129. Cyclizations of the C_{10} Fatty Acids from the Mandibulary Glands of the Honeybee *Apis mellifica* L.: Queen Substance and Royal Jelly Acid

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This publication is dedicated to the memory of the late Professor *Frantisek Sörm*

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Summary

Series of assays have been performed to cyclize the two main C_{10} aliphatic acids, (*E*)-9-oxo-dec-2-enoic acid (**3**) (queen substance) and (*E*)-10-hydroxy-dec-2-enoic acid (**7**) (royal jelly acid) produced by the mandibulary glands of the honeybee *Apis mellifica* L. Queen substance **3**, resistant to thermocyclization, was cyclized by the action of polyphosphoric acid to the fulvene ketone **5**. Triphenylphosphine-diethyl azodicarboxylate treatment of royal jelly acid **7** mainly furnished the dilactide **6** together with a series of cyclic oligomers up to the pentamer. Attempts to favour the formation of the lactone **11** through other conditions failed, and thus the reaction does not seem suitable for the preparation of lactones using α,β-unsaturated hydroxy acids.

Substances **3**, **7**, **12**, **13**, **14**, **15**, were submitted to *in vitro* antibacterial as well as growth inhibition tests but all results were negative at the concentrations reported.

Introduction. – The honeybee *Apis mellifica* L. produces in its mandibulary glands a series of C_{10} aliphatic acids which have been widely investigated since 1957, and among which queen substance **3** [1–5] and royal jelly acid **7** [6] [7] are characteristic components. Queen substance ((*E*)-9-oxo-dec-2-enoic acid, **3**) is secreted specifically by the mandibulary glands of the queen honeybee and is responsible for several biological properties [7–12] such as inhibition of ovarian development, attraction of males, inhibition of construction of royal cells, *etc.*, thus showing a variety of pheromonal activities. Royal jelly acid ((*E*)-10-hydroxy-dec-2-enoic acid, **7**) is produced in the worker honeybee mandibulary glands [6] [7] [12–15] and is a determinant food constituent given to the young during the first larval stage.

We report here the results of attempts to cyclize **3** and **7**. *Conia* [16] [17] failed to cyclize queen substance **3** by thermal treatment and we decided to try other methods. As royal jelly acid **7** has bacteriostatic effects *in vitro* [18–20] we also prepared some cyclic esters in order to search for possible cytostatic activity. The antibiotics pyrenophorin and norpyrenophorin [21] are dilactides derived from similar α,β-unsaturated hydroxy fatty acids in C_7 and C_8.

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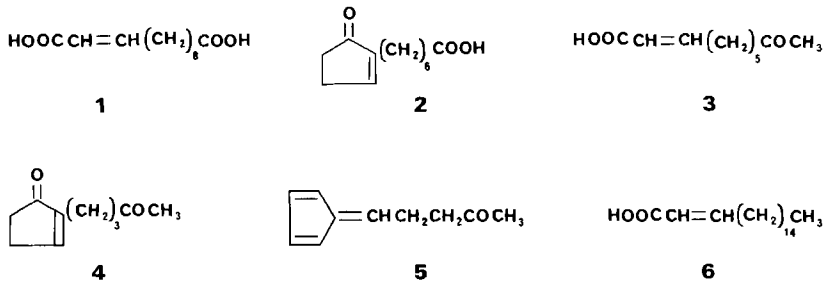
4-(6-Fulvenyl)-2-butanone (**5**). – The polyphosphoric acid cyclization of an unsaturated aliphatic acid gives the corresponding ketone or lactone [22]. Traumatic acid ((*E*)-dodec-2-enoic acid **1**) leads to the prostanoid synthon **2** [23]. Attempts to cyclize **3** by *Conia*'s thermal treatment [16] [17] or by reaction of the methyl ester with $NaOCH_3$, failed. Thermocyclization of the lower homologue of **3** ((*E*)-8-oxo-



1 **2** **3**

4 **5** **6**

non-2-enoic acid) [3] [5] to 2-acetylcyclopentylacetic acid has, however, been successful [16] [17] with a 90% yield. Consequently, we applied polyphosphoric treatment to queen substance **3** under conditions reported [23] for the cyclization of traumatic acid **1**. After 1 h at 100°, only traces of new products absorbing in UV. light (TLC.) were produced. The best conditions for maximum yield of transformation products with a minimum of degradation, were 110° for 2 h.

