Utility of the Cleavage of Nitrosamides for the Preparation of Chiral Acids after Chromatographic Separation of Their Diastereomeric Amides

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Optical Resolution, Nitrosamide, Amide Cleavage

A method for optical resolution of chiral acids is described. It consists of the conversion of racemic acids to diastereomeric amides, their chromatographic separation and subsequent deamination via the nitrosamide route. Reaction conditions for cleavage of amide derivatives of phenylalanine and methylbenzylamine are given. No or only negligible racemization of carboxylic acids, chiral in α-position takes place under those conditions. The extent of E,Z-isomerization of double bonds is very small, as is the extent of double bond migration from the α-position into conjugation with the carboxyl function. Enantiomerically pure R- or S(2-3H)2-methylbutanoic acid and (2)-methyl-3(p-chlorophenyl)-2-chloropropionate (Bidisin®) were prepared by this procedure.

Separation of the enantiomers of chiral carboxylic acids presents still a problem, which frequently can only be solved by fractionated crystallization of diastereomeric salts obtained with chiral bases. Although some progress has been made in the optical resolution of chiral acids by preferential crystallization1-2, by enzymatic methods3,4 or even more so by use of chiral chromatography phases5,6 these methods are so far rather limited in scope. More generally applicable procedures, like the formation of diastereomers and their separation by modern chromatographic technics are therefore still of importance. One of these methods is the amidation of racemic acids with commercially available chiral amines like L-phenylalanine ester, α-methylbenzylamine or phenylglycine ester and subsequent chromatographic separation of the diastereomeric amides. In certain cases this even permits the deduction of optical configurations7. In spite of the obvious advantages of this resolution method, only very few preparative examples have been documented in the literature8, mainly due to the lack of appropriate procedures, which cleave the separated amides without racemization8. Our investigations on the stereospecific hydrogenation of unsaturated acids by microorganisms9,10 required the synthesis of both enantiomers of [2-3H]2-methylbutanoic acid. Since the procedure for optical resolution of this acid11 did not appear suitable for the synthesis of the tritiated derivatives in terms of yield and effort resolution via diastereomeric amides offered a reasonable alternative provided high yield and mild cleavage conditions could be found. The second prerequisite, the chromatographic separation of diastereomeric amides on a preparative scale, can generally be achieved by HPLC.

Preliminary amide hydrolysis experiments had shown, however, that under standard conditions (4 N HCl, reflux) [2-3H]-2-methylbutyrphenylalanine lost 30% of its tritium in 4 h with hydrolysis far from being complete. Enzymatic hydrolysis with common peptidases (subtilisin novo EC 3.4.4.16; carboxypeptidase A EC 3.4.2.1) could not be achieved and hog renal acylase (EC 3.5.1.14) which is frequently used in analogue cases3 displays substrate specificity for acylated aliphatic amino acids only and would not attack acylphenylalanine derivatives. Therefore a mild chemical cleavage reaction was necessary for the optical resolution. HUTSCHENRoth's studies12,13 on nitrosamides and their thermal or alkaline decomposition led us to suspect amide cleavage via nitrosamides to meet the...
requirements as to mildness and applicability. We were particularly interested in the extent of racemization of chiral centers adjacent to a carboxyl function and in the configurational and positional stability of $\Delta^2$ or $\Delta^3$ double bonds under the cleavage conditions.

**Results and Discussion**

Nitrosamides can be split by two different reaction pathways (Scheme 1): Thermal cleavage is based on the rearrangement of the nitrosamide (2) to the diazoester (3), which decomposes to a close ion pair 4. Depending on $R'$ the ion pair may decay either by nitrogen extrusion to the ester 7 or undergo proton tautomerisation to give the acid 5 and the diazo compound 6 (route II and I in Scheme 1). The product ratio of 5 to 7 can be expected to be highest if $R'$ stabilizes the diazo group, because the rate of nitrogen elimination would be lowered and therefore reaction II disfavoured. Thus thermal decomposition of N-nitroso derivatives of acylphenylalanines (2a-21) or acylphenylglycines, in which an adjacent ester function can stabilize the diazo group, should give better yields of acid 5 than the corresponding phenethylamine derivatives (2g-2m).

