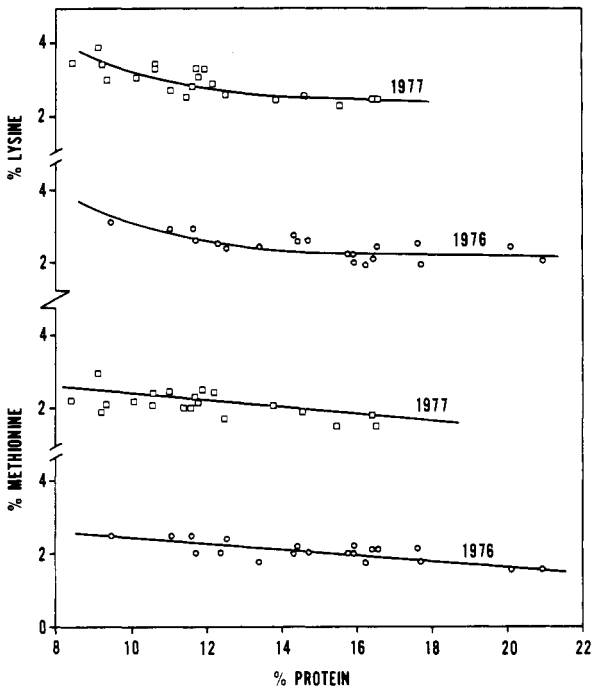

Figure 2. Lysine and methionine variation with protein content for pearl millet inbreds and hybrids.

the situation at the higher protein concentrations in pearl millet.

Mineral Content. Mineral assays are presented in Tables IV and V. The predominate mineral in all five millets was potassium, followed by phosphorus, and approximately equal amounts of calcium and magnesium. Increased nitrogen fertilizer had little effect on mineral concentrations. Concentrations of copper, iron, zinc, and manganese varied between about 2 and 6 ppm, and the amount of these minerals was little affected by fertilizer level, season, or millet type. Magnesium, reported to increase with increased fertilizer, varied between 20 and 128 ppm, a somewhat higher level than that reported by Shukla and Bhatia (1971), but showing no particular relationship to amount of fertilizer. The amount of bromine remained fairly constant at about 12 ppm, and the concentration of strontium varied between 13 and 66 ppm for all of the millets over the two seasons.

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Mass Spectral Characterization of 2,4-Disubstituted 1,3-Dioxolanes Found in Flavors

Ronald C. Welch* and G. L. K. Hunter

A number of 2,4-disubstituted-1,3-dioxolanes, only a few of which are approved for food use, have been found in commercial flavors. These 2,4-disubstituted-1,3-dioxolanes can be readily characterized by mass spectroscopy. The mass spectra of the acetals of the more common flavoring aldehydes are presented here for the first time.

The presence of substituted 1,3-dioxolanes, which are frequently found in commercial flavors, can result from the condensation of flavoring aldehydes with the solvent propylene glycol. A gas chromatogram of a typical commercial flavor containing such condensation products is presented in Figure 1. Because many of these substituted dioxolanes are not permitted in food products nor characterized in the literature, a procedure using mass spectroscopy has been developed for their rapid positive identification. Their mass spectra and a procedure for structural elucidation are presented.

EXPERIMENTAL SECTION

Preparation of Substituted Dioxolanes. The parent 1,3-dioxolane was purchased from Phatz and Bauer (Stamford, CT). The remaining derivatives were synthesized according to the method of Lucas and Guthrie (1950) by reacting the aldehyde with 1,2-propylene glycol in the presence of a mineral acid.

Combined Gas Chromatography–Mass Spectroscopy. The substituted 1,3-dioxolanes from commercial flavors and from synthetic mixtures were separated and identified by using a Varian/Aerograph Series 1200 instrument fitted with a flame ionization detector and coupled to a Hitachi RMU-6L single focusing magnetic sector mass spectrometer, respectively. A small fraction of the column effluent was bypassed through a fine metering valve into a single stage glass jet separator (McFadden, 1973) leading into the ion source. Both the molecular separator and valve were maintained at 200 °C with a convection circulated air oven.

