mixture of sterols was chromatographed on silica gel–$AgNO_3$ (hexane–Et_2O, 7:3) and gave **5M** (6 mg) whereas hexane–Et_2O (3:2) gave a mixture of four sterols which were separated through reversed phase argentation HPLC (Lichrosorb RP-18, 7 μm, 250 mm × 10 mm i.d. MeOH–H_2O, 17:3, 0.1 N $AgNO_3$). **7M** (11 mg) had MS *m/z* (rel. int.): 412 (50), 397 (27), 379 (10), 328 (13), 285 (100), 269 (30), 245 (10), 241 (15), 227 (23). **4N** (8 mg) had MS *m/z* (rel. int.): 412 (42), 397 (100), 379 (12), 328 (15), 285 (65).

Hydrogenation of **7M** and **4N**. Pure samples of **7M** (6 mg) and **4N** (5 mg) dissolved in EtOAc were treated with H_2 over PtO_2 for 2 hr to give the monounsaturated sterols **5M** and 4α,24*S*-dimethyl-5α-cholest-8(14)en-3β-ol.

Acknowledgement—This work was supported by M.P.I. (Ministero della Pubblica Istruzione).

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Phytochemistry, Vol. 28, No. 2, pp. 631–633, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

2,5-DIMETHYL-4-HYDROXY-3(2*H*)-FURANONE GLUCOSIDE: ISOLATION FROM STRAWBERRIES AND SYNTHESIS

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(*Received in revised form* 15 *July* 1988)

Key Word Index—*Fragaria ananassa*; Rosaceae; glucoside; 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone glucoside.

Abstract—2,5-Dimethyl-4-hydroxy-3-(2*H*)furanone β-glucoside has been isolated from strawberry juice and synthesized. Both the natural and synthetic material exist as a mixture of diastereoisomers.

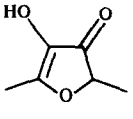
INTRODUCTION

For more than 20 years Furaneol® [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, **1**] has been known as an important aroma compound of many fruits, notably pineapples [1] and strawberries [2], in which it occurs to the extent of 2.2–7.4 ppm, depending on the source and length of time of storage [3]. It seemed likely that this compound could occur in the plant as a glycoside, and this paper presents the successful outcome of a search for Furaneol glycoside in strawberries.

RESULTS AND DISCUSSION

Recently a reverse-phase HPLC method was described for use in the quantification of Furaneol in pineapple and grapefruit juice [4], although the peak assigned to Furaneol was not checked spectroscopically by isolation. We based our search for Furaneol glucoside (**2**) in strawberry juice on a similar method.

The juice was prepared by macerating commercial strawberries with a small amount of water followed by centrifugation. The supernatant was subjected to a series of membrane filtrations, using decreasing pore sizes (if fine-bore membranes are used immediately, they become rapidly clogged). A final lyophilization yielded 21.6 g of solid residue from 725 g of strawberries. This material



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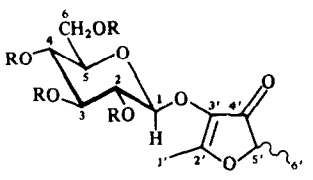

2 R = H
3 R = Ac

*The ^1H NMR values, particularly for the protons H-6 and H-1 are close to those of phenolic β-glucosides [5].

was then purified by fractionation over reverse-phase silica gel on a medium pressure liquid chromatography column. Finally the material was purified by preparative HPLC, allowing isolation of 67 mg of somewhat impure glycoside (**2**). A second preparative HPLC purification step gave 49 mg of material which was still impure, and a final separation on an Aminex column enabled 27 mg of pure glycoside to be isolated, with $[\alpha]_{589}^{20} - 50°$. The spectra (NMR, FAB/MS, and UV) were identical with synthetic material prepared as follows.

