Sulfonyl Halides as Modifying Reagents for Polypeptides and Proteins

IV. On the Conversion of 2-Thio-Aryl-Tryptophan to 2-Hydroxy-Tryptophan

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The conversion of 2-thio-aryl-tryptophan and related derivatives to the corresponding 2-hydroxy-compounds by acidic hydrolysis was investigated. The thioether function is introduced into the tryptophan residue by the selective reaction of sulphenyl halides with the indole nucleus. The substitution occurs under mild conditions only when the amino group of the tryptophan residue is free. The assistance of the amino group was confirmed using model compounds. Thus 2-thio-(2-nitrophenyl)-β-indolyl-propionic acid is completely stable under the same conditions. The conversion of 2-thio-aryl-tryptophan units linked in a polypeptide chain to the corresponding 2-hydroxy-tryptophan occurs only under conditions during which hydrolysis of the peptide bond occurs. It was also observed that the 2-nitro-thiophenols which are released during the hydrolysis of 2-thio-(2-nitrophenyl)-tryptophan and related compounds are converted under the conditions of the reaction to 2-amino-benzene sulfonic acids.

Recent work 1-3 has shown that sulfonyl halides are selective modifying reagents for polypeptides and proteins. In acidic solution the only aminoacids encountered in the preparation of analytically pure symmetric 2,2'-disulfides by reaction of tryptophan peptides and sulfur dichloride.

This behaviour is related to that observed by Wieland and Witkop 4 and Wieland et al. 5-7 in polypeptides of Amanita Phalloides, in which the tryptophan residue is linked to the sulfur atom of cysteine in the 2-position of the indole nucleus. Hydrolysis of these peptides in aqueous sulfuric acid yields Trp-OH and cysteine.

The introduction of the hydroxy-function into the 2-position of tryptophan by mild acid hydrolysis of the corresponding symmetric 2,2'-disulfides was early used to prepare Trp-OH 8 and 2-hydroxy-tryptamine 9. Our experience, in agreement with the results of Freter et al. 10, indicates that difficulties are encountered in the preparation of analytically


* Abbreviations: Trp-OH, 2-hydroxy-tryptophan; Trp(NPS), 2-thio-(2-nitrophenyl)-tryptophan; Trp(pNPS), 2-thio-(4-nitrophenyl)-tryptophan; Trp(DNPS), 2-thio-(2,4-dinitrophenyl)-tryptophan; Z, carbobenzoxy; NPS, 2-nitrophenyl-sulfenyl; NPS, 4-nitrophenylsulfenyl; DNPS, 2,4-dinitrophenyl-sulfenyl. The other abbreviations are those recomme

sulfenyl chloride (DNPS-Cl) react quantitatively with tryptophan-containing peptides, we investigated the possibility of converting these 2-thio-aryl-compounds to their corresponding Trp-OH-derivatives.

First the influence of pH and temperature on the displacement of the thioaryl-function of tryptophan was examined.

Fig. I shows the influence of pH on the conversion of Trp(pNPS) to Trp-OH after a 30 minutes period of hydrolysis in sealed tubes at 100°C in buffered solutions, as determined by spectrophotometric reading of Trp-OH after chromatographic separation of the reaction mixture. The rate of conversion is fastest at pH 1–2. Similar results were obtained in the hydrolysis of Trp(NPS).

![Fig. I. The pH-profile of the yield of Trp-OH by hydrolysis of Trp(pNPS). The experiments were carried out as described in the text.](image)

Hydrolysis could be equally well performed in 20% acetic acid. These conditions were preferred not only for the better solubility of the tested compounds, but also because the interference of the salts of the buffer during the chromatographic separation was avoided.

These mild acidic conditions of hydrolysis were routinely used on a preparative scale; Trp-OH was isolated in high yields and in analytically pure form from Trp(pNPS) or Trp(NPS).

The rate of hydrolysis of 2-thio-aryl-tryptophan derivatives at 95°C and 120°C is shown in Fig. II. Whereas Trp(pNPS) and Trp(NPS) release Trp-OH practically at the same extent, Trp(DNPS) is hydrolyzed comparatively slowly.

