Table 1. Tyramine content of *Magnolia* species

Species	Collection time	Plant part	Tyramine content (mg/100 g)
M. denudata	mid September	Leaves	8·0*
M. liliiflora	mid September	Leaves	2·5*
M. liliiflora	late May	Buds	7·8†
M. obovata	late May	Leaves	2·8*

* Method A: content per 100 g of plant parts was calculated based on the weight of crystalline tyramine monohydrochloride isolated.

† Method B: calculated from amino acid analyser.

Investigation of leaves, buds and flowers of 5 *Magnolia* species revealed the presence of tyramine as a common component besides several of the normal protein amino acids. This unusual occurrence of tyramine, known as an important adrenergic drug [1, 2], may be responsible for the pharmacological action of the oriental folk medicines mentioned above. The tyramine content of the plants examined are shown in Table 1. In addition, qualitative tests on TLC of aqueous extracts from flowers and leaves of *M. kobus* and leaves of *M. grandiflora* also showed the presence of tyramine in appreciable amounts.

It was shown that tyramine was not formed by enzymatic decarboxylation of tyrosine during the isolation procedure, since the amounts of the amine did not vary after either immediate boiling the fresh leaves of *M. denudata* or keeping the homogenate at room temperature for several hours.

The identity of the isolated sample with authentic tyramine monohydrochloride was confirmed by elemental analysis, amino acid analysis (15 cm column, 0·24 N borate buffer of pH 11·3; retention time; 29 min), IR, UV and NMR spectra.

EXPERIMENTAL

Isolation of tyramine. The fr. leaves (500 g) of *M. obovata* collected in the end of May were macerated and extracted 2× with boiling H_2O. The combined filtrate (3 l.) was chromatographed on an Amberlite CG-50 (NH_4^+ form) column 3 × 50 cm. The eluate with 2 N-NH_4OH (0·6 l.) was evaporated *in vacuo*. Repetition of the column chromatography with gradient elution of NH_3 gave pure tyramine which was crystallized from EtOH as mono HCl-ide (14 mg) as leaflets, mp 245–255° (dec.). The same method was used for the other 2 species. IR, ν 3180, 1620, 1600 cm^{-1}; UV, $\lambda_{max}^{EtOH-H_2O}$ 282, 276, 223, 198 nm (ϵ 1420, 1670, 7990, 2770); NMR (D_2O) 100 MHz, δ7·2 (4H, *q*, aromatic proton), 3·2 (4H, A_2B_2 type). Anal. found: C, 54·90, H, 6·96; N, 7·95; Cl, 20·26. Calcd for $C_8H_{12}ONCl$: C, 55·34; H, 6·97; N, 8·07; Cl, 20·42%.

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α-SPINASTEROL GLUCOSIDE AND OTHER CONSTITUENTS OF *MAESA CHISIA**

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Key Word Index—*Maesa chisia*; Myrsinaceae; fatty acids; fatty alcohols; long-chain ketones; α-spinasterol; α-spinasterol-β-D-glucoside; stigmasta-8(14),22-dien-3β-ol; β-amyrin.

Plant. Maesa chisia D. Don. *Source.* Sub-Himalayan region. The specimen is available in the herbarium of Central Drug Research Institute. Lucknow. *Uses.* Insecticide [1]. *Previous work.* Nil.

Present work. Leaves and branchlets. Light petrol extracts on chromatography yielded (a) a mixture of long chain methyl ketones, mp 76–77° (IR 1720 cm^{-1}, positive DNP test) with C-31 and

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C-33 predominating (MS); (b) a mixture of fatty alcohols, mp 87–88°, with C-30 and C-32 predominating (IR, GC, MS); (c) mixture of fatty acids, mp 79–80°, with C-24 and C-26 predominating (IR, MS); (d) α-spinasterol and (e) β-amyrin.

From the *alcoholic extract* were isolated α-spinasterol, β-amyrin and α-spinasterol-β-D-glucoside (**1**) [2], $C_{35}H_{58}O_6$ (M^+ 574), mp 282–283°; *tetraacetate* (**2**), mp 157–158°. The MS of both the free glycoside and its tetraacetate showed fairly stable molecular ions, the spectrum of the acetate distinctly showing characteristic fragmentation of Δ^{22} steroids and hexopyranoside tetraacetate.

Acid hydrolysis of **1** yielded glucose (identified by PC) and a mixture of 2 steroids resolved as acetates through argentized Si gel chromatography. One of them was identified as α-spinasterol. The other one responded to Tortelli–Jaffé test for a tetrasubstituted nuclear double bond and could also be obtained in good yield by acid catalysed isomerization [3] of α-spinasterol. It was thus identified as stigmasta-8(14),22-dien-3β-ol (**3**) [4], in excellent agreement with all the spectral data. It was, however, proved to be an artefact formed during acid hydrolysis, since the NMR spectrum of **2** showed only one set of methyl signals corresponding to that of α-spinasterol.

