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PREPARATION OF TRIMETHYLSILYL DERIVATIVES OF THIAMPHENICOL

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SUMMARY

The silylation of thiamphenicol has been investigated. Treatment of thiamphenicol with hexamethyldisilazane or N-trimethylsilylimidazole, either alone or in the presence of trimethylchlorosilane, in acetonitrile or pyridine yields the bis(trimethylsilyl) (TMS) ether derivative. These procedures, however, cause to some extent the formation of 1-(*p*-methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol. Silylation with N,O-bis(trimethylsilyl)acetamide gives, depending on the solvent used, the mono-, bis- and tris-TMS derivatives.

INTRODUCTION

The determination of thiamphenicol by the gas chromatography (GC) of its trimethylsilyl (TMS) derivative is more specific than the UV spectrophotometric assay. Several workers have reported the preparation of the TMS derivative of thiamphenicol with hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) in pyridine[1–3] and the reaction product has been considered by some workers to be the bis-TMS ether[1] and by others to be the N,O,O-tris-TMS derivative[4]. Recently, Nakagawa *et al.*[5] silylated thiamphenicol with N,O-bis(trimethylsilyl)acetamide (BSA) in acetonitrile and identified the reaction product as the bis-TMS ether. As opposed to our results[6], Nakagawa *et al.*[5] also reported that the silylation of chloramphenicol with BSA in acetonitrile produces the bis-TMS ether exclusively. Previously the application of this procedure to the gas chromatographic determination of chloramphenicol as bis-TMS ether had been advocated by Margosis[7,8]. However, we observed the formation of a mixture of the bis- and tris-TMS derivatives of chloramphenicol under these conditions[6]. Further, we ascertained that the silylation of chloramphenicol with HMDS and TMCS in pyridine affords only the bis-TMS ether[6]. In view of these diverging results, we checked the silylation of thiamphenicol by these procedures.

EXPERIMENTAL

Apparatus

GC analyses were performed on a Pye Series 104 chromatograph, equipped

with a flame-ionization detector. Coiled glass columns, 5 ft. × 4 mm I.D., packed with 3% QF-1, 3% OV-1 or 3% OV-17 on Gas-Chrom Q (100–120 mesh) (Applied Science Labs., State College, Pa., U.S.A.) were used. The carrier gas was helium or nitrogen at a flow-rate of 60 ml/min.

The identification of the TMS derivatives was performed by combined gas chromatography and mass spectrometry (GC–MS). The mass spectra were recorded on a single-focusing AEI MS-12 mass spectrometer, operated at accelerating voltage 8 kV, trap current 100 μA and ionization energy 70 eV. A 1:2 stream splitter divided the column effluent between the flame-ionization detector and the mass spectrometer. A membrane separator (Varian, Type V5620) allowed the eluted substances to flow into the ion source. The temperature of the separator and ion source was maintained 30–40° above the column temperature.

Reagents

The silylating reagents BSA, TMCS, HMDS, N-trimethylsilylimidazole (TSIM), Tri-sil (a solution of HMDS and TMCS in pyridine) and Tri-sil Z (a solution of TSIM in pyridine) were purchased from Pierce (Rockford, Ill., U.S.A.). N-Methyl-N-trimethylsilyltrifluoroacetamide was obtained from Macherey, Nagel & Co. (Düren, G.F.R.). The solvents were of reagent grade, and were obtained from Union Chimique Belge (Drogenbos, Belgium) (acetonitrile), Merck (Darmstadt, G.F.R.) (pyridine, ethyl acetate) and Pierce (acetonitrile). Thiamphenicol was obtained from Zambon (Milan, Italy). The DL-*erythro* isomer of thiamphenicol and 1-(*p*-methylsulphinylphenyl)-2-dichloroacetamido-1,3-propanediol were supplied by Prof. D. Della Bella (Zambon). Chloramphenicol was obtained from Lepetit (Milan, Italy). D-*threo*-1-(*p*-Methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol and D-*threo*-1-(*p*-nitrophenyl)-2-monochloroacetamido-1,3-propanediol were prepared by acylation of the corresponding amines with monochloroacetyl chloride[9]. Methyl phenyl sulphone was prepared from thioanisole[10].

