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SORPTION OF SAPONINS ON ION-EXCHANGE RESINS

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UDC 547.918

The capacity of a number of ion-exchange resins for the saponins isolated from the seeds of the tea plant, soapwort, horse chestnut and Iberian cyclamen has been studied. IA-1 anion-exchange resin possessed the highest sorption capacity. The influence of a number of technological parameters on the capacity of the sorbent has been considered. It has been shown that increases in the diameter of the resin grain, in the rate of feed of the solution to the column, and in the concentration of alcohol in the sorbate solution lead to a fall in sorption capacity. At the same time, the type of lyophilic alcohol in the range of C_1-C_4 scarcely affects the capacity. In the case of impure extracts of the saponin-containing plants, the capacity of IA-1 anion-exchange resin fell by a factor of 3-4, but ion-exchange treatment of such extracts led to better results.

In publications on the ion-exchange purification of glycoside-containing extracts from the impurities accompanying them, nothing is ever said about the magnitude of the sorption of the desired product [1-5]. In the case of saponin-containing extracts, this is connected with the fact that sufficiently reliable rapid methods of determining them quantitatively have been developed only in recent years [6-8].

We have studied the sorption activity of ion-exchange resins marketed industrially in the sorption of the saponins obtained from the fruit of the horse chestnut (*Aesculus hippocastanum L.*), soapwort (*Saponaria officinalis* L.), the tuberous roots of the Iberian (or Caucasian) cyclamen (*Cyclamen ibericum* Stev.), and the seeds of the tea plant (*Camelia sinensis* O. Ktze). The saponins so used had physicochemical properties agreeing completely with the constants given in the literature [9-11].

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Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 56-59, January-February, 1983. Original article submitted January 26, 1982.

0009-3130/83/1901-0052$07.50

TABLE 1. Sorption of Saponins

Saponins	Solvent	MDEC, g/g				
		KU-2	EDE-10P	AV-17	AN-31	IA -1
Iberian cyclamen	80% isopropanol	0,020	0,09	0,08	0,05	0,10
	Water					0,18
Fruit of the horse chestnut	80% isopropanol	0,020	0,07	0,06	0,03	0,2
Soapwort	Water					0,06
	80% isopropanol	0,010	0,03	0,02	0,01	0,03
Tea seeds	Water					0,14
	80% isopropanol	0,009	0,06	0,06	0,03	0,080

TABLE 2. Influence of the Rate of Feed of the Solution to the Columns (IA-1 resin)

Saponins	MDEC (g/g) at the following rates of feed of the solution, ml/min/cm^2			
	1,13	2,26	3,39	4,52
Iberian cyclamen	0,10	0,09	0,07	0,06
Fruit of the horse chestnut	0,20	0,15	0,12	0,08
Soapwort	0,06	0,04	0,03	0,02
Tea seeds	0,14	0,09	0,07	0,05

TABLE 3. Influence of the Diameter of the Grain of the Ion-Exchange Resin (IA-1) on the Capacity of the Sorbent

Saponins	MDEC (g/g) at the following grain diameters, mm				
	0,25	0,25–0,5	0,8–1,0	1,25–1,6	1,6–2,0
Iberian cyclamen	0,13	0,10	0,09	0,06	—
Fruit of the horse chestnut	—	0,20	0,17	0,11	0,09
Soapwort	0,12	0,06	0,03	0,025	—
Tea seeds	0,18	0,14	0,09	0,06	—

IA-1 resin possessed the greatest capacity (Table 1). Since, according to the results of our previous investigations, it is just this resin that gave the best results in the purification of saponins from the ballast impurities accompanying them in the plant raw material [4], the subsequent investigations of the influence of a number of technological parameters on the sorption process were carried out using IA-1 anion-exchange resin.

With a rate of feed of the solutions to the column greater than 1.13 ml/min/cm^2, the capacity of the IA-1 resin fell considerably, regardless of the type of sorbate, and at 4.52 ml/min/cm^2 the anion-exchanger sorbed 2-3 times less of the desired product (Table 2).

This effect is characteristic for the sorption of large organic molecules with the gel mechanism of diffusion kinetics. A similar effect of a fall in the sorption capacity of IA-1 anion-exchange resin is observed with an increase in the size of the grains in the column (Table 3).

It was not possible to obtain an unambiguous answer to the question of the influence of the type of alcohol on the course of sorption of the saponins (Table 4). In this case, the main value is apparently possessed not by the polarity of the medium but by the solubility of the saponin. We can only mention a slight influence of the sorbate on the course of the process. At the same time, the dependence of the sorption activity of IA-resin on the concentration of the alcohol used can be clearly traced, appearing as a lowering of the capacity of the resin with a rise in the concentration of alcohol (Table 5).

On passing to extracts of saponin-containing plants, it was found that because of the sorption of competing compounds [5], the sorption of the saponins on the IA-1 resin fell by a factor of 3-4. Preliminary filtration of an aqueous alcoholic extract through KU-2 resin in the H^+ form led to its desalting and to an increase in quality.

