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# RECENT RESULTS IN THE USE OF PHOSGENE AS A DERIVATIZING REAGENT PRIOR TO GAS CHROMATOGRAPHY OF AMINO ALCOHOLS

OLLE GYLLENHAAL* and JÖRGEN VESSMAN
*Analytical Chemistry, AB Hässle, S-431 83 Mölndal (Sweden)*

## SUMMARY

Recent kinetic experiments concerning the reaction of phosgene with the amino alcohol metoprolol and different related 1-alkylamino-3-(aryloxy)-2-propanol compounds in dichloromethane show that the amino group substituent influences the reaction rate to a high degree, whereas the aromatic part is of little importance. The relative rate is 50 times lower for a *tert.*butylamino group than for an isopropylamino group. Some new examples of compounds of pharmaceutical interest that undergo ring-closure reaction with phosgene are also presented. These include ketamine, mefloquine, and tryptamine.

## INTRODUCTION

Many of the chemical reactions of phosgene are similar to those of acid chlorides and chloroformates. However, a unique property is its ability to form cyclic derivatives with bifunctional compounds, *i.e.* compounds with two functional groups in appropriate proximity to each other in the molecule[1]. Nevertheless, the number of published papers on using phosgene as a reagent is not large.

2-Aminoalcohols such as metoprolol readily form cyclic oxazolidine-2-ones (oxazolidones). Also 6- and 7-membered rings can be formed from corresponding 3- and 4-aminoalcohols[2]. Tocainide, a 2-aminopropanoic acid amide, gives a hydantoin derivative[3,4]. Phenols do also react. Simple phenolic compounds can be derivatized by the simultaneous addition of methanol to the reaction mixture to form a methyl carbonate[5,6]. The aqueous alkaline conditions used for derivatization with phosgene leaves functional groups such as alcohols and carboxyls intact[7–9]. Using anhydrous conditions, cyclic derivatives were obtained from 1,2- and 1,3-diols and from α-hydroxy acids[10]. Several liquid chromatographic enantiomeric separations, preceded by derivatization with phosgene, have also been published[11–14].

Despite hydrolysis, derivatization of amino alcohols such as metoprolol with phosgene can conveniently be performed both in water, as well as in organic solvents. A two-phase system was prefered for the purpose of analyzing metoprolol in plasma samples, because the proteins tended to form a gel by the action of phosgene and the agitation of the tubes. Suitable pH values were in the range 7.5–12, and 10 μl of 2 *M* phosgene was sufficient for a consistent yield of the derivative[2]. The derivatization

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of phenols[5] or the presence of carboxyl groups[7] necessitated more alkaline conditions, pH 11.5–12, and repeated additions of reagent gave a higher yield of the desired derivative. The reaction appears to be almost instantaneous[2], but to reduce the risk of phosgene exposure, one should wait at least 10 min in order to let excess phosgene be rendered harmless by hydrolysis.

The absolute yield of the method for metoprolol in plasma was 85%[2]. For metoprolol and metabolites in water the yields were about 90%, but only about 56% for the acid metabolite when urine was present[7]. These data indicate that other reactions compete with the ring-closure reaction.

The aim of the present study is to improve our understanding of phosgene as a chromatographic derivatization reagent. Some recent results concerning the relative derivatization rates of amino alcohols are presented as well as some new examples of compounds that react to yield cyclic compounds with phosgene are presented. Some results are also reported that show the importance of the pH and influence from competing compounds such as dimethylamine and phenol.

## EXPERIMENTAL

### *Gas chromatography*

A Varian 3700 gas chromatograph equipped with a flame ionization detector was used. The capillary column was connected to the injector with adaptors to allow split or splitless injections and to introduce make-up gas into the detector. The inlet pressure of the nitrogen carrier gas was 100 kPa, giving a flow-rate of about 60 cm/s. The oven temperature was kept at 100°C for 1 min after the injection and then increased at a rate of 20°C/min to 280°C. The fused-silica column (25 m × 0.32 mm I.D.) used was coated with CP-Sil 8 from Chrompack (Middelburg, The Netherlands). The chromatograms were recorded with a Hewlett-Packard 3290A integrator.

