A SPECTROPHOTOMETRIC METHOD OF DETERMINING MOLECULAR WEIGHTS OF TRITERPENE GLYCOSIDES

G. L. Genkina, E. S. Kondratenko, L. G. Mzhel'skaya, and N. K. Abubakirov

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 6, pp. 501–503, 1969

UDC 547.597+547.918

At present, there are no reliable methods for determining the molecular weights of triterpene glycosides, particularly those with a large number of sugar residues. Methods for determining the molecular weights of acetates of the glycosides by isothermal distillation have been described in the literature [1, 2]. The molecular weights of glycosides with free carboxyl groups can be determined by potentiometric titration [3]. Satisfactory results have been obtained in determining molecular weights from the yields of genins as a result of quantitative acid hydrolysis of oligosides [4]. However, the latter method is not applicable to glycosides the aglycones of which are unstable to acids, it requires a large amount of substance, and not infrequently gives an analytical error equal to the molecular weight of one monosaccharide residue [4].

To determine the molecular weights of a number of glycosides (of gypsogenin and hederagenin) we have used the absorption in the UV region caused by the presence of a trisubstituted olefinic group. The maximum of this absorption is between 198–204 mμ, depending on the solvent used. The investigations carried out with triterpene glycosides of established structure have shown that within the limits of the error of the spectrophotometric measurements (2–3%) the molar extinction coefficients of the aglycones and glycosides coincide. This characteristic was established previously for steroid glycosides which, however, have absorption of a different nature [5, 6]. The absence of an influence of the size of the sugar chain on the molar extinction coefficient enables the molecular weights of the triterpene glycosides to be determined.

The table gives the molecular weights of a number of known compounds calculated on the basis of a comparison of the optical densities of the absorption of the aglycone and the glycoside. On the basis of the results for leontoside B, gypsoside, and kalopanax saponins A and B it can be seen that the presence or absence of a carbohydrate chain attached to the carboxyl group of the genin has no appreciable significance. A difficulty arises because of the insolubilities of the aglycones and glycosides in ethanol. Consequently the measurements must be carried out in 70% ethanolic solution or the molecular weights not of the glycosides but of their acetates must be determined, as is shown for the case of analyses of the acetates of leontosides B, D, and E. When dilute aqueous ethanol is used, the absorption maximum undergoes some hypsochromic shift.

It was shown by special experiments that the method can be extended to other triterpene glycosides with an olefinic bond (those of oleanolic acid, echinocystic acid, and others) and with an α, β-unsaturated ketone grouping (glycyrrhetic acid).

EXPERIMENTAL

The measurements were carried out on a "Hitachi" recording spectrophotometer at wavelengths of 195–210 mμ in 1-cm cells. The calculations were carried out with the readings of the optical density at the point of maximum absorption.

The concentrations of the glycosides studied and the aglycone were selected in such a way that the ratio of their optical densities at the point of measurement approximated to unity and the absolute values of the densities fell in that part of the scale where the error of the determination is a minimum (D = 0.4–0.7).

For the aglycones good results were obtained with the dissolution of 4 mg (weighed accurately on a microanalytical balance) of the substance in 100 ml of 70% or 95% optically pure ethanol. The concentration of the glycoside being determined must be selected in accordance with the assumed molecular weight. For diglycosides the most suitable concentration is 0.07–0.08% mg/ml, and for tetraosides and pentaosides 0.12–0.14 mg/ml, and so on.

The molecular weight of the substances under investigation, M_1, was calculated from the formula

$$M_1 = \varepsilon_0 \frac{c_1}{D_1},$$

where ε_0 is the molar extinction coefficient of the genin;

c_1 is the concentration of the glycoside solution; and

D_1 is the optical density of the solution of the substance being determined.

