Anal. Chem. **2002,** *74,* 3802−3808

Mass Spectrometric Behavior of Thiazide-Based Diuretics after Electrospray Ionization and Collision-Induced Dissociation

Mario Thevis,*[,†,‡] Hans Schmickler,[§] and Wilhelm Schänzer[†]

Institute of Biochemistry, German Sport University Cologne, Carl-Diem-Weg 6, 50933 Cologne, Germany, and Institute of Organic Chemistry, University of Cologne, Greinstrasse 4, 50939 Cologne, Germany

The mass spectrometric behavior of 21 thiazide-based compounds after electrospray ionization in the negative ion mode and collision-induced dissociation was investigated on a triple-stage quadrupole mass spectrometer. The mass spectra show individual and common fragmentation patterns, the generations of which are discussed based on comparable molecular structures of commercially available substances and the synthesis of unlabeled, deuterated, and ^{15}N-labeled analogues. The synthesis of deuterated thiazides is perfomed by condensation of 4-amino-6-chloro-1,3-benzenedisulfonamide with appropriately labeled aldehydes, while the introduction of ^{15}N into the sulfonamide groups of thiazides was achieved by the synthesis of 4-amino-6-chloro-1,3-benzenedisulfonamide($^{15}N_2$) from 3-chloroaniline via 4-amino-6-chloro-1,3-benzenedisulfonyl chloride. The most common fragments determined are *m*/*z* 269, 205, and 126 for 6-chloro-7-sulfamoyl-3-alkyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxides and *m*/*z* 303, 239, and 160 for 6-trifluoromethyl-7-sulfamoyl-3-alkyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxides. Individual fragmentation behaviors were found that mainly depended on the C-3-linked side chain.

The analysis of products based on the structure of 6-chloro-7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide (hydrochlorothiazide, Figure 1) has gained attention ever since the diuretic effect of thiazides was observed with chlorothiazide by Novello and Sprague in 1957.[1] Owing to the wide variety of analogous products developed in the following years,[2−7] the clinical, forensic, and antidoping analysts require informative and unambiguous data about administered pharmaceuticals. Therefore, different analytical techniques, such as high-performance liquid chromatography (HPLC),[8−12] gas chromatography (GC),[13−15] and capillary electrophoresis (CE)[16] coupled to ultraviolet (UV) detectors or mass spectrometers (MS), were developed. Especially the use of LC/MS/MS has proven to be an appropriate means to provide detailed information about thiazide-based diuretics and other compounds belonging to this class of remedies. In particular, the doping analysis in human and equine sports profits from avoiding time-consuming sample preparation steps and the possibility of sensitive and selective measurements of thiazides.[17,18]

Due to the acidic character of thiazides and sulfonamides in general, the use of negative ion electrospray ionization proved to be reasonable. Some gas-phase reactions of organic compounds after hydrogen abstraction were reviewed by Bowie,[19] showing possible rearrangements, elimination, and fragmentation routes as a result of collision-induced dissociation. The knowledge of gas-phase reactions of negatively charged ions after collisional activation is of analytical significance and important for the determination and identification of compounds by mass spectrometry. The goal of the present study is the elucidation of the mass spectrometric behavior of thiazides after negative ion electrospray ionization in correlation to their individual structures by means of a triple-stage quadrupole mass spectrometer. The mass spectra of 21 compounds with a 7-sulfamoyl-1,2,4-benzothiadiazine 1,1-

† German Sport University Cologne.

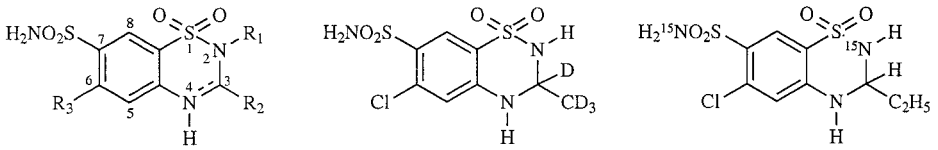
‡ Current address. Department of Chemistry and Biochemistry, University of California at Los Angeles, 3021 Young Hall, 607 Charles E. Young Drive E., Los Angeles, CA 90095. Phone: 310-794-7308. Fax: 310-206-7286. e-mail: mthevis@chem.ucla.edu..

§ University of Cologne.

