Novel 2-Acylcyclohexane-1,3-diones in the Mandibular Glands of Lepidopteran Larvae. Kairomones of *Ephestia kuehniella* Zeller

By **Alan Mudd**, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ

Novel 2-acylcyclohexane-1,3-diones and 4-hydroxy-2-acylcyclohexane-1,3-diones hitherto unknown in animals have been identified in the larval mandibular glands of *Ephestia kuehniella* Zeller. These compounds act as kairomones, causing the parasite *Venturia canescens* (Grav.) of *E. kuehniella* to oviposit.

THE secretion from the larval mandibular glands of some Lepidoptera influences the population density of the larvae on their food substrate by affecting the dispersal of the larvae and the oviposition behaviour of both the adult moth and the larval parasite *Venturia* (= *Nemeritis*) *canescens* (Grav.).[1-5]

The isolation and identification of two novel natural products, 2-oleoylcyclohexane-1,3-dione and 4-hydroxy-2-oleoylcyclohexane-1,3-dione, from the larval mandibular glands of *Ephestia* (= *Anagasta*) *kuehniella* Zeller has been briefly described.[6] Further analysis of the mandibular gland contents has now revealed the presence of several more novel β-triketones. This is the first time such compounds have been found in animals; similar compounds have insecticidal, fungicidal, and antibiotic properties.[7-11]

RESULTS

Dissected mandibular glands (1 415 pairs) were ground with ether. Drying ($MgSO_4$), filtration, and evaporation of this extract gave a pale yellow gum (110 mg). Isolation of active material was monitored using the oviposition response [1,3] of the parasite *V. canescens* (Grav.).

Active fractions, I (30 mg) and II (10 mg), were isolated by h.p.l.c. on silica using dichloromethane–formic acid as eluant. These were each further separated into three major active fractions, A, B, and C, by reverse-phase h.p.l.c. on an octadecylsilane column using water–methanol–formic acid as eluant. Each fraction was examined by electron-impact (e.i.) and chemical-ionisation (c.i.) mass spectrometry, and by u.v., i.r., and ^1H n.m.r. spectroscopy. Where sufficient material was available ^{13}C n.m.r. spectra were also obtained. The results (Tables 1—4), show that of the six fractions, five contained single compounds and the sixth (IIB) was a four-component mixture (Figure 1).

DISCUSSION

High-resolution mass spectra established the molecular constitution of all components (Table 2), and the identity of all molecular ions was confirmed by chemical-ionisation mass spectra using isobutane as the reactant gas. Evidence for the presence and nature of the enolic ring systems is discussed first, followed by that for side chains.

Enolic Ring System.—The presence of a β-triketone system (Figure 1) in all components was indicated by peaks at 233 and 274 nm in their u.v. spectra (Table 1, intensities vary with solvent, concentration, and pH because hydrogen bonding and ionisation of the enol is affected [12]) and at $\nu_{max.}$ 1 665 (conjugated carbonyl) and 1 565 cm^{-1} (conjugated chelated carbonyl) in their i.r. spectra.

The high-resolution mass spectra of all components of fraction I showed significant fragments at *m/e* 167, 154, and 139 with compositions $C_9H_{11}O_3$, $C_8H_{10}O_3$, and $C_7H_7O_3$, and corresponding fragments at $C_9H_{11}O_4$,

