Hydrazide of α-diethylphosphinylphenylacetic acid (III). A mixture of 8.9 g (0.033 mole) of ester (I), 4 ml (0.08 mole) of hydrazine hydrate and 5 ml of ethanol was boiled for 3 h. When cool, 5.7 g (67%) of (III) were isolated, mp 171-172.5°C (alcohol). IR spectrum, ν, cm^{-1}: 3370 (NH), 1690 (C=O), 1600 (C_6H_5), 1150 (P=O). Found, %: N 11.17, 11.06; P 11.87, 11.84. $C_{12}H_{19}O_2N_2P$. Calculated, %: N 11.00; P 12.20.

Hydrazide of α-diphenylphosphinylphenylacetic acid (IV). A solution of 3.6 g (0.01 mole) of ester II and 2 ml (0.04 mole) of hydrazine hydrate in 20 ml of n-amyl alcohol was boiled for 5 h. The yield was 2.5 g (71%) of hydrazide IV, mp 249-251°C (n-amyl alcohol). IR spectrum, ν, cm^{-1}: 3300 (NH), 1670 (C=O), 1620, 730, 700 (C_6H_5), 1180 (P=O). Found, %: N 8.05, 7.85; P 8.83, 8.67. $C_{20}H_{19}O_2N_2P$. Calculated, %: N 8.02; P 8.88.

m-Nitrobenzylidenehydrazide of α-diethylphosphinylphenylacetic acid. A mixture of 0.25 g (0.001 mole) of hydrazide III, 0.15 g (0.001 mole) of m-nitrobenzaldehyde, 5 ml of ethanol, and a few drops of 1 N HCl was boiled for $2^1/_2$ h. The yield of the product was 0.25 g (64%), mp 226-228°C (from aqueous DMFA). IR spectrum, ν, cm^{-1}: 3180 (NH), 1690 (C=O), 6140 (C=N), 1540, 1370 (NO_2), 1150 (P=O). Found, %: N 10.70, 10.56; P 3.20. $C_{19}H_{22}O_4N_3P$. Calculated, %: N 10.75; P 8.03.

m-Nitrobenzylidenehydrazide of α-diphenylphosphinylphenylacetic acid. Similarly, from 0.18 g (0.0005 mole) of hydrazide IV and 0.08 g (0.0005 mole) of m-nitrobenzaldehyde, 0.15 g (63%) of a product was obtained, mp 261-263°C (from aqueous DMFA). Found, %: N 6.40, 6.31; P 9.27. $C_{27}H_{22}O_4N_3P$. Calculated, %: N 6.42; P 8.70.

Mixed probes of the hydrazones with initial hydrazides show a considerable depression of the melting point.

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EFFECTS OF CERTAIN FLAVONOIDS ON THE ULCEROGENIC ACTION OF RESERPINE IN MICE

O. D. Barnaulov, O. A. Manicheva, G. G. Zapesochnaya, V. L. Shelyuto, and V. I. Glyzin

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According to data of S. V. Anichkov et al. [1, 2], one of the leading mechanisms of the formation of neurogenic dystrophies of the stomach is exhaustion of the catecholamine depots in its tissues and an inhibition of the trophic function of the sympathetic nervous system. There is also information on the ability of flavonoids to inhibit catechol ortho-methyl transferase (COMT) — one of the enzymes responsible for the inactivation of epinephrine and norepinephrine — and thus to prolong the action of catecholamines [3, 4]. Data on antiulcer activity of flavonoids [5, 6, 7] and preparations containing them [8, 9] agree with this information. The purpose of the present work was a comparative estimation of the effects of 11 flavonoids and a COMT inhibitor, depaverine [10], on the formation of destruction of the gastric mucosa, induced by reserpine in mice. The selection of reserpine as the ulcerogenic agent was due to the fact that it labilizes norepinephrine and epinephrine in the sympathetic ter-

V. L. Komarov Botanical Institute, Academy of Sciences of the USSR, Leningrad. All-Union Scientific-Research Institute of Medicinal Plants, Moscow Province.

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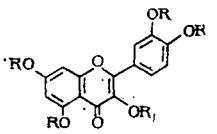
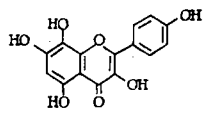
minals, exhausting their content. The use of mice permits a sharp reduction of the number of preparations from plants necessary for the experiments [8, 9].

EXPERIMENTAL CHEMICAL PART

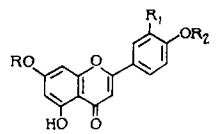
Quercetin pentacetate (II) was produced by acetylation of quercetin (3,5,7,3',4'-pentahydroxyflavone, I) with acetic anhydride in the presence of pyridine for 24 h at 20°C. A commercial preparation of rutin (III) was used.