Procedure

The compounds (5–7 mg) were dissolved in 0.5 ml of a mixture of either 1 ml of BSA, HMDS, TMCS or TSIM in 9 ml of solvent or 1 ml of HMDS or TSIM in 8.5 ml of solvent containing 0.5 ml of TMCS, or in 1 ml of Tri-sil or Tri-sil Z. The solutions in acetonitrile and pyridine were kept at room temperature; with ethyl acetate, a solution was obtained only after stirring and heating at 70° for 20 min for thiamphenicol and about 60 min for the *erythro* isomer. A volume of 1 μl or less was injected directly into the gas chromatograph.

RESULTS AND DISCUSSION

Silylation

A gas chromatogram on OV-17 of the reaction mixture of thiamphenicol with BSA in acetonitrile or pyridine, kept at room temperature, shows two peaks between which the baseline is not reached (Fig. 1). Combined GC–MS identified the first compound to emerge as the tris-TMS derivative of thiamphenicol, the two hydroxyl groups and the amide function being silylated, and established the identity of the second compound as the bis-TMS ether. A similar chromatogram was obtained on QF-1,

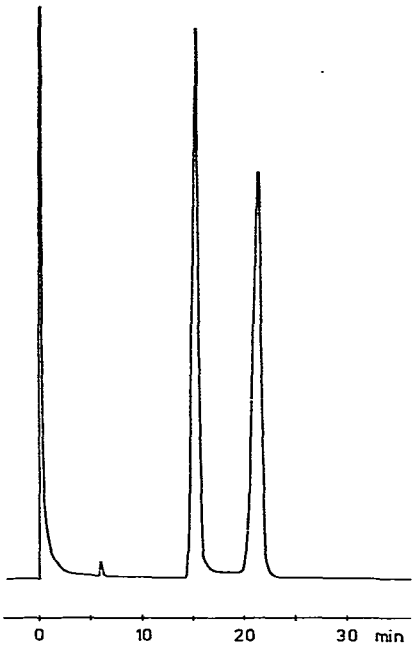


Fig. 1. Gas chromatogram of the silylation mixture of thiamphenicol with BSA in acetonitrile; 5-ft. 3% OV-17 column, 221°, nitrogen as carrier gas at a flow-rate of 60 ml/min.

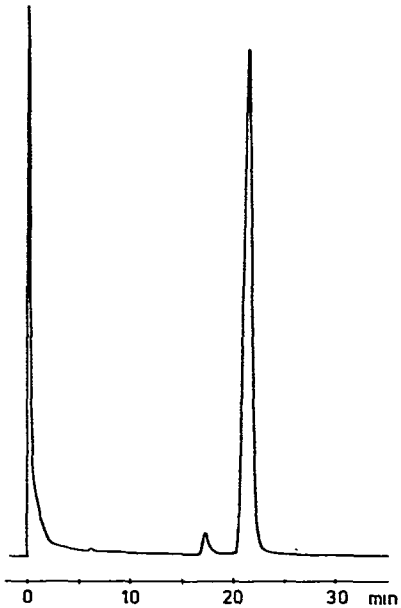


Fig. 2. Gas chromatogram of the silylation mixture of thiamphenicol with HMDS and TMCS in pyridine; 5-ft. 3% OV-17 column, 221°, nitrogen as carrier gas at a flow-rate of 60 ml/min.

although an unstable baseline prior to elution of the tris-TMS derivative indicates the presence of some decomposition products. There is no separation of these derivatives on OV-1. The retention times on these phases are given in Table I. These chromatograms exhibit in addition a small extraneous peak (retention time 6.2 min at 218.5° on a 5-ft. 3% OV-17 column and 8.7 min at 200.5° on a 5-ft. 3% OV-1 column with helium as carrier gas at a flow-rate of 60 ml/min), which could not be identified (see below). The amount of the tris-TMS derivative of thiamphenicol increases with reaction time to a roughly constant value. This value, however, varies between 15 and 50%.

The results of a typical experiment are indicated in Table II. The formation of the tris-TMS derivative of thiamphenicol by this procedure was not observed by Nakagawa *et al.*[5]. As the tris-TMS derivative of thiamphenicol is contaminated with the bis-TMS ether, the variation of the amount of tris-TMS derivative formed and its absence in the cited case[5] may be caused by rapid hydrolysis to the bis-TMS ether during manipulation or chromatography.

