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# CONVERSION OF INDOLE INTO 3-S-(CYSTEINYL)INDOLES AND 2-S-(CYSTEINYL)TRYPTOPHANS. AN APPROACH TO TRYPTATHIONINES§

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Abstract - The 3-S-(cysteinyl)indoles **8a** and **8b**, easily derived (ca 95% yield) from indole and the sulfenyl chlorides **7a** and **7b**, respectively, have been converted (ca 50%) into oximino derivatives of tryptophan **9a,b**. Reduction gave a mixture of two diastereomeric 2-S-(cysteinyl)tryptophan derivatives - *i.e.* **10** and its $H_2N$-C(α)epimer - which could be separated. One of these is a derivative of tryptathionine, the characteristic structural element of toxic principles (*e.g.* Phalloin (**5**)) of members of the genus *Amanita*.

## INTRODUCTION

Recently, we reported[1] a novel reaction observed in the cycloaddition of the nitroso olefin **3** with indoles having a 3-alkylthio substituent, *i.e.* **1** (Scheme 1). This reaction gave a rearranged product **4** in which the thioalkyl group had migrated from C(3) to C(2). As part of a further exploration of this cycloaddition-rearrangement reaction we report now the synthesis of the tryptathionine derivative **10**. This 2-S-(cysteinyl)tryptophan derivative is related to the central chromophoric dipeptide system of the phallotoxins **5** (Figure 1), toxic principles of the mushroom *Amanita phalloides*[2].

## RESULTS

The first problem to be faced was the synthesis of the 3-S-(cysteinyl)indole derivative **8** (Scheme II). An obvious approach seemed to be the reaction of a sulfenyl halide (*e.g.* **7**) derived from cysteine, with indole (**6**). Surprisingly, the reaction of sulfenyl halides with indole to yield 3-(alkylthio)-indoles has not yet been reported[3], although the reaction of 3-substituted indoles, *e.g.* tryptophans, with sulfenyl halides to yield the corresponding 2-(alkylthio)-tryptophans is well-studied[3,4].

Scheme I

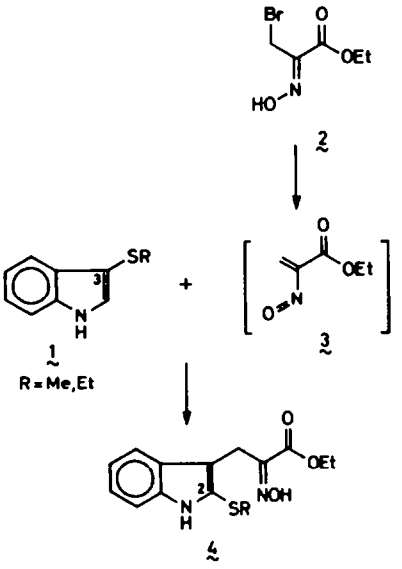
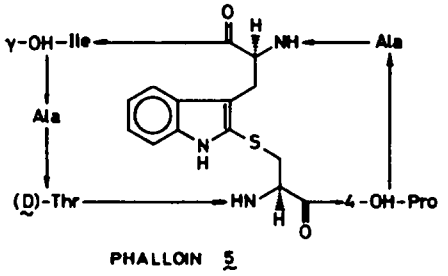
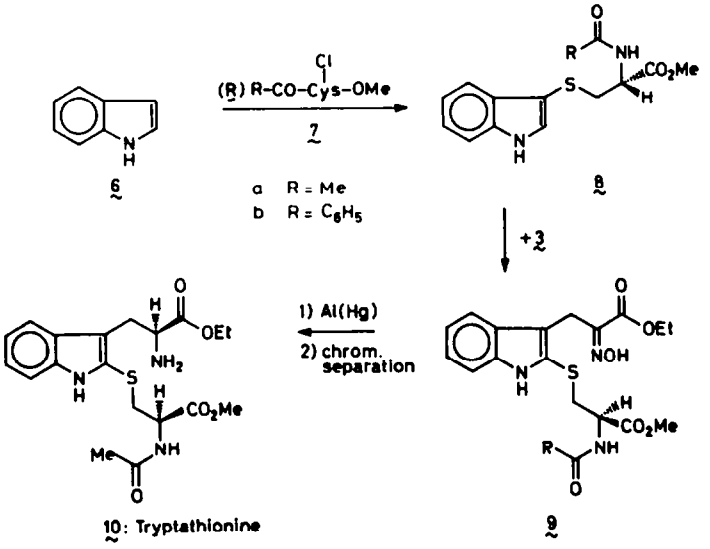