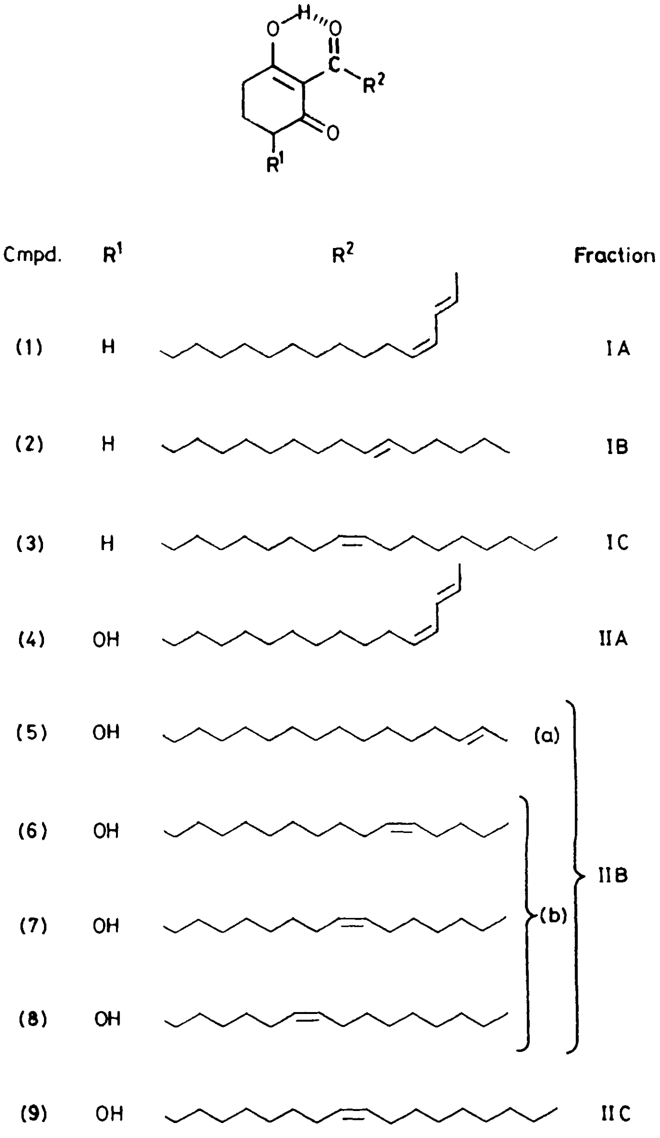


FIGURE 1 Active fractions obtained from h.p.l.c. of mandibular gland extract

TABLE 1

Fractions obtained from h.p.l.c. of mandibular gland extract

Fraction	H.p.l.c. retention ratio [a] Silica	H.p.l.c. retention ratio [a] ODS	Appearance (20 °C)	U.v. spectrum (hexane) ϵ (274 nm)	U.v. spectrum (hexane) ϵ (233 nm)	I.r. spectra/cm^{-1} (micro-KBr disc)	Ozonolysis product(s) [b]
IA		0.22	pale yellow wax	10 000	33 400	1 665, 1 565, 979	
IB	0.65	0.16	yellow oil	10 600	9 100	1 665, 1 565, 965	hexanal
IC		0.13	yellow oil	10 800	9 200	1 665, 1 565	nonanal
IIA		0.34	white wax	10 600	33 600	1 665, 1 565, 979	
IIB(a)	0.35	0.26	yellow wax	9 300	10 000	1 665, 1 565, 965	acetaldehyde
IIB(b)		0.24	yellow oil	9 200	9 900	1 665, 1 565	pentanal, heptanal, nonanal
IIC		0.20	yellow oil	9 500	10 700	1 665, 1 565	nonanal

[a] Retention ratio = t_0/t_r where t_0 = retention time for non-sorbed peak, and t_r = retention time of component. [b] Identified by g.l.c.–mass spectral comparison with authentic aldehydes.

TABLE 2

Mass spectra of fractions

Fraction	Molecular ion [a] Observed mass [b]	Molecular ion [a] Calculated mass	Other significant ions
IA	346.2494	346.2507 ($C_{22}H_{34}O_3$)	328 ($M - H_2O$), 167 (D), 154 (E), 139 (F), 81, 68, 67
IB	348.2590	348.2662 ($C_{22}H_{36}O_3$)	330 ($M - H_2O$), 167 (D), 154 (E), 139 (F)
IC	376.2996	376.2977 ($C_{24}H_{40}O_3$)	358 ($M - H_2O$), 167 (D), 154 (E), 139 (E)
IIA	362.2475	362.2456 ($C_{22}H_{34}O_4$)	344 ($M - H_2O$), 183 (D), 170 (E), 155 (F), 81, 68, 67
IIB	364.2613	364.2632 ($C_{22}H_{36}O_4$)	346 ($M - H_2O$), 183 (D), 170 (E), 155 (F), 55, 41
IIC	392.2923	392.2926 ($C_{24}H_{40}O_4$)	374 ($M - H_2O$), 183 (D), 170 (E), 155 (F)

