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DAPHNANE DITERPENES OF *THYMELAEA HIRSUTA*

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Key Word Index—*Thymelaea hirsuta*; Thymelaeaceae; 12-hydroxy-daphnane esters.

Abstract—Five 12-hydroxy-daphnane esters were isolated from the leaves and twigs of Egyptian *Thymelaea hirsuta*. These compounds were identified as gnidicin, gniditrin, genkwadaphnin, the aliphatic C-12 ester, 12-*O*-heptadecenoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate and the novel aliphatic C-12 ester 12-*O*-butenyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13-14-orthobenzoate.

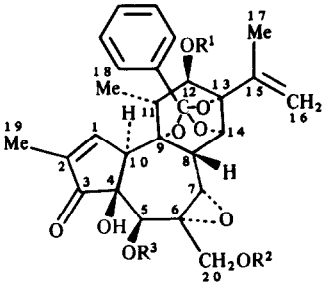
INTRODUCTION

Thymelaea hirsuta L. is a thymelaeaceous shrub which grows in abundance in the western Egyptian desert [1] and is the only species of *Thymelaea* that is indigenous to Egypt [2]. The plant is not grazed by livestock and locally it has a reputation for its toxic nature [1]. A previous short report [3] on this plant revealed the presence of two toxic diterpenes based upon the daphnane nucleus. The compounds were shown to induce intense inflammation of the skin, the eyes and the mucus membranes of the mouth. These daphnanes were considered to be the major toxic principles of this species but the recent availability of considerably greater quantities of plant material has enabled a more complete examination of the extract for toxic daphnane derivatives. In this communication we report on the isolation and structure elucidation of five 12-hydroxy-daphnane *ortho*-esters from this poisonous Egyptian species.

RESULTS AND DISCUSSION

This re-examination of the poisonous Egyptian shrub *Thymelaea hirsuta* has enabled the isolation of five 12-hydroxy-daphnane diterpenes from the leaves and twigs of this species. Three of these compounds, viz. gnidicin (**1**), gniditrin (**3**) and genkwadaphnin (**5**) were previously known from plants of other genera of the family Thymelaeaceae [4, 5] but two related aliphatic C-12 derivatives of 12-hydroxydaphnetoxin, compounds **2** and **5**, were daphnanes currently restricted to this *Thymelaea* species. Compound **2** has previously been characterized from this species [3] whereas compound (**5**) is a novel daphnane ester. A selective base-catalysed hydrolysis reaction of compounds **1**–**5** suggested the presence of a 12-hydroxydaphnetoxin parent nucleus in each case. This was confirmed by the semi-synthesis of the 5,12,20-triacetate derivative from each hydrolysed product. All triacetates were shown to be identical with 12-hydroxydaphnetoxin 5,12,20-triacetate as determined from spectroscopic and chromatographic data.

Compounds **1**–**4** were identified conclusively by comparison of their mass spectral and ^{1}H NMR data with previously published data [3–5]. The novel ester **5** was identified from its mass spectral and ^{1}H NMR data which were characteristic for 12-hydroxydaphnetoxin esters [6]. A molecular ion at *m/z* 566 was observed in the EIMS with the subsequent loss of a molecule of water. Fragment ions at *m/z* 480 and *m/z* 358 were evident and represent the loss of the esterifying acid followed by loss of the benzoate ester moiety directly from the molecular ion. The ^{1}H NMR spectrum of **5** was characteristic of



1 R^1 = OC.CH:CH.C_6H_5, R^2 = R^3 = H
2 R^1 = OC.CH:CH.$(CH_2)_{13}$.Me, R^2 = R^3 = H
3 R^1 = OC.$(CH{:}CH)_3$.$(CH_2)_2$.Me R^2 = R^3 = H
4 R^1 = OC.C_6H_5, R^2 = R^3 = H
5 R^1 = OC.CH:CH.CH_2.Me, R^2 = R^3 = H
6 R^1 = R^2 = R^3 = H
7 R^1 = R^2 = R^3 = O.C.Me

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daphnane esters [6] and suggested the presence of a single olefinic bond with two protons being exhibited as doublets at δ7.33 and 6.15. The large coupling constant of 14.5 Hz suggested that the bond was in a *trans*-configuration. A broad three proton signal was also observed at δ1.27 consistent with the presence of a methyl group attached to an olefinic bond. Accordingly, **5** was 12-*O*-butenyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate.