(1) Novello, F. C.; Sprague J. M. *J. Am. Chem. Soc.* **1957**, *79*, 2028−2029.
(2) Werner, L. H.; Halamandaris A.; Ricca S., Jr.; Dorfman L.; DeStevens G. *J. Am. Chem. Soc.* **1960**, *82*, 1161−1166.
(3) DeStevens, G.; Werner, L. H.; Barret, W. E.; Chart, J. J.; Renzi, A. H. *Experientia* **1960**, *16*, 113−114.
(4) Novello, F. C.; Bell, S. C.; Abrams, E. L. A.; Ziegler, C.; Sprague, J. M. *J. Org. Chem.* **1960**, *25*, 965−970.
(5) Sherlock, M. H.; Sperber, N.; Topliss, J. *Experientia* **1960**, *16*, 184−185.
(6) DeStevens, G.; Werner, L. H.; Halamandaris, A.; Ricca, S., Jr. *Experientia* **1958**, *14*, 463.
(7) Whitehead, C. W.; Traverso, J. J.; Sullivan, H. R.; Marshall, F. J. *J. Org. Chem.* **1961**, *26*, 2814−2818.
(8) Rosado-Maria, A.; Gasco-Lopez, A. I.; Santos-Montes, A.; Izquierdo-Hornillos, R. *J. Chromatogr., B* **2000**, *748*, 415−424.
(9) Smith, R. M.; Murilla, G. A.; Hurdley, T. G. *J. Chromatogr.* **1987**, *384*, 259−278.
(10) Leahey, W. J.; McMeekin, J. R. *J. Chromatogr.* **1987**, *421*, 401−406.
(11) Cooper, S. F.; Massé, R.; Dugal, R. *J. Chromatogr.* **1989**, *489*, 65−88.
(12) Tisdall, P. A.; Moyer, T. P.; Anhalt, J. P. *Clin. Chem.* **1980**, *26*, 702−706.
(13) Lisi, A. M.; Trout, G. J.; Kazlauskas, R. *J. Chromatogr.* **1991**, *563*, 257−270.
(14) Ahnoff, M.; Ervik, M.; Lagerström, P. O.; Person, B. A.; Vessman, J. *J. Chromatogr.* **1985**, *340*, 73−138.
(15) Maurer, H. H. *J. Chromatogr., B* **1999**, *733*, 3−25.
(16) Jumppanen, J.; Sirén, H.; Riekkola, M. L. *J. Chromatogr., A* **1993**, *652*, 441−450.
(17) Thieme, D.; Grosse, J.; Lang, R.; Mueller, R. K.; Wahl, A. *J. Chromatogr., B* **2001**, *757*, 49−57.
(18) Garcia, P.; Popot, M. A.; Bonnaire, Y.; Tabet, J. C. *Proc. 11th Int. Conf. Racing Analysts Veterinarians* **1996**, 498−502.
(19) Bowie, J. H. *Mass Spectrom. Rev.* **1990**, *9*, 349−379.

10.1021/ac020020e CCC: $22.00
Published on Web 06/22/2002

A) B) C)



Compound	Molecular weight (Da)	R_1	R_2	R_3	C-3–N-4 double bond
Chlorothiazide	295.7	H	H	Cl	√
Hydrochlorothiazide	297.7	H	H	Cl	
"Methiazide"	311.7	H	CH_3	Cl	
Ethiazide	325.8	H	C_2H_5	Cl	
"Prothiazide"	339.8	H	C_3H_7	Cl	
"n-Buthiazide"	353.8	H	C_4H_9	Cl	
Butizide	353.8	H	H_2C–	Cl	
"n-Penthiazide"	367.8	H	C_5H_{11}	Cl	
Cylcopenthiazide	379.9	H	H_2C–	Cl	
Cyclothiazide	389.9	H	HC=	Cl	
Bemetizide	401.9	H	HC–	Cl	
Hydroflumethiazide	331.3	H	H	CF_3	
Bendroflumethiazide	421.4	H	H_2C–	CF_3	
Methylclothiazide	360.2	CH_3	CH_2Cl	Cl	
Trichlormethiazide	380.6	H	$CHCl_2$	Cl	
Teclothiazide	415.1	H	CCl_3	Cl	
Althiazide	383.9	H	$CH_2SCH_2CH{=}CH_2$	Cl	
Epithiazide	425.8	H	$CH_2SCH_2CF_3$	Cl	
Benzthiazide	431.9	H	$CH_2SCH_2C_6H_5$	Cl	
Polythiazide	439.9	CH_3	$CH_2SCH_2CF_3$	Cl	√
"Methylthioethiazide"	371.9	H	$CH_2CH_2SCH_3$	Cl	

Figure 1. (A) Structures of commercially available and synthesized thiazide-based compounds. Structure names, INN; if others, in quotes. (B) Structure of methiazide-d_4. (C) Structure of [$^{15}N_2$]-ethiazide.

dioxide structure (Figure 1) are discussed, and elimination steps and rearrangements for the generation of common and individual fragment ions are proposed, based on the synthesis and analysis of different alkylated thiazides in addition to deuterated and ^{15}N-labeled analogues.

EXPERIMENTAL SECTION

Chemicals. 3-Chloroaniline, chlorosulfonic acid, 4-amino-6-chloro-1,3-benzenedisulfonamide, acetaldehyde, propionaldehyde, butyraldehyde, valeraldehyde, capronaldehyde, 3-methylmercaptopropionaldehyde, acetaldehyde-d_4, D_2O, DCl (6 N in D_2O), ethanol-*d*, ammonium deuteride (12 N in D_2O), and [^{15}N]-ammonium hydroxide were purchased from Aldrich. Ethanol, toluene (dried, over molecular sieve), *n*-heptane, and sodium chloride were obtained from Merck (Darmstadt, Germany), *tert*-butyl methyl ether (distilled before use) was from KMF (St. Augustin, Germany), and acetonitrile was from J. T. Baker (Deventer, Netherlands).

