A Facile Synthesis of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone [2- (or 5-) methyl ^{14}C] (Furaneol [2- (or 5-) methyl ^{14}C])

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SUMMARY

A convenient, one-step synthesis of ^{14}C-labelled 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (furaneol) was accomplished by a piperidine acetate catalyzed Maillard-type reaction of commercially available fucose L-[1-^{14}C]. This important aroma compound found in strawberry fruits, obtained in ≥ 90 % radiochemical purity, should prove useful in *in-vivo* metabolism studies.

Key Words: Synthesis, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, furaneol, carbon-14, aroma

INTRODUCTION

Recently, interest has developed in the biosynthesis and metabolism of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (1), an important flavour constituent of many fruits such as strawberry, pineapple, tomato and mango (1) as well as processed food (2). Due to its pleasant caramel-like taste and low odour threshold (3) it is produced on industrial scale and widely used as flavouring agent for food and beverages. Although 1 is a naturally occurring compound it is difficult to isolate it in large amounts, and synthetic 1 is generally employed.

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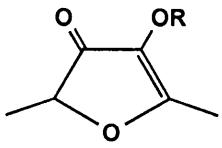
However, the use of synthetic products as 'nature-identical' substances in food is not always well accepted by consumers, so that the biotechnological production of 1 is of economic interest. Such a procedure implies detailed knowledge of its biosynthesis and metabolic transformations to its methoxy derivative 2 (4), glucoside 3 (5), malonylated glucoside 4 (6), and glucuronide 5 (7)



1 R = H
2 R = CH_3
3 R = ß-D-glucopyranose
4 R = 6-O-malonyl ß-D-glucopyranose
5 R = ß-D-glucuronic acid

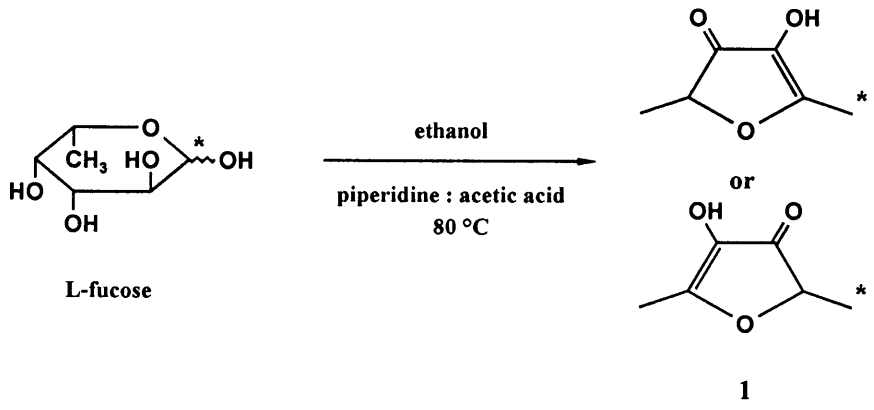
In toxicological investigations 1 showed mutagenicity to a *Salmonella typhimurium* TA100 strain (8). In other studies however, 1 inhibited benzo[a]pyrene-induced forestomach neoplasia in mice, indicating that 1 could act as a potent anticarcinogen (9).

The ^{14}C-labeling of 1 at the methyl carbon at position 2 or 5 of the furanone ring provides the opportunity to investigate the metabolism of 1 *in-vivo*, in order to gain more information about the biosynthesis of 2-4 in plant tissues and to assess the bioactivity of 1 for man. The recent commercial availability of fucose L-[1-^{14}C] has permitted the straightforward preparation of ^{14}C-labeled 1.

RESULTS AND DISCUSSION

A method for the preparation of 1 was initially developed following the procedure of Wong et al (10) using unlabelled L-fucose. L-fucose was converted into 1 in ca. 40 % yield, by a Maillard-type reaction using a mixture of piperidine and acetic acid (6:10 by weight) as catalyst in absolute ethanol. Traces of water reduced the yields of 1 significantly (data not shown). The identity of 1 was confirmed by its spectral data and retention time obtained by means of high performance liquid chromatography-diode array detection (HPLC-DAD) and

capillary gas chromatography-mass spectrometry (GC-MS) analysis and by comparison with those of the commercially available unlabelled compound 1.



L-fucose **1**

Reaction scale down to eventually employ fucose L-[1-^{14}C] was performed using unlabelled L-fucose in a 1 ml-vial at 80°C, in the dark. Periodic sampling of the reaction mixture by HPLC-UV detection at 284 nm revealed the time course of formation of 1 (Figure 1).

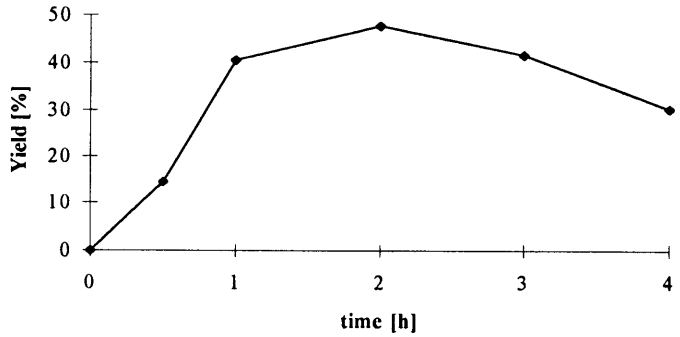


Figure 1. Time course of the formation of 1 in the Maillard-type reaction of L-fucose with piperidine:acetic acid (6:10 by weight) as catalyst.

Maximum yield of 1 was obtained after ca. 2 hours. Following, the yield decreased gradually due to the already known instability of 1 (11). Purification of 1 was achieved by HPLC separation using a RP18 column and subsequent solid phase extraction with diethyl ether elution. The yields of 1 obtained after each purification step are shown in Table 1.

