Isolation and Identification of a Series of α,β-Unsaturated Aldehydes from Valencia Orange Peel Oil

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The isolation of a series of seven α,β-unsaturated aldehydes, consisting of various self- and mixed-condensation products of octanal, nonanal, and decanal from orange peel oil is reported. The compounds possess the structure of dehydrated aldol condensation products, α,β-dialkyl acroleins. Five of the seven compounds, not previously reported as constituents of natural products are: α-hexyl-β-heptyl, α-hexyl-β-octyl, α-heptyl-β-heptyl, α-octyl-β-heptyl, and α-hexyl-β-nonyl acrolein. The other two are probably α-octyl-β-octyl and α-heptyl-β-nonyl acrolein. Structure proof was accomplished by synthesis using the appropriate combinations of octanal, nonanal, and decanal in a base-catalyzed aldol condensation; isolation of the various mixed- and self-condensation products by gas-liquid chromatography; and proof of structure by ozonolysis and mass spectrometry. The possibility that these compounds are artifacts produced during the isolation procedure by condensation of the aldehydes is contradicted by the absence of appreciable amounts of the decanal self-condensation product.

As part of continuing investigations by this laboratory on the constituents of orange peel oil, a study of the "carbonyl" fraction of the volatile portion of Valencia peel oil was undertaken. This "carbonyl" fraction contains the aldehydes, ketones, and esters and is considered to contain the major portion of the basic flavor components of orange. Of the 17 aldehydes and ketones and three esters in this fraction, the most abundant were found to be the saturated, straight-chain aldehydes octanal, nonanal, and decanal. Lesser amounts of the other saturated straight-chain aldehydes were also found in this fraction. Similar results with respect to the relative concentrations of the saturated straight-chain aldehydes in orange oil are reported by Stanley *et al.* (1961), except that the relative amount of nonanal reported by Stanley is considerably lower than that observed by us.

During this study, five peaks were observed in the section of the gas-liquid chromatogram at longer retention times, that is, after most of the other components had been eluted. The similarity of the infrared spectra suggested that these were a series of compounds of very similar structure. Since this group of compounds represented approximately 5 to 10% of the total "carbonyl" fraction, an investigation of the structures of the series was undertaken.

PROCEDURE

All gas-liquid chromatographic analyses were performed on an F & M Model 810 gas chromatograph equipped with a thermal conductivity detector using a $^1/_4$-inch × 20-foot column packed with 20% Carbowax 20M on 60- to 80-mesh Chromosorb P. The helium flow rate was 60 ml. per minute and the column was programmed from 135° to 225° C. at 1° per minute.

Mass spectra were obtained with a Bendix Model 3012 (TOF) mass spectrometer at an ionizing voltage of 70 eV. and ion source temperature of 100° C. NMR spectra were obtained on a Varian Model A60. Samples were collected in glass capillaries from the GLC and introduced into the mass spectrometer through the liquid sampling system.

Isolation. Cold-pressed Valencia orange oil (4750 ml.) was distilled at 36° C. and 2 mm. of Hg in rotary evaporator until most of the volatile portion came over. The residue (197 ml.) was distilled on a Nester/Faust 10-mm. × 36-inch spinning band column, and a fraction weighing 35 grams collected at a head temperature of 35° to 115° C. at 1 to 0.7 mm. of Hg.

Ten grams of the distillate from the spinning band column were separated into three fractions on a 2.5- × 45-cm. column of Fisher activity II neutral alumina. The fractions were eluted by washing the column successively with 300-ml. portions of hexane, ethyl ether, and ethanol. The weight of material in grams in each of the fractions was as follows: hexane, 6.2; ether, 1.5; ethanol, 2.3. Most of the carbonyl-containing compounds were in the ether fraction based on the relative intensity of the carbonyl stretching band in the infrared spectrum.

Analysis of the ether fraction from the column by gas-liquid chromatography produced 27 major peaks. Peaks 20, 23, 25, 26, and 27 represented the unknown aldehydes and were designated by letters A to E. Retention times of these aldehydes were 91, 106, 126, 150, and 182 minutes, respectively.

