of composition $C_{17}H_{14}N_5O_2$, $C_{14}H_{16}N$, $C_8H_8N_4O_2$, $C_{10}H_9N_2$, C_9H_8N and $C_5H_6N_3$) and comparison with the mass spectra of 11a,15-dideutero-, 19,20-dihydro-, and 3,12-dihydroroquefortines. In the dideutero-compound, the first and third fragment ions are increased by two mass units, the fourth and sixth by one mass unit, while the second and fifth remain unchanged.

Structure I proposed for roquefortine contains the same ring system as the dethio derivatives of sporidesmins[10], verticillins[11], and chaetocin[12], but arises by coupling of tryptophan and dehydrohistidine. Oxaline[8] is the only other fungal metabolite known to contain a dehydrohistidine unit, which has a *cis* double bond. The stereochemistry of the 3,12 double bond in roquefortine remains to be established. Also the configuration of the 11a proton relative to the 5a proton or the side chain at position 10b could not be determined from coupling constants observed at 240 MHz for the 11,11a $CH_2 \cdot CH$ grouping ($J_{11,11a} = 5$ Hz, $J_{11',11a} = J_{11',11} = 12.5$ Hz).

The minor metabolite isofumigaclavine A, $C_{18}H_{22}N_2O_2$ (found: C, 72.32; H, 7.41; N, 9.40. Calc. for $C_{18}H_{22}N_2O_2$: C, 72.45; H, 7.43; N, 9.39%), had m.p. 190–193° (from benzene), $[\alpha]_D^{22} - 54.1°$ (*c* 0.67, $CHCl_3$), λ_{max} (95% EtOH) 226 (log ε 4.42), 277 (sh, log ε 3.77), 283 (log ε 3.81), and 293 (log ε 3.75) nm, ν_{max} ($CHCl_3$) 3495, 1743, 1605 cm^{-1}, and principal mass spectral peaks at *m/e* 298, 239, and 154. The ^1H NMR-spectrum ($CDCl_3$) confirmed the presence of an acetate group (δ 2.22 ppm), and a multiplet at 5.18 ppm indicated that it was secondary; CH_3CH (0.97d, $J = 7$ Hz) and N–CH_3 (2.46s) groups were also present. Alkaline hydrolysis yielded isofumigaclavine B, $C_{16}H_{20}N_2O$[4], m.p. 278–282° (dec.), whose mass spectrum was virtually identical with that of the clavine alkaloid umigaclavine B[2,3]. In addition to fumigaclavine B, low yields of isofumigaclavine B were also obtained during hydroboration of agroclavine[2] under certain conditions. Thus the two compounds are stereoisomers (III). The stereochemistry of fumigaclavine B has been established by Bach et al.[13].

The metabolites of *Penicillium roqueforti* were readily detected by TLC on silica gel layers (Schleicher & Schüll F 1500/LS 254) developed with chloroform-methanol – c. ammonium hydroxide (85:15:1; v/v/v). Detection was by spraying with 50% H_2SO_4 and heating at about 100° for 10 min; roquefortine formed a blue spot and isofumigaclavine A a mauve spot at respective Rf values of approximately 0.46 and 0.62.

Roquefortine possesses neurotoxic properties[14]. What appears to be roquefortine has been recently isolated as roquefortine C from *Penicillium roqueforti* by OHMOMO et al.[15], who did not propose a structure. Isofumigaclavines A and B may be the same as their roquefortines A and B, although different structural formulae were suggested[15].

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Transfer of Cantharidin (1) During Copulation[1] from the Adult Male to the Female *Lytta vesicatoria* ('Spanish flies')[2]

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Summary. Adult males of the 'spanish fly' *Lytta vesicatoria* (Meloidae, Coleoptera) are able to biosynthesize cantharidin (**1**) 'in copula' after injection of E,E-11'-(^3H,^{14}C)-farnesol and 2-^{14}C-methylfarnesoate. During mating, the cantharidin biosynthesized in the males is transferred to the females, who are unable to biosynthesize cantharidin from any terpenoid precursors. After injection of (^3H,^{14}C)-cantharidin into males during copulation, more than 10% is transferred into the female sex organs. Males injected with 2-(^3H,^{14}C)-mevalonate 24–30 h prior to copulation transferred 93–98% of the biosynthesized cantharidin to the females during mating. After mating, the males continue to produce cantharidin. It seems that in the male the biosynthesis of cantharidin is stimulated during copulation.

Insects of the family Meloidae contain the physiologically important substance cantharidin (**1**). We have investigated *Lytta vesicatoria* from Sicily and found that the adult males *Lytta* contain an average of 1.8% cantharidin (calculated from dry weight), whereas the females from the same population (1975) contain only 1.0% (see also[5–8]).

