6 h. The solvent was allowed to evaporate and excess reagent was removed by immersing the plates in toluene (15 min) and $CH_3OH$ (1 h). After drying at 120 °C (weight gain 0.40 g) the plates were immersed in a 5% solution (by weight) of Dow-Corning 704 diffusion pump fluid in ether overnight. The solvent was allowed to evaporate, after which the plates were kept at 120 °C for 1 h. By this treatment the plates had gained an additional 1.0 g in weight. The analogues were spotted as 1 μL each of a $CH_2Cl_2$ solution (3 mg/mL). The plates were developed in an acetone–water solution (3:1 v/v), saturated with the Dow-Corning fluid. Spots were visualized by spraying with 2% aqueous $AgNO_3$. The $R_m$ values reported are averages of five determinations and are calculated by means of the formula: $R_m = \log (1/R_f - 1)$.

**Reversed-Phase LC.** As stationary phase a CO:PELL ODS (stationary phase, octadecylsilane; Reeve Angel, Clifton, N.J.) was used; column dimensions were 90 cm × 2.1 mm i.d. The mobile phase was MeOH–$H_2O$ (2:1), while the flow rate was kept at 0.4 mL/min. The eluate was monitored by UV absorption (Waters Associates, Model 440). The elution time of $CH_3CN$ was defined as $t_0$. The log $k'$ values reported are averages of two determinations and are calculated by means of the formula: $k' = (t_r - t_0)/t_0$.

**Acknowledgment.** We thank Mr. H. Ketelaars, Professor R. J. F. Nivard, and Mr. J. Diersmann for valuable discussions. Part of this work was supported by the Koningin Wilhelmina Fonds.

## References and Notes

(1) H. M. Temin and S. Mizutani, *Nature (London)*, **226**, 1211 (1970).
(2) D. Baltimore, *Nature (London)*, **226**, 1209 (1970).
(3) T. H. Maugh, *Science*, **184**, 970 (1974).
(4) (a) R. G. Smith and R. C. Gallo, *Life Sci.*, **15**, 1711 (1974); (b) P. Chandra, L. K. Steel, U. Ebener, M. Woltersdorf, H. Laube, B. Kornhuber, B. Mildner, and A. Götz, *Pharmacol. Ther., Part A*, **1**, 231 (1977).
(5) H. C. J. Ottenheijm, J. D. M. Herscheid, G. P. C. Kerkhoff, and T. F. Spande, *J. Org. Chem.*, **41**, 3433 (1976).
(6) E. De Clercq, A. Billiau, H. C. J. Ottenheijm, and J. D. M. Herscheid, *Biochem. Pharmacol.*, **27**, 635 (1978).
(7) For a review on natural epidithiodioxopiperazines, see A. Taylor in "Microbial Toxins", Vol. VII, S. Kadis, A. Ciegler, and S. J. Ajl, Ed., Academic Press, New York, N. Y., 1971, p 337; see also references cited in ref 5.
(8) S. Mizutani and H. M. Temin, personal communication, 1975.
(9) (a) H. C. J. Ottenheijm, T. F. Spande, and B. Witkop, *J. Am. Chem. Soc.*, **95**, 1989 (1973); (b) G. H. L. Nefkens and G. I. Tesser, private communication.
(10) H. C. J. Ottenheijm and J. H. M. de Man, *Synthesis*, 163 (1975).
(11) J. H. Noordik, J. D. M. Herscheid, M. W. Tijhuis, and H. C. J. Ottenheijm, *Recl. Trav. Chim. Pays-Bas*, **97**, 91 (1978).
(12) G. L. Biagi, A. M. Barbero, M. F. Gamba, and M. C. Guerra, *J. Chromatogr.*, 41, 371 (1969).
(13) A. Leo, C. Hansch, and D. Elkens, *Chem. Rev.*, **71**, 525 (1971).
(14) C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, **208**, 537 (1965).
(15) A. N. Tischler, F. M. Thompson, L. J. Libertini, and M. Calvin, *J. Med. Chem.*, **17**, 948 (1974).
(16) This technique has been used earlier in reversed-phase column chromatography; see, e.g., H. G. Cassidy in "Technique of Organic Chemistry", Vol. X, A. Weissberger, Ed., Interscience, New York, N.Y., 1957, p 119.
(17) J. M. McCall, *J. Med. Chem.*, **18**, 549 (1975).
(18) K. C. Murdock, *J. Med. Chem.*, **17**, 827 (1974).
(19) E. De Clercq and P. J. Claes, *Biochim. Biophys. Acta*, **33**, 328 (1973).

# Gliotoxin Analogues as Inhibitors of Reverse Transcriptase. 2.[1] Resolution and X-ray Crystal Structure Determination

Harry C. J. Ottenheijm,* Jacobus D. M. Herscheid, Marian W. Tijhuis, Rutger J. F. Nivard,

*Department of Organic Chemistry, University of Nijmegen, Toernooiveld, Nijmegen, The Netherlands*

Erik De Clercq,

*Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium*

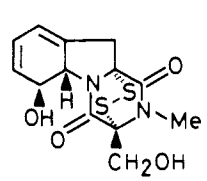
and Peter A. J. Prick

*Department of Crystallography, University of Nijmegen, Toernooiveld, Nijmegen, The Netherlands.*
*Received December 12, 1977*

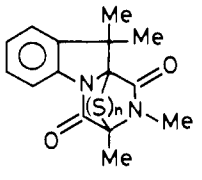
A novel, simple, and efficient method for the chemical resolution of epidithiodioxopiperazines is reported, which is based upon covalent formation of diastereomers. This method might be a general one for the resolution of chiral cyclic disulfides. Dithiol **5**, prepared from **2** by reduction with $NaBH_4$, was allowed to react with the disulfenyl chloride **8** to yield **9** and **10**, which were separated by short-column chromatography on silica gel. From these, the optically pure enantiomers **11** and **12**, respectively, were obtained by reduction with $NaBH_4$, followed by reoxidation with $I_2$–pyridine. In this way the precursor **7** of the resolving agent could also be recovered. The absolute configurations of **11** and **12** were derived from CD spectra. Kinetic asymmetric transformation of the gliotoxin analogue **2** with the diphosphine **6** gave a 19% enrichment in one enantiomer of the starting material. Surprisingly, both enantiomers were found to inhibit reverse transcriptase, the RNA-dependent DNA polymerase, to the same degree, indicating that there is no relation between this property of epidithiodioxopiperazines and their bridgehead configurations. From the X-ray crystal structure determination it can be seen that there is a considerable torsional and conformational strain in compound **2**, which might enhance the ease of cleavage of the S–S bond. A possible relationship between this property and the biological activity of **2** is discussed.

