7. C. H. Briskorn and T. Pfeufer, Chem. Ber., 100, 1998 (1967).
8. D. P. Popa and A. M. Reinbol'd, Khim. Prirodn. Soedin., 67 (1972); 31 (1973); 321, 589 (1974).
9. D. P. Popa, A. M. Reinbol'd, and A. I. Rezvukhin, Khim. Prirodn. Soedin., 169 (1973).
10. E. Fugita, I. Ichida, T. Fugita, N. Masaki, and K. Osaki, J. Chem. Soc. Chem. Comm., 20, 793 (1973).
11. H. Budzikiewiez, C. Djerassi, and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, Holden-Day, San Francisco, Vol. 2 (1964).
12. L. Novotny, J. Jizba, V. Herout, F. Sorm, L. H. Zalkow, S. Hu, and C. Djerassi, Tetrahedron, 19, 1101 (1963).
13. D. H. R. Barton and D. Elad. J. Chem. Soc., 2085 (1956).
14. G. V. Lazur'evskii, I. V. Terent'eva, and A. A. Shamshurin, Practical Work in the Chemistry of Natural Compounds [in Russian], Moscow (1966), p. 283.
15. K. S. Rybalko, N. N. Ban'kovskaya, and R. I. Evstratova, Med. Prom. SSSR, No. 3, 13 (1962).

SPECTROPHOTOMETRY OF GLYCOSIDES OF OLEANOLIC ACID AND HEDERAGENIN IN CONCENTRATED SULFURIC ACID

G. L. Genkina, L. G. Mzhel'skaya,
T. T. Shakirov, and N. K. Abubakirov

UDC 547.914.4

In recent years, investigations of triterpene glycosides have been performed on a broad scale. The structures of about 200 such compounds have been demonstrated. Glucosides of oleanolic acid and of hederagenin have proved to be the most widely distributed – they make up more than half the compounds discovered. Some triterpene glycosides have found use in medicine. In view of this, the development of methods for their analytical determination in preparations, medicinal forms, and the plant raw material is a matter of considerable interest.

Triterpenoids with an isolated double bond have an absorption maximum in the UV region of the spectrum in the range between 193 and 205 nm [1]. We have previously [2] made use of this property for determining the molecular weights of glycosides of hederagenin and gypsogenin. Unfortunately, this region of the spectrum is unsuitable for the quantitative determination of triterpenoids and their glycosides. Consequently, in the present work we have investigated the possibility of determining triterpene glycosides spectrophotometrically in concentrated sulfuric acid.

The halochromic reaction in concentrated sulfuric acid has been widely studied for a whole series of natural compounds. It has been used for the identification and quantitative analysis of steroid and triterpenoid sapogenins [3-13]. With concentrated sulfuric acid, the latter show characteristic absorption peaks in the UV region of the electronic spectrum which can be used for analytical determination [3, 10, 12]. It has been found that to obtain reproducible results the main reaction conditions – the concentration of the sulfuric acid, the time (rate) of the reaction, and the temperature – must be strictly controlled.

At the same time for a long period there was no single opinion among the authors making use of the halochromic reaction in sulfuric acid concerning the conditions for performing this reaction: some performed it at room temperature [14, 15] and others [4, 10, 11, 13] recommended thermostating at 40, 60, 70°C, etc. There was no agreement on the time of performance of the reaction, either.

V. F. Semenchenko et al. [11] studied the reaction of triterpenoids with concentrated sulfuric acid and, making use of the method of mathematical planning, concluded that the optimum conditions for this reaction are heating the reaction mixture at 70°C for 60 min. Karting et al. [13] came to the same conclusion.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 220-227, March-April, 1977. Original article submitted December 17, 1976.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.