Only a limited number of ^{13}C NMR spectra have been recorded to date for daphnane polyol esters [6]. In the present study, the previously unreported ^{13}C NMR spectra for compounds **1** and **3** were run and assigned by comparison with data from previously characterised daphnane esters and with ^{13}C NMR spectra obtained from the esterifying acids. The two spectra were essentially similar and differed only in the resonances of their esterifying acids at C-12.

The daphnane diterpenes [7] occur only in the families Euphorbiaceae and Thymelaeaceae in a manner similar to the biosynthetically related phorbol derivatives [8]. In particular 12-hydroxy-daphnane diterpenes are characteristic of genera of the Thymelaeaceae [7], from which they were originally isolated and their structure elucidated by X-ray methods [8]. All of the species so far investigated are known toxic plants and these compounds were originally isolated as irritant and stage 2 tumour-promoting agents [9] as determined in modified Berenblum experiments. In addition, they are known to activate protein kinase C [10] the tumour-promoting receptor site. Three of the compounds isolated here, **1**, **4** and **5** are claimed to possess anti-tumour activities *in vivo* against P-388 leukemia in mice [4, 5]. Accordingly, 12-hydroxy-daphnane derivatives are currently of biochemical interest as specific probes for the investigation of structural relationships concerning tumour-promotion versus tumour inhibition.

EXPERIMENTAL

Plant material. Thymelaea hirsuta L. leaves and twigs were collected in the Egyptian Western desert, air-dried and powdered. Samples were authenticated by Professor Batanouny (Department of Botany, Cairo University).

Extraction. Powdered plant material (20 kg) was exhaustively extracted by cold maceration in Me_2CO for 1 week. Following filtration, the Me_2CO was removed by red. pres. distillation below 45° to yield a green tar which was dissolved with the aid of gentle heat in 1 l 40% MeOH. The MeOH soln was partitioned with hexane (5 × 200 ml) to remove green pigments and lipids and then re-extracted with Et_2O (5 × 200 ml). The Et_2O phase was washed with 0.5% Na_2CO_3 soln (2 × 50 ml) followed by distilled H_2O (1 × 100 ml), dried over Na_2SO_4 and the Et_2O removed by distillation as before to produce a cream coloured resin (15.8 g).

Separation. The resin was fractionated by means of gradient elution CC using activated Florosil as adsorbent. Fractions were collected in 300 ml aliquots by elution with toluene (1 l), EtOAc (1 l), Me_2CO (1 l) and MeOH (1 l). Those fractions exhibiting a positive response in the mouse ear erythema assay as previously described [11] were evapd to resins and used for further purifications.

