Synthesis of [131-I]-Iodinated Quercetin

María Graciela Barolli and Alicia B. Pomilio*

PROPLAME-CONICET, Departamento de Química Orgánica,
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires,
Pabellón 2, Ciudad Universitaria,
1428 Buenos Aires, Argentina.

Abstract

Two iodination procedures *via* thallation were applied to quercetin leading to the formation of 2'-iodoquercetin and 2',6,8-triiodoquercetin; their structures being elucidated by FT-IR and NMR spectral analysis. The preparation of mono- and polyiodinated products is discussed here on the basis of the experimental details and spectral data.This is the first report on the radiolabelling of quercetin, which is here selectively performed with 131-iodine. The radiochemical purity was of 99%.

Key words: iodination, quercetin, 2'-iodoquercetin, 2',6,8-triiodoquercetin, radiolabelling, 131-iodine.

Introduction

Flavonoids possess a variety of biochemical and pharmacological activities, and are especially well known as antioxidants, enzyme inhibitors and growth regulators (1,2). Some plant flavonoids have been also reported to activate bacterial nodulation genes involved in control of nitrogen fixation, thus suggesting important relationships between particular flavonoids and the activation and expression of genes (2, 3).

In particular, quercetin is widespread in nature usually as a glycoside, *e.g.* quercetin 3-*O*-rutinoside (rutin). Quercetin affects the activity of many mammalian enzyme systems *in vitro* and remarkable structure-activity relationships have been determined in most cases. Early reports suggested that histidine and DOPA decarboxylases, lactic dehydrogenase and pyruvate kinase were inhibited by quercetin (1).

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* To whom correspondence should be addressed.

CCC 0362–4803/97/011927–07$17.50
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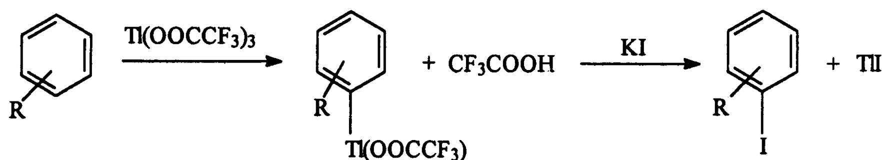
Received 13 May 1997
Revised 29 June 1997

Inhibition of aldose reductase, non-activated phosphorylase kinase (2), phospholipase A_2 (4), ornithine decarboxylase (5), rat pancreatic acinar cell amylase secretion (6), transcription with RNA polymerase II (7), reverse transcriptases (8), anionic and cationic glutathione *S*-transferase isozymes (9), and xantine oxidase (10) have been recently reported as properties of quercetin. Quercetin also affects the function of the immune system and inflammatory cells, *e.g.* T-cells, B-cells, mast cells and basophils (1). Moreover, quercetin shows effects on smooth muscle, antitoxic and hepatoprotective, antiviral, endocrine, on lipid peroxidation and oxyradical production, antiproliferative, and an important effect on xenobiotic metabolism, i.e. dietary quercetin was shown to induce hepatic aminopyrine demethylase activity in rats (1,2,3). Studies *in vivo* may be easily carried out with labelled flavonoids, however little is known on this subject. Furthermore, the lack of reports on iodinated flavonoids prompted us to carry out the synthesis of [131-I] iodinated quercetin, which is reported here for the first time.

Results and Discussion

Iodination of aromatic compounds is usually performed by the Sandmeyer reaction, which consists of preparing the diazonium salt followed by the iodination step in the presence of Cu (I) salts. We present here an alternative to the classic synthetic procedure of halogenation based on the iodination *via* thallation. In fact, the thallation reaction was earlier applied to simple aromatic compounds as well as mono- and disubstituted heterocycles.

In the synthesis reported herein (method 1, scheme 1) the first step is the thallation of the aromatic compound using Tl (III) trifluoroacetate to yield the corresponding arylthallium ditrifluoroacetate. This compound then reacts quickly with KI to give the iodinated aromatic compound and TlI (11).



Scheme 1.

Under these neutral conditions quercetin yields only one iodinated product in position 2' because electrophilic aromatic substitution is the most favoured in the thallation step. These results are in agreement with the spectral data. Hence, the FT-IR spectrum of the iodinated product showed the absorptions typical for an aromatic compound with free *meta* protons (H-6 and H-8 of ring A) and free *ortho* protons (H5'and H-6' of ring B) with the usual out-of-the-plane deformations pattern in the region of aromatic substitutions. The respective signals in the ^{1}H-NMR spectrum in DMSO-d_6, two doublets in the δ 6.0-6.5 region each with a coupling constant of 2 Hz (*meta*-coupling)

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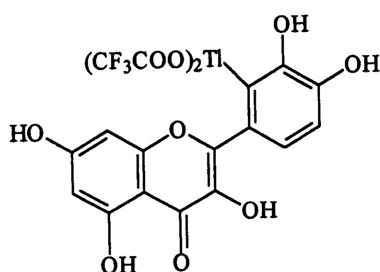
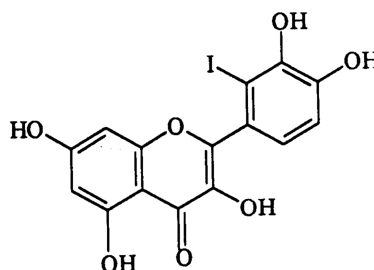
due to H-6 and H-8, and two doublets at δ 6.80 and 7.35 each with a coupling constant of 8 Hz (*ortho*-coupling) due to H-5'and H-6', further confirmed the 2'-iodinated product.

