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Polyhydroxy Chalcones and Flavanones as Antioxidants for Edible Oils

Stanley Z. Dziedzic & Bertram J. F. Hudson

Department of Food Science, University of Reading,
London Road, Reading RG1 5AQ, Great Britain

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ABSTRACT

A series of 11 polyhydroxy chalcones, and in 4 cases, flavanones derived from them by isomerisation, some of which occur naturally in plant tissues, have been shown to be potent antioxidants for lard. Most of these compounds, not being commercially available, were prepared synthetically. Two of them, 3',3,4-trihydroxy- and 2',4',2,3-tetrahydroxy chalcone, are described for the first time.

3,4-Dihydroxy chalcones, such as butein and okanin, are particularly effective antioxidants in the range of concentrations 0·025–0·1%, as judged by induction period measurements. Chalcones are more effective than the corresponding flavanones.

The hypothesis is put forward that the effectiveness of 3,4-dihydroxy chalcones is dependent on the formation of extended forms of resonance-stabilised free radicals.

INTRODUCTION

Recent studies (Pratt, 1977; Hudson & Lewis, 1983*a*, 1983*b*) have revived the interest in polyhydroxy flavonoid natural products as antioxidants in lipid systems. Particular attention has centred on the polyhydroxy flavonols and flavanols, on account of their ubiquitous occurrence, both as glycosides and aglycones, in vegetable tissue, especially in leaves and heartwoods. It seemed unlikely, however, that antioxidant activity should be confined to these two classes of flavonoids, and in a subsequent study it

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was shown (Dziedzic & Hudson, 1983) that the closely related polyhydroxy isoflavones also exhibited antioxidant properties.

In our first communication (Hudson & Lewis, 1983*a*) we included in our study the naturally occurring chalcone, butein, which is found in *Acacia* heartwood and, as its 4′-glucoside, in the petals of *Coreopsis* spp. (Geissman, 1941). Butein has a very close structural relationship to the tetrahydroxy flavone, luteolin. It proved to be a very potent antioxidant for lard, being about three times as active, weight for weight, as luteolin.

The yellow chalcones are biochemical precursors of the flavone and dihydro flavone series, being transient intermediates between the hydroxy cinnamic acids and the flavones. They are scarcer, as natural products, than either of these classes of compounds but it seemed appropriate, in view of the observations with butein, to investigate them further as a class. Moreover, they are relatively simple molecules, readily accessible through synthesis when not available by extraction from natural sources. Finally, it was hoped that, by comparing the activities of different hydroxy and polyhydroxy chalcones, some further understanding could be reached regarding the relationship between structure and antioxidant activity in this class of compounds.

Certain flavanones also had logically to be included in this study since 2′-hydroxy chalcones readily isomerise under acidic conditions to yield the corresponding colourless flavanones. Although such chalcones are sufficiently stable to be handled as such in the absence of acid, under the conditions of test it is likely that they are converted to flavanones.

MATERIAL AND METHODS

As substrate for the oxidation studies, pure dried rendered lard, free from additives and not chemically processed, was used.

2′,4′,6′,3,4-Pentahydroxy chalcone and eriodictyol were purchased from Apin Chemicals Ltd, Abingdon, Oxon. The other chalcones and flavanones, which were not commercially available, were synthesised, either (a) by condensation in strong aqueous alkali of the appropriate phenolic acetophenones and benzaldehydes, or (b) by condensation of the appropriate methyl esters in 2 M sodium methoxide, followed by demethylation by pyridinium hydrochloride. Flavanones were prepared by acid or base catalysed isomerisation of 2′-hydroxy chalcones.

All potential antioxidants were repeatedly recrystallised to chromato-

TABLE 1
Synthetic Chalcones and Flavanones

	Melting point (°C)	
	Observed	*From the literature*
Chalcones		
4-Hydroxy[a]	182	183–184 (Shriner & Kurosawa, 1930)
3,4-Dihydroxy[a]	203–204	204–205 (Kurth, 1939)
2',4'-Dihydroxy[b]	150	150 (Saiyad *et al.*, 1937)
3',4'-Dihydroxy[a]	186–187	187 (Klinke & Gibian, 1961)
2',3,4-Trihydroxy[b]	183–185	185–186 (Kurth, 1939)
3',3,4-Trihydroxy[b]	202–204	Not recorded[c]
4',3,4-Trihydroxy[b]	202–204	203–204 (Klinke & Gibian, 1961)
2',4',2,3-Tetrahydroxy[b]	208–210	Not recorded[d]
2',4',3,4-Tetrahydroxy[b]	213–214	213–214 (Geissman, 1941)
2',3',4',3,4-Pentahydroxy[b]	248–249	249 (Kurth, 1939)
Flavonones		
3',4'-Dihydroxy	185–187	188 (Kurth, 1939)
7,3',4'-Trihydroxy	223–225	224–226 (Saiyad *et al.*, 1937)
7,8,3',4'-Tetrahydroxy	252–254	252–254 (Kurth, 1939)

[a] Condensation by method (b).
[b] Condensation by method (a).
[c] Deep yellow needles from aqueous ethanol, $\lambda_{max(EtOH)}$ 396 nm and 272 nm.
[d] Orange prisms from aqueous ethanol, $\lambda_{max(EtOH)}$ 391 nm and 257 nm (inflexion).

graphic standards of purity as judged by TLC on Merck silica F_{524} plates (elution with toluene/ethyl acetate/acetic acid, 25:3:1 v/v/v). Chromatograms were visualised under ultraviolet light, then reinspected after spraying with 10% H_2SO_4 in ethanol followed by brief heating at 110 °C, or after dipping plates in 1% ethanolic $FeCl_3$.

