DIOL LIPIDS

COMMUNICATION 27.* IDENTIFICATION OF C_2-C_4-DIOLS IN THE FORM OF CYCLIC ESTERS OF PHENYLBORIC ACID BY THE METHOD OF COMBINED GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

S. G. Batrakov, A. G. Panosyan,
A. N. Ushakov, B. V. Rozynov,
and L. D. Bergel'son

UDC 543.544.25+543.51:547.422:547.915.5

Lipid derivatives of lower C_2-C_4-diols are rather often present as minor components in neutral lipids, isolated from various natural sources [2]. Recently diol analogs of glycerolipids were also detected in the phospholipid fractions of the rat liver [3, 4] and in the phospholipids of microorganisms [1].

The first step in the identification of diol lipids is the study of the polyol composition of a hydrolyzate or methanolyzate of one lipid fraction or another. According to the scheme of investigation posed earlier [5] polyols are separated from lipophilic products of hydrolysis (or methanolysis) by thin layer chromatography (TLC) or by distribution between hexane and aqueous methanol. The mixture of polyols purified in this way is analyzed by the method of gas chromatography (GC), or, which is more effective, by a combined method of GC and mass spectrometry in the form of acetyl or trimethylsilyl derivatives. However, acetates and trimethylsilyl esters of lower diols cannot be considered sufficiently convenient derivatives for GC and mass spectrometric investigation. The peaks of the molecular ions (M^+) are absent in the mass spectra of diol acetates, while certain peaks of importance for structural analysis have low intensity, which hinders the identification of C_3- and C_4-diols with various positions of the OH groups. Mass spectrometric analysis of trimethylsilyl esters also does not give reliable information in the case of isomeric compounds. We should add that in the acetylation of diols, as well as in the case of silylation, frequently unidentified side products are formed, the peaks of which in GC are sometimes superimposed upon the peaks of the diol derivatives. Another shortcoming of the indicated scheme of investigation is the substantial loss of lower diols during the evaporation and drying of their solutions before silylation or acetylation [5], as well as in the case of concentration of solutions of the derivatives obtained.

The recently proposed method of chromato-mass-spectrometric determination of diols in the form of cyclic acetals of hexadecanal [6] has a number of advantages in comparison with the analysis of acetates and trimethylsilylesters, but a shortcoming of it is its multistep nature.

In this communication we propose a new method of identification of small quantities of C_2-C_4-diols, based on combined GC-mass spectrometry of their phenylborate esters (PBE).† The main advantage of the method is the possibility of obtaining the indicated esters directly in an aqueous or methanol solution of diols. As our investigations showed, when such solutions are treated with phenylboric acid, followed by evaporation under vacuum, there is practically no loss of the diols and their PBE (see "Experimental Method" section).

---

*For Communication 26, see [1].

†For information on the gas-chromatographic and mass spectrometric identification of higher 1,2-, 1,3-, and 1,4-diols, as well as hydroxyacids and hydroxyketones in the form of derivatives of phenylboric acid, see, for example, [7] and the studies cited in this article.

M. M. Shemyakin Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR, Moscow. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 8, pp. 1739-1747, August, 1974. Original article submitted October 15, 1973.

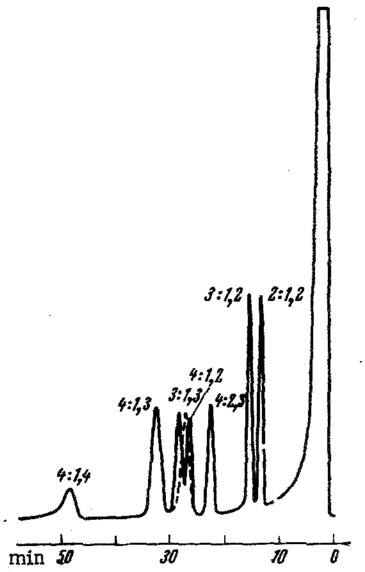
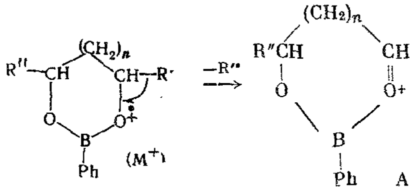


Fig. 1. GC of phenylborate esters of C_2-C_4-diols (for the conditions, see "Experimental Method" section). The dotted line represents the peak of the acetate of the phenylborate ester of glycerin.