Characterization of compounds

***Compound* 1. 12-*O*-Cinnamoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate.** This fraction was purified by means of prep. TLC on silica gel GF_{254} 0.5 mm layers developing with $CHCl_3$–EtOAc (2:1, R_f 0.37), and finally by partition prep. TLC on Kieselguhr G/20% diethylene glycol and developing with cyclohexane–butanone (19:1, R_f 0.13). Removal of diethylene glycol by partition between H_2O–CH_2Cl_2 and evapn of the CH_2Cl_2 phase produced 67.2 mg of a clear glassy resin. UV λ_{max}^{MeOH} nm (ε): 203 (35 796), 215 (sh), 221 (27 632), 279 (34 854); CD $\Delta\varepsilon_{237} = -14.3°$, and $\Delta\varepsilon_{274} = +12.4°$. High resolution accurate mass EIMS (190°, 40 eV), measured 628.2311, calculated 628.2308 for $C_{36}H_{36}O_{10}$; FDMS (ST = 80°, accel. voltage = 3 kV), *m/z* (rel. int.): 629 $[M+1]^{+\cdot}$ (53), 628 $[M]^{+\cdot}$ (100), 610 $[M-18]^{+\cdot}$ (4); 481 $[M-R.COO]^{+}$ (5), 148 [R.COOH] (6). ^{13}C NMR ($CDCl_3$, 62.9 MHz, TMS, DEPT): δ209.33 (C-3), 165.80 (C = 0, cinnamate), 160.25 (C-1), 145.93 (C-2″), 142.86 (C-15), 136.88 (C-2), 135.25 (C-4″), 134.00 (C-2′), 130.62 (C-7″), 129.62 (C-5′), 128.90 (C-5″, C-9″), 128.23 (C-4′, C-6′), 128.04 (C-6″, C-8″), 126.01 (C-3′, C-7′), 117.84 (C-1′), 117.19 (C-3″), 113.76 (C-16), 84.0 (C-13), 80.77 (C-14), 78.60 (C-4), 78.53 (C-12), 72.26 (C-9), 71.82 (C-5), 65.23 (C-20), 64.32 (C-7), 60.59 (C-6), 47.44 (C-10), 44.16 (C-11), 35.54 (C-8), 18.86 (C-19), 18.29 (C-17), 9.83 (C-18) ppm. ^{1}H NMR and IR data have previously been reported [3, 4] so have not been included. Compound **1** was accordingly gnidicin previously isolated from *Gnidia lamprantha* [4].

***Compound* 2. 12-*O*-Heptaecenoyl-5-hydroxy-6,7-epoxy-resiniferonol-l9,13,14-orthobenzoate.** Purified by prep. adsorption TLC as before using $CHCl_3$–EtOAc (2:3, R_f 0.45) as solvent and then by means of partition TLC with cyclohexane–butanone (4:1, R_f 0.30) as solvent. Evaporation of the recovered band afforded 67.0 mg of a clear glassy resin. UV λ_{max}^{MeOH} (ε): 205 (10 173), 212 (sh) 248 (8 228), 264 (sh), 303 (7 330) nm. ^{1}H NMR and CIMS data have previously been reported [3] for this compound.

