Novel Pyrazines from the Myxobacterium *Chondromyces crocatus* and Marine Bacteria

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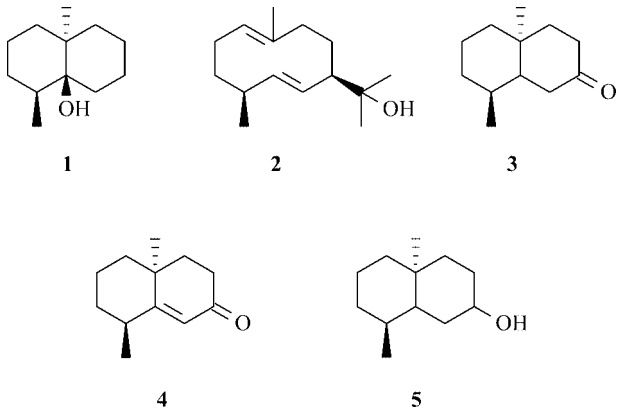
The volatiles released by two strains of the myxobacterium *Chondromyces crocatus* and seven strains of marine Alphaproteobacteria from the North Sea were collected using the CLSA or SPME headspace methods and analysed by GC-MS. In the extracts of *C. crocatus* 27 pyrazines were identified, belonging to different classes. 2,5-Dialkylpyrazines and related 3-methoxy-2,5-dialkylpyrazines dominated. Several pyrazines like 2-(1-methylethenyl)-5-(1-methylethyl)-pyrazine (**7**) and 3-methoxy-2,5-dialkylpyrazines with methyl, isopropyl, isobutyl or *sec*-butyl side-chains were obtained from natural sources for the first time. It was essential for the identification to rely on synthetic reference materials, which were obtained using Fürstner's iron-catalysed coupling of chloropyrazines with Grignard reagents or condensation of azido ketones as key steps. The synthetic material allowed the identification of two previously unknown attractants of bacterial origin for the pineapple beetle *Carpophilus humeralis*, namely 3-methoxy-2-(1-methylpropyl)-5-(2-methylpropyl)pyrazine (**17**) and 3-methoxy-2,5-bis(1-methylpropyl)pyrazine (**52**). Several 2,5-dialkylpyrazines were identified in the extracts of the marine Alphaproteobacteria. The unique 2,5-dimethyl-3-(methylsulfanyl)pyrazine (**67**) represents a new type of natural pyrazine. Our results, together with literature reports, show that pyrazines are an important class of bacterial volatiles which might be more widespread than previously thought.

Introduction

Myxobacteria are known for their unique social life that is expressed by the formation of swarms, fruiting bodies and myxospores.[1] The swarms glide in a film over substrate surfaces, while single cells normally do not leave the swarm. Multicellular fruiting bodies are formed under starvation conditions, and the vegetative cells in these fruiting bodies may convert into myxospores, which are desiccation and heat resistant. These myxospores represent the survival strategy of the myxobacteria. Intercell communication is necessary for all these processes, and several signalling systems have been found in myxobacteria.[2] Stigmolone, for example, was isolated from fruiting *Stigmatella aurantiaca* and is the aggregation pheromone of this species.[3,4]

Myxobacteria have a large genome associated with a broad metabolic potential. During the last two decades several secondary metabolites with promising pharmacological activities have been isolated from different species of myxobacteria.[5–8] Nevertheless, only a few studies deal with the analysis of their volatile compounds, which are normally lost during the isolation procedure of more polar metabolites. Geosmin (**1**) was the first volatile identified in the myxobacterium *Nannocystis exedens* (Figure 1),[9] and it is also released by several other species. Recently, more systematic studies on the volatiles of *Chondromyces crocatus*,[10] *Myxococcus xanthus*[11] and *Stigmatella aurantiaca*[12] have been carried out, but the role of these volatiles in cell-to-cell communication still remains unclear.



1 **2** **3** **4** **5**

Figure 1. Terpenoid compounds from *Chondromyces crocatus*.

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Among the myxobacteria, *Chondromyces crocatus* exhibits a unique odour that is different from that of other myxobacteria. During several years of work with myxobacteria we noticed that this characteristic odour can be used with some confidence to distinguish this species from other myxobacteria. Recently, the volatiles released by the myxobacterium *Chondromyces crocatus* grown on different culture media were investigated by us using CLSA (closed-loop stripping apparatus) or SPME (solid-phase microextraction) headspace methods in combination with GC-MS analyses.[10] The bouquet of the released volatiles was composed of different sesquiterpenes {e.g. (–)-germacrene D and [1(10)*E*,5*E*]-germacradien-11-ol (**2**)}, sesquiterpene degradation products {e.g. **1**, (1*R**,6*R**,10*R**)-6,10-dimethylbicyclo[4.4.0]decan-3-one (**3**) and related compounds **4** and **5**}, aromatic compounds (e.g. 2-phenylethanol, methyl salicylate and methyl anthranilate), and pyrazines (Figure S1 in the Supporting Information to this article). Nevertheless, the identity of several compounds emitted by *C. crocatus*, preferentially pyrazines, remained unknown.

Pyrazines are also present in the bouquet of volatiles released by the Alphaproteobacteria *Loktanella* sp. (strain BIO-204), which occurs in biofilms, and the dinoflagellate associated species *Dinoroseobacter shibae* (strain DFL-27).[13] Therefore the production of pyrazines may be more widespread than previously thought. Seven additional strains of Alphaproteobacteria were investigated for the production of pyrazines. The results of these investigations as well as the identification and synthesis of the unknown *Chondromyces* volatiles are presented here.

Results

The volatiles emitted by two different strains of *C. crocatus* (Cm c2 and Cm c5) grown on two different media (yeast and peptone medium) were analysed by the CLSA or SPME headspace methods as described previously.[10] The results of the headspace analyses are summarised in Table S1 (see Supporting Information), and total ion chromatograms of both strains grown on yeast medium are presented in Figure 2. Besides the compounds already reported,[10] several additional pyrazines were identified in the headspace extracts (Figure 3). Their structures were elucidated by analysis of their mass spectra and verified by comparison with synthetic compounds. The number and total amount of pyrazines present in the headspace extracts was especially high for bacteria grown on yeast medium. The mass spectra of the pyrazines showed fragment ions in the low mass region typically arising from aromatic compounds and exhibited even-numbered molecular ions pointing to an even number of nitrogen atoms. The most intense fragment ions were found in the higher mass region, as is expected for (hetero)cyclic compounds. A comparison with library spectra led to the suggestion that the unknown volatiles were alkylated pyrazine derivatives, with some of them bearing an additional methoxy function. The pyrazines were suggested to be generated from the amino acids alanine, valine, leucine or isoleucine, probably via cyclic dipeptides. Therefore, the volatiles are most likely 2,5-dialkylpyrazines containing methyl, isopropyl, isobutyl and *sec*-butyl groups and, in some cases, an additional methoxy substituent. The presence of an isobutyl group is indicated by the neutral loss of propene [$M^+ - 42$] by McLafferty rearrangement, whereas a *sec*-butyl group shows the neutral loss of ethene [$M^+ - 28$]. Isopropyl pyrazines also exhibit an intense [$M^+ - 28$] ion.[10] All three methyl-branched alkyl groups strongly favour the loss of a methyl fragment. These fragmentation patterns are presented for two exemplary pyrazine derivatives in Figure 4. Structural proposals derived from prominent fragment ions for several pyrazines

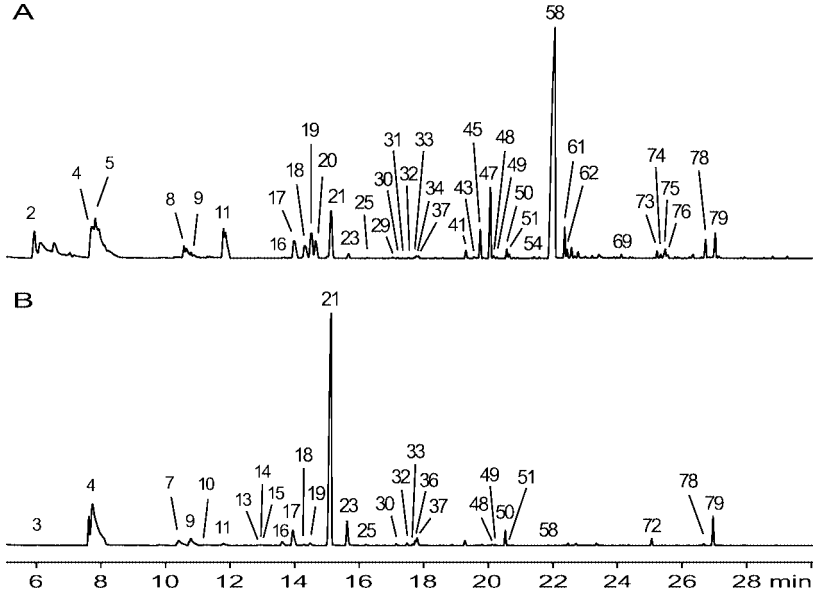


Figure 2. Total ion chromatograms of volatiles collected from strain Cm c2 (A, experiment 5 in Table S1) and strain Cm c5 (B, experiment 6 in Table S1) grown on yeast medium. Peak numbers refer to numbers in Table S1.

