NATURALLY OCCURRING PYRAZINES AND THEIR MASS SPECTROMETRIC CHARACTERISATION

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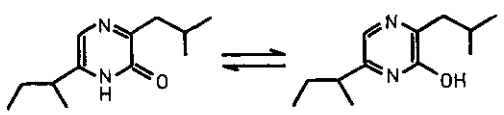
Abstract

The occurrence of pyrazines in plants and animals - terrestrial and marine - is reviewed. Pyrazines detected in raw and cooked foods are also listed. Aspects of the biosynthesis and biogenesis of the pyrazines are considered, and attention is drawn to the role of various groups of pyrazines in food chemistry, as insect pheromones and in luminescence processes in marine organisms. Pyrazines occur in trace proportions, so their chemical characterisation by mass spectrometry is considered in detail. Salient features of the mass spectra of the various classes of naturally occurring pyrazines are reviewed.

Introduction

Pyrazine derivatives have been isolated from widely differing sources: from micro-organisms, plants, animals, especially insects, and more recently, from marine organisms. They are well-known as flavour components in foodstuffs. This review is concerned primarily with the chemical characterisation of the naturally occurring compounds which often are present only in trace proportions. Some consideration is also given to the origin and biological activity of the various groups of pyrazines.

Orginally, the isolation of aspergillic acid and related metabolites from a wide range of fungi focussed attention on the hydroxypyrazines and the derived hydroxamic acids. Typical members of this series are deoxyaspergillic acid (1), aspergillic acid (2) and hydroxyaspergillic acid (3).



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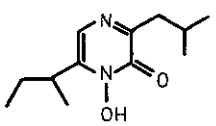

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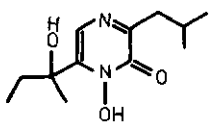

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In this series the hydroxydialkylpyrazines, e.g. (1) do not show antibiotic activity: the presence of the hydroxamic acid group would appear essential for effective antagonism to micro-organisms. The chemistry of the pyrazines of microbiological origin has been extensively reviewed by Sammes.[1] In the present review consideration of these pyrazines is essentially for comparative purposes. Moreover, the biosynthesis of the hydroxypyrazines of microbiological origin has been studied in detail.[2-5]

The 2-methoxy-3-alkylpyrazines, of which the 3-isobutyl compound (4) was the first to be isolated from a plant source,[6] have also received considerable attention,[7] as the methoxypyrazines are major contributors to the odour character of green vegetables, peas, peppers, etc. Quite recently a benzoylmethoxypyrazine was isolated from the mushroom *Septoria nodorum* Berk.[8]

Comparably, a range of pyrazines having methyl and higher alkyl substituents, often di- and tri-substituted have been reported from processed foods. Again, these pyrazines contribute to the aroma of the various foodstuffs, including coffee, cocoa, tea and cooked meats.[9] Several thioalkyl pyrazines have also been isolated from roasted coffee.[10] The known pyrazines obtained from animal and plant sources, including fresh and processed foods are listed in the Appendix.

A series of trialkylpyrazines have been reported from insects.[11-15] These compounds are considered to function as alarm pheromones for various species of ants. The biological activity, if any, of the unusual styrylpyrazines from the Argentine ant, is unknown. Pyrazines have also been isolated from the scent glands of the Canadian beaver[16] and from several marine animals. In the latter case, they are the actual light emitters in bioluminescence processes.[17] Pyrazines from the *Animalia* are listed in Table 1.

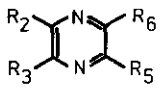
Occurrence and Identification of the Naturally Occurring Pyrazines

Isolation and identification has been achieved in most cases by the use of gas chromatographic-mass spectrometric techniques. Thus, the major component of bell pepper oil, 2-methoxy-3-isobutylpyrazine (4), was identified on the basis of mass spectrometric and n.m.r. data.[6] The pyrazine comprises 16% of the oil which in turn represents only 1 p.p.m. of the whole pepper. Comparably, Murray, Shipton and Whitfield isolated three components; 3-isopropyl (5), 3-secbutyl (6) and 3-isobutyl-2-methoxypyrazine (4) from green peas.[18] The two former compounds each represent 1 part in 10^{11}, and the latter, 1 part in 10^{12} of the fresh peas.

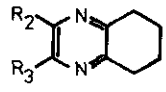
The benzoylmethoxypyrazine isolated from the mushroom *Septoria nodorum* Berk[8] has been assigned structure (7) or (8) on the basis of n.m.r. evidence, as yet the position of the methoxy group has not been defined.

TABLE 1

Pyrazines of Animal Origin



No.		R_2	R_3	R_5	R_6	Source	Ref.
18	(122/7)*	Me	Me	Me		G	16
14	(136/11)	Me	Et	Me		F	12
12	(136/12)	Me	Et		Me	C, D	11
19	(136/14)	Me	Me	Me	Me	G	16
17	(150)	Me	Pr	Me		E	13
11	(150/13)	Me	n Pr		Me	C, D	11
10	(164/11)	Me	n Bu		Me	C, D	11
9	(178/1)	Me	n-pentyl		Me	C, D	11
13	(178/3)	Me	iso-pentyl	Me		A, B, E H, I	11, 13, 14
	(192/1)	Me	n-hexyl		Me	D	11
16	(210/1)	Me	*trans*-styryl	Me		E	13
15	(210/2)	Me	*cis*-styryl	Me		E	13



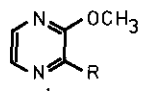
30	(134/7)	H	H			G	16
21	(148/8)	Me	H			G	16
22	(162/6)	Me	Me			G	16
23	(188/3)	$-CH_2CH_2CH_2CH_2-$				G	16

* This code refers to the Appendix

Source

A	*Odontomachus hastatus*	B	*O. clarus*	C	*O. brunneus*
D	*Odontomachus* unknown	E	*Iridomyrmex humilis* (Argentine ant)	F	*Atta sexdens rubropilosa*
G	*Castor fiber* (Canadian beaver)	H	*Hypoponera opacior*	I	*Ponera pennsylvania*

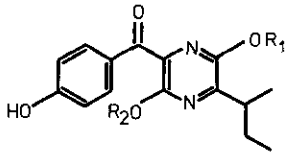
See also *Addendum*



4 : R = i Bu

5 : R = i Pr

6 : R = *sec* Bu



7 : R_1 = H, R_2 = CH_3

8 : R_1 = CH_3, R_2 = H

The trialkylpyrazines obtained from some insect sources are available in relatively greater amounts than in fresh vegetables. The large ponerine ants of the genus *Odontomachus*, when disturbed, discharge a secretion which has a characteristic odour similar to that of chocolate.[11] Following on a gas chromatographic-mass spectrometric examination of solvent extracts of the heads of various *Odontomachus spp.*, a series of 3-*n*-alkyl-2,6-dimethylpyrazines were characterised as major components of the mandibular gland secretion.[11] For example, *O. brunneus* was found to contain the 3-pentyl- (9), 3-butyl- (10), 3-propyl- (11) and 3-ethyl-2,6-dimethylpyrazines (12) in the ratio of 91:7:1.4:0.6. However, it is difficult to distinguish between a 3-alkyl-2,5-dimethylpyrazine and the corresponding 3-alkyl-2,6-dimethylpyrazine by mass spectrometry alone, though pairs may sometimes be distinguished by gas chromatography. Conversion of the dimethylalkylpyrazines into the corresponding mono-N-alkyl-piperazines, by quaternisation with methyl iodide and reduction with sodium borohydride, has enabled mass spectrometric differentiation in the case of the pyrazines from *O. brunneus*.[11] In *O. hastatus* and *O. clarus* the major component was 2,5-dimethyl-3-isopentylpyrazine (13). Two additional species of ponerine ants, *Hypoponera opacior* and *Ponera pennsylvania*, also contained 2,5-dimethyl-3-isopentylpyrazine in their mandibular glands. Identification was based on GC-MS data.[14] Again, this mandibular gland secretion is thought to function as an alarm pheromone.

