

---

---

# Pyridine-Containing Schiff Base Derivatives for the Structural Determination of Long-Chain Aldehydes by Gas Chromatography Combined with Mass Spectrometry

D. J. Harvey

Department of Pharmacology, University of Oxford, Oxford, United Kingdom

---

Long-chain aldehydes, encountered as insect pheromones, were converted into Schiff bases with 3-aminopyridine, 3-(aminomethyl)pyridine, or 2-aminopyrimidine to provide derivatives suitable for revealing the alkyl chain structure by mass spectrometry. The two pyridine-containing derivatives were satisfactory in initiating a radical-induced cleavage of the chain to give a series of fragment ions, the masses and relative abundance of which revealed the chain structure. The derivatives were applied to aldehydes having straight, branched (*iso* and *anteiso*), and unsaturated ( $\delta$ -7,  $\delta$ -9,  $\delta$ -11, and  $\delta$ -13) structures; these all gave the fragmentation patterns that have been seen earlier for similar pyridine-containing derivatives of fatty acids (picolinyl esters) and alcohols (nicotines). Of the two derivatives, those from 3-aminopyridine gave slightly simpler spectra. Derivatives formed from 2-aminopyrimidine were less satisfactory in revealing chain structure. (*J Am Soc Mass Spectrom* 1991, 2, 245-249)

---

Long-chain aldehydes and ketones are frequently encountered as pheromones of both insects and lower vertebrates (see for example [1, 2]). The alkyl chains of these compounds can have various structural features, such as unsaturation, that are difficult to determine by mass spectrometry without preparation of group-specific derivatives such as thiomethyl ethers of double bonds. Following the successful applications of pyridine-containing derivatives to the structural elucidation of fatty acids (picolinyl esters) [3-5], fatty alcohols (nicotines) [6, 7], and glycerol ethers (nicotinylidene derivatives) [8], we extended this type of derivative to long-chain aldehydes by preparation of the Schiff base derivatives 1, 2, and 3 from 3-aminopyridine, 3-(aminomethyl)pyridine, and 2-aminopyrimidine, respectively. Like the earlier pyridine-containing derivatives, these fragment under electron impact by charge localization on the pyridine residue and by abstraction of a hydrogen atom from the alkyl chain, to leave a radical site which then initiates a radical-site-induced fragmentation. This results in the spectrum containing a series of ions formed by cleavage at each carbon-carbon bond. The masses and relative abundances of these ions carry the required structural information. The advantage of derivatives of this type is that they

involve derivatization of the functional group of the compound rather than the structural features of the alkyl chain, such as unsaturation or branching, and are thus applicable to all chain types. This article describes derivatives of this type applicable to long-chain aldehydes.

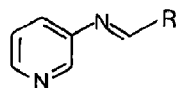
## Experimental

### Materials

Straight-chain primary alcohols ( $C_{10}$ ,  $C_{11}$ ,  $C_{12}$ ,  $C_{14}$ ,  $C_{16}$ , and  $C_{18}$ ), *cis*-7-tetradecenal, *cis*-9-hexadecenal, *cis*-11-hexadecenal, *cis*-13-octadecenal, and *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) were obtained from Sigma Chemical Co. (Poole, Dorset, UK). 3-Aminopyridine, 3-(aminomethyl)pyridine, and nonanal were from Aldrich Chemical Co. (Gillingham, Dorset, UK). Branched-chain fatty acids (*iso* and *anteiso*- $C_{14}$ - $C_{21}$ ) were obtained from Applied Science Laboratories (State College, PA) and were reduced to the corresponding alcohols with lithium aluminum hydride in ether. 2-Aminopyrimidine was obtained from the Dyson Perrins Laboratory (Oxford, UK). The straight-chain and branched-chain aldehydes were synthesized from the corresponding alcohols by oxidation with chromium trioxide in pyridine for 30 min at room temperature followed by solvent (ethyl acetate) extraction.

