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GAS PHASE ION CHEMISTRY OF COUMARINS: *AB INITIO* CALCULATIONS USED TO JUSTIFY NEGATIVE CHEMICAL IONIZATION TRENDS

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ABSTRACT. The gas phase ion chemistry of coumarins using electron ionization (EI), positive chemical ionization (PCI) and negative chemical ionization (NCI) in a time of flight and quadrupole mass spectrometer (qMS) coupled to a gas chromatograph is outlined. The observations in NCI mode were complimented with *Ab initio* calculations. This was because NCI gave enhanced signals in contrast to EI and PCI and therefore could serve as a potential ionization method for quantitative analysis. For the two classes of analytes that were examined here, i.e. methyl and acetyl coumarin derivatives, experimental data showed that the methyl derivatives underwent dissociative electron capture to produce ions of the type $[M–H]^-$ while the acetyl derivatives underwent resonance electron capture to produce ions of the type $M^{\cdot-}$. The $M^{\cdot-}$ type of ions were more intense than the earlier. *Ab initio* calculations showed higher electron affinities in the acetyl than the methyl derivatives.

KEY WORDS: Coumarins, *Ab Initio* calculations, Electron ionization, Positive chemical ionization, Negative chemical ionization

INTRODUCTION

Coumarin (2H-1-benzopyran-2-one, CAS no. 91-64-5), shown in Figure 1, is a naturally occurring product which has a fused benzene and α-pyrone rings and was first isolated from Tonka beans in 1822 [1]. It is a white crystalline solid with a fresh, hay-like, sweet, spicy and strong fragrant odor and has found use as sweetener, perfume fixative and as a flavor enhancer. Coumarins have been isolated from at least 60 plants that include lavender, vanilla beans, sweet woodruff, sweet clover, citrus oils, oil of cassia and balsum of Peru [2]. Despite their natural occurrence, the principal source of coumarins is laboratory synthesis [3].

Mass spectrometry has been used in several instances for the qualitative identification and quantification of coumarins. Methods employing liquid chromatography-mass spectrometry (LC-MS) and in some cases gas chromatography mass spectrometry (GC-MS) in different matrices have been reported [4-16]. Fragmentation patterns of some coumarins using high and low resolution mass spectrometry in electron ionization (EI) mode have been reported in the literature [17-18]. A study on the analytical characteristics of some synthetic and naturally occurring coumarins in a LC-MS system operated using ion trap technology, gas chromatography (GC) and polarography has also been reported [19]. Suffice to mention that there is a very large number of possibilities of ionization/fragmentation patterns that are possible in the ion chemistry of any given chemical compound, coumarins inclusive. Classically, from the Franck–Condon principle, the electronic transition that leads to ionization of any molecule occurs much faster than any changes in the bonding or positions of the atoms of the molecule. The structure of the molecular ion thus remains the same as the structure of the molecule from which it is formed from. The fragmentation of the molecular ion thus depends on the internal energy that they contain which in turn depends on the thermal energy of the molecule just before ionization [20]. Due to the numerous vibrational levels between which transitions can occur, a

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mass spectrum is thus a statistical average of all possible transitions. It is thus imperative to compliment plausible ionization/fragmentation patterns with some other analytical techniques or theoretical calculations in order to consolidate fragmentation observations.

This study outlines some ion chemistry characteristics of the coumarin derivatives shown in Figure 2 in the EI, PCI and NCI modes of ionization in a quadrupole mass spectrometer. Further, *Ab Inito* calculations are used to consolidate the proposed ionization and fragmentation characteristics including the observed trends using NCI of the coumarins under study.

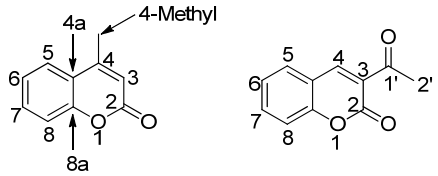
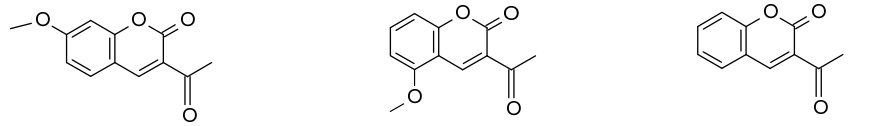


Figure 1. Generic chemical structures of the coumarins in this study.



3-Acetyl-7-methoxycoumarin (A) 3-Acetyl-5-methoxycoumarin (B) 3-Acetylcoumarin (C)

4-Methyl-5,7-dimethoxycoumarin (D) 4,6-Dimethylcoumarin (E)

Figure 2. Specific chemical structures of coumarins studied.

