EXTRACTION OF GOSSYPOL

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UDC 665.3/35:665.1.03

The conditions for the extraction of gossypol from cottonseed flakes and from isolated gossypol glands have been investigated. It has been established that the amount of gossypol extracted by hexane is affected mainly by the degree of stirring, the moisture content, the material, and the temperature. By steeping the flakes first with concentrated miscella and then with hexane it is possible to extract about 60-65% of the gossypol. It has been shown that hexane and miscella extract practically no gossypol either from dry or from moistened gossypol glands, and only acetone extracts it almost completely.

In the complex processing of cotton seeds for the production of high-quality oil and meal from which food protein could be extracted, the gossypol must be eliminated.

Gossypol dissolves readily in the oil but is practically insoluble in the aliphatic hydrocarbons used in the industrial extraction of oils [1-3]. In the extraction of crude cottonseed flakes with gasoline under the usual conditions, about 10-12% of the free gossypol passes into the miscella [4]. Attempts to increase the passage of gossypol into gasoline miscellas have been made by a number of workers [5-12]. They have shown that in the extraction of cotton seeds with hexane or gasoline the amount of gossypol passing into the miscella depends on a number of factors: the speed of the stirring, the thickness and moisture content of the flakes, the liquid/solid ratio, etc. According to Rzhekhin [7], in the stepwise treatment (first with 20-30% miscella and then with gasoline) of cottonseed flakes brought to a moisture content of 12-15%, it is possible to extract about 60-70% of the gassypol.

Grigorchuk et al. [8] have expressed the opinion that "... the careful preparation of the kernels for extraction...should ensure the passage of the maximum amount of gossypol present in the seed kernel into the miscella ...". However, this has not been confirmed by experimental results. Furthermore, the drying of the heavily moistened flakes with hot air recommended by these authors leads to the binding of the free gossypol to proteins and, consequently, to a worsening of the quality of the meal.

In the process of extracting gossypol from isolated gossypol glands it was established that at 20°C hexane extracts practically no gossypol from the intact glands, but at 60°C its extractability rises to 12% [13].

We have investigated the extraction of gossypol by hexane and by miscellas of various concentrations from cottonseed kernels, and have also extracted it from isolated gossypol glands by acetone with the aim of investigating the influence of a number of factors on the completeness of extraction of the gossypol.

In a first series of experiments we considered the influence of stirring, the temperature of the process, and the presence of water in the solvent on the degree of extraction of gossypol:

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 157-160, March-April, 1980. Original article submitted December 17, 1979.

 0009-3130/80/1602-0114$07.50

Expt. No.	Conditions of extraction	Concentration of the miscella obtained, %	Meal		
			oil content, %	moisture content, %	free gossypol,* %
1	Steeping at 20°	10.00	11.00	8.00	1.88
2	Steepint at 40°	11.10	8.50	8.40	1.73
3	Stirring at 4000 rpm, 20°C	10.74	12.22	7.45	1.79
4	Stirring with solvent containing added water at 20°C	6.57	16.40	12.03	1.60

The results obtained show that these factors lead to a more complete extraction of the gossypol, but a high amount of this substance still remains in the meal.

Previously, the influence of the moisture content of the flakes on the extraction of gossypol had been studied. It had been found that the maximum amount of gossypol is extracted from cottonseed flakes with a moisture content of 12-15% [7-14]. However, there are reports that the same amount of gossypol is extracted with different moisture contents of the kernels: 6.5, 8.7, and 11.0% [15].

We performed experiments with flakes in which various amounts of water had been introduced with the aid of a sprayer and which, after being kept for two hours, were extracted three times with hexane, after which the meal was dried under a current of air:

Expt. No.	Moisture content (%) of the		Concentration of the miscella after the first extraction, %	Meal	
	flakes	meal		oil content, %	free gossypol, %
5	7.60	7.80	15.80	5.10	2.28
6	15.30	8.00	13.20	5.60	2.20
7	17.50	8.30	12.60	7.00	2.13
8	21.90	9.00	11.00	8.40	1.85

As the results obtained witness, a fall in the amount of free gossypol in the meal by 0.43% (experiment 8) takes place only with a high degree of moistening of the flakes. However, under these conditions the amount of oil extracted falls appreciably which is due to an inhibition of the process of the diffusion of the oil from the flakes into the external medium [16, 17]. The slight fall in the gossypol content of the meal apparently takes place because moistening alone with no change in the other parameters is insufficient.

To improve the extractability of the gossypol we tried the use of miscellas of various concentrations:

Expt. No.	Concentration of the miscella, %		Meal		
	before extraction	after extraction	moisture content, %	oil content, %	free gossypol, %
9	0	14.7	8.10	18.90	2.38
10	5	17.0	6.50	22.20	3.31
11	10	20.0	6.70	26.60	2.30
12	15	23.8	6.60	26.80	2.25
13	20	29.8	6.00	29.40	2.16
14	25	31.5	5.90	34.10	2.13
15	25	-	8.50	6.70	1.58
16	84	-	7.50	12.50	1.46

*The amount of free gossypol in the meal is given on the absolutely dry and defatted substance.

