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COMPOSITION OF THE TRITERPENE ALCOHOL FRACTION OF HORSE CHESTNUT SEED

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Abstract—The triterpene alcohol fraction was isolated from the seed oil of horse chestnut; it amounted to 42.2% of the unsaponifiable matter, i.e. 0.93% of the oil. The components were identified as taraxerol, β-amyrin, butyrospermol, parkeol, 5α-tirucalla-8,23-dien-3β-ol, α-amyrin and 24-methylenecycloartenol. Butyrospermol and 5α-tirucalla-8,23-dien-3β-ol were the major components and amounted to 60% of the fraction. The latter has not been identified in any vegetable oils yet.

INTRODUCTION

In a recent paper we described the separation of the unsaponifiable matters of horse chestnut seed into six fractions: Δ^7-sterols, Δ^5-sterols, 4-methylsterols, triterpene alcohols, tocopherols and hydrocarbons, and the compositions of the Δ^5- and Δ^7-sterol fractions were determined [1]. The present paper describes the identification of seven triterpene alcohols of which one is a new plant sterol. The distribution of these sterols in horse chestnut seed was also determined. Triterpene alcohols which have been found in horse chestnut seed are butyrospermol, taraxerol and fridelin, as was reported elsewhere [2].

RESULTS AND DISCUSSION

Triterpene alcohols were extracted and separated from horse chestnut seed [1] and then analysed by gas chromatography and by GC/MS. GLC analysis on a glass capillary column demonstrated the presence of at least 19 components in this fraction, while the analysis of the TMSi derivatives enabled the detection of two more components, **5** and **10** (Table 1).

On the basis of GLC and mass spectral evidence, compounds **6**, **8**, **10** and **13** were identified as β-amyrin, parkeol, α-amyrin and 24-methylenecycloartanol [3–6]. A discussion of their mass spectral data was given in one of our earlier papers [3]. The mass spectrum of **4** was characterized by the ions at m/z 302 (4%, $[M-C_9H_{16}-Me]^+$) and m/z 269 (11%, $[M-C_9H_{16}-Me-H_2O]^+$), which was formed by retro-Diels–Alder fragmentation of ring D, as well as by the ion at m/z 189 (24%, $[M-C_{15}H_{24}-Me-H_2O]^+$), which arose by ring C fragmentation [6]. On the basis of the mass spectral ions, component **4** was identified as taraxerol.

Components **7** and **9** could not be identified on the basis of their mass spectra alone and they were separated in pure form and their ^{1}H NMR spectra were recorded. The separation was performed by acetylating the triterpene alcohol fraction (acetic anhydride–pyridine) and the resulting acetate fraction was further fractionated by $AgNO_3$–silica gel TLC. Band one (R_f 0.20) contained only the acetate of **9** and band two (R_f 0.68) the acetate of **7**. The mass spectrum of **9** indicated the molecular formula $C_{30}H_{50}O$ for this triterpene alcohol. The presence of ions at m/z 313 (5%, $[M-SC-2H]^+$) and m/z 259 (20%, $[M-SC-C_3H_6-CH_2]^+$) showed that this triterpene alcohol contained a monounsaturated side chain [7] and also a monounsaturated skeleton with an additional C-32 methyl group [8]. The ^{1}H NMR spectrum of the unknown alcohol showed only one olefinic proton (δ5.54), which indicated that one of the two double bonds was tetrasubstituted. It must be situated in the steroid skeleton because the only possible position of the tetrasubstituted double bond in the side chain ($\Delta^{17(20)}$) was easily excluded on the basis of the absence of a signal in the vinyl methyl group region (δ1.5–1.7). This fact aided considerably the determination of the position of the double bond in the side chain. The absence of a vinyl methyl group signal in the ^{1}H NMR spectrum of **9** excluded unambiguously the Δ^{24}-, Δ^{25}- and $\Delta^{20(22)}$-positions for the double bond [9]. As the absence of a signal for methylene protons excluded the $\Delta^{20(21)}$-position, the double bond position came down to a choice between the Δ^{22}- and Δ^{23}-positions. Signals due to two allylic methyl groups (26,27-methyls) [10, 11], which appeared at δ0.99 and 1.09, indicated the Δ^{23}-position of the double bond. This position of the double bond was strongly supported by the mass spectrum of **9**, because there were no ions characteristic of the Δ^{22}-bond [7]. The presence of an axial proton at C-3 with a broad multiplet (δ4.48) confirmed the 3β-configuration of the hydroxyl group [12].