EXPERIMENTAL

Materials and instruments

HPLC analytical grade acetone, hexane, dichloromethane, methanol, toluene, ethyl acetate and acetonitrile were all purchased from Sigma-Aldrich, (St. Louis, USA). 99.9% pure chlorpromazine which was used as an internal standard in gas chromatography was bought from Sigma-Aldrich (Germany). The coumarins (3-acetyl-7-methoxycoumarin, 4-methyl-5,7-dimethoxycoumarin, 4,6-dimethylcoumarin, 3-acetylcoumarin, and 3-acetyl-5-methoxy-coumarin) were donated by the Department of Chemistry-Synthetic Organic Chemistry Laboratory at the University of Botswana, Botswana. A 12-port model Supelco-Visiprep^{TM} 20" Hg Vacuum SPE manifold (USA) was used for solid phase extraction studies. A Sartorious super micro balance GmbH purchased from Goettingen, Germany, was used for weighing of the

solid analytes. An ultrasonic bath from Sigma-Aldrich (USA) was used to dissolve the coumarins in tap water during the sample preparation stage prior to SPE experiments.

High resolution mass spectra

High resolution mass spectra were obtained using the Waters GCT Premier Time of Flight (ToF) mass spectrometer in the electron ionization (EI) mode. The lock mass was *m/z* 218.9856 from the perfluorotributylamine (PFTBA) tune solution. The acquisition software was Masslynx Version 4.1.

Gas chromatography-mass spectrometry

GC 7890A GC system coupled with 5975C Inert XL EI/CI MSD with Triple-Axis Detector from Agilent Technologies (USA) with an autosampler, injector and column compartment was used for introduction of the analytes into the mass spectrometer for all low resolution experiments. All positive and negative chemical ionization (PCI and NCI) experiments were done using this system. The GC injector temperature was set at 260 ^{o}C and pressure was 64.69 kPa while the flow rate of the carrier gas was 1 mL/min. The initial oven temperature was set at 80 ^{o}C for 2 min then ramped at 10 ^{o}C/min to 220 ^{o}C for 15 min. A HP5MS capillary column from Agilent Technologies was used for separation in the GC. The solvent delay time was 4 min and the MS source temperature was set at 230 ^{o}C while that of the quadrupole was 150 ^{o}C. The autotune function set the filament emission current to 34 μA, the electron energy to 70 eV and the electron multiplier voltage to 1420 V in EI mode. The scan range was set from *m/z* 50 to 350 under full scan acquisition mode. 1 μL of each analyte was introduced into the GC-MS through the liquid autosampler under splitless injection mode.

PCI and NCI experiments

Methane was used as the reagent gas at a flow rate of 0.4 mL/min and all the optimized GC parameters were kept constant throughout the analysis. Other parameters were automatically set using the PCI autotune function. The GC effluent was introduced into the mass analyzer via the interface into the mass spectrometer source pressurized with the reagent gas. In the ion source, methane underwent EI producing a series of reagent gases but the mass spectrometer scan function is set to optimize the intensity of m/z 29, i.e. $C_2H_5^+$ that afforded the ion molecule reactions between itself and the neutral coumarin molecules. The NCI autotune function was used to set all other parameters for optimum production of the thermalized electrons.

Ab Initio calculations

Ab initio calculations were performed for all the coumarins and their molecular anions to get further insight into their NCI behavior. The geometries were optimized at the DFT/B3LYP level of theory using the 6-31+G(d) basis set. Vibrational frequencies were calculated for each minimized structure to ensure that the located structure is a true global minimum. Single point energy calculations of the DFT/B3LYP/6-31+G(d) optimized geometries were calculated with the larger basis set 6-311+G(d,p). Electrostatic properties (electron densities and electrostatic potential) were also calculated for the anions. Geometry optimizations were performed using Qchem Quantum Chemistry package [21] and electrostatic properties were calculated using Gaussian 03 Software suite [22].

RESULTS AND DISCUSSION

The ion chemistry of coumarins using EI

3-Acetylcoumarin derivatives

In this category three compounds namely 3-acetyl-7-methoxycoumarin (A), 3-acetyl-5-methoxycoumarin (B) and 3-acetylcoumarin (C) were examined. The high resolution EI mass spectrum of 3-acetyl-7-methoxycoumarin, i.e. compound A (MM = 218.0681) is given in Figure 3(a).