Figure 1




Scheme II



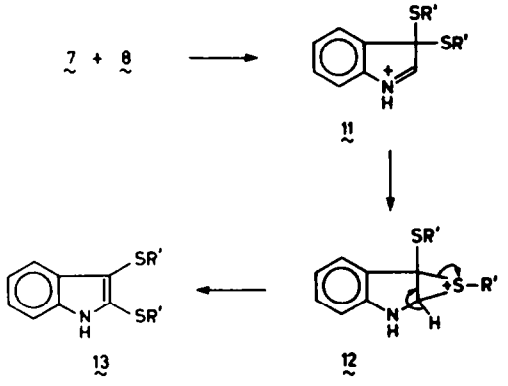

We found that exposure of two equivalents of indole (6) to one equivalent of the sulfenyl chlorides 7a or 7b gave the desired 3-S-(cysteinyl)indole derivatives 8a and 8b, respectively, in excellent yields (>95%). This reaction and the preparation of 7 deserve further explanation.

The sulfenyl chlorides 7a and 7b - heretofore troublesome to prepare[5] - were obtained by reaction of the corresponding *L*-cystine derivative with sulfuryl chloride. The progress of the reaction can be followed by observing the color

changing from colorless to deep-yellow. Addition of a base *e.g.* triethylamine appeared detrimental to the reaction. We tried also to generate these sulfenyl chlorides from the corresponding *L*-cysteine derivatives and N-chlorosuccinimide according to literature procedures[6]. Due to decomposition of the product in the subsequent coupling with the indole the resulting reaction mixture gave, however, unsatisfactory results.

The molar ratio of the sulfenyl chlorides 7 *versus* indole 6 is crucial for the preparation of 8; two or more molar equivalents of indole are required. When a solution of 6 was treated with 7a or 7b in equimolar amounts the 2,3-di-S- (cysteinyl)indoles 13a and 13b, respectively, were isolated in high yields (ca 85%). The formation of 13a and 13b might be rationalised as depicted in Scheme III. Initial formation of 8 might be followed by reaction with a second molecule of the sulfenyl chloride 7. The resulting indolenine 11 forms the episulfonium 12

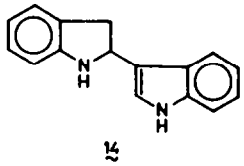
Scheme III




a R' = (*L*)-$CH_2CH(NHCOCH_3)CO_2Me$
b R' = (*L*)-$CH_2CH(NHCOC_6H_5)CO_2Me$

which undergoes rearomatisation to yield 13. The formation of 8 is accompanied by the liberation of HCl, which causes the well-documentated[6] dimerisation of 6 to give 14. We could indeed isolate this dimer. This reaction of 6 might explain why 8 is formed efficiently only in the presence of a molar excess of 6, the dimer of which *i.e.* 14 fortuitously serves as a proton-acceptor.

Figure 2




14

Reaction of 8a and 8b with the transient nitroso olefin 3 - prepared *in situ*[1] from 2 - proceeded as expected from Scheme I. The desired 2-S-(cysteinyl)indole derivatives 9a and 9b were obtained in ca 50% yield[7]. The mechanism of this rearrangement has been rationalized before[1]; it features a cycloaddition followed by C(3)→C(2) migration of the thioalkyl substituent leading to re-opening of the dihydro-1,2-oxazine ring and re-aromatisation. Of all solvents used in this reaction ($CH_3CN$, $CH_2Cl_2$, $CHCl_3$, $CCl_4$), $CCl_4$ was found to give the best res-

ults. We impute the relatively low yield of this reaction to the formation of a cycloadduct from 3 and 9[7] and to an intermolecular exchange of the migrating thioalkyl substituent[8].