We investigated the GC separation of PBE of dihydric alcohols on a model mixture, compiled from the PBE of seven C_2-C_4-diols and glycerin. The PBE were produced by treatment of acetone (method 1, see "Experimental Method" section), methanol (method 2), or aqueous (method 3) solutions of the alcohols with an excess of phenylboric acid. In the selection of the optimum conditions of separation of the model mixture, polyethylene glycol succinate, rheoplex-400, carbowax, SE-30, and SKTV were used as the stationary phases. Better results were obtained on columns with polysiloxanes SE-30 and SKTV (Fig. 1). Under the conditions of GC that we tested, the PBE of glycerin remains on the chromatographic column. The acetylated PBE of glycerin is more volatile, but it is eluted almost simultaneously with the PBE of 1,2-butanediol. Therefore, when the joint determination of diols and glycerin in polyol fractions of lipid hydrolyzates is necessary, it is advisable to preliminarily remove glycerin by preparative thin-layer chromatography [5].

The PBE of C_2-C_4-diols proved to be extremely convenient derivatives for mass spectrometric identification. The interpretation of their mass spectra (Fig. 2a-g) is facilitated by the fact that two isotopic peaks, shifted by one mass unit relative to one another, correspond to the boron-containing fragments. The intensities of these peaks are in a 1 : 4 ratio, which correspond to the relative distribution of the isotopes of boron in nature ($^{10}B : ^{11}B = 1 : 4$). Earlier [8], with the aid of high-resolution mass spectrometry, the structures of the hydrocarbon ions arising in the decomposition of M^+ of PBE of lower diols were established. Quite recently the same authors considered certain basic directions of fragmentation of M^+ of PBE of most of the C_2-C_4-diols under electron impact [9]; however, no experimental data were cited in this communication.

The mass spectra of all the PBE that we studied contain intense peaks of M^+, which permits a determination of the size of the hydrocarbon chain of the diol. The positions of the oxygen functions in the molecules of isomeric C_3- and C_4-diols can be judged on the basis of the following peculiarities of the fragmentation of the M^+ of their PBE. In the spectra of PBE of diols 3 : 1,2; 4 : 1,2; 4 : 1,3; and 4 : 2,3*; i.e., of compounds containing aliphatic substituents in the ring, one of the most intense peaks corresponds to the ion A, formed by the elimination of this substituent (R' or R")



---

*A code according to which the first number denotes the number of C atoms in the carbon chain of the diol, while the numbers after the colon represent the positions of the OH groups in the molecule, is used to denote the diols.