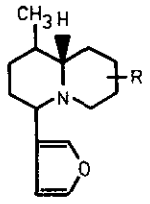
Quite recently, a major component of the trail pheromone of the myrmecine leaf-cutting ant, *Atta sexdens ssp. rubropilosa*, was identified as 2,5-dimethyl-3-ethylpyrazine (14). It is a constituent of the poison gland, and represents approx.25 p.p.b.(2.5×10^{-8}) of the body weight of the insect. This compound was identified on the basis of gas chromatographic and mass spectral comparisons with the synthetic 2,5-dimethyl-3-ethylpyrazine and 2,6-dimethyl-3-ethylpyrazine: the final identification was made by a comparison of the fingerprint region in the i.r. spectra.[12]

In the *Dolichoderinae*, 2,5-dimethyl-3-isopentylpyrazine (13) and a mixture of the (Z)- and (E)-2,5-dimethyl-3-styrylpyrazines (15), (16) were characterised from the heads of the Argentine ant, *Iridomyrmex humilis*.[13] They represent approximately 70 and 200 p.p.m. of body weight respectively. Mass spectral data suggests that a fourth pyrazine, a dimethylpropyl derivative

(7), is also present at approximately 5 p.p.m. The *cis*- and *trans*-styrylpyrazine structures were assigned on the basis of mass, n.m.r. and u.v. spectral data. The styryl moiety was confirmed by micro-ozonolysis. Microhydrogenation gave the 2,5-dimethyl-3-phenethylpyrazine identical with the synthetic compound. As the ready conversion of the (E)-styryl into the (Z)-styryl derivative in sunlight was noted in the course of the above studies, an extraction of *I. humilis* workers was carried out in the dark. This extract showed a (*Z*)- to (*E*)-ratio of approximately 1:3. The biological significance, if any, of this configurational change has yet to be assessed.

A series of pyrazines have also been identified in the mandibular gland secretion of a formicine ant, a *Calomyrmex spp.*[15] Pyrazines are widespread among the *Formicinae* and, no doubt, are likely to be found elsewhere among the *Insecta*.

Finally, a series of two pyrazines (18), (19) and four tetrahydroquinoxalines (20), (21), (22), (23) have been isolated from the scent gland of the Canadian beaver, *Castor fiber*.[16] The pyrazines are very minor components of the secretion each representing approx. 0.001% of the gland contents. The major components are nine alkaloids, primarily 4(3-furyl)-1-methylquinolizodine derivatives (24).



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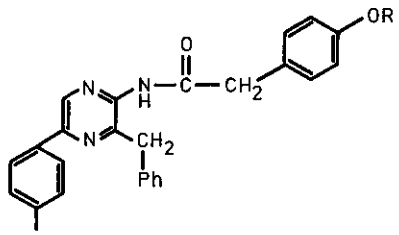
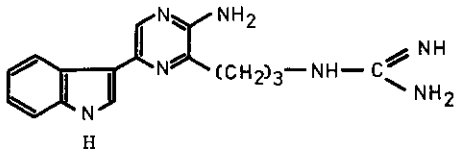
Aminopyrazines have been identified as light emitters in bioluminescence reactions of some coelenterates: in the living animal they are bound to protein. Coelenteramide (25, R=H) has been isolated from the families *Hydrozoa* (the jellyfish, *Aequorea*), *Anthozoa* (the sea cactus, *Cavernularia*; sea pansy *Renilla*; sea pen *Leioptilus*) and *Scyphozoa* (the jellyfish, *Pelagia*).[17,19,20] The corresponding sulphate (26, R=SO_3H) has been isolated from the firefly squid, *Watasenia scintillans*,[21] while from the crustacean, *Cypridina hilgendorfii*, the indoylpyrazine (27) was obtained.[22]

These compounds are involved in both the Ca^{2+} triggered photoprotein, and the "luciferin-luciferase" type of luminescence.[17]



25 : R = H
26 : R = SO_3H



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The amounts of the material involved are extremely small, e.g. 6 x 10^{-9} mole of (25) could be isolated from the sea cactus, *Cavernularia obesa*, which weighs ~ 30 g.[17] The structural identification of the isolated products has been heavily dependent on u.v. and mass spectrometry and confirmation in some cases has been provided by synthesis of the oxidised (i.e. pyrazine) and/or reduced species.

A vast number of pyrazines have been isolated from foodstuffs after some form of heat treatment (see Appendix). The chemistry of these compounds has been reviewed by Maga and Sizer[9] and, more recently, by Ohloff and Flament.[23] They are briefly mentioned here.

Pyrazines have been reported from many food sources: from bread, flour and the ubiquitous sponge cake,[24] from meat of most types, mushrooms, whisky, fusil oil,[25] beer,[26] cheese and from vegetables.[7] The heat treatment that the foodstuff undergoes would seem to determine the amount and number of pyrazines formed. For example, some thirty-five pyrazines were found in roast beef, but only three in boiled beef.[27] Whilst the pyrazines contribute towards the flavour of the meat they are not solely responsible for it. Lactones, other oxygenated aliphatic compounds and hydrocarbons are also present.[27]

Where foodstuffs have not undergone heat treatment, for example, dried mushrooms,[28] fresh vegetables,[7,29] green coffee,[30] and barley,[31] the majority of the pyrazines are alkoxy derivatives. These compounds are only present in trace proportions; however, they have very low odour thresholds and hence contribute to a large extent to the aroma of the material from which they were isolated. 3-Isobutyl-2-methoxypyrazine (4),[32] from green peas, has an odour threshold in water of 2 parts in 10^{12}. The threshold in water for alkyl pyrazines is considerably higher being 3.5 parts in 10^{8} for 2-isobutyl-3-methylpyrazine (28)[32] and 6 parts in 10^{5} for methylpyrazine (29).[33]

