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RECENT ADVANCES IN DOPING ANALYSIS (4)

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Trimethylsilylation - Aspects for Derivatisation

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Trimethylsilylation, the most suitable derivatisation for gas chromatography, can be performed with a lot of reagents. First trimethylhalosilanes were used, later on TMS-amines, TMS-esters of organic acids, TMS-amides and more recently TMS-esters of inorganic acids. In doping analysis trimethylsilylation is generally performed with N-TMS-amides to which a catalyst is often added. Here I will give a small review of some special aspects of trimethylsilylation with N-TMS-amides.

The synthesis of N,N-bis-trimethylsilyl-acetamide (BSA,Fig.1) was published in 1963 by L.Birkofer who also published in 1965 the synthesis of N-trimethylsilyl-imidazole (TMSImi), which can be seen as a TMS amidine and so as a related compound. In 1967 the synthesis of N-methyl-N-trimethylsilyl-acetamide (MSA) was published by L.Birkofer and M.Donike. They described the silylation of amino acids, fatty acids, polyalcohols, carbohydrates, amines and their hydrochlorides, and of phenol-alkylamines with MSA which, in nearly all cases, can act as its own solvent.

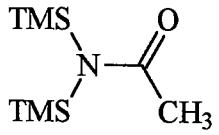
The synthesis of N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) was published by D.L. Stalling in 1968 and that of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) in 1969 by M.Donike. BSTFA and MSTFA are today the most important and most commonly used silylating agents for the analytical derivatisation of nearly all classes of compounds. This is based on the fact that not only the silylation powers are higher than that of other N-TMS-amides but also that they and their by-products are very volatile and do not interfer with early eluting substances in the chromatogram.

Moreover the presence of fluorine atoms results in less fouling of the flame ionisation detector (FID) and especially of the nitrogen-phosphorus detector (NPD) by deposits of silica. The ratio of F/Si = 3 in MSTFA is already double that of BSTFA: an advantage of MSTFA over BSTFA. Additionally, in 1971 M.Donike synthesised N-methyl-N-trimethylsilyl-heptafluorobutyramide (MSHFB: F/Si = 7) to enhance the performance of screening procedure II for the XX. Olympic Games in Munich 1972, since at that time a NPD was used for monitoring silylated nitrogen containing substances.

Fig. 1: Formulas of some N-TMS-amides

N,N-Bis-trimethylsilyl-acetamide

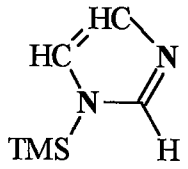
1963 L.Birkofer et. al.
Angew. Chem. *75*, 93



BSA

N-Trimethylsilyl-imidazole

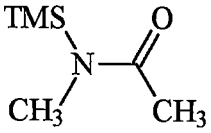
1965 L.Birkofer,A.Ritter
Angew. Chem. *77*, 414



TMSImi

N-Methyl-N-trimethylsilyl-acetamide

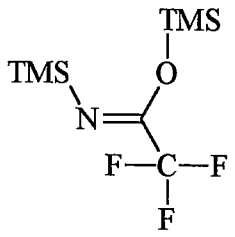
1967 L.Birkofer, M.Donike
J.Chromatogr. *26*, 270



MSA

N,O-Bis-trimethylsilyl-trifluoroacetamide

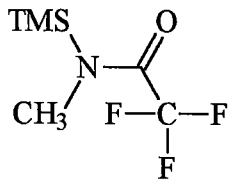
1968 D.L.Stalling et. al.
Biochem. Biophys. Res. Commun. *31*, 616



