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Dear Sir,

Characterization and Differentiation of Heterocyclic Isomers. Part 5†—Mass Spectra and Library Entries of Benz(is)oxazoles

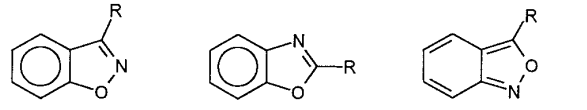
The identification and structural characterization of isomeric compounds are very important problems in chemistry and biochemistry. Among different analytical techniques, mass spectrometry is a very powerful tool for the unambiguous identification and assignment of the structure of an unknown compound. To help in this aim, some collections of electron ionization mass spectra have been compiled, and more and more sophisticated search algorithms have been proposed and implemented.[2,3]

In the ambit of a general project aimed at characterizing and differentiating heterocyclic isomers,[4,5] after having studied methyloxazolopyridines,[6] we recently started to investigate their benzo derivatives. Although these compounds are fairly common, each of them has been studied independently of the others and their behaviours in the gas phase have been not compared.

We report here on a study in the gas phase of the two isomers 3-methyl-1,2-benzisoxazole (**1**) and 2-methylbenzoxazole (**2**) and on errors found in commercially available mass spectral databases regarding their mass spectra and those of related compounds, i.e. 3-methyl-2,1-benzisoxazole (**3**) and benz(is)oxazole-derivatives **4**–**6** (Scheme 1).

The electron ionization mass spectra of **1** and **2** were obtained under the same experimental conditions using a double sector instrument and a quadrupole ion trap, and compared in Fig. 1. Although conventional mass spectrometry generally does not allow one to differentiate isomers, in this case compounds **1** and **2** produce different mass spectra. Similarly to other analogous heterocycles such as 3-methyl-1,2-isoxazole- and 2-methyloxazolopyridines,[7] the compounds exhibit high stability under electron ionization, their molecular ions being the base peak of the mass spectra. As observed for 1,2-benzisoxazole[8] and benzoxazole,[9,10] the elimination of a hydrogen radical is slight from both **1** and **2**. On the other hand, the $[M - H]^+$ ion is one of the most abundant ions in the mass spectra of related heterocycles, such as isoxazole[11] and benz(is)othiazoles.[12,13]

Isomers **1** and **2** follow common fragmentation pathways, but considerable differences in the relative abundances of fragment ions occur. The main fragmentation pathways involve the elimination of CO and ·CHO yielding ions at *m/z* 105 and 104, respectively, whose relative abundances allow the two isomers to be clearly distinguished. In fact, whereas with **1** fragmentations occurring in the source are dominated by ions due to the loss of CO (*m/z* 105, 76%) and ions at *m/z* 104 are significantly less abundant, isomer **2** produces both of



1 R=CH_3 **2** R=CH_3 **3** R=CH_3

4 R=H **5** R=H **6** R=H

Scheme 1

† For Part 4, see Ref. 1.