A 2 mm (i.d.) × 3 m glass column packed with 5% Carbowax 20M on 60–80 mesh acid-washed DMCS Chro-

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Table I. A Series of 2,4-Disubstituted-1,3-dioxolanes

compound	mass spectra data, *m/e* (intensity)
2-ethyl-4-methyl-1,3-dioxolane	M^+ 116, 87 (100), 59 (75), 41 (64), 31 (58), 57 (45), 72 (36), 42 (25), 27 (15)
2-propyl-4-methyl-1,3-dioxolane	M^+ 130, 87 (100), 59 (80), 41 (67), 71 (65), 31 (60), 43 (53), 27 (45), 57 (29)
2-butyl-4-methyl-1,3-dioxolane	M^+ 144, 87 (100), 59 (75), 41 (60), 31 (56), 71 (45), 57 (33), 27 (31), 42 (22)
2-pentyl-4-methyl-1,3-dioxolane	M^+ 158, 87 (100), 59 (71), 41 (55), 31 (53), 43 (35), 27 (27), 71 (27), 88 (20)
2-hexyl-4-methyl-1,3-dioxolane	M^+ 172, 87 (100), 59 (67), 41 (53), 31 (45), 43 (24), 27 (16), 55 (13), 88 (13)
2-heptyl-4-methyl-1,3-dioxolane	M^+ 186, 87 (100), 59 (80), 41 (68), 31 (59), 57 (50), 88 (48), 43 (46), 55 (42)
2-octyl-4-methyl-1,3-dioxolane	M^+ 200, 87 (100), 59 (70), 41 (55), 31 (47), 88 (30), 43 (27), 55 (24), 57 (22)
2-nonyl-4-methyl-1,3-dioxolane	M^+ 214, 87 (100), 59 (75), 41 (64), 88 (60), 31 (58), 43 (56), 55 (49), 57 (40)
2-decyl-4-methyl-1,3-dioxolane	M^+ 224, 87 (100), 59 (76), 41 (63), 31 (46), 55 (46), 88 (45), 67 (27), 43 (21)
2-(1,5-dimethyl-4-hexenyl)-4-methyl-1,3-dioxolane	M^+ 198, 87 (100), 59 (80), 41 (61), 31 (40), 127 (37), 58 (37), 116 (27), 82 (17)
2-(2,6-dimethyl-5-heptenyl)-4-methyl-1,3-dioxolane	M^+ 212, 87 (100), 59 (72), 41 (70), 127 (65), 121 (59), 69 (50), 95 (33), 31 (27)
2-(*cis*-2,6-dimethyl-1,5-heptadienyl)-4-methyl-1,3-dioxolane	M^+ 210, 87 (100), 41 (60), 59 (52), 69 (46), 55 (21), 31 (19), 140 (15), 119 (12)
2-(*trans*-2,6-dimethyl-1,5-heptadienyl)-4-methyl-1,3-dioxolane	M^+ 210, 87 (100), 41 (71), 59 (69), 69 (43), 55 (40), 141 (27), 31 (14), 27 (10)

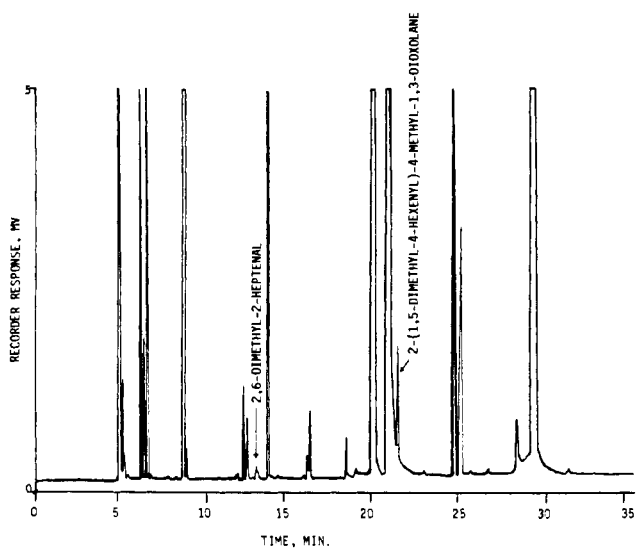

Figure 1. Gas chromatogram of a typical commercial flavor containing 2,4-disubstituted-1,3-dioxolane.

mosorb W was used throughout the analyses. The column was operated at a programmed rate of 2 °C/min from 60–190 °C with a helium flow of 25 mL/min. Both the injector and detector temperatures were maintained at 230 °C. All mass spectra were obtained at 90 eV and a filament current of 80 μA with an ion source temperature of 200 °C.