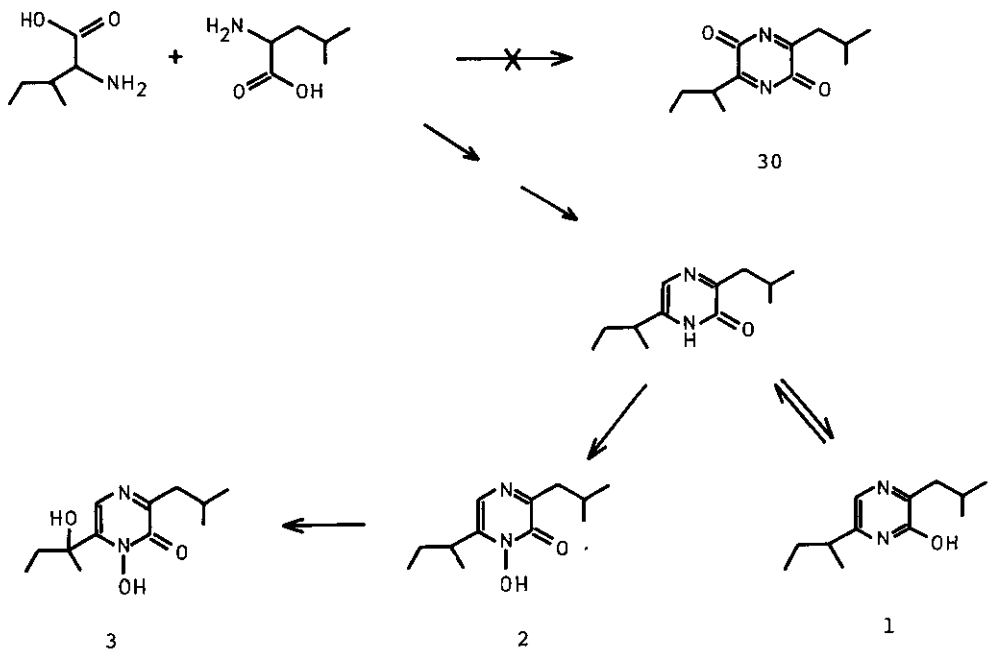
Synthesis of pyrazines

The chemistry of the pyrazines has been reviewed.[25,34] Generally, the synthesis of a pyrazine involves either condensation of a 1,2-diamine with a 1,2-dicarbonyl compound, or the self-condensation of a 2-aminocarbonyl system. (For a comprehensive review of synthetic methods

to 1971 see ref. 25). Improved syntheses of acyl and alkylpyrazines, useful for flavouring purposes, have been reported.[35-37] A series of cyclopenta[b]pyrazines, some of which were isolated from roast beef,[38] were synthesised from the appropriately substituted ethylene diamine and cyclopenta-1,2-dione.[38-40] The alkoxypyrazines of green peas and other vegetables were synthesised *via* the proposed biosynthetic route (see below).[6,7,18] A synthesis of the *Cypridina* etioluciferin, involved in the bioluminescence reaction in *Cypridina halgendorfii* also proceeded *via* the α-diketone and ethylene diamine route.[42]

Biogenesis and Biosynthesis of Naturally Occurring Pyrazines

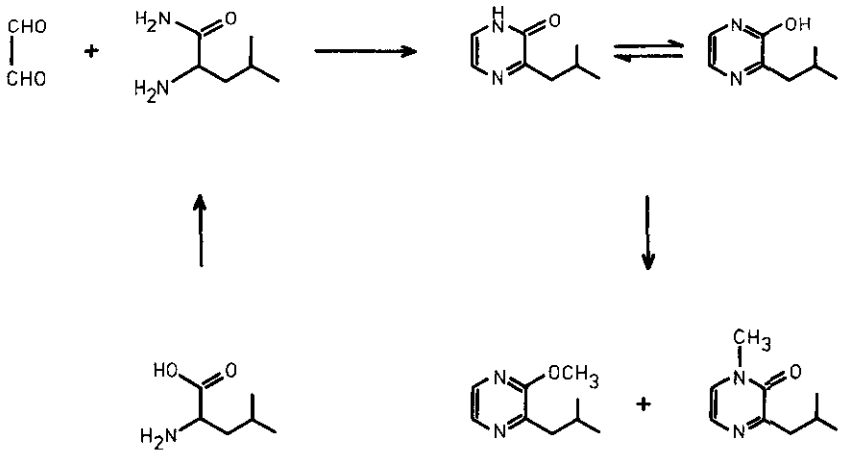
The extensive biosynthetic studies of McDonald and his co-workers[2-5] have established the route by which the aminoacids, valine, leucine and isoleucine, are converted into the corresponding 2-hydroxy-3,5-dialkylpyrazines in various species of *Aspergillus*, and in related micro-organisms. Subsequent hydroxylation of the hydroxydialkylpyrazine gives the hydroxamic acid. The pathway is typified in the conversion of isoleucine and leucine into deoxyaspergillic acid (1), and thence to aspergillic (2) and hydroxyaspergillic acid (3), as shown in Scheme 1.



Scheme 1

In general, the biosynthesis of the mould metabolites does not proceed *via* the diketopiperazine[5] (30). Rather it would seem that the amino acids, for example, leucine and isoleucine, are metabolised into a "pyruvate" pool before incorporation.[2]

A biogenesis has been proposed[18] for the 2-methoxy-3-alkylpyrazines which have been isolated from green foodstuffs. This scheme leading to the intermediate 2-hydroxy-3-alkylpyrazine is not inconsistent with the pathway determined for the mould metabolites. This route (Scheme 2) has been simulated in the synthesis of the 2-methoxy-3-alkyl derivatives, including 2-methoxy-3-isobutylpyrazine (4).



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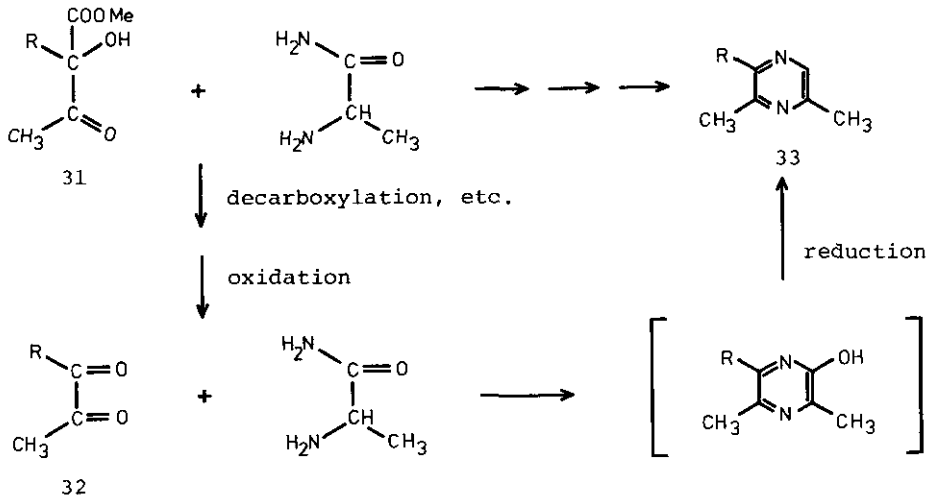
Scheme 2

A comparable biogenesis (see Scheme 3) may accommodate the formation of the 3-alkyl-2,6-dimethylpyrazines from insect sources.[11] These pyrazines, isolated from various species of *Odontomachus* ants have a normal alkyl chain, C_2-C_6, indicative of a fatty acid precursor. Condensation of the pyruvate (RCOCOOMe or its equivalent) with "active" acetate would give an acyloin (e.g. 31). The acyloin, or the derived 2,3-dione (32), may then condense with the amide of alanine, ultimately giving the 3-alkyl-2,6-dimethylpyrazine (33) (Scheme 3).



