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AN IMPROVED DEGRADATION PROCEDURE FOR DETERMINATION OF THE ABSOLUTE CONFIGURATION IN CHIRAL ISOQUINOLINE AND β-CARBOLINE DERIVATIVES*†

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Key Word Index—ruthenium(VIII)-catalysed oxidative degradation; tetrahydroisoquinolines; dihydroisoquinolines; naphthylisoquinolines; michellamines; tetrahydro-β-carbolines; structural elucidation.

Abstract—A reliable method for the determination of the absolute configuration of chiral isoquinolines, naphthylisoquinolines, michellamines and β-carbolines is described. The ruthenium-mediated oxidative degradation of the di- or tetra-hydropyridine heterocycle in these structures provides simple amino acid derivatives, which can be analysed by GC after Mosher-type derivatization. The method allows for the secure determination of the absolute configuration at the stereocentres C-1 or C-3 in a single step. By using a mass-selective detector instead of a flame ionization detector for GC analysis of the amino acid derivatives, the procedure offers a more sensitive detection and unambiguous characterization of the degradation products. Because of this improved method, the required stereo-information is reliably available with as little as 0.5 mg of the analysate, even for complex natural products, such as the michellamines. The broad applicability of the procedure is demonstrated by a variety of examples taken mainly from the field of natural products chemistry. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Tetrahydropyridine rings (see Fig. 1) and their dihydro analogues are present in a broad diversity of natural products, but mainly isoquinoline and β-carboline alkaloids from various sources, predominantly from plants [3]. They may be simple, with a stereogenic centre exclusively at C-1, such as salsolinol (**1**) [4, 5] or eleagnine (**2**) [3, 5]. However, they may also be more complex, such as the naphthylisoquinoline alkaloid dioncophylline A (**3**) [6, 7], which has a characteristic 1,3-dimethyl substitution pattern, and thus two stereocentres, and in addition a stereogenic axis. More recently, related dimeric naphthylisoquinolines were discovered, named michellamines [1, 8–10], such as michellamine A (**4**), which has four stereocentres, two stereogenic axes and a freely rotating, and thus configurationally unstable, central axis between the two molecular moieties.

Elucidation of the absolute configuration of these chiral natural products is an important and sometimes difficult task, particularly if interpretation of the CD spectra is hampered by the additional presence of largely dominating biaryl axes (such as in compounds **3** and **4**) [7, 11], or the lack of comparison data for an empirical interpretation. Often, no crystalline material of a quality suitable for an X-ray structure analysis is available, thereby invoking the need to involve heavy atom substitution or derivatization with a chiral auxiliary of known absolute configuration. Consequently, the absolute configuration of such natural products can sometimes be established only by total syntheses, which may often be lengthy and tedious. For the rapidly growing class of naphthylisoquinoline alkaloids, we have recently introduced a simple method for the reliable and unambiguous determination of the absolute configuration at the stereocentres of *N*-unsubstituted 1,3-dimethyltetra- and di-hydroisoquinolines [12]. The method is based on a smooth, ruthenium-mediated oxidative degradation to provide the simple and easy-to-analyse amino acids 3-aminobutyric acid and alanine (see Scheme 1). After esterification and Mosher-type derivatization [13] with the acid chloride of (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (i.e. with (*R*)-MTPA-Cl), the absolute configuration can be easily determined by gas chromatographic comparison with authentic amino acid derivatives of known configuration.

*Part 76 in the series 'Acetogenic Isoquinoline Alkaloids'. For Part 75, see ref. [1]. Part 27 in the series 'Endogenous alkaloids in man'. For Part 26, see ref. [2].

†Dedicated to Prof. Burchard Franck, on the occasion of his 70th birthday.