TABLE 1. Extrema in the UV Spectra of Hederagenin in Concentrated Sulfuric Acid

Reaction conditions	Time of thermostating, h						
	0,5	1	2	3	4	16	24
Room temperature	300(4,1) 400(3,3)	300(4,26) 400(3,64)	308(4,29) 400(3,82)	310(4,32) 400(3,85)	310(4,32) 400(3,85)	320(4,56) 400(4,23)	316—324(4,58) 400(4,28)
Thermostating at 70°C, immediate determination							
immediately after cooling		300(3,74) 400(4,22)	302(4,17) 400(4,27)	300(4,18) 400(4,26)			
3 h after thermostating			302(4,19) 400(4,27)				
12 h after thermostating			302(4,24) 400(4,26)				

In our view, the reaction in concentrated sulfuric acid depends on the structure of the compounds being determined and group methods are unsuitable here; consequently it is necessary to develop the optimum conditions applicable to a concrete material. It is impossible to postulate that the maximum at 310 nm is characteristic only for triterpenoids and is not found in steroids and that steroids and terpenoids can be identified from their spectra in concentrated sulfuric acid when they are present simultaneously [12]. Such sterols as cholesterol, ergosterol, stigmasterol, and β-sitosterol have absorption maxima at 306-318 nm in sulfuric acid [13] and therefore there is no necessity for the development of universal methods of determining steroids and triterpenoids. Furthermore, both steroids and triterpenoids are found in nature mainly in the form of glycosides, and such widely distributed sugars as D-glucose, D-galactose, L-rhamnose, D-xylose, and L-arabinose have, in concentrated sulfuric acid, independent and fairly intensive extrema in the 320-330 nm region. This absorption is inevitably superimposed on the spectra of the aglycones [16, 17].

We have investigated the triterpene sapogenins – oleanolic acid hederagenin, and their glycosides – found in *Ladyginia bucharica* Lipsky [18], *Scabiosa soongorica* Schrenk [19], and *Leontice ewersmannii* Bge [20].

Of course, the rate of formation of the reaction products and their stability and, in addition to this, the position and intensity of the absorption peaks depend above all on the concentration of the sulfuric acid. With an increase in concentration the extrema shift to longer wavelengths, the reaction takes place faster, and the stability of the products formed rises. We used 94% sulfuric acid (sp. gr. 1.835). To determine the optimum conditions we first recorded the absorption spectrum of hederagenin in concentrated sulfuric acid and traced its dependence on the temperature and time of the reaction (Table 1).

As can be seen from Table 1, at room temperature the reaction of hederagenin with concentrated sulfuric acid is not complete in 24 h. With an increase in the time of the reaction a bathochromic shift of the absorption peak of the first band (300-324 nm) takes place and the intensity of absorption of the second band (400 nm) rises. Thermostating at 70°C leads to the formation of stable reaction products after only two hours, and regardless of whether the determination is performed immediately after the reaction mixture has been cooled or 3 or 12 h later the pattern of the spectrum does not change. For reliability, it is desirable to prolong the heating for one more hour and to measure the intensity of absorption immediately after cooling. Taking these conditions as the optimal, we recorded and analyzed the spectra of oleanolic acid and hederagenin and their natural glycosides (Table 2).

Simultaneously, we recorded the spectra of the sugars most frequently found in natural terpene glycosides (Table 3).

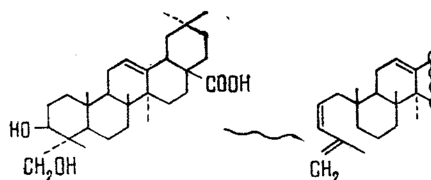
In concentrated sulfuric acid, triterpenoids show specific absorption in the form of two intense bands at 300-320 nm and 400-405 nm. The spectrum of oleanolic acid contains two maxima – in the 300-306 nm and 375-380 nm regions with log ε 4.17 and 3.64, respectively. The hederagenin molecule contains a CH_2OH group at C-23 instead of the CH_3 group of oleanolic acid. The dehydration of the hydroxymethyl group under the action of sulfuric acid creates the conditions for the formation of a new chromophore. It is not excluded that as the result of the simultaneous splitting off of the hydroxy groups at C-3 and C-23 degradation products may arise that contain a diene grouping in ring A. In the UV spectrum of hederagenin, this appears in the form of a bathochromic shift of the second band in the 400-405 nm region together with an increase in its intensity to log ε 4.29. (see Scheme on following page.)