Earlier investigations have shown that, when ^{14}C-acetate, -mevalonate or -farnesol is injected into adult male *Lytta vesicatoria*, it is, as a rule, incorporated into cantharidin by an amount of 1%[8–10]. Female insects which are only 14 days old and are of the same population did not, however, incorporate the above precursors at all[8,11,12]. In view of these findings, the question was earlier raised as to where the cantharidin found in female *Lytta* actually came from[13]. Some of it is probably of larval origin[13], but the further possibilities exist that freshly hatched female *Lytta* are still able to produce cantharidin, or that cantharidin is transferred from the male to female insects during copulation. The sex organs of male Meloids are particularly rich in cantharidin[5,14]. According to CARREL[14], the cantharidin content of freshly hatched male *Epicauta amaicha* (Meloidae), cultured in the laboratory, appears to rise steeply during the first 7 days, whereas in the female insects the level remains more or less unchanged. Compared to the level before

Table I. Experiment with sodium-2-(^{3}H,^{14}C)-mevalonate

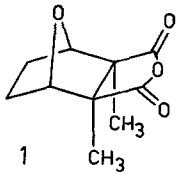
Lytta vesicatoria (couples)		Isotope	Total activity (dpm)	Cantharidin dpm/mg	Cantharidin Total activity (dpm)	Cantharidin ^{3}H/^{14}C	Incorporation rate[a] (%)	Transferred total activity (%)	Transferred cantharidin (%)
5	♂	^{3}H	6.5×10^6	1 087 ± 9	$(5.51\pm0.045)\times10^4$	2.12 ± 0.02	0.68		
		^{14}C	1.2×10^6	512 ± 7	$(2.60\pm0.035)\times10^4$		1.45		
	♀	^{3}H	1.6×10^6	14 025 ± 133	$(7.14\pm0.067)\times10^5$	2.32 ± 0.03	8.83	19.8	92.8
		^{14}C	5.9×10^5	6 035 ± 113	$(3.07\pm0.057)\times10^5$		17.14	33.0	92.3
9	♂	^{3}H	5.5×10^6	715 ± 14	$(3.23\pm0.063)\times10^4$	2.27 ± 0.04	0.35		
		^{14}C	9.9×10^5	315 ± 3	$(1.42\pm0.013)\times10^4$		0.67		
	♀	^{3}H	3.5×10^6	38 000 ± 134	$(1.72\pm0.066)\times10^6$	2.24 ± 0.01	19.02	38.9	98.3
		^{14}C	1.1×10^6	16 952 ± 65	$(7.66\pm0.029)\times10^5$		36.04	52.6	98.2

[a] Radioactivity of cantharidin obtained from the experiments × 100 divided by the activity found after work-up in the water- and methylene chlorid-phase = total activity.

Table II. Transfer of (^{3}H,^{14}C)-cantharidin, (^{3}H,^{14}C)-1 from male to female *Lytta vesicatoria* at copulation

Lytta vesicatoria (couples)		Isotope	Injected cantharidin Amount (mg)	Spec. activity (dpm/mg)	^{3}H/^{14}C	Reisolated cantharidin Amount (mg)	Spec. activity (dpm/mg)	^{3}H/^{14}C	Original body-content of cantharidin (mg)	Specific cantharidin content[a] (%)
25	♂	^{3}H	5.42	115 619 ± 922	10.0 ± 0.13	15.25	36 850 ± 848	10.04 ± 0.02	10.39	1.1
		^{14}C		11 525 ± 97			3 668 ± 77			
	♀	^{3}H				20.62	3 122 ± 73	9.97 ± 0.04	20.06	⩾ 1.0
		^{14}C					313 ± 7			

[a] Calculated for an average dry weight of 37.7 mg for a ♂ and of 83.7 mg for a ♀ insect respectively.



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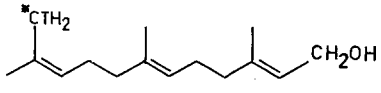

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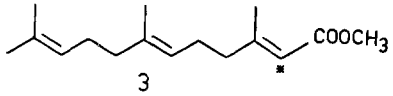

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copulation the cantharidin content in the sex organs of the male insects was found to be reduced, whereas in the female it was considerably increased.

Thus we have injected 2-(^{3}H,^{14}C)-mevalonate under CO_2-narcosis into male *L. vesicatoria* (Sicily 1975) 24 to 30 h prior to copulation. After an average copulation period of 20–24 h, the insects separated. They were killed by deep-freezing, separated and processed as follows: the insects were crushed, inactive cantharidin was added and the whole mass was boiled under reflux in 2 *N* HCl. Finally the water suspension was extracted exhaustively with methylene chloride. The radioactivity of both the water- and of the methylene chloride-phases was then measured (sum:total activity). The cantharidin was isolated from the methylene chloride-phase and purified until constant specific radioactivity. The results are reported in Table I.

