INVESTIGATION OF THE FATTY OIL OF THE SEEDS OF PRANGOS PABULARIA LINDL

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Prangos pabularia Lindl. (the hay plant) belongs to the family Umbelliferae, which numbers more than 140 genera and 2500 species represented in the USSR [1, 2]. Of these, only a few (about 20 species), are known as oil-bearers. Only the seed of coriander is used in practice for the industrial production of fatty and essential oils. Its content of fatty oil is 19-21%.

Parsley seed contains as much oil. Both these oils, like the oils of the other Umbelliferae, contain petroselinic (octadec-6, 7-enoic) acid, which is apparently characteristic for plants of the family Umbelliferae, though there are exceptions.

Many representatives of this family contain resins dissolved in the essential oils in the roots, stems, and pods.

The genus Prangos includes about 25 species found in the region of the Mediterranean sea, in Asia Minor, Transcaucasia, Central Asia, Iran, Afghanistan, and eastern India.

The hay plant is a perennial herbaceous plant up to 1 m in height. It grows on clayey and clayey-stony mountain slopes, forming fairly considerable thickets [3]. It flowers, depending on the region of distribution, in May-June and the seeds ripen in July-August. The life of the plant is about 50 years.

In the roots and subterranean parts it contains 13-20% of resin. Its chemical composition has been studied inadequately [2, 4, 5]. The following substances, among others, have been isolated from the resin: oxypeucedanin ($C_{16}H_{14}O_5$), prangenin ($C_{15}H_{14}O_4$), and osthol ($C_{15}H_{16}O_3$). Many of these substances are toxic; some (for example, osthol) are used as drugs.

A bright yellow essential oil with a weak and uncharacteristic odor is isolated from the fresh plant by distillation. Yield 0.08-0.19% [6, 7].

Experimental

We investigated seeds of Prangos collected by the expedition of the Institute of the Chemistry of Plant Substances to the Bostandyk district of Tashkent province in the upper parts of the Kainarsai.

The seeds were in the form of straw-colored, elongated cylinders with raised ribs. The kernel is firmly fixed to the husk. The content of kernel is 47.66 and of husk 52.34%. Dimensions of the seeds: length 12.0-18.5 mm, width 5.0-10.8 mm, thickness 2.0-4.9 mm; weight of 1000 seeds - 50.24 g, bulk density 86.93 g/l. The total weight of substances extractable with petroleum ether, on an absolute dry-matter basis, was 17.36%.

The air-dried seeds were comminuted and extracted with petroleum ether (bp 40-60°). The extract was concentrated by distilling the solvent in a current of carbon dioxide in several stages (see scheme). Partial evaporation of the extract yielded a heavy sticky yellow resinous product (A); the extract was poured off from this deposit and was further evaporated, but not to completion: white crystals mixed with a brown amorphous product (A_2) deposited; in the last stage, the third evaporation, the same crystals as in fraction A_2 were obtained almost pure; their melting point was 82° (A_3).