Finally, the oximino group of 9a had to be reduced to an amine function. Aluminum amalgam in moist diethyl ether was found to work most effectively (69% yield). The resulting diastereomeric tryptathionines - formed in a 1:1 ratio - were separated by careful preparative HPLC-chromatography to yield 10 and its $H_2N$-C(α)epimer. No attempts were made to establish which of the two isolated materials is 10 and which its epimer.

In conclusion the reaction of 6 with 7 provides a new and simple synthesis of 3-S-(cysteinyl)indoles 8a,b as well as 2,3-di-S-(cysteinyl)indoles 13a,b. The reaction sequence 1+3→4 can be applied to compounds 8a,b of which 8a is converted into tryptathionine derivative 10. Previous approaches to tryptathionines start either from tryptophan using sulfenyl chlorides[6], or iodides[9], or from hexahydropyrrolo[2,3-b]-indoles[10] or tetrahydropyrrolo[2,3-b]-indoles[11] derived from tryptophan using sulfides. Our approach starting from indole is more general, as it allows the preparation of tryptathionines having substituents in the indole nucleus.

## EXPERIMENTAL SECTION

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin-Elmer spectrometer, Model 555.

Proton magnetic resonance spectra were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer. Chemical shifts are reported as δ-values (parts per million) relative to tetramethyl silane as an internal standard. Mass spectra were obtained with a double-focussing VG 7070E spectrometer. Thin layer chromatography (TLC) was carried out by using Merck precoated silicagel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, $Cl_2$-TDM[12], cinnamaldehyde/HCl for indole detection[13], $AgNO_3/Na_2CrO_7$ for the detection of sulfides[14] or ninhydrine. A miniprep LC (Jobin Yvon) was used for preparative HPLC; as stationary phase Merck silicagel H (Type 60) was used. Merck silicagel (Type 60) was used for flash chromatography.

### N-acetyl-S-(indol-3-yl)-(*L*)-cysteinyl methyl ester (8a)

To a well stirred solution of N-acetyl-(*L*)-cystine methyl ester (5 mmol, 1.76 g) in $CH_2Cl_2$ (50 ml) was added sulfuryl chloride (5 mmol, 675 mg) at room temperature and under argon atmosphere. After stirring for 2 minutes the mixture was added dropwise to a solution of indole (20 mmol, 2.34 g) in $CH_2Cl_2$ (25 ml). After 2 hrs the white precipitate was removed by filtration. Evaporation of the solvent and recrystallization (ethyl acetate/n-hexane) afforded 8a in 97% yield (2.83 g), m.p. 118-119 °C. $[\alpha]^{21}=83°$ (c=0.27, $CH_2Cl_2$). UV (MeOH) $\lambda_{max}$=286, 278, 271 (sh), 216 nm; $\lambda_{min}$=282, 252 nm. EIMS (70 eV) m/e=292 ([M]$^+$, 100%), 233

([M-$C_2H_3O_2$]$^+$, 18%), 148 ([$C_8H_6NS$]$^+$, 50%), 144 ([$C_6H_{10}NO_3$]$^+$, 62%); exact mass calcd. for $C_{14}H_{16}N_2O_3S$ 292.0882, found: 292.0897; $^1$H-NMR (90 MHz, $CDCl_3$) δ=8.90 (s(br), 1H, indole NH), 7.80-7.10 (m, 5H, indole C(2)H) and C(4)-C(7)H), 6.30 (d(br), 1H, NH), 4.75 (X part of ABX spectrum, 1H, S-$CH_2CH$), 3.45 (s, 3H, $OCH_3$), 3.15 (AB part of ABX spectrum, 2H, $SCH_2$-CH), 1.80 (s, 3H, $COCH_3$). Anal. calcd. for $C_{14}H_{16}N_2O_3S$ (M=292.357) C 57.52; H 5.52; N 9.58, found: C 57.50; H 5.50; N 9.55. The white precipitate was diindole•HCl **14**. EIMS m/e=233/235 ([M]$^+$). $^1$H-NMR (60 MHz, $CDCl_3$) δ=7.8 (s(br), 1H, indole NH), 7.7-6.4 (m, 8H, indole C(4)-C(7)H and indoline C(4)-C(7)H), 5.15 (X part of ABX spectrum, 1H, indoline C(2)H), 3.7 (s, 1H, indoline NH), 3.3 and 3.7 (AB part of ABX spectrum, 2H, indoline C(3)$H_2$.

