Tetrahedron Letters 52 (2011) 4738–4740

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

An expeditious synthesis of quercetin 3-*O*-β-D-glucuronide from rutin

Mohammed Kajjout, Rajae Zemmouri, Christian Rolando *

Université de Lille 1, Sciences et Technologies, USR CNRS 3290 Miniaturisation pour la synthèse, l'Analyse & la Protéomique, 59655 Villeneuve d'Ascq Cedex, France

ARTICLE INFO

Article history:
Received 20 May 2011
Revised 25 June 2011
Accepted 27 June 2011
Available online 2 July 2011

Keywords:
Rutin (quercetin-3-rutinoside)
Quercetin 3-*O*-β-D-glucuronide
TEMPO oxidation

ABSTRACT

We report the synthesis of the major human metabolite of quercetin, quercetin 3-*O*-β-D-glucuronide, from rutin (quercetin-3-rutinoside), which is commercially available at low cost. This straightforward synthesis is based on the key intermediate 3′,4′,5,7-tetra-*O*-benzyl-quercetin which is obtained in only two steps by the total benzylation of rutin followed by acid hydrolysis of the rutinoside residue. Glycosylation of the free 3 hydroxyl group by 1-bromo-3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside yields the protected glucoside. TEMPO-mediated oxidation of primary alcohol on the deprotected glucoside gives access to the benzylated glucuronide. Removal of the benzyl groups which protect the quercetin hydroxyl groups by H_2 (10% Pd/C) yields quercetin 3-*O*-β-D-glucuronide.

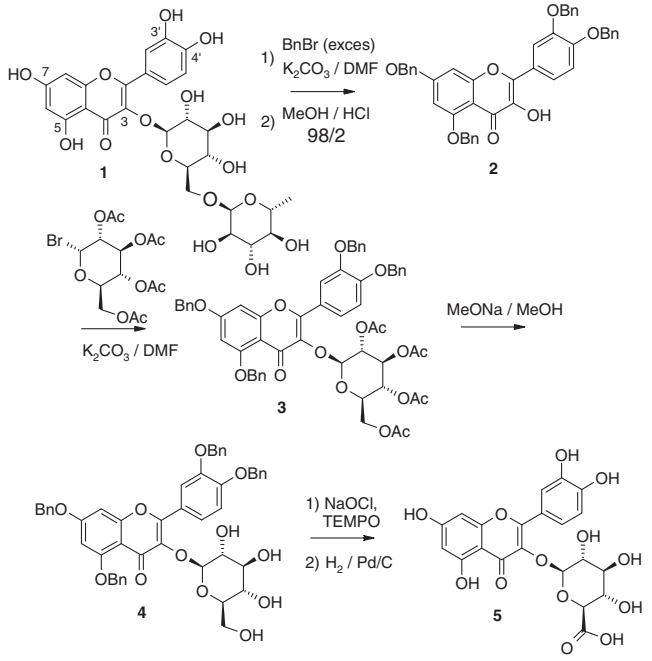
Flavonoids are the most important family of plant polyphenolic compounds and are diverse both in chemical structures and characteristics. Flavonoids are categorized into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, dihydroflavonols, and chalcones. Quercetin is the major individual flavonol in fruits, vegetables, and beverages and the human daily consumption ranges between 10 and 100 mg according to eating and drinking habits.[1–4] Quercetin is an antioxidant and appears to be active in many diseases related to aging like cardiovascular,[5] cancer[6,7] and neurodegenerative diseases.[8] Quercetin glucuronides and sulfates are the main circulating metabolites of quercetin along with methylated quercetins and their glucuronide and sulfate conjugates.[9] In humans quercetin 3-*O*-β-D-glucuronide and quercetin 3′-*O*-sulfate are the most abundant quercetin metabolites and have even more potent biological activities than quercetin itself.[10] Quercetin 3-*O*-β-D-glucuronide, which is not commercially available for biological studies, has been isolated from green beans[11] and from *Reynoutria sachialinensis*.[12] We developed a novel synthesis of quercetin 3-*O*-β-D-glucuronide from rutin. Our approach is based on the protection of all hydroxyl groups and mild hydrolysis of the 3-*O*-rutinoside natural protecting group, leading to 3′,4′,5,7-tetrabenzylquercetin. Glycosylation of the free hydroxyl at the 3 position, oxidation of the primary alcohol of the glucoside catalyzed by TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) and the deprotection of hydroxyl groups gave quercetin 3-*O*-β-D-glucuronide. This synthetic pathway gives straightforward access to quercetin functionalized at the position 3 via the key intermediate **2**.