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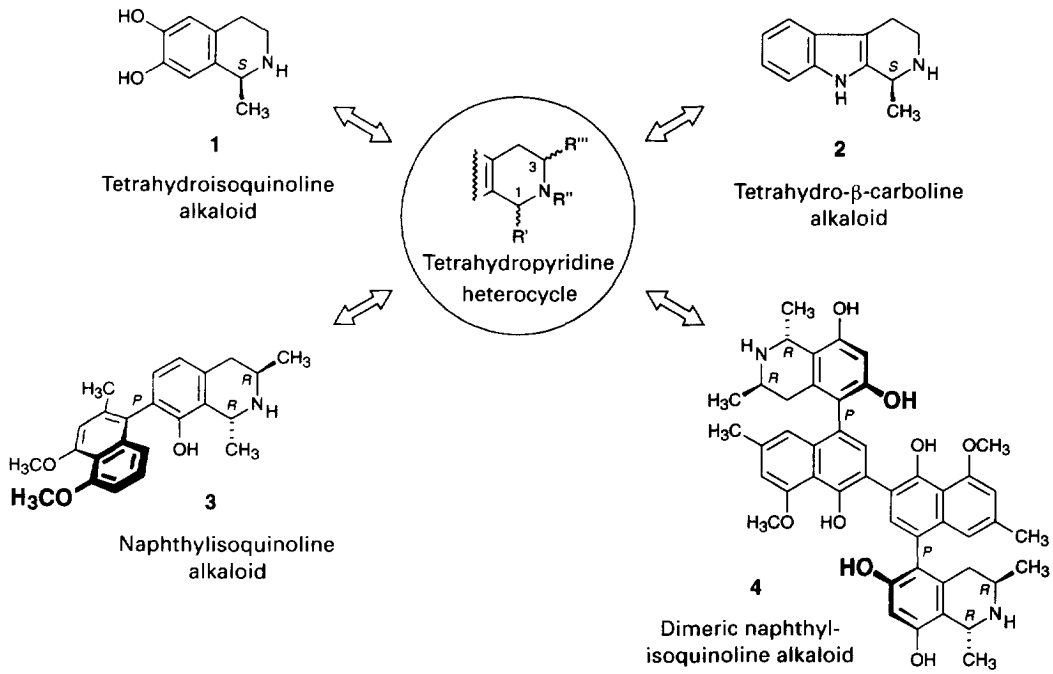
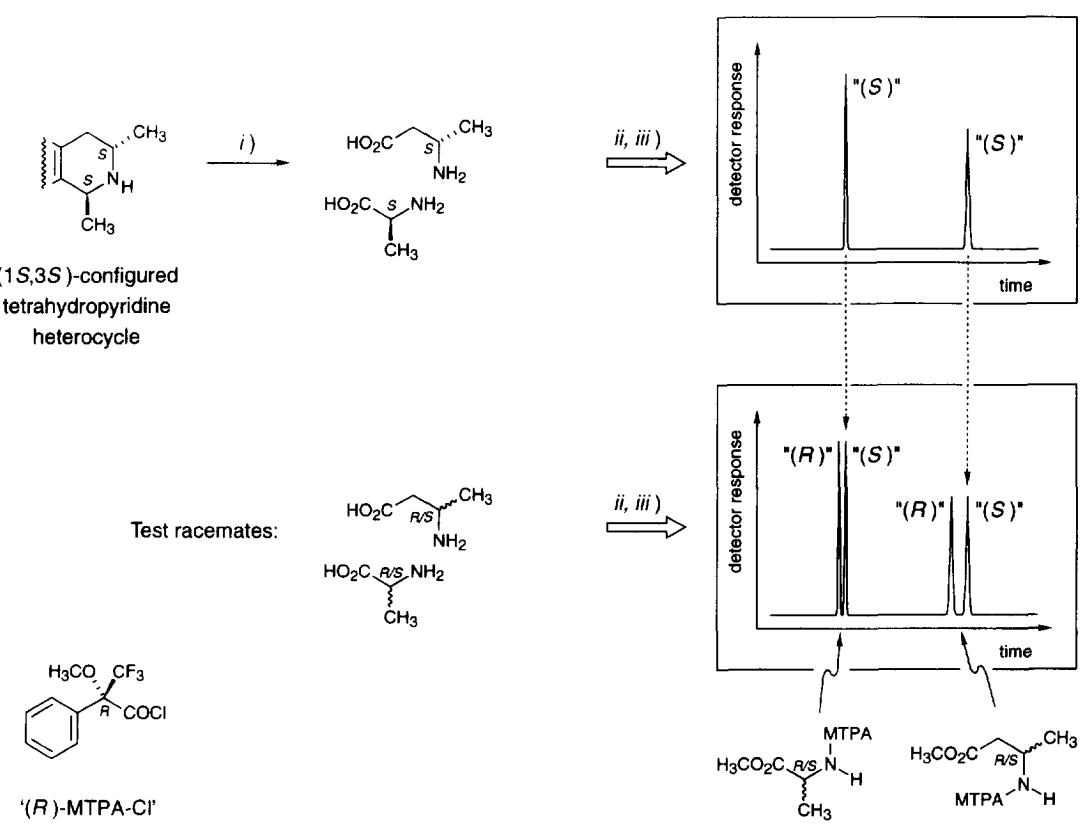


Fig. 1. Chiral tetrahydropyridine heterocycle with stereocentres C-1 and/or C-3 as a structural element of many natural products.



Scheme. 1. Ruthenium-mediated oxidative degradation of the tetrahydropyridine heterocycle and schematic chromatogram of the resulting amino acids after Mosher-type derivatization. (i) $RuCl_3$, $NaIO_4$; (ii) MeOH, $SOCl_2$; (iii) '(*R*)-MTPA-Cl' {'(*R*)-Mosher's chloride' [13]}, NEt_3.

This efficient methodology has become one of the crucial standard tools in the elucidation of the absolute configuration of numerous naphthylisoquinoline alkaloids [14–20]. It proved to be applicable even to the michellamines [9, 10], whose structures had previously been described only with respect to their relative configurations [8]. More recently, we have reported on initial attempts to extend the procedure to the degradative analysis of the similarly naturally occurring *N*-methylated 1,3-dimethyltetrahydroisoquinolines [20, 21].

In view of the many further naphthylisoquinoline alkaloids occurring only in trace amounts, and the great importance of 'normal' (i.e. 3-unsubstituted) isoquinoline alkaloids (e.g. compound **1**) and the similarly large group of β-carbolines (e.g. compound **2**), including 3-carboxy-substituted representatives, we have now further elaborated the procedure, *inter alia* by using a mass-selective detector (MSD) instead of a flame ionization detector (FID), thereby allowing the analytical procedure to be performed with quantities as little as 0.3 mg of alkaloid. We have also extended the procedure to tetrahydroisoquinoline and β-carboline alkaloids in general. The scope and limitations of the procedure are demonstrated with the aid of selected examples.

RESULTS AND DISCUSSION

Before the extension of the degradation procedure to other (i.e. 3-unsubstituted) isoquinoline derivatives and to β-carbolines, an important task was to improve the sensitivity of the procedure and therefore to lower the quantity of analysate required to achieve reliable and unambiguous analytical results. This was attained both by an optimized technique for the degradation procedure and the derivatization of the resulting amino acids, and by a further elaboration of the analytical system, involving a GC–MSD technique.

From such a GC–MSD combination, an enhanced sensitivity of our analytical procedure was anticipated because the MSD allows the detection of pg down to fg quantities and, thus, offers a better response than the FID method previously used [12]. This is of importance both for alkaloids, which are available in small quantities, and for analysates that show formation of one of the two expected amino acids in very low yields, such as *cis*-configured *N*-unsubstituted 1,3-dimethyltetrahydroisoquinolines, which give very little or no alanine [12]. Further, the MSD permits the identification of GC peaks by their mass spectra.