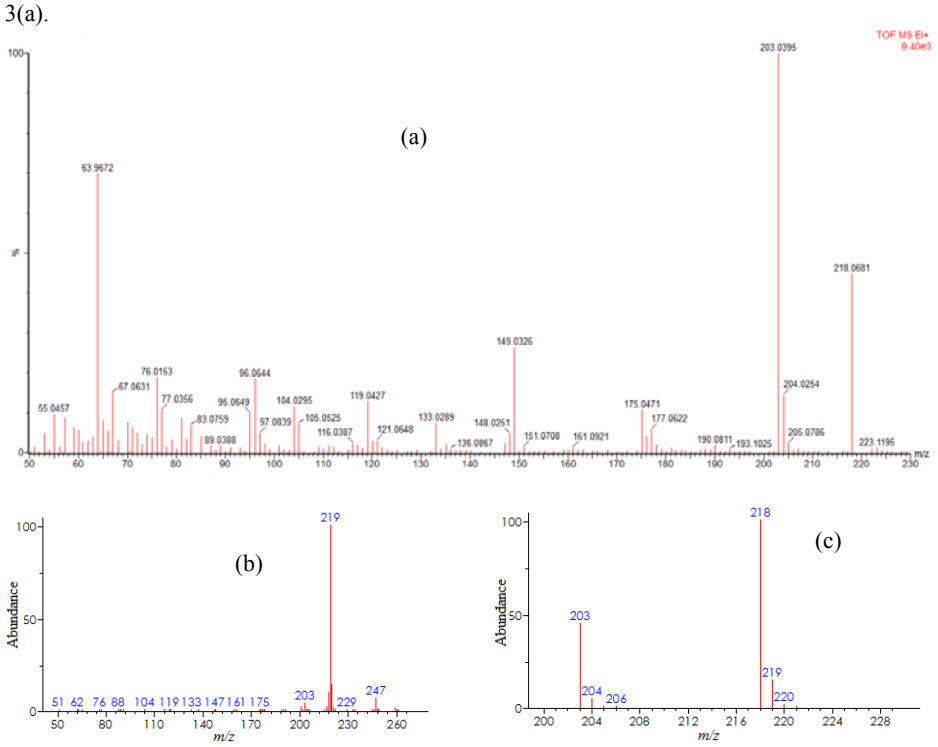
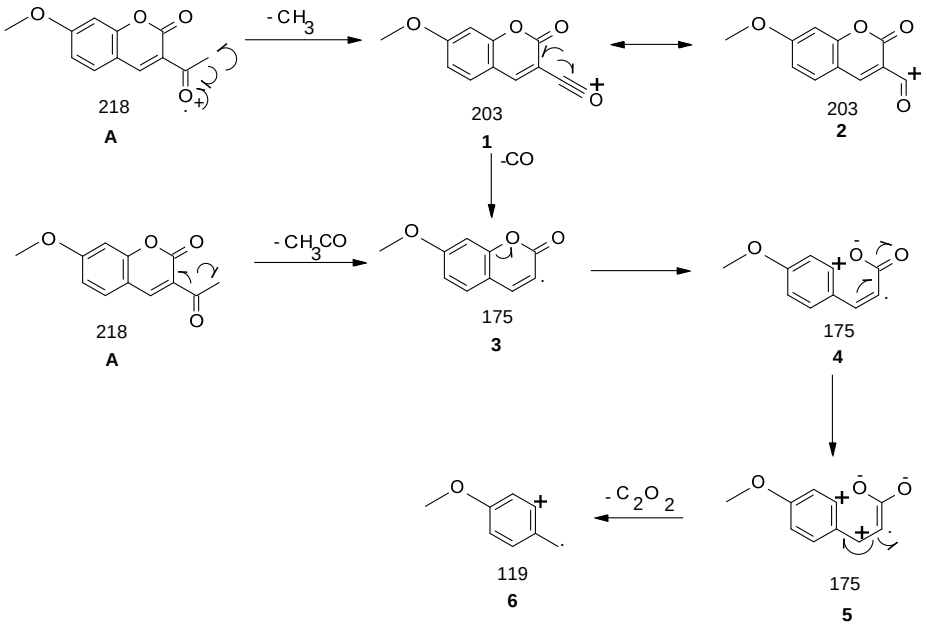


Figure 3. (a) EI high resolution mass spectrum, (b) PCI and (c) NCI mass spectra of 3-acetyl-7-methoxycoumarin (MM = 218).

Scheme 1 depicts a plausible fragmentation pathway in EI leading to the observed ions. The base peak at *m/z* 203.0395 is due to a loss of the methyl group. The observed abundance of the ion could be partly due to its stability which results from the delocalization of the charge at the carbon atom as shown between ion **1** and ion **2**. Subsequent loss of CO gave ion **3** with *m/z* 175.0471 with a relative abundance of 10%. Ion **3** could also be produced by the direct loss of CH_3CO from the parent molecule **A**. Further probable fragmentation involves opening of the pyrone ring and cleavage of the C3-C4 bond as shown in ion **5** leading to loss of C_2O_2 to give ion **6** with *m/z* 119.0427 and relative abundance of 15%. These observations were made in both the low and high resolution mass spectrometers.

