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URSADIOL: A NEW TRITERPENE DIOL FROM *CALENDULA OFFICINALIS* FLOWERS

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Key Word Index—*Calendula officinalis*; Compositae; triterpene diols; ursadiol; structure.

Abstract—A new triterpene diol was isolated from dry *Calendula officinalis* flowers. Its structure was proved as 3,21-di-OH-ursa-12-en.

INTRODUCTION

IN THE flowers of *Calendula officinalis* the following triterpene diols have been previously identified: faradiol, arnidiol,[1] brein, erythrodiol and calenduladiol.[2] Observations on the fractions obtained by column chromatography in our earlier studies[2] suggested the presence of an unknown triterpene diol with the ursane skeleton. In this paper, a more detailed characterization of the compound is presented.

RESULTS AND DISCUSSION

The triterpene pentacyclic diol fraction was obtained from dry flowers of *Calendula officinalis* as described previously.[2] The diols were acetylated and subsequently separated on alumina column. Fractions containing a compound migrating between diacetates of brein and faradiol on silica gel plates impregnated with $AgNO_3$ were obtained. The compound was isolated by preparative TLC.

The new triterpene compound has been named ursadiol. Elemental analysis of ursadiol diacetate gave results for C and H corresponding to the content of these elements in a pentacyclic triterpene diol diacetate ($C_{34}H_{54}O_4$).

MS provided further confirmation of the pentacyclic triterpene diol structure proposed for ursadiol. No peak was found at *m/e* 526 which corresponds to the M ion of ursadiol diacetate. On the other hand, peaks were observed at *m/e* 406 (100·0) corresponding to M-2AcOH and *m/e* 391 (20·0) corresponding to M-(2AcOH + Me). Analogous peaks *m/e* 406 (100·0) and *m/e* 391 (25·5) and lack of the M ion peak was found also for brein diacetate.

Additionally for ursadiol diacetate peaks were observed at *m/e* 216 (12·0) and *m/e* 190 (16·0). These peaks are typical fragments for α- or β-amyrin derivatives (Δ^{12}) formed as a result of retro-Diels–Adler fragmentation.[3,4] Analogous peaks at *m/e* 216 (16·5) and *m/e* 190 (21·2) were found also for brein diacetate.

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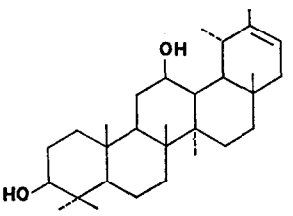
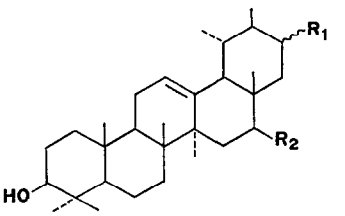
[2] Z. KASPRZYK and J. PYREK, *Phytochem.* **7**, 1631 (1968).
[3] C. DJERASSI, H. BUDZIKIEWICZ and J. M. WILSON, *Tetrahedron Letters* **263** (1962).
[4] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

In order to establish the type of skeleton for ursadiol, oxidation of the diacetate was performed with selenium dioxide. This compound oxidizes β-amyrin derivatives to oleana 11,13 (18)-diene[5,6] and does not oxidize α-amyrin derivatives.[7] It was found that under conditions ensuring oxidation of β-amyrin acetate and erythrodiol diacetate to the corresponding diene derivatives, neither ursadiol diacetate nor brein diacetate were oxidized. The suggestion that ursadiol is an α-amyrin derivative was further confirmed by IR: the spectra of ursadiol and α-amyrin are closely similar in the 1050–1400 cm^{-1} range—analogous bands at 1095, 1150 and 1188 cm^{-1} and a characteristic split band of identical intensity at 1370–1390 cm^{-1}. The IR spectrum of brein is not as close to the α-amyrin spectrum. This is probably related to different localization of hydroxyls in the ursadiol and brein molecules.



Faradiol



Ursadiol, R_1 = OH; R_2 = H
Brein, R_1 = H; R_2 = OH

A preliminary indication that two secondary hydroxyls occur in the ursadiol molecule was found in the results of oxidation with chromic anhydride. This reaction resulted in conversion of ursadiol into a compound which chromatographically resembled brein diketone. Using a previously described procedure,[8] it was also possible to obtain two different monoacetoxy-monoketo derivatives of ursadiol. The above results indicate that ursadiol is the second diol, after brein, with the α-amyrin skeleton, occurring in the flowers of *Calendula officinalis*.

