metric method it is possible to determine the type of linkage of the dihydropyrane ring with the coumarin ring for each pair of linear and angular isomers in this series of substances, to determine the presence of hydroxy or methoxy groups in position 4' of their molecules, and to distinguish the presence of the acyl residue of senecioic acid from that of angelic acid in the products of the partial hydrolysis and methanolysis of 3',4'-diacyloxydihydropyranocoumarins.

LITERATURE CITED

1. P. I. Zakharov, P. B. Terent'ev, G. K. Nikonov, and A. I. Ban'kovskii, Khim. Prirodn. Soedin., 704 (1971).
2. L. G. Avramenko, G. K. Nikonov, and M. G. Pimenov, Khim. Prirodn. Soedin., 190 (1970).
3. F. M. McLafferty, Mass Spectrometry of Organic Ions, Academic Press, New York (1963), p. 318.
4. P. I. Zakharov, P. B. Terent'ev, G. K. Nikonov, and A. I. Ban'kovskii, Khim. Prirodn. Soedin., 431 (1972).
5. P. I. Zakharov, V. S. Kabanov, A. I. Ban'kovskii, G. M. Nikonov, and N. E. Ermatov, Khim. Prirodn. Soedin., 398 (1971).
6. M. Shipchandler and T. O. Soine, J. Pharm. Sci., 2062 (1968).
7. A. P. Johnson, A. Pelter, and P. Stainton, J. Chem. Soc., (C), 192 (1966).

FLAVONOID INHIBITORS OF Na^+,K^+-ATPase

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Among natural compounds, cardiac glycosides are the most effective [1, 2] but not the only inhibitors of the active transport of Na^+ and K^+ and of Na^+,K^+-ATPase. A number of metabolic poisons (oligomycin, ethacrynic acid, cassaine) can also suppress these processes. In spite of the considerable difference in the structures of the cardiac glycosides and the compounds mentioned above, their inhibiting action on Na^+,K^+-ATPase is equally prevented by high concentrations of potassium.

Among more than 100 derivatives of α- and β-unsaturated ketones, Kobashi [3] found a new inhibitor of Na^+,K^+-ATPase – luteolin (3',4',5,7-tetrahydroxy flavone), which possesses the highest affinity for the transport enzyme after its specific inhibitor ouabain. The mechanism of the action of luteolin on Na^+,K^+-ATPase is different from that of the action of ouabain. Recently, the action of isoflavonoids on aerobic glycolysis on mitochondrial ATPase and on ATPase from the plasmatic membranes of tumors has been studied [4]. Of the flavonoids investigated an inhibiting effect on the ATPase of the plasmatic membranes was possessed by tetra- and penta-hydroxyflavones with the hydroxy groups in positions 3, 3', 4, 5, and 7 but the strongest of them was 2,4',5',6'-tetrahydroxychalcone.

We have studied the action on Na^+,K^+-ATPase from the microsomal cell fractions of rat and bovine cerebral cortex with a specific activity of 600-700 μmole of P_{inorg}/mg of protrin/h of four flavone aglycones and eight glycosides mainly of the same aglycone. Of the compounds investigated, the highest inhibiting activity was shown by myricetin (3,3',4',5,-5',7-hexahydroxyflavone). At a concentration of $1 \cdot 10^{-4}$ M, this aglycone completely suppresses transport ATPase. The inhibiting action can be traced fairly clearly at concentrations of $1 \cdot 10^{-5}$ and $5 \cdot 10^{-6}$ M but falls off sharply at a dilution of $1 \cdot 10^{-6}$ M (Table 1). Quercetin (3,3',4',5,7-pentahydroxyflavone) and luteolin (3',4',5,7-tetrahydroxyflavone), in spite of the differences in the number of substituting hydroxy groups, behave in approxi-

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TABLE 1. Dependence of the Inhibiting Activity of Flavone Aglycones and Glycosides on Their Concentration (% inhibition of transport ATPase)

Flavonoid	Concn., M				
	$1 \cdot 10^{-4}$	$5 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$5 \cdot 10^{-6}$	$1 \cdot 10^{-6}$
Aglycones					
Myricetin (3,3',4',5,5',7-hexahydroxyflavone)	100	92	90	63	34
Quercetin (3,3',4',5,7-pentahydroxyflavone)	89	89	69	53	24
Luteolin (3',4',5,7-tetrahydroxyflavone)	88	88	67	50	16
Kaempferol (3,4',5,7-tetrahydroxyflavone)	43	39	28	0	0
Glycosides					
Myricetin 3'-glucoside	88	88	67	45	—
Quercetin 3'-glucoside	20	16	0	0	0
Quercetin 3-glucoside	66	46	40	0	0
Quercimeritrin (quercetin 7-glucoside)	50	39	34	0	0
Luteolin 7-glucoside	22	18	0	0	0
Astragalin (kaempferol 3-glucoside)	11	8	0	0	0
Kaempferol 3,7-dirhamnoside	8	7	0	0	0
Gossypitrin (gossypetin 7-glucoside)	88	88	63	5	0

mately the same way. Apparently, the main role is played not by the number of hydroxyls in the chromone moiety of the molecule (ring A) but by those hydroxy groups that are present in ring B. It is not excluded that the circumstance that the hydroxyls are in the ortho positions to one another also has no little significance. In actual fact, when we pass kaempferol (3,4',5,7-tetrahydroxyflavone) the inhibiting activity falls sharply, so that the last-mentioned compound can hardly be included among the inhibitors of Na^+,K^+-ATPase.

