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Extractive Methylation of Clopamide and Indapamide: Structures of the Derivatives

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Extractive methylation of clopamide and indapamide gives rise to the formation of several derivatives. Their positive and negative ion mass spectra are described and their respective structures established. This derivatization procedure seems to be unsuitable for a quantitative analysis of these two diuretics, unless a deuterated analogue or a compound with the same chemical functions is used as the internal standard.

INTRODUCTION

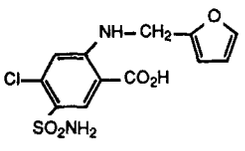
Diuretics are drugs very frequently used in diseases whose treatment requires water and/or sodium loss, such as hypertension or cardiac failure. They may be assayed by several techniques [high-performance liquid chromatography (HPLC),[1–4] gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS)[2,3,5–8]], but as most of them contain a sulphonamide group which lowers their volatility, derivatization is necessary to increase this volatility when they have to be analysed by GC, GC/MS or even mass spectrometry with direct introduction; this can be done, for instance, by methylation of these compounds during their extraction from aqueous solutions, such as urine. Using the extraction–methylation method described by Fagerlund *et al.*[5] and modified by Lisi *et al.*,[9] we obtained the methylated derivatives of 19 diuretics and determined some of the characteristics of their negative ion mass spectra[10] by direct introduction:

(i) When an aromatic chlorine atom is present in the molecule, such as in furosemide or in hydrochlorothiazide (Fig. 1), the base peak in the electron capture negative ion mass spectrum corresponds always to $[M - 43]^{-\cdot}$.

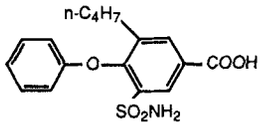
(ii) In contrast, when there is no aromatic chlorine atom present, such as in bumetanide or hydroflumethiazide (Fig. 1), the base peak corresponds to $M^{-\cdot}$ or to $[M - 44]^{-\cdot}$.

(iii) In the case of acetazolamide (Fig. 1), two derivatives were always observed: the expected trimethyl compound and the derivative which arises from preliminary hydrolysis of the acetamide bond before the methylation reaction, wrongly described as monomethylacetazolamide.[2]

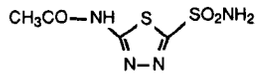
In the case of clopamide and indapamide (Fig. 1), several peaks were observed, when using the extraction–methylation method.[10] In order to determine the structure of these derivatives, we derivatized about 30 mg (instead of 100 μg) of either compound and purified them by column chromatography on SiO_2. For clopamide, we obtained two major compounds (**C1**, **C3**) and sometimes a minor one (**C2**), but only two were observed for indapamide (**I1** and **I3**). No derivative of indapamide corresponding to **C2** was observed.



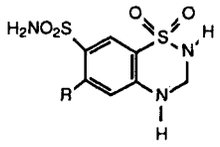
Furosemide



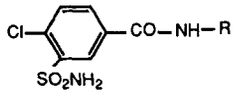
Bumetanide



Acetazolamide



R = CF_3 Hydroflumethiazide
R = Cl Hydrochlorothiazide



R = 2,6-dimethyl-1-piperidinyl Clopamide
R = 2-methyl-1-dihydroindolyl Indapamide

Figure 1. Structures of the diuretics mentioned in the present article

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MATERIALS AND METHODS

Positive ion electron impact (EI) and negative ion electron capture mass spectra were recorded on an LKB-2091 mass spectrometer. The ion source was set at 200 °C and the electron energy at 30 eV in the EI mode, and at 170 °C and 50 eV in the electron capture mode with ammonia as moderating gas.

Tandem mass spectrometry (MS/MS) experiments were performed on a Finnigan-MAT TSQ-70 mass spectrometer. The collision gas was argon, and the collision energy was set between −10 and −20 eV (for positive ions) and +10 eV (for negative ions); parent and/or daughter ions were recorded.

Extraction–derivatization

Clopamide or indapamide (30 mg) was dissolved in 5 ml water containing 0.3 ml 5 N NaOH and 0.3 ml 0.2 N tetrahexylammonium hydrogen sulphate solution in 1 N NaOH and reacted with 1 ml of iodomethane in 6 ml toluene for 3 h. The pH of the aqueous solution was about 12.5. After washing of the toluene phase twice with 1 ml of a saturated silver sulphate solution, the organic phase was evaporated and the residue dissolved in methylene chloride. This was then absorbed on silica and eluted with mixtures of cyclohexane, ethyl acetate and methanol.

Decomposition of C3 and I3

A solution of **C3** or of **I3** in toluene containing a small quantity of methanol was heated overnight at 150 °C. After cooling, the solution was evaporated and the residue chromatographed on a silica column after dissolution into CH_2Cl_2.