Gliotoxin (**1**), the sporidesmins,[2] and chaetocin[3] belong to the class of fungal metabolites containing an epidithiodioxopiperazine ring system. The first two compounds have the *R* configuration at the bridgehead carbons and exhibit selective antiviral properties, whereas the antipodal chaetocin does not show this activity. Recently, we reported a synthesis[4] of a racemic gliotoxin analogue **2**, which inhibits reverse transcriptase,[5] the RNA-dependent DNA polymerase of RNA tumor viruses, and whose activity is of the same order as that of gliotoxin. As the antiviral activity of epidithiodioxopiperazines might be related to their bridgehead configurations, we wanted to examine the

0022-2623/78/1821-0799$01.00/0 



1



2 n = 2
3 n = 1

enantiomers of 2 separately. A practical method for the resolution of 2, which might be of general applicability to other chiral disulfides,[6] has been developed. The resulting enantiomers 11 and 12 were tested as inhibitors of reverse transcriptase activity.

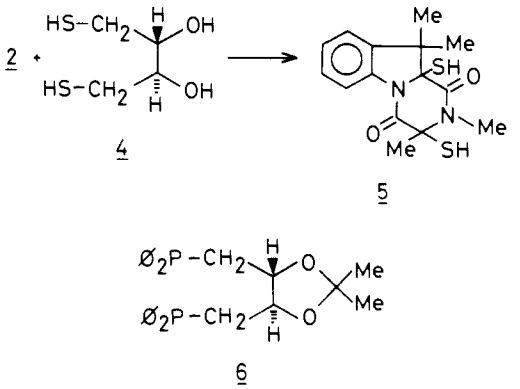
The antiviral activity of 1 and related products appears to reside in the epidithiodioxopiperazine moiety of the molecules.[7-11] Interestingly, only the native, disulfide form is active; the reduced dithiol form is not.[9] In this respect, it was not unexpected to find that only the disulfide form 2 is active in inhibiting reverse transcriptase.[5] Thus, conformational information on 2, and especially on the spatial arrangement of the dioxopiperazine ring with the disulfide bridge, is of importance to gain insight into the structure–activity relationship of 2 and probably all other gliotoxin analogues. Therefore, the crystal structure of 2 was determined by X-ray diffraction.

**Resolution of 2.** Chemical resolution of racemic cyclic disulfides, devoid of convenient handles for conversion into diastereomers, has not been reported. In general, resolution of 2 which lacks such a reactive handle, might be achieved[12] by crystallization, chromatographic,[13] or kinetic methods. Initially we attempted a kinetic asymmetric transformation by two routes, viz. reduction of 2 to 5 with the optically active dimercapto compound 4 (Scheme I) and partial desulfurization of 2 to 3 with the chiral phosphine 6. The former approach is based upon a general reaction of disulfides, viz. their reduction with thiols,[9] whereas partial desulfurization of 2 and 3 has been performed[4] by treatment with $(C_6H_5)_3P$.

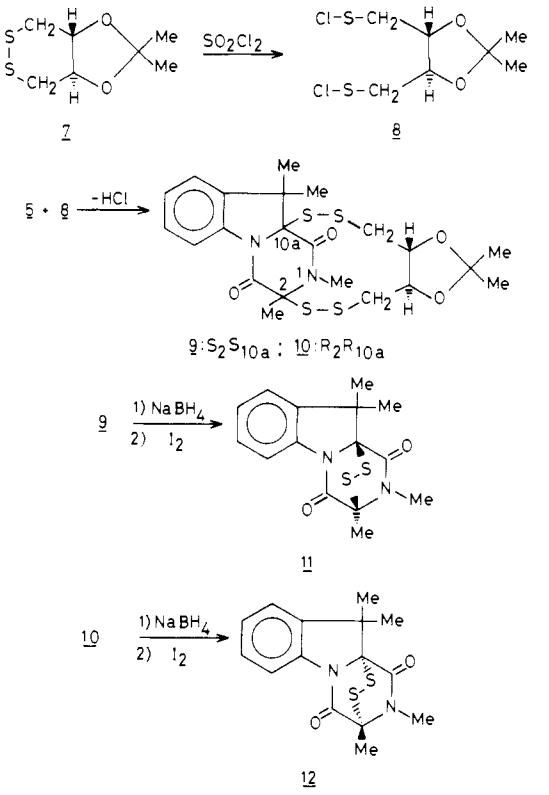
When 2 was treated with 0.5 equiv of the optically active Cleland's reagent 4,[14] only racemic mixtures of 2 and 5 were isolated. However, when 2 was reacted with 0.25 equiv of the diphosphine 6, (–)-Diop,[15] a 19% enrichment in one enantiomer in the isolated starting material was observed. This enantiomeric purity was determined by $^1H$ NMR spectroscopy: racemic 2 showed two, well-separated signals for the N(1)-methyl as well as for the C(2)-methyl group when a chiral shift reagent was used. As this enrichment was too small for our needs, we turned to resolution via covalent formation of diastereomers. The reaction scheme for the synthesis[4] of 2 proceeds via the stable intermediate 5; the latter can also be obtained quantitatively by reduction of 2 with $NaBH_4$.[4] This dithiol could be converted into diastereomers and each transformed into 2 without racemization as will be shown below. The resolving agent was selected on the basis of the following considerations: (1) a bifunctional agent was selected, as diastereomers with a high rigidity would allow an optimal separation;[16] (2) the minimum number of diastereomers (two) would result if this bifunctional reagent possessed an axis of symmetry. These features are present in a derivative of 4, viz. the disulfenyl chloride 8. This was prepared quantitatively from 7[17] with $SO_2Cl_2$ and a trace of pyridine (Scheme II).

