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Regio- and stereoselective synthesis of the major metabolite of quercetin, quercetin-3-*O*-β-D-glucuronide

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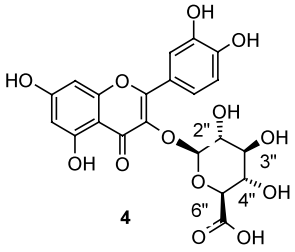
Abstract—Protected quercetin was first transformed selectively in its 3-*O*-β-D-glucoside. Further protection of the remaining phenolic hydroxyl group allows a clean oxidation of the glucoside by TEMPO/NaOCl/NaBr under phase transfer conditions into the corresponding glucuronide which was cleanly deprotected to quercetin-3-*O*-β-D-glucuronide.

Flavones and flavonols are plant secondary metabolites found in many foods, especially in fruits and vegetables.[1,2] The daily consumption of flavonoids ranges from 6 mg in Finland to 70 mg in Japan. Quercetin and its glucosylated forms are the major individual molecules among polyphenols which are not polymeric and represent 60–75% of the flavonoid intake.[3]

Quercetin is a very efficient antioxidant[4] and appears to be active in many diseases related to aging like cancer,[5] cardiovascular[6] and neurodegenerative[7] diseases. However many questions are still open on the origin of this activity.[8,9] Both quercetin aglycone **1** and glucosylated forms like quercitrin **2a** (quercetin 3-*O*-α-rhamnopyranoside), rutin **2b** (quercetin 3-*O*-β-D-rutinoside) or isoquercetin **3** (quercetin 3-*O*-β-D-glucoside), are absorbed through the intestine.[10] However their metabolism is quite different as glucosylated forms are absorbed directly whereas quercetin free aglycone is first glucuronidated.[11] The analysis of plasma of volunteers fed with quercetin supplemented diet shows that quercetin is mainly circulating as glucuronide, mainly quercetin-3-*O*-β-D-glucuronide and 3′-methylquercetin-3-*O*-β-D-glucuronide.[12,13] The same metabolism is observed in rat and pig with variation on the site of methylation.[14–16]



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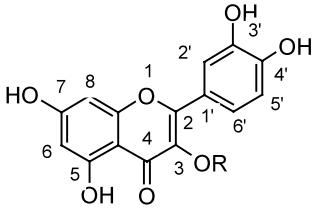
In vitro studies showed that the activity of quercetin derivatives on model enzymatic system is very dependent upon the substitution pattern on quercetin.[17,18]



1 R=H quercetin
2a R= α-L-rhamnopyranose quercitrin
2b R= β-rutinoside rutin
3 R= β-D-glucose isoquercetin

Keywords: quercetin; *O*-glucoside; *O*-glucuronide; TEMPO oxidation; chemical synthesis.

