Adduct formation of steroids in APCI and its relation to structure identification

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Adduct ion formation is a frequently observed phenomenon in mass spectra originating from atmospheric pressure chemical ionisation processes. In this study the relation between the relative intensities of the deprotonated molecule and the acetate adduct ion and the chemical structure of some 22 corticosteroids was investigated. The ratio between the ion intensities of both ions changes upon the presence and the position of the hydroxy group in the steroid molecule. That is compounds without any hydroxy have a low tendency towards acetate adduct ion formation whereas its position influences the deprotonation of the molecule. From the data of this limited study it can be concluded that knowledge on the mechanism of adduct ion formation will be of help for structure identification studies.

Introduction

The major breakthrough of LC-MS took place when the thermospray interface was introduced in the mid 80's [1]. In the 90's this "spray" interface was replaced by the Heated nebulizer [2], also known as APCI, and the electrospray/ionspray interface [3]. In contrast to the less famous but still applied "trace enrichment" particle beam interface, the spray interface make use of the LC effluent during the nebulization and ionisation processes.

Due to this involvement of the eluent solvents, adduct ions consisting of the analyte bounded to one or more solvent molecules have been observed in LC-MS mass spectra and reported in many papers [4-9]. Nevertheless, a minority of them was dedicated to the explanation of such ions in the typical dynamic LC-MS interfaces [10]. This is in contrast to the extensive reports concerning adduct ion formation in ion sources under static chemical ionisation conditions (no spraying of solvents) [11-13].

The formation of abundant methanol and acetonitrile adduct ions with aromatic amines were observed in thermospray mass spectra and systematically investigated [10]. The difference in behaviour towards the formation of specific adducts could well be explained with the dissociation energies (DE) of the complexes [14]. The DE is related to the proton affinity (PA) of the constituent base by DE = a-bΔPA where a and b are constants and ΔPA is the absolute PA difference [10]. Therefore it can be argued that the DE hypothesis used for the thermospray data can also be used for the explanation of the adduct formation in APCI.

As the PA, and consequently the DE, of a molecule is dependent on its structure, the tendency of a molecule to form adduct ions can be used for structural identification purposes. A major problem with this theory is the presence of the collisionally induced dissociation processes occurring in the sampling region of the atmospheric pressure source (somewhat like the effect of the repeller in the thermospray interface) which are, ironically enough, meant to de-cluster the adduct ions. The following, limited study, discusses the data from the acetate adduct ions with steroid molecules (see Fig. 1 for a general structure and the numbering of the C atoms) in the negative ion mode. Abundant adduct ions for these type of molecules have been reported both with the thermospray [7] as with the HN [8,9] interface. These types of compounds are chosen because the MSMS data are considered hard to interpret [15]. That is hydrogen transfer

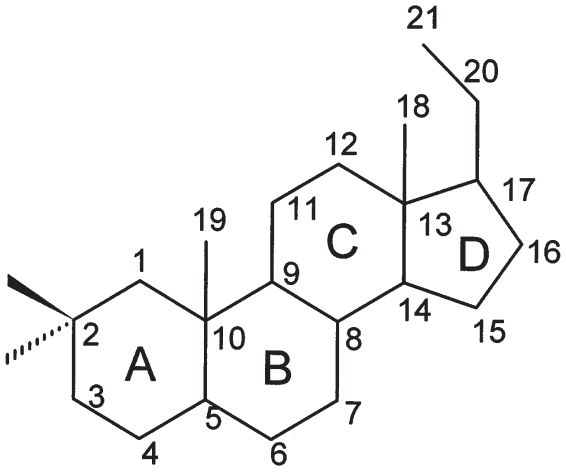

Figure 1. General structure and C atom numbering of a steroid molecule.

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reactions make the assignment of the fragment ion structure for these molecules extremely difficult and in contrast to EI mass spectra little [15] is known about the fragmentation mechanisms of steroids in the MS/MS mode. The APCI data show that adduct ions can tell us more about the structure of the steroid molecule.