Reaction of 8 in $CCl_4$ with 5 in the presence of 2 equiv of pyridine gave, besides 2, the diastereomeric disulfides 9 and 10. Separation by column chromatography on silica gel gave 9 and 10 (28% yield of each) whose $^1H$ NMR spectra were nearly identical (for the ketalic $CH_3$ groups,

Scheme I



Scheme II



two singlets at $\delta$ 1.35 and 1.43 for 9; broad singlet at 1.40 for 10). Reduction of 9 or 10 with $NaBH_4$ in ethanol, followed by reoxidation with $I_2$–pyridine, gave the enantiomers 11 ($[\alpha]^{22}_D$ +507°) or 12 ($[\alpha]^{22}_D$ –502°), in 73 and 70% yield, respectively, as well as the precursor 7 of the resolving agent. The $^1H$ NMR spectra of 11 or 12 in the presence of a chiral shift reagent showed that the optical purity is higher than 90%. The absolute configurations of the two enantiomers were derived from CD spectra (Figure 1).

Gliotoxin (1), having the *R* configuration in the epidithiodioxopiperazine moiety, shows a negative Cotton effect at 230 nm.[18] From this the tentative conclusion is drawn that 11 possesses the *S* configuration, while 12 has the *R* configuration.

This method of resolution via covalent formation of diastereomers meets all of the principal desirable features as formulated by Wilen.[12] Application to other racemic, cyclic disulfides and to other chiral compounds having two reactive groups is under investigation.

**Antireverse Transcriptase Activity.** The two enantiomers 11 and 12 were tested as inhibitors of the RNA-directed DNA polymerase (reverse transcriptase)

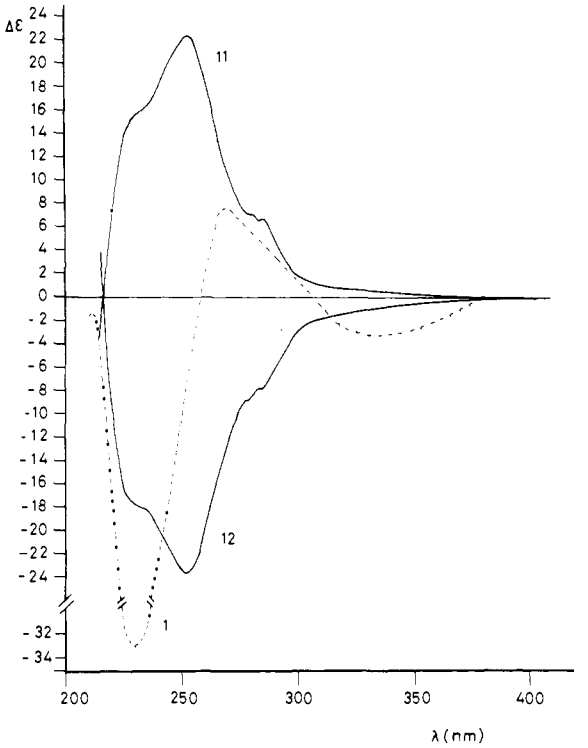



**Figure 1.** Circular dichroism curves of **1**, **11**, and **12**.

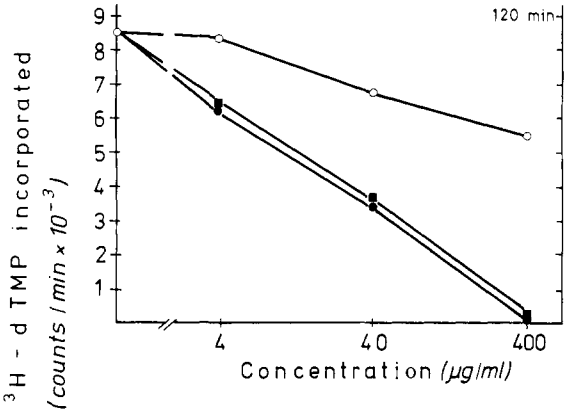



**Figure 2.** Effect of the *R* and *S* enantiomers on reverse transcriptase activity associated with murine (Moloney) leukemia virus: *R* enantiomer (●); *S* enantiomer (■); $Me_2SO$ control (○). At analogue concentrations of 4, 40, and 400 μg/mL, the $Me_2SO$ concentration was 0.2, 2, and 4% (v/v), respectively. The data represent average values for two separate determinations.

activity associated with murine leukemia virus (Figure 2). The methodology for measuring the reverse transcriptase activity has been described previously.[19] Irrespective of the concentrations at which they were assayed, and of the time they were incubated with the DNA polymerase reaction mixture, the two enantiomers, *R* and *S*, caused a similar, if not identical, inhibition of DNA synthesis.

The two diastereomers, **9** and **10**, were also tested for antireverse transcriptase activity. Interestingly, both compounds showed identical activity which was of the same order as those of a series of gliotoxin analogues described earlier[1,20] [log ($I_{50}$ × $10^5$) = 0.796].

**X-ray Structure Determination.** The crystal structure of **2** was determined by X-ray diffraction. Fractional coordinates and thermal parameters are listed in Table I (see paragraph at end of paper regarding supplementary material). Bond lengths and angles are shown in Figure 3 and Table II, respectively. A projection of the molecular structure, showing a pronounced boat conformation of the dioxopiperazine ring, is given in Figure 4.

