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A SIMPLE PROCEDURE FOR THE DEUTERIATION OF PHENOLS

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SUMMARY

A simple procedure for the deuteriation of a large range of phenols has been developed using Amberlyst 15, a polymer supported acid catalyst. A number of phenols present in olive oil have been successfully deuteriated. The aromatic protons of hydroxytyrosol are 100% exchanged under the conditions used. This method is applicable for the tritiation of phenols.

Keywords: Olive oil, hydroxytyrosol, deuteriation, tritiation, polymer supported acid catalyst.

INTRODUCTION

In recent years the popularity of olive oil has increased mainly due to its reported nutritional and health benefits. Epidemiological studies have linked the high dietary intake of natural antioxidants with lower incidence of coronary heart disease (1, 2) and certain cancers (3, 4). Olive oil contains a number of polyphenolic compounds, which are known to be antioxidants (5). The major polyphenolic compounds present in olive oil are hydroxytyrosol I and oleuropein II (6, 7). However, evidence of the absorption and disposition of these phenols in humans has not been reported (8). The

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biological fate of phenolic compounds could be determined if the appropriate analytical techniques are developed and if the sensitivity of their detection in biological matrices is increased. One way of achieving this is with the use of radiolabelled compounds. Tritium labels can be introduced post-synthetically and high specific activities can be obtained, consequently they have numerous applications in biomedical sciences. Due to their high specific activities, they are used in preference to unlabelled compounds as they can be used in small quantities, and their analysis is quicker than conventional non-radiometric methods of analysis.

It was thus of biological interest if a straightforward process for the tritiation of phenols, namely hydroxytyrosol I, could be found. Acid or base catalysed exchange reactions have previously been used for the proton exchange of phenols (9). However, the aromatic protons of hydroxytyrosol I did not exchange under these conditions.

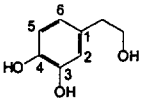
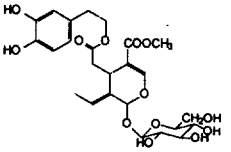
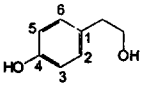
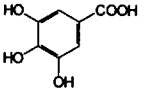
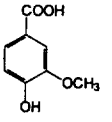
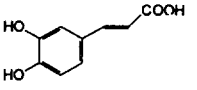
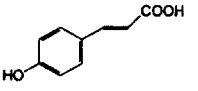
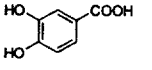
Over the past few decades a range of one step catalytic hydrogen isotope exchange procedures have been developed to deuteriate and tritiate compounds. One important discovery was by Brewer *et al*, who used ion exchange resins, in the acid form, to deuteriate and tritiate a large number of molecules (10). We have recently improved and simplified this reaction and have applied the modification to deuteriate a large number of phenols. We have initially deuteriated rather than tritiated the compounds, this is principally due to the wide availability, high isotopic purity and affordability of deuteriated water and the fact that the reactions can be monitored by ^{1}H NMR spectroscopy.

RESULTS AND DISCUSSION

The need for straightforward reaction conditions and a simple work-up procedure required modification of the procedure that had been previously published by *Brewer et al* (10). This modification removes the work-up procedure, and the desired compound is obtained after removal of the resin and freeze-drying of the remaining solution. This reaction is simple to perform, no complex glassware is required, and it can be successfully carried out on as little as 5 milligrams of substrate. The modification was only attempted with Amberlyst 15 as the polymer supported acid catalyst.

The results for some phenolic constituents in olive oil are shown in Table 1. In all cases, the extent of deuterium incorporation was determined by ^{1}H NMR spectroscopy, with complete disappearance of the signal indicating that the proton had completely exchanged.

Table 1 – Deuteriation of some phenolic constituents of olive oil.

Compound	Structure	Time (hrs)	Position	% Incorporation at each site
I		24	2,5,6	100
II		24	Did not survive conditions	-
III		24	2, 6 3, 5	10 100
IV		24	2, 6	100†
V		23	2 5 6	10 10 25
VI		24	Did not survive conditions	-
VII		24	Did not survive conditions	-
VIII		24	2 5 6	99† 98 98

Reaction mixture: substrate (5-15 mg), Amberlyst 15 resin (5-15 mg) and deuteriated water (0.2 mL). All reactions were carried out at 90°C. All reactions were carried out in a Hewlett Packard™ HPLC vial (2 mL capacity) with a magnetic stirrer (7 mm).

† In the cases where all the protons were completely exchanged analysis of the NMR solution by HPLC or TLC was required to confirm the substrate had survived the reaction.

The results show that the successful deuteriation of a number of phenols present in olive oil has been achieved. It was discovered that uniform stirring was required for optimisation of the percentage of deuterium incorporation. Oleuropein II, caffeic acid VI and *p*-coumaric acid VII gave a number of rearrangement products when heated to 90°C in the presence of Amberlyst 15. It was initially thought that these substrates may not survive the reaction conditions as the double bonds present in the substrates may react with the strong acid from the resin.