The elaboration of such a GC–MSD analysis requires the availability of comparison material of the corresponding amino acids expected from the degradation reactions. For this reason, we have synthesized the Mosher derivatives **5–11** of alanine, *N*-methylalanine, glycine, 3-aminobutyric acid and its *N*-methyl homologue, β-alanine, and aspartic acid (see Table 1) and recorded their gas chromatograms and EI-mass spectra. The mass numbers of the most abundant ions, together with the GC retention times, are given in Table 1. Fortunately, all of these derivatives of **5–11** can be represented by only four base ions at *m/z* 102, 116, 130, and 160. A reconstructed ion current (RIC) chromatogram, based on the full mass range from *m/z* 50 to 650 is shown in Fig. 2a. By extracting the four ion traces from the RIC, the MSD serves as an efficient filter.

The great selectivity of this filter is impressively documented in the analysis of compounds (*R*)-**8** and **9**, i.e. the derivatives of (*R*)-3-aminobutyric acid and β-alanine, which show virtually identical retention times, thus giving a joint peak in the RIC, but which can clearly be distinguished by their different base ions at *m/z* 102 and 130, respectively (see (a) RIC and (b) extracted ion traces *m/z* 102 and 130 in Fig. 2).

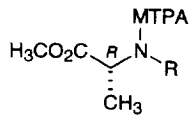
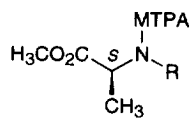
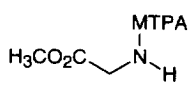
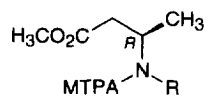
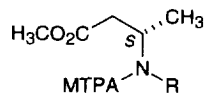
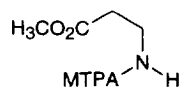
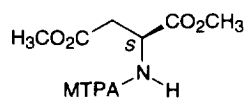
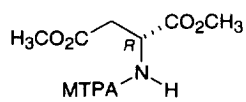
For complex molecules, such as michellamine A (**4**), the degradation procedure leads to significant, additional degradative byproducts. In the GC analysis, their peaks can interfere with those of the relevant amino acid derivatives (see Fig. 3b, top). By using the MSD, the characteristic base ion traces (here for *m/z* 102, see Fig. 3b bottom) can now easily be extracted from the reconstructed ion current. This provides a clear and unambiguous chromatogram of the desired amino acid derivatives, with all the previously interfering peaks being eliminated.

For special purposes, e.g. if only very small quantities of a sample are available for degradation, it is advisable to record the significant base ion traces for the expected amino acid derivatives exclusively, i.e. to analyse in the selected ion monitoring mode (SIM mode), which further enhances the sensitivity of the MSD. Figure 3c represents the unambiguous SIM chromatogram resulting from the oxidative degradation of only 0.5 mg (1.3 μmol) of dioncophylline A (**3**), a typical and well-investigated representative of the naphthylisoquinoline alkaloids [7].

In addition, the 'chemical part' of the analytical method, in particular the derivatization procedure, was further optimized. The amino acids originating from the oxidation of the corresponding alkaloid with $NaIO_4$ in the presence of catalytic amounts of ruthenium(III)-chloride were previously esterified with methanolic HCl [12]. We have now found it much more convenient to perform the esterification by adding thionyl chloride to an ice-cooled methanolic solution of the residue from the oxidation reaction after evaporation of the solvent. This method has practical advantages and gives more reproducible results. In agreement with literature reports [22], no racemization of the resulting amino acid derivatives is observed. After evaporation of the solvent, the second derivatization step is performed by treating the residue with a solution of Mosher's chloride in dichloromethane and triethylamine. The resulting solution can be used directly for GC analysis.

The improved technique was then applied to the stereoanalysis of several selected compounds, starting with some mono- and dimeric naphthylisoquinoline alkaloids. Application of the degradative analysis to

Table 1. Structures, GC retention times (R_t), and most intensive GC–EI-mass spectrum fragment ions (70 eV) of the Mosher derivatives **5–11** prepared from (*R*)-MPTA-Cl

Structure		*R*	R_t (min)	*m/z*	(Rel. int)
	(*R*)-**5**	H	11.4	189 $[M - C_5H_8NO_3]^+$	(39)
	(*S*)-**5**	H	11.6	130 $[M - C_9H_8F_3O]^-$	(78)
				102 $[M - C_{10}H_8F_3O_2]^+$	(100)
	(*R*)-**7**	CH_3	14.2	189 $[M - C_6H_{10}NO_3]^+$	(70)
	(*S*)-**7**	CH_3	14.3	144 $[M - C_9H_8F_3O]^+$	(69)
				116 $[M - C_{10}H_8F_3O_2]^+$	(100)
	**6**		12.5	189 $[M - C_4H_6NO_3]^+$	(42)
				116 $[M - C_9H_8F_3O]^+$	(100)
				88 $[M - C_{10}H_8F_3O_2]^+$	(93)
	(*R*)-**8**	H	15.2	144 $[M - C_9H_8F_3O]^+$	(85)
	(*S*)-**8**	H	15.9	102 $[M - C_{11}H_{10}F_3O_2]^+$	(100)
				59 $[M - C_{13}H_{15}F_3NO_2]^+$	(79)
	(*R*)-**10**	CH_3	19.4	158 $[M - C_9H_8F_3O]^+$	(51)
	(*S*)-**10**	CH_3	19.6	116 $[M - C_{11}H_{10}F_3O_2]^+$	(100)
				59 $[M - C_{14}H_{17}F_3NO_2]^+$	(79)
	**9**		15.3	189 $[M - C_5H_8NO_3]^+$	(16)
				130 $[M - C_9H_8F_3O]^+$	(100)
				88 $[M - C_{11}H_{10}F_3O_2]^+$	(55)
	(*S*)-**11**		25.0		
	(*R*)-**11**		25.9		
				189 $[M-C_7H_{10}NO_5]^+$	(50)
				188 $[M - C_9H_8F_3O]^+$	(68)
				160 $[M - C_{10}H_8F_3O_2]^+$	(100)

dioncophylline A (**3**) gives two significant peaks in the GC–MSD chromatogram mentioned above (see Fig. 3c). By comparison with authentic samples, these two peaks at retention times of 11.4 min and 15.2 min were attributed to (*R*)-**5** and (*R*)-**8**, respectively, i.e. the derivatives of D-alanine and (*R*)-3-aminobutyric acid, thus clearly confirming the 1*R*,3*R*-configuration of this natural product as previously established by spectroscopy, synthetic and X-ray crystallographic procedures [6, 7, 23, 24].