### N-benzoyl-S-(indol-3-yl)-(*L*)-cysteinyl methyl ester (8b)

This compound was prepared from N-benzoyl-(*L*)-cystine methyl ester (2.5 mmol, 1.19 g) sulfuryl chloride (2.5 mmol, 340 mg) and indole (10 mmol, 1.17 g) as described for the preparation of **8a**. Compound **8b** was obtained as an oil (1.7 g, 96%) which was homogeneous on TLC. UV (MeOH) $\lambda_{max}$=286, 277, 271 (sh), 216 nm; $\lambda_{min}$=283, 252 nm. EIMS (70 eV) m/e=354 ([M]$^+$, 39%), 206 ([M-$C_8H_6NS$]$^+$, 60%), 149 ([M-$C_{11}H_{11}NO_3$]$^+$, 20%); 148 ([$C_8H_6NS$]$^+$, 21%), 105 ([$C_7H_5O$]$^+$, 100%); exact mass calcd. for $C_{19}H_{18}N_2O_3S$ 354.1038, found: 354.1035. $^1$H-NMR (90 MHz, $CDCl_3$) δ=8.50 (s(br), 1H, indole NH), 7.80-7.15 (m, 10H, indole C(2)H and C(4)-C(7)H, $COC_6H_5$), 6.80 (d(br), 1H, CONH), 5.00 (X part of ABX spectrum, 2H, S-$CH_2CH$), 3.52 (s, 3H, $OCH_3$), 3.34 and 3.28 (AB part of ABX spectrum, 2H, $SCH_2CH$).

### Ethyl α-(hydroxyimino)-β-[2-S-(N-acetyl-(*L*)-cysteinyl methyl ester)indol-3-yl-propanoate (9a)

A solution of ethyl α-(hydroxyimino)-β-bromopropanoate **2**[1] (18 mmol, 3.8 g) in dry $CCl_4$ (50 ml) was added dropwise to a stirred solution of N-acetyl-(S-indol-3-yl)-(*L*)-cysteinyl methyl ester **8a** (6 mmol, 1.75 g) and $Na_2CO_3$ (24 mmol, 2.54 g) in dry $CCl_4$ (50 ml) at room temperature and under argon atmosphere. The mixture was stirred for 3 days. To remove $Na_2CO_3$ and NaBr the mixture was filtered through a thin layer of silicagel 60. The resulting solution was washed with 1 N HCl and brine and dried over $Na_2SO_4$. The residue obtained after evaporation of the solvent was subjected to HPL chromatography (MeOH/$CH_2Cl_2$, 2/98, v/v) and subsequently recrystallized twice from $CH_2Cl_2$/n-hexane to give **9a** in 51% (1.28 g) yield. M.p. 134-135 °C; $[\alpha]^{22}$=-99° (c=0.12, $CH_2Cl_2$). UV (MeOH) $\lambda_{max}$=299(sh), 290, 220 nm; $\lambda_{min}$=255 min. EIMS (70 eV) m/e=421 [M]$^+$, 20%), 144 ([$C_6H_{10}NO_3$]$^+$, 100%); exact mass calcd. for $C_{19}H_{23}N_3O_6S$ 421.1308, found 421.1279. $^1$H-NMR (90 MHz, $CDCl_3$) δ=9.70 (s(br), 2H, indole NH and NOH), 7.70-7.00 (m, 4H, indole C(4)-C(7)H), 6.50 (d(br), $^3J_{NH-X}$=8.7 Hz, 1H, NHCO), 4.90 (X part of ABX spectrum, $^3J_{AX}$=3.6 Hz, $^3J_{BX}$=8.7 Hz, $^3J_{NH-X}$=8.7 Hz, 1H, $SCH_2CH$), 4.22 (s, 2H, indole C(3)-$CH_2$), 4.20 (q, 2H, $OCH_2CH_3$), 3.65 (s, 3H, $OCH_3$), 3.40 and 2.85 (AB part of ABX spectrum, $^3J_{AX}$=3.6 Hz, $^3J_{BX}$=8.7 Hz, $^2J_{AB}$=14 Hz, 2H, $SCH_2$-CH), 1.93 (s, 3H, $COCH_3$), 1.21 (t, 3H, $OCH_2CH_3$). Anal. calcd. for $C_{19}H_{23}N_3O_6S$; C 53.26; H 5.52; N 9.98, found C 54.15; H 5.50 N 9.97.

