Heteroaromatic polyphenolic systems based on chitosan

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Abstract

Chitosan is a biodegradable, biocompatible and non-toxic natural polymer derived from chitin. It presents a structure that provides with a wide number of features which could be interesting for its applicability in different fields. [1] On the other hand, polyphenolic compounds, such as hydroxytyrosol, have shown a marked anti-inflammatory and antioxidant properties directly related to cardiovascular diseases, neurodegeneration, metabolic syndrome or cancer. [2]

Preparation of different chitosan derivates, particulary polyphenolic systems, will be described in this work. All the new compounds have been characterized by NMR and MS techniques.

Finally, in order to study the applicability of these compounds, photophysical properties, antioxidant capacity and glutathione peroxidase mimetics will be studied.

Keywords

Chitosan; Polyphenolic systems; Pyrylium salts; 3-desoxyanthocyanins; Flavonoid; Pyridinium salts; Pyrylium salts; Quinolinium salts

Introduction

Nowadays, among the major challenges faced by Society are the search for solutions against cancer and Alzheimer´s disease, both diseases having in common the presence in patients of a large oxidative stress, that is, an imbalance between the concentration of pro-oxidant species and antioxidant compounds.

A preventive effort against these pathologies could be based on the intake of antioxidant such as polyphenols, that can be found in the Mediterranean diet, in which extra virgin oil is an important pillar. [2] It could be a suitable option due to the most polyphenols have anti-inflamatory properties, and there is a clear correlation between chronic inflammation, the development of certain tumors and complications associated with diabetes mellitus.

Therefore, synthesis of polyphenolic systems based on chitosan could be a suitable option not only to take advantage of beneficial properties of both type of compounds but also for the multivalent presentation of these polyphenols by attaching to the biopolymeric backbone. This purpose will be carried out by taking advantage of the reactivity of chitosan´s amino group. These new compounds consist of quaternized salts with structure of pyrylium or quinolinium salts depending on they were synthesized by reaction of chitosan with polyphenolic pyrylium salts or polyhydroxysubstituted 3-desoxyanthocianines salts respectively . [3] Pyrylium and anthocyanin salts used to obtain such systems were previously synthesized by means of a methodology optimized by this research group. [4]

Experimental Methods

All chemicals were purchased and used without further purification. Evaporations were conducted under reduced pressure. TLC was performed on silica gel plates (DC-Alufolien F254, E. Merck). All new compounds were synthesized following the below general procedure. Detection of compounds was accomplished with UV light (254 nm) and by charring with H_2SO_4 and characterization by ^1H and ^{13}C- NMR spectroscopy and Mass spectrometry.

General Procedure for synthesis of Pyrylium salts, 3-Desoxyanthocyanins and Flavonoids [5]

$BF_3\cdot Et_2O$ or $HClO_4$ (2.6 equiv.) was added dropwise under a Ar atmosphere to a solution of *o,m* or *p*-substituted benzaldehyde (1 equiv.) and *o,m* or *p*-substituted acetophenone (2.5 equiv.) in toluene. The mixture was heated to 40 ºC until the reaction was complete, monitored by TLC (1:4 EtOAc–hexane). The residue was treated with H_2O and EtOH. Crystallization from CH_2Cl_2 gave the corresponding pure pyrylium or 3-desoxyanthocyanin. An example of these kind of compound is the following pyrylium salt (Figure 1):

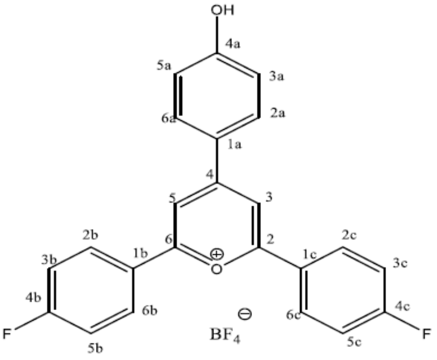


Figure 1. Example of synthesized pyrylium salt

^{1}H NMR (500 MHz, DMSO-d6): δ 8.9 (2H, s, H-3 y 5), 8.63 (6H, dd, J 8.42 Hz,H-2b, 6b,2c, 6c,3a 18y 5a), 7.63 (4H, t, J 8.0 Hz, H-3b, 5b, 3c y 5c) y 7.12 (2H, d, J 8.74 Hz ,H-2a y 6a). ESIHRMS: m/z 361.10, calculated for $C_{23}H_{14}O_2F_2{}^+$: 360.35286

