

Further Novel 2-Acylcyclohexane-1,3-diones from Lepidopteran Larvae

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Eight further natural 2-acylcyclohexane-1,3-diones, hydroxylated and unhydroxylated at C-4, were identified in cultures of *Ephesia kuehniella* Zeller. These compounds had C₁₆ or C₁₈ acyl side-chains with unsaturation at C-7', -9', -11' (Z) or C-14' (E) or diunsaturation at C-9' and C-11' (Z,Z) or C-7' and C-9' (Z,Z) or (Z,E).

Recently a series of novel natural 2-acylcyclohexane-1,3-diones were identified^{1,2} in the mandibular gland secretion of larvae of the Mediterranean Flour Moth (*Ephesia kuehniella* Zeller). These compounds had a wide range of activity as kairomones³ causing oviposition movements in the Hymenopteran parasite *Venturia canescens* (Grav.). With larger amounts of material from whole-culture extracts and improved h.p.l.c. (high-performance liquid chromatography) separ-

ation techniques eight new naturally occurring 2-acylcyclohexane-1,3-diones were identified.

Results and Discussion

After extensive preparative rechromatography on silica columns to remove contaminating fatty acids *etc.*, two fractions [I and II (Figure 1), separated as previously²]

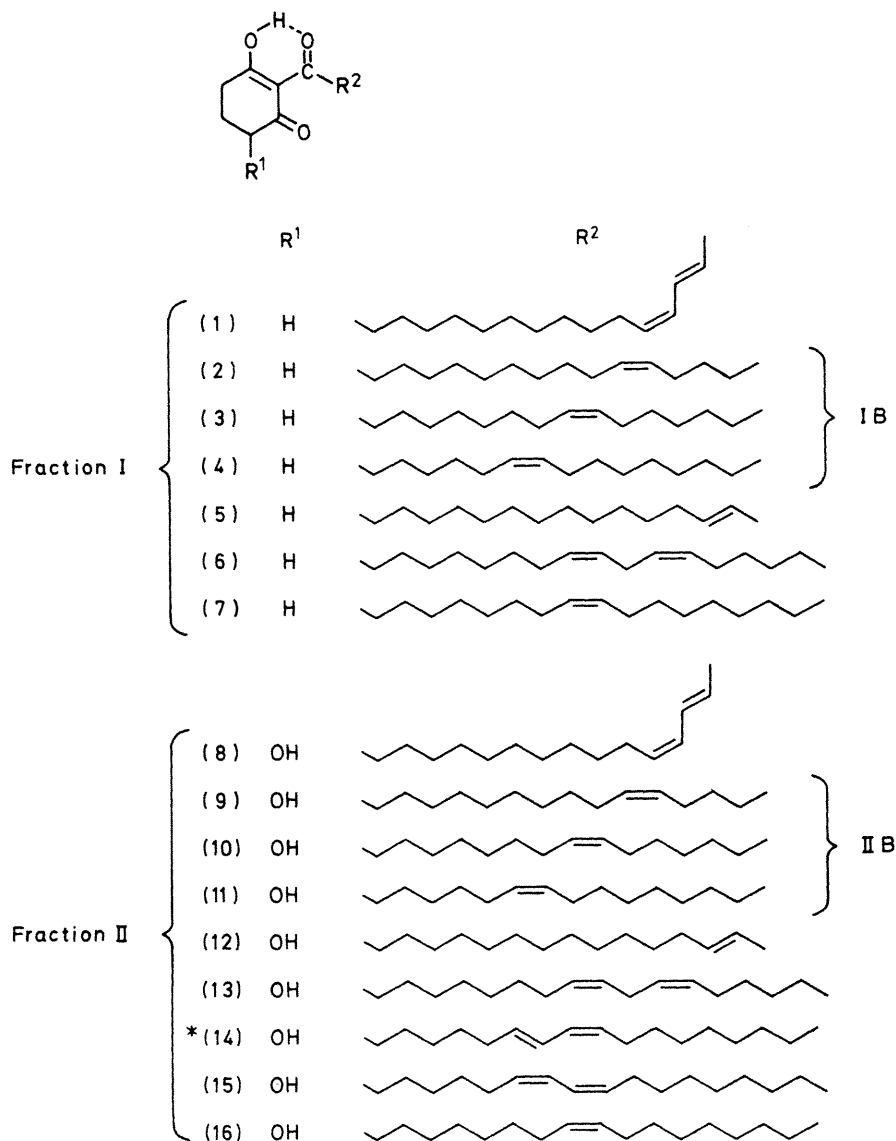


Figure 1.

