PREPARATION AND QUANTITATIVE DETERMINATION OF BENZOYLMARMESIN

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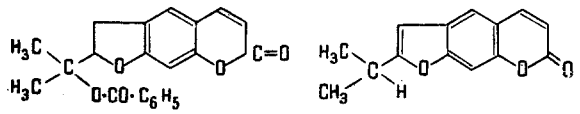
UDC 542,91+543,062+547,99

In 1967, during the synthesis of biologically active acylated coumarins, we obtained benzoylmarmesin (I) identical with felamedin, natural benzoylmarmesin, concerning which a report appeared in the press somewhat later [1]. Felamedin, with mp 132-134°C, $[\alpha]_D^{20}$ − 98.9° (c 0.2, chloroform) was isolated from the roots of Ferulago meoides (L.) Boiss. It differs in optical activity and melting point from the compound that we found, apparently being a racemic mixture of the d and *l* forms.

The existence of pharmacological activity induced us to develop methods for obtaining this substance and determining it quantitatively.

The starting material for the synthesis of benzoylmarmesin was marmesin with mp 190-191°C, $[\alpha]_D^{20}$ +29.8° (c 0.84, chloroform), isolated from wastes of the production of ammifurin.



I II

The marmesin was acylated by heating it with benzoyl chloride in benzene. Esterification of the tertiary alcohol group was accompanied by dehydration with the formation of the anhydro derivative (II), which complicated the purification of the final product. In dilute solutions dehydration took place more slowly than acylation, as a result of which the yield of benzoylmarmesin rose to 50% of the calculated figure. The benzoylmarmesin obtained after repeated recrystallization had mp 109-111°C (Kofler), $[\alpha]_D^{20}$ 0° (c 1.29, chloroform), and the IR spectrum shown in Fig. 1.

We have developed a spectrophotometric method for determining benzoylmarmesin and anhydromarmesin in the pure state and in mixtures. In the latter case, the substances can be separated on a thin-layer chromatogram (silica gel) in a benzene–petroleum ether (70-100°C)–methanol (88 : 10 : 2) system. In this system benzoylmarmesin and anhydromarmesin have R_f 0.29 and 0.41, and possess a violet and a pale blue fluorescence, respectively, in UV light.

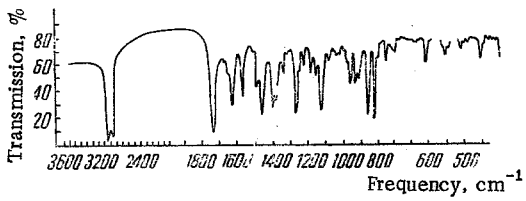


Fig. 1. IR spectrum of benzoylmarmesin (mull in paraffin oil).

A number of strong bands appear in the UV spectra of benzoylmarmesin and anhydromarmesin (Fig. 2).

The long-wave absorption maximum (335 nm) is used for the separate determination of the compounds under consideration.

The specific absorption coefficients of benzoylmarmesin and anhydromarmesin at this wavelength are, respectively, 726 ± 1.32 and 281 ± 0.92 (mean of 20 independent determinations).

All-Union Scientific-Research Institute of Medicinal Plants. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 526-531, September-October, 1970. Original article submitted June 20, 1970.

TABLE 1. Determination of Benzoylmarmesin and Anhydromarmesin in Synthetic Mixtures

Amount taken, mg		Found, mg		Relative error, %	
benzoylmarmesin	anhydromarmesin	benzoylmarmesin	anhydromarmesin	benzoylmarmesin	anhydromarmesin
0,0170	0,0090	0,0169	0,0089	−0,58	−1,11
0,0255	0,0135	0,0254	0,0134	−0,39	−0,77
0,0340	0,0180	0,0342	0,0181	−0,59	+0,55
0,0425	0,0255	0,0423	0,0252	−0,47	−1,17
0,0510	0,0270	0,0511	0,0268	+0,19	−0,74
0,0595	0,0315	0,0591	0,0317	−0,67	+0,63

TABLE 2. Specific Absorption Coefficients of Benzoylmarmesin and Anhydromarmesin at 290, 335, and 307 nm

Substance	Wavelength, nm		
	290	335	307
Benzoylmarmesin	223±1,14	726±1,32	353±0,54
Anhydromarmesin	452±1,19	281±0,92	353±0,57

TABLE 3. Determination of Benzoylmarmesin and Anhydromarmesin in Synthetic Mixtures