The spectra of the individual monosaccharides present in the carbohydrate moities in the triterpene glycosides, taken under the same conditions, show that their absorption maxima are mainly superposed on the first band of the sapogenins in the 300-306 nm region. This leads to the situation that in the glycosides this band undergoes a bathochromic shift by 15-20 nm, and its intensity rises (Fig. 1).

TABLE 2. Extrema of the Electronic Absorption Spectra of Oleanolic Acid and Hederagenin and their Glycosides in Concentrated Sulfuric Acid

Compound	Composition mol. wt.	Carbohydrate moiety	λ_{max}(log ε), nm	λ_{min}(log ε). nm
Oleanolic acid	$C_{30}H_{48}O_3$, 456,68		300–306(4,17) 375–380(3,64)	250(3,62) 334(3,40)
Glycosides				
Ladyginoside A	$C_{42}H_{66}O_{14}$, 794,94	D-Glc:D-GlcUA; 1:1	310–320(4,42) 400(3,99)	270(4,15) 340–350(4,10) plateau 350(3,90)
Ladyginoside C	$C_{47}H_{74}O_{18}$, 927,06	D-Glc : L-Ara : : D-GlcUA; 1 : 1 : 1	260(4,21) 316–320(4,63) 390–405(3,77)	280(4,12) 360(3,67)
Ladyginoside E	$C_{72}H_{116}O_{39}$, 1605,64	D-Glc : D-Gal : : D-GlcUA; 5 : 1 : 1	260(4,47) 318–322(5,02) 390–400(3,87)	280(4,27) 360(3,85)
Songoroside G	$C_{51}H_{82}O_{19}$ 999,16	L-Rha : D-Xyl;1 : 3	260(4,38) 304–324(5,33) 370–380(3,88)	270(4,28) 360(3,84)
Songoroside O	$C_{68}H_{110}O_{33}$, 1455,56	L-Rha : D-Glc : : D-Xyl; 1 : 2 : 4	260(4,85) 300–310(4,72) – plateau 320–324(5,02) 385(4,02)	280(4,38) 370–380(3,71)
Hederagenin Glycosides	$C_{30}H_{48}O_4$, 472,68		300(4,14) 400–405(4,29)	260(3,93) 330(3,80)
Leontoside A	$C_{35}H_{56}O_8$, 604,79	L-Ara; 1	306(4,15) 400–405(4,07)	250(3,92) 350(3,75)
Leontoside B	$C_{41}H_{66}O_{13}$, 766,93	L-Ara : D-Glc; 1 : 1	260(4,12) 312–318(4,49) 400–405(4,29)	270(4,10) 354(3,87)
Leontoside C	$C_{47}H_{76}O_{18}$, 929,07	L-Ara : D-Glc; 1 : 2	260(4,23) 316–318(4,67) 400–405(4,18)	274(4,16) 360(3,87)
Leontoside D	$C_{59}H_{96}O_{27}$, 1237,35	L-Ara : D-Glc : L-Rha; 1 : 3 : 1	256–260(4,28) 320–322(4,77) 400–405(4,20)	270(4,20) 358(3,85)
Ladyginoside B	$C_{42}H_{66}O_{15}$, 810,94	D-Glc : D-GlcUA; 1 : 1	252–256(4,18) 312–316(4,47) 400–405(4,24)	270(4,15) 360(3,81)
Ladyginoside D	$C_{60}H_{96}O_{30}$, 1297,36	D-Glc : D-Gal : D-GlcUA; 3 : 1 : 1	258(4,40) 316–320(4,79) 400–405(4,45)	280(4,34) 360(3,99)
Ladyginoside F	$C_{72}H_{116}O_{40}$, 1621,64	D-Glc : D-Gal : D-GlcUA; 5 : 1 : 1	256(4,50) 316–320(4,93) 400–405(4,46)	278(4,37) 350(4,06)

Glc) glucose; Gal) galactose; Ara) arabinose; Xyl) xylose; Rha) rhamnose; GlcUA) glucuronic acid.



