EPR and Antioxidant Efficiency studies of Resveratrol, Quercitin and Fisetin

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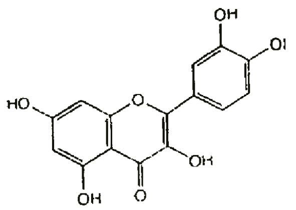
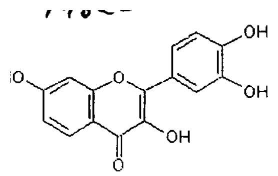
Resveratrol (a stilbene), Quercitin and Fisetin (flavonols) are polyphenols found in red wine, the last to a small extent. They behave differently physiologically in humans. Can EPR and antioxidant efficiency tests distinguish between them? The antioxidant efficiency was effectively 100% in all: both the flavonoids showed a similar free radical signal, but the Fisetin sample was clearly contaminated by transition metal ions. The Quercitin free-radical signal was markedly different.

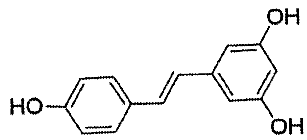
1. Introduction



Quercitin



Fisetin



trans-resveratrol

EPR (**E**lectron **P**aramagnetic **R**esonance: also ESR, E **S**pin R) is a well established spectroscopic technique exploiting the precession of electron spins about a constant magnetic field [1]. It can reveal free radicals and certain paramagnetic ions. Its use in the wine industry has been established by this group[2]. Stable free radicals in wines occur mainly on polyphenols, [2], known to be antioxidants. The most potent antioxidant in red wine is the stilbene Resveratrol [3]. Quercitin is the most abundant flavonol found in red wine [4]. Both these compounds now appear in 'dietary supplements'. The flavonol Fisetin is also in red wine but to a much lesser extent. It has recently been shown to improve the memory of laboratory mice, which Quercetin does not do. Is this a matter of different free radical behaviour, or of the stereochemistry? How different are the antioxidant efficiencies? These were the problems we set out to investigate in these three molecules.

2. Sample preparation

Resveratrol powder (99% pure) was obtained from China. Both Quercetin and Fisetin were purchased in solid powder form (99% pure) from Sigma. A specimen of each was placed in a standard EPR quartz tube (Wilmad), and the EPR measurements done at room temperature on all 3, and at 77k for Resveratrol. A Bruker X-band (~ 9.4 GHz) spectrometer was used.

The sample preparation and the antioxidant efficiency test are described in [5]. Briefly, free radicals are created in a solution at a constant rate, giving an increasing absorption in the UV. An antioxidant will decrease the absorption, so an efficiency can be calculated. Since Resveratrol not attached to a sugar is almost insoluble in water, the antioxidant efficiency tests were carried out in methanol instead of aqueous solution.

3. Results

3.1 EPR measurements

The EPR spectrum of Resveratrol over a 100 Gauss sweep was a single structureless line ~8 Gauss wide, which at room temperature did not reach maximum amplitude (saturate) until the power was 100mW. To test for temperature broadening of the line, the measurements were repeated at 77k, but the linewidth stayed the same. This is quite different to the flavonol behaviour described below.

The EPR spectrum of Quercitin over a 100 Gauss sweep was a single structureless line, ~ 4 Gauss wide, which reached maximum signal strength at 2 mW power, and then decreased with power increase (saturation.). The 1mW power spectrum of the Fisetin sample is shown in Fig. 1: the free radical line is marked.

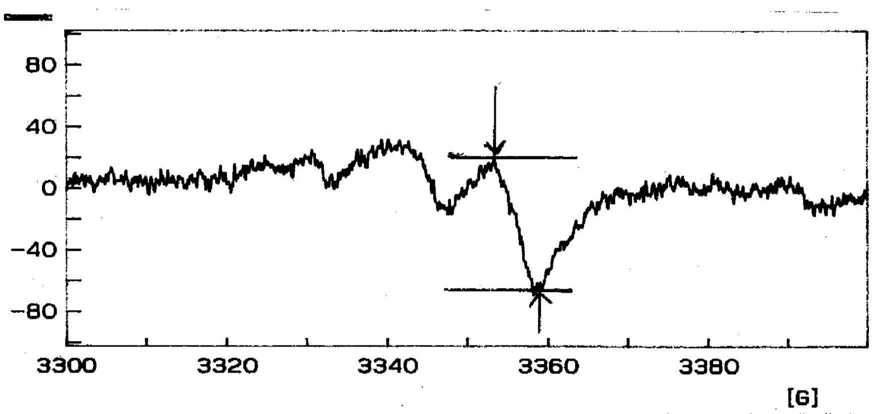


Figure. 1 EPR spectrum of 'fisetin', power 1mW. Axes: horizontal, magnetic induction, Gauss; vertical, signal strength, arbitrary units.