* The present data do not show which bond is *cis* and which *trans*

Table 1.

Fraction or compound IB = (2) + (3) + (4)	H.p.l.c. retention ratio ^a			Appearance 20 °C	U.v. spectrum (hexane)		I.r. spectrum (thin film) ($\nu_{\max.}/\text{cm}^{-1}$)	Ozonolysis products
	Silica	ODS-3	Ag ⁺		ϵ (274 nm)	ϵ (233 nm)		
	0.65	0.23	—	oil	10 500	9 400	1 665, 1 565	pentanal heptanal nonanal
(5)	0.65	0.18	0.38	yellowish solid	10 400	9 500	1 665, 1 565, 965	acetaldehyde
(6)	0.65	0.18	0.15	oil	10 400	9 500	1 665, 1 565	hexanal
(13)	0.35	0.15	0.20	oil	10 800	9 900	1 665, 1 565	hexanal
(14)	0.35	0.14	0.55	oil	9 200	33 400	1 665, 1 565, 979	nonanal
(15)	0.35	0.14	0.50	white solid	9 400	33 900	1 665, 1 565	nonanal

^a Retention ratio = t_o/t_r where t_o = retention time for non-sorbed peak and t_r = retention time of component.

Table 2. ¹H N.m.r. data (δ_{H}) for the side-chain (R²) ^a

Compound or fraction IB = (2) + (3) + (4)	2'-CH ₂	Olefinic H	Allylic H ^b	Methylene chain	Terminal CH ₃
	3.01 (2 H, m)	5.33 (2 H, m)	1.95—2.01 (ca. 4 H, m)	1.27 (ca. 16 H)	0.88 (3 H, m)
(5)	3.02 (2 H, m)	5.42 (2 H, m)	1.95—2.02 (2 H, m)	1.26 (ca. 16 H)	1.62 (ca. 3 H, d, barely split)
(6)	3.02 (2 H, m)	5.35 (4 H, m)	1.96—2.02 (ca. 4 H, m)	1.21—1.32 (ca. 16 H) 2.77 (2 H, m, 11'-CH ₂)	0.89 (3 H, m)
(13)	3.01 (2 H, m)	5.36 (4 H, m)	1.95—2.03 (ca. 4 H, m)	1.33 (ca. 16 H) 2.77 (2 H, m, 11'-CH ₂)	0.89 (3 H, m)
(14)	3.04 (2 H, m)	5.31—6.29 (4 H, see text)	1.94—2.11 (ca. 4 H, m)	1.28—1.4 (ca. 16 H)	0.88 (3 H, m)
(15)	3.04 (2 H, m)	5.44 (2 H, dt, <i>J</i> 7 and 9.6 Hz) 6.25 (2 H, d, <i>J</i> 9.6 Hz)	1.94—2.04 (ca. 4 H, m)	1.28 (ca. 16 H)	0.88 (3 H, m)

^a Spectra for ring protons given in an earlier publication (ref. 2). ^b Superimposed on methylene envelope.

corresponding to unhydroxylated and hydroxylated 2-acylcyclohexane-1,3-diones, respectively, were isolated and then separated into four and five further subfractions, respectively. One of the Fraction I subfractions contained (¹³C n.m.r. monitoring) two components [(5) and (6), Figure 1] and two of the Fraction II subfractions each contained two components [(12) and (13) and also (14) and (15), Figure 1]. These pairs separated on a strongly acidic ion-exchange column loaded with silver ions using methanol as eluant.

Results from electron-impact (e.i.) and chemical-ionisation (c.i.) mass spectrometry, u.v., i.r., and ¹H- and ¹³C-n.m.r. spectroscopy of the fractions and from micro-ozonolysis studies are given in Tables 1—3. Fraction I contained four discrete 2-acylcyclohexane-1,3-diones and a mixture (IB) of three [(2), (3), and (4) Figure 1]. From Fraction II six 4-hydroxy-2-acylcyclohexane-1,3-diones and a mixture (IIB) of three [(9), (10), and (11), Figure 1] were obtained.