As an alternative cleavage reaction we used alkaline methanolysis, which yields the corresponding methyl esters (9). Catalytic quantities of methanol were sufficient to solvolyze nitrosophenethylamides (2g-2m), because the decomposition of the primarily formed unstabilized diazotates regenerates the initially used methanolate. In contrast equimolar quantities alkali were necessary for cleavage of compounds 2a-2f due to stabilization of their diazo groups.

The third possibility of nitrosamide cleavage — acid hydrolysis — was not studied, because regardless of $R'$ deamidation is always accompanied by denitrosation and thus low yields of acids 5 can be anticipated.

The amides 1a-1k were prepared by standard procedures of the Schotten-Baumann reaction or — for the more sensitive ones — the DCC-N-hydroxysuccinimide coupling (cf. Experimental). According to our experiences nitrosation was best achieved by treating the amides with an excess of nitrosating agent, prepared by reaction of NOCl with anhydrous potassium acetate in ether at 0 °C. These conditions gave comparable results in yield and purity of product as dinitrogentetroxide, which has been recommended by White. The results of the amide cleavage sequence are summarized in Table I.

Neither thermal nor alkaline cleavage conditions shift a double bond of an aliphatic acid from the $\Delta^3$-position into conjugation with the carboxyl function (expts. 1, 2). In contrast to the excellent yields of reisolated acid in experiments 1 and 2, the yields of the structurally related $\Delta^3$-4-phenylbutenoic acid are only moderate (expts. 3-7). Thermal decomposition of the isolated and purified nitrosamide (2b), however, yielded $\Delta^3$-4-phenylbutenoic acid quantitatively (expt. 5). Obviously not cleavage but nitrosation was the yield limiting step of the overall reaction. Apparently the reagent attacked the amide nitrogen as well as the particularly activated methylene group and was thus
Tab. I. Reaction conditions and yields of the amide cleavage via nitrosamides.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Substance</th>
<th>Cleavage conditions</th>
<th>T [°C]</th>
<th>t [min]</th>
<th>Yield [%]</th>
<th>Yield of isomers [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>Benzene</td>
<td>80</td>
<td>80</td>
<td>87b</td>
<td>&lt; 0.02 A²-acid</td>
</tr>
<tr>
<td>2</td>
<td>1g</td>
<td>CH₃ONa/CH₃OH</td>
<td>20</td>
<td>2</td>
<td>95c</td>
<td>&lt; 1 A²-ester</td>
</tr>
<tr>
<td>3</td>
<td>1b</td>
<td>Xylene</td>
<td>150</td>
<td>5</td>
<td>68d</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1b</td>
<td>K₂CO₃/CH₃OH</td>
<td>20</td>
<td>60</td>
<td>45c</td>
<td>&lt; 1 A²-ester</td>
</tr>
<tr>
<td>5</td>
<td>1b</td>
<td>Benzene</td>
<td>80</td>
<td>90</td>
<td>100% e</td>
<td>&lt; 0.1 A²-ester</td>
</tr>
<tr>
<td>6</td>
<td>1h</td>
<td>K₂CO₃/CH₃OH</td>
<td>20</td>
<td>15</td>
<td>57c</td>
<td>&lt; 1 A²-ester</td>
</tr>
<tr>
<td>7</td>
<td>1h</td>
<td>Benzene</td>
<td>80</td>
<td>130</td>
<td>24d</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1e</td>
<td>Toluene</td>
<td>80</td>
<td>90</td>
<td>91d</td>
<td>4 trans d</td>
</tr>
<tr>
<td>9</td>
<td>1e</td>
<td>CH₃ONa/CH₃OH</td>
<td>20</td>
<td>30</td>
<td>74d</td>
<td>3 trans d</td>
</tr>
<tr>
<td>10</td>
<td>1i</td>
<td>Toluene</td>
<td>80</td>
<td>30</td>
<td>42g f</td>
<td>2 trans d</td>
</tr>
<tr>
<td>11</td>
<td>1i</td>
<td>CH₃ONa/CH₃OH</td>
<td>20</td>
<td>3</td>
<td>70c</td>
<td>3 trans e</td>
</tr>
<tr>
<td>12</td>
<td>1d</td>
<td>Benzene</td>
<td>80</td>
<td>120</td>
<td>91b</td>
<td>&lt; 4 antipode g</td>
</tr>
<tr>
<td>13</td>
<td>1k</td>
<td>Toluene</td>
<td>110</td>
<td>900</td>
<td>53b</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1e</td>
<td>Xylene</td>
<td>150</td>
<td>5</td>
<td>74b</td>
<td>2.3 trans</td>
</tr>
<tr>
<td>15</td>
<td>1l</td>
<td>CH₃ONa/CH₃OH</td>
<td>30</td>
<td>3</td>
<td>70b</td>
<td>4 antipode</td>
</tr>
<tr>
<td>16</td>
<td>1f</td>
<td>Toluene</td>
<td>100</td>
<td>60</td>
<td>85b</td>
<td>&lt; 1 zero antipode</td>
</tr>
<tr>
<td>17</td>
<td>1m</td>
<td>CH₃ONa/CH₃OH</td>
<td>30</td>
<td>3</td>
<td>81b</td>
<td></td>
</tr>
</tbody>
</table>

