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SESQUITERPENE LACTONES OF *OTANTHUS MARITIMUS*

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Key Word Index—*Otanthus maritimus*, Anthemideae, Compositae, sesquiterpene lactones, guaianolides

Abstract—Three sesquiterpene lactones have been isolated from the aerial parts of *Otanthus maritimus* The compounds, all guaianolides, are identified as C-8 esters of 11,13-dihydroartecanin

INTRODUCTION

Otanthus maritimus [1] is an indigenous Egyptian herb that is reputed among the Bedouins to be an effective antiasthmatic drug In previous publications [2, 3], we reported the isolation from this herb of sesamin, 5-hydroxy-6,7,3′,4′,5′-pentamethoxyflavone, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone and the flavone glycoside acacetin 7-*O*-neohesperidoside, in addition to α-amyrin, sitosterol and sitosteryl glycoside In a continuation of our investigations on this plant, we present here the isolation and characterization of three sesquiterpene lactones Lactone B was shown to have antiasthmatic properties on preliminary testing

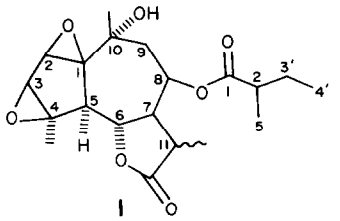
RESULTS AND DISCUSSION

Fresh leaves and unexpanded flowerheads of *Otanthus maritimus* were extracted with ether and chloroform Chromatographic separation of the purified extracts gave three sesquiterpene lactone esters, A, B and C The different qualitative and spectral data indicated that they were structurally related but differed in the ester part They were identified as C-8 esters of 11,13-dihydroartecanin The ester side chain in A was 2-methylbutyrate and in B and C it was probably hydroxy tiglate and 2,3-dihydroxy-2-methylpropanoate, respectively The principal basis for the assignment of structures of the lactones lies in the close correspondance of their spectra with those of artecanin [4, 5], except for the features associated with the ester side chain at C-8 and the C-13 methyl All compounds exhibited a prominent loss of the ester side chain from the molecular ion (MW 380 for A, 394 for B and *m/z* 380 $[M-H_2O]^+$ for C) to yield similar ions at *m/z* 295, 278, 260 and a base peak at *m/z* 111 suggesting that the three compounds have common structural features The interpretation of the *m/z* 111 fragment has been previously reported [6] Their UV spectra all showed λ_{max}^{MeOH} 209 nm In the IR spectra peaks were observed for hydroxyl, γ-lactone, epoxy and ester groupings

Lactone A, mp 163–165°, $[\alpha]_D^{21}$ +43 5°($CHCl_3$, *c* 0 127), had an elementary analysis indicating a molecular formula of $C_{20}H_{28}O_7$, MS *m/z* 380 $[M]^+$, IR ν_{max}^{KBr} cm^{-1} 3480 (hydroxyl), 1785 (γ-lactone), 1695 (C = O of ester) and 1165 (–C–O–C–epoxy) The structure and stereochemistry of lactone A was established from ^1H NMR ($CDCl_3$) data and by careful spin decoupling which allowed the assignment of all signals and couplings The nature of the ester residue followed from the characteristic signals (C-2′), δ 2 21 (*m*, C-3′), 1 23 and 1 165 (*m*, C-4′), 0 94 (*t*, *J* = 6 Hz, C-5′), 1 34 (*d*, *J* = 7 5 Hz), as was compared with published data [7] for 2-methylbutyrate The presence of 11,13-dihydrolactone was indicated by the doublet at 1 25 (*J* = 7 5 Hz) and the absence of typical signals for methylene protons This was confirmed by spin decoupling, as irradiation at 2 53 (H-7) collapsed the methyl doublet to a singlet Two additional methyl singlets were visible at 1 133 (Me-10) and at 1 5 (Me-4) The protons at C-2 and C-3 ($J_{2\,3}$ = 1 3 Hz) gave a pair of narrow doublets at 3 28 and 3 43 The proton at C-5 appeared as a double doublet at 3 05, coupled with H-6 ($J_{5,6}$ = 10 Hz) and was broadened by small couplings (*J* = 1 3 Hz) with H-2 and H-3 The C-6 proton coupled with H-5 ($J_{5\,6}$ = 10 Hz) and H-7 ($J_{6,7}$ = 11 Hz) and the C-7 proton coupled with H-6, H-11 and H-8 ($J_{6,7}$ = 11 Hz, $J_{7,11}$ = 12 75 Hz and $J_{7\,8}$ = 4 5 Hz) appeared as a doublet of doublets at 4 15 and 2 53, respectively These couplings were seen by irradiation at the mean resonance of the protons on C-5, C-6 and C-7 Irradiation at δ 3 05 collapsed the narrow doublets at 3 28 and 3 43 to two singlets, while irradiation at 3 43 reduced the doublet at 3 28 to a singlet and the signal at 3 05 to a doublet, revealing the existence of a small coupling to both H-2 and H-3