**Table I.** Fractional Coordinates and Standard Deviations

| Atom | *x* | *y* | *z* |
|---|---|---|---|
| S(1) | 0.44546 (2) | 0.13018 (5) | 0.96157 (4) |
| S(2) | 0.41003 (3) | −0.00556 (5) | 1.04416 (5) |
| O(1) | 0.44465 (7) | 0.37390 (13) | 1.13441 (11) |
| O(2) | 0.26064 (6) | 0.09078 (12) | 0.99293 (11) |
| N(1) | 0.39295 (7) | 0.20110 (14) | 0.94852 (11) |
| N(2) | 0.33116 (6) | 0.21617 (12) | 0.94852 (11) |
| C(1) | 0.39213 (7) | 0.26026 (15) | 0.97764 (13) |
| C(2) | 0.41232 (8) | 0.28754 (16) | 1.09863 (14) |
| C(3) | 0.36030 (8) | 0.09479 (15) | 1.10968 (14) |
| C(4) | 0.31106 (8) | 0.13284 (15) | 1.01280 (14) |
| C(5) | 0.39489 (8) | 0.36622 (17) | 0.89511 (14) |
| C(6) | 0.34340 (8) | 0.32858 (16) | 0.80292 (14) |
| C(7) | 0.32841 (10) | 0.37157 (19) | 0.69710 (16) |
| C(8) | 0.27512 (11) | 0.33289 (20) | 0.62990 (16) |
| C(9) | 0.23752 (10) | 0.25376 (20) | 0.66749 (16) |
| C(10) | 0.25131 (8) | 0.20846 (17) | 0.77394 (15) |
| C(11) | 0.30511 (8) | 0.24832 (15) | 0.83912 (13) |
| C(12) | 0.42229 (11) | 0.19671 (27) | 1.27798 (17) |
| C(13) | 0.33616 (10) | 0.00929 (18) | 1.18492 (16) |
| C(14) | 0.37718 (11) | 0.49150 (19) | 0.93781 (19) |
| C(15) | 0.45552 (11) | 0.37799 (28) | 0.86384 (23) |
| H(7) | 0.3522 (8) | 0.4252 (17) | 0.6748 (14) |
| H(8) | 0.2627 (10) | 0.3667 (20) | 0.5566 (17) |
| H(9) | 0.2019 (9) | 0.2269 (18) | 0.6244 (16) |
| H(10) | 0.2252 (8) | 0.1536 (15) | 0.8006 (14) |
| H(121) | 0.4490 (11) | 0.1252 (23) | 1.2891 (19) |
| H(122) | 0.3935 (10) | 0.1848 (21) | 1.3242 (18) |
| H(123) | 0.4462 (11) | 0.2668 (24) | 1.2987 (19) |
| H(131) | 0.3713 (10) | −0.0247 (19) | 1.2422 (17) |
| H(132) | 0.3081 (9) | 0.0506 (19) | 1.2204 (15) |
| H(133) | 0.3170 (9) | −0.0633 (20) | 1.1455 (17) |
| H(141) | 0.3389 (9) | 0.4806 (18) | 0.9650 (16) |
| H(142) | 0.3724 (9) | 0.5504 (20) | 0.8796 (17) |
| H(143) | 0.4086 (11) | 0.5233 (21) | 0.9929 (20) |
| H(151) | 0.4669 (10) | 0.3031 (22) | 0.8261 (18) |
| H(152) | 0.4858 (12) | 0.3933 (21) | 0.9229 (20) |
| H(153) | 0.4552 (11) | 0.4495 (25) | 0.8130 (21) |

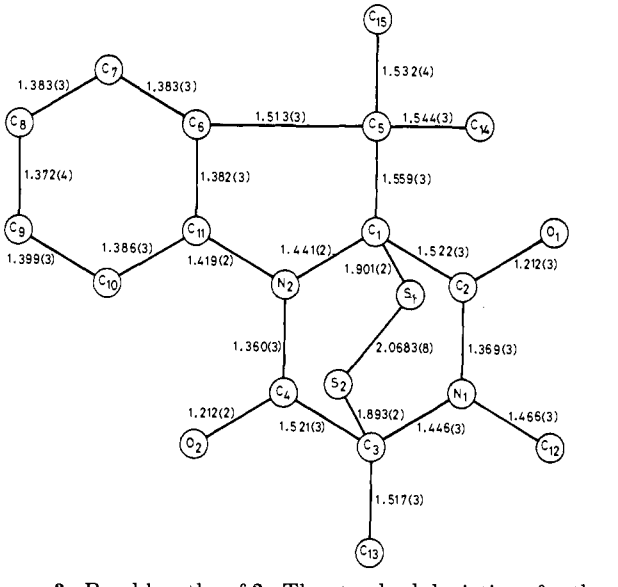



**Figure 3.** Bond lengths of **2**. The standard deviations for the distances are given in parentheses.

Bond lengths and angles in the epidithiodioxopiperazine moiety of **2** are in good agreement with those found in gliotoxin (**1**).[21] The dioxopiperazine ring has a markedly folded conformation: the dihedral angle between the planes (see Figure 3) through the atoms [N(2),C(1),C(2)] and [C(4),C(3),N(1)] and the least-squares plane through the atoms [N(1),C(2),N(2),C(4)] are 36 and 41°, respectively. Corresponding angles in the C(1)-mercapto-