### *Mass spectrometry*

Mass spectra of the derivatives formed were recorded with a Varian MAT 44S gas chromatograph–mass spectrometer at an ionization voltage of 70 eV. The gas chromatographic set up was the same as above and the transfer line to the ion source was kept at 250°C.

### *Reagents and chemicals*

Mefloquine, tryptamine, ketamine and Organon 6001 (3α-amino-5α-androstan-2β-ol-17-one) were available as hydrochlorides. The structures of the first three are given in the Results and discussion section. N-Desethylchloroquine was used as a 400 $\mu M$ aqueous solution. Metoprolol and analogues as well as other β-blocking drugs were obtained as salts from the Department of Organic Chemistry, Hässle. Phosgene in toluene (2 *M*) was from Fluka (Buchs, Switzerland). Buffers were prepared from sodium phosphate to an ionic strength of 1.

### *Methods*

*Determination of relative reaction rates for metoprolol and related compounds in dichloromethane.* To determine chromatographic response factors $10^{-6}$ mol each of the compounds under investigation and of an inert internal standard were dis-

solved in 1 ml of dichloromethane. Then 10 μl of 0.01 *M* phosgene in toluene was added and allowed to react for 10 min before evaporation under a stream of nitrogen. The residue was dissolved in 1 ml of ethyl acetate and analyzed by gas chromatography, 3 μl being injected under splitless conditions.

To determine relative reaction rates samples were prepared with a reference (metoprolol) and the compound under study. When different relative reaction rates were encountered (*e.g.*, higher than 5 and lower than 0.2) the concentrations of the substrates were adjusted accordingly. In some cases, metoprolol was substituted by alprenolol to facilitate the chromatographic separation of the derivatives formed. The relative reaction rates were calculated with metoprolol as reference.

*Screening for compounds that might yield cyclic products with phosgene.* The following general procedure was used: 0.1 ml of a solution of the compound (1 mg/ml) was mixed with 1 ml buffer of pH 8, and 20 μl of a 2 *M* phosgene solution in toluene was added under vigorous agitation. Next, 2 ml of dichloromethane was added and any derivatives formed were extracted. An aliquot was withdrawn, evaporated, and dissolved in ethyl acetate before gas chromatographic analysis. The peaks obtained from the derivatives were then subjected to mass spectral analysis for identification.

## RESULTS AND DISCUSSION

### *Relative reaction rates in dichloromethane*

Attempts to study the reaction of metoprolol with phosgene in dichloromethane on the basis of pseudo-first order kinetics failed, owing to the very high reaction rate, which makes it difficult to terminate the reaction momentarily. Instead, relative rates were determined in order to gain some understanding of how different structural environments influence the overall reaction. Equimolar amounts of the reference and the test compound were mixed and then derivatized with phosgene in a 10-fold molar excess. From the observed area ratio quotient the relative ratio was calculated. This procedure is based on the assumptions that the cyclization reaction is complete in each case and that no side reactions occur. Differences in detector responses were corrected for by assaying separate samples of the reference and the test compound. Major deviations from the expected area relative to the internal standard were only observed in a few cases.

*Metoprolol and some of its analogues.* The observed relative reaction rates are given in Table I. The alkyl substituent on the nitrogen atom was changed from a hydrogen atom in increasing order of methylene units to a *tert.*-butyl group. Relative to metoprolol, the rate is higher with hydrogen and with the smaller and straight-alkyl chain substituents. On the other hand, with the bulky *tert.*-butyl group the rate is approximatively 50 times slower. A low relative rate was also observed with an analogue of metoprolol. In this instance, the alcohol group is a primary one (Table I), and this apparently reduces the overall rate.