Substance	Taken, mg	Found, mg	Error, %
Benzoylmarmesin	2,498	2,511	+0,52
Anhydromarmesin	0,642	0,621	−3,27
Total	3,140	3,132	−0,25
Benzoylmarmesin	1,697	1,708	+0,65
Anhydromarmesin	0,514	0,536	−4,28
Total	2,211	2,244	+1,49
Benzoylmarmesin	1,593	1,606	+0,82
Anhydromarmesin	0,328	0,310	−5,49
Total	1,921	1,916	−0,26
Benzoylmarmesin	2,236	2,247	+0,49
Anhydromarmesin	1,197	1,259	+5,18
Total	3,433	3,506	+2,12
Benzoylmarmesin	4,810	4,782	−0,58
Anhydromarmesin	0,792	0,834	+5,68
Total	5,602	5,616	+0,25

The completeness of the elution of the compounds from the silica gel was checked by chromatography with subsequent spectrophotometric determination of the pure substances. When benzoylmarmesin is eluted, approximately 2.6% of the amount of material deposited on the chromatogram remains on the silica gel, and for anhydromarmesin the corresponding figure is 3.1%. This permitted the introduction of correction factors which took this loss into account in the equations used to calculate the amounts of these substances.

The results of the determination of benzoylmarmesin and anhydromarmesin in synthetic mixtures (mean of two duplicate determinations) are given in Table 1. The relative error of the determination did not exceed 1.20%.

We have developed a variant of the separate spectrophotometric determination of benzoylmarmesin and anhydromarmesin in solutions without their preliminary separation. In this process the optical density of the solution is measured at two wavelengths (290 and 335 nm). By solving a system of two equations, the amount of benzoylmarmesin is determined and, simultaneously, the combined content of benzoylmarmesin and anhydromarmesin in the mixture is found by measuring the optical density of the solution at 307 nm, where the specific absorption coefficients of the two substances are equal (Table 2, means of 20 independent determinations). The amount of anhydromarmesin is found by difference [2].

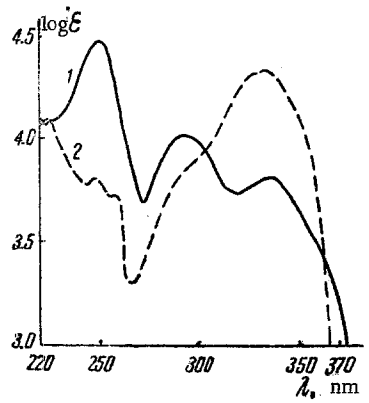


Fig. 2. UV spectra of (1) anhydromarmesin and (2) benzoylmarmesin.

Results of the combined determination of the substances concerned (means of 3 determinations) are given in Table 3.

The error in the determination of the total does not exceed ±2.1%, while that for benzoylmarmesin is ±0.8% and for anhydromarmesin, ±5.5%.

EXPERIMENTAL

The IR spectrum of benzoylmarmesin was taken on a UR-10 spectrophotometer (mull in paraffin oil). A spectrophotometric determination was carried out on an SF-4A spectrophotometer.

Preparation of Benzoylmarmesin, 5'-(1-Benzoyloxy-1-methylethyl)-4',5'-dihydrofuro-2',3':7,6-coumarin. A mixture of 4.9 g (0.04 mole) of benzoic acid and 4.8 g (0.04 mole) of thionyl chloride was left for a day. Then 50 ml of benzene and 5 g (0.02 mole) of marmesin were added, and the resulting reaction mixture was heated under reflux for 10 h. The solution was treated with 4% aqueous sodium hydrogen carbonate and then with water, after which it was evaporated to small volume, and the precipitate was recrystallized from a benzene–petroleum ether mixture (1:4). The resulting precipitate was separated off, washed with saturated sodium bicarbonate solution, and recrystallized [from 50% aqueous methanol and from benzene–petroleum ether (1:4)]. This gave 2.5 g of crystals with mp 109-111°C, $[\alpha]_D^{20}$ 0° (c 1.29, chloroform). Yield 50%. Found %: C 72.00; H 5.27. $C_{21}H_{18}O_5$. Calculated %: C 71.99; H 5.18.