The ^{1}H NMR spectrum of the unknown alcohol showed five tertiary methyl groups which must be localized in the steroid skeleton with singlets at δ0.83 (3H), 0.88 (3H), 0.90 (3H), 0.92 (3H) and 0.99 (3H). The chemical shifts of these methyl groups were close to those found for 5α-tirucalla-8,24-dienol (Table 2) [13]. This fact may be correcily interpreted by regarding the steroid skeleton of the unknown alcohol as identical to that of the steroid

Table 1. Composition of the triterpene alcohol fraction of horse chestnut seed

Component No.	Methylene indices	Content (%) of triterpene fraction	Content (%) of USM*	Content (mg/100 g) in oil	Content (mg/100 g) in seed	Name
1	31.64	1.9	0.8	17.6	0.5	Unidentified
2	31.85	tr	tr	tr	tr	Unidentified
3	32.28	tr	tr	tr	tr	Unidentified
4	32.41	12.5	5.3	116.0	3.5	Taraxerol
5	—	tr	tr	tr	tr	β-Amyrin
6	32.65	tr	tr	tr	tr	Unidentified
7	32.72	20.8	7.8	193.1	5.8	Butyrospermol
8	32.96	6.5	2.8	60.3	1.8	Parkeol
9	33.20	39.9	16.8	370.4	11.1	5α-Tirucalla-8,23-dien-3β-ol
10	—	tr	tr	tr	tr	α-Amyrin
11	38.41	1.4	0.6	13.0	0.4	Unidentified
12	33.55	tr	tr	tr	tr	Unidentified
13	33.65	3.8	1.6	35.3	1.1	24-Methylene-cycloartenol
14	33.72	1.4	0.6	13.0	0.4	Unidentified
15	33.79	1.1	0.5	10.2	0.3	Unidentified
16	34.00	3.9	1.6	36.2	1.1	Unidentified
17	34.29	tr	tr	tr	tr	Unidentified
18	34.45	5.4	2.3	50.1	1.5	Unidentified
19	34.65	tr	tr	tr	tr	Unidentified
20	34.84	tr	tr	tr	tr	Unidentified
21	35.16	tr	tr	tr	tr	Unidentified

*USM = Unsaponifiable matter; tr ⩽ 1% of the triterpene fraction.

Table 2. ^{1}H NMR chemical shifts* of proton signals of 5α-eupha and 5α-tirucalla steryl acetates

Compound	Methyl groups 18	19	30	31	32	21	26	27	3β-OAc	3α-CH	Others
Eupha-8,24-dienol	0.76	0.96	0.88	0.88	0.88	0.85	1.62	1.69	2.05	4.50 *m*	5.08 *t* (24-CH)
Eupha-7,24-dienol	0.82	0.78	0.96	0.85	1.00	—	1.62	1.70	2.05	4.56 *m*	5.10 *m* (24-CH) 5.21 *m* (7-CH)
Tirucalla-8,24-dienol	0.76	0.99	0.81	0.89	0.89	0.96	1.62	1.69	2.05	4.48 *m*	5.11 *m* (24-CH)
Tirucalla-7,24-dienol	0.82	0.79	0.94	0.87	0.99	0.93	1.62	1.70	2.05	4.54 *m*	5.10 *m* (24-CH) 5.25 *m* (7-CH)
7	0.83	0.79	0.97	0.87	0.99	0.91	1.62	1.71	2.07	4.53 *m*	5.10 *m* (24-CH) 5.25 *m* (7-CH)
9	0.83	0.99	0.92	0.88	0.90	0.92	0.99	1.09	2.07	4.48 *m*	5.54 *m* (23-CH) (24-CH)

*Chemical shifts in δ (ppm) in $CDCl_3$ with TMS as internal standard.

skeleton of a Δ^8-alcohol of the euphane–tirucallane series. The fact that the double bond in the steroid skeleton must be tetrasubstituted strongly supports the proof already provided for its Δ^8-position.

The mass spectrum of 7, as in the case of 9, indicated the molecular formula $C_{30}H_{50}O$ for this triterpene alcohol. The presence of ions at *m/z* 313 (7%, $[M-SC-2H]^+$) and *m/z* 259 (14%, $[M-SC-C_3H_6-CH_2]^+$) suggested that this unknown alcohol possessed a monounsaturated side chain [7] and also a monounsaturated steroid skeleton with an additional C-32 methyl group [8]. The ^{1}H NMR spectrum of the unknown alcohol showed five tertiary methyl groups which must be localized in the steroid skeleton with singlets at δ0.83 (3H), 0.79 (3H), 0.97

(3H), 0.87 (3H) and 0.99 (3H). The chemical shifts of these methyl singlets lay close to those for 5α-eupha-7,24-dienol (Table 2). This fact may be correctly interpreted by regarding the steroid skeleton of the unknown alcohol as identical to that of the steroid skeleton of a Δ^7-alcohol of the euphane–tirucallane series. The ^{1}H NMR spectrum of 7 showed two olefinic protons (δ5.10 and 5.25) of which one (δ5.10) must be on the isopropyledene group of the side chain, because the vinyl methyl group signals (26,27-methyls) were recorded in the spectrum at δ1.62 and 1.71 [13]. The intensity of an ion at m/z 69 (100%, $[C_5H_9]^+$) in the mass spectrum of 7, which arose from allylic cleavage of the C(22)–C(23) bond, strongly supported the presence of the double bond at C-24 and the presence of the isopropyledene group in the side chain of 7 [14]. The presence of an axial proton at C-3 which gave a broad multiplet (δ4.53) indicated the 3β-configuration of the hydroxyl group [12]. Therefore, the structure of a 7,24-dienol of the euphane–tirucallane series can be ascribed to sterol 7 and the structure of an 8,23-dienol of the euphane–tirucallane series can be given to sterol 9. Distinction of C-20 epimers (5α-euphane/5α-tirucallane) is not possible on the basis of mass spectral and ^{1}H NMR evidence, so it is necessary to analyse their GLC retention data [13]. Thus, 7 showed an identical mobility on TLC (R_f 0.68) and GLC (MU = 32.72) to that of the standard sample of butyrospermol. Compound 7 was therefore identified as butyrospermol, i.e. 5α-eupha-7,24-dien-3β-ol. If the sterol 9 had belonged to the euphane series, it would, considering the distribution of double bonds, have eluted from GLC before 7 [13]. However, as 9 had a retention time (MU = 33.20) considerably greater than 7 (MU = 32.77), it is obvious that it belonged to the tirucallane series and it can be identified as 5α-tirucalla-8,23-dien-3β-ol.

