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Studies on the sex pheromone of *Dacus oleae*. Analysis of the substances contained in the rectal glands[1]

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Summary. 17 substances contained in the rectal glands of *Dacus oleae* have been identified by GC-MS techniques. The synthesis of 3 isomeric spiroketals allows us definitively to assign the structure of 1,7-dioxaspiro-[5,5]-undecane to the most volatile compound.

During the last few years we have developed studies on the substances emitted by *Dacus oleae* (Gmelin), (Trypetidae)[3-8]. This fly (olive fruit fly) is responsible for great damage to olive oil production, especially in the Mediterranean area, and until now, no efficient method for the control of this pest has been found.

We started our work by studying the complex mixture of volatile substances emitted by the female during the period of major sexual activity, and also analyzing the content of the rectal glands of the insect. Morphological studies[9,10] have shown that the anal gland of this insect is the producing and accumulating centre for substances with attractive activity.

In a recent publication[6] we have described all the substances identified in a 'cold trap' condensate of the emission of virgin female *Dacus oleae*, and the attractiveness displayed by some of them. In this paper we want to summarize the results of our analyses on the rectal glands of the insect.

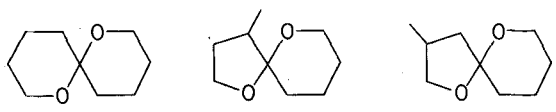
6-day-old virgin females were immobilized by keeping them in a refrigerator (− 10 °C) for some minutes. With the aid of a stereomicroscope the ovipositor was pulled out and the rectal glands excised from the abdominal organs.

3-5 freshly isolated glands were introduced in a glass capillary tube and in a solid-sample injection system connected with a GLC-MS apparatus. Best resolution of peaks was achieved with a SCOT glass capillary column CW 20 M (40 m, 0.25 mm ID) connected with a LKB 2091 mass spectrometer equipped with a Digital LKB 2130 data system.

A typical total ion current plot is shown in the figure. Table 2 shows the main MS fragmentations of the eluted peaks.

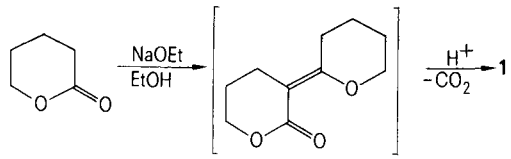
All the identified substances, with the one exception of the most volatile one (RT = 17.11 min), are fatty acids with the number of carbons ranging from C_6 to C_{18} esterified with C_1, C_2, C_3, C_4 aliphatic primary alchohols. Their structure was confirmed by comparison with commercially available or easily synthesized samples.

The sharp peak with retention time 17.11 min showed fragmentations (m/z 156 (22%), 128 (18), 111 (18), 101 (100), 100 (53), 98 (87), 83 (30)) which suggested the structure of spiroketal of MW 156[11]. An accurate analysis of the MS-spectrum of this compound in comparison with those described in the literature[12,13] allowed us to propose structures **1**, **2a**, **2b** as the most probable.



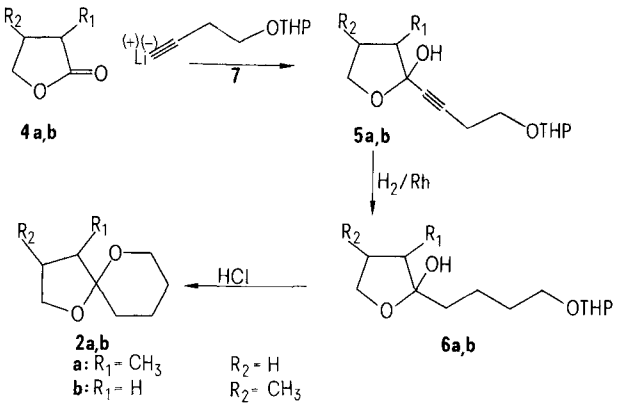
(1) **(2a)** **(2b)**

To confirm unambiguously the structure of the natural substance we started synthesizing the 3 compounds. **1** was synthesized by the method of Erdmann[14], untilizing the following reaction sequence:



Spectroscopic properties were in agreement with those reported in the literature[16].

2a and **2b**, which, to the best of our knowledge, are new compounds, were synthesized as a mixture of diastereoisomers after the general method of Phillips et al.[15] by the following scheme:



4b was the main product from controlled $LiAlH_4$ reduction of methyl succinic anhydride[17].

