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Reviewers have suggested various alternative research schemes and experiments which they feel may more effectively establish the conclusions drawn. As it seems impractical at this time to suggest extensive additional experimentation to answer the questions raised by the reviewers, we are publishing the papers essentially as delivered [Editor]. Other papers presented at the symposium were "Dietary Fiber in Cereals: Chemical, Enzymatic, and in Vivo Observations" (R. M. Saunders, E. Hautala, and E. A. Elliston), "Cereal Brans as Dietary Fiber Sources and Their Physiological Effects in Humans" (G. E. Inglett), "Changes in Dietary Fiber of Cereals during Processing" (S. K. Skarsauna and D. R. Schaller), "A Systems View of the Structure and Function of Dietary Fiber" (Ingemar Falkehag), and "The Physico-chemical Properties of Lignins and Their Potential Roles in Dietary Fiber" (B. A. Pethica and P. Zuman).

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## ARTICLES

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### Synthesis and Assessment of Three Compounds Suspected as Egg Aroma Volatiles

Victor Gil and Alexander J. MacLeod\*

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Previous work provided evidence of the occurrence of three novel compounds in the aroma volatiles of the hen's egg, namely, 1,2,4-trithiolane, 2-butylandan, and *O*-decylhydroxylamine. Here the synthesis of these three compounds is reported, but only the first two were confirmed as genuine components of egg volatiles. This is the first report of 1,2,4-trithiolane as a food volatile.

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During our analysis of the volatile flavor components of the hen's egg, 65 compounds were positively identified (MacLeod and Cave, 1975), but in addition two novel and interesting components were only very tentatively assigned and could not therefore be reported, namely, 1,2,4-trithiolane and *O*-decylhydroxylamine. Stronger evidence was obtained for another component, 2-butylandan, but since this compound was somewhat unexpected, confirmation was desirable. All identifications were originally accomplished by GC-MS and included initially computer matching of spectra with a library file. The matching process was based on the 10 most intense peaks in the spectrum but was limited within a specified molecular weight range determined by inspection of the unknown spectrum. In addition, the selection of reference spectra was further limited to those in which the base peak of the unknown spectrum was also present as one of the six most intense peaks of the library spectrum. Although *O*-decylhydroxylamine showed an excellent match on this basis (0.85, with 1.0 representing perfect agreement between unknown and reference spectra), since the system was relatively unsophisticated this high score was mainly

due to the alkyl fragments which dominate the spectra of many compounds possessing long-chain hydrocarbon substituents. Hence, the only certain deduction from the computer matching was that this egg component contained a labile long-chain alkyl group (probably decyl) and there was little real evidence for the particular class of compound suggested. This emphasizes the deficiency of these types of computer matching systems for mass spectra. However, more detailed examination of the full spectrum in question showed that the assignment from the computer matching was not entirely unreasonable and was certainly worthy of further consideration. Clearly, though, such a compound would be very unexpected as an aroma volatile.

With regard to the alkylandan, the computer matching was again very high (0.84), but for the decyl derivative. This seemed particularly unreasonable on the basis of its expected low volatility, and on careful inspection of the sample spectrum it seemed more likely that the unknown component might be a butylandan. It transpired that 2-decylandan was the only 2-alkylandan in the library file.

Although 3,5-dimethyl-1,2,4-trithiolane is well documented as a volatile of a number of foods, e.g., beef (Brinkman et al., 1972), potatoes (Buttery et al., 1970), and roasted filbert nuts (Kinlin et al., 1972), the computer match for the parent compound, previously undetected in

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food volatiles, was low (0.43), so doubt applied to this assignment as well. In order to prove or disprove the presence of these interesting novel compounds in the aroma volatiles of eggs, all three were synthesized so that their mass spectra could be more directly compared with those obtained during GC-MS of egg volatiles (Table I gives summaries of the mass spectra of the three egg components in question). Furthermore, and more important, agreement on GC retention data would then positively confirm the identifications.

#### EXPERIMENTAL SECTION

IR spectra were recorded on a Pye-Unicam SP 200 instrument,  $^1\text{H}$  NMR on a Perkin-Elmer R12B operating at 60 MHz, and mass spectra on an AEI MS 30 operating at 70 eV and equipped with an AEI DS 50 data processing system. A Pye-Unicam 104 with heated fid was used for GC.