Table II. Bond Angles[a]

| atoms | angle, deg | atoms | angle, deg |
|---|---|---|---|
| C(1)-S(1)-S(2) | 97.72 | C(3)-C(4)-O(2) | 124.24 |
| S(1)-S(2)-C(3) | 99.15 | N(2)-C(4)-O(2) | 123.99 |
| S(1)-C(1)-C(2) | 101.20 | C(4)-N(2)-C(1) | 120.34 |
| S(1)-C(1)-N(2) | 109.69 | C(4)-N(2)-C(11) | 128.27 |
| S(1)-C(1)-C(5) | 110.23 | C(1)-N(2)-C(11) | 109.67 |
| S(2)-C(3)-N(1) | 110.81 | C(1)-C(5)-C(6) | 100.11 |
| S(2)-C(3)-C(4) | 102.23 | C(1)-C(5)-C(14) | 110.79 |
| S(2)-C(3)-C(13) | 105.48 | C(1)-C(5)-C(15) | 114.21 |
| C(2)-C(1)-C(5) | 118.75 | C(6)-C(5)-C(14) | 106.50 |
| C(2)-C(1)-N(2) | 112.09 | C(6)-C(5)-C(15) | 114.63 |
| N(2)-C(1)-C(5) | 104.80 | C(14)-C(5)-C(15) | 110.04 |
| C(1)-C(2)-N(1) | 113.11 | C(5)-C(6)-C(7) | 129.31 |
| C(1)-C(2)-O(1) | 122.95 | C(5)-C(6)-C(11) | 110.81 |
| N(1)-C(2)-O(1) | 123.85 | C(7)-C(6)-C(11) | 119.57 |
| C(2)-N(1)-C(3) | 118.33 | C(6)-C(7)-C(8) | 118.89 |
| C(2)-N(1)-C(12) | 118.14 | C(7)-C(8)-C(9) | 120.70 |
| C(3)-N(1)-C(12) | 120.29 | C(8)-C(9)-C(10) | 121.90 |
| N(1)-C(3)-C(4) | 111.51 | C(9)-C(10)-C(11) | 116.07 |
| N(1)-C(3)-C(13) | 114.80 | C(6)-C(11)-C(10) | 122.87 |
| C(4)-C(3)-C(13) | 111.12 | C(6)-C(11)-N(2) | 108.54 |
| C(3)-C(4)-N(2) | 111.72 | C(10)-C(11)-N(2) | 128.58 |

[a] Standard deviations for the bond angles are 0.06° for those involving S and between 0.10 and 0.20° for other angles.

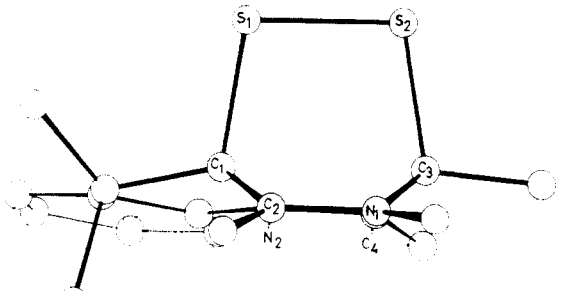



Figure 4. Projection of 2.

C(3)-methylene compound[23] are 23 and 14°, respectively, showing a more pronounced boat conformation in the dioxopiperazine moiety of 2. An alternative way to express the "fold" in the dioxopiperazine ring is the magnitude of the dihedral angle between the least-squares planes through the atoms [C(1),N(2),C(4),C(3)] and [C(1),-C(2),N(1),C(3)]. This angle is 134° for 2, a value very similar to that for gliotoxin[21] (1), where this angle has a value of 129 and 132° in the two symmetry-independent molecules. In the C(1)-mercapto-C(3)-methylene compound the "fold", expressed in this way, is 158°.

A comparison of the dihedral angle between the planes through the atoms [N(2),C(1),C(2)] and the least-squares plane through the atoms [N(1),C(2),N(2),C(4)] in 2 (36°) and the C(1)-mercapto-C(3)-methylene compound[23] (23°) contradicts the conclusion of Karle et al.[22] that addition of a disulfide bridge to the dioxopiperazine ring "does not change appreciably the geometry of the ring". Karle et al. came to this conclusion from a comparison of the "fold" in *cyclo*-(L-Pro-L-Leu) and 3,4-dehydroproline anhydride on one hand and several natural epidithiodioxopiperazines on the other hand, compounds which, in our opinion, are not comparable for the conclusion to be drawn, since the disulfide bridge is not the only factor in which they are different from each other.

The attachment of the disulfide group to C(1) and C(3) restricts the normal open-chain torsion angle about the S–S bond (ca. 100°) to the small value of 13° for [C(1),S(1),-S(2)]/[S(1),S(2),C(3)], similar to that found[21] in gliotoxin (1) (8.8 and 15.8°). This geometric constraint causes significant torsional strain. The S–S distance of 2.068 Å is typical for a disulfide with single bond character, so this strain is not eased by a lengthening of the S–S bond, as has been observed in 1,2-dithiocyclopentanes.[24]

The chirality of the CSSC group[25] in the *R* enantiomer of 2, i.e., 12, is left handed as has been found[21] in 1.

## Conclusions

A facile method has been described for the resolution of the racemic epidithiodioxopiperazine 2, which might be of general applicability to other chiral, cyclic disulfides.

Both enantiomers of 2, viz. 11 and 12, were found to have the same antireverse transcriptase activity, indicating that there is no relation between this biologic property of epidithiodioxopiperazines and their bridgehead configurations.

The X-ray crystal structure determination of 2 shows a markedly folded conformation for the dioxopiperazine ring, caused by the addition of the disulfide bridge. In addition, attachment of this disulfide group leads to considerable torsional strain in the disulfide, whose dihedral angle is restricted to the small value of 13°. These conformational and torsional strains will undoubtedly enhance the ease of cleavage of the S–S bond in 2 and its analogues. From these observations one is tempted to speculate that at least part of the action of the gliotoxin analogue 2 on the reverse transcriptase is due to a disulfide–thiol interchange reaction.[26]

This hypothesis is supported by the earlier made observation[5] that only the disulfide form of 2 is active in inhibiting reverse transcriptase. In this respect, the high activities of 9 and 10 are also noteworthy.[27]

This hypothesis is under investigation by the synthesis of radioactive-labeled 2.