By SiO_2 preparative TLC., the main product (17.5%) was isolated together with traces of two other substances. As about 60% of **3** was recovered from the TLC., a non-negligible part of the material was lost in volatile, non-UV. absorbing, or water-soluble substances.

The mass spectrum of the isolated oil shows a molecular ion at *m/z* 148 (high resolution MS.: 148.0883, calc. for $C_{10}H_{12}O$: 148.0888) and the UV. spectrum in CH_3OH had λ_{max} at 225 nm (ε 4,500) and 279 nm (ε 11,000), eliminating a structure such as **4** and suggesting the possibility of a fulvene [24]. The mass spectrum *(Fig. 1* and *2)* is in agreement with the fulvene structures [25] in connection with the particular fragmentation pattern due to the ions issued from the pseudo-aromatic group. The presence of the methylketone is demonstrated by the fragment in MS. at *m/z* 133 $(M-CH_3)^+$, and also by the preparation of a 2,4-dinitrophenylhydrazone and a semicarbazone. The MS. of these derivatives still contain the fragments compatible with the fulvene structure (in particular, the tropylium ion at *m/z* 91). The ^{1}H-NMR. spectrum ($CDCl_3$) confirms the existence of 5 fulvene protons with two multiplets centered at 6.60 and 6.28 ppm for the H–C(1,4) and H–C(2,3), respectively. The vinylic proton at C(6) appears as a multiplet centered at 5.58 ppm and the $COCH_3$ group gives a singlet at 2.2 ppm. Similar attributions for a 6-substituted fulvenic structure have been reported [26].

The substance **5** is relatively unstable, only 50% being recovered from a preparative TLC. after 48 h at 0°, and at the same temperature, it is totally transformed to colourless and polar polymers (from MS.) after 6 days. Consequently, all determinations, on **5** and its derivatives, were performed immediately after the TLC.

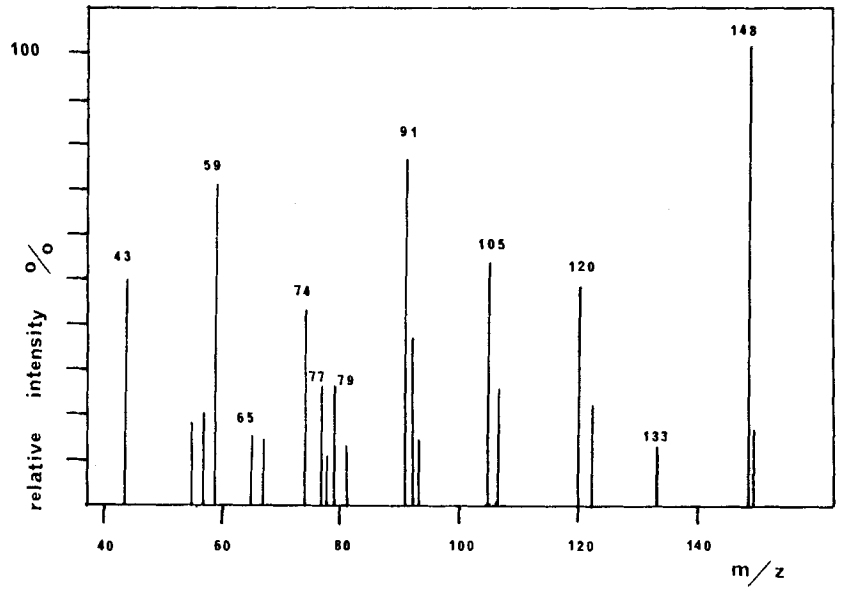


Fig. 1. *Mass spectrum* (from an ether solution) *of 4-(6-fulvenyl)-2-butanone* (**5**) *obtained through the polyphosphoric acid cyclization of queen substance*