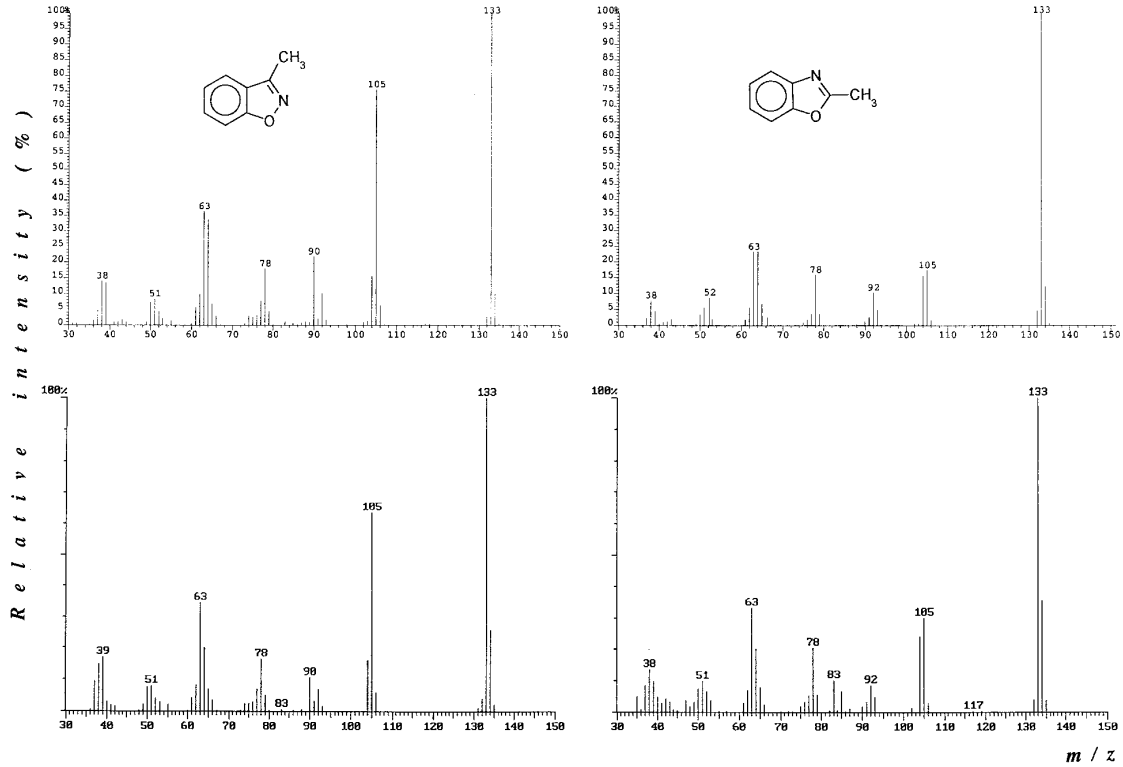


Figure 1. Comparison between electron ionization mass spectra of 3-methyl-1,2-benzisoxazole (**1**) and 2-methylbenzoxazole (**2**) obtained with a double sector instrument (top row; VG 70-250S, EI^+, 70 eV, 180 °C) and with an ion trap (bottom row; Varian Star 3400 CX-Varian Saturn 4D GC/MS system; EI^+, 70 eV, 180 °C).

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these ions with similar relative abundances. High-resolution measurements have shown that ions at m/z 105 are entirely due to the loss of CO, and no contribution of $[M - H_2CN]^+$ ions is observed with either of the isomers.

Differences are also found for the ions at m/z 90 and 92, attributable to $[M - (CO, CH_3)]^+$ and $[M - CH_3CN]^{+\cdot}$, respectively. Whereas the latter ions have similar relative abundances for both isomers, the ions at m/z 90 represent an important fragmentation pathway of **1**, while their abundance is 1.2% for **2**. The fragment ions at m/z 78 are attributable to $[M - (CO, HCN)]^{+\cdot}$. Ion species at m/z 63 are reasonably produced by loss of HCN by the ion at m/z 90, and the ions at m/z 64 are due to the loss of CO from the ion at m/z 92. Preliminary collision-induced dissociation data confirmed these pathways. Similarly to sulfur-containing analogues,[12] compounds **1** and **2** do not show abundant loss of HCN from the molecular ions.

The presence of the aromatic system and two heteroatoms allows the delocalization of positive charges. In the mass spectrum of derivative **2** doubly charged species, corresponding to M^{2+} (m/z 66.5, 2.6%) and $[M - CO]^{2+}$ (m/z 52.5, 4.5%), are present. For isomer **1** the relative abundances of doubly charged ions are below 1%.

The mass spectra of **1** and **2** obtained with the quadrupole ion trap are similar to those obtained with the sector instrument and they are still distinctive for each compound (Fig. 1). Whereas for **1** fragment ions produced in the sector instrument have generally higher relative abundances than those obtained in the ion trap, the opposite situation occurs for **2**. As an example, whereas for **1** ions at m/z 105 have relative abundances of 75.8 and 60.7% in the mass spectra obtained with the sector instrument and by the ion trap, respectively, for **2** these values are 17.1 and 29.6%, respectively. Although differences in the relative abundances of the fragment ions occur, their ratios are very close. For example, the ratios between the relative abundances of ions at m/z 105/104 are to 4.8 (sector instrument) and 4.2 (ion trap) for **1** and they are 1.1 and 1.3, respectively, for **2**.

After having obtained the mass spectra of isomers **1** and **2**, we performed a library search to compare them with those contained in mass spectral databases. As regards isomer **2**, the Nist-Nistrep library report[14] suggests the entry corresponding to 2-methylbenzoxazole as the third one. This is the only entry for which eight peaks are matched; the purity and mixture indexes are 909 and 919, respectively. The Wiley Library, 6th edition,[15] contains three different entries for compound **2**. Whereas two of these (6196 and 70925) have similar mass spectra, the molecular ion being the base peak and the $[M - H]^+$ ions having low relative abundances, the mass spectrum of entry 70926,[16] obtained at electron energy of 80 eV, shows the base peak at m/z 132 ($[M - H]^+$) and the molecular ion has a relative abundance of 17%.

