by reticuloendothelial cells, or by an asialofragment, or by a major hormonal increase, remains uncertain.

The role of hormones (growth hormone[92], ACTH[93], cortisol[94] alone or associated with insulin[95]) is well documented in vivo and in vitro, but again the role of substances eliciting an increased hormonal response remains uncertain. There is another impressive number of factors, the most significant and similar deriving from leukocytes (leukocytic endogenous mediator, LEM[96–98]; endogenous pyrogen, EP[99,100]; supernatant fraction of Darcy[101]) that also, most likely indirectly, are strongly influencing the synthesis of 'acute phase' glycoproteins.

Thus the regulation of synthesis of glycoproteins can be envisaged at present as regulated in at least two ways: a protein-specific regulatory function could be accomplished by the same protein fragment that, if desialylated, could reach its target more promptly and influence the basal synthesis, while a second aspecific mechanism may variably influence the synthesis in emergency conditions.

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SPECIALIA

Roquefortine and Isofumigaclavine A, Metabolites from *Penicillium roqueforti*

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Summary. The structures of two metabolites from *Penicillium roqueforti*, designated roquefortine and isofumigaclavine A, have been determined by chemical and spectroscopic studies.

Extraction of mycelium from 2-week-old cultures of *Penicillium roqueforti* (strain CS1) on yeast extract-sucrose medium[2] has yielded 2 crystalline nitrogen-containing metabolites. The major metabolite (20–30 mg/l) is designated roquefortine and assigned structure I on chemical and spectroscopic evidence. The minor substance is a stereoisomer of fumigaclavine A (II)[3].

Isolation of the compounds from chloroform-methanol (9:1, v/v) extracts of lyophilized mycelium was accomplished by partition from ethyl acetate into 0.5 *N* hydrochloric acid followed by addition of ammonium hydroxide and re-extraction with chloroform. The metabolites were separated on a column of Florisil by elution with chloroform-methanol (97:3, v/v).

The more polar product, roquefortine, crystallized from methanol-water as colourless needles, m.p. 195–200° (dec.), $[\alpha]_D^{22} - 703°$ (c 1.0, $CHCl_3$). The molecular formula was $C_{22}H_{23}N_5O_2$[4] (found: C, 65.33; H, 6.29, N, 16.87. Calc. for $C_{22}H_{23}N_5O_2 \cdot CH_3OH$: C, 65.54; H, 6.46, N, 16.62%). UV- and IR-spectral properties were λ_{max} (95% EtOH) 209 (log ε 4.47), 240 (log ε 4.21), and 328 (log ε 4.43) nm; ν_{max} ($CHCl_3$) 3430, 3380, 3190, 1685, 1665, and 1608 cm^{-1}. The ^{13}C NMR-spectrum (off-resonance decoupled)[2] (Table) showed the presence of 2 CH_3, 1 CH_2, 2 CH, 1 CH_2=, 8 CH=, 2 sp^3 and 4 sp^2 fully substituted C, and 2 C=O groups and accounted for 20 protons. Only 2 of the remaining 3 protons were observed as D_2O exchangeable NH protons in the ^1H NMR-spectrum.

Catalytic reduction (H_2/Pt/acetic acid) of roquefortine yielded 19,20-dihydroroquefortine, $C_{22}H_{25}N_5O_2$[4], m.p. 185–187°, $[\alpha]_D^{22} - 740°$ (c 0.15, $CHCl_3$), whose mass spectrum showed a strong peak at *m/e* 320 (M-71). The suggested isoprene unit in roquefortine (M-69 fragment ion) was shown to be an inverted γ,γ-dimethylallyl group by comparison of the relevant portions of the ^1H NMR-spectra of roquefortine (I) and 19,20-dihydroroquefortine

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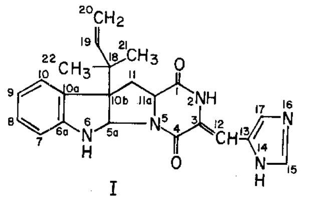
^{13}C and ^{1}H nmr-absorptions of roquefortine and 19,20-dihydroroquefortine[a]