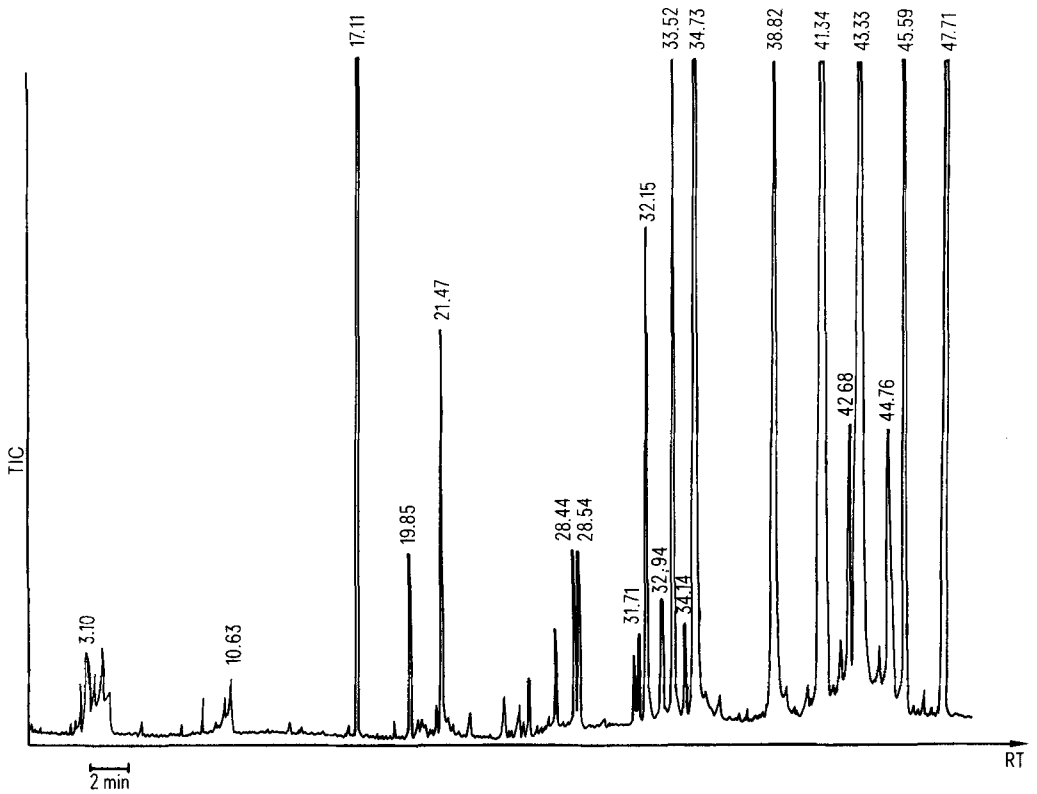
5a was obtained in an overall yield of 26% and showed ^{1}H-NMR ($CDCl_3$) δ: 1.15 (d, 3H), 1.2 (d, 3H), 1.6 (m, 9H), 2.6 (t, 2H), 2.75 (s, OH), 3.6 (m, 6H), 4.5 (bs, 1H); IR (liquid film): 1045, 1075, 1125, 1140, 1675, 3460 cm^{-1}.

6a (93.6%): ^{1}H-NMR ($CDCl_3$) δ: 1.1 (d, 3H), 1.5 (bs, 13H), 2.6 (m, 6H), 4.5 (bs, 1H); I.R. (liquid film): 1035, 1080, 1125, 1140, 1715, 3460 cm^{-1}.

2a (49.6%): b.p. 96–97 °C, 23 mm Hg; ^{1}H-NMR ($CDCl_3$) δ: 0.9 (d, 3H), 1.0 (d, 3H), 1.6 (m, 9H), 3.7 (m, 4H); IR (liquid film): 815, 895, 1010, 1025, 1080, 1160, 1215, 1270, 1380, 1460 cm^{-1}.

