THE MONITORING OF THE PRODUCTION OF ECDYSTERONE

M. R. Yakubova, A. U. Mamatkhanov,
M.-R. I. Shamsutdinov, and T. T. Shakirov

UDC 615.322+543.544.4+543.420.
62+581.192+547.92.02

A method for monitoring the production of ecdysterone from the roots of Rhaponticum carthamoides (Willd.) and the inflorescences of Rh. integrifolium C. Winkl. is given.

Steroid substances – phytoecdysteroids – have been isolated from various species of Rhaponticum [1-7], and also from other plants possessing a tonic action [8-12]. Their biological activity, especially the biological activity of ecdysterone, has been studied [13].

Ecdysterone possesses tonic and adaptogenic properties and anabolic activity, and it stimulates erythropoiesis under conditions of experimental anemia [13-16]. The influence of ecdysterone on the central nervous system of experimental animals and on their dynamic capacity for work and adaptation to a high temperature has been considered. As a result of pharmacopoeial tests, new medicinal preparations have been created from ecdysterone.

In view of this, the development of methods for the industrial isolation of ecdysterone and for determining it in substances, medicinal forms, and plant raw material and the monitoring of production over the stages of the industrial process are acquiring great importance.

We give the results of the stagewise monitoring of the production of ecdysterone from the inflorescences of Rh. integrifolium C. Winkl., and the roots of Rh. carthamoides (Willd.) Iljin (family Asteraceae). The methods of obtaining ecdysterone from the materials mentioned have been given in the literature [17, 18]. The proposed method of analysis is based on the quantitative determination of ecdysterone in plant raw material [18], the essence of which consists in the chromatographic separation of the combined extractive substances in a thin layer of Alusil (a mixture of alumina and silica gel) in the chloroform–methanol–acetone (6:2:1) solvent system, eluting the zones containing the ecdysterone, and determining it spectrophotometrically in the eluate at a wavelength of 241 nm.

The ecdysterone was obtained from the inflorescences and roots by the following scheme: The comminuted raw material was extracted with ethanol, the extract was evaporated, the residue was diluted with water, and the hydrophobic impurities were eliminated by treatment with chloroform. The ecdysterone was extracted from the purified aqueous layer with a mixture of chloroform and isopropanol (1:1). After evaporation, the chloroform–isopropanol residue was transferred to a column of alumina and the ecdysterone was eluted with

TABLE 1. Dynamics of the Extraction of Ecdysterone by Methanol from Plant Raw Material

Object analyzed	Amount of ecdysterone, %			
	inflorescences of Rh. integrifolium		roots of Rh. carthamoides	
	on wt. of raw material	on amt. ecdysterone in raw material	on wt. of raw material	on amt. ecdysterone in raw material
Raw material	0,490	100,000	0,160	100,000
Extraction 1	0,178	36,260	0,770	48,125
2	0,090	18,286	0,028	17,375
3	0,062	12,755	0,017	10,437
4	0,054	11,143	0,009	5,688
5	0,043	8,918	0,0088	5,500
6	0,016	3,061	0,0056	3,500
	0,443	90,423	0,145	90,625

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 322-324, May-June, 1983. Original article submitted February 26, 1972.

0009-3130/83/1903-0305$07.50

TABLE 2. Quantitative Indices of the Control of the Production of Ecdysterone according to the Stages of the Industrial Process

Object analyzed	Amount of ecdysterone, %			
	inflorescences of Rh. integrifolium		roots with rhizomes of Rh. carthamoides	
	on the weight of the raw material	on the amount of ecdysterone in the raw material	on the weight of the raw material	on the amount of ecdysterone in the raw material
Initial raw material	0.490	100.00	0.160	100.00
Combined extraction	0.440	89.80	0.14	87.50
Meal	0.048	9.80	0.012	7.50
Chloroform extraction	0.0285	5.85	0.015	9.38
Chloroform–isopropanol extraction	0.355	72.50	0.124	77.50
Chloroform–methanol eluate	0.340		0.110	
Aqueous mother liquor	0.0210	4.30	0.001	0.69
Spent sorbent	0.036	7.35	0.006	3.75
Technical ecdysterone	0.255	52.10	0.08	49.75
Ecdysterone after recrystallization	0.248	50.60	0.076	47.8
Mother liquor from the first crystallization	0.082	16.7	0.037	23.3
Unaccounted losses	0.008	1.66	0.003	1.5
		5		4

chloroform–methanol (2:1). After evaporation of the eluate and recrystallization of the residue from ethyl acetate, the desired product was obtained.

The amounts of ecdysterone obtained after six extractions from the inflorescences of Rh. integrifolium and from the roots with rhizomes of Rh. carthamoides were 0.44 and 0.14% of the weight of the raw material, respectively (Table 1).

The yield of finished product in the process of producing ecdysterone amounted to 50%, the accounted losses of ecdysterone being 45% and the unaccounted losses 5% (Table 2).

The proposed method permits the reliable analysis of the plant raw material, the production intermediates, and the finished preparation.

EXPERIMENTAL

The analysis of the plant raw material was carried out as described previously [19].

Analysis of the Amount of Ecdysterone in the Various Stages of the Industrial Process. The extraction of the ecdysterone was carried out with methanol, six times from 20 kg of the inflorescences of Rh. integrifolium and from 50 kg of the roots with rhizomes of Rh. carthamoides. The liquor ratios of the process were 1:45 and 1:15, respectively. The results of the analysis of the extractions are given in Table 1.

