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THE USE OF QUERCETIN SULPHATES FROM *FLAVERIA BIDENTIS* AS INHIBITORS OF THE ENZYMIC REDUCTION OF CARBONYL GROUPS

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Key Word Index—*Flaveria bidentis*; Compositae; enzyme inhibitors; sulphated flavonoids; quercetin; aldehydes; dehydrogenases.

Abstract—Quercetin tetrasulphate and quercetin acetyl trisulphate from *Flaveria bidentis* are non-competitive inhibitors of the reduction of carbonyl groups catalysed by alcohol, lactic and malic dehydrogenases with K_i/K_m values of 3×10^{-3} to 2.0. They do not affect the dehydrogenation of alcohol groups nor do they inactivate these enzymes.

INTRODUCTION

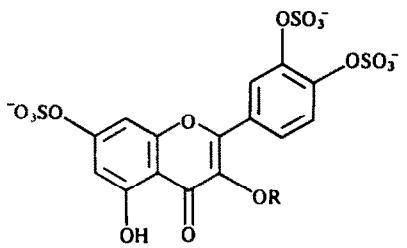
The biosynthesis of *E*- and *Z*-sesquiterpenoids occurs either through stereospecific synthesis of the isomeric pyrophosphates or through a redox isomerization of prenols mediated by dehydrogenase–reductase activities [1–4]. The coexistence of these pathways finds support in experiments with an aldehyde trapping reagent [2, 5]. It was interesting to investigate the effect of more specific compounds on the enzymic steps involved in this redox mechanism and we thought that some known secondary metabolites could be useful inhibitors of dehydrogenases.

Several flavonoids inhibit aldose reductase (EC 1.1.1.21) with $I_{0.5}$ values of 10^{-6}–10^{-8} M [6–9]. The effect is not specific for aldose reductase [10, 11] since flavonoid inhibition of other reductases has been reported, and there are also negative reports on the effects on dehydrogenases and glycolytic enzymes [6, 8, 9]. Most studies were only performed either on the oxidation or on the reverse reduction.

Since the specificity pattern of flavonoid inhibition was not clear, it seemed necessary to test their effect on different dehydrogenases in both directions, before employing them in the study of prenol isomerization [1–5, 12]. The model enzymes tested were selected according to their availability in a purified state. We also considered similarities and differences with the well known reaction catalysed by aldose reductase, as well as with the redox interconversion of prenols [1]. We used two sulphated flavonoids (1 and 2) which are inhibitors of lens aldose reductase [13], to avoid solvent effects [14] and because they can be easily isolated from plants of the South American flora [13, 15].

RESULTS AND DISCUSSION

The effect of the two sulphated quercetin derivatives, QTS and QATS, on the forward and reverse reaction



1 QTS R = SO_3^-
2 QATS R = Ac

catalysed by different dehydrogenases is summarized in Table 1. They are non-competitive or mixed type inhibitors of alcohol, lactic and cytosolic malic dehydrogenases, only when the enzyme is acting as a carbonyl reductase.

The effectiveness of sulphated flavonoids as estimated by the K_i/K_m ratio is maximal for the reduction of acetaldehyde to ethanol by yeast alcohol dehydrogenase with a K_i/K_m ratio of 2.9×10^{-3}, for the carbonyl substrate, i.e. comparable to their effect on the reduction of glyceraldehyde to glycerol [7, 9, 13] by aldose reductase from lens. In the three enzymes inhibited by QATS or QTS the value of K_i for NADH was substantially similar to its K_m.

QTS and QATS (10^{-4} M) have no inhibitory effect on polyol dehydrogenase (EC 1.1.1.14) from *Candida utilis* in either direction. This may be due to the fact that the K_m of 0.2 M for fructose does not allow a high enough ratio of inhibitor/substrate concentration. This consideration is not valid for NADH (Table 1). The oxidation of glyceraldehyde-3-phosphate (muscle enzyme) and glucose-6-phosphate (yeast enzyme) is not inhibited by 10^{-4} M QTS or QATS, which agrees with other negative reports [8, 9]. The inhibition was not modified if the order of addition of substrates and enzyme was changed.

