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Approaches to Analogs of Anhydrogliotoxin. 3.[1,2] Synthesis of a Desthiomethylene Analog

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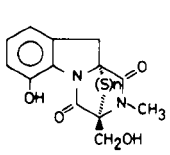
Received February 24, 1975

The reaction of *N*-benzyloxycarbonyl-L-cysteine (**7**) with the ethyl indolenino-2-carboxylate (**6**) gave two diastereomeric addition products, **8a** and **8b**. Deprotection of the amino group of **8a** yielded **9a**. On basification the free amine **10a** underwent ring closure by intramolecular aminolysis to yield **5**, a desthiomethylene analog of anhydrogliotoxin. The diastereomeric amine **10b** could also be converted into **5** in a reaction believed to proceed via **10a** by an autocatalyzed epimerization at C-9. Support for this mechanism was obtained by deuterium exchange studies. Recrystallization of **5** yielded a racemic and an optically active fraction. A CD spectrum of the latter supports an R_2S_9 absolute configuration, which is identical with that of gliotoxin **11**. The stereochemistry of the addition reaction is discussed: optically active product **5** results from a chiral component **7** whose configuration inverts during the reaction. The racemic, as well as the optically active compound **5**, is devoid of antiviral and antibacterial activity. This indicates that in natural products containing an epidithiodioxopiperazine moiety the disulfide bridge is essential for activity and the three-dimensional structure is of secondary importance.

Dehydrogliotoxin (**1**, n = 2) and sporidesmin (**2**, R = H) belong to a group of fungal metabolites characterized by a bridge of sulfur atoms across a dioxopiperazine ring.[3] Recently, a simple synthetic homolog, 3,6-epidithio-1,4-dimethyl-2,5-dioxopiperazine (**3**),[4-6] was found to have biological properties which are characteristic of this class of compounds.[5] On the other hand, the conversion of the complex natural products into their dithioalkylated derivatives is accompanied by complete loss of biological activity. From these observations Taylor concluded[4] that the sulfur bridged dioxopiperazine moiety, or a metabolite of it, might be responsible for the activity of these compounds. In this respect the activities of sporidesmin C, **4** (R = H),[7] having a methylene–disulfide bridge, and of monodesthiodehydrogliotoxin (**1**, n = 1)[8] are of interest. Taylor argues[3] that the low activity of the diacetate of **4** (R = $COCH_3$),[9] which is about 100 times less active than sporidesmin diacetate (**2**, R = $COCH_3$), can be accounted for by a contamination of **4** (R = $COCH_3$) with **2** (R = $COCH_3$).



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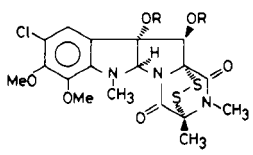

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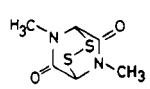

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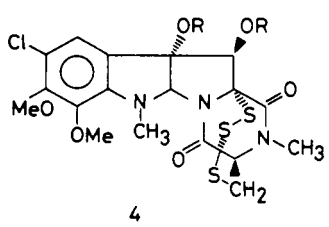

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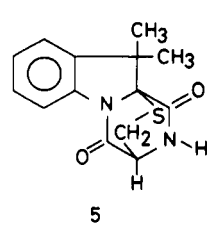


