niques may thus be useful in further fractionating these molecules. That some polypeptide bands do not segregate cleanly into cryoprecipitate and supernatant fractions may be due to the presence of several polypeptides of the same molecular weight but with different properties. This has been demonstrated for zein I polypeptides by isoelectric focusing (Valentini et al., 1979). The observation that removal of the cryoprecipitate proteins from zein II results in an amino acid composition very close to that of zein I supports the hypothesis of Landry and Moureaux (1970) and of Sodek and Wilson (1971) that zein II consists of zein I polypeptides and some other polypeptides with higher contents of methionine, proline, glycine, and tyrosine and lower contents of aspartic acid (asparagine), leucine, isoleucine, and phenylalanine. This contention is further supported by the similarity of isoelectric focusing patterns of zein I and zein II polypeptides (Gianazza et al., 1976).

The cryoprecipitate proteins may be regarded as zeins although a more restricted use of the term zein has been proposed (Landry, 1979). They do have amino acid compositions closely related to the zein I polypeptides, in particular the high glutamic acid (glutamine) and proline contents. In addition, it has been shown that protein body messenger RNA codes for the synthesis of at least one of the low molecular weight methionine-rich polypeptides when translated in vitro (Melcher, 1979). Sequestration in protein bodies is one characteristic that differentiates zeins from glutelins which are deposited in the cytoplasmic matrix. It is interesting to speculate that these methionine-rich polypeptides serve as sulfur storage proteins while the bulk of the zeins serve as nitrogen storage proteins. The production of specific sulfur storage proteins may be an adaptation that allows the plant to survive variations in the level of sulfur available to it, as has been suggested for pea storage proteins (Millerd et al., 1979).

ACKNOWLEDGMENT

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2-Hexyl-3-methylmaleic Anhydride: An Unusual Volatile Component of Raisins and Almond Hulls

2-Hexyl-3-methylmaleic anhydride has been identified in the volatile oils of California raisins and almond hulls by using capillary GLC–MS, IR, and ^{1}H NMR spectrometries. An authentic sample for comparison was synthesized by the Stobbe condensation of hexanal with diethyl methylsuccinate, followed by hydrolysis, anhydride formation, and acid-catalyzed double-bond rearrangement. This seems to be the first detection of a natural volatile acid anhydride in a food.

The authors are involved in a study to determine the identities of the volatile components of raisins (and other products) and to test whether these components attract certain insect pests. Some of the authors had already reported on a study of the volatile components of almond hulls (Buttery et al., 1980). An unusual component previously detected in almond hulls was also found in raisins. The present communication reports the identification of this compound.

EXPERIMENTAL SECTION

Materials. High-quality raisins (dried Thompson's seedless grapes) were obtained from a local raisin processor in the Fresno area of California. Raisins were also obtained from local markets. Starting materials for the synthesis of the anhydride were obtained from Aldrich Chemical Co.

Isolation of Volatile Oil from Raisins. Raisins (1500 g) were placed in a 12-L round-bottomed flask together with 6 L of odor-free water. A Likens-Nickerson steam distillation continuous extraction head was attached to the flask. Purified hexane (100 mL) was placed in a 250-mL flask attached to the solvent arm of the head. The extraction head condenser was cooled with a water–ethanol mixture at 0 °C. A dry ice cooled reflux condenser was

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attached to the outlet of the extraction head. The isolation was carried out for 3 h under reduced pressure (100 mmHg) with the raisin mixture at ~45–50 °C. After the isolation, the hexane extract was dried by freezing out any water and concentrated by using low holdup Vigreux distillation columns to give the raisin volatile oil.

Capillary Gas–Liquid Chromatography–Mass Spectrometry (GLC–MS) Analysis. The components of the raisin volatile oil were separated by GLC on a 150 m long by 0.64 mm i.d. Pyrex glass capillary column coated with Carbowax 20-M. The temperature programming conditions used were to hold the column at 50 °C for 30 min after injection and then to program at 1 °C/min from 50 to 170 °C, holding at the upper limit. The column inlet pressure was 16 psi He (flow velocity 28 cm/s). A single-stage Lewellyn-Littlejohn silicone rubber membrane molecular separator was used to couple the end of the capillary column to the mass spectrometer (a modified Consolidated 21-620, cycloidal type). Electron ionization was 70 eV.