The silylation of the *erythro* isomer of thiamphenicol with BSA proceeds in the same way (Tables I and II). Our sample of *erythro* isomer contained a minor impurity, which was identified by GC–MS as 1-(*p*-methylsulphinylphenyl)-2-dichloroacetamido-1,3-propanediol and is present as bis- and tris-TMS derivative in this silylation mixture. This compound was also detected by thin-layer chromatography[9], and probably results from incomplete oxidation of the methylthio intermediate, as the *erythro* isomer is prepared by the same process as the *threo* compound[11,12].

TABLE I

RETENTION TIMES (min) OF THE TMS DERIVATIVES OF THIAMPHENICOL, THE *erythro* ISOMER AND SOME RELATED COMPOUNDS
Column: 5 ft. × 4 mm I.D. Carrier gas: helium, flow-rate 60 ml/min.

Compound	*Derivative*	*Stationary phase*			*Silylating reagent*
		3% QF-1	*3% OV-1*	*3% OV-17*	
Thiamphenicol	Tris-TMS	22.5 (218°)		15.4 (218.5°)	BSA–CH_3CN
	Bis-TMS	44.9 (218°)	31.4 (200.5°)*		BSA–CH_3CN
		33.8 (223°)	30.2 (200.5°)	21.6 (218.5°)	Tri-sil
				9.4 (237°)	BSA–EtOAc
	Mono-TMS		42.1 (200.5°)*	23.9 (237°)*	BSA–EtOAc
erythro-Thiamphenicol	Tris-TMS	21.7 (218°)	29.6 (200.5°)**	14.2 (218.5°)	BSA–CH_3CN
	Bis-TMS	45.1 (218°)	32.5 (200.5°)**		BSA–CH_3CN
		34.1 (223°)	31.7 (200.5°)	23.9 (218.5°)	Tri-sil
				10.0 (237°)	BSA–EtOAc
	Mono-TMS		37.6 (200.5°)*	21.5 (237°)*	BSA–EtOAc
1-(*p*-Methylsulphonylphenyl)-2-monochloro-acetamido-1,3-propanediol	*threo* isomer, bis-TMS	29.0 (223°)	23.1 (200.5°)	16.9 (218.5°)	Tri-sil
	erythro isomer, bis-TMS	28.0 (223°)	23.3 (200.5°)	18.1 (218.5°)	Tri-sil
1-(*p*-Methylsulphinylphenyl)-2-dichloro-acetamido-1,3-propanediol	*erythro* isomer, tris-TMS			12.4 (218.5°)**	BSA–CH_3CN
	erythro isomer, bis-TMS	23.0 (223°)	28.7 (200.5°)**	20.2 (218.5°)	Tri-sil

* Asymmetrical peak.
** Partially resolved peak.

TABLE II

AMOUNTS OF THE BIS- AND TRIS-TMS DERIVATIVES OF THIAMPHENICOL AND THE *erythro* ISOMER AFTER DIFFERENT REACTION TIMES UPON SILYLATION WITH BSA

The figures indicate the area of the peak in question, expressed as a percentage of the total area of the bis- and tris-TMS derivative peaks of a chromatogram on 3% OV-17 at 218.5°.

Compound	*Solvent*	*TMS derivative*	*Time (h)*				
			1/4	*1*	*3*	*6*	*24*
Thiamphenicol	Acetonitrile	Bis	99	94	76	69	74
		Tris	1	6	24	31	26
	Pyridine	Bis	100	96	87	82	76
		Tris	—	4	13	18	24
erythro-Thiamphenicol	Acetonitrile	Bis	96	81	60	43	50
		Tris	4	19	40	57	50

The silylation of thiamphenicol with BSA in ethyl acetate was also investigated. Because of its low solubility in this solvent, the mixture was heated at 70° and dissolution occurred after 20 min. Chromatograms of this mixture on OV-1 and OV-17 show, behind the important bis-TMS ether peak, large poorly resolved peaks, probably representing both mono-TMS ethers of thiamphenicol and, as implied from GC–MS data, some of their decomposition products. Longer heating of this mixture reduces the amount of the mono-TMS ethers and, after 3–4 h, the mixture contains only the bis-TMS ether. Keeping this solution for a further 24 h at room temperature yields only a very small amount of the tris-TMS derivative.