a Yields of carboxylic acids or methylesters, respectively, based on starting amide.
b Isolated acid or ester, respectively.
c Determined by quantitative GC-analysis.
d Determined by UV-absorption after separation by high performance liquid chromatography.
e After esterification with diazomethane.
f 42% of ester 7h isolated.
g Estimated by tritium exchange.

Responsible for considerable resinification. The small yield of the thermal decomposition of compound 1h (expt. 7) reflects the preferential formation of ester 7h.

For investigation of the question, whether Z-double bonds retain their configuration under deamidation conditions, the particularly easily isomerizable Z-cinnamic acid derivatives were subjected to the cleavage reactions. Thermal as well as alkaline decomposition gave high yields of Z-cinnamic acid and less than 4% isomerization to the E-isomer (expts. 8-11).

For estimation of racemization of α-branched carboxylic acids the exchange of tritium from the α-position of 2-methylbutanoic acid was to be measured. The required [2-³H]2-methylbutanoic amides (1d) and (1k) were obtained by the following sequence (Scheme 2).

Scheme 2. Synthesis of R(−)- or S(+)[2-³H]2-methylbutanoic acid.

![Scheme 2](image-url)
The specific radioactivity of \( \text{Id} \) was estimated after careful chromatographic purification and distillation. The \((2R)[2-\text{3}^\text{H}]2\)-methylbutanoic acid as well as the \((2S)\) enantiomer obtained after nitrosoation, thermal deamidation, chromatography on silica gel and steam distillation showed the identical specific radioactivity (expt. error \( \leq 4\% \)) (expt. 12). Thermal decomposition of \( \text{Ik} \) gave only 55\% 2-methylbutanoic acid due to the ester formation already mentioned and the yield could not be increased by prolonged refluxing, which had been successful in analogous cases\(^{17}\). Since no racemization could be detected on deamidation of \( \text{Id} \), the diastereomeric amides \( \text{Id} \) were separated by preparative TLC and subjected to the amide cleaving sequence. After chromatographic purification 0.6 mMol each of the enantiomeric \([2-\text{3}^\text{H}]2\)-methylbutanoic acid were obtained (spec. act. 1.39 mCi/mMol; \( S(+)\)[\(\alpha\])\(D\) \(+ 19.6\); \( R(−)\)[\(\alpha\])\(D\) \(− 19.2\)). The estimation of racemization by tritium exchange is inherently falsified by kinetic isotope effects, which gear down the true results but are not exactly known \textit{a priori}. In contrast racemization tests based on measuring the optical rotation do not suffer from this flaw and in addition may be very sensitive if the substrates possess high specific rotations. To check the limits of the deamidation method via nitrosamides which are marked by racemization of chiral carboxylic acids we chose \(Z\)\(13\)-2-ethyl-4-phenylbutanoic acid (12), because of its high racemization sensitivity due to the special activation of the \(\alpha\)-position. In addition \(E/Z\) isomerization and double bond migration could be tested likewise, which made 12 a valuable touchstone for our method. The \(R(−)\) enantiomer of this acid is one hydrogenation product of racemic 2-ethyl-4-phenylallencarboxylic acid\(^{18}\) (10) with hydrogen gas and \textit{Clostridium kluyveri}\(^{10}\), the other being the acid 11 with the same configuration at carbon-2 but with the alternative \(E\)-geometry of the double bond. 12 may be separated cleanly from the accompanying \(E\)-isomer by HPLC. Presumably based on a chiral torsion of its \(\pi\)-system 12 possesses the extraordinarily high specific rotation of \([\alpha]\)\(_{D} = −2500\).
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(p-chlorophenyl)-2-chloropropionate in 81% yield (expt. 17). Biological experiments with this material are in progress.