Physical Properties. Aldehyde A to E had similar infrared spectra characterized by absorption bands at 3.7 μ (C—H on aldehyde carbonyl); 5.9 μ (conjugated $\overset{O}{\overset{\|}{C}}$) and 6.2 μ (—C = C—). A typical infrared spectrum is shown in Figure 1.

The mass spectra indicated molecular weights of 238, 252, 266, 280, and 280, respectively, for aldehydes A, B, C, D, and E. The UV spectra of A to E in ethanol indicated a high intensity absorption maximum at 230 mμ in each case which could be assigned to a disubstituted α,β-unsaturated aldehyde chromophore. An NMR spectrum was obtained of aldehyde C in CCl_4 with multiplet signals at 0.9, 1.3, 1.7, and 2.2 and a singlet at 9.3 (δ values in parts per million relative to TMS).

Synthesis. Aldehyde A was synthesized by sodium ethoxide catalyzed dimerization of octanal according to the procedure of Villani and Nord (1947). The following properties were observed for the product: n_D^{25} 1.4563; 2,4-DNP m.p. 98–100°; λ max 230 mμ, $\epsilon = 1.24 \times 10^4$ (EtOH). Shoruigin *et al.* (1933) reported n_D^{20} 1.4612 for the aldehyde, and Hennion and Hanzel (1960) reported m.p. of 103.5–104.5° for

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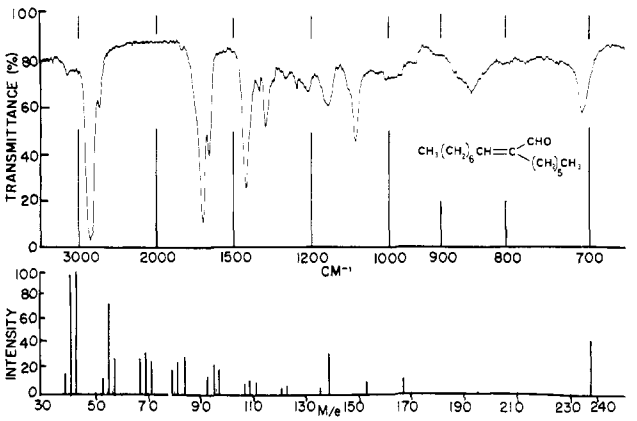

Figure 1. Infrared and partial mass spectrum of aldehyde A (α-hexyl-β-heptyl acrolein)

Table I. Mass Spectra of Aldehydes A to C and Labeled Analogs of Aldehyde A[a]

Major Fragment Peaks	Compound				
(m/e)	A	A-D_2[b]	A-O^{18}[c]	B	C
41	96	76	97	80	84
43	100	100	100	100	100
55	74	63	86	77	85
57	29	44	42	44	65
67	28		34	32	
69	33	34	45	44	53
71	26	34	30	33	
79	17				
81	25		28	23	35
83	29	34	34	36	35
84	13	28			
85		20			
95	24	31	29	26	28
97	19	28	22	21	28
98		24			
109				11	14
110					14
111				11	
123					11
127				14	
139	33	98	30	16	22
141			17		
153	9		10	25	11
154		20			
155		19	5		
167	12		12	15	19
168		19			
169		27	7		
181				7	7
238	43		38		
240		10	15		
252				66	
266					39

[a] In percentage of base peak. Base peak is at m/e 43 in all cases.
[b] 4,4-d_2-2-hexyl-2-decenal.
[c] O^{18}-labeled synthetic aldehyde A.

the 2,4-DNP of α-hexyl-β-heptyl acrolein. The infrared spectrum (neat) was identical to that of aldehyde A (Figure 1) as was the GLC retention time. Its mass spectrum, identical to that of aldehyde A, is reproduced in Figure 1 and Table I. The M-99 fragment (m/e 139) is the most prominent peak in the high mass range.