[a] Confirmed by c.i. mass spectroscopy using isobutane. [b] By e.i. mass spectroscopy.

TABLE 3

^1H N.m.r. spectra of fractions

Fraction	Ring: Enolic OH	Ring: 4-H_2	Ring: 4-OH	Ring: 5-H_2	Ring: 6-H_2	Side-chain: 2′-CH_2	Side-chain: Olefinic H	Side-chain: Allylic H′ [b]	Side-chain: Methylene chain	Side-chain: Terminal CH_3
IA	18.3	2.49 (2 H, m)		2.1 [a]	2.66 (2 H, m)	3.02 (2 H, m)	5.3—6.2 (4 H, see text)	1.9—2.1 (*ca.* 2 H, m)	1.27 (*ca.* 16 H)	1.77 (*ca.* 3 H, d, *J* 7 Hz) [b]
IB	18.3	2.49 (2 H, m)		2.1 [a]	2.66 (2 H, m)	3.02 (2 H, m)	5.35 (2 H, m)	1.9—2.1 (*ca.* 4 H, m)	1.26 (*ca.* 16 H)	0.89 (3 H, m)
IC	18.3	2.49 (2 H, m)		2.1 [a]	2.66 (2 H, m)	3.02 (2 H, m)	5.35 (2 H, m)	1.9—2.1 (*ca.* 4 H, m)	1.26 (*ca.* 20 H)	0.88 (3 H, m)
IIA	18.3	4.09 (1 H, dd, *J* 13 and 4 Hz)	4.04br (1 H, s)	1.95 [a]	2.77 (2 H, m)	3.02 (2 H, m)	5.3—6.3 (4 H, see text)	1.9—2.1 (*ca.* 2 H, m)	1.28 (*ca.* 16 H)	1.77 (*ca.* 3 H, d, *J* 7 Hz) [b]
IIb(a)	18.3	4.09 (1 H, dd, *J* 13 and 4 Hz)	4.03br (1 H, s)	1.95 [a]	2.76 (2 H, m)	3.03 (2 H, m)	5.35 (2 H, m)	1.95 (*ca.* 2 H, m)	1.27 (*ca.* 16 H)	1.63 (*ca.* 3 H, d, barely split) [b]
IIB(b)	18.3	4.09 (1 H, dd, *J* 13 and 4 Hz)	4.03br (1 H, s)	1.95 [a]	2.77 (2 H, m)	3.02 (2 H, m)	5.41 (2 H, m)	1.9—2.1 (*ca.* 4 H, m)	1.29 (*ca.* 16 H)	0.89 (3 H, m)
IIC	18.3	4.09 (1 H, dd, *J* 13 and 4 Hz)	4.04br (1 H, s)	1.95 [a]	2.76 (2 H, m)	3.03 (2 H, m)	5.35 (2 H, m)	1.9—2.1 (*ca.* 4 H, m)	1.26—1.3 (*ca.* 20 H)	0.88 (3 H, m)

[a] Obscured by methylene envelope, presence confirmed by spin-decoupling experiments. [b] Superimposed on methylene envelope.