Packed Column GLC–Infrared Spectral (IR) Analysis. Components were separated from the volatile oil by GLC using a 3 m long by 0.64 cm o.d. stainless steel column packed with 80–100 mesh Chromosorb G-DMCS coated with 2% Carbowax 20-M. Samples were collected in dry ice cooled Pyrex tubes (3 mm o.d. × 14 cm long) which were sealed and stored at -20 °C until the spectra could be recorded. IR spectra were measured as thin films of the pure material by using ultramicro salt plates with a Perkin-Elmer Model 237 instrument.

High-Resolution Mass Spectrometry. This was measured on both the sample isolated from raisins and on the synthetic sample by using a Consolidated 21-110 A instrument.

Nuclear Magnetic Resonance (NMR) Spectra. NMR spectra were determined on the packed column GLC separated samples. ^{1}H spectra were recorded at 90 MHz by using a Varian EM 390 instrument with CDCl$_3$ as the solvent and Me$_4$Si as the reference standard. ^{13}C spectra were obtained at 23 MHz from a JEOL PFT 100 equipped with an EC 100 data system.

Synthesis of 2-Hexylidene-3-methylsuccinic Acid Monoethyl Ester (I). Diethyl methylsuccinate (14.5 g) and freshly distilled hexanal (4.0 g) were mixed and added (together) dropwise to a refluxing solution of potassium *tert*-butoxide (prepared from 2.5 g of potassium) in dry *tert*-butanol (80 mL). The mixture was refluxed for 2 h. Most of the *tert*-butanol was then distilled off, and the solution was cooled and acidified with 3 N hydrochloric acid. The remaining *tert*-butanol was then removed by distillation. The residual solution was extracted with ether (4 × 25 mL). The ether extract was washed with water (20 mL) and then extracted with 10% sodium carbonate solution (5 × 20 mL). The carbonate extract was then acidified with 12 N HCl. The organic portion was separated and the aqueous portion was extracted with ether (3 × 25 mL). The organic portion together with the ether extract was dried over anhydrous CaSO$_4$ and distilled under vacuum (1 mmHg) to give 2-hexylidene-3-methyl succinic acid monoethyl ester (I, 3.3 g).

Synthesis of 2-Hexyl-3-methylmaleic Anhydride (III). The monoethyl ester from above (3.3 g) was hydrolyzed by refluxing with a solution of KOH (5 g) in water (10 mL) for 2 h. The ethanol formed was distilled off and the solution was refluxed an additional 30 min. The remaining solution was cooled to 5 °C and acidified with sulfuric acid and then extracted with ether (3 × 25 mL). The ether extract was dried over anhydrous sodium sulfate
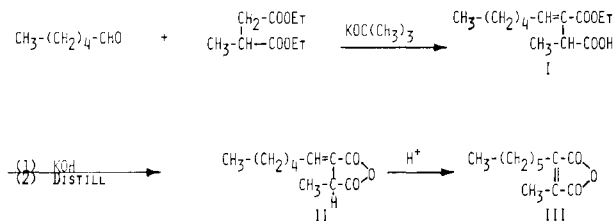

Figure 1. Outline of synthesis.

and then the ether removed by distillation. This gave the 2-hexylidene-3-methylsuccinic acid (1.7 g). Distillation of the acid under vacuum (20 mmHg), bp 160–170 °C, gave the crude 2-hexylidene-3-methylsuccinic anhydride (II). This was dissolved in benzene, a trace (~3%) of *p*-toluenesulfonic acid was added, and the mixture was refluxed for 4 h. The *p*-toluenesulfonic acid was removed by shaking with a little solid sodium bicarbonate and filtering. Removal of the benzene gave the crude 2-hexyl-3-methylmaleic anhydride (III, 1 g). The crude material was purified by GLC separation using the packed Carbowax 20-M column described above.