In the literature quercetin 3-*O*-β-D-glucuronide has been synthesized first by direct glucuronidation of 4′,7-di-*O*-benzylquercetin using methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide in the presence of silver oxide.[13] This synthesis has been improved by Needs et al. using a desiccant (either calcium sulfate or molecular sieves) in pyridine at 0 °C, which prevents scrambling of the acetate group protecting the glucuronate residue during the alkylation step. After debenzylation and hydrolysis of the acetate protecting the sugar secondary alcohols and of the methyl ester protecting the carboxylic acid, quercetin 3-*O*-β-D-glucuronide has been obtained in 11% yield from quercetin.[14] The preparation of quercetin-3-*O*-β-D-glucuronide has also been previously described in our group in modest yield (25% from protected quercetin) by selective oxidation of the glucoside by NaOCl/TEMPO in the presence of potassium bromide and tetrabutylammonium bromide.[15,16] The required 3-*O*-β-D glucoside quercetin protected at all phenolic positions was obtained in five steps. First the quercetin B catechol ring was protected by neat heating at 180 °C with dichlorodiphenylmethane in low yield. The intermediate was glycosylated under phase transfer conditions at the more reactive 3 position. Before TEMPO oxidation the 5 and 7 positions were protected by benzylation and the glucose moiety was deprotected using sodium methylate. The deprotection of the ketal was more difficult than expected and the diphenylmethylene group required a strong catalyst such as 30% palladium on charcoal.

Our present method provides an efficient and easy approach to access of quercetin 3-*O*-β-D-glucuronide **5** in just four steps from rutin as depicted in Scheme 1, taking advantage of the natural protection of the 3 position in rutin which is readily available and cheap. Huang et al.[17] obtained 3′,4′,7 tribenzyl-quercetin after treatment of rutin **1** using 3.35 equiv of benzyl bromide in *N*,*N*′-dimethylformamide (DMF) at 60 °C and hydrolysis by HCl/ethanol (15:85, v/v) during 2 h at 70 °C. In our approach all hydroxyl groups must be protected during the oxidation step. We previously described that totally benzylated quercetin is obtained by alkylating quercetin with 6 equiv of benzylbromide.[18] So, we expected that rutin would be

* Corresponding author. Tel.: +33 320434977; fax: +33 320336136.
E-mail address: Christian.rolando@univ-lille1.fr (C. Rolando).

0040-4039/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2011.06.108



Scheme 1. Synthesis of quercetin 3-*O*-β-D-glucuronide.

tetrabenzylated under the same conditions. We observed also in our previous work[16,18] that the cleavage of the benzyl group at position 5, during the attempted selective deprotection of catechol ring of 3,5,7-tribenzylated quercetin whose B cycle was protected by the diphenylmethane ketal under acidic conditions using a mixture of acetic acid/water (80:20) at reflux. So in order to avoid an additional step of rebenzylation of the 5-hydroxy group, we decided to work under diluted acidic conditions to cleave the rutinoside group. The four free hydroxyl groups of rutin were benzylated using an excess quantity (8 equiv) of benzyl bromide and potassium carbonate in DMF during 10 h at room temperature. Then the hydrolysis of tetrabenzylated rutin was performed using a mixture of HCl/methanol (2/98, v/v) at reflux (ca 65 °C) which led to the desired product 3′,4′,5,7-tetrabenzylated quercetin **2** in 60% yield in gram quantities.[19]

The O-glucosylation was achieved by condensing 1-bromo-3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (acetobromoglucose) on protected quercetin **2** in DMF at room temperature using simply potassium carbonate as a base. Then the glycosyl moiety was deprotected by the transesterification of the four acetate groups with sodium methylate.[20] Finally oxidation of the protected quercetin-glucoside to the corresponding glucuronic acid was accomplished by sodium hypochlorite (NaOCl) catalyzed by TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) in the presence of potassium bromide and tetrabutylammonium bromide. This step, which is sensitive to the precise structure of the molecule to be oxidized,[16,21] proceeded smoothly and was not affected by the change of the protecting group on the B catechol cycle from diphenylmethyleneketal to dibenzylethers. The synthesis ended by the cleavage of the benzyl groups by a mild hydrogenolysis using palladium hydroxide at 10% on charcoal in a THF/EtOH mixture. It must be pointed out that a higher content of palladium (30%) was required to deprotect the diphenylmethyleneketal group we used previously. The desired quercetin 3-*O*-β-D-glucuronide **5** was obtained after a final purification on a C18 reverse phase, solid phase extraction (SPE) cartridge using a mixture of methanol/water as the eluent, and was identified by its ESI mass spectrum and its ^{1}H and ^{13}C NMR spectra.[22] Glucuronide **5** was obtained in 28% yield from rutin **1**.