The reactions described above consequently are gentle procedures for cleavage of secondary carboxylic amides, which proceed without racemization, give good yields of acids or methyl esters, respectively, and do not isomerize double bonds. Together with the efficient chromatographic separation of diastereomeric salts this method combines to a rather useful procedure for optical resolution of chiral acids. Although chirality of the amine moiety is lost during reaction, the enantiomerically pure amines are sufficiently inexpensive not to inhibit broad application. We consider this procedure superior to the time consuming and high-loss optical resolution of chiral acids via crystallization of diastereomeric salts.

Experimental

Materials and methods

All chemicals used were reagent grade. The solvents were carefully dried and distilled. NMR spectra were recorded on a Varian T-60 instrument, with TMS as internal standard. ORD measurements were performed on a J-5 spectropolarimeter of Japan Spectroscopic Co. For radioactivity measurement a Betascint 5000 liquid scintillation counter was used. Aqueous samples were counted in a pump equipped with a U6K injector. The preparative separation of the diastereomeric pair (Im) was achieved according to the parameters given in Table II. All separations on an analytical column (4 x 1000 mm) with a resolution > 1.2 can be done on a preparative scale, too.

General procedure for nitrosation of amides 1a-k

All operations were conducted in the cold room with prechilled reagents. A solution of 0.5 mMol amide in 2 ml diethylether of 0 °C was added under magneting stirring to a prereacted mixture (30 min, 0 °C) of 1.5 mMol NOCl (300 Mol%) and 2 mMol powdered anhydrous potassium acetate in 2 ml ether. After 25 min at 0 °C the nitrosation was stopped by addition of 2 mMol urea and the ethereal suspension transferred to a 15 ml centrifugetube. Shaking the yellow suspension with 2 M bicarbonate solution (2 ml), separation of layers by centrifugation, washing the ethereal phase with saturated NaCl.

Table II. Chromatographic data of the separation of diastereomeric amides and of E- and Z-cinnamic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Size μMol/solvent</th>
<th>Flow rate ml/min</th>
<th>Pressure bar/psi</th>
<th>Retention time [min]</th>
<th>Capacity factor k' = (t—t0)/t0</th>
<th>Resolution^</th>
<th>Lowest detect. amount (nMol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1d</td>
<td>3.2/5 μl n-Propanol 3.60</td>
<td>220/3100</td>
<td>[S, S]</td>
<td>33.7</td>
<td>6.02</td>
<td>1.35</td>
<td>150</td>
</tr>
<tr>
<td>1m</td>
<td>0.25/10 μl CCl4 2.0</td>
<td>245/3500</td>
<td>[R, S]</td>
<td>23.3</td>
<td>4.26</td>
<td>1.28</td>
<td>10</td>
</tr>
<tr>
<td>1e</td>
<td>0.32/25 μl CCl4 2.0</td>
<td>245/3500</td>
<td>[S, S]; [R, S]</td>
<td>28.5</td>
<td>4.00</td>
<td>0.83</td>
<td>60</td>
</tr>
<tr>
<td>Cinnamid^d</td>
<td>0.025/5 μl CH3OH 0.70</td>
<td>190/2700</td>
<td>Z</td>
<td>17.2</td>
<td>1.58</td>
<td>1.44</td>
<td>5</td>
</tr>
</tbody>
</table>

^a Resolution = 2 (t2—t1)/6w2 + w1, w = band width at the intersection of the tangents of the inflection points of Gaussian peaks with the base line.
^b Merckosorb Si 100/10 μm particle size; column 4 mm i.d. x 1000 mm; eluent: iso-octan + 0.5% n-propanol.
^c Maximum sample size for one separation on a preparative column (12 mm i.d. x 500 mm) about 60 μMol.
^d Aminex A-25; Cl-form; column 4 mm i.d. x 500 mm; eluent: 0.2 M NaCl in CH3OH:H2O = 70:30 adjusted with HCl to pH = 3.0.
^e The aeryl residue was obtained by biological hydrogenation^9 (results to be published).
solution was followed by drying with MgSO$_4$ and evaporation of the solvent in vacuo. The residue was subjected directly to one of the decomposition conditions. The workup should be completed within 10–15 min to keep spontaneous decomposition of sensitive nitrosamides at minimum.