RESULTS AND DISCUSSION

Figure 2 shows the fragmentation pattern of 2,4-dimethyl-1,3-dioxolane under electron impact which has been studied and reported by Conde-Caprace and Collin (1972). Although the abundance of the ions reported for 1,3-dioxolane differs from that found for the 2,4-disubstituted derivatives, the mode of fragmentation does not. Conde-Caprace and Collin reported the fragmentation of 1,3-dioxolane to proceed in two different ways: (a) the primary fragmentation step being the ejection of a hydrogen atom from the molecular ion to yield the cyclic ion $(C_3H_5O_2)^+$ which is the base peak, followed by the loss of CO to give the cyclic ion $(C_2H_5O)^+$ and (b) the loss of CH_2O from the molecular ion resulting in the $(C_2H_4O)^+$ fragment. The present study deals with the mass spectral identification of 1,2-propylene glycol condensation products with both saturated and olefinic, straight and branch chained aldehydes. The resulting 2,4-disubstituted-1,3-dioxolanes follow the fragmentation pattern shown by Conde-Caprace and Collin for 1,3-dioxolane, thus permitting the characterization of these derivatives by direct comparison. The cracking pattern for these condensation products is shown in Figure 3. In all cases the *m/e* 87 fragment is the base peak for these derivatives and, when accompanied by the *m/e* 59 fragment, indicates the alcohol in the condensation product to be 1,2-propylene glycol. The last peak in the spectra represents the molecular weight minus one. The value of *R* can be readily deduced by subtracting *m/e* 87 from the molecular ion, the latter obtained by adding one to the last peak.

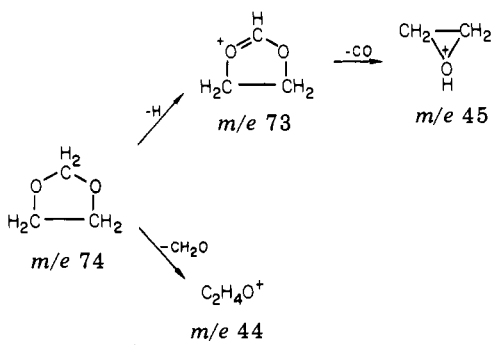

Figure 2. Fragmentation pattern of 1,3-dioxolane under electron impact.

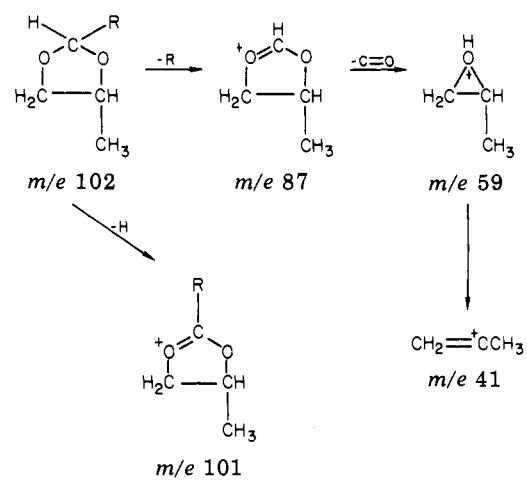

Figure 3. Cracking pattern of 2,4-disubstituted-1,3-dioxolane.

The mass spectra of propylene glycol acetals of the more common nonaromatic aldehydes used in flavorings are presented in Figure 4 and Table I. It can be seen from these spectra that all of the 2,4-disubstituted-1,3-dioxolanes contain the three peaks *m/e* 87, 59, and 41.

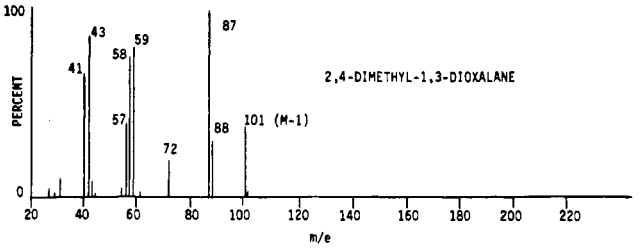

Figure 4. Mass spectra of 2,4-dimethyl-1,3-dioxolane.