General Procedure for synthesis of Pyridinium and Quinolinium salts [6,7]

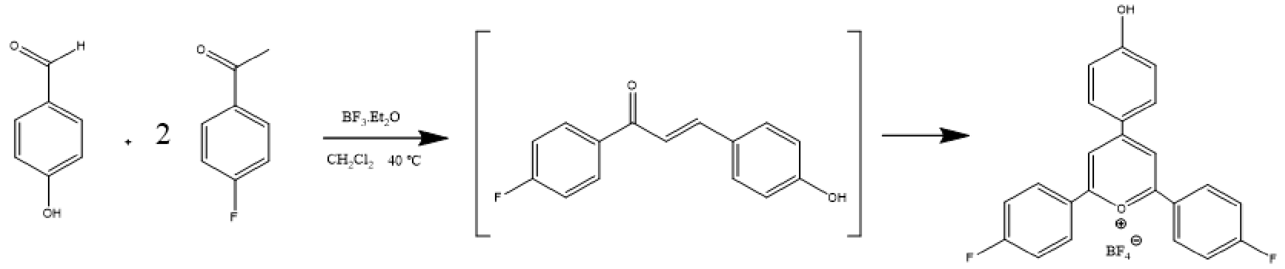
To a solution of chitosan (100 mg) in aqu. acetic acid solution (25 mL), a solution of pyrylium tetrafluoroborate salt (0.30 mmol) in MeOH (100 mL) was added. The resulting solution was heated to 65 ºC. The reaction mixture was left to reach the room temperature and then was treated with 4 M NaOH. The resulting solid was flittered off and successively washed with H_2O (2 x 2 mL), EtOH (2 x 4 mL), CH_2Cl_2 (2 x 2 mL) and acetone (2 x 2 mL) to give the corresponding pure chitosan-pyridinium or qinolinium salt.

Results and discussion

First aim was the synthesis of polyphenolic pyryliums salts, but the presence of any other kind of compounds was detected in some cases by NMR and HRMS: 3-desoxyanthocyanins and flavonoids.

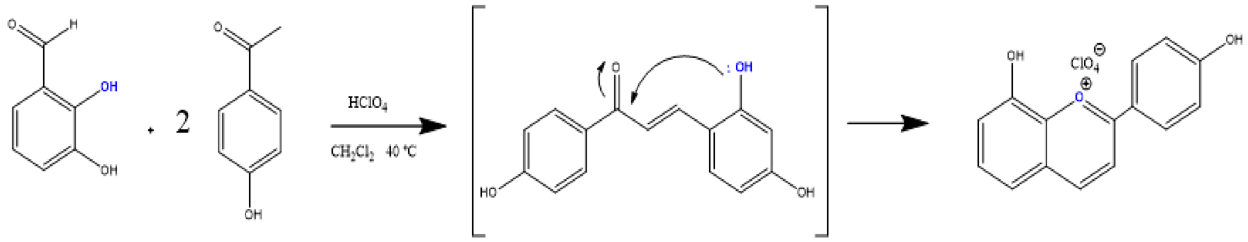
Pyrylium salts, 3-desoxyanthocyanins and flavonoids used to obtain polyphenolic systems based on chitosan were previously synthesized by means of a methodology optimized by this research group. [4] It consists of using ortho, meta and/or para-substituted acetophenones as well as *ortho, meta* and / or *para*-substituted benzaldehyde in the presence of a Lewis acid, which mediates in the dehydration and cyclation process.

Firstly, when starting benzaldehydes and acetophenones have hydroxy groups in any position except *ortho* one, pyrylium salts or chalcones are obtained depending on the reactivity of acetophenone. In the first step, Claisen-Schmidt condensation occur leading to the α,β-unsaturated carbonyl compound (chalcone). Then, the second mol of reactive acetophenone attacks chalcone by Michael addition to give 1,5-dicarbonilyc compound that evolves to pyrylium salt by means of an intramolecular reaction (Scheme 1).



