Flavonoid Antagonism of the Spasmogenic Effect of Angiotensin, Bradykinin, and Eledoisin on Guinea Pig Ileum

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Abstract □ Investigation of the antagonism of the spasmogenic effect of three polypeptides by 12 flavonoids revealed that generally, the aglucones were more potent than the glycosides. Against angiotensin the order of potency was homoeriodictyol, quercetin, morin, rhamnetin, and 4-methylesculetin; against bradykinin it was quercetin, rhamnetin, and homoeriodictyol; and against eledoisin it was quercitrin, morin, rhamnetin, and catechin. The other flavonoids showed varying degrees of activity but they tended to show less activity as their doses were increased. Structure-activity evaluation revealed that the most active compounds had free phenolic hydroxyl groups in the 5,7-positions of the γ-benzopyrone nucleus. Other structural requirements were also discussed.

Keyphrases □ Angiotensin, spasmogenic activity—flavonoid effect □ Bradykinin, spasmogenic activity—flavonoid effect □ Eledoisin, spasmogenic activity—flavonoid effect □ Structure-activity relationship—flavonoid antagonism polypeptide activity

In 1966, Gascon and Walaszek (1) showed that the flavonoid compound, osajin [5,8-dihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano(3,2-*b*)xanthen-6-one], could counteract the spasmogenic effects of angiotensin on the guinea pig ileum. Later, Leme and Walaszek (2) observed similar effects of the flavonoids, apiin (4′,5,7-trihydroxyflavone-7-apioseglucoside) and hesperidin (3′,5,7-trihydroxy-4′-methoxyflavanone-7-rhamno glucoside), as antagonists for bradykinin and eledoisin. With the view to establish possible structure-activity relationships for this antagonism of the spasmogenic activity of the three synthetic polypeptides, twelve other flavonoid compounds have been investigated.

METHODS AND MATERIALS

Segments of guinea pig ileum, 30 mm. in length, were suspended in a 40-ml. bath containing Locke-Ringer solution (NaCl, 9 g.; KCl, 0.42 g.; $CaCl_2$, 0.24 g.; $MgCl_2$, 0.2 g.; $NaHCO_3$, 0.5 g.; glucose, 0.5 g., and distilled water, 1 l.) thermostatically regulated to 37.5 ± 0.2°. The pH of the bathing solution was 8.2. The flavonoids were solubilized with a few drops of 0.1 *N* NaOH, then diluted with Locke-Ringer solution. The polypeptides were also dissolved in Locke-Ringer solution, and their spasmogenic doses per milliliter were: angiotensin, 1 mcg.; bradykinin, 1 mcg.; and eledoisin, 80 ng. The following flavonoids were all tested in the dosage range of 0.65 to 2,000 mcg./ml.: esculetin, esculin, homoeriodictyol, 4-methylesculetin, morin, phloridzin, quercetin, quercitrin, rottlerin, and xanthorhamnin. The dosage range for catechin was 1,000 to 30,000 mcg. The flavonoids were allowed to act for 2 min. prior to the addition of the polypeptides. Five ileal strips were used for each flavonoid and, where possible, the results analyzed statistically by the Litchfield-Wilcoxon method (3).

RESULTS

Antagonism of Angiotensin—Table I indicates the following order of descending potency: homoeridictyol, quercetin, morin, rhamnetin, and 4-methylesculetin. It was not possible to calculate the ED_{50} for xanthorhamnin, quercitrin, or phloridzin because

Table I—Antagonism of Angiotensin

Common Name	Chemical Name	Type	ED_{50} and Range, mcg./ml.	Slope and Range	Potency
Homoeriodictyol	4′,5,7-Trihydroxy-3′-methoxy-flavanone	Aglucone	100 (24–413)	11.5 (6.4–22.42)	
Quercetin	3,3′,4′,5,7-Pentahydroxy-flavone	Aglucone	290 (112–750)	3.4 (.66–17.34)	
Morin	2′,3,4′,5,7-Pentahydroxy-flavone	Aglucone	600 (200–1800)	5.9 (1.76–17.7)	
Rhamnetin	3,3′,4′,5-Tetrahydroxy-7-methoxy flavone	Aglucone	1000 (285–3500)	6.9 (1.1–42.78)	
4-Methylesculetin	4-Methyl-6,7- dihydroxy-coumarin	Aglucone	1450 (725–2800)	2.7 (1.5–5)	
Quercitrin	3,3′,4′,5,7-Pentahydroxy-flavone 3-L-rhamnoside	Glycoside			29% max. at 25 mcg.
Xanthorhamnin	3,3′,4′,5-Tetrahydroxy-7-methoxy flavone, 3-trirhamnoside	Glycoside			34% max. at 500 mcg.
Phloridzin	2-Glycoside of the chalcone of 2,4,6,4′-tetrahydroxy-flavanone	Glycoside			20% max. at 1,000 mcg.
Rottlerin	Structure unknown contains phloroacetophenone group $C_{31}H_{30}O_8$ or $C_{30}H_{28}O_8$	Aglucone			No block
Esculetin	6,7-Dihydroxycoumarin	Aglucone			No block
Esculin	6,7-Dihydroxycoumarin 6-glycoside	Glycoside			No block