Table 1

RT (min)	Area (%)	Name
3.10	0.7005	
10.63	7.7500	
17.11	6.0038	1,7-dioxaspiro-[5,5]-undecane
19.85	0.7317	n-butyl hexanoate
21.47	2.0010	methyl dodecanoate
28.44	0.7729	ethyl dodecanoate
28.54	1.3495	methyl tetradecanoate
32.15	3.1059	ethyl tetradecanoate
32.94	0.8178	cis-methyl hexadecenoate
33.52	5.4025	ethyl hexadecenoate
34.14	0.9088	methyl hexadecanoate
34.73	12.2752	n-butyl tetradecanoate
38.82	10.5030	ethyl hexadecanoate
41.34	17.6928	n-butyl hexadecanoate
42.68	3.5316	n-propyl hexadecanoate
43.33	19.0144	n-butyl hexadecenoate
44.76	4.3372	ethyl octadecanoate
45.59	9.7548	methyl cis-9-octadecenoate
47.71	11.9875	n-butyl octadecenoate

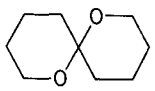
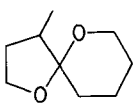
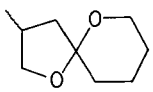


Total ion current plot of a sample of 5 whole rectal glands of *Dacus oleae* females. Conditions: glass capillary column SCOT (40 m, 0.25 mm ID) 3% CW 20 M on chromosorb. Carrier H_2 0.54 kg/cm^2; col. flow 1.55 ml/min; split flow 50 ml/min. prog. 50 $\xrightarrow{5c/'}$ 180 °C.

Table 2

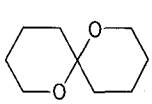
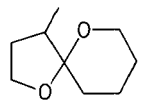
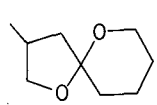
RT (min)	Main fragmentations	RT (min)	Main fragmentations
17.11	156 M+ (22%), 128 (18), 111 (18), 101 (100), 100 (53), 98 (97), 83 (30).	38.82	284 M+ (65%), 255 (10), 241 (42), 239 (51), 101 (85), 88 (51), 70 (100), 69 (92).
19.85	172 M+ (3%), 117 (5), 98 (36), 71 (100), 57 (78).	41.34	314 M+ (6%), 257 (72), 256 (62), 239 (100), 85 (59), 69 (81).
21.47	214 M+ (7%), 183 (10), 87 (100), 74 (87).	42.68	298 M+ (20%), 255 (31), 239 (53), 102 (90), 83 (61), 57 (100).
28.44	228 M+ (10%), 183 (18), 101 (90), 88 (100).	43.33	310 M+ (9%), 237 (58), 236 (32), 83 (63), 69 (92), 55 (100).
28.54	242 M+ (29%), 211 (24), 199 (41), 143 (98), 69 (100).	44.76	312 M+ (3%), 283 (18), 281 (9), 111 (42), 97 (88), 88 (84), 56 (100).
32.15	256 M+ (65%), 213 (60), 211 (62), 157 (92), 101 (98), 88 (67), 68 (100).	45.59	310 M+ (15%), 265 (40), 264 (42), 88 (80), 83 (100), 57 (95).
32.94	268 M+ (2%), 237 (5), 83 (30), 69 (79), 55 (100).	45.71	338 M+ (17%), 265 (70), 264 (72), 83 (100), 73 (70), 69 (71).
33.52	282 M+ (10%), 237 (17), 101 (88), 88 (100).		
34.14	270 M+ (22%), 239 (12), 227 (27), 88 (28), 69 (70), 55 (100).		
34.73	284 M+ (2%), 229 (33), 211 (53), 85 (31), 71 (65), 56 (100).		

Table 3. Retention times (min) of the synthetic compounds in comparison with the natural sample from female *Dacus oleae* glands

	OV 101	CW 20 M
Natural sample	16.23	23.31
 (1)	16.23	23.31
 (2a)	13.55	19.27
	15.11	23.31
 (2b)	14.26*	20.22*
	WCOT OV 101 20 m	SCOT CW 20 M on Chromosorb 40 m
	Carrier H_2 0.45 kg/cm^2	Carrier H_2 0.54 kg/cm^2
	Split flow 50 ml/min	Split flow 50 ml/min
	Col. flow 1.5 ml/min	Col. flow 1.55 ml/min
	Prog. 50 (4′) $\xrightarrow{2°C/1}$ 180 °C	Prog. 50 (4′) $\xrightarrow{2°C/1}$ 180 °C
	Chart speed 0.5 cm/min	

*The 2 diastereoisomers do not separate in either of the stationary phases.