The properties of the ursadiol and brein derivatives are compared in Table 1. Examination of the UV spectrum of the diketo derivative of ursadiol does not show coupling between the keto groups. Localization of the secondary hydroxyls in the ursadiol molecule was established by analysis of NMR, ORD and CD spectra of the derivatives of this compound.

NMR spectra were obtained for ursadiol, brien and faradiol diacetates. In the brein diacetate molecule, acetoxyl groups at positions 3 and 16 are located in the vicinity of Me groups, whereas acetoxyl at position 12 of faradiol diacetate is located in an environment distinct from that of the OAc group at position 3 in this molecule, since it is not close to a Me group. In the NMR spectrum of brein diacetate, protons of both acetoxyls[11] give a single band (τ8·03), whereas the proton signal of these groups in the faradiol diacetate is clearly split into two bands (τ8·02, 8·06). It was established that in the NMR spectrum of ursadiol diacetate, protons of both acetoxyls give the single band (τ8·03) as in brein. Thus,

[5] G. R. RETTIT, H. KLINGER, N. OTTO, N. JORGENSEN and J. OCCOLOWITZ, *Phytochem.* **5**, 301 (1966).
[6] G. H. STOUT and K. L. STEVENS, *J. Org. Chem.* **28**, 1259 (1963).
[7] E. J. COREY and J. URSPRUNG, *J. Am. Chem. Soc.* **78**, 183 (1956).
[8] J. ŚLIWOWSKI and Z. KASPRZYK, *Tetrahedron* **28**, 991 (1972).
[9] P. BOITEAU, B. PASICH and A. RAKOTO RATSIMAMANGA, *Les Triterpenoides*, Paris (1964).
[10] *Encyclopedia of Organic Chemistry*, Ser. III, Vol. 14 Supplement, *Triterpenes*, Elsevier, Amsterdam (1952).
[11] N. S. BHACCA and D. H. WILLIAMS, *Application of NMR Spectroscopy in Organic Chemistry*, Holden-Day, San Francisco (1964).

both the secondary hydroxyls of ursadiol molecule are located in the vicinity of Me groups. Consequently, the following positions can be taken into consideration for localization of the hydroxyls: C1, C3, C7, C15, C21 and C22. Position C16 for a hydroxyl group can be eliminated since it occurs in brein.

TABLE 1. THE PROPERTIES OF URSADIOL AND BREIN DERIVATIVES

Ursadiol derivatives Compound	m.p. (°)	$[\alpha]_D^{25°}$(°)	Brein derivatives Compound	m.p. (°)	$[\alpha]_D^{25°}$(°)
Ursadiol	240–242	−48·0	Brein	218–221 216–223[9]	69·5 65·0[9]
Ursadiol diacetate	214–217	6·5	Brein diacetate	195–197 197–202[9]	73·0 72·0[9]
Ursadion	278–280	4·0	Briendion	210–213[8] 159–160[10]	82·0
3-oxo-21-acetoxy ursa-12-en	207–210	5·5	3-Oxo-16-acetoxy ursa-12-en	200–204[8]	—
3-Acetoxy-21-oxo ursa-12-en	215–218	4·0	3-Acetoxy-16-oxo ursa-12-en	208–210[8] 206–206·5[10]	—

The diketo derivative and two different monoacetoxy-monoketo derivatives of ursadiol were examined by ORD and CD. The spectra obtained are reproduced in Fig. 1. Our previous investigations[8] showed that 3-oxo-X-acetoxy derivatives of pentacyclic triterpenes with ursane, lupane and oleanane skeletons give positive ORD Cotton and CD effects. Of