**Synthesis of 1,2,4-Trithiolane (1).** A solution of  $\text{Na}_2\text{S}_{2.5}$  was prepared from  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  (100 g) and sulfur (20 g) in 330 mL of water. It was necessary to stir the mixture for some time until the sulfur dissolved and the solution became orange red. After filtration,  $\text{CH}_2\text{Cl}_2$  (330 mL) was added and the mixture was stirred at room temperature for 7 h, after which the organic layer was separated, washed with water, and dried (anhydrous  $\text{Na}_2\text{SO}_4$ ). Excess  $\text{CH}_2\text{Cl}_2$  was removed on the rotary evaporator and the remaining oily liquid (24.4 g) distilled under reduced pressure to yield 2.0 g (47%) of 1,2,4-trithiolane as a yellow oily liquid with a strong garlic-like odor: bp 55–57 °C/0.5 mmHg;  $n_{\text{D}}^{20}$  1.6998; IR (Nujol) 4350, 2250, 1180, 1080, 840, 720, 690  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  4.2 (s); for the mass spectrum, see Table I.

**Synthesis of *O*-Decylhydroxylamine (2).** *Potassium Benzohydroxamate (3).* Hydroxylamine hydrochloride (46.7 g, 0.67 mol) was dissolved with heating in methanol (240 mL). Potassium hydroxide (56.1 g, 1 mol) was also dissolved with heating in methanol (140 mL), and after being cooled to 30–40 °C, the solutions were mixed. Ethyl benzoate (4; 50 g, 0.33 mol) was added with thorough mixing and the resultant precipitate removed by filtration. The filtrate was allowed to stand at room temperature for 48 h after which the crystals which had formed were collected by filtration at the pump and dried. The salt 3 was thus obtained in 70% yield (41 g).

*Benzohydroxamic Acid (5).* A mixture of 27 g (0.15 mol) of the potassium salt (3) and of 1.25 N acetic acid (125 mL) was stirred and heated until a clear solution resulted. After cooling in ice, crystals of 4 deposited which were collected and recrystallized from ethyl acetate to give 13.3 g (63%) of the acid as a white crystalline solid: mp 125–126 °C; IR (Nujol) 3350, 2750, 1620, 1575, 1160, 1030, 910, 700  $\text{cm}^{-1}$ .

*O-Decylbenzohydroxamic Acid (6).* Benzohydroxamic acid (5; 5 g, 0.036 mol) was refluxed for 2 h with decyl bromide (11 g, 0.05 mol) in alcoholic NaOH (30 mL). After the mixture was cooled, the solvent was removed on the rotary evaporator, 20 mL of water was added to the residue, and it was extracted with  $\text{CHCl}_3$  (4  $\times$  25 mL). After the solution was dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), the solvent was removed on the rotary evaporator to yield an oily liquid which solidified on standing overnight. Recrystallization from benzene provided 9.6 g (96%) of 6 as a white crystalline solid: mp 54 °C; IR (Nujol) 3250, 1650, 1315, 1160, 1050, 1030, 910, 810, 700  $\text{cm}^{-1}$ .

*O-Decylhydroxylamine Hydrochloride (7).* *O*-Decylbenzohydroxamic acid (6; 5 g, 0.018 mol) was refluxed for 2 h with 4.5 mL of concentrated HCl in 30 mL of EtOH. After dilution with water (20 mL), the mixture was extracted with  $\text{CHCl}_3$  (4  $\times$  25 mL). The extract was dried

(anhydrous  $\text{Na}_2\text{SO}_4$ ), and the solvent was removed on the rotary evaporator to give a glassy solid which was recrystallized from ethyl acetate-EtOH (1:1) to yield 7 as a white crystalline solid, mp 135 °C, in 45% yield (1.7 g): IR (Nujol) 2700, 1600, 1190, 1160, 1080, 1025, 910, 875, 720  $\text{cm}^{-1}$ .