Thiazides. Chlorothiazide was purchased from Sigma (Deisendorf, Germany), benzthiazide from Knoll AG (Ludwigshafen, Germany), bendroflumethiazide from ICI Pharma (Plankstadt, Germany), trichlormethiazide from Merck, polythiazide from Pfizer (Karlsruhe, Germany), methyl chlothiazide from Abbott Laboratories Ltd. (Queensborough, England), butizide from Boehringer (Mannheim, Germany), and bemetizide from Schwarz Pharma GmbH (Monheim, Germany). The reference materials althiazide, ethiazide, epithiazide, cyclothiazide, cyclopenthiazide, and hydroflumethiazide were kindly provided by Simon Biddle from HFL, UK. Methiazide, methiazide-d_4, prothiazide, *n*-buthi-

azide, *n*-penthiazide, and [$^{15}N_2$]-ethiazide were synthesized in our laboratory.

Synthesis of 6-Chloro-7-sulfamoyl-3-alkyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-Dioxides. The preparation of not commercially available alkylated analogues of hydrochlorothiazide (e.g., methiazide, methiazide-d_4, [$^{15}N_2$]-ethiazide, prothiazide, *n*-buthiazide, and *n*-penthiazide) was performed according to the method of Whitehead et al.[7] The synthesis of methiazide is described in the following: In a volume of 40 mL of warm ethanol and 6 N aqueous HCl (50:50, v/v) 2.85 g of 4-amino-6-chlorobenzene-1,3-disulfonamide (**I**) was suspended. A total of 1.5 equiv of acetaldehyde (0.66 g, 840 μL) was added, and the mixture was stirred for 1 h. After storage at 4 °C for 10 h, the precipitate was collected on a medium-porosity frit and washed with bidistilled water until the residue of mineral acid was removed. The resulting methiazide (1.6 g, 52%) was dried in a desiccator over phosphorus pentoxide under reduced pressure. Smaller amounts of synthesized diuretics were extracted from the reaction mixture by dilution with a 5-fold volume of bidistilled water and subsequent solid-phase extraction of the thiazide. To do this, the mixture was transferred onto a PAD-I column (bed height 3 cm, inner diameter 1 cm), washed with distilled water, and the product was eluted with methanol.

For the preparation of deuterated methiazide-d_4 (Figure 1B) the acetaldehyde was replaced by its deuterated analogue and the condensation with **I** was performed in ethanol-*d* and DCl in D_2O.

Synthesis of [$^{15}N_2$]-Ethiazide. To introduce selectively two ^{15}N-atoms into the thiazide structure, the intermediate product of **I**, 4-amino-6-chlorobenzene-1,3-disulfonyl chloride, was prepared in accordance to the method described by Novello et al.[4] 3-Chloroaniline (6.4 g, 0.05 mol) was added dropwise to 67 g of chlorosulfonic acid (0.6 mol) placed in an ice-cooled three-necked flask under constant stirring. Further, 35 g of sodium chloride (0.6 mol) was added portionwise. The mixture was heated slowly to 90 °C and maintained at this temperature for 1 h. After cooling to ambient temperature, the product was extracted with 500 mL of *tert*-butyl methyl ether, the organic layer was evaporated to dryness by means of a rotary evaporator under reduced pressure, and the residue was dried in a desiccator over phosphorus pentoxide in vacuo for 12 h. The crude crystals were finally purified by recrystallization from toluene/*n*-heptane (yield: 2.4 g, 15% of the theory).

A total of 500 mg of the resulting 4-amino-6-chlorobenzene-1,3-disulfonyl chloride (**II**) was treated with 6 N [^{15}N]-ammonium hydroxide at 80 °C under argon for 1 h. After being cooled to ambient temperature, the mixture was stored at 4 °C for 10 h, the precipitate was collected on a medium-porosity frit and washed with bidistilled water (yield: 380 mg, 85% of the theory). The doubly nitrogen-labeled 4-amino-6-chlorobenzene-1,3-disulfonamide was then condensed to the corresponding [$^{15}N_2$]-ethiazide with propionaldehyde as described above.

Mass Spectrometry. All analyses were performed on a PE Sciex API2000 triple quadrupole mass spectrometer (PE Biosystems, Foster City, CA) equipped with an electrospray interface. The flow rate of the solution containing the analyte was 10 μL/min, introduced by a syringe pump into the ESI interface. All analytes were solved in a mixture of acetonitrile/water (50:50, v/v) at a concentration of 5 μg/mL. The ionization mode was negative and variable parameters of orifice, ring electrode, and interquadrupole lenses were optimized for maximum abundance of the ions of interest $(M - H)^-$. The collision gas was nitrogen obtained from a Whatman 75-72 K727 nitrogen generator, and the collision offset voltage was 25 eV for all product ion scans.

Table 1. ^{1}H and ^{13}C Chemical Shifts of Synthesized Methiazide-d_4

carbons	δ	hydrogens	δ
C-3	63.19	N-2	7.65
C-5	118.31	N-4	8.00
C-6	136.65	C-5	6.90
C-7	129.45	C-8	8.08
C-8	127.34	SO_2NH_2	7.44
C-9	119.81		
C-10	148.41		
C-11	19.25		

Nuclear Magnetic Resonance Spectroscopy. Structure confirmation of the synthesized methiazide-d_4 was performed on a Bruker DRX 500 instrument with a solution of the analyte in CD_3OD or CD_3OH. The one- and two-dimensional experiments were ^{1}H, ^{1}H Watergate (at 20, −10, and − 44 °C), ^{13}C, HMBC (CD_3OH, −44 °C), and ROESY (CD_3OH, −44 °C).

