During the course of this investigation, tormentic acid and euscaphic acid were also obtained, as their methyl esters. Full details of the isolation and spectral identification of the known compounds are available on request to the authors.

Acknowledgements—One of us (B.L.S.) would like to thank Prof. J.-C. Jacquesy (University of Poitiers, France) for the ^{1}H NMR (200 MHz) and the mass spectra measurements, and Mr B. Mpom (Cameroon National Herbarium, Yaoundé) for the identification of the plant material. The research programme was partially supported by the University of Yaoundé research grant.

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TRITERPENE FATTY ACID ESTERS AND FLAVONOIDS FROM *INULA BRITANNICA*

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Key Word Index—*Inula britannica*; Compositae; triterpene fatty acid esters; 3β,16β-dihydroxylupeol 3-palmitate; 3β,16β-dihydroxylupeol 3-myristate; flavonoids; quercetin 3-sulphate; 6-hydroxykaemperol 3-sulphate.

Abstract—Two new triterpene fatty acid esters, 3β,16β-dihydroxylupeol 3-palmitate and 3β,16β-dihydroxylupeol 3-myristate, and a new kaempferol derivative, 6-hydroxykaempferol 3-sulphate were isolated from the aerial parts of *Inula britannica*. Furthermore, *epi*-friedelinol, β-amyrin palmitate, olean 13(18)-en 3-acetate, sitosteryl 3-glucoside and quercetin 3-sulphate were also identified.

INTRODUCTION

Inula britanica is a widespread plant growing in the western part of Turkey. In earlier articles, sesquiterpene lactones, thymol derivatives and flavonoids [1–3] were reported from this plant. Our reinvestigation of *I. britanica* afforded two new triterpene fatty acid esters, 3β,16β-dihydroxylupeol 3-palmitate **1** and 3-myristate **1a** besides the known compounds epi-friedelinol **2** [4], β-amyrin palmitate **3** [5], olean 13(18)-en 3-acetate **4** [6] and sitosteryl 3-glucoside.

From an ethanolic extract of the aerial parts, in addition to the known flavonoids quercetin 3-glucoside, 6-methoxyquercetin 7-glucoside, quercetin 7-glucoside, 6-methoxyluteolin 7-glucoside and quercetin 3-sulphate [7], a new kaempferol derivative 6-hydroxykaempferol 3-sulphate **5** was also isolated.

RESULTS AND DISCUSSION

The residue from a petrol–ether extract of the dried plant material was separated by CC on silica gel and triterpenes were obtained. The first fractions gave olean 13(18)-en 3-acetate **4**, *epi*-friedelinol **2** and β-amyrin palmitate **3**.

Compound **4** was found to be olean 13(18)-en 3-acetate **4** by comparing its ^{1}H NMR and mass spectrum to that of an authentic sample.

Compound **2** was identified as *epi*-friedelinol by

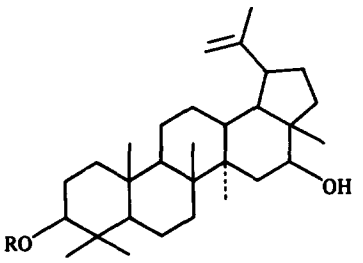
^{1}H NMR spectra as well as mmp and co-TLC with an authentic sample.

Compound 3 was β-amyrin palmitate. After alkaline hydrolysis the triterpene part was characterized as β-amyrin by spectral methods as well as by TLC comparisons. The aliphatic acid part was isolated and identified as palmitic acid by GC after esterification with CH_2N_2.

