corresponding to severn *tert.* C-methyl groups. TLC, GLC and IR revealed that unknown 2 is very similar to oleanolic acid and the MS data of the monoacetate showed exactly the same fragments as those of oleanolic acid monoacetate. Furthermore, the NMR spectra showed that one proton of unknown 2 at δ3.44 ppm was shifted to the lower field at δ4.68 ppm (t, J = 8 Hz) after acetylation. However, the compound (mp 247–249°) was not identical with any known 3-monohydroxy compounds, e.g. oleanolic acid (mp 305–310°) or 3-epi-oleanolic acid (mp 298°). A dimorphic form of oleanolic acid was not proved in this case.

The IR spectrum of the third unknown indicated that it is a tetracyclic triterpene and with mp 133–134°; it is probably tirucallol (mp 133–134.5[8]) but its identity could not be confirmed owing to the unavailability of authentic sample.

EXPERIMENTAL

The wood sample was collected near Cairns, Queensland. Ground heartwood (3.5 kg) was extd with MeOH (3 days) and the extract concentrated in vacuum. A crystalline ppt. (32 g) was collected, recrystallized from hot MeOH and identified as ellagic acid by direct comparison with an authentic sample [9]. The ether soluble neutrals (14 g) were obtained from the MeOH filtrate after washing with 5% $NaHCO_3$ and sat. Na_2CO_3 solutions successively. The Et_2O soluble neutrals (3.7 g) were chromatographed on thick TLC (Si gel GF_{254}, 0.75 mm thickness) plates with EtOAc–$CHCl_3$–HCOOH, (2:10.1) to give 5 opaque bands with R_f values of 0.20 (fraction 1), 0.29 (2), 0.38 (3), 0.63 (4) and 0.72 (5). After repeated crystallization, finally, fraction 1 (unknown **1**; 115 mg), 2 (maslinic acid; 82 mg), 4 (unknown **2**; 113 mg) and (probably tirucallol; 20 mg) were obtained.

Acknowledgements—The author thanks Dr. W. E. Hillis and Dr. J. A. Lamberton for their invaluable comments on this work, Dr. J. A. Lamberton and Dr. F. E. King for samples of oleanolic and arjunolic acids, Mr. N. K. Hart for $[\alpha]_D$ determination, Mr. R. I. Willing for the NMR spectra and Dr. J. A. Wunderlich for mass spectra. Analyses were done by the Australian Microanalytical Service.

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Phytochemistry, 1977, Vol. 16, pp. 139–140. Pergamon Press. Printed in England.

STEROLS AND TRITERPENES OF *ILEX AQUIFOLIUM*

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(*Received* 2 *August* 1976)

Key Word Index—*Ilex aquifolium*; sterols; triterpenes; 24-ethylcholesterol.

Abstract—Sterols of male and female flowers from *Ilex aquifolium* were found to be mainly 24-ethylcholesterol, with trace amounts of cholesterol, 24-methylcholesterol and 24-ethyl-5,22-cholestadien-3β-ol. Several pentacyclic triterpenes were partially characterized.

INTRODUCTION

The involvement of sterols in the flowering process has been inferred from work by Bonner *et al.* [1] and Biswas *et al.* [2]. To our knowledge no study of the sterols of flowers placing emphasis on sexual differentiation has been recorded. *Ilex aquifolium* (holly) is a dioecious species and we report here on the sterols of flowers and leaves of male (staminate flowers) and female (carpellate flowers) trees of this species.

RESULTS

GLC data on sterol-triterpene fractions from holly harvested from two locations are summarized in Table 1. Identification of sterols was by comparison of GLC and MS data with those from authentic sterols and with literature data [3]. The principal sterol in all cases was 24-ethylcholesterol (80% of all the sterol in all cases examined). This compound is presumed to be sitosterol although no evidence for the stereochemistry at C-24 is presented. With the same reservations, the other sterols were identified as stigmasterol (10%), campesterol (4%) and cholesterol (trace). Only in male flowers could 24-ethylidenecholesterol be detected (by GLC and MS), in all other cases peaks at this point in the chromatograms appeared by MS to be triterpenes of the oleanane type (*m/e* 218 as base peak) [4].

Leaves also presented similar chromatograms from both types of tree but in these cases a considerable amount of triterpene (presumably oleanane skeletons, as all had base peaks in their mass spectra at *m/e* 218) was present overlapping on TLC with the sterol fraction.

DISCUSSION

No consistent differences in sterol-triterpene composition between flowers or leaves from male or female trees could be noted except possibly for the presence of 24-ethylidenecholesterol in the first harvest of male flowers. This could represent newly synthesized sterol not yet fully reduced to sitosterol or else could be associated with anthesis, since compounds with unsaturated side-chains occur frequently in large amounts in pollen [5].