On the other side hydrolysis was not observed in the case of tryptophan derivatives in which the α-amino group was acylated or aminoacylated. Thus while acidic hydrolysis (20% acetic acid, 2–4 hrs, 100°C) of Trp(NPS)-OMe·HCl* or Trp(NPS)-Gly yielded the 2-hydroxy-derivatives, as judged by chromatographic analysis and UV spectra, N-acetyl-Trp(NPS), N-carbobenzoxy-Trp(NPS), N-carbobenzoxy-Trp(DNPS), Phe-Val-Gln-Trp(NPS)-Leu, and Phe-Val-Gln-Trp(DNPS)-Leu were completely stable under the same conditions. In the case of Ala-Trp(NPS)-OMe·HCl the thio-aryl-moiety was stable under the above conditions, although conversion to the corresponding diketopiperazine was observed.

These results indicate that the conversion of 2-thioaryl-tryptophan to Trp-OH occurs only when the amino group of the tryptophan residue is free. The assistance of the amino group was further confirmed using 2-thio-(2-nitrophenyl)-tryptamine, which was smoothly converted to 2-hydroxy-tryptamine, whereas 2-thio-(2-nitrophenyl)-β-indolyl-propionic acid was completely stable under the same conditions.

These results which suggest the assistance of the α-amino group explain the difficulties encountered by Free et al. in the conversion of the disulfide of lysergic acid and its diethylamide to the hydroxy-derivatives.

This interpretation was further supported by the conversion of N-acetyl-Trp(NPS), Ala-Trp(NPS)-OMe·HCl and Phe-Val-Gln-Trp(NPS)-Leu to Trp-OH under conditions (25% H2SO4, 15 hrs, 110°C) which cause hydrolysis of the peptide bond with the liberation of the amino group of the tryptophan residue. The lower yields (32–65%; see Table I) of Trp-OH are due to the instability of this compound in strongly acidic media.

* This compound by hydrolysis in this conditions is converted to Trp-OH.
was possible to obtain 4,4'-dinitrophenyl-disulfide in quantitative yield. On the other hand 2,2'-dinitrophenyl-disulfide was obtained only in small amount (7% yield) in the hydrolysis of Trp(NPS), whereas 2-aminobenzenesulfonic acid was the main product (72% yield). This compound was separated by chromatography and identified by elemental analysis, UV spectra and colour reactions.

The same compound was obtained from 2-nitrothiophenol in 20% acetic acid at 110°, whereas from 4-nitrothiophenol no formation of sulfanilic acid was observed under the same conditions. In addition 2-amino-4-nitrobenzene sulfonic acid was formed in the hydrolysis of Trp(DNPS) as well by heating 2,4-dinitrothiophenol.

These results suggest that the thiol and o-nitro group undergo an intramolecular oxidation-reduction in aqueous solvents at high temperature as shown below:

\[
\begin{align*}
\text{SH} & \quad \text{NO}_2 \\
+ \quad \text{H}_2\text{O} & \quad \rightarrow \\
\text{SO}_3\text{H} & \quad \text{N}_2\text{H}_2
\end{align*}
\]

with: \( R = -\text{H}; \quad -\text{NO}_2 \).

In this connection it may be noted that aminobenzene sulfonic acids were also formed among other products (disulfides, thiosulfinates, thiosulfonates), during the hydrolysis of 2-nitro- and 2,4-dinitrophenylsulfonyl chloride\(^{16}\). More recently Barton et al.\(^{17}\) observed analogous oxidation-reduction by UV irradiation of 2,4-dinitrophenyl-sulfenamides and sulfenic esters.

**Experimental**

**Materials.** NPS-Cl\(^{18}\) and DNPS-Cl\(^{19}\) were obtained from Fluka AG (Basle, Switzerland) and recrystallized from ethyl ether (mp 75° - 76°) and chloroform-ethyl ether (mp 94° - 95°) respectively; pNPS-Cl was prepared according to the literature\(^{20}\) and recrystallized from anhydrous petroleum ether (mp 50° - 52°). The Folin-Ciocalteu's Reagent was obtained