RESULTS AND DISCUSSION

Nuclear Magnetic Resonance Spectroscopy. The NMR experiments provided unambiguous information about the structure of the synthesized methiazide-d_4. The ^{1}H and ^{13}C chemical shifts are shown in Table 1.

Mass Spectrometry. *3-Hydrogenated and 3-Alkylated 7-Sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-Dioxides.* On the basis of the mass spectrum of the synthesized methiazide (Figure 2), fundamental fragmentation steps after collision-induced dissociation of the quasimolecular ion are discussed.

In all product ion scan spectra of $(M - H)^-$ of hydrochlorothiazide and 3-alkylated 7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides, the fragments *m*/*z* 269, 205, 126, and 78 are present (Table 2, Figure 3A), which are missing in atmospheric pressure chemical ionization (APCI) mass spectra as reported in the literature.[20] The generation of *m*/*z* 269 is proposed to be initiated by the fission of the C-3−N-4 bond and a subsequent elimination of R−CH=NH. Here, the presence of a single bond between atoms 3 and 4 and the following cleavage of the linkage seems to be essential for the existence of *m*/*z* 269. Two of the investigated compounds, chlorothiazide and benzthiazide, do contain double bonds at this particular position and do not generate the common fragment *m*/*z* 269 or any other corresponding ion (Table 2). The proposal that hydrogens are shifted within the suggested fragmentation is supported by the appearance of *m*/*z* 272 and 271 in the spectrum of methiazide-d_4 (Table 2). Thus, two or three deuteriums of C-3 or its neighboring methyl group have to migrate from the leaving group to the anion. Other studies dealing with stably labeled compounds report a comparable "scrambling" of hydrogens in a negatively charged gas-phase ion during its dissociation.[21] The position of charge could not be determined, but it is known from the literature[19,21] that, with chemical and electrospray ionization in the negative mode, generally the most

(20) Garbis, S. D.; Hanley, L.; Kalita, S. *J. AOAC Int.* **1998**, *81*, 948−957.
(21) Bowie, J. H. *Mass Spectrom. Rev.* **1984**, *3*, 161−207.

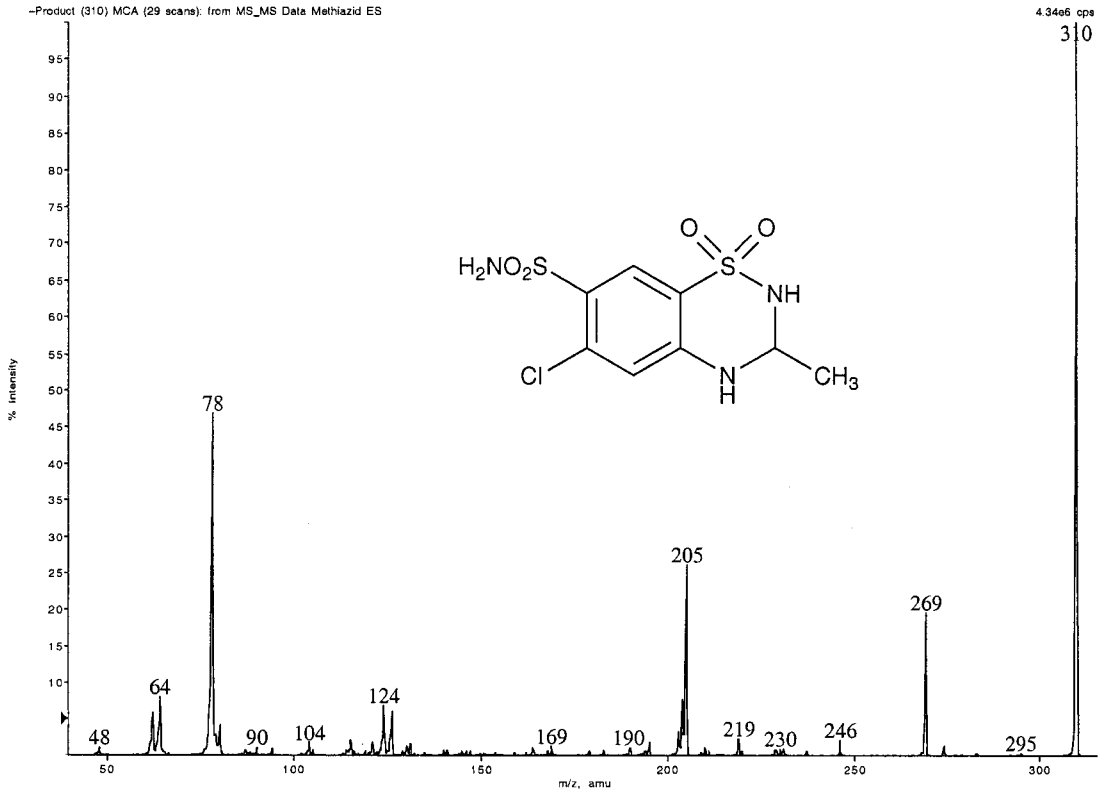


Figure 2. ESI product ion scan of *m*/*z* 310 of synthesized methiazide.