The fragmentation pattern of 3-acetyl-5-methoxycoumarin, i.e. compound B (MW = 217.9900), was found to be the same as that of compound A, involving loss of a methyl group followed by loss of CO which was stabilized by resonance. This ion is also generated by direct

loss of CH_3CO from the parent molecule. Further probable fragmentation involves opening of the pyrone ring and cleavage of the C3-C4 bond leading to loss of C_2O_2 to give an ion with *m/z* 119.0056 and relative abundance lower than in compound A. The high resolution EI mass spectrum of compound B is given in Figure 4.



Scheme 1. Plausible EI fragmentation pattern of 3-acetyl-7-methoxycoumarin.

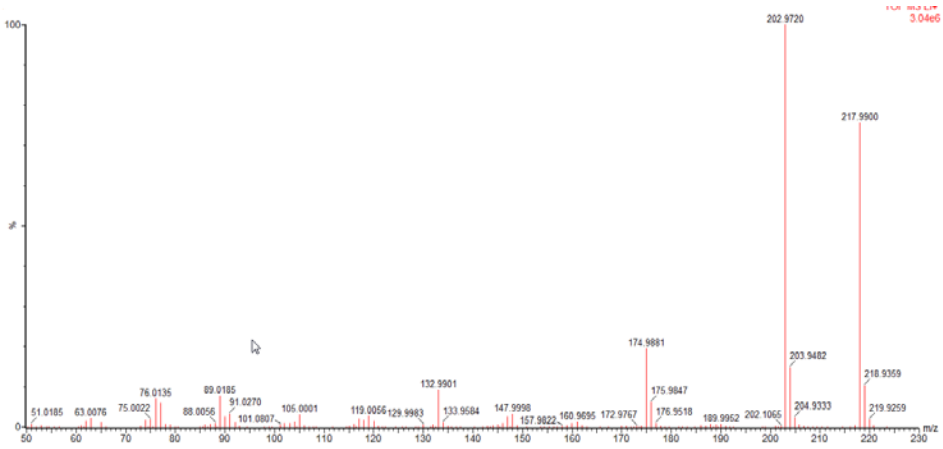


Figure 4. EI high resolution mass spectrum of 3-acetyl-5-methoxycoumarin.

Similar fragmentation characteristics were exhibited by compound C, i.e. 3-acetylcoumarin (MW = 188.0526), wherein, a loss of a methyl group occurred to give a

base peak at *m/z* 173.0303. The CO group was subsequently lost from the base peak to give an ion with *m/z* 145.0350 which could also be generated by direct loss of CH_3CO from the parent molecule. The pyrone ring probably opens and C3-C4 bond cleaves leading to loss of :C=C=O to give an ion with *m/z* 101.0448 with a relative abundance of 10%. The high resolution EI mass spectrum of compound C is given in Figure 5.

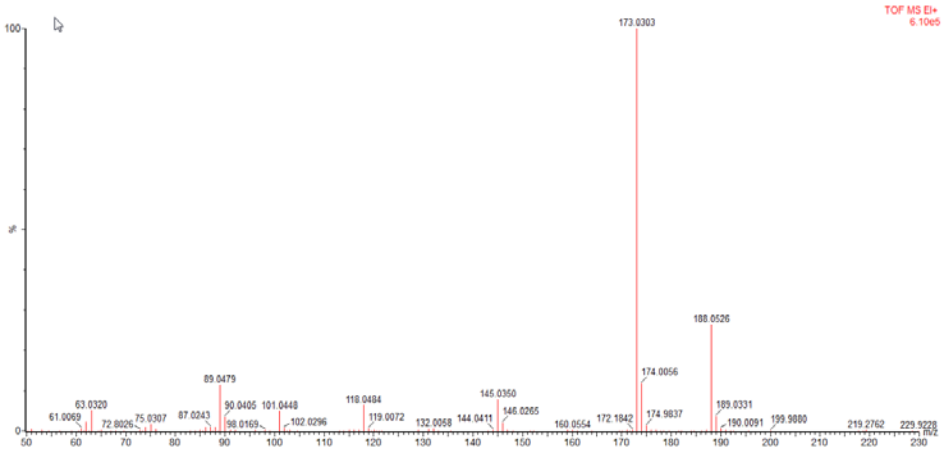


Figure 5. EI high resolution mass spectrum of 3-acetylcoumarin.