Surprisingly, when we search for the mass spectrum of isomer **1**, none of the first 42 entries found in the Nist-Nistrep library[14] search suggests 3-methyl-1,2-benzisoxazole as a probable candidate. On the other hand, the library contains one entry (5976) for isomer **1**, but its mass spectrum is very different from that which we obtained. In fact, the library spectrum shows the base peak at m/z 133 and abundant fragment ions at m/z 104 (28%) and 43 (53%) (Fig. 2), reasonably attributed to $[M - CHO]^+$ and $[CH_3CO]^+$, respectively. This behaviour appears anomalous for **1**, and the expected abundant loss of CO, observed for analogous pyridine derivatives,[7] is very poor in the library spectrum. Furthermore, the presence of abundant $[CH_3CO]^+$ ions should suggest a very favourable rearrangement process, difficult to rationalize for isomer **1**. All this indicates that the library spectrum is probably not produced by compound **1**, but by its isomer **3**. Suspecting this wrong entry, we performed an extensive search for all entries for compound **1** contained in the Wiley library. We found two entries (6197 and 70927) whose mass spectra are significantly different from each other. In fact, the mass spectrum of entry 6197 resembles that reported in entry 5976 in the Nist-Nistrep library, described above. The bibliographic source code (O 2-1269-5)[17] given in the Wiley entry allows one to establish unambiguously that the mass spectrum of these two entries is produced by isomer **3** and not, as erroneously reported, by **1**. The mass spectrum of entry 70927 is completely different, but it is in good agreement with that we measured for isomer **1**. The bibliographic source code (H 62-321-0)[18] confirms that this mass spectrum is really produced by **1**. Summarizing, both entry 5976 of the Nist-Nistrep library and entry 6197 of the Wiley library contain parameters (chemical names and formulae, Chemical Abstracts Serial (CAS) number, Wisswesser line notations[19]) referred to isomer **1**. However, these are inappropriate because the mass spectra associated with the entries are produced by isomer **3**, and not by **1**. On the other hand, entry 70927 of the Wiley library contains the mass spectrum produced by **1**, but the Wisswesser notation is referred to **3**.

As regards isomer **3**, the Nist-Nistrep library has an entry (5964) whose mass spectrum shows abundant fragment ions at m/z 104 (28%) and 43 (53%), attributable to $[M - CHO]^+$ and $[CH_3CO]^+$, respectively, in agreement with the molecular structure. This mass spectrum is very close to that of entry 5976 and with that of entry 6197 of the Wiley library, the last two erroneously attributed to isomer **1**. This further confirms that the mass spectra of entries 5976 (Nist-Nistrep) and 6197 (Wiley) are produced by compound **3** and not by isomer **1**. Also, the Wiley library contains one entry for compound **3** (entry 6205) with a poor (127) quality index. In addition, fragment ions differing by 13 u from the molecular ion, present with high abundance (34%), also described in the original paper,[20] should be reasonably due to some mistakes.[21]

In the course of this investigation, library entries for 1,2-benzisoxazole (**4**), benzoxazole (**5**) and 2,1-benzisoxazole (**6**) were also examined. As regards 1,2-benzisoxazole (**4**), the Wiley library reports two entries. Unfortunately, also in this case, different parameters (chemical names, mass spectra, etc.) relevant to distinct compounds, in particular to isomers **4** and **6**, are mixed in the same entry. Although the two mass spectra show the molecular ion (m/z 119) as the base peak, they are very different one from the other. In fact, while the most abundant fragment ions in the mass spectrum of entry 3920 are at m/z 92 ($[M - HCN]^{+\cdot}$), in that of entry 69990 they are at m/z 91, and reasonably due to $[M - CO]^{+\cdot}$. By comparison with previous mass spectrometric data of analogous derivatives,[7] ions $[M - CO]^{+\cdot}$ are expected in high abundance from the fragmentation pathways of isomer **4**, whereas the loss of HCN should be of much lower abundance. For this reason, the mass spectrum of entry 3920 seems unlikely to be produced by compound **4**, but by a compound having a different arrangement of the heteroatoms in the five-membered ring. In fact, the bibliographic code (O 2-1269-4)[17] confirms that this mass spectrum is produced by 2,1-benzisoxazole (**6**) and not by **4**. On the other hand, the mass spectrum of entry 69990 is really produced by isomer **4**, but it contains fragment ions at m/z 105, differing by 14 u from the molecular ion. Reasonably these are due to some mistakes, and they are absent in another published mass spectra of 1,2-benzisoxazole.[8] Misassignments of chemical names are also present. In fact, both entries contain the same terms, i.e. '1,2-benzisoxazole' and '2,1-benzisoxazole,' that, as indicating two distinct isomers, cannot be mixed together. Entries 3920 and 69990 in the Wiley library correspond to entries 3640 and 3644, respectively, in the Nist-Nistrep library. For these latter, the same remarks as made above for the two entries in the Wiley library are still valid.