Consequently, it is undesirable to perform the analysis of triterpene glycosides at the 300-320 nm band, as certain authors recommend [13]. The intensity of this band depends substantially on the qualitative and quantitative composition of the sugars participating in the formation of the glycosides.

We have considered the possibility of using another band for analytical determinations, namely that at 400-405 nm. In this region the glycosides have an absorption peak that is close to the sapogenin peak and has a relatively constant intensity regardless of the qualitative and quantitative composition of the carbohydrate chain (see Table 2).

TABLE 3. Extrema of the UV Absorption Spectra in Concentrated Sulfuric Acid of the Sugars Found in Triterpene Glycosides

Name	Composition, mol. wt.	λ_{max}(log ε) nm	λ_{405}, nm	λ_{min}(log ε), nm
L-Arabinose	$C_5H_{10}O_5$, 150,14	280—284(3,73) 314—316(3,73)	2,43	
D-Xylose	$C_5H_{10}O_5$, 150,14	255(3,77) 320(4 24)	2,09	280(3,40)
L-Rhamnose	$C_6H_{12}O_5$, 164,16	240—250(3,68) 320—322(4 05)	2,14	270(3 62)
D-Fucose	$C_6H_{12}O_5$, 164,16	250—254(3,70) 322(3,99)	2,00	272—274(3,54)
D-Glucose	$C_6H_{12}O_6$, 180,16	256—258(3,71) 320—326(4,27)	2,38	280(3,62)
D-Glactose	$C_6H_{12}O_6$, 180,16	260(3,58) 320(4,07)	2,34	280(3,34)
D-Glucuronic acid	$C_6H_{10}O_7$, 194,14	260(3,70) 320(3,70)	2,83	250(3,65) 280(3,47)
D-Galacturonic acid	$C_6H_{10}O_7$, 194,14	316—320(4,17)	2,87	240—245(3,71)

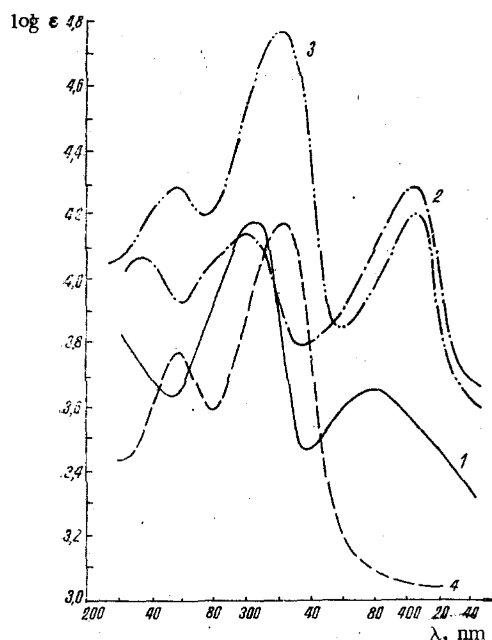


Fig. 1. UV spectra in concentrated sulfuric acid of oleanolic acid (1), hederagenin (2), the hederagenin glycoside leontoside D (3), and a model mixture of the sugars of leontoside D (4) taken in molar ratio.

It appears possible to use the absorption at 400-405 nm for the quantitative determination of individual triterpene glycosides or the aglycone in a mixture of several glycosides with different sugar compositions.

For oleanolic acid and its glycosides the absorption band at 380-405 nm appears in the form of a broadened plateau of the maximum with an intensity of log ε 3.64-4.02. For all the sugar-containing compounds of this sapogenin the Lambert–Beer law is observed at this wavelength – for oleanolic acid in the range from 60 to 200 μg and for its glycosides, especially ladyginoside E, from 120 to 360 μg. The sensitivity of the halochromic reaction is 60 μg for oleanolic acids and 120 μg for ladyginoside E.