Methyl coumarin derivatives

Two compounds were examined in this category. These were 4-methyl-5,7-dimethoxycoumarin, i.e. compound D and 4,6-dimethylcoumarin, i.e. compound E. The high resolution EI mass spectrum of 4-methyl-5,7-dimethoxycoumarin, i.e. compound D (MM = 220.0827) is given in Figure 6.

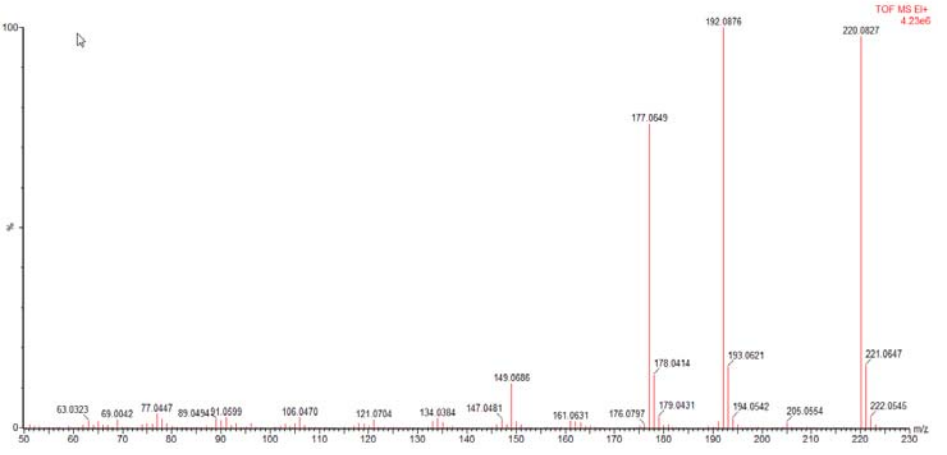
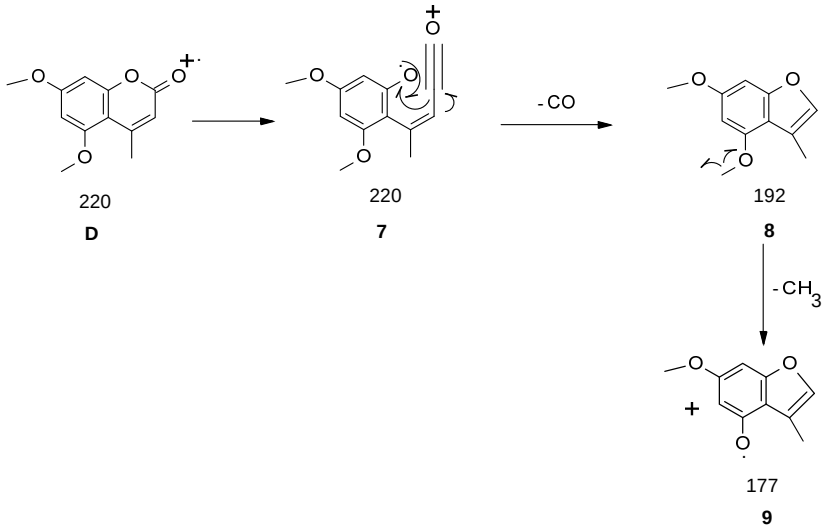


Figure 6. EI high resolution mass spectrum of 4-methyll-5,7-dimethoxycoumarin.

Scheme 2, shows the fragmentation of 4-methyl-5,7-dimethoxycoumarin involved loss of CO group to give the base peak ion **8** with *m/z* 192.0876 converting the 6-membered pyrone ring to the aromatic 5-membered furan ring. The aromaticity of ion **8** makes it stable hence it gives the base peak. The base peak ion further lost a methyl group to give an ion **9** with *m/z* 177.0649 as shown in Scheme 2.



Scheme 2. Plausible EI fragmentation pattern of 4-methyl-5,7-dimethoxycoumarin.