12 R. E. Cantfield, J. Biol. Chemistry 238, 2698 [1963].
16 N. Kharasch, W. King, and Th. C. Bruce, J. Amer. chem. Soc. 77, 932 [1955].
19 N. Kharasch, G. I. Gleason, and C. M. Buess, J. Amer. chem. Soc. 72, 1796 [1950].
20 Th. Zincke and S. Lenhardt, Liebigs Ann. Chem. 400, 2 [1913].
from Merck AG (Darmstadt, Germany). Tryptamine was a chromatographically pure compound obtained from Lab. Plan SA (Aire - Genève, Switzerland). 2-Aminobenzene sulfonic acid was purchased from Merck AG. Amberlite XE-64 and Amberlite IR-120 were obtained from Bio-Rad. The pentapeptide Phen-Val-Gln-Trp-Leu, corresponding to the 22 — 26 sequence of glucagon, was a gift of Dr. E. Wünsch (Max-Planck-Institut für Eiweiss und Lederforschung, Munich, Germany). Lysozyme, trypsin, and α-chymotrypsin were obtained from Bovine pancreas. Lysozyme (from chicken egg whites) was obtained from Worthington Biochem. Corp. were used without further purification.

**Methods.** UV spectra were obtained with a Beckman Model DB spectrophotometer connected to a Sargent recorder. Absorptions at single wavelengths were determined with a Beckman Model DU spectrophotometer. The IR spectra were obtained with a Perkin-Elmer Model 141 polarimeter; concentrations are given in grams per 100 ml of solvent. The melting points were determined with Tottol's apparatus (Büchi, Flawil, Switzerland) and are uncorrected.

Thin layer chromatography (SiO₂) was performed using the following solvent mixtures: n-butyl alcohol : water : glacial acetic acid (3:1:1) (R₁₁); ethyl acetate : pyridine : water : glacial acetic acid (60:20:14:6) (R₁₂); chloroform : benzene : glacial acetic acid (85:10:5) (R₁₃). Paper chromatography was performed on Whatman paper n. 1 using n-butyl alcohol : water : glacial acetic acid (4:5:1) as eluent; for bidimensional chromatography this solvent was used for the first run and water for the second one. The compounds were in turn detected by ninhydrin spray, hypochlorite-starch-KI test, and reaction with p-dimethylaminobenzaldehyde.

Amino acid analyses were carried out in the laboratory of Dr. E. Wünsch with a Beckman-Spinco Model 120 B amino acid analyzer, according to the standard procedure of Spackman et al. 11

A. Preparation of 2-thio-aryl-tryptophan derivatives and related compounds

2-Thio-(2-nitrophenyl)-tryptamine monohydrate. To a solution of 6.9 g (43 mmoles) of tryptamine in 100 ml of glacial acetic acid, 8.6 g (45 mmoles) of NPS-Cl were added with stirring. After 2 hrs at 22 — 24°, the mixture was filtered from undissolved material and evaporated in vacuo. The residual oil was treated with ethyl ether which caused crystallisation. The yellow product was filtered, recrystallized from ethanol-anhydrous ethyl ether and dried in vacuo over P₂O₅. The yield was 12.5 g (84%).

The hydrochloride (1 g; 28 mmoles) was dissolved in 100 ml of water and precipitated by adjusting the pH to 9 with 5% Na₂CO₃ solution. The yellow precipitate was collected by filtration, washed several times with water and dried in vacuo (10⁻² torr) over P₂O₅. The yield was 0.7 g (76%), mp 87 — 90°; R₁₂ 0.64 (single yellow, ninhydrin-positive spot).

**Anal.** Calcd for C₁₆H₁₅N₃O₃S·H₂O (331.2): N, 12.64; S, 9.65. Found: N, 12.45; S, 9.66.

2-Thio-(4-nitrophenyl)-l-tryptophan. Trp(pNPS). To a suspension of 2.04 g (10 mmoles) of l-tryptophan in 20 ml of glacial acetic acid, 2.09 g (11 mmoles) of NPS-Cl in 15 ml of acetic acid were added with stirring at room temperature. After 3 hours, the solution was concentrated in vacuo. The residual oil, after washing with ethyl ether, was dissolved in 5 ml of acetic acid. After adding 200 ml of water, the precipitate was collected by filtration, washed with water and ethyl ether, and dried in vacuo over P₂O₅ at 50° (3.2 g; yield 80%), mp 195° dec; [a]ρ +30.3 (c 1, 100 ml of MeOH).