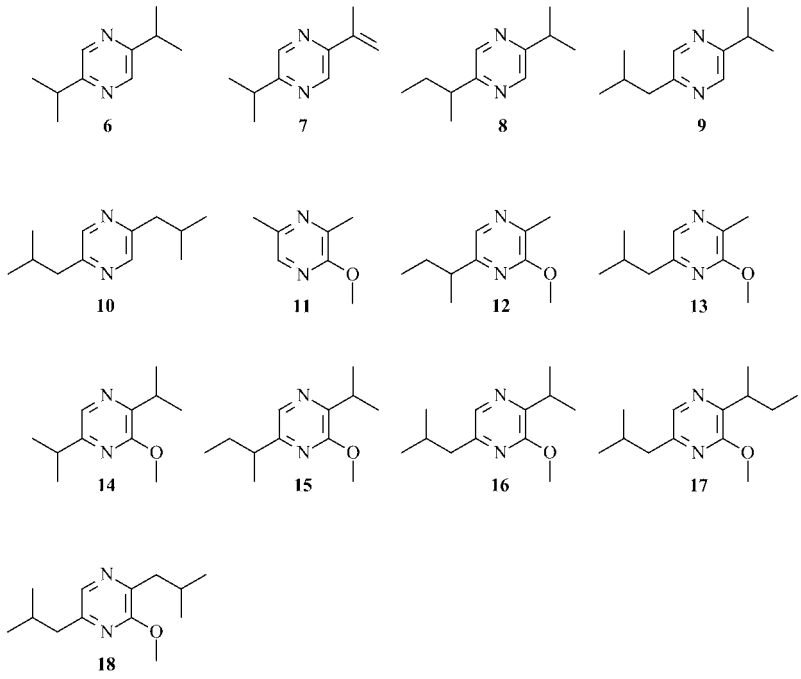


Figure 3. Pyrazines from *Chondromyces crocatus* identified in this study.

present in the headspace extracts are summarised in Table 1. Nevertheless, the exact structures for all unknown pyrazines emitted by *C. crocatus* could not be derived from their mass spectra because the arrangement of substituents on the pyrazine ring cannot be deduced. As outlined above, a 2,5-dialkyl substitution pattern seemed to be most likely, but 2,6-dialkylated pyrazines are also known from *C. crocatus*.[10] Because the mass spectra and retention indices of pyrazines with different arrangement of the substituents are very similar, a synthesis of several regioisomers was carried out to unambiguously identify the volatiles released by this species.

One sample of strain Cm c2 grown on yeast medium contained a volatile with a molecular ion at *m*/*z* = 180 corre-

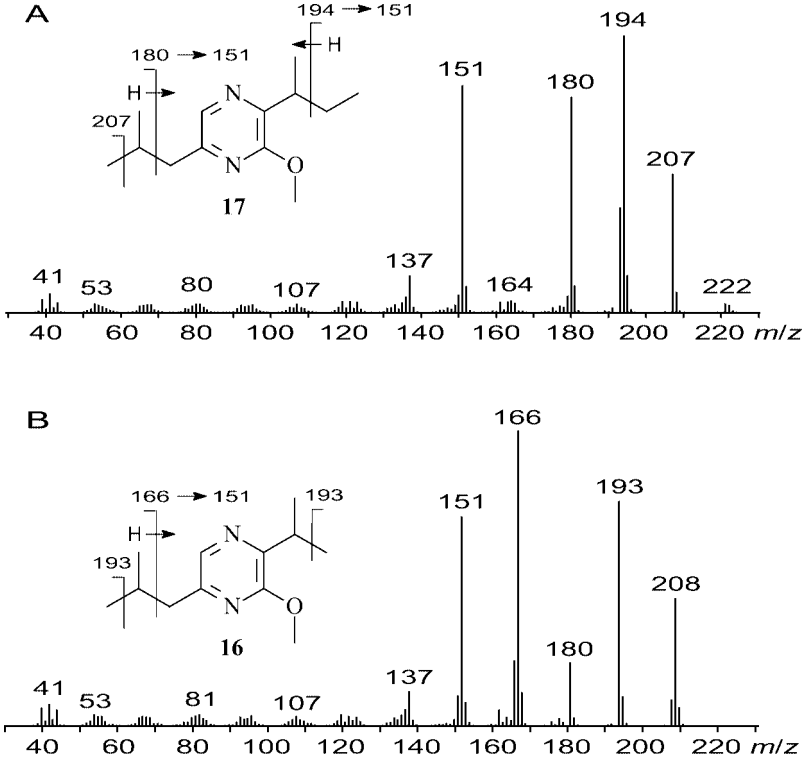


Figure 4. Mass spectra and fragmentation pattern of **17** (A) and **16** (B).

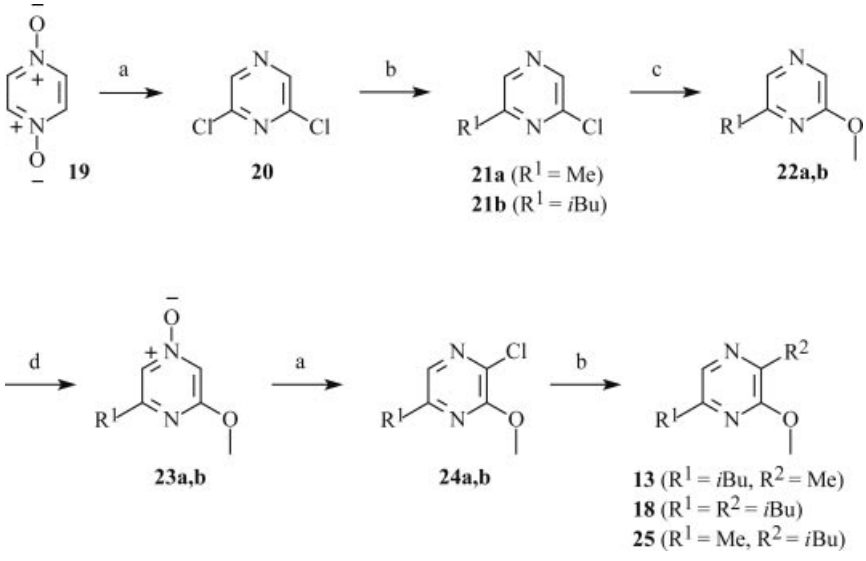
Table 1. Structural proposals derived from molecular ions and important fragment ions in the mass spectra of unknown compounds.

Entry	[M$^+$]	Formula[a]	FI[b]	Substituents[c]
1	180	$C_{10}H_{16}N_2O$	165, 138	Me, *i*Bu
2	222	$C_{12}H_{22}N_2O$	207, 180, 137	2 × *i*Bu, OMe
3	192	$C_{12}H_{20}N_2$	177, 150, 107	2 × *i*Bu
4	194	$C_{11}H_{18}N_2O$	179, 166	2 × *i*Pr, OMe
5	178	$C_{10}H_{16}N_2$	163, 150, 135	*i*Pr, *sec*Bu
6	208	$C_{12}H_{20}N_2O$	193, 180, 165	*i*Pr, *sec*Bu, OMe
7	178	$C_{10}H_{16}N_2$	163, 136, 121	*i*Pr, *i*Bu
8	208	$C_{12}H_{20}N_2O$	193, 180, 166, 151	*i*Pr, *i*Bu, OMe
9	222	$C_{12}H_{22}N_2O$	207, 194, 180, 151	*i*Bu, *sec*Bu, OMe
10	180	$C_{10}H_{16}N_2O$	165, 152	Me, *sec*Bu, OMe

[a] Suggested molecular formulae that are in accordance with the molecular ions. [b] Important fragment ions in the mass spectra of unknown compounds. [c] Presence of substituents derived from mass spectra.

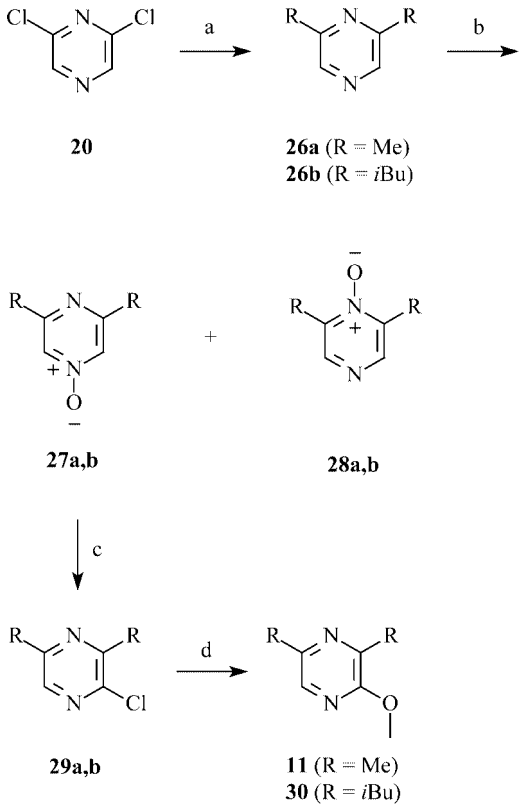
sponding to, for example, $C_{10}H_{16}N_2O$, and major fragment ions at *m*/*z* = 165 and 138 indicating the presence of an isobutyl group (Table 1, entry 1). The unknown volatile was suggested to be one of the regioisomers of methoxy(methyl)(2-methylpropyl)pyrazine, most likely with a 2,5-dialkyl substitution pattern. A synthesis of the two respective methoxypyrazines was started with pyrazine-*N*,*N*′-dioxide (**19**), which was transformed into 2,6-dichloropyrazine (**20**) in boiling $POCl_3$ with high yield (Scheme 1).[14,15] Chloropyrazines can be alkylated with Grignard reagents by $Fe(acac)_3$ catalysis in a procedure developed by Fürstner et al.[13,16] The 2-chloro-6-alkylpyrazines **21a**,**b** were prepared by this method using 1.2 equivalents of methylmagnesium bromide or 2-methylpropylmagnesium bromide, respectively. Reaction with NaOMe in MeOH at 120 °C provided the methoxypyrazines **22a**,**b**.[17] The *N*-oxidation with sodium perborate afforded only one regioisomer (**23a** or **23b**).[18] Analysis of the ^{13}C NMR spectra showed which of the two possible regioisomers was formed in these reactions. While the aromatic carbons in **22a** appear at δ = 132.1 (CH), 135.5 (CH), 150.3 (C), and 159.7 ppm (C), the CH signals of the *N*-oxide are shifted upfield (δ = 120.0 and 126.8 ppm). The signals for the quaternary carbons show a downfield shift (δ = 153.8 and 162.9 ppm). Similar results were obtained for **22b** and the respective *N*-oxide. These observations were expected for **23a** and **23b** because, compared to **22a** and **22b**, the electron density is increased on the aromatic CH carbons whereas it is decreased on the aromatic quaternary carbons. The transformation into the chloropyrazines in refluxing $POCl_3$ also proceeded with high regioselectivity to give only **24a** or **24b**, respectively. The structures of these chloropyrazines were elucidated by 2D NMR experiments. The HMBC spectrum of **24a** shows a coupling of the aromatic proton to the methyl carbon attached to the aromatic ring, to the aromatic C(Cl), and to C(Me), while no cross-peak between this proton and C(OMe) occurs. Analogous results were obtained for **24b**. The alkylation of **24a** following Fürstner's method with isobutylmagnesium bromide gave **25**, whereas its regioisomer **13** was obtained by the alkylation of **24b** with methylmagnesium bromide. The structures of **13** and **25** were rigorously assigned by extensive 2D NMR experiments (H,H-COSY, HSQC and HMBC). A comparison of the retention indices and mass spectra clearly proved the bacterial volatile to be identical with **13**, whereas **25** has a similar mass spectrum but a different retention index.