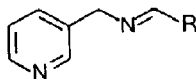
---

Address reprint requests to D. J. Harvey, Department of Pharmacology, South Parks Road, Oxford, OX1 3QT, UK.

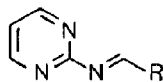


R = Alkyl chain

Structure 1



Structure 2



Structure 3

### Schiff Base Derivatives of Long-Chain Aldehydes

The aldehyde (about 0.1 mg) was dissolved in acetonitrile and heated for 15 min with about 1 mg of either 3-(aminomethyl)pyridine, 3-aminopyridine, or 2-aminopyrimidine. Aliquots of these solutions were injected directly into the gas chromatography combined with mass spectrometry (GC/MS) instrument.

### Gas Chromatography Combined with Mass Spectrometry

Gas chromatography combined with mass spectrometry data were recorded with a VG 70/70F mass spectrometer, (Manchester, UK) interfaced to a Varian 2440 gas chromatograph (Palo Alto, CA) that was fitted with an SGE (Ringwood, Australia) split-splitless injection system operated in the split mode with a split ratio of 10:1. The column was a 25 m  $\times$  0.2 mm OV-1 bonded-phase fused-silica capillary, film thickness 0.33  $\mu$ m terminating 10 mm inside the mass spectrometer ion source. Helium at 1 mL/min (measured in the absence of the mass spectrometer vacuum) was used as the carrier gas. Operating conditions were as follows: injector, transfer line, and ion source temperatures, 300, 300, and 280  $^{\circ}$ C, respectively, column

oven temperature programmed in the range 200–350  $^{\circ}$ C at 4  $^{\circ}$ C/min to give a retention time of about 5 min for each sample; accelerating voltage, 4 kV; electron energy, 70 and 25 eV; trap current, 1.0 mA; scan speed, 1 sec/decade. Spectra were acquired and processed with a VG 11/250 data system.

## Results and Discussion

### Derivatives with 3-Aminopyridine

These compounds formed in near quantitative yield and were examined by GC/MS by direct injection of the reaction mixture into the chromatograph. Gas liquid chromatography properties were generally good with little tailing. To demonstrate stability towards trimethylsilylating reagents, an aliquot was blown to dryness, dissolved in BSTFA, and a sample was injected. This gave the same result as before. The reaction worked equally well at the low microgram level and was thus suitable for use with biological samples.

*Straight-chain aldehydes.* The 25-eV electron impact mass spectrum of the 3-aminopyridine Schiff base derivative of *n*-octadecanal is shown in Figure 1. The expected radical-induced fragmentation gives a series of ions separated by 14 u (see Scheme I), but the relative abundance of these was not as great as in the corresponding spectra of picolinyl [3–5] or nicotinate [6, 7] derivatives of acids and alcohols, respectively (note the  $\times 10$  multiplication factor above mass 140 in Figure 1). Use of 25 eV rather than 70 eV reduced the relative abundance of the low mass ions by a factor of 2–3, but there was no significant loss in sensitivity. The molecular ion, on the other hand, was satisfactorily abundant. The base peak at  $m/z$  120 appeared to have been formed by hydrogen migration to the pyridine moiety with cleavage to give the allylic radical ion *b* (Scheme II). This was supported by corresponding base peaks in the spectra of the other Schiff base derivatives discussed below; the source of the migrating hydrogen atom was not determined. The high relative abundance of the ion at  $m/z$  133 can be accounted for by formation of the conjugated ion *c* (Scheme III). From here to the  $[M - 1]^+$  ion at  $m/z$