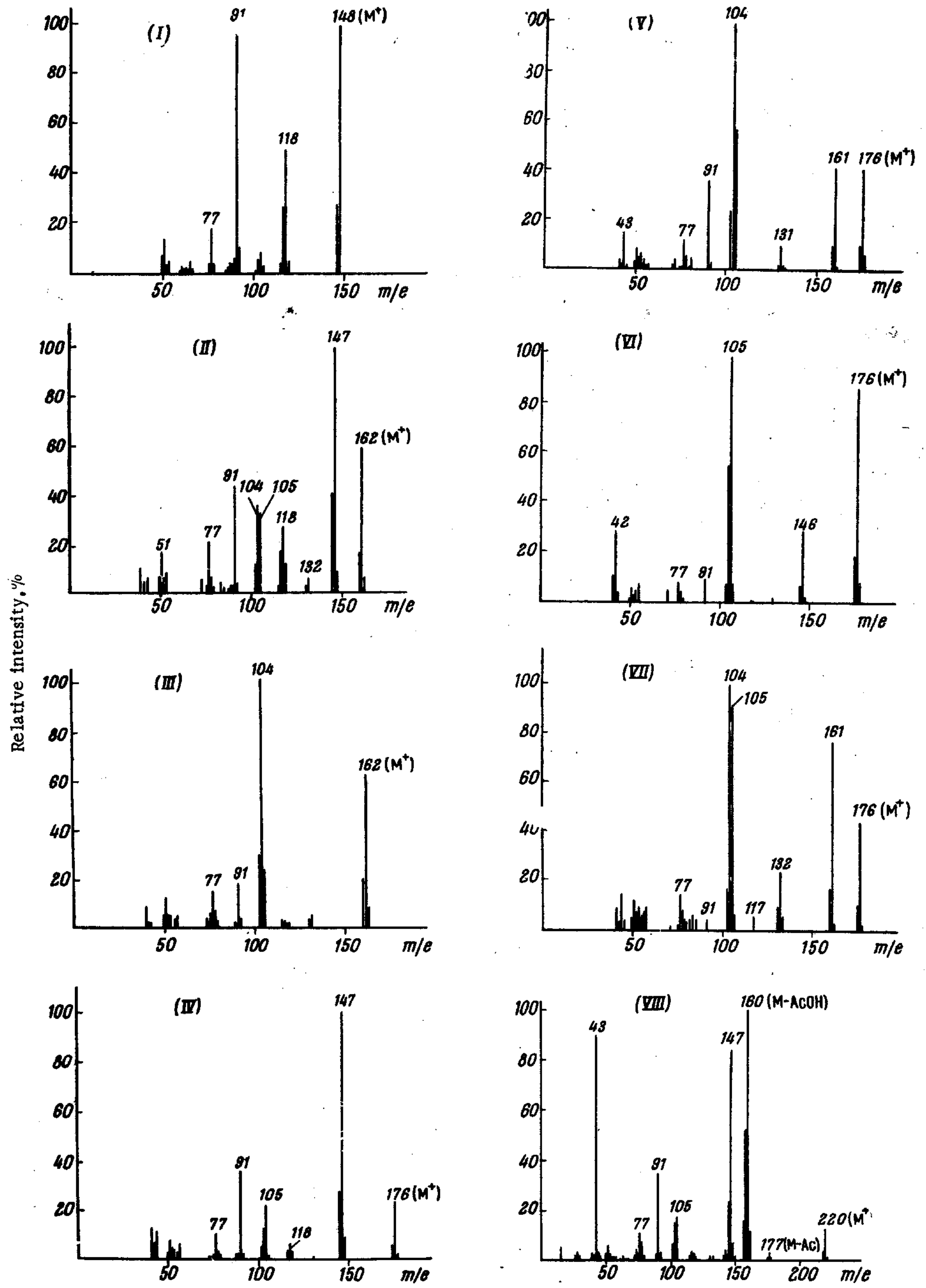
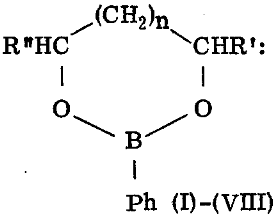


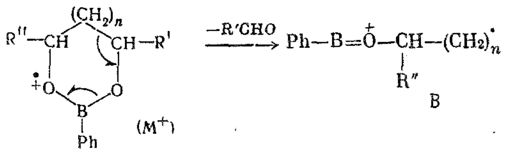
Fig. 2. Mass spectra of phenylborate esters of diols (I)-(VI) and the acetate of the phenylborate ester of glycerin (VIII)

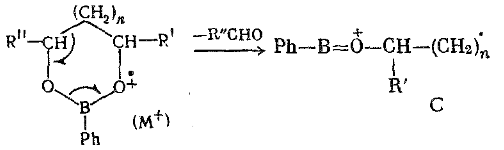


(I)-(VIII): I) R' = R" = H, n = 0; II) R' = Me, R" = H, n = 0; III) R' = R" = H, n = 1; IV) R' = Et, R" = H, n = 0; V) R' = Me, R" = H, n = 1; VI) R' = R" = H, n = 2; VII) R' = R" = Me, n = 0; VIII) R' = CH_2OAc, R" = H, n = 0.