Scheme 3

The 3-isopentyl-2,5-dimethyl (13) and/or 3-styryl-2,5-dimethylpyrazines (15, 16) has not been isolated from a species which contains the 3-*n*-alkyl-2,6-dimethylpyrazines and vice versa.[11-14] In these circumstances, a separate biogenesis is envisaged for the 2,5-dimethyl isomers. It would seem likely that the precursors to the 3-isopentyl and 3-styryl substituents in these compounds may be introduced into a symmetrical intermediate arising *via* dimerisation of alanine.

The various alkylpyrazines, often di- and tri-alkyl derivatives which have been isolated from cooked foods are, of course, essentially of secondary origin. The products are most likely formed from α-hydroxycarbonyl or α-dicarbonyl compounds[43] - themselves degradation products of carbohydrates and the amino acids. Despite observations that free ammonia and reducing sugars, when heated together, can form pyrazines,[44,45] it has been reported by Koehler[46] that the pyrazines formed in sugar - amino acid systems are dependent upon the nature of the amino acid used. It was concluded that the nitrogen was still bound to the amino acid during, at least, part of the synthesis of the pyrazine.

The source of the carbon atoms in the pyrazine was reported to be almost exclusively from the sugar and not from the amino acids.[46] ^{14}C labelling of the amino acids on carbon 1 or 2 showed no incorporation into the pyrazines, while labelling of the sugar in either the C_1, C_6 or C_3 and C_4 positions resulted in incorporation of the label into the pyrazine. The results seemed to indicate a degradation of the sugar into C_2 and C_4, or two C_3, units which were then incorporated into the pyrazine. It was concluded from this study that both the ring and substituent carbons in the pyrazine came from the sugar, and that none originated from the amino acid.[46]

Pyrazines have also been isolated from heating some hydroxy-amino compounds in the presence of air.[47] Ethanolamine, glucosamine, serine, threonine, 4-amino-3-hydroxybutyric acid and alanylserine, all produced mixtures of pyrazines when heated at ~ 200° in air for about 4 hr. Serine, for example, when heated produced eleven pyrazines ranging from pyrazine itself to a pyrazine with a molecular weight of 178. A large number of amino acids, including most of the natural ones did not, however, produce any pyrazines under these conditions.

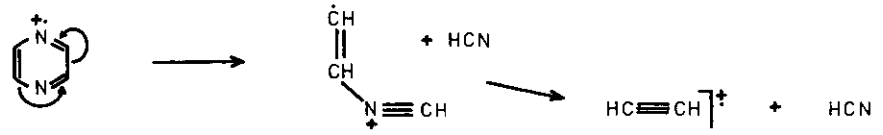
The fate of pyrazines once they have been ingested has been examined by Hawksworth and Scheline.[48] In rats, alkyl substituted pyrazines were oxidised to the corresponding carboxylic acids which were excreted in the urine either free or as their glycine conjugates. An increase in the number of alkyl groups caused a decrease in the extent of carboxylic acid formation while ring hydroxylation also occurred. Methoxypyrazines underwent *O*-demethylation and ring hydroxylation.

Mass Spectrometry of Pyrazines

As a result of the very small quantities of pyrazines that are available from natural sources, maximum reliance has to be placed on chromatographic and spectrometric techniques for their identification. Because of its sensitivity and specificity, mass spectrometry has been used quite successfully in determining gross structural details of the naturally occurring pyrazines and it can, in favourable circumstances, lead to definitive structures. The following is a discussion of the major features in the mass spectra of various classes of naturally occurring pyrazines.

Since the pyrazine nucleus itself is relatively stable and resistant to fragmentation,[50] the mass spectra of substituted pyrazines tend to be influenced by the nature of the substituents. Pyrazines of natural origin can be grouped by substitution pattern as follows: (A) methyl (or polymethyl), (B) ethyl, (C) propyl and higher alkyl, (D) alkoxy and acetyl, and (E) cycloalkyl pyrazines. These five groups exhibit differing fragmentation pathways and each will be dealt with separately. Mass spectral data for monofunctional short chain pyrazines has been reported on previously,[49,50] and is now compared with that for the various classes of pyrazines.

Pyrazine itself has a mass spectrum consisting of three principal ions: m/z 80 (100%), 53 (45%), 26 (40%). A rationale for the formation of these three ions is set out below; both steps being supported by the appropriate metastable ions.[50]



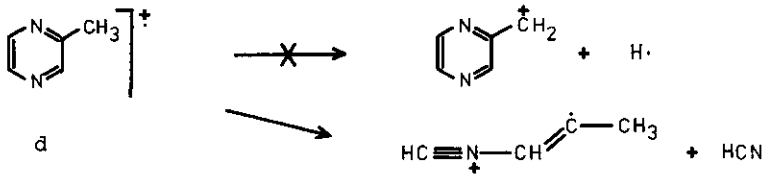
m/a 80 m/z 53 m/z 26

a b c

Unlike the pyridine nucleus in which the 2, 3, and 4 ring positions are different, each ring position in the pyrazine nucleus is equivalent. Differences in the mass spectra of the three methylpyridines (picolines) can be accounted for by variations in the electron density at a particular point. For example, the largest $(M-1)^+$ ion (40%) occurs in 3-methylpyridine in which a formal positive charge is not placed on the N atom.[51,52] In the 2- and 4-methyl pyridine the $(M-1)^+$ ion is much smaller at 20%.

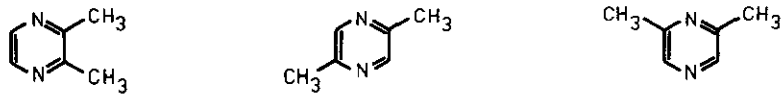
(A) *Methylpyrazines*

The pyrazine nucleus has four equivalent positions and, in theory, each of these could resemble the 2- and the 3-substituted pyridine. But the methylpyrazine (29) behaves more like 2-methylpyridine, *viz:* there is no $(M-1)^+$ ion, the base peak being the molecular ion. The only large peak in the spectrum corresponds to the loss of HCN from the parent ion.[50]



d e

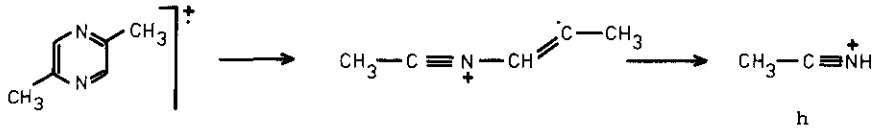
The ion at m/z 42 corresponding to protonated acetonitrile is, in this case, quite small (17%).