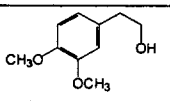
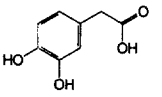
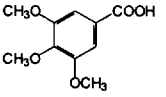
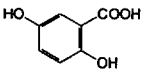
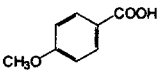
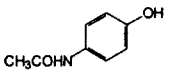
The percentage deuterium incorporation was dependent on the functional groups on the aromatic ring. The solubility of the substrate increased with the number of hydroxyl groups and the percentage of deuterium incorporated also increased. Hydroxytyrosol I gave 100% deuterium incorporation after heating at 90°C for 24 hours. However, when the reaction was stopped after 15 hours the incorporation was 100% at C6, 70% at C2 and 15% at C5. From this result, and the fact that the C2 position in tyrosol III only incorporated 10% deuterium, it can be said that the difference in the rate of deuteriation is due to the relationship of each of the protons to the hydroxyl groups present in the substrate. In these compounds the *ortho* and *para* positions are mesomerically activated and the protons in these positions will exchange preferentially, this is in accord with an electrophilic substitution type mechanism.

To test the limits of the reaction a number of phenols and aromatic ethers were reacted under the same conditions. The results are shown in Table 2.

The majority of the substrates gave good incorporation of deuterium under the reaction conditions used. Substrates XI, XIII and XV were not soluble in the reaction mixture at 90°C and this could be why incorporations of only 10-20% were obtained. However, even a poor incorporation of 20% will give a satisfactorily high specific activity for many pharmacokinetic and metabolism studies.

In conclusion, this procedure provides a straightforward and simple method for the incorporation of a deuterium label in a number of phenols. This reaction can be directly applied to the tritiation of phenols and provides a route for the synthesis of radiolabelled hydroxytyrosol I.

Table 2 – Deuteriation of aromatic compounds.

Compound	Structure	Time (hrs)	Position	% Incorporation
IX		17	2 5 6	33 66 100
X		24	2,6 overlapping 5	42 65
XI		24	2, 6	20
XII		24	3,4,6	100†
XIII		24	2,6 3,5	20
XIV		24	3,4,5,6	100†
XV		24	2,6 3,5	10 10

Reaction mixture: substrate (5-15 mg), Amberlyst 15 resin (5-15 mg) and deuteriated water (0.2 mL). All reactions were carried out at 90°C. All reactions were carried out in a Hewlett Packard™ HPLC vial (2 mL capacity) with a magnetic stirrer (7 mm.).

† In the cases where all the protons were completely exchanged analysis of the NMR solution by HPLC or TLC was required to confirm the substrate had survived the reaction.

EXPERIMENTAL

Materials. The substrates were used as received from Sigma-Aldrich, except for hydroxytyrosol I which was synthesised from 3,4-dihydroxyphenyl acetic acid X by the procedure of Capasso *et al* (11). Deuterium oxide (99.9%) was used as received from Aldrich. The Amberlyst 15 resin was available commercially (Aldrich). Prior to use it was dried over anhydrous phosphorus pentoxide at 50°C under high vacuum for 8 hours.

Hydrogen isotope exchange reaction. The substrate (5-15 mg), Amberlyst 15 (5-15 mg), deuterium oxide (0.2 mL), and a magnetic stirrer (7 mm) were introduced into a Hewlett Packard™ HPLC vial (2 mL capacity). The flask was evacuated with N_2, the top was screwed on, and the flask was placed in an oil bath. The reaction was heated at 90°C, with continuous stirring, for the desired reaction time (normally 24 hours). On completion the tube was cooled, the solution removed and water (0.2 mL) was added to the flask to rinse the resin, this solution was removed and added to the initial solution. This procedure was repeated twice, to ensure complete removal of the substrate. The recovered substrate was obtained after the combined solutions were freeze-dried.

In the cases where the substrate was not soluble in cold water (compounds IV, XI and XII), diethyl ether (2×0.2 mL) was added to the reaction vessel instead of water, to ensure complete removal of the substrate from the resin. The diethyl ether was removed in *vacuo* and the residual solution was freeze-dried.

The regiospecificity of the deuteriationwas determined by ^{1}H NMR spectroscopy on a Varian spectrometer, with an operating frequency of 200 MHz. ^{1}H resonances are quoted in parts per million downfield from the ^{1}H resonance of tetramethylsilane (TMS). Depending on the solubility of the substrate *d*-chloroform, d_6-acetone or d_2-water were used as the solvents. In the cases when all the protons were completely exchanged analysis of the NMR solution by HPLC or TLC was required to confirm that the substrate had survived the reaction conditions

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