**Anal.** Calcd for C₂₁H₁₇N₃O₅S (399.20): C, 57.16; H, 4.41; N, 11.90; S, 8.47.

N-Acetyl-2-thio-(2-nitrophenyl)-l-tryptophan. N-acetyl-Trp(pNPS). To a solution of 2.46 g (10 mmoles) of N-acetyl-Trp 22 in 100 ml of glacial acetic acid, 2 g (10.5 moles) of NPS-Cl were added. The solution was stirred at 22 — 24° for 2 hrs and then evaporated in vacuo to dryness. The residual oil was dissolved in water (200 ml) with the equivalent amount of 1N NaOH and then precipitated again by acidification (pH 2) with 1N HCl. The yellow precipitate was collected, washed several times with water and dried in vacuo over P₂O₅. The yield was 3.15 g (79%), mp 135 — 138°; [a]ρ +6.1 (c 1, MeOH); R₁₃ 0.46 (single yellow, ninhydrin-positive spot).

**Anal.** Calcd for C₁₄H₁₂N₃O₅S (357.2): C, 57.16; H, 4.46; N, 11.75; S, 8.97. Found: C, 56.81; H, 4.41; N, 11.90; S, 8.47.

N-Carbobenzyoxy-2-thio-(2-nitrophenyl)-l-tryptophan. Z-Trp(pNPS). This compound was prepared from N-carbobenzyoxy-l-Trp 23 and NPS-Cl in glacial acetic acid as described above for N-acetyl-Trp(pNPS). The yield was 82%, mp 83 — 85°; R₁₃ 0.46; [a]ρ +6.1 (c 1, MeOH); R₁₃ 0.46 (c 0.5, MeOH).

**Anal.** Calcd for C₁₃H₁₄N₃O₅S (491.2): C, 51.11; H, 4.27; N, 8.54; S, 6.51. Found: C, 50.43; H, 4.52; N, 8.23; S, 6.57.

N-Carbobenzyoxy-2-thio-(2,4-dinitrophenyl)-l-tryptophan. Z-Trp(DNPS). This compound was prepared from N-carbobenzyoxy-l-Trp and DNPS-Cl in glacial acetic acid in the same way as reported above for N-acetyl-Trp(pNPS). The yield was 84%, mp 142 — 144°, R₁₃ 0.37; [a]ρ +8.4 (c 0.5, MeOH).

Anal. Calcd for C_{25}H_{20}N_{4}O_{8}S (536.2): C, 55.98; H, 3.72; N, 10.43; S, 5.96. Found: C 55.40; H, 3.90; N, 10.45; S, 5.73.

2-Thio-(2-nitrophenyl)-L-tryptophan methyl ester hydrochloride·Trp(NPS)-OMe·HCl. To a solution of 2.5 g (10 mmoles) of Trp-OMe·HCl\(^{24}\) in 60 ml of glacial acetic acid, 2 g (10.5 mmoles) of NPS·Cl were added with stirring. After 3 hrs at room temperature the solvent was removed in vacuo and the residual oil recrystallized from ethanol-anhydrous ethyl ether. The yellow product was then dried in vacuo over P\(_2\)O\(_5\) at 50\(^\circ\)C. The yield was 3.65 g (90%), mp 144 — 146\(^\circ\); \(R_f\) 0.65; \([\alpha]_D^9 + 22.0\) (c 0.5, MeOH).

Anal. Calcd for C\(_{18}\)H\(_{11}\)N\(_3\)O\(_3\)S (407.6): C, 53.03; H, 4.42; Cl, 8.71; N, 10.32; S, 7.86. Found: C, 52.46; H, 4.64; Cl, 8.65; N, 10.25; S, 7.49.

2-Thio-(2-nitrophenyl)-L-tryptophanyl-L-leucine bihydrate. Phe-Val-Gln-acetic acid in the same way was previously reported by Scoffone et al.\(^{2}\) on an antanona.

The preparation of NPS-proteins (Figs. I and II). The extent of hydrolysis was also followed by the disappearance of the 2-thio-aryl-tryptophan derivatives. These compounds (yellow spots, dark under UV light) were eluted with ethanol and determined spectrophotometrically in the range of 280 — 360\(\mu\)m.