Synthesis of 2-Butyl-3-methylmaleic Anhydride. This was carried out by using essentially the same procedure as for the hexyl compound except that butanal was used instead of hexanal.

RESULTS AND DISCUSSION

The vacuum steam volatile oil from raisins amounted to 6 parts per million (ppm) of the raisins. Analysis by capillary GLC–MS showed that most of the components were of the types commonly encountered in foods (aliphatic aldehydes, alcohols, ketones, and acids) except for one component which formed ~5% of this oil. This compound had a mass spectrum consistent with that of an unidentified "lactone" previously found in the volatile oil of almond hulls (Buttery et al., 1980) and originally thought to have a molecular weight of 168 but later found to have a very weak molecular ion at *m/e* 196. An IR spectrum of the almond hull compound had shown an absorption at 5.7, indicating a possible lactone. ^{1}H NMR data had shown one probable methyl group on a double bond and another at the end of an aliphatic chain.

In the present study, the unknown's mass spectrum was evaluated by the Cornell University PBM and STIRS computer mass spectrometry identification system. This indicated a number of compounds with probable similar structural features. One of these was 2-(carboxymethyl)-3-hexylmaleic anhydride. Comparison of our unknown's IR and ^{1}H NMR spectra with that of methylmaleic anhydride as a model compound further indicated that our compound was a derivative of maleic anhydride. The molecular weight of 196 (together with the ^{1}H NMR information that there was a methyl group on the double bond and only one other methyl group at the end of an aliphatic chain) indicated that our unknown had the structure 2-hexyl-3-methylmaleic anhydride (III).

To our knowledge, this compound has not been found in nature or synthesized before. The synthesis is outlined in Figure 1. By use of the Stobbe condensation [cf. Johnson and Daub (1951) and Overberger and Roberts (1949)], hexanal was condensed with methylsuccinic acid diethyl ester in the presence of potassium *tert*-butoxide to give 2-hexylidene-3-methylsuccinic acid monoethyl ester (I). Hydrolysis followed by distillation gave the corresponding cyclic anhydride (II). Refluxing with *p*-toluenesulfonic acid in benzene rearranged the double bond to the desired position in the anhydride ring (III). The synthetic compound (III) was purified by GLC. The

mass, IR, and ¹H NMR spectra and GLC retention data of the synthesized compound were identical with that of the unknown.

Very few of these types of compounds seem to have been reported in the literature. In the present work, for comparison, the homologue 2-butyl-3-methylmaleic anhydride was also synthesized by using the same procedure as that for the hexyl compound. 2-Isoamyl-3-methylmaleic anhydride had been synthesized by Auden et al. (1899) and 2-isobutyl-3-methylmaleic anhydride by Purdie and Holt (1965) using more involved synthetic procedures.