Position[b]	Group	Roquefortine ^{13}C nmr[c]	Roquefortine ^{1}H nmr[d]	19,20-Dihydroroquefortine ^{1}H nmr[d]
21	CH_3	22.4q	1.06s	0.98s
22	CH_3	22.9q	1.17s	
19,20	CH_3CH_2	–	–	0.73–1.67
11	CH_2	36.8t	2.58m[e]	2.58m
11a	CH	58.8d[f]	4.12dd[f]	4.00m
5a	CH	78.3d	5.70s	5.74s
18	C	40.9s	–	–
10b	C	61.5s	–	–
20	$CH_2=$	114.7t	5.04dd, 5.28s	–
19	CH=	143.4d	6.07m	–
12	CH=	109.2[g]d	6.40s	6.39s
7	CH=	110.9[g]d	6.58–7.30	6.5–7.3
9	CH=	119.1d		
10	CH=	125.2d		
8	CH=	129.1d		
17	CH=	134.3d		
15	CH=	136.5d[f]	7.73s[f]	7.73s
3	C=	122.3s	–	–
13	C=	125.8s	–	–
10a	C=	128.7s	–	–
6a	C=	150.1s	–	–
4	C=O	159.4s	–	–
1	C=O	167.0s	–	–
6	NH	–	5.10s[h]	5.00br[h]
2	NH	–	10.22s[h]	9.78[h]

[a] $CDCl_3$ solution, δ in ppm from TMS internal standard. [b] Numbering scheme for structure I. [c] 22.63 MHz. [d] 60 MHz. [e] Singlet in 11a,15-dideuteroroquefortine. [f] Signal absent in dideuteroroquefortine. [g] Assignments may be reversed. [h] Disappears on D_2O exchange.

and by ^{13}C NMR-signals of roquefortine at 143.4 (d), 114.7 (t), 40.9 (s), 22.9 (q), and 22.4 (q) ppm (Table). Placement of this group on the remaining fully substituted quaternary carbon (δ 61.5 ppm) allows the formulation of an indoline unit whose ^{13}C chemical shifts (Table) compare closely with those for the corrresponding carbons of hodgkinsine[5] and 16-epivindolinine[6].



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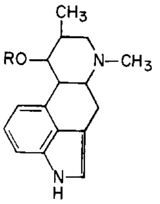

II, R = $COCH_3$
III, R = H

Further support for the indoline moiety in roquefortine is provided by a singlet in the ^{1}H NMR-spectrum at δ 5.70 assigned to the methine hydrogen at position 5a and (at 240 MHz[2]) a pattern similar to that in chaetocin diacetate[7] for the 4 adjacent aromatic protons (positions 7–10).

Reduction of roquefortine with Zn in acetic acid at 70° afforded 2 amorphous 3,12-dihydro derivatives $C_{22}H_{25}N_5O_2$[4] with indoline UV-spectra and lacking the one proton singlet at 6.40 ppm in the ^{1}H NMR-spectra. Both yielded histidine (identified by TLC, paper electrophoresis, and automatic amino acid analysis[2]) on hydrolysis with 5 *N* HCl at 105°, but roquefortine itself did not and hence must contain a dehydrohistidine unit. ^{13}C NMR shifts for C-3, C-12, C-13, C-15 and C-17 of roquefortine (Table) correspond closely with those of the dehydrohistidine moiety of oxaline[8,9].

Heating roquefortine with CH_3OD and D_2O yielded 11a,15-dideuteroroquefortine ($M^+ = 391$), which lacked ^{13}C NMR signals at 58.8 and 136.5 ppm and ^{1}H NMR peaks at 4.12 and 7.73 ppm present in the spectra of roquefortine (Table). The 11a methine proton in roquefortine at δ 4.12 ppm forms part of a $CH_2 \cdot CH \cdot CO$ grouping, since the CH_2 multiplet at δ 2.58 ppm simplifies to a broad singlet in the ^{1}H NMR-spectrum of dideuteroroquefortine.

Strong evidence in support of structure I for roquefortine was provided by its high resolution mass spectrum (characterized by the molecular ion and fragment ions[4]

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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 fumigaclavine 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'-(^{3}H,^{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 (^{3}H,^{14}C)-cantharidin into males during copulation, more than 10% is transferred into the female sex organs. Males injected with 2-(^{3}H,^{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