The pH dependence of the hydrolysis was determined by heating Trp(pNPS) for 30 minutes in 1 N HCl, 0.1 N HCl, 0.01 M citrate and 0.01 M phosphate buffers. The yields were calculated on the basis of the expected amount of Trp-OMe·HCl.

\(\nu\)-Alanyl-2-thio-(2-nitrophenyl)-L-tryptophan diketopiperazine. A solution of 0.24 g (0.5 mmoles) of Ala-Trp(NPS)-OMe·HCl\(^{28}\) in 20 ml of 20% acetic acid was heated in a sealed tube at 110\(^\circ\)C for 4 hrs. The precipitate (yellow needles) was collected by filtration, washed with 50% acetic acid and dried in vacuo over P\(_2\)O\(_5\). The yield was 0.2 g (92%), mp over 250\(^\circ\); \(R_f\) 0.93 (yellow, ninhydrin-negative spot); \([\alpha]_D^9 - 48.0\) (c 0.5, glacial acetic acid).

The absorption spectrum of this compound in 80% acetic acid was the same as the spectrum of the starting material (\(\lambda\) max 280\(\mu\)m and 363\(\mu\)m); IR \(\nu\) max 3340, 1675, 1340 cm\(^{-1}\) (KBr).

Anal. Calcd for C\(_{29}\)H\(_{18}\)N\(_4\)O\(_4\)S (410.22): C, 58.55; H, 4.39; N, 13.65; S, 7.83. Found: C, 58.52; H, 4.27; N, 13.75; S, 7.60.

Hydrolysis in 25% H\(_2\)SO\(_4\). (Table I). The Trp(NPS)-derivatives, N-acetyl-Trp(NPS), Ala-Trp(NPS)-OMe·HCl\(^{28}\) and Phe-Val-Gln-Trp(NPS)-OMe·HCl were heated in 25% H\(_2\)SO\(_4\) (about 20 mg in 3 ml) in a sealed tube at 105\(^\circ\)C for 24 hrs. After this time the solution was filtered from insoluble by-products, diluted to 60 ml with water and the sulfate precipitated as the barium salt with 2 N Ba(OH)\(_2\) (indicator phenolphthalein). The suspension, after addition of 5 ml of 1 N HCl, was heated on a steam bath for 30 minutes, filtered and evaporated in vacuo under a nitrogen stream. The


\(^{27}\) O. Folin and V. Ciocalteu, J. biol. Chemistry 73, 627 (1927).
residue was redissolved in water and an aliquot analysed for Trp-OH content by paper chromatography, as described above.

The NPS-proteins were hydrolysed in the same way and, after elimination of sulfate and evaporation in vacuo, the residue was redissolved in citrate buffer (pH 2.2) and applied directly to the amino acid analyser. The columns were developed according to Spackman et al. 11.

2-Hydroxy-tryptophan. a) From 2-thio-(4-nitropheryl)-1-tryptophan. 1.4 g (39 mmoles) of Trp(pNPS) were dissolved in 150 ml of 20% acetic acid, and heated for 10 hrs at 110° in a sealed tube. Oxygen was bubbled through the solution and the precipitate was collected (0.56 g; 95% yield) and identified as 4,4′-dinitrophenyldisulfide from its melting point (mp 193 to 194°, lit. 3 1 195°) was removed and the clear solution was passed through a column (15 x 0.9 cm) of Amberlite XE-64 in order to remove NH₃, and the column washed with 100 ml of water. All effluent was lyophilized, yielding 0.6 g (53%) of analytically pure compound.

2-Hydroxy-tryptamine hydrochloride. A solution of 1.15 g (34 mmoles) of 2-thio-(2-nitropheryl)-tryptamine in 40 ml of 15% acetic acid was heated in a sealed tube at 110° for 10 hrs. A precipitate of 2,2′-dinitrophenyl-disulfide (0.14 g; mp 193 – 194°, lit. 31 195°) was removed, and the solution passed through a 15 x 0.9 cm column of Amberlite IR-120 (H⁺). The column was washed with water and all effluent evaporated in vacuo to dryness. The residual product (0.37 g; theory 0.58 g) was 2-aminobenzene-sulfonic acid already obtained in the hydrolysis of Trp(NPS).