*Metoprolol and some homologues.* Increasing the distance between the amino and alcohol groups reduces the relative rate (Table II) but only to a minor extent. Raw data also indicate that with H 170/40, having four methylene groups between the amine and the hydroxylic carbon, the reaction is not complete as the observed area ratio was *ca.* 40% lower than the expected ratio. Hence, the reaction may, in fact, be slower than suggested in the table.

TABLE I

RELATIVE REACTION RATES OF PHOSGENE WITH METOPROLOL AND SOME OF ITS ANALOGUES IN DICHLOROMETHANE

Structures of compounds correspond to $CH_3OCH_2CH_2$–$C_6H_5$–$OCH_2CH(OH)CH_2NH$–R with the exception of H 84/79 which corresponds to the following: $CH_3OCH_2CH_2$–$C_6H_4$–$OCH_2CH(NH$–R)–$CH_2OH$. Method: see Experimental section.

| *Compound* | *R* | *Relative rate* |
|---|---|---|
| Metoprolol | Isopropyl | 1.0 |
| H 98/52 | H | 8.9 |
| H 100/11 | Methyl | 5.3 |
| H 173/09 | Ethyl | 5.7 |
| H 117/78 | *n*-Propyl | 5.9 |
| H 105/29 | *tert.*-Butyl | 0.02 |
| H 84/79 | Isopropyl | 0.03 |

*Metoprolol and some of its metabolites.* These results (Table III) show a negligible influence of the hydroxyl groups. Prior to gas chromatography, the remaining hydroxyls were silylated[7].

*Metoprolol and some common β-adrenoreceptor blocking drugs.* Table IV lists the relastive rates for some other structurally related compounds used as β-blockers in clinical practise. The variations are only minor. The relative rates are mostly within 20% of that of metoprolol, except for timolol which has a *tert.*-butyl substituent on the amino group (*cf.* Table I). These findings are consistent with the observation (Table III) that structural modifications far from the amino alcohol group exert only a minor influence on the relative reaction rate.

*Interferences with the phosgene derivatization reaction*

The reaction of phosgene with metoprolol in the presence of plasma gives an absolute yield of 85%[2]. The yield of derivatizated metoprolol and metabolites in urine varied between 93 and 56%[7]. These figures indicate losses in the overall reaction. A possible reason for this might be the presence of amino and phenol and/or other reactive compounds in the sample that compete with the chlorocarbonylamine intermediate in the ring-closure reaction. To shed some light on this hypothesis, some

TABLE II

RELATIVE REACTION RATES OF PHOSGENE WITH METOPROLOL AND SOME HOMOLOGUES IN DICHLOROMETHANE

Structures of compounds correspond to $CH_3OCH_2CH_2$–$C_6H_4$–$OCH_2$–R–$CH(CH_3)_2$. Method: see Experimental section.

| *Compound* | *R* | *Relative rate* |
|---|---|---|
| Metoprolol | $CH(OH)CH_2NH$ | 1.0 |
| H 170/31 | $CH(OH)CH_2CH_2NH$ | 0.66 |
| H 170/40 | $CH(OH)CH_2CH_2CH_2NH$ | 0.77* |

* See comment in the text.

TABLE III

RELATIVE REACTION RATES OF PHOSGENE WITH METOPROLOL AND SOME OF ITS METABOLITES

Structures of compounds correspond to $R–C_6H_4–OCH_2CH(OH)CH_2NH–CH(CH_3)_2$. Method: see Experimental section, silylation before gas chromatography.

