(2H, *m*, H-1, 3), 2 12 (OAc), 2 × 1 05, 2 × 0 92 (CMe) ^{13}C NMR δ174 2*s*, 170 6*s*, 169 6*s*, 145 6*s*, 142 8*d*, 140 8*d*, 120 8*s*, 112 0*t*, 109 8*d*, 81 8*s*, 80 4*d*, 77 9*d*, 75 5*d*, 68 3*d*, 51 9*d*, 51 2*q*, 45 5*s*, 41 3*s*, 40 3*s*, 35 3*d*, 33 5*t*, 32 1*t*, 29 6*t*, 27 7*q*, 23 8*t*, 21 4*q*, 21 2*q*, 20 9*q*, 14 1*q*

Oxidation with Jones' reagent gave the corresponding ketone in the ^{1}H NMR spectrum of which H-1 and H-2 appeared as doublets δ5 60 (*d*, H-2, J = 4 Hz), 3 70 (*d*, H-1) Acetylation with pyridine–Ac_2O produced no change

Another fraction gave E P 2, not obtained quite pure (^{1}H NMR identical with the spectrum of a partially synthetic sample [6]), while a third gave E P 3, apparently pure but not crystalline [^{1}H NMR δ7 42, 7 36 (H-21, 23), 6 38 (H-22), 5 39 (H-17), 5 29, 5 13 (2H-30), 4 64 (H-15), 3 69 (CO_2Me), 1 04, 0 97, 0 91, 0 86 (4 × CMe)]

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COMPOSITION OF THE STEROL FRACTION IN HORSE CHESTNUT

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Key Word Index—*Aesculus hippocastanum*, Hippocastanaceae, sterols

Abstract—The Δ^7- and Δ^5-sterol fractions were isolated from the unsaponifiable matter of ripe, air dried, chestnut seed The Δ^7-sterol fraction amounted to 18 4% and the Δ^5-sterol fraction amounted to 8 2% of the unsaponifiable matter In the Δ^7-sterol fraction three components were identified as Δ^7-campesterol, α-spinasterol and Δ^7-stigmastenol One component was probably $\Delta^{5,7}$-stigmastadienol and five components remained unidentified Δ^7-stigmastenol and α-spinasterol were the major components and amounted to 74 8% of the fraction In the Δ^5-sterol fraction at least ten components were found Five of them were identified as cholesterol, campesterol, stigmasterol, sitosterol and Δ^4-stigmasten-3-one Stigmasterol and sitosterol amounted to 73 6% of the fraction

One of the first important results obtained in the investigation of the constitution of horse chestnut was the isolation of escine present in the mixture of acylated triterpene glycosides of the β-amirin type [1, 2] Flavonoid glycosides were identified later [3–6]. Fiedler and Hildebrand [7] isolated adenine, adenosine, guanine and uric acid Damm [8] identified filoguinone in horse chestnut seed and Haenel [9] identified vitamin B complex, methionine and holine Windaus and Bock [10] first isolated a mixture of sterols in which they identified provitamin D in less than 2% of the mixture, and Allan and coworkers [11] succeeded in isolating α-spinasterol, butyrospermol, fridelin, taraxasterol and triacontane The unsaponifiable constituents of horse chestnut oil remained largely unidentified, and although they constitute only a very small part of the oil (2–3%), they comprise of sterols, 4-methylsterols, triterpenes and aliphatic alcohols, vitamins, hydrocarbons, pigments, etc Recently the composition of the sterol fractions of some vegetable oils have been analysed Δ^5-Sterols, such a stigmasterol, campesterol, sitosterol and Δ^5-avenasterol were found to be the major components of most vegetable oils, while in the Δ^7-sterol fractions α-spinasterol and Δ^7-stigmastenol mostly predominated [1–6] In the present study the sterol fraction obtained from a ripe seed dried in air was investigated by GC/MS The Δ^7-sterol fraction amounted to 17% of the unsaponifiable matter which is 0 01% of the dry weight of the seed On the basis of methylene indices