QTS and QATS are not time-dependent inactivators. Preincubation of alcohol, malic or lactic dehydrogenases with 2 mM QTS or QATS, under the same conditions of the assay but in the absence of substrates, does not

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Table 1. Effect of QTS and QATS on the activities of different dehydrogenases

Enzyme	Substrate	K_m (M)	K_i (M)		K_i/K_m	
			QTS	QATS	QTS	QATS
Alcohol dehydrogenase, yeast (EC 1.1.1.1)	Ethanol	1.7×10^{-5}	n.i.	n.i.	—	—
	NAD^+	4.6×10^{-4}	n.i.	n.i.	—	—
	Acetaldehyde	1.1×10^{-3}	n.i.	3.2×10^{-6} (NC)	—	2.9×10^{-3}
	NADH	3.9×10^{-5}	n.i.	6.0×10^{-6} (NC)	—	0.15
Lactic dehydrogenase, rabbit muscle (EC 1.1.1.27)	Lactate	5.5×10^{-3}*	n.i.	n.i.	—	—
	NAD^+	1.0×10^{-5}*	n.i.	n.i.	—	—
	Piruvate	4.0×10^{-4}	n.i.	3.8×10^{-5} (NC)	—	9.5×10^{-2}
	NADH	3.9×10^{-5}	n.i.	2.4×10^{-5} (NC)	—	0.62
Malic dehydrogenase (porcine heart) cytosolic‡ (EC 1.1.1.37)	Malate	9.0×10^{-4}†	n.i.	n.i.	—	—
	NAD^+	1.0×10^{-5}†	n.i.	n.i.	—	—
	Oxalacetate	2.2×10^{-5}	7.0×10^{-5} (MX)	4.8×10^{-5} (MX)	3.2	2.2
	NADH	3.7×10^{-5}	2.8×10^{-5} (NC)	7.5×10^{-5} (NC)	0.76	2.02
Polyol dehydrogenase, *Candida utilis* (EC 1.1.1.14)	Sorbitol	7.2×10^{-2}	n.i.	n.i.	—	—
	NAD^+	2.0×10^{-3}	n.i.	n.i.	—	—
	Fructose	0.2	n.i.	n.i.	—	—
	NADH	2.9×10^{-5}	n.i.	n.i.	—	—

* From ref. [19].
† From ref. [20].
‡ The effect on the mitochondrial enzyme is very similar.
MX, Mixed type inhibition; NC, non-competitive inhibition; n.i., no inhibitory effect observed with 10^{-4} M flavonoids. The concentrations of flavonoids tested ranged from 0.3 to 110×10^{-6} M.

diminish the enzyme activity after sufficient dilution of the inhibitor.

The inhibition shows a peculiar pattern: some dehydrogenases were not affected at all and thus these flavonoids are not general inhibitors of nicotinamide-linked dehydrogenases. Inhibitory effects were observed only in one direction. The non-competitive or mixed type of inhibition shows that these sulphated flavonoids do not bind exclusively to the free enzyme, but to some form of enzyme–substrate complex [16].

The absorption spectra of QTS or QATS were not modified by the addition of acetaldehyde, pyruvate, oxaloacetate or NADH in the absence of enzyme, and any observed increase in absorbance due to the added substrate was simply additive. This excludes the possibility of a direct interaction of the sulphated flavonoids with the substrates, that could have reduced their concentration and simulated an inhibition.

The one-directional effectiveness of QTS and QATS may be used to explore the kinetic mechanism of other dehydrogenases or reductases. Since aldehydes participate in the redox mechanism of prenols [1–4, 12], QTS and QATS may be conveniently used in the study of this pathway and experiments along this line are in progress in this laboratory.

EXPERIMENTAL

Substrates, nicotinamide dinucleotides and the dehydrogenases were purchased from Sigma. QTS and QATS were extracted from *Flaveria bidentis* O. Kuntze and their purity checked as described [13, 15]. They were added as aq. solns, pH 7.8, to the reaction medium. Their absorbance at 340 nm (log ε for QTS = 3.92; for QATS = 4.11) does not permit concns above 10^{-4} M in the assay. The forward and/or reverse reaction of dehydrogenases were assayed recording the changes of absorbance at 340 nm as described under the heading of each particular enzyme in refs [17] and [18]. Constant spectrophotometer slit was used in all assays. Kinetic parameters and K_i values were calculated through double reciprocal and secondary plots [16]. Spectra of the incubation media (200–420 nm) were run in the absence of the enzyme; non enzymic controls were performed with heat inactivated enzyme.

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