J Forensic Sci, Apr. 1979, Vol. 24, No. 2

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Chemical Ionization Mass Spectrometry of Morphine Derivatives

Chemical ionization (CI) mass spectrometry is being used with increasing frequency for studying various classes of organic compounds. Molecular weight data and information relating to the loss of labile substituent groups have been obtained from organic compounds as diverse as biogenic amines [*1*], phenothiazines [*2*], polytertiary alkylamines [*3*], quaternary amines [*4*], esters of carboxylic acids [*5*], trinitroaromatics [*6*], and carbohydrates [*7,8*].

The major advantage of using CI for the structural determination of organic compounds is the flexibility it gives the operator in altering the nature and complexity of the mass spectrum. Ion-molecule reactions with reagent gases such as methane and isobutane will produce relatively simple spectra dominated in many cases by a protonated molecular ion (MH^+). In these situations the degree of fragmentation for the compound of interest is dependent on the exothermicity of the proton transfer reaction occurring between the sample molecule and reagent gas ion. Increasing the exothermicity will result in greater fragmentation. Conversely, lowering the exothermicity of the proton transfer reaction will decrease the extent of fragmentation and enhance the abundance of the MH^+ ion. While the simplicity of protonic CI spectra discourages its use for elucidating detailed structural information, it does provide molecular weight data and information relating to the presence of acid-labile functional groups.

When some types of aprotic gases are used as reagent gases under CI conditions, electron transfer from the sample molecule to the reagent gas ion may occur. This charge exchange reaction arises when the recombination energy of the reagent ion exceeds the ionization potential of the sample molecule. Often charge exchange mass spectra will resemble conventional electron impact (EI) spectra yielding comparable structural details. For example, Jardine and Fenselau [*9,10*], using a nitrogen-nitrous oxide reagent gas mix, have reported on the charge exchange ionization spectra of morphine derivatives. These spectra are comparable to their EI counterparts with the added presence of a predominant molecular ion.

In our continuing investigation of compounds of forensic interest, our laboratory had occasion to study the CI mass spectra of morphine and a number of its derivatives. The purpose of the present study was to illustrate the influence of two reagent gases, isobutane and ethylenediamine, on the CI pattern of one particular class of organic compounds, morphine derivatives.

Experimental Procedure

All CI spectra were taken on a Du Pont 21-490 single focusing mass spectrometer. Spectra were obtained at a source temperature of 200 °C and an ionizing voltage of 70 eV.

Received for publication 27 July 1978; accepted for publication 1 Sept. 1978.

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All materials were introduced into the source with a direct insertion probe. The instrument was scanned over the mass region of interest as the direct probe was heated until the sample volatilized into the source. In this study the recorded spectra were obtained at a probe temperature between 250 and 300°C.

Isobutane reagent gas was kept at a source pressure of 0.5 to 1.0 torr (66.6 to 133.3 Pa). Ethylenediamine reagent gas was introduced into the source via a heated batch inlet. A 2-μl injection of ethylenediamine was injected into a 2-cm^3 batch inlet prior to the introduction of the sample. The ethylenediamine (obtained from MC & B) had a label purity of 98%.

Synthesis

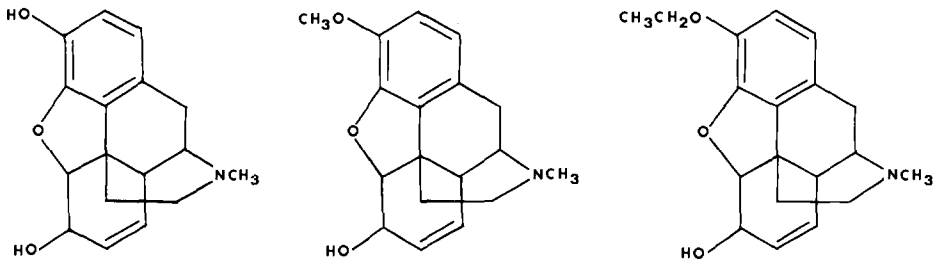
Diacylmorphine compounds, acylcodeine compounds, and acylethylmorphine derivatives were synthesized according to the methods described by Splies and Shellow [*11*]. The O^3-monoacylmorphine compounds were prepared according to the method described by Welsh [*12*]. The O^6-monoacylmorphine compounds were prepared according to the procedures described by Wright [*13*] from the appropriate diacylmorphine compound. Preparation of the mixed diacylmorphine derivatives was accomplished by the acylation of O^3-monoacylmorphine with the appropriate anhydride.