The synthesised chalcones and flavanones are listed in Table 1, and characterised by melting point (Electrothermal Apparatus, uncorrected). Antioxidant activity was evaluated by measurement of induction periods after incorporation in lard using the Metrohm Rancimat (Hudson & Lewis, 1983*a*).

RESULTS

Induction periods (Rancimat) at 100 °C are listed for a series of hydroxy and polyhydroxy chalcones in Table 2, each compound being evaluated at

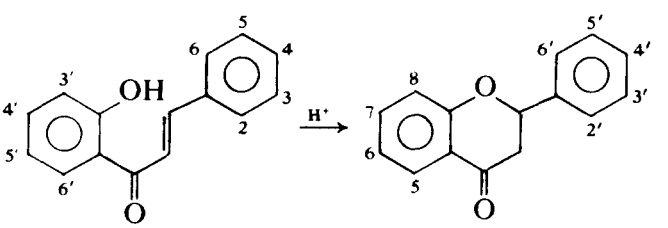
TABLE 2
Induction Periods at 100 °C (Rancimat) of Hydroxy Chalcones in Lard
(The Induction Period for Lard Without Additives is 1·3 h)

Hydroxy chalcone	*Induction periods (h) at stated concentrations*		
	0·025%	*0·05%*	*0·1%*
4-Hydroxy	2·4	3·4	5·1
3,4-Dihydroxy	40	72	126
2′,4′-Dihydroxy	1·3	1·3	1·3
3′,4′-Dihydroxy	17	33	56
2′,3,4-Trihydroxy	48	84	135
3′,3,4-Trihydroxy	50	74	122
4′,3,4-Trihydroxy	49	88	110
2′,4′,2,3-Tetrahydroxy	44	70	103
2′,4′,3,4-Tetrahydroxy[a]	50	94	161
2′,3′,4′,3,4-Pentahydroxy[b]	60	97	152
2′,4′,6′,3,4-Pentahydroxy	22	40	73

[a] Butein.
[b] Okanin.

three concentrations in lard. Corresponding data for certain related flavanones are given in Table 3.

2′-Hydroxy chalcones are converted in the presence of protons to the isomeric flavanones:



Chalcone Flavanone

Chalcones lacking 2′- or 6′-hydroxyl substituents are unable to undergo this transformation. In the course of the test, carboxylic acids are formed as degradation products. Hence, it is to be expected that 2′-hydroxy chalcones will be converted to the corresponding flavanones and induction periods for both starting materials will be rather similar. For this reason, the data in Table 3 should be compared with those of the

TABLE 3
Induction Periods at 100°C (Rancimat) of Flavanones in Lard

Flavanones	*Induction periods (h) at stated concentrations*		
	0·025%	*0·05%*	*0·1%*
3′,4′-Dihydroxy[a]	27	48	90
7,3′,4′-Trihydroxy[b]	29	49	79
5,7,3′,4′-Tetrahydroxy[c]	22	35	79
7,8,3′,4′-Tetrahydroxy[d]	50	84	146

[a] From 2′,3,4-trihydroxy chalcone.
[b] Butin, from butein.
[c] Eriodictyol, from 2′,4′,6′,3,4-pentahydroxy chalcone.
[d] From okanin.

