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# PREPARATION OF TRIMETHYLSILYL DERIVATIVES OF CHLORAMPHENICOL FOR GAS–LIQUID CHROMATOGRAPHY

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## SUMMARY

Treatment of chloramphenicol or its *erythro* isomer with N,O-bis(trimethylsilyl)-acetamide gives, depending on the solvents used, the mono-, bis- and tris(trimethylsilyl) derivative. Silylation with a mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine yields only the bis(trimethylsilyl)ether.

## INTRODUCTION

Gas–liquid chromatography (GLC) of chloramphenicol appears to be an excellent method of analysis of this antibiotic, provided that a suitable derivative is used. In several instances, the silylation method of Bentley *et al.*[1], involving the use of hexamethyldisilazane and trimethylchlorosilane in pyridine, was applied[2–4] and recently the use of N,O-bis(trimethylsilyl)acetamide (BSA) as silylating reagent in acetonitrile has been described[5].

However, when the BSA method was used in this laboratory, the application of the reaction mixture on to an OV-1 column showed a chromatographic peak with a slight inflection (Fig. 1). On lowering the column temperature, this peak was split into two partially resolved peaks. When BSA, acetonitrile and chloramphenicol samples of different origin were used and even on substitution of N,O-bis(trimethylsilyl)trifluoroacetamide[6] (BSTFA) for BSA, this phenomenon persisted. On the other hand, under the same chromatographic conditions, the trimethylsilyl (TMS) derivative of chloramphenicol prepared by the method of Bentley *et al.*[1] exhibited a single symmetrical peak.

Similarly, silylation of the *erythro* isomer of chloramphenicol with BSA or BSTFA in acetonitrile afforded two partially resolved peaks, whereas with the procedure of Bentley *et al.*[1] the chromatogram showed one symmetrical peak. Furthermore, for both isomers, the relative heights of the two peaks changed with reaction time and a rough estimation of their retention times suggested an isomerization of the *threo* to the *erythro* isomer and *vice versa*.

The unexpected behaviour of the silylation of chloramphenicol and its *erythro* isomer with BSA or BSTFA prompted us to determine the structure of the products formed.

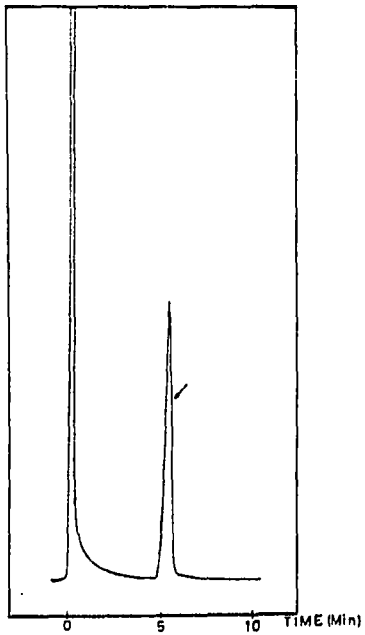



Fig. 1. Gas chromatogram of the silylation mixture of chloramphenicol with BSA in acetonitrile; 5 ft., 3% OV-1, 205°, helium carrier gas.

## EXPERIMENTAL

### *Apparatus*

Gas chromatographic (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 Laboratories) were used. The carrier gas was helium or nitrogen at a flow-rate of 60 ml/min.

The mass spectra were recorded on a single-focusing AEI-MS 12 mass spectrometer, operated at 8 kV accelerating voltage, 100 $\mu$A trap current and 70 eV ionization energy. The source temperature was 120° when using the direct insertion technique. For the combined gas chromatography–mass spectrometry, a 1:2 stream splitter divided the output of the column 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 silylation reagents BSA, BSTFA, and "Tri-sil", a solution of hexamethyldisilazane and trimethylchlorosilane in pyridine, were purchased from Pierce Chemical Co., U.S.A. BSA was also obtained from Merck, Darmstadt, G.F.R. The solvents were of reagent grade and were purchased from Union Chimique Belge and Merck, Darmstadt. Acetonitrile was obtained from Pierce Chemical Co. Chloramphenicol

samples were obtained from Lepetit, Italy, and from Mr. M. Margosis, F.D.A. Washington, D.C., U.S.A.