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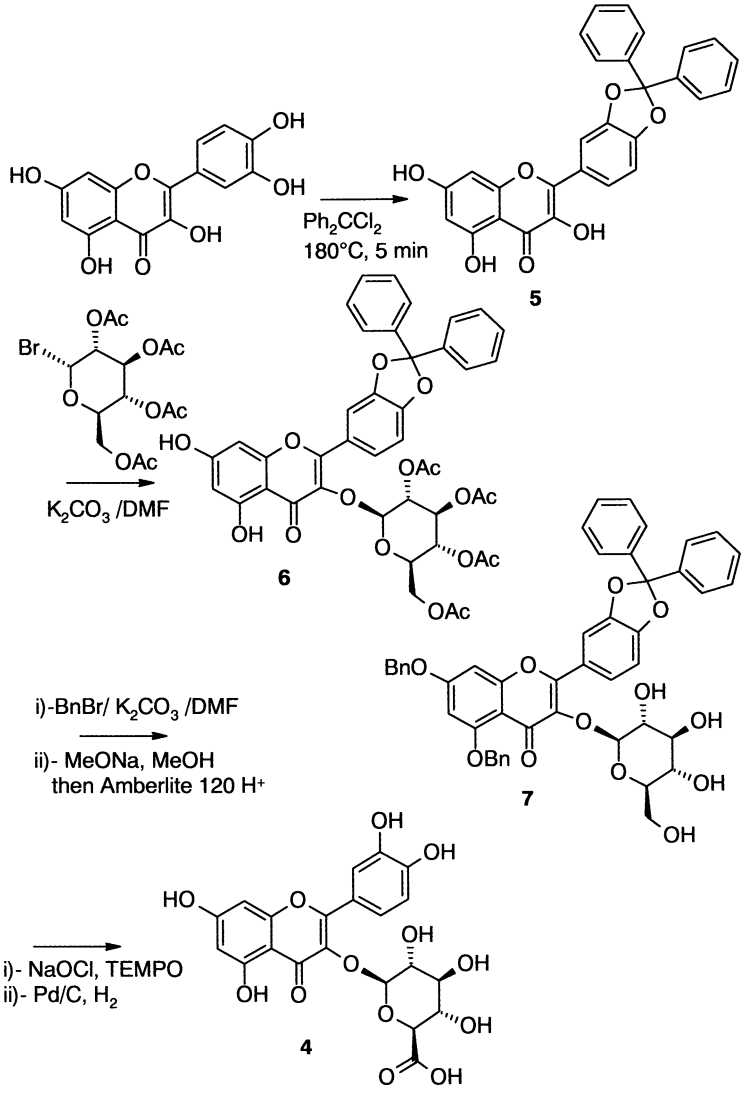
However most of the in vitro biological studies for assessing the properties of quercetin are performed on quercetin and not on their metabolites as they are not readily available from commercial sources. For example quercetin-3-*O*-β-D-glucuronide must be isolated from green beans[19] which contains in the most suitable variety 15 mg per kg of fresh beans or prepared in low yield by direct glucuronidation of quercetin and separated by tedious preparative HPLC procedures.[16] As pointed out by many authors, glucuronidation is by far more difficult than glucosylation.[20] This trend is enforced in the case of phenols which requires special care even for glucosylation. For example alkylation of 3,3′,4′,5-tetrabenzoylquercetin by glucuronic acid methyl ester bromide triacetate gave only a 8% yield of quercetin-7-glucuronide.[21] Similar results were reported previously for the synthesis of normorphine-3 and 6-*O*-β-D-glucuronide.[22] The recently described efficient acid condensation of methyl 2,3,4-tri-*O*-isobutyryl-trichloroacetimidoyl-α-D-glucopyranuronate catalyzed by BF_3–Et_2O for synthesizing morphine-3,6-diglucuronide[23] is not a general approach for flavonoids, as many of them like catechin are very sensitive in acidic medium.[24] On the other hand, glucosylation of phenols can be performed in mild conditions using phase transfer catalyst such as tetrabutylammonium bromide[25] or tris(2-(2-methoxyethoxy)ethyl)amine (TDA-1) in good to fair yields.[26] Glucoside can be selectively oxidized to the corresponding glucuronide by TEMPO in catalytic amount in the presence of sodium hypochlorite which regenerates the free radical.[27,28] So we choose to synthesize quercetin glucuronide by oxidation of the corresponding glucoside. This provides an easy access to both of these compounds, including labeled molecules, needed in metabolic studies, starting from commercially available labeled glucose. Using the protecting strategy we developed to access selectively to each hydroxyl function of quercetin,[29] we synthesized selectively quercetin-3-*O*-β-D-glucoside, which was subsequently transformed in the corresponding glucuronide.

