tions, H. G. Haring and F. Dukers for organizing the threshold value measurements, N. van der Plasse for determining infrared spectra, and P. J. de Valois for supplying the mass spectral data.

Supplementary Material Available. Descriptions of the syntheses as well as the ir, MS, and NMR data of the pyrazines mentioned will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-638.

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Received for review December 10, 1974. Accepted March 17, 1975.

Mass Spectrometry of Some Ethane- and Propanediol Diesters

Paul R. LeTellier and Wassef W. Nawar*

Mass spectral data of 9 ethanediol diesters and 18 propanediol diesters are presented. Ions arising from the two-step expulsion of aldehydes from the diol portion of the molecule can be used to differentiate between the 1,2-propanediol diesters and their 1,3 isomers. The ions at *m/e* 86 in the spectra of ethanediol diesters and at *m/e* 100 for the propanediol diesters are characteristic and can be used to distinguish between the two homologous series. Also distinctive are the fragments corresponding to the ion $[RCOCH_2OC(OH)=CH_2]^+$. These are formed in ethanediol diesters and 1,2-propanediol diesters but not in 1,3-diol diesters.

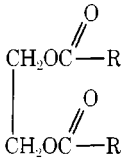
In our work on the radiolysis of simple triglycerides, we identified some medium-chain esters of diols (LeTellier and Nawar, 1972). In certain respects, the mass spectral fragmentation patterns of these compounds were found to differ from those reported by Baumann et al. (1969) for the longer chain compounds, and also from those established by Sasâki et al. (1967) for acyclic α-glycol diesters. To clarify these anomalies, the homologous series of ethanediol diesters of C_2–C_{10} fatty acids and those of propanediol diesters of C_2–C_8 fatty acids were synthesized and their spectra carefully studied.

Department of Food Science and Nutrition, University of Massachusetts, Amherst, Massachusetts 01002.

EXPERIMENTAL SECTION

The 1,2-ethanediol diesters, 1,2-propanediol diesters, and 1,3-propanediol diesters were synthesized, according to Mattson and Volpenhein (1962), by treating the appropriate acyl chlorides with 1,2-ethanediol, 1,2-propanediol, and 1,3-propanediol, respectively. A better than 99% purity of the diesters was obtained by preparative gas chromatography on a 10% SE 30 column. The mass spectra were determined with a Hitachi Perkin-Elmer combined gas chromatograph–mass spectrometer, Model RMU-6A. The ionizing voltage was 80 eV.

Table I. Relative Intensities in the Mass Spectra of Ethanediol Diesters[a]



Ion	R = CH_3 mol wt 146	R = C_2H_5 mol wt 174	R = C_3H_7 mol wt 202	R = C_4H_9 mol wt 230	R = C_5H_{11} mol wt 258	R = C_6H_{13} mol wt 286	R = C_7H_{15} mol wt 314	R = C_8H_{17} mol wt 342	R = C_9H_{19} mol wt 370
RCO	100	100*	100	100	100*	100	100	100	100
(M − RCO) + 2H	0	0.1	0.5	1	1	2	6	11	14
M − RCOO	1	5	14	18	26	34	49	59	67
m/e 57	0.4	100*	0.2	48*	2	13	68	76	58
m/e 84	0	0	0	3	0.6	24	26	44	49
m/e 98	0	0	0	1	10	0.6	22	29	47
m/e 112	0	0	0	0	1	8	1	15	15
M − CH_2O	6	1	0.3	0.1	0	0	0	0	0
M − $2CH_2O$	14*	7	2	0.6	0.1	0.2	0	0	0
$CH_2OC(=O)R$	6	7	2	0.3	0.6	0.5	0.2	0.3	0.3
$RC(=O)O(H)C(=O)R$	3	3	1	0.1	0.2	0	0	0	0
McLafferty rearr.	0	0	0.3	0.5	0.8	0.5	2	1	1
m/e 146	0	0	0	0.3	1	1	2	2	2
m/e 86	14*	0	7	20	25	32	48	55	59
M − RCOOH	14*	13	4	2	2	3	6	5	7
m/e 99	0	0	2	4	100*	8	17*	37	48
$RC(=O)CH_2OC(OH)=CH_2$	0	0	1	2	2	1	0.6	0.7	0.3
R	3	49	60	48*	38	25	17*	7	3

[a] The values with asterisks, within each vertical column, refer to one peak in the spectrum which may result from more than one mode of fragmentation.