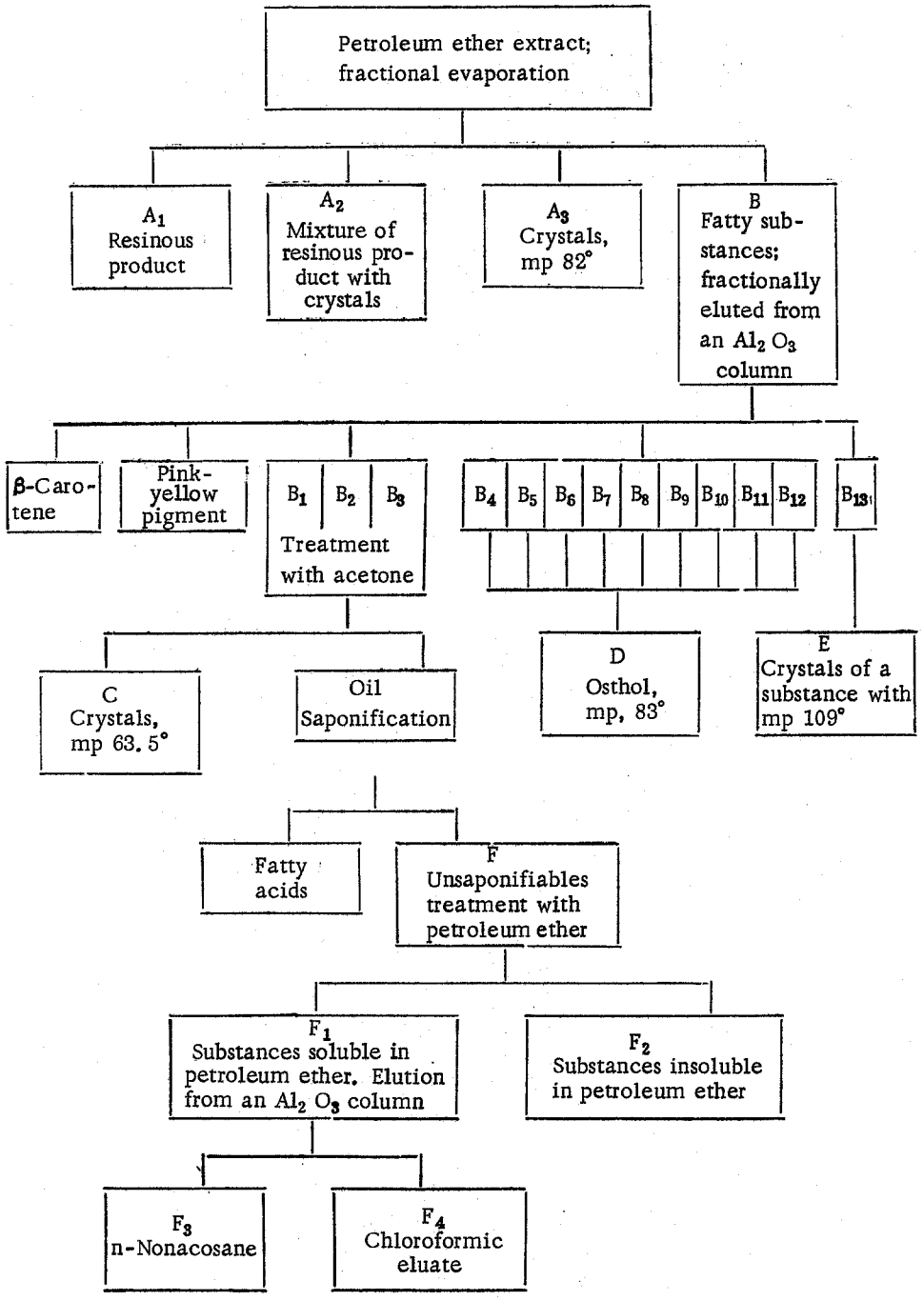
The concentrated extract was again decanted off and evaporated until it was completely free from solvent. The residue (C) was dried in a vacuum drying chamber.

The product obtained was dissolved in an equal weight of petroleum ether. The solution was subjected to chromatographic separation on a column containing ten times as much alumina by weight as the solution. Development of the chromatogram with petroleum ether gave two colored rings moving with the solvent: the lower ring was yellow and the upper ring pinkish-yellow. A stationary diffuse pinkish brown zone remained at the top of the column.

The petroleum ether eluates were collected in fractions approximately equal in volume to the initial solution of substance B. In all, 13 eluate fractions were collected. After the solvent had been distilled off in a current of carbon dioxide, a fatty substance was found in the first three fractions, the characteristic indices of which are given in Table 1.

All three fractions were slightly opalescent. On saponification with alcoholic alkali, fractions B_1 and B_2 did not darken, while fraction B_3 darkened somewhat. When the fractions were dissolved in acetone, the solution deposited white lamellar crystals with a nacreous luster; mp of the crystals 63.5-63.8°. The crystals were separated by filtration,

Scheme of Separation of the Extract from Prangos Seeds



and the acetone was distilled off from the solution to leave a straw-yellow oil with a characteristic odor and with a slightly burning taste.