RESULTS AND DISCUSSION

Clopamide derivatives

The R_F values of **C1**, **C2** and **C3** on thin-layer chromatography (TLC) silica plates with ethyl acetate as eluent were 0.73, 0.52 and 0.05, respectively.

Table 1 shows the negative ion electron capture mass spectra of clopamide and of derivatives **C1** and **C2** obtained after methylation with either iodomethane or with trideuteroiodomethane. The spectrum of clopamide itself is very simple as it shows only the molecular peak (*m/z* 345) and a peak corresponding to M − 111 (loss of tetrahydrodimethylpyridine).

In comparing **C1a** and **C1b**, it is clear that deuteromethylation shifted all of the peaks, except two very small ones, by 4 mass units. The two small peaks (*m/z* 275 and 241 in **C1a**) were increased by 9 mass units, indicating that they contain the total label arising from three deuteromethyl groups. If our assumption for the other chlorinated sulphonamide diuretics is applied here,[10] then the peak at *m/z* 344 must correspond to the loss of $CH_2{=}N{-}CH_3$ from the molecular ion peak of a trimethylated derivative (*m/z* 387); deuteromethylation then results in a peak at *m/z* 348 as five of the nine deuterium atoms are lost as $CD_2{=}N{-}CD_3$. Subsequently, the radical anion at *m/z* 344 loses the chlorine atom to give the fragment at *m/z* 309. The radical anion at *m/z* 233 corresponds to the loss of 43 ($CH_2{=}N{-}CH_3$) and 111 mass units from the molecular ion; however MS/MS measurements show that the ion at *m/z* 344 is not a parent ion. Thus it is probable that the molecular radical anion first loses 111 mass units and that the intermediate radical anion (*m/z* 276) is not stable enough to be detected.

Very small peaks (intensities less than 5%) corresponding to M − 111 − H$^\cdot$ (*m/z* 275) and M − 111 − Cl$^\cdot$ (*m/z* 241) support this hypothesis. The radical anion at *m/z* 233 then loses either HCl (*m/z* 197) or SO_2 (*m/z* 169).

In comparing **C2a** and **C2b**, it appears that only one hydrogen atom of the introduced methyl groups remains in the different negative ions of compound **C2** (except the peak at *m/z* 227). Assuming that a dimethylaminosulphonyl group loses $CH_2{=}N{-}CH_3$ in our mass spectrometric conditions, we may then conclude that compound **C2** should be a dimethylclopamide with the two methyl groups introduced into the sulphonamide function. The ion radical at *m/z* 330 (M − 43) fragments by losing either HCl (*m/z* 294) or SO_2 (*m/z* 266).

MS/MS recordings show that the radical anion *m/z* 219 is not a daughter of radical anion *m/z* 330, but that it probably results from an undetected ion at M − 111 (*m/z* 262). Here also a small ion at *m/z* 227 (233 for **C2b**)

Table 1. Negative ion electron capture mass spectra of clopamide, C1, C2, C4 and C5 obtained with iodomethane (a) and deuteroiodomethane (b). Fragments containing the chlorine atom are labelled (*)

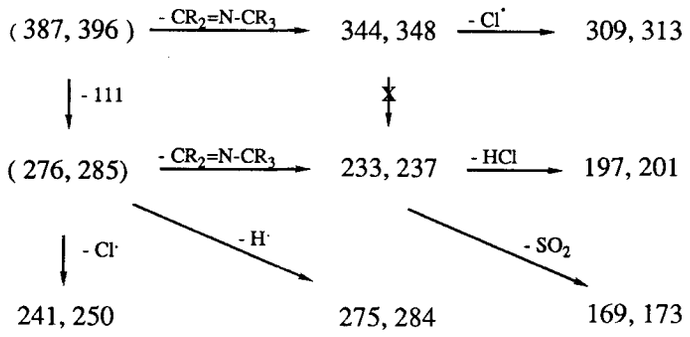
	C1		C2		C4		C5	
Clopamide	a	b	a	b	a	b	a	b
345	387	396	373	379	292	298	387	396
345 (100)*	344 (100)*	348 (100)*	330 (45)*	331 (79)*	249 (100)*	250 (100)*	344 (38)*	348 (74)*
	309 (6)	313 (20)	294 (28)	295 (15)	217 (18)*	218 (10)*		
	275 (<5)*	284 (<5)*			185 (36)*	186 (20)*	308 (9)	312 (18)*
			266 (100)*	267 (100)*			280 (54)*	284 (100)*
	241 (<5)	250 (15)	227 (6)	233 (<5)				
234 (23)*	233 (45)*	237 (50)*	219 (23)*	220 (17)*			219 (100)*	220 (64)*
	197 (6)	201 (20)						
	169 (63)*	173 (70)*	155 (31)*	156 (15)*			155 (96)*	156 (60)*

corresponding to M − 111 − Cl$^{\cdot}$ can be seen. Radical anion *m/z* 219 then loses SO_2 to give *m/z* 155.