Furaneol glucoside tetraacetate (**3**) was prepared from a solution of a slight excess of Furaneol in ethanolic potassium hydroxide at 80° and acetobromoglucose in toluene. For this reaction to be successful, it is imperative that it be carried out rapidly (Furaneol decomposes in alkaline solution). Even so, yields are not good, although *ca* 7–10% of the tetraacetate (**3**) could be obtained as a mixture of diastereoisomers. The spectra were entirely consistent with the structure shown. The acetoxy groups were removed by hydrolysis with a catalytic amount of sodium hydroxide in methanol. The Furaneol glucoside prepared in this way contained glucose and free Furaneol, and was purified by preparative reverse-phase chromatography. Two further impurities were seen on TLC of the eluted fractions. These gave the same ^{1}H NMR spectrum in solution, and were identified as α- and β-glucopyranose 6-acetate.

The synthetic glucoside had $[\alpha]_{589}^{20} - 62.8°$, an additional proof that the natural material is also of the D-series.

NMR spectra

The synthetic Furaneol glucoside tetraacetate (**3**) (measured in $CDCl_3$ with tetramethylsilane as shift reference) had the following ^{1}H NMR signals corresponding to methyl groups: δ 1.435 and 1.450 (each *d*, appearing as a *t*), 2.015, 2.03, 2.07, 2.11 and 2.12, and 2.20 (each *s*). After chromatography on silica gel, a fraction was obtained where the ^{1}H NMR signal at δ 1.435 was much more important than that at 1.45; likewise that at 2.12 was now more important than that at 2.11. This was interpreted as showing that the two high-field doublets corresponded to the C-5′ methyl signals of the two epimers of the tetraacetate, and the singlets at δ 2.11 and 2.12 corresponded to the C-2′ methyl signals. The other singlets were the signals of the acetoxy groups in the sugar moiety, these being very similar in both epimers. The remaining signals were as follows: δ 3.73 (1H, *br*, H-5), 4.15 (1H, *dd*, $J = 13$ Hz, H-6), 4.22 (1H, *m*, H-6), 4.44 and 4.45 (each *q*, $J = 7$ Hz, after silica gel chromatography the second became more important, H-5′), 3H are in a *m*, *ca* 5.07–5.15, and 1H in a *t* (with further coupling) at 5.24. This means that the glycoside linkage must be β-, because otherwise there should be a doublet at lower field.* This assignment is supported by the spectra of the glucoside (**2**). The ^{1}H NMR spectrum was measured in D_2O with sodium trimethylsilyltetradeuteriopropionate as shift reference: δ 1.46 (*d*, Me-C-5′), 2.35 (*s*, Me-C-2′), 4.7–4.8 (H-5′). The latter signal overlaps with the residual HOD signal, preventing observation of the expected quartet. Indirect proof of the assignment was made by irradiating the multiplet at 4.7–4.8, when the doublet at 1.46 became a singlet. Compared with free Furaneol (**1**) in D_2O, the signals of both methyl groups appear at slightly lower field in the glucoside. The D_2O solution of the glucoside (**2**) was kept at room temperature, and further ^{1}H NMR spectra were measured after 24 hr and 5 days. No deuterium incorporation had occurred after 24 hr; after 5 days, the C-5′ doublet appeared slightly unsymmetrical, with one of its branches overlapping with the singlet of the DC-5′ methyl group. Furaneol incorporates deuterium at C-5′ and both methyl groups in less than 2 hr at 75° and pH 8.1 (F. Mazenod Firmenich SA, personal communication).