The spectral data for the 2-hexyl-3-methylmaleic anhydride are given below. The mass spectrum showed (two most intense ions each 14 mass units above m/e 34, intensities in parentheses, molecular ion in boldface) the following: 41 (33), 43 (50); 53 (12), 55 (14); 67 (12), 69 (9); 79 (4), 81 (10); 95 (12), 98 (25); 111 (4), 112 (6); 126 (100), 127 (9); 139 (9), 140 (13); 151 (3), 153 (4); 168 (6), 169 (1); **196** (3). High-resolution mass spectrometry molecular weight was found to be 196.1092 ($C_{11}H_{16}O_3$ requires 196.1099). The IR spectrum (film) showed absorption maxima (in microns between 5.0 and 16.0) as follows: strong (5.7, 7.8, 10.8, 13.5); medium (5.4, 5.5, 6.0, 6.8, 7.2, 8.9, 11.1); weak (8.2, 8.4, 9.7, 12.6, 14.6, 14.9). The ¹H NMR spectrum (90 MHz, CDCl₃, 34 °C) showed: δ 0.87 (t, 3 H, —$(CH_2)_5CH_3$), 1.28 (br s, 6 H, —$CH_2(CH_2)_3CH_3$), 1.55 (pt, 2 H, =CCH_2CH_2—) 2.05 (s, 3 H, =CCH_3), 2.44 (t, 2 H, =CCH_2CH_2—). The ¹³C NMR spectrum (25.03 MHz, CDCl₃, 30 °C) showed δ 9.48 (q, 1 C, CH_3C=), 13.99 (q, 1 C, CH_3CH_2—), 22.46 (t, 1 C, CH_3CH_2—), 24.44 (t, 1 C, —CH_2C=), 27.55 (t, 1 C, —CH_2CH_2C=), 29.11 (t, 1 C, —$CH_2CH_2CH_2C$=), 31.36 (t, 1 C, $CH_3CH_2CH_2$—), 140.4 (s, 1 C, —CH_2—C=), 144.8 (s, 1 C, CH_3C=), 165.9 (s, 1 C, —C=O), 166.3 (s, 1 C, —C=O). The spectrum was compared with those of 2-methylmaleic anhydride and 1-octene as models (Johnson and Jankowski, 1972). The GLC Kovats retention index on the Pyrex Carbowax 20-M coated capillary was 2090. Carbon and hydrogen microanal. Calcd for $C_{11}H_{16}O_3$: C, 67.37; H, 8.22. Found: C, 67.1; H, 8.40.

The mass spectrum found for 2-butyl-3-methylmaleic anhydride showed the following: 41 (98), 43 (100); 53 (33), 55 (26); 67 (25), 69 (12); 79 (9), 81 (23); 95 (14), 98 (32); 111 (11), 112 (2); 125 (11), 126 (86); 139 (9), 140 (39); **168** (3).

Possible Mechanism of Formation and Role in Odor. We have been unable to come up with a likely mechanism for the formation of 2-hexyl-3-methylmaleic anhydride in raisins and almond hulls. We have, however, speculated on some remotely possible mechanisms. One of these is that methylmaleic anhydride is first formed in the dried fruits by dehydration of citric acid (this can be

brought about in the laboratory) and then reacts with a hexyl free radical from lipid autoxidation. Another speculation is that the keto group of pyruvic acid (CH_3C-OCOOH) condenses with the α-methylene group in octanoic acid [$CH_3(CH_2)_5CH_2COOH$; present in raisins and almond hulls] and the dicarboxylic acid so formed dehydrates to the anhydride. Both of these postulated mechanisms would be difficult to bring about in the laboratory in high yield but might possibly be facilitated in the dried fruit by special conditions or special forms (or derivatives) of these elementary reactants.

In the author's opinion, the 2-hexyl-3-methylmaleic anhydride has very little odor and is unlikely to contribute to the aroma of raisins as far as humans are concerned. The olfactory senses of insects are, however, quite different from that of humans. The compound will be tested for attractancy with certain insects along with other raisin components in a study already under way with the Stored Product Insects Laboratory, USDA, Fresno, CA.

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An Intensely Sweet Analogue of Kynurenine: 3-(4-Chloroanthraniloyl)-DL-alanine

A new kynurenine derivative, 3-(4-chloroanthraniloyl)-DL-alanine, was synthesized from 6-chloro-DL-tryptophan. The former compound was found to be ~80 times sweeter than sucrose by organoleptic tests.

Excessive consumption of sugar has been implicated in several diseases such as diabetes, hyperlipidemia, obesity, cardiac infarction, and dental caries. Meanwhile, there is a continued uncertainty over the safety of well-known artificial sweeteners: cyclamate and saccharin. Thus, there

is a great insistent need for a noncaloric, biologically safe sweetener.

A number of amino acids or their derivatives are known to have sweet taste (Ariyoshi, 1976). Although sweetness intensity of amino acids are generally weak, aromatic am-