The same sample of Cm c2 contained another volatile with a molecular ion at *m*/*z* = 222 (e.g. $C_{12}H_{22}N_2O$) and major fragment ions at *m*/*z* = 207, 180 and 137, pointing to two isobutyl groups, while no fragment ion at *m*/*z* = 194 was present, thus excluding the presence of a *sec*-butyl group (Table 1, entry 2). This volatile was proposed to be 3-methoxy-2,5-bis(2-methylpropyl)pyrazine (**18**) or, less



Scheme 1. Synthesis of **13**, **18** and **25**: a) $POCl_3$, reflux; b) R^1MgBr, $Fe(acac)_3$, THF, NMP, 0 °C; c) NaOMe, MeOH, 120 °C; d) $NaBO_2{\cdot}H_2O_2{\cdot}3H_2O$, AcOH, 80 °C.

likely, its regioisomer 2-methoxy-3,5-bis(2-methylpropyl)-pyrazine (**30**). For a synthesis of **18** the chloropyrazine **24b** was alkylated with isobutylmagnesium bromide as outlined above, while **30** was prepared from **20** (Scheme 2). Alkylation with 2.4 equivalents of isobutylmagnesium bromide afforded **26b** in moderate yield. The *N*-oxidation with sodium perborate gave a mixture of two regioisomeric *N*-oxides **27b** and **28b** in a 63:27 ratio, which were separable by column chromatography. The *N*-oxide **27b** was chlorinated in refluxing $POCl_3$ to give **29b**, and reaction with NaOMe furnished **30**. Compound **18** was also prepared by a route that is presented below, thus corroborating the conclusions about the regioselectivity of the oxidation and chlorination steps outlined above. The volatile of strain Cm c2 was unambiguously identified as **18**.

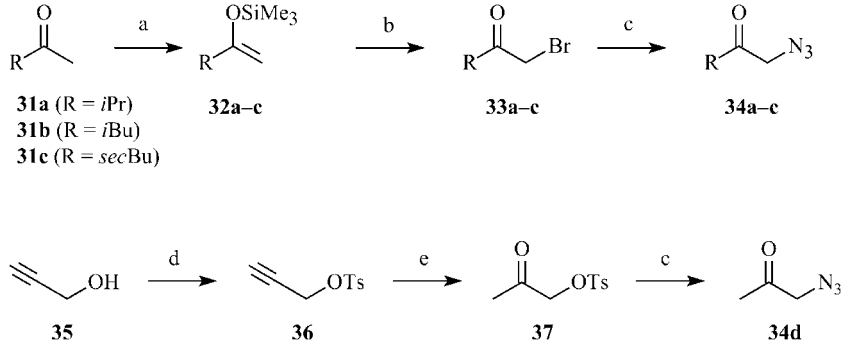


Scheme 2. Synthesis of **11** and **30**: a) RMgBr, $Fe(acac)_3$, THF, NMP, 0 °C; b) $NaBO_2{\cdot}H_2O_2{\cdot}3H_2O$, AcOH, 80 °C; c) $POCl_3$, reflux; d) NaOMe, MeOH, 120 °C.

2-Methoxy-3,5-dimethylpyrazine (**11**), tentatively identified from its mass spectrum, was prepared by the same route, but with methylmagnesium bromide instead of isobutylmagnesium bromide. The synthetic material proved to be identical to the volatile emitted by *C. crocatus*.

The introduction of secondary alkyl groups into chloropyrazines with Fürstner's method turned out to be difficult. Furthermore, 2,5-dialkylpyrazines lacking the methoxy substituent, such as 2,5-bis(2-methylpropyl)pyrazine (**10**), could not be obtained regioselectively with the methods described above because the regioselectivity in the *N*-oxidation step to **23** as well as the chlorination step to **24** (Scheme 1) proved to be strongly influenced by the methoxy group. In addition, the alkylation of alkylpyrazines with Grignard reagents proceeds in low yields to furnish a mixture of 2,3-, 2,5- and 2,6-dialkylpyrazines.[10] Therefore, a known dimerisation procedure with α-azido ketones[19] was used for the preparation of the symmetrically substituted 2,5-dialkylpyrazines **6** and **10**, while the asymmetrically substituted 2,5-dialkylpyrazines **8** and **9** were obtained from mixtures of two different α-azido ketones. The α-azido ketones were prepared from methyl-branched methyl ketones **31** (Scheme 3). Their kinetic enolates, generated by treatment with LDA in THF at –90 °C, were trapped with chlorotrimethylsilane to yield the silylenol ethers **32**.[20] Treatment with bromine[21] and subsequent nucleophilic substitution with NaN_3 provided the α-azido ketones **34**.[22]

The presence of 2,5-bis(1-methylethyl)pyrazine (**6**) in the headspace extracts of *C. crocatus* had already been ascertained by preparation of a mixture of all regiosomeric bis(1-methylethyl)pyrazines.[10] A regioselective synthesis of **6** is possible by a reaction of **34a** with triphenylphosphane (Scheme 4).[19] This volatile is one of the major compounds in the headspace extracts, especially of strain Cm c5; its regioisomer 2,6-bis(1-methylethyl)pyrazine is present in lower amounts. Another volatile released by the bacteria had a molecular ion of m/z = 192 and major fragment ions at m/z = 177, 150 and 107, indicating the presence of two isobutyl groups (Table 1, entry 3). Compound **10** was synthesised from **34b** by the dimerisation procedure (Scheme 4). Its regioisomer **26b** was obtained en route to



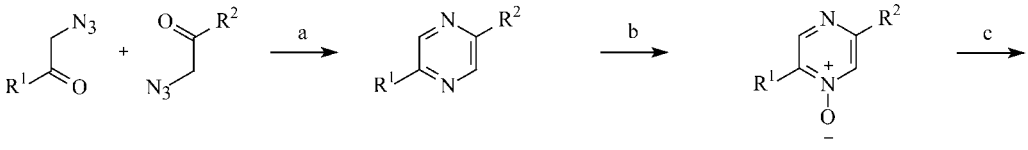
Scheme 3. Synthesis of **34a**–**d**: a) LDA, Me_3SiCl, THF, –90 °C; b) Br_2, pentane, –78 °C; c) NaN_3, AcOH, EtOH, H_2O, 0 °C; d) KOH, *p*-TsCl, Et_2O, 0 °C; e) $HgSO_4$, H_2SO_4, MeOH, H_2O, 60 °C.

30 (Scheme 2). The natural volatile proved to be identical to **10**.

In addition, the headspace extracts of strain Cm c2 grown on yeast contained a volatile that was assumed to be a methoxypyrazine from its mass spectrum. The unknown compound had a molecular ion at *m*/*z* = 194 according to a composition of $C_{11}H_{18}N_2O$, for example, and major fragment ions at *m*/*z* = 179 and 166, indicating the presence of two isopropyl groups (Table 1, entry 4). In conclusion, this compound was suggested to be the methoxy derivative of **6**, 3-methoxy-2,5-bis(1-methylethyl)pyrazine (**14**). A synthesis of **14** was started with **6** (Scheme 4), which was transformed via the *N*-oxide **42a** into the chloropyrazine **47a** by the methods described above. Reaction with NaOMe afforded **14**, which had the same retention index and mass spectrum as its natural counterpart. Compound **18** could also be obtained from **10** (Scheme 4) and was found to be identical to the final product obtained in the sequence outlined in Scheme 1, further corroborating the conclusions regarding the regioselectivity of the *N*-oxidation and the chlorination step as outlined above. Furthermore, **38** and its methoxy derivative **52** were synthesised from **34c** (Scheme 4). Neither compound was detected in the headspace extracts.

The synthesis of asymmetrically substituted 2,5-dialkylpyrazines proved to be more difficult. One trace compound emitted by both strains was characterised by a molecular ion at *m*/*z* = 178 (corresponding to $C_{11}H_{18}N_2$). The fragment ion at *m*/*z* = 136 was present in low abundance only, thus ruling out the presence of an isobutyl group, whereas prominent fragment ions at *m*/*z* = 163, 150 and 135 were in accordance with an isopropyl and a *sec*-butyl group (Table 1, entry 5). Asymmetrically substituted 2-(1-methylethyl)-5-(1-methylpropyl)pyrazine (**8**) was prepared from a 1:1 mixture of **34a** and **34c** (Scheme 4). The dimerisation procedure yielded a mixture of **6**, **8** and **38** (in a ratio of 22:47:31) that was inseparable by column chromatography. Compound **8** was found to be identical to the natural volatile.