Table 1. Chemical (a) and radiochemical yield (b) of 1 in percentage after each purification step following the conversion of labelled or unlabelled L-fucose by Maillard type reaction, respectively. (n.d. not determined).

	L-fucose	
	unlabelled[a]	labelled[b]
reaction mixture	47	n.d.
after HPLC separation	43	28
after SPE	32	10

When using ^{14}C-labelled L-fucose the yield of labelled 1, obtained by HPLC-radiodetection and liquid scintillation counting, was lower compared with the values achieved with unlabelled L-fucose (Table 1). The work-up provided ^{14}C-1 with a radiochemical purity of ≥ 90% by HPLC- and TLC-radiodetection. Due to keto-enol tautomerism (12), 1 carries the ^{14}C-label at the methyl group at carbon 2 or 5 of the furanone ring (13). Compound ^{14}C-1 was even more unstable than unlabelled 1, since radiochemical purity of ^{14}C-1 dropped from 95 % to 30 % within 3 days of storage in aqueous solution at -20°C. It has been already reported that 1 is unstable in air and in aqueous solutions and sensitive towards photooxidation (14).

Summarizing, a facile, one-step procedure for the preparation of ^{14}C-1 by a Maillard-type reaction of L-fucose was described. This aroma compound should prove useful for *in-vivo* metabolism studies.

EXPERIMENTAL

HPLC-DAD UV spectra were recorded with a Hewlett Packard photodiode detector 1040A connected to a HPLC system composed of two Knauer 64 pumps, Knauer programmer 50, Rheodyne injector 7125 and an Europher 100-C18 column 250 x 40 mm, 5 µm. The profiling HPLC gradient was conducted in two linear steps at a flow rate of 1 ml/min, utilizing acetonitrile and 0.05 % formic acid. The gradient proceeded from 8% to 40% acetonitrile in 10 min followed by 40% to 90% acetonitrile in 2 min and remained at 90% acetonitrile for an additional 3 min. GC-MS analysis was performed by a Varian gas chromatograph using a fused

silica WCOT column (30 m x 0.25 mm, df = 0.25 μm) coated with Carbowax 20M. The column was programmed from 50 °C, 3 min isothermal, then at 4 °C/min to 240 °C and held for 5 min; carrier gas He 2 ml/min. EIMS was determined by a MAT 44 at 70 eV, scanning from m/z 41 to 499 with total ion monitoring. TLC was performed on 0.2 mm silica gel 60 F_{254} plastic plates from Schleicher & Schuell and diethyl ether:methanol 95:5 as developing solution. Plates were scanned by a Berthold TLC radiodetector Linear Analyzer with Ar:CH_4 90:10 as counting gas at 1381 V. HPLC-radiodetection was conducted on a Waters system equipped with two model 510 pumps, gradient controller, Rheodyne injector 7125, an Europher 100-C18 column 250 x 40 mm, 5 μm , UV detector SOMA UV-vis S-3702 operated at 284 nm, and a Canberra Packard A100 Flow-one/beta radiodetector. The same gradient elution as mentioned above was employed. ^{14}C-labelled compounds were recorded by means of a 300 μl YtSi solid scintillator cell. Aliquots of liquid samples were added to 10 ml of scintillation cocktail Emulsifier-Safe (Canberra-Packard). All measurements of ^{14}C were carried out by means of a liquid scintillation counter LKB Rackbeta 1214 after decaying of the chemiluminescence counts.

Fucose L-[1-^{14}C] was obtained from Biotrend, reference 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, piperidine, acetic acid from Aldrich and L-fucose from Fluka.

Synthesis of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (1). In each of six 1 ml vials a solution consisting of 60 μg of L-fucose dissolved in 30 μl abs. ethanol was added to a solution of 8.4 μg of piperidine and 14 μg of acetic acid in 70 μl of abs. ethanol. The solutions were covered with argon. The vials were closed and heated to 80 °C, in the dark. After defined time periods, as indicated in Figure 1, vials were cooled to room temperature and aliquots were analyzed by HPLC-UV detection at 284 nm and HPLC-DAD. R_t of 1: 6.5 min, UV max at 284 nm. The fraction eluting at 6.5 min was collected, extracted with diethyl ether and analyzed by GC-MS: R_i of 1: 1995, m/z (%) 57 (100), 128 (M^+, 69), 85 (30), 55 (26), 56 (14), 72 (11), 84 (5), 58 (5). Amounts of 1 were calculated by means of a calibration curve.

Isolation and purification of 1 was achieved as follows: In an additional experiment the reaction mixture was fractionated by 3 independent HPLC separations, the pooled fractions

eluting at 6.5 min were diluted by a factor 2 with water and subjected to solid phase extraction using a RP18 cartridge (Supelco, 100 mg). After washing of the cartridge with 1 ml of water, 1 was eluted by 3 ml of diethyl ether and analyzed by GC-MS.

Synthesis of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone [2- (or 5-) methyl ^{14}C] (^{14}C-1). In a 1 ml vial a solution consisting of 60 µg of fucose L-[1-^{14}C] (20 µCi, 55 mCi/mmol) dissolved in 30 µl abs. ethanol was added to a solution of 8.4 µg of piperidine and 14 µg of acetic acid in 70 µl of abs. ethanol. The solution was covered with argon. The vial was closed and heated to 80 °C in the dark. After 2 hours the vial was cooled to room temperature and the reaction mixture fractionated by 3 independent HPLC separations. Solid phase extraction of the pooled fractions containing 1 followed by diethyl ether elution yielded ^{14}C-labelled 1, exhibiting a radiochemical purity of ≥ 90% by HPLC- and TLC-radiodetection.

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