*O-Decylhydroxylamine (2).* The hydrochloride (7; 1 g, 0.0048 mol) was heated with 10% aqueous KOH (10 mL) until the solution cleared. Water (20 mL) was added and the mixture extracted with  $\text{CHCl}_3$  (4  $\times$  15 mL). The extract was dried (anhydrous  $\text{Na}_2\text{SO}_4$ ) and the solvent removed in vacuo. Distillation of the residue yielded 0.75 g (91%) of 2 as a colorless liquid: bp 83–86 °C/0.5 mmHg;  $n_{\text{D}}^{20}$  1.4372; IR (liquid) 3400, 2950, 1610, 1495, 1400, 1205, 1080, 1020, 740  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  0.8 (t, 3 H), 1.2 (s, 16 H), 3.5 (t, 2 H), 4.8 (s, 2 H); for the mass spectrum, see Table I.

**Synthesis of 2-Butylindan (8).** *Diethyl Benzylmalonate (9).* This was prepared by a well-established method (Marvel, 1955) and was obtained in 65% yield.

*Diethyl Benzylbutylmalonate (10).* Diethyl benzylmalonate (9; 120 g, 0.48 mol) was added to a solution of sodium ethoxide and warmed for 30 min. Butyl bromide (68.5 g, 0.5 mol) was added dropwise and the mixture refluxed for 6 h. After removal of the solvent, the residue was extracted with a mixture of ether and aqueous  $\text{NaHSO}_3$  solution. The ethereal fraction was collected and dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), and the solvent removed on the rotary evaporator. The residue was distilled to yield 104 g (71%) of 10 as a colorless liquid: bp 121 °C/0.5 mmHg;  $n_{\text{D}}^{20}$  1.4839; IR (liquid) 3000, 1750, 1605, 1505, 1460, 1380, 1340, 1200, 1100, 1040, 875, 760, 700  $\text{cm}^{-1}$ .

*2-Butyl-3-phenylpropanoic Acid (11).* Diethyl benzylbutylmalonate (10; 86 g, 0.28 mol) was refluxed with aqueous KOH (160 g in 600 mL of water) for 12 h. After the mixture was cooled, ice (100 g) was added and the solution slowly acidified to Congo Red with concentrated HCl. The mixture was extracted with ether (4  $\times$  200 mL) and dried ( $\text{CaCl}_2$ ), and the solvent removed on the rotary evaporator to give a yellowish oil which was crude acid 12. Without any purification this was heated to 170 °C for 30 min to effect decarboxylation. Distillation under reduced pressure afforded 44 g (76%) of 11 as a colorless liquid: bp 116–118 °C/0.5 mmHg;  $n_{\text{D}}^{20}$  1.4952; IR (liquid) 2950, 1720, 1605, 1505, 1460, 1425, 1390, 1300, 1250, 1080, 1040, 950, 750, 705  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  0.9 (t, 3 H), 1.3 (s, 6 H), 2.7 (s, 1 H), 2.9 (d, 2 H), 7.6 (s, 5H).

*2-Butylindan-1-one (13).* 2-Butyl-3-phenylpropanoic acid (20.6 g, 0.1 mol) and  $\text{SOCl}_2$  (16 g, 0.13 mol) were refluxed together for 2 h. The flask was then connected to a water pump and heated on a steam bath for 15 min and finally on a small flame for 5 min in order to remove the excess  $\text{SOCl}_2$ . The acid chloride (14) thus obtained was a reddish liquid which was used directly and not further purified. It was cooled in ice, and  $\text{CS}_2$  (80 mL) was added, followed by 20 g (0.15 mol) of crushed  $\text{AlCl}_3$ . A reflux condenser was fitted, and after the initial rapid evolution of HCl, the mixture was refluxed with stirring for 10 min. It was then cooled and ice (50 g) was added, followed by concentrated HCl (25 mL). Extraction with benzene (3  $\times$  100 mL) and removal of solvent in vacuo gave a yellow liquid which was distilled under reduced pressure to yield a colorless product: bp 97 °C/0.5 mmHg;  $n_{\text{D}}^{20}$  1.5346. The indanone 13 was thus obtained in 93% yield (17.5 g): IR (Nujol) 3050, 1720, 1620, 1480, 1340, 1300, 1205, 1150, 1100, 1020, 760, 720  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  0.9 (t, 3 H), 1.3 (s, 6 H), 3.0 (m, 3H), 7.6 (m, 4 H).