In conclusion, we developed an efficient methodology for the synthesis of quercetin 3-*O*-β-D-glucuronide **5** based on the readily available rutin. The reported strategy based on easy access to the key intermediate 3′,4′,5,7-tetrabenzylated quercetin **2** which can be very easily deprotected and which is not prone to protecting group scrambling could be applied to the synthesis of 3-*O*-methylquercetin[18] or labile derivatives of quercetin like sulfate which are compatible with the hydrogenolysis of the *O*-benzyl protecting group.[14]

Acknowledgments

The NMR and Mass Spectrometry facilities used in this study are funded by the European community (FEDER), the Région Nord-Pas de Calais (France), the CNRS, and the Université de Lille 1, Sciences et Technologies. Part of this work was funded by the European Contract QLK1-CT-1999-00505 POLYBIND. The authors thank Dr. Maria van Agthoven for her help with editing this manuscript.

References and notes

1. Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. *J. Agric. Food Chem.* **1992**, *40*, 2379–2383.
2. Hertog, M. G. L.; Hollman, P. C. H.; van de Putte, B. *J. Agric. Food Chem.* **1993**, *41*, 1242–1246.
3. Hollman, P. C. H.; Katan, M. B. *Food Chem. Toxicol.* **1999**, *37*, 937–942.
4. Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
5. Hollman, P. C. H.; Geelen, A.; Kromhout, D. *J. Nutr.* **2010**, *140*, 600–604.
6. Gibellini, L.; Pinti, M.; Nasi, M.; De Biasi, S.; Roat, E.; Bertoncelli, L.; Cossarizza, A. *Cancers* **2010**, *2*, 1288–1311.
7. Wang, L.; Lee, I. M.; Zhang, S. M.; Blumberg, J. B.; Buring, J. E.; Sesso, H. D. *Am. J. Clin. Nutr.* **2009**, *89*, 905–912.
8. Spencer, J. P. E.; Vauzour, D.; Rendeiro, C. *Arch. Biochem. Biophys.* **2009**, *492*, 1–9.
9. Mullen, W.; Edwards, C. A.; Crozier, A. *Br. J. Nutr.* **2006**, *96*, 107–116.
10. Chao, C. L.; Hou, Y. C.; Lee Chao, P. D. L.; Weng, C. S.; Ho, F. M. *Br. J. Nutr.* **2009**, *101*, 1165–1170.
11. Price, K. R.; Colquhoun, I. J.; Barnes, K. A.; Rhodes, M. J. C. *J. Agric. Food Chem.* **1998**, *46*, 4898–4903.
12. Zhang, X.; Thuong, P. T.; Jin, W.; Su, N. D.; Sok, D. E.; Bae, K.; Kang, S. S. *Arch. Pharm. Res.* **2005**, *28*, 22–27.
13. Wagner, H.; Danninger, H.; Seligmann, O.; Farkas, L. *Chem. Ber.* **1970**, *103*, 3674–3677.
14. Needs, P. W.; Kroon, P. A. *Tetrahedron* **2006**, *62*, 6862–6868.
15. Bouktaib, M.; Atmani, A.; Rolando, C. *Tetrahedron Lett.* **2002**, *43*, 6263–6266.
16. Kajjout, M.; Rolando, C. *Tetrahedron* **2011**, *67*, 4731–4741.
17. Huang, H.; Jia, Q.; Ma, J.; Qin, G.; Chen, Y.; Xi, Y.; Lin, L.; Zhu, W.; Ding, J.; Jiang, H.; Liu, H. *Eur. J. Med. Chem.* **2009**, *44*, 1982–1988.
18. Bouktaib, M.; Lebrun, S.; Atmani, A.; Rolando, C. *Tetrahedron* **2002**, *58*, 10001–10009.
19. Procedure for the synthesis of the key intermediate 5,7-bisbenzyloxy-2-(3,4-bisbenzyloxyphenyl)-3-hydroxyl-4*H*-chromen-4-one **2**. All commercially available products were purchased from Aldrich (Saint-Quentin Fallavier, France) and used as received. To a solution of rutin hydrate **1** (5.00 g, approximately 8.0 mmol) in DMF (60 ml), potassium carbonate (9.04 g, 65.52 mmol) and benzyl bromide (7.79 ml, 65.52 mmol) were added under argon. The reaction mixture was stirred vigorously at room temperature for 10 h. The resulting mixture was diluted with 200 ml of ethyl acetate and was washed with water (2 × 150 ml). The residue obtained after evaporation of the solvent was solubilized in a mixture of MeOH/HCl (98:2). The solution was refluxed until a precipitate was formed. After cooling the precipitate was filtered off and was washed with 50 mL of cold MeOH to afford **2** (3.20 g, 60% yield). ^{1}H NMR ($CDCl_3$): 5.08 (s, 2H, OCH_2Ph), 5.20 (s, 2H, OCH_2Ph), 5.22 (s, 2H, OCH_2Ph), 5.23 (s, 2H, OCH_2Ph), 6.45 (d, J = 1.9 Hz, 1H, aromatic H), 6.55 (d, J = 1.9 Hz, 1H, aromatic H), 7.01 (d, J = 8.5 Hz, 1H, aromatic H), 7.19–7.47 (m, 20H, aromatic H), 7.59 (dd, J = 8.5, 1.9 Hz, 1H, aromatic H), 7.75 (d, J = 1.9 Hz, 1H, aromatic H). ^{13}C NMR ($CDCl_3$): 70.4 (OCH_2Ph), 70.6 (OCH_2Ph), 70.9 (OCH_2Ph), 72.5 (OCH_2Ph), 93.6, 97.5, 106.7, 114.0, 114.1, 121.2, 124.3, 126.7, 127.2, 127.5, 127.7, 127.8, 127.9, 128.5, 128.6, 128.7, 128.9, 135.6, 136.2, 136.8, 137.1, 137.7, 141.9, 148.6, 150.1, 158.7, 159.3, 163.2, 171.7 (C=O). Elemental Anal. Calcd for $C_{43}H_{34}O_7$: C, 77.94; H, 5.14; O, 16.92. Observed C, 77.96; H, 5.16; O, 16.94.
20. *Procedure for the synthesis of 5,7-bisbenzyloxy-2-(3,4-bisbenzyloxyphenyl)-3-O-β-D-glucopyranosyloxy-4H-chromen-4-one* **4**: (1.00 g, 1.51 mmol) of compound **2**, (1.24 g, 3.02 mmol) of acetobromoglucose and (417 mg, 3.02 mmol) of potassium carbonate were dissolved in 20 ml of DMF under argon. The mixture was agitated for 6 h. The reaction mixture was diluted with 150 ml of ethyl acetate and was washed with water (2 × 75 ml). The organic phase was