Morphine (I), codeine (II), and ethylmorphine (III) (Fig. 1) were reduced to their respective dihydro derivatives in a hydrogenator with platinum black as a catalyst.

All compounds studied were purified by several recrystallizations from suitable solvents. The purities (98% or greater) were confirmed by gas chromatography.

Results and Discussion

The isobutane CI data of morphine and a number of its derivatives are presented in Table 1. Morphine's base peak at *m/e* 268 arises from the loss of a protonated hydroxy group from the C-6 position. The relatively higher abundance of this ion compared to MH$^+$ is attributed to the formation of a stabilized allylic carbonium ion [*14*]. Similarly, the resultant formation of a highly unstable aryl carbonium ion precludes the elimination of a hydroxy group from morphine's C-3 position. The O-3 and O-6 substituted derivatives of morphine behave in an analogous fashion. Protonation by the reagent ion and subsequent loss of acetic acid or propionic acid from the MH$^+$ ion signifies the presence of either an acetyl or propionyl group on the O-6 position that consistently yields the dominant ion in the spectrum. Comparable elimination does not take place on the unreactive C-3 site of the molecule. Not unexpectedly, this behavior is also observed for codeine and ethylmorphine as well as for their respective O-6 acetyl and propionyl derivatives.



I II III

FIG. 1—*Structure of morphine* (I), *codeine* (II), *and ethylmorphine* (III).

TABLE 1—*Isobutane CI data of morphine compounds.* [a]

Compound	Molecular Weight	MH^+	$MH^+ - H_2O$	$MH^+ - CH_3COOH$	$MH^+ - CH_3CH_2COOH$	$MH^+ - CH_3CHO$
Morphine (I)	285	10	100	...	...	...
Dihydromorphine	287	100	3	...	...	...
O^3-Monoacetylmorphine	327	5	100	...	...	...
O^3-Monacetyldihydromorphine	329	100	15	...	...	...
O^6-Monoacetylmorphine	327	12	...	100	...	...
O^6-Monoacetyldihydromorphine	329	100	...	10	...	...
Diacetylmorphine (heroin)	369	10	...	100	...	...
Diacetylhydromorphine	371	100	...	20	...	...
O^3-Monopropionylmorphine	341	12	100	...	...	...
O^3-Monopropionyldihydromorphine	343	100	10	...	...	...
O^6-Monopropionylmorphine	341	30	...	...	100	...
O^6-Monopropionyldihydromorphine	343	100	...	...	10	8
Dipropionylmorphine	397	8	...	...	100	...
Dipropionyldihydromorphine	399	100	...	...	12	10
O^3-Acetyl-O^6-propionylmorphine	383	15	...	...	100	...
O^3-Acetyl-O^6 propionyldihydromorphine	385	100	...	...	16	5
O^3 Propionyl-O^6 acetylmorphine	383	10	...	100	...	...
O^3-Propionyl-O^6-acetyldihydromorphine	385	100	...	16	...	...
Codeine (II)	299	12	100	...	...	...
Dihydrocodeine	301	100	33	...	...	...
Acetylcodeine	341	15	...	100	...	...
Acetyldihydrocodeine	343	100	...	12	...	...
Propionylcodeine	355	10	...	...	100	...
Propionyldihydrocodeine	357	100	...	...	20	7
Ethylmorphine (III)	313	12	100	...	...	...
Dihydroethylmorphine	315	100	30	...	...	...
Acetylethylmorphine	355	15	...	100	...	...
Acetyldihydroethylmorphine	357	100	...	12	...	...
Propionylethylmorphine	369	20	...	...	100	...
Propionyldihydroethylmorphine	371	100	...	...	15	15

[a] All intensities are expressed as a percentage of the base peak (100). Ions containing the isotope ^{13}C are not included.

