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TRITERPENIC ALCOHOLS OF *CALENDULA OFFICINALIS* L. FLOWERS

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Abstract—A number of alcohols representing different types of pentacyclic triterpenes were identified in the flowers of *Calendula officinalis*. In the group of monohydroxyalcohols were identified: α-amyrin, β-amyrin, taraxasterol and lupeol in addition to previously isolated ψ-taraxasterol. In the group of dihydroxyalcohols: brein and calenduladiol (a new diol of lupeol type) in addition to the previously isolated arnidiol and faradiol. The presence of four other diols of the α-amyrin, β-armyrin and ψ-taraxasterol types was noticed. Alcohols of ψ-taraxasterol type, possessing three and four hydroxyl groups, were also isolated as well as a small amount of oleanolic aldehyde.

INTRODUCTION

IT HAS been reported that a number of triterpenes is present in the flowers of *Calendula officinalis*. Besides the glycosides of oleanolic acid,[1,2] dihydroxyalcohols arnidiol and faradiol were identified by Zimmermann.[3] The works of Gedeon[4] and Herout and Suchy[5] suggested the presence of other diols in this material. Monohydroxyalcohol "calendol" was detected in it by Kasprzyk,[6] and was identified as ψ-taraxasterol by Stevenson.[7] The presence of β-amyrin and taraxasterol was observed on thin-layer chromatograms.[8]

The results of the present work indicate that the calendula flowers contain a mixture of triterpenic monols, diols and polyalcohols.

RESULTS

The non-saponifiable fraction from 4 kg of dry flowers of *Calendula officinalis* was fractionated by counter-current extraction between hexane and 80 per cent ethanol. Upper layers contained hydrocarbons, triterpenic monols, methylsterols and sterols. Triterpenic diols and more polar compounds were found in the lower phase. The majority of hydrocarbons were removed by co-crystallization with urea. ψ-Taraxasterol (Ia) and faradiol (Ib) were then obtained from the upper and lower phase respectively and purified by crystallization from ethanol. Mother liquors from these compounds were fractionated on columns of alumina to yield the various polar groups of compounds. Each step was checked by thin-layer chromatography. The compounds in each group of the same polarity were separated by means of argentation chromatography[9] using a mixture of hexane containing