The *erythro* isomer of chloramphenicol was prepared from the D-*threo* isomer. After acetylation with acetyl chloride in pyridine of the primary hydroxyl group[7], the secondary hydroxyl group was oxidized with chromic oxide[8]. Reduction of the keto function with sodium borohydride[9] and selective O-deacylation[10] gave a mixture of D-*threo*- and D-*erythro*-chloramphenicol. Repeated recrystallizations of this mixture afforded the pure D-*erythro* isomer. It is possible that some of the D-ketone was racemized but the specific rotation of our *erythro*-chloramphenicol agrees well with the reported values[11,12]. Recently an elegant method for obtaining D-*threo*-1-deuteriochloramphenicol, together with the D-*erythro* isomer, was described, involving oxidation with N-bromosuccinimide and subsequent reduction with $Ca(BD_4)_2$[13].

*Chloramphenicol 3-acetate.* To a solution of 19.4 g of chloramphenicol in 180 ml of pyridine, 4.7 ml of acetyl chloride were added dropwise and the resulting mixture was kept at room temperature for 3 h. It was then poured into ice-water and the oily precipitate was extracted with ether. The ethereal solution was washed with dilute hydrochloric acid and water and then dried. After evaporation of the solvent, chloramphenicol acetate was obtained as an oil in almost quantitative yield. It could not be obtained in crystalline form (in ref. 7, a m.p. of 82–84° is reported), but showed only one spot on thin-layer chromatography (TLC) with ether as eluting solvent (pre-coated TLC plates of silica gel, with fluorescence indicator, 0.25 mm layer thickness; Merck, Darmstadt).

D-*2-(2,2-Dichloroacetamido)-3-acetoxy-4'-nitropropiophenone.* To a solution of 20 g of crude chloramphenicol 3-acetate in 100 ml of glacial acetic acid a solution of 5.47 g of $CrO_3$ in a mixture of 5.5 ml of water plus 50 ml of glacial acetic acid was added dropwise with stirring while the mixture was maintained at 20° by cooling. The solution was further stirred at room temperature for 2 h. After removal of the excess of $CrO_3$ with aqueous $NaHSO_3$, the mixture was poured into water and the oily precipitate was extracted with ether. The ethereal solution was washed successively with aqueous $NaHCO_3$ and water and then dried. Removal of the solvent left a crystalline residue, which was recrystallized from benzene–ligroin, yielding 9 g of the ketone, m.p. 78–80°; mass spectrum: (M+1) ion at *m/e* 363; (M–HOAc) ion at *m/e* 302. A m.p. of 124° is reported for the racemate[14].

D-*Erythro-chloramphenicol.* A solution of 1.1 g of $NaBH_4$ in 100 ml of absolute ethanol was added dropwise with stirring to a solution of 10 g of 2-(2,2-dichloroacetamido)-3-acetoxy-4'-nitropropiophenone in 100 ml of absolute ethanol, and the resulting solution was maintained at room temperature for 1 h. It was then acidified with dilute HCl and after evaporation of most of the ethanol under vacuum the precipitate was extracted with ether and the ethereal solution was washed with water and dried. Removal of the ether left 8 g of an oily residue, which was dissolved in 65 ml of acetone and the solution cooled to −15°. NaOH (1*N*, 21.9 ml) was added with stirring over 5–10 min and the reaction mixture was maintained at this temperature for 1 h and then neutralized with dilute HCl. Evaporation of acetone under vacuum left an oily residue, which was extracted with $CH_2Cl_2$. The organic layer was washed with water and dried, and removal of the solvent gave a solid residue. GC analysis of this mixture on 3% OV-17, after conversion to the bis-TMS derivative

with "Tri-sil", as described below, showed the presence of *erythro*- and *threo*-chloramphenicol in about equal amounts. Recrystallization from ethylene dichloride and water, successively, yielded 550 mg of pure D-*erythro*-chloramphenicol, m.p. 175–177°, $[\alpha]_D^{17} = -12.4$ ($c = 1$, in EtOH). The reported physical constants are m.p. 179–180°, $[\alpha]_D^{20} = -13.9$ (EtOH)[11], and m.p. 175–176°, $[\alpha]_D^{25} = -11.8$ (EtOH)[12]. The mother liquors, containing the *threo* isomer, were discarded.