34 35 36

The three dimethylpyrazines, (34), (35) and (36) do not exhibit an $(M-1)^+$ ion.

The molecular ion is the base peak in the case of (35) and (36), and 95% in (34). A loss of CH_3CN from the molecular ion of (34), corresponding to the loss of HCN from (29), once again, yields an ion at m/z 67 and this is the base peak in the mass spectrum of (34). In the spectra of (35) and (36) there is a small ion ~ 15% at m/z 81 corresponding to the loss of HCN. Also in the spectra of (35) and (36) there is a large ion (~ 70%) at m/z 42 corresponding to C_2H_4N (protonated acetonitrile) (h); this ion is only 20% in the case of (34). In the case of (35) and (36), however, metastable transitions indicate that the ion at m/z 42 is formed at least in part from the molecular ion (m/z 108) via the ion at m/z 81 (g).[53]

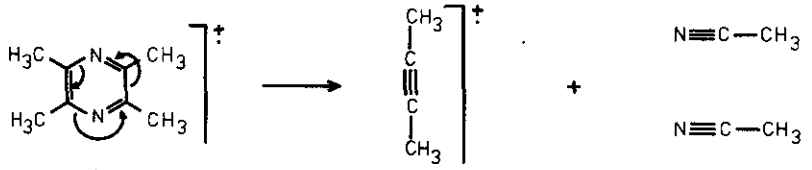


f g h

+ HCN

Trimethylpyrazine (18), analogous to the 2,5-isomer (35), gives a mass spectrum with a base peak at m/z 42, a small ion at m/z 81 and the parent ion (80%) at m/z 122; these being the principal ions in the mass spectrum.[53]

Tetramethylpyrazine (19) has 3 principal ions : at m/z 42 (37%), 54 (100%), and the parent ion m/z 136 (85%) (i).[10] The ion at m/z 54 is C_4H_6 (j) which can be derived directly from the parent ion in an electrocyclic process.[54]



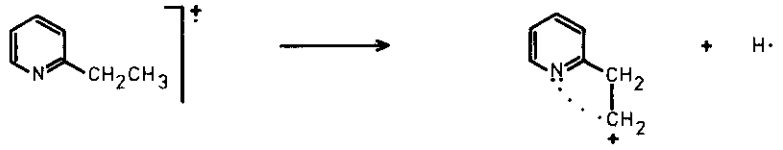
i j m/z 54

This ion at m/z 54 is small (10%) in the trimethylpyrazine (18), and almost insignificant (5%) in the 2,3-dimethylpyrazine (34).

Thus, for all methylpyrazines there is no significant $(M-1)^+$ ion. Fragmentation proceeds mainly by the loss of HCN or CH_3CN to yield a (presumably) acyclic species which may fragment further.

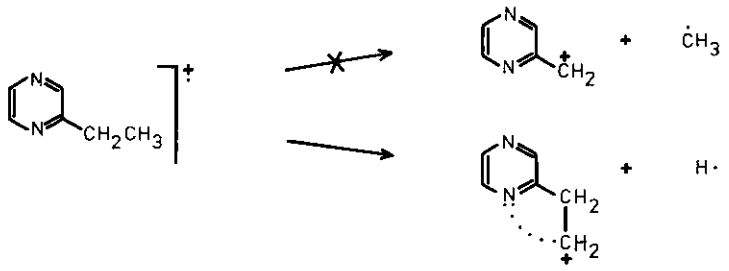
(B) *Ethylpyrazines*

The mass spectrum of 2-ethylpyridine exhibits an intense peak for the $(M-1)^+$ ion. This has been shown by deuterium labelling to be due to the loss of β-hydrogen; the resulting primary carbonium ion is, presumably, stabilised by the lone pair of electrons on the nitrogen (k).[55]



k

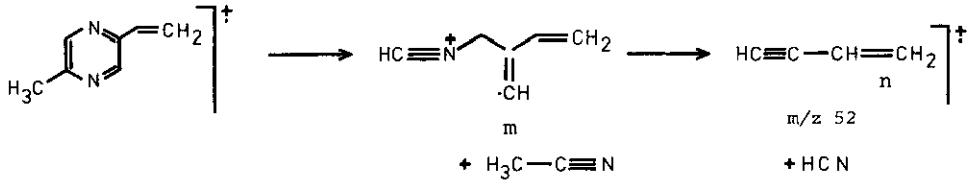
Ethylpyrazines also exhibit a very strong $(M-1)^+$ (l) ion (usually the base peak), probably for the same reason. There is virtually no $(M-15)^+$ ion which is consistent with the lack of $(M-1)^+$ ion from the methylpyrazines.[53]



l

The presence of the $(M-1)^+$ (l) ion might also indicate a longer chain than an ethyl group, though in this case, there is a characteristic McLafferty type rearrangement, which will be discussed in the next section. Fragmentation involving the pyrazine ring follows a similar pattern to that for the corresponding methyl compounds.[10]

The vinylpyrazines which are found in foods do not exhibit this strong $(M-1)^+$ ion, presumably, because of the difficulty in removing a vinyl proton. There is, however, a moderately intense ion (~ 50%) at m/z 52 (n), attributed to the fragmentation of the nucleus.[53]

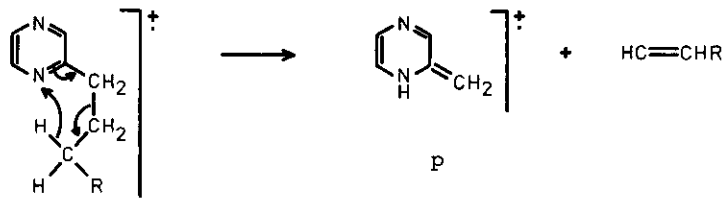


m

m/z 52

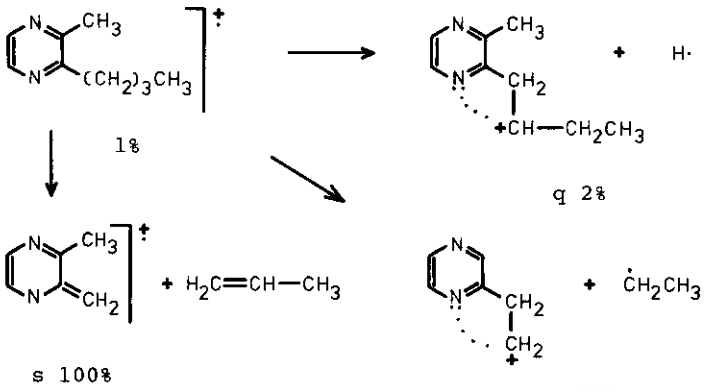
(C) *Propyl and Higher Alkyl Pyrazines*

Once the alkyl chain extends to 3 carbon atoms a McLafferty type rearrangement is possible in which a γ-hydrogen atom is transferred to the pyrazine nitrogen via a 6-membered transition state (o) and a neutral olefin is lost. The rearranged species (p) is usually the base peak of the spectrum.[49,50]



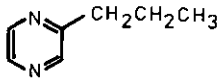
o p

As the chain length gets longer the proportion of charge carried by such ions as $M^{+\cdot}$, $(M-1)^+$, $(M-15)^+$, etc. gets smaller. It is quite characteristic for an alkyl pyrazine in which the alkyl group is greater than 4 carbons to have $M^{+\cdot}$ and $(M-1)^+$ each less than 5% and a base peak resulting from the McLafferty type rearrangement.[10] There is usually a small peak due to β cleavage and loss of a portion of the alkyl group (see Section B). Ions in the mass spectrum of 2-*n*-butyl-3-methylpyrazine[50] (37) are shown below.