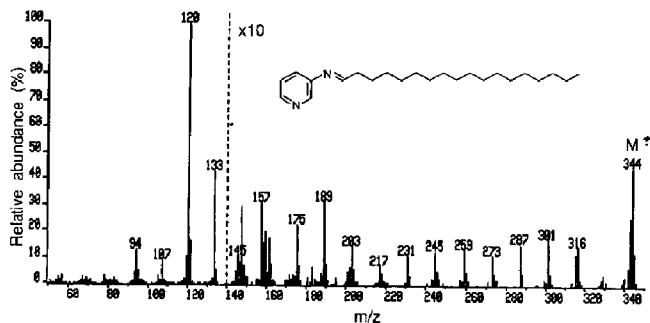
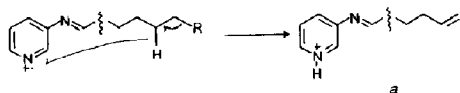
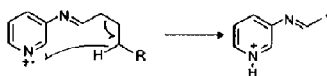


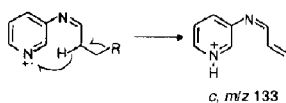
Figure 1. Electron impact mass spectrum (25 eV) of the 3-aminopyridine Schiff base derivative of *n*-octadecanal.



Scheme I



Scheme II



Scheme III

343, the diagnostic series of ions is  $m/z$  147, 161, 175, 189, 203, 217, 231, 245, 259, 273, 287, 301, 315, and 329. The structures of the two ions at  $m/z$  157 and 159 are unclear, although they appeared to contain the pyridine ring.

**Branched-chain aldehydes.** The spectrum of the 3-aminopyridine Schiff base derivative of *iso*-octadecanal (16-methyl-heptadecanal) is shown in Figure 2. This shows the same general features as the spectrum of the straight-chain compound discussed above, with the expected change in the pattern of ions revealing the position of the branchpoint [3]. This structural feature is apparent by (1) the low abundance of the ion at  $m/z$  315, reflecting the fact that the molecule cannot lose 29 u from the end distal to the pyridine ring and (2) the relatively high abundance of the adjacent ions at  $m/z$  301 and 329 caused by cleavage at the tertiary carbon atom. In this respect the fragmentation is strictly analogous to that of the picolinyl and nicotinate derivatives discussed earlier. *Ante*-iso-aldehydes showed similar behavior, with the missing ion corresponding to  $[M - 43]^+$  (Figure 3).

**Unsaturated aldehydes.** The spectrum of *cis*-13-octadecenal is shown in Figure 4. The ions in the lower half of the spectrum were the same as those in the

spectra of the saturated compounds just discussed, but those in the upper half of the spectrum showed the pattern expected for monounsaturations [3]. Thus, the molecular ion was abundant, and the 40 rather than 42 u difference between the ions  $d$   $m/z$  245 and  $f$   $m/z$  285 located the position of unsaturation. The relatively high abundance of the characteristic ions at  $m/z$  245 ( $d$ ), 299 ( $g$ ), and 313 ( $h$ ) can be accounted for by the mechanisms shown in Schemes IV-VI, respectively. Ion  $d$  involves the loss of an allylic radical, whereas ions  $j$  and  $h$  can be rationalized as involving abstraction of one of the allylic hydrogen atoms. The spectra of the homologous unsaturated aldehydes (see Materials) gave similarly diagnostic spectra but with a drop in the relative abundance of ion  $g$ . It has been observed [9, 10] with the spectra of the picolinyl derivatives of the fatty acids that unsaturation closer to the oxygenated function than C-7 results in a loss in the elevated relative abundance of this ion although the increased abundance of ion  $h$  remains unchanged.

#### Derivatives with 3-(Aminomethyl)pyridine

The spectra of these derivatives were strictly analogous to those of the 3-aminopyridine derivatives just discussed (see Figure 5) with the addition of several relatively abundant ions in the lower half of the spectrum. These were produced as shown in Scheme VII. The shift in the base peak from  $m/z$  120 in the spectra of the 3-aminopyridine derivatives to  $m/z$  134 in the spectra of these 3-(aminomethyl)pyridine Schiff bases supports the structure for  $m/z$  120 proposed above. The ion at mass 203 corresponds to that at  $m/z$  189 in the above spectra.

