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THE STEROLS OF *ARGANIA SPINOSA* SEED OIL

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Key Word Index—*Argania spinosa*; Sapotaceae; Argan seed oil; sterols; 5α-stigmast-7-en-3β-ol; 5α-stigmast-7,22(*E*)-dien-3β-ol; schottenol; spinasterol.

Abstract—The sterol fraction of Argan seed oil (*Argania spinosa*) contains two main compounds, 5α-stigmast-7-en-3β-ol (schottenol) and 5α-stigmast-7,22(*E*)-dien-3β-ol (spinasterol).

INTRODUCTION

Argan (*Argania spinosa*, Sapotaceae) is a typical tree of south-west Morocco. Its seed oil, which is prepared on a small scale by native craftsmen, is highly appreciated in Morocco. The chemical composition and physical properties of this oil have been studied by several authors [1–4]. We have now examined a Moroccan oil, obtained by treatment of seed flour by hot water, followed by decantation [1]. The unsaponifiable part constituted about 0.8% of the oil by weight, and contains hydrocarbons, tocopherols, triterpene alcohols, sterols and xanthophylls. We have studied the sterol fraction, obtained either by digitonin precipitation and pyridine regeneration or by preparative thin layer chromatography.

RESULTS AND DISCUSSION

According to Huyghebaert and Hendrickx [4], the sterol fraction of argan seed oil contains four compounds: campesterol (4.3%), stigmasterol (39.3%), sitosterol (54.6%), and stigmast-7-en-3β-ol (1.9%). GLC analysis confirmed the presence of four sterols, **1** (4.0%), **2** (41.5%), **3** (47.5%) and **4** (7.0%). However, the relative retention times did not correspond exactly to the literature values for the sterols previously identified [5, 6]. GC/MS of the sterol trimethylsilyl ethers showed that the derivatives had molecular ions at *m/z* 484 and 486 respectively and the sterols are thus C_{29}-compounds, with two and one double bond respectively.

Peaks at *m/z* 343 and 345 in the spectra correspond to the loss of a C_{10}-lateral chain showing that these sterols belong to the stigmastane series. The *m/z* 345 peak in **2** (loss of 139; $C_{10}H_{19}$) showed that this compound had a double bond in the side chain. The partial hydrogenation of this bond during fragmentation (to yield *m/z* 343) by H_2 transfer from the D-ring [7], proved that the double bond was in the C-22, C-23 position. According to Brooks *et al.* [8] the peaks at *m/z* 255 (M^+ – TMSOH – side chain) and 213 (255 – C_3H_6) are characteristic of Δ^7-sterols. Moreover, there was no peak at *m/z* 129 or M^+ – 129, typical of Δ^5-sterols [9–12]. A peak corresponding to a retro Diels–Alder fragmentation (*m/z* at 300) was either small (**2**) or absent (**3**) and such a pattern is typical of Δ^7-sterols.

Thus, the main sterols of argan seed oil were identified as stigmasta-7,22-dien-3β-ol and stigmast-7-en-3β-ol. The mass spectrum of **3** was identical to that given by Brooks *et al.* [8] for this compound.

To confirm the position of the ring double bond, the mixture of sterols was hydrogenated. In neutral medium, only the C-22, C-23 double bond was reduced, and the GLC peak of **3** increased at the expense of **2**. In acidic medium, reduction of both compounds resulted in a migration of the Δ^7-double bond to the $\Delta^{8(14)}$-position and the stigmast-8(14)-en-3β-ol thus obtained had a retention time consistent with that given by Homberg [6]. This behaviour during hydrogenation in acidic conditions is typical of Δ^7-sterols [13].

The mass spectrum of the TMS derivative of the minor product **1** had the molecular peak at *m/z* 472 showing it was a C_{28}-sterol of the ergostane series containing one double bond. The (M^+ – 127) fragment showed that the side chain was saturated while the peaks at *m/z* 255 and 213 are characteristic of Δ^7-sterols. Thus compound **1** seems to be ergost-7-en-3β-ol.