Results of Determinations of the Molecular Weights of Triterpene Glycosides

Compound	Content of ethanol, %	λ, mμ	c, mg/ml	D	ε	M calculated	M found
Gypsogenin	70	198–199	0.0426	0.715	7907	470.7	—
$C_{30}H_{46}O_4$	70	198–199	0.0488	0.808	7810	—	—
			Mean		7859		
Gypsoside [7,8]	70	198–199	0.1620	0.725	8000	1791.9	1752.6
$C_{80}H_{126}O_{44}$	—	—	0.1724	0.775	8050	—	1748.6
Hederagenin	95	201–203	0.0387	0.535	6550	472.7	—
$C_{30}H_{48}O_4$	—	—	0.0476	0.665	6600	—	—
	—	—	0.0294	0.405	6500	—	—
			Mean		6550		
Hederagenin	70	198–200	0.0397	0.450	5320	—	—
	—	—	0.0480	0.540	5300	—	—
			Mean		5310		
Leontoside B [9] $C_{41}H_{66}O_{13}$	70	199–200	0.0772	0.530	5260	767.0	774.0
Leontoside B heptaacetate [9] $C_{41}H_{59}O_6(CH_3COO)_7$	95	203–204	0.0650	0.410	6700	1061.2	1038.4
Leontoside D hexaacetate [9] $C_{59}H_{80}O_{11}(CH_3COO)_{16}$	95	203–204	0.2040	0.690	6250	1910.0	1936.5
Leontoside E nonadecaacetate [9] $C_{65}H_{87}O_{13}(CH_3COO)_{19}$	95	203–204	0.1460	0.450	6790	2198.3	2125.1
Kalopanax saponin A [10] $C_{41}H_{66}O_{12}\cdot H_2O$	95	201–202	0.0846	0.685	6227	769.0	808.9
Kalopanax saponin B [10] $C_{59}H_{96}O_{26}$	70	200–201	0.1364	0.590	5270	1221.4	1227.6

In view of the fact that the molar extinction coefficients vary over fairly wide ranges according to the temperature and the other conditions, the optical densities of solutions of the aglycone and of the glycoside must be determined simultaneously under the same conditions. In this case, the molecular weight of the substance under investigation (M_1) is calculated from the formula

$$M_1 = M_0 \frac{c_1 \cdot D_0}{c_0 \cdot D_1},$$

where M_0 is the molecular weight of the aglycone;

c_0 is the concentration of the aglycone solution, mg/ml;

D_0 is the optical density of the aglycone solution;

c_1 is the concentration of the solution of the substance under investigation, mg/ml; and

D_1 is the optical density of the glycoside solution.

The samples of kalopanax saponins A and B were kindly supplied by A. G. Ven'yaminova.

CONCLUSIONS

A spectrophotometric method for determining the molecular weights of triterpene glycosides with an olefinic group has been developed.

REFERENCES

1. E. P. Clark, Ind. Eng. Chem. Anal. Ed., **13**, 820, 1941.
2. J. Jonaš, J. Kansky, and O. Knessl, Coll., **26**, 2073, 1961.
3. R. Signer, Ann., **478**, 246, 1960.
4. N. K. Kochetkov and A. Ya. Khorlin, DAN SSSR, **150**, 1289, 1963.
5. N. K. Abubakirov and G. L. Genkina, Uzb. khim. zh., no. 6, 63, 1960.

6. N. K. Abubakirov and S. D. Nikonovich, Khim. farm. zh., no. 1, 40, 1967.

7. N. K. Kochetkov, A. Ya. Khorlin, and Yu. S. Ovodov, ZhOKh, **32**, 782, 1962; izv. AN SSSR, ser. khim. 83, 90, 1436, 1964; Tetrah. Let., 477, 1963.

8. K. Amanmuradov, E. S. Kondratenko, and N. K. Abubakirov, KhPS [Chemistry of Natural Compounds], **1**, 143, 1965.

9. L. G. Mzhel'skaya and N. K. Abubakirov, KhPS [Chemistry of Natural Compounds], **3**, 101, 218, 1967; 4, 153, 216, 1968.

10. A. Ya. Khorlin, A. G. Ven'yaminova, and N. K. Kochetkov, DAN SSSR, **155**, 619, 1964; Izv. AN SSSR, ser. khim., 1447, 1964; 1588, 1966.

15 July 1968

Institute of the Chemistry of Plant Substances AS UzSSR