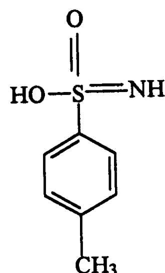
A second method (method 2) of iodination was undertaken taking into account the method used for iodination of peptide compounds with iodide and chloramine T. This method involves iodination in aqueous solution in the presence of chloramine T as oxidant, and KI as precursor for the electrophilic I^+. The reaction was carried out in alkaline medium in order to allow the dissolution of quercetin, which is a salt at pH 10. Then, quercetin had the phenyl groups dissociated due to the strong alkaline medium and consequently both rings A and B were activated for iodination. 2',6,8-Triiodoquercetin was obtained in agreement with spectral data. The ^{1}H-NMR spectrum in D_2O showed only two doublets at δ 7.77 and 7.33, each with an *ortho*-coupling, due to H-5'and H-6', supporting the 2'-substitution. Both H-6 and H-8 of ring A gave no signal in the spectrum, both being substituted by iodine. Therefore, method 2 yielded a 2',6,8-triiodinated quercetin.



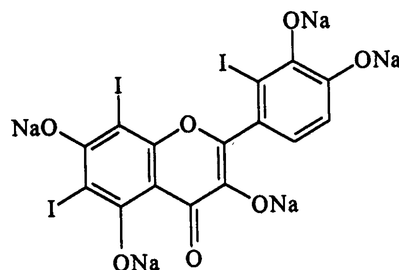
2'-Thalliumditrifluoroacetate quercetin



2'-Iodoquercetin



p-Toluensulfonamide



2',6,8-Triiodoquercetin sodium salt

The quercetin was iodinated by both methods and the compounds were isolated (see experimental). However, only the iodination procedure with iodide and chloramine T was selected for the labelling of quercetin as it is simple, easy and quick enough for labelling with ^{131}I.

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The ^{131}I derivative was prepared by this method with a radiochemical purity of 99%. However, the fact that the Rf of the labelled compound was the same as that of ^{131}I, using a variety of solvent systems and/or stationary phases, made correct quality control difficult. Therefore, we tried a different TLC separation. Quercetin, through its *o*-dihydroxy groups in ring B, formed known complexes with $AlCl_3$ (12), which allowed a neat separation of iodinated quercetin from ^{131}I in a silica gel layer using methanol as developing solvent.
Prior to the chromatographic run ^{131}I and $AlCl_3$ were kept in alkaline medium for 10 minutes, likewise the reaction mixture. $AlCl_3$ was added to an aliquot of the reaction mixture which was also kept at 25°C as mentioned above. An appreciable Rf difference was obtained (Rf ^{131}I = 1, Rf iodinated flavonoid = 0.7).

Experimental

General.

Solvents of maximum purity were checked by gas chromatography (GC) and high-performance liquid chromatography (HPLC). Quercetin was purchased from Aldrich, carrier-free $Na^{131}I$ from Amersham. TLC analyses were performed on aluminum-backed silica gel 60 F_{254} plates (0.2 mm) obtained from Merck and were visualised using ultraviolet light (254 nm) or iodide chamber. Electron impact mass spectra (EI-MS) were achieved on a Trio2 VG spectrometer operating at 70 eV. Melting points (mp) were recorded on a Fisher-Jones apparatus and are uncorrected. All ^{1}H-NMR spectra were recorded on a Bruker ACE 200 either in D_2O, DMSO-d_6 or methanol-d_4 as stated in each spectrum. Resonances are reported downfield from internal standard (tetramethylsilane). Infrared spectra (KBr) were recorded on a Mattson 3000 FT-IR spectrometer. Samples were counted using an automatic gamma detector (Clinigamma Pharmacia).

Thallation of Quercetin.

Quercetin (0.2 g ; 0.6 mmol) was dissolved in acetonitrile (16 mL). Thallium trifluoroacetate (0.3 g; 0.6 mmol) was added to the solution, which was stirred at 25°C for 24 h, and further heated at 60°C for 2 h giving a brown solution. The reaction was followed by TLC using ethyl acetate-n-hexane (10:1.5, in volume). An aliquot of the reaction mixture was kept at 25°C yielding a crystalline beige-coloured compound identified as 2'-thalliumtrifluoroacetate quercetin. Mp 150°C (dec.).