acidic hydrogen is abstracted. Therefore, the charge is typically located at the sulfonamide group. The following elimination of sulfur dioxide (−64 amu) from *m*/*z* 269 generates *m*/*z* 205. This fragment was also observed in the mass spectrum of 4-amino-6-chlorobenzene-1,3-disulfonamide (Table 2), which can initially lose SO_2NH (−79 amu) under hydrogen rearrangement, producing the same ion *m*/*z* 205. The exchange of all nitrogen-bonded hydrogens by deuteriums led to the expected corresponding fragment *m*/*z* 209. A further loss of SO_2NH produces the common ion *m*/*z* 126 with the structure of deprotonated 3-chloroaniline, which was demonstrated in an earlier study.[18] In case of 6-trifluoromethylated thiazides, such as hydroflumethiazide and bendroflumethiazide, the counterparts to *m*/*z* 269, 205, and 126 are detected with *m*/*z* 303, 239, and 160, respectively. Obviously, the kind of substitution of C-6 (Cl or CF_3) does not essentially influence the presented fragmentation process. The composition of *m*/*z* 78 is proposed to be SO_2N^- since the fragment shifts to *m*/*z* 79 with ^{15}N-labeling but is constant under the conditions of deuterium labeling.

In Figure 3B, the generation of ions with a comparatively decreased abundance is proposed, e.g., for ethiazide. All mass spectra of 3-*n*-alkylated thiazides contain a fragment of $(M - H - 64)^-$, due to the loss of SO_2 from the quasimolecular ion and a possible formation of a five-membered ring structure. Subsequently, the elimination of HCN is observed, which includes exclusively the nitrogen N-2. This was proven by the synthesis of ^{15}N-labeled ethiazide, in which only the nitrogens of the sulfonamide group and N-2 were isotopically substituted. The analysis of [$^{15}N_2$]-ethiazide showed the fragment *m*/*z* 262 (corresponding to *m*/*z* 260) after removal of SO_2 from $(M - H)^-$ and the following loss of 28 mass units producing the fragment *m*/*z* 234, owing to the elimination of $HC^{15}N$. Further, the hydrogen involved in the rearrangement of the HCN elimination definitely originates from C-3 and not from any nitrogen. Evidence to this effect was obtained by the analysis of methiazide-d_4, which also expels 28 mass units with DCN (Table 2). Thus, the removal of HCN of 3-alkylated thiazides includes the migration of the alkyl group from C-3, possibly to N-4, as demonstrated with ethiazide and its fragment ion *m*/*z* 233 in Figure 3B.

In general, the 3-alkylated and 6-chlorinated thiazides such as methiazide, ethiazide, prothiazide, *n*-buthiazide, butizide, *n*-penthiazide, cyclopenthiazide, bemetizide, and cyclothiazide eliminate HCl (Table 2), the hydrogen of which obviously originates from different sources. With methiazide-d_4, the loss of HCl and DCl is observed in equal intensities, and also 4-amino-6-chlorobenzene-1,3-disulfonamide, without the typical thiazide cyclization and alkylation at C-3, eliminates HCl. A comparable phenomenon (elimination of HCF_3 or HF) is not present for those thiazides bearing a trifluoromethyl group at C-6.

Another common fragment of 3-hydrogenated or 3-alkylated and 6-chlorinated thiazides is *m*/*z* 190 (Table 2). Its proposed structure is shown in Figure 4A. The fact that N-4 remains in the fragment was proven by [$^{15}N_2$]-ethiazide, which does not generate an ion *m*/*z* 191 owing to the absence of the amine group of the sulfonamide unit. In the mass spectra of the 6-trifluoromethylated thiazides, bendroflumethiazide and hydroflumethiazide, the fragment *m*/*z* 224, corresponding to *m*/*z* 190, is also observed.

A distinction between 3-alkylated thiazides with identical masses but different side-chain constituents such as butizide and *n*-buthiazide or an open-chain structure compared to a cyclic substitute appears to be difficult by means of mass spectrometry.

(22) Martinez-Alvarez, R.; Martin, N.; Seoane, C.; Suarez, M.; Perez, R.; Nour Kayali, H. R. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 758−762.

Table 2. Individual and Common Fragment Ions (Intensities in % Relative to Base Peak) of Compounds Based on the Structure of 3-Hydrogenated or 3-Alkylated 7-Sulfamoyl-1,2,4-benzothiadiazine 1,1-Dioxides