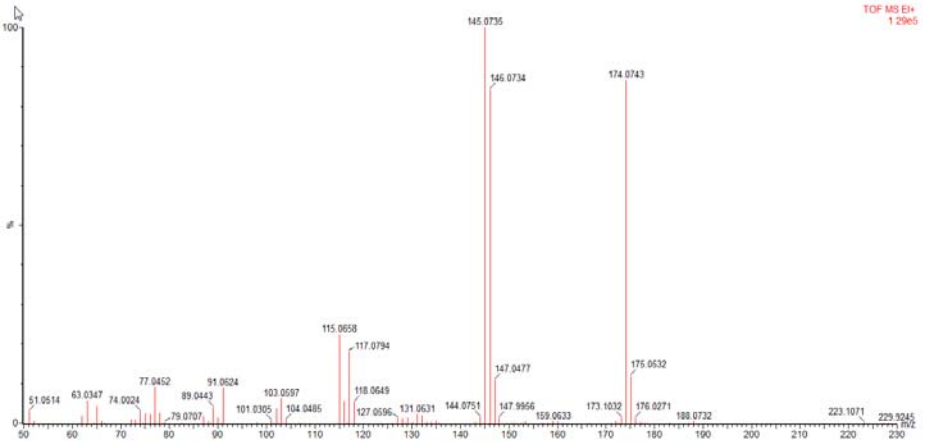


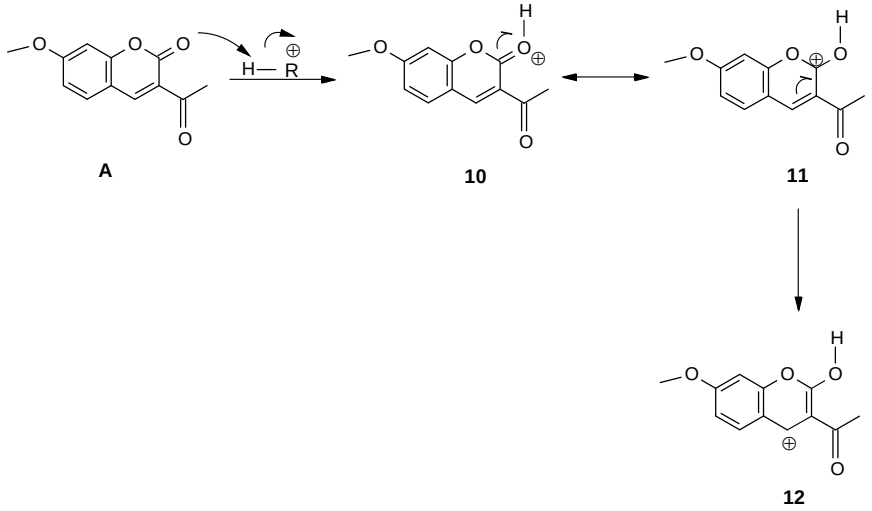
Figure 7. EI high resolution mass spectrum of 4,6-dimethylcoumarin.

In a plausibly and similar fashion, 4,6-dimethylcoumarin (MW = 174.0743) lost the CO group from the pyrone ring to give most likely a benzofuran derivative with *m/z* 146.0734 as seen from the high resolution EI mass spectrum in Figure 7. Interestingly the base peak was consistent with loss of CHO from the parent molecule or loss of a hydrogen from the benzofuran derivative and gave a base peak at *m/z* 145.0735. Subsequent loss of a methylene groupfrom the molecule responsible for the base peak gave *m/z* 131.0631. The *m/z* 131.0631 peak can also be

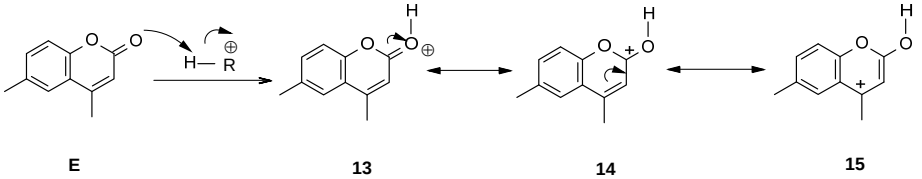
due to the loss of a methyl group from the benzofuran derivative. Subsequent opening of the furan ring and loss of CO group gave an ion with *m/z* 117.0794 which further lost C_2H_2 and CH_2 groups respectively to give ions with *m/z* 91.0624 and 77.0452, respectively.

The ion chemistry of coumarins using PCI

Proton transfer from the reagent methane gas ion to the analyte was observed in all the five coumarins regardless of whether they were acetyl or methyl substituted. Representative PCI mass spectra of 3-acetyl-5-methoxycoumarin and 4-methyl-5,7-dimethoxycoumarin are given in Figure 3(b) and Figure 3(c). From Figure 3(b), it is clear that the proton affinity (PA) of the coumarins in this study is higher than that of methane which is 543.5 kJ/mol [10]. The common electron accepting group in all the coumarins under study is the ester carbonyl group, C=O, in the pyrone ring and it is speculated that carbonyl groups in both 3-acetylcoumarins and 4-methylcoumarins accept protons from the reagent methane to give cation **10** and **13** in Scheme 3 and 4, respectively. The positive charges generated on cations **10** and **13** are stabilized by resonance hence proton transfer is feasible.



Scheme 3. Plausible PCI mechanism for proton transfer in 3-acetyl-7-methoxycoumarin.