Table II—Antagonism of Bradykinin

Common Name	Chemical Name	Type	ED_{50} and Range, mcg./ml.	Slope and Range	Potency
Quercetin	3,3′,4′,5,7-Pentahydroxy-flavone	Aglucone	36 (20–66.6)	3.12 (1.6–6.2)	
Rhamnetin	3,3′,4′,5-Tetrahydroxy-7-methoxyflavone	Aglucone	380 (170–814)	3.8 (1–14.25)	
Homoeriodictyol	4′,5,7-Trihydroxy-3′-methoxy-flavanone	Aglucone	1550 (738–3,255)	2.9 (.53–15.96)	
Morin	2′,3,4′,5,7-Pentahydroxy-flavone	Aglucone			47% max. at 50 mcg.
Esculin	6,7-Dihydroxycoumarin 6-glycoside	Glycoside			40% max. at 5 mcg.
Catechin	3,5,7,3′,4′-Pentahydroxy-flavan	Aglucone			No block
Xanthorhamnin	3,3′,4′,5-Tetrahydroxy-7-methoxy-3-trirhamnoside	Glycoside			No block
Rottlerin	Contains phloroacetone group $C_{31}H_{30}O_8$ or $C_{30}H_{28}O_8$	Aglucone			No block
Quercitrin	3,3′,4′,5,7-Pentahydroxy-flavone 3-L-rhamnoside	Glycoside			No block
Esculetin	6,7-Dihydroxycoumarin	Aglucone			No block
4-Methylesculetin	4-Methyl-6,7-dihydroxy-coumarin	Aglucone			No block
Phloridzin	2-Glycoside of the chalcone of 2,4,6,4′ tetrahydroxy-dihydroflavanone	Glycoside			No block

their effectiveness decreased as their dose increased. However, these compounds had some blocking activity, in contrast to esculin, esculetin, and rottlerin which were entirely inactive. This lack of effect of the coumarin compounds was surprising in view of the effectiveness of 4-methylesculetin.

Antagonism of Bradykinin—Table II indicates the following order of descending potency: quercetin, rhamnetin, and homoeriodictyol. Although morin and esculin showed some activity, it was again observed that increasing the dose did not produce a greater antagonistic effect. All of the other compounds were ineffective within the dosage range used.

Antagonism of Eledoisin—Table III indicates the following order of descending potency: quercitrin, morin, rhamnetin, and catechin. Quercetin, xanthorhamnin, rottlerin, homoeriodictyol, and phloridzin were active but showed decreasing effectiveness with increasing dosage. All of the coumarin derivatives were ineffective in the dosage range employed.

DISCUSSION

Antagonism of the spasmogenic effect of angiotensin by flavonoid compounds appears to involve the presence of free phenolic hydroxyl groups in the 5,7-positions of the γ-benzopyrone nucleus of the aglucone. The addition of either rhamnose or glucose in the 3-position decreases or completely abolishes antispasmodic activity (quercetin *versus* quercitrin and rhamnetin *versus* xanthorhamnin). Methoxylation in the 7-position also causes a decrease in activity (quercetin or morin *versus* rhamnetin). The contribution of the dihydric phenolic substituent in Position 2 of the γ-benzopyrone nucleus is difficult to assess because both active and inactive compounds have such a substitution. However, methoxylation in the 3′-position gives the highest activity (homoeriodictyol) and moving the second hydroxyl group from the 3′- to the 2′-position decreases activity twofold (quercetin *versus* morin). Shifting the keto group of the parent nucleus to the

Table III—Antagonism of Eledoisin

Common Name	Chemical Name	Type	ED_{50} and Range, mcg./ml.	Slope and Range	Potency
Quercitrin	3,3′,4′5,7-Pentahydroxy-flavone 3-L-rhamnoside	Glycoside	16 (5–57.75)	10.35 (2–36.5)	
Morin	2′,3,4′,5,7-Pentahydroxy-flavone	Aglucone	107 (36–321)	7.93 (7.3–17.23)	
Rhamnetin	3,3′,4′,5-Tetrahydroxy-7-methoxy flavone	Aglucone	600 (250–1680)	5.5 (.5–55)	
Catechin	3,5,7,3′,4′-Pentahydroxy-flavan	Aglucone	10,000 (5,500–18,000)	3.7 (1.3–10.36)	
Xanthorhamnin	3,3′4′,5-Tetrahydroxy-7-methoxyflavone 3-trirhamnoside	Glycoside			50% max. at 2.5 mcg.
Quercetin	3,3′,4′,5,7-Pentahydroxy-flavone	Aglucone			40% max. at 2.5 mcg.
Rottlerin	Contains phloroacetophenone group $C_{31}H_{30}O_8$ or $C_{30}H_{28}O_8$	Aglucone			36% max. at 2.5 mcg.
Phloridzin	2-Glycoside of the chalcone of 2,4,6,4′-tetrahydroxy-dihydroflavanone	Glycoside			47% max. at 5 mcg.
Homoeriodictyol	4′,5,7-trihydroxy 3′-methoxy-flavanone	Aglucone			69% max. at 6,000 mcg.
Esculetin	6,7-Dihydroxycoumarin	Aglucone			No block
4-Methylesculetin	4-Methyl-6,7-dihydroxy-coumarin	Aglucone			No block
Esculin	6,7-Dihydroxycoumarin 6-glycoside	Glycoside			No block