***Compound* 3. 12-*O*-*n*-Deca-2,4,6-trienoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate.** This compound was purified by prep. TLC as before using EtOAc–$CHCl_3$ (3:2, R_f 0.72) as solvent and then re-purified using $CHCl_3$–EtOAc (2:1, R_f 0.31) as solvent. After elution the residue was dissolved in 5 ml EtOH and absorbed onto an activated charcoal column. Compound **3** was eluted with 50 ml EtOH. The solvent was removed by evapn to yield 49.6 mg of a clear glassy resin. IR ν_{max}^{KBr} cm^{-1}: 3450, 2960, 2930, 2880, 1740, 1620, 1450, 1270, 1130, 1080, 1010, 750. High resolution accurate mass EIMS (190°, 40 eV) Measured 646.2779, calcd 646.2776 for $C_{37}H_{42}O_{10}$; FDMS (ST = 80°, accel. voltage 3 kV), *m/z* (rel. int.): 647 $[M+1]^{+\cdot}$ (59), 646 $[M]^{+}$ (100), 628 $[M-18]^{+\cdot}$ (9), 481 $[M-R^2.COO]^{+\cdot}$ (99), 358 $[M-R^2.COOH-R^1.COOH]^{+\cdot}$ (14), ^{1}H NMR ($CDCl_3$, 80 MHz, TMS): δ7.82–7.28 (*m*, 5H-aromatic, 1H-olefinic), 7.51 (*s*, H-1), 7.16–5.63 (*br m*, 5H-olefinic), 5.12 (*s*, H-12), 5.01 (*s*, 2H-16), 4.92 (*d*, *J* = 2.60 Hz, H-14), 4.26 (*m*, H-5, D_2O exchange 1 × OH), 3.91 (*m*, 2H-20, H-10, D_2O exchange 1 × OH), 3.63 (*br s*, H-7, H-8), 3.44 (*s*, D_2O exchange 1 × OH), 2.52 (*q*, *J* = 7.59 Hz, H-11), 2.14 (*m*, 2H-acyl), 1.87 (*s*, 3H-17), 1.78 (*m*, 3H-19), 0.90 (*br m*, 3H-18, 3H-acyl). ^{13}C NMR ($CDCl_3$, 62.9 MHz, TMS, DEPT): δ209.46 (C-3), 166.04 (C = 0, *n*-deca-2,4,6-trienoate), 160.40 (C-1), 146.08 (C-2″), 142.93 (C-15), 142.26 (C-3″), 141.28 (C-4″), 136.92 (C-2), 135.34 (C-2′), 130.90 (C-5″), 129.63 (C-5′), 128.07 (C-4′, C-6′), 127.39 (C-6″), 126.05 (C-3′, C-7′), 118.88 (C-7″), 117.87 (C-1′), 113.74 (C-16), 84.25 (C-13), 80.85 (C-14), 78.66 (C-4), 78.38 (C-12), 72.28 (C-9), 72.00 (C-5), 65.18 (C-20), 64.36 (C-7), 60.59 (C-6), 47.54 (C-10), 44.19 (C-11), 35.58 (C-8), 35.05 (–CH_2–CH_2–Me), 22.16 (–CH_2–CH_2–Me), 18.92 (C-19), 18.29 (C-17), 13.68 (–CH_2–CH_2–Me), 9.86 (C-18). Compound **3** was accordingly gnidiltrin previously isolated from *G. lamprantha* [4]

***Compound* 4. 12-*O*-Benzoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate.** This compound was purified by

adsorption TLC as before using EtOAc–$CHCl_3$ (3:2, R_f 0.57) as solvent. Further purification was achieved using a second prep. stage and developing twice with EtOAc–$CHCl_3$ (7:2, R_f 0.30). Final purification was achieved by means of prep. partition TLC using cyclohexane–butanone (3:2, R_f 0.64) as solvent. Following recovery in the normal manner 25.8 mg of a clear glassy resin was obtained. UV λ_{max}^{MeOH} nm (ε): 203 (13 587), 223 (13 451), 231 (13 451), 252 (sh), 276 (7065); EIMS (200°, 70 eV) *m/z* (rel. int.): 602 $[M]^{+\cdot}$ (1), 481 $[M - R^2.COO]^+$ (0.5), 480 $[M - R^2.COOH]^{+\cdot}$ (1), 358 $[M - R^2.COOH - R^1.COOH]^{+\cdot}$ (2), 340 $[M - R^2.COOH - R^1.COOH - 18]^{+\cdot}$ (1), 322 $[M - R^2.COOH - R^1.COOH - 36]^{+\cdot}$ (1), 122 $[R^2.COOH]$ (4), 105 $[R^2CO.]$ (100); ^{1}H NMR data have previously been reported for this compound [5]. Accordingly, compound **4** was identified as genkwadaphnin previously obtained from *Daphne genkwa* [5].