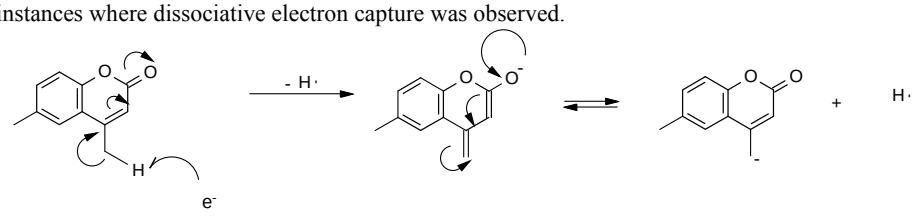
Scheme 4. Plausible PCI mechanism for proton transfer in 4,6-dimethylcoumarin where RH is $C_2H_5^+$ from the reagent gas.

The ion chemistry of coumarins using NCI

Methyl coumarin derivatives

Dissociative electron capture was observed in 4-methylcoumarin derivatives: 4,6-dimethylcoumarin and 4-methyl-5,7-dimethoxycoumarin. The NCI mass spectrum of 4-methyl-5,7-dimethoxycoumarin is given in Figure 3(c).

A plausible mechanism is that an electron abstracts a proton from the neutral molecule at the carbonyl position shown in Scheme 5 for molecule E, which in turn loses a hydrogen radical to give an even electron negatively charged ion **16**, Scheme 5. This even electron negatively charged ion is stabilized by delocalization which plausibly leads to ring opening to give the even electron negatively charged ion as depicted by **17**. With the electron affinity (EA) of the hydrogen atom at 0.754 electron volts (eV), [23] it can be speculated that the dissociation energy of the hydrogen atom from the coumarin should be greater than the EA of the H atom for the overall dissociative process to be driven in the forward direction i.e. endothermic in both instances where dissociative electron capture was observed.

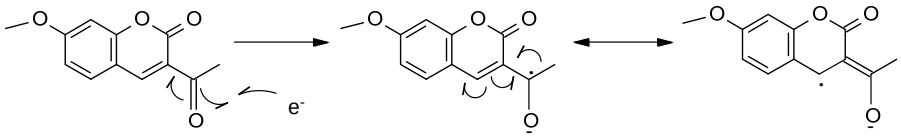


E **16** **17**

Scheme 5. Plausible NCI mechanism for electron capture in 3-acetyl-7-methoxycoumarin.

3-Acetylcoumarin derivatives

Electron capture was observed in acetyl substituted coumarins: 3-acetyl-7-methoxycoumarin, 3-acetylcoumarin and 3-acetyl-5-methoxycoumarin to form odd electron negative molecular ions M^{-}. A representative NCI mass spectrum of 3-acetyl-7-methoxycoumarin is shown in Figure 3(c). From Figure 3(c) and the results observed thus far, it can be inferred that that methane was able to produce free thermal electrons whose energies were less than the ionization energies of the neutral coumarins and capable of attaching themselves to these neutrals. It is logical to infer that the keto group rather than the ester carbonyl group is responsible for the electron capture as this was observed in 3-acetylcoumarin derivatives but not in the 4-methylcoumarins. Scheme 6 shows a plausible mechanism of electron capture in the 3-acety-7-methoxylcoumarin which is representative of other acetyl substituted coumarins. An electron from the source is captured by the analyte molecule **A** to give a radical anion **18** which is stabilized by resonance to give radical anion **19**.



A **18** **19**

Scheme 6. Plausible NCI mechanism of proton abstraction in 4,6-dimethylcoumarin.

The formation of an odd electron negative molecular ion as observed with the acetyl substituted coumarins above can be influenced by a number of factors including ease of reduction, stability of the molecular anion with respect to fragmentation and stability relative to the neutral precursor molecule. In the case of the methyl and acetyl substituted coumarins, enhanced resonance driven stability of the acetyl substituted coumarin anions was supported by the calculated electron affinities shown in Table 1. These were consistently larger for the acetyl-substituted than the methyl-substituted coumarins.

Table 1. Calculated electron affinities in (eV) for the five coumarins.

Molecule	Calculated electron affinities (zero-point energy corrected)	
	DFT/6-31+G(d)	DFT/6-311+G(d,p)
4,6-Dimethylcoumarin	0.73	0.77
4-Methyl-5,7-dimethoxycoumarin	0.45	0.49
3-Acetyl-7-methoxycoumarin	1.41	1.44
3-Acetylcoumarin	1.57	1.60
3-Acetyl-5-methoxycoumarin	1.43	1.47

