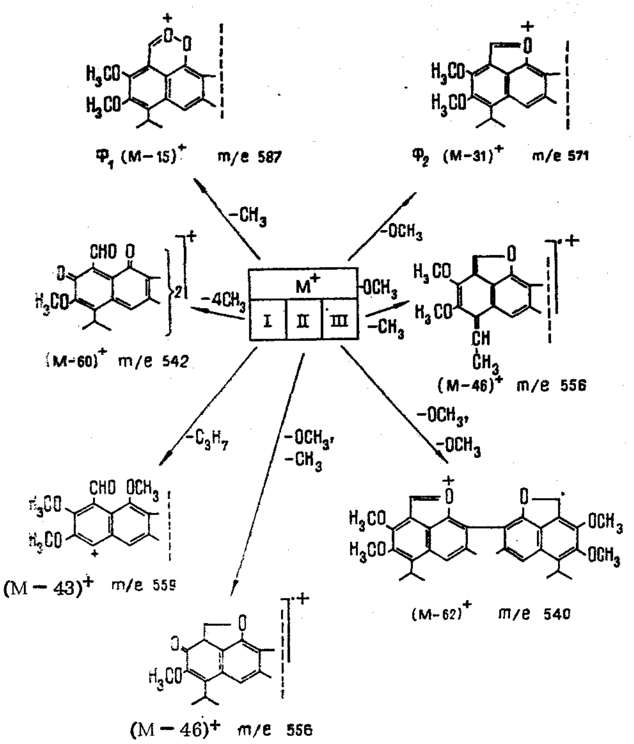

Scheme 1. Scheme of fragmentation of the M^+ ions of the gossypol hexamethyl ethers (I-III).

The presence of fragmentary ions corresponding to the splitting out of methyl (Φ_1) and methoxy (Φ_2) groups is common for the DADI spectra of the M^+ ions of all the tautomeric forms of GHME under consideration. In the DADI spectrum of the M^+ ion of (I), the peak of the Φ_1 ion has the maximum intensity, while in the spectra of (II) and (III) it has a very low intensity. In the spectra of these compounds the opposite pattern is observed for the intensity of the peak of the Φ_2 ion (Fig. 1, a–c).

The high intensity of the peak of the Φ_2 fragment in the DADI spectra of M^+ for (II) and (III) shows that the ejection of a methoxy group of the lactol ring takes place far more readily than that of a methyl group.

In addition to those mentioned above, compound (I) gives a $(M - 60)^+$ fragmentary ion which, in all probability, corresponds to the simultaneous splitting out of four methyl groups. The energetic suitability of such an ejection is possibly due to the stability of the quinoid structure ascribed to this ion (Scheme 1). On the other hand, the presumable structure of the $(M - 60)^+$ ion agrees well with the properties of gossypol and some of its derivatives, which, under certain conditions, readily pass into substances with structures analogous to that of the $(M - 60)^+$ ion. The $(M - 43)^+$ ion is formed as the result of the splitting out of an isopropyl radical. The spectra of compounds (II) and (III) show the peaks of a $(M - 46)^+$ ion with m/e 556. This ion is formed by the simultaneous ejection of a methoxy group from the lactone ring and of a methyl group either from methoxy side groups or from an isopropyl radical with the formation of a quinoid structure.

Among all the fragmentary ions, the most striking is the $(M - 62)^+$ ion with m/e 540 of compound (III) which does not appear in the spectra of the other forms of GHME. The formation of this ion is apparently connected with the simultaneous presence of aldehyde and lactol groups. The peak of this ion is characteristic of compound (III) exclusively.

The similar volatilities and also the differences shown in the DADI spectra of the M^+ ions of the GHMEs can be used for answering the question of the ratio of the isomers in the products of the methylation of gossypol.

With this aim we have studied the DADI spectra of a model mixture of the GHMEs (I-III) prepared in a ratio of 1 : 1 : 1 and of their native mixture obtained by methylating gossypol with dimethyl sulfate in the presence of alkali, without purification and without separation on a column (Fig. 1, d, e). As can be seen from Figure 1, the DADI spectra of the mixtures consist of a combined pattern of the DADI spectra of all three isomers.

The absolute intensities of the peaks of the ions detected in the DADI spectra of the M^+ ions of (I-III) and model and native mixtures of them are given below (mV):