5

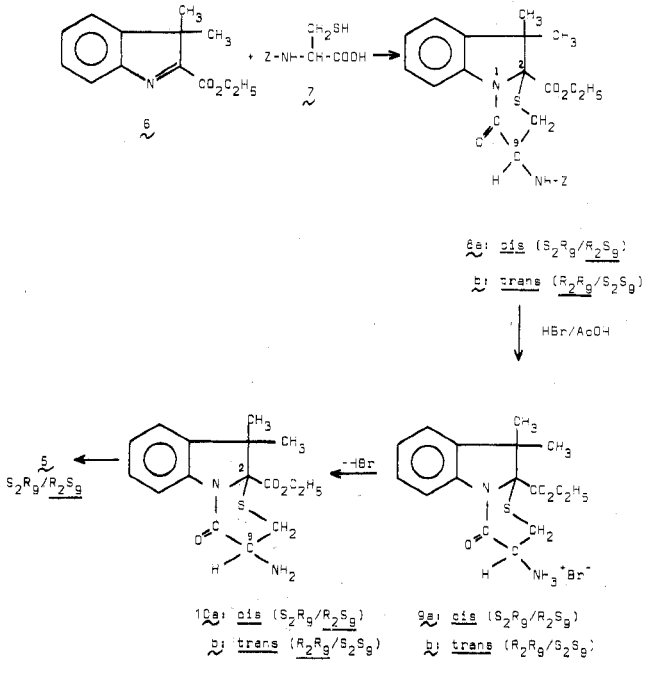
Here we wish to report on the synthesis of an anhydrogliotoxin analog **5** possessing a methylene sulfide bridge instead of a disulfide bridge. The three-dimensional structure will not be significantly altered by such a substitution and examination of **5** for biological activity might shed more light on the importance of the disulfide bridge in the activity of compounds such as **1–3**.

As we have previously reported,[2] β-mercaptopropionic acid reacts with the ethyl indolenino-2-carboxylate **6** to yield an indoline tetrahydrothiazone derivative. In an analogous reaction addition of *N*-benzyloxycarbonyl-L-cysteine[10] (**7**) to **6** gave in 95% yield a mixture of two compounds that could be separated by column chromatography. Both products, one an oil ($[\alpha]^{20}D$ −13.8°), the other crystalline ($[\alpha]^{20}D$ −19.2°) (ratio 5:6) were optically active. Elemental analyses and spectral data supported the diastereomeric indoline tetrahydrothiazone structures **8a** and **8b** (Scheme I). These compounds had nearly the same mass spectra, having parent peaks at m/e 454, differing only in that the oily component had a peak at m/e 408 (M − C_2H_5OH) in its spectrum which was missing in the spectrum of the crystalline material. Because intramolecular loss of C_2H_5OH seems more probable in a cis isomer, it was assumed that structure **8a** belongs to the oily material and the trans configuration **8b** to the crystalline product. This assumption could be substantiated as will be discussed below.

The *N*-benzyloxycarbonyl group was smoothly removed

from **8a** with HBr in acetic acid, yielding the crystalline hydrobromide **9a.** The free amino group was produced either by ion-exchange chromatography, or preferably, by adjusting the pH of a methanolic solution to 9.5 with sodium hydroxide followed by desalting on Sephadex LH-20. Surprisingly, **10a** could not be isolated as such, as it spontaneously underwent partial ring closure to **5.** This intramolecular aminolysis could be completed by refluxing the mixture in ethanol for 1 hr. This facile ring closure supports the assignment of the cis configuration **8a** to the oily fraction. Compound **5** thus obtained was found to be optically active: $[\alpha]^{20}D$ −10.2°. Structure proof is based on elemental analysis and the usual spectral data. Recrystallization yielded a racemic, crystalline product (mp 212–213°) leaving an optically active, amorphous residue, $[\alpha]^{20}D$ −63°.

Scheme I



When the trans compound **8b** was subjected to the same operations, homogeneous free amino **10b** could be isolated. It was anticipated that **10b** would not react further, since the ester and amino group are in a trans position. Surprisingly, this amine could also be ring closed, though slowly, by refluxing in ethanol for 20 hr. The compound thus obtained was identical in all respects with that prepared from **10a,** except for the rotation, this being $[\alpha]^{20}D$ −52°. Recrystallization of this material again yielded a racemic, crystalline fraction (mp 213–214°) but the optically active amorphous residue had $[\alpha]^{20}D$ −156°.