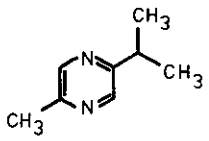


1% q 2% s 100% r 13%

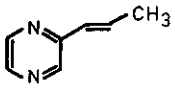
In the mass spectrum of n-propylpyrazine[53] (38) the base peak (McLafferty rearrangement) is at m/z 94 $(M-28)^{+\cdot}$. In 2-isopropyl-5-methylpyrazine (39) this peak is only 39% $(M-28)^{+\cdot}$, and must arise via a prior rearrangement, the base peak is, in fact, at m/z 121 $(M-15)^+$ [53]. In the mass spectrum of prop-1-enyl pyrazine (40) the rearrangement peak is only 5% $(M-26)^{+\cdot}$. Instead the base peak is $(M-1)^+$, undoubtedly due to a combination of allylic stability, and stabilisation of the charge by the nitrogen lone pair.[53]



38



39

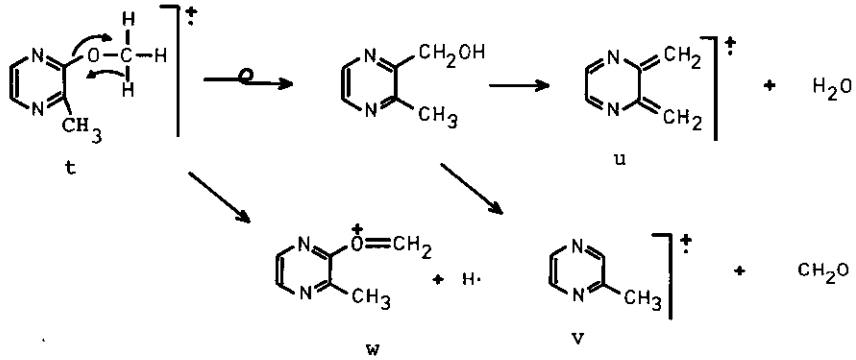


40

The fragmentation of the pyrazine nucleus itself does not yield much diagnostic information.

(D) *Alkoxy and Thioalkylpyrazines*

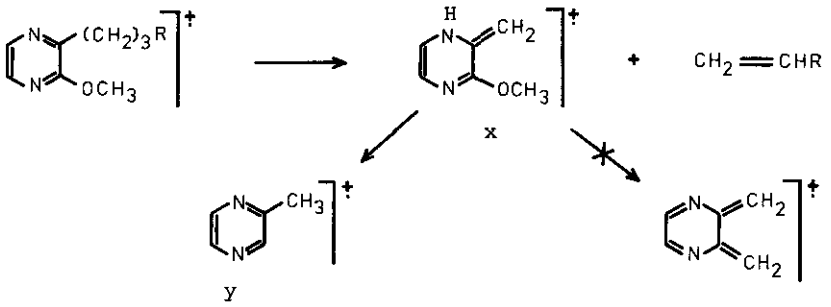
No unsubstituted alkoxypyrazine has yet been isolated from natural sources; the simplest member of this class (from nature) is 2-methyl-3-methoxypyrazine (41).[30] In the mass spectrum of this compound the parent ion is the base peak, a feature characteristic of the short chain compounds. There are also ions for $(M-1)^+$, $(M-18)^{+\cdot}$ and $(M-30)^{+\cdot}$. These latter two peaks have been shown by deuterium labelling of the methoxy group to arise possibly through a prior rearrangement of the methoxy to a hydroxymethyl group.[56]



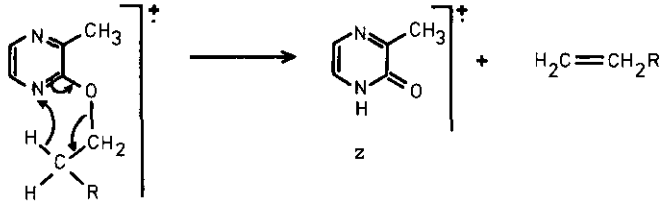
t u w v

This rearrangement surprisingly does not appear to occur in the case of 2-methoxy-3,6-dimethylpyrazine.[9] 2-Methyl-3-thiomethylpyrazine (42), the thio analogue of (41) also loses H_2S and CH_2S from the parent ion. These losses are possibly due to the above rearrangement.

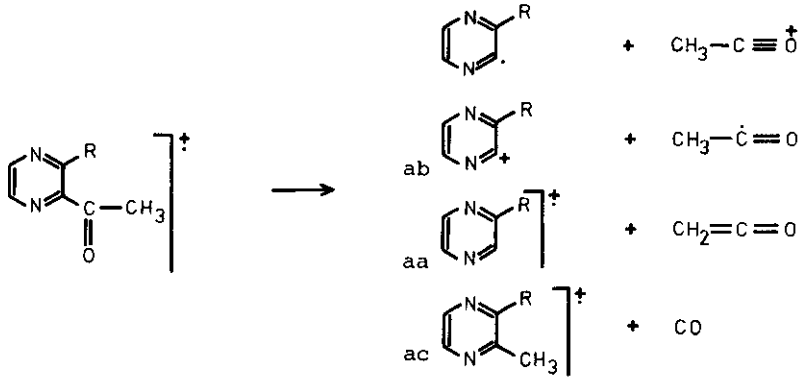
2-Alkyl-3-methoxypyrazines, in which the alkyl group is greater than three carbons, are capable of undergoing the McLafferty type rearrangement to yield the tautomer of (w). This ion at m/z 124 (x) is the base peak of the mass spectrum of compound (41), but there is no evidence of the loss of water from this ion. A peak corresponding to the loss of formaldehyde from this ion (x) is, however, normally present.[10]



For alkoxypyrazines in which the alkoxy chain is 2 carbons or longer the McLafferty type rearrangement can occur through the alkoxy group. This rearrangement occurs in most of these compounds, and constitutes the base peak (Z) if no other rearrangement is possible.[9] Where both the alkyl and alkoxy chains are of sufficient length to allow the rearrangement the process occurs with both chains, but tends to be concentrated in the alkyl (as opposed to alkoxy) chain.[9]

