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# PENTACYCLIC TRITERPENE TRIOLS FROM *CALENDULA OFFICINALIS* FLOWERS

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**Key Word Index**—*Calendula officinalis*; Compositae; triterpene triols.

**Abstract**—From the flowers of *Calendula officinalis* five pentacyclic triterpene trihydroxyalcohols were isolated and identified as: olean-12-ene-3$\beta$,16$\beta$,28-triol, lup-20(29)-ene-3$\beta$,16$\beta$,28-triol, tarax-20-ene-3$\beta$,16$\beta$,22$\alpha$-triol, tarax-20-ene-3$\beta$,16$\beta$,30-triol and ursa-12-ene 3$\beta$,16$\beta$,21-triol, by chromatographic and spectroscopic means.

## INTRODUCTION

*Calendula officinalis* flowers are a very rich source of pentacyclic triterpene alcohols possessing different numbers of hydroxyl groups. In 1968 Kasprzyk and Pyrek [1] published a paper describing the mono- and dihydroxy-alcohols of this plant. In the group of mono-ols were identified $\alpha$-amyrin, $\beta$-amyrin, taraxasterol, $\psi$-taraxasterol and lupeol and amongst the diols they found faradiol, brein, arnidiol, erythrodiol and calenduladiol. A few years later the structure of a new diol and a new triol were elucidated [2, 3]. Later Pyrek [4–6] revised the structures of faradiol, arnidiol, calenduladiol and ursadiol and presented evidence to show that the position of the second hydroxyl group was at C-16. Pyrek [4] also suggested that the above mentioned triol could be a faradiol epoxide, but this seemed to be an erroneous suggestion, because a comparison of the mass spectral patterns of these compounds showed some differences. In the course of our study on the triterpene constituents of *Calendula officinalis* flowers we have now focused on more polar pentacyclic triterpene alcohols. The aim of the present work was the isolation and structure determination of the pentacyclic triterpene triols from the title plant.

## RESULTS AND DISCUSSION

The dry flowers of *Calendula officinalis* were extracted with diethyl ether. The ether extract was evaporated *in vacuo* to dryness and hydrolysed with 10% sodium hydroxide in methanol. The methanol solution was partly evaporated, diluted with water and adsorbed onto silica gel. Chromatography over a silica gel column gave a mixture of triterpene alcohols more polar than triterpene diols. The mixture of polar triterpene alcohols was acetylated and TLC separation of this fraction gave four bands. Three of them were individual compounds when examined by $AgNO_3$–silica gel TLC, whereas the lowest band divided into two compounds. Hence, after a combination of normal silica gel TLC and $AgNO_3$–silica gel TLC five individual triacetates of triterpene alcohols were obtained. GLC analysis confirmed their purity. Free alcohols obtained after hydrolysis of the acetates gave a negative reaction with diazomethane proving the lack of a carboxyl group. However, positive tetranitromethane tests showed the presence of unsaturation.

The mass spectrum of the triacetate with lowest polarity exhibited a molecular ion $[M]^+$ at $m/z$ 584 and three peaks at $m/z$ 524, 464 and 404 formed by the successive losses of acetic acid units. The appearance of intense peaks at $m/z$ 203, 201 base and 189 is typical for retro-Diels–Alder fragmentation, which has been recognized as a characteristic feature of the mass spectra of $\Delta^{12}$-unsaturated oleananes and ursanes [7]. The greater relative intensities of the peaks at $m/z$ 203 and 201 compared to the peak at $m/z$ 189 suggested the oleanane skeleton. The peak at $m/z$ 451 indicated the presence of a primary hydroxyl group. The mass spectrum was identical with that of an authentic sample of longispinogenine triacetate. Longispinogenine is the triterpene triol isolated first by Djerassi *et al.* [8], who confirmed its structure by a partial synthesis from echinocystic acid [9]. Co-chromatography with the authentic sample confirmed the identity of the investigated compound with longispinogenine triacetate, i.e. olean-12-ene-3$\beta$,16$\beta$,28-triol triacetate.

The mass spectrum of the next compound exhibited the loss of three acetic acid molecules ($m/z$ 524, 464, 404) from the molecular ion ($m/z$ 584). The peak at $m/z$ 451 indicated the presence of a primary hydroxyl group. The very intense peak at $m/z$ 189 is characteristic for all triterpenes with the lupane skeleton irrespective of the nature of substitution in rings A, B, D and E [10]. The peak at $m/z$ 201 can originate from the rings D and E together with an isopropenyl group. Other peaks do not possess diagnostic value in this type of skeleton. The $^1H$ NMR spectrum showed the presence of three acetyl groups ($\delta$2.02, 9H, 3 × Ac) and five tertiary methyl groups (singlets in the region $\delta$1.25). The vinylic methyl group absorbed at $\delta$1.65 and the vinylidene group at the region 4.50–4.80 [11]. Hence, for this compound the structure proposed is lup-20(29)-ene-3$\beta$,16$\beta$,28-triol triacetate.

The mass spectrum of the third compound exhibited significant peaks at $m/z$ 289, 274, 271, 229, 189 and 187 which are identical with those in the mass spectrum of heliantriol F [12]. Moreover, the molecular ion at $m/z$ 584 and ions at $m/z$ 524, 464 and 404 confirmed that three acetyl groups were present in the molecule. The presence

of three acetyl groups was confirmed by the $^1$H NMR spectrum (singlets at $\delta$1.98–2.02). A doublet of an olefinic proton appeared at $\delta$5.18. Signals at $\delta$4.42 *s* and 4.65 *dd* were derived from the 3α- and 16α-protons, respectively. These signals are well correlated with those of authentic heliantriol F triacetate, i.e. tarax-20-ene-3β,16β,30-triol triacetate [12].