Similarly, formation of the derivatized products (*R*)-**5** and (*R*)-**8** in the degradative analysis of michellamine A (**4**, see Fig. 3b) indicates this antiviral dimeric naphthylisoquinoline to be *R*-configured at all its four stereogenic centres. More recently, related, constitutionally unsymmetric michellamines with two different isoquinoline moieties and, thus, four individual stereocentres, have been analysed unambiguously in a single degradative experiments starting from a sample of less than 1 mg (i.e. $<1.3\ \mu$mol) of these complex molecules [6].

N-Methyldioncophylline A (**12**), a naphthylisoquinoline alkaloid occurring, for example, in *Ancistrocladus abbreviatus* [25] and a typical *trans*-configured 1,3-dimethyltetrahydroisoquinoline with a tertiary nitrogen atom, gives rise to the expected *N*-methyl amino acid derivatives **7** and **10** and, in addition, to the corresponding *N*-demethylated products **5** and **8**; all are exclusively in an *R*-configured form, thus confirming the absolute configuration of this natural product, as established earlier [25]. This enhanced variety of degradative products, which hints at the additional

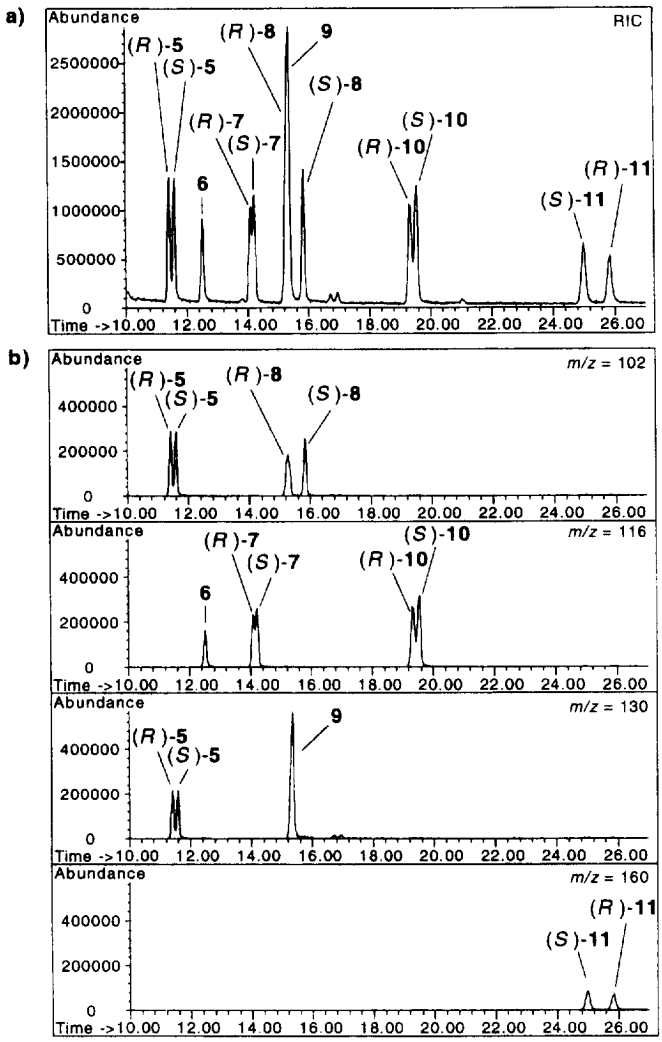


Fig. 2. Chromatogram of amino acid derivative standards illustrated (a) by reconstructed ion current (RIC) and (b) by extracted ion traces of base ions at *m/z* 102, 116, 130 and 160, respectively.

cleavage of the exocyclic CN-bonds, does not diminish the value of the method. In contrast, it gives further support for the 1*R*,3*R*-configuration of compound **12**, and, thus, enhances the significance of the stereochemical attribution.

As an example of 3,4-dihydroisoquinolines with a stereocentre at C-3, ancistrocladinine (**13**) was degraded, clearly confirming the previously established [26] 3*S*-configuration of this natural product, as indicated by the occurrence of compound *S*-**8** in the chromatogram. Unexpectedly, the chromatogram also shows the alanine derivative (*S*)-**5**, in small, but significant amounts, probably arising from the 'C-4/C-3/Me-3/N portion' of the alkaloid, by an extended degradation reaction with additional loss of a carbon atom. With the less sensitive GC-FID method previously used, this phenomenon had not been detected. It again underlines the larger analytic value and significance of the stereoanalysis of C-3 compared with that of C-1, which may be hampered by the additional alanine formation from C-3 and its environment.

A similar effect is observed for isoancistrocladine (**14**), the first natural *cis*-configurated, *N*-unsubstituted tetrahydroisoquinoline [27]. Because of rapid oxidation of C-1 to give compound **13**, compounds of this type had previously been analysed to give 3-aminobutyric acid, exclusively, but no alanine [12]. Figure 4c clearly shows, in addition to the formation of **S-8**, the occurrence of alanine, the *R*-form apparently coming from C-1, the (more abundant) *S*-form arising from an over-oxidation of the 'C-3 part' of the molecule.

The examples show that all sorts of naturally occurring naphthylisoquinoline derivatives with one or two stereocentres and with or without an *N*-methyl group can be analysed stereochemically in a rapid and reliable manner, in all cases with reliable results for C-3 and, for all *trans*-diastereomers, also for C-1.