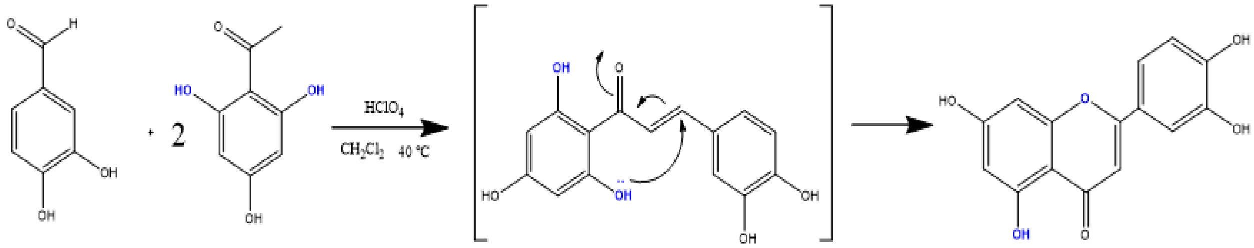
Scheme 1. Synthesis of pyrylium salts

When the starting aldehyde has an hydroxy group in *ortho* possition chalcone evolves to a new structure called 3-desoxyanthocyanin. It occurs as a consequence of an intramolecular reaction in which chalcone carbonyl group is attacked by *ortho* hydroxy group of benzaldehyde leading to anthocyanin (Scheme 2).



Scheme 2. Synthesis of 3-desoxyanthocyanins

In cases in which acetofenone has an hydroxy group in *ortho* possition, it has been noted that chalcone has lead to a flavonoid system due to the intramolecular nucleophilic attack of this hydroxy group previously called (Scheme 3).



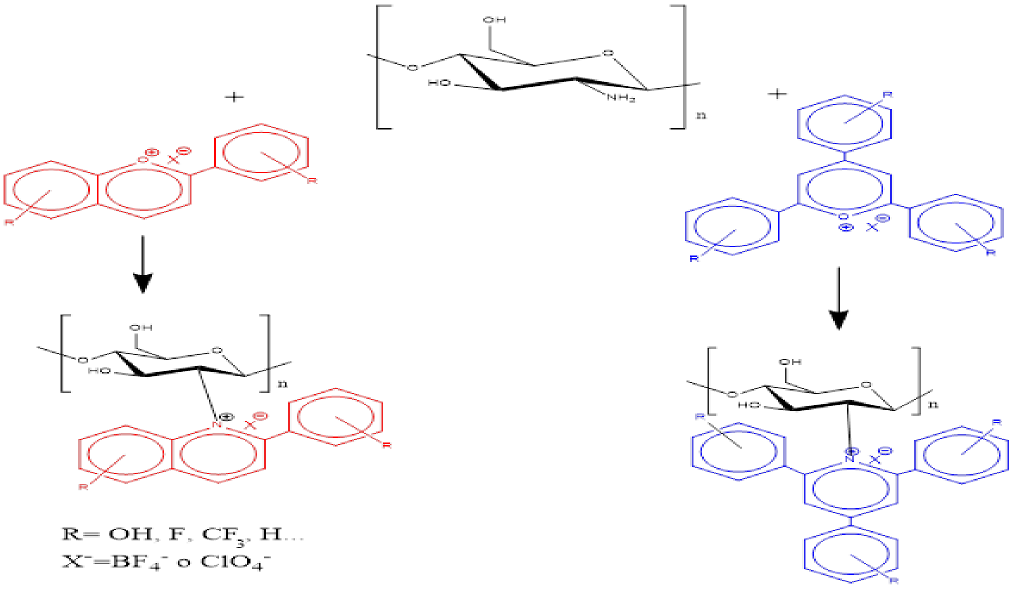
Scheme 3. Synthesis of flavonoids

The presence of symmetric Pyrylium cation are confirmed by a singlet for H-3 and H-5 at 9 ppm approximately observed in ^{1}H NMR.