The fourth, lowest, TLC band separated on $AgNO_3$–silica gel TLC into two compounds. The higher one appeared to be identical in all chromatographic and spectroscopic properties with an authentic sample of heliantriol C [12]. Its mass spectrum exhibited significant peaks at *m/z* 584, 524 (base), 509, 482, 464, 449, 389 and 289, confirming the structure of a triterpene triol triacetate derivative with a taraxane skeleton. In the $^1$H NMR spectrum a doublet at $\delta$5.20 corresponded to an olefinic proton. The structure of heliantriol C was clearly demonstrated by Pyrek [12] to be tarax-20-ene-3β,16β,22α-triol.

The lower compound obtained from the $AgNO_3$–silica gel TLC was identical in its mass and NMR spectra with a previously reported triol [3]. We postulated for it the structure urs-12-ene-3,16,21-triol in spite of the absence in the $^1$H NMR spectrum of the signal derived from an olefinic proton. However, we find now a doublet at $\delta$4.90. Co-chromatography of the newly obtained triol with the previous sample of ursatriol revealed their identity.

The results obtained indicate that in all the triols identified in *Calendula officinalis* flowers, except for longispinogenine, the third hydroxylation occurs either in ring E or between rings D and E. Only in longispinogenine which is derived from erythrodiol (second hydroxyl group at C-28) does the third hydroxylation occur at C-16. Thus, all marigold triols have hydroxyl groups in positions C-3 and C-16; they differ from one another at the third centre of hydroxylation and in the type of skeleton.

## EXPERIMENTAL

*Extraction and isolation.* The dry ligulate marigold flowers (400 g) were extracted with boiling $Et_2O$ for 3 days. The extract was evaporated *in vacuo* and the crude lipid fraction (52 g) was hydrolysed by refluxing with 10% NaOH in MeOH. The reaction mixture was concd under red. pres. and diluted with $H_2O$. The extraction with $Et_2O$ and evaporation of extract *in vacuo* gave the unsaponified fraction (28 g) which was adsorbed onto silica gel and applied to a silica gel column. Compounds were eluted with a mixture of hexane–$CHCl_3$ (0–100% of $CHCl_3$). Crude triterpene polyols were obtained from the eluate containing 80–100% of $CHCl_3$ (2.1 g). Acetylation of this fraction was carried out in the usual manner with $Ac_2O$ and pyridine at room temp giving after TLC separation the mixture of triol triacetates (220 mg). Triol triacetates were separated by silica gel TLC and $AgNO_3$–silica gel TLC using the solvent systems: $CHCl_3$–MeOH (75:1) and $CHCl_3$ (free from MeOH)–$Et_2O$ (50:1), respectively. The purity of the isolated triol triacetates was checked by GLC on an SE-30 column [13].

*Olean-12-ene-3β,16β,28-triol triacetate (longispinogenine).* MS (70 eV) *m/z* (rel. int.): 584 $[M]^+$ (2), 524 $[M-AcOH]^+$ (56), 464 $[M-2AcOH]^+$ (1), 451 $[M-CH_2OAc-AcOH]^+$ (2), 404 $[M-3AcOH]^+$ (1), 391 $[M-3AcOH-CH_2]^+$ (0.7), 274 (19), 203 (9), 201 (100), 189 (17), 187 (17), 43 (48). MS (15 eV) *m/z* (rel. int.): 584 $[M]^+$ (0.5), 524 (6), 464 (100), 451 (11), 404 (2), 391 (15), 274 (28), 203 (7), 201 (62), 189 (10), 187 (7), 43 (0.5).

*Lup-20(29)-ene-3β,16β,28-triol triacetate (lupenetriol).* MS (70 eV) *m/z* (rel. int.): 584 $[M]^+$ (0.5), 524 (30), 464 (75), 451 (14), 404 (10), 391 (12), 249 (6), 201 (40), 189 (52), 187 (39), 43 (100). MS (15 eV) *m/z* (rel. int.): 584 $[M]^+$ (1), 524 (46), 464 (100), 451 (18), 404 (13), 201 (24), 189 (32), 187 (21), 43 (3).

*Tarax-20-ene-3β,16β,30-triol triacetate (heliantriol F).* MS (15 eV) *m/z* (rel. int.): 584 $[M]^+$ (0.6), 524 (6), 509 (1), 496 (5), 464 (9), 451 (5), 449 (5), 404 (3), 389 (3), 289 (20), 274 (16), 271 (11), 189 (100), 187 (58), 43 (28).

*Tarax-20-ene-3β,16β,22α-triol triacetate (heliantriol C).* MS 70 eV *m/z* (rel. int.): 584 $[M]^+$ (0.4), 524 (21), 464 (17), 422 (3), 404 (5), 389 (4), 201 (12), 191 (16), 189 (9), 43 (100). MS (15 eV) *m/z* (rel. int.): 584 $[M]^+$ (1), 524 (100), 482 $[M-AcOH-ketene]^+$ (31), 464 (46), 422 (6), 404 (11), 389 (1), 201 (11), 189 (34), 43 (5).

*Ursa-12-ene 3,16,21-triol triacetate (ursatriol).* MS (70 eV) *m/z* (rel. int.): 584 $[M]^+$ (0.5), 524 (0.2), 464 (4), 404 (1), 248 (7), 189 (34), 43 (100), MS (15 eV) *m/z* (rel. int.): 584 $[M]^+$ (0.8), 524 (2), 464 (15), 404 (3), 201 (17), 189 (100), 187 (23), 43 (26).

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