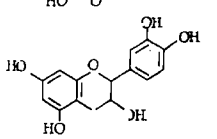
I Quercetin R = R_1 = H
II Quercetin acetate R = R_1 = CH_3CO
III Rutin R = H, R_1 = rutinosyl [rutinose(6-O-α-L-rhamnopyranosyl)-β-D-glucopyranose]
IV Glycerin R = H, R_1 = β-D-galactopyranosyl
V Isoquercitrin R = H, R_1 = β-D-glucopyranosyl
VI Quercitrin R = H, R_1 = α-L-rhamnopyranosyl



VII Gerbacetin



VIII Luteolin R = H, R_1 = OH, R_2 = H
IX Cinaroside R = β-D-glucopyranosyl, R_1 = OH, R_2 = H
X Linarin R = rutinosyl, R_1 = H, R_2 = CH_3



XI (+)-Catechin [rutinose-(6-O-α-L-rhamnopyranosyl)-β-D-glucopyranose]

Glycerin (quercetin-3-O-β-D-galactopyranoside, IV) was isolated from leaves of golden rhododendron (*Rhododendron aureum* Georgi) [11]. Isoquercitrin (quercetin-3-O-β-D-glycopyranoside, V) was isolated from leaves of the mountain ash *Sorbus tianschanica* Rupr. [12]. (+)-Catechin (3,5,7,3',4'-pentahydroxyflavone, XI) was isolated from an ether extract of an evaporated methanol extract of leaves *Aflatunia ulmifolia* (Franch.) Vass. [13], while quercitrin(quercetin-3-O-α-E-rhamnopyranoside, VI) was isolated from an ethyl acetate extract of the same extract [14]. Gerbacetin (3,5,7,8,4-pentahydroxyflavone, VII) was produced by acid hydrolysis of the total flavonoids isolated from roots of *Rhodiola algida* (Ledeb.) Fisch. et Mey [15, 16]. Linarin (acacetin-7-O-rutinoside, X) was isolated from leaves of the thistle *Cirsium oleraceum* L. [17]. Luteolin (5,7,3',4'-tetrahydroxyflavone, VIII) was produced from cinaroside (IX) by acid hydrolysis. IX (luteolin-7-O-β-D-glycopyranoside) was isolated from leaves of the sharp-leaved willow *Salix acutifolia* L.

EXPERIMENTAL PHARMACOLOGICAL PART

The flavonoids were dissolved in dimethyl sulfoxide, then 95% by volume water was added. The preparations or suspensions of the preparations obtained were administered to mice internally twice 5-6 h before and simultaneously with intraperitoneal injection of reserpine (2.5 mg/kg) in doses equimolar to 50 mg/kg I, while depaverine was administered in a dose of 200 mg/kg [10]. The mice were kept in the cold for 0.5-2 h, depending upon the temperature in the vivarium, to obtain a standard ulcerogenic lesion. According to the method that we developed [8, 9], after the injection of reserpine the mice were kept at 7°C for a limited period, which sharply increases their ulcerogenic effect. Gastric lesions were counted after 17-18 h. The experiments were conducted by a blind method in September 1980 to February 1981 on 920 mice of both sexes of the SHR stain. To study the histological picture of gastric lesions, the stomach tissue was imbedded in paraffin, followed by staining of sections according to Van Gieson. In addition, the adrenopotentiating properties of flavonoids in concentrations equimolar to $1 \cdot 10^{-5}$ g/ml I and depaverine ($1 \cdot 10^{-5}$ g/ml) were studied on an isolated segment of rabbit large intestine.

In the series of derivatives of I, a similar moderate ability to prevent the formation of severe striated destruction was exhibited by I, IV, and VI, which reduced the average number of lesions per animal in the group from 0.98 ± 0.27 in the control to 0.55 ± 0.21, 0.53 ± 0.28, and 0.46 ± 0.18, respectively (here and henceforth, values of x ± Sx·t are cited). III reduced only the relative proportion of striated destructions from 12 to 6.7%. II and V did not exhibit activity. In repeated series of experiments, ineffectiveness of II, III, and V was noted. The activity of preparations I, IV, and VI ranged from weak to distinct for I, from the absence of significant differences from the control to distinct for IV, while VI consistantly decreased only the average number of severe striated ulcers.

TABLE 1. Comparitive Evaluation of the Influence of Certain Flavonoids and a COMT Inhibitor, Depaverine, on the Formation of Destruction of the Gastric Mucosa in Reserpinized Mice