The large coupling constants exhibited by signals corresponding to the C-5, C-6 and C-7 protons indicate that the C-6 proton bears a transdiaxial relationship with both the C-5 and C-7 protons Assuming the C-7 side chain is β-oriented as in all guaianolides [4, 8, 9], the compound should possess the stereochemistry at C-5, C-6 and C-7 as shown in **1** The α-position of the H-5 proton, as well as the small coupling of H-5 with H-2 suggest that both epoxide groups are β-oriented as in artecanin [5] This was further supported by the rather downfield position of the C-6 proton signal The spin decoupling allowed the assignment of the C-11 proton through C-7, as irradiation at δ 4 15 changed the double doublet at 2 53 to a quartet ($J_{7\,11}$ = 12 75 Hz, $J_{11\,13}$ = 7 5 Hz), thus revealing the H-11 proton obscured by H-7 The C-8 proton appeared as a double double doublet at 5 05 ($J_{7\,8}$ = 4 5 Hz, $J_{8\,9}$ = 3 Hz and $J_{8,9}$ = 6 Hz) The C-9 proton gave a broadened doublet of doublets at 2 1 coupled ($J_{8\,9}$ = 3 Hz, $J_{8,9}$ = 6 Hz) with H-8 This was confirmed by irradiation at δ 5 05 which collapsed the

signal at 2 1 to a broadened singlet while irradiation of the latter changed the signal at 5 05 to a sharp doublet The sharp singlet at 1 6 was the signal of a tertiary hydroxyl group as shown by deuterium exchange This tertiary hydroxyl group must be placed at C-10 as only a guaianolide of type **1** is in agreement with all the data The stereochemistry at C-8 was deduced from the small coupling ($J_{7,8} = 4.5$ Hz) while the α-orientation of the hydroxyl at C-10 followed from the normal position of the C-6 proton



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Lactones B and C show in their IR spectra a strong band at 3520 cm^{-1} and another broad band between 3240–3320 cm^{-1} indicating the presence of associate and dimeric alcoholic groups These were further proved in their mass spectra by the appearance of low intensity peaks at *m/z* 360 and at 345 resulting from successive splitting of two hydroxyls and two waters plus a hydroxyl from the parent ion peaks of B and C, respectively Moreover, the loss of fragments *m/z* 115 and 119 from their parent ions suggests that the ester side chains in B and C are $C_5H_7O_3$ and $C_4H_7O_4$, respectively These could be attributed to hydroxy tiglate and 2,3-dihydroxy-2-methylpropanoate The latter was further supported by the presence of a mass spectral peak at *m/z* 349 [380 $-31]^+$ characteristic for a loss of CH_2OH

The isolation of these lactones that are related to artecanin and rupins isolated from *Artemisia* species [4–6] may be an indication of a relationship between the genus *Otanthus* and *Artemisia* In addition, the previously isolated flavones from *O maritimus* appear to be also related to those recorded before from members of the genus *Artemisia*

EXPERIMENTAL

Mps were determined on a Koffler's heating stage microscope ^{1}H NMR spectra were determined in $CDCl_3$ and D_2O at 90 and 170 MHz, using TMS as int standard MS were obtained by direct inlet, 70 eV Analytical and prep TLC were done on Si gel G Al_2O_3 (grade I) was used for CC separations Plates were visualized by spraying with anisaldehyde reagent and heating

Plant material Aerial parts of *O maritimus* Hoffmgg et Link were collected near Alexandria Its identity was confirmed by the Late Professor Dr V Tackholm (Cairo University) A voucher sample is kept in the Faculty of Pharmacy, Alexandria University