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[6] Z. KASPRZYK, *Prece Głównego Instytutu Chemii Przemysł* **1**, 39 (1951).
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[8] Z. KASPRZYK, Z. GRZELCZAK and J. PYREK, *Bull. Acad. Polon. Sci., Ser. Sci. Biol.* **13**, 661 (1965).
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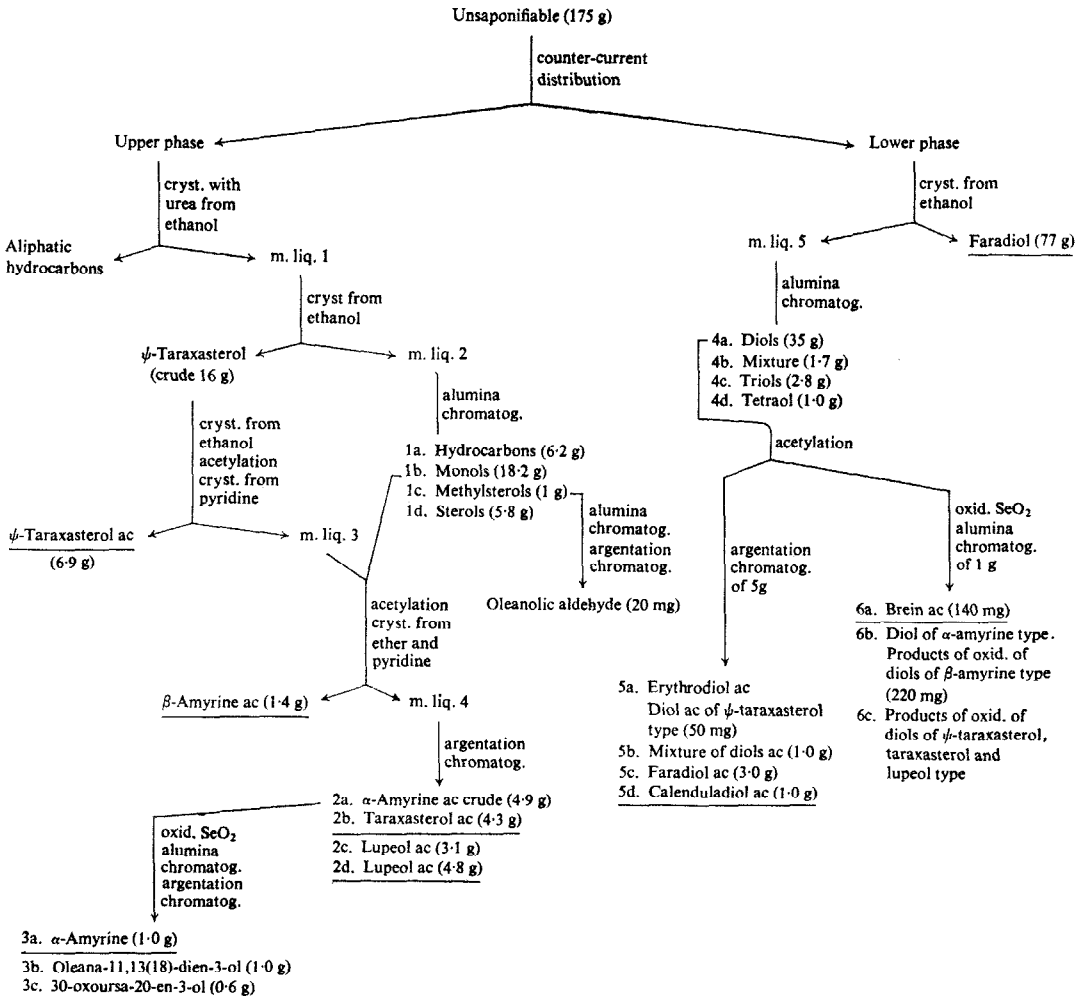


FIG. 1. SCHEME OF FRACTIONATION OF TRITERPENIC ALCOHOLS FROM THE FLOWERS OF *Calendula officinalis*.

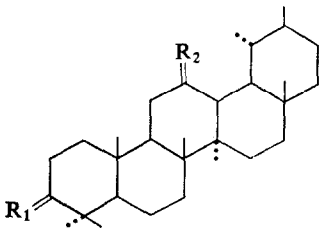
TABLE 1. THE PROPERTIES OF THE TRITERPENIC ALCOHOLS ISOLATED FROM THE FLOWERS OF *Calendula officinalis* AND OF THEIR DERIVATIVES