RESULTS AND DISCUSSION

The characteristic fragmentation ions and their relative abundances are given in Tables I and II. It can be seen that for all the compounds studied, the molecular ion is absent and the acylium ions $[RCO]^+$ give rise to the base peaks (with the exception of 1,3-propanediol dinonanoate and 1,3-propanediol didecanoate. This is in contrast to the longer chain compounds (Baumann et al., 1969) where the base peaks corresponded to the ions $[M - RCOO]^+$. With decreasing molecular weights, the ions $[(M - RCO) + 2H]^+$ and $[M - RCOO]^+$, *m/e* 57, become less and less intense. As expected, however, the mass peaks at *m/e* 84, 98, and 112, which have been assigned to ions of cycloalkenol structures, can be observed if the fatty acid chain contains five or more carbon atoms.

The ions arising from the two-step expulsion of aldehydes from the diol portion of the molecule (M − CH_2O and M − $2CH_2O$ for ethanediol diesters and M − C_2H_4O and M − C_2H_4O − CH_2O for 1,2-propanediol diesters) are characteristic and may be used to differentiate between the 1,2-propanediol diesters and their 1,3 isomers. Their intensity is decreased, however, with increased molecular weights. Fragments resulting from C_1–C_2 fission in the diol moiety and those corresponding to the protonated anhydride $RC(=O)O(H^+)C(=O)R$ are only of significance in the lower homologs.

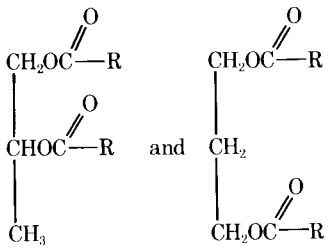
Both ethanediol and propanediol diesters exhibit McLafferty rearrangements, caused by 2,3 cleavage and a hydrogen transfer from the fourth carbon to the carbonyl oxygen. A double rearrangement involving both acyl groups gives rise to characteristic peaks at *m/e* 146 for ethanediol diesters and at *m/e* 160 for propanediol diesters.

Other distinctive peaks not previously reported are observed at *m/e* 86 in the spectra of ethanediol diesters and at *m/e* 100 for the propanediol diesters. These ions can be used to distinguish between ethanediol and propanediol diesters. They are present in compounds having fatty acids longer than C_3 and become more intense with increasing chain length (*m/e* 100 becomes the base peak for the dinonanoate and didecanoate of 1,3-propanediol). Furthermore, the peak at *m/e* 100 may also serve to differentiate between the 1,2-propanediol diesters and their 1,3 isomers, since this ion is usually about twice as intense in the case of the latter series of compounds. It is speculated that both peaks (*m/e* 86 and 100) arise from the molecular ion via the loss of the acid group RCOOH plus a single McLafferty rearrangement. Ions at *m/e* 99 for ethanediol diesters and *m/e* 113 for propanediol diesters are similarly distinctive and probably result from the molecular ion by loss of one RCOOH group plus 3,4 cleavage of the fatty acid chain. The latter cleavage is analogous to that producing the ion *m/e* 87 in the spectra of methyl esters (Sharkey et al., 1959).

Fragments corresponding to the ion $[RCOCH_2OC(O-H)=CH_2]^+$, which may result from an aldehyde expulsion and a McLafferty rearrangement, can also provide valuable means of identification. They are formed in ethanediol diesters and 1,2-propanediol diesters but not in 1,3-diol diesters.

The alkyl ion $[R]^+$ is relatively intense in the spectra of the diesters containing C_2–C_8 fatty acids but insignificant for the higher or lower homologs. This ion is useful in identifying the fatty acid moiety and appears to arise from the decomposition $[RCO]^+ \rightarrow [R]^+$ as confirmed by metastable peaks observed at *m/e* 14.8 for the propionates, 26.0 for the butyrates, 38.2 for the pentanoates, 50.9 for the hexanoates, and 63.0 for the heptanoates.