The synthesis of a deuterium labeled analog of aldehyde A (4,4-d_2-2-hexyl-2-decenal) was accomplished by the use of a base catalyzed dimerization of octanal very similar to the Villani and Nord procedure. A solution of 9 mg. of sodium in 3 ml. of CH_3OD was prepared, octanal (500 mg.) was added, and the mixture was allowed to stand at room temperature overnight. The solvent was removed by distillation at reduced pressure and the residue distilled at a pot temperature of 100° to 210° C. at 0.3 mm. of Hg to yield 250 mg. of colorless oil. The infrared spectrum and GLC retention time of the product was identical to that of aldehyde A. The mass spectrum had a molecular ion peak at m/e 240 and a strong fragment peak at m/e 139, representing a loss of 101 mass units. The salient features of the mass spectrum are tabulated in Table I.

An O^{18}-labeled analog of aldehyde A was produced by an acid-catalyzed exchange procedure. To a mixture of 30 mg. of H_2O^{18} (97% enrichment) in 2 ml. of dry, reagent grade dioxane containing 2×10^{-4} eq. of HCl per ml. was added 10 mg. of synthetic aldehyde A. The mixture was allowed to stand for 16 hours at room temperature, excess reagent removed by distillation at 0.3 mm. of Hg, and the residue distilled at a pot temperature of 100 to 200° C. at 0.2 mm. of Hg to give a colorless oil. The infrared spectrum and GLC retention time of this product were identical to that of aldehyde A. From the ratio of molecular ions at m/e 238 and 240 in the mass spectrum of the product, it can be seen that approximately 30% exchange was achieved. Reference to Table I shows that 30 to 40% of the m/e 139 fragment peak has shifted to 141.

The remaining aldehydes B to E were synthesized by the Villani and Nord procedure by condensation of equivalent quantities of: octanal and nonanal, octanal and decanal, and nonanal and decanal. In each of these reactions, mixtures of self- and mixed-condensation products were produced. Gas liquid chromatographic analysis of the product mixtures revealed three peaks in the case of the first two reactions and four in the last reaction. Infrared spectra of all of the fractions were similar and resembled closely that reproduced in Figure 1.

Retention times of the middle peaks from the octanal-nonanal condensation product and from the octanal-decanal product corresponded with those of aldehydes B and C, respectively. The mass spectra of the synthetic aldehydes B and C, included in Table I, were identical with the mass spectra of the products isolated from orange oil.

The GLC retention times of the two middle peaks in the chromatogram of the nonanal-decanal condensation product matched those of aldehydes D and E. The mass spectra of aldehydes D and E could never be obtained clean and free of contamination from residues in the mass spectrometer, so useful information, other than the molecular weight, could not be obtained by means of mass spectrometry.

Ultraviolet spectra in ethanol were obtained on all of the synthetic aldehydes B to E and all possessed a high intensity absorption maximum at 230 mμ.

Ozonolysis Experiments. Small-scale ozonolysis experiments were carried out on synthetic aldehydes A, B, and C using the apparatus described by Beroza and Bierl (1967). The products were identified, on the basis of retention time and mass spectrometry, as octanal from A, a mixture of approximately equal amounts of octanal and nonanal from B, and a mixture of equal amounts of octanal and decanal from C. The fragment containing the α-alkyl moiety was apparently converted into a product which did not show up in the gas liquid chromatogram.

Table II. Summary of Physical Data Obtained on Synthetic Condensation Products

		$CH_3(CH_2)_nCH{=}C(CHO)(CH_2)_mCH_3$		IR	UV	NMR	MS	Retention Time
		n	m					
(A)		6	5	X	X		X	X
(B)		{6, 7}	{6, 5}	X	X		X	X
		7	6	X				X
(C)		{6, 8}	{7, 5}	X	X	X	X	X
(D)	or	7	7					
		8	6	X	X		X(M.W.)	X
(E)	or	8	6					
		7	7	X	X		X(M.W.)	X
		8	7	X				X

RESULTS AND DISCUSSION

The structures of aldehydes A, B, and C can be unequivocally assigned from the data, according to the arguments presented in this section. Probable structures only can be inferred for aldehydes D and E because of the inadequate mass spectra obtained on these compounds.