Compound		Melting point		$[\alpha]_D^{25°}$ (in chloroform)		Found (%)		Required (%)		Formulae
		Found	Reported	Found	Reported	C	H	C	H	
ψ-Taraxasterol	Ia	208–211°	217–221°[12]	48·0°	47·5 ± 2·5°[12]					
ψ-Taraxasterol ac		236–239°	225–247°[12]	55·5°	54·0 ± 2·0°[12]	81·95	11·05	82·0	11·2	$C_{32}H_{52}O_2$
ψ-Taraxasterol bz		270–279°	273–292°[12]	—	—					
Epoxide of ψ-taraxasterol ac		279°	263–267°[21]	—	—					
30-Oxoursa-20-en-3β-ol ac	IIa	269°	—	84·0°	—	79·44	10·28	79·6	10·5	$C_{32}H_{50}O_3$
β-Amyrine	IVa	194–200°	194–200°[12]	81·5°	80·0 ± 2·0°[12]					
β-Amyrine ac		241–242·5°	237–242°[12]	79·5°	80·0°[12]	81·99	11·15	82·0	11·2	$C_{32}H_{52}O_2$
β-Amyrine bz		231–236°	230–236°[12]	—	—					
Epoxide of β-amyrine ac		250–252°	—	—	—					
Oleana-11,13(18)-dien-3β-ol ac	Va	228–229°	227–228°[11]	−67·0°						
α-Amyrine	VIa	186–190°	183–187°[12]	78·5°	82·0°[12]					
α-Amyrine ac		222–225°	220–227°[12]	75·0°	83·0°[12]	82·01	10·92	82·0	11·2	$C_{32}H_{52}O_2$
Taraxasterol	IIa	213–219°	217–227°[12]	82·0°	95·0°[12]					
Taraxasterol ac		234–248°	245–248°[12]	95·0°	101·0°[12]	82·00	11·00	82·0	11·2	$C_{32}H_{52}O_2$
Lupeol	VIIa	214–216·5°	212–216°[12]	28·7°	28·0 ± 1·0°[12]					
Lupeol ac		210–216·5°	213–220°[12]	46·0°	46·0°[12]	81·95	10·80	82·0	11·2	$C_{32}H_{52}O_2$
30-Oxolupa-20(30)-en-3β-ol ac	VIIIa	215–225°	224–226°[18]	—	—	76·85	9·82	76·6	10·4	$C_{32}H_{50}O_3$
Oleanolic aldehyde	IVc	182–189°	186°[12]	—	—					
Faradiol	Ib	235–236°	235–240°[12]	43·5°	43·0°[12]					
Faradiol diac		156–166°	140–167°[12]	55·0°	50·0°[12]	76·95	9·65	77·6	10·3	$C_{34}H_{54}O_4$
12-Epifaradiol	Ic	—	—	−8·7°	—					
30-Oxoursa-20-en-3β,12β-diol	IIb	—	—	—	—	75·62	9·61	75·55	9·63	$C_{34}H_{52}O_5$
Faradion	Ie	232–243°	249–250°[12]	28·0°	22·0°					
3-Oxoursa-20-en-12β-ol	If	195–207°	—	69·0°	—					
12-Oxoursa-20-en-3β-ol	Id	218–221°	—	−17·3°	—					
Ursa-20-en-12β-ol	Ig	185–186°	—	39·0°	—					
Calenduladiol	VIIb	213–219°	—	17·0°	—					
Calenduladiol diac		189–194°	—	37·0°	—	77·38	10·08	77·6	10·3	$C_{34}H_{54}O_4$
Calenduladion		163°	—	25·0°	—					
Lupa-20(30)-en		162–166°	163–166°[12]	—	—					
Brein	VIb	215–219°	216–223°[12]	45·0°	65·0°					
Brein diac		192–197°	197–202°[12]	72·0°	72·0°	75·68	9·87	75·1	10·3	$C_{34}H_{54}O_3H_2O$
Triols of ψ-taraxasterol type		272–282°	—	—	—	77·92	10·56	78·6	11·0	$C_{30}H_{50}O_3$
Tetraol of ψ-taraxasterol type		279–281°	—	—	—	76·10	10·50	76·0	10·6	$C_{30}H_{50}O_4$

* ac = acetate; diac = diacetate; bz = benzoate.

increasing quantities of benzene for elution. This method allowed the separation of the acetates of triterpenic monols and diols into individual compounds. The fractionation scheme is shown in Fig. 1 and the properties of isolated compounds in Table 1.

Crude ψ-taraxasterol (Ia) was acetylated and crystallized from pyridine and identified by its properties[10,12] and i.r. spectrum. Oxidation of ψ-taraxasterol acetate with selenium



