QUANTITATIVE DETERMINATION OF TRITERPENOIDS OF THE DAMMARANE SERIES BY THE DENSITOMETRY OF THIN-LAYER CHROMATOGRAMS

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The leaves of some species of Far Eastern birch contain tetracyclic triterpenoids: betulafolienetriol (I), betulafolienetriol oxide (II) [1], betulafolienetetraol (III), and betulafolienetetraol oxide (IV) [2] – the starting materials for the production of glycosides [3] similar to panaxosides [4]. The amount of triterpenoids present varies considerably according to the periods [5] and sites of collection [6]. It is necessary to develop fast and sufficiently accurate methods for the quantitative estimation of the individual triterpenoids in mixtures obtained from plant sources.

At the present time, spectrophotometric and chromatographic methods are used for this purpose. The use of spectrophotometry for the analysis of a natural mixture of panaxosides and their genins without the separation of the substances to be determined has been reported by Hiai et al., [7]. The method is based on differences in the absorption spectra of the products of a color reaction of the genins with vanillin and sulfuric acid. Its applicability is limited to mixtures of panaxosides the genins of which obtained by hydrolysis consist of triterpenoids of only two types (panaxadiol and panaxatriol) and have differences in their absorption spectra. N. E. Mashchenko et al., [8] have reported the determination of the total cucurbitacins in cucumbers without their preliminary separation. When extracts were treated with formaldehyde, the cucurbitacins present fluoresced, and the fluorescence of the solutions was measured spectrophotometrically. Thus, the use of spectrophotometric methods without the separation of the substances to be determined is limited, since it is based either on the specific composition of the mixture being investigated or on the common nature of the properties of the group of compounds being analyzed.

Ordinary liquid adsorption chromatography (LAC) is unsuitable for routine analyses because of the lengthiness of the process. Gas–liquid chromatography (GLC) of natural mixtures of triterpenoids is used mainly in order to identify the individual components [9-11]. Its possibilities have been shown by Ikekawa [12]. Quantitative GLC has not so far been widely used [13, 14], apparently because of the complexity of the natural mixtures studied.

The applicability of thin-layer chromatography in the investigation of natural mixtures of the pentacyclic triterpenoids from Opilia celtidifolia followed by the estimation of the substance in the spot by optical densitometry has been discussed by Dawidar et al., [15]. A review of various methods of quantitatively estimating thin-layer chromatograms has been given by Kirchner [16], and also by Perry et al., [17]. In comparison with the GLC, LAC, and spectrophotometric methods, the densitometry of thin-layer chromatograms has a number of advantages: the speed of separation, low consumption of standard substances, and the possibility of analyzing heat-labile compounds.

It appeared to us to be desirable to use this method for quantitative evaluation of chromatograms of ethereal extracts from birch leaves. Because of the complexity of the mixture to be separated (10-14 components) and the close chromatographic mobilities of triterpenoids (I-IV), we used various solvent systems for their separation. The spots were revealed with antimony trichloride, which colors triterpenoids various shades of red-brown. The colors of the triterpenoids (I) and (II) did not change with time, while those of (III) and (IV) weakened during the first 15 min, subsequently remaining fairly constant. On the chromatograms of some extracts, in addition to the sharp spots of individual substances, a series of "trails" – background due to im-

