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THE STEROLS OF STRAWBERRY FRUIT

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Key Word Index—*Fragaria ananassa*; Rosaceae; strawberry; silylation; sitosterol; campesterol; isofucosterol; phytosterols

Abstract—The present paper describes silylation of a plant sterol mixture directly on the silica gel after purification by thin layer chromatography The procedure was applied to sterols extracted from strawberry fruits Sitosterol and an unknown, tentatively identified as isofucosterol, were the main free sterols in the strawberry fruits, with lesser amounts of campesterol and traces of cholesterol and stigmasterol The isofucosterol represented up to 40% of the free sterols of the fruits and accounted for 3% of the free sterols in the leaves

INTRODUCTION

Free plant sterols are often analysed by the method of Grunwald [1], which includes digitonin precipitation and derivatization before determination by GLC They can also be determined simultaneously with other lipid fractions after separation of a total lipid extract on a silicic acid column or by TLC followed by elution and derivatization of the dry sample [2, 3] This procedure involves the risk of selective elution and losses during transfer and is time-consuming The present paper describes the silylation of sterols directly on the silica gel after TLC The procedure was applied to the analysis of strawberry fruit sterols and showed sitosterol (>50%) and an unknown, tentatively identified as isofucosterol (up to 40%), as the main sterols in this material

RESULTS AND DISCUSSION

Evaluation of sterol silylation directly on the silica gel

Comparison of the silylation of the plant sterol mixture on the silica gel with silylation after elution and silylation of the original mixture showed complete recovery of the sterols by all three procedures (Table 1). The composition of the sterol mixture was not affected Silylation of sterols directly on the silica gel after TLC is therefore a reliable and time-saving procedure

Analysis of strawberry sterols

Silylation directly on the silica gel yielded as much sterol as silylation after elution from the silica gel (Table 2) Analysis of the sterols without TLC purification (procedure *c*) was unsatisfactory Only three sterols were detected in significant amounts sitosterol, an unknown, and campesterol, traces of cholesterol and stigmasterol were also detected The proportions of the three main sterols were the same following the two analytical procedures

Tentative identification of the unknown sterol by GC-MS

The identity of campesterol [(24*R*)-ergost-5-en-3β-ol] and sitosterol (stigmast-5-en-3β-ol), established tentatively by comparing their retention time with that of authentic standards, was confirmed by mass spectrometry The unknown sterol which amounted to 10 to 40% of the free sterols of the strawberry fruit, was tentatively identified by its mass spectrum as fucosterol [stigmasta-5,24(28)*E*-dien-3β-ol] or isofucosterol [stigmasta-5,24(28)*Z*-dien-3β-ol] The probability based match (PBM) [4] with the library mass spectrum was 93% However, fucosterol is typical for brown algae, while isofucosterol is normally found in higher plants [5] In the absence of an isofucosterol standard, co-chromatography of authentic fucosterol with reference sitosterol and with strawberry fruit sterols showed that fucosterol had the same retention time as sitosterol, but that it did not co-chromatograph with the unknown The stereo-

Table 1 Effect of silylating a plant sterol mixture after TLC (*a*) directly on the silica gel, (*b*) after elution from the gel, or (*c*) without TLC, on the composition* and the amounts† of the sterols recovered

Sterols	Silylation procedure (*a*)	(*b*)	(*c*)
Cholesterol	1 5±0 1*	1 6±0 1	1 7±0 1
Brassicasterol	13 4±0 2	13 1±0 5	12 3±0 3
Campesterol	23 6±0 3	23 8±1 0	23 6±0 5
Stigmasterol	7 2±0 1	7 7±0 1	7 2±0 2
Sitosterol	53 6±0 8	52 1±2 7	53 6±1 2
Total	1 77±0 03†	1 72±0 08	1 68±0 04

*% of total sterols in the plant sterol mixture
†mg

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Table 2 Effect of the silylation procedures (*a*) and (*b*) (see Table 1) on the composition* and the amounts† of sterols in the strawberry fruit

Sterols	Silylation procedures (*a*)	(*b*)
Campesterol	1 8±0 6*	2 4±0 8
Sitosterol	57 4±1 5	58 3±1 4
Unknown	40 7±1 2	39 2±1 9
Total	4 76±0 13†	4 71±0 10

*% of total free sterols
†mg/100 g fresh weight

chemistry (*Z* or *E*) of the $\Delta^{24(28)}$-double bond cannot easily be determined by mass spectrometry [6], therefore it is assumed that the unknown sterol is likely isofucosterol or a closely related compound

To our knowledge, the sterols of strawberry fruit have not been analysed previously The sterols of the strawberry leaves have been examined by O'Neill *et al.* [7] by GLC on a packed column Sitosterol, cholesterol, campesterol and stigmasterol were detected and the sitosterol represented 95% of the total free sterols In our samples of strawberry fruit sitosterol and the tentatively identified isofucosterol together accounted for 93% of the free sterols The sterol mixture from the leaves contained 3% of isofucosterol. The use of a capillary column with a greater resolution than the packed column used by O'Neill *et al* [7] allowed the separation of the isofucosterol from sitosterol with retention times of 22 39 and 22.63 min, respectively. Mukherjee *et al.* [8] analysed the sterols in whole plants of *Fragaria indica* and also identified sitosterol as the main sterol.

EXPERIMENTAL

Material. For sterol analysis strawberries (*Fragaria* × *ananassa*) were bought at a local supermarket and intact healthy fruits were selected Strawberry (cv Kent) fruits and leaves were harvested locally for comparing sterols from these tissues. The plant sterol mixture used for the development of the method was from Supelco. The internal standard cholestane and the reference sterols were obtained from Sigma TLC was performed on 250 μm silica gel plates (silica gel G, Fisher Scientific, Québec, Qc) Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was purchased from Supelco

Strawberry fruit and leaf lipid extraction. The lipids were extracted by the method of ref [9] as described previously [10]

Preparation of the plant sterol mixture for GLC Three procedures were compared (*a*) The sterols were chromatographed on thin layer plates in hexane–Et_2O–HOAc (80 20 1) The sterol band was detected with I_2 vapour, using sitosterol as a reference. The sterols were scraped off the plate and silylated directly on the silica gel in BSTFA-dimethylformamide (1 6) (*b*) The sterols were eluted from the silica gel with 20 ml $CHCl_3$–MeOH–H_2O (10·5 1) before derivatization (*c*) A 1 75 mg sample of the original plant sterol mixture was silylated without previous TLC For each procedure cholestane (10 μg/2 9 g fr wt) was added as an internal standard prior to derivatization, to the silica gel scraped from the plate for procedure (*a*) and (*b*), and to the sterol mixture for (*c*) The trimethylsilyl derivatives were separated by GLC on a 12 m high performance methyl silicone cross-linked capillary column (Hewlett-Packard) The temp program included holding at 175° for 2 min, increasing the temp to 250° at 5° per min, then increasing to 275° at 2° per min and holding for 5 min Injector and detector (FID) temps were held at 310 and 325°, respectively. The split ratio was 50 1 and the linear velocity was 50 cm/sec The peaks were identified by comparison of the R_ts with authentic standards The peak areas were determined with a 3392A Hewlett-Packard integrator Three injections were made for each replication The results are means of three replications ± s d The efficiency of silylation of strawberry sterols on the silica gel was compared with the yield from a similar sample which had been eluted from the gel

Peak identification by GC-MS The MS detector used was a hyperbolic quadrupole mass filter (Hewlett-Packard model 5970) It was coupled to the GC described above The identity of the sterols was established by the Probability Based Matching (PBM) algorithm of ref [4]

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