TABLE 4

^{13}C N.m.r. spectra [a,b] of fractions and synthetic compounds

	Ring atoms						Side-chain (R^2) atoms																	
Fraction or compound	1	2	3	4	5	6	1′	2′	3′	4′	5′	6′	7′	8′	9′	10′	11′	12′	13′	14′	15′	16′	17′	18′
I [c]	195.1	113.0	198.5	38.8	19.1	33.3	206.2	40.5	24.7	29.4	29.4	29.4	29.4	27.2	130.0	130.0	27.2	29.4	29.4	29.4	29.4	32.0	22.7	14.1
IIA	195.5	110.3	197.9	71.7	31.4	27.2	206.0	40.3	24.6	29.5	29.5	29.5	29.5	29.5	29.5	29.5	27.7		129.9	128.9		18.3		
																			128.7	127.2				
IIB [c]	195.5	110.0	197.8	71.7	31.3	27.2		40.2	24.6	29.7	29.7	29.7	29.7	27.2	130.3	130.3	27.2	29.7	29.7	32.0	22.7	14.1		
IIC	195.5	110.3	197.8	71.6	31.4	27.2	206.0	40.2	24.6	29.3	29.3	29.3	29.3	27.2	130.0	130.0	27.2	29.3	29.3	29.3	29.3	32.0	22.7	14.0
Synthetic IC [d]	194.9	113.0	198.3	38.8	19.2	33.3	206.1	40.5	24.7	29.3	29.3	29.3	29.3	27.2	130.2	130.2	27.2	29.3	29.3	29.3	29.3	32.0	22.7	14.1
2-Octanoylcyclohexane-1,3-dione	195.1	113.0	198.6	38.8	19.2	33.3	206.2	40.6	24.7	29.2	29.4	31.8	22.7	14.1										
2-Acetylcyclohexane-1,3-dione	195.1	113.4	198.6	38.6	19.1	33.3	202.6	28.6																

[a] In p.p.m. from Me_4Si (=0.00). [b] Partially decoupled spectrum for fraction II gave the expected multiplicities for all observable peaks. [c] Insufficient material for ^{13}C n.m.r. spectra of individual components. Peaks listed for side-chain are those for major component. [d] 1 : 1 mixture of *cis*- and *trans*-isomers; extra peak of 32.6 p.p.m. (IC) due to *trans*-isomer.

$C_8H_{10}O_4$, and $C_7H_7O_4$ were found in the spectra of all components of fraction II. The presence of the $C_7H_7O_4$ fragment strongly suggested that all the oxygen atoms were on or adjacent to a ring system. The difference in h.p.l.c. retention ratios on silica between fractions I and II (Table 1) suggested that the compounds in fraction II had an extra hydroxy-group. On this basis the series of oxygenated ions above can be represented by the fragmentation schemes outlined in Figure 2.

The occurrence of a 2-acylcyclohexane-1,3-dione system was confirmed by the ^1H n.m.r. spectra which detected an enolic proton [δ 18.3 (1 H, s)] in all compounds and established a $CO{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CO$ system common to fraction I components and a $CO{\cdot}CH_2{\cdot}CH_2{\cdot}CHOH{\cdot}CO$ system for the II series (see Table 3). The expected $4\text{-}H_2\text{-}5\text{-}H_2$(or H)$\text{-}6H_2$ and $2'\text{-}H_2\text{-}3'\text{-}H_2$ couplings were confirmed for both I and II series by irradiation experiments, establishing the assignments shown in Table 3.