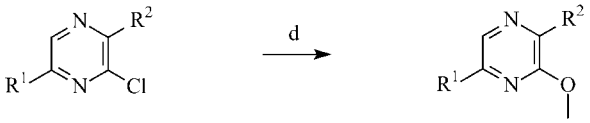
A methoxy derivative of **8** also seemed to be present in the headspace extracts of strain Cm c2. The mass spectrum



34a ($R^1 = R^2 = i$Pr)
34b ($R^1 = R^2 = i$Bu)
34c ($R^1 = R^2 = sec$Bu)
34d (R^1 = secBu, R^2 = Me)

6 ($R^1 = R^2 = i$Pr)
8 ($R^1 = sec$Bu, $R^2 = i$Pr)
9 ($R^1 = i$Bu, $R^2 = i$Pr)
10 ($R^1 = R^2 = i$Bu)
38 ($R^1 - R^2 - sec$Bu)
39 ($R^1 = i$Bu, $R^2 = sec$Bu)
40 ($R^1 = sec$Bu, R^2 = Me)
41 ($R^1 - R^2$ – Me)

42a ($R^1 = R^2 = i$Pr)
42b ($R^1 = R^2 = i$Bu)
42c ($R^1 = R^2 = sec$Bu)
43a ($R^1 = sec$Bu, $R^2 = i$Pr)
43b ($R^1 - i$Pr, $R^2 - sec$Bu)
44a ($R^1 = i$Bu, $R^2 = i$Pr)
44b ($R^1 = i$Pr, $R^2 = i$Bu)
45a ($R^1 - i$Bu, $R^2 - sec$Bu)
45b ($R^1 = sec$Bu, $R^2 = i$Bu)
46 (R^1 = Me, $R^2 = sec$Bu)



47a ($R^1 = R^2 = i$Pr)
47b ($R^1 = R^2 = i$Bu)
47c ($R^1 = R^2 = sec$Bu)
48a ($R^1 = sec$Bu, $R^2 = i$Pr)
48b ($R^1 = i$Pr, $R^2 = sec$Bu)
49a ($R^1 = i$Bu, $R^2 = i$Pr)
49b ($R^1 = i$Pr, $R^2 = i$Bu)
50a ($R^1 = i$Bu, $R^2 = sec$Bu)
50b ($R^1 = sec$Bu, $R^2 = i$Bu)
51a (R^1 = Me, $R^2 = sec$Bu)
51b ($R^1 = sec$Bu, R^2 = Me)

12 ($R^1 = sec$Bu, R^2 = Me)
14 ($R^1 = R^2 = i$Pr)
15 ($R^1 = sec$Bu, $R^2 = i$Pr)
16 ($R^1 = i$Bu, $R^2 = i$Pr)
17 ($R^1 = i$Bu, $R^2 = sec$Bu)
18 ($R^1 = R^2 = i$Bu)
52 ($R^1 = R^2 = sec$Bu)
53 ($R^1 = i$Pr, $R^2 = sec$Bu)
54 ($R^1 = i$Pr, $R^2 = i$Bu)
55 ($R^1 = sec$Bu, $R^2 = i$Bu)
56 (R^1 = Me, $R^2 = sec$Bu)

Scheme 4. Synthesis of 2,5-dialkyl-3-methoxypyrazines: a) PPh_3, benzene, 20 °C; b) $NaBO_2 \cdot H_2O_2 \cdot 3H_2O$, AcOH, 80 °C; c) $POCl_3$, reflux; d) NaOMe, MeOH, 120 °C.

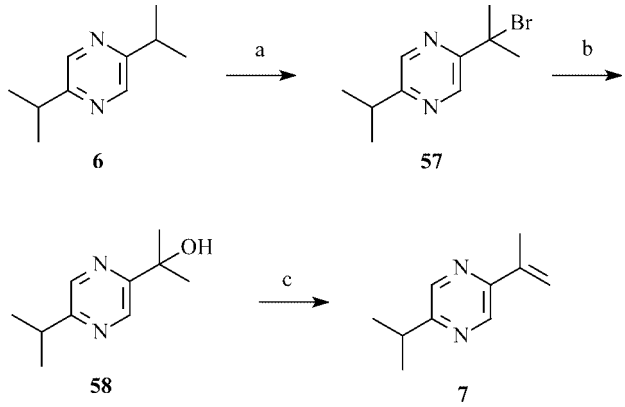
of this compound was characterised by a molecular ion at *m*/*z* = 208 ($C_{12}H_{20}N_2O$) and strong fragment ions at *m*/*z* = 193, 180 and 165 consistent with an isopropyl and a *sec*-butyl group (Table 1, entry 6). For the synthesis of the two regioisomeric methoxy-2-(1-methylethyl)-5-(1-methylpropyl)pyrazines a mixture of **6**, **8** and **38** was used (Scheme 4). The *N*-oxides **42a** and **42c** could be separated from the mixture of **43a** and **43b** (45:55) by column chromatography. This mixture was used for the preparation of chloropyrazines **48a** and **48b** (54:46) and methoxypyrazines **15** and **53** (60:40), which were inseparable by column chromatography. The latter were separated by preparative HPLC, and their structures were rigorously confirmed by extensive 2D NMR experiments. The bacterial volatile was identified as **15**.

C. crocatus emitted two other volatiles, one eluting slightly later in the GC than **8** and the other slightly later than **15**. The mass spectrum of the first one showed a molecular ion at *m*/*z* = 178 and major fragment ions at *m*/*z* = 163, 136 and 121, while the second compound was characterised by a molecular ion at *m*/*z* = 208 and prominent fragment ions at *m*/*z* = 193, 180, 166 and 151 (Table 1, entries 7 and 8). The first compound was suggested to be 2-(1-methylethyl)-5-(2-methylpropyl)pyrazine (**9**), while the second was assumed to be a methoxy derivative thereof. A synthesis of **9** was performed using a 1:1 mixture of **34a** and **34b** (Scheme 4). The reaction with triphenylphosphane provided a mixture of **6**, **9** and **10** (16:52:32), which were not separable by column chromatography. The unknown volatile was identical to **9**. Its two regioisomeric methoxy derivatives were prepared from a mixture of 2,5-dialkylpyrazines. *N*-Oxidation with sodium perborate gave an inseparable mixture of four different *N*-oxides, whereas the chlorination step afforded two fractions after column chromatography. The first one contained **47a** and **49a** (36:64), and the second was composed of **47b** and **49b** (60:40). These two mixtures were treated with NaOMe to furnish the respective methoxypyrazines [mixture of **14** and **16** (34:66) as well as **18** and **54** (60:40)]. The volatile released by strain Cm c2 was shown to be **16**.

The main component emitted by strain Cm c2 grown on yeast medium, but also released in small amounts by strain Cm c5, showed a mass spectrum with a molecular ion at *m*/*z* = 222 (according to $C_{13}H_{22}N_2O$) and strong fragment ions at *m*/*z* = 207, 194, 180 and 151, thus indicating the presence of an isobutyl and a *sec*-butyl group (Table 1, entry 9). The two regioisomeric methoxypyrazines with a 2,5-dialkyl substitution pattern were synthesised from a mixture of **34b** and **34c** (Scheme 4). The three 2,5-dialkylpyrazines **10**, **38** and **39** were separable by column chromatography. Oxidation of **39** furnished a mixture of the *N*-oxides **45a** and **45b** (70:30), which could not be separated, whereas the chloropyrazines **50a** and **50b** were separable. Reaction with NaOMe yielded the methoxypyrazines **17** and **55**. Compound **17** proved to be identical to the bacterial volatile.

Another trace component emitted by strain Cm c2 grown on yeast medium showed a molecular ion at *m*/*z* = 180 (supporting the formula $C_{10}H_{16}N_2O$) and important fragment ions at *m*/*z* = 165 and 152 (Table 1, entry 10). The compound was assumed to be one of the regioisomers of methoxy(methyl)(1-methylpropyl)pyrazine, most likely with 2,5-dialkyl substitution. Therefore azidoacetone (**34d**) was synthesised (Scheme 3). Hydroxyacetone *p*-toluenesulfonate (**37**) was used for its preparation because bromoacetone is highly lachrymatory. Propargyl alcohol (**35**) was tosylated to yield **36**.[23] An oxymercuriation[24] furnished **37**, and treatment with NaN_3 gave **34d**. The 1:1 mixture of **34c** and **34d** provided three different 2,5-dialkylpyrazines **38**, **40** and **41**, which could easily be separated by column chromatography (Scheme 4). Compound **40** was used in the oxidation step to **46**. Its regioisomeric *N*-oxide was not formed, probably due to the steric hindrance of the *sec*-butyl group. Nevertheless, chlorination in refluxing $POCl_3$ gave a mixture of two chloropyrazines **51a** and **51b** in a ratio of 68:32. In contrast to the chloropyrazines, the respective methoxypyrazines **56** and **12** were separable by column chromatography. The unknown volatile of strain Cm c2 was found to be identical to **12**.

As outlined in the previous report on the volatiles released by *C. crocatus*,[10] 2-(1-methylethenyl)-6-(1-methylethyl)pyrazine, 2-(1-hydroxy-1-methylethyl)-6-(1-methylethyl)pyrazine and 2-(1-methylethenyl)-5-(1-methylethyl)-pyrazine (**7**) were tentatively identified in the headspace extracts from their mass spectra. To corroborate these assumptions, **6** was brominated in a radical reaction with *N*-bromosuccinimide (NBS) and azobisisobutyronitrile (AIBN) to provide **57** (Scheme 5). The reaction with AgOH furnished **58**, which did not match the retention index of the compound present in the extracts, but had a similar mass spectrum. Therefore, the identity of the volatile as 2-(1-hydroxy-1-methylethyl)-6-(1-methylethyl)pyrazine was further corroborated. Treatment of **58** with acetic acid and concentrated sulfuric acid (4:1, v:v) yielded the elimination product **7**, which was identical to the later eluting isomer (GC) of the (1-methylethenyl)(1-methylethyl)pyrazines. Because of the great similarities between the mass spectra of **7** and tentatively identified 2-(1-methylethenyl)-6-(1-methylethyl)pyrazine, the structural proposal for the first eluting regioisomer seems also to be correct. The alternative structures of 2-(1-methylethenyl)-3-(1-methylethyl)pyrazine and



Scheme 5. Synthesis of **7**: a) NBS, AIBN, CCl_4, reflux; b) AgOH, H_2O, 30 °C; c) AcOH, H_2SO_4, 70 °C.

2-(1-hydroxy-1-methylethyl)-3-(1-methylethyl)pyrazine are unlikely because no 2,3-dialkylpyrazines are produced by *Chondromyces crocatus*, whereas 2,5- and 2,6-dialkylpyrazines are present in the extracts.