The mono- and bis-TMS ethers are also obtained on treatment of the *erythro* isomer of thiamphenicol with BSA in ethyl acetate. Heating of this mixture for about 4 h yields only the bis-TMS ether.

The silylation of thiamphenicol with N-methyl-N-TMS-trifluoroacetamide in acetonitrile at room temperature similarly yields a mixture of the bis- and tris-TMS derivatives.

These results correspond to those observed for the silylation of chloramphenicol and *erythro* isomer with BSA[6]. Using acetonitrile or pyridine as solvent, a mixture of the bis- and tris-TMS derivatives is obtained and in ethyl acetate the mono- and bis-TMS derivatives are formed.

The silylation of chloramphenicol with a mixture of HMDS and TMCS in pyridine gives only the bis-TMS ether[6]. GC–MS indicated that silylation of thiamphenicol with that reagent also yields the bis-TMS ether. However, gas chromatograms of this mixture, obtained on OV-1, OV-17 or QF-1 as the stationary phase, show a small extraneous peak (Fig. 2). This peak occurs on silylation with different batches of the reagent, which was the commercial, prepared Tri-sil solution from Pierce. Its area corresponds to about 5% of that of the bis-TMS ether of thiamphenicol and does not increase upon standing for 24 h. A mass spectrum and the retention time identified the compound as the bis-TMS ether of *threo*-1-(*p*-methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol.

The separation of thiamphenicol and its monochloro analogue is readily accomplished by thin-layer chromatography on silica gel using ethyl acetate or ethyl acetate containing a few per cent of methanol as the mobile phase[9]. However, no monochloro compound could be detected on a thin-layer chromatogram of even