Evidently, the diastereomers **10a** and **10b** are interconvertible, with the cis product **10a** being removed from the mixture because of irreversible ring closure (**10a** → **5**). A priori, epimerization of **10b** might happen at either C-2 or C-9. To distinguish between these possibilities, the amino deuterated trans compound **10b** was refluxed in deuterated methanol after which compound **5** was isolated with 100% deuterium at C-9. Proton exchange after ring closure of **10a** was ruled out by the following experiment. Reflux of a solution of **5** in deuterated methanol with 1 equiv of amino deuterated aniline did not lead to incorporation of deuterium. Thus epimerization of **10b** probably occurs at C-9, with the amino group functioning as an autocatalyst.

The specific rotations of the amorphous residues of **5** differed, but were in both cases negative, indicating that in

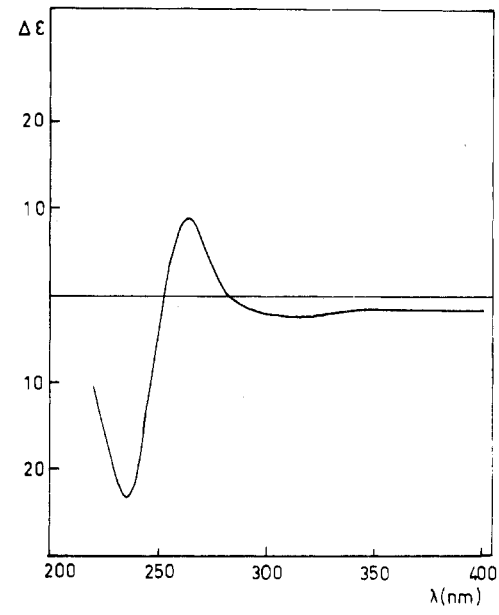
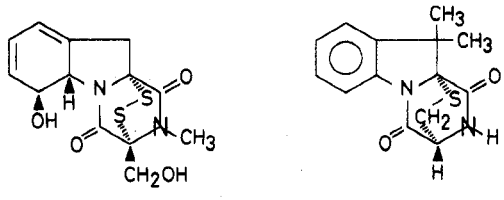


Figure 1. Circular dichroism curve of **5,** $[\alpha]^{20}D$ −156°, 0.2325 mg/ml of absolute MeOH, path length 2 mm.

both fractions the same enantiomer of **5** predominated. The absolute configuration of the fraction of **5** having $[\alpha]D$ −156° was derived from a CD spectrum (Figure 1).

The negative Cotton effect at 235 nm ($\Delta\epsilon_{max}$ −23.3) is due to a n,π^* transition of a peptide bond.[11] The natural product gliotoxin **11,** similarly having two chiral centers in the dioxopiperazine ring,[12] also shows a negative Cotton effect at 230 nm ($\Delta\epsilon_{max}$ −33).[13] From this, the tentative conclusion is drawn that compound **5,** with $[\alpha]^{20}D$ −156°, consists mainly or entirely of the enantiomer **5**-R_2S_9. In the



11 5-R_2S_9

partially racemic end product **5,** whether obtained directly from the cis isomer **10a** or via epimerization at C-9 from the trans isomer **10b,** the levorotating R_2S_9 isomer apparently predominates. Thus the intermediate **10a** must have contained more R_2S_9 isomer. Similar enantiomer ratios must also have been present in the addition products **8a** and **8b** after separation, because alterations in these ratios after that stage are not possible by epimerization but only via racemization, which is very improbable under the experimental conditions used.

On the other hand, the initially formed addition products from the achiral starting compound **6** and optically pure (*R*)-cysteine can only have been the diastereomers (R_2R_9)-**8** and (S_2R_9)-**8** since benzyloxycarbonylamino acids are known for their low tendency toward racemization.[14] Apparently the addition step proceeds rather stereoselectively, leading mainly to the R_2R_9 isomer, which undergoes epimerization at C_9 before it is separated from the minor product S_2R_9. The occurrence of epimerization at C_9 in the addition product **8** under the experimental conditions of the addition reaction could be demonstrated by refluxing a pure sample of **8b** in benzene. Chromatography of the solution revealed that a new spot, having an R_f value equal to that of **8a,** had appeared. Treatment of **8b** with sodium deuteroxide in deuterated methanol gave complete ex-

change of one proton (mass spectrum). The NMR spectrum showed that the C_9 proton had been exchanged and that the isomers **8a** and **8b** were present in the ratio 3:2. The relatively facile epimerization of **8** may be caused by the formation of a dehydroalanine derivative via β-elimination.[15] Thus it can be concluded that optically active product **5** results from a chiral component **7**, whose configuration inverts during the reaction.