In a recent investigation on the volatiles released by marine bacteria from the North Sea we showed that the complex headspace bouquets of *Loktanella* sp. (strain BIO-204) and *Dinoroseobacter shibae* (strain DFL-27) are composed of several compound classes including pyrazines.[13] Two alkylated pyrazines, 3-butyl-2,5-dimethylpyrazine (**64**) and 2,5-dimethyl-3-(3-methylbutyl)pyrazine (**66**), are emitted by strain BIO-204, while strain DFL-27 produces **64** (Table 2, Figure 5). These compounds were synthesised from **41** via 2,5-dimethylpyrazine *N*-oxide (**68**) and 2-chloro-3,6-dimethylpyrazine (**69**) according to the procedures described above (Scheme 6). Alkylation by Fürstner's method using the respective Grignard reagents furnished **64** and **66**.[13] We then analysed seven other strains from the North Sea, all of which release pyrazines along with other compounds. 2-Ethyl-5-methylpyrazine (**59**), 5-methyl-2-(1-methylethyl)-pyrazine, 3-ethyl-2,5-dimethylpyrazine (**60**) and tetramethylpyrazine (**61**) are emitted by most strains, whereas ethyltrimethylpyrazine (**62**) is only produced by one strain (BIO-007). These pyrazines were tentatively identified from their mass spectra. Compounds **64** and **66** are released by two other strains (BIO-007 and BIO-205). Strain BIO-007 emits two more pyrazines, one eluting shortly before **64** and one shortly before **66** in the GC. From their mass spectra and retention indices these compounds were suggested to be 2,5-dimethyl-3-(2-methylpropyl)pyrazine (**63**) and 2,5-dimethyl-3-(2-methylbutyl)pyrazine (**65**). A synthesis from **69** confirmed these suggestions. Furthermore, 2,5-dimethyl-3-(methylsulfanyl)pyrazine (**67**) was identified from its mass spectrum in the extract of strain BIO-007 and prepared

Table 2. Pyrazines identified in the headspace extracts of North Sea bacteria.

Compd.	I[a]	Ident[b]	BIO-007[c]	BIO-204[c,d]	BIO-205[c]	PIC-68[c]	PIC-70[c]	DFL-11[c]	DFL-27[c,d]	HEL-26[c]	HEL-45[c]
			Sulfitobacter pontiacus	*Loktanella hongkongensis*	*Sulfitobacter dubius*	*Roseobacter gallaeciensis*	*Sulfitobacter sp.*	*Stappia marina*	*Dinoroseobacter shibae*	*Jannaschia helgolandensis*	*Oceanibulbus indolifex*
59	1015	ms	xx[e]	x	–	x	x	x	x	–	x
A	1067	ms	x	x	–	x	x	x	x	x	x
60	1088	ms	xx	x	xx	x	xx	–	x	–	x
61	1096	ms	x	x	x	–	–	x	x	–	–
62	1164	ms	x	–	–	–	–	–	–	–	–
63	1207	syn	x	–	–	–	–	–	–	–	–
67	1260	syn	x	–	–	–	–	–	–	–	–
64	1263	syn	x	x	x	–	–	–	x	–	–
65	1308	syn	x	–	–	–	–	–	–	–	–
66	1321	syn	x	x	x	–	–	–	–	–	–

A: 5-methyl-2-(1-methylethyl)pyrazine, **59**: 2-ethyl-5-methylpyrazine, **60**: 3-ethyl-2,5-dimethylpyrazine, **61**: tetramethylpyrazine, **62**: ethyltrimethylpyrazine, **63**: 2,5-dimethyl-3-(2-methylpropyl)pyrazine, **67**: 2,5-dimethyl-3-(methylsulfanyl)pyrazine, **64**: 3-butyl-2,5-dimethylpyrazine, **65**: 2,5-dimethyl-3-(2-methylbutyl)pyrazine, **66**: 2,5-dimethyl-3-(3-methylbutyl)pyrazine

[a] Retention index. [b] Identification from mass spectrum (ms) or by comparison to reference compounds (syn). [c] Strain names of different species of North Sea bacteria. [d] Results from literature.[13] [e] Relative amounts. –: not detected; x: 0–2%; xx: 2–8%; xxx: >8% of total area in GC.

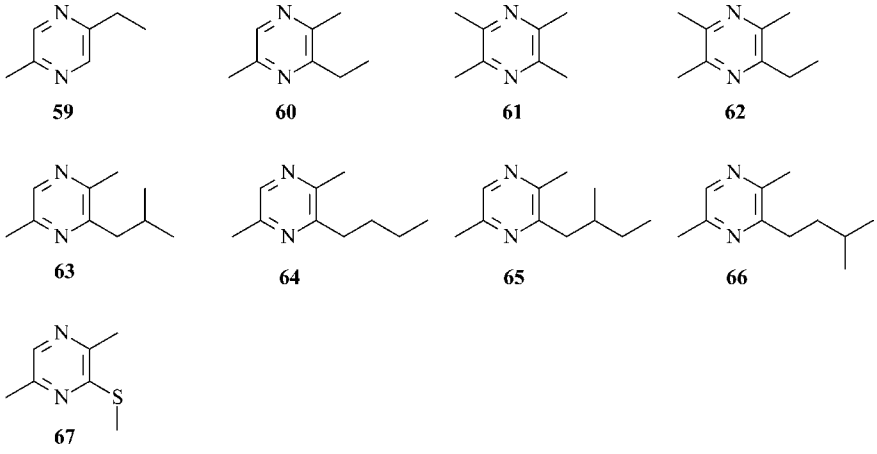
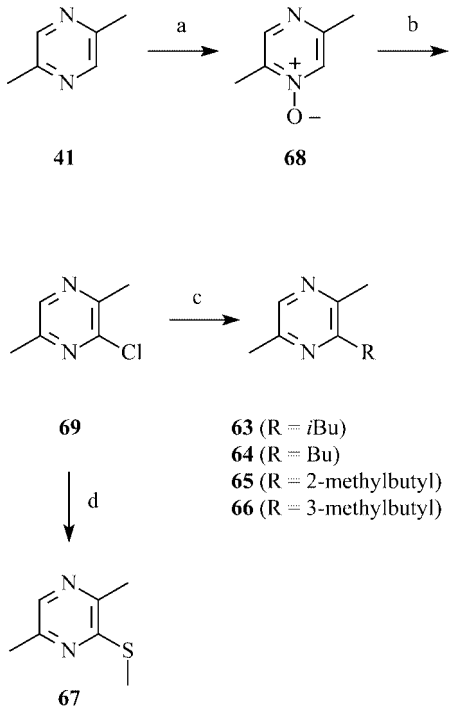


Figure 5. Pyrazines from North Sea bacteria.

from **69** by a reaction with NaSMe similar to the known procedure for the synthesis of methoxypyrazines (Scheme 6).



Scheme 6. Synthesis of **63**–**66** and **67**: a) $NaBO_2 \cdot H_2O_2 \cdot 3H_2O$, AcOH, 80 °C; b) $POCl_3$, reflux; c) RMgBr, $Fe(acac)_3$, THF, NMP, 0 °C; d) NaSMe, DME, 150 °C.

Discussion

In the present and previous work[10] on the volatiles emitted by *Chondromyces crocatus*, 27 different pyrazines were identified, mostly by comparison with synthetic samples. It is so far the only myxobacterium known to produce pyrazines. The occurrence of regioisomeric pyrazines with similar mass spectra, for example **6** and 2,6-bis(1-methylethyl)-pyrazine or **7** and 2-(1-methylethenyl)-6-(1-methylethyl)pyrazine, shows the need for synthetic reference material to unequivocally establish the structure of a given compound. The target compounds can be conveniently synthesised using Fürstner's Grignard coupling as the key step.[16] In the case of secondary alkylmagnesium halides, which proved to be of low reactivity in our hands, defined mixtures can be obtained by condensation of different azido ketones in good yields.

Pyrazines exhibit strong odour properties and are relatively widespread in nature. They are important flavouring components and have been identified from several bacteria,[25,26] mostly originating from fermenting food. Methyl and ethyl derivatives like 2,5-dimethylpyrazine are particularly common and may serve a communicative role in plants in some instances.[27,28] Higher dialkylpyrazines occur less often in nature. The regioisomers **6** and 2,6-bis(1-methylethyl)pyrazine have been identified as metabolites of the bacterium *Paenibacillus polymyxa*, whereas **8**–**10** have been tentatively identified in this species based on mass spectral evidence.[29] The pyrazine **6** is also an important constituent of the attractant of the pineapple beetle *Carpophilus humeralis* and is produced by microorganisms living on rotting pineapples.[30] The unsaturated analogues **7** and 2-(1-methylethenyl)-6-(1-methylethyl)pyrazine have not been reported from nature before. The trialkylpyrazines **63**–**66** are found in several insects, such as flies[31] and ants.[32] Pyrazine **64** has been found as a fermentation product of cocoa,[33] while **63** was tentatively identified from *P. polymyxa*.[29]

The second pyrazine class of *C. crocatus* consists of methoxypyrazines, of which the monoalkyl compounds 2-methoxy-3-methylpyrazine, 2-methoxy-3-(1-methylethyl)-pyrazine, 2-methoxy-3-(2-methylpropyl)pyrazine and 2-methoxy-3-(1-methylpropyl)pyrazine, which have been reported previously,[10] are relatively widespread in nature. They can be detected in very low concentrations by humans by their characteristic odour. Occurrences include different plants,[34–36] microorgansims[37,38] and insects. The isopropyl derivative is a pheromone of ladybird beetles (*Coccinella septempunctata*).[39] Furthermore, their wide occurrence in aposematic coloured insects has led to the theory that they serve as general warning odours of toxic insects.[36] The unique benzyl derivative 2-benzyl-3-methoxypyrazine has not been reported elsewhere so far, while the hydroxyalkylpyrazines 2-(1-hydroxy-1-methylethyl)-3-methoxypyrazine, 2-(1-hydroxy-1-methylpropyl)-3-methoxypyrazine, 2-(1-hydroxy-2-methylpropyl)-3-methoxypyrazine and 2-(1-hydroxy-1-methylethyl)-6-(1-methylethyl)pyrazine have been discussed previously by us.[10]

Dialkylmethoxypyrazines are important constituents of *C. crocatus*. The methoxypyrazine **11** is present in raw arabica coffee,[40] aerobic Gram-negative bacteria,[41] and is responsible for the musty odour of wine corks.[42] Only a few 2,5-dialkyl-3-methoxypyrazines have been identified so far from bacteria.[43] The derivatives **12**–**18** have been identified from natural sources for the first time and are probably derived from the amino acids alanine, valine, leucine and isoleucine, respectively, via cyclic dipeptides. Besides **6**, the pineapple beetle *C. humeralis* is attracted to two additional pyrazines whose structures were not elucidated.[30] By comparison with our mass spectra, we can assign these compounds to structures **17** and **52**. The latter compound is not released by *C. crocatus*, but obviously by the bacteria on rotting pineapple. The identified pyrazines are closely related to demethylated analogues that occur in the fungus *Aspergillus*.[44]

Finally, the (methylsulfanyl)pyrazine **67**, which occurs in strain BIO-007, represents the first member of a new class of natural pyrazines. Generally, pyrazines seem to constitute one of the major classes of volatiles released by bacteria. This is exemplified by the fact that all of the seven randomly chosen marine isolates contain them.