Physical and chemical characteristics of the oil: d_4^{20} 0.9133; n_D^{20} 1.4746; Ostwald viscosity 0.425 poise; relative viscosity 6.14°E; saponification number 173.37 mg of KOH; acid number 0.52 mg of KOH; iodine number by Kaufmann's method 117.69%; thiocyanogen number 88.48%.

The petroleum ether eluates B_4-B_{12} yielded white needle-shaped crystals with mp 83°, and the eluate B_{13} bright yellow crystals with mp 106°. The latter were dissolved in a mixture of petroleum ether and chloroform, repurified by passage through a column of alumina, and recrystallized, giving a substance with mp 109°.

Table 1

Characteristics of the Fatty Fractions of the Extracts

Fraction	Yield, %		Characteristics of the products			
	In relation to product C	In relation to the weight of the absolutely dry seeds	Acid number, mg of KOH	Iodine number by Kaufmann's method, %	Color	Consistency
B_1	6.05	0.75	1.22	83.99	Bright yellow	Greasy
B_2	23.40	2.92	0.37	87.85	Yellow	Oily
B_3	13.54	1.69	0.48	107.91		

Investigation of the fatty oil. In order to establish the quantitative amount of fatty oil in the Prangos seeds, 122 g of the seeds (calculated at the absolutely dry substance) was comminuted, and extracted with petroleum ether in a Soxhlet apparatus. The total yield of extractable substances was 19.24% of the weight of the seeds.

Then the extracted substances were treated in accordance with the scheme described above, the fatty oil being isolated. Its yield was 34% of the weight of the extractable substances; hence, the oil content of the seeds was 6.54%.

Fractions B_1-B_3 were combined and saponified with 2 N alcoholic caustic potash; after separation of the unsaponifiables, the soap solution was treated with sulfuric acid and the liberated fatty acid was extracted with petroleum ether. The yield of unsaponifiables was 4.30% of the weight of the fatty fraction.

The solid acids were obtained from the mixture of fatty acids by Twitchell's and the saturated acids by Bertram's method (Table 2).

Table 2

Characteristics of the Fatty Acid Fractions

Index	Total fatty acids	Saturated acids	Total solid acids	Saturated solid acids
Yield in relation to the total acids, %	—	14.89	15.92	9.75
Neutralization number, mg of KOH	201.29	216.90	187.86	181.03
Mean molecular weight	278.75	258.69	298.67	309.95
Iodine number, %	104.16	—	34.86	—
Thiocyanogen number, %	82.30	—	—	—

In order to determine the qualitative composition of the acids, mixtures of them, and the saturated acid fractions were subjected to paper chromatography by Alimova's method [8]. A striking fact is the considerable amount of lauric acid in the saturated acid fractions (Fig. 1). This is in accord with the high iodine number of the total fatty acids which, of course, is due to the presence here of a considerable amount of petroselinic acid, which is solid at room temperature (mp 32-34°). The oxidative degradation of this acid gives lauric and adipic acids. From the iodine number of the total solid acids, the content of petroselinic acid can be calculated as 38.79%, which corresponds to 6.17% of the total mixture of fatty acids of the oil.

The quantitative composition of the total fatty acids of the oil was determined by thiocyanometric and spectrophotometric methods (Table 3). The results of the two methods agreed fairly satisfactorily.

Investigation of the pigments. When the petroleum ether solution of substance B was fractionated by chromatography, two pigments were eluted successively. Judging from its maxima in the UV spectrum at 450 and 480 mμ, one of them, yellow, is β-carotene [9]; the second exhibited four maxima in the spectrum, at 400, 455, 460 and 485 mμ. The

nature of the latter pigment had not been established.

Investigation of the unsaponifiables (fraction F). The unsaponifiables consisted of a brownish yellow semi-solid mass with a sharp characteristic odor. When they were treated with petroleum ether, 89% of them dissolved (fraction F_1) and 11% of insoluble matter was left (F_2).

The petroleum ether solution of fraction F_1 was passed through a column of alumina. On elution, part of the substance issued in the petroleum ether (F_3) and part in chloroform (F_4) (Fig. 2).