*Procedure*

Chloramphenicol or *erythro*-chloramphenicol (5–7 mg) was dissolved in either 0.5 ml of a mixture of 1 ml of BSA plus 9 ml of the appropriate solvent or in 1 ml of "Tri-sil", and the resulting solution was maintained at room temperature. A volume of 1 $\mu$l or less was injected directly into the gas chromatograph.

## RESULTS AND DISCUSSION

The TMS derivative of chloramphenicol or its *erythro* isomer, prepared by the procedure of Bentley *et al.*[1], gave one symmetrical peak on a QF-1, OV-1 or OV-17 column. The retention times on these columns are given in Table I. In contrast to the previously observed complete separation of both isomers on a QF-1 column[15], a mixture of the isomers appeared as one peak on this column, whereas the chromatogram on the OV-1 and OV-17 columns showed two partially resolved peaks. The silylation reaction is fast, as no mono-TMS derivative was present after reaction for

TABLE I

RETENTION TIMES (min) OF THE TMS DERIVATIVES OF CHLORAMPHENICOL AND ITS *erythro* ISOMER

Carrier gas: helium.

| *Derivative* | *Column* | | |
|---|---|---|---|
| | *5 ft., 3% QF-1* | *5ft., 3% OV-1* | *5ft., 3% OV-17* |
| *Chloramphenicol* | | | |
| Bis-TMS | 22.6/203°** | 19.7/193°** | 17.2/196°** |
| | | 27.4/181°**** | 6.3/217°**** |
| Tris-TMS | 11.0/203°*** | | 11.3/196°*** |
| Mono-TMS C-1 | | 35.9/181°*** | 14.5/217°**** |
| Mono-TMS C-3 | | | 16.7/217°**** |
| *Erythro-chloramphenicol* | | | |
| Bis-TMS | 22.5/203°** | 20.5/193°** | 18.8/196°** |
| | | 38.3/181°**** | 7.7/217°**** |
| Tris-TMS | 10.6/203°*** | | 11.4/197°*** |
| Mono-TMS | | 43.0/181°**** | 17.1/217°****§ |

* Silylation performed with "Tri-sil".
** Silylation performed with BSA in acetonitrile.
*** Silylation performed with BSA in ethyl acetate.
§ Asymmetrical peak.

15 min at room temperature. As opposed to previous observations[1,5], only one symmetrical peak could be observed on an OV-17 column after keeping the reaction mixture at room temperature for 24 h. Even the addition of a small amount of methanol to the silylation mixture, which should accelerate the isomerization[15], did not yield another peak.