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TABLE 1

ple*	Species of birch	Date of collection of leaves (1975)	Amt. of unsaponifiable fraction, %		Betulafolienetriol (I), %		Betulafolienetriol oxide (II), %		Betulafolienetetraol (III), %		Betulafolienetetraol oxide IV, %	
			in the fresh leaves	in the air-dry leaves	in unsaponifiable fract. of ethereal extract	calc. on the leaves	in unsaponifiable fract. of ethereal extract	calc. on the leaves	in unsaponifiable fract. of ethereal extract	calc. on the leaves	in unsaponifiable fract. of ethereal extract	calc. on the leaves
1	Betula costata Trautv.	21.05	0,38	—	1,81±0,16	0,007	Traces		5,91±0,54	0,022	11,15±1,34†	0,042
2	"	3.06	0,39	—	2,43±0,08	0,010	Traces		17,03±0,82	0,66	10,31±0,42	0,040
3	"	24.06	—	1,08	1,55±0,09	0,017	None		4,56±0,22	0,049	4,56±0,17	0,049
4	B. mandshurica (Regel) Nakai	21.05	—	1,20	7,42±0,39	0,089	3,08±0,26	0,037	None		None	
5	"	3.06	0,38	—	13,40±0,33	0,051	15,00±0,63	0,057	None		None	
6	"	24.06	—	2,13	17,70±0,60	0,378	3,23±0,30	0,069	None		None	
7	B. dahurica Pall.	21.05	0,21	—	6,89±0,67	0,014	4,62±0,18	0,010	None		None	
8	"	26.05	—	1,10	7,60±0,37	0,084	None		None		None	
9	"	3.06	0,18	—	8,20±1,16	0,015	15,00±0,60	0,027	None		None	
10	B. platyphylla Sukacz.	26.05	—	2,80	4,40±0,20	0,123	Traces		None		None	
11	"	5.06	—	1,47	9,95±0,39‡	0,146	Traces		None		None	
12	"	29.06	—	2,16	5,67±0,31	6,122	Traces		None		None	
13	"	9.07	—	1,92	7,09±0,32	0,136	3,02±0,36	0,058	None		None	
14	B. kamtschatica (Regel) Jansson (B. platyphylla, s. l.)	12.08	—	3,10	2,70±0,19	0,084	None		None		None	

*Samples 1-7 and 9 were collected in the valley of the R. Gryaznaya, Khasan region, Maritime Territory; 8 and 10-13 in the environs of the village of Verkhne-Blagoveshchensk, Amur oblast; and 14 in the environs of the settlement of Khutor, Elizovo region, Kamchatka oblast.
†Results of two determinations.
‡Results of four determinations.

purities – was observed. Attempts to get rid of these impurities by boiling with activated carbon and by filtration through a layer of silica gel or Sephadex LH-20 [18] were unsuccessful. This background does not substantially affect the results of determinations when the concentration of the substance under investigation in a solution of the extract is greater than 1-2%. A linear relationship was observed between the densitometer signal and the weight of the substance in the spot in the range from 0.1 to 1.4 μg/spot. Calibration curves were plotted for each determination, since these curves taken for one substance but with different plates differed somewhat in slopes and did not always pass through the origin. The results obtained (averaged values and mean square deviation were calculated from five determinations as described by Yamamoto et al., [19]) are given in Table 1. The mean square deviations of the measurements ranged mainly between 4 and 9%. The results of the smallest scatter were obtained for the sharper and more compact spots.

The figures given in the Table reflect the dynamics of the accumulation of the triterpenoids that we have studied in birch leaves. The work was carried out both with freshly collected and with air-dry leaves. The loss of weight on drying the raw material amounted to 60-80%. The maximum amount of the combined triterpenoids (I, III, and IV) in freshly gathered leaves of Betula costata was found at the beginning of June (collection 2). A sample of B. costata (collection 3) was taken in the air-dry state, and a marked fall in the amount of triterpoids in the unsaponifiable fraction of the ethereal extract was observed. The amount of triterpenoids in the leaves of B. costata probably begins to fall at the end of June, but the possibility of their partial decomposition during the drying of the leaves is not excluded.

A maximum amount of (I) and (II) calculated on the air-dry leaves in B. mandshurica was observed in collection 6. Betulafolienetetraol (III) and its oxide (IV) were detected only B. costata.

For B. platyphylla, all the samples of which were used only in the form of the air-dry leaves, the amount of (I) did not vary substantially (0.12-0.15%). The low concentration of (II) in the leaves of collections 10-12 did not permit their quantitative determination; it can be observed only that the amount of the triterpenoid (II) in the unsaponifiable fraction of the ethereal extracts from collections 10-12 was less than 1%.

For B. dahurica, the maximum of amount of (I) and (II) in the freshly collected leaves was found in collection 9. In a sample of B. dahurica (collection 8), beturafolienetriol oxide (II) was completely absent.

In the leaves of B. kamtschatica, the amount of betulafolienetriol (I) was 0.084%, but in view of the fact that the sample was collected in the middle of August, it may be assumed that this amount of (I) in the leaves of this species is not the maximum.