Activity Tests. The racemic compound **5**, as well as the optically active residue with $[\alpha]D$ −156°, were found to be devoid of antiviral and antibacterial activity.

The activity of both fractions toward the reverse transcriptase[16] which is known to be involved in the integration of viral genomes into the host DNA was tested according to Verma et al.[17] At the concentration used (6.1×10^{-5} mol/l.) no inhibition was observed with either of the compounds (1 and 4 ml of each solution, containing 16 and 64 μg, respectively), whereas the reference compound, chetomin,[18] reduced the activity to about 85 and 20%, respectively (10^{-5} mol/l., using 7 and 28 μg, assumed mol wt 710). The same result was obtained when the endogenous reaction[19] of dissociated Rauscher leukemia virus was used for the activity test. The antibacterial activity was also tested.[20] No growth-inhibitory activity against *Bacillus subtilis, Sarcina lutea,* or *Pseudomonas solanacearum* was found. The compounds did not show antifungal activity in a test with *Candida utilis.*

These results support the supposition that in the natural products containing the epidithiodioxopiperazine moiety the disulfide bridge is essential for the biological activity and the three-dimensional structure might be of secondary importance.

Experimental Section

Infrared spectra were measured with a Perkin-Elmer spectrophotometer, Model 257. Proton magnetic resonance spectra were measured on a Varian Associates Model A-100 spectrometer. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent. Mass spectra were obtained with a double-focusing Varian Associates SMI-B mass spectrometer. CD spectra were measured with a Cary 60 using a 8.5×10^{-4} *M* solution (MeOH) in a 2-mm cell. Melting points were taken on a Kofler hot stage and are uncorrected. Thin layer chromatography (TLC) was carried out using Merck precoated silica gel F-254 plates, thickness 0.25 mm. Spots were visualized with a uv hand lamp, iodine vapor, a 0.1% solution of ninhydrin in methanol–1-butanol–2 *N* acetic acid (20:10:1 v/v) or according to Reindel and Hoppe.[21] Solvent systems used: A, 8% ethanol in toluene; B, hexane–2-butanol–acetic acid (3:1:1 v/v); C, 1-butanol–acetic acid–water (4:1:1 v/v). For column chromatography Sephadex LH-20 (Pharmacia) was used (up to 150 mg—column A, 2.4 × 90 cm, eluent 80% methanol, flow rate 18 ml/hr, 3.6-ml fractions; up to 2.0 g—column B, 3.4 × 167 cm, eluent 83% methanol, flow rate 52 ml/hr, 15 ml fractions). The eluent was monitored continuously by measuring the uv absorption at 254 nm with an LKB Uvicord 8300A apparatus. Fractions were collected with a remote-controlled LKB-Ultrarac fraction collector 7000-2.

Benzyloxycarbonyl-L-cysteine (7). This compound was prepared from *N,N'*-dibenzyloxycarbonylcystine[22] according to Wieland and Sarges.[10] The crystalline material (mp 61–63°, yield 98%) thus obtained showed only one spot on TLC (system B) which was sodium nitroprusside positive. By treatment of a sample of **7** with iodine in ethyl acetate, optically pure starting material was obtained quantitatively [$[\alpha]D$ −88° (AcOH); starting material −90.6°; lit.[22] −91.7°] indicating that no racemization had occurred during reduction.