Selective protection of the north-east catechol of quercetin **1** by dichlorodiphenylmethane led to the ketal **5** (Scheme 1) which gave an entry in the series substituted on the 3 position.[29,30] Indeed protected quercetin ketal **5** still exhibits three free hydroxyl groups. How-



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

ever the higher reactivity of position 3 allows the selective alkylation by methyl iodide or benzyl bromide in DMF using 1 equiv. of K_2CO_3 as base along with the formation of dialkylated products in 3, 7 positions (in 50 and 25% ratio for methyl and benzyl, respectively).[29] Under the same conditions glucosylation take place very cleanly at the 3 position leading to **6** in 54% yield. Only traces of di-glucosylated quercetin are formed. On the other hand we observed that glucosylation takes place in the 7 position as reported by Dangles and co-workers under more basic conditions involving phase transfer by tris[2-(2-methoxyethoxy)ethyl]amine, saturated hydrogenocarbonate and dichloromethane.[30] Moreover, the cross peak from H-1″ (δ 5.77) to C-3 (δ 136.29) in the HMBC spectrum confirmed that the glucusodation takes place in the 3 position. The large proton coupling ($J=7.5$ Hz) observed for the anomeric proton demonstrates that the glucoside has the expected β-configuration. Cleavage of the ketal by hydrogenolysis catalyzed by palladium hydroxide on charcoal led to quercetin-3-*O*-β-D-glucoside (isoquercetin) **3** in 86% isolated yield. Before oxidation by TEMPO of the glucoside to the glucuronide, the free hydroxyl functions in position 5 and 7 must be protected, as phenols are reactive under such conditions. Treatment of the partially protected glucoside with benzyl bromide in excess (K_2CO_3, DMF, room temperature) led to the fully protected quercetin-3-glucoside **6**. Classical deprotection of the acetoxy protecting group on the sugar residue (sodium methylate then neutralization by anionic resin in protonated form) led to the unprotected glucoside **7**.

Oxidation of the protected quercetin-3-*O*-β-D-glucoside to the corresponding glucuronic acid was performed by NaOCl catalyzed by TEMPO in presence of NaBr. The low solubility of protected quercetin glucoside **7** was circumvented using phase transfer catalysis by tetrabutylammonium between CH_2Cl_2 and saturated sodium hydrogenocarbonate.[27] After 2 hours, TLC showed a complete disappearance of the starting glucoside. Then the glucuronide was precipitated by the addition of ethyl acetate. This phase transfer protocol avoids the use of an oxidant soluble in organic medium as *t*-butyl hypochlorite required for the oxidation of phenyl-*O*-β-D-glucuronide.[31] The crude product was purified by chromatography on silica gel column. We plan to remove both diphenylmethylene and benzyl protecting groups in one step by catalytic hydrogenation. However, the deprotection of the ketal was more difficult than expected and the diphenylmethylene group was not cleaved by the usual catalyst (10% Pd–C). The cleavage required a more reactive catalyst such as 30% palladium on charcoal. Final purification on LC-18 SPE cartridge using a methanol, water gradient as eluent, afforded analytically pure quercetin-3-*O*-β-D-glucuronide **4** according to NMR and LC-MS/MS analysis in millimolar amount (27% isolated yield from **5**).

The quercetin-3-*O*-β-D-glucoside **3** and quercetin-3-*O*-β-D-glucuronide **4** were fully characterized by their ESI mass spectra and their ^1H and ^{13}C NMR spectra.[32,33] The ^1H NMR spectrum of compound **3** showed two doublets (δ 6.15 and 6.33, $J=2.0$ Hz) consistent with the *meta* protons H-6 and H-8 on the resorcinol A-ring, whereas the catechol B-ring protons are characterized by the ABX system (δ 6.75, $J=8.6$ Hz; 7.57, $J=8.6$ and 2.0 Hz; 7.70, $J=2.0$ Hz). The ^1H NMR spectrum of compound **4** is similar to that of compound **3**. The complete attribution of the glucuronic acid moiety as well as the structure of the aglycone moiety was established by ^1H and ^{13}C NMR and HMBC analyses. Besides the signal attributed to the quercetin structure, the ^{13}C NMR spectrum of **4** showed six carbon signals assigned to the glucuronic acid, including the anomeric carbon (δ 104.3) and one carboxyl carbon (δ 176.2) (Table 1). The obtained ^{13}C NMR spectrum for the synthesized quercetin-3-*O*-β-D-glucuronide **4** is very similar to the spectra previously published from natural sources.[16,19] This hemisynthesis of quercetin-3-*O*-β-D-glucoside **3** and quercetin-3-*O*-β-D-glucuronide **4** is very complementary of the total synthesis of labeled quercetin-4′-*O*-β-D-glucoside recently described.[34]

We are now studying the synthesis of other quercetin glucuronides and labeled quercetin glucuronides and glucosides, where the labels are on the sugar moiety and/or in the polyphenol portion, as well as the extension of the present methodology starting from selectively protected catechin.

