DETERMINATION OF THE MOLECULAR WEIGHT OF FLAVONOIDS

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Reduction is widely used in the identification of individual types of flavonoids. In view of the specificity of the cyanidin reaction for flavones and flavonols, we have studied the absorption spectra of the products of their reduction in the UV region without the isolation of the latter from the reaction mixture. The products of the reduction of flavones and flavonols are characterized by the presence of two absorption bands – in the 350-370 nm region (band I) and at 253, 257, or 260 nm (band II).

Band II is common to all the compounds investigated and corresponds to a wavelength of 253 nm for flavones and 257 nm for flavonols. It is known that the glycosidation of flavonoids causes no shift in the maximum but has an effect on its intensity. By comparing the absorption spectra of the products of the reduction of monosides, biosides, and aglycones, it has been established that the intensity of the absorption depends on the molar ratio of the aglycone and the sugar residues in the glycosides studied. This rule was found previously by V. I. Litvinenko in a comparison of the absorption spectra of rutin and of glyphoside, isolated from common liquorice, and its aglycone [1].

Since absorption band II of the reduction products is within a narrow range for all glycosides and aglycones, we have attempted to use its intensity for determining the molecular weights (M) of flavonoid glycosides, having previously studied the optimum conditions for performing the cyanidin reaction by the method of the mathematical planning of experiments.

TABLE 1. Determination of the Molecular Weights of Flavonoids

Substance*	D_1	D_2	D_3	D_4	D_5	D_6	D_{cp}	M found	M theor.	Error, %
Quercetin	0,940	0,918	0,964	0,964	0,930	0,966	0,945	—	302	—
Quercitrin	0,643	0,655	0,654	0,657	0,646	0,653	0,650	440	448	−1,80
Guaiaverin	0,657	0,658	0,657	0,658	0,694	0,700	0,687	430	434	−0,90
Quercimeritrin	0,638	0,618	0,638	0,615	0,605	0,610	0,620	460	464	−0,86
Hyperoside	0,601	0,599	0,601	0,612	0,605	0,611	0,605	469	464	+1,10
Quercetin 3-rhamnoside	0,632	0,627	0,630	0,650	0,636	0,642	0,636	449,6	448	+0,30
Rutin	0,469	0,471	0,473	0,448	0,452	0,452	0,461	620	610	+1,60
3-Methoxy-quercetin	0,846	0,872	0,861	0,846	0,864	0,878	0,861	—	316	
Stizoliside	0,580	0,585	0,586	0,577	0,573	0,575	0,580	469	478	−1,80
Tamarixetin	0,895	0,879	0,880	0,896	0,897	0,895	0,890	—	316	—
Tamarixin	0,648	0,655	0,652	0,650	0,645	0,650	0.650	431,7	434	−0,53
Kaempferol	0,780	0,786	0,783	0,771	0,810	0,785	0,786	—	286	—
Kaempferol 3-rhamnoside	0,542	0,548	0,538	0,543	0,565	0,574	0,551	431	432	−0,23
Luteolin	0,777	0,764	0,748	0,773	0,808	0,792	0,777	—	286	—
Luteolin 7-glucoside	0,500	0,512	0,498	0,510	0,505	0,486	0,500	442	448	−1,10
Chrysoeriol	0,830	0,820	0,832	0,830	0,824	0,772	0,818	—	300	—
Salicapriin	0,538	0,542	0,543	0,540	0,540	0,541	0,540	453	462	−1,70
Salicaprin	0,418	0,417	0,423	0,422	0,416	0,420	0,419	584,5	594	−1,60

* For all the substances, λ_{max} – 257; for chrysoeriol, salicapriin, and salicaprin – 253 nm.

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In taking band II of the products of the reduction of the flavonoids as a basis, we started from the following considerations:

1) neither band has a single maximum in the absorption spectra of the unreduced flavonoids;

2) the absorption band at a wavelength of 253 or 257 nm in the reduction products is free from the superposition of absorption bands of other components of the molecule; and

3) it possesses a fairly high absorption index for each individual compound.

In view of the direct proportionality between the optical density and the amount of aglycone present in the glycosides, we used the formula

$$M_{glycoside} = \frac{D_1 \cdot M_{aglycone}}{D_2}$$

where D_1 is the optical density of the aglycone, and D_2 is the optical density of the glycoside.

Table 1 gives the results of the determination of the molecular weights of several flavone and flavonol glycosides. To obtain statistically reliable results, in each case six determinations were made. It can be seen from the Table that the maximum error of the determination is 0.3-2.0%.

The method of determining molecular weights of flavonoids that we have developed possesses a number of advantages in comparison with existing methods: the necessity for using such constants as the specific and molar extinctions is eliminated.

EXPERIMENTAL

Solutions of the substances in 95% ethanol were made at a concentration of 1 mg in 10 ml. Metallic magnesium was weighed out into dry test-tubes: 60 mg for aglycones, 40 mg for monosides, and 30 mg for biosides. To each of these test-tubes was added 1 ml of the previously prepared solutions of the substances under investigation, and also a mixture of conc. HCl and 95% ethanol in the following respective amounts (ml): for the aglycones, 0.72 and 4.28; for the monosides 0.44 and 4.56; and for the biosides and diglycosides 0.36 and 4.64. (The first figure in each case corresponds to the amount of HCl and the second to the amount of ethanol.) A blank test was performed on 1 ml of 95% ethanol to which the respective amounts of metallic magnesium and of HCl and ethanol given above were added.

The spectra were recorded half an hour after the beginning of the reaction in an SF-4A spectrophotometer.

SUMMARY

A method of determining the molecular weights of flavonoid glycosides from the absorption spectra of their reduction products has been proposed. The error of the determination is from 0.3 to 2.0%.

LITERATURE CITED

1. V. I. Litvinenko, Rast. Res., 11, 4, 531 (1966).