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THYMELACEAE

ISOLATION OF DAPHNORETIN AND β-SITOSTEROL-β-D-GLUCOSIDE FROM *THYMELEA HIRSUTA*[*]

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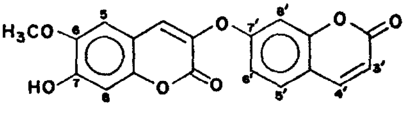
Abstract—The reportedly 'new' constituent (thymelol) of *Thymelea hirsuta* (L) Endl has been proved to be daphnoretin Another component, identified as β-sitosterol-β-D-glucoside, was isolated

INTRODUCTION

SEVERAL species of the Thymelaceae had been reported to contain coumarins.[1] Daphnetin (7,8-dihydroxycoumarin) occurs as daphnin (7-β-glucosyloxy-8-hydroxycoumarin) in *Daphne alpina L* , *D. kiusiana*, *D. laureola L* , *D. odora Thunb* , *D mezereum L* , as well as in *Gnidia polycephala*, *Wikstoroemia ganpi* and *Edgeworthia papyrifera* [2–5] Umbelliferone (7-hydroxycoumarin) had been early isolated from *Daphne mezereum*,[6] while daphnoretin (6-methoxy-7-hydroxy, 3,7′-dicoumarin ether) was separated by Tschesche *et al.*[7] from both *Daphnopsis racemosa* Griseb and *Daphne mezereum* L as well as from *Daphne cannabina*.[8] The glucoside daphnorin (daphnoretin-7-β-glucoside) was isolated from *Daphne mezereum* by Tschesche *et al* [7] Kosheleva and Nikonov[9] isolated from *Daphne mezereum* L. a new coumarin glucoside, identified as 7-hydroxycoumarin-8-β-D-glucopyranoside and a new phenol glycoside named daphnoside (3-methoxy-4-hydroxybenzyl-4-α-glucopyranoside), in addition to daphnin, daphnoretin and umbelliferone.

The family Thymelaceae is represented in Egypt by only one species, viz. *Thymelea*



Daphnoretin

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[*] The IR spectrum of the component A was identical with that of authentic daphnoretin (isolated from Thymelaceae plants[7]) kindly supplied by Professor T Tschesche (Bonn), but again differs very slightly from daphnoretin (isolated from *Ruta graveolens* by Reisch *et al* [14]) kindly supplied by Dr K Szendrei (Hungary) This may be the reason for the difference in the NMR of our component and that reported by Reisch *et al*

[†] The plant was kindly authenticated by Dr K H Batanouny, Faculty of Sciences, Cairo University.

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hirsuta (L.) Endl. (= *Passerina hirsuta* L),[10] which grows in the Mediterranean coastal strip and the desert Saleh *et al*[11] reported the isolation from this plant of a crystalline substance of the empirical formula C_3H_2O, m.p 242·5–243 5° and suggested the name thymelol for this 'new' compound. Later, Gharbo *et al*[12] isolated from the same plant stigmasterol, β-sitosterol and two other unidentified crystalline substances (aliphatic alcohol and lactone) The present work deals with the identification of the thymelol as well as the separation and identification of the substance found with it

RESULTS AND DISCUSSION

Investigation of the light petroleum-insoluble fraction of the ether extract of *Thymelea hirsuta* (the source of thymelol, as described by Saleh *et al*[11]) by TLC revealed the presence of a non-fluorescent component in addition to the fluorescent one corresponding to thymelol

Column chromatographic fractionation of the purified mixture on silica gel and elution with chloroform, followed by methanol afforded the separation of component A (daphnoretin) which corresponds to thymelol and component B (β-sitosterol-β-D-glucoside) respectively

The molecular formula, functional groups, m p of the component A and its derivatives (monoacetate and methyl ether) and the mass spectrum are in agreement with the data reported for daphnoretin [7,13,14] Though the NMR data for H-4′ and H-3′ differ slightly from that reported by Reisch *et al*[14] for daphnoretin, isolated from *Ruta graveolens* (found H-4′ 8·08, H-3′ 6 40 ppm, reported 7·85 and 6·25 respectively), yet the identity of the substance as daphnoretin was shown by direct comparison with authentic daphnoretin (UV, TLC, mixed m p , IR*)