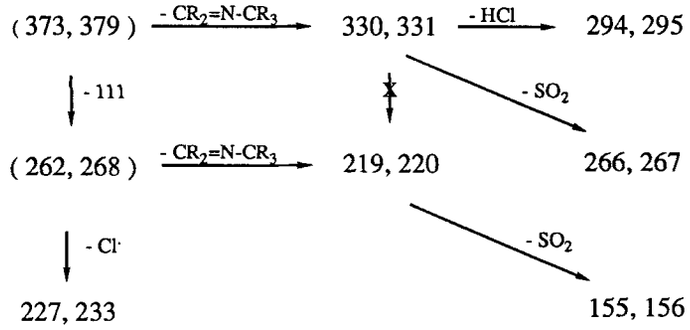
Schemes 1 and 2 summarize the possible fragmentations of **C1** and **C2** in the negative ion mode.

To better determine the structures of these derivatives, we studied their positive EI spectra after methylation and deuteromethylation (Table 2). These spectra are more complicated than the negative ion spectra and most of the ions clearly arise from the non-aromatic part of the molecules, as they do not contain the chlorine atom. The spectra of derivatives **C1** and **C2** are similar to those of underivatized clopamide (with some peaks shifted following derivatization).

The peak at *m/z* 111, which is the base peak in all of the compounds except **C1a**, probably arises from the molecular ion by a McLafferty rearrangement, with charge retention at the olefinic fragment. In the case of **C1a**, the base peak is at *m/z* 112, but in **C1b** this peak is split into a doublet at *m/z* 112 and 113: this means that part of the *m/z* 112 peak of **C1a** is due to the transfer of a hydrogen atom from the carboxamido *N*-methyl group to the piperidine nitrogen. The other peaks arise from $M^{+\cdot}$ by cleavage of either the amide or the nitrogen–nitrogen bond.



Scheme 1. Fragmentation of **C1a**, **C1b** in the electron capture negative ion mode. Figures in parentheses correspond to hypothetical non-detected ions.



Scheme 2. Fragmentation of **C2a**, **C2b** in the electron capture negative ion mode. Figures in parentheses correspond to hypothetical non-detected ions.

Scheme 3 shows a possible fragmentation pattern of clopamide, **C1a**, **C1b**, **C2a** and **C2b**.

The case of compound **C3** isolated as a pure substance from the silica column is more complicated in that it decomposes into at least two compounds when it is heated in the ion source of the mass spectrometer. When a toluene solution of **C3**, containing a few drops of methanol, was heated in a sealed tube overnight at 150 °C, it was transformed into two much less polar compounds **C4** and **C5**, detected on fluorescent TLC plates. They were purified by silica column chromatography; their R_F on silica thin-layer plates were 0.90 and 0.57, respectively, compared to 0.05 for **C3** (eluent: ethyl acetate). But GC/MS of the heated toluene solutions of **C3a** or **C3b** also allowed for the detection of 1,2,6-trimethylpiperidine (mol. wt *m/z* 127; base peak *m/z* 112) or 1-trideuteromethyl-2,6-dimethylpiperidine (mol. wt *m/z* 130; base peak *m/z* 115). The negative and positive ion mass spectra of these two derivatives are shown in Tables 1 and 2.

We conclude that **C4** is 2-chloro-5-methoxycarbonylamino-*N*,*N*-dimethylbenzenesulphonamide (Scheme 4) for the following reasons:

(i) Its molecular ion contains two methyl group arising from either CH_3I or from CD_3I (*m/z* 292 for **C4a** and 298 for **C4b**).

(ii) The presence of an M − 43 (or M − 48) and of an M − 43 − 64 (or M − 48 − 64) ion in the negative ion spectrum of **C3a** (or **C3b**) confirms the presence of a dimethylaminosulphonyl group.