Table 3

Fatty Acid Composition of the Oil

Fatty Acids	Composition of the mixture of acids (by the given methods), %	
	Thiocyanometric	Spectrophotometric
Saturated	9.58	9.75
Oleic	58.90	59.25
Petroselinic	6.17	6.17
Linoleic	25.35	24.83

On evaporation, fraction F_3 gave a paraffin-wax-like substance with mp 46-49°. On treatment with sulfuric acid it partially resinified. In order to free it from resinous impurities, the substance was again dissolved in petroleum ether and purified by passage through a column of silica gel. This gave a substance with mp 63.5-64° (from acetone); it did not react with H_2SO_4 and $KMnO_4$. Found: C 85.60, 85.30; H 14.90, 14.80%; molecular weight 410.00 (cryoscopic). Calculated for $C_{29}H_{60}$: C 85.29; H 14.71%; molecular weight 408.77.

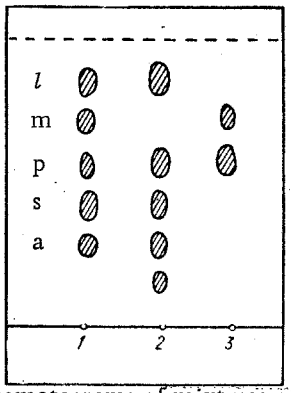


Fig. 1. Chromatograms of mixtures of fatty acids of Prangos oil: 1) reference acids: a – arachidic, s – stearic, p – palmitic, m – myristic, l) lauric; 2) mixture of saturated acids; 3) total mixture of acids of the oil.

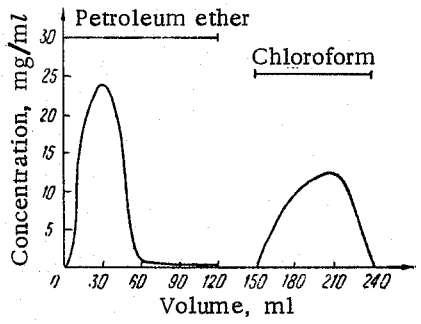


Fig. 2. Chromatogram of the unsaponifiables fraction of Prangos oil.

The IR spectrum of the hydrocarbon (Fig. 3) showed bands at 2962 and 2872 cm^{-1} corresponding to the symmetrical and anti-symmetrical stretching valence vibrations of methyl groups, and well-defined bands at 2926 and 2853 cm^{-1} corresponding to the same vibrations of methylene groups; there was no band at 2890 cm^{-1}, characteristic of tertiary carbon $\rangle$CH.

Thus, the hydrocarbon that we have found among the unsaponifiables has been identified as n-nonacosane, for which an mp of 63.8° is given in the literature [10].

Substance C, isolated from the combined fractions B_1-B_3 by treatment with acetone also proved to be n-nonacosane.

Investigation of fraction D. The substance with mp 83°, on testing on paper [11], proved to be chromatographically pure. A single spot with a persistent yellow color and R_f 0.92 was obtained.

The system used was a mobile phase of n-hexane-benzene-methanol (5: 4: 1) and a stationary phase consisting of a 10% solution of formamide in methanol.

The UV spectrum of a solution of the substance in ethanol showed absorption maxima at 258 and 322 mμ (Fig. 4), which indicates its coumarinic nature.

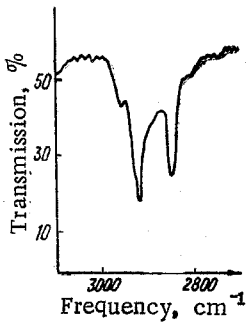


Fig. 3. IR spectrum of n-nonacosane. Preparation compressed with KBr in a ratio of 1: 75. LiF prism.

Found: C 74.10, 73.90; H 6.80, 6.67%; molecular weight 243.13 (cryoscopy). Calculated for $C_{15}H_{16}O_3$: C 73.77; H 6.60%; molecular weight 244.00.

On the basis of these results, the substance with mp 83° was identified as osthol, for which a mp of 83-84° is given in the literature. This substance was found in the roots of the hay plant by Pigulevskii and Kuznetsova [12], but,

as has been shown by the above results, it is also present in the seeds of this plant.