*2-Butylindan (8).* 2-Butylindan-1-one (13; 15.8 g, 0.084

Table I. Mass Spectra of Suspected 1,2,4-Trithiolane, *O*-Decylhydroxylamine, and 2-Butylindan in Egg Flavor Volatiles and of Synthesized Reference Compounds

compd		mass spectrum								
1,2,4-trithiolane										
suspected:	<i>m/e</i>	45	46	124	60	78	59	56	44	84
	% rel int <sup>a</sup>	100	80	77	55	39	35	32	25	12
synthesized:	<i>m/e</i>	45	78	124	46	60	59	44	56	64
	% rel int	100	94	82	77	47	25	25	21	8
<i>O</i> -decylhydroxylamine										
suspected:	<i>m/e</i>	57	43	71	29	41	85	55	56	70
	% rel int	100	97	52	48	41	24	15	12	11
synthesized:	<i>m/e</i>	43	57	71	41	85	55	29	56	70
	% rel int	100	91	84	62	49	38	33	28	22
2-butylindan										
suspected:	<i>m/e</i>	117	104	174	55	57	41	43	91	131
	% rel int	100	76	45	39	37	35	32	29	20
synthesized:	<i>m/e</i>	117	104	41	43	174	55	57	131	91
	% rel int	100	65	49	42	40	37	34	32	27

<sup>a</sup> Percent relative intensity.

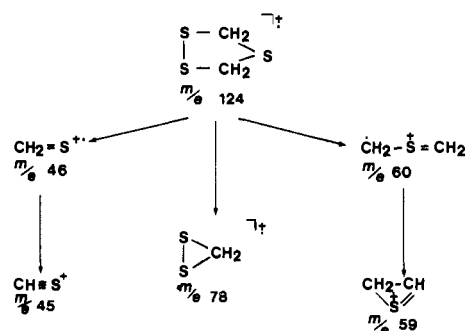
mol) in EtOH (125 mL) was added slowly during 6 h to a boiling mixture of amalgamated zinc (100 g), concentrated HCl (105 mL), EtOH (10 mL), and water (35 mL). Concentrated HCl (50 mL) was then added and the mixture refluxed with stirring for 24 h. The mixture was then poured into water and extracted with benzene (3 × 50 mL). On removal of solvent in vacuo a liquid was obtained which was distilled under reduced pressure by using a Vigreux column to give 9.4 g (64%) of 8 as a colorless liquid: bp 60–62 °C/0.5 mmHg;  $n_D^{20}$  1.5097; IR (liquid) 3050, 1500, 1480, 1400, 1040, 755  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$   $\delta$  0.9 (t, 3 H), 1.3 (s, 6 H), 2.6 (m, 5 H), 7.2 (s, 4H); for the mass spectrum, see Table I.

**Gas Chromatography.** Relative retention times of synthesized 1,2,4-trithiolane and *O*-decylhydroxylamine were determined mainly by using a 1.5 m × 4 mm i.d. glass column packed with 10% PEG 20M coated on 100–120 BSS mesh acid-washed Diatomite C. The nitrogen carrier gas flow rate was 50 mL/min, and the column temperature program was 7 min at 60 °C, followed by an increase of 8 °C/min to a final temperature of 110 °C. For the less volatile 2-butylindan a 100 ft × 0.02 in. metal SCOT column containing PEG 20M was used with a carrier gas flow rate of 3 mL/min. The column temperature was increased from 60 to 150 °C at 3 °C/min. A similar SCOT column containing OV-101 was also used.

## RESULTS AND DISCUSSION

**1,2,4-Trithiolane.** While a number of substituted 1,2,4-trithiolanes have been synthesized, data on the parent compound are rather more limited and confused. Davis (1953, 1955) claimed that it could be prepared from bis(chloromethyl) sulfide and sodium tetrasulfide. A polymer was first formed which yielded the monomer on steam distillation in the presence of base. However, no properties of the monomer were described. Asinger and co-workers (Asinger and Thiel, 1958; Asinger et al., 1958, 1959) reported a more general method of synthesis of 1,2,4-trithiolanes involving reaction of an aldehyde or ketone with a solution of sulfur in an amine solvent saturated with  $\text{H}_2\text{S}$ , but the method failed with formaldehyde (which would give the parent compound). In this project 1,2,4-trithiolane was synthesized in one step by a modification of the general method of Morita and Kobayashi (1967). A solution of sodium disulfide ( $\text{Na}_2\text{S}_{2.5}$ ) was reacted with dichloromethane, and the required product was eventually obtained in a very pure state (>99% by GC). It had an intense garlic odor, although to date it has not been reported as a component of that plant. It was found that the compound was relatively unstable and very readily