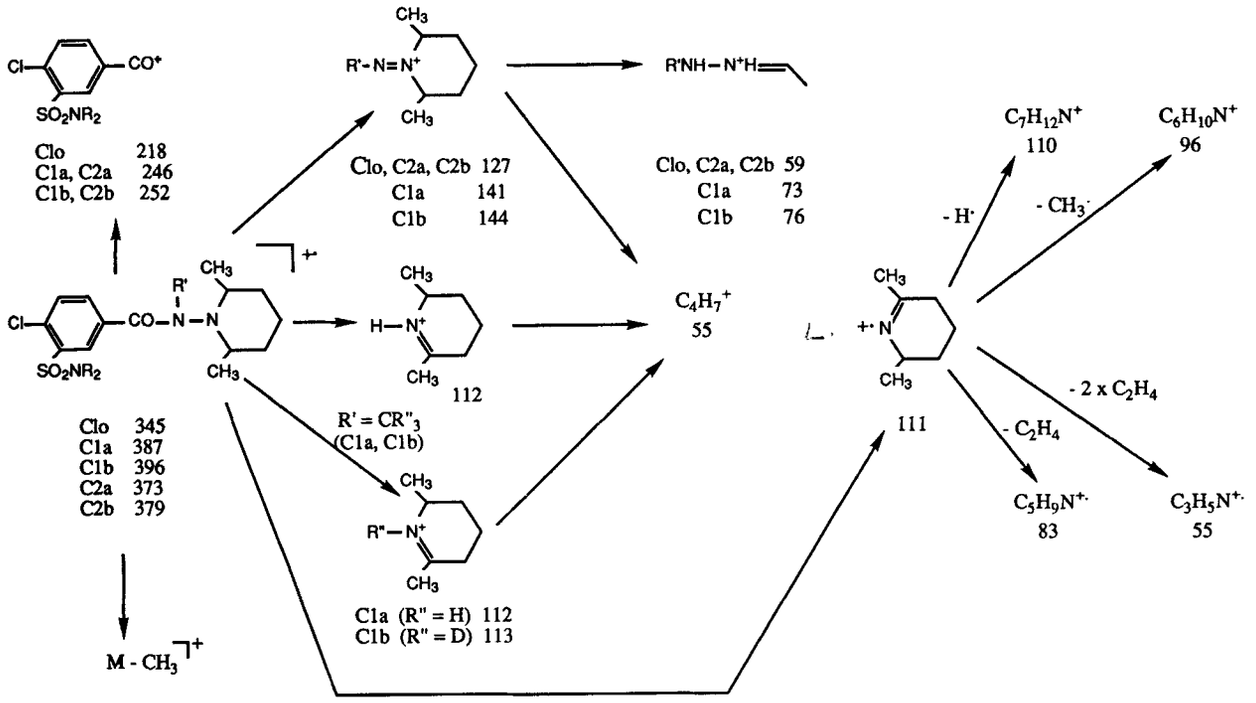
(iii) As the molecular ion mass is even, the compound contains an even number of nitrogen atoms.

(iv) A chlorine atom is present.

(v) The *m/z* 246 (or 252) ion, corresponding to the 3-dimethylaminosulphonyl-4-chlorobenzoyl group, is no longer present in this spectrum.

Table 2. Positive ion EI mass spectra of clopamide, C1, C2, C4 and C5 obtained with iodomethane (a) and deuteroiodomethane (b). Fragments containing the chlorine atom are labelled (*)

	C1		C2		C4		C5	
Clopamide	a	b	a	b	a	b	a	b
345	387	396	373	379	292	298	387	396
330 (<5)*	372 (<5)*	381 (<5)*	358 (7)*	364 (5)*	292 (55)*	298 (50)*	387 (10)*	396 (<5)*
218 (6)*	246 (<5)*	252 (<5)*	246 (6)*	252 (6)*	260 (10)*	266 (7)*	372 (<5)*	381 (<5)*
127 (62)	141 (98)	144 (95)	127 (79)	127 (65)	257 (<5)	263 (5)	318 (95)*	327 (37)*
		113 (21)			185 (100)*	186 (100)*	246 (20)*	252 (10)*
112 (12)	112 (100)	112 (88)	112 (12)	112 (14)	184 (25)*	184 (28)*	141 (61)	144 (40)
111 (100)	111 (86)	111 (100)	111 (100)	111 (100)	153 (11)*	154 (13)*	126 (20)	129 (15)
83 (14)	83 (18)	83 (12)	83 (15)	83 (14)	44 (95)	50 (65)	125 (40)	128 (23)
59 (14)	73 (11)	76 (11)	59 (21)	59 (10)		49 (50)	110 (36)	113 (14)
							73 (33)	76 (31)
							71 (57)	74 (58)
55 (17)	55 (15)	55 (14)	55 (12)	55 (11)			55 (100)	55 (100)
							45 (82)	48 (79)



Scheme 3. EI fragmentation of clopamide (Clo, R = R' = H), trimethylclopamide (**C1a**, R = R' = CH_3), nonadeuterotrimethylclopamide (**C1b**, R = R' = CD_3), dimethylclopamide (**C2a**, R = CH_3, R' = H) and hexadeuterodimethylclopamide (**C2b**, R = CD_3, R' = H).

(vi) Both **C4a** and **C4b** lose methanol from the molecular ion (to give *m/z* 260 or 266); but **C4a** loses the dimethylaminosulphonyl group with transfer of a hydrogen atom (*m/z* 185), whereas **C4b** loses the hexadeuterodimethylaminosulphonyl group with transfer of a deuterium atom (*m/z* 186).

(vii) When methanol was not added to toluene, we detected the corresponding isocyanate together with the aniline resulting from its hydrolysis followed by thermal decarboxylation.