The 2-hydroxy-tryptamine was then eluted with 1 N HCl, the effluent analyzed by thin layer chromatography and the fractions containing the compound were lyophilized. The residue (0.54 g; 74%), recrystallized from ethanol, melted at 247 – 249° (lit. 9 237°). Anal. Calcd for C₁₆H₁₄N₂O₄·HCl: C, 56.47; H, 6.12; N, 13.17. Found: C, 56.80; H, 6.19; N, 13.05.

C. Oxidation-reduction of nitrothiophenols

a) The 2-nitro 32, 4-nitro 33 and 2,4-dinitrothiophenol 34 (20 μmoles) were dissolved in 20% acetic acid (6 ml). The solutions were deaerated in vacuo and heated in sealed tubes at 110° for 2 hrs.

From 2-nitrothiophenol it was obtained 2-aminobenzene sulfonic acid, identified by paper chromatography in n-butyl alcohol: water : glacial acetic acid (4:5:1), yellow spot by the p-dimethylaminobenzaldehyde reaction.

On the other hand from 4-nitrothiophenol no 4-aminobenzene sulfonic acid (sulfanilic acid) was formed as judged by paper chromatography.

Analysis of the reaction mixture from 2,4-dinitrothiophenol by paper chromatography showed the formation of 2-amino-4-nitrobenezene sulfonic acid. This compound was identified by comparing its chromatographic properties.
behaviour and UV spectrum with that of 2-amino-4-nitrobenzene sulfonic acid, prepared following the procedure of Kharasch et al.\textsuperscript{16}.

b) 2-Aminobenzene sulfonic acid. A solution of 46 mg (30 \mu moles) of 2-nitrothiophenol\textsuperscript{32} in 10 ml of 80\% acetic acid was heated in a sealed tube for 2 hrs at 110\(^\circ\)C. The solution was decolorised with charcoal, evaporated \textit{in vacuo} and the residue recrystallized from hot water. The yield was 25 mg (50\%) of 2-amino-benzene sulfonic acid, identified by elemental analysis, colour reactions, chromatographic behaviour and UV spectrum.

We wish to thank Prof. Ernesto Scoffone for his interest in this work and Dr. Eloisa Celon for the elemental analysis.

Fig. III. Elution profile of an acid hydrolysate of NPS-lysozyme on chromatography on the short column of the Beckman-Spinco Model 120 B amino acid analyser at 25\(^\circ\). The column was developed according to the procedure of Spackman et al.\textsuperscript{14}.

Seitenketten-Fragmentierung und Anthracen-Synthese bei Friedel-Crafts-Reaktionen von \(N\)-Alkyl-\(N\)-hydroxy-guanidin-\(O\)-sulfonsäuren

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Schon früher hatten wir gezeigt, daß die Hydroxy-guanidin-\(O\)-sulfonsäure und ihre \(N\)'-alkylierten Derivate in Gegenwart von viel Aluminiumchlorid in der Lage sind, ihren Guanidinrest auf Aromaten zu übertragen. Das Isomerenverhältnis beim Tolylguanidin und die relativen Guanidierungs-Geschwindigkeiten von Toluol und Benzol forderten dazu einen elektrophilen Mechanismus\textsuperscript{1}.

Bei der Verwendung von \(N\)-Alkyl-\(N\)-hydroxy-guanidin-\(O\)-sulfonsäure (1 und 2) änderte sich das Reaktionsbild völlig. Während beim Methyl-Derivat (1) noch in mäßiger Umfang eine Guanidierung ablief, trat diese beim Butyl-Derivat (2) kaum noch ein.

\[ \text{NH}_2-C-N-O-SO_2H ] \]

\[ \text{NH} \]

Als völlig unerwartete Produkte fanden wir bei der Reaktion von 1 mit Benzol/\(\text{Al}_2\text{Cl}_6\) in der organischen Phase höhere, neutrale Kondensationsprodukte: Anthracen und Diphenylmethan. Auch beim Umsatz mit Toluol erfolgte diese Synthese: wir erhielten ein Gemisch von 2.6- und 2.7-Dimethylanthracen, wie es auch bei der Friedel-Crafts-Reaktion von Toluol mit Trioxymethylen entsteht\textsuperscript{2}.

Da auch das Butyl-Derivat 2 nur unsubstituiertes Anthracen lieferte, vermuteten wir, daß das C-Atom