**Ethyl α-(hydroxyimino)-β-[2-S-(N-benzoyl-(*L*)-cysteinyl methyl ester)indol-3-yl]-propanoate (9b)**

This compound was prepared from ethyl α-(hydroxyimino)-β-bromopropanoate **2**[1] (4.6 mmol, 960 mg), $Na_2CO_3$ (4.6 mmol, 475 mg) and N-benzoyl-(S-indol-3-yl)-*(L)*-cysteinyl methyl ester **8b** (1.16 mmol, 410 mg) as described for the preparation of **9a**. A pure product **9b** was obtained in 47% (263 mg) yield after careful HPL chromatography ($MeOH/CH_2Cl_2$, 1/99, v/v) and recrystallization M.p. 169-170 °C ($CH_2Cl_2$/n-hexane). UV (MeOH) $\lambda_{max}$=300 (sh), 290, 217 nm; $\lambda_{min}$=248 nm. EIMS (70 eV) m/e=483 ($[M]^+$, 66%), 466 (9%), 206 ($[C_{11}H_{12}NO_3]^+$, 100%), 105 ($[C_7H_6O]^+$, 60%); exact mass calcd. for $C_{24}H_{25}N_3O_6S$ 483.146, found: 483.144. $^1$H-NMR (90 MHz, $CDCl_3$) δ=9.50 (s(br), 1H, NOH), 8.70 (s(br). 1H, indole NH), 7.60-7.00 (m, 10H, indole C(4)-C(7)H, $C_6H_5$, CONH), 5.06 (X part of ABX spectrum, 1H, $SCH_2CH$), 4.20 (s, 2H, indole C(3)$CH_2$), 4.06 (q, 2H, $OCH_2CH_3$), 3.60 (s, 3H, $OCH_3$), 3.40 and 3.34 (AB part of ABX spectrum, 2H, $SCH_2CH$), 1.15 (t, 3H, $OCH_2CH_3$).

**Ethyl 2-S-(N-acetyl-(*L*)-cysteinyl methyl ester)tryptophanoate (10)**

To a solution of **9a** (0.5 mmol, 212 mg) in moist ether (50 ml), excess aluminum amalgam was added in small portions at room temperature and under an argon atmosphere. After 6 hours the reaction mixture was filtered and the solvent was evaporated. The residue was washed with 0.1 N HCl and brine, and subsequently dried ($Na_2SO_4$) and filtered. The solvent was removed *in vacuo* to give a mixture of the two diastereomeric tryptathionines **10** in 69% (141 mg) yield. Separation by HPL chromatography (silicagel 60H; $MeOH/CH_2Cl_2$, 1/99, v/v) gave equal amounts of diastereomers (oils).