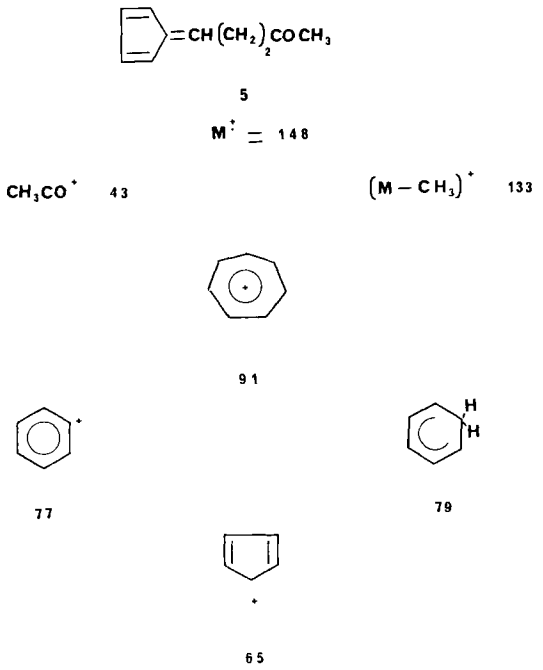
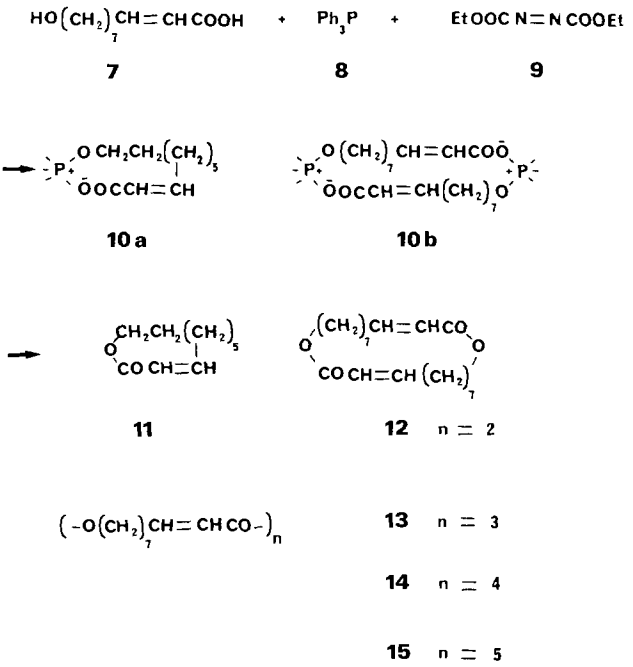


Fig. 2. *Characteristic ions [25] observed in the mass spectrum of* **5** (molecular ion analyzed in high resolution)

The conditions used to obtain **5** from queen substance **3** were checked with traumatic acid **1** and in this case, the keto acid **2** was obtained as described [23]. Attempts to cyclize (*E*)-octadec-2-enoic acid (**6**) failed, the substance being recovered after 2 h. Apparently other functional groups in the fatty acids participate in the attack by polyphosphoric acid, and particularly in the case of queen substance **3** the oxo group seems responsible for the formation of the fulvene structure from the α,β-unsaturated carboxylic C-atoms. We intend to prepare homologues of **3** to see whether a longer distance between the carboxyl and the keto groups would reduce the yield of the fulvene formation as we expect.

Triphenylphosphine-diethyl azodicarboxylate cyclization of royal jelly acid **7**. (*E*)-10-Hydroxy-dec-2-enoic acid (**7**) was treated by triphenylphosphine (**8**) and diethyl azodicarboxylate (**9**) in anhydrous benzene, in presence of molecular sieves 4 Å as a dehydrating agent. This reaction gives lactones and dilactides from hydroxy acids [27] [28]. After 72 h at 20°, several cyclization products were isolated using SiO_2 column chromatography monitored by TLC. on fluorescent SiO_2 films and final preparative TLC.

The main substance (24%) was the easily crystallised dilactide **12**. Concentration of the mother-liquors gave a small amount of the lactone **11**, MS.: 168 (M^+), which could not be isolated pure by chromatography. The dilactide **12**, as well as the trilactide **13**, the tetralactide **14**, and the pentalactide **15** were characterized by their UV., IR. and MS. data (absence of OH group, and absorption of CH=CH–COOR groups at 1700 and 1640 cm^{-1}). Small quantities of more polar, higher molecular weight compounds were also noticed from TLC. and MS. but could not be isolated



and were not further investigated. The hydrolysis of these products gave quantitatively the starting acid **7**.

Less than stoechiometric amounts of triphenylphosphine in order to favour the formation of monophosphonium complex **10a** also cyclized **7** to the dilactide **12** and the trilactide **13**, both isolated in small amounts. Dilution considerably lowered the yield of the expected cyclized products. Excess of the reagents did not modify the appearance of the oligomers, nor facilitate the formation of the lactone **11**. Owing to the conjugated carbonyl function in royal jelly acid **7** the cyclic phosphonium complex **10a** is probably less stable than polymolecular complexes of the **10b** type, and this would explain the formation of the series of lactides and the poor yield of the lactone **11**.