Scheme I. Proposed Mass Spectral Fragmentations of 1,2,4-Trithiolane

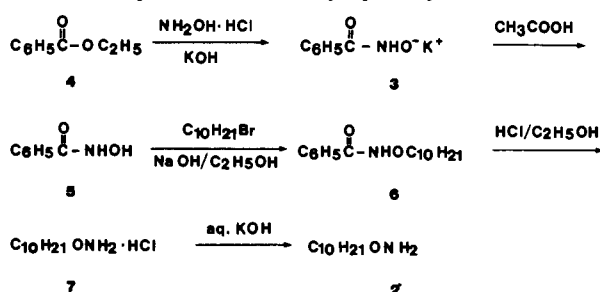


polymerized if not kept well sealed from the atmosphere and well cooled ( $\leq 4$  °C). Its identity was confirmed from its IR and  $^1\text{H NMR}$  spectra. The latter exhibited a single peak at  $\delta$  4.2 since both methylene groups are equivalent. The resonance remained unchanged even upon cooling to  $-90$  °C, which indicated a very low barrier to pseudorotation. The mass spectrum is given in Table I and it can be rationalized as suggested in Scheme I. The spectrum of the suspected 1,2,4-trithiolane of egg volatiles (also given in Table I) was very similar except that it did not possess a very significant peak at *m/e* 78 while that ion was particularly prominent in the spectrum of the genuine compound. Clearly, this would be one of the main reasons why the original computer match was relatively low.

Comparison of the GC relative retention time of the synthesized 1,2,4-trithiolane with that of the suspect peak in the sample of egg volatiles confirmed the identification. Under exactly the same GC conditions, both eluted after 42.5 min from a 1.5-m 10% PEG 20M column. Similar agreement was obtained with a 100-ft OV-101 SCOT column, so there is little doubt that 1,2,4-trithiolane is a constituent of egg volatiles.

To the best of our knowledge this is the first positive report of the parent trithiolane as an aroma volatile of a food, although it was tentatively identified in the volatile oil of the Shiitake mushroom (*Lentinus edodes*), together with the more common lenthionine and 1,2,4,6-tetra-thiepane, by Kameoka and Higuchi (1976). It was also located together with other cyclic polysulfides in the red alga *Chondria californica* by Wratten and Faulkner (1976). More recently Sakaguchi and Shibamoto (1978) identified significant amounts of the compound together with about 40 other products from the reaction of  $\text{H}_2\text{S}$  with D-glucose. This would suggest that it certainly could have wider occurrence in certain food systems.

***O*-Decylhydroxylamine.** This would be a most un-

Scheme II. Synthesis of *O*-Decylhydroxylamine

likely flavor volatile, but the computer match for the mass spectrum of the component in the egg sample was exceptionally high, and although the usual long-chain alkyl fragments provided little detailed characterization, there was some indication of the hydroxylamine nucleus (in particular a very small peak at  $m/e$  173 which could have been the molecular ion).

A number of syntheses of *O*-alkylhydroxylamines have been devised and reported [e.g., Brady and Peakin (1930)], particularly in more recent decades due to interest in their antibacterial activity (Fuller and King, 1947). The most successful methods involve hydrolysis of alkylbenzohydroxamates [e.g., George et al. (1970)], and in this project *O*-decylbenzohydroxamate (6) was readily obtained by treating benzohydroxamic acid (5) with decyl bromide. The acid was itself prepared from ethyl benzoate (4) and hydroxylamine. A summary of the overall synthesis is given in Scheme II.