dried over $MgSO_4$ and the solvent was evaporated. The residue obtained was purified by flash column chromatography using a mixture of CH_2Cl_2/EtOAc (85/15) as the eluent to give 1.19 g of **3** (79% yield). Then (500 mg, 0.50 mmol) of the obtained compound **3** was dissolved in 30 ml of a mixture of MeOH/THF (50/50). A solution sodium methylate, prepared from 10 mg of sodium metal in methanol (10 ml) was added at room temperature. When the deprotection was completed, the solution was neutralized by adding 2.0 g of an ion-exchange resin (H^+ form). The agitation was maintained for 30 min, and then the resin was filtered off. Methanol was eliminated by evaporation under vacuum, at room temperature to give **4**, which was used without further purification in the next step, TEMPO mediated oxidation (383 mg, 93% yield).

21. Wang, P.; Zhang, Z.; Yu, B. *J. Org. Chem.* **2005**, *70*, 8884–8889.
22. *Procedure for the preparation of 3-O-β-D-glucuronide* **5**: to a solution of compound **4** (50 mg, 0.06 mmol) in CH_2Cl_2 were added 1 ml of solution saturated in potassium carbonate, 1.5 mg of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy), 5 mg of potassium bromide, and 2.0 mg tetrabutylammonium bromide. Then 10 ml of a solution of 5.5% NaOCl were added dropwise. After 1 h the resulting mixture was diluted with 30 ml of ethyl acetate and 30 ml of water. The precipitate thus formed was filtered off. The solid obtained was dissolved in a mixture of EtOH (20 ml) and THF (20 ml), and was treated with palladium hydroxide (10%, 30 mg) under a hydrogen flow for 12 h. The reaction mixture was then filtered on Celite and rinsed with EtOH (30 ml). The residue obtained after evaporation of the solvent was purified by chromatography on a C18 reverse phase SPE (solid phase extraction) cartridge using a mixture of MeOH/water (40/60) as the eluent pushed through the cartridge by a slight argon pressure to afford **5** (18 mg, 63% yield). Data for quercetin 3-*O*-β-D-glucuronide **5**: ^1H NMR (CD_3OD) 3.45–3.83 (m, 4H), 5.28 (d, J = 7.2 Hz, 1H), 6.18 (d, J = 2.2 Hz, 1H), 6.42 (d, J = 2.2 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 7.62 (dd, J = 8.5 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H). ^{13}C NMR (CD_3OD) 73.1, 75.5, 77.5, 78.4, 95.0, 100.4, 104.1, 106.0, 116.1, 118.3, 122.0, 123.1, 135.7, 146.0, 149.7, 157.9, 160.0, 162.9, 165.7, 171.8, 178.6. HR-FAB-MS (*m/z*): observed, 479.0799; calcd for $C_{21}H_{19}O_{13}$, 479.0826 $[M+H]^+$.