(viii) The formation of **C4** can be explained from **C3** by the elimination of the carboxamido hydrogen to give a nitrene, which rearranges to an isocyanate, which, in turn, is transformed into **C4** by methanol (Scheme 7). Formation of an isocyanate has already been described for other nitrogen derivatives of acids like amides (Hofmann rearrangement[11]), azides (Curtius rearrangement[12]) or *O*-acetyl derivatives of hydroxamic acids (Lossen rearrangement[13]).

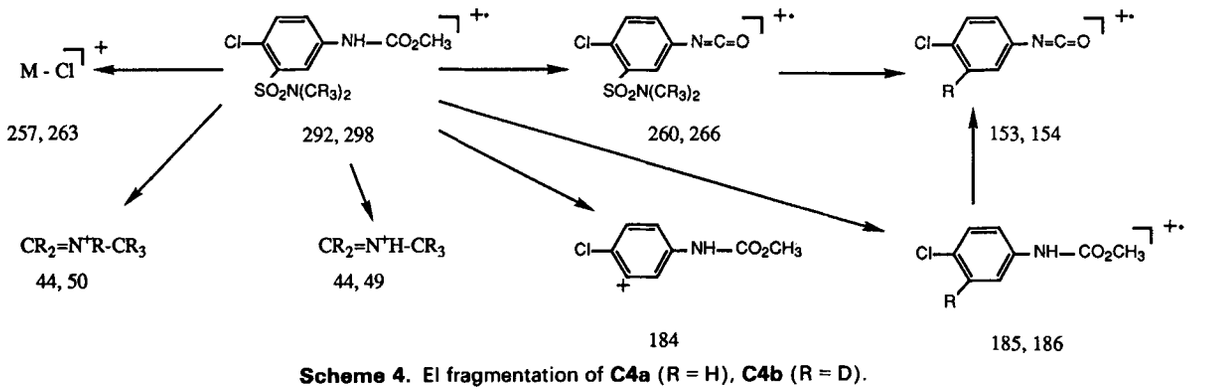
The negative ion mass spectrum of **C5** shares some characteristics with the spectra of **C1** and **C2**:

(i) It shows a mass peak at *m/z* 344 (348 after deuteromethylation), which indicates that it is probably a trimethylated derivative, as **C1**.

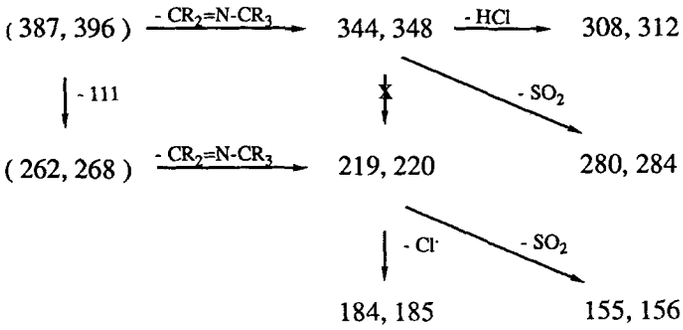
(ii) An ion at *m/z* 233 was not detected, but the methyl derivative **C5a** gives *m/z* 219, whereas the deuteromethyl derivative **C5b** gives *m/z* 220. This means that the third methyl group has been added to the piperidine ring nitrogen; instead of losing 111 followed by 43 mass units as in **C1a** or **C2a**, the molecular ion of **C5a** loses 125 mass units and then 43 mass units (128 and 48 in **C5b**).

This is confirmed by the fact that the ion at *m/z* 344 is not a parent of the ion at *m/z* 219 in the spectrum of **C5a**. But MS/MS experiments show that the ion at *m/z* 344 (or 348) loses HCl and SO_2 to give ions at *m/z* 308 (or 312) and 280 (or 284), respectively, and that the ion at *m/z* 219 (or 220) loses SO_2 to give an ion at *m/z* 155 (or 156) (Scheme 5).

In the case of compound **C5a**, positive ion MS/MS experiments show that peaks at *m/z* 372, 318, 246 and 141 (*m/z* 381, 327, 252 and 144 in **C5b**) are daughter



Scheme 4. EI fragmentation of **C4a** (R = H), **C4b** (R = D).



Scheme 5. Fragmentation of **C5a**, **C5b** in the electron capture negative ion mode. Figures in parentheses correspond to hypothetical non-detected ions.

ions of the molecular ion: the presence of an ion at *m/z* 318 means that the piperidine ring has been split off. Thus this ion can be explained by the classical rupture of a C—C bond alpha to the ex-piperidine ring nitrogen (Scheme 6).

All of the other peaks, except *m/z* 55 ($C_4H_7^+$), contain the N—CH_3 group because the labelling with deuterium shifts them by 3 mass units; the structure of some of them has yet to be clarified, but it is probable that some rearrangements, due to the presence of the double bond in the side-chain, occur.