The presence of an enolic ring system in all components, either hydroxylated (R¹ = OH) at C -4 or unhydroxylated (R¹ = H) was demonstrated by their u.v., i.r., ¹H n.m.r., and

¹³C n.m.r. spectra and by their e.i. mass spectra as discussed in previous publications ^{1,2} (see Tables 1—3). The molecular ions of all new components were confirmed by c.i. mass spectrometry and all gave diagnostically significant ions ² in their e.i. spectra: ($M - \text{H}_2\text{O}$)⁺, m/z 167, 154, and 139 for unhydroxylated (Fraction I) components and m/z 183, 120, and 155 for hydroxylated (Fraction II) components.

The new components [(2)—(6) and (13)—(15), Figure 1] differed from previously described ² 2-acylcyclohexane-1,3-diones in the nature of their side-chains. Evidence for the structures of their side-chains is discussed below.

Components (2), (3), and (4).—Mass spectrometry gave the molecular formula C₂₂H₃₆O₃ for Fraction IB indicating a mono-unsaturated C₁₅H₂₉CO side-chain. A peak at δ_c 27.2 p.p.m. in the ¹³C n.m.r. spectrum, corresponding to two carbon atoms flanking a *cis* double bond, indicates its configuration. This is supported by the absence of both an i.r. band $\nu_{\max.}$ 965 cm⁻¹ in its i.r. spectrum and a peak at δ_c 32.6 p.p.m. in the ¹³C n.m.r. spectrum.

Table 3. ^{13}C N.m.r. data (δ_{C} /p.p.m.) for the side-chain (R²)^{a, b}

Compound or fraction	1'	2'	3'	4'	5'	6'	7'	8'	9'	10'	11'	12'	13'	14'	15'	16'	17'	18'
(1)	206.3	40.6	24.7	29.5	29.5	29.5	29.5	29.5	29.5	29.5	27.7		129.8, 128.8, 128.6, 127.2	18.3				
(2) + (3) + (4) = IB	206.3	40.6	24.8	29.5	29.5	29.5	129.9, 129.9, 27.2, 27.2, 29.5, 29.5, 29.5, 29.5	29.5	29.5	29.5	29.5		29.5	32.0	22.4	14.0		
(5)	206.3	40.6	24.7	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6	32.6	131.7	124.5	17.9		
(6)	206.3	40.6	24.7	29.4	29.4	29.4	29.4	27.2	130.1	128.0	25.7	128.0	130.1	27.2	29.3	31.6	22.6	14.1
(7)	206.5	40.7	24.7	29.4	29.4	29.4	29.4	27.3	129.8	129.8	27.3	29.4	29.4	29.4	29.4	32.0	22.8	14.1
(8)	206.1	40.3	24.6	29.5	29.5	29.5	29.5	29.5	29.5	29.5	27.7		129.9, 128.9, 128.5, 127.2	18.3				
(9) + (10) + (11) = IIB	206.0	40.3	24.6	29.4			129.8, 129.8, 27.2, 27.2, 29.4, 29.4, 29.4, 29.4						29.4	32.0	22.4	14.0		
(12)	206.0	40.2	24.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6	32.6	131.7	124.5	17.8		
(13)	206.0	40.2	24.6	29.3	29.3	29.3	29.6	27.2	130.1	128.0	25.6	128.0	130.1	27.2	29.3	31.5	22.6	14.1
(14)	206.0	40.2	24.4	29.2	29.7	32.6	134.1, 130.3, 128.5, 125.9				27.7	29.7	29.7	29.7	29.7	31.9	22.6	14.1
(15)	206.0	40.2	24.5	29.4	29.4	27.5	132.3, 131.5, 124.0, 123.5				32.6	29.4	29.4	29.4	29.4	31.9	22.7	14.1
(16)	206.0	40.1	24.5	29.4	29.4	29.4	27.2	129.7	129.9	129.9	27.2	29.7	29.4	29.4	29.4	31.8	22.6	14.1

^a δ_{C} -Values in p.p.m. from Me₄Si (δ_{C} 0.00) ^b Shifts of ring C atoms are given in an earlier publication (ref. 2).