| *Compound* | *R* | *Relative rate* |
|---|---|---|
| Metoprolol | $CH_3OCH_2CH_2$ | 1.0 |
| H 105/22 | $CH_2CH_2OH$ | 0.94 |
| H 119/66 | $CH(OH)CH_2OCH_3$ | 1.1 |
| H 119/68 | $CH_2OH$ | 0.93 |
| H 119/72 | $CH(OH)CH_2OH$ | 0.60 |

experiments were performed with metoprolol and its *n*-propyl and *tert.*-butyl analogues with dimethylamine and phenol, respectively, as possible competing agent in increasing concentrations. The results and experimental details are given in Table V. Only the *n*-propyl compound is significantly influenced by the presence of dimethylamine, but no concentration dependence can be deduced within the range studied. The relative yield was about 30% lower than in the absence of amine. Metoprolol (isopropyl) and the *tert.*-butyl compound always gave a relative yield about 10% lower than the reference sample derivatized in the absence of amine. A possible explanation for the interference is that the cyclization reaction proceeds in two steps: first chlorocarbonylation and then ring-closure. The ring-closure step in the presence of an *n*-alkyl substituent on the nitrogen atom is likely to be slower than when sterically more bulky groups are present and thus the former compounds are more susceptible to interferences. However, as discussed above, the overall reaction with phosgene is faster and the present results indicate that the initial chlorocarbonylation is the rate-determining step. Corresponding experiments with phenol present instead of

TABLE IV

RELATIVE REACTION RATES OF PHOSGENE WITH METOPROLOL AND SOME OTHER β-BLOCKING DRUGS

Structures of compounds correspond to $Ar–CH_2CH(OH)CH_2NH–CH(CH_3)_2$ with Ar as listed in the table. Method: see Experimental section.

| *Compound* | *Relative rate* | *Structure (Ar)* |
|---|---|---|
| Metoprolol | 1.0 | See Table II |
| Alprenolol | 1.2 | 2-Allylphenoxy |
| Oxprenolol | 1.2 | 2-Allyloxyphenoxy |
| Propranolol | 0.84 | 1-Naphtyloxy |
| Pindolol | 1.2 | 4-Indolyloxy |
| Timolol* | 0.02 | 4-Morfolino-1,2,5-tiadiazol-3-yl-oxy |
| "Isopropyl timolol" | 0.62 | |
| Acebutolol | 0.67 | 2-Acetyl-4-*n*-butyramidophenoxy |

* *tert.*-butylamino.

TABLE V

POSSIBLE INTERFERENCE FROM DIMETHYLAMINE WHEN DERIVATIZING 2-AMINOALCOHOLS WITH PHOSGENE IN AQUEOUS SOLUTION pH 12

Method: see Experimental section; $1.9 \cdot 10^{-5}$ moles of each compound. Structures of the compounds in Table II.

| *Amine* ($M \cdot 10^5$) | *Relative yield (%)* | | |
|---|---|---|---|
| | *Isopropyl (metoprolol)* | *tert.-Butyl (H 105/29)* | *n-Propyl (H 117/78)* |
| 0 | 100 | 100 | 100 |
| 1.9 | 95 | 99 | 74 |
| 5.6 | 93 | 100 | 74 |
| 9.4 | 88 | 95 | 68 |
| 18.9 | 89 | 94 | 71 |
| 47 | 90 | 94 | 69 |
| 94 | 89 | 91 | 71 |

dimethylamine showed no influence on any of the compounds in the concentration range studied.

As mentioned in a previous section, a bulky amine substituent gives rise to a lower relative reaction rate. The implication of this fact was demonstrated by derivatizing the three compounds at pH 8 and 12 with 1, 2, 3, and 4 10-μl volumes of phosgene reagent. The results are given in Table VI and show that the reaction proceeded smoothly for all compounds at pH 12, but at pH 8 the *tert.*-butyl compound gave low and irreproducible yields when 1 and 2 portions of phosgene reagent were added. At this pH, the amines are largely ionized and therefore in a less reactive form. It is reasonable to assume that the slowest compound (Table I) is more dependent on the pH and consequently more prone to incomplete reaction when the conditions are unfavourable.