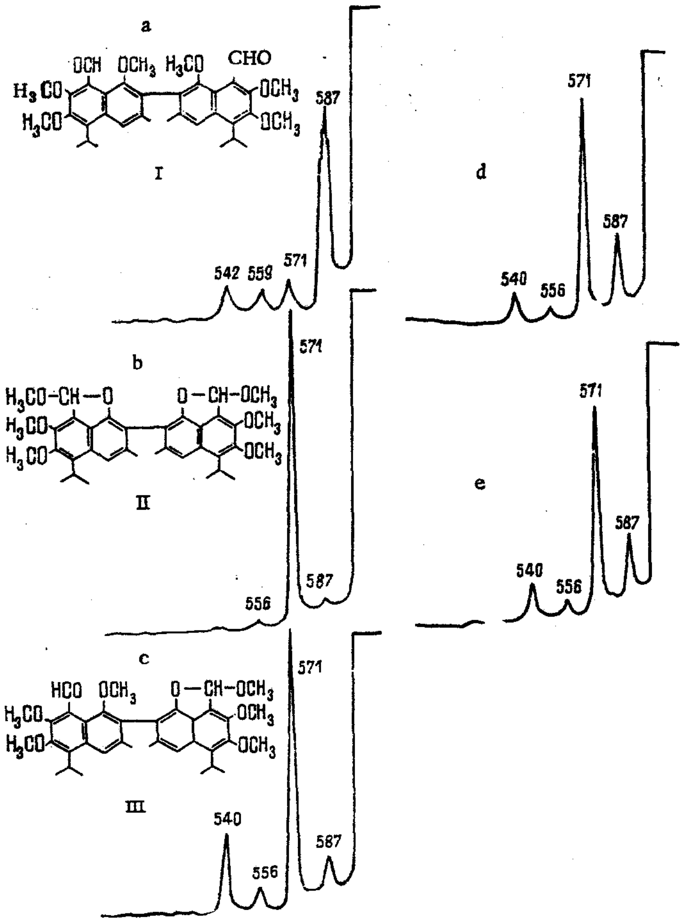

Fig. 1. DADI spectra of M^+ with m/e 602 of the hexamethyl ethers of: a) the dialdehyde form of GHME (I); b) the dilactol form of GHME (II); c) the monoaldehyde-monolactol form of GHME (III); d) a model mixture of (I-III); e) a mixture obtained by methylating gossypol with dimethyl sulfate in the presence of alkali.

Compound	Ion with m/e			
	587	*571*	*556*	*540*
I	25,40	5 20	—	—
II	1,62	40,00	1,33	—
III	4,30	33,80	3,00	9,75
Model mixture (1 :1 :1)	10,20	26,50	1,45	3,30
Native mixture of (I-III)	8,35	25,25	2,68	4,7

With identical intensities of the maternal ions (M^+), the absolute intensities of the daughter ions Φ_1 and Φ_2 depend on the contributions made by each component of the mixture of (I-III). But the amounts only of compounds (II) and (III) appear in the intensities of the peaks of the ions with m/e 556 and 540. The presence of (III) is reflected in its DADI spectrum by the peak of the ion with m/e 540.

Since in the DADI spectrum of M^+ for (III) the ratio of the intensities of the peaks of the ions Φ_1 and Φ_2 and of that with m/e 556 to the intensity of the peak of the ion with m/e 540 is constant, being 0.44, 3.45, 0.3, respectively, by measuring the intensity of the last-mentioned peak in the DADI spectrum of the mixture under study it is possible to determine the amount of (III) in it. Then, by calculating the contribution made by the (III) to the total pattern of the DADI spectrum of the mixture and performing the analogous calculations for the DADI spectra of M^+ for (II) with respect to the same ions it is possible to find the amounts of the other two isomers.

As a result of repeated measurements and calculations the amounts of (I), (II), and (III) in the model and native mixtures have been established (34.34, 33.07, and 32.59%, and 27.09, 20.81, and 52.10%, respectively).

EXPERIMENTAL

The mass spectra and DADI spectra were taken on a MAT-311 instrument (GFR) at an energy of the ionizing electrons of 70 eV with a cathode emission current of 300 μA and an accelerating voltage of 3 kV. The temperature of the ionization chamber was 100°C. The temperature of evaporation of the samples was varied from 130 to 200°C but no appreciable changes in the ratios of the peaks of the DADI were observed. We also checked the volatility of each of the samples in this range of temperatures by recording their evaporation curves at identical amounts of the samples at times of evaporation within this range of temperatures. It was found that the volatilities were identical.

The mixture of isomers in a ratio of 1 : 1 : 1 was prepared by dissolving 0.21 mg of each of them in 1 ml of absolute methanol.

The absolute intensities of the peaks in the DADI spectra were measured in millivolts and are given in the form of the arithmetic mean values from several measurements at different temperatures, the relative error not exceeding 4-6%.

At a constant magnitude of the peak of the maternal ion, the true (absolute) intensity of the peak of each daughter ion in the DADI spectra also remains constant. For an accurate comparison of the DADI spectra of (I-III) and a measurement of the intensities of the peaks in them, the intensities of the peaks of the maternal ions were adjusted to 6 mV for all the samples recorded.

The isomeric GHMEs were obtained as described previously [8], and the mixture was purified and separated into the individual isomeric forms as described by ourselves and others [3, 4].

SUMMARY

On the basis of the results of a study of the DADI spectra of the M^+ ions of the hexamethyl ethers of the tautomeric forms of gossypol and of mixtures of them, the amounts of each form in a native mixture have been determined.

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