**Thermal decomposition of nitrosamides 2a–f**

The residue obtained from nitrosation of the amides 1a–f was dissolved and heated under reflux for the periods stated in Table I. Generally, the reaction was monitored by TLC (hexane/ether 1:1 vol) by disappearance of the nitrosamide spot and appearance of diazoester (6). After completion of the decomposition the chilled intensely yellow solution was extracted with icecold 1 M sodium carbonate solution (5 × 1 ml) (emulsions were broken by centrifugation). Washing of the aqueous phase with benzene (2 × 3 ml) and subsequent cautious acidification (4 N H$_2$SO$_4$) resulted in precipitation of the acid 5, which was dissolved in ether. Washing the etherphase with water (2 × 2 ml), drying (MgSO$_4$) and warming to 50 °C for 4 h. The upper layer was taken up in 1 ml THF and added slowly to 1.62 g (7.5 mMol; 150 Mol%) L-phenylalanine methylester hydrochloride, dissolved in 10 ml pyridine and 1.05 ml triethylamine. The amidiﬁcation was stopped after 30 min at 0 °C and another hour at room temperature by stripping off the solvent in vacuo. The oily residue was partitioned between CH$_2$Cl$_2$ and water (20/20 ml), the organic layer was washed with 1 N HCl (3 × 10 ml), 2 M bicarbonate (2 × 10 ml), saturated NaCl solution and dried with MgSO$_4$. Evaporation of the solvent left a residue which on Kugelrohr distillation led to 1.016 g (3.87 mMol; 77%) of a colourless slowly solidifying oil.

Chromatographic separation was achieved on thick layer plates (2 mm; Kieselgel PF$_{254}$, Merck, load: 1.5 mg mixture/cm) on threefold development with hexane/ether (1:1 vol) at 4 °C. The product bands were scraped off and eluted with ether.

The S,S-diastereomer of 1d moved faster under these conditions than the R,S-isomer.

**NMR (CDCl$_3$, ppm):**

7.24 (m, 5 H, arom. H); 6.07 (broad d, J = 7 Hz, 1 H, N–H); 4.90 (doublet J = 8 Hz of tripletts J = 6.5 Hz, 1H, CH–COOCH$_3$); 3.69 (8 H, COOCH$_3$); 3.11 (d, J = 6 Hz, 2 H, NH–CH–CH$_2$); 2.1 (m, 1 H, CH$_2$–CH–CH$_3$); 1.40 (m, 2 H, CH$_3$–CH$_2$–CH) 1.06 (d, J = 6.5 Hz, 3 H, CH–CH$_3$); 0.85 (t, J = 7 Hz, 3 H, CH$_3$–CH$_3$).

Both diastereomeric amides gave nearly identical NMR spectra, with the only exception, that the terminal methyl group of the R,S-isomer showed up 0.05 ppm upfield of the corresponding signal of the S,S-isomer.

Each of the separated diastereomeric amides (0.9 mMol) were subjected to the general thermal cleavage procedure described above. Purification of the crude acid was achieved by chromatography on silicic acid (from 20 g Kieselgel “Malinckrodt” and 10.5 g H$_2$O) according to Marvel and Rand.$^{19}$ The main fractions containing 2-methylbutanoic acid were collected, titrated with 0.1 N NaOH and the solvent evaporated. Subsequent steam distillation from a solution consisting of 50 ml saturated MgSO$_4$ solution, 5 ml concentrated sulfuric acid and 10 ml water gave ~0.6 mMol each of the enantiomeric [2-3H]2-methylbutanoic acids, which were pure by GC on a 2 m NPGS-column.

$R$ (−) [α]$_D$ = +19.6° [specific radioactivity]

S (+) [α]$_D$ = −19.2° [3.09 × 10$^{-6}$ dpm/mMol].

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