			common product ions							individual product ions		
compound	$(M-H)^-$	$(M-H-HCl)^-$	$(M-H-SO_2)^-$	$(M-SO_2-HCN)^-$	269	205	190	126	78			
4-amino-6-chlorobenzene-1,3,disulfonamide	284 (100)	248 (3.1)				5.5			34.2	169 (6.3)	136 (4.1)	120 (2.6)
hydrochlorothiazide	296 (100)	-	232 (1.0)	-	30.5	22.4	1.3	7.0	31.6			
methiazide	310 (100)	274 (1.4)	246 (2.1)	219 (2.4)	19.6	26.2	1.1	6.0	46.8			
methiazide-d_4	314 (100)	277 (0.6)/278 (0.6)	250 (1.7)	222 (2.2)	272 (9.8)/271 (8.2)	207 (12.8)/208 (10.6)	192 (0.5)	128 (2.1)	36.6			
ethiazide	324 (100)	288 (1.4)	260 (1.3)	233 (5.0)	14.4	17.6	1.7	3.7	40.5			
[$^{15}N_2$]-ethiazide	326 (100)	290 (1.5)	262 (1.1)	234 (5.6)	270 (13.2)	206 (21.2)	1.5	4.1	32.4			
prothiazide	338 (100)	302 (1.5)	274 (1.3)	247 (3.2)	14.4	17.2	1.6	4.6	36.2			
n-buthiazide	352 (100)	316 (1.0)	288 (1.1)	261 (3.5)	14.3	16.4	1.0	4.1	30.8			
butizide	352 (100)	316 (1.3)	288 (1.1)	261 (2.4)	21.8	23.3	2.3	5.5	41.0			
n-penthiazide	366 (100)	330 (1.8)	302 (1.4)	275 (5.7)	19.3	25	2.6	7.0	51.0			
cyclopenthiazide	378 (100)	342 (1.1)	314 (1.0)	287 (2.3)	19.1	22.2	1.5	4.9	38.5			
cyclothiazide	388 (100)				35.7	24.1		5.8	37.5	322 (19.3)	258 (4.6)	158 (4.2)
bemetizide	400 (100)				6.9	8.2			23.4	294 (53.4)	277 (3.8)	195 (10.4)
compound	$(M-H)^-$	$(M-H-HCl)^-$	$(M-H-SO_2)^-$	$(M-H-SO_2-HCN)^-$	303	239	224	160	78			
hydroflumethiazide	330 (100)		266 (4.7)		28.3	39.7	5.9	35.2	43.4			197 (6.3)
bendroflumethiazide	420 (100)				7.1	12.1	14.1	13.1	35.4	328 (18.2)	289 (25.3)	197 (24.2)

Figure 3. (A) Proposed generation of the fragment ions *m*/*z* 269, 205, and 126 of 6-chloro-7-sulfamoyl-3-alkyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides and hydrochlorothiazide. (B) Postulated successive elimination of SO_2 and HCN from ethiazide.

However, the influence on the fragmentation process of a double bond or a complete functional unit such as a phenolic ring (e.g., bemetizide, Figure 1) is intense and informative with respect to its structure. So, cyclothiazide (Figure 1) generates an ion *m*/*z* 322 (Table 2) from the quasimolecular ion by a classical retro-Diels−Alder rearrangement (Figure 4B), in addition to the common fragments of 3-alkylated thiazides. In contrast, bemetizide produces an ion $(M - H - 106)^-$ by the elimination of ethylbenzene at the expense of the common fragmentation pattern. So, *m*/*z* 269, 205, and 126 appear with decreased intensities and further individual ions are generated (Table 2). The mass spectrum of bendroflumethiazide, which bears a benzyl group at C-3, contains all common ions (with respect to the shift owing to the substitution of Cl by CF_3) and the ion *m*/*z* 328, the origin of which is the elimination of toluene from the quasimolecular ion.

3-Chloroalkylated 6-Chloro-7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-Dioxides. The halogenation of the C-3 alkyl side chain results in a fragmentation pattern different from the one described for 3-hydrogenated and 3-alkylated 7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides. Typical representatives of this class of thiazides are methylclothiazide, trichlormethiazide, and teclothiazide (Figure 1). The mass spectra of those compounds are dominantly characterized by the elimination of HCl and the subsequent loss of SO_2 (Table 3). In contrast to those thiazides

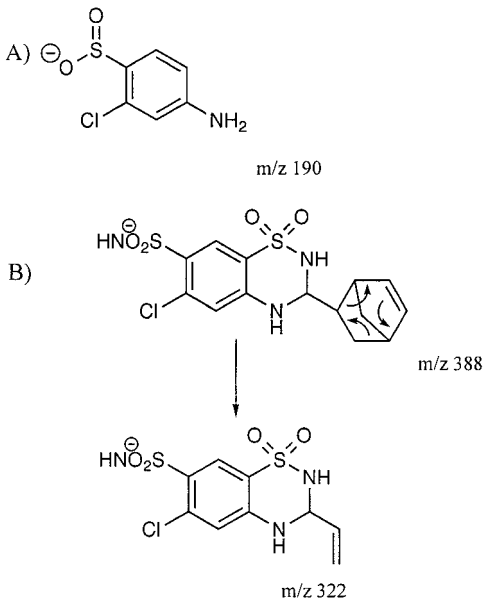


Figure 4. (A) Proposed fragment ion structure of *m*/*z* 190 of 6-chloro-7-sulfamoyl-3-alkyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides. (B) Possible retro-Diels−Alder rearrangement of the quasi-molecular ion of cyclothiazide (*m*/*z* 388) to *m*/*z* 322.

without chlorination of the 3-methyl group (methiazide) or with an alkyl side chain in general, the base peak is not $(M - H)^-$. Also, the fragmentation to *m*/*z* 269, 205, and 126 is not observed, probably due to the initial formation of double bonds into the thiazide structure by neutral loss of hydrochloric acid. The lack of those common fragment ions was also reported for chlorothiazide and benzthiazide, both of which contain a 3−4-double bond.