Experimental

Chemicals

For the experiments Milli Q water and LC grade methanol were used. The steroids were from the internal stock and their purity, higher than 90 %, was checked before application by LC-APCI-MS. Acetic and formic acid were purchased from Merck (Darmstadt, Germany).

Instrumentation

An HP1100 LC system was used for the delivery of the mobile phase, consisting of 80 % methanol and 20 % water, at a flow-rate of 1 ml/min. From samples with an approximate concentration of 20 μg/ml, 25 μl were injected into the eluent. An Hypersil ODS 5um (60 × 4.6 mm) column was applied in order to guarantee complete mixing of the compound with the eluent. The MS experiments were performed with a Sciex API 365 equipped with a heated nebulizer interface. The MS settings of the system are summarised in table I.

Results and discussion

Adduct ions of acetic or formic acid with analyte present in the negative ion mass spectra have been reported before [8,9]. Although the DE have been calculated for positive charged adduct ions [6], it can be argued that the abundance of acetate adduct ions, $[M\text{-}CH_3COO]^-$ is also related to the dissociation bond energy, and thus the proton affinity, of the bonded molecules [10]. This could mean that adduct ion formation of a steroid can tell us something about its structure, especially when the PA of a hydroxy group in a steroid molecule depends on its position and the three dimensional structure of the molecule.

Table I. Listing of the main MS settings.

Parameter	*Value*
Ionisation mode	Neg. Ion
Scan range	150-550
Nebulizer flow	8
Curtain gas flow	10
Orifice	–11 V
Ring potential	–120 V

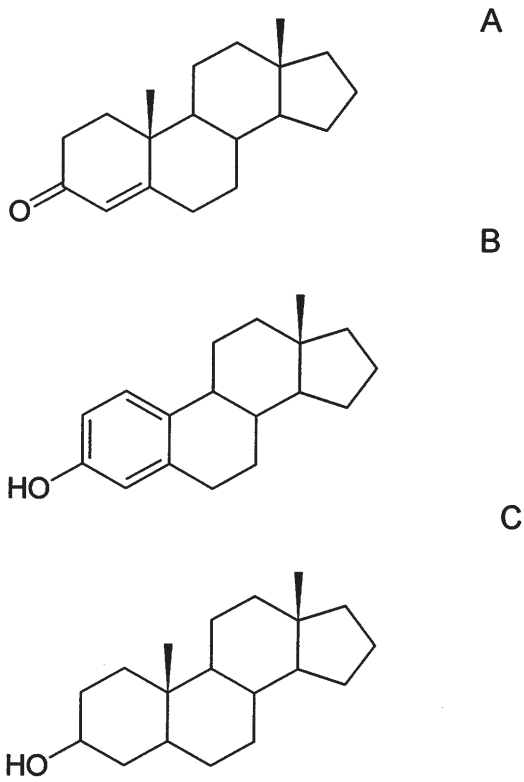

Figure 2. The 3-keto-Δ4 steroids, 3-OH aromatic A ring steroids and the 3-OH aliphatic A ring steroids.

In a limited project we intended to explore this hypothesis by starting off with the analysis of some 30 steroid compounds, being variations of three groups of compounds (see Fig. 2). These groups are: 3-keto-delta-4, aromatic A ring with a hydroxy on 3 and an aliphatic A ring with a hydroxy on three. The first group showed a good response for the protonated molecule in the positive ion mode. In the negative ion mode no significant signals were obtained, except for two compounds. Better response in this mode was observed for the compounds with the aromatic A ring. But then again, these compound lack significant ions in the positive ion-mode. Group three did not generate any abundant ions in both modes. Steroids lacking keto groups show a low tendency to the formation of protonated molecules, whereas acidic hydroxy groups, *e.g.* a hydroxy on an aromatic ring or next to a COCH2OH substituent on the 17 position (corticosteroids), give rise to abundant deprotonated and/or acetate adduct ions.

Consequently the tendency to form deprotonated molecules and acetate adduct ions was investigated in more detail for some 22 corticosteroids. A general structure of these 22 compounds can be found in figure 3.