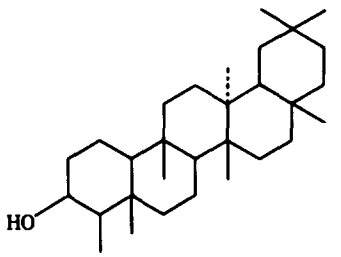
Compounds 1 and 1a were obtained as a mixture from later column fractions. IR indicated an ester carbonyl at 1730 and 1250 cm^{-1} and unsaturation at 1639 cm^{-1}. The structures of 1 and 1a were identified from the ^{1}H NMR data which were similar to those of lupeyl acetate. The NMR spectrum showed besides the typical peaks for 3β,16β-dihydroxylupeol (calenduladiol), a pattern of signals which were assigned to fatty acid esters with a triplet at δ0.87 for one terminal methyl of Me-CH_2-, a broad singlet at δ1.24 for (-CH_2- n) and a triplet at δ2.28 for methylene protons attached to a carbonyl function. A double doublet for H-3 was shifted downfield to δ4.46 due to the ester group. The position of the second oxygen function could be deduced by comparison with the spectrum of 16-hydroxylupeol.

The nature of the ester group followed from the mass spectrum. Showing two $[M]^+$ peaks the spectrum clearly indicated the presence of two long-chain fatty acids in this fraction. $[M]^+$ ions at m/z 680 and 652 gave the molecular formula $C_{46}H_{80}O_3$ for 3,16-dihydroxylupeol 3-palmitate 1 and $C_{44}H_{76}O_3$ for 3,16-dihydroxylupeol 3-myristate 1a. The most important peak was at m/z 424 for the loss of ester moiety $[M - RCO_2H]^+$ for both compounds and the other fragments at m/z 406, 381, 203 and 189 supported this conclusion.

6-hydroxykaempferol 3-sulphate 5 was obtained from the ethanol extract of aerial parts of *I. britannica*. The presence of a 6-OH group in 5 was first suspected from the purple colour under UV light which remained unchanged when exposed to ammonia vapour. The UV band I shift of 27 nm in aluminium chloride/hydrochloric acid relative to band I in methanol also indicated a flavonoid with a 6-OH group. The presence of band III and a 5 nm shift of band II in sodium acetate suggested the presence of a free OH group at C-7. The lack of a shift of band I with sodium acetate/boric acid showed that there was no *ortho*-dihydroxy group in the B-ring but the UV spectrum observed in methanol after adding six drops concentrated



1 R = Me—$(CH_2)_{14}$—CO
1a R = Me—$(CH_2)_{12}$—CO



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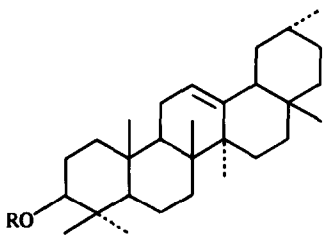
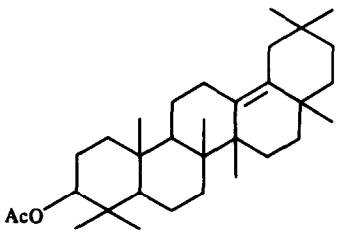
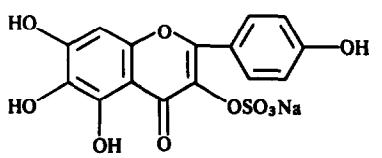


3 R = Me—$(CH)_{14}$—CO



4



5

hydrochloric acid gave band I at 355 nm in 15 minutes indicating the presence of a free 3-OH group. The ^{1}H NMR spectrum showed only 6-hydroxykaempferol protons. Since the NMR exhibited no sugar protons and acidic hydrolysis failed to give any sugar, the water layer was treated with barium chloride and a white precipitate was obtained. Electrophoretic migration indicated the presence of a monosulphate by comparing with standard monosulphates of quercetin and kaempferol. The UV findings required that the sulphate group is at C-3.

EXPERIMENTAL

I. britannica L. was collected from western Turkey in August 1985. It was identified by Dr Neriman Özhatay (University of Istanbul). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 56125).

Isolation of triterpenoids. Air-dried and powdered aerial parts (2 kg) were extd with petrol (bp 40–60°) Et_2O (2:1). After filtration the ext was concd *in vacuo* at room temp and subjected to CC over silica gel. Elution was started with petrol and Et_2O was gradually added up 100%. The fractions gave **2** (5 mg), **3** (7 mg), and **4** (4 mg). Further fractions eluted with 75% Et_2O yielded **1** and **1a** (29 mg).