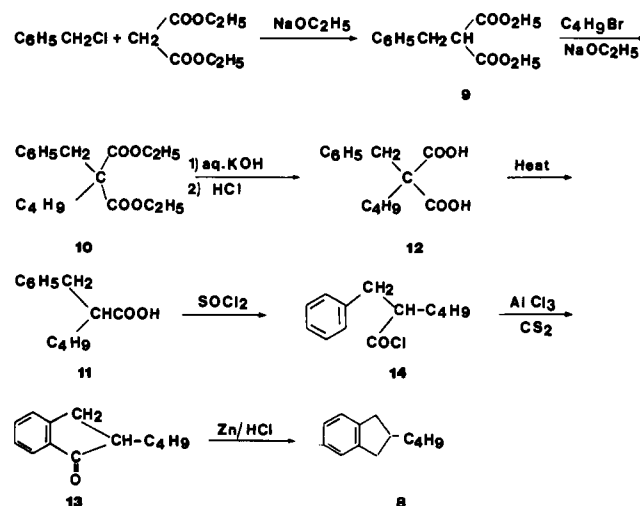
IR and  $^1\text{H}$  NMR spectra confirmed that the required compound had been synthesized. Details of its mass spectrum are given in Table I, and it can be seen that there is very good agreement with the suspected component of egg volatiles, which explains the very high computer matching of spectra.

Perhaps surprisingly the GC relative retention times of the suspect peak in the egg sample and the synthesized *O*-decylhydroxylamine were very close. Under exactly the same GC conditions, the egg volatile eluted at 58 min from a 10% PEG 20M column, while during a number of replicate injections the pure compound always eluted between 54.5 and 55.5 min. Similar data were obtained by using other stationary phases. Although slight, the discrepancy is, however, sufficient to prove that *O*-decylhydroxylamine is not a genuine constituent of egg volatiles. It is probable that the unknown component did contain a relatively long alkyl chain, but little further information can be deduced concerning its identity.

**2-Butylindan.** This was a rather unexpected aroma volatile although indan itself and methylindan have been detected before respectively in roasted filberts (Kinlin et al., 1972) and in roasted peanuts (Walradt et al., 1971). Also, some confusion existed concerning the deductions that could be drawn from the excellent computer match between the mass spectrum of the suspect egg volatile and the reference spectrum of 2-decylindan. 2-Butylindan seemed a more likely possibility for a number of reasons. In particular, the sample mass spectrum exhibited a significant peak at  $m/e$  174 which would be the molecular ion peak for butylindan. In addition, the butyl derivative seemed more reasonable than the decylindan on the grounds of volatility, in that it seemed unlikely that under the conditions of analysis employed, the latter compound would have a retention time near that of the egg component in question, even if it were sufficiently volatile to be eluted from the GC column at all.

A number of methods have been reported for the synthesis of 2-alkylindans, including an intriguing procedure in which diazonium salts derived from *o*-(2-substituted

## Scheme III. Synthesis of 2-Butylindan



alkyl)anilines undergo thermal decomposition in aqueous acid solution (Martinson, 1968). However, the most widely used approach involves condensation cyclization of the appropriate 2-alkyl-3-phenylpropanoic acid to give a 2-substituted indan-1-one which can then be made to undergo Clemmensen reduction to give the required product (Elsner and Parker, 1957). Consequently, a synthesis based on this general procedure was adopted for 2-butylindan and it is summarized in Scheme III. The critical step is the cyclization which is best accomplished by using the acid chloride and a Friedel-Crafts reagent. This reaction has been the subject of much study [e.g., see Johnson (1944)], and based on such previous work our procedure gave an excellent yield of 93%.

IR and  $^1\text{H}$  NMR spectra confirmed the success of the synthesis, and details of the mass spectrum of the butylindan are given in Table I. It can be seen that there is very good agreement with the spectrum of the suspect egg component, also given in Table I. Under identical GC conditions using a PEG 20M SCOT column, the synthesized compound had virtually the same relative retention time as the suspect peak in the sample of egg volatiles (86.5 min compared with 87 min) so this identification is also confirmed. The aroma of the synthesized indan was not intense and was undistinguished, being sweet and fruity with a slight oily nuance.

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## Volatile Flavor Components of Soursop (*Annona muricata*)

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Representative samples of the aroma volatiles of soursop—a tropical fruit—were obtained by means of a modified Likens and Nickerson apparatus by using 2-methylbutane as the solvent. Extracts were concentrated by a low-temperature-high-vacuum distillation procedure, and components of resultant essences were identified as far as possible by GC-MS using both EI mass spectrometry and CI mass spectrometry. Most aroma components were esters (~80% of the sample), and they constituted a chemically closely related series. Methyl hexanoate (~31%) and methyl hex-2-enoate (~27%) were the two most abundant components and together amounted to ~0.7 mg/kg of fruit.