Incidentally, the reported [5] positive Tortelli–Jaffé test by α-spinasterol obtained *via* acid hydrolysis of its glycoside must have been due to the presence of the $\Delta^{8(14)}$ isomer (**3**) as the pure Δ^7 sterols have since been shown [6] to be irresponsive to such a colour reaction, an observation we confirm. Again, the NMR spectrum (60 MHz) of **2** showed the signal for C-5 sugar proton at δ 3·62, considerably upfield from that of methyl-α-D-glucose tetraacetate [7]. The sugar linkage was thus assigned the β-configuration since the H-5 signal in β-glucosides is known [8] to be shifted upfield from that in the α-anomers and that it remains unaffected [9] by the nature of the aglycone.

EXPERIMENTAL

α-Spinasterol-β-D-glucoside (**1**). Needles from C_6H_6–EtOH, mp 282–283°; $[\alpha]_D^{C_5H_5N}$ −35° (*c* 0·4); ν_{max}^{Nujol} 3200 (OH), 970 () cm^{-1}; MS: *m/e* (rel. intensity) 574 (M^+ 1·3), 559 (0·3), 556 (0·1), 531 (0·3), 433 (4), 412 (16), 394 (40), 379 (10), 369 (6), 351 (12), 300 (10), 299 (9), 273 (15), 271 (43), 255 (65), 253 (32) and 55 (100). (Found: C, 72·0; H, 10·4. Calc. for $C_{35}H_{58}O_6$: C, 73·1; H, 10·1%).

α-Spinasterol-β-D-glucoside tetraacetate (**2**). Prepared from **1** with Ac_2O–C_5H_5N and crystallized from MeOH, mp 157–158°. ν_{max}^{Nujol} 1750 and 1230 (OAc), 970 ( ) cm^{-1}; MS: *m/e* (rel. intensity) 742 (M^+, 0·6) 725 (0·3), 699 (0·02), 682 (0·1), 601 (0·2), 412 (2·5), 394 (70), 379 (8), 331 (55), 271 (25), 255 (48), 253 (27), 229 (12), 211 (16), 169 (100), 115 (20), 109 (60); NMR: (δ in $CDCl_3$) 0·55 (18-Me), 0·80 (19-Me), 1·05 *d* (21-Me), 0·7–1·00 (25-Me, 26-Me, 29-Me), 2·02, 2·05, 2·09 (4 × OAc), 3·62 *m* (H-5′ and H-3), 4·20 *m* (H_2-6′) 4·4–5·4 *m* (H-1′, H-2′, H-3′, H-4′ and H-7, H-22, H-23).

Stigmasta-8(14),22-dien-3β-ol (**3**). Obtained by acid hydrolysis (2N H_2SO_4, EtOH, reflux) of **1** or by acid rearrangement (2N H_2SO_4, EtOH, reflux) of α-spinasterol. Separated from α-spinasterol by chromatography of the acetate over $AgNO_3$ (15%) impregnated Sil gel. Crystallized from MeOH, mp 162–163°; $[\alpha]_D^{CHCl_3}$ −56° (*c* 0·25); ν_{max}^{Nujol} 3200 (OH). MS: *m/e* (rel. intensity) 412 (M^+, 70), 397 (8), 394 (6), 383 (1·5), 379 (5), 369 (14), 351 (18), 327 (3), 314 (7), 300 (28), 273 (21), 272 (22), 271 (40), 255 (45), 229 (15), 213 (16) and 55 (100).

Stigmasta-8(14),22-dien-3β-yl acetate (**4**). Crystallized from MeOH, mp 136–137 °; $[\alpha]_D^{HCl_3}$ (−42° (*c* 0·8); ν_{max}^{Nujol} 1730, 1250 (OAc) 970 ( ) cm^{-1}. MS: *m/e* (rel. intensity) 454 (M^+ 8), 439 (2), 411 (2), 394 (79), 379 (8), 351 (12), 342 (2), 313 (25), 282 (12), 273 (4), 255 (62), 228 (12), 213 (16). NMR: (δ in $CDCl_3$) 0·78 (18-Me), 0·91 (19-Me) 1·08 *d* (21-Me) 0·86–1·08 *m* (25-Me, 26-Me, 29-Me), 2·08 (OAc), 4·73 *m* (H-3), 5·30 *m* (H-22, H-23). (Found: C, 82·20; H, 11·34. Calc. for $C_{31}H_{50}O_2$: C, 81·93; H, 11·01%).

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