Table 1 shows the qualitative and quantitative composition of the triterpene alcohol fraction of horse chestnut seed. This fraction consists of 42.2% of the unsaponifiable matter, i.e. *ca* 0.93% of the oil and 0.028% of the seed. It contains at least 21 components, of which seven have been identified. The triterpene alcohols from the euphane/tirucallane series amounted to 60% of the fraction; β-amyrin was present only in traces, although it is the basis of escine, the major triterpene glycoside of horse chestnut seed.

EXPERIMENTAL

The unsaponifiable matter of horse chestnut seed oil was separated by TLC on silica gel (0.5 mm), as described in our previous paper [1]. The purified triterpene fraction was acetylated by Ac_2O–pyridine and the resulting acetate fraction was further fractionated by $AgNO_3$–silica gel (1:7) TLC (0.5 mm); the acetates of 7 and 9 were obtained in pure form.

GLC was performed with a glass capillary column (30 m × 0.3 mm) coated with OV-101 liquid phase and programmed to 2°/min from 160° to 300° with H_2 as the carrier gas (0.75 kg/m^2) and a flame ionization detector (FID) (H_2, 0.5 kg/m^2; air, 1.4 kg/m^2). The retention indices were determined by applying *n*-alkanes (C_{18}–C_{36}). GC/MS analysis was performed on a Varian 3700 gas chromatograph–mass spectrometer (MAT 312) combination equipped with a computer system (MAT 200) and at an ionization voltage of 70 eV. The operating conditions of the gas chromatogram were the same as those in the case of performing only GLC analysis. ^{1}H NMR spectra were determined at 250 MHz in $CDCl_3$ with TMS as the internal standard. The chemical shifts are given in ppm and the coupling constants in Hz.

Triterpene alcohol identification. Butyrospermol (7): MS m/z (assignment, rel. int.): 426 $[M]^+$ (22), 411 $[M-Me]^+$ (84), 393 $[M-Me-H_2O]^+$ (38), 313 $[M-SC-2H]^+$ (7), 259 $[M-SC-C_3H_6-CH_2]^+$ (14), 69 $[C_5H_9]^+$ (100).

Butyrospermyl-3β-acetate (7a): ^{1}H NMR (250 MHz, $CDCl_3$): δ0.83 (3H, *s*), 0.79 (3H, *s*), 0.97 (3H, *s*), 0.87 (3H, *s*), 0.99 (3H, *s*), 0.91 (3H, *d*, J = 6, 5), 1.62 (3H, *s*), 1.71 (3H, *s*), 2.07 (3H, *s*), 4.53 (H, *m*), 5.10 (H, *m*), 5.25 (H, *m*).

5α-Tirucalla-8,23-dien-3β-ol (9): MS m/z (assignment, rel. int.): 426 $[M]^+$ (32), 411 $[M-Me]^+$ (100), 393 $[M-Me-H_2O]^+$ (48), 313 $[M-SC-2H]^+$ (5), 259 $[M-SC-C_3H_6-CH_2]^+$ (20), 69 $[C_5H_9]^+$ (58).

5α-Tirucalla-8,23-dienyl-3β-acetate (9a): ^{1}H NMR (250 MHz, $CDCl_3$): δ0.83 (2H, *s*), 0.99 (3H, *s*), 0.92 (3H, *s*), 0.88 (3H, *s*), 0.90 (3H, *s*), 0.92 (3H, *d*, J = 6, 5 Hz), 0.99 (3H, *d*, J = 6, 8 Hz), 1.09 (3H, *d*, J = 6, 8 Hz), 2.07 (3H, *s*), 4.48 (H, *m*), 5.54 (2H, *m*)

Nomenclature. β-Amyrin = 5α-olean-12-en-3β-ol; α-amyrin = 5α-ursan-12-en-3β-ol; parkeol = 5α-lanosta-9(11)-24-dien-3β-ol; 24-methylenecycloartanol = 24-methylene-9,19-cyclo-5α-lanostan-3β-ol; butyrospermol = 5α-eupha-7,24-dien-3β-ol; taraxerol = 5α-taraxeren-14-en-3β-ol.

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