IDENTIFICATION OF ALIPHATIC 2- AND 3-HYDROXY ACIDS IN THE FORM OF CYCLIC PHENYLBORONATES BY GLC-MASS SPECTROMETRY

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A method is prepared for identifying aliphatic 2- and 3-hydroxy acids which is based on the GLC-mass spectrometry of the corresponding phenylboronates. The phenylboronates are sufficiently volatile for analysis with the aid of GLC and give simple and informative mass spectra.

Aliphatic 2- and 3-hydroxy acids are components of the lipids of various classes (see, for example, [1-5]). For the unambiguous identification of these acids, use is usually made of the combined GLC-mass spectrometry of some or other of their derivatives: methyl esters containing a free hydroxy group [5, 6], and O-acetylated [5, 7] and O-trimethylsilylated [8, 9] methyl esters. In the mass spectra of the derivatives mentioned, the relative intensities of the peaks of the molecular ions and the other characteristic ions in the region of high mass numbers of the spectrum are fairly low [10], which complicates the identification of substances insufficiently separated on GLC from accompanying impurities, and this also applies where the mass spectrum of the substance being determined and the background from the stationary phase of the column become comparable in intensity (for example, with a high temperature of the column, when the concentration of the substance is low, etc).

Recently, for the analysis of bifunctional compounds by the methods of GLC and GLC-mass spectrometry, use has frequently been made of their cyclic derivatives — cyclic acetals, ketals, alkyl- and arylboronates, alkylphosphonothionates, etc. (see the review [11]). Since two functions of the compound being analyzed participate in the formation of rings with these reagents, the selectivity of the analytical method rises. As a rule, the mass spectra of cyclic derivatives is more informative than the mass spectra of derivatives with an acyclic structure. Thus, in the mass spectra of the isopropylidene derivatives of the C_{12}-C_{18} hydroxy acids the relative intensities of the peaks of the molecular and other diagnostically important ions amount to 30-40%. The cyclic butylboronates of low-molecular-weight 2- and 3-hydroxy acids give extremely specific mass spectra [13], but the intensities of the characteristic peaks in the spectrum fall sharply with an increase in the length of the hydrocarbon chain of the acids up to C_{10} and beyond. We have found that the cyclic phenylboronates of acids of this type are not only free from this drawback but also have a number of advantages from the point of view of mass spectrometry. The results of a study of the mass-spectrometric and gas-chromatographic behavior of the phenylboronates of aliphatic 2- and 3-hydroxy acids are described in the present communication.

The phenylboronates are obtained by treating the 2- and 3-hydroxy acids with triphenylborazole in an aprotic solvent. The method is distinguished by its simplicity and does not require the expenditure of large amounts of time (see the Experimental part and [13]).

In the development of the method of analysis discussed below, as model compounds we used the phenylboronates of 2-hydroxydodecanoic (Ia), 2-hydroxytetradecanoic (Ib), 2-hydroxyhexadecanoic (Ic), 2-hydroxyoctadecanoic (Id), and 3-hydroxyoctadecanoic (II) acids.

The volatility of these phenylboronates is somewhat lower than that of the corresponding butylboronates, and therefore the gas-chromatographic analysis of the phenylboronates must be carried out at higher temperature. When columns containing polysiloxane stationary phases (OV-17 and OV-101) were used, the phenylboronates investigated were recorded on the chromatograms in the form of sharp symmetrical peaks. Figure 1 shows the dependence of the relative retention volumes (V_R^{rel}) of the phenylboronates on the number of carbon atoms in the chains of