Isolation of flavonoids. Dried and powdered aerial parts (1 kg) were extd with 80% EtOH in a Soxhlet. After evapn the ext. was dild with an equal vol. of H_2O and partitioned with *n*-hexane, $CHCl_3$ and EtOAc, successively. The remaining aq. phase and the EtOAc phase contained the flavonoids. Quercetin 3-sulphate and 6-hydroxykaempferol 3-sulphate **5** (13 mg) were obtained from the aq phase by polyclar CC using MeOH–H_2O for elution. The EtOAc phase gave the other flavonoids.

3β,16β-Dihydroxylupeol 3-palmitate **1** *and 3-myristate* **1a**. Colourless oil IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3442 (OH), 3071, 1730 (COOR), 1639 (C=C), 1465, 1380, 1250, 1109, 943, 883, 722. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87, 0.82, 0.84, 0.97, 1.02 (each 3H, *s*, C-24, C-26, C-27, C-25, C-23, C-28, respectively), 1.68 (3H, *s*, C-30) 0.86 (3H, *t*, J = 7 Hz, Me–CH_2–) 3.60 (1H, *dd*, J = 5 and 11 Hz, H-16), 4.46 (1H, *dd*, J = 6 and 11 Hz, H-3), 2.53 (2H, *t*, J = 7.5 Hz, –CH_2–COO) 1.24 (*br s*, –CH_2– *n*). MS (70 eV); *m/z* (rel. int): $[M]^+$ 680 (5), 662 (5) $[M-H_2O]^+$, 424 (20) $[M-RCO_2H]^+$ for **1**, $[M]^+$ 652 (8), 634 (6) $[M-H_2O]^+$, 424 (20) $[M-RCO_2H]^+$ for **1a** and the other fragments 406 (11) $[M-RCO_2H-H_2O]^+$, 381 (10), 363 (10), 256 (9), 228 (5), 216 (18), 203 (29), 189 (32).

β-Amyrin palmitate **3**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2930, 2858, 1730 (COOR), 1464, 1379, 1250, 1050, 980. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.79, 0.85, 0.86, 0.95, 0.99, 0.88 (*t*, J = 7 Hz, Me–CH_2–), 2.29 (*t*, J = 7 Hz, –CH_2–COO–), 5.25 (*m*, H-12) 4.50 (*dd*, J = 5.5 and 10 Hz, H-3). *Hydrolysis of* **3**. **3** (5 mg), 2 ml of 5% KOH in MeOH and 15 ml of C_6H_6 were refluxed for 24 hr. After removing the triterpene with $CHCl_3$ the alkaline soln was acidified and extd with EtOAc. The obtained fatty acid was esterified with CH_2N_2 in C_6H_6.

6-Hydroxykaempferol 3-sulphate **5**. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2936, 1657 (C=O), 1607, 1562, 1510, 1450, 1363, 1050, 773. UV λ_{max}^{MeOH} nm: 268, 295, 345; NaOMe: 273, 315, 400 (incr. int.); $AlCl_3$: 275, 303, 350, 392; $AlCl_3$/HCl: 269, 290, 335, 376; NaOAc: 273, 300, 355; NaOAc/H_3BO_3: 266, 300, 350. ^{1}H NMR (DMSO-d_6) δ: 7.15 (*d*, J = 8 Hz, H-3′ and H-5′), .765 (*d*, J = 8 Hz, H-2′ and H-6′), 6.55 (*s*, H-8)

Hydrolysis of **5**. **5** (6 mg) was heated at 100° with 5 ml of 0.1 N TFA for 30 min. The cation was identified as Na by atomic absorption spectroscopy. Electrophoresis (200 V) was carried out on Schleicher & Schüll No. 2043 paper (30 × 18 cm) for 24 hr at pH 2.2 (HOAc–HCO_2H–H_2O, 29:52:920).

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