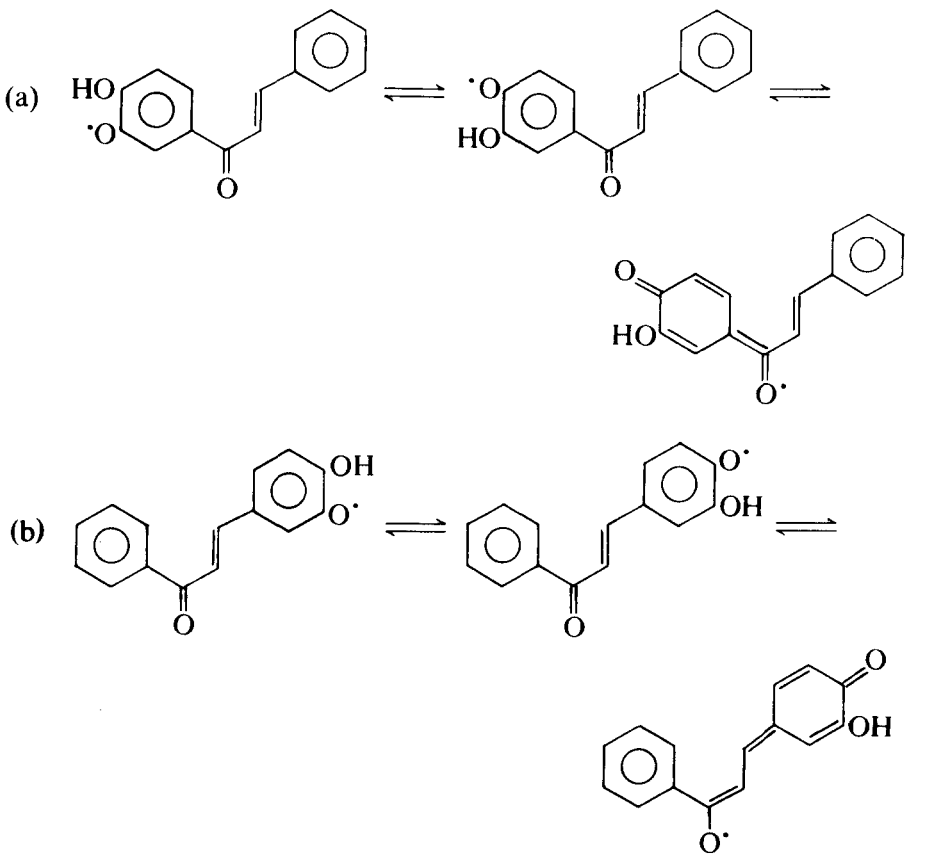
corresponding chalcones in Table 2. Care must be taken to note the rather confusing numbering systems for chalcones vis-à-vis flavanones.

DISCUSSION

From Tables 2 and 3 it is clear that a single hydroxy group generates little or no antioxidant activity in chalcones or flavanones (2′,4′-dihydroxy chalcone is probably converted to 4-hydroxy flavanone in the course of the test). The 3,4-dihydroxy chalcones are, however, fully effective, as is also, though to a much reduced extent, 3′,4′-dihydroxy chalcone. 3′,4′-Dihydroxy flavanone is also effective, though significantly less so than the 2′,3,4-trihydroxy chalcone from which it is derived. The 3′ and 4′ isomers of the latter show a similar order of activity. Further hydroxylation, as in butein and okanin, leads to slight enhancement of activity, butein being somewhat more active than its 2-analogue.

The data obtained with 3,4- and, to a lesser extent, 3′,4′-dihydroxy chalcones, incorporating the catechol configuration, illustrate the overriding importance of this grouping. A comparison of the highly active okanin (2′,3′,4′,3,4-pentahydroxy chalcone), which contains two such groupings, with its 2′,4′,6′,3,4 isomer which contains only one and is markedly less active, reinforces this point. The same feature can be noted in the polyhydroxy flavanones, eriodictyol being much less active than the isomeric 7,8,3′,4′-tetrahydroxy flavanone.

The significance of the catechol configuration in relation to the molecule as a whole probably resides in the possibility of resonance-stabilised free radical formation, a mechanism that is generally accepted as important for primary antioxidant activity. The extended resonance form shown in reaction scheme (b) below is likely to be more stable than the shorter form in reaction scheme (a), in line with the superiority of 3,4- over 3′,4′-dihydroxy chalcone as an antioxidant, and of butein over 2′,4′,2,3-tetrahydroxy chalcone.



The catechol configuration occurs in a number of known natural antioxidants, e.g. in gallic and caffeic acids, in hydroxy chavicol and in nordihydroguaiaretic acid. A particularly interesting recent observation has been the identification of another substituted catechol, carnosic acid, the highly active antioxidant in oil of rosemary (Bracco *et al.*, 1981).

Views have been expressed, based on studies with polyhydroxy flavones and dihydroflavones, that the presence in the molecule of 3- and/or 5-hydroxy groups favours, or even may be essential for, antioxidant activity. Such hydroxy groups can interact by hydrogen bonding with the carbonyl or with trace, possibly pro-oxidant, metals such as copper to form chelates (Letan, 1966; Pratt, 1977; Hudson & Lewis, 1983*a*). Such views are not strengthened by our work on chalcones and flavanones since some of the most active compounds, e.g. 3,4-dihydroxy chalcone and 7,8,3′,4′-tetrahydroxy flavanone cannot support any such interactions. It must be concluded that in the well known antioxidants quercetin and taxifolin, for example, the antioxidant activity is basically due to the 3′,4′-dihydroxy configuration, and that the 3- and 5-hydroxyl group are of no special significance.

In conclusion, some of the polyhydroxy chalcones and flavanones are amongst the most active antioxidants, under suitable conditions, yet studied. Butein, for example, at 0·02 % in lard at 100 °C is approximately twice as active, weight for weight, as quercetin and twice as active as α-tocopherol, though slightly less active than γ-tocopherol or δ-tocopherol. Compared with synthetics under the same conditions, it is about 6 times as active as BHT.

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