fatty acids obtained from triterpenoid esters is clearly distinct from those of the total fractions of bound and free fatty acids obtained from the flowers of calendula. Those latter fractions contain considerable proportions of C_{18} acids. The analyses performed gave the following percent composition of these fractions; bound acids—C_8-traces, C_{10}-traces, C_{12}-10, C_{14}-36, C_{16}-43, C_{18} (combined saturated and unsaturated)-11, free acids—C_{12}-2, C_{14}-17, C_{16}-50, C_{18}-31.

EXPERIMENTAL

Chromatography. Column chromatography was carried out using alumina (Fluka 507 C) inactivated by adding water to give activity II. The column was eluted consecutively with light petroleum (40–60°), light

[16] J. ŚLIWOWSKI, private communication.

petroleum–benzene, benzene and benzene–Et_2O. TLC was carried out on silica gel (Kieselgel G, Merck) or silica gel/10% $AgNO_3$. The chromatograms were developed with: $CHCl_3$–Et_2O (19:1) (I), cyclohexane–benzene (1:1) (II), light petroleum (40–60°)–benzene (1:1) (III), *n*-heptane–$CHCl_3$–MeOH (60:30:3) (IV), $CHCl_3$ (V), and benzene (VI).

Chemical operations. Hydrolysis. Ester samples were heated with 10% methanolic KOH in sealed ampoules at 100° for 12 hr. Acetylation of alcohols with Ac_2O (in pyridine) and methylation of fatty acids with CH_2N_2 were performed according to usual procedures. Oxidation of diol monoesters to monoketo-monoacyl derivatives was carried out using CrO_3 in pyridine[17] at 18° for 24 hr.

Quantitative determinations. Triterpenoid alcohols were determined colorimetrically as $CoCl_2$ complexes.[18] Calibration curves were drawn for each of the compounds studied. Fatty acids C_8–C_{20} (as methyl esters) were determined by GLC on a Gas-Chrom-2 Chromatograph at 197°. Stationary phase: 20% Apiezon L on Kieselgur (Merck). ORD and CD measurements were made using a Jasco UV (ORD) CD-5 spectrophotometer in methanol at room temp.

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Key Word Index—Calendula officinalis; Compositae; steroids; triterpene alcohol esters; fatty acids.