The structure of compound **C5a** is confirmed by nuclear magnetic resonance (NMR), which shows the presence of four methyl groups (2.86 ppm, 6H, s, SO_2—N(CH_3)$_2$; 2.65 ppm, 3H, s, N—CH_3; 1.06 ppm, 3H, d, CH_3—CH) and of one vinyl group (4.9 ppm, 2H, m, CH_2=CH and 5.75 ppm, 1H, m, CH_2=CH—CH_2).

Compound **C3** is therefore a quaternary ammonium (mol. wt 388), which is transformed in the ion source (i) into 1,2,6-trimethylpiperidine, (ii) into an isocyanate, which gives the urethane **C4** following treatment with methanol, and (iii) into the unsaturated tertiary amine **C5**. The formation of such a tertiary amine **C5** from a quaternary ammonium is an intermediate reaction in the well-known Hofmann degradation.[14]

Indapamide derivatives

The R_F values of the two derivatives **I1** and **I3** on silica TLC plates were 0.90 and 0.12, respectively (eluent: ethyl acetate). Tables 3 and 4 show the negative ion and the positive ion mass spectra of indapamide and of its derivatives. In Table 3, it appears that the fragmentation of **I1** in the negative ion mode is basically the same as that of the clopamide derivative **C1**. The fact that both **C1a** and **I1a** give ions at *m/z* 233 and 169 proves that these ions do not contain the heterocycle of clopamide or indapamide.

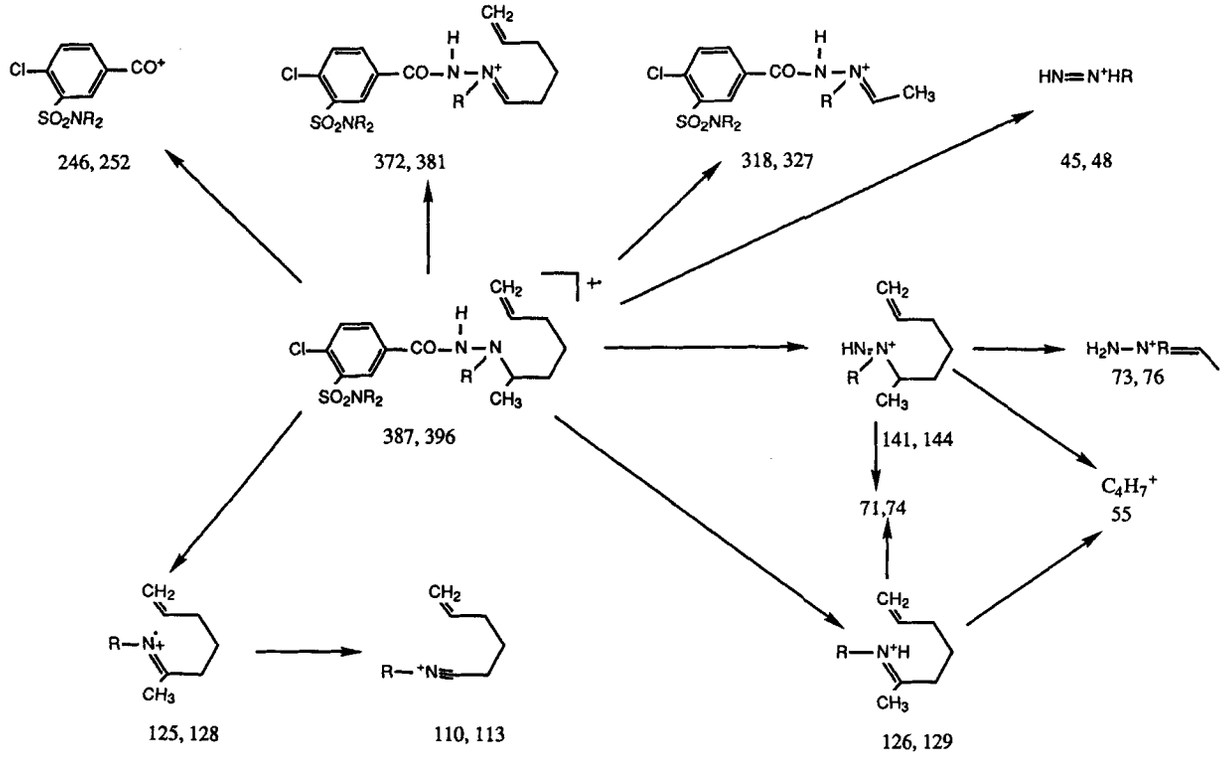
In the positive ion mode, compound **I1a** shows relatively little fragmentation, with ions at *m/z* 161, 132, 131, 130 analogous to the ions at *m/z* 141, 112, 111, 110 of trimethylclopamide **C1a**. There are no low-mass ions present as the 2-methyldihydroindolyl group of indapamide does not fragment as easily as the 2,6-dimethylpiperidinyl group of clopamide (Scheme 8).