## Experimental Section

Infrared spectra were measured with a Perkin-Elmer spectrophotometer, Model 257. Proton magnetic resonance spectra were measured on a Varian Associates Model T-60 spectrometer. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer. Melting points were taken on a Köfler hot stage (Leitz-Wetzlar) 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, and, in the case of sulfur-containing products, by spraying with 2% aqueous $AgNO_3$.[10]

**Reaction of 2 with (–)-Diop (6).** To a stirred solution of 320 mg (1 mmol) of 2[4] in 15 mL of dry dioxane was added at room temperature 124 mg (0.25 mmol) of (–)-Diop.[15] Stirring at room temperature was continued for 1 h, after which time the solvent was removed in vacuo and the residue column chromatographed on 50 g of Merck silica gel PF-254 in $CCl_4$–$CHCl_3$ (1:1 v/v), yielding the monosulfide 3 besides unreacted starting material 2. Of the latter the enantiomeric enrichment was determined by $^1H$ NMR spectroscopy in $CCl_4$; in the presence of tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium(III) the N(1)-$CH_3$ as well as the C(2)-$CH_3$ groups showed two signals; integration of the latter gave an enrichment of 19% in one enantiomer (peak ratio 27:40).

***S,S*-Dichloro-2,3-*O*-isopropylidene-1,4-dithio-L-threitol (8).** To an ice-cooled, stirred solution of 1.279 g of 7[14] [6.65 mmol; $^1H$ NMR ($CCl_4$) δ 3.55 (m, 2 H), 3.10 (m, 4 H), and 1.42 (s, 6 H)] in 17 mL of $CCl_4$ was added dropwise 0.897 g (6.65 mmol) of $SO_2Cl_2$, followed by 1 drop of pyridine. The reaction mixture, whose color changed from colorless to yellow as soon as the base was added, was used immediately in the following reaction: NMR ($CCl_4$) δ 4.30 (m, 2 H), 3.42 (m, 4 H), and 1.42 (s, 6 H).

**Diastereomers 9 and 10.** To an ice-cooled, stirred solution of 2.145 g (6.65 mmol) of 5[4] in 500 mL of alcohol-free $CH_2Cl_2$ was

added a $CCl_4$ solution of 6.65 mmol of **8**, prepared as described above, subsequent to the addition of 1.05 g (13.3 mmol) of pyridine. After 10 min of stirring at room temperature, TLC ($CH_2Cl_2$) showed the presence of two spots, corresponding to **9** ($R_f$ 0.29) and **10** ($R_f$ 0.23). The pyridine salts were removed by filtration, and the filtrate was evaporated to dryness. The residue was column chromatographed on 200 g of Merck silica gel H in $CCl_4$–$CH_2Cl_2$ (1:1 v/v) under slightly increased pressure (about 10 cmHg); in order to prevent acid-catalyzed deketalization a thin layer of solid $Na_2CO_3$ was placed on top of the column.

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 **9** and **10**, 950 mg (28%) of each was isolated. **9**: $[\alpha]^{22}_D$ −96.6° (*c* 2.565, $CHCl_3$); NMR $\delta$ 1.17 (s, 3 H), 1.35 (s, 3 H), 1.43 (s, 3 H), 1.83 (s, 3 H), 2.13 (s, 3 H), 3.13 (s, 3 H), 2.3–4.0 (m, 4 H), 4.00–4.38 (m, 2 H), 7.30 (m, 3 H), and 7.87 (m, 1 H). **10**: $[\alpha]^{22}_D$ −5.05° (*c* 2.020, $CHCl_3$); NMR ($CDCl_3$) nearly identical with that for **9**, except for the absence of the signals at $\delta$ 1.35 and 1.43 and the presence of a broad singlet at $\delta$ 1.40 (6 H).

**(2*S*,9a*S*)-9,9a-Dihydro-1,2,9,9-tetramethyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11) and Its 2*R*,9a*R* Isomer 12.** To an ice-cooled, stirred solution of 729 mg (1.42 mmol) of **9** in a mixture of 100 mL of ethanol and 50 mL of dimethoxyethane was added 360 mg (9.5 mmol) of $NaBH_4$. After stirring for 10 min the same amount of $NaBH_4$ was added. Stirring was then continued for 20 min at 0 °C and for 15 min at room temperature. After evaporation of the solvent and addition of water and $CH_2Cl_2$, the pH of the aqueous layer was adjusted at 7 with 1 N $H_2SO_4$. The aqueous layer was washed with $CH_2Cl_2$, after which the combined organic layers were dried ($Na_2SO_4$) and filtrated. To the reaction mixture thus obtained was added dropwise a 2.5% solution of $I_2$ in $CH_2Cl_2$, containing 2 molar equiv of pyridine, until the reaction mixture remained colored. The reaction mixture was washed with water, to which were added a few crystals of $Na_2S_2O_3$, and was then dried ($Na_2SO_4$). Filtration, followed by evaporation and column chromatography of the residue on 100 g of Merck silica gel H in $CCl_4$–$CH_2Cl_2$ (1:1 v/v), yielded, besides **7**, 331 mg (73%) of **11**: mp (MeOH) 140–143 °C; $[\alpha]^{22}_D$ +507° (*c* 2.010, $CHCl_3$); TLC, NMR, and IR were identical with those of **2**.[4]

**Compound 12** was prepared as described for **11** in 70% yield (315 mg): $[\alpha]^{22}_D$ −502° (*c* 2.345, $CHCl_3$).

**Reverse Transcriptase Assay.** The method and materials used are described in the accompanying article.[1]

**X-ray Structure Determination. Crystal Data:** colorless crystals from methanol–water; formula $C_{15}H_{16}N_2O_2S_2$. Cell dimensions were determined from diffractometer readings (Mo K$\alpha$, $\lambda$ = 0.71069 Å): monoclinic; $a$ = 22.892 (3), $b$ = 10.794 (2), $c$ = 12.638 (2) Å; $\beta$ = 103.12 (1)°; $Z$ = 8; space group $C2/c$; $D_m$ = 1.41 (1) g cm$^{-3}$; $D_x$ = 1.41 g cm$^{-3}$.