Saturation of morphine's C-7 double bond removes the allylic carbonium ion as the primary driving force for elimination of the C-6 substituent. All of the dihydromorphine compounds studied showed predominant MH^+ ions in their isobutane CI spectra. While the loss of protonated C-6 substituents from dihydromorphine derivatives does occur, it always yields an ion of lower intensity compared to MH^+.

Bowen and Field [*15*] have reported using an ethylenediamine-isobutane mixture as a reagent gas in a CI source. The utility of ethylenediamine stems from its increased basicity relative to other common reagent gases, namely isobutane and ammonia. By using a reagent gas having a relatively high proton affinity, ethylenediamine CI can be expected to show little, if any, fragmentation. This situation will undoubtedly facilitate the acquisition of molecular weight data from compounds failing to show predominant MH^+ ions with isobutane, as is the case with some morphine derivatives.

While ammonia is useful as a reagent gas for characterizing highly labile compounds of forensic science interest [*16*], its use may be accompanied by gas handling and ventilation problems [*15*]. Our experience with ethylenediamine shows it to be a valuable and innocuous reagent gas. Substituting this reagent for isobutane in our CI source, we were able to achieve a "soft" ionization of morphine and its derivatives. The gas was introduced into the spectrometer's source via a heated batch inlet prior to the insertion of our sample with a direct probe.

The ethylenediamine CI data of some representative morphine derivatives are listed in Table 2. As expected, enhancement of the relative intensity of MH^+ as compared to isobutane CI is achieved. Significantly, the lower exothermicity of the proton transfer reaction markedly reduced the extent of elimination of the C-6 substituent. For each compound studied, the MH^+ ion is by far the most abundant ion in the spectrum. The formation of the adduct ion $(M+61)^+$ between the molecule under investigation and protonated ethylenediamine conforms with the previously reported tendency of reagent ions to form adducts with sample molecules as the reagent gas' proton affinity increases [*15,17*]. Another characteristic of the ethylenediamine CI spectra of morphine derivatives is the consistent appearance of a metastable ion arising from the decomposition $(M+61)^+ \rightarrow MH^+$. Its presence offers additional confirmation of the identity of the protonated molecular ion.

Summary

Isobutane CIMS is useful for determining the molecular weight of morphine and its derivatives, as well as for identifying labile acyl substituents on morphine's O-6 position. Furthermore, this technique will provide information relating to the presence of absence of pi-bonding on the C-7 carbon. The spectra of morphine derivatives can be further simplified by employing ethylenediamine as a reagent gas. This approach proves useful for eliciting or confirming molecular weight information from the CI spectrum. In our laboratory extended use of ethylenediamine has been accomplished without any deleterious

TABLE 2—*Ethylenediamine CI data of morphine compounds.* [a]

Compound	MH^+	MH^+-H_2O	$(M+C_2H_9N_2)^+$	MH^+-CH_3COOH
Morphine (I)	100	9	6	...
O^6-Monoacetylmorphine	100	...	4	28
O^3-Monoacetylmorphine	100	14	31	...
Diacetylmorphine (heroin)	100	...	26	13
Codeine (II)	100	7	16	...

[a] Isotopic contributions are not included in this table.

effect on the mass spectrometer's source or its vacuum system. The utility of isobutane and ethylenediamine CI rests with its ability to supply the analyst with structure elucidation data that may be used to complement more detailed information extractable from either EI or CE spectra. This aspect of mass spectrometry is especially useful when one is dealing with an unknown member of a particular class of organic compounds.

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