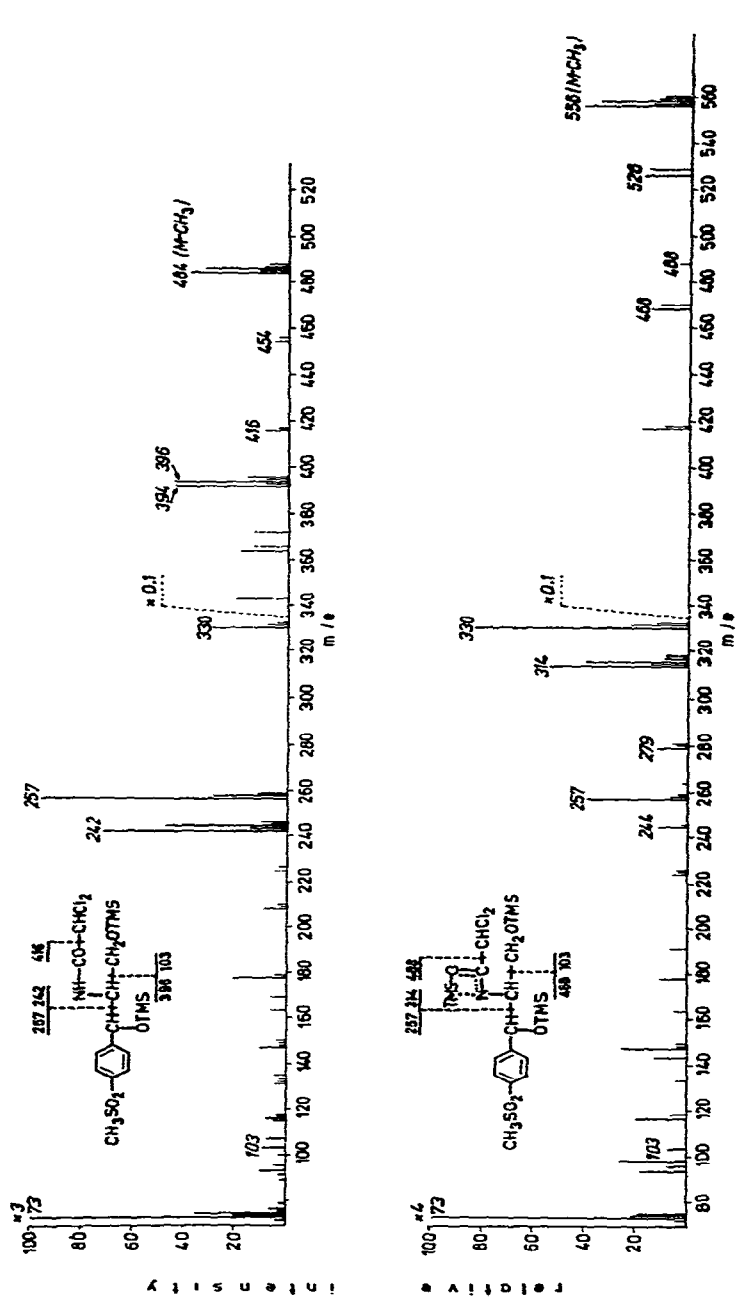


Fig. 3. Mass spectra of bis-TMS-thiamphenicol (above) and tris-TMS-thiamphenicol (below).

250 μg of our sample of thiamphenicol, although 1–2 μg of the former can be detected. This result indicates that this compound is formed during silylation or subsequent chromatography.

As GC of the silylation mixture of chloramphenicol with this reagent on the same chromatographic columns does not reveal the presence of the corresponding monochloro compound, the methylsulphone group in thiamphenicol could be responsible for this artifact. However, the addition of an amount of methyl phenyl sulphone equal to that of thiamphenicol does not increase the extent of dechlorination. Similarly, the addition of neither methyl phenyl sulphone nor thiamphenicol induces the dechlorination of chloramphenicol by Tri-sil.

When, however, the same Tri-sil silylation mixture of thiamphenicol was injected under the same conditions on different columns of OV-17, some of the chromatograms obtained show as much as 5% and others only trace amounts of the monochloro compound. This result indicates that the dechlorination is induced on the column packing. It occurs on both used and newly packed and conditioned columns, even after silanization.

The influence of the silylating reagent on the extent of dechlorination was checked by silylation with HMDS and TMCS in acetonitrile and with TSIM, either alone or in the presence of TMCS, in pyridine or acetonitrile. Only the TSIM–pyridine silylation mixture gives no or only trace amounts of the monochloro analogue, but all other mixtures exhibit some dechlorination, the extent of which seems to be slightly increased by the presence of TMCS. The influence of TMCS on the dechlorination was checked by treatment of thiamphenicol with this reagent in pyridine. However, no silylation takes place, even after heating the mixture at 60° for 20 h. Removal of solvent and reagent and silylation of the residue with TSIM in pyridine yields only the bis-TMS ether. Similar results were obtained upon silylation of the *erythro* isomer of thiamphenicol with these reagents. Gas chromatograms of these silylation mixtures sometimes show the same extraneous peak as that present in the BSA reaction mixtures.

Careful examination of the chromatograms of the silylation mixtures of chloramphenicol with these reagents occasionally showed the presence of a trace compound with the same retention time as the monochloroacetamido analogue.

All of these silylations are rapid and are completed in 15 min at room temperature as no mono-TMS ether is present at that moment. The retention times on QF-1, OV-1 and OV-17 as stationary phases are given in Table I. No isomerization of the *threo* to the *erythro* isomer and *vice versa* occurs on keeping the reaction mixture at room temperature for 24 h. Occasionally, after standing for this period a solution showed small secondary peaks.

The silylation of thiamphenicol with HMDS in acetonitrile or pyridine at room temperature proceeds very slowly: complete conversion to the bis-TMS ether is achieved only after about 6 and 30 h, respectively; a very small amount of the monochloro analogue is also formed.

Mass spectrometry

The structures of the TMS derivatives were determined by GC–MS. The mass spectra of the bis-TMS ethers of 1-(*p*-methylsulphonylphenyl)-2-monochloroacetamido- and 1-(*p*-methylsulphinylphenyl)-2-dichloroacetamido-1,3-propanediol were

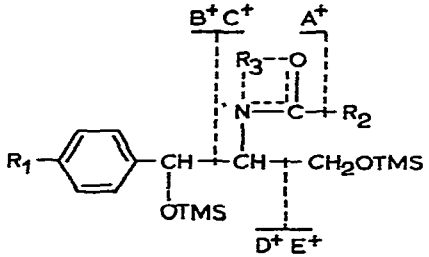
compared with those of authentic samples, which were silylated with a mixture of HMDS and TMCS in pyridine.