BSTFA

N-Methyl-N-trimethylsilyl-trifluoroacetamide

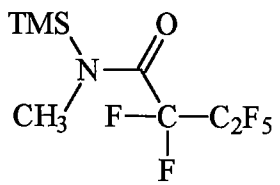
1969 M.Donike
J. Chromatogr. *42*, 103



MSTFA

N-Methyl-N-trimethylsilyl-heptafluorobutyramide

1971 M.Donike
unpublished



MSHFB

The MSHFB synthesis was not promptly published nor were the results of a systematic investigation of the silylation potentials of N-TMS-amides by Gerhard Schroers for his thesis 1973 by proposal of M.Donike. An extract is given in Table 1. The equilibrium constants (K_C) of the reactions MTFA + TMS-amide $\longleftrightarrow$ MSTFA + amide were determined from at least 6 analyses using gas chromatography. The silylation potential has been defined relative to that of MSTFA/MTFA. The relatively low silylation potential of BSTFA may be explained with the assumption that the first leaving TMS group is that bonded to the oxygen atom.

Table 1: Equilibrium constants and negative relative silylation potentials of some N-TMS-amides with N-methyl-trifluoroacetamide (MTFA)
from: Gerhard Schroers, thesis 1973, University Cologne, Germany

Trimethylsilyl-donor	Abbr.	K_C	$-\mu°$ [cal]	S.D.
N-TMS-acetamide	SA	0.0007	-4340	± 68
N-TMS-trifluoroacetamide	STFA	0.0024	-3589	±156
N-Methyl-N-TMS-acetamide	MSA	0.171	-1048	± 33
N-Methyl-N-TMS-trifluoroacetamide	**MSTFA**	**1**	**0**	
N,O-Bis-TMS-trifluoroacetamide	BSTFA	1.81	349	± 36
N-Methyl-N-TMS-heptafluorobutyramide	MSHFB	2.16	455	± 19
N,N-Bis-TMS-acetamide	BSA	3.13	676	± 8
N-t-Butyl-N-TMS-acetamide		20.6	1789	± 33

K_C = [Amide] x [MSTFA] / [N-TMS-amide] x [MTFA] $\qquad \mu° = -RT\ln K_C$

The silylation capacity of the N-TMS-amides is of interest too. In Table 2 the theoretical transferable amount of TMS groups in mmoles/ml is calculated from the density and the molecular weight. Regarding BSA and BSTFA only one TMS group is taken into account because the second group is not transferred under correct conditions for analytical purposes (excess of the reagent). By the way, M.Donike demonstrated in 1975 (M.Donike, J.Chromatogr. *115*, 591) that the silylation capacity can be checked by the use of methylorange, a procedure which is helpful for the selective derivatisation of phenolalkylamines to their N-acyl-O-TMS-ethers.

The properties of the N-TMS-amides are very similar. They all can act more or less as their own solvent. A typical difference can be demonstrated with crystallised sucrose: while MSA dissolves it within some minutes under complete silylation, pure MSTFA will not dissolve it; even not by heating at 80°C for two days. After addition of pyridine the crystals dissolve within some minutes under complete silylation. So often pyridine, trifluoroacetic acid and acetonitrile are recommended as solvents. Addition of a solvent reduces of course the silylation capacity. In most cases MSTFA can be applied without the aid of a solvent.

Table 2: Theoretical transferable amount of TMS groups of some N-TMS-amides

Compound	M.W.	Density	mmol TMS-/ml	rel. capacity
BSA*	203.4	0.832	4.1	76.1
TMSImi	140.3	0.957	6.8	126.9
MSA	145.3	0.901	6.2	115.3
BSTFA*	257.4	0.970	3.8	70.1
MSTFA	199.4	1.072	5.4	**100**
MSHFB	299.1	1.255	4.2	78.1

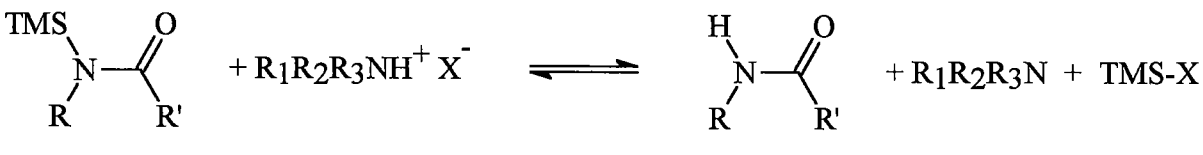
* only one TMS group taken into account

see also M.Donike, "Control of trimethylsilylation potential and trimethylsilylation capacity by the use of colour indicators", J.Chromatogr. *115*, 591 (1975)