As a first representative of the 'ordinary', i.e. only

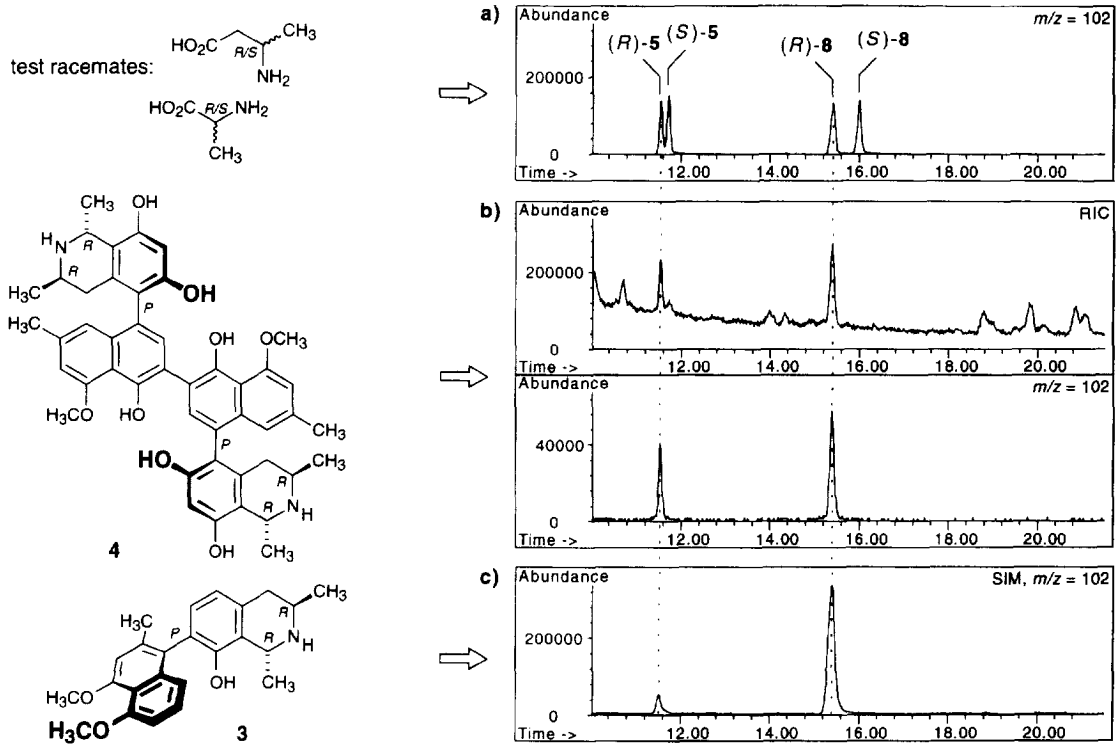


Fig. 3. GC–MSD chromatograms of (a) a test racemate and (b) degradation samples of michellamine A (**4**), illustrated as reconstructed ion current (RIC) and as extracted ion trace at *m/z* 102, respectively, and (c) of dioncophylline A (**3**), recorded in the SIM mode, starting from only 0.5 mg of the alkaloid.

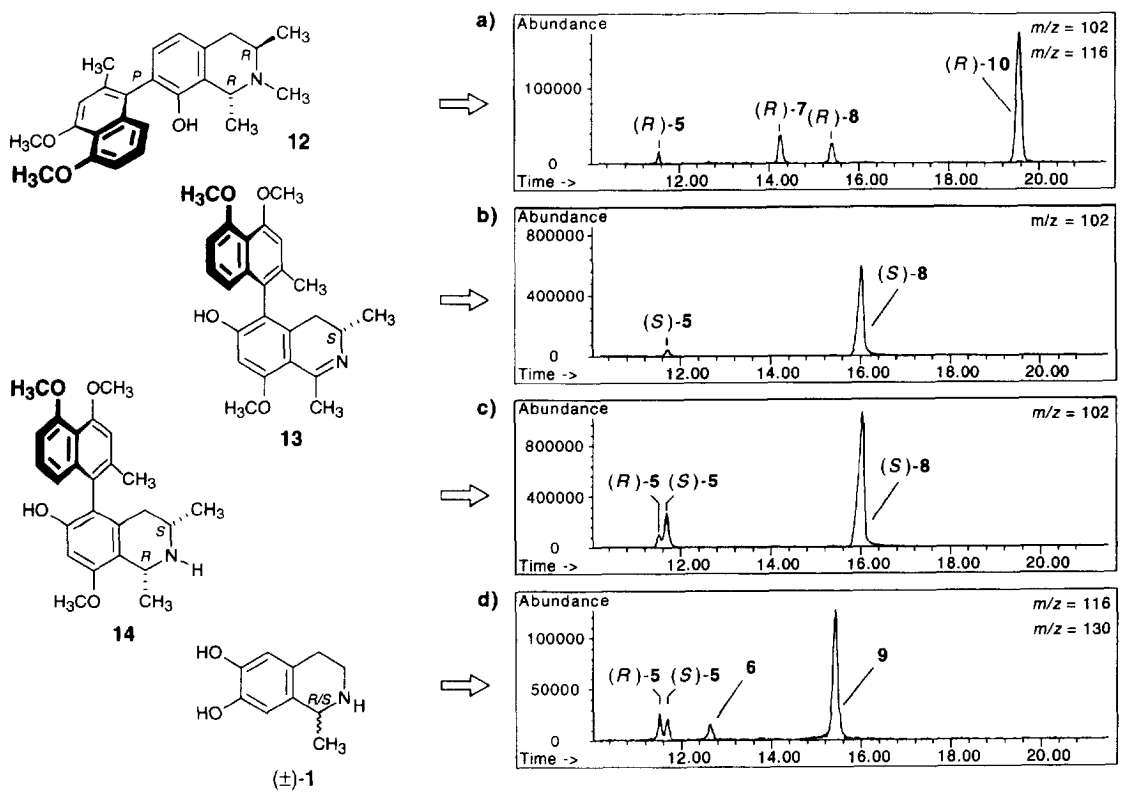


Fig. 4. Degradation of (a) *N*-methyldioncophylline A (**12**), (b) ancistrocladinine (**13**), (c) isoancistrocladine (**14**), and (d) racemic salsolinol (**1**).