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Table 1. ^{13}C NMR spectra of isoquercetin **3** and 3-glucuronide quercetin **4** (75 MHz, CD_3OD, δ ppm) and comparison with spectra from natural products

Carbon		This work	Ref. 19	Ref. 16
	3	**4**	**4**	**4**
2	159.00	159.22	159.01	158.5
3	135.65	135.76	134.84	135.4
4	179.41	179.46	179.19	166.0
5	162.93	163.02	163.06	159.1
6	99.98	99.90	99.87	99.9
7	165.91	166.06	165.71	163.0
8	94.75	94.70	94.83	94.8
9	158.37	158.52	158.38	158.5
10	105.67	105.73	105.61	105.7
1′	123.04	122.72	122.83	122.9
2′	117.62	118.12	117.21	117.3
3′	145.76	145.89	145.90	145.9
4′	149.81	149.85	149.86	149.9
5′	116.00	116.17	116.00	116.1
6′	123.23	122.81	123.52	123.5
1″	104.32	104.33	104.32	104.3
2″	75.73	75.62	75.37	75.4
3″	78.34	78.13	77.58	77.1
4″	71.19	73.41	72.82	72.9
5″	78.10	77.68	77.02	77.6
6″	62.55	176.46	172.14	179.3

lah (Fès-Atlas, Morocco), Faculté des Sciences Dhar Mehraz for a sabbatical leave.

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32. Selected data for compound **3**: $[\alpha]_D^{20}$ −9 (*c* 1.0 g L^{-1}, MeOH) ^{1}H NMR (CD_3OD): 3.18–3.78 (H2″, H3″, H4″, H5″, H6″ m, 6H), 5.23 (H1″, d, $J=7.7$, 1H); 6.15 (H8, d, $J=2.0$ Hz, 1H), 6.33 (H6, d, $J=2.0$ Hz, 1H), 6.75 (H5′, d, $J=8.6$ Hz, 1H), 7.57 (H6′, dd, $J=8.6$, 2.0 Hz, 1H), 7.70 (H2′, d, $J=2.0$ Hz, 1H). ^{13}C NMR (CD_3OD): 62.55, 71.19, 75.73, 78.10, 78.34, 94.75, 99.88, 104.32, 105.67, 116.00, 117.62, 123.04, 123.23, 135.65, 145.76, 149.81, 158.37, 159.00, 162.93, 165.91, 179.41. ESI MS (m/z)= 465 $[M+H]^+$.
33. Selected data for compound **4**: ^{1}H NMR (CD_3OD): 3.85–3.48 (H2″, H3″, H4″, H5″, m, 4H), 5.38 (H1″, d, $J=7.2$, 1H); 6.22 (H8, d, $J=2.0$ Hz, 1H), 6.42 (H6, d, $J=2.0$ Hz, 1H), 6.76 (H5′, d, $J=8.4$ Hz, 1H), 7.64 (H6′, dd, $J=8.4$, 2.0 Hz, 1H), 7.73 (H2′, d, $J=2.0$ Hz, 1H). ^{13}C NMR (CD_3OD): 73.41, 75.62, 77.68, 78.13, 94.70, 99.90, 104.33, 105.73, 116.17, 118.12, 122.72, 122.80, 135.76, 145.89, 149.85, 158.52, 159.22, 163.02, 166.06, 176.27, 179.46. ESI MS (m/z)=479 $[M+H]^+$.
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