Constituents of Egyptian Euphorbiaceae, I

Triterpenoids and Related Substances of *Euphorbia paralias*

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The study of the triterpenoids and their related substances of *Euphorbia paralias* resulted in isolation of ursolic acid, cycloartenol, 24-methylenecycloartenol, uvaol, a hydrocarbon fraction (n-C_{29} – n-C_{35}), alcohol fraction (hexacosanol and octacosanol) and a sterol fraction (β-sitosterol, stigmasterol, campesterol and cholesterol).

Introduction

The family Euphorbiaceae is represented in Egypt by seven genera *viz. Euphorbia, Chrozophora, Jatropha, Mercurialis, Securinega, Phyllanthus* and *Andrachne*[1]. None of the 33 *Euphorbia* species growing in Egypt, had been yet investigated. This work represents the first part of a series dealing with the constituents of these species.

Euphorbia paralias L. is one of the most common *Euphorbia* species, growing in Egypt. Previous work on the species growing in Spain, revealed the presence of amyrin, sitosterol, uvaol, betulin, oleanolic and ursolic acids[2]. The work on the same species, growing in Greece, resulted in the isolation of esculetin, a sterol and the detection of flavonoids[3].

Results and Discussion

Concentration of the light petroleum extract of the whole plant deposited ursolic acid (m.m.p., R_F, superimposable IR, NMR, MS of the triterpenoid and its acetate). It was also obtained from the ether extract of the defatted powder.

Fractionation of the unsaponifiable matter, by column chromatography (using alumina) and preparative TLC (Silica gel G impregnated with $AgNO_3$) succeeded in the separation of the different constituents.

The hydrocarbon fraction (C, H; IR), eluted with light petroleum (fractions 1 – 18) was proved by GLC and MS to be a mixture of *n*-alkanes: *n*-hentriacontane ($C_{31}H_{64}$) represents the major constituent (76.0%), *n*-nonacosane $C_{29}H_{60}$ (6.3%), *n*-triacontane $C_{30}H_{62}$ (1.5%), *n*-dotriacontane $C_{32}H_{66}$ (0.6%), *n*-tritriacontane $C_{33}H_{68}$ (15.0%) and *n*-pentatriacontane $C_{35}H_{72}$ (0.6%).

The combined fractions 265 – 312 (light petroleum – benzene 70:30), gave upon crystallisation from methanol, a colourless substance m.p. 75 °C. Though TLC of this compound showed it to be one spot, both GLC and MS showed that this substance is a mixture of four substances. GLC of its acetate derivative showed that the main component could be hexacosanol acetate; in addition to another two unrelated substances which possess the same retention times as cycloartenol acetate and 24-methylenecycloartenol acetate. The MS of the substance confirmed these results and showed ions at m/e 382, 410, 426 and 440 which corresponded to hexacosanol ($C_{26}H_{54}O$), octacosanol ($C_{28}H_{58}O$), cycloartenol ($C_{30}H_{50}O$) and 24-methylenecycloartenol ($C_{31}H_{52}O$) respectively. On examining the acetyl derivative by reactive TLC (using silica gel G impregnated with 10% $AgNO_3$) three spots were detected, upon spraying with 50% H_2SO_4; two of which possess the same R_F as cycloartenol and 24-methylenecycloartenol acetates while the third corresponds to ceryl acetate. They were then fractionated on preparative TLC (reactive) using petroleum ether-benzene (50:50). The first fraction (R_F 0.26) yielded on hydrolysis (1 N KOH) 24-methylenecycloartenol (m.m.p., TLC, MS)[4,5] and the second fraction (R_F 0.34) was identified as cycloartenol acetate (m.m.p., MS)[6]. On the other hand, the third fraction was shown by MS and GLC to consist mainly of hexacosanol acetate with

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octacosanol acetate as minor constituent. The alcohols, isolated from *Euphorbia* species, had been shown to be mixtures (as proved by GLC of the acetates) [7].

The sterol substance (eluted with light petroeum-benzene 50:50; fractions 547 – 582) was subjected to GLC both in the free state and as its TMS derivative. The results obtained showed that it is a mixture of β-siosterol (95.0%), stigmasterol (2.6%), campesterol (2.0%) and cholesterol (0.4%). These findings were confirmed by MS.

Uvaol was isolated from the benzene-methanol eluent (97:3) (fractions 833 – 882) and was identified (m.m.p., TLC, IR, NMR, MS).

Oleanolic, betulin and β-amyrin, isolated from the same species growing in Spain were not detected.

Experimental

Material

E. paralias L. was collected from Marsa Matrooh in May. The plant was kindly authenticated by Prof. V. Täckholm, Faculty of Science, Cairo University.