When derivative **I3** is heated in the same way as **C3**, it also decomposes into several less polar compounds:

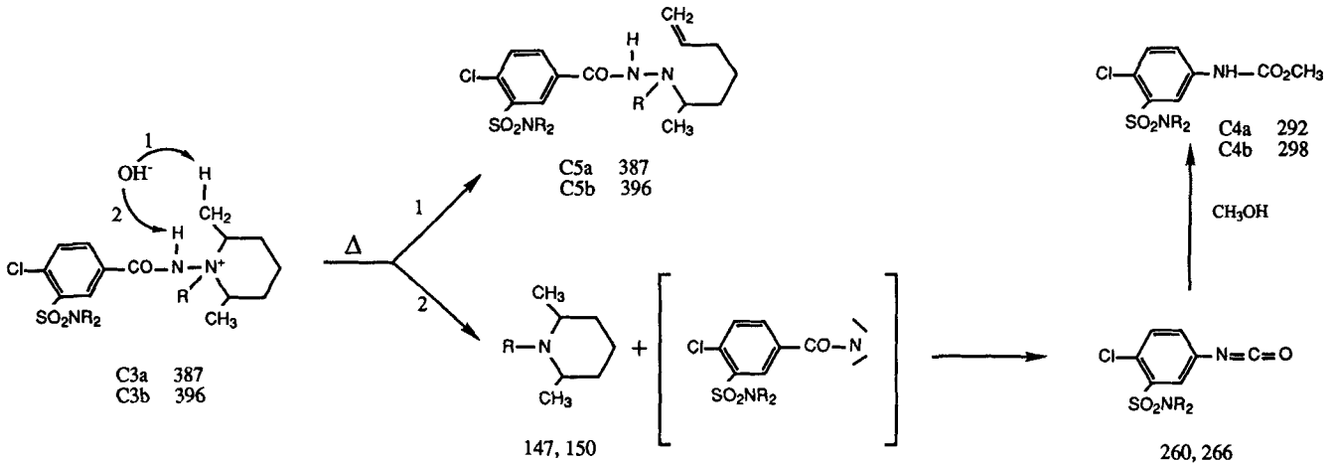
(i) 1,2-Dimethyl-2,3-dihydroindole[15] (mol. wt 147, base peak 132) or its trideuterated analogue (mol. wt 150, base peak 135).

(ii) Derivative **I4**, which is in fact identical to **C4**.

(iii) Derivative **I5**: its structure can be deduced from its negative and positive ion spectra as was done for **C5**. As in the later compound, the heterocycle has been split between the nitrogen and the adjacent non-aromatic carbon atom with formation of a vinylic double bond.



Scheme 6. EI fragmentation **C5a** (R = CH_3), **C5b** (R = CD_3).



Scheme 7. Thermal decomposition of **C3a** (R = CH_3), **C3b** (R = CD_3).

As for derivative **C5**, this structure can be confirmed by NMR which shows three methyl groups [3.21 ppm, s, 3H, N—$\underline{CH_3}$; 2.9 ppm, s, 6H, SO_2—N($\underline{CH_3}$)$_2$] and one vinyl group (4.94–5.0 ppm, m, 2H, $\underline{CH_2}$=CH— and 5.9–6.0 ppm, m, 1H, CH_2=$\underline{CH}$—CH_2).

But the proportion of **I5** versus **I4** is much smaller than that of **C5** versus **C4**. This is probably due to the fact that the carboxamido hydrogen of indapamide is more acidic (it is easier to remove by a base) than that of clopamide. Consequently pathway 2 (Scheme 7) is probably preferred with indapamide.