Compound **5**. 12-*O*-Butenyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate. Compound **5** was purified by adsorption prep. TLC as before using EtOAc–$CHCl_3$ (3:2, R_f 0.57) as solvent and then further purified in the same system using $CHCl_3$–EtOAc (7:2, R_f 0.16) as solvent. Final purification was achieved using partition prep. TLC as before and developing with cyclohexane–butanone (7:3, R_f 0.54) as solvent; 16.20 mg of a clear glassy resin were finally produced. UV λ_{max}^{MeOH} nm (ε): 201 (sh), 210 (8 830), 229 (12 480), 312 (14 793) nm; EIMS (200°, 70 eV), *m/z* (rel. int.): 566 $[M]^{+\cdot}$ (1), 548 $[M - 18]^{+\cdot}$ (1), 480 $[M - R^2.COOH]^{+\cdot}$ (1), 358 $[M - R^2.COOH - R^1.COOH]^{+\cdot}$ (3), 340 $[M - R^2.COOH - R^1.COOH - 18]^{+\cdot}$ (2), 322 $[M - R^2.COOH - R^1.COOH - 36]^{+\cdot}$ (2), 105 $[R^1.CO]$ (52), 57 (100). ^{1}H NMR ($CDCl_3$,80 MHz, TMS: δ7.65–6.65 (*m*, 5H-aromatic), 7.44 (*s*, H-1), 7.33 (*d*, *J* = 14.5 Hz, 1H-olefinic), 6.15 (*d*, *J* = 14.5 Hz, 1H-olefinic), 5.06 (*s*, H-12), 5.00 (*s*, 2H-16), 4.73 (*br s*, H-14), 4.22 (*s*, H-5), 3.93–3.47 (*m*, 2H-20, H-10, H-7, H-8, D_2O exchange 1 × OH), 2.44 (*m*, H-11), 1.95 (*s*, 3H-17), 1.84 (*br s*, H-19), 1.27 (*br s*, –Me), 0.87 (*m*, 3H-18).

Base catalysed selective hydrolysis. *Ca* 5 mg of compounds **1–5** were separately hydrolysed with 0.7 ml 1% NaOMe in dry MeOH overnight under N_2 at room temp. The reactions were terminated by the addition of ice-cold H_2O and the hydrolysis product extracted by partition into CH_2Cl_2 (3 × 25 ml). The residue (**6**) was purified by means of adsorption prep. TLC using EtOAc–$CHCl_3$ (3:2, R_f 0.11) as solvent; EIMS (170°, 40 eV), *m/z* 498 $[M]^{+\cdot}$ (1), 470 $[M - 18]^{+\cdot}$ (3), 376 $[M - R^1.COOH]^{+\cdot}$ (12), 358 $[M - R^1.COOH - 18]^{+\cdot}$ (15), 105 $[R^1CO.]$ (100). Chromatographically this compound was identical to 12-hydroxydaphnetoxin [6], and was the C-12 selective hydrolysis product of compounds **1–5**. Upon acetylation with an excess of Ac_2O–pyridine (0.5 ml) at room temp. for 4.5 hr and following purification of the product by means of prep. adsorption TLC using EtOAc–$CHCl_3$ (3:2, R_f 0.48) as solvent, the acetate [6] was produced in 90% yield; FDMS (ST = 80°, accel. voltage = 3 kV), *m/z* (rel. int.): 625 $[M+1]^{+\cdot}$ (47), 624 $[M]^{+\cdot}$ (100), 581 $[M - Ac]^{+\cdot}$ (9), 495 $[M - 3 \times Ac]^{+\cdot}$ (5). ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ7.72 (*m*, 2H-aromatic), 7.48 (*br s*, H-1), 7.39 (*m*, 3H-aromatic), 5.56 (*s*, H-5), 5.04 (*s*, 2H-16), 5.00 (*s*, H-12), 4.90 (*d*, *J* = 2.42 Hz, H-14), 4.80 (*d*, *J* = 12.09 Hz, 1H-20), 4.06 (*m*, 1H-10), 3.67 (*d*, *J* = 2.42 Hz, H-7), 3.62 (*d*, *J* = 12.09 Hz, 1H-20), 3.52 (*s*, H-8), 2.37 (*q*, *J* = 6.05 Hz, H-11), 2.20 (*s*, Me.CO), 2.02 (*s*, 2 × Me.CO), 1.88 (*s*, 3H-17), 1.75 (*m*, 3H-19), 1.30 (*d*, *J* = 7.32 Hz, 3H-18). Accordingly, compound **7** was 12-hydroxydaphnetoxin-5,12,20-triacetate.

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