2-Carbethoxy-3,3-dimethylindolino[2,1-*b*]-9-carbobenzoxyaminotetrahydrothiazone-8 (8a and 8b). A solution of **7** (25.9 g, 100 mmol) in 125 ml of refluxing, dry benzene was allowed to react with the indolenine ester **6** (1.30 g, 6 mmol) for 10 days in a simplified Soxhlet apparatus, filled with sodium sulfate as drying agent. The apparatus was filled with argon. After 10 days no further progress of the reaction could be detected on TLC (system A; two new spots with R_f values higher than and identical with that of **6**, respectively). After removal of the solvent under vacuum, ethyl acetate and water were added; the organic layer was washed with 5% $NaHCO_3$ solution, water, 1 *N* HCl solution, water until neutral, and finally brine.[23] After drying (Na_2SO_4), filtration, and removal of the solvent under vacuum, 7.7 g of a light-yellow oil was isolated which was subjected to column chromatography on Sephadex LH-20 in five aliquots, column B. After elution of **6**, two partially overlapping peaks were observed in the uv diagram with elution volumes of 1500 and 1610 ml. The compound with the smaller elution volume had the lower R_f value on TLC (system A) and was assigned structure **8a;** the other peak contained **8b.** Only those fractions were pooled which showed a single spot on TLC, so that rechromatography of the fractions in the overlapping region was necessary. Of **8a,** 1.22 g (2.47 mmol, 41.5%) was isolated as a chromatographically pure, colorless oil, which could not be brought to crystallization. The trans compound **8b** (1.470 g, 3.24 mmol, 54%) was obtained in a crystalline form, mp 140–141° (ethanol–hexane). Structures were assigned on the following basis.

Cis compound 8a: ir ($CHCl_3$) 3400 (NH), 2950, 1720 (br, ester and urethane), 1660 (amide), 1495, 1480, 1452, and 1390 cm^{-1}; NMR ($CDCl_3$) δ 8.24 (m, 1 H, C_7 H), 7.20 (m, 8 H, C_{4-6} H and C_6H_5), 6.10 (d, 1 H, NH, *J* (NH–C_9 H) = 5 Hz), 5.10 (s, 2 H, $C_6H_5CH_2$), 4.48 (six lines, 1 H, C_9 H, *J* (C_9 H–C_{10} H_A) = 12.5 Hz, *J* (C_9 H–C_{10} H_B) = 5 Hz, ABCX spectrum[24]), 4.08 (q, 2 H, CH_2CH_3, *J* = 7 Hz), 3.34 (four lines, 1 H, C_{10} H_A, *J* (C_{10} H_A–C_{10} H_B) = 14 Hz), 2.80 (four lines, 1 H, C_{10} H_B), 1.44 (s, 3 H, C_3 $C_\alpha H_3$), 1.32 (s, 3 H, C_3 $C_\beta H_3$), 1.10 (t, 3 H, CH_2CH_3); mass spectrum (70°) *m/e* 454 (M^+), 408 ($M^+ - C_2H_5OH$), 381 ($M^+ - CO_2C_2H_5$), 346 ($M^+ - C_6H_5CH_2O$), 274 ($M^+ - C_6H_5CH_2OCOOC_2H_5$) (base peak); $[\alpha]^{20}D$ −13.8° (MeOH).

Anal. Calcd for $C_{24}H_{26}N_2O_5S$: C, 63.42; H, 5.77; N, 6.16. Found: C, 63.42; H, 5.71; N, 6.13.

Trans compound 8b: ir ($CHCl_3$) identical with that for **8a,** except for the position of the amide carbonyl absorption at 1670 cm^{-1}; NMR ($CDCl_3$) δ 8.19 (m, 1 H, C_7 H), 7.22 (m, 8 H, C_{4-6} H and C_6H_5), 6.26 (d, 1 H, NH, *J* (NH–C_9 H) = 5 Hz), 5.12 (s, 2 H, $C_6H_5CH_2$), 4.50 (five lines, 1 H, C_9 H, *J* (C_9 H–C_{10} H_A) = 6 Hz, *J* (C_9 H–C_{10} H_B) = 11 Hz, ABCX spectrum), 4.22 (q, 2 H, CH_2CH_3, *J* = 7 Hz), 3.52 (four lines, 1 H, C_{10} H_A, *J* (C_{10} H_A–C_{10} H_B) = 11 Hz), 2.64 (three lines, 1 H, C_{10} H_B), 1.47 (s, 3 H, C_3 $C_\alpha H_3$), 1.31 (s, 3 H, C_3 $C_\beta H_3$), and 1.21 (t, 3 H, CH_2CH_3); mass spectrum (75°) identical with that for **8a,** except for the absence of *m/e* 408 ($M^+ - C_2H_5OH$); $[\alpha]^{20}D$ −19.2° (MeOH).