Hederagenin and its glycosides are characterized by a sharper extremum at 400-405 nm having a high intensity (log ε 4.30-4.45) with complete observance of the Lambert–Beer law – for hederagenin between 15 and 60 μg and for its glycosides, such as ladyginoside F, between 30 and 130 μg. The sensitivity of the spectrophotometric determination of hederagenin in concentrated sulfuric acid is 4-5 times higher than that of oleanolic acid and amounts to 15 μg for hederagenin and 30 μg for ladyginoside F.

To show the absence of an influence of a sugar fragment at 400–405 mm, we recorded the differential spectrum of leontoside D (see Fig. 1). For this purpose we made a model mixture of monosaccharides corresponding qualitatively and quantitatively to the natural glycoside.

In view of the spectrophotometric behavior of the triterpene glycosides in concentrated sulfuric acid we have developed a method for standardizing a leontoside preparation obtained from L. ewersmannii bulbs. The preparation consists of a combination of the individual leontosides A, B, C, D, and E. Since all these glycosides, like hederagenin, have an absorption maximum at 400–405 nm, from the magnitude of this absorption it is possible to determine the amount of sapogenin in them. As already mentioned, to obtain reproducible results it is necessary strictly to control the concentration of acids and the time and temperature of thermostating. The results of the analysis of six batches of the preparation are given below

Sample no.	Replicate	Hederagenin content, %	Arithmetic mean, %	Deviation from the mean, %
I	1	44.22	44.68	−1.02
	2	44.22		−1.02
	3	45.62		+2.10
II	1	47.51	47.38	+0.27
	2	47.07		−0.65
	3	47.51		+0.27
III	1	44.69	44.93	−0.53
	2	45.14		+0.46
	3	44.97		+0.08
IV	1	42.70	43.60	−2.06
	2	43.49		−0.26
	3	44.60		+2.29
V	1	47.02	47.41	+0.82
	2	47.44		−0.06
	3	47.78		+0.78
VI	1	46.33	46.61	−0.60
	2	47.18		+1.22
	3	46.33		−0.60

The maximum deviation from the mean hederagenin content for six batches of the preparation did not exceed ± 2.29%, which shows the satisfactory reproducibility of the method.

EXPERIMENTAL

A 2-3 mg (hederagenin) or 4-6 mg (hederagenin glycoside) sample of the substance previously dried to constant weight was dissolved in methanol in a 25-ml measuring flask. With a pipette, 0.3-0.5 ml was transferred to a 25-ml flask of a rotary evaporator and the solvent was evaporated off to dryness at 40°C. The residue was dried to completion in a thermostat at 105°C for 1 h. It is important to ensure that the solvent has been eliminated completely. With a pipette, exactly 4 ml of concentrated sulfuric acid (sp. gr. 1.835) was added to the flask with the residue and the contents were shaken until the substance being determined had dissolved completely. The resulting solution was thermostated in the water bath at 70°C for 3 h. The reaction mixture was cooled to room temperature and the spectrum of the colored solution (pale pink–pinkish brown, depending on the concentration of the substance being determined) was recorded in the 220–420 nm region relative to concentrated sulfuric acid in cells with a layer thickness of 1 cm.

In the case of oleanolic acid and its glycosides, the same method was used but the sample was 4-5 times larger (10-12 mg for the aglycone).

Quantitative determination of the sapogenin in a leontoside preparation was performed relative to a standard sample of hederagenin (mp 328°C) by a comparison of optical densities. For this purpose, 2 mg of hederagenin (accurately weighed) was dissolved in methanol in a 25-ml measuring flask. A 0.2-0.4 ml portion of the resulting solution was evaporated to dryness. The residue, after being dried at 105°C for 1 h, was dissolved in 4 ml of concentrated sulfuric acid. The solution was thermostated at 70°C for 3 h and, after cooling, the optical density was measured in a 1-cm cell at 405 nm relative to concentrated sulfuric acid.