3-Alkylthiomethyl-6-chloro-7-sulfamoyl-1,2,4-benzothiadiazine 1,1-Dioxides. The mass spectrum of epithiazide is shown as an example for the substances of this class (Figure 5). All compounds contain a side chain including a thioether functionality. Except those compounds bearing a 3−4-double bond (benzthiazide) or a 2-*N*-methyl group (polythiazide), the diuretics of this section (Table 3) generate the common fragments *m*/*z* 269, 205, 126, and 78. The thioether linkage turned out to be of great interest since it obviously represents a preferred position of dissociation. With epithiazide, benzthiazide, and polythiazide, fragmentation occurs on both sides of the sulfur atom, generating characteristic fragments for this particular thiazide structure. The dissociation of the side chain, including the elimination of the sulfur atom, involves the migration of a hydrogen from the leaving group to the remaining ion (e.g., *m*/*z* 310 of epithiazide, Figure 5). The ion *m*/*z* 341 of althiazide and epithiazide (Table 3) obviously results from a homologous fission of the S−C bond remote from the thiazide nucleus. The corresponding fragments of benzthiazide (*m*/*z* 339, owing to its 3−4-double bond) and polythiazide (*m*/*z* 355, owing to its 2-*N*-methyl group) are present, too. Such a fragmentation requires the generation of two radicals from an even-electron anion. Although the "even-electron rule" is commonly accepted[23] and appropriate for many cases of mass spectrometry, the literature reports several exemptions,[24] the number of which increases proportional to published fragmenta-

Table 3. Individual and Common Fragment Ions (Intensities in % Relative to Base Peak) of Compounds Based on the Structure of 3-Chloroalkylated 7-Sulfamoyl-1,2,4-benzothiadiazine 1,1-Dioxides and Analogous 3-Thio Ether 7-Sulfamoyl-1,2,4-benzothiadiazine 1,1-Dioxides

	common product ions					individual product ions					
compound	$(M-H)^-$	$(M-H-HCl)^-$	$(M-H-2xHCl)^-$	$(M-H-HCl-SO_2)^-$	$(M-H-2xHCl-SO_2)^-$	269	205	126	78				
methylclothiazide	358 (35.4)	322 (100)		258 (41.5)					17.2	243 (3.8)	222 (13.4)	208 (5.3)	194 (25.7)
trichlormethiazide	378 (10.1)	342 (1.1)	306 (38.5)		242 (100)				2.4	215 (11.3)	198 (2.7)	178 (3.1)	142 (5.7)
teclothiazide	414 (13.1)		340 (3.8)		276 (4.6)				1.5	294 (100)	214 (30.9)	179 (20.9)	
compound	$(M-H)^-$	$(M-H-HF)^-$	$(M-H-2xHF)^-$	$(M-H-3x\ HF)^-$									
althiazide	382 (43.6)				341 (100)	92.7	39.4	12.1	47.9	260 (17.6)	213 (9.7)	150 (4.9)	115 (8.5)
epithiazide	424 (100)	404 (37.6)	384 (29.1)	364 (25.0)	341 (23.6)	70.3	51.4	18.5	80.6	310 (45.1)	300 (58.6)	279 (12.1)	260 (12.4)
benzthiazide	430 (59.1)				339 (2.2)					308 (100)	228 (36.0)	193 (8.2)	175 (7.2)
polythiazide	438 (100)	418 (37.4)	398 (82.5)	378 (14.4)	355 (14.0)				57.2	324 (82.9)	278 (30.7)	260 (43.2)	229 (18.7)
methylthioethiazide	370 (30.0)					35.1	24.1	6.9	32.7	322 (100)	258 (10.2)	222 (3.9)	158 (5.7)

(23) McLafferty, F. W.; Turecek, F. *Interpretation of Mass Spectra*, 4th ed.; University Science Books: Mill Valley, CA, 1993; Chapter 8.

(24) Karni, M.; Mandelbaum, A. *Org. Mass Spectrom.* **1980**, *15*, 53−64.