$R_f$ 0.21 ($MeOH/CH_2Cl_2$, 6/94, v/v), $[\alpha]^{21}$=-110 °C (c=0.200, $CH_2Cl_2$), UV (MeOH) $\lambda_{max}$=299 (sh), 290, 220 nm; $\lambda_{min}$=255 nm. EIMS (70 eV) m/e=407 ($[M]^+$, 2%), 305 ($[M-C_4H_8NO_2]^+$, 88%), 162 ($[C_9H_8NS]^+$, 79%), 144 ($[C_6H_{10}NO_3]^+$, 100%), exact mass calcd. for $C_{19}H_{25}N_3O_5S$ 407.1515; found 407.1516. $^1$H-NMR (90 MHz, $CD_2Cl_2$) δ=10.00 (s(br), 1H, indole-NH), 7.90 (d(br), $^3$J=8.4 Hz, 1H, CONH), 7.60-6.95 (m, 4H, indole C(4)-C(7)H), 4.90 (X part of ABX spectrum, $^3J_{NH-CH}$=8.4 Hz, $^3J_{AX}$=6.6 Hz, $^3J_{BX}$=3.6 Hz, 1H, $SCH_2CH$), 4.20 (q, 2H, $OCH_2CH_3$), 3.85 (X' part of A'B'X' spectrum, $^3J_{A'X'}$=9.6 Hz, $^3J_{B'X'}$=4.3 Hz, 1H, indole C(3)-$CH_2$-CH), 3.75-2.80 (m, 2×AB part of ABX spectrum, 4H, indole C(3)-$CH_2$ and S-$CH_2$), 3.70 (s, 3H, $OCH_3$), 1.90 (s(br), 2H, $NH_2$), 1.55 (s, 3H, $COCH_3$), 1.24 (t, 3H, $COCH_2CH_3$).

$R_f$ 0.18 ($MeOH/CH_2Cl_2$, 6/94, v/v), $[\alpha]^{21}$=-102° (c=0.155, $CH_2Cl_2$), UV (MeOH) $\lambda_{max}$=299 (sh), 290 , 220 nm; $\lambda_{min}$=255 nm. EIMS (70 eV) m/e=407 ($[M]^+$, 2%), 305 ($[M-C_4H_8NO_2]^+$, 100%), 162 ($[C_9H_8NS]^+$, 79%), 144 ($[C_6H_{10}NO_3]^+$, 99%), exact mass calcd. for $C_{19}H_{25}N_3O_5S$ 407.1515, found 407.1519. $^1$H-NMR (90 MHz, $CD_2Cl_2$) δ=10.0 (s(br), 1H, indole NH), 7.60-6.95 (m, 4H, indole C(4)-C(7)H), 6.86 (d(br), 1H, CONH), 4.80 (X part of ABX spectrum, $^3J_{AX}$=9.0 Hz,$^3J_{BX}$=3.8 Hz, 1H, S-$CH_2$-CH), 4.10 (q, 2H, $OCH_2CH_3$), 3.85 (X' part of A'B'X' spectrum, $^3J_{A'X'}$=8.5 Hz, $^3J_{B'X'}$=5.0 Hz, 1H, indole C(3)$CH_2$-CH), 3.64 (s, 3H, $OCH_3$), 3.80-2.80 (2×AB part of ABX spectrum, 4H, indole C(3)-$CH_2$ and S-$CH_2$), 1.90 (s, 3H, $COCH_3$), 1.80 (s(br), 2H, $NH_2$), 1.20 (t, 3H, $OCH_2CH_3$).

### 2,3-Di-S-((*L*)-cysteinyl)indole derivative (13a)

To a stirred solution of N-acetyl-(*L*)-cystine methyl ester (0.5 mmol, 176 mg) in $CH_2Cl_2$ (10 ml) was added sulfuryl chloride (0.5 mmol, 70 mg) at room temperature and under an argon atmosphere. After stirring for 2 minutes, one equivalent of indole (1.0 mmol, 117 mg) in $CH_2Cl_2$ (5 ml) was added dropwise to room temperature. The solution was stirred under argon atmosphere and the progress of the reaction was monitored by tlc. After completion of the reaction (2 h) the white precipitate was filtered off. The organic layer was washed twice, dried over $Na_2SO_4$, filtered, and then concentrated to dryness *in vacuo*. The residue was recrystallized (methanol/ethyl acetate) to give **13a** in 85% (200 mg) yield[§].

### 2,3-Di-S-((*L*)-cysteinyl)indole derivative (13b)

This compound was prepared from N-benzoyl-(*L*)-cystine methyl ester (5 mmol, 2.4 g) sulfuryl chloride (5 mmole, 680 mg) and indole (10 mmol, 1.17 g) as described for the preparation of **13a**. The pure product was obtained after recrystallization (methanol/ethyl acetate) in 84% (2.5 g) yield[§].

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