TABLE VI

DERIVATIZATION OF 2-AMINOALCOHOLS IN AQUEOUS SOLUTION WITH PHOSGENE

Method: $1.9 \cdot 10^{-5}$ *M* of each compound in buffer. Duplicate samples were derivatized with phosgene in 2 min intervals as indicated in the table. Other details as in the Experimental section. Structures of the compounds in Table II.

| *Buffer pH* ($\mu = 1$) | *10 μl 2 M phosgene* | *Relative yield (%)* | | |
|---|---|---|---|---|
| | | *Isopropyl (metoprolol)* | *tert.-Butyl (H 105/29)* | *n-Propyl (H 117/78)* |
| 8 | 1 | 97 | 57, 7 | 98 |
| 8 | 2 | 101 | 68,81 | 102 |
| 8 | 3 | 101 | 100 | 102 |
| 8 | 4 | 99 | 100 | 98 |
| 12 | 1 | 100 | 100 | 97 |
| 12 | 2 | 100 | 100 | 100 |
| 12 | 3 | 99 | 99 | 101 |
| 12 | 4 | 100 | 101 | 101 |

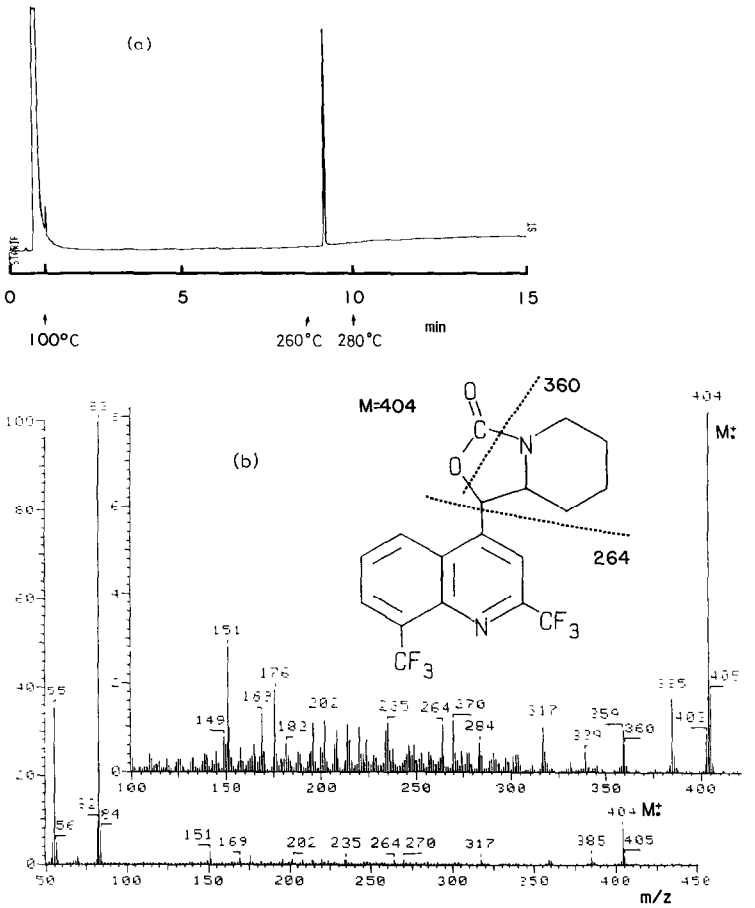


Fig. 1. The mefloquine derivative after phosgene derivatization. (a) Gas chromatogram; (b) mass spectrum at 70 eV.