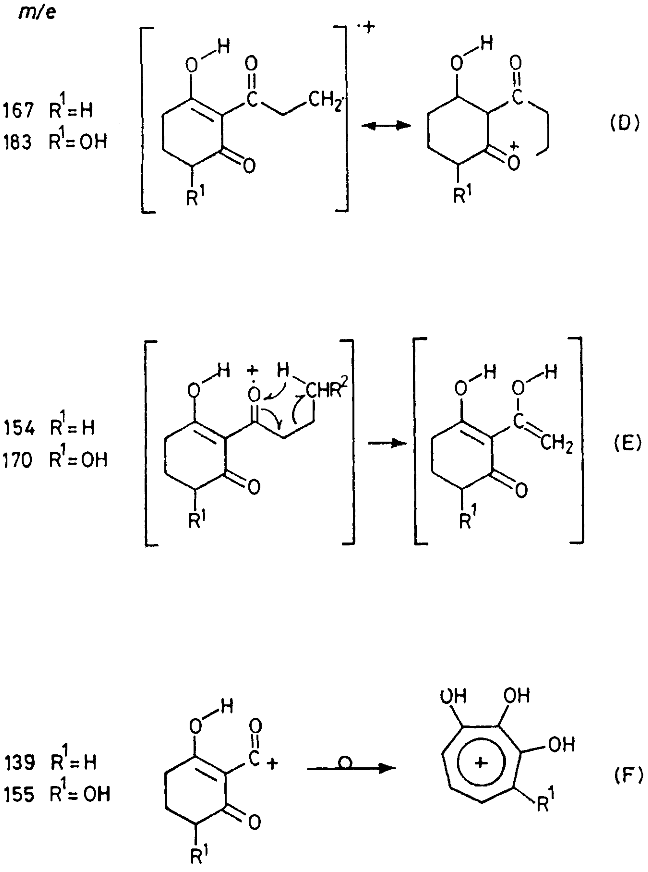
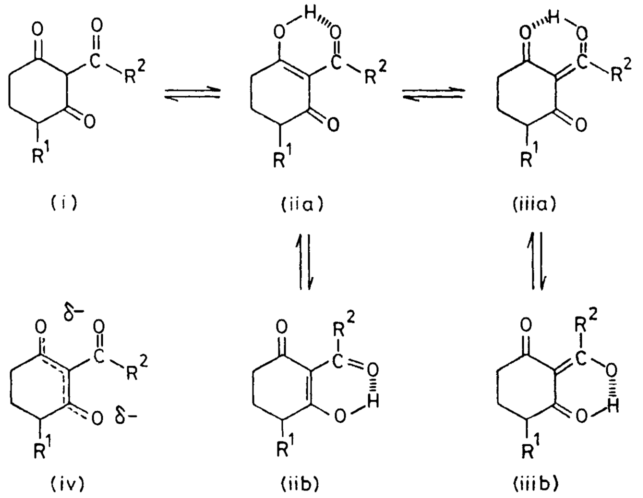


FIGURE 2 Fragmentation schemes for components of fractions I and II

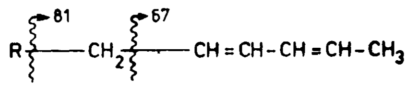
The ^1H n.m.r. spectra of the components of fractions I and II showed only a single enol signal [δ 18.3 (1 H)] even in the unsymmetrical cases where two signals might be expected from two enols in equilibrium (iiia and b; R = OH) as in other unsymmetrical β-triketones.[13,14] *Whether the* absence of two signals is due to more rapid intermolecular exchange of enolic protons[15] or to the predominance of one form due to hydrogen bonding is not clear. No direct evidence for alternative positions of the enolic double bond [*i.e.* (ii) or (iii)] is available. However, because the addition of triethylamine caused the peaks at δ 38.8 (C-4) and 33.3 (C-6) in the ^{13}C n.m.r. spectrum of IC to coalesce at δ 36.5 whereas the C-2 peak at δ 40.5 was virtually unaffected, the enolate anion is assumed to be best represented by structure (iv).



The ^{13}C n.m.r. spectra of three synthetic compounds (Table 4) 2-acetyl-, 2-octanoyl-, and 2-oleoyl-cyclohexane-1,3-dione, confirmed the presence of this ring system in the natural products since this part of the ^{13}C n.m.r. spectra was indistinguishable from that observed for the natural fraction I compounds (^{13}C n.m.r. spectra on unseparated fraction I only). In addition, ^{13}C shifts for fraction II components (fraction IIB unseparated) were consistent with the presence of a 4-hydroxy-group (Table 4).