Anal. Calcd for $C_{24}H_{26}N_2O_5S$: C, 63.42; H, 5.77; N, 6.16. Found: C, 63.29; H, 5.74; N, 6.02.

(S_2R_9/R_2S_9)-2-Carbethoxy-3,3-dimethylindolino[2,1-*b*]-9-aminotetrahydrothiazone-8 Hydrobromide (9a). A solution of 1.05 g (2.31 mmol) of **8a** in 12 ml of a 40% solution of hydrobromic acid in acetic acid was stirred at room temperature for 60 min with exclusion of moisture. Solvent and excess reagent were removed in vacuo, after which the yellow residue was treated twice with dry ether, yielding 755 mg (1.89 mmol, 81%) of a crystalline material (mp 114–117°, methanol–ether) which was subjected to column chromatography on Sephadex LH-20, column B, to yield 648 mg (1.62 mmol, 70%) of a chromatographically pure (TLC, system C), ninhydrin-positive product: ir ($CHCl_3$) 3600 (sh) and 3420 (br, NH_3), 2960, 1730 (ester, sh), 1665 (amide), 1480, 1405, and 1205 cm^{-1}; NMR ($CDCl_3$) δ 8.74 (br s, 3 H, NH_3), 8.20 (m, 1 H, C_7 H), 7.04 (m, 3 H, C_{4-6} H), 4.73 (m, 1 H, C_{10} H_α), 4.15 (q, 2 H, CH_2CH_3), 3.62 (m, 2 H, C_9 H + C_{10} H_α), 1.36 (s, 3 H, C_3 $C_\alpha H_3$), 1.20 (s, 3 H, C_3 $C_\beta H_3$), 1.02 (t, 3 H, CH_2CH_3).

(R_2R_9/S_2S_9)-2-Carbethoxy-3,3-dimethylindolino[2,1-*b*]-9-aminotetrahydrothiazone-8 Hydrobromide (9b). A solution of 955 mg (2.11 mmol) of **8b** in 12 ml of a 40% solution of hydrobromic acid in acetic acid was treated and worked up as described for the preparation of **9a,** yielding 720 mg (1.80 mmol, 78%) of a crystalline (mp 134–135°, methanol–ether) material. After chromatography on Sephadex LH-20 (column B) 592 mg (1.48 mmol, 70%) of a ninhydrin-positive material was isolated, which showed only one spot on tlc (system C, R_f value identical with that of **9a**): ir ($CHCl_3$) 3600 (sh) and 3420 (br, NH_3), 1735 (ester, sh), 1675 (amide), 1490, 1415, and 1240 cm^{-1}; NMR ($CDCl_3$) δ 8.16 (br s, 4 H, NH_3 and C_7 H), 7.05 (m, 3 H, C_{4-6} H), 4.68 (m, 1 H, C_{10} H_α), 4.15 (q, 2 H, CH_2CH_3), 3.70 (m, 2 H, C_9 H + C_{10} H_α), 1.39 (s, 3 H, C_3 $C_\alpha H_3$), 1.29 (s, 3 H, C_3 $C_\beta H_3$), 1.09 (t, 3 H, CH_2 CH_3).

(S_2R_9/R_2S_9)-9,9a-Dihydro-2,9a-epimethylenethio-3,10-diketo-9,9-dimethylpiperazino[4,3-*a*]indole (5). Deprotonation of **9a** could be achieved by ion-exchange chromatography (Amberlite IRA-400) or by adjusting the pH to 9.5 with sodium hydroxide.