The silylation potential of all N-TMS-amides is so high that all hydroxyl groups should be silylated by them, but for the silylation of tertiary hydroxyl groups catalysts are required. It seems that the N-TMS-amides are to voluminous to react with sterically hindered functions. As catalysts are recommended TMSImi and trimethyliodosilane (TMIS). The preparation of TMS-enol ethers requires in most cases catalysts, e.g. TMIS or potassium acetate. The latter acts as a very strong base which forwards the enolisation of the carbonyl function.

Catalysts are used also for the silylation of amino functions mainly to enhance the reaction velocity. Commonly, trimethylchlorosilane (TMCS) is recommended. All catalysts with a TMS group react with the substrate and are regenerated by reaction of their by-product by the silylating reagent (if not they cannot be used as catalysts !). Thus also hydrogen chloride is sometimes applied but is equivalent to TMCS. TMSImi which darkens easily and is difficult to purify can be replaced by imidazole itself. Normally the catalysts are added in amounts of 1 to 5 % but sometimes amounts up to 50% have been used with BSTFA.

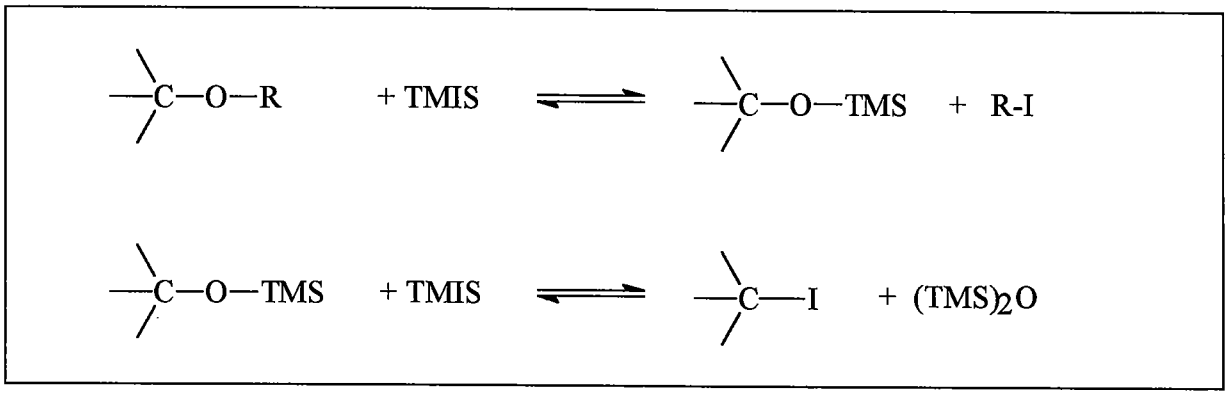
Fig. 2: Reaction of N-TMS amides with amine hydrohalides



All N-TMS-amides are strong bases and react with amine hydrohalides (Fig. 2) assuming they are soluble thereby generating the corresponding trimethylhalosilanes. This displacement can be used for the in situ generation of less stable catalysts like trimethylbromosilane (TMBS) and especially TMIS. TMIS is one of the most reactive silylating agents (a clear colorless

liquid) but extremely sensitive. It decomposes in air and by light generating iodine, it is very moisture sensitive, and its storage is not desired. In preparative organic chemistry it is used for the cleavage of ethers, esters, carbamates and ketals and for the synthesis of iodides by cleavage of TMS-ethers (Fig. 3).