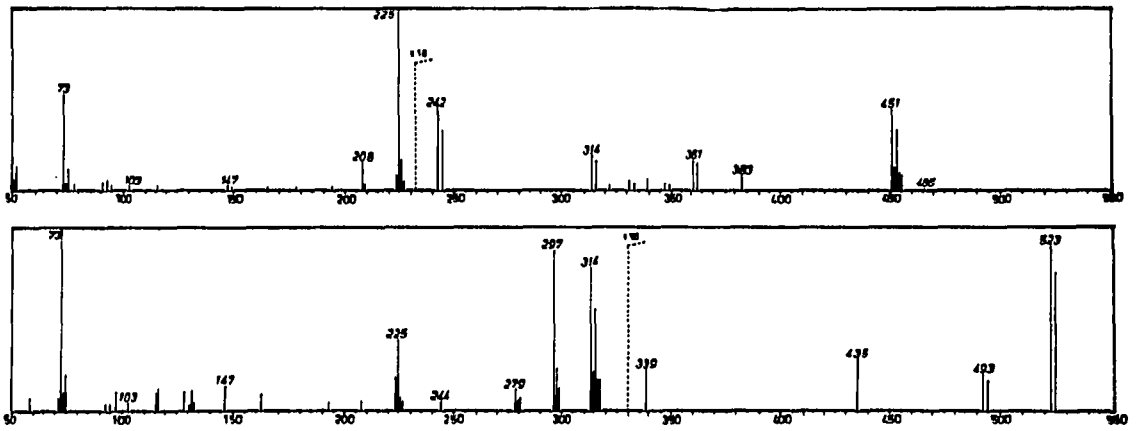
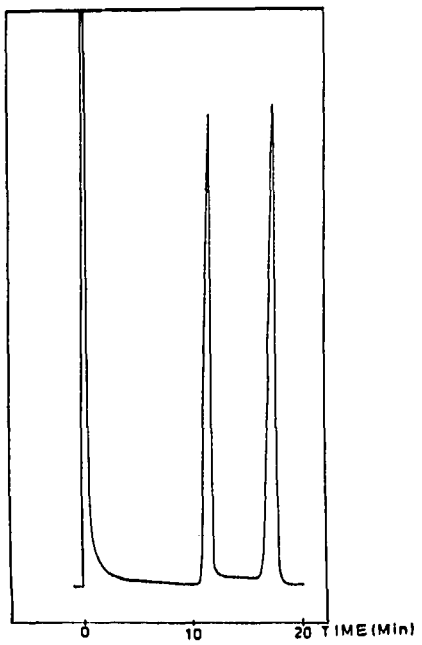


Fig. 2. Mass spectra of bis-TMS-chloramphenicol (above) and tris-TMS-chloramphenicol (below).

The mass spectrum of the TMS derivative of chloramphenicol (Fig. 2), prepared by this method, shows that the bis-TMS ether was obtained. The molecular ion is very weak but an important ($M-CH_3$) ion at *m/e* 451, characteristic of TMS derivatives, is present. Loss of the dichloromethyl radical from the molecular ion gives a weak fragment at *m/e* 383. Elimination of trimethylsilanol from the *m/e* 451 ion produces the *m/e* 361 ion. A broad metastable peak at *m/e* 290, due to the coalescence of two metastable peaks at *m/e* $361^2/451 = 289.0$ and *m/e* $363^2/453 = 290.9$ for each of the two chlorine isotopes, is associated with this elimination. The base peak at *m/e* 225 and important fragment ions at *m/e* 224 and *m/e* 242 are formed, with simultaneous gain of one hydrogen for the *m/e* 225 ion, by cleavage between C-1 and C-2. A metastable peak at *m/e* 192.3 (calculated, $208^2/225 = 192.3$) demonstrates the loss of a hydroxyl group from the *m/e* 225 ion with the production of the intense *m/e* 208 ion. Cleavage between C-2 and C-3 produces a fragment ion at *m/e* 103. The abundant *m/e* 73 ion originates from the *m/e* 225 and *m/e* 103 ions, as shown by corresponding metastable peaks at *m/e* 23.7 (calculated, $73^2/225 = 23.7$) and at *m/e* 51.7 (calculated, $73^2/103 = 51.7$), respectively. It is interesting to note the different fragmentation patterns of *p*-nitrobenzyl TMS ether[16] (molecular ion at *m/e* 225) and the *m/e* 225 ion in the mass spectrum of chloramphenicol bis-TMS ether. This may be caused by the fact that the ions have different structures. The mass spectrum of the bis-TMS ether of the *erythro* isomer of chloramphenicol, prepared by the method of Bentley *et al.*[1], is similar to that of the bis-TMS derivative of chloramphenicol. It shows only minor differences in the relative abundance of certain fragment ions.

