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SHORT COMMUNICATION

THE SQUALENE CONTENT OF PLANT TISSUES

R W. LEWIS

CSIRO Division of Food Research, Box 52, Ryde, New South Wales 2113, Australia

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Abstract—A method for determining squalene gave adequate recoveries when tested on a concentration similar to that of plants A preliminary survey of ten fruits and vegetables showed squalene contents ranging generally from 0 11 ppm to 2 5 ppm *

INTRODUCTION

DESPITE the widespread occurrence of squalene in nature as a precursor in the biosynthesis of sterols, there is very little information on the squalene content of plant tissues Except for analyses of vegetable oils, there appear to be only three reports of the squalene content of plant tissues and the results from these were given as approximations. Assorted plant leaves contained squalene within the range of 0·5–1 ppm,[1] tomato fruits and seeds contained an estimated 1 ppm,[2] and the content of horse-chestnut leaves was likewise estimated to range between 2–32 mμmoles/g tissue (0 8–13 ppm) depending on age.[3]

A previous study[4] has shown that an analytical method which used an internal standard and a combination of TLC and GLC gave a recovery of 85% on a concentration of 5 ppm squalene This method was used in a further study of the squalene content of animal tissues [5] Because of the limited and approximate data from plants, the present investigation was undertaken to test the method on smaller concentrations of squalene (< 1 ppm) and, if it proved satisfactory, to conduct a preliminary survey of the squalene content of some common plant materials.

RESULTS AND DISCUSSIONS

Testing the Method

Recoveries of squalene from a sample of hydrogenated tallow known to contain 52·8 ppm squalene (relative to lipid weight) were 49·8, 42 3 and 45 3 ppm from three GLC analyses of the same hydrocarbon fraction obtained by preparative TLC. These values give an average of 45·8 ppm for a recovery of 86 7% and a coefficient of variance of 8·2% The squalene content of the tallow can be converted into a hypothetical tissue content for comparison with the values from plant materials by using the average lipid content (0 45%) of all the plant materials except the avocado The calculation gives a value of 0 24 ppm

* Unless otherwise specified, ppm will be relative to tissue weight

[1] S Q ALAM, J BROSSARD and G MACKINNEY, *Nature, Lond* **194**, 479 (1962)
[2] M YAMAMOTO and G MACKINNEY, *Nature, Lond* **213**, 799 (1967)
[3] A R WELLBURN and F W HEMMING, *Phytochem* **5**, 969 (1966)
[4] R W LEWIS, *Nature, Lond* **224**, 1220 (1969)
[5] R W LEWIS, *Int J Biochem* in press

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squalene relative to tissue Only three of the plant tissues had lower concentrations of squalene.

In the tests of reproducibility, duplicate lipids from eggplant and green bananas (obtained by dividing the lipid extract into two portions) were analysed independently The squalene contents were found to be 2 5 and 2 3 ppm and 0 159 and 0 168 ppm, respectively, giving coefficients of variance of 5·4% and 3 7%. These variations are less than the 8 2% coefficient of variance found in the three analyses of hydrogenated tallow and are about the best that was achieved with the present method. However, repeated TLC and GLC analyses of other lipids showed coefficients of variance ranging from 3% to 10%. Thus it may be assumed that the results from this study represent recoveries of 87% and coefficients of variance of up to 10% On this basis, a true tissue concentration of 0 5 ppm would yield an average value of 0·43 ppm from a range of 0 39 ppm to 0 47 ppm. Since the concentrations of squalene found in the plant tissues of this study cover a much wider range, it appears that they are valid findings and not the result of experimental error

Preliminary Survey

The first GLC analyses of plant material revealed that hydrocarbons from cuticular waxes obscured the squalene peak and may have contributed to the internal standard peak Thus analysis by this method in the presence of cuticular wax was impossible However, plant materials which did not appear to possess cuticular wax (carrot root and immature green peas), or from which it could be removed by solvents (eggplant, apple, squash) or peeling (banana, avocado) presented no problems Of the ten fruits and vegetables analysed, six had chromatographic traces which showed only minor differences from similar traces from the analysis of animal tissues Four (green and ripe bananas, apple and squash) showed a small hydrocarbon leading the squalene peak but not contributing to it in such a way as to complicate the calculations of peak area

The results are listed in Table 1 and show that except for the avocado, which had 44 4 ppm, most of the materials contained between 0 11 ppm and 2 5 ppm squalene These values generally agree with those from two previous studies which reported a range of 0 5–1 ppm for *Acanthus* leaves, carrot leaves and roots, elderbery, and lettuce[1] and approx 1 ppm