C-1-substituted tetrahydroisoquinoline alkaloids, we chose salsolinol (**1**), a well-known alkaloid from plant material [28], and which is also the most prominent representative of endogenous alkaloids in man, formed, for example, in the course of alcoholism [4, 5, 28]. With the absence of a substituent at C-3, the question was whether this compound would, like all *trans*-configurated representatives of the naphthylisoquinoline alkaloids, deliver sufficient amounts of alanine from C-1, or whether it would behave like the *cis*-derivatives, by being rapidly oxidized to the corresponding dihydroisoquinoline, so that no alanine formation would occur. The chromatogram (see Fig. 4d) of its degradation mixture shows the derivatives **9** and **5** of the expected amino acids, β-alanine and alanine, as the most abundant peaks, the latter in an expected 1:1 ratio, because salsolinol (**1**) had been used in its racemic (commercially available) form. Surprisingly, the glycine derivative **6** also appears in the chromatogram, clearly confirming that an additional C, C-bond cleavage in the C-4/C-3/N part of the molecule can occur, which, however, does not weaken the analytical information.

As a first simple model for the structurally related, tryptamine- or tryptophan-derived β-carboline alkaloids, we submitted the parent compound, tryptoline (**15**), to our optimized degradation procedure. The GC–MSD spectrum (see Fig. 5a) clearly shows the Mosher derivatives **6** and **9** of the expected degradation products, glycine and β-alanine, demonstrating that the analysis can likewise be applied to β-carboline derivatives. This is emphasized by the degradation of the 1-methylated analogue eleagnine (tetrahydroharman, **2**), an alkaloid occurring in *Eleagnus angustifolia* L. [29, 30], in its racemic, and thus easily available, form. The chromatogram (Fig. 5b) shows the expected 1:1 mixture of the alanine derivatives (*R*)-**5** and (*S*)-**5**, in addition to the anticipated β-alanine derivative **9**. Again, the formation of glycine, as indicated by compound **6**, hints at an additional C, C-bond cleavage, which does not however affect the diagnostically essential, peaks of the alanine derivatives.

An interesting β-carboline derivative and simultaneously a test case for the applicability for our degradation method to β-carbolines with two stereogenic centres, at C-1 and C-3, is the ester (1*S*,3*S*)-**16**, which was synthesized according to a published procedure [31]. The stereochemical analysis of the *cis*-configured stereoisomer is unambiguous. On the basis of the extraction ion traces of base ions at *m/z* 102 and 160, the formation of two amino acids can be stated, namely L-alanine, as indicated by compound (*S*)-**5**, and L-aspartic acid methyl ester, as shown by the occurrence of compound (*S*)-**11**. This is the first extension of our degradative procedure to stereochemically homogeneous β-carboline derivatives and, in addition, to 3-carboxylated heterocycles of this type. The analysis of a *trans*:*cis* mixture of (1*R*,3*S*)-**16** and (1*S*,3*S*)-**16** shows an additional peak for (*R*)-**5**, thus hitting at the 1*R*-configuration of the minor *trans*-diastereomer. The

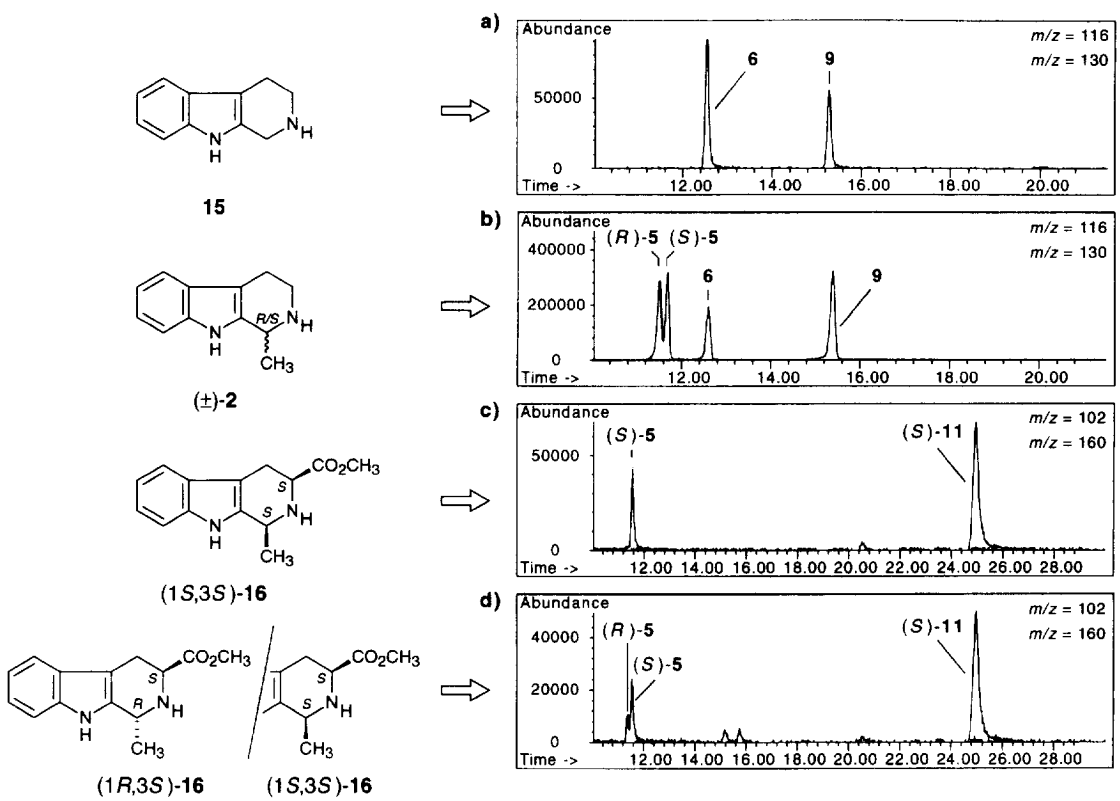


Fig. 5. Degradation of 1- and 1,3-substituted tetrahydro-β-carbolines.