The latter method is the preferred one, as considerable losses of material occurred owing to irreversible binding to the ion-exchange resin.

From 9a. The pH of a stirred solution of **9a** (630 mg, 1.57 mmol) in 20 ml of 50% methanol was adjusted to 9.5 with a 0.5 *N* NaOH solution. This solution was evaporated under vacuum to a final volume of about 5 ml (water bath temperature kept below 40°) and subjected to column chromatography on Sephadex LH-20 (column B). Fractions in the peaks with elution volumes of 1500 ml (fraction 1) and 1900 ml (fraction 2) were pooled separately, to yield 330 mg (1.04 mmol) of **10a** and 100 mg (0.365 mmol) of ring-closed product **5.** A solution of fraction 1 in 10 ml of ethanol was refluxed for 1 hr, and the resulting solution, combined with fraction 2, was chromatographed on Sephadex LH-20, column B. Fractions in the peak with an elution volume of 1900 ml were pooled, yielding 245 mg (0.9 mmol, 57%) of a compound which on TLC (systems A or B) showed only one ninhydrin-negative, Reindal–Hoppe positive spot, $[\alpha]^{20}D$ −10° (MeOH). Recrystallization yielded crystalline, racemic crystals, mp 212–213° (methanol), and an amorphous residue, which was optically active, $[\alpha]^{20}D$ −63° (MeOH). Both fractions were chromatographically homogeneous.

Uv λ_{max} (MeOH) 286, 277, and 242 nm (ϵ 2.92, 3.60, and 9.5 × 10^3, respectively); ir ($CHCl_3$) 3380 (NH), 2960, 1705 (br, 2 amide), 1600, 1480, 1455, 1390, 1290, 1140, and 1105 cm^{-1}; NMR ($CDCl_3$) δ 7.95 (m, 2 H, disappearance of 1 H after addition of D_2O, C_7 H and NH), 7.25 (m, 3 H, C_{4-6} H), 4.56 [br s, 1 H, C_9 H; after addition of D_2O only slight sharpening up, indicating that J (C_9 H–NH) must be negligibly small; irradiation of signals at 3.12 and 3.54 revealed the presence of four overlapping lines [J (C_9 H–C_{10} H_A) = 3.5, J (C_9 H–C_{10} H_B) = 1 Hz], 3.54 [four lines, 1 H, C_{10} H_A, J (H_A–H_B) = 11 Hz], 3.12 (four lines, 1 H, C_{10} H_B), 1.68 (s, 3 H, C_3 $C_\alpha H_3$), and 1.54 (s, 3 H, C_3 $C_\beta H_3$); mass spectrum (100°) *m/e* 274 (M^+, base peak), 231 (M^+ − CONH), 228 (M^+ − SCH_2), 216 (M^+ − CONH, CH_3), and 213 (M^+ − SCH_2, CH_3).

Anal. Calcd for $C_{14}H_{14}N_2O_2S$: C, 61.29; H, 5.14; N, 10.21. Found: C, 61.59; H, 5.17; N, 10.25.

From 9b. 9b (580 mg, 1.28 mmol) was treated as described above. Column chromatography on Sephadex LH-20, column B, yielded 441 mg (1.26 mmol) of the trans amine **10b,** after pooling of the fractions in the peak having an elution volume of 1500 ml. A solution of this material in 10 ml of ethanol was refluxed for 20 hr, and the resulting solution was chromatographed on Sephadex LH-20, column B. Fractions in the peak with an elution volume of 1900 ml were pooled, yielding 190 mg (0.695 mmol, 55%) of a compound which had spectral properties identical with those described above, except for $[\alpha]^{20}D$ −52° (MeOH). Recrystallization of this material yielded a racemic crystalline fraction (mp 213–214°) and an optically active amorphous residue, $[\alpha]^{20}D$ −156° (MeOH). Both fractions were chromatographically homogeneous. A CD dispersion curve of the optically active fraction showed a positive and a negative Cotton effect at 235 and 263 nm, respectively (Figure 1).