Iodination of Quercetin (method 1).

The raw product of the thallation reaction was iodinated with KI in a 2:1 molar rate (KI: thalliated compound) under stirring at 30°C for 4 h. An orange-coloured solution containing a

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yellow solid in suspension was obtained. The reaction was followed by TLC using methanol as solvent. The iodinated compound gave a yellow spot under visible and UV light, while the thalliated quercetin was only visualised under UV light. The free iodine was destroyed with $Na_2S_2O_3$. The yellow solid (TlI) was filtered off *in vacuo*. Evaporation *in vacuo* to dryness yielded a crystalline yellow-greenish solid iodinated compound identified as 2'-iodoquercetin.

2'-Iodoquercetin: Mp. 160°C (dec.). ^{1}H-NMR (DMSO-d$_6$): δ 6.20 (d, 2H, J = 2 Hz, H-6), 6.41 (d, 2H, J = 2 Hz, H-8), 6.80 (d, 2H, J = 8 Hz, H-5'), 7.35 (d, 2H, J = 8 Hz, H-6').
FT-IR : 965 cm^{-1}, 980 cm^{-1}

Iodination of quercetin (method 2).

a) To a solution of quercetin (0.1 g; 0.3 mmol) in conc NaOH (violet solution), chloramine T (0.075 g; 0.3 mmol) in NaOH and then KI (0.06 g; 0.3 mmol) were added at 25 °C and stirred at that temperature for 30 min. The reaction was followed by TLC using methanol as solvent. After 30 min a white solid appeared, and the suspension was filtered *in vacuo* giving a white solid (**A**) and a violet-brownish solution. The latter was concentrated nearly to dryness giving a violet solid (**B**) and a brownish solution. Compounds (**A**) and (**B**) were identified by mp, ^{1}H-NMR, IR and EI-MS as *p*-toluensulfonamide and 2',6,8-triiodoquercetin sodium salt, respectively.

p-Toluensulfonamide (**A**): Mp= 120-123 °C. ^{1}H-NMR (methanol-d$_4$): δ 2.4 (s, 3H, aromatic methyl group), 7.3 (d, 2H, *para*-aromatic protons), 7.7 (d, 2H, *para*-aromatic protons). EI-MS: [M^+] = 171.

The structure of (**A**) is in agreement with the reduction product of chloramine T.

2',6,8-Triodoquercetin sodium salt (**B**) : Mp > 200°C. ^{1}H-NMR (D_2O): δ 7.33 (d, 2H, J= 8 Hz), 7.77 (d, 2H, J= 8 Hz).

b) The reaction was performed under controlled pH according to the procedure described in a). When the chloramine T and KI were added to the alkaline solution of quercetin the pH of the solution was 10. After stirring at 25 °C for 30 min the colour of the solution was still violet, but the pH decreased to 8. The solution was acidified to pH 6.5 by addition of 2 % HCl, then changing the colour of the reaction from violet to brown. KIO_3 was added and I_2 was extracted with CCl_4. The organic layer was evaporated giving a white solid, which was analysed by IR, NMR, EI-MS and mp, concluding that it was (**A**). The violet-coloured aqueous layer was evaporated giving the iodinated quercetin, identified as the sodium salt of 2',6,8-triiodoquercetin. Identification was performed by NMR, IR, mp. The spectral data were as indicated above for compound (**B**).

Labelling of quercetin with ^{131}I.

The procedure described in a) was used for the labelling of quercetin, because it is a simple and rapid labelling procedure. Thus, a solution of quercetin (10 mg) in conc NaOH was prepared. To

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this solution an equimolar amount of chloramine T was added under stirring at 25 °C until dissolution. Finally, an equimolar amount of carrier-free ^{131}I was added and the reaction was over in 15 min. The reaction was followed by TLC using methanol as solvent. The radiochemical purity was 99%.

Conclusions

Since quercetin is a flavonoid substituted in both rings A and B iodination is easily performed by method 1 as well as by method 2. In method 1 quercetin under neutral pH conditions yields an iodinated product because electrophilic aromatic substitution is the most favoured in the thallation, thus giving rise to a 2'-iodinated product in agreement with the spectral data obtained. In the case of method 2 quercetin had the phenyl groups dissociated due to the strong alkaline medium and consequently both rings A and B were activated for iodination. Therefore, polysubstitution could be achieved as demonstrated by the spectral data. Accordingly, 2',6,8-triiodoquercetin was obtained.

The labelling procedure described here gives rise to compounds of high radiochemical purity sufficient for biological studies such as those required for determining metabolic routes or receptor labelling.

Acknowledgements

Thanks are due to CONICET (Argentina) and Universidad de Buenos Aires (Argentina) for financial support. A.B.P. is a Research Member of the National Research Council of Argentina (CONICET). One of us (María Graciela Barolli) also thanks CONICET for a research fellowship.

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