R:*S* ratio of the alanine derivatives **5** of 1:2.6, as determined by integration of the GC peaks very reproducibly reflects the 1:23 diastereomeric ratio of the alkaloids, as previously determined by NMR, showing that stereoisomeric mixtures can also be analysed by our method.

The fact that in this experiment (as in all degradation experiments for naphthylisoquinoline alkaloids) virtually no glycine was formed, clearly shows that the glycine resulting from compounds **1** and **2**, must have come from the 'upper half' (i.e. from C-3 and C-4) of these alkaloids. The hypothesis of such an additional degradation of the C-3/C-4 fragment is rigorously confirmed by the additional formation of aminomalonic acid methyl ester in the degradation of **16**, as identified by its retention time (20.4 min) and the expected mass peaks (*m*/*z* 189, 174 and 146) of the Mosher derivative. As Fig. 5c and Fig. 5d clearly show, this does not influence the stereochemical information deduced from the presence of compounds (*S*)-**5** and (*S*)-**11**.

As an example of alkaloids with chemically less stable side-chains, we chose the chloral-derived β-carboline and isoquinoline derivatives **17**–**19**. These compounds are of considerable current interest because they constitute novel potent neurotoxins acting on the dopaminergic system [32]. In addition, they can be formed by a spontaneous *in vivo* Pictet–Spengler condensation [33] of the corresponding biogenic amines and chloral, which is still administered therapeutically. A reliable stereoanalysis at C-1 would be an important analytical tool for an investigation of whether the condensation reaction *in vivo* occurs spontaneously (hence without stereocontrol) or with enzymatic assistance.

By analogy with the 1-methyl substituted heterocycles degraded above, compounds **17**–**19** should give 3,3,3-trichloroalanine. Unfortunately, however, this highly halogenated amino acid could not be identified in any of these degradation reactions; this may be because of the pronounced instability of this amino acid [34] and of the alkaloid, itself, which is known [2] to easily undergo dehydrohalogenation reactions. However, this does not affect the 'upper part' (i.e. the C-3/C-4 part) of the molecule, as clearly shown by the β-alanine derivative **9** from both compounds **17** and **18**—again accompanied by the further degraded glycine derivative **6**. Of positive stereochemical value is the identification of the (*S*)-aspartic acid derivative (*S*)-**11** from the diastereomeric mixture (1*R*/*S*,3*S*)-**19** and vice versa of compound (*R*)-**11** from its D-tryptophan-derived enantiomers (1*R*/*S*,3*R*)-**19**, clearly showing that this analytical device can be used for the stereoanalysis of C-3, even if C-1 is linked to a chemically unstable group.

In summary, the ruthenium-mediated oxidative degradation with subsequent esterification, Mosher-type

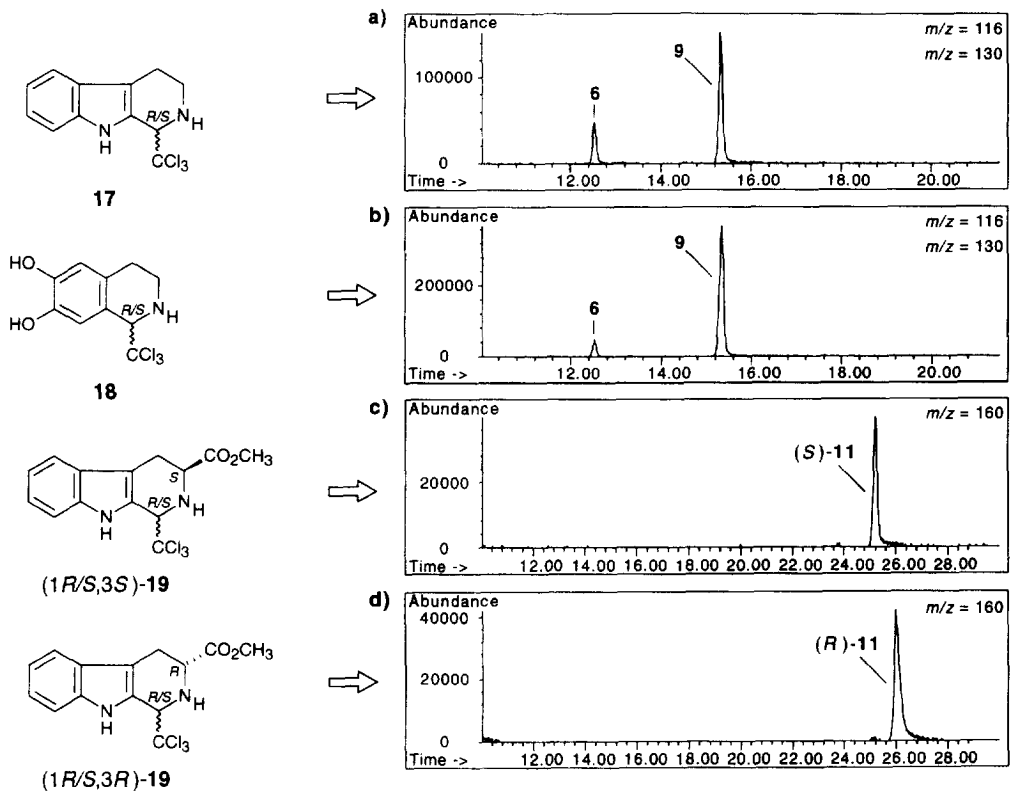


Fig. 6. Degradation of chloral-derived tetrahydro-β-carbolines and isoquinolines: (a) racemic 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (**17**), (b) racemic 1-trichloromethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**18**), and (c) and (d) methyl 1,2,3,4-tetrahydro-1-trichloromethyl-β-carboline-3-carboxylate (**19**).

derivatization and GC–MSD analysis of the resulting amino acids, has become an efficient tool for the unambiguous determination of the absolute configuration of both isoquinoline and β-carboline alkaloids. Thus, the absolute configuration at C-3 and/or C-1 can be established, giving reliable results with quantities of the analysate down to even *ca* μmol quantities. The examples given show that the method is not limited to alkyl (represented by methyl) substituents, but also works for carboxymethyl groups. Even if the stereoanalysis is hampered by the presence of sensitive functional groups (here the trichloromethyl substituent), it still works for the aliphatic remainder of the molecule, as shown for the tryptophan/chloral-derived β-carboline **19**.