Table II. Relative Intensities in the Mass Spectra of 1,2- and 1,3-Propanediol Diesters[a]



	R = CH_3 mol wt 160		R = C_2H_5 mol wt 188		R = C_3H_7 mol wt 216		R = C_4H_9 mol wt 244		R = C_5H_{11} mol wt 272		R = C_6H_{13} mol wt 300		R = C_7H_{15} mol wt 328		R = C_8H_{17} mol wt 356		R = C_9H_{19} mol wt 384	
Ion	1,2	1,3	1,2	1,3	1,2	1,3	1,2	1,3	1,2	1,3	1,2	1,3	1,2	1,3	1,2	1,3	1,2	1,3
RCO	100	100	100*	100*	100	100	100	100	100	100	100*	100*	100	100	100	95	100	87
(M − RCO) + 2H	0	0	0	0	0.2	0.6	0.3	0.8	0.3	1	0.7	2	3	8	5	19	9	24
M − RCOO	0.2	2	8	2	8	17	15	24	13	35	20	41	34	54	36	84	48	92
m/e 57	0.9	13	100*	100*	1	3	44*	57*	2	4	15	17	92	99	49	73	40	49
m/e 84	0	0	0	0	0.2	0	4	4	0	0.4	15	30	21	34	19	42	16	42
m/e 98	0	0	0	0	0	0	4	0.3	7	12	0.2	0.5	18	27	14	27	21	46
m/e 112	0	0	0	0	0	0	0	0.2	4	0.4	9	9	0.5	1	5	14	8	16
M − C_2H_4O	6	0	4	0	3	0	2	0	0.8	0	0.8	0	1	0	1	0	1	0
M − C_2H_4O − CH_2O	5	0	13	0	2	0	1	0	0.4	0	0.2	0	0.3	0	0	0	0	0
$C_2H_4OC(=O)R$	12	7	12	1	7	2	4	1	2	1	2	0.7	2	1	2	0.7	1	2
$CH_2OC(=O)R$	2	8	2	2	1	3	0.3	0.4	0.7	0.2	0.2	0.2	0	0	0	0.4	0.4	0.2
RC(=O)O(H)-C(=O)R	1	10	0.4	3	0.1	1	0.1	0.6	0	0.2	0	0	0	0	0	0	0	0
McLafferty rearr.	0	0	0	0	0.1	0.7	0.4	1	0.5	1	0.5	1	1	4	2	8	2	5
m/e 160	0	0	0	0	0	0.1	0.4	1	0.5	2	1	3	1	4	2	7	2	4
m/e 100	5*	18*	0	0	5	16	15	33	23	57	22	59	47	82	36	100	48	100
M − RCOOH	5*	18*	13	11	5	13	4	7	2	7	3	10	5	17	9	26	15	36
m/e 113	0	0	0	1	2	3	4	7	5	15	100*	100*	19	25	44*	35*	65	43
RC(=O)CH_2OC-(OH)=CH_2	0	0	0	0	3	0	4	0	5	0	4	0	4	0	2	0	3	0
R	1	0.2	42	28	69	59	44*	57*	36	46	29	35	2	2	44*	35*	5	5

[a] The values with asterisks, within each vertical column, refer to one peak in the spectrum which may result from more than one mode of fragmentation.

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Received for review February 14, 1975. Accepted April 21, 1975. Paper No. 1039, Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst. This work was supported in part from Experimental Station Project No. 198 and in part by U.S. Public Health Service Grant No. FD-00053.

Quantitative Evaluation of Lachrymatory Factor in Onion by Thin-Layer Chromatography

Gyanendra M. Tewari and Chiranjib Bandyopadhyay*

A thin-layer chromatographic technique and an incubation method have been developed for quantitative evaluation of lachrymatory factor in onion. Both these methods were essentially based on color forming reaction of lachrymators with glycine–formaldehyde reagent followed by measurement of the color at 520 mμ. A linear relationship between the amounts of lachrymator and corresponding optical densities has been ascertained by both these methods. Total lachrymator content of white and red globe as well as Madras varieties of onion was determined by the incubation method, while the relative abundance of individual lachrymators within the varieties was quantitized by thin-layer chromatography.