(Ia) R_1 = OH, H; R_2 = H, H

(Ib) OH, H; OH, H

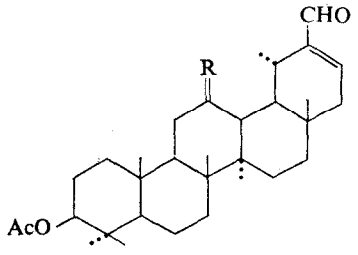
(Ic) OH, H; H, OH

(Id) OH, H; =O

(Ie) =O; =O

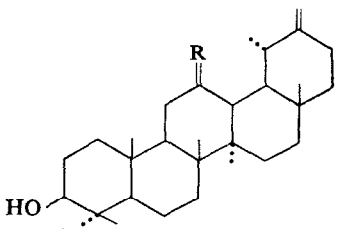
(If) =O; OH, H

(Ig) H, H; OH, H



(IIa) R = H, H

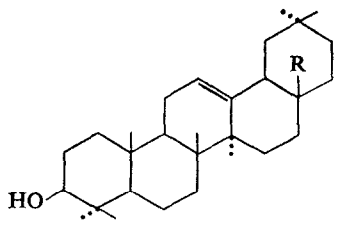
(IIb) OAc, H



(IIIa) R = H, H

(IIIb) OH, H

(IIIc) H, OH



(IVa) R—CH_3

(IVb) —CH_2OH

(IVc) —CHO

(IVd) —COOH

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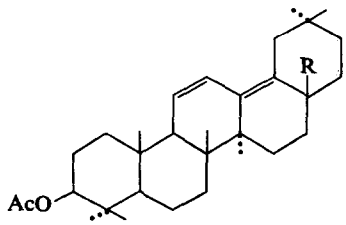
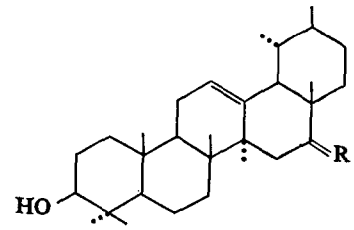
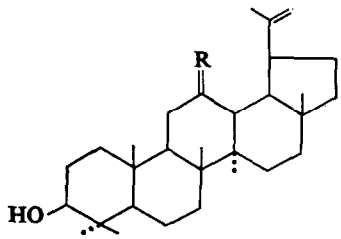
[12] P. BOITEAU, B. PASICH and A. RAKOTO RATSIMAMANGA, *Les Triterpenoides*, Paris (1964).



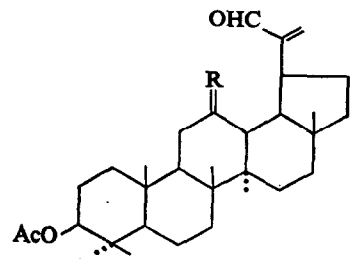
(Va) R—CH_3
(Vb) —CH_2OAc



(VIa) R: H, H
(VIb) OH, H



(VIIa) R: H, H
(VIIb) OH, H



(VIIIa) R: H, H
(VIIIb) OAc, H

dioxide yielded 30-oxoursa-20-en-3β-ol acetate (IIa) with λ_{max}^{EtOH} 233 (3·86), 228 nm (reported[10] for 30-oxoursa-20-en λ_{max}^{EtOH} 234 nm (4·09)). I.r. spectrum shows bands 1240, 1630, 1670, 1709 and 2600 cm^{-1} characteristic for esters and α,β-unsaturated aldehydes.

Crystallization from ethyl ether of compounds in mother liquor 3 (Fig. 1), together with the acetylated monols (1b), yielded 2·6 g of material which, after re-crystallization from pyridine, was identified as β-amyrine (IVa) acetate by its properties,[12] i.r. spectrum and comparison with a standard. Oxidation of β-amyrine acetate with selenium dioxide yielded 11,12(18)-dien-3β-ol acetate (Va) with λ_{max}^{EtOH} 241 (4·58), 250 (4·62), 259 nm (4·41) (reported[11,12] λ_{max}^{EtOH} 244 (4·47), 252 (4·53), 262 (4·33)).

The substances in mother liquor 4 (Fig. 1) were fractionated by means of argentation chromatography. Fraction 2a contained the acetates of α- and β-amyrine. In order to isolate α-amyrine acetate, a part of the fraction 2a was oxidized with selenium dioxide, which oxidizes derivatives of β-amyrine[13,14] but not those of α-amyrine.[15] The reaction product was separated on a column of alumina followed by an argentated column into unchanged α-amyrine (VIa) acetate and the dien acetate (Va). α-Amyrine acetate and the α-amyrine obtained from it by decomposition with lithium aluminium hydride were identified by their properties,[12] i.r. spectrum and by comparison with the standards.