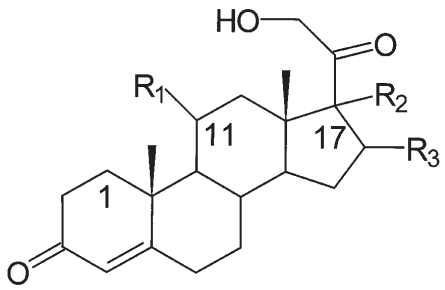

Figure 3. General structure of the tested compounds.

Table II. The inter-day reproducibility of the ion intensities (mean intensity (*n* = 5) for compounds 5 and 8 on three consecutive days).

Compound (day)	*$[M+H]^+$*	*$[M+H-60]^+$*	*$[M-H]^-$*	*$[M+59]^-$*
5(1)	5.8	3.8	1.7	3.9
5(2)	6.1	3.7	2.0	4.2
5(3)	6.0	3.3	1.9	4.4
8(1)	3.4	2.9	1.2	2.8
8(2)	5.2	4.5	1.4	3.8
8(3)	5.3	4.1	1.4	4.5

In order to generate good analytical data, the APCI interface was optimised and tested for its reproducibility. For compounds 5 and 8 the mean (n = 5) was analysed on three consecutive days. In table II the results are listed. From these data we concluded that the data were reliable.

In table III the results and the exact structures of the tested compounds are listed. Unless mentioned explicitly, all molecules contain a COCH2OH group on the 17 and beta orientated.

The intensities of both the types of ions is low for the compounds 1, 6 and 13, whereas the "hydroxy compounds" generated abundant ions. Therefore it is clear that the hydroxy at C21 has no function during the ionisation process. Looking at the intensities of the compounds having an additional hydroxy group, it is clear that abundant depro-

Table III. Results and structure of the tested compounds.

Number	*R1*	*R2*	*R3*	*Δ1*	*$[M-H]^-$*	*$[M+59]^-$*	*ratio*	*Remark*
1	-	-	-	-	0.012*	0.18	15	
2	α-OH	-	-	-	0.12	4.3	36	
3	β-OH	-	-	-	0.20	4.0	20	
4	β-OH	-	-	-	0.13	4.5	35	α-$COCH_2OH$
5	-	-OH	-	-	2.4	5.3	2	
6	-	-$C_5H_9O_2$	-	-	0.068	0.62	9	
7	-	-$C_2H_3O_2$	-	-	0.37	1.6	4	
8	α-OH	-OH	-	-	1.7	5.0	3	
9	β-OH	-OH	-	-	1.3	5.0	4	
10	=O	-OH	-	-	2.9	4.8	3	
11	-	-OH	α-CH_3	-	3.2	5.1	2	
12	-	-OH	β-CH_3	-	2.2	5.9	3	
13	-	-CH_3	-CH_3	-	0.0047	0.048	10	
14	-	β-OH	-	-	0.6	0.8	1	17α-$C_4H_7O_2$
15	-	-OH	-	-	1.4	4.3	3	17-$C_4H_5O_3$
16	-	-OH	-	+	2.8	5.3	2	
17	α-OH	-OH	-	+	2.1	4.9	2	
18	β-OH	-OH	-	+	1.9	5.0	3	
19	=O	-OH	-	+	3.3	4.6	1	
20	β-OH	-OH	α-OH	+	3.5	2.7	1	
21	α-OH	-OH	b-CH_3	+	1.9	4.6	2	
22	β-OH	-CH_3	-CH_3	+	0.13	6.2	48	
23	β-OH	-OH	α-CH_3	+	3.9	5.1	1	9-Fluor ipv. 9-H
24	β-OH	-OH	β-CH_3	+	2.9	5.1	2	9-Fluor ipv. 9-H

* = 10^6 counts per second (cps)

tonated molecules are formed with the 17-OH compounds (5, 8-12, 14, 15, 16-21, 23 and 24). Its orientation (α or β) does not influence the ratio, whereas the orientation of an additional methyl group on the 16 position shows a significant change of the [M-H]- intensities (Compound 11,12 and 23,24). Compounds with the hydroxy exclusively on the 11 position do not form deprotonated molecules. This difference is very predominant between compounds 4 and 5. The intensity of the acetate adduct ions does not change much (not more then a factor of 5) by altering the position of the hydroxy group and maintaining the identity of the 17 substituent. The site of adduct formation must take place at both the C11 and C17 hydroxy groups (Compounds 2-4 and 14,15). Compound 22 is also in line with this observation. More work on this dependence must be performed.