The ^{13}C NMR spectrum clearly showed that Furaneol glucoside was a mixture of diastereoisomers. Since the ^{13}C NMR spectra of both synthetic glucoside and that isolated from strawberry juice are identical, natural Furaneol glucoside occurs as two epimers, differing in the configuration at C-5′: 16.57 (*q*, CH_3–C-2′), 18.08 and 18.17 (*q*, CH_3–C-5′), 84.72 (*d*, C-5′), 136.08 (*s*, C-3′), 188.70 (*s*, C-2′), 203.30 (*s*, C-4′), 63.17 (*t*, C-6), 71.98 (*d*, C-2), 75.78 (*d*, C-4), 78.25[a] (*d*, C-3), 78.91[a] (*d*, C-5), 106.00 (*d*, C-1) ([a]indicates the values are interchangeable). Assignments of the methyl group signals were checked by a COSY experiment using free Furaneol. In the ^{13}C NMR spectrum of the glucoside (**2**), only the signals of the C-5′ methyl group exhibit resolution of the diastereoisomers in aqueous solution. The signals of the glucoside arising from the Furaneol part of the molecule have similar chemical shifts to those of the corresponding carbon atoms of Furaneol, with the exception of C-2′, which is at δ 180.7 in Furaneol (**1**). The pattern of the signals for the glucose half of the molecule is in agreement with β-substitution on C-1, when compared with the signals of free glucose in D_2O [*cf.* 6].

EXPERIMENTAL

General. Preparative and analytical HPLC was carried out on a Spectra-Physics model SP 8700 instrument on either an Aminex Carbohydrate HPX 87 C column (300 × 7.8 mm, Biorad Company) at 85° in H_2O (0.6 ml/min) or a Nucleosil C_{18} column (250 × 4 mm, Macherey–Nägel, Düren, F.R.G.) in H_2O (with trifluoroacetic acid to pH 3.5)–MeOH (3:1) for 8 min, then to 100% MeOH, 0.8 ml/min. Other details of HPLC are given below. NMR spectra were measured at 360 MHz for ^{1}H NMR spectra and 90 MHz for ^{13}C NMR spectra. Chemical shifts are in ppm downfield from TMS for spectra measured in $CDCl_3$, and downfield from sodium trimethylsilyltetradeuteriopropionate for spectra measured in D_2O.

Isolation of Furaneol glucoside from strawberries. Strawberries purchased from the local supermarket (1.3 kg) were blended with H_2O (650 ml) in a mixer. The juice was centrifuged (8000 rpm) and the decanted liquid passed through a filter-press (Seitz–Filterschichten Supra 5500 and Supra 300) to obtain 1750 ml of clear juice. Analytical HPLC showed this juice to contain both Furaneol and its glucoside (by comparison with authentic material in the case of Furaneol, and with the synthetic glucoside described below). A portion of the juice (607 ml) was lyophilized, when it gave 103 g of viscous syrup, still containing some water. After addition of 100 ml H_2O, the juice was filtered (using an Amicon Corp. apparatus), through the following membranes in order: Schleicher & Schüll (room temp.) (1) AE 99, 8 μm; (2) AE 98, 5 μm; (3) AE 91, 0.8 μm; (4) BA 85, 0.45 μm; (5) BA 83, 0.2 μm; then Amicon (at 6°) (6) PM 30 (cutoff *ca* 30,000); (7) PM 10 (cutoff *ca* 10,000); (8) YM 2 (cutoff *ca* 1000); (9) YCO 5 (cutoff *ca* 500). The filtrate was lyophilized to yield 21.6 g of solid residue, corresponding to *ca* 3% of the weight of the strawberries as low M_r (< 500) compounds. The solids (21.6 g) in H_2O (20 ml) were introduced into a medium pressure preparative column