2-position and removing the dihydric phenolic substituent abolishes activity (homoeriodictyol *versus* esculin and esculetin). However, the addition of a methyl group in this position restores part of the activity (homoeriodictyol *versus* 4-methylesculetin).

Antagonism of the spasmogenic activity of bradykinin by flavonoid compounds also appears to involve the phenolic hydroxyl groups in Positions 5 and 7 because methoxylation in Position 7 decreases activity ten times (quercetin *versus* rhamnetin). However, the dihydric phenolic substituent in Position 2 of the γ-benzopyrone nucleus must also contribute to activity because shifting one hydroxy from the 3′- to the 2′-position decreases activity (quercetin *versus* morin). In the coumarin derivatives, the glycoside linkage seems to be necessary for activity (esculin *versus* esculetin and 4-methylesculetin). In general, the aglucones have greater activity than the glycosides (quercetin *versus* quercitrin and rhamnetin *versus* xanthorhamnin).

Antagonism of the spasmogenic activity of eledoisin by the flavonoid compounds again demonstrated the requirement of free phenolic hydroxyls in the 5,7-positions of the γ-benzopyrone nucleus. Methoxylation of the 7-position resulted in a large decrease in activity (quercitrin and morin *versus* rhamnetin). In general, all compounds, except the coumarin derivatives, showed some degree of activity against eledoisin but structure-activity relationships are somewhat obscure.

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Sulfones of Potential Medicinal Value I: Diazonium Coupling Products of Ethyl *p*-Toluenesulfonylacetate

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Abstract □ A coupling of various aryldiazonium salt solutions with ethyl *p*-toluenesulfonylacetate has been effected at pH 7.5–8.5. Experimental details and physical properties of the products of this reaction are given. In order to establish whether the coupled product exists as an azo or as the isomeric hydrazo, NMR and IR spectroscopic data were obtained. Both sets of data confirm that the azo form is the proper structural assignment.

Keyphrases □ Ethyl *p*-toluenesulfonylacetate diazonium derivatives—synthesis □ TLC—separation □ IR spectrophotometry—identity, structure □ NMR spectroscopy—identity, structure

The present project was begun in order to determine what spectrum of pharmacological activity one might expect from compounds containing the sulfone group, either as an active or ancillary moiety. The sulfone group, which in some ways resembles the ketone moiety, has received very little consideration in prior pharmacological studies.

DISCUSSION

For preparation of the anhydrous sodium *p*-toluenesulfinate from the commercial hydrous form,[1] the drying procedure recommended by Panizzi and Nicolaus (1) was used. A modification of the procedure of Ashley and Shriner (2) was employed for preparation of ethyl *p*-toluenesulfonylacetate.

Early in the present studies an attempt to couple diazonium salts with α-arylsulfonyl-substituted propionic acids was made. Rather than affording the expected Japp-Klingemann reaction product the process gave only tarry products from which the isolation of pure compounds was not feasible. In an attempt to simplify the product mixture the corresponding ester, *i.e.*, ethyl α-*p*-toluenesulfonylpropionate, was used based on an hypothesis that the decarboxylation which results from Japp-Klingemann condensations could be a major complicating factor in the present example. While considerable qualitative improvement appeared to result, TLC on silica gel revealed the presence of at least eight compounds among the products.

At this point, it became obvious that additional studies were required in order to determine the conditions which were optimum for such coupling reactions and to determine the precise structure and chemical properties of the coupling products. This report is the first in a series which deals with this phase of the overall objective.

An attempt to couple benzenediazonium chloride with ethyl *p*-toluenesulfonylacetate in alcoholic solution at 5–10° according to the procedure of Bülow and Neber (3) gave unsatisfactory results. In this procedure buffers such as sodium acetate are not employed and the initially formed mixture is therefore strongly acidic (about pH 2.0). Cautious addition of 5% potassium hydroxide solution led to formation of a sticky product in poor yield. A crystalline product could not be obtained by this procedure.

In a subsequent trial run, the pH of the diazonium salt solution was adjusted to pH 5.0 before addition and then immediately adjusted to pH 8.0 after addition to the sulfone solution. Im-

[1] Aldrich Chemical Co.