#### Derivatives with 2-Aminopyrimidine

The 2-aminopyrimidine group did not abstract a hydrogen atom from the aliphatic chain as effectively as the pyridine ring and, consequently, although the spectra showed the normal series of radical-induced ions, these were relatively weak and did not adequately reflect the position of methyl branch points as can be seen in Figure 6. However, the molecular ions were abundant. Ions of the type discussed above,

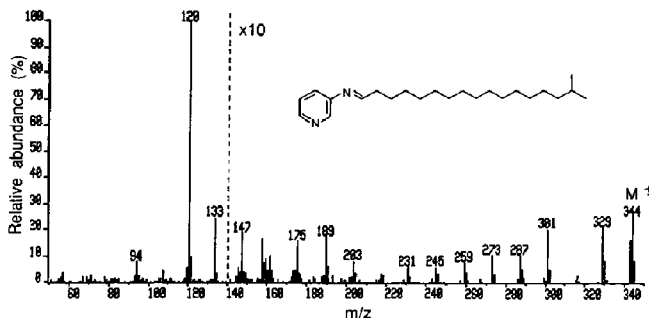


Figure 2. Electron impact mass spectrum (25 eV) of the 3-aminopyridine Schiff base derivative of *iso*-octadecanal.

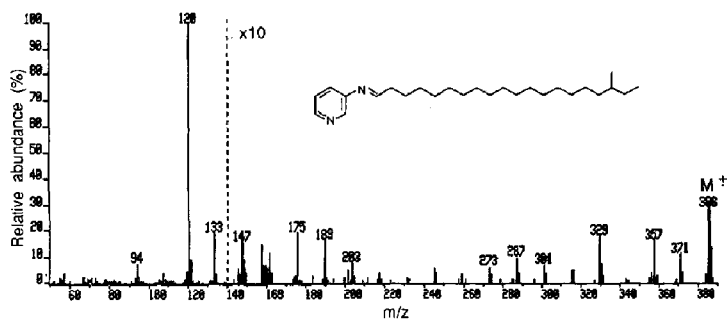


Figure 3. Electron impact mass spectrum (25 eV) of the 3-aminopyridine Schiff base derivative of *anteiso*-heneicosanal.

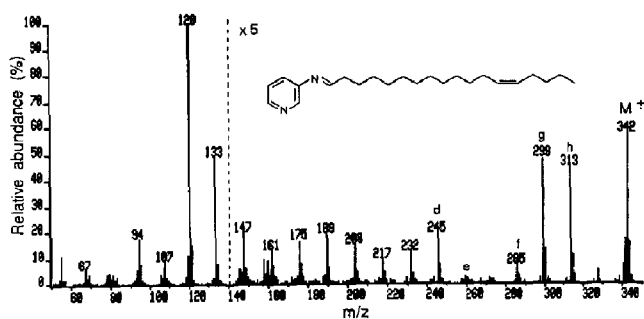
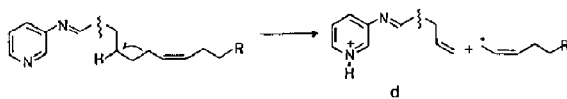
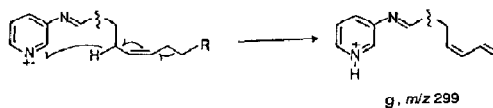


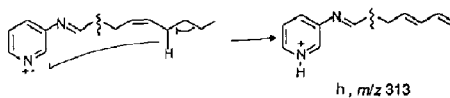
Figure 4. Electron impact mass spectrum (25 eV) of the 3-aminopyridine Schiff base derivative of *cis*-13-octadecenal.