From the concentrated extract, after it had been diluted with water, treatment with chloroform (5× 10 liters) removed oils and tanning and resinous substances. The total ecdysteroids were extracted from the purified aqueous solution with a mixture of chloroform and isopropanol (1:7) (7× 10 liters). Pigments were eliminated by chromatography on alumina (Brockmann activity grade II). The ratio of total material to sorbent was 1:12. A mixture of methanol and chloroform (1:2) was used in an amount of 30 ml per 1 g of total material. The eluate was evaporated to dryness and the residue was recrystallized from a mixture of methanol and ethyl acetate (1:9).

Depending on the concentration of ecdysteroid in the sample being analyzed, for each case the amount of solution deposited on a glass plate with dimensions of 24 cm with a fixed layer of Alusil was selected individually in such a way that the amount of ecdysteroid was within the limits of the sensitivity of the determination.

SUMMARY

Conditions have been developed for determining ecdysterone in the intermediates of its production from the inflorescences of Rhaponticum integrifolium and from the roots of Rh. carthamoides and for monitoring the stages of the industrial process.

LITERATURE CITED

1. U. Baltaev, M. B. Gorovits, S. A. Khamidkhodzhaev, and N. K. Abubakirov, Khim. Prir. Soedin., 406 (1974).
2. N. K. Abubakirov, Khim. Zhizn', No. 11, 57 (1975).
3. E. A. Krasnov, A. S. Saratikov, and G. D. Yakunina, Khim. Prir. Soedin., 550 (1976).
4. U. Bataev, M. B. Gorovits, N. D. Yakunina, and N. K. Abubakirov, Khim. Prir. Soedin., 813 (1977).
5. U. Bataev, M. B. Gorovits, Ya. V. Rashkes, and N. K. Abubakirov, Khim. Prir. Soedin., 463 (1978).
6. M. B. Gorovits, I. L. Zatsny, and N. K. Abubakirov, Rast. Res., 10, 261 (1974).
7. U. Baltaev, M. B. Gorovits, N. D. Abdullaev, Ya. V. Rashkes, M. R. Yagudaev, and N. K. Abubakirov, Khim. Prir. Soedin., 457 (1978).
8. B. Z. Usmanov, M. B. Gorovich, and N. K. Abubakirov, Khim. Prir. Soedin., 535 (1971).
9. Z. Saatov, B. Z. Usmanov, and N. K. Abubakirov, Khim. Prir. Soedin., 422 (1977).
10. I. L. Zatsny, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 840 (1971).
11. I. L. Zatsny, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 175 (1973).
12. I. L. Zatsny, M. B. Gorovits, Ya. V. Rashkes, and N. K. Abubakirov, Khim. Prir. Soedin., 155 (1975).
13. V. N. Syrov and A. G. Kurmukov, Farmakol. Toksikol., 690 (1976).
14. V. N. Syrov, M. I. Aizikov, and A. G. Kurmukov, Dokl. Akad. Nauk Uzb. SSR, No. 8, 37 (1975).
15. V. N. Syrov, R. A. Ashrapova, and A. G. Kurmukov, in: Current Questions of Obstetrics and Gynecology [in Russian], No. 1 (1976), p. 62.
16. V. N. Syrov and A. G. Kurmukov, Dokl. Akad. Nauk Uzb. SSR, 12 (1977).
17. A. U. Mamatkhanov, M.-R. I. Shamsutdinov, and T. T. Shakirov, Khim. Prir. Soedin., 667 (1970).
18. A. U. Mamatkhanov, M.-R. I. Shamsutdinov, and T. T. Shakirov, Khim. Prir. Soedin., 528 (1980).
19. M. R. Yakubova, G. L. Genkina, T. T. Shakirov, and N. K. Abubakirov, Khim. Prir. Soedin., 737 (1978).

SYNTHESIS OF ACETYLATED GLYCOSIDES OF HYDROXYNAPHTHOQUINONES

S. G. Polonik, A. M. Tolkach, and N. I. Uvarova

UDC 547.656+547.455+616-006.6

A method is proposed for the synthesis of acetylated glycosides of hydroxynaphthoquinones. The condensation of D-glucose and D-galactose (tert-butyl orthoacetate)s with lawsone and lapachol has given the tetra-O-acetyl-β-D-glucopyranosides of lawsone and of lapachol and the tetra-O-acetyl-β-galactopyranoside of lawsone. The structures of the glycosides obtained have been confirmed by IR and ^{1}H and ^{13}C NMR spectroscopy. The structure of the lawsone acetylgalactopyranoside described previously has been corrected.

The majority of investigation of recent years in the field of the synthesis of glycosides of hydroxynaphthoquinones have been connected with the creation of water-soluble hydroxynaphthoquinone derivatives, which are necessary for studying the influence of the carbohydrate moieties on their biological activity. The tetra-O-acetyl-β-D-glucopyranosides of lawsone (40%) and menoctone (56%) with the o-quinoid structure of the aglycone [1], and the tetra-O-acetyl-D-glucopyranosides of lawsone (30%) and of lapachol (16%) and the tetra-O-acetyl-D-galactopyranoside of lawsone (28%) with the p-quinoid structure of the aglycone [3] have been obtained by using various modifications of the Koenigs–Knorr method. The acetates of glycosides of lawsone and lapachol exhibited antitumoral activity [2, 3]. The reduction of an acetylated D-glucose residue leads to a marked increase in the immunodepressive action of lawsone [4].

The biological activity of glycosides of hydroxynaphthoquinones makes necessary a search for new and more effective methods for their synthesis.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok.

Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 324-327, May-June, 1983. Original article submitted March 19, 1982.

0009-3130/83/1903-0307$07.50