The biosynthesis of pyrazines is a controversial topic in the literature. One proposed pathway proceeds through the amidation of an amino acid, followed by condensation with

an α,β-dicarbonyl compound and subsequent methylation to give methoxypyrazines. Following this pathway, compounds 2-methoxy-3-(1-methylethyl)pyrazine, 2-methoxy-3-(2-methylpropyl)pyrazine and 2-methoxy-3-(1-methylpropyl)pyrazine were suggested to arise by condensation of valine, leucine and isoleucine, respectively, with glyoxal[34] or glyoxylic acid.[35] Alternatively, the biosynthesis of pyrazines might proceed via cyclic dipeptides.[45–47]

The function of the volatiles, especially their role in cell–cell communication, and the biosynthesis of pyrazines are currently under investigation.

Experimental Section

Strains and Culture Conditions: The strains and culture conditions for *Chondromyces crocatus* were described previously.[10] The isolation and phylogenetic affiliation of the strains investigated here have been described by Allgaier et al.[48] Strains BIO-007, BIO-204, and BIO-205 were isolated from biofilms grown on a glass plate exposed to the North Sea for 14 d.[48] Analysis of the almost complete 16S rRNA sequence showed that BIO-204 was 98% similar to *Loktanella hongkongensis* and thus can be assumed to be a new strain of this species or a new species within this genus. Strains BIO-007 and BIO-205 are new strains of the species *Sulfitobacter pontiacus* (99%) and *S. dubius* (99%), respectively. Strains PIC-68 and PIC-70 were enriched from filtered North Sea water. PIC-68 is a strain of the species *Roseobacter gallaeciensis* (97%), while PIC-70 affiliates with various *Sulfitobacter* species (around 96%) and thus can be assumed to be a new genus or species within the *Roseobacter* group. Strain DFL-11 was isolated from cells of the dinoflagellate *Alexandrium lusitanicum* and is described as *Stappia alexandrii*.[49] Strain DFL-27 was isolated from single cells of the dinoflagellate *Alexandrium ostenfeldii* and was recently described as *Dinoroseobacter shibae*.[50] HEL-26 and HEL-45 were isolated from a North Sea water sample and represent two new genera, *Jannaschia helgolandensis*[51] and *Oceanibulbus indolifex*.[52] Cultivation conditions for headspace analysis have been described previously.[13]

Headspace Analysis: For investigations with *Chondromyces crocatus* the CLSA and SPME headspace methods were used, whereas all strains of the marine bacteria were investigated by CLSA. These headspace methods were carried out as described previously.[10,11]

GC-MS: GC-MS analyses were carried out on an HP 6890 Series GC System connected to a HP 5973 mass-selective detector (Hewlett–Packard) fitted with a BPX5 fused-silica capillary column (25 m × 0.22 mm i. d., 0.25 μm film, SGE). Conditions were as follows: inlet pressure: 77.1 kPa, He 23.3 mL min^{-1}; injection volume: 1 μL; transfer line: 300 °C; electron energy: 70 eV. The GC was programmed as follows: 5 min at 50 °C increasing at 5 °C min^{-1} to 320 °C, and operated in splitless mode (60 s valve time). The carrier gas was He at 1 mL min^{-1}. Retention indices, *I*, were determined from a homologous series of *n*-alkanes (C_8–C_{38}). Identification of compounds was performed by comparison of mass spectra to the Wiley 6 Library and the Essential Oils Library (Massfinder) and by comparison with synthetic standards (see Table 1 for details).

General Remarks: Chemicals were purchased from Fluka Chemie GmbH (Buchs, Switzerland) or Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and used without further purification. Solvents were purified by distillation and dried according to standard methods. All reactions were carried out under an inert atmosphere of N_2 in oven-dried glassware. Thin-layer chromatography was carried out using 0.2 mm pre-coated plastic sheets Polygram Sil G/UV$_{254}$ (Marcherey–Nagel) and viewed by UV (254 nm). Column chromatography was performed with Merck silica gel 60 (70–200 mesh). ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AMX400 spectrometer. The structures of regioisomeric pyrazines were assigned by 2D NMR methods (H,H-COSY, HSQC, and HMBC). Physical and spectroscopic data are given for exemplary compounds. The respective data of the other synthesised compounds are given in the Supporting Information.

Preparation of Chloropyrazines: Similar to the method of Sato and Fujii,[15] the pyrazine *N*-oxide was added in small portions to freshly distilled, ice-cooled $POCl_3$ until the concentration reached 2 mol L^{-1}. The mixture was heated under reflux for 6 h and then poured onto ice. The aqueous layer was made alkaline by the addition of 6 N NaOH and then extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel to give the chloropyrazine.

2,6-Dichloropyrazine (20): This compound was synthesised from **19** (Scheme 1). Yield: 84% (24.9 g, 167 mmol). TLC (pentane/diethyl ether, 20:1): R_f = 0.41. GC: *I* = 1017. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.53 (s, 2 H, 2 × CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 142.4 (2 × CH), 147.8 (2 × C) ppm. EI-MS (70 eV): *m*/*z* (%) = 148 (100) [M$^+$], 113 (71), 86 (52), 60 (49), 51 (58), 38 (11).

Preparation of Alkylpyrazines: According to the method of Fürstner et al.,[16] a solution of the alkylmagnesium bromide, freshly prepared from the respective alkyl bromide (1.2 equiv.) and magnesium (1.2 equiv.) in THF (1 mol L^{-1}), was added to an ice-cooled solution of the chloropyrazine (1.0 equiv.) and $Fe(acac)_3$ (5 mol-%) in dry THF/NMP, (10:1, 0.25 mol L^{-1}). For the introduction of a methyl group a commercially available solution of MeMgCl in THF (1 mol L^{-1}) was used. The reaction mixture was stirred for 15 min at 0 °C and then quenched by the addition of water. The aqueous layer was extracted three times with diethyl ether. The combined organic layers were dried ($MgSO_4$) and the solvents were removed under reduced pressure. Column chromatography on silica gel yielded the alkylated pyrazine.

2-Chloro-6-methylpyrazine (21a): This compound was synthesised from **20** (Scheme 1). Yield: 82% (6.30 g, 49.2 mmol). TLC (pentane/diethyl ether, 5:1): R_f = 0.39. GC: *I* = 986. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.57 (s, $^1J_{C,H}$ = 128.5 Hz, 3 H, CH_3), 8.37 (s, $^1J_{C,H}$ = 183.4 Hz, 1 H, CH), 8.42 (s, $^1J_{C,H}$ = 192.7 Hz, 1 H, CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 21.1 (CH_3), 141.5 (CH), 142.2 (CH), 148.3 (C), 154.1 (C) ppm. EI-MS (70 eV): *m*/*z* (%) = 128 (100) [M$^+$], 101 (8), 93 (24), 87 (35), 66 (60), 60 (32), 51 (14), 39 (43).

Preparation of Methoxypyrazines: As described by Ohta et al.,[17] a stainless steel autoclave was charged with the chloropyrazine (1 equiv.) and a solution of freshly prepared NaOMe in MeOH (0.5 mol L^{-1}, 5 equiv.). The reaction mixture was heated to 120 °C for 3 h. The solvent was then removed under reduced pressure and the residue was diluted with water. The aqueous layer was extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated. Purification by column chromatography on silica gel afforded the methoxypyrazine.

2-Methoxy-6-methylpyrazine (22a): This compound was synthesised from **21a** (Scheme 1). Yield: 69% (1.12 g, 9.03 mmol). TLC (pentane/diethyl ether, 5:1): R_f = 0.30. GC: *I* = 994. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.44 (s, $^1J_{C,H}$ = 127.6 Hz, 3 H, CH_3), 3.95 (s, $^1J_{C,H}$ = 146.3 Hz, 3 H, CH_3), 7.98 (s, $^1J_{C,H}$ = 182.0 Hz, 1 H, CH), 8.02 (s, $^1J_{C,H}$ = 186.4 Hz, 1 H, CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 21.0 (CH_3), 53.3 (CH_3), 132.1 (CH),

135.5 (CH), 150.3 (C), 159.7 (C) ppm. EI-MS (70 eV): *m/z* (%) = 124 (100) [M^+], 109 (2), 95 (55), 81 (8), 67 (21), 54 (38), 40 (33).