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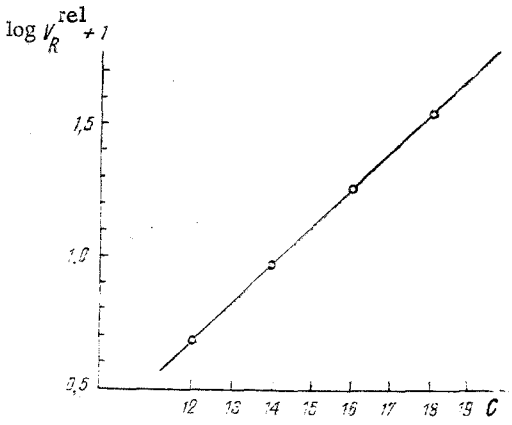
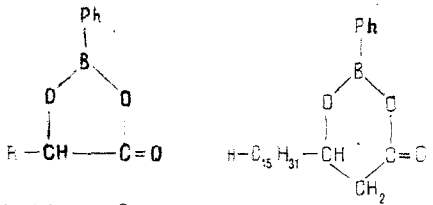


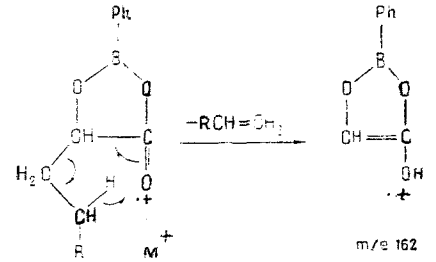
Fig. 1. Logarithm of the relative retention volumes (V_R^{rel}) of the phenylboronates of normal saturated aliphatic 2-hydroxy acids in GLC on polysiloxane OV-101 (for conditions, see the Experimental part); C — number of carbon atoms in the chain of the hydroxy acid. The values of V_R^{rel} were calculated relative to V_R for triphenylborazole.

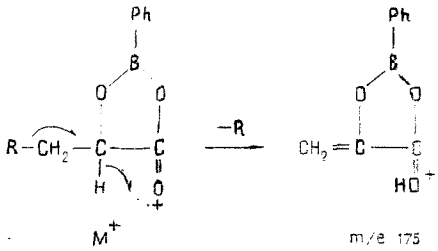
the 2-hydroxy acids in GLC on a column containing OV-101. On the above-mentioned polysiloxane stationary phase, the phenylboronates of 2-hydroxyoctadecanoic (Id) and of 3-hydroxyoctadecanoic (II) acids are separated satisfactorily, a greater retention volume corresponding to the last-mentioned acid. At the same time, the excess of triphenylborazole that is present in the sample being analyzed is eluted fairly close to the phenylboronate of 2-hydroxytetradecanoic acid (Ib). With a sufficiently high concentration of the former in the mixture being separated, the peaks of these substances may overlap on the chromatograms. Although this does not prevent the identification of the phenylboronate of (Ib) from the mass spectrum (see below), its quantitative determination becomes difficult. However, it must be mentioned that in natural materials aliphatic 2-hydroxy acids with 5-14 carbon atoms are found comparatively rarely.

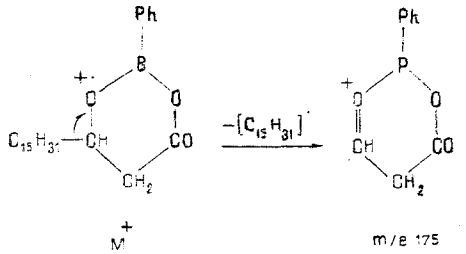


The main advantage of the mass spectra of the phenylboronates of the 2-hydroxy acids consists in their extreme simplicity and their high intensity of the peaks of the characteristic ions. The latter is connected with the fact that in the structures of these fragments, because of the presence of the aromatic nucleus, the charge is considerably delocalized, as a result of which the stability of the fragments rises sharply in comparison with the corresponding ions formed in the mass spectrometry of the alkylboronates and, in particular, the butylboronates of the 2-hydroxy acids. In the spectra of the phenylboronates of (Ia-d) the peaks of the molecular ions and those of the ions with m/e 175, 162, 105, and 104 have the highest intensities (see Table 1). The peaks at m/e 104 and 105 corresponding to the two pairs of ions $[C_8H_8]^{\cdot+}$ and $[Ph{-}B{=}O]^{\cdot+}$, and $[C_8H_9]^+$ and $[Ph{-}B{=}\overset{+}{O}H]$, respectively, are present in the mass spectra of phenylboronates with the most diverse structures (see, for example, [14-16]) and show that the compound under investigation is an ester of phenylboronic acid. The ion with m/e 162 (see scheme) is obviously produced by the splitting out of the alkyl residue from the dioxaborolane ring of the molecular ion, accompanied by the migration of a hydrogen atom to the charged fragment. As a result of the cleavage of the bond of the same residue present in the β position to the ring, an ion with m/e 175 arises. The peaks of the