Thus, among the samples studied the highest amount of betulafolienetriol (I) and its oxide (II) was found in the unsaponifiable fraction of the ethereal extracts from B. mandshurica (collections 6 and 5), and of betulafolienetetraol (III) and its oxide (IV) in B. costata (collections 1 and 2).

EXPERIMENTAL

The betulafolienetriol (I) isolated from the leaves of B. platyphylla (1974 collection), mp 196-197°C (acetone), gave no depression of the melting point with an authentic sample [6]. Betulafolienetriol oxide (II) was also obtained from the leaves of B. platyphylla, mp 236-238°C (petroleum ether); the results of IR and NMR spectroscopy corresponded to those of Nagai et al., [1]. Betulafolienetetraol (III), mp 168-170°C (acetone) and betulafolienetetraol oxide (IV), mp 250-251.5°C (acetone) were isolated from the leaves of B. costata (1973 collection) as described previously [2]. The unsaponifiable fraction of the ethereal extracts from the birch leaves was obtained by the method of Fischer and Seiler [5]. Densitometry was carried out on a Shimadzu CS-900 TLC-scanner (Japan), the construction and working principle of which has been described in a paper by Yamamoto et al., [19]. The choice of the wavelength λ_S (length of the luminous wave absorbed by the chromatographic spot) and λ_R (length of the comparison luminous wave not absorbed by the spot) was made in accordance with the recommendations of the manufacturers. Since the absorption maxima of the triterpenoids investigated were close and poorly characteristic, in all cases we used the same wavelengths of λ_S 550 and λ_R 730 nm. After chromatography and staining, the plates were scanned perpendicularly to the direction of the light slit (width 0.20 mm and height ~0.9 diameter of the smallest spot). The rate of scanning was 10 mm/min and the rate of movement of the recorder chart 24 mm/min. The area of each peak was calculated by multiplying its height by its width measured at half-height. The solvent systems used were: To determine (I) (R_f 0.23) petroleum ether–chloroform–methyl ethyl ketone–ethanol (16:4:6:1); for (II) (R_f 0.31) petroleum ether–chloroform–methyl ethyl ketone–ethanol (16:4:5:1); and for (III) (R_f 0.24) and (IV) (R_f 0.31) petroleum ether–chloroform–ethanol (9:11:2). A microsyringe with a capacity of 10 μl modernized as described by Perry et al., [17] was used. TLC was performed on "Silufol" plates (Czechoslovakia) with sizes of 5 × 15, 7 × 15, and 10 × 15 cm. All the solutions were prepared in chloroform. The chloroform was made absolute and was stabilized with 2% of ethanol. On the plates were deposited four spots (2 μl each) of standard solutions containing 0.4, 0.6, 0.8, and 1.0 μg/spot and, depending on the width of the plate, 1-3 portions (2 μl each) of the extract. The concentration of the extract was made such that the weight of the spot investigated came within the levels shown. The plates were chromatographed in cylindrical glass chambers 18 cm high and 8 and 10 cm in diameter. They were stained by spraying with a solution of antimony trichloride in chloroform followed by heating in the drying chest at 110°C for 15-20 min.

SUMMARY

Using the optical densitometry of thin-layer chromatograms, the amounts of triterpenoids of the dammarane series have been determined in the unsaponifiable fractions of ethereal extracts from the leaves of Far Eastern species of birch: Betula costata, B. mandshurica, B. dahurica, B. platyfolia, and B. kamtschatica.