Table 4

MS fragments*	Natural sample	 (1)	 (2a)		 (2b)
156 M$^+$	22	18	14	16	12
141	-	-	-	-	7
128	18	10	12	12	5
111	18	17	20	20	27
101	100	100	100	100	100
100	53	42	36	52	40
98	87	91	56	75	72
83	30	35	20	25	25

*Relative abundance of MS fragments of the synthetic compounds in comparison with the natural sample from females *Dactus oleae* glands. For **2a** and **2b** are shown the fragmentations of the 2 diastereoisomers.

5b (22.4%) ^{1}H-NMR δ: 0.9 (d, 3H), 0.95 (d, 3H), 2.2 (m, 9H), 2.6 (t, 2H), 3.7 (m, 7H), 4.6 (bs, 1H); I.R. (liquid film): 1065, 1125, 1135, 1670, 2220, 3460 cm^{-1}.
6b (99.7%): ^{1}H-NMR δ: 0.9 (d, 3H), 1.9 (bs, 13H), 2.4 (m, 3H), 3.5 (m, 6H), 4.5 (bs, 1H); I.R. (liquid film) 1030, 1080, 1120, 1710, 3400 cm^{-1}.
2b (54.5%): b.p. 99 °C 24 mm Hg: ^{1}H-NMR δ: 1.1 (d, 3H), 1.15 (d, 3H), 1.9 (m, 9H), 3.75 (m, 4H): I.R. (liquid film): 800, 845, 1005, 1040, 1120, 1380, 1440 $cmxl^{-1}$.
The compounds synthesized so far were first submitted to a GLC analysis utilizing the same capillary column as described above, but a more accurate temperature program. The results are summarized in table 3; the peaks corresponding to compound **1** and to one of the diastereoisomers of compound **2a**, and that of the natural product, displayed the same retention time.
Looking at the main fragmentations (table 4) of the same substances, again no appreciable differences were detectable, therefore the structure of the natural spiroketal could not hitherto be assigned.
A further GLC analysis, employing a less polar stationary phase (WCOT glass capillary column, OV 101, 25 m, 0.2 mm ID) achieved a satisfactory resolution of all the synthesized substances; compound **1** showed the same RT of the natural product and their identity was confirmed by an injection of the 2 combined samples.
While our work was in progress, a paper appeared by Baker et al.[16] in which the identification of the same spiroketal **1** in female rectal glands was described. Such a compound is claimed by the authors to be the major sex pheromone component emitted by the fly.
An analogous GLC-MS analysis of the rectal glands of *Dacus oleae* males did not show any of the substances found in the females. Only some peaks corresponding to C_{10}-C_{18} aliphatic hydrocarbons, which were not further investigated, were detected.

1 Part of this work has been presented at ESOC II, Stresa, June 1981, and at the II Meeting on *Dacus oleae,* Perugia, March 1982.

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Inability of thiamine phosphates transport in isolated rat hepatocyte[1]

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Summary. The hapatic transport of thiamine phosphates was studied in isolated rat hepatocytes. No significant radioactivity of ^{14}C-thiamine monophosphate and ^{14}C-thiamine pyrophosphate was incorporated into liver cells freshly isolated using the collagenase-perfusion technique. The result indicates that neither thiamine phosphates nor their thiamine moiety are available for liver cells.

Evidence has been accumulated which shows that the uptake of thiamine by various living cells occurs by active transport. Two reports recently have appeared on thiamine transport in isolated rat hepatocytes, in which thiamine was shown to be transported into the cells in an unaltered form by an active, sodium dependent process[3,4]. Thiamine is known to be present in living cells in 4 forms: free, mono-, pyro- and tri-phosphate[5,6]. Nakayama and Hayashi provided evidence that both thiamine monophosphate and thiamine pyrophosphate can be utilized intact by *Escherichia coli* mutants auxotrophic for thiamine phosphates[7]. Consequently Nishimune and Hayashi clearly demonstrated using ^{32}P-labeled thiamine pyrophosphate that the coenzyme was accumulated by *E. coli* without dephosphorylation[8]. On the other hand, no conclusive evidence has been obtained on the availability of thiamine phosphates for animal cells. With respect to this problem, Schaller and Höller suggested that splitting of thiamine pyrophosphate at the surface on the intestinal mucosa or in mucosal fluid and active transport of thiamine are interrelated with each