The use of BSA as a silylating reagent gave different results according to the solvents used. For chloramphenicol, the results can be summarized as follows:

GLC on an OV-17 column showed that after standing for 15 min at room temperature, the silylation in acetonitrile or pyridine gave two distinct peaks (at 11.3 and 17.2 min; 196°), although the baseline was not reached between these peaks, indicating decomposition of the first emerging compound into the second compound during GLC (Fig. 3). The composition of the reaction mixture changed with time: in aceto-



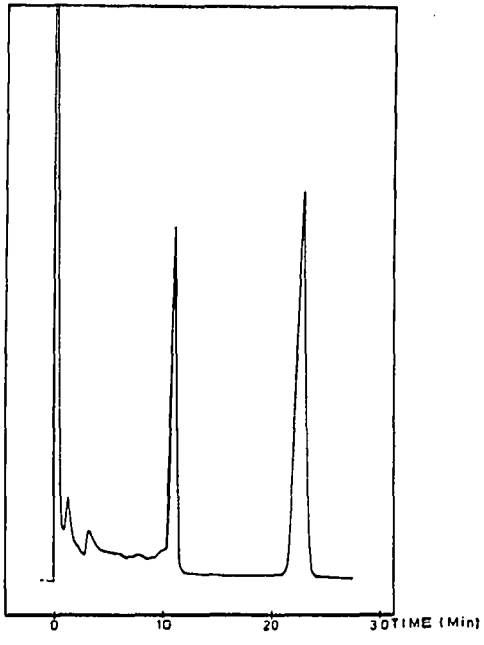




Fig. 3. Gas chromatogram of the silylation mixture of chloramphenicol with BSA in acetonitrile after 3 h; 5 ft., 3% OV-17, 196°, helium carrier gas.

Fig. 4. Gas chromatogram of the silylation mixture of chloramphenicol with BSA in acetonitrile after 60 min; 5 ft., 3% QF-1, 203°, helium carrier gas.

nitrile, the area of the first peak amounted to 6% of the total area after 15 min and this value increased to a constant value of approximately 45% after 6 h (Table II). When this reaction mixture was chromatographed on a QF-1 column, a similar chromatogram was obtained (peaks at 11 min and 22.6 min; 203°) (Fig. 4).

Combined GLC and mass spectrometry was used to determine the structure of these products. A mass spectrum of the 11.3-min peak of a chromatogram on OV-17 identified the product as the tris-TMS derivative of chloramphenicol, the third TMS group being located at the amide function. No molecular ion is present but an ($M-CH_3$) ion at *m/e* 523 and a weak ($M-CHCl_2$) ion at *m/e* 455 indicate the molecular weight. The *m/e* 455 ion decomposes further by elimination of trimethylsilanol (*m/e* 365). As for chloramphenicol, scission between C-1 and C-2 gives important fragment ions at *m/e* 314 and, with simultaneous gain of a TMS group, at *m/e* 297. Loss of chlorine from the *m/e* 314 ion produces a fragment ion at *m/e* 279 and, as affirmed by a metastable peak at *m/e* 213.4 (calculated, $244^2/279 = 213.4$), the latter loses the second chlorine atom (*m/e* 244).

TABLE II

COMPOSITION OF THE REACTION MIXTURES AT DIFFERENT TIMES UPON SILYLATION OF CHLORAMPHENICOL AND ITS *erythro* ISOMER WITH BSA IN VARIOUS SOLVENTS

The figures indicate the area of the peaks expressed as a percentage of the total area of the peaks present on the chromatogram, except for the calculation of the area of mono-TMS C-1 and C-3, where the total area of the mono-TMS derivative peaks is taken.