Preparation of Pyrazine *N*-Oxides: As described by Ohta and Ohta,[18] a solution of the pyrazine (1 equiv.) and $NaBO_2{\cdot}H_2O_2{\cdot}3H_2O$ (1.2 equiv.) in glacial acetic acid (0.25 mol L^{-1}) was heated to 80 °C for 5 h. The solvent was removed under reduced pressure and the residue was diluted with 2 N NaOH. The aqueous layer was extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel with diethyl ether to give the pyrazine *N*-oxides. In the case of 2,6-dialkylpyrazines or asymmetrically substituted 2,5-dialkylpyrazines two regioisomeric dialkylpyrazine *N*-oxides were obtained, while a methoxy substituent led to only one product with the *N*-oxide function in the *meta*-position relative to the methoxy group.

3-Methoxy-5-methylpyrazine 1-Oxide (23a): This compound was prepared from **22a** (Scheme 1). Yield: 62% (0.78 g, 5.57 mmol); m.p. 133 °C. TLC (diethyl ether/methanol, 20:1): R_f = 0.35. GC: *I* = 1375. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.41 (s, $^1J_{C,H}$ = 128.9 Hz, 3 H, CH_3), 3.98 (s, $^1J_{C,H}$ = 147.3 Hz, 3 H, CH_3), 7.70 (s, $^1J_{C,H}$ = 193.0 Hz, 1 H, CH), 7.71 (s, $^1J_{C,H}$ = 191.4 Hz, 1 H, CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 21.4 (CH_3), 54.4 (CH_3), 120.0 (CH), 126.8 (CH), 153.8 (C), 162.9 (C) ppm. EI-MS (70 eV): *m/z* (%) = 140 (100) [M^+], 123 (12), 110 (38), 95 (14), 81 (15), 66 (29), 54 (28), 42 (53). $C_6H_8N_2O_2$ (140.14): calcd. C 51.42, H 5.75, N 19.99; found C 51.47, H 5.91, N 19.81.

Preparation of Silylenol Ethers: According to the method of Bach and Jödicke,[20] a solution of MeLi in diethyl ether (62.5 mL, 1.6 mol L^{-1}, 0.10 mol) was added dropwise over 1 h to an ice-cooled solution of *i*Pr_2NH (10.1 g, 0.10 mol) in dry diethyl ether (150 mL). The solution was cooled to –78 °C and then a solution of Me_3SiCl (11.8 g, 0.12 mol) in dry diethyl ether (30 mL) was added dropwise. The mixture was cooled to –90 °C and a solution of the respective methyl ketone (0.10 mol) in dry diethyl ether (30 mL) was added dropwise. The reaction mixture was stirred at this temperature for 30 min and then allowed to warm to room temperature. The solvent was removed under reduced pressure. Pure silylenol ethers were obtained by distillation as colourless liquids.

3-Methyl-2-trimethylsilyloxybut-1-ene (32a): This compound was prepared from **31a** (Scheme 3). Yield: 69% (10.9 g, 69.1 mmol). B.p. 25–27 °C (50 mbar). GC: *I* = 808. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 0.00 (s, 9 H, 3×CH_3), 0.82 (d, $^3J_{H,H}$ = 6.8, $^1J_{C,H}$ = 126.1 Hz, 6 H, 2×CH_3), 2.00 (dsept, $^4J_{H,H}$ = 0.4, $^3J_{H,H}$ = 6.7 Hz, 1 H, CH), 3.76 (d, $^2J_{H,H}$ = 1.0 Hz, 1 H, CH_2), 3.83 (m, 1 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 0.05 (3×CH_3), 20.8 (2×CH_3), 34.8 (CH), 87.4 (CH_2), 165.1 (C) ppm. EI-MS (70 eV): *m/z* (%) = 158 (25) [M^+], 143 (59), 130 (3), 115 (3), 101 (3), 85 (2), 75 (100), 73 (65), 59 (6), 45 (18).

Preparation of α-Bromo Ketones: As described by Blanco et al.,[21] a solution of bromine (1 equiv.) in pentane (3 mol L^{-1}) was added dropwise at –78 °C to a solution of the silylenol ether in pentane (0.25 mol L^{-1}). The reaction mixture was stirred for 30 min at –78 °C, warmed to room temperature, and then water (300 mL) was added. The aqueous layer was extracted with pentane (3×200 mL). The combined organic layers were dried with $MgSO_4$ and concentrated. The residue was purified by distillation to give the α-bromo ketones as colourless liquids. ***Caution!*** *The α-bromo ketones are highly lachrymatory.*

1-Bromo-3-methylbutan-2-one (33a): This compound was prepared from **32a** (Scheme 3). Yield: 79% (16.6 g, 105 mmol). B.p. 87 °C (70 mbar). GC: *I* = 930. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.17 (d, $^3J_{H,H}$ = 6.9, $^1J_{C,H}$ = 128.0 Hz, 6 H, 2×CH_3), 2.99 (sept, $^3J_{H,H}$ = 6.9 Hz, 1 H, CH), 3.99 (s, $^1J_{C,H}$ = 150.1 Hz, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 18.4 (2×CH_3), 33.0 (CH_2), 38.2 (CH), 205.5 (CO) ppm. EI-MS (70 eV): *m/z* (%) = 164 (3) [M^+], 121 (5), 93 (10), 79 (2), 71 (71), 55 (2), 43 (100).

Preparation of α-Azido Ketones: As described by Boyer and Straw,[22] a solution of the respective α-bromo ketone (1 equiv.) and acetic acid (2 equiv.) in ethanol (1 mol L^{-1}) and a solution of NaN_3 (2 equiv.) in water (5 mol L^{-1}) were cooled to 0 °C. The two solutions were combined and allowed to stay at 0 °C for 20 h. The solvents were removed under reduced pressure. Water (100 mL) was added and the aqueous layer was extracted with diethyl ether (3×100 mL). The combined organic extracts were dried with $MgSO_4$ and concentrated to yield the pure α-azido ketones as pale-yellow liquids.

1-Azido-3-methylbutan-2-one (34a): This compound was prepared from **33a** (Scheme 3). Yield: 100% (5.18 g, 40.8 mmol). GC: *I* = 992. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.15 (d, $^3J_{H,H}$ = 7.0, $^1J_{C,H}$ = 128.0 Hz, 6 H, 2×CH_3), 2.68 (sept, $^3J_{H,H}$ = 6.9 Hz, 1 H, CH), 4.02 (s, $^1J_{C,H}$ = 140.4 Hz, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 18.2 (2×CH_3), 38.9 (CH), 55.9 (CH_2), 208.2 (CO) ppm. EI-MS (70 eV): *m/z* (%) = 71 (50) [M^+ – CH_2N_3], 55 (3), 43 (100), 41 (59), 39 (24).

Preparation of 2-Propynyl *p*-Toluenesulfonate (36): As described by Burton et al.,[23] KOH (56.0 g, 1.0 mol) was added in small portions to a cooled (–10 °C) solution of **35** (5.60 g, 0.10 mol) and *p*-toluenesulfonyl chloride (22.8 g, 0.12 mol) in dry diethyl ether (150 mL, Scheme 3). The reaction mixture was stirred for 30 min at 0 °C and then poured onto ice water. The aqueous layer was extracted three times with diethyl ether. The combined organic layers were dried and concentrated. The residue was purified by column chromatography on silica gel with pentane/diethyl ether (5:1) to yield **36** (17.9 g, 85.2 mmol, 85%) as a colourless liquid. TLC (pentane/diethyl ether, 5:1): R_f = 0.25. GC: *I* = 1666. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.45 (s, $^1J_{C,H}$ = 127.4 Hz, 3 H, CH_3), 2.49 (t, $^3J_{H,H}$ = 2.5, $^1J_{C,H}$ = 254.3 Hz, 1 H, CH), 4.70 (d, $^3J_{H,H}$ = 2.5, $^1J_{C,H}$ = 155.0 Hz, 2 H, CH_2), 7.36 (d, $^3J_{H,H}$ = 8.1 Hz, 2 H, 2×CH), 7.81 (d, $^3J_{H,H}$ = 8.3 Hz, 2 H, 2×CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 21.3 (CH_3), 57.0 (CH_2), 75.0 (C), 77.0 (CH), 127.8 (2×CH), 129.5 (2×CH), 132.5 (C), 144.9 (C) ppm. EI-MS (70 eV): *m/z* (%) = 210 (4) [M^+], 155 (12), 139 (15), 130 (32), 118 (20), 107 (3), 91 (100), 77 (10), 65 (41), 51 (8), 39 (30).

Preparation of Hydroxyacetone *p*-Toluenesulfonate (37): According to the method of Prib and Yasinskii,[24] **36** (17.9 g, 85.2 mmol) was added in one portion to a mixture of methanol (50 mL), water (5 mL), conc. H_2SO_4 (1.5 mL) and $HgSO_4$ (2.0 g, 6.7 mmol) at 60 °C (Scheme 3). A yellowish solid precipitated after stirring for 10 min that later on turned to grey. The reaction mixture was stirred for 2 h and then another portion of $HgSO_4$ (2.0 g) was added. The mixture was stirred for an additional 2 h at 60 °C and then concentrated under reduced pressure. The residue was diluted with water (100 mL). The aqueous layer was extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated to give pure **37** (17.2 g, 75.4 mmol, 89%) as a colourless liquid. GC: *I* = 1778. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.20 (s, $^1J_{C,H}$ = 128.7 Hz, 3 H, CH_3), 2.46 (s, $^1J_{C,H}$ = 127.4 Hz, 3 H, CH_3), 4.50 (s, $^1J_{C,H}$ = 149.2 Hz, 2 H, CH_2), 7.36–7.39 (m, 2 H, 2×CH), 7.79–7.83 (m, 2 H, 2×CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 21.2 (CH_3), 26.1 (CH_3), 71.6 (CH_2), 127.6 (2×CH), 129.6 (2×CH), 131.9 (C), 145.1 (C), 200.6 (CO) ppm. EI-MS (70 eV): *m/z* (%) = 228 (5) [M^+], 198 (3), 155 (28), 150

(29), 139 (3), 119 (14), 108 (3), 91 (77), 77 (5), 65 (41), 51 (6), 43 (100).