Thin-Layer chromatography

Adsorbent: Silica gel G. Solvent systems: 1. Benzene-acetone (6:2); 2. hexane-ethyl acetate (75:25; 85:15); 3. benzene-ethyl acetate (60:20); 4. chloroform-acetone (90:10). Spraying reagents: *p*-anisaldehyde or 50% H_2SO_4, followed by heating at about 120 °C for few minutes.

Gas-liquid chromatography

For Hydrocarbons: Column 4 m SE-40 3%; temp. program 230 °C/4 °C per min.

For Alcohols: Column glass 6 feet, 3.5 mm (i.d.), SE-30 3%; gas chromosorb Q (100 – 120 mesh); temp. isothermal then using temp. program; column 255 – 300 °C (injector 295 °C, detector 300 °C *or* column 260 – 300 °C, injector 290 °C, detector 300 °C; carrier gas N_2; detector type FID, H_2 24 lb 42 ml/min, air 80 lb 576 ml/min. For sterols: column 6 feet × 0.3 mm (i.d.); non-polar (2% SE-33) or polar (2% silicone QF-1) on gas chromosorb Q (100 – 120 mesh); temp.: for polar column 250 °C, injection 300 °C; non-polar column 285 °C, injection 300 °C; carrier gas N_2 30 lb/inch; H_2 16 and air 20.

Extraction and fractionation

Three Kg. of the dried powdered herb were extracted with light petroleum (b.p. 60 – 80 °C). On leaving the extract at room temperature, a crystalline precipitate separated (ursolic acid). About 27 g of the unsaponifiable matter (prepared in the usual manner) were chromatographed on alu- 27 g of the unsaponifiable matter (prepared in minium oxide (1500 g). Elution was affected with light petroleum, followed by mixture of light petroleum-benzene (70:30; 50:50; 30:70), benzene and benzene-methanol (97:3). Fractions (50 ml each) were collected.

Ursolic acid

After crystallisation from methanol, it melted at 285 – 286 °C; reported [8] 285 – 286 °C.

$C_{30}H_{48}O_3$ (456)
Calcd: C 78.94 H 10.52,
Found: C 78.76 H 10.53.

The m.p. of the acetate showed no depression upon admixture with the authentic material.

$C_{32}H_{50}O_4$ (498)
Calcd: C 77.11 H 10.04,
Found: C 77.06 H 10.10.

IR showed $\nu_{\max}$ 3390 (OH), 1695 (COOH), 1389, 1379, 1361 and 1307, 1274, 1250 (ursolic acid) [8, 9], 833 and 812 cm^{-1} (trisubstituted double bond); and its NMR spectrum is identical with that reported [10]. The MS showed *m/e* at 456 and 498 for the triterpenoid and its acetate respectively.

Hydrocarbon fraction

The hydrocarbon fraction, after crystallisation from chloroform-methanol, melted at 66 °C. Found: C 85.16, H 14.85.

The MS showed *m/e* at 408, 422, 436, 450, 460, 462 and 492 corresponding to the molecular ions of $C_{29}H_{60}$, $C_{30}H_{62}$, $C_{31}H_{64}$, $C_{32}H_{66}$, $C_{33}H_{68}$ and $C_{35}H_{72}$ respectively.

24-Methylenecycloartenol

The substance, crystallised from methanol, melted at 112 – 114 °C; reported 112 – 113.5 °C [11]. MS of the acetate showed *m/e* at 482 and fragmentation ions at 467 ($-CH_3$); 422 ($-$Ac); 407 ($-CH_3-$Ac); 379, 357 ($-$side chain); 353, 300 ($-$ring A) and 297 ($-$side chain $-$ Ac) [4, 5].

Cycloartenol

The acetate melted at 120 – 122 °C, reported [12, 13] 119 – 122 °C, 118 – 122 °C. MS showed *m/e* at 468 fragmentation ions at 453 ($-CH_3$); 408 ($-$Ac); 393 ($-CH_3-$Ac); 365, 357 ($-$side chain); 359, 297 ($-$side chain $-$ Ac) and 286 ($-$ring A) [6].

Octacosanol and Hexacosanol

Both alcohols were proved by GLC and MS.

Sterol fraction

The sterol, crystallised from chloroform-methanol, melted at 133 °C. It was further purified by precipitation with digitonin[14] and then subjected to GLC (as sterol and after preparation of its trimethylsilyl derivative).

Uvaol

It melted at 234 – 235 °C (undepressed); reported 233 °C[15].

$C_{30}H_{50}O_2$ (442)

Calcd:	C 81.44	H 11.31,
Found:	C 81.29	H 11.30.

The diacetate melted at 156 – 158 °C (undepressed).

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