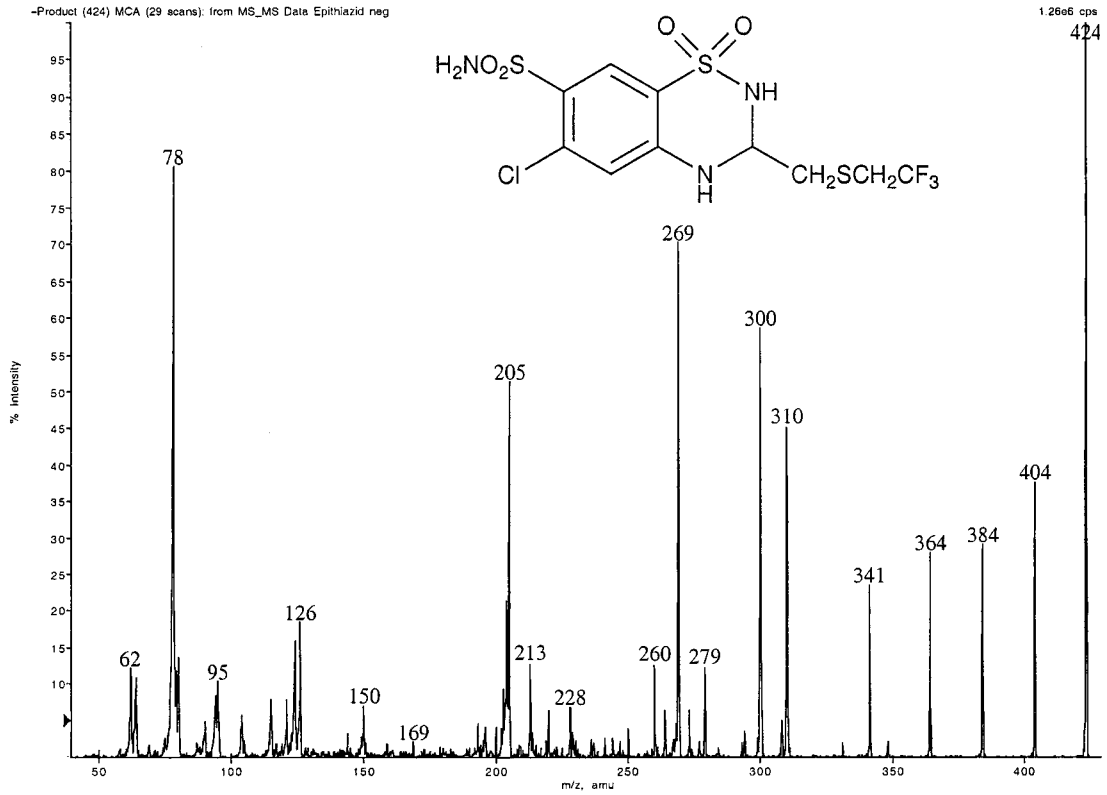


Figure 5. ESI product ion scan of *m*/*z* 424 of epithiazide.

tion studies.[25−29] In the case of althiazide, only the fission of the S−C-bond remote from the thiazide nucleus is observed, and not a neutral loss of the side chain including the sulfur atom. This may be explained by the formation of a leaving group, which is able to stabilize the generated radical by an allylic double bond. In contrast, the mass spectrum of methyl thioethiazide does not contain an ion resulting from the elimination of a methyl radical, which would correspond to the homologous fragmentation described above. Here, only the neutral loss of methylene sulfide from $(M - H)^-$ is found (−48 Da, Table 3), generating *m*/*z* 322.

The presence of a trifluoroethyl group in the side chain of epithiazide and polythiazide intensively influences the fragmentation process. Besides the ions resulting from eliminations including or excluding the sulfur atom, the multiple loss of HF (−20 Da) is observed from $(M - H)^-$ producing *m*/*z* 404, 384, and 364, respectively (Figure 5). After removal of three HF molecules, the subsequent elimination of SO_2 generates *m*/*z* 300. The hydrogens expelled from the thiazides as HF obviously do not originate from the nitrogens N-2 and N-4 since the product ion spectrum of epithiazide after exchange of nitrogen-bonded hydrogens by deuteriums still presents the loss of three HF, but no DF molecules.

CONCLUSION

The collision-induced dissociation of thiazides after electrospray ionization in the negative ion mode gives rise to mass spectra that contain common and individual fragment ions, the generation of which is dominantly influenced by the N-4-linked side chain. This consists of *n*-, iso-, or cyclic alkyl groups, phenolic groups, mono-, bis-, or trischlorinated methyl groups, or thioether chains. The alkylated thiazides mainly generate the common fragments *m*/*z* 269, 205, and 126 besides their quasimolecular ion, except those bearing a 3−4-double bond in the thiazide nucleus. The exchange of the C-6-linked chlorine atom by a trifluoromethyl group results in a shift of 34 mass units to the fragments *m*/*z* 303, 239, and 160, respectively, in accordance to the Biemann shift rule. The introduction of double bonds or heteroatoms such as sulfur or halogen atoms into the side chain generates fragmentation patterns that show individual ions, especially those originating from a homologous fission of linkages from an even-electron ion or those generated by the elimination of hydrogen halides.

The possibility of differentiation of thiazide-based compounds by mass spectrometry enables the characterization and classification of compounds belonging to this class of drugs. The individual and diagnostic fragment ions support the identification of substances, e.g., in biological matrixes of doping control samples, and the knowledge of fragmentation behavior should facilitate the identification of derivatives of these particular remedies.

(25) Gioacchini, A. M.; Czarnocki, Z.; Arazny, Z.; Munari, I.; Traldi, P. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1592−1599.
(26) Smyth, W. F.; McClean, S.; Ramachandran, V. N. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2061−2069.
(27) Domingues, M. R. M.; Domingues, P.; Gajewski, M.; Czuchajowski, L.; Ferrer-Correia, A. J. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 908−914.
(28) Justesen, U. *J. Mass Spectrom.* **2001**, *36*, 169−178.
(29) Qin, X. Z. *J. Mass Spectrom.* **2001**, *36*, 911−917.

ACKNOWLEDGMENT

We thank Simon Biddle from HFL, UK, for supplying different reference compounds and the Bundesinstitut für Sportwissenschaft, Bonn, for financial support.

Received for review January 9, 2002. Accepted April 5, 2002.

AC020020E