One way to corroborate the presence of chalcones is through the study of the aliphatic double bond. There are two doublets in H-NMR for 1H each one, which have coupling constants of approximately 15 Hz, characteristics of the protons that are in trans-disposition.

For the rest of the compounds: assymetric pyrylium salts, anthocyanins and flavonoids structures can be checked by the same procedure above. For each compound there is a set of different signals so they do not have characteristic protons of the structures as in the case of the previous pyrylium salts. Therefore, a detailed study must be done for each of the compounds.

Finally, Based on the reactivity of positions 2 and 6 which can be attacked by a nucleophile, amino groups of chitosan have been used to obtain pyridinium/quinolinium-chitosan derivatives (Scheme 4).



Scheme 4. Synthesis of quinolinium and pyrydinium salts

Chitosan derivatives have been characterized by using ^{1}H-NMR experiments and ^{13}C-CPMAS-NMR in solid state. An effective attachment to chitosan backbone has been corroborated on the basis of diffusion NMR experiments.

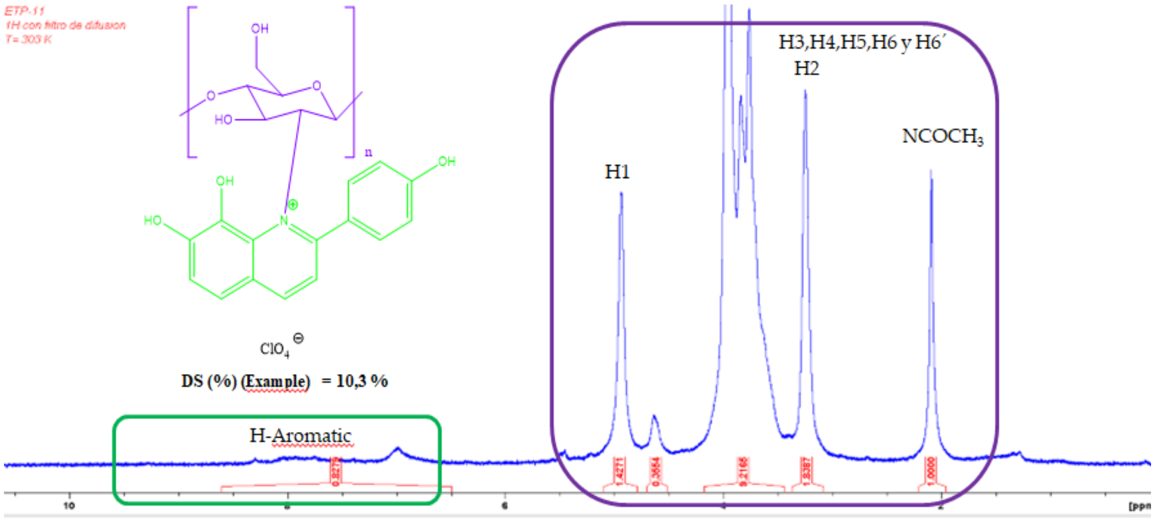


Figure 2. ^{1}H-RMN (500 MHz) of chitosan derivate solved in CD_3COOD/D_2O

In these spectra, the characteristic signals of chitosan are observed, as well as those corresponding to aromatic protons at 8.5-7 ppm. Finally, these spectra have been used to calculate the degrees of incorporation / substitution (DS). For instance: (Figure 2) .

Conclusions

Reaction of polyhydroxysubstituted acetophenones with substituted benzaldehydes not only lead to pyrylium salts as expected, but 3-desoxyanthocyanins and flavonoids are also obtained. Benzaldehydes with an hydroxy group in *ortho* possition lead to 3-desoxhyanthocyanins while acetophenones with an hydroxy group in same situation originate flavonoids. Furthermore, new quaternized derivatives of chitosan have been synthesized, specifically pyrydinium and quinolinium salts derived from chitosan by reaction of the biopolymer with the corresponding salts of pyrylium and cromylium, respectively. Finally, as future work photophysical properties and antioxidant capacity of these new compounds must be studied.

Acknowledgements

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