metastable ions m* 101.4, 92.8, 85.5, and 79.4 present in the mass spectra of the phenylboronates of (Ia-d), respectively, show that the direct precursors of the fragment with m/e 175 are the molecular ions. The peaks of the ions with m/e 162 and 175 accompany peaks at m/e 161 and 174 corresponding to fragments of analogous structure containing the isotope ^{10}B. The presence in the mass spectrum of a particular component of a mixture of phenylboronates of fatty acids of strong peaks at m/e 162 and 175 together with peaks with m/e 161 and 174 with about a quarter of their intensities (the natural ratio of the isotopes ^{11}B: $^{10}B \approx 4$) gives sufficient grounds for considering that the substance is the phenylboronate of a 2-hydroxy acid. The size of the hydrocarbon chain of the acid is readily determined on the basis of the mass number of the molecular ion.







The dominating peak in the mass spectrum of the phenylboronate of 3-hydroxyoctadecanoic acid (II) is the peak of an ion with m/e 175, which, most probably, represents an isomer of the ion with the same mass number observed in the spectra of the phenylboronates of the 2-hydroxy acids. This ion is formed as the result of the loss of the alkyl residue by the molecular ion (see scheme). The intensity of the peak of the molecular ion in the spectrum of the phenylboronate of (II) is considerably less than in the spectrum of the phenylboronate of 2-hydroxyoctadecanoic acid (Id), and the peak at m/e 162 discussed above is inconsiderable, which permits the phenylboronates of 3-hydroxy acids to be distinguished from the isomeric phenylboronates of 2-hydroxy acids. Thus, the ion with m/e 162 that is characteristic for the mass spectra of the phenylboronates of aliphatic 2-hydroxy acids can be taken as diagnostic in the analysis of these derivatives by the method of mass fragmentography. In the spectrum of the phenylboronate of (II) the peaks of the ions $[C_6H_6]^{\cdot+}$ and $[Ph-B=O]^{\cdot+}$ with m/e 104 and of $[C_6H_9]^+$ and $[Ph-B=\overset{+}{O}H]$ with m/e 105 have comparatively high intensities.

The relative intensities of the peaks in the mass spectra of the phenylboronates of (Ia-d) that have not been considered above, and those in the spectrum of phenylboronate of (II) do not exceed 3% in the m/e 100-200 region and 0.2% in the m/e 200-400 region. This fact permits a preliminary evaluation of the homologous composition of a mixture of 2- and 3-hydroxy acids under investigation to be made directly from the mass spectra of their phenylboronates and, namely, from the mass numbers and intensities of the peaks of the molecular ions of the components of the mixture of phenylboronates. The results of such an evaluation

TABLE 1. Relative Intensities (%) of the Main Peaks in the Mass Spectra of the Phenylboronates of the 2-Hydroxy Acids (Ia-d) and of 3-Hydroxyoctadecanoic Acid (II)

Ion, m/e	Phenylboronate				
	Ia	Ib	Ic	Id	II
104	25	20	16	14	6
105	52	49	36	30	13
162	100	90	73	61	
175	93	90	91	81	100
M^+:					
302	69				
330		100			
358			100		
386				100	6

considerably accelerate the choice of conditions for the subsequent gas-chromatographic analysis of the phenylboronates or other derivatives of the hydroxy acids.

The results presented above permit the conclusion that the cyclic phenylboronates of aliphatic 2- and 3-hydroxy acids are a readily available and convenient form for determining the latter with the aid of GLC-mass spectrometry. The method described can compete successfully with other methods used at the present time for similar purposes.