*Miscellaneous compounds of pharmaceutical interest that yield cyclic compounds with phosgene*

A few odd compounds were investigated for the formation of cyclic compounds with phosgene. The first example illustrated is mefloquine, which is used therapeutically to treat malaria. A typical chromatogram is shown in Fig. 1a. The identity of the product was confirmed by electron impact mass spectrometry at 70 eV which yielded a spectrum with the expected molecular ion (Fig. 1b). Some diagnostic ions are indicated in the proposed structure. The next example involves ketamine, which is used as an anesthetic. The derivatization with phosgene leads to a cyclic derivative because the ketone is in equilibrium with its enol form. The chromatogram is free from any significant side-products (Fig. 2a). The mass spectrum (Fig. 2b) supports the suggested cyclic structure. The last example illustrated here is tryptamine. The chromatogram shown (Fig. 3a) has a single dominant peak with some tailing, which is due to the remaining labile hydrogen (Fig. 3b). As discussed in the Introduction section 5-, 6- and 7-membered rings can be formed from amino

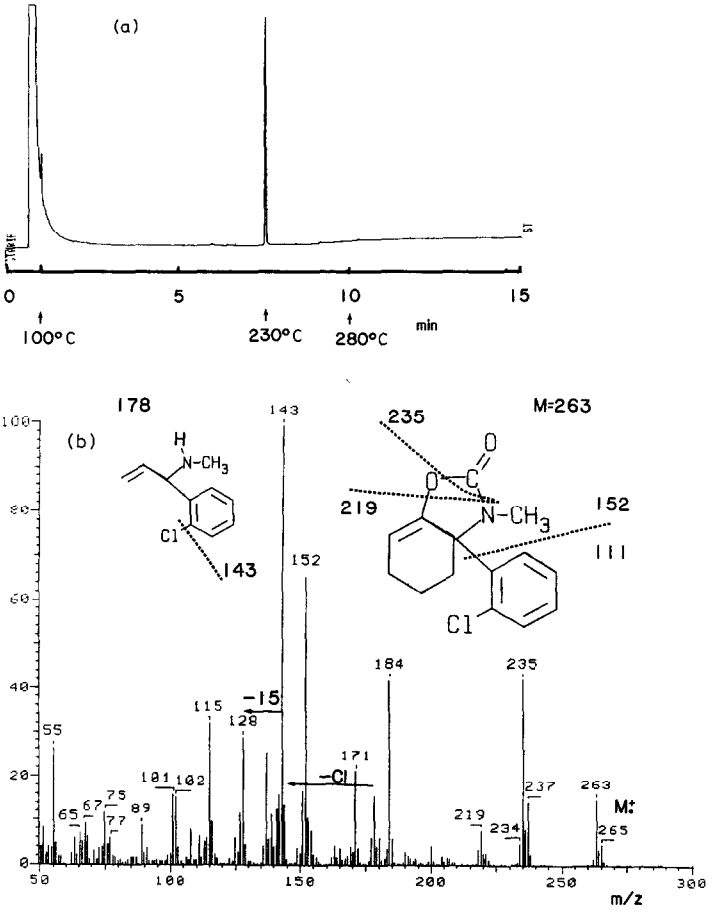


Fig. 2. The ketamine derivative after phosgene derivatization. (a) Gas chromatogram; (b) mass spectrum at 70 eV.

alcohols. Even if the amino and alcoholic groups are parts of a cyclic ring and positioned in $\alpha$ and $\beta$ positions, a cyclic carbamate can be formed, as was shown with Organon 6001[15] as model compound. The configuration of the cyclic ring makes the distance between the amino group and the alcoholic one closer than the structural formula would indicate. N-Desethylchloroquine also yielded cyclic products with phosgene in a buffer of pH 8. Here the chlorocarbonylamine reacts with a secondary aromatic amine and a 7-membered ring is formed.