(Büchi, 47 × 7 cm) of LiChroprep RP18 (40–63 μm, Merck). The flow rate was 42 ml/min under a pressure of *ca* 2 bar. The eluting solvent was H_2O with increasing amounts of MeOH, taking fractions of 500 ml. Furaneol glucoside and Furaneol were eluted in Fractions 5 to 8, when the solvent contained 15–30% MeOH. Fractions were selected by HPLC analysis, combined and lyophilized to yield 1.27 g solids. This material was subjected to prep. HPLC by repeated injections of *ca* 400 mg amounts on a Nucleosil column (7 μm, C_{18} 250 × 20 mm) in H_2O–MeOH (3:1), flow rate 9 ml/min, with UV detection at 276 nm. In this way, 67 mg of still impure Furaneol glucoside was obtained. A second preparative HPLC purification under the same conditions produced 49 mg of the glucoside, which was apparently 42% pure, and prep. HPLC of this on an Aminex column enabled collection of 27 mg of pure material having NMR spectra (^1H and ^{13}C) and a FAB/MS spectrum identical with those of the synthetic material. The UV spectrum was measured by a stop-flow technique on Aminex, and the maximum was at 288 nm. Synthetic **2** gave the same result.

Based on the 49 mg batch of material of 42% purity, the concentration of Furaneol glucoside in these strawberries was *ca* 29 ppm. From the prep. HPLC, there was obtained a fraction from which 8 mg of Furaneol was obtained, corresponding to 11 ppm in the fresh fruit.

Glucofuraneol tetraacetate (**3**). A soln of Furaneol (17 g, 133 mmol) in a preheated (to 80°) soln of KOH in EtOH (1N, 128 ml) was stirred under N_2 while 1-bromo-1-deoxyglucose tetraacetate (50 g, 122 mmol) in toluene (50 ml) was added rapidly (10 min). After 30 min, the soln had become orange-coloured and had pH *ca* 5. The mixture was poured onto ice and the product extracted into $CHCl_3$. After washing ($NaHCO_3$, NaCl), drying and concentrating, there was obtained 58 g of material. Chromatography of 16.2 g of this on silica gel in cyclohexane–EtOAc (6:4, increasing the amount of EtOAc) led to elution of, first, a small amount of glucose pentaacetate, followed by 3.8 g of the title product as a clear non-crystalline solid. The NMR spectra are described in the Results.

Furaneol β-glucoside (**2**). A soln of NaOMe made from 0.2 g Na in 30 ml MeOH was added slowly to a soln of Furaneol glucoside tetraacetate (8 g) in MeOH (50 ml). The soln became orange, and was stirred at room temp. for 4 hr, then neutralized with Amberlyst 15 resin, filtered and concd, to yield 2.8 g of free glucoside, which was 39% pure (by HPLC). Another experiment using 1.4 g of purified tetraacetate yielded 330 mg of glucoside of 46% purity. These two products were combined and purified by chromatography on 70 g LiChroprep, flow rate 10 ml/min, with 5 ml fractions going from H_2O (100%, Fractions 1–52), H_2O + 5% MeOH (Fractions 53–78), (to H_2O + 10% MeOH (Fractions 131–156). From Fractions 86–140, 1.05 g of the glucoside (**2**) of 92% purity was isolated; the remaining 8% was Furaneol. For analytical measurements, this material was re-chromatographed under the same conditions; $[\alpha]_{589} - 62.8°$ (H_2O; 1.8%). The NMR spectra are discussed in the Results. FAB/MS was carried out by Dr K. Rose by applying an aq. soln (1 μl) to a glycerol (1 μl)–HOAc (0.5 μl) matrix. There was a clear signal at *m/z* 291 corresponding to the protonated molecular ion, $C_{12}H_{19}O_8^+$, of **2**. A second strong signal appeared at *m/z* 129, corresponding to protonated Furaneol, $C_6H_9O_3^+$. We assume that the latter fragment results from a true fragmentation process, since there was no free Furaneol in the sample (by NMR spectrometry) and traces of Furaneol would not give rise to such a prominent fragment, Furaneol being rather insensitive to FAB-measurements in a glycerol–HOAc matrix.

Acknowledgements—We are grateful to Dr K. Rose (Département de Biochimie Médicale, Centre Médical de l'Université de Genève) for the FAB mass spectrometric measurements. Thanks are also extended to Mr P. Deladoey, Mr J.-C. Kuhn and Ms F. Rey for invaluable practical assistance.

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