Within the last few years, especially since the recent advances in chromatographic techniques, increasing attention has been given to an objective method of evaluating the pungent and lachrymatory components of onion. Both these factors arise as a result of the interaction of S-substituted L-cysteine sulfoxide derivatives and enzyme of the alliinase type when the integrity of the onion tissue is destroyed by comminution (Schwimmer et al., 1960; Virtanen and Matikkala, 1959). Pungency of onion is attributed mainly to various disulfides (Bernhard, 1968), while the lachrymatory factor (LF) is a thiopropanal *S*-oxide (Brodnitz and Pascale, 1971).

In recent years several approaches have been made to assess the volatile flavor compounds of onion and onion products by gas–liquid chromatography (Boelens et al., 1971; Saghir et al., 1964) as well as thin-layer chromatography (Bandyopadhyay et al., 1970; Lukes, 1971). However, there have been no objective methods for quantitative evaluation of LF in onion. Thin-layer chromatographic separation of LF in onion in the form of a cysteine derivative on a cellulose plate (Lukes, 1971) and the gas–liquid chromatographic method of isolation of thiopropanal *S*-oxide (Brodnitz and Pascale, 1971) from onion extract have been reported. Recently, Bandyopadhyay and Tewari (1973) have investigated the color-developer (Lukes, 1959; Shannon et al., 1967) involved in pinking of onion purees by thin-layer chromatography, where they have shown for the first time that LF of onion is in fact the color-developer compound(s) consisting of at least three components, all of which are indeed related to the tear-causing factor of onion. Although Shannon et al. (1967) have demonstrated that the color-developer compound reacts with glycine–formaldehyde reagent resulting in a pink color having an absorption maximum at 520 mμ, quantitative data regarding this aspect are not available.

In the present investigation the color-forming reaction of lachrymators with glycine–formaldehyde reagent has been utilized to develop primarily a thin-layer chromatographic (TLC) technique and also an incubation method for quantitative evaluation of LF in onion.

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085, India.

EXPERIMENTAL SECTION

White and red globe (Nasik, Maharashtra) as well as small Madras variety onions believed to have been stored at ambient temperature (30–34°) for about 4 months after harvest were purchased from a local market. All solvents and reagents were analytical grade. The solvents were redistilled before use.

Glycine–Formaldehyde Reagent. Glycine (0.11 *M*) and formaldehyde (3×10^{-3} *M*) solutions were prepared in distilled water. Freshly prepared solutions were used.

Onion Extract. Onion extract containing the flavor compounds as well as lachrymatory agents was obtained by extracting the respective variety of onions with cold peroxide-free diethyl ether at 0°. Prior to extraction the onions were kept at 0° overnight. Skin- and disk-free onions (200 g) were cut into pieces and macerated with 200 ml of distilled water in a Waring Blendor and the juice was collected by filtering through four layers of mull cloth. The juice was then extracted repeatedly with cold ether (3 × 200 ml) in a separating funnel. The residual pulp was macerated twice with 100 ml of ether each time. Ether layers from both pulp and juice were pooled together after centrifugation, dried over anhydrous sodium sulfate, and filtered. The bulk of the ether was removed in a rotary evaporator at room temperature and the crude extract was then transferred quantitatively into a small glass stoppered container, from which the solvent was finally removed by blowing a stream of nitrogen at room temperature. A 2% chloroform solution of each extract was prepared and kept at −20° under nitrogen until further use.

Reference Lachrymator. A lachrymatory compound identified as thiopropanal *S*-oxide was isolated from the ether extract of white onion purees by high vacuum distillation at 40° and purified as a single component by preparative silica gel TLC according to the method described elsewhere (Bandyopadhyay and Tewari, 1973). This served as a reference compound since a synthetic compound of