The mass spectrum of the bis-TMS ether of thiamphenicol is shown in Fig. 3. No molecular ion is observed, but an intense $M-CH_3$ ion at *m/e* 484, which is characteristic of TMS derivatives, and a weak $M-CHCl_2$ ion at *m/e* 416 clearly determine the molecular weight. Elimination of trimethylsilanol from the *m/e* 484 fragment ion produces the *m/e* 394 ion. A broad metastable peak at *m/e* 322, due to the coalescence of two metastable peaks at *m/e* $394^2/484 = 320.7$ and *m/e* $396^2/486 = 322.7$ for each of the two chlorine isotope-containing fragments confirm the elimination. A peak at *m/e* 454 probably corresponds to expulsion of formaldehyde from the *m/e* 484 fragment. Benzylic cleavage with charge retention on either fragment results in intense fragments at *m/e* 242 and 257. The abundance of the *m/e* 258 ion indicates that this cleavage is accompanied to some extent by the rearrangement of one hydrogen atom. Similarly, the important *m/e* 330 ion is probably formed by benzylic cleavage and rearrangement of a TMS group. Weak fragment ions at *m/e* 103 and 396, or at least a part of the latter ion, are formed by cleavage of the 2–3 bond. The base peak at *m/e* 73, representing the TMS cation, is derived, at least partially, from the *m/e* 257 and 103 ions, as shown by corresponding metastable peaks at *m/e* 20.7 (calculated, $73^2/257 = 20.7$) and at *m/e* 51.7 (calculated, $73^2/103 = 51.7$), respectively. The fragmentation pattern is characteristic of the other TMS derivatives analyzed

TABLE III

SIGNIFICANT FRAGMENTS IN THE MASS SPECTRA OF THE TMS DERIVATIVES OF THIAMPHENICOL, THE *erythro* ISOMER AND SOME RELATED COMPOUNDS

Each entry gives the *m/e* value with the corresponding relative intensity for each diastereoisomer immediately below. For each compound the base peak occurs at *m/e* 73 (relative intensity 100).



R_1	R_2	R_3	$(M-CH_3)^+$	$(M-CH_3-TMSOH)^+$	$(M-CH_3-CH_2O)^+$
CH_3SO_2	$CHCl_2$	H	484/486	394/396	454/456
	threo		1.3/1.1	1.5/1.5*	0.18/0.14
	erythro		1.5/1.1	1.8/1.4*	0.31/0.19
CH_3SO_2	$CHCl_2$	TMS	556/558	—	526/528
	*threo***		1.05/0.89	—	0.45/0.40
	*erythro***		0.42/0.37	—	0.45/0.40
CH_3SO_2	CH_2Cl	H	450/452	360/362	420
	threo		2.5/1.1	1.9/1.7*	trace
	erythro		2.7/1.8	2.7/1.2*	trace
CH_3SO	$CHCl_2$	H	468/470	378/380	438/440
	erythro		0.93/0.73	0.59/0.50	0.42/0.33

* Composite peak.

** Composite peak: isotope peak of B^+ and $(B+H)^{+\cdot}$.

*** These values are corrected for the presence of the bis-TMS ether.

during the course of this work. Table III is a compilation of the peaks in the mass spectra of these TMS derivatives. Each entry in the table gives the *m/e* value with the corresponding relative intensity for each diastereoisomer immediately below. In each mass spectrum the base peak occurs at *m/e* 73.

The tris-TMS derivatives of thiamphenicol (Fig. 3) and the *erythro* isomer do not eliminate trimethylsilanol from the $M-CH_3$ ion, but they show, in addition to the fragmentation discussed above, successive losses of a chlorine atom from the *m/e* 314 ion, yielding ions of moderate intensity at *m/e* 279 and 244. A metastable peak at *m/e* 213.4 is associated with the latter transition (calculated, $244^2/279 = 213.4$). As a result of their decomposition, the mass spectra of the tris-TMS derivatives were contaminated with peaks originating from fragmentation of the bis-TMS ethers.