Earlier work[16] reported very low yields of β-triketones when cyclohexane-1,3-dione was acylated with anhydrides of longer-chain fatty acids (up to C_8) in the presence of boron trifluoride at room temperature. However, in the present work, using higher temperatures, much higher yields were obtained even for oleic anhydride (C_{18}).

Side Chains.—Fractions IA and IIA both contain a $C_{15}H_{27}CO$ acyl side-chain as indicated by subtraction of their ring contributions from their molecular formulae; they give ions at *m/e* 81, 68, and 67 suggesting a side-chain with 12,14-conjugated unsaturation.[17] Their u.v. spectra (Table 1) support the presence of a conjugated diene since both have increased absorption at 233 nm (ε 33 500) indicating a conjugated diene chromophore [$\lambda_{max.}$ 233 nm (ε 23 000)[18]] superimposed on a β-triketone [typically $\lambda_{max.}$ 273 (ε 10 000) and 233 nm (10 000)]. Analysis of the ^1H n.m.r. spectra confirms the presence of



$R\text{—}CH_2\text{—}CH{=}CH\text{-}CH{=}CH\text{-}CH_3$

this group and the coupling constants define its stereochemistry as 12*Z*,14*E*. Similarly the ^{13}C n.m.r. shifts for these six carbon atoms in IIA correspond with those from (*ZE*)-dodeca-8,10-dienyl acetate; the (*EE*)-, (*EZ*)-, (*ZE*)-, and (*ZZ*)- isomers give different ^{13}C spectra (unpublished observations).

A band at $\nu_{max.}$ 979 cm^{-1} in the i.r. spectra of both compounds indicates a *cis,trans*-conjugated diene.[19] Thus fractions IA and IIA are, respectively, 2-(*ZE*)-hexadeca-12′,14′-dienoylcyclohexane-1,3-dione (1) and its 4-hydroxy-derivative (4) (see Figure 1).

Similarly, from their molecular formulae, fractions IC and IIC both have $C_{17}H_{33}CO$ side-chains; the presence of the signal for a terminal methyl group in their ^{1}H n.m.r. spectra at δ 0.88 (3 H, m) shows that the isolated double bond (δ 5.35) is internal in a linear chain ($[CH_2]_n$ δ 1.26—1.32). The absence of an i.r. band at $\nu_{max.}$ 965 cm^{-1} suggests a *cis*-double bond, confirmed by the ^{13}C n.m.r. spectrum of pure IIC and crude IC. The allylic carbons in oleic and elaidic acids flanking the *cis*- and *trans*-double bonds give signals at δ 27.0 and 32.5 respectively.[20] The corresponding signals in IC and IIC appear at δ 27.2. The structure of IC was also supported by comparing its spectra with those of a synthetic sample made by BF_3-catalysed acylation of cyclohexane-1,3-dione with oleic anhydride. Although stereochemically pure anhydride was used, the product was a mixture of equal parts of *cis*- and *trans*-IC (peaks at δ 27.2 and 32.4 integrating for only 1 C each) but in all other respects the ^{13}C n.m.r. spectrum was identical with that of the natural product as were the u.v., ^{1}H n.m.r., and mass spectra. The double bond was finally proved to be in the 9,10 position by micro-ozonolysis [21] which gave nonanal (identified by g.l.c.–mass spectroscopy). Thus IC and IIC are respectively 2-oleoylcyclohexane-1,3-dione (3) and its 4-hydroxy-derivative (9).