Further, the intensity of these three peaks appears in decreasing order of magnitude in the order listed. Neral and geranial are exceptions in that the three peaks appear in the decreasing order m/e 87, 41, 59. The appearance of these three peaks in the mass spectrum serves to identify the presence of 2,4-disubstituted-1,3-dioxolanes since they are specific for this type of compound.

These substituted 1,3-dioxolanes do not appear in all mixtures containing aldehydes and propylene glycol, although it has been noted that the frequency of their appearance in flavors has been increasing. The reason may be due to the increasing use of organic acids as flavorants. However, with the growing availability of mass spectroscopic instrumentation in commercial laboratories the above procedure will provide a rapid means for detection so that corrective action can be taken.

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New Tryptamine Derivatives Isolated from Wax of Green Coffee Beans

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Some unknown 5-hydroxytryptamine derivatives were found to be present in the cuticular wax of green coffee beans. They were isolated by polyamide column chromatography and high-pressure liquid chromatography on a reversed-phase column and were identified by mass spectrometry, infrared spectrometry, proton magnetic resonance, and ultraviolet spectrometry as N_β-(20-hydroxyarachidoyl)-5-hydroxytryptamine and N_β-(22-hydroxybehenoyl)-5-hydroxytryptamine.

Investigation of the chemical composition of the cuticular wax of green coffee beans is part of our research interest (Folstar, 1976). It was previously found that the wax could be separated into two fractions: a fraction which is soluble in petroleum ether (40–60 °C) and a fraction which is insoluble in petroleum ether. The chemical composition of the petroleum ether soluble fraction has been established, and it was found to be largely identical with the composition of coffee bean oil (Folstar et al., 1975a,b). The petroleum ether insoluble part of the wax was fractionated by polyamide column chromatography and high-pressure liquid chromatography (LC); four homologues of N_β-alkanoyl-5-hydroxytryptamine (C-5-HT), in which the alkanoyl group is a stearoyl (C_{18}-5-HT, Ia), arachidoyl (C_{20}-5-HT, Ib), behenoyl (C_{22}-5-HT, Ic), or lignoceroyl (C_{24}-5-HT, Id), were found to be the main constituents; moreover, caffeine and some fractions consisting of unknown compounds were found (Folstar et al., 1979). In this paper we describe the isolation and structural identification of two of these unknown compounds: N_β-(20-hydroxyarachidoyl)-5-hydroxytryptamine (IIa) and N_β-(22-hydroxybehenoyl)-5-hydroxytryptamine (IIb).

EXPERIMENTAL SECTION

Materials. Polyamide-6S for column chromatography was obtained from Riedel de Haën AG, Seelze-Hannover, Germany. Commercially prepared silica gel $60F_{254}$ plates

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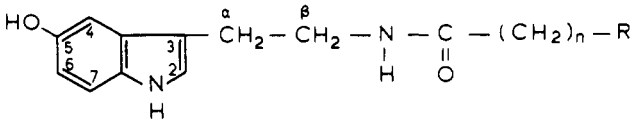

Ia R = CH_3 ; n = 16
Ib R = CH_3 ; n = 18
Ic R = CH_3 ; n = 20
Id R = CH_3 ; n = 22
IIa R = CH_2OH ; n = 18
IIb R = CH_2OH ; n = 20

were obtained from Merck AG, Darmstadt, Germany. LiChrosorb 10RP18 columns for LC were purchased from Chrompack BV, Middelburg, The Netherlands. Green coffee beans (Santos coffee) were kindly supplied by D.E.J. International Research Co., B.V. Utrecht, The Netherlands. Reference samples of 5-hydroxytryptamine and 6-hydroxytryptamine, both as creatinine sulfate complex, were from Sigma, St. Louis, MO.

Isolation and Fractionation of Coffee Wax. Coffee wax was obtained by extracting 10 kg of unground green beans with methylene chloride. The extraction was carried out by heating and stirring portions of 500 g of beans with 1 L of the solvent each, using a 5-L flask which was equipped with a condenser. The flask was heated on a hot plate with a built-in magnetic stirrer. The methylene chloride solutions were combined, and the solvent was evaporated in a rotating vacuum evaporator at 30 °C. Next the residue was extracted with 500 mL of petroleum ether

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