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LIPOPHILIC COMPONENTS OF COTTONSEED TAR

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Purified components of the biologically active substances have been isolated from cottonseed tar by treatment with calcium hydroxide.

The tar that is a multitonnage by-product from the distillation of the fatty acids of cottonseed oil soapstock contains about 18% of unsaponifiable substances, including a series of biologically active compounds [1].

The unsaponifiable substances are usually extracted with diethyl ether after the oil has been saponified with potassium hydroxide solution [2]. However, KOH corrodes the apparatus and partially decomposes tocopherols and other biologically active components.

Since our task was to isolate purified biologically active compounds from the tar for possible use, for its saponification we have employed $Ca(OH)_2$ [3], and, for the extraction of the unsaponifiables, ethanol, in which the resinous substances do not dissolve [4]. In addition, by changing the conditions for the saponification of the tar with $Ca(OH)_2$, we have succeeded in obtaining components with a composition differing from that resulting from classical saponification.

The alcoholic extract was saponified by column chromatography and preparative TLC on silica gel. The yield of products was 12%. For comparison, we saponified the tar with potassium hydroxide under the same conditions.

Table 1 gives the sets of components obtained by the known and the proposed methods. The mass fraction of tocopherols, sterol esters, and triterpenols obtained by the proposed method was higher than by the known method. In addition, the extract obtained by the proposed method contained another biologically active component — sterol glycosides.

The compounds were identified by their chromatograpic mobilities and those of model compounds, by qualitative reactions, and from literature information and spectral characteristics [5].

In the glycosides, the main components identified were sterol glycosides, which were purified by preparative TLC and were then subjected to acid hydrolysis [6]. The hydrolysis products were β-sitosterol, galactose, and an unidentified carbohydrate.

The mass spectrum of the hydrocarbons showed the peaks of molecular ions with *m/z* 394, 408, 422, 450, 392, 406, 420, 462, 390, 401, 418, 432, 402, 416, 430, 444, 458, 400, 414, 428, 442, 456, 398, 412, 426, 440, and 454. The most intense were the ions with M^+ 458, 454, 444, 440, 416, and 412. On the basis of the mass-spectral results, it was established that the hydrocarbons consisted of C_{28}-C_{32} paraffins, C_{28}-C_{33} monoenes, C_{28}-C_{31} dienes, C_{29}-C_{33} trienes, C_{29}-C_{33} tetraenes, and C_{28}-C_{33} pentaenes.

The mass spectrum of the tocopherols included the peaks of molecular ions (M^+ 430, 416, and 402) corresponding to α-, γ-, and δ-tocopherols and also the peaks of molecular ions (M^+ 858 and 830) characteristic for tocopherol dimers; the latter could have been formed during the technological treatment of the oil.

On the basis of mass-spectral results, among the sterols we identified β-sitosterol (M^+ 414), stigmasterol (M^+ 412), campesterol (M^+ 400), and a 4-monomethylsterol (MC^+ 428). The main component was β-sitosterol.

In the triterpene alcohol fraction, together with the peak of a molecular ion (M^+ 426) there were fragmentary ions with *m/z* 411, 408, 393, 365, 297, 271, 218, 203, and 189, showing the presence of four main compounds: α- and β-amyrins, cycloartenol, and cyclostadienol. In addition, the presence of M^+ 440 and of fragmentary ions with *m/z* 425, 422, 407, 315, and 300 indicated the presence of 24-methylenecycloartenol, while ions with *m/z* 313, 297, 295, 273, 270, 259, 255, and 241 showed the presence of 24-methylenelanosterol in the mixture.

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TABLE 1. Composition of the Unsaponifiable Fraction Isolated from Cottonseed Tar

Components	Amount, % by weight	
	Known method	Proposed method
Hydrocarbons	8.2	11.4
Sterol and triterpenol esters	14.3	40.4
Tocopherols	4.6	5.8
Free fatty acids	Tr.	Tr.
Sterols	1.6	1.2
Triterpenols	36.9	7.1
Pigments + unidentified components	34.4	21.0
Sterol glycosides	–	13.1

In the mass spectra of the esters of fatty acids with alcohols there were the peaks of molecular ions with *m/z* 704, 694, 692, 680, 678, 676, 666, 664, 652, 648, 638, 624, 620, 606, 604, 602, 592, 578, and 564, and also those of acyl fragments with *m/z* 285-229 (18:0-14:0), 283 (18:1), 281 (18:2) $[RCOOH_2]^+$, and 267-211, 265, and 263 $([RCO]^+$. The presence of acyls of the acids indicated was confirmed by the GLC of the methyl esters of the fatty acids obtained as the result of the acid hydrolysis of the esters [8] (% by weight): 12:0 — 1.1; 14:0 — 4.0; 16:0 — 46.6; 16:1 — 2.8; 18:0 — 17.1; 18:1 — 24.7; 18:2 — 3.7. The highest intensities in the mass spectrum were possessed by ions of the $[M - RCOO]^+$ and $[R - RCOOH]^+$ types, with *m/z* 397, 396 (100%) for β-sitosterol, 395 (38%), 394 (83%) for stigmasterol, and 383 (19%), 382 (42%) for campesterol, while ions with *m/z* 411 (16%), 410 (23%), and 288 (24%) for cycloartenol were less intense. The intensities of the fragments for other triterpene alcohols (M^+ 426) and the $C_{22:0}$-$C_{26:0}$ aliphatic alcohols amounted to less than 1%.

EXPERIMENTAL

The biologically active components were isolated with alcohol after saponification of the tar with calcium hydroxide.

The tar (100 g) was heated to 70°C, and powdered calcium hydroxide (12 g) was added. After heating for half an hour a stable pasty mass was formed that hardened after cooling. The hardened mass was ground, and the biologically active components were extracted with alcohol.

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