EXPERIMENTAL

Capillary GC. GC–MSD analyses were performed on a Hewlett Packard 5890 Series II GC with a Hewlett Packard 5971A MSD. Transfer-line temp. was maintained at 280° resulting in a source temp. of 180°. GC–EI-MS was performed with an ionizing energy of 70 eV. RIC was measured in the mass range *m/z* 50 to 650 and SIM at *m/z* 102, 116, 130, and 160 as given in the text. A nonpolar fused silica capillary column Hewlett Packard Ultra 2, 25 m × 0.32 mm (i.d.) × 0.52 μm (film thickness), crosslinked 5% diphenyl and 95% dimethylpolysiloxane with an on column injector maintained at 210° was used for chromatographic separation of amino acid derivatives. He was used as carrier with a column head pressure of 40 kPa. Column temp. was prog. 100°–160° at 30° min^{-1}, then 160°–190° at 1° min^{-1} and finally increased at 40° min^{-1} to 270°.

Materials and reference compounds. The natural products dioncophylline A (**3**) [7], michellamine A (**4**) [10], *N*-methyldioncophylline A (**12**) [25], ancistrocladinine (**13**) [26] and isoancistrocladine (**14**) [27] were isolated from plant material, respectively, or prepd by partial or total syntheses [23, 25, 35, 36]. (±)-Salsolinol (**1**, HBr salt) and tryptoline (**15**, free base) were purchased from Aldrich. (±)-Eleagnine (**2**) was synthesized from 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid [37] by decarboxylation in boiling HCl. Epimers of Me 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**16**) were synthesized from L-tryptophan Me ester and acetaldehyde according to ref. [31]. Chloral-derived β-carbolines were available from previous synthetic work. 1-Trichloromethyl-1,2,3,4-tetrahydro-β-carboline (**17**) [38] and methyl 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**19**) [39] were synthesized from tryptamine, L- or D-tryptophan Me ester and chloral, respectively, according to procedures described earlier. 1-Trichloromethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**18**) was prepd by condensation of dopamine with chloral [40]. The catalyst $RuCl_3 \cdot H_2O$ was purchased from Heraeus Feinchemikalien und Forschungsbedarf GmbH, Germany. 0.1 M Na-Pi buffer was prepd by dissolving 1.38 g (10 mmol) $NaH_2PO_4 \cdot H_2O$ in 100 ml water and adjusting pH to 6 with 0.1 M NaOH. A soln of (*R*)-α-methoxy-α-trifluormethylphenylacetic acid chloride [(*R*)-MTPA-Cl] was prepd by refluxing 469 mg (2 mmol) (*S*)-α-methoxy-α-trifluormethylphenylacetic acid [(*S*)-MTPA] in 10 ml freshly dist. $SOCl_2$ for 1.5 hr. After evapn of $SOCl_2$, the residue was dissolved in 10 ml dry CH_2Cl_2 resulting in a *ca* 0.2 M soln of (*R*)-MTPA-Cl. (Note, in this publication the α-methoxy-α-trifluormethylphenylacetic acid chloride, freshly prepared from (*S*)-α-methoxy-α-trifluormethylphenylacetic acid was used. Because of the Cahn–Ingold–Prelog rules, the corresponding chloride has to be denoted as the (*R*)-, not the (*S*)-isomer.) Alanine, *N*-methylalanine and aspartic acid in (±)- or enantiomerically pure form, (±)-3-aminobutyric acid, glycine, aminomalonic acid DiMe ester and β-alanine were purchased from Sigma. (*S*)-3-aminobutyric acid was prepd as described in ref. [12]. (±)-*N*-methyl-3-aminobutyric acid was prepd by methylation of the corresponding (±)-3-aminobutyric acid. Assignment of the (*R*)- and (*S*)-enantiomer was done by degrading configuratively known (since synthetically prepd [25]) *N*-methyldioncophylline A (**12**) with subsequent Mosher-type derivatization and GC co-chromatographic expts, providing (*R*)-*N*-methyl-3-aminobutyric acid. All GC standards were prepd by treatment of the amino acids according to the described derivatization procedure.

General procedure for oxidative degradation on an analytical scale. Reactions were performed in 2.5 ml Wheaton screw-cap vials. Analyte (10 μmol) and 0.1 mg (0.4 μmol) $RuCl_3 \cdot H_2O$ (as catalyst) were added with stirring to a two-phase mixt. consisting of 100 μl MeCN, 100 μl CCl_4 and 200 μl 0.1 M Na-Pi buffer (pH 6) at room temp. Over a period of 60 min, 25.7 mg (120 μmol) $NaIO_4$ were added in several portions and the mixt. stirred at room temp. for another 1.5 hr. For extraction of the resulting amino acids, 700 μl of H_2O were added and after a short period of additional stirring, the aq. phase was sepd, washed ×2 with 300 μl portions of $CHCl_3$ and subsequently lyophilized. The residue was extracted with 1.5 ml of dry MeOH followed by sepn of the insol. inorganic salts by centrifugation, thus yielding a MeOH soln of amino acids, which was used for derivatization.

Derivatization procedure. For esterification, 70 μl (0.1 mmol) of freshly dist. $SOCl_2$ were added dropwise at 0° to the MeOH soln of the amino acids with vigorously stirring. The mixt. was then allowed to stand at room temp. for 12 hr and another portion of 70 μl of $SOCl_2$ was added in the same manner. After 6 hr standing at room temp. and evapn of solvent, the residue was suspended in 500 μl dry CH_2Cl_2 and 100 μl of a 0.2 M soln of (*R*)-MTPA-Cl in CH_2Cl_2 were added followed by up to 20 μl NEt_3 until the mixt. became alkaline. After stirring for 30 min, 1 μl of the resulting mixt. was used directly for GC analysis.

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