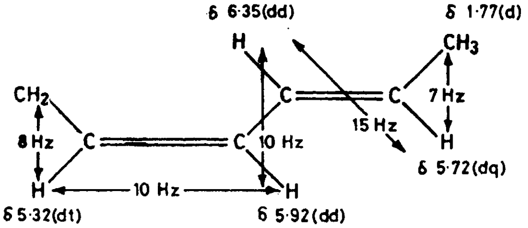


FIGURE 3 ^{1}H N.m.r. assignments and coupling constants for fractions IA and IIA

Similar ^{1}H n.m.r. and mass spectral evidence establishes that the side-chain in IB is $C_{15}H_{29}CO$. An i.r. band at $\nu_{max.}$ 965 cm^{-1} and the identification (g.l.c.–mass spectroscopy) of hexanal after ozonolysis show that the side chain is *trans*-hexadec-10-enoyl. Therefore IB is 2-(*E*)-hexadec-10′-enoylcyclohexane-1,3-dione (2).

Fraction IIB contained four components, one of which, IIB(a), was separated by rechromatography (see Table 1). Ozonolysis of IIB(a) produced acetaldehyde, and peaks at $\nu_{max.}$ 965 cm^{-1} and at *m/e* 55 and 41 in its e.i. mass spectrum indicated a terminal *trans*-disubstituted double bond. This was confirmed by its ^{1}H n.m.r. spectrum which was similar in this respect to that of (*E*)-dodec-10-enyl acetate. Thus IIB(a) is 4-hydroxy-2-(E)-hexadec-14′-enoylcyclohexane-1,3-dione (5).

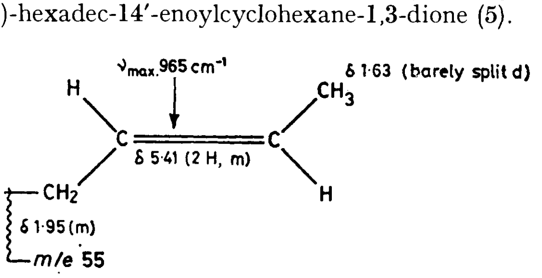


FIGURE 4 ^{1}H N.m.r. assignments for fraction IIB(a)

The remainder of fraction IIB, IIB(b), was a mixture of three very closely related compounds with spectra (except the mass spectrum) indistinguishable from the homologue (9). However ozonolysis gave respectively pentanal, heptanal, and nonanal showing that IIB(b) is a mixture of 4-hydroxy-(2*Z*)-hexadec-7′-, -9′-, and 11′-enoylcyclohexane-1,3-diones (6), (7), and (8).

EXPERIMENTAL

I.r. spectra were determined with a Perkin-Elmer Infracord spectrophotometer with polystyrene calibration at 1 601 cm^{-1}. Insufficient material was available for solution spectra and the spectra from micro-KBr discs (25 μg per component using beam condenser) of those components which are oils at room temperature were inadequately resolved. A small hydroxy-peak ($\nu_{max.}$ 3 600—3 200 cm^{-1}) in freshly fused KBr also tended to obscure the OH peak in fraction II components; nevertheless the main features of the spectra of all components (Table 1) were clear. U.v. spectra were determined in hexane (50 μg ml^{-1}) with a Unicam SP1800 spectrophotometer.

For g.l.c.–mass spectral (Pye 204–VG Micromass 70-70F) identification of aldehydes produced by ozonolysis, a 2 m × 4.5 mm (i.d.) 2½% Carbowax 20M column was used, programmed from 50—200 °C at 8 °C min^{-1}, using a jet-separator at 200 °C. For g.l.c.–mass spectral identification of acetaldehyde, a 1 ft × 1/8 in (i.d.) Poropak Q column was used isothermally at 80 °C in addition to the Carbowax column.

Aldehydes produced by ozonolysis were identified by g.l.c.–mass spectroscopic comparison with authentic samples. Except for acetaldehyde, the M^+, $M - 18$, $M - 28$, $M - 44$, and *m/e* 29 ions were observed and the corresponding ion (and total ion current) chromatograms generated had the same retention times as the authentic aldehydes. Acetaldehyde was identified by its retention time (*m/e* 29 ion chromatogram) on two g.l.c. columns (Carbowax 20M and Porapak Q).