There are very many tropical fruits which are little known in the Western World yet whose flavor would undoubtedly appeal to the Western palate. Very few of these fruits have been studied to determine the nature of the volatile components responsible for their characteristic flavor, although Alves and Jennings (1979) recently reported a preliminary survey of some fruits of the Amazon region. Soursop (*Annona muricata*) is a tropical fruit native to and common in tropical America and the West Indies, although it is grown in some other countries including Sri Lanka. It is a member of the annonaceous fruits which are sometimes collectively known as "custard apples" from the custard-like flavor of many. Soursop, however, is rather more acid and less sweet than most other members of the group. It can grow to a large size and may weigh up to 4 kg. Its very pleasant flavor is unique and the fruit has potential for development as a processed product. Normally the juicy, fibrous pulp is consumed as such, but it can be used to prepare an ice cream and it can be mixed with water and sugar to provide an extremely refreshing drink. This paper describes the results of a project aimed at determining the nature of the compounds mainly responsible for the characteristic soursop flavor.

### EXPERIMENTAL SECTION

Fresh Soursop fruits were transported by air from Sri Lanka, but it was found essential to pack them in an ethylene adsorbent to prevent overripening and spoilage en route. Fruits were then ripened in the laboratory in an atmosphere of 0.1% ethylene in nitrogen for 24 h at 30 °C.

**Sample Preparation.** Fruit pulp (350 g), separated from embedded seeds, was mixed with water (100 mL) and extracted for 1.5 h in a Likens and Nickerson (1964) apparatus as modified by MacLeod and Cave (1975) by using 2-methylbutane (10 mL) as the solvent. At the end of this time the residue did not possess any appreciable aroma. The extract was concentrated to 0.5 mL by using the low-temperature-high-vacuum distillation procedure devised by MacLeod and Cave (1975). The resultant essence possessed a strong aroma characteristic of the fruit.

**Gas Chromatography.** Essences were examined by gas chromatography using a Pye-Unicam 204 instrument equipped with a heated FID. Most work was carried out

by using an 18 ft × 4 mm i.d. glass column packed with 10% PEG 20M coated on 100-120 BSS mesh acid-washed Diatomite C. Nitrogen carrier gas was used (60 mL/min), and the best temperature program was 60 °C for 5 min, followed by an increase of 12 °C/min to 160 °C for the remainder of the run. Detector and injection temperatures were 250 °C, and typically 4  $\mu$ L of sample was injected.

**Gas Chromatography-Mass Spectrometry.** Components in the essence were identified as far as possible by GC-MS using a Kratos MS 25 instrument linked on-line to a Kratos DS 50 data processing system. The same GC conditions as described above were employed but using helium as the carrier gas and a slightly lower flow rate (40 mL/min). A single-stage, all-glass jet separator was used at 250 °C. Both electron impact (EI) mass spectrometry and chemical ionization (CI) mass spectrometry were performed, and at various times the MPM unit and the retrospective single ion monitoring facility of the data system were employed to good advantage. Significant operating parameters of the mass spectrometer during EI work were as follows: ionization potential, 70 eV; ionization current, 100  $\mu$ A; source temperature, 200 °C; accelerating voltage, 1.5 kV; resolution, 600; scan speed, 1 s/decade (repetitive throughout run). Identical conditions were employed during CI mass spectrometry except for the following: reagent gas, methane (or isobutane or ammonia); ionization potential, 100-110 eV; emission current, 5 mA.

**Quantitative Assessment.** Sample preparation and concentration were conducted with quantitative accuracy so that a known aliquot of the fruit sample was analyzed. Quantitative data were then derived both from the trace obtained from the TIC monitor during GC-MS and from the FID trace during routine GC. Known amounts of a selection of identified components (particularly the esters) were injected under the same analytical conditions to assess the response factors of the detectors to the various classes of identified compounds.

**Odor Assessment.** Aromas of the separated components of the essence were assessed at an odor port following GC using a Pye-Unicam 104 instrument. An outlet splitter set at 10:1 diverted the major fraction of the eluant through a heated line to the outside of the oven for aroma assessment by a total of three subjects. An injection volume of 10  $\mu$ L was necessary.

### RESULTS AND DISCUSSION

Various extraction methods using a number of different

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