The component B was identified as β-sitosterol-β-D-glucoside (IR, MS,[15,16] NMR, tetraacetate[17–20]) Acid hydrolysis gives glucose (paper chromatography) and β-sitosterol (TLC, m.m p)

EXPERIMENTAL

Plant material *Thymelea hirsuta* (L) Endl was collected from Borg El-Arab in March †

Apparatus NMR Varian A 60 (in $DMSO\text{-}d_6$, TMS as internal standard, δ-value), MS Atlas CH 7, IR Leitz-Unicam SP 200 G, UV Zeiss PMQ II, m p Kofler (uncorrected)

Chromatography Plates coated with SiO_2 gel using different solvents[7 20,21] were applied The coumarin spots were detected by UV light, while β-sitosterol and its glucoside were revealed by spraying with phosphoric acid reagent [22] Glucose was detected by spraying the paper chromatogram with aniline-oxalic acid reagent [23]

* Dedicated to the memory of Prof Z F Ahmed

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Isolation of the components A and B 3 kg of the powdered leaves were extracted with ether and the solvent evaporated *in vacuo* at 40° The residue was then refluxed 5 times (each of 800 ml) with petroleum (b p 40–60°), filtered and the filtrate discarded The petroleum-insoluble residue was dissolved in boiling ethanol (1500 ml), filtered while hot, concentrated *in vacuo* to about 200 ml and left to stand at room temp for 48 hr The pale greenish crystalline deposit was filtered, washed with cold ethanol, dissolved in about 300 ml $CHCl_3$ and passed through a column of SiO_2 gel (70 cm × 1 cm) Elution was first carried out with $CHCl_3$ then with methanol

Component A (daphnoretin) Component A (obtained from the $CHCl_3$ eluate) gave on crystallisation ($CHCl_3$–petroleum) pale yellowish white needles m p 252° ($C_{19}H_{12}O_7$ (352) required C, 64 83, H, 3 44, found C, 64 64, H, 3 78%) The monoacetate (Ac_2O-pyridine at 100° for 3 hr) melted at 242° and the methyl ether (Me_2SO_4 and K_2CO_3 in dry acetone at 60° for 5 hr) at 238° (m m p)

Component B (β-sitosterol-β-D-glucoside) Component B (methanol eluate), after crystallization from large volume of methanol melted at 296–298° ($C_{35}H_{60}O_6$ required C, 72 87, H, 10 48, found C, 72 20, H, 10 05%) The tetraacetate (Ac_2O-pyridine at 100° for 1 hr) melted at 167–169° Hydrolysis of 20 mg (in 20 ml ethanol containing 1 ml conc HCl under reflux for 8 hr) afforded glucose and β-sitosterol

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Key Word Index—*Thymela hirsuta*, Thymelaceae, coumarin, daphnoretin, β-sitosterol glucoside

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UMBELLIFERAE

EXTRACTIVES OF *ANGELICA GLAUCA*

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Plant Angelica glauca Edgw. *Occurrence*. Higher regions of Kashmir.[1] *Uses* Medicinal.[2] *Previous work*. Investigation of fruits[3] and roots [4]

Present work Extraction with hexane followed by separation on alumina afforded three crystalline products, isoimperatorin (I) m p 109–110, prangolarin (II) m.p. 103–104 and a new coumarin m p. 119–120 $C_{17}H_{18}O_6$ to which structure (III) has been assigned on the basis of spectral data. λ_{max}^{EtOH} 220, 250, 266 and 300 nm, ν_{max}^{KBr} 3500, 1725, 1375, 1380 and 1265 cm^{-1}. In the NMR spectrum (60 Mc $CDCl_3$) the furocoumarin nucleus is defined by the doublets at 2·41; 2·94 and 1 78, 3·78τ having $J = 2$ and 9 cps respectively There is evidence of only one free position in the benzene ring and that this must be at C-8 is shown by the singlet at 3·01τ, the resonance of the C-5 proton occurring usually at 2·6 to 2·75τ This leaves position C-5 for attachment of the C-6 side chain. The side chain methoxyl is

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