TABLE 4. Influence of the Type of Alcohol (IA-1 ion-exchange resin) on the Capacity of the Sorbent

Saponins	MDEC (g/g) on the use of			
	methanol	ethanol	n-propanol	isopropanol
Iberian cyclamen	0,12	0,11	0,11	0,10
Fruit of the horse chestnut	0 22	0,20	0,19	0,20
Soapwort	0,06	0,05	0,05	0,06

TABLE 5. Influence of the Concentration of the Alcohol on the Capacity of the Sorbent (solvent isopropanol; IA-1 resin)

Saponins	MDEC (g/g) at the following concentrations of isopropanol, %				
	H_2O	20	40	60	80
Iberian cyclamen	0,18	0,13	0,12	0,10	0,10
Soapwort	0 06	0,06	0,05	0,04	0,02
Tea seeds	0,14	0,10	0,09	0,08	0,07

The results of the investigations performed and of earlier work [5, 12] show the desirability of using, at the stage of purification of the concentrated extracts from fragments and phenolic compounds in the production of saponins, a system of KU-2 (H^+ form) and IA-1 (OH^- form) ion-exchange resins at a rate of feed of the solution to the column of 1-2 ml/min/cm^2 with a diameter of the grains of the KU-2 cation-exchange resin of 0.5-0.8 mm [5, 12] and of the IA-1 anion-exchange resin of 0.25-0.5 mm, and a ratio of the column parameters H : D = 8 : 1. The type of alcohol and its concentration must be correlated with the optimum conditions for the extraction of the raw material. Such ion-exchange purification of saponin-containing extracts with the minimum losses of saponins (as compared with the methods of reprecipitation, recrystallization, and extraction in a liquid—liquid system) will permit practically colorless extracts to be obtained from which the subsequent isolation of the desired product will be substantially simplified [12].

EXPERIMENTAL

The comparative investigation of the sorption capacity of the ion-exchange resins was performed under dynamic conditions using 1% aqueous alcoholic solutions of the saponins. In order to observe definite parameters, the loading of the column amounted to 3 g of resin in the case of ion-exchangers with a gel structure and 1 g (absolutely dry weight) for the macroporous IA-1 anion-exchanger, and in both cases the mean grain diameter was 2.5-0.5 mm.

Forms of the resins: OH^- for the anion-exchange resins and H^+ for the cation-exchange resins. The rate of feed of the solution to the column ranged between 1.13 and 4.52 ml/min/cm^2.

The extracts of saponin-containing plants were obtained by treating the raw material with 80% isopropanol at the boiling point of the solvent in a flask with a reflux condenser for 1 h twice, the ratio of raw material to solvent being 1 : 6. Samples of filtrate issuing from the column (aqueous or aqueous alcoholic solutions) were analyzed photocolorimetrically in a mixture of sulfuric acid and acetic anhydride.*

The amounts of saponins in the extracts were determined densitometrically.

SUMMARY

1. It has been shown that IA-1 anion-exchange resin possesses the greatest activity with respect to saponins among a number of ion-exchange resins produced industrially.

With increase in the rate of feed of the solutions to the columns in the diameter of the resin grain, and in the concentration of alcohol in the solutions the capacity of the resins decreased. However, a change in the type of lyophilic alcohol in the range of C_1-C_4 had practically no effect on the capacity of the sorbent.

*The method of analysis was developed by Ts. P. Sulakvelidze and É. P. Kemertelidze.

2. It has been established that in the treatment of extracts of saponin-containing plants it is desirable to use IA-1 resin in combinations with KU-2 resin for the preliminary desalting of the extract.

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GLYCOSIDES OF MARINE INVERTEBRATES.

XVI. CUCUMARIOGENIN FROM GLYCOSIDES OF THE HOLOTHURIAN *Cucumaria fraudatrix*

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UDC 547.996:593.96

A new genin of the holostane series has been isolated by the acid hydrolysis of the glycosidic fraction of the holothurian *Cucumaria fraudatrix*. It has been shown that this genin is the native alycone of glycoside G_1 from the same holothurian. The structure of the genin has been established as 16β-acetoxyholosta-7,24-dien-3β-ol.

Continuing an investigation of the glycosides of marine invertebrates, we have studied the structure of the native genin from the glycosides of the Far Eastern holothurian *Cucumaria fraudatrix*. The structure of 16β-acetoxyholosta-7,24-dien-3β-ol (Ia) for this compound was proposed on the basis of the following facts. Cucumariogenin was obtained in the form of the acetate (Ib) from the glycosidic fraction of the holothurian *Cucumaria fraudatrix* after acid hydrolysis of this fraction and acetylation of the hydrolysis products. Cucumariogenin acetate (Ib) had seven methyl groups, since its ^{1}H NMR spectrum had seven 3H singlets in the 0.9-1.68 ppm region. The IR spectrum of (Ib) showed an acetate and a lactone function ($\nu_{C=O}$ 1727 cm^{-1}, ν_{C-O-C} 1253 cm^{-1}, $\nu_{C=O}$ 1755 cm^{-1}). There was no absorption of free hydroxy groups in the spectrum.

The ^{13}C NMR spectrum of cucumariogenin acetate contained three signals of carbonyl carbons (168.9, 170.9, and 179.0 ppm) corresponding to two acetate groups and a γ-lactone, and also four signals of carbon atoms for two tri-substituted double bonds (120.3 d, 144.8 s,

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Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 59-64, January-February, 1983. Original article submitted January 22, 1982.

0009-3130/83/1901-0055$07.50