Anal. Calcd for $C_{14}H_{14}N_2O_2S$: C, 61.29; H, 5.14; N, 10.21. Found: C, 61.48; H, 5.12; N, 10.27.

Ring Closure of 10b to C-9 Deuterated 5. To a solution of 49 mg (1.54×10^{-4} mol) of **10b** in 2 ml of CH_3OD was added 0.5 ml of D_2O. The resulting solution was evaporated to dryness. This procedure was repeated, after which a solution of the residue in 5 ml of CH_3OD was refluxed for 5 days. After evaporation the reaction mixture was subjected to column chromatography on Sephadex LH-20 (column A), yielding in addition to the deuterated starting material (5 mg, v_e 250 ml, M^+ *m/e* 321) and the corresponding methyl ester (4 mg, v_e 360 ml, M^+ *m/e* 307), 25 mg (59%, v_e 414 ml) of ring closed, C_9 deuterated material **5,** NMR ($CDCl_3$) identical with that described above, except for the absence of the four lines at δ 4.56 (C_9 D) and the presence of a simplified AB pattern at δ 3.53 and 3.11 (C_{10} H_A and C_{10} H_B, J (H_A–H_B) = 11 Hz), mass spectrum (110°) *m/e* 275 (M_D^+, base peak), 232 (M_D^+ − CONH), 229, 217, 214.

Deuterium Exchange in 8b and Its Epimerization. To an ice-cooled, stirred solution of 3 mmol of sodium deuterioxide in 2 ml of deuterated methanol [prepared by addition of 162 mg (3 mmol) of freshly prepared sodium methoxide to 0.5 ml of deuterium oxide in 1.5 ml of deuterated methanol] was added 90 mg (0.198 mmol) of **8b.** The reaction mixture was kept at 0° for 30 min, after which the reaction was stopped by the addition of acetic acid. The mixture was extracted with ethyl acetate subsequent to the addition of water; the organic layer was washed with 5% $NaHCO_3$ solution, water, 1 *N* HCl solution until neutral, and finally with saturated brine to yield 83 mg of an oil, which on TLC (system A) showed a spot on the origin, besides the two spots of the diastereomers **8a** and **8b.** Purification was achieved by column chromatography on Sephadex LH-20 (column A) to yield 42 mg (0.093 mmol, 47%) of a mixture of C_9-deuterated **8a** and **8b** in a ratio of 3:2 as an NMR spectrum showed. A mass spectrum indicated that the exchange was complete. NMR ($CDCl_3$), essentials only: δ 6.25 (s, 0.4 H, NH-**8b**), 5.97 (s, 0.6 H, NH-**8a**), 3.54 [two lines, 0.4 H, C_{10} H_A of **8b,** J (H_A–H_B) = 11 Hz], 3.36 [two lines, 0.6 H, C_{10} H_A in **8a,** J (H_A–H_B) = 14 Hz], 2.83 (two lines, 0.6 H, C_{10} H_B in **8a**), and 2.67 (two lines, 0.4 H, C_{10} H_B in **8b**); mass spectrum (120°) *m/e* 455 (M^+), 409 (M^+ − C_2H_5OH, characteristic for the cis compound), 382 (M − $CO_2C_2H_5$).

Acknowledgment. We are grateful to Drs. A. J. M. Berns and A. Taylor for performing the bioassays and to Dr. T. F. Spande for making the CD spectrum and valuable discussions.

Registry No.—(S_2R_9/R_2S_9)-**5,** 55400-05-0; (R_2S_9)-**5,** 55281-65-7; **6,** 41296-09-7; **7,** 53907-29-2; **8a,** 55281-66-8; **8b,** 55281-67-9; **9a,** 55281-68-0; **9b,** 55281-69-1; **10a,** 55281-70-4; **10b,** 55281-71-5.

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