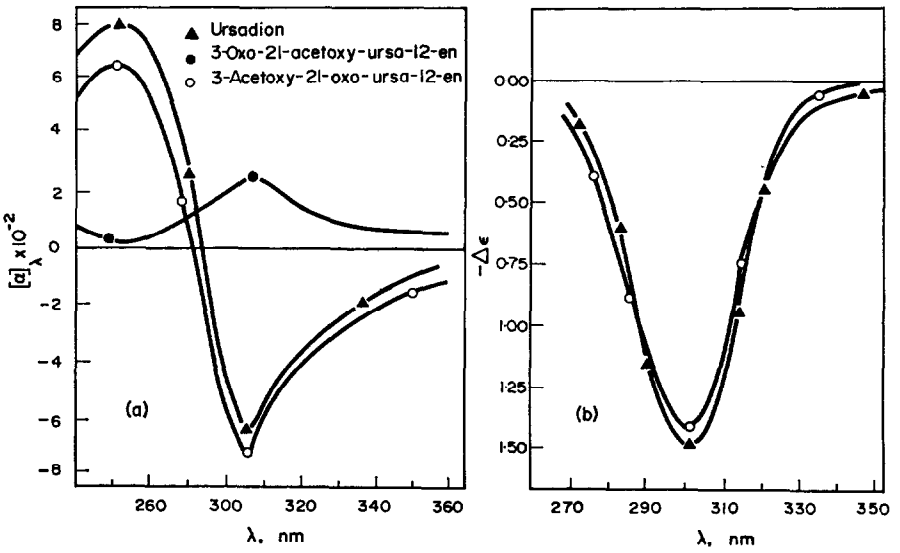


FIG. 1. ORD (a) AND CD (b) CURVES OF URSADION, 3-OXO-21-ACETOXY-URSA-12-EN and 3-ACETOXY-21-OXO-URSA-12-EN.

the two monoketo derivatives of ursadiol, one shows a positive ORD Cotton effect and a zero CD effect; this is probably a consequence of an intramolecular compensation between CO and OAc groups. The other derivative gives a negative ORD Cotton effect and a negative CD effect. For the diketo derivative, a negative ORD Cotton effect and a negative CD effect were also observed. This indicates[8] that a keto group (consequently also a hydroxyl) is located at position 3 of the skeleton, as in a great majority of triterpenes. The second CO group in the ursadiol diketo derivative is responsible for the strong, negative rotation and the negative CD effect. It should be stressed that ursadiol also shows a negative rotation in the UV range: $[\alpha]_{250}$ −453°, $[\alpha]_{300}$ −279°, $[\alpha]_{350}$ −160° in contrast to brein: $[\alpha]_{250}$ +940°, $[\alpha]_{300}$ +650°, $[\alpha]_{350}$ +360°. It is also significant that the diketo derivative of brein shows positive rotation above 347 nm.[8] whereas this effect is not observed for ORD of the ursadiol diketo derivative.

Djerassi *et al.*[12] performed ORD spectra for oleanane derivatives with keto groups at C15, C21 and C22. Comparison of the molecular amplitudes obtained for ursadiol keto derivatives with the values given by those authors indicates that the localization of the second hydroxyl at C15 is unlikely. On the other hand, the character of ORD Cotton effects and molecular amplitudes of these effects indicate the likelihood of positions C21 and C22. A small positive optical rotation at 589 nm for the diketo derivative and for the 3-acetoxy-X-oxo derivative of ursadiol seem to favour localization of the second OH group of ursadiol at position C21. The results obtained suggest that ursadiol is: 3,21-di-OH-ursa-12-en.

EXPERIMENTAL

From 4·5 kg of dry *Calendula officinalis* flowers, 104 g triterpene diol and polyol fraction was obtained From this material, after acetylation and column chromatography, 0·52 g of crude ursadiol diacetate was obtained. Ursadiol diacetate was 3 × crystallized from 96% EtOH. Elemental analysis of ursadiol diacetate (Found: C, 77·70; H, 10·45. Required: C, 77·6; H, 10·3%). The conditions for column and TLC were analogous to those used previously.[2,8] Ursadiol keto derivatives were obtained in the manner described previously.[8] M.ps (uncorrected) were determined on a heated microscopic plate. MS were obtained using an LKB-9000 apparatus; samples were introduced by the direct inlet. The IR spectra were obtained in KBr discs using a Unicam SP 200 spectrophotometer. NMR spectra were obtained in a High Resolution NMR Instrument INH-C-6OH against TMS in 0·1% CCl_4 solution. ORD and CD spectra were obtained with a JASCO UV/ORD/CD 5 spectrophotometer under the conditions described previously.[8] $[\alpha]_D$ was determined in 0·02–0·03% $CHCl_3$ solutions.

[12] C. DJERASSI, J. OSIECKI and W. CLOSSON, *J. Am. Chem. Soc.* **81**, 4587 (1959).