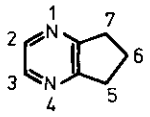


Acetylpyrazines are easily recognised by the intense ion at m/z 43 arising from the ion $CH_3C{\equiv}O^+$. Their spectra also contain intense ions for the molecular ion, $(M-42)^{+\cdot}$ (aa) and $(M-43)^+$ (ab) and an ion at $(M-28)^{+\cdot}$ (ac), presumably due to the loss of carbon monoxide. Once the ketene group has been lost, $(M-42)^{+\cdot}$, the ion is a tautomer of parent pyrazine. The mass spectrum from m/z (M-42) to low masses is similar (but not identical) to that of the parent pyrazine.[9]

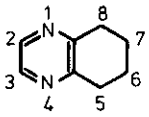


(E) *Cycloalkylpyrazines*

This class of pyrazines falls into two main groups, 6,7-dihydro-5H-cyclopentapyrazines (43) and 5,6,7,8-tetrahydroquinoxalines (44). A series of these compounds has been isolated from coffee by Vitzthum *et al.*, and their spectra reported therein.[57]



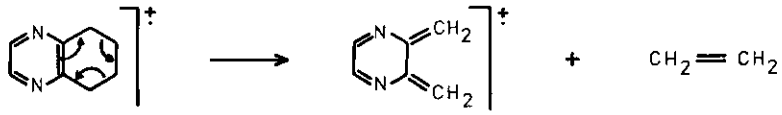
43



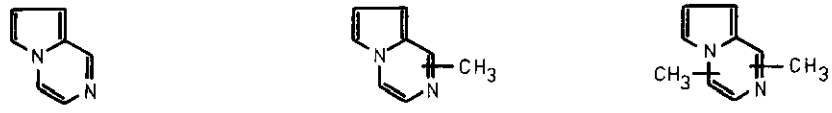
44

In the cyclopentapyrazines (43) there is a strong molecular ion and a comparable (M-1) ion. If a methyl group is present in either the 2 or 3 position the (M-15) ion is very small, if it is in the cyclopentane ring the (M-15) ion is usually the base peak. Note the similarity to the corresponding acyclic analogues, e.g. (39). Ethyl groups also cause different fragmentations depending on their position; if substituted in the pyrazine ring the normal (M-1) ion is the base peak, but if in positions 5 or 7 in the cyclopentane ring, the (M-28) ion is the base peak (rearrangement peak). Substituents in both rings lead to a combination of all these processes.[57]

The tetrahydroquinoxaline derivatives (44) all show strong molecular ions and (M-1) ions. There is also an ion (10-20%) for the reverse Diels-Alder fragmentation of the saturated ring (ae). (M-15) ions occur in all reported spectra, even in the spectrum of the unsubstituted compound (44)[57,58] presumably due to ring contraction.



ad ae



45 46 47

A series of seven pyrrolo(1,2,a)pyrazines have been isolated from roast meat.[41] They fall into three types, viz. unsubstituted (45), monomethyl (46), and dimethyl (47) pyrazines.

The three isomers of type (46) all exhibit very similar spectra; the principal ions being $M^{+\cdot}$ 100%, $(M-1)^{+}$ 60% and $(M-28)^{+\cdot}$ 40%. The three compounds for type (47) also show similar mass spectra; the main peaks being at $(M)^{+\cdot}$ 100%; $(M-1)^{+}$ ~ 60%; $(M-15)^{+}$ ~ 30%; $(M-28)^{+\cdot}$ ~ 25%; $(M-42)^{+\cdot}$ ~ 40%. The mass spectra within any one of these classes are very similar; presumably due to scrambling and no structural information, within a class was obtained from them.

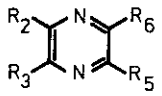
Mass spectrometry has played an important role in the structural elucidation of the etioluciferins[59-61] isolated from aquatic animals.

Overall, the mass spectra of pyrazines are useful in the determination of the types of groups attached to the pyrazine nucleus but not necessarily their position. In the case of the methyl and polymethyl pyrazines, the mass spectrum of the compound is definitive. The main fragmentation is of the pyrazine nucleus itself. In ethylpyrazines there is a strong peak at (M-1) which is usually the base peak, the molecular ion is also intense. There is also fragmentation of the nucleus but the overall spectrum is not definitive of any particular isomer.

Alkyl groups of 3 or more carbons attached to the pyrazine nucleus produce the very characteristic McLafferty type rearrangement and the ion resulting is the base peak of the spectrum. There are very small peaks (the longer the chain the smaller) for the molecular and (M-1) ions. Fragmentation of the nucleus itself does not throw much, if any, light on the substitution pattern though it should be possible to determine the gross structure of the aliphatic groups. Dihydrocyclopentapyrazines and tetrahydroquinazolines show strong molecular and (M-1) ions. An idea of the position of methyl substitution can be gained from the height of the (M-15) ion. For tetrahydroquinazolines there is usually a small but significant ion present arising from the reverse Diels-Alder fragmentation. Acetyl pyrazines are easily recognised by the large ion, usually the base peak, at m/z 43.

ADDENDUM: *Pyrazines of Animal Origin*

Since this review was written a number of trialkylpyrazines have been characterised from additional species of ponerine and formicine ants, viz.:



No.	R_2	R_3	R_5	R_6	Source	Ref.
14 (136/11)	Me	Et		Me	J	76
49 (164/12)	Me	i Bu	Me		K, L	76, 77
50 (164/14)	Me	sec Bu	Me		L	76
10 (164/11)	Me	n Bu		Me	J, L, M	76
51 (164/13)	Me	i Bu		Me	L	76
52 (164/18)	Me	sec Bu		Me	L	76
9 (178/1)	Me	n pentyl		Me	J, M	76
53 (178/6)	Me	n pentyl	Me		L	76
13 (178/3)	Me	iso pentyl	Me		K	77
54 (178/7)	Me	2-methylbutyl	Me		K	77
55 (192/1)	Me	n-hexyl		Me	J	76

Source

J *Odontomachus troglodytes* (workers and males)

K *Calomyrmex* sp No. 1 ANIC (? *splendidus*)

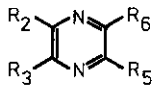
L *Anochetus sedilloti*

M *Brachyponera sennaarensis*

In all cases, the pyrazines were found in the mandibular glands. The pyrazines of the *Calomyrmex* sp are implicated in alarm reaction. The *Odontomachus troglodytes* males retreated and hid when subjected to minute amounts (< 0.1 ant equivalent) of the pyrazines contained in the mandibular gland of either the males or workers. On the other hand, *O. troglodytes* workers were attracted to, and attacked the pheromone source when presented in small amounts; larger amounts (> 5 ant equivalent) repelled the workers.