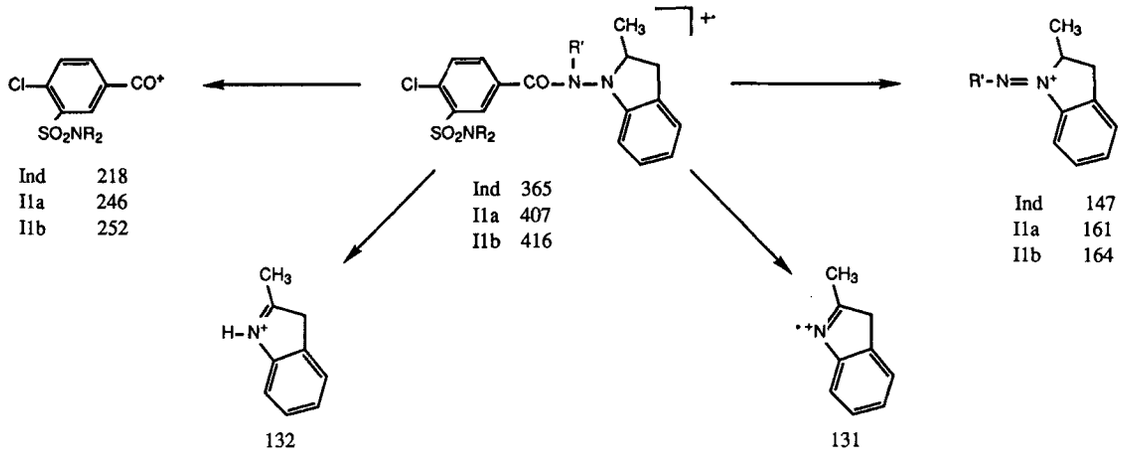
The formation of two or even three derivatives during this derivatization reaction may be a drawback when performing quantitative studies. The methylation of the different nitrogen atoms depends on their respective pK and on the pH of the aqueous solution. The pK_a of the sulphonamide group can be estimated at about 8.45,[16] whereas that of the hydrazide is much higher. When methylation was done at a pH of 10–10.5, only dimethylclopamide **C2** was formed in moderate yield, but as it still contains an exchangeable hydrogen atom, its GC properties were expected to be very poor. When

Table 3. Negative ion electron capture mass spectra of indapamide, I1, I4 and I5 obtained with iodomethane (a) and deuteroiodomethane (b). Fragments containing the chlorine atom are labelled (*)

	I1		I4		I5	
Indapamide 365	a 407	b 416	a 292	b 298	a 407	b 416
365 (27)*	364 (37)*	368 (14)*	249 (100)*	250 (100)*	364 (7)*	368 (34)*
	329 (<5)	333 (<5)	217 (56)*	218 (23)*	328 (<5)	332 (7)
	276 (6)*	285 (17)*	185 (66)*	186 (26)*	300 (41)*	304 (52)*
	275 (35)*	284 (100)*				
	241 (24)	250 (18)			261 (31)*	267 (47)*
234 (22)*	233 (100)*	237 (90)*			227 (<5)	233 (<5)
233 (100)*					219 (74)*	220 (100)*
	197 (<5)	201 (8)				
	169 (87)*	173 (90*)			155 (100)*	156 (75)*

Table 4. Positive ion EI mass spectra of indapamide, I1, I4 and I5 obtained with iodomethane (a) and deuteroiodomethane (b). Fragments containing the chlorine atom are labelled (*)

	I1		I4		I5	
Indapamide 365	a 407	b 416	a 292	b 298	a 407	b 416
365 (14)*	407 (12)*	416 (16)*	292 (50)*	298 (48)*	407 (32)*	416 (18)*
234 (<5)*			260 (6)*	266 (5)*	246 (10)*	252 (5)*
218 (7)*	246 (<5)*	252 (<5)*	257 (6)	263 (5)	161 (28)	164 (19)
147 (100)	161 (100)	164 (100)	185 (100)*	186 (100)*	147 (22)	150 (10)
132 (10)	132 (45)	132 (28)	184 (28)*	184 (20)*	146 (80)	149 (50)
131 (31)	131 (14)	131 (17)	153 (10)*	154 (15)*	145 (70)	148 (26)
130 (14)	130 (10)	130 (10)	44 (91)	50 (56)	144 (100)	147 (100)
	117 (7)	117 (5)		49 (50)	132 (51)	135 (31)
					130 (54)	130 (30)
					117 (49)	117 (20)



Scheme 8. EI fragmentation of indapamide (Ind, R = R′ = H), trimethylindapamide (**II a**, R = R′ = CH_3) and nonadeuterotrimethylindapamide (**II b**, R = R′ = CD_3).

the pH is above 12, essentially compound **C1** and **C3** are formed; we were never able to obtain **C1** alone. In the case of indapamide, we never, even at pH 10, observed the dimethyl derivative, as the hydrazide hydrogen is more acidic than in clopamide.

Stueber *et al.*[7] recovered clopamide from plasma by solid-phase extraction with C18 Sep-Pak cartridges and derivatized clopamide with Methelute (trimethylanilinium hydroxide in methanol) in the presence of furosemide as internal standard, before a single-ion GC/MS analysis. When we reacted clopamide or indapamide with this reagent and analysed the resulting mixture by direct introduction, we detected derivative **C1** or **II**, but also the quaternary ammonium derivatives. This means that in the above-mentioned study, only a part of the clopamide might have been determined as only the 'normal' trimethylclopamide was measured. This has led us to conclude that, when clopamide or indapamide has to be quantified by GC, it is necessary to use an internal standard which will behave strictly like these two diuretics during the extraction–methylation procedure (e.g. a stable isotope labelled analogue or a compound which contains in its structure all the chemical functions of clopamide or indapamide).

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