| *Compound* | *Solvent* | *Column* | *Carrier gas* | *TMS derivative* | *Time (h)* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *1/4* | *1* | *3* | *6* | *20* | *24* | *28* |
| Chloramphenicol | $CH_3CN$ | OV-17/195° | He | Bis | 94 | 84 | 67 | 56 | | 59 | |
| | | | | Tris | 6 | 16 | 33 | 44 | | 41 | |
| | $C_5H_5N$ | OV-17/193° | He | Bis | 95 | 86 | 74 | 70 | | 56 | |
| | | | | Tris | 5 | 14 | 26 | 30 | | 44 | |
| | EtOAc | OV-1/180.5° | $N_2$ | Mono | 65 | 47 | | | 5 | | |
| | | | | Bis | 35 | 53 | | | 94 | | |
| | | | | Tris | — | — | | | 1 | | |
| | | OV-17/217° | He | Mono C-1 | 4 | | | | | | |
| | | | | Mono C-3 | 96 | | | | | | |
| | $CHCl_3$ | OV-1/180.5° | $N_2$ | Mono | 61 | 36 | | | | | — |
| | | | | Bis | 39 | 64 | | | | | 99 |
| | | | | Tris | — | — | | | | | 1 |
| *Erythro*-chloramphenicol | $CH_3CN$ | OV-17/195° | He | Bis | 83 | 62 | 37 | 28 | | 32 | |
| | | | | Tris | 17 | 38 | 63 | 72 | | 68 | |
| | EtOAc | OV-1/191.5° | He | Mono | 50 | 33 | 18 | | | — | |
| | | | | Bis | 50 | 67 | 82 | | | 100 | |
| | | | | Tris | — | — | — | | | — | |

Cleavage between C-2 and C-3 produces fragment ions at *m/e* 435 and *m/e* 103. The base peak occurs at *m/e* 73. This mass spectrum was contaminated with small peaks derived from fragmentation of the bis-TMS ether, the latter being present as a decomposition product. In Fig. 2 the mass spectrum of the tris-TMS derivative of chloramphenicol, obtained by direct introduction of the silylation mixture with BSA in acetonitrile and corrected for the presence of the bis-TMS ether, is represented. The direct insertion technique allows a lower source temperature to be used, which causes small changes in the relative abundance of some fragment ions, *e.g.*, the disappearance of the weak *m/e* 455 and *m/e* 365 ions.

As already implied from the retention time, a mass spectrum confirmed the identity of the 17.2-min peak of the same chromatogram as the bis-TMS ether of chloramphenicol. In the latter mass spectrum, the base peak also occurs at *m/e* 73. Probably the higher source temperature promotes the formation of the TMS cation.

Recording of the mass spectra of the silyl derivatives as they emerge from an OV-1 column indicated a gradual transition of the bis-TMS to the tris-TMS derivative of chloramphenicol.

GC analysis on an OV-1 column of the silylation mixture of chloramphenicol with BSA in ethyl acetate or chloroform showed the presence of two symmetrical peaks (at 27.4 and 35.9 min; 181°). When the solution in ethyl acetate was injected

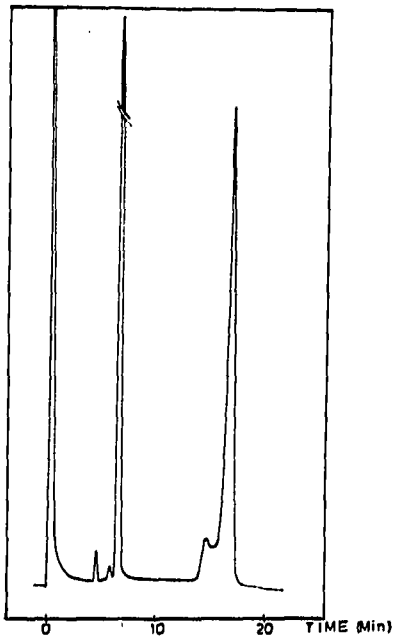



Fig. 5. Gas chromatogram of the silylation mixture of chloramphenicol with BSA in ethyl acetate after 15 min; 5 ft., 3% OV-17, 217°, helium carrier gas.

on to an OV-17 column, two peaks (at 6.3 and 16.7 min; 217°) were also obtained but the second peak was preceded by a small partially resolved peak (at 14.5 min) (Fig. 5). The 6.3-min peak of the chromatogram on OV-17 gives a mass spectrum identical with that of the bis-TMS derivative of chloramphenicol. A mass spectrum of low intensity was obtained from the small 14.5-min peak and it exhibits only two fragments, at *m/e* 73 and *m/e* 225. Comparison with that of the bis-TMS ether of chloramphenicol shows that the presence of the *m/e* 225 ion indicates silylation of the C-1 hydroxyl group. The product is probably the 1-mono-TMS derivative of chloramphenicol.