APPENDIX

Pyrazines Isolated from Terrestrial Animal and Plant Sources

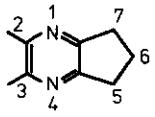


MW/No.		R_2	R_3	R_5	R_6	Ref.
80/1		H	H	H	H	62
94/1	(29)	Me				63
106/1		vinyl				63
108/1		Et				64
108/2	(34)	Me	Me			64
108/3	(35)	Me		Me		64
108/4	(36)	Me			Me	64
110/1		Me		OH		65
120/1	(40)	*t*-propenyl				53
120/2		*c*-propenyl				66
120/3		Me	vinyl			67
120/4		Me		vinyl		66
120/5		Me			vinyl	53
122/1	(38)	Pr				66
122/2		i Pr				64
122/3		acetyl				63
122/4		Me	Et			66
122/5		Me		Et		67
122/6		Me			Et	67
122/7	(18)	Me	Me	Me		67
134/1		Me		*t*-propenyl		62
134/2		Me			*t*-propenyl	62
134/3		Et			vinyl	10
134/4		Me	vinyl	Me		68
136/1		Me		n Pr		66
136/2		Me	i Pr			66

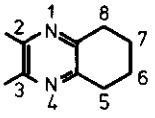
136/3 (39)	Me		i Pr		53
136/4	Me			i Pr	66
136/5	Me	acetyl			69
136/6	Me		acetyl		66
136/7	Me			acetyl	44
136/8	Et		Et		64
136/9	Et			Et	53
136/10	Me	Me	Et		66
136/11 (14)	Me	Et	Me		12
136/12 (12)	Me	Et		Me	16
136/13	Me	Me		Et	66
136/14 (19)	Me	Me	Me	Me	64
138/1	CH_2OH	Me	Me		44
146/1	2'-furyl				66
148/1	Me	butenyl			68
150/1	i-pentyl				68
150/2	2-oxopropyl				70
150/3 (37)	Me	n Bu			10
150/4 (28)	Me	i Bu			10
150/5	Me		i Bu		66
150/6	Me			i Bu	66
150/7	Et			Pr	66
150/8	Et		i Pr		66
150/9	Et	acetyl			67
150/10	Et		acetyl		70
150/11	Et			acetyl	66
150/12	Me	n Pr	Me		64
150/13 (11)	Me	n Pr		Me	10
150/14 (48)	Me	i Pr	Me		66
150/15	Me	i Pr		Me	66
150/16	Me	Me		acetyl	69
150/17	Me	acetyl	Me		69
150/18	Me	acetyl		Me	69
150/19	Et	Me	Et		66

150/20	Et	Me		Et	66
150/21	Et	Et	Me		66
150/22	Me	Me	Me	Et	64
160/1	Me	2'-furyl			10
160/2	Me		2'-furyl		66
160/3	Me			2'-furyl	66
164/1	Me	n-pentyl			10
164/2	Me		n-pentyl		10
164/3	Me			n-pentyl	62
164/4	Me	i-pentyl			66
164/5	Me		i-pentyl		68
164/6	Me			i-pentyl	64
164/7	Me	*sec*-pentyl			66
164/8	Me			*sec*-pentyl	64
164/9	acetyl		acetyl		71
164/10	Me	n Bu	Me		66
164/11 (10)	Me	n Bu		Me	66
164/12 (49)	Me	i Bu	Me		64
164/13 (51)	Me	i Bu		Me	64
164/14 (50)	Me	*sec*-Bu	Me		64
164/15	Et	Et	Et		66
164/16	Me	Et	Me	Et	64
164/17	Me	Et	Et	Me	64
170/1	Me		Ph		65
174/1	Me			2'-(4'-methylfuryl)	
174/2	Me	2'-furyl		Me	69
174/3	Me		Me	2'-furyl	69
174/4	Me	Me	2'-furyl		69
174/5	Me	2'-(4'-methylfuryl)			69
174/6	Me		2'-(4'-methylfuryl)		69
174/7	Me			2'-(4'-methylfuryl)	69
174/8	Me	2'-(5'-methylfuryl)			69
174/9	Me		2'-(5'-methylfuryl)		69
174/10	Me			2'-(5'-methylfuryl)	69

176/1	Me	2'-thiophenyl			10
178/1 (9)	Me	n-pentyl		Me	11
178/2	Me	Me	i-pentyl		64
178/3 (13)	Me	i-pentyl	Me		64
178/4	Me	i-pentyl		Me	64
178/5	Me	Me	*sec*-pentyl		64
188/1	Me			2'-[3',4'(5')-dimethylfuryl]	66
188/2	Me			2'-(4',5'-dimethylfuryl)	69
192/1(55)	Me	n-hexyl		Me	11
192/2	Me	Me	Me	i-pentyl	64
192/3	Me	Me	Me	*sec*-pentyl	64
210/1 (15)	Me	*t*-styryl	Me		13
210/2 (16)	Me	*c*-styryl	Me		13
124/1 (41)	OMe	Me			10
138/2	OMe	Et			30
138/3	OEt	Me			10
140/1 (42)	SMe	Me			10
152/1	OMe	n Pr			10
152/2 (5)	OMe	i Pr			7
152/3	OMe	acetyl			71
154/1	SEt	Me			10
166/1	OMe	n Bu			10
166/2 (4)	OMe	i Bu			7
166/3 (6)	OMe	*sec*-Bu			7
166/4	OMe	acetyl	Me		72
166/5	OMe	i Pr	Me		7
180/1	OMe	n-pentyl			10
180/2	OMe	i-pentyl			10
182/1	OMe	2-OH i Pr	Me		72
196/1	OMe	i Pr	Me	OMe	7

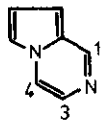


No.	R_2	R_3	R_5	R_6		R_7	R_8	Ref.
118/1					=			73
120/6								57
132/1	Me	(Me)			=			73
132/2				Me				73
132/3					=	Me		73
134/5	Me							57
134/6			Me					57
146/2	Me	(Me)		Me	=	(Me)		73
148/2	Et							57
148/3				Et				57
148/4	Me	Me						57
148/5	Me		Me					63
148/6		Me	Me					63
148/7			Me					57
162/1	Me	Et						57
162/2	Et		Me					74
162/3	Me	Me	Me					57
162/4	Me		Me			Me		57
176/2	acetyl		Me					44
192/4	acetyl	OH	Me					71



130/1		H	H		=	=		75
134/7	(21)	H	H					57

144/1		Me			=			=		66
144/2				Me	=			=		71
144/3					=	Me		=		75
148/8	(22)	Me								57
148/9				Me						57
158/1		Me	Me		=			=		66
160/4		Et					=			44
162/5		Et								57
162/6	(23)	Me	Me							57
162/7		Me		Me						66
162/8				Me			Me			58
162/9				Me					Me	58
174/11		Et	Me				=			44
176/3		Et	Me							44
176/4		Et		Me						44
176/5		Et				Me				44
176/6				Me			$(Me)_2$			58
188/3	(24)	$-CH_2CH_2-CH_2CH_2-$								16



	R_1	R_3	R_4					
118/2	H	H	H					41
132/4	Me							41
132/5		Me						41
135/6			Me					41
146/3	Me	Me						41
146/4	Me		Me					41
146/5		Me	Me					41
160				C_3H_7				41
174				C_4H_9				41

* The position of the double bond in unsaturated cycloalkyl pyrazines is indicated by placing "=" between the appropriate substituents in the table.

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