TABLE 1 SQUALENE AND LIPID CONTENT OF FRUITS AND VEGETABLES

Material	Per cent lipid (w/w)	Squalene	
		% in lipid	ppm
Avocado (*Persea gratissima*)	26 7	0 0166	44 4
Carrot root (*Daucus carota*)	0 19	0 050	0 96
Apple (*Malus communis*)	0 34	0 018	0 62
Eggplant (*Solanum melongena*)	0 63	0 0396	2 5
duplicate	0 63	0 0363	2 3
Squash flesh (*Cucurbita pepo*)	0 099	0 0158	0 15
Squash seeds	0 65	0 0129	0 78
Green banana (*Musa sapientum*)	0 22	0 0075	0 159
duplicate	0 22	0 0077	0 168
Ripe banana	0 168	0 0067	0 113
Green peas, frozen (*Pisum sativum*)	0 87	0 0142	1 24
Mushroom (*Agaricus campestris*)	0 86	0 0035	0 30

for tomato fruit and seeds.[2] The third report gave values extending from 0 8 ppm to 13 ppm (recalculated) for horse-chestnut leaves [3]

In seeking reasons for the differences in squalene concentrations found in plants, it is immediately apparent that lipid content is a major factor For example, the avocado had by far the largest squalene content, 44 4 ppm, but this was due almost entirely to the high lipid content (27%), for the per cent squalene in its lipid was nearly in the middle of the range found in the other plant materials In contrast, squash had about the same percentage of squalene in its lipid, but reached only 0 15 ppm because of its extremely low lipid content, 0 099% Green peas had nearly the same per cent squalene in their lipids, but eight times more lipid than squash, thus accounting almost exactly for the increase in squalene content to 1·24 ppm

However, lipid content is not the only factor, for the percentages of squalene in lipid also differ considerably. Two of the materials, carrot root and eggplant, contained up to 2·5 times more squalene in their lipids than average, whereas others, such as mushroom and bananas, had much less No explanations can be offered for these differences at the present time.

EXPERIMENTAL

Materials In view of the exploratory nature of this study, it was deemed unnecessary to harvest the materials freshly, most of the fruits and vegetables were purchased from local markets The green bananas were of the 'Dwarf Cavendish' variety grown in northern New South Wales and were judged to be half mature, they had been removed from the tree several days previously The skins of the ripe bananas were largely brown and the flesh was turning soft The squash ('custard marrow' variety) had a diameter of 18 cm The eggplant was 15 cm long and the middle third was taken for analysis The mushrooms had caps up to 4 cm in diameter and appeared to be no more than half mature The avocado (Haas variety) was in the early stages of edible ripeness, only the flesh was analysed The seeds of the apple (Jonathan variety) were excluded from analysis

Analytical procedures The eggplant, apple and squash are covered by a relatively impermeable skin which permitted them to be rinsed in $CHCl_3$–MeOH (2 1, v/v) to remove cuticular wax and any contaminating sebum which may have come from handling The carrot was washed and the green peas were analysed without prior treatment, neither showed significant amounts of cuticular hydrocarbons The avocado, bananas, and mushrooms were peeled The weighed tissues were homogenized in $CHCl_3$–MeOH (2 1, v/v) and filtered The extract was washed in 0 2 vol of 0 2 N NaCl and the $CHCl_3$ layer was dried (Na_2SO_4) before being taken to dryness The lipid residues were subjected to high vacuum prior to weighing

Thereafter, the analysis followed the method previously described [4,5] This consisted in the addition of an internal standard, *n*-tetracosane, to the lipid to comprise 0 25% of the lipid weight The hydrocarbon fraction was then separated by preparative TLC on 350 μ layers of Silica Gel G (Merck) in a solvent system of hexane–Et_2O–HOAc (95 3 1, by vol) The hydrocarbon fraction was eluted from the silica gel, concentrated, and analysed by GLC using a 156 cm stainless steel column containing 3% APL on 100/120 mesh Aeropak operated at a temp of 260° and a N_2 flow of 60 ml/min Squalene was identified by co-chromatography with authentic squalene Peak areas were calculated by peak height times width at half height The area of the squalene peak could be related to the amount of lipid and tissue through the internal standard, permitting calculation of the squalene content in ppm relative to tissue weight

This method had previously given a recovery of 85% from a tissue containing a known squalene concentration of 5 ppm In order to test this method at less than 1 ppm, squalene was added to a squalene-free sample of hydrogenated tallow (I value of 0 5) to give a concentration of 52 8 ppm *relative to lipid weight* As mentioned earlier, this would be the equivalent of approximately 0 24 ppm relative to plant tissue weight.

Key Word Index—Angiosperms, Fungi, squalene, quantitative determination