EXPERIMENTAL

Preparation of the Phenylboronates of 2- and 3-Hydroxy Acids. A mixture of 0.2-1.0 mg of a hydroxy acid or mixture of hydroxy acids, 0.4-1.5 mg of triphenylborazole, and 0.5 ml of anhydrous dioxane or toluene was heated with stirring at 45-50°C until it was fully homogeneous, after which it was evaporated in vacuum at 35-40°C to dryness. The residue was dissolved in 1 ml of dioxane or toluene, and the solution was evaporated to dryness under the same conditions; this operation was repeated, and the dry residue obtained was dissolved in one of the solvents mentioned (concentration: 5-10 mg of phenylboronate/ml of solution) and was analyzed with the aid of GLC and GLC-mass spectrometry.

The GLC of the phenylboronates was performed on a Pye-Unicam 104 (model 24) chromatograph fitted with a double flame-ionization detector and a glass column (1500 × 4 mm) containing 1.5% of OV-101 on Chromosorb W (60-80 mesh) under isothermal conditions at 205°C, with argon as the carrier gas (50 ml/min).

The mass spectra were obtained on a LKB 9000 chromatomass spectrometer at an energy of the ionizing electrons of 70 eV and an accelerating voltage of 3.5 kV. The samples to be analyzed were introduced into the injector of the glass chromatographic column (1500 × 4 mm), which was filled with Chromosorb W (80-100 mesh) impregnated with 1.5% of OV-17. The temperature regime of the column was: 2 min at 240°C, and then a linear rise in the temperature (4 deg/min) to 300°C. The carrier gas was helium (25 ml/min). The mass spectra of the substances eluted from the column were recorded at the moments of the maximum ion current.

SUMMARY

The cyclic phenylboronates of aliphatic 2- and 3-hydroxy acids form readily accessible derivatives which are sufficiently volatile in GLC and give simple and informative mass spectra. In view of this, these derivatives can be used successfully for the analysis by the GLC-mass-spectrometric method of mixes of 2- or 3-hydroxy acids formed as the result of the degradation of natural lipids.

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EPOXY AND HYDROXY ACIDS OF THE SEED OIL OF *Galeopsis bifida*

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The lipids of the seeds of the hemp nettle, which is toxic for ruminants have been studied. The composition of the fatty acids of five classes of lipids and the position-species composition of the triacylglycerols have been determined. The presence of epoxyacyl- and hydroxyacylglycerols in the oil and the structures of the acids of these lipids have been established. Squalene has been isolated from the oil.

Galeopsis L. (hemp nettle) is a widespread annual weed plant of the family Labiatae. Five species of hemp nettle are found on the territory of the USSR.

The amount of oil in the seed of this plant, which belongs to the semidrying group [1], is fairly high (42-46%). The high toxicity of hemp nettle oil is well known [2, 3].

It is not clear precisely what substances are responsible for the toxicity of the hemp nettle. A hypothesis exists according to which they are alkaloids, but the results of the analysis of several samples of seeds for the presence of alkaloids are contradictory [1]. However, it has been established that the poisonous components of the hemp nettle are stable on heating and accumulate in the fatty tissue [2].

The chemical composition of the fatty oil of the seeds of *Galeopsis* species has not been studied. Only some constants for hemp nettle oil are given in the literature [1, 4].

We have investigated the composition of the seed oil of *G. bifida* Boenn. The oil was extracted from the comminuted seeds with petroleum ether. The yield of extract was 42% on the weight of the seeds. A number of indices of the oil were determined by standard methods, and the total acids were isolated from it:

The qualitative reaction of the hemp nettle oil with tungstosilicic acid for the presence of alkaloids was negative [5]. From the defatted meal a base was isolated which was identi-

	Oil	Acids
Density, d_4^{20}, g/cm^3	0.9228	0.9003
Refractive index, n_D^{20}	1.4784	1.4685
Iodine No., % I_2	157.94	164.92
Acid No., mg KOH/g	2.0	–
Saponification No., mg KOH/g	190.09	–
Neutralization No., mg KOH/g	–	198.38
Amount of unsaponifables, %	1.07	–

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