**Intensity Data, Structure Determination, and Refinement.** The intensities of 6784 reflections with $\theta < 27°$ were collected on a Nonius CAD4 diffractometer (monochromated Mo K$\alpha$ radiation). Of the 3294 independent reflections, 2176 had $I > 3\sigma_c(I)$ [$\sigma_c(I)$ based on counting statistics] and were used in the least-squares refinement. No absorption corrections were applied ($\mu$ = 3.5 cm$^{-1}$, crystal dimensions, 0.5 × 0.5 × 0.5 mm$^3$). The structure was solved by direct methods using the sign–correlation procedure.[28,29] This procedure gave two-phase sets, one of which gave the three-dimensional structure. The hydrogen atoms were found by successive difference–Fourier syntheses. Atomic coordinates and anisotropic thermal parameters for S, O, N, and C and isotropic thermal parameters for H were refined by means of a full-matrix least-squares program,[30] minimizing the function $\Sigma w[|F_o| - K|F_c|]^2$ with $w = [\sigma_c^2(F_o) + (0.0085F_o)^2]^{-1}$. The final $R$ value on 2176 reflections is 0.029 (0.0389 including weak reflections).

**Acknowledgment.** The authors thank Dr. J. H. Noordik and Dr. R. O. Gould for their participation in the structure analysis. Part of this work was supported by the Koningin Wilhelmina Fonds.

**Supplementary Material Available:** $U_{ij}$ values of Table I and structure factors from the X-ray crystal structure determination (18 pages). Ordering information is given on any current masthead page.

## References and Notes

(1) For paper 1 of this series, see H. C. J. Ottenheijm, J. D. M. Herscheid, M. W. Tijhuis, M. Oosterbaan, and E. De Clercq, *J. Med. Chem.*, preceding paper in this issue.
(2) For a review, see A. Taylor in "Microbial Toxins", Vol. VII, S. Kadis, A. Ciegler, and S. J. Ajl, Ed., Academic Press, New York, N.Y., 1971, p 337.
(3) D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, **53**, 1661 (1970).
(4) H. C. J. Ottenheijm, J. D. M. Herscheid, G. P. C. Kerkhoff, and T. F. Spande, *J. Org. Chem.*, 41, 3433 (1976).
(5) E. De Clercq, A. Billiau, H. C. J. Ottenheijm, and J. D. M. Herscheid, *Biochem. Pharmacol.*, **27**, 635 (1978).
(6) Part of this work was communicated by H. C. J. Ottenheijm, J. D. M. Herscheid, and R. J. F. Nivard, *J. Org. Chem.*, **42**, 925 (1977).
(7) J. K. McDougall, *Arch. Gesamte Virusforsch.*, **27**, 225 (1969).
(8) P. W. Trown, *Biochem. Biophys. Res. Commun.*, **33**, 402 (1968).
(9) P. W. Trown and J. A. Bilello, *Antimicrob. Agents Chemother.*, **2**, 261 (1972).
(10) K. C. Murdock, *J. Med. Chem.*, **17**, 827 (1974).
(11) H. C. J. Ottenheijm, J. A. M. Hulshof, and R. J. F. Nivard, *J. Org. Chem.*, **40**, 2147 (1975).
(12) S. H. Wilen, *Top. Stereochem.*, **6**, 107 (1971).
(13) Conformational enantiomers of cyclic disulfides have been resolved by chromatography, followed by crystallization: A. Lüttringhaus, U. Hess, and H. J. Rosenbaum, *Z. Naturforsch. B*, **22**, 1296 (1967).
(14) M. Carmack and C. J. Kelley, *J. Org. Chem.*, **33**, 2171 (1968).
(15) H. B. Kagan and T. P. Dang, *J. Am. Chem. Soc.*, **94**, 6429 (1972).
(16) A possible relation between rigidity in diastereomers and their ease of separation can be deduced from Woodward's statement that separation of diastereomeric salts is more likely to succeed when the chiral centers are as close as possible in space: R. B. Woodward et al., *Tetrahedron*, **19**, 247 (1963), and footnotes on p 259.
(17) This compound is prepared in 50% overall yield from L-(+)-tartaric acid; see ref 14.
(18) The Cotton effect at 230 nm is due to an n,$\pi^*$ transition of a peptide bond; see, e.g., R. Nagarajan and R. W. Woody, *J. Am. Chem. Soc.*, **95**, 7212 (1973).
(19) E. De Clercq and P. J. Claes, *Biochim. Biophys. Acta*, **231**, 328 (1973).
(20) Analogues **9** and **10** and those described in ref 1 were tested under the same conditions, so that their activities may be compared.
(21) J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, **23**, 439 (1967).
(22) I. L. Karle, H. C. J. Ottenheijm, and B. Witkop, *J. Am. Chem. Soc.*, **96**, 539 (1974).
(23) J. H. Noordik, J. D. M. Herscheid, M. W. Tijhuis, and H. C. J. Ottenheijm, *Recl. Trav. Chim. Pays-Bas*, **97**, 91 (1978).
(24) J. A. Kice in "Sulfur in Organic and Inorganic Chemistry", Vol. I, A. Senning, Ed., Marcel Dekker, New York, N.Y., 1971, p 153.
(25) J. A. Schellman in "Optical Rotatory Dispersion. Applications to Organic Chemistry", C. Djerassi, Ed., McGraw-Hill, New York, N.Y., 1960, section 15-4.
(26) Z. M. Bacq introduced the expression "substances thioloprives" for compounds that possess active groupings which may react with the SH–enzyme systems of microorganisms [Z. M. Bacq, *Experientia*, **2**, 349, 385 (1946)]. As early as 1948 H. O. Huisman postulated that the biological activity of gliotoxin might be due to its ability to oxidize thiols to the corresponding disulfides (H. O. Huisman, Ph.D. Thesis, Groningen, The Netherlands, 1948).