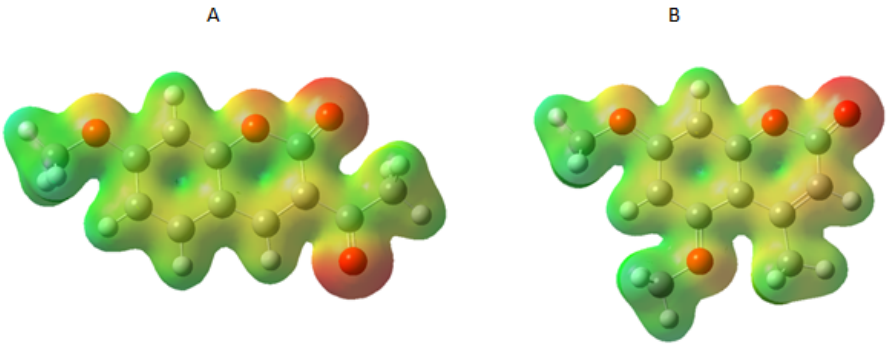


Figure 8. Ab initio electron density surfaces color mapped with the electrostatic potential for 3-acetyl-7-methoxycoumarin anion (A) and 4-methyl-5,7-dimethoxycoumarin anion (B). The isovalue used is 0.02. Red (online) shows higher electron density.

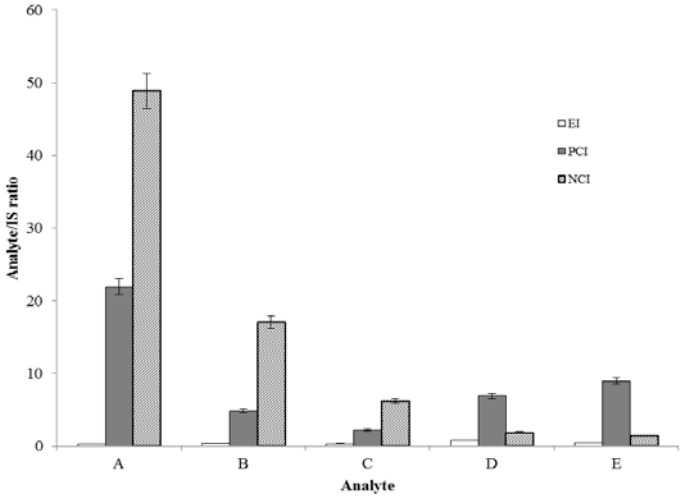


Figure 9. Comparison of SIM signal intensities in EI, PCI and NCI. EI all ion types = $M^{\cdot+}$; PCI all ion types = $[M+H]^+$; NCI compounds A, B and C ion types = $M^{\cdot-}$, compounds D and E ion types = $[M-H]^-$. IS = Internal standard.

Further evidence of resonance enhanced stabilization came from the electron densities of M^- for the two groups of anions. These showed that most of the negative charge for the methyl substituted coumarin anions lies on the ester carbonyl oxygen. In acetyl coumarin anions, significant amount of negative charge is centered on the ester carbonyl oxygen and the keto

oxygen as seen in Figure 8. The overall effect is a more stable acetyl substituted coumarin anion.

Further experiments showed that compounds that underwent electron capture as opposed to dissociative electron capture showed higher signal intensities than the latter. These observations were made when NCI was done on the analytes followed by selected ion monitoring (SIM) where the intensities of the $M^{\cdot -}$ ions and the $[M–H]^-$ were compared using compound D as the internal standard (IS) for others and in turn compound E as the internal standard for compound D.

Acetyl derivatives which underwent resonance electron capture to yield $M^{\cdot -}$ ions showed much higher intensities compared to methyl derivatives that underwent dissociative electron capture, i.e. the analyte/IS ratios were as follows (values in brackets): A(49); B(17); C(6.2); D (1.9) and E (1.4). Figure 9 shows a comparison of ion intensities using EI, PCI and NCI in SIM mode.

CONCLUSION

Plausible EI, PCI and NCI fragmentation patterns were discerned with particular interest laid on the NCI ionization trends for the five coumarins under study. Generally, there was no difference between the spectra obtained in the high and low resolution mass spectrometers. The 3-acetylcoumarin derivatives fragmented by losing the acetyl group (either directly or by loss of CH_3 followed by CO group) prior to opening of the pyrone ring and further fragmentation. In contrast, the 4-methylcoumarin derivatives fragmented by first opening of the pyrone ring and subsequent loss of CO group accompanied by formation of a furan ring. Depending on the substitution pattern of the 4-methylcoumarin, the furan ring can open leading to further fragmentation. Negative electron density distribution and electron affinity values obtained by *Ab Initio* calculations favoured ionization by resonance electron capture for acetyl coumarin derivatives and dissociative electron capture by methyl substituted coumarins. Those coumarins that underwent resonance electron capture showed much higher intensities of $M^{\cdot -}$ than $[M– H]^-$ ions. These observations will be useful in designing quantitative methods to improve detectability of the analytes.

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