As a rule, glycosides possess no appreciable inhibiting action. For example, at a concentration of $1 \cdot 10^{-4}$ M luteolin inhibits to the extent of 88%, luteolin 7-glucoside only 22%, quercetin 89%, and quercetin 3'-glucoside 20%. Of the glucosides, only myricetin 3'-glucoside has an activity approaching that of quercetin and luteolin. The absence of inhibiting activity in flavones substituted by sugar residues is possibly due to steric factors — the larger molecule approaches the active center of the enzyme with greater difficulty.

However considerable the inhibiting action of flavone aglycones on transport ATPase may be, it is a whole order of magnitude lower than that of the cardiac glycosides. While for the majority of natural cardiotonic glycosides the concentration of substance causing 50% inhibition (I_{50}) of Na^+,K^+-ATPase is between $1 \cdot 10^{-6}$ and $1 \cdot 10^{-7}$ M [2], for the flavone aglycones the analogous index is in the range from $1 \cdot 10^{-5}$ to $1 \cdot 10^{-6}$ M.

The property of the flavonoids that has been found can apparently be used in an investigation of the receptive zones of Na^+,K^+-ATPase. No appreciable difference was found in the behavior of the microsomal fractions from the rat and bovine cerebral cortices.

EXPERIMENTAL

The microsomal fraction of the cells of rat and bovine cerebral cortices, which contain Na^+,K^+-ATPase, were isolated by a modification of Skou's method [5]. From this fraction we then, by treatment with NaI, Triton X-100, and gel filtration on Sephadex G-200 obtained transport ATPase with a specific activity of 600-700 μmole of P_{inorg}/mg of protein/h [6]. The kinetics of the ATPase activity was measured by recording the acidification of the medium. The incubation medium contained 3 mmole of tris-HCl (pH 7.9), 100 mmole of NaCl, 20 mmole of KCl, 2 mmole of $MgCL_2$, and 2 mmole of ATP. The flavones were treated in concentrations of $1 \cdot 10^{-4}$ to $5 \cdot 10^{-7}$ M, but since the last concentration had no appreciable influence on the transport ATPase it is not given in the Table.

SUMMARY

A comparative investigation of the inhibiting influence on transport Na^+,K^+-ATPase of four flavone aglycones and eight of their glycosides has been performed on the microsomal fraction of the cells of the cerebral cortex.

It has been shown that in concentrations of $1 \cdot 10^{-4}$ to $5 \cdot 10^{-6}$ M myricetin, quercetin, luteolin, and myricetin 3'-glucoside possess an appreciable inhibiting effect. For kaempferol and its glycosides, as for the glycosides of quercetin and luteolin, the inhibiting effect is extremely feeble.

LITERATURE CITED

1. A. Schwartz, G. E. Lindenmayer, and J. C. Allen, Pharmacol. Rec., 27, 1 (1975).
2. N. M. Mirsalikhova, F. T. Umarova, N. Sh. Pal'yants, and N. K. Abubakirov, Khim. Prirodn. Soedin., 188 (1976).
3. K. Kobashi and J. Hase, Ninth International Congress of Biochemistry. Abstracts Book, Stockholm (1973), p. 277.
4. E. M. Snolinna, R. N. Buchsbaum, and E. Racker, Cancer Res., 35, 1865 (1975).
5. I. C. Skou, Biochim. Biophys. Acta, 58, 318 (1962).
6. R. A. Ziyamukhamedov and N. M. Mirsalikhova, Uzb. Biol. Zh., No. 1, 74 (1976).
7. G. N. Zatsepina and S. É. Shnol', Biofizika, 10, 37 (1965).

DITERPENES OF *Lagochilus pubescens*

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The plant *Lagochilus pubescens* (family Labiatae) is widely grown but has been little studied [1, 2]. From this plant we have previously isolated the hydrocarbon nonacosane, β-sitosterol, the flavonoid 5-hydroxy-4',7-dimethoxyflavone, and diterpenes — lagochilin and 3,18-O-isopropylidenelagochilin [3, 4].

By chromatography on alumina and silica gel followed by elution with various solvents, from an ethereal fraction of the chloroform extract [4] we have isolated five individual diterpenes.

Diterpene (I), $C_{26}H_{44}O_5$, mp 118-119°C (from ether), $[\alpha]_D$ −50° (c 1; ethanol); M^+ 436.

The IR spectrum of (I) had bands at 1095 cm^{-1} characteristic for an ether bond. The mass spectrum showed the peak of the molecular ion with m/e 436 and peaks with m/e 238, 225, and 212, which shows that (I) belongs to the labdane group with a grindelane skeleton [5]. The PMR spectrum has signals in the 1.1-1.3 ppm regions due to the methyl groups of an isopropylidene part of the molecule. The signals of methyl groups at C_4, C_8, and C_{10} are located, just as in lagochilin, in the 0.65-0.85 ppm region. Protons located adjacent to oxygen (—CH_2—O—, >CH—O—) resonate in the 3.2-3.7 ppm region. The acid hydrolysis of (I) led to the formation of lagochilin (V).

On the basis of its IR, PMR, and mass spectra and chemical transformation, (I) was identified as di-O-isopropylidenelagochilin [4, 6].

Diterpene (II), $C_{27}H_{44}O_7$, mp 120-121°C (from ether), M^+ 480.

The IR spectrum of (II) has an absorption band at 1735 cm^{-1} which is characteristic for an ester group. In the PMR spectrum, the signals of the methyl groups of the main skeleton

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