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WAXES, TRITERPENES AND FREE ACIDS IN *ANTHYLLIS SERICEA*

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Anthyllis sericea Lag. (Papilionoideae) was collected in April 1974 at La Cañada (Valencia). No previous work on *A. sericea* has been published but some papers, mostly referring to *A. vulneraria*, have appeared: free acids [1, 2], cyclitols [3], monosaccharides [4] and flavonoids [5–9]. From a petrol extract of *A. sericea* a wax precipitated by cooling to room temp. was found (IR and column chromatography on Al_2O_3) to contain almost exclusively aliphatic long-chain esters. By saponification the acidic and alcoholic components were separated. GC–MS analysis of the appropriate derivatives (Me esters and acetates respectively) gave the following results—saturated fatty acids (homologue, relative amount %). C_{22} 9.0, C_{24} 10.8. C_{28} 21.9, C_{30} 22.3. C_{32} 13.5; 1-alkanols, C_{24} 16.9, C_{26} 441, C_{28} 19.3, C_{30} 7.9. The homologue composition in both series is remarkably different and it would appear that acids and alcohols are independently biosynthesized.

From a $CHCl_3$ extract, γ-sitosterol and ursolic acid (as its Me ester) were isolated and identified by their spectral properties [10–14]. This is the first report of these compounds in the genus *Anthyllis*. As in other instances [15], γ-sitosterol is a mixture of the closely related sterols β-sitosterol, stigmasterol and campesterol.

From an EtOH extract an acidic fraction ($NaHCO_3$) was analysed. It contained (IR) both acids and hydroxyacids and was thus submitted to acetylation and methylation analysis. GC–MS of these derivatives enabled the identification of the following 18 compounds—phenylacetic, *cis*- and *trans*-cinnamic, *cis*- and *trans*-4-methoxycinnamic, 3- and 4-methoxybenzoic, 2- and 4-hydroxybenzoic, 2-hydroxy-4-methoxybenzoic, 2,4-dimethoxybenzoic, palmitic, adipic, pimelic, suberic, azelaic, sebacic and undecandioic acids.

EXPERIMENTAL

IR spectra were recorded in KBr discs and PMR spectra (90 MHz) in $CDCl_3$ soln with TMS as int. standard. GC–MS analysis was performed at 70 eV. Optical rotations were determined in $CHCl_3$ soln. Mps (uncorr.) were recorded on a Reichert apparatus. Column chromatography was performed on Si gel (0.063–0.2 mm) All compounds were identified by spectral and direct comparison with authentic samples.

Extraction. Air-dried stems and leaves (3 kg) of *A. sericea* were Soxhlet extracted with 95% EtOH. The extract was adsorbed on Si gel (1.5 kg) dried at room temp. and the powder re-extracted with petrol (bp 50–70°), $CHCl_3$ and EtOH. By concentrating the petrol extract to 1 l. vol and cooling to room temp a crude wax precipitated out. By repeated crystallization from EtOAc a white product (8.8 g, mp 75–80°) was obtained and submitted to analysis as described in text. The $CHCl_3$ extract was divided into neutral and acidic fractions. Column chromatography of the neutral fraction gave mainly long-chain saturated alcohols, and from hexane–Me_2CO (20:1) γ-sitosterol (1.088 g), mp 144–146° (from hexane), $\{\alpha\}_D^{20}$ −42° IR ν_{max}: 3350, 1055 (OH), 1640, 833, 795 cm^{-1} (C=CH—); MS (probe), *m/e* (rel. int.): 414 (25), 412 (18), 400 (8), 273 (14), 43 (100). It was found to be identical (GLC) with a mixture of β-sitosterol ($M^+ = 414$), stigmasterol ($M^+ = 412$) and campesterol ($M^+ = 400$). The acidic fraction was boiled with hexane to eliminate long-chain compounds, the residue treated with excess CH_2N_2 and chromatographed on Si gel. With hexane–EtOAc (10:1), Me ursolate (0.195 g) was eluted: mp 168–170° (from hexane), $\{\alpha\}_D^{20}$ +60°, IR ν_{max}: 3350, 1041, 1025 (OH), 1732 (COOMe), 1650, 825, 798 cm^{-1} (C=CH—); PMR (δ ppm): 0.6–2.4 (45H, *br* band), 3.2 (1H, *m*, C-3), 3.6 (3H, *s*, COOMe), 5.3 (1H, *brt*, $J = 5$ Hz, C-12); MS (probe), *m/e* (rel. int.): 470 M^+ (9), 411 (M^+-59, 5), 410 (6), 262 (86), 207 (30), 203 (100), 189 (26), *Anal.*: Found: C, 78.97; H, 10.88. Calc. for $C_{31}H_{50}O_3$: C, 79.15; H, 10.64. By the usual method (Ac_2O–Py) an acetylated derivative was also prepared: mp 244–246° (lit. [14] 245–246°); $\{\alpha\}_D^{20}$ +62° (lit. [14] +63°).

The EtOH extract was evaporated to dryness and the residue boiled with Et_2O. The Et_2O-soluble part was extracted with 5% aq $NaHCO_3$ and the aq layer acidified and re-extracted with Et_2O. After removal of solvent, the residue was successively acetylated (Ac_2O–Py), methylated (CH_2N_2) and percolated through a Si gel column (hexane–Et_2O 2:1) to eliminate highly polar impurities. The eluted material was analysed by GC–MS and the compounds described in the text identified.

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