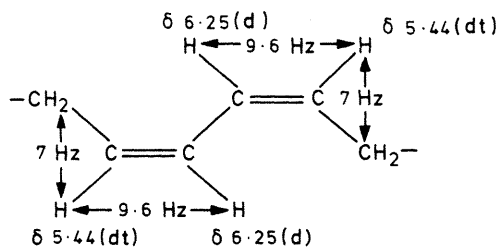


Figure 2.

Although spectroscopy and chromatography indicated that Fraction IB was a single compound, ozonolysis produced a mixture of pentanal, heptanal, and nonanal showing that Fraction IB was a mixture of 2-(*Z*)-hexadec-7'-, and -9'-, and -11'-enoylcyclohexane-1,3-diones.

Component (5).—Similar mass spectral evidence showed that component (5) had a mono-unsaturated C₁₅H₂₉CO acyl side-chain. Peaks at δ_{C} 32.6, 131.7, 124.5, and 17.9 p.p.m. in its ^{13}C n.m.r. spectrum indicated ^a a *trans*-disubstituted double bond at the end of the side-chain. This was confirmed by ozonolysis, which gave acetaldehyde, by the ^1H n.m.r. methyl signal which occurred at δ_{H} 1.62 as a barely split doublet, and by the i.r. spectrum which showed a band at 965 cm⁻¹ indicating a *trans* double bond. Component (5) is therefore 2-(*E*)-hexadec a -14'-enoylcyclohexane-1,3-dione.

Components (6) and (13).—A doubly unsaturated C₁₇H₃₁CO acyl side-chain was indicated by mass spectrometry for both components after subtraction of their ring contributions to their molecular formulae. This is supported by the ^{13}C n.m.r. spectrum which closely resembles that of linoleic acid ^a when the contribution of the ring carbon atoms is subtracted. Particularly diagnostic are the shifts for four carbon atoms of the double bonds (δ_{C} 128.0 and 130.1 p.p.m.), those flanking them (δ_{C} 27.2 p.p.m., indicating *cis* configurations), and that of the methylene group between them (δ_{C} 25.7 p.p.m.). This was confirmed by ozonolysis which gave hexanal and ^1H n.m.r. spectroscopy which showed isolated double bonds δ_{H} 5.36 (4 H, m). Components (6) and (13) are, respectively, 2-(*Z,Z*)-octadeca-9',12'-dienoylcyclohexane-1,3-dione and its 4-hydroxy derivative.

Component (15).—From the molecular formula (C₂₄H₃₈O₄) deduced by mass spectrometry, a doubly unsaturated C₁₇H₃₁CO acyl side-chain was present. The ^{13}C n.m.r. spectrum showed four separate olefinic C atoms (δ_{C} 132.3, 131.5, 124.0, and 123.5 p.p.m.) and only two carbon atoms (δ_{C} 27.5 p.p.m.) flanking *cis* double bonds. There was no peak at δ_{C} 25.5 p.p.m. for a methylene group between the double bonds [*cf.* components (6) and (13)] suggesting the unusual *cis,cis*-conjugated diene system. Conjugation was confirmed by both u.v. and ^1H n.m.r. spectra. The u.v. absorption at 233 nm (ϵ 34 000), approximately three times more intense than that at 274 nm (ϵ 10 000), indicated a conjugated chromophore superimposed on a β -triketone [typically λ_{max} 273 (ϵ 10 000) and 233 nm (10 000)], similar to the u.v. spectra of components (1) and (8) in an earlier publication.²

The olefinic region of the ^1H n.m.r. spectrum was simple (Figure 2), consisting of a doublet (δ_{H} 6.25, 2 H, *J* 9.6 Hz) and a double triplet (δ_{H} 5.44, 2 H, *J* 9.6 and 7 Hz) reflecting the symmetry of the diene. Because the chemical shifts of the central protons are virtually identical, the coupling between them does not influence the appearance of the spectrum. Also the chemical shifts of the outer protons are similar so only one

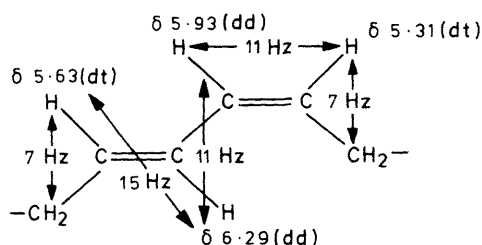


Figure 3.

double triplet is observed. Ozonolysis of (15) produced nonanal, showing that the double bonds were at C-7' and C-9' in the side-chain. Component (15) is therefore 4-hydroxy-2-(*Z,Z*)-octadeca-7', 9'-dienoylcyclohexane-1,3-dione.