Here and henceforth, PBE: 2:1,2 R' = R" = H, n = 0; 3:1,2 R' = Me, R" = H, n = 0; 3:1,3 R' = R" = H, n = 1; 4:1,2 R' = Et, R" = H, n = 0; 4:1,3 R' = Me, R" = H, n = 1; 4:1,4 R' = R" = H, n = 2; 4:2,3 R' = R" = Me, n = 0. The peak of the ion A–$[M-Me^{\cdot}]^+$ (m/e 147) is a maximum in the spectrum of PBE 3:1,2, while in the spectrum of PBE 3 :1,3 this peak is not observed. In the mass spectrum of PBE 4 :1,2, the peak of the ion $A-[M-Et^{\cdot}]^+$ (m/e 147) also possesses the greatest intensity; however, the peak at this value of m/e is absent in the spectra of PBE 4 :1,4; 4 : 2,3; and 4 :1,3; in the spectra of the last two PBE, the ion A corresponds to the peak m/e $161-[M-Me^{\cdot}]^+$.

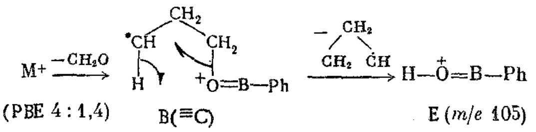
Another important direction of decomposition of M^+ of PBE of diols from the standpoint of identification is the elimination of an aldehyde molecule, which evidently proceeds according to the following scheme:





This type of fragmentation is characteristic of all the PBE that we studied. The mass spectra of PBE possessing aliphatic substituents in the ring, moreover, contain peaks of the ions $[M-R^{\cdot\prime}-R''CHO]^+$ (or $[M-R^{\cdot\prime\prime}-R'CHO]^+$) (D), the intensity of which is comparatively low. According to the presence of the peak m/e 117, corresponding to the ion $[M-Me^{\cdot}-MeCHO]^+$, in the mass spectrum of PBE 4 :2,3, this compound can easily be distinguished from PBE 4 :1,3, in the spectrum of which the indicated peak is absent, while the peak with m/e 131 corresponds to the ion D ($[M-Me^{\cdot}-CH_2O]^+$).

On the basis of the values of m/e of the ions considered above (M^+ and A–D), we can draw an unambiguous conclusion on the structure of the investigated PBE, and, consequently, on the structure of the initial diol. In addition to the peaks of these fragments, the mass spectra of all the PBE contain intense peaks of ions with m/e 105, 104, 91, and 77. It was shown earlier [8] that one of the directions of decomposition of the molecular ions of PBE 3 :1,2; 4 :1,3; and 4 :2,3 under electron impact is the formation of the hydrocarbon ion $C_8H_9^+$ (m/e 105), while in the case of the PBE of the remaining C_3-C_4-diols, this ion was not observed. However, as can be seen from Fig. 2, in the mass spectra of almost all the PBE of diols that we studied (with the exception of PBE 2 :1,2), the peaks with m/e 105 possess rather high intensity. Moreover, the relative intensity of the indicated peaks in the spectra of the three enumerated PBE is significantly higher than that which might have been attributed to the ion $C_8H_9^+$ (compare, for example, the intensities of the peaks with m/e 105 relative to the peaks with m/e 91 according to the data of [8] and Table 1). The peak of the metastable ion (m/e 75.5) present in the mass spectrum of PBE 4 :4,1 indicates that the precursor of the fragment with m/e 105 is the ion B (≡C), on the basis of which we can assume the following pathway of formation of the former:



Probably the ions E arise analogously in the decomposition of M^+ of the remaining PBE of C_3-C_4-diols, and in the mass spectra of PBE 3 :1,2; 4 :1,3; and 4 :2,3, the peak of the fragment (E) coincides with the peak of the ion $C_8H_9^+$.

As it follows from [8, 9], the peaks with m/e 91 in the mass spectra of PBE of diols correspond to the fragment $C_7H_7^+$, while the peaks with m/e 104 correspond to the ions $C_8H_8^+$ and $PhB=O^{\cdot +}$ (F). Judging by the intensities of the isotopic ions (m/e 103) (see Fig. 2), the basic contribution to the peak with m/e 104 is made by the ion F. The absence of the corresponding isotopic peak (m/e 76) indicates that the ion with m/e 77 does not contain boron and is more likely the phenyl ion $C_6H_5^+$.

