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PENTACYCLIC TRITERPENOIDS OF *EUCLEA NATALENSIS**

FAUSTO SCHIAFFELLA, LUCIANO FRATINI, TARCISIO MEZZETTI and VITO BELLAVITA

The Institute of Pharmaceutical Chemistry and Toxicology of the University of Perugia, Italy

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Key Word Index—*Euclea natalensis*; Ebenaceae; α-amyrin; uvaol ursolic acid, ursolic acid lactone and 3-β-acetoxy-ursa-11,12-ene-28-oic-13(28)-lactone and lupeol, betulin, betulinic acid, sitosterol.

Plant. Euclea natalensis A.DC. (Voucher A. Batelli No. 6271a specimen is deposited in the Herbarium of this University). *Source.* Fountains Valley, Pretoria, South Africa. *Uses.* In African folk-medicine, the plant infusion is used for abdominal complaints. Despite the fact that the plant is thought to be poisonous, the Zulus use it as a remedy for scrofula. The charred and powdered root is applied to skin lesions in leprosy [1]. *Previous work.* None.

Part examined. Branches and leaves were investigated separately because, as previously observed in our paper on *Euclea kellau* [2], branches and leaves do not contain the same triterpenoids.

Comparison between the two plants and the percentage of triterpenoids in each is shown in Table 1. We are now working on full identification of the minor triterpenoids of the mixture.

EXPERIMENTAL

Materials for column chromatography: Silica gel 922, 800 and Silica alumina 113 (GRACE). TLC was performed on silica gel plates with and without $AgNO_3$ impregnation using (a): C_6H_6–EtOAc (9:1); (b) C_6H_6–EtOAc (7:3). Triterpenoids were detected with 10% H_2SO_4 and heating to 100°.

Extraction: The dried and powdered leaves were first percolated with *n*-hexane (3 l/kg) and then with $CHCl_3$–EtOAc (1:1) (4·5 l/kg). After evaporation of the solvents, the first extract yielded a dark sticky residue (1·7%) and the second, a solid whitish–green residue (4·6%). The first residue was composed mainly of hydrocarbons, waxes, free and esterified α-amyrin and some uvaol, and the second of ursolic acid, some uvaol and other partially identified triterpenoids. The branches gave 0·6% of the first residue and 0·5% of the second.

Separation: The first residue was first chromatographed on silica gel 922, to rid of non-polar components and then hydrolyzed (5% KOH–MeOH) and re-chromatographed on silica gel 800. Separation of the α-amyrin-lupeol, uvaol-betulin and ursolic acid–betulinic acid pairs, was achieved with 2% $AgNO_3$ on silica gel. The second residue was acetylated and chromatographed on silica gel 800.

α-Amyrin. From $(Me)_2CO$: m.p.s and $[\alpha]_D$ of compound and acetate and IR and NMR spectra, were identical to those of authentic samples. *Lupeol.* $(Me)_2CO$: m.p., $[\alpha]_D$, IR and NMR spectra of compound and acetate. *Uvaol.* Isolated as diacetate and partially hydrolysed to 3-acetoxy derivative; IR and NMR spectra were identical to those obtained from authentic samples. *Betulin.* As diacetate, m.p. $[\alpha]_D$; IR and NMR spectra. *Ursolic acid.* As acetate and its methyl ester, m.p. $[\alpha]_D$, IR and NMR spectra. *Betulinic acid.* Acetate (m.p. and $[\alpha]_D$). The two 3-acetoxy-28-methyl esters (separable on TLC). IR. *Ursolic acid lactone.* The mother liquors from ursolic acid acetate crystallization were chromatographed on

* Part II in the series "Triterpenoids of the Euclea Species". For Part I see *Planta Medica* **19**, 30.

Table 1

Component	% Of each component			
	Euclea natalensis		*Euclea kellau*	
	Branches	Leaves	Branches	Leaves
α-Amyrin	0·2	16·8	—	37
Lupeol	39·5	—	20	—
Uvaol	0·2	13·3	—	15
Betulin	11·4	—	13	—
Ursolic acid	37	59	66	45
Betulinic acid	2	—	—	—
Ursolic acid lactone (saturated)	—	0·1	0·2	0·5
Unsaturated ursolic acid lactone	—	0·1	—	—
Unidentified triterpenoids	7	10·7	0·7	1·5
Sitosterol	2·7	—	0·10	1
Total yield of triterpenoids (% dried material)	0·64	6·5	4	5·2