Preparation, dose	Number of animals	See note	Destruction of stomach: point	distinct	striated	Total
Control: 5% solution of dimethyl sulfoxide, 0.5 ml/10 g twice	58	1	8,6±2	2,09±0,6	1,17±0,65	11,86±2,6
		2	72,5	17,6	9,9	100
		3	4	15	20	2
VIII; 474 mg/kg twice	65	1	5,8±2,2	1,58±0,65	0,85±0,46	8,2±2*
		2	71,1	18,6	10,3	100
		3	6	23	34*	3
XI; 72.5 mg/kg twice	41	1	9,4±2,2**	2,7±1**	0,9±0,37	13±3,6**
		2	72,4	20,7	6,9	100
		3	2	16	22	3
Control; 5% solution of dimethyl sulfoxide, 0.5 ml/10 g twice	48	1	5,52±2,3	1,65±0,55	1,33±0,37	8,5±1,8
		2	65	19,4	15,6	100
		3	9	12	18	5
XI; 49.7 mg/kg twice	55	1	5,85±1,94	1,94±0,74	1,8±0,65	9,6±2,0
		2	61	20,3	18,7	100
		3	4	16	19	3
VIII; 47.4 mg/kg twice	50	1	4,36±1,8	1,9±0,74	1±0,5	7,28±2,6
		2	60	26,2	13,8	100
		3	6	15	24	3
I; 50 mg/kg twice	46	1	5,28±2,4	1,98±0,37	0,8±0,27*	8,1±2,5
		2	65,5	24,5	10*	100
		3	9	11	24	3
VI; 50 mg/kg twice	53	1	5,06±2,7	1,7±0,74	0,68±0,27*	7,45±2,7
		2	67,8	23	8,1*	100
		3	6	14	34*	4
Control; water 0.5 ml/10 g twice	41	1	7,46±2,5	2,41±0,74	1,19±0,37	11,06±2,97
		2	67,4	21,8	10,8	100
		3	2	6	12	1
Depaverine; 200 mg/kg twice	40	1	5,82±2,78	1,8±1,2	0,8±0,46	8,42±1,45
		2	69,1	21,4	9,5	100
		3	7	9	23*	3

Note. 1) Average number of destructions per animal in the group (here and in the text the values of $\bar{x} \pm S\bar{x} \cdot t$ are cited); 2) relative proportion of destructions of different severity (in % of their total number); 3) number of animals without destruction; *differences from the control are statistically significant at $P < 0.05$; **differences from the group that received luteolin are statistically different.

Preparation VIII, in comparison with IX (see Table 1), significantly decreased the average number of point, distinct, and summary destructions per animal in the group. However, in a repeated series of experiments VIII had no significant antiulcerogenic effect (see Table 1). X and XI were inactive. Among the aglycones, higher activity was exhibited by VII, which halved the average number of severe striated destructions, their relative proportion among all the lesions, and the number of animals with severe striated destructions. The COMT inhibitor depaverine was ineffective (see Table 1). Consequently, the COMT-inhibiting mechanism of action, possibly characteristic of flavonoids [3, 4], provides for only a slight increase in the resistance of the gastric mucosa to the ulcerogenic action of reserpine. Not one of the preparations studied exhibited antagonism to reserpine with respect to its neuroleptic action; adynamia, ptosis, and hypothermia in the mice were not reduced.

In experiments on an isolated segment of rabbit small intestine, no increase in the sensitivity of the object to the relaxing effect of subthreshold concentrations of epinephrine ($1 \cdot 10^{-9}$ g/ml) were detected under the action of depaverine and flavonoids. Depaverine ($1 \cdot 10^{-5}$ g/ml) lengthened the time of restoration of the tonus of a segment of intestine to the initial level after administration of epinephrine in concentrations of $1 \cdot 10^{-8}$ and $1 \cdot 10^{-7}$ g/ml, respectively, from 39 ± 12 to 90 ± 60 min (230%) and from 2.7 ± 0.5 to 6.4 ± 2.8 min (233%). The most demonstrative was the prolonging by flavonoids of the relaxing effect of epinephrine in a concentration of $1 \cdot 10^{-7}$ g/ml: I up to 105% of the control, II up to 326%, III up to 197%, IV up to 185%, V up to 147%, VI up to 165%, VII up to 170%, IX up to 167%, X up to 96% (coincides with the control), XI up to 190%. In most of the experiments VIII exhibited distinct

spasmolytic properties, and therefore its action on the relaxing effect of epinephrine was not studied. In these experiments most of the flavonoids exhibited effects similar to those of depaverine. The data cited do not permit the establishment of a distinct correlation between the prolonging by flavonoids of the action of epinephrine on an isolated intestinal segment and their weakening of the ulcerogenic action of reserpine on mice. The adrenoprolonging effect was also exerted by preparations that did not exhibit antiulcerogenic properties. Despite the moderate antiulcer activity of flavonoids, it can be noted that the most constant effect was exhibited by the aglycones VII and I, and to a lesser degree VIII. The tendency for a decrease in the activity in this series corresponds to the decrease in the number of hydroxyl groups in these compounds. The presence of rutinose in the 3- and 7-positions of III and X, respectively, removes the antiulcer activity. However, not only the 7-rutinoside (X), but also the 7-glucoside of luteolin (XI), did not reduce the ulcerogenic action of reserpine. The 3-glucoside and acetate of quercetin (V and II) were also ineffective. The relationship noted between the structure of the flavonoids and their ability to decrease the ulcerogenic effect of reserpine is in need of further study, bringing in other compounds.

Histological investigations have revealed that in mice the destruction of the secretory portion of the stomach, induced by reserpine, is based upon massive hemorrhage in the submucous membrane. As a result of necrotic and autolytic processes, there is no mucous membrane above the hemorrhages. As a result of contact with the hydrochloric acid of gastric juice, the lesion is marked by hematin hydrochloride.

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