[13] G. R. Rettit, H. Klinger, N. Otto, N. Jorgensen and J. Occolowitz, *Phytochem.* **5**, 301 (1966).
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Fraction 2b (165 mg) was separated on preparative argentated plates to give ψ-taraxasterol and 32 mg of taraxasterol (IIIa) acetates. The latter was identified by comparison with taraxasterol obtained from *Eupatorium cannabinum*,[16] by its properties and i.r. spectrum. Oxidation of taraxasterol acetate by selenium dioxide yielded three products. They were separated on thin-layer chromatograms and their u.v. spectra measured; λ_{max}^{EtOH} 222 nm for the least polar, 232 nm for the intermediate compound (identical with 30-oxoursa-20-en-3β-ol (IIa) acetate) and 236 nm for the most polar.

Fractions 2c and 2d contained lupeol acetate with traces of taraxasterol acetate. Lupeol (VII) acetate was obtained by crystallization from ethanol and the free alcohol and other derivatives possessed properties identical with those reported.[12] As expected, oxidation with selenium dioxide yielded 30-oxolupa-20(29)-en-3β-ol acetate (VIIIa)[17] with λ_{max}^{EtOH} 225 (3·8–3·9), 325 nm (1·3–1·6). I.r. spectrum possessed bands 930, 1240, 1620, 1684, 1721 and 2660 cm^{-1} characteristic for acetate and α,β-unsaturated aldehyde with $C=CH_2$ group.

The fraction of methylsterols free of triterpenic monols obtained from fraction 1c by chromatography on alumina was separated on argentated plates to give 20 mg of a fraction with highest R_f value showing i.r. bands characteristic for aldehyde (1710, 2680 cm^{-1}) and alcohol (1040, 3400 cm^{-1}) groups. As the result of transformation of this compound into β-amyrin (IVa) by Wolff–Kishner reduction, and into erythrodiol (IVb) by reduction with sodium borohydride, it was identified as oleanolic aldehyde (IVc).

Faradiol (Ib) obtained by crystallization had properties similar to those reported previously.[12] It was not possible to separate arnidiol (IIIb) from this compound by means of argentation chromatography, nor by multiple crystallization. The presence of a small quantity of arnidiol was however confirmed by a very weak band characteristic for vinyl group at 890 cm^{-1} observed in the i.r. spectrum. Similarly, the compounds could not be separated from the mixture obtained from arnica,[18] in which arnidiol constitutes up to 30 per cent.[19] Faradiol, oxidized with selenium dioxide, yielded 30-oxoursa-20-en-3β,12β-diol diacetate (IIb) with λ_{max}^{EtOH} 232 nm (4·0) and had in the i.r. spectrum bands 1240–1260, 1640, 1720 and 2700 cm^{-1} characteristic for acetate and α,β-unsaturated aldehyde. Reduction of this compound, which was expected to give arnidiol, gave a mixture of faradiol and arnidiol. This was indicated by its intermediate m.p. 220–241° and $[\alpha]_D$ 66° (m.p. 235–240°, $[\alpha]_D$ 43° for faradiol; and m.p. 249–257°, $[\alpha]_D$ 82° for arnidiol). The properties of the mixture did not change after four crystallizations. I.r. spectrum showed characteristic faradiol bands 790 and 845 cm^{-1} and characteristic bands for the $C{=}CH_2$ group in arnidiol at 890 and 1640 cm^{-1}. Faradiol is a derivative of ψ-taraxasterol and arnidiol of taraxasterol. The second hydroxyl group is located at C-12 in both and its configuration remains unknown. Obtaining a dihydroderivative having a m.p. differing by 9° from both compounds may indicate that the hydroxyl group at C-12 is different in the two cases.[12]