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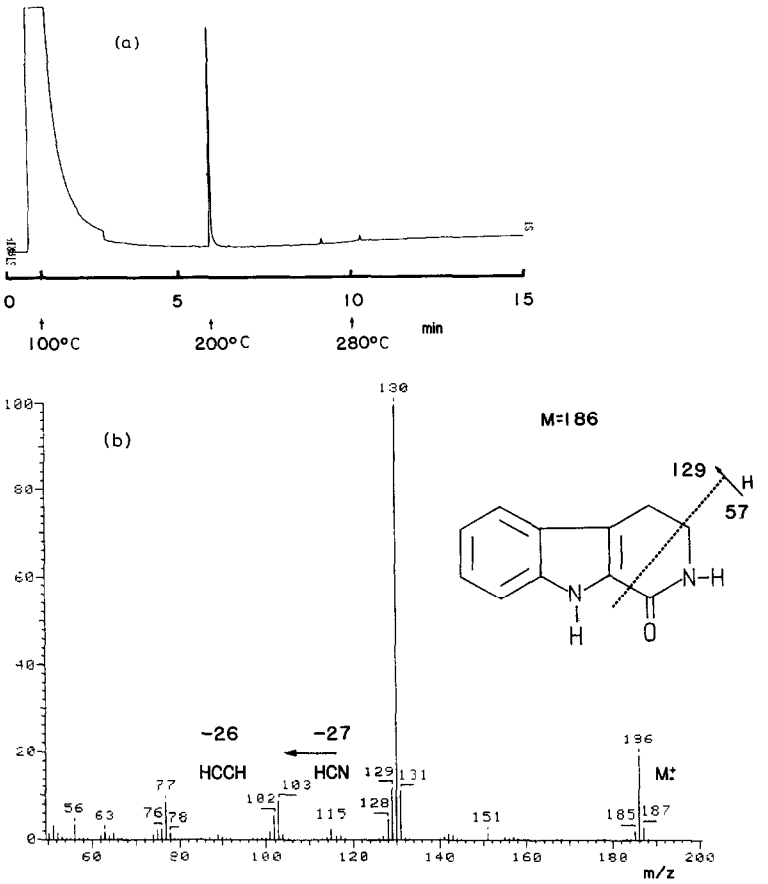


Fig. 3. The tryptamine derivative after reaction with phosgene. (a) Gas chromatogram; (b) mass spectrum at 70 eV.

## REFERENCES


1 C. F. Poole and A. Zlatkis, *J. Chromatogr.*, 184 (1980) 99.
2 O. Gyllenhaal and J. Vessman, *J. Chromatogr.*, 273 (1983) 129.
3 R. Venkataramanan, F. S. Abbot and J. E. Axelson, *J. Pharm. Sci.*, 71 (1982) 491.
4 L. Johansson and J. Vessman, *J. Chromatogr.*, 239 (1982) 323.
5 O. Gyllenhaal, *J. Chromatogr.*, 349 (1985) 447.
6 O. Gyllenhaal, *J. Chromatogr.*, 413 (1987) 270.
7 O. Gyllenhaal and K.-J. Hoffmann, *J. Chromatogr.*, 309 (1984) 317.
8 O. Gyllenhaal and K. J. Hoffman, presented at *Symposium on Analytical Techniques in Studies on Metabolism, Stockholm, Sweden, April 1985.*
9 K.-J. Hoffmann, O. Gyllenhaal and J. Vessman, *Biomed. and Envir. Mass Spectrom.*, (1987) in press.
10 W. A. König, E. Steinbach and K. Ernst, *J. Chromatogr.*, 301 (1984) 129.
11 I. W. Wainer, T. D. Doyle, K. H. Donn and J. R. Powell, *J. Chromatogr.*, 306 (1984) 405.
12 I. W. Wainer, T. D. Doyle, Z. Hamidzadeh and M. Aldridge, *J. Chromatogr.*, 268 (1983) 107.
13 J. Hermansson, *J. Chromatogr.*, 325 (1985) 379.
14 R. Isaksson and B. Lamm, *J. Chromatogr.*, 362 (1986) 436.
15 J. Vink, H. J. M. van Hal and C. J. Timmer, *Biomed. Mass Spectrom.*, 7 (1980) 592.