As royal jelly **7** possesses some *in vitro* antibacterial and cytostatic activities [18–20], tests were performed with compounds **3**, **7**, **12**, **13**, **14**, and **15**. They were submitted to the agar diffusion tests for antibiotic activities on *Bacillus subtilis*, *Straphylococcus aureus* and *Candida albicans*, at concentrations within the range 10–20 ppm, but all the results were negative. Tests of inhibition of germination and growth on water-cress seedlings and bio-assays on *Artemia salina* eggs development, up to concentrations of 100 ppm, were negative. It seems very likely that the length of the C_7 or C_8 α,β-unsaturated hydroxy fatty acid and the 4-oxo group in the pyrenophorins [21] are determinant for the biological activity.

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Experimental Part

General. Melting points (m.p.) were measured on the *Kofler* apparatus and are corrected, the mass spectra on *AEI* MS 9 and MS 50 apparatus and the ^{1}H-NMR. on a *Cameca* 250 MHz apparatus in $CDCl_3$, with TMS. as an internal standard.

4-(6-Fulvenyl)-2-butanone (**5**) *from queen substance.* Fresh polyphosphoric acid was prepared following [23]. Queen substance **3** (200 mg) was poured into 5 g polyphosphoric acid, the mixture being kept at 110° in an electric oven with frequent shaking for 2 h. After 30 min, a red homogeneous solution was observed, which became deeper in colour. After cooling, excess crushed ice was added and the mixture extracted with ether (2×20 ml). The combined ether solutions were washed with water, dried (Na_2SO_4) and concentrated. TLC. was performed on fluorescent SiO_2 film *Schleicher-Schüll*, with hexane/ethyl acetate/methanol 7:3:1 (observing in UV. light at 254 nm, *Desaga* apparatus). The recovered SiO_2 bands (**3** Rf 0.30; **5** Rf 0.60), were extracted with ether and the solutions were evaporated after filtration on a cotton plug, giving: recovered **3**, 120 mg (40% transformation) and **5**, 35 mg (17.5%) yellow oil, UV., NMR. and MS. of **5**: see text and *Figure 1* and *2*.

2,4-dinitrophenylhydrazone of **5**: orange-red amorphous powder, m.p. 51–54° (methanol/water). – MS.: 328 (M^+).

$C_{16}H_{16}O_4N_4$	Calc.	C 58.53	H 4.91	O 19.49	N 17.07%
(328.32)	Found	„ 58.41	„ 4.80	„ 19.70	„ 16.92%

Semicarbazone of **5**: orange-yellow leaflets, m.p. 170–180° (methanol/water). – MS.: 205 (M^+).

Triphenylphosphine-diethyl azodicarboxylate cyclization of royal jelly acid **7**. To a solution of (*E*)-10-hydroxydec-2-enoic acid (**7**) (1 mmol) in 10 ml anhydrous benzene was added triphenylphosphine (**8**) (1.5 mmol) and diethyl azodicarboxylate (**9**) (1.5 mmol) in presence of 1 g activated molecular sieves 4 Å. After 72 h at 20°, the mixture was chromatographed on SiO_2 (monitored by TLC. on *Schleicher-Schüll*

SiO_2 fluorescent films) with cyclohexane/ethyl acetate 9:1. The main products were purified by preparative TLC. in the same conditions (observations in UV. light at 254 nm). The main substance (24%), Rf 0.50, was the dilactide **12**, m.p. 94–96° (prisms from cyclohexane). – MS.: 336 (M^+).

$C_{20}H_{32}O_4$ (336.46) Calc. C 71.39 H 9.59% Found C 71.30 H 9.54%

The trilactide **13**, Rf 0.43, amorphous (waxy), $C_{30}H_{48}O_6$, MS.: 504 (M^+) represents 11% of the mixture, the tetralactide **14**, Rf 0.40, amorphous, $C_{40}H_{64}O_8$, MS.: 672 (M^+) about 9%, and the pentalactide **15**, Rf 0.30, amorphous, $C_{50}H_{80}O_{10}$, MS.: 840 (M^+) 7%. The products showed analytical results similar to **12**.

The oligomers were hydrolyzed by refluxing in 2N NaOH/benzene/ethanol.

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