The data that we obtained in a study of the mass spectra of the PBE of C_2-C_4-diols are summarized in Table 1, which cites the values of m/e and relative intensity of the basic ions formed in the decomposition of M^+ of the PBE, as well as the values of m/e of metastable ions, which permit a judgment of the origin of certain characteristic fragments of decomposition.

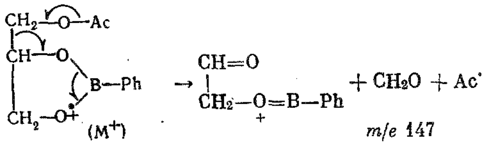
TABLE 1. Basic Ions Formed in the Fragmentation of Molecular Ions of PBE of C_2-C_4-Diols under Electron Impact*

Type of ion	Diol						
	2 : 1,2	3 : 1,2	3 : 1,3	4 : 1,2	4 : 1,3	4 : 1,4	4 : 2,3
M^+	148(100)	162(60)	162(68)	176(23)	176(44)	176(87)	176(45)
A	—	147(100) [133,4 from M^+]	—	147(100) [122,7 from M^+]	161(43) [147,3 from M^+]	—	161(78) [147,3 from M^+]
B	118(50)	132(6)	132(3)	146(28)	132(2)	146(30)	132(24) [121,1 from M^+]
C	118(50)	118(27)	132(3)	118(7)	—	146(30)	132(24) [121,1 from M^+]
D	—	117(17)	—	117(5)	131(10)	—	117(6)
E or E + $C_8H_9^+$	105(2)	105(33)	105(21)	105(21)	105(58) [84,2 from B]	105(100) [75,5 from B]	105(92) [83,5 from B]
F + $C_8H_9^+$	104(8)	104(36)	104(100)	104(13)	104(100)	104(56) [61,5 from M^+]	104(100) [61,5 from M^+]
$C_7H_7^+$	91(100) [70,2 from B]	91(44) [70,0 from C]	91(15)	91(36) [56,3 from A]	91(36) [51,4 from A]	91(10)	91(5)
$C_6H_5^+$	77(18)	77(20)	77(13)	77(10)	77(12)	77(8)	77(15) [33,7 from M^+]

---

*The values of m/e of ions are indicated, in parentheses the relative intensity, in brackets the value of m/e of the corresponding metastable ion and ion precursor.

Under the action of phenyboric acid, glycerin forms a 1,2-derivative [10], and thus the structure 2-phenyl-1-acetoxymethyl-1,3,2-dioxaborolane should correspond to the acetate of the 1,2-derivative. The mass spectrum of the acetate of the PBE of glycerin (see Fig. 2h) contains a noticeable peak of M^+ (m/e 220). The maximum in the spectrum is the peak of the ion $[M-AcOH]^+$ (m/e 160); the peak of the fragment A – $[M-{}^{\bullet}CH_2OAc]^+$ (m/e 147) also possesses substantial intensity. We should mention that the ion with m/e 147 may also arise as a result of the so-called h-cleavage [11], analogously to cyclic acetals of glycerin



The intensity of the peaks of ions of the type of B – $[M-AcOCH_2CHO]^+$ (m/e 118) and of type D – $[M-{}^{\bullet}CH_2OAc-CH_2O]^+$ (m/e 117) in the mass spectrum of the acetate of the PBE of glycerin is low, while the peak of $[M-CH_2O]^+$ (C) is practically absent.

EXPERIMENTAL METHOD

The PBE of diols were produced according to a modified method of [10].

Method 1 (in acetone solution). A solution of 5-6 mg of the diol (or mixture of diols) in 0.8 ml abs. acetone was treated at 20° with 10-15 mg phenylboric acid. The mixture was mixed until homogenization and left at 20° for 30 min, after which an aliquot of the mixture was analyzed by gas chromatography.

Method 2 (in methanol solution). A solution of 5-6 mg of the diol (or mixture of diols) in 4 ml MeOH was treated with 10-15 mg phenylboric acid. The mixture was heated until homogenization (~40°) and left for 30 min at 20°, MeOH was distilled off under vacuum at a temperature <40°, and the residue was dissolved in acetone and analyzed by gas chromatography.