In a further experiment, (^{3}H,^{14}C)-cantharidin (^{3}H,^{14}C)-(**1**) in acetone was injected into the males of 25 copulating couples (Sicily 1975). After a copulation period of 4–8 h, the partners were separated and the total cantharidin contained in the male insects was isolated. The ovary and the receptaculum of the females were dissected and the cantharidin contained in these organs was extracted. The results are reported in Table II. This experiment demonstrates that, of the cantharidin injected into the

[1] III. Communication on the Biosynthesis of Cantharidin; II. Communication[16].

[2] Dedicated to Professor KURT MOTHES' (Halle/Saale, DDR) 75th birthday, 4.11.1975.

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Table III. Experiment with E,E-11'-(^{3}H,^{14}C)-farnesol (**2**)

Lytta vesicatoria (couples)		Isotope	Life-time after copulation (h)	Total activity (dpm)	Cantharidin dmp/mg	Cantharidin Total activity (dpm)	^{3}H/^{14}C	Incorporation rate[a] (%)	Transferred total activity (%)	Transferred cantharidin (%)
4	♂	^{3}H	36	3.1×10^7	33 856 ± 224	$(1.69\pm0.011)\times10^6$	9.67 ± 0.07	3.6		
		^{14}C		9.3×10^5	3 500 ± 3	$(1.75\pm0.001)\times10^5$		13.9		
	♀	^{3}H	36	1.5×10^7	28 021 ± 36	$(1.40\pm0.002)\times10^6$	9.75 ± 0.04	3.0	32.8	45.2
		^{14}C		3.2×10^5	2 873 ± 9	$(1.44\pm0.004)\times10^5$		11.4	25.5	45.0

[a] See Table I.

Table IV. Experiment with E,E-2-^{14}C-methylfarnesoate (**3**)

Lytta vesicatoria (couples)		Life-time after copulation (h)	Total activity (dpm)	Cantharidin dpm/mg	Cantharidin Total activity (dpm)	Incorporation rate[a] (%)	Transferred total activity (%)	Transferred cantharidin (%)
7	♂	31	1.0×10^8	2 3507 ± 114	$(1.18\pm0.006)\times10^6$	1.1		
	♀	31	3.0×10^6	9 486 ± 3	$(4.74\pm0.002)\times10^5$	0.5	2.9	28.7

[a] See Table I.

males, 10.3% is transferred into the female sex organs during the short lasting copulation.

The results in Table I show that 93–98% of the cantharidin biosynthesized from 2-(^{3}H,^{14}C)-mevalonate (injection 24–30 h prior to copulation) is transferred from the male *Lytta* to the females during a copulation period of 20–24 h (in particular the ^{3}H/^{14}C-ratio of the isolated cantharidins from the males and females are equal). Of the total activity (100%) present after copulation, only 33.0–52.6% was found in the female *Lytta*. The high rate of incorporation of 18.6–36.7% of 2-^{14}C-mevalonate is remarkable, and it would appear that, in the male, the biosynthesis of cantharidin is stimulated during copulation. Both experiments provide strong evidence against the transfer of an intermediate species, formed from mevalonate during the cantharidin biosynthesis, to the female at copulation.

In further experiments E,E-11'-(^{3}H,^{14}C)-farnesol (**2**)[15] and E,E-2-^{14}C-methylfarnesoate (**3**)[12] were injected into male *L. vesicatoria* from Sicily (1972–1974) during copulation. After an average copulation time of 20 ± 2 h, the couples separated. The males and females were kept alive separately for 30–40 h and then processed. The results of these experiments are presented in Tables III and IV.

Of the total cantharidin biosynthesized from radioactive precursors **2** and **3** by the male *Lytta* during and after copulation, the percentages found in the females were 45.0% and 28.7% respectively. A comparison of these values with those for total activity transferred from male to female during copulation only, show that there is a preferential transfer of cantharidin at copulation. The fact that the value is lower, compared to that in experiment 1, shows that the male insects continue to synthesize cantharidin after copulation from labelled precursors still present in their bodies. Indications suggest that cantharidin is stored in the accessory glands of the male sexual organs. Our observations demonstrate that an almost complete transfer of cantharidin occurs at copulation. Thus it appears very likely that these glands are the site of cantharidin biosynthesis.

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Amino Acid Requirements of the Bug *Dysdercus similis* Freeman (Hemiptera: Heteroptera)

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Summary. The amino acid requirements for moulting, growth and development of *Dysdercus similis* have been investigated. The insects could not moult and reach maturity when given 10 essential amino acids only. However, if these were supplemented either with glutamic acid, glycine or aspartic acid growth and moulting was found to be normal. *Dysdercus* has an unusual synthetic mechanism for converting tyrosine into phenylalanine.

A number of papers deal with the amino acid requirements of insects[2–6]. Still we know very little about the amino acid requirements and their role in the growth and moulting physiology of insects[7–9]. Therefore an attempt has been made to elucidate the role of different amino acids during the moulting cycle of *Dysdercus similis*.

Material and methods. Nymphs and the adults of *D. similis* were collected from the fields and reared all the