The 16.7-min peak was identified as the TMS derivative of the C-3 hydroxyl group. A fragmentation pattern similar to that of the bis-TMS ether was obtained. No molecular ion is present, but $(M-CH_3)$ and $(M-CHCl_2)$ ions are present at *m/e* 379 and *m/e* 311, respectively. The *m/e* 379 ion eliminates water (*m/e* 361). Apart from the base peak at *m/e* 73, intense fragment ions at *m/e* 242 and, with gain of a hydrogen atom, at *m/e* 153 derive from cleavage between C-1 and C-2. The base peak in the mass spectrum of chloramphenicol appears at *m/e* 153 (ref. 15). The presence of the *m/e* 153 and *m/e* 242 ions and of the $CH_2=\overset{+}{O}$–TMS ion at *m/e* 103, formed by cleavage between C-2 and C-3, clearly proves silylation of the hydroxyl group at C-3. In the spectra of both mono-TMS derivatives, the *m/e* 147 pentamethyldisiloxonium ion, characteristic of compounds that contain more than one trimethylsilyl group[17–20], is absent. The relative abundances of the fragment ions of 3-mono-TMS-chloramphenicol are given in parentheses: *m/e* 379 (2.3), 361 (1.4), 311 (1.7), 242 (31), 153 (32), 103 (17) and 73 (100).

The composition of the BSA silylating mixture in ethyl acetate or chloroform changed with time. Analysis on OV-1 showed a slow conversion of the mono-TMS ether into the bis-TMS derivative and even a small amount of the tris-TMS derivative (Table II).

Silylation of the *erythro* isomer of chloramphenicol with BSA in acetonitrile similarly gives the bis- and tris-TMS derivatives, whereas the use of ethyl acetate as solvent yields a mixture of the bis- and mono-TMS ethers. Retention times of the silyl derivatives and composition of the reaction mixtures are given in Tables I and II, respectively. A chromatogram on OV-17 of the silylation mixture in ethyl acetate showed an asymmetrical peak at 17.1 min, indicating the presence of both monosilyl ethers.

The mass spectrum of tris-TMS-*erythro*-chloramphenicol is very similar to that of the tris-TMS derivative of chloramphenicol. Only small differences occur in the relative abundance of certain fragments.

The silylation of amides is well known. With TMS-*tert*.-butylamine or hexamethyldisilazane, one TMS group is introduced and with trimethylchlorosilane and triethylamine one or two TMS groups may be introduced[21]. N-Haloamides have been silylated with various silylamides[21] and silyl derivatives of N-alkylamides have been prepared by using trimethylchlorosilane[21] or dialkyldichlorosilanes[22] in the presence of triethylamine. Various ring-substituted acetanilides have been silylated with BSA in acetonitrile[21,23] and the silylation of the amide function in phenoxymethyl- and benzylpenicillin esters[24], phenoxymethyl- and benzylpenicillin sulphoxide esters[25] and their 6-epimers has been accomplished with BSA. In the light of the above, silylation of the amide function in chloramphenicol with BSA, being a very powerful silyl donor[23,26], is not unusual.

As the use of BSA as a silylating reagent for chloramphenicol does not yield a single derivative in the solvents used, difficulties may be experienced in the quantitative determination of the antibiotic. On the other hand, silylation with a mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine gives only the bis-TMS derivative and can therefore, in spite of some practical drawbacks[5], be used as a procedure for derivative formation in the GC analysis of chloramphenicol. If the presence of the *erythro* isomer in chloramphenicol is to be detected, an OV-17 or an OV-1 column can be used, but not a QF-1 column.

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