(27) Interestingly, Murdock (see ref 10) found that the bis(methyl disulfide) analogue of acetylaranotin had a higher anti-RNA polymerase activity than acetylaranotin itself, whereas the bis(methylthio) analogue was considerably less active.
(28) P. T. Beurskens, *Acta Crystallogr.*, 17, 462 (1964).
(29) Th. E. M. van den Hark, Thesis, University of Nijmegen, 1976.
(30) The X-Ray System, Technical Report TR-192 of the Computer Science Center, University of Maryland, College Park, Md., June 1972.

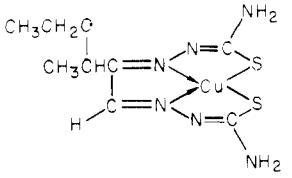
# Comparative Analysis of the Cytotoxicity of Substituted [Phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelates. 2. Parabolic Correlations and Their Implications for Selective Toxicity[1]

Eugene A. Coats,* Stanley R. Milstein, Michael A. Pleiss, and Jeffrey A. Roesener

*College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.* *Received February 22, 1978*

The synthesis of an extended series of para-substituted [phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) chelates is reported. Subsequent biological evaluation and regression analysis have been performed, correlating $pI_{50}$ with extrathermodynamic substituent parameters. Parabolic correlations with $\pi$ have resulted which predict optimum lipophilic character of the para substituent with respect to Ehrlich ascites cytotoxicity ($\pi_0 = -2.13$) and with respect to ascites vs. liver slice cytotoxicity ($\pi_0 = -1.31$). Results indicated clearly that the chelate most toxic to the tumor cell model may not be the most selective.

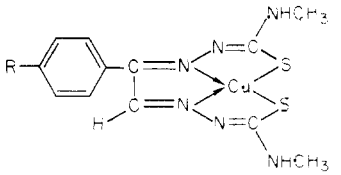
Considerable interest in recent years has been shown in the development of metal chelates and chelating agents as potential antineoplastic agents. Aside from complexes of platinum,[2] some work has focused upon chelates of copper(II) such as Cu(II) KTS [[2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone)]copper(II) chelate; [kethoxal bis(thiosemicarbazone)]copper(II) chelate]] (1) and its analogues.[3]



1

The mechanism of action of Cu(II) KTS as an antitumor agent has been the subject of intense study and some controversy. Several workers[4] have promulgated the view that the copper chelate is a lipophilicity-potentiated form of $Cu^{2+}$, better able to transport the latter species into the tumor cell, whereupon dissociation of the chelate and copper(II)-mediated cytotoxic effects upon enzymes involved in DNA synthesis occurs. An adjunct to this hypothesis has been advanced[5] more recently in the suggestion that while the proposed "shuttle" of copper chelate may well occur, the *primary* effect of Cu(II) KTS on tumor cells is that of interfering with their energy transport system; that is, inhibition of DNA synthesis might very well be observed as a secondary effect resulting from a lack of ATP. Unpublished investigations have indicated that Cu(II) KTS may have the ability to uncouple oxidative phosphorylation in isolated rat liver mitochondria at concentrations below those required for solutions of copper(II) ions alone.[5b] It is also known that Cu(II) KTS inhibits the respiration of both Ehrlich ascites cells and rat liver slices. These observations could be accounted for by the recent findings of Petering,[5] who demonstrated the ability of Cu(II) KTS to undergo a sluggish reduction to Cu(I) by thiols such as coenzyme A and lipoic acid. Such processes might be expected to interfere with various mitochondrial functions (i.e., pyruvate dehydrogenase complex, $\alpha$-ketoglutarate dehydrogenase, etc.), ultimately having an effect on observed cellular respiration.

Based upon these considerations, our laboratory recently reported a preliminary study,[6] in which a series of substituted [phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) chelates, **2**, was synthesized as more easily



2

accessible and systematically variable analogues of Cu(II) KTS. The study, whose purpose was to investigate via quantitative structure–activity relationship (QSAR) techniques the design of potential antineoplastic chelates which would exhibit selective toxicity for a tumor cell model vs. a normal cell model, yielded some significant insights into the structural requirements needed to realize the desired goal. These are summarized in eq 1–3. The

$$pI_{50}\ (\text{liver}) = 0.14\ (\pm 0.09)\,E_s + 2.26\ (\pm 0.10) \quad (1)$$
$$n = 8;\ s = 0.12;\ r = 0.85$$

$$pI_{50}\ (\text{ascites}) = -0.69\ (\pm 0.33)\,\pi - 1.17\ (\pm 0.74)\,\sigma_p + 5.08\ (\pm 0.29) \quad (2)$$
$$n = 8;\ s = 0.28;\ r = 0.95$$

$$pI_{50}\ (\text{ascites}) - pI_{50}\ (\text{liver}) = -0.54\ (\pm 0.36)\,\pi - 1.03\ (\pm 0.81)\,\sigma_p + 2.42\ (\pm 0.31) \quad (3)$$
$$n = 8;\ s = 0.30;\ r = 0.92$$

negative coefficients associated with $\pi$ and with $\sigma_p$ in eq 3 suggested that the desired selective cytotoxicity against Ehrlich ascites tumor cells with minimal cytotoxicity to rat liver slice could be achieved by introducing substituents into **2** which would enhance water solubility while stabilizing the chelate against premature dissociation by electron donation through the conjugated ring system. However, it was reasonable to assume that continued increases in hydrophilic character would eventually lead to a drop in activity, thus establishing an optimum for this parameter. Based upon this projection, further analogues

0022-2623/78/1821-0804$01.00/0 