Isolation of the three lactones Fresh leaves and unexpanded flowerheads (10 kg) were exhaustively extracted with EtOH at room temp The concd extract was shaken with petrol then successively re-extracted with Et_2O and $CHCl_3$ Evaporation of the Et_2O extract gave 2 5 g of dark syrup This was dissolved in EtOH acidified with HOAc, then shaken with petrol and Et_2O The latter was washed with a 10% soln of Na_2CO_3, then with H_2O, dried and the solvent evaporated under red pres The purified syrup (1 2 g) was chromatographed over 35 g Al_2O_3 The following fractions (20 ml each) were collected 1–4 (C_6H_6), 5–20 (C_6H_6–$CHCl_3$, 3 1, 1 1 and 1 3), 21–26 ($CHCl_3$) The eluates were monitored by TLC Fractions 21–26 gave oily crystals that were purified by prep TLC (EtOAc–C_6H_6, 1 1), then recrystallized from a $CHCl_3$–petrol mixture to yield 70 mg of plates of lactone A Concn of the mother liquor of the above recrystallization furnished after purification (prep TLC) 10 mg of minute prisms of lactone B The $CHCl_3$ extract was similarly treated The purified syrup (2 3 g) was fractionated on an Al_2O_3 column (50 g) using the same solvent and solvent mixtures for elution (14 fractions, 40 ml each) Purification (prep TLC), then crystallization of fraction 13 afforded 20 mg of lactone C, in the form of needles

8β-(2-Methylbutyryloxy)-11,13-dihydroartecanin (lactone A, **1**) Mp 163–165°, Found C, 64 0, H, 7 69, O, 28 31 $C_{20}H_{28}O_7$ requires C, 63 14, H, 7 42, O 29 44% MS *m/z* (rel int) 380 $[M]^+$ (weak), 362 $[M-H_2O]^+$ (8), 296(7), 295 $[M-C_4H_9CO]^+$ (5), 281 $[296-Me]^+$ (12), 278 $[M-C_4H_9CO_2H]^+$ (20), 263 $[278-Me]^+$ (18), 261 (46), 260 $[278-H_2O]^+$, 205(27), 111(100), 85 $[C_4H_9CO]^+$ (23), 57 $[85-CO]^+$ (75)

8β-Hydroxytiglinoyloxy-11,13-dihydroartecanin (lactone B) Mp 91–92° ($CHCl_3$–petrol), $[\alpha]_D^{20}$ + 44 8° ($CHCl_3$, *c* 0 124) IR ν_{max}^{KBr} cm^{-1} 3520, 3340–3220 (OH), 1760 (γ-lactone), 1722, 1650 (C = CCO_2R), 1190, 1165, 1130 Found C, 60 71, H, 7 44, O, 31 85 $C_{20}H_{26}O_8$ requires C, 60 90, H, 6 64, O, 32 45% MS *m/z* (rel int) 394 $[M]^+$ (weak), 360 $[M-2OH]^+$ (2), 296(11), 295 $[M-C_4H_7OCO]^+$ (6), 281 $[296-Me]^+$ (8), 278 $[M-C_4H_7OCO_2H]^+$ (24), 260 $[278-H_2O]^+$ (19), 205(27), 111(100), 99 $[C_4H_7OCO]^+$ (5), 71 $[99-CO]^+$ (20)

8β-(2, 3-Dihydroxy-2-methylpropyloxy)- 11, 13-*dihydroartecanin (lactone C)* Mp 176–177° ($CHCl_3$–petrol), $[\alpha]_D^{20}$ + 39 9° ($CHCl_3$, *c* 0 143) IR ν_{max}^{KBr} cm^{-1} 3520, 3340–3240 (OH), 1760 (γ-lactone), 1722 (CO_2R), 1195, 1165 Found C, 57 19, H, 6 51, O, 36 3 $C_{19}H_{26}O_9$ requires C, 57 28, H, 6 58, O, 36 14% MS *m/z* (rel int) 380 $[M-H_2O]^+$ (1), 362 $[M-2H_2O]^+$ (4), 345 $[362-OH]^+$ (2), 296(9), 295 $[M-C_3H_7O_2CO]^+$ (5), 281 $[296-Me]^+$ (15), 278 $[M-C_3H_7O_2CO_2H]^+$ (23), 261(15), 260 $[278-Me]^+$ (20), 205(24), 111(100), 103 $[C_3H_7O_2CO]^+$ (9)

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