Sterol nomenclature used in this paper cholesterol = cholest-5-en-3β-ol, campesterol = (24*R*)-24-methylcholest-5-en-3β-ol, stigmasterol = (24*S*)-24-ethylcholest-5,22-dien-3β-ol, Δ^7-campesterol = (24*R*)-24-methylcholest-7-en-3β-ol, sitosterol = (24*R*)-24-ethylcholest-5-en-3β-ol, α-spinasterol = (24*S*)-24-ethylcholest-7,22-dien-3β-ol, $\Delta^{5,7}$-stigmastadienol = (24*R*)-24-ethylcholest-5,7-dien-3β-ol, Δ^4-stigmasten-3-one = (24*R*)-ethylcholest-4-en-3-one, Δ^7-stigmastenol = (24*R*)-24-ethylcholest-7-en-3β-ol

and mass spectra of the free sterols and their TMSi-derivatives three components were identified as Δ^7-campesterol, α-spinasterol and Δ^7-stigmastenol [12–16] Five components remained unidentified On the basis of the mass spectrum of the TMSi-derivative one component was identified as $\Delta^{5,7}$-stigmastadienol Qualitative and quantitative compositions of the Δ^7-sterol fraction are given in Table 1

The Δ^5-sterol fraction amounted to 6 5% of the unsaponifiable matter which is 0 004% of the seed At least ten components were found by GLC On the basis of methylene indices and mass spectra of the free sterols and their TMSi-derivatives five of them were identified as cholesterol, campesterol, stigmasterol, sitosterol and Δ^4-stigmasten-3-one [17, 18] The last compound is possibly produced by enzymatic oxidation [19, 20] The action of cholesterol oxidase on 3β-hydroxy-Δ^5-steroids yields, for example, Δ^4-3-keto steroids [21] The composition of the Δ^5-sterol fraction is given in Table 2

EXPERIMENTAL

Plant material Horse chestnut originated from the district of Leskovac (Yugoslavia) Ripe seed was obtained from horse chestnut which had been picked in the second half of September It was dried in air until the moisture content had decreased to less than 10%

Oil extraction Horse chestnut seed was extracted twice with 30 ml petrol under reflux for 30 min The seeds were filtered off, the solvent removed by distillation and the residue dried for 24 hr in a desiccator over conc H_2SO_4

Saponification The oil (100 g) in 1 l alcoholic 1 0 N KOH were refluxed for 1 hr under N_2 The reaction mixture was diluted with 2 l of H_2O and the unsaponifiables were extracted with one 1 l portion and three 800 ml portions of Et_2O The Et_2O extracts were combined, washed ×3 with H_2O and dried over dry Na_2SO_4, and the Et_2O removed by evaporation under N_2

Thin-layer chromatography The unsaponifiables were fractionated on alkaline modified silica gel G (30 g silica gel + 65 ml 0 2 N KOH) developed with a hexane–Et_2O (1 1) for 1 hr The plate was sprayed with 0 2% 2,7-dichlorofluorescein soln in EtOH, and the bands were observed under UV light Six zones were marked under UV light (254 nm) and then scraped off, and quantitatively extracted with Et_2O The R_f values for the zones were as follows Δ^7-sterols R_f = 0 16, Δ^5-sterols R_f = 0 23, 4-monomethylsterols R_f = 0 35, 4,4-dimethylsterols R_f = 0 42, tocopherols R_f = 0 58 and hydrocarbons R_f = 0 98

Gas-liquid chromatography GLC was on a glass capillary column (30 m × 0 3 mm ID) coated with OV-101, programmed at 2°/min from 160 to 300°, with H_2 as the carrier gas, and with a