Scheme IV



Scheme V



Scheme VI

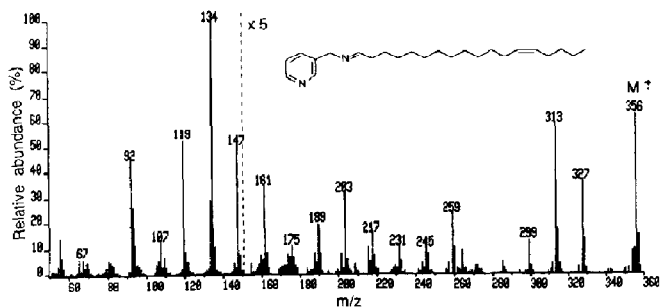
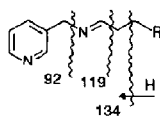


Figure 5. Electron impact mass spectrum (25 eV) of the 3-(aminomethyl)pyridine Schiff base derivative of *cis*-13-octadecenal.



Scheme VII

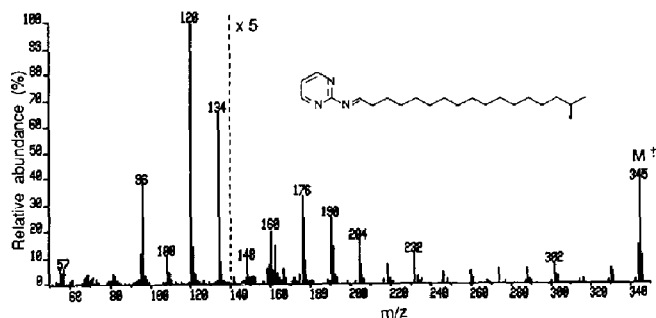


Figure 6. Electron impact mass spectrum (25 eV) of the 2-aminopyrimidine Schiff base derivative of iso-eicosanal.

reflecting unsaturation, were present in the spectra of unsaturated aldehydes, but were relatively weaker than those in the spectra of the other Schiff base derivatives. Therefore, these derivatives were not investigated further. Ions in the lower part of the spectrum reflect predictable cleavages adjacent to the pyrimidine ring.

## Conclusions

In conclusion, Schiff base derivatives from both 3-aminopyridine and 3-(aminomethyl)pyridine were suitable for revealing the structure of straight-, branched- and unsaturated-long-chain aldehydes by initiating radical-induced cleavages of the alkyl chain in a manner similar to that observed previously for analogous derivatives of long-chain acids and alcohols. Although both derivatives gave fragment ions of comparable relative abundance, the simpler spectra produced by the 3-aminopyridine Schiff bases made them marginally better as analytical derivatives. Because the fragmentation of these compounds was found to be strictly analogous to that displayed by corresponding pyridine-containing derivatives of fatty acids and alcohols, it would be expected that other

alkyl chain structures, such as cyclopropane rings, could be similarly identified.

## Acknowledgments

The mass spectrometer was originally purchased with the aid of a grant from the Medical Research Council. Funds were also provided by the Wellcome Trust.

## References

- Brand, J. M.; Young, J. C.; Silverstein, R. M. *Fortschr. Chem. Org. Naturst.* **1979**, *37*, 1-190.
- Aldrich, J. R.; Lusby, W. R.; Kochansky, P. *Experientia* **1986**, *42*, 583-585.
- Harvey, D. J. *Biomed. Mass Spectrom.* **1982**, *9*, 33-38.
- Harvey, D. J. *Biomed. Mass Spectrom.* **1984**, *11*, 187-192.
- Harvey, D. J. *Biomed. Mass Spectrom.* **1984**, *11*, 340-347.
- Vetter, W.; Meister, W. *Org. Mass Spectrom.* **1981**, *16*, 118-122.
- Harvey, D. J.; Tiffany, J. M. *Biomed. Mass Spectrom.* **1984**, *11*, 353-359.
- Harvey, D. J. *Biomed. Environ. Mass Spectrom.* in press.
- Christie, W. W.; Brechany, E. Y.; Holman, R. T. *Lipids* **1987**, *22*, 224-228.
- Harvey, D. J. *Biomed. Environ. Mass Spectrom.* **1989**, *18*, 719-723.