New derivatives of faradiol were obtained in the present work. Faradiol, oxidized with chromium trioxide, yielded three products separated on the column with alumina: faradione (Ie); 3-oxoursa-20-en-12β-ol (If), and 12-oxoursa-20-en-3β-ol (Id). Reduction of the products 1–3 by Wolff–Kishner reaction yielded respectively ψ-taraxen, ursa-20-en-12β-ol (Ig) and ψ-taraxasterol. Reduction of 12-oxo-ursa-20-en-3β-ol (Id) with sodium borohydride gave a diol of R_f value higher than faradiol (TLC) with $[\alpha]_D$ −8·7°. Ketone (Id) with steric hindrance

[16] J. GRZYBOWSKA, Z. JERZMANOWSKA and H. WITKOWSKI, *Roczniki Chem.* **28**, 197 (1954).
[17] L. RUZICKA and G. ROSENKRANZ, *Helv. Chim. Acta* **23**, 1311 (1940).
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[19] J. O. SANTER and R. STEVENSON, *J. Org. Chem.* **27**, 3204 (1962).

between 12-C=O and 19-CH_3 should, in this reaction, yield the compound with an axial hydroxyl group at C-12.[20] Faradiol (Ia) and the new diol (12-epifaradiol, Ic) oxidized with chromium trioxide both yielded the 3-keto compound, but 12-epifaradiol was also oxidized to the 12-keto and 3,12-diketo compounds. The faster oxidation rate of 12-epifaradiol under these conditions indicate that it has the 12-OH group axial, and thus faradiol has this group equatorial. The equatorial position of this group in faradiol was also confirmed by NMR spectrum.

The configuration β for 12-OH group in arnidiol can be confirmed by the comparison of a contribution of this group in molecular rotation of arnidiol, faradiol and 12-epifaradiol. $[M]_D$ calculated by substracting molecular rotations of ψ-taraxasterol and taraxasterol from those of corresponding diols are: $-27°$ for faradiol, $-243°$ for 12-epifaradiol and $-27°$ for arnidiol. Since the values of $[M]_D$ for arnidiol and faradiol differ significantly from the value for 12-epifaradiol, one can assume that 12-OH group in arnidiol has also configuration β (see also Table 2).

TABLE 2. CONTRIBUTION OF SECOND HYDROXYL GROUPS (OTHER THAN 3-OH) OF DIOLS TO THE $[M]_D$ VALUES

Compound	Contribution*		
	12 hydroxyl	12 carbonyl	12-O-acetyl
Calenduladiol	$-45°$	$-170°$	$+3°$
Faradiol	$-43°$	$-222°$	$+11°$
Arnidiol	$-27°$	$-126°$	$-55°$

* Calculated by subtracting the molecular rotations of 3-monohydroxyalcohols: lupeol, ψ-taraxasterol and taraxasterol and their derivatives from the corresponding molecular rotations of diols and their derivatives.

A part of mother liquor 4a (Fig. 1) was separated on an argentated column. The first fraction, 5a, after several crystallizations from ethanol, was chromatographically identical with erythrodiol (IVb). After oxidation with selenium dioxide the product showed not only λ_{max}^{EtOH} 240 (3·76), 250 (3·62) and 260 nm (3·68) characteristic for the oxidation products of β-amyrin type, but also λ_{max}^{EtOH} 233 nm (3·77) characteristic for C=C—C=O grouping formed in the oxidation products of the compounds of ψ-taraxasterol and taraxasterol type. In the i.r. spectrum bands were found at 760, 910, 990, 1135, 1240, 1370, 1390, 1460, 1726 and 2910 cm^{-1}. Lack of adsorption at 890 cm^{-1} indicates that the mixture 5a does not contain taraxasterol derivatives. It is thus a mixture of erythrodiol and a diol of ψ-taraxasterol type different from faradiol (having R_f values on TLC chromatograms like 12-epifaradiol). The quantity of faradiol calculated from intensity of bands, amounts to 20 per cent of 5a (i.e. 0·2% of 4a).

The fraction 5b was a mixture of diol acetates with $[\alpha]_D$ 46–66°, which could not be separated, and fraction 5c was faradiol diacetate.