silica-alumina 113 (in hexane and eluted with hexane 3% EtOAc). Crystallized from EtOAc; m.p. 253°; $[\alpha]_D^{20}$ +13 ($CHCl_3$). The IR spectrum was identical to that of the sample obtained by HCl reaction on ursolic acid [3]. *Unsaturated ursolic acid lactone.* Separated from the same column and isolated as its acetate crystallized from EtOAc in microcrystals; m.p. 252°; $[\alpha]_D^{20}$ +46° ($CHCl_3$). IR and NMR spectra showed that the natural lactone was identical to the lactone obtained by $LiAlH_4$ reduction of 3-acetoxy-11-keto-ursolic acid [4]. It was thus proved to be 3β-acetoxy-ursa-11,12-ene-oic-13,(28)-lactone. *Sitosterol.* Found only in the first residue from the branches: m.p. 137–138; $[\alpha]_D^{20}$ −36° ($CHCl_3$). Identified by co-TLC, IR and NMR spectra.

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REFERENCES

1. Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *The Medicine and Poisonous Plants of Southern and Eastern Africa*, 2nd edn, p. 390, Livingston, London.
2. Orzalesi, G., Mezzetti, T., Rossi, C. and Bellavita, V. (1970) *Planta Medica*, **19**, 30.
3. Barton, D. H. R. and Holness, N. J. (1952) *J. Chem. Soc.* 78.
4. Mezzetti, T., Orzalesi, G. and Bellavita, V. (1971) *Planta Medica*, **20**, 244.

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ISOLATION OF PHORBOL FROM *EUPHORBIA FRANCKIANA*

A. D. KINGHORN and F. J. EVANS

Department of Pharmacognosy (University of London), The School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX

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Key Word Index—*Euphorbia franckiana*; Euphorbiaceae; Diterpene; phorbol.

Plant material. Euphorbia franckiana is a succulent species indigenous to Southern Africa, which produces copious latex on incision of the leaves. During biological screening tests involving the mice ear irritant assay [1] we were able to show that the latex had a short term irritant effect reaching a maximum within four hours. This is in contrast to several other ingenol and phorbol ester containing *Euphorbia* and *Croton* species which have a maximum irritant activity only after 24 hr.

Present work. E. franckiana latex was collected from Kew Gardens into alcohol and immediately dried below 40°. The acetone extract of two samples of latex had ID50's on mice of 70 μg/5 μl and were non-irritant after 24 hr. Extraction of the polar extract with *n*-hexane removed the lipid and triterpenoid compounds and the irritants were extracted with CH_2Cl_2. The biologically active fraction was hydrolysed with KOH in MeOH to produce a resin a component of which (M^+ $C_{20}H_{28}O_6$) was acetylated [2] and purified by TLC [3]. The recovered solid was recrystallized from MeOH m.p. 120–1° and was chromatographically pure by TLC [2] and GLC [4]. The high resolution MS gave parent ion *m/e* 490 (M^+ $C_{26}H_{34}O_9$); fragment ions at *m/e* 430 (M-60); 388 (M-60 + 42); 387; 370 (M-120); 352 (M-120 + 18); 328; 310 (M-180); 292 (M-180 + 18); 282; 267; 227; 215; 199; 173;159; 145; 133; 125; 121; 109; 95; 93; 91; 83 (base peak). The NMR spectrum (60 MHz), $CDCl_3$ (TMS $\delta = 0{\cdot}00$) exhibited resonances at δ 0·93 (3Hd-18); 1·22 (2Me-16, 17); 1·76 (3Hd-19); 2·05 (3MeCO-12, 13, 20); 2·45 (2Hm-5); 2·72 (1 OH deuterium exchange); 3·22 (2H broad-8 and 10); 4·43 (2H-20); 5·27 (Hd-12); 5·45 (1OH deuterium exchange); 5·70 (Hd-7); 7·54 (Hm-1)ppm: C.D.(MeOH); 204 nm [8] = −27291; 229 nm = +53295; 270 nm = −5181; 340 nm = −3984, confirming the presence of phorbol, isolated as its triacetate. This compound has been isolated from *Croton tiglium* seed oil [5], but from our own unpublished results of screening approximately 60 *Euphorbia* species the common diterpene of this genus is ingenol [3]. It occurs in the latex together with one or more of ingol [6], 16-hydroxy-ingenol [7] or 5-deoxyingenol [8]. Phorbol has