Unlike the bis-TMS ether of thiamphenicol, benzylic cleavage of *erythro*-1-(*p*-methylsulphinylphenyl)-2-dichloroacetamido-1,3-propanediol proceeds almost exclusively with charge retention on the aromatic moiety, *m/e* 241, and is accompanied to some extent by the rearrangement of one hydrogen atom, *m/e* 242. The abundance of the *m/e* 244 ion indicates that, if any, only a small amount of the *m/e* 242 ion arises from α-fission of the 1–2 bond, triggered by removal of an electron from the amide-nitrogen. A metastable peak at *m/e* 209.2 (calculated, $225^2/242 = 209.2$) establishes the loss of a hydroxyl group from the aromatic *m/e* 242 ion to the very intense *m/e* 225 ion.

A^+	B^+	$(B + TMS)^{+\cdot}$	C^+	D^+	E^+	$(C - Cl)^{+\cdot}$	$(C - 2Cl)^+$	$(B + H - OH)^+$
416	257/258	330	242/244	396/398	103	—	—	—
0.31	32/9.5**	10	24/16	1.5*/0.55	2.7	—	—	—
0.30	34/10**	11	26/18	1.4*/0.43	2.8	—	—	—
488	257/258	330	314/316	468/470	103	279/281	244	—
0.10	9.9/1.7	21	13.5/10	0.38/0.29	1.8	3.1/1.5	2.9	—
0.11	8.9/1.5	19	13/8.7	0.27/0.20	2.8	3.0/2.1	2.3	—
416	257/258	330	208/210	362/364	103	—	—	—
trace	19/4.7**	8.5	71/27	1.7*/0.39	3.0	—	—	—
—	23/5.4**	8.5	65/23	1.2*/0.27	2.9	—	—	—
400	241/242	—	242/244	—	103	—	—	225
0.22	42/11*	—	11*/1.1	—	3.8	—	—	76

Owing to simultaneous elution of other products, no clear mass spectrum could be obtained of the tris-TMS sulphoxide, but its formation was established by the presence of the $M-CH_3$ ion at *m/e* 540 and the important fragment ions at *m/e* 241 and 225.

A low-intensity mass spectrum was obtained from the small extraneous peak in the BSA silylation mixture of thiamphenicol or the *erythro* isomer. The absence of any chlorine-containing fragment ion is noteworthy. Significant peaks occur at *m/e* (relative intensity) 384 (2.3), 354 (4.4), 285 (3.9), 268 (3.2), 257 (49), 215 (4.9), 176 (4.4), 103 (4.9), 84 (6.3) and 73 (100). However, with these data, no plausible structure could be assigned to this compound.

The mass spectra of the mixture of both mono-TMS ethers of thiamphenicol or of the *erythro* isomer show the characteristic $M - CH_3$ ion at *m/e* 412. In analogy with the bis-TMS ether, the *m/e* 382 fragment is probably formed by elision of formaldehyde from the *m/e* 412 fragment. Silylation of the C-3 hydroxyl group is implied from the presence of the *m/e* 242 fragment ion, whereas a fragment at *m/e* 153, also containing two chlorine atoms, and the *m/e* 257 ion establish silylation of the C-1 hydroxyl group. The *m/e* 153 ion is derived from benzylic cleavage with simultaneous loss of the hydroxyl group and is the base peak in the mass spectrum of thiamphenicol. The absence of the *m/e* 330 ion and of the *m/e* 147 pentamethyldisiloxonium ion, characteristic of compounds that contain more than one TMS group, further prove silylation of only one hydroxyl group[13].

CONCLUSION

As the silylation of thiamphenicol with TSIM in pyridine usually yields one derivative, this procedure can be used in the GC determination. Because silylation with BSA affords, depending on the solvents used, a mixture of the mono-, bis- and tris-TMS derivatives, this reagent is not suitable for such determinations.

As opposed to previous observations[4], treatment of thiamphenicol with HMDS and TMCS in pyridine does not yield the N,O,O-tris-TMS derivative but rather the bis-TMS ether derivative. As silylation of thiamphenicol with HMDS and TMCS in pyridine is attended to some extent by dechlorination, the significance of the detection of 1-(*p*-methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol as a metabolite[4] can be questioned.

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