Changing the orientation of the hydroxy on the 11 position for compounds 8-9 and 17-18, does not result in large differences in the ratio. A little effect is observed for compounds 21, 23 and 24. Changing the identity of the substituent on the 11 position does influence the ratio due to a slide increase of $[M-H]^-$ and slide decrease of $[M+59]^-$. Introduction of a carbonyl group at the 11 position increases, although little the abundance of the deprotonated molecule (compare 16 with 19). Another little effect, decrease of the intensity of the adduct ion, is observed when a hydroxy group (compound 20) is incorporated at the 16 position. This effect is specific for this hydroxy group, because compound 23 shows a significant higher tendency towards adduct formation.

Conclusion

From the first survey it became clear that the response of these compounds in the negative ion mode is rather low, except for the corticosteroïds. Concluding, if good response for a steroid is obtained in the negative ion mode, the compound must have an rather acidic hydroxy group and compounds with exclusively a keto, an aromatic or aliphatic hydroxy on C3 can be excluded. Further investigation of some twenty compounds showed that the intensities of both the deprotonated molecule and the acetate adduct, and thus the ratio, is dependent on the structure of the molecule. Acetate adduct ions are formed with those (cortico)steroids which contain hydroxy groups. Furthermore, the [M-H]- ion is intense when the proton of the hydroxy is acidic, *e.g.* on the 17 position and not on the 11 position. Change of the orientation from α to β does not lead to a significant difference in the ratio. Further investigation, such as the calculation of the bond energies by the application of collision-induced dissociation processes [16-17], will be of help to set up some rules of thumb.

References

1. Vestal, M.L.; Fergusson, G.J. *Anal. Chem.* **1985**, *57*, 2373-2378.
2. Niessen, W.M.A.; Tinke, A.P. *J. Chromatogr. A* **1995**, *703*, 37-57.
3. Bruins, A.P. *Mass Spectrom. Rev.* **1991**, *10*, 53-77.
4. Parker, C.E.; Smith, R.W.; Gaskell, S.J.; Bursey, M.M. *Anal. Chem.* **1986**, *58*, 1661-1664.
5. Volmer, D.; Levsen, K.; Honing, M.; Barceló, D.; Abían, J.; Gelpí, E.; van Baar, B.L.M.; Brinkman, U.A.Th. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 913-
6. Tong, H.; Bell, D.; Tabei, K.; Siegel, M.M. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 1174-1187.
7. Kim, Y.; Kim, T.; Lee, W. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 863-868.
8. Ma, Y.-C.; Kim, H.-Y. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 1010-1020.
9. Volmer, D.A.; Hui, J.P.M. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1926-1934.
10. Honing, M.; Barceló, D.; van Baar, B.L.M.; Brinkman, U.A.Th *J. Mass Spectrom.* **1996**, *31*, 527-536.
11. Aviyente, V.; Zhang, R.; Varnali, T.; Lifshitz, C. *Int. J. Mass Spectrom. Ion Proc.* **1997**, *161*, 123-132.
12. Feng, W.Y.; Lifshitz, C. *J. Phys. Chem.* **1994**, *98*, 3658-3663.
13. Feng, W.Y.; Lifshitz, C. *J. Phys. Chem.* **1994**, *98*, 6075-6081.
14. Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1984**, *106*, 1257-1264.
15. Williams, T.M.; Kind, A.J.; Houghton, E.; Hill, D.W. *J. Mass Spectrom.* **1999**, *34*, 206-216.
16. Graul, S.T.; Squires, R.R. *Int. J. Mass Spectrom. Ion Proc.* **1989**, *94*, 41-61.
17. Haas, M.J.; Harrison, A.G. *Int. J. Mass Spectrom. Ion Proc.* **1993**, *124*, 115-134.