Determination of Benzoylmarmesin and Anhydromarmesin (with preliminary separation). A 5-mg quantity of the sample (accurately weighed) was dissolved in a 5-ml measuring flask. The resulting solution (0.01-0.03 ml) was deposited on a chromatogram and chromatographed for 30 min in a non-fixed thin layer of KSK silica gel (2 g of silica gel in 7 ml of isopropanol on a plate) in a benzene–petroleum ether–methanol (88:10:2) system. The chromatogram was examined in UV light, and the spots with R_f 0.29 (benzoylmarmesin, violet fluorescence) and 0.41 (anhydromarmesin, pale blue fluorescence) were marked. The marked sections of the silica gel were transferred quantitatively into 15- to 20-ml flasks with ground stoppers, 10 ml of ethanol was added to each, and they were left overnight. The eluate (6-7 ml) was taken with a pipette and filtered through dense filter paper into a cell 1 cm thick. The optical densities of the solutions obtained were determined at a wavelength of 335 nm against the eluate from an equal amount of silica gel from the same plate.

The percentage content of benzoylmarmesin (X_1) and that of anhydromarmesin (X_2) were calculated (%) from the following equations:

$$X_1 = \frac{1000 \cdot V_1 \cdot V_3 \cdot D_{335} \cdot 1.027}{\left(D_{1\,cm}^{1\%}\right)_{335} \cdot V_2 \cdot P},$$

$$X_2 = \frac{1000 \cdot V_1 \cdot V_3 \cdot D_{335} \cdot 1{,}032}{\left(D_1^{1\%}\right)_{335} \cdot V_2 \cdot P},$$

where V_1 is the volume of the solution of the preparation, ml; V_2 is the volume of the solution deposited on the chromatogram, ml; V_3 is the volume of the eluate, ml; P is the weight of the sample, mg; and 1.027 and 1.032 are the correction factors for benzoylmarmesin and anhydromarmesin.

Determination of Benzoylmarmesin and Anhydromarmesin (with previous separation). A 2-mg quantity of the sample (accurately weighed) was dissolved in ethanol in a 25-ml measuring flask (solution A); 1 ml of solution A was transferred with a pipette into a 25-ml flask, and 10 ml of ethanol was added (solution B). The optical densities of solution B at wavelengths of 290, 307, and 335 nm were determined in a layer 1-cm thick on an SF-4A spectrophotometer. The percentage content of benzoylmarmesin and anhydromarmesin together was determined from the equation

$$X = \frac{1000 \cdot V \cdot n \cdot D_{307}}{D_{1\,cm}^{1\%} \cdot P};$$

where X is the percentage of benzoylmarmesin and anhydromarmesin combined in the sample; V is the volume of the solution, ml; D_{307} is the optical density of the solution at λ 307 nm; P is the weight of the sample, mg; n is the dilution factor; and $D^{1\%}_{1\,см}$ is the specific absorption coefficient of benzoylmarmesin and anhydromarmesin at 307 nm.

The percentage of benzoylmarmesin in the mixture was calculated by means of the following equation:

$$X_1 = \frac{\left[D^{335}\left(D^{1\%}_{1\,cm}\right)^{290}_{anh} - D^{290}\left(D^{1\%}_{1\,cm}\right)^{335}_{anh}\right]\cdot 1000\cdot n\cdot V}{\left[\left(D^{1\%}_{1}\right)^{335}_{anr}\left(D^{1\%}_{1\,cm}\right)^{290}_{6м} - \left(D^{1\%}_{1\,см}\right)^{290}_{anh}\cdot\left(D^{1\%}_{1}\right)^{335}_{bm}\right]\cdot P};$$

where D^{335} and D^{290} are the optical densities of solution B at 335 and 290 nm; $\left(D^{1\%}_{1\,см}\right)^{335}_{bm}$ and $\left(D^{1\%}_{1\,cm}\right)^{335}_{anh}$ are the specific absorption coefficients of benzoylmarmesin and anhydromarmesin at 335 nm (analogous symbols apply to the specific absorption coefficients at 290 nm); P is the weight of the mixture of substances, mg; n is the dilution factor; and V is the volume of solution A, ml.

When the numerical values are substituted, this equation becomes

$$X_1 = \frac{452\cdot D^{335} - 281\cdot D^{290}}{0{,}965\cdot P}.$$

CONCLUSIONS

1. A method for preparing benzoylmarmesin has been proposed.

2. Two variants of the spectrophotometric determination of benzoylmarmesin and the anhydromarmesin obtained as an impurity have been developed.

LITERATURE CITED

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2. M. E. Perel'son and G. K. Nikonov, Med. prom. SSSR, No. 3, 42 (1965).