It is possible that the crystalline substance present in fraction A_3 and in admixture with the resinous product in fraction A_2 is also osthol.

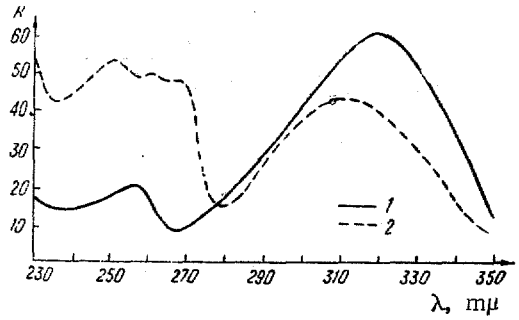


Fig. 4. UV spectra of the coumarin derivatives of Prangos oil: 1) osthol; 2) compound with mp 109°. Solvent ethanol; concentration 0.02 g/l.

Investigation of the crystalline substance E isolated from fraction B_{13}. After purification, this substance gave, with the same system of solvents on paper [11], one spot with a non-permanent crimson coloration, R_f 0.92.

The UV spectrum (cf. Fig. 4) showed absorption maxima at 252 and 312 mμ. The similarity of the UV and IR spectrum of osthol and substance E (Fig. 5) permits the latter to be assigned to the class of coumarin derivatives.

Found: C 71.70, 71.40; H 5.37, 5.74; molecular weight 259.70% (Rast). Calculated for $C_{16}H_{14}O_4$: C 71.11; H 5.18; molecular weight 270.00%.

The structure of the substance has not been established.

The UV spectra were taken by S. D. Nikonovich and the IR spectra by Ya. V. Rashkes.

Summary

The seeds of the resin-bearing plant Prangos pabularia Lindl. contain 17-19% of a complex mixture of substances extractable with petroleum ether, including 6.54% of fatty oil. The following saturated acids have been found in the oil: behenic, arachidic, stearic, and palmitic; and the following unsaturated acids: linoleic, oleic, and petroselinic, which is characteristic for the family Umbelliferae.

The non-glyceride complex of the oil has been shown to contain β-carotene and another pigment of undetermined structure, the hydrocarbon n-nonacosane, and, two coumarin derivatives, osthol and another with mp 109°, the structure of the latter not having been established.

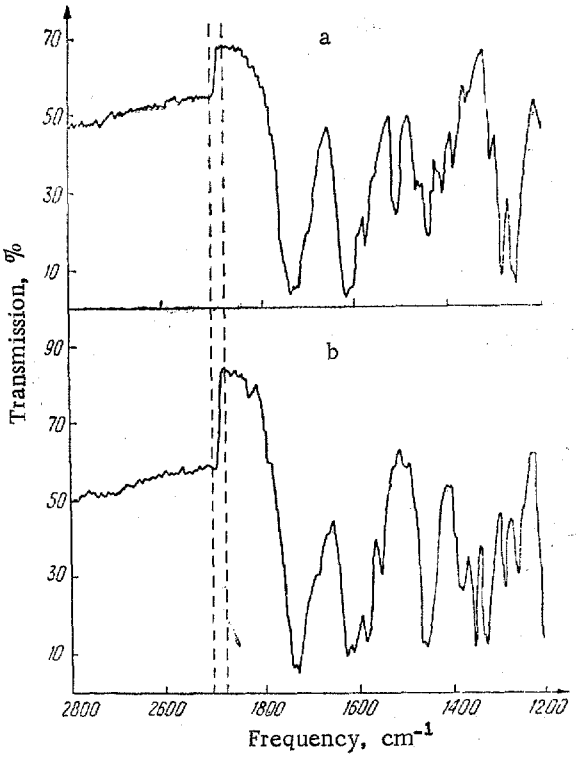


Fig. 5. IR spectra of the coumarinic derivatives of Prangos oil: a) osthol; b) compound with mp 109°. Preparations compressed with KBr in a ratio of 1:75. NaCl prism up to 2000 cm^{-1}; LiF prism above 2000 cm^{-1}.

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