Preparation of 2,5-Dialkylpyrazines: According to the method of Zbiral and Stroh,[19] a solution of PPh_3 (1 equiv.) in dry benzene (1 mol L^{-1}) was added to a solution of the α-azido ketone (1 equiv.) in dry benzene (1 mol L^{-1}). In the case of asymmetrically substituted 2,5-dialkylpyrazines two different α-azido ketones (each 0.5 equiv.) were used. The reaction mixture was stirred at room temperature for 24 h and then concentrated. The residue was purified by column chromatography on silica gel with pentane/diethyl ether (2:1) to obtain the 2,5-dialkylpyrazines as pale-yellow liquids.

2,5-Bis(1-methylethyl)pyrazine (6): This compound was prepared from **34a** (Scheme 4). Yield: 40% (660 mg, 4.02 mmol). TLC (pentane/diethyl ether, 2:1): R_f = 0.57. GC: I = 1198. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.33 (d, $^3J_{H,H}$ = 6.9, $^1J_{C,H}$ = 126.7 Hz, 12 H, 4×CH_3), 3.08 (sept, $^3J_{H,H}$ = 6.9 Hz, 2 H, 2×CH), 8.39 (s, $^1J_{C,H}$ = 178.5, $^3J_{C,H}$ = 9.2 Hz, 2 H, 2×CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 22.2 (4×CH_3), 33.5 (2×CH), 141.8 (2×CH), 159.3 (2×C) ppm. EI-MS (70 eV): *m*/*z* (%) = 164 (25) [M^+], 149 (100), 136 (43), 121 (19), 107 (5), 94 (4), 80 (4), 67 (7), 53 (11), 41 (13).

Preparation of 2-(1-Bromo-1-methylethyl)-5-(1-methylethyl)pyrazine (57): A solution of **6** (450 mg, 2.74 mmol), AIBN (45 mg, 0.27 mmol) and NBS (400 mg, 2.2 mmol) in dry CCl_4 (20 mL) was heated under reflux for 4 h (Scheme 5). The precipitated succinimide was removed by filtration and the filtrate was concentrated. The residue was purified by column chromatography on silica gel with pentane/diethyl ether (10:1) to yield **57** (280 mg, 1.15 mmol, 42%), 2,5-bis(1-bromo-1-methylethyl)pyrazine (80 mg, 0.25 mmol, 9%) and recovered starting material (180 mg, 1.09 mmol, 40%).

2-(1-Bromo-1-methylethyl)-5-(1-methylethyl)pyrazine (57): TLC (pentane/diethyl ether, 10:1): R_f = 0.29. GC: I = 1459. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.34 (d, $^3J_{H,H}$ = 6.9, $^1J_{C,H}$ = 126.9 Hz, 6 H, 2×CH_3), 2.23 (s, $^1J_{C,H}$ = 129.6 Hz, 6 H, 2×CH_3), 3.13 (sept, $^3J_{H,H}$ = 6.9, $^1J_{C,H}$ = 128.2 Hz, 1 H, CH), 8.42 (d, $^5J_{H,H}$ = 1.3, $^1J_{C,H}$ = 181.6 Hz, 1 H, CH), 8.87 (d, $^5J_{H,H}$ = 1.3 Hz, 1 H, CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 22.1 (2×CH_3), 33.6 (2×CH_3), 33.7 (CH), 61.4 (C), 141.0 (CH), 141.1 (CH), 156.4 (C), 160.7 (C) ppm. EI-MS (70 eV): *m*/*z* (%) = 163 (100) [M^+ – Br], 148 (26), 133 (9), 120 (4), 107 (4), 92 (4), 80 (5), 65 (11), 52 (14), 39 (30). $C_{10}H_{14}BrN_2$ (243.14): calcd. C 49.40, H 6.22, N 11.52; found C 49.54, H 6.04, N 11.60.

2,5-Bis(1-bromo-1-methylethyl)pyrazine: TLC (pentane/diethyl ether, 10:1): R_f = 0.41. GC: I = 1718. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.24 (s, $^1J_{C,H}$ = 129.8 Hz, 12 H, 4×CH_3), 8.90 (s, $^1J_{C,H}$ = 183.5, $^3J_{C,H}$ = 9.0 Hz, 2 H, 2×CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 33.5 (4×CH_3), 60.8 (2×C), 140.4 (2×CH), 157.5 (2×C) ppm. EI-MS (70 eV): *m*/*z* (%) = 241 (35) [M^+ – Br], 241 (40), 161 (100), 147 (30), 133 (10), 119 (10), 106 (6), 92 (8), 80 (10), 65 (26), 51 (25), 39 (61). $C_{10}H_{14}Br_2N_2$ (322.04): calcd. C 37.30, H 4.38, N 8.70; found C 37.22, H 4.43, N 8.32.

Preparation of 2-(1-Hydroxy-1-methylethyl)-5-(1-methylethyl)pyrazine (58): A solution of **57** (254 mg, 1.05 mmol) in acetone (1 mL) was added to a suspension of freshly prepared AgOH (0.26 g, 2.1 mmol) in water (20 mL, Scheme 5). The reaction mixture was stirred at 30 °C for 4 h. The aqueous layer was extracted with diethyl ether (3×50 mL). The combined organic layers were dried with $MgSO_4$ and concentrated. Purification by column chromatography with pentane/diethyl ether (1:1) gave **58** (110 mg, 0.61 mmol, 58%) as a colourless liquid. TLC (pentane/diethyl ether, 1:1): R_f = 0.24. GC: I = 1326. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.34 (d, $^3J_{H,H}$ = 7.0 Hz, 6 H, 2×CH_3), 1.60 (s, $^1J_{C,H}$ = 127.0 Hz, 6 H, 2×CH_3), 3.13 (sept, $^3J_{H,H}$ = 6.9 Hz, 1 H, CH), 4.21 (br. s, 1 H, OH), 8.38 (d, $^5J_{H,H}$ = 1.4, $^1J_{C,H}$ = 180.1 Hz, 1 H, CH), 8.69 (d, $^5J_{H,H}$ = 1.4, $^1J_{C,H}$ = 181.2 Hz, 1 H, CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 22.1 (2×CH_3), 30.3 (2×CH_3), 33.6 (CH), 71.3 (C), 140.1 (CH), 140.3 (CH), 158.5 (C), 160.2 (C) ppm. EI-MS (70 eV): *m*/*z* (%) = 180 (7) [M^+], 165 (100), 147 (20), 132 (8), 122 (19), 107 (26), 94 (13), 80 (10), 67 (9), 59 (20), 52 (19), 43 (47). $C_{10}H_{16}N_2O$ (180.25): calcd. C 66.63, H 8.95, N 15.54; found C 66.49, H 8.80, N 15.27.

Preparation of 2-(1-Methylethenyl)-5-(1-methylethyl)pyrazine (7): A solution of **58** (110 mg, 0.61 mmol) in acetic acid (0.8 mL) and conc. H_2SO_4 (0.2 mL) was heated to 70 °C for 2 h (Scheme 5). The reaction mixture was made alkaline with 2 N NaOH and extracted with diethyl ether (3×10 mL). The combined extracts were dried ($MgSO_4$) and concentrated. The crude product was purified by column chromatography on silica gel with pentane/diethyl ether (1:1) to yield **7** (77 mg, 0.48 mmol, 78%) as a colourless liquid. TLC (pentane/diethyl ether, 1:1): R_f = 0.79. GC: I = 1272. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.33 (d, $^3J_{H,H}$ = 6.9 Hz, 6 H, 2×CH_3), 2.22 (d, $^4J_{H,H}$ = 0.8 Hz, 3 H, CH_3), 5.34–5.36 (m, 1 H, CH_2), 5.88 (d, $^2J_{H,H}$ = 0.6 Hz, 1 H, CH_2), 8.42 (s, 1 H, CH), 8.69 (s, 1 H, CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 19.7 (CH_3), 21.8 (2×CH_3), 33.3 (CH), 116.0 (CH_2), 140.0 (CH), 140.5 (C), 141.2 (CH), 150.6 (C), 159.9 (C) ppm. EI-MS (70 eV): *m*/*z* (%) = 162 (38) [M^+], 147 (100), 134 (49), 119 (6), 106 (4), 92 (6), 80 (5), 65 (24), 52 (19), 39 (46). $C_{10}H_{16}N_2O$ (180.25): calcd. C 66.63, H 8.95, N 15.54; found C 66.49, H 8.80, N 15.27.

Preparation of 2,5-Dimethyl-3-(methylsulfanyl)pyrazine (67): ***Caution!*** *NaSMe forms highly malodorous MeSH in contact with moisture.* Therefore, the reaction should be carried out in a well-ventilated hood. A stainless-steel autoclave was charged with **69** (1.00 g, 7.04 mmol), NaSMe (986 mg, 14.1 mmol) and dry dimethoxyethane (20 mL, Scheme 6). The reaction mixture was stirred at 150 °C for 12 h and then quenched by the addition of water (100 mL). The aqueous layer was extracted with diethyl ether (3×100 mL). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with pentane/diethyl ether (5:1) to give **67** (1.04 g, 6.75 mmol, 96%) as a pale-yellow liquid. TLC (pentane/diethyl ether, 5:1): R_f = 0.36. GC: I = 1260. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.45 (s, $^1J_{C,H}$ = 127.8 Hz, 3 H, CH_3), 2.46 (s, $^1J_{C,H}$ = 127.5 Hz, 3 H, CH_3), 2.55 (s, $^1J_{C,H}$ = 141.6 Hz, 3 H, CH_3), 7.95 (s, $^1J_{C,H}$ = 180.4 Hz, 1 H, CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 12.4 (CH_3), 20.8 (CH_3), 20.9 (CH_3), 137.1 (CH), 147.8 (C), 150.0 (C), 154.4 (C) ppm. EI-MS (70 eV): *m*/*z* (%) = 154 (100) [M^+], 139 (26), 121 (83), 107 (24), 98 (15), 80 (21), 71 (14), 52 (12), 39 (36).

Supporting Information Available: The structures of all pyrazines already identified in a previous study (Figure S1),[10] complete results of the headspace analysis of *Chondromyces crocatus* (Table S1), and physical and spectroscopic data for all synthesised compounds that are not given here.

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