LITERATURE CITED

1. M. Nagai, N. Tanaka, O. Tanaka, and S. Ishikawa, Chem. Pharm. Bull., 21, 2061 (1973).
2. N. I. Uvarova, G. V. Malinovskaya, Yu. N. El'kin, V. V. Isakov, A. K. Dzizenko, and G. B. Elyakov, Khim. Prirodn. Soedin., 659 (1975).
3. N. I. Uvarova, G. I. Oshitok, A. K. Dzizenko, V. V. Isakov, and G. B. Elyakov, Zh. Organ. Khim., 12, 984 (1976).
4. O. Tanaka, M. Nagai, and S. Shibata, Chem. Pharm. Bull., 14, 1150 (1966).
5. F. G. Fischer and N. Seiler, Ann. Chem., 626, 185 (1959).
6. P. G. Gorovoi, N. I. Uvarova, G. I. Oshitok, and G. B. Elyakov, Rast. Res., 11, 97 (1975).
7. S. Hiai, H. Oura, Y. Odaka, and T. Nakajima, Planta Medica, 28, 363 (1975).
8. N. E. Mashchenko, P. K. Kintya, I. P. Dragalin, G. V. Lazur'evskii, T. V. Demakova, and L. I. Guseva, Khim. Prirodn. Soedin., 265 (1976).
9. M. Suzuki and N. Ikekawa, Chem. Pharm. Bull., 14, 1049 (1966).
10. A. T. Glen, W. Lawrie, J. McLean, and M. El-Garby Younes, Chem. Ind. (London), 1908 (1965).
11. G. A. Fokina and N. V. Belova, Khim. Prirodn. Soedin., 735 (1975).
12. N. Ikekawa, Methods in Enzymology, 15, 200 (1969).
13. I. Sakamoto, K. Morimoto, and O. Tanaka, Yakugaki Zasshi, 95, 1456 (1975).

14. T. Tsukamoto, A. Yagi, K. Mihashi, and Y. Mori, Chem. Pharm. Bull., 16, 2123 (1968).
15. A. M. Dawidar, A. A. Saleh, and M. M. Abdel-Malek, Z. Anal. Chem., 273, 127 (1975).
16. J. G. Kirchner, J. Chromatogr., 82, 101 (1973).
17. S. A. Perry, R. Amos, and P. I. Brewer, Practical Liquid Chromatography, Plenum, London (1972).
18. T. Suzuki and K. Hasegawa, Agr. Biol. Chem., 38, 871 (1974).
19. H. Yamamoto, T. Kurita, Y. Suzuki, R. Hira, K. Nakano, H. Makabe, and K. Shibata, J. Chromatogr., 116, 29 (1976).
20. K. Doerfel, Z. Anal. Chem., 185, 1 (1962) [Russian translation as book under the title Statistics in Analytical Chemistry], Moscow (1969), p. 27.

STEROLS AND STEROL GLYCOSIDES OF Bryonia alba

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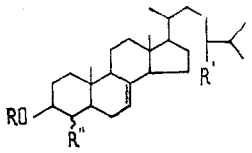
The roots of Byronia alba have long been widely used in the folk medicine of many countries. In view of this, we are systematically studying the chemical composition of extracts of the roots of this plant [1].

The present paper describes the identification of the least polar glycosidic fraction J (TLC, reagents a, b, and d), and also of fractions of free sterols S not differing in their chromatographic mobilities and the red coloration of the spot on TLC (reagent d) from the aglycones of the glycosides J.

The compositions and amounts of the phytosterols and their glycosides in fractions S and J are as follows:



				Amount, %	Fraction
I	R=D-glucopyranosyl,	R'=H, R"=H		1	J
II	"	Me	"	4	
III	"	Et	"	40	
IV	"	=CH_2	"	7	
V	"	=$CHCH_3$	"	42	
VI	"	Et	Me	2	
VII	"	=$CHCH_3$	"	4	
VIII	R = H	H	H	1	S
IX	"	Me	"	5	
X	"	Et	"	46	
XI	"	=CH_2	"	3	
XII	"	=$CHCH_3$	"	42	
XIII	"	Et	Me	2	
XIV	"	=$CHCH_3$	"	1	

Glycoside (I), detected on TLC in the form of a homogeneous spot, was isolated by column chromatography of a chloroform extract on silica gel, followed by recrystallization, in the form of white crystals melting at 211-213°C.

As early as 1911, Power and Moore [2] in a study of the composition of the roots of Bryonia isolated a substance with the same melting point which they named "bryonol." For it they established the empirical formula $C_{22}H_{36}O_4$, but the structure remained unknown. Later, Klein put forward the hypothesis that "bryonol" was actually a "glycoside of the phytosterol group" [3].

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