Regarding isomer **6**, both the Nist-Nistrep and the Wiley libraries contain two different entries each. All the spectra are

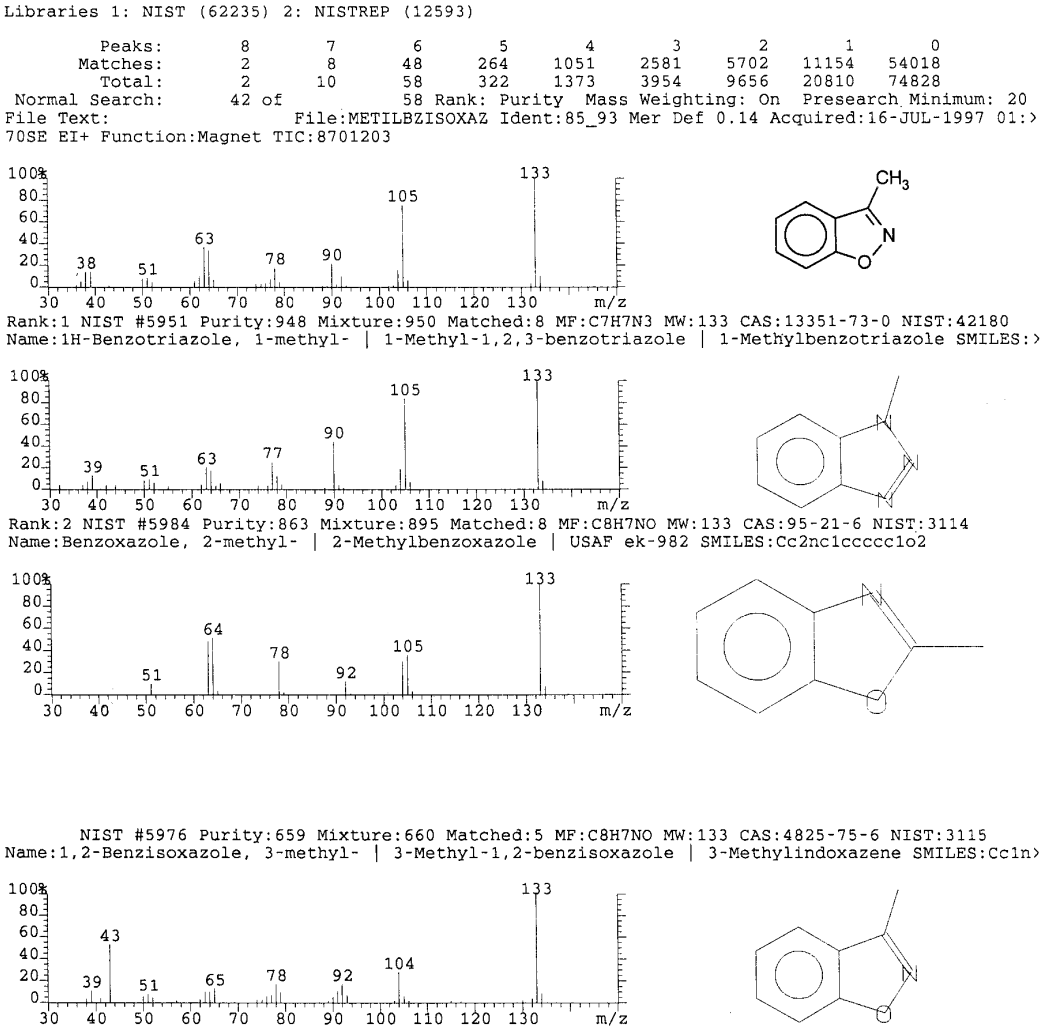

Figure 2. Nist-Nistrep library report and the first two entries found in a library search of the mass spectrum of 3-methyl-1,2-benzisoxazole (**1**). The library entry for 3-methyl-1,2-benzisoxazole (5976) is also reported, but is relevant to 3-methyl-2,1-benzisoxazole (**3**) (see text).

consistent with each other and with the behaviour in the gas phase of compound **6**. On the other hands both entries in the Wiley library (3922[22] and 69992[20]) report the name '3,4-benzisoxazol,' incorrect for identifying isomer **6**. It is noteworthy that the mass spectrum of entry 2272 in the Nistrep library, produced by isomer **6**, is superimposable on that of the entry 3640, that is misassigned to isomer **4** (see above).

Serious errors in mixing up nomenclature, CAS numbers, chemical formulae and spectra of isomers in mass spectral databases, as found in this work, might mislead and create confusion in the identification and structure elucidation of unknown compounds when the aid of a library search is invoked.

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Yours,

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