Table 1. Sterols and triterpenes from holly flowers and leaves

Peak	r*	M^+(m/e)	Structural assignment	% of total fraction‡ Kilbarchan flowers ♂30	Kilbarchan flowers ♂3	Kilbarchan flowers ♀3	Kilbarchan leaves ♂3	Kilbarchan leaves ♀3	Garscube leaves ♂	Garscube leaves ♀
1		458	cholesterol	tr			tr	4.2	—	2.2
2	4.2	472	24-methylcholesterol	1.5	1.3	3.9	1.1	—	tr	—
3	4.5	484	24-ethyl-5,22-cholestadien-3β-ol	3.2	5.5	11.3	3.9	2.8	1.2	1.4
4	5.05	(a) 486 (b) 484	24-ethylcholesterol 5,25-diene?	62.6	79.1	84.8	56.3	61.8	29.9	27.4
5	5.86	(a) 486 (b) 484	24-ethylcholesterol† 24-ethylidenecholcsterol¶	8.9	tr	—	4.7	9.0	1.7	4.5
6	6.0	498	pentacyclic triterpene§ (amyrin type)	16.2	6.3	tr	29.7	18.8	8.6	23.4
7	6.6	498	pentacyclic triterpene§ (amyrin type)	1.35	2.4	—	tr	tr	7.7	7.6
8	7.3	496	pentacyclic triterpene§ (amyrin type)	6.25	5.4	—	5.3	3.4	50.8	33.5

* $C_{28}H_{58}$ = 1.0. † Carried over into mass spectrometer from previous peak. ‡ By triangulation, tr = trace, —not detected. ¶ In leaves this peak appears to largely consist of triterpenoid material. § Usually a broad peak (2 or more isomeric compounds).

EXPERIMENTAL

Holly was collected from 3 sources, a male tree, Tandlehill Road, Kilbarchan (30th May 1976: ♂30) National Grid Reference NS 409623, and trees in the garden of Riversdale, Tandlehill Road, Kilbarchan, National Grid Reference NS 409623 (3rd June 1976: ♂, ♀3) and the Garscube Estate, Glasgow (8th June 1976), National Grid Reference NS 551704. Leaf material was air-dried (90°) and crushed, whereas flowers were stored in MeOH. Extraction ($CHCl_3$–MeOH), sterol isolation (TLC on Si gel $CHCl_3$) and GLC (of TMS ethers on OV-17) were by standard methods. GC–MS was performed on a V.G. Micromass 16F GC–MS (jet separator) with an OV-17 column. Peaks 6 and 7 both exhibited molecular ions at *m/e* 498 with base peak *m/e* 218 and prominent ions at *m/e* 203, 190, 189. Peak 8 had M^+ 496, and base peak *m/e* 216.

Acknowledgements—We thank Professor W. A. Harland for permission to use the GC–MS and Dr J. Oliver for the analyses.

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Phytochemistry, 1977, Vol. 16, pp 140–141. Pergamon Press. Printed in England

STEROLS OF LILIACEAE

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(*Received* 23 *July* 1976)

Key Word Index—*Cordyline indivisa; Allium cepa; A. fistulosum* var. *caespitosum*; Liliaceae; sterols.

Cordyline indivisa Kunth, an ornamental plant, was collected on the campus of Tokyo Gakugei University in November 1974. There has been no previous work on the sterols of this plant.

Preparative Si gel TLC [1] of the unsaponifiable matter (2.20 g) of the lipid (89 g), Soxhlet extracted by CH_2Cl_2 from dried and ground seeds (616 g), gave 4,4-dimethyl- (110 mg), 4-monomethyl- (210 mg) and 4-desmethyl- (530 mg) sterol fractions. The following sterols were tentatively identified by GLC (OV-17) [2] and GC–MS: cycloartanol (approximately 44%), cycloartenol (33%), and 24-methylenecycloartanol (3%) in the 4,4-dimethylsterol fraction [3,4]; 31-norlanostenol and 4α-methylzymostenol (unresolved by GLC, 13%) [5,6], lophenol (56%) [5,6], 31-norcycloartenol (14%) [5,7], gramisterol (24-methylenelophenol) [8] and cycloeucalenol [9] (unresolved, 2%), and citrostadienol (1%) [8] in the 4-monomethylsterol fraction; and cholesterol (13%), cholest-7-enol (12%), campesterol (9%), stigmasterol (17%), sitosterol (49%), and 28-isofucosterol (trace) in the 4-desmethylsterol fraction [10]. The 4-desmethylsterol fraction was further resolved as its acetate by preparative $AgNO_3$–Si gel TLC. Traces of cholestanol (M^+, *m/e* 430), campestanol (M^+, *m/e* 444), stigmastanol (M^+, *m/e* 458) and an unidentified sterol (M^+, *m/e* 442) also were found as their acetates in the fraction from the least polar faint zone.

Allium cepa L. (onion) has previously been reported to contain cholesterol, brassicasterol, campesterol, stigmasterol and sitosterol in the bulbs [11]. In the present work preparative Si gel TLC of the unsaponifiable matter (1.35 g) of the lipid (5.2 g), extracted from the milled and dried bulbs (337 g) by CH_2Cl_2, afforded 4,4-dimethyl- (210 mg), 4-monomethyl- (230 mg) and 4-desmethyl-