The unsplit aldehyde C—H signal at 9.3 eliminated the possibility of a proton on the alpha carbon of aldehyde C. At this point one could assign the general formula

$$R{-}CH{=}\underset{\displaystyle R'}{\underset{|}{C}}{-}CHO$$

to aldehyde C where R and R′ are acyclic saturated hydrocarbon radicals. Assuming that the other members of the series likewise lack a proton at the alpha carbon, they would also possess the above general formula and would differ from one another by the number of $-CH_2-$units in R and R′.

The presence of relatively large amounts of the saturated straight-chain aldehydes octanal, nonanal, and decanal suggested that the unknowns were dehydrated aldol condensation products of these aldehydes.

A summary of the mixed and self-condensation products of octanal, nanonal and decanal which were obtained on each compound or mixture synthesized is included in Table II. The compounds are listed in order of increasing retention time. Bracketed compounds indicate that mixtures only were isolated.

From the experimental procedure, it can be seen that the infrared spectra and GLC retention times of the aldehydes isolated from orange oil matched those of some of the synthetic condensation products.

The results from the ozonolysis of the corresponding synthetic products led to the structural assignments shown in Table II. Since ozonolysis experiments were not performed on aldehydes D and E and partial mass spectra indicating molecular weight only were obtained on the two compounds, the structures of D and E can only be inferred from the coincidence of the infrared spectra and retention times.

The mass spectra provided important confirming evidence for the structures A to C. In the mass spectrum of the octanal self-condensation product (aldehyde A), the major fragment in the high mass region is at M-99 (*m/e* 139). This fragment must arise by cleavage next to the double bond with loss of a C_7H_{15} fragment because of the shift of this fragment

$$R\text{-}CHO + R'CH_2CHO \overset{H^+}{\rightleftharpoons} R\text{-}\underset{OH}{CH}\text{-}\underset{R'}{CH}\text{-}CHO \overset{H^+}{\rightleftharpoons} R\{CH{=}\underset{R'}{C}\,CHO + H_2O$$

Figure 2. Aldol condensation equilibria

peak to M-101 (*m/e* 139) with the deuterated analog coupled with the fact that the fragment remains M-99 (*m/e* 141) in the mass spectrum of the O^{18} labeled analog (Table I). This characteristic fragmentation is illustrated in Figure 2, where the wavy line represents the point of cleavage. From the position of this major fragment peak, it is possible to differentiate any of the various aldehyde condensation products, since the two possible mixed condensation products, even though they will have the same molecular weight, will have different numbers of carbons in the chain on the beta carbon and this characteristic peak will appear at a different mass number. This approach can be used to interpret the mass spectra of B and C. In the mass spectrum of B, instead of a predominate peak at M-99 (*m/e* 153) or M-113 (*m/e* 139) there are two moderate-sized fragment peaks at these mass numbers, and in the mass spectrum of C, instead of a large peak at either M-99 (*m/e* 167) or M-127 (*m/e* 139), there are two medium-sized peaks at these mass numbers (Table I). The percentage composition of aldehyde mixtures B and C can only be estimated at roughly 50 to 50 with respect to each possible mixed condensation product from the mass spectral and ozonolysis data.

These aldehydes are formally related to the straight-chain saturated aldehydes present in relatively large quantities in orange oil, octanal, nonanal, and decanal, by acid-catalyzed aldol condensation according to the set of equilibria shown in Figure 2. Whether or not such an equilibrium exists and the extent to which it influences the flavor of natural orange products has not been established. The α,β-unsaturated aldehydes themselves contribute little to the flavor other than a faint, burnt, fatty quality.

The question of the origin of these compounds, whether they are artifacts produced by condensation of the relatively abundant aldehydes octanal, nonanal, and decanal at some point in the isolation procedure or whether they are produced biogenetically and are present in the natural oil cannot be answered by the present data. The fact that only a relatively small peak was observed in the gas liquid chromatogram at the retention time of the nonanal and decanal self-condensation products would be evidence against the theory that they are artifacts, since nonanal and decanal are quite abundant in the oil and any random condensation of the aldehydes during the isolation should result in appreciable amounts of the nonanal and decanal self-condensation products along with the others.

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