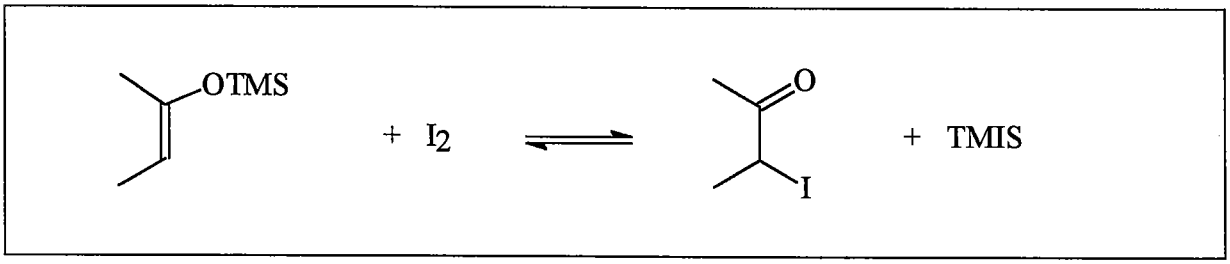
Fig. 3: Scheme for cleavage reactions with TMIS



In 1980 M.Donike and J.Zimmermann published results showing that TMIS is the only catalyst which converts steroidal ketones to isomerically pure TMS enol ethers. The use of this feature not only enhanced the detection limit for anabolic steroids and their metabolites but is also a prerequisit for an effective steroid profiling. Subsequently normal ranges of endogenous steroids have been determined, and especially the ratio testosterone/epitestosterone became the tool to detect misuse of testosterone. Also the misuse of 5α-dihydrotestosterone can meanwhile be detected by means of steroid profiling.

The use of TMIS as a catalyst bears some problems. First one has to take into account that TMS ethers can be cleaved by TMIS generating iodated compounds. So a very low amount (0.2 %) was choosen. The second problem arises from the fact that iodine is generated also in the solution in MSTFA. Like enol acetates, TMS enol ethers react with iodine to α-iodo-ketones most probably under regeneration of TMIS (Fig. 4). This iodo ketones or their TMS enol ethers are not detected when they are not monitored.

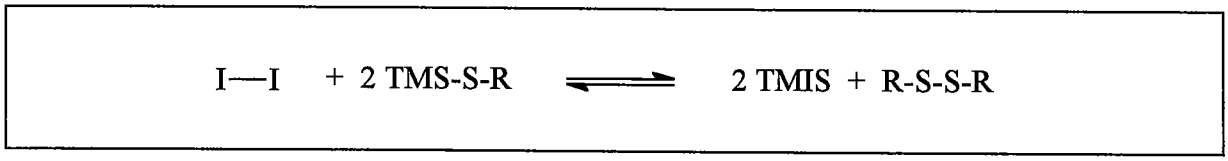
Fig. 4: Scheme for iodination of TMS enol ethers



So it is necessary to suppress the formation of iodine or to remove it. Iodine is reduced by thiols and also by S-TMS thiols under regeneration of TMIS so that the amount of the catalyst

remains constant as far as the thiol is in excess (Fig. 5). Dithioerythreitol (DTE, Cleland's reagent) was first choosen because it is easy to handle, does not smell and is described to form a cyclic disulfide. But its use in steroid profiling led to some samples which could not be evaluated due to interfering peaks in the chromatogram. These peaks were associated to DTE by subsequently performed mass spectrometric analysis.

Fig. 5: Scheme of the reduction of iodine by S-TMS-thiols



DTE obviously reacts not only to the desired cyclic disulfide but also with another molecule to a disulfide of higher molecular weight. As a bifunctional compound it furthermore meets the prerequisite for polymerisation, and under unfortunate conditions the chromatograms showed large peaks in the region of interest. Consequently DTE was replaced by a monofunctional substance. M.Donike decided to use S-TMS-ethane-thiol, though it smells terrible, but it does not produce heavy volatile substances nor consume any silylating reagent for the derivatisation of hydroxyl functions.

This small review neither covers all aspects of trimethylsilylation nor contains all newer developments , but its contents are very close to the scientific work of Manfred Donike.

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