The e.i. mass spectra of all components were determined (VG Micromass 70-70F) as probe-tip samples at 70 eV with a source temperature of 200 °C. C.i. mass spectra were determined using isobutane (5 × 10^{-5} Torr) as reactant gas; all components gave $M + 1$ ions. High-resolution mass spectra were determined using a Kratos MS 30 (with DS 50 data system) and by peak matching with a Hitachi–Perkin-Elmer model RMU 6E double-focusing spectrometer at 70 eV.

^{1}H and ^{13}C N.m.r. spectra were recorded (in $CDCl_3$) with a

JEOL JNM-PFT-100 spectrometer using Me_4Si as an internal standard; for identification of synthetic compounds, a JEOL JNM-PMX60 n.m.r. spectrometer was used.

Fractions were separated with an Applied Chromatography Systems Ltd. apparatus using a Cecil Instruments CE 212 variable wavelength u.v. monitor at 273 or 233 nm. Fractions I and II were separated on a Zorbax SIL Column (Dupont; 250 × 7.9 mm i.d.) with 99 : 1 (v/v) dichloromethane–formic acid (double glass-distilled) at 2 ml min^{-1} as eluant. Components of fractions I and II were separated on a Waters μ Bondapak C18, octadecylsilane column (300 × 3.9 mm i.d.) using water–methanol–formic acid (double glass-redistilled) (89 : 10 : 1 v/v) as eluant at 2.1 ml min^{-1}.

2-*Octanoylcyclohexane*-1,3-*dione*.—A mixture of cyclohexane-1,3-dione (5.15 g), octanoic anhydride (10 g), and octanoic acid (10 g) was heated to 100 °C, and boron trifluoride gas was passed rapidly through the mixture for 2 min. The mixture was poured into cold aqueous sodium acetate (35 g in 50 ml) and extracted into ether. After washing three times with saturated aqueous sodium hydrogen-carbonate the ethereal extract was dried and evaporated. Distillation gave 2-octanoylcyclohexane-1,3-dione (3.6 g, 25.8%), b.p. 138—142 °C at 0.6 mmHg (lit.,[16] yield 0.9%; b.p. 100 °C (bath-temp). at 0.1 mmHg), δ_C (60 MHz, $CDCl_3$) (see Table 4) 17.4 (1 H, s), 3.02 (2 H, m), 2.7 (2 H, m), 2.49 (2 H, m), 1.35 (*ca.* 12 H), and 0.87 (3 H, m); $\nu_{max.}$ (liquid film) 1 565 and 1 670 cm^{-1}; $\lambda_{max.}$ (hexane) 233 (ε 10 500) and 274 nm (9 800); *m*/*e* 238 (M^+), 220, 167, 154, and 139.

2-(Z)- *and* (E)-*Octadec*-9′-*enoylcyclohexane*-1,3-*dione*.—A mixture of cyclohexane-1,3-dione (2 g) and oleic anhydride (10 g) was heated under nitrogen as above until the dione had dissolved and then treated with boron trifluoride gas. This time, however, the fatty acid solvent was omitted and after work-up as before a yellow oil was obtained, preparative h.p.l.c. of which on a Zorbax SIL column (Dupont; 250 × 7.9 mm i.d.) using 99 : 1 (v/v) dichloromethane–formic acid gave a 1 : 1 mixture of 2-(*E*)- and (*Z*)-octadec-9′-enoylcyclohexane-1,3-dione (1.3 g, 18%). δ_C (60 MHz, $CDCl_3$) (see Table 4) 18.5 (1 H, s), 5.35 (2 H, m), 3.02 (2 H, m), 2.5 (2 H, m), 2.66 (2 H, m), 1.9—2.1 (*ca.* 4 H, m), 1.28 (*ca.* 20 H), and 0.88 (3 H, m); *m*/*e* 376 (M^+), 358, 167, 154, and 139; $\nu_{max.}$ (liquid film) 1 665 and 1 565 cm^{-1}; $\lambda_{max.}$ 233 (ε 10 900) and 274 nm (9 930).

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