How was this line identified? - by its behaviour in the 15 mW spectrum (Fig. 2). The marked line has decreased in size, while the other lines have increased. The other lines therefore are not from the phenolic radical, and are due to transition metal ion impurities.

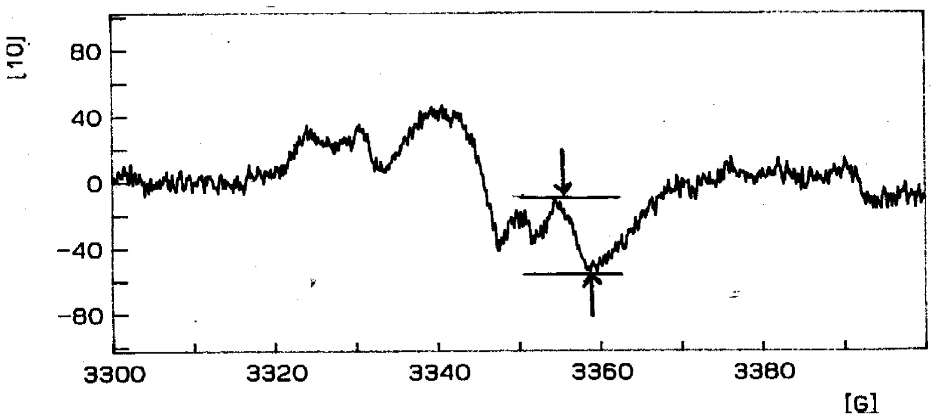


Figure.2. Same as for Fig.1, but power 15mW.

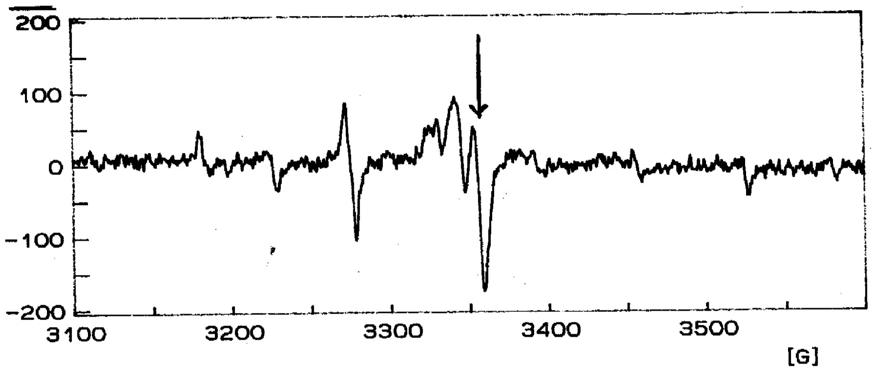


Figure.3 Broader magnetic induction range for fisetin, power 2mW. Axes as for Fig.1.

Fig. 3 shows a broader sweep spectrum at 2mW. Some of the smaller lines (regularly spaced) can be attributed to Mn(2+): the larger line left of centre can be assigned to Cu(2+), and the large line next to the free radical to Fe(3+), by g-values (See details for individual ions in [1]).

3.2 Antioxidant efficiency.

The antioxidant efficiency results for both flavonoid specimens were 100 +/- 5%. That for vitamin E was its usual 95 +/- 5%. The Result for Resveratrol in methanol was 96%, while for vitamin E was 91%, both +/- 5%.

4. Discussion.

The EPR behaviour of Resveratrol is very different from that of the flavonoids. The high antioxidant efficiency is to be expected from previous studies [2]. It may well be that the EPR behaviour, as well as the structure of this molecule, are responsible for its antioxidant and other properties.

The Quercetin and Fisetin EPR behaviours are very similar, perhaps to be expected from their structural similarity. The powerful antioxidant behaviour of Quercetin is well known. The problem is, how much of the measured antioxidant efficiency of the Fisetin sample was due to the metal antioxidant impurities? We need a pure sample to know.

However, to our knowledge, the purity of commercially available samples is measured via Thin Layer Chromatograpy or High Performance Liquid Chromatography, which previous experience has shown DO NOT DETECT PARAMAGNETIC IONS!

Note added during Conference

Professor G. Scollary, Wine Science and Agriculture, Charles Sturt University, informed us that the performance of the well known antioxidant, ascorbic acid, which is used in the wine industry, is affected by the presence of transition metal ions!

References

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