Before analysis, the preparation was dried to constant weight and the qualitative composition of the glycosides [20] was checked in the chloroform–methanol–water (65 : 35 : 8) system in a thin layer of silica gel. A sample of the preparation (5 mg) was dissolved in 10 ml of methanol, 0.2 ml of this methanolic solution was evaporated to dryness, and the subsequent proceedure was the same as in the determination of the optical determination of hederagenin.

The percentage of hederagenin in the preparation was calculated from the formula

$$X = \frac{DC_{st} V_{st} 100}{D_{st} CV},$$

where D is the optical density of the solution of the preparation; D_{st} is the optical density of the solution of the standard sample of hederagenin; C_{st} is the concentration of the methanolic solution of hederagenin, mg/ml; V_{st} is the volume of the solution of standard sample of hederagenin, ml; C is the concentration of the methanolic solution of the preparation, mg/ml; V is the volume of the solution of preparation taken for analysis, ml.

SUMMARY

The UV spectra in concentrated sulfuric acid of hederagenin and oleanolic acid and their glycosides has been studied. For analytical purposes it is proposed to use the extremum at a wavelength of 405 nm, which is distinguished by constancy of position and intensity of absorption for the genins and their glycosides.

A spectrophotometric method is proposed for the determination of the triterpene glycosides in a preparation from the bulbs of Leontice ewersmannii Bge.

LITERATURE CITED

1. P. S. Ellington and G. D. Meakins, J. Chem. Soc., 697 (1960).
2. G. L. Genkina, E. S. Kondratenko, L. G. Mzhel'skaya, and N. K. Abubakirov, Khim. Prirodn. Soedin., 501 (1969).
3. T. Karting and G. Mikula, Arch. Pharm., 303, 767 (1970).
4. M. A. Walens, A. Turner, and M. E. Wall, Anal. Chem., 26, 825 (1954).
5. K. Bauer, Die organische Analyse, Second ed., Geest und Portig, Leipzig (1950).
6. C. H. Brieskorn and L. Capuano, Chem. Ber., 86, 866 (1953).
7. C. H. Brieskorn and H. Herrig, Arch. Pharm., 292/64, 485 (1959).
8. É. T. Oganesyan, A. L. Shinkarenko, and V. A. Bandyukova, Khim. Prirodn. Soedin., 212 (1968).
9. I. A. Murav'ev, and V. F. Semenchenko, Khim. Prirodn. Soedin., 17 (1969).
10. V. D. Ponomarev, É. T. Oganesyan, and V. F. Semenchenko, Khim. Prirodn. Soedin., 147 (1971).
11. V. F. Semenchenko, É. T. Oganesyan, V. D. Ponomarev, and V. I. Frolova, Khim. Prirodn. Soedin., 294 (1971).
12. A. V. Simonyan, A. L Shinkarenko, and É. T. Oganesyan, Khim. Prirodn. Soedin., 293 (1972).
13. T. Karting, R. Danhofer-Nöhammer, and O. Wegscheider, Arch. Pharm., 305, 515 (1972).
14. S. Bernstein and H. Lenhard, J. Org. Chem., 18, 1146 (1953); 19, 1296 (1954); 25, 1405 (1960).
15. L. Smith and W. H. Muller, J. Org. Chem., 23, 960 (1958).
16. M. Okawa and C. Neimann, J. Biol. Chem., 180, 923 (1959).
17. S. D. Nikonovich and N. K. Abubakirov, Zh. Obshch. Khim., 33, 3920 (1963).
18. M. Patkhullaeva, L. G. Mzhel'skaya, and N. K. Abubakirov, Khim Prirodn. Soedin., 485 (1970), 466 (1972); 36, 733, 803 (1973).
19. A. Akimaliev, P. K. Alimbaeva, L. G. Mzhelskaya, and N. K. Abubakirov, Khim. Prirodn. Soedin., 472, 476 (1976).
20. L. G. Mzhel'skaya and N. K. Abubakirov, Khim. Prirodn. Soedin., 421 (1966); 101, 218 (1967); 153, 216 (1968).