Fraction 5d did not change its properties when crystallized four times from diluted ethanol, and was a new diol named calenduladiol (VIIb) diacetate. Its i.r. spectrum showed bands at 885, 1630 and 3020 cm^{-1} characteristic for a C=CH_2 group. The diketone obtained

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from calenduladiol by oxidation with chromium trioxide was reduced by the method of Wolff–Kishner to lupa-20(30)-en with properties already reported.[12] Oxidation of calenduladiol diacetate with selenium dioxide yielded 30-oxo-lupa-20-en-3β,12β-diol diacetate (VIIIb) which migrated on chromatograms with 30-oxoursa-20-en-3β,12β-diol diacetate and had λ_{max}^{EtOH} 224 and 340 nm similar to the corresponding derivative of lupeol (VIIIa), and a second less polar compound with λ_{max}^{EtOH} 361 nm. The above reactions confirm that calenduladiol belongs to lupeol type of triterpenes. Comparison of molecular rotations (Table 2) suggest that the second hydroxyl group in calenduladiol occurs also at position 12β as in arnidiol and faradiol. The deformation of ring E in calenduladiol and faradiol may be responsible for creation of a steric hindrance between 12-OH and 19-CH_3 groups in these compounds. It is reflected in the differences of $[M]_D$ values for 12-carbonyl and 12-O-acetyl groups in faradiol and calenduladiol as compared with arnidiol possessing undeformed ring E.

In order to separate diols of the α-amyrin type, 1 g of the fraction 5b was oxidized with selenium dioxide and the products separated on the column of alumina. The first fraction was identified as brein (VIb) diacetate by its properties[12] and i.r. spectrum.

Fraction 6b, with m.p. 206–219° and $[\alpha]_D$ 1°$-$8·5°, represents a mixture of two compounds, one, unoxidized, was a second diol of α-amyrin type (differing in R_f value from brein) and the second had λ_{max}^{EtOH} 241 (4·2–4·4), 249 (4·2–4·4) and 258 nm (4·0–4·2) characteristic for oleana-11,13(18)-dien-3β,12β-diol diacetate (Vb). The extinction values indicate that this compound constitutes 30 per cent of fraction 6b (i.e. 66 mg/g of 4a). This is a much greater quantity than that of erythrodiol (2 mg/g of 4a) obtained earlier and which also gave diene (Vb) on oxidation with selenium dioxide. It is thus concluded that the second component of 6b is a diol of β-amyrine type different from erythrodiol.

Fraction 6c contained oxidation products of the diols of ψ-taraxasterol, taraxasterol and lupeol type.

Fractions more polar than diols were eluted from the column with ethyl ether (4b), ethanol (4c) and methanol (4d) and precipitated from each eluate with light petroleum. Fraction 4b was not examined. Fraction 4c on thin-layer chromatograms was separated into two spots. Its elementary composition indicate the presence of three OH groups for C_{30}. The substance obtained from fraction 4d was chromatographically homogenous and had sharp m.p. Its composition corresponds to formula $C_{30}H_{50}O_4$. The i.r. spectrum of both the triol and the tetrol is almost the same as that of faradiol, implying that these alcohols are triterpenic alcohols of ψ-taraxasterol type.

METHODS

Chromatography

Silica gel (Serva) was used for TLC either in water or in 10 per cent (in regard to the quantity of gel) nitrate solution. The plates were activated before use at 120° for 2 hr. After developing, the chromatograms were sprayed with 50 per cent H_2SO_4. Column chromatography was carried out using alumina (Woelm) inactivated by adding water to give activity II or III. Argentation chromatography was performed on silica gel (Serva) impregnated with solution of $AgNO_3$ (7–15 per cent with regard to the quantity of gel) in methanol–water, 1 : 1. After mixing and removal of the solvent by distillation under lower pressure, the gel was activated at 120° for 20 hr.

Physical Properties

Melting points (corrected) were determined on a heated microscopic plate. All optical rotations were measured for 0·5–1·5% $CHCl_3$ solution at 25°, the error of determination being 1·5 per cent. I.r. spectra were taken using Unicam SP 200 spectrophotometer, in KBr plates.