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EFFECTS OF SUBSTITUENTS IN THE A-RING ON THE PHYSIOLOGICAL ACTIVITY OF FLAVONES

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Key Word Index—Flavones; scutellarein; quercetagetin; gossypetin; indole-3-acetic acid oxidase; ATP formation.

Abstract—The flavonoids quercetagetin, gossypetin and scutellarein 7-glucoside which have *o*-dihydroxyl groups in the A ring inhibit indole-3-acetic acid oxidase. Since scutellarein 7-glucoside has no such grouping in the B-ring it is clear that it is the hydroxyls in the A-ring that cause the inhibition. The presence of *o*-dihydroxyls in the A-ring also decreases the inhibitory effect upon ATP formation in mitochondria.

The potential effects of flavonoids upon the enzymatic destruction of indoleacetic acid through "IAA oxidase" are well established. Substances with two hydroxyl groups in *o*-positions in the B-ring are inhibitors whereas substances with one hydroxyl in the 4'-position normally are stimulators [1]. The effect of the hydroxyls in the A-ring is not equally clear. Substances lacking substituents in the B-ring which possess single hydroxyls in the A-ring or two hydroxyls at the "normal" 5 and 7 positions do not seem to be stimulatory [2] (e.g. monosubstituted anthocyanidins, the flavone chrysin and the flavanones pinocembrin and pinobanksin). Thus, it is not clear whether flavonoids with two or more hydroxyls at adjacent positions in the A-ring are inhibitors. Therefore some substances with extra hydroxyl groups in addition to the normal 5- and 7-hydroxyls were tested for their effects upon IAA oxidase (Tables 1 and 2). Gossypetin and quercetagetin as well as scutellarein-7-glucoside proved to be strong inhibitors of IAA oxidase.

In the case of quercetagetin (6-hydroxyquercetin) and gossypetin (8-hydroxyquercetin) it is not clear whether the hydroxyls in the A-ring contribute to the inhibition, since the parent substance quercetin is inhibitory due to its two hydroxyls in the B-ring. The result with scutellarein, or 6-hydroxy-apigenin is significant since apigenin itself is distinctly stimulatory to IAA oxidase [2]; in this case the two *o*-positioned 5- and 6-hydroxyls in scutellarein-7-glucoside must give rise to the inhibition of IAA oxidase. Substitution with glucose at position 6 in apigenin-7-glucoside to give the *C*-glucoside saponarin does not reverse the stimulatory affect of apigenin (Table 3). It is possible that the 4'-hydroxyl is necessary for or increases the inhibitory activity as it may facilitate the binding of the molecule at some critical site. It has not yet been possible to test this since a sample of baikalein (5,6,7-trihydroxyflavone) has not been available.

Many flavonoids inhibit ATP production. The substances with extra hydroxyl groups in the A-ring were also tested for their effects upon ATP formation in plant mitochondria. From tests with other flavonoids, it is obvious that increasing hydroxylation often leads to a less pronounced inhibition of ATP formation [3]. This

Table 2. Effect of scutellarein 7-glucoside upon the destruction of IAA by IAA oxidase in the presence of phloridzin or 2,4-dichlorophenol

Conc. of scutellarein 7-glucoside, M	Stimulator added		
	Phloridzin 10^{-5} M	2,4-dichlorophenol 10^{-6} M	2,4-dichlorophenol 3×10^{-6} M
10^{-6}	101	—	104
3×10^{-6}	99	88	108
6×10^{-6}	76	69	—
10^{-5}	17	47	47
3×10^{-5}	0	0	0

The values expressed as % of destruction in control with only phloridzin or 2,4-dichlorophenol added.

Table 1. Effect of some flavonols upon the destruction of IAA by IAA oxidase in the presence of phloridzin (10^{-5} M)

Conc. of flavonol, M	Flavonol added		Quercetagetin 7-glucoside
	Gossypetin	Quercetagetin	
10^{-6}	57	69	—
3×10^{-6}	14	7	—
10^{-5}	5	0	8
3×10^{-5}	0	0	0

The values expressed as % of destruction of IAA in control medium with only phloridzin added.

Table 3. Effect of saponarin upon the destruction of IAA by IAA oxidase

Conc. of saponarin				
0	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M
107	121	140	199	276

The values expressed as nmol IAA destroyed/sample.

Table 4. Effect of some flavones on the formation of ATP in mitochondria from hypocotyls of cucumber

Flavone added	Conc. 10^{-4} M	Conc. 3×10^{-4} M
Quercetin	40	12
Quercitrin	79	64
Quercetagetin	74	39
Quercetagetin 7-glucoside	94	86
Gossypetin	75	51
Apigenin	28	
Scutellarein	45	
Scutellarein 7-glucoside	85	

The values expressed as % of ATP formation in control medium without addition of flavone.

conclusion is further supported by the results with quercetagetin, gossypetin and scutellarein which are all less inhibitory than the corresponding parent substances quercetin and apigenin (Table 4). Quercetagetin 7-glucoside and scutellarein 7-glucoside are still less active than the corresponding aglucones, which agrees well with results obtained with other flavonoids. For many flavonoids tested in various processes it has been found that the glycosides are less inhibitory than the aglycones [4]. The inhibitory activity against ATP formation seems to increase with increasing lipophilic properties as the hydrophilic sugars and hydroxyl groups diminish the inhibitory activity. The activity against IAA oxidase is not connected to the structure in the same manner, because the enzyme activity in this case is not membrane-bound.

EXPERIMENTAL

The effects upon IAA oxidase prepared from wheat roots and upon ATP formation in mitochondria from cucumber hypocotyls were studied as described elsewhere [5].

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