We could not obtain the mass spectrum of **4** (4.7%) but it could possibly be stigmasta-7,24-dien-3β-ol which has been reported previously by Homberg [6].

EXPERIMENTAL

GLC was carried out on a 2 m column containing 3% OV-17 on W-HP chromosorb; the temp. was 270°. Mass spectra were determined at 70 eV.

Unsaponifiable lipid was obtained from 5 g of argan oil. The sterol fraction was obtained either by digitonin complexation, followed by pyridine regeneration [14] or by preparative TLC (Si gel, 2 mm thick, hexane–EtOAc, 70:30).

Mass spectra of sterol-TMS derivatives. **2**-TMS *m/z* (rel. int.): 484 $[M]^+$ (36.6), 469 (18.6), 372 (17.7), 345 (28.3), 343 (94.5), 300 (2), 255 (100), 213 (34.4). **3**-TMS *m/z* (rel. int.): 486 $[M]^+$ (100), 471 (11.1), 345 (6.8), 255 (90.1), 229 (20.4), 213 (27.5).

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STEROLS OF ROOTS AND NITROGEN-FIXING ROOT NODULES OF SOME NON-LEGUMINOUS SPECIES

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Key Word Index—*Alnus glutinosa*; *A. rubra*; *A. cordata*; Betulaceae; *Casuarina cunninghamiana*; Casuarinaceae; roots; nodules; sterols.

Abstract—Sterols of both roots and nodules of three species of *Alnus* were found to consist only of sitosterol, whereas *Casuarina cunninghamiana* contained substantial amounts of campesterol, stigmasterol and sitosterol. In all four cases more sterol was extracted from nodules than from roots.

INTRODUCTION

In a previous report, we described the sterols of roots and their associated nitrogen-fixing nodules from *Vicia faba* [1]. Qualitatively, the same sterols were present in both tissues but a greater amount of sterol per unit fresh weight was recorded for nodules. The three sterols characterized were 24-methylcholesterol, 24-ethylcholesterol and 24-ethyl-5,*E*22-cholestadien-3β-ol.

In the present work we report on the sterols of three species of *Alnus* (Betulaceae) and one of *Casuarina* (Casuarinaceae), which are all species of plants bearing nitrogen-fixing nodules formed in association with a class of micro-organism (Actinomycetes) not closely related to *Rhizobium*, the endophyte of the Leguminosae.

RESULTS

On the basis of relative GLC and of MS data for TMSi derivatives, it was found that all three species of *Alnus* examined (*A. glutinosa*, *A. rubra*, *A. cordata*) afforded only one sterol, identical in each case with 24-ethylcholesterol. A sample isolated from *A. glutinosa* afforded ^{1}H NMR data at 90 MHz identical with data found for authentic sitosterol (24*R*) 24-ethylcholesterol and distinguishable from that for the (24*S*)-isomer clionasterol [2–4]. (Found for *A. glutinosa* sterol: δ 0.682 *s*: C-18; 0.837 *d*, J = 7.5 Hz: C-26/27; 0.85 *t*, J = 7.0 Hz: C-29; 0.923 *d*, J = 6.5 Hz: C-21; 1.009 *s*: C-19). Three peaks were observed in GLC of sterol extracts from *Casuarina cunninghamiana* roots and nodules. In each case the data for TMSi derivatives were identical to those recorded previously by us [1, 5] for respectively 24-methylcholesterol, 24-ethyl-5,*E*22-cholestadien-3β-ol and 24-ethycholesterol. Quantitative data are listed in Table 1 for the four species examined.

DISCUSSION

It is generally assumed that where 24-ethylcholesterol occurs in nature, it is the (24*R*)-isomer (sitosterol) in higher plants and the (24*S*)-isomer (clionasterol) in lower plants and micro-organisms [6]. If this correlation is generally true, then the presence of the (24*R*)-isomer sitosterol in both roots and nodules of *A. glutinosa* can be taken as a strong indication that the probable source of sterols in both roots and nodules is the host plant. This is in agreement with our earlier work for *V. faba* where no sterol could be detected in pure cultures of the endophyte *Rhizobium leguminosarum*. Currently, our attempts to