Table 1 Composition of the Δ^7-sterol fraction of horse chestnut

GC peak no	Compound	MW	Methylene index	Percentage composition of the fraction
1	Δ^7-Campesterol	400	31, 76	0 9
2	Unidentified	414	31, 88	1 2
3	Unidentified	—	—	tr
4	α-Spinasterol	412	32, 28	26 4
5	$\Delta^{5,7}$-Stigmastadienol	412	32, 59	tr
6	Unidentified	—	—	—
7	Δ^7-Stigmastenol	412	32, 90	65 4
8	Unidentified	414	33, 03	5 3
9	Unidentified	—	33, 28	0 7

tr, Trace

Table 2 Composition of the Δ^5-sterol fraction of horse chestnut

GC peak no	Compound	MW	Methylene index	Percentage composition of the fraction
1	Cholesterol	386	30, 37	tr
2	Unidentified	—	30, 83	tr
3	Campesterol	400	31, 41	2 0
4	Stigmasterol	412	31, 75	18 7
5	Unidentified	414	32, 14	4 6
6	Sitosterol	414	32, 37	59 9
7	Unidentified	—	32, 54	0 2
8	Unidentified	414	32, 61	0 2
9	Δ^4-Stigmasten-3-one	412	32, 89	3 3
10	Unidentified	426	34, 56	9 9

tr, Trace

flame ionization detector Peak area percentages of each chromatogram were calculated by approximating the total area to 100%, by the use of an electronic integrator (Spectra Physics Auto Lab System) Retention indices were determined using *n*-alkanes (C_{26}–C_{36}) at an initial temp of 160° programmed at 2°/min The sterols were analysed as free alcohols and TMSi-derivatives TMSi ethers were prepared in the usual manner

Combined gas chromatography mass spectrometry The analyses were performed on a Varian 3 700 gas chromatograph–mass spectrometer (MAT 312) combination, equipped with a MAT 200 computer system

Authentic materials A sterol fraction consisting of campesterol, stigmasterol and sitosterol was supplied by the Riken Vitamin Oil Co, Tokyo, Japan Cholesterol was supplied by Fluka AG, Buch SG, and brassicasterol was supplied by Prof T Matsumoto (College of Science and Technology, Nihon University, Japan)

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QUAESITOL, A PHENOL FROM *GARCINIA QUAESITA*

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Key Word Index—*Garcinia quaesita*, Guttiferae, bark, hermonionic acid, quaesitol, α-spinasterol

Abstract—The petrol extractives of the bark of *Garcinia quaesita* gave hermonionic acid, its decarboxylated product and a new phenol, quaesitol

Bark extractives of *Garcinia quaesita* were chemically investigated by us [1] and we reported the isolation of hermonionic acid (**1**) and its decarboxylated product (**2**) We now report the characterisation of the most polar pigment, quaesitol (**3**), isolated from the hot petrol extractives of *G quaesita*

Quaesitol (**3**) had a molecular formula of $C_{33}H_{44}O_5$ (high resolution mass spectrum) The ^{1}H NMR spectrum showed the presence of the following signals (a) δ1 75, 1 65 and 1 55 (seven methyl groups of the type Me–C=), (b) 1 90 (two pairs of allylic protons), (c) 3 10–3 40 (six benzylic protons of the type $ArCH_2CH$=), (d) 3 70 (one methoxyl group), (e) 4 98–5 20 (four olefinic protons), (f) 5 90 (two aromatic protons) and (g) 6 30 (one aromatic proton) These data indicate the presence of two isoprenyl and one geranyl side chains as in **1** Acetylation of **3** with acetic anhydride–pyridine gave a triacetate showing the presence of three phenolic hydroxyl groups The mass fragmentation of **3** (Fig 1) showed significant fragments at *m/z* 465, 464, 397, 396, 327 and 175 The fragments at *m/z* 327 for **3** and for **1** isolated from the same source shows that both have identical ring A substitution The methoxyl group, the geranyl and isoprenyl chains of quaesitol should therefore be oriented in ring A The

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