Method 3 (aqueous solution). A solution of 5-6 mg of the diol (or mixture of diols) in 4 ml of water was treated with 10-15 mg phenylboric acid. The mixture was heated to homogenization (~60°), left for 30 min at 20°, the water distilled off under vacuum at a temperature <40°, the residue dissolved in acetone and analyzed by gas chromatography.

Gas Chromatography of PBE of Diols. GC was conducted on a Pye Instrument, equipped with a β-ionization detector and column (1300 × 4 mm) with 3% SE-30 on Chromosorb W (60-80 mesh). The temperature of the column was 110°, consumption of the carrier gas (argon) 20 ml/min, voltage on the electrodes of the detector 1250 V.

Determination of Losses of PBE of Diols in the Case of Production in Methanol and Aqueous Solutions. Solutions of the same amounts (2.0 mg) of ethylene glycol in acetone (0.4 ml), MeOH (5 ml), and in water (5 ml) were treated with 6 mg phenylboric acid each; samples of the PBE were prepared for gas –liquid chromatography by the methods described above (1, 2, and 3, respectively). An acetone solution of PBE 3 : 1,3, produced according to method 1, was introduced into the samples as an internal standard and GC analysis conducted. The yield of PBE 2 : 1,2 in each of the three experiments, which was estimated according to the ratio of the peaks of PBE 2 : 1,2 and PBE 3 : 1,3 on the chromatograms, proved to be practically the same (differences <1%). The losses of the remaining PBE were determined analogously, and the same results were obtained. In an analysis of the losses of PBE 3 : 1,3, PBE 2 : 1,2 was used as an internal standard in GC.

Combined GC–Mass Spectrometry of PBE of Diols. The PBE of diols were analyzed by a combined method of GC–mass spectrometry on an LKB-9000 instrument. To separate the mixtures into components we used a column (2000 × 4 mm) with 3% SE-30 on Chromosorb W (60-80 mesh) at 100 → 250° (3 deg/min), carrier gas helium (30 ml/min). Energy of the exciting electrons 70 eV. Temperature of the ion source 270-290°.

CONCLUSIONS

1. A new method was proposed for the analysis of small quantities of C_2-C_4-diols, formed in the cleavage of lipids, based on combined gas-chromatography and mass spectrography of their phenylborate esters.

2. Phenylborate esters of diols can be obtained without appreciable losses in aqueous or methanol medium. The mass spectra of phenylborate esters permit an unambiguous identification of the investigated diols.

LITERATURE CITED

1. S. G. Batrakov, A. G. Panosyan, I. V. Konova, and L. D. Bergel'son, Dokl. Akad. Nauk SSSR, 211, 722 (1973).
2. L. D. Bergelson, Fette, Seifen, Anstrichmittel, 75, 89 (1973).
3. V. A. Vaver, V. A. Shchennikov, and L. D. Bergel'son, Biokhimiya, 32, 1027 (1967).
4. L. D. Bergelson, V. A. Vaver, N. V. Prokasova, A. N. Ushakov, B. V. Rozynov, K. Stefanov, L. I. Ilyukhina, and T. N. Simonova, Biochim. et Biophys. Acta, 260, 571 (1972).
5. V. A. Vaver, V. V. Dorogov, and L. D. Bergel'son, Izv. Akad. Nauk SSSR, Ser. Khim., 2681 (1967).
6. E. Schupp and W. J. Baumann, J. Lipid Res., 14, 121 (1973).
7. C. J. W. Brooks and J. Maclean, J. Chromatogr. Sci., 9, 19 (1971).
8. J. R. McKinley and H. Weigel, Chem. Commun., 1022 (1970).
9. J. R. McKinley and H. Weigel, Chem. Commun., 1051 (1972).
10. E. J. Bourne, E. M. Lees, and H. Weigel, J. Chem. Soc., 3798 (1965).
11. O. S. Chizhov, L. S. Golovkina, and N. S. Vulfson, Carbohydrate Research, 6, 138 (1968).