Component (14).—Similar mass spectral data also indicated a doubly unsaturated $C_{17}H_{31}CO$ acyl side-chain for this component. Its ^{13}C n.m.r. spectrum was very similar to that of component (15) except for signals for olefinic carbon atoms (δ_c 134.1, 130.3, 128.5, and 125.9 p.p.m.) and for one of the carbon atoms (δ_c 32.6 p.p.m.) flanking the double bonds, indicating that one of the double bonds had the *trans* configuration. The absence of a peak (δ_c 25.5 p.p.m.) for a methylene group between the olefins suggested a *cis,trans*-conjugated diene. Again this was substantiated by its u.v. and 1H n.m.r. spectra. The u.v. spectrum was similar to that of component (15); however, the olefinic region of the 1H n.m.r. spectrum was more complex than that of (15) (Figure 3) and was consistent with a *cis,trans*-conjugated diene.

As with (15), ozonolysis of (14) produced nonanal, showing that the double bonds were in similar positions in the side-chain (*i.e.* C-7' and C-9'). The present data do not show which bond is *cis* and which *trans*.

A component, 2(*E*)-hexadec-10'-enoylcyclohexane-1,3-dione, described previously² was not detected in this study and was possibly an unresolved mixture of components (5) and (6) which did not separate using the techniques then available. Further doubt is cast on the structure since it does not occur in the hydroxylated series, whereas the rest [components (1)–(7)] do appear. The similarity of the side-chains in the 4-H and 4-OH series suggests that the dienes (14) and (15) probably have their counterparts in the unhydroxylated series but these were not detected.

There is no direct evidence for the mode of biosynthesis of these compounds. The fatty acid side-chains are clearly derived from head-to-tail linkage of acetate units and this process could continue, to give cyclic triketones, as suggested⁵ by Birch and Donovan to account for the formation of acylphloroglucinols.

Experimental

Experimental details have been given previously² with the following exceptions. Whole cultures (typically 1 kg) containing larvae, pupae, moths, and flour substrate were extracted with diethyl ether (2 l) and after filtration and evaporation a crude extract (12 g) was obtained. Extracts were prepared and purified at low temperatures, low light intensity and, where practicable, under nitrogen. The crude extract was dissolved in diethyl ether and washed three times, first with saturated aqueous sodium hydrogen carbonate, and then twice with saturated aqueous sodium carbonate. The combined sodium carbonate washings were acidified with ice-cold hydrochloric acid and extracted with diethyl ether to give, after having been washed (water) and dried ($MgSO_4$) an acidic fraction (*ca.* 2 g). Repeated preparative h.p.l.c. of this acidic fraction on a Jobin-Yvon Chromatogram Prep 10 using Merck TLC silica 60H with dichloromethane-formic acid (99 : 1) as eluant gave Fraction I (*ca.* 30 mg) and Fraction II (*ca.* 60 mg). Subsequent h.p.l.c. separations were similar to those described² except that Whatman Magnum 9 Partisil-10 and Partisil-10, ODS-3 columns (50 cm \times 9.4 mm) were used with eluants dichloromethane-trifluoroacetic acid (100 : 0.1) and methanol-water-trifluoroacetic acid (90 : 10 : 0.1), respectively. Three pairs of components [(5) and (6), (12) and (13), (14) and (15), Figure 1] which chromatographed as single peaks on the silica and ODS3 columns were separated by h.p.l.c. on an ion-exchange column loaded with silver ions (Chromopack, Nucleosil 10 SA Ag^+ ; 25 cm \times 4.6 mm). Aldehydes produced by ozonolysis were identified by g.l.c. peak enhancement on a 3 m \times 4.6 mm (*i.d.*) 10% Carbowax 20M column and by g.c.-m.s. as described² but using a capillary column (Flexsil OV101; 25 m \times 0.2 mm). Both columns were programmed from 50–200 $^\circ C$ at 12 $^\circ C$ min^{-1} after a 5 min isothermal period at 50 $^\circ C$.

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