Concentrations of miscella of from 5 to 25% had little effect on the extraction of gossypol (experiments 10-14), and only a subsequent extraction with hexane after the steeping of the flakes with miscella permitted the extraction of 1.5 times more gossypol than extraction with miscella alone (experiments 15 and 16).

In study of the influence of the time of the process on the completeness of the extraction of gossypol (experiments 17-20) it was found that the bulk of the gossypol is extracted during the first half-hour (gossypol content of the meal 1.77%) while with longer steeping of the flakes with hexane for 60, 90, and 120 min the amounts of gossypol in the meal were 1.72, 1.71, and 1.70%, respectively.

We have investigated the extraction of gossypol from isolated gossypol glands with various solvents. After steeping for two hours, the amount of free gossypol that had passed into the solution was determined:

	Expt. No.					
	21	22	23	24	25	26
gossypol, %	0.0	0.04	25.80	24.0	25.80	0.40

These figures show that gossypol is not extracted at all by hexane and only very slightly by 30% miscella from dry gossypol glands.

Nevertheless, a very small amount of gossypol does pass into hexane from moistened glands, apparently as the result of the breakdown of the walls of the glands by the water. However, the extract obtained on the steeping of dry glands with aqueous acetone and of moist glands with dry acetone contained the same amount of free gossypol; again, on treatment of the unmoistened glands with dry acetone a large mount of gossypol was extracted.

EXPERIMENTAL

The initial cottonseed kernels contained 7.85% of moisture, 35.74% of oil, and 2.40% of free gossypol. For each of experiments 1-4 we used 50 g of ground kernels, from which the oil was extracted with 150 g of hexane for 15 min. In each of experiments 5-8 we used 50 g of flakes, which were extracted successively with 100, 100, and 80 ml of hexane, the filtrate being separated on a Büchner funnel after every 30 minutes' steeping.

In each of experiments 9-14 the flakes were steeped in 120 ml of miscella for 30 min. In experiments 15 and 16 the conditions were the same as in 9-14, but after the steeping in the miscella the flakes were extracted with 100 ml of dry hexane twice for 30 min. In each of experiments 17-20, 60 g of cotton flakes was extracted with 200 ml of hexane and after a predetermined time the mixture was filtered on a Büchner funnel. In experiments 21-24, respectively 0.0353 g of dry glands was covered with 50 ml of hexane, 30% miscella, 70% aqueous acetone, and dry acetone. In experiments 25 and 26 we used moistened glands, which were covered with dry acetone and with hexane, respectively. The amounts of free gossypol were determined by the p-anisidine method [4]. Its amount in the initial glands was 28.9%.

SUMMARY

The conditions for the extraction of gossypol from the cotton flakes by hexane and miscellas have been studied. It has been established that the extraction of gossypol is affected mainly by the degree of stirring, the moisture content of the material, and the temperature. When flakes are first steeped in concentrated miscella and are then treated with pure hexane it is possible to extract 60-65% of gossypol. It has been shown that the bulk of the gossypol is extracted in the first half-hour.

2. On steeping, hexane and miscellas extract practically no gossypol either from dry or moistened isolated glands, and it is extracted almost completely only by acetone.

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ANTHRAQUINONES OF THE LICHEN *Asahinea chrysantha*

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UDC 547.673+547.976+582.29

From a hexane extract of the dry lichen we have isolated six anthraquinones: chrysophanol (I), islandicin (II), cynodontin (III), emodin (IV), a tetrahydroxymethylanthraquinone (V), and a pentahydroxymethylanthraquinone (VI). The structures of (I) and (IV) were confirmed by direct comparison with authentic samples. The structures of (II) and (III) were established by the aid of UV, IR, PMR, and mass spectra. Pigments (V) and (VI) were isolated from a carbonate extract. Pigment (V): mp > 320°C; UV spectrum (nm) 258, 283, 310, 447, 500, 533; mass spectrum: 286 (M^+ 100%), 270, 258, 257, 241, 229, 216, 213, 212, 211, 201, 161, 155, 137, 115, 105, 97. Pigment (VI): mp 315°C; UV spectrum (nm): 247, 261, 302, 500, 540, 565, 578; IR spectrum (cm^{-1}): 1587, 3492; mass spectrum: 302 (M^+, 100%), 286, 274, 245, 228, and the metastable ions 248.6, 219.1, and 192.5. The positions of the β-hydroxyls in the molecules of (V) and (VI) have not been definitively established.

In the territory of the Soviet Union, the genus *Asahinea* is represented by two mass species: *A. chrysantha* (Tuck.) Culb. et Culb. and *A. scholanderi* (Llono) Culb. et Culb., occupying enormous areas of the northeastern part of the country. Both species are char-characteristic for mountain tundras [1]. The high-mountain lichens are arousing special interest because of their capacity for protecting the cells of their organisms from ultraviolet radiation. It is assumed that the colored lichen substances act as filters protecting the phycobionts from the effects of radiation [2].

It is known that lichens of the genus *Asahinea* contain usnic, alectoronic and α-collatolic acids and pink pigments the nature of which has not so far been established [3]. In the present paper we consider the isolation of the pigments of the lichen *Asahinea chrysantha* and the determination of their structures.

Pacific Ocean Institute of Bioorganic Chemistry of the Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 160-165, March-April, 1980. Original article submitted October 12, 1979.

0009-3130/80/1602-0117$07.50