29, 87939-13-7; 30, 78903-83-0; 31, 87939-03-5; 32, 87939-15-9; 33, 84209-43-8; 34, 87939-16-0; 35, 87939-17-1; 36, 74274-71-8; 36·HCl, 75167-16-7; 36·$MeSO_3H$, 75167-17-8; 37, 74274-72-9; 38, 84209-34-7; 39, 84209-33-6; 40, 87939-26-2; 41, 84424-27-1; ethyl 1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate, 87938-88-3; ethyl 7-chloro-1-ethyl-4-oxo-1,8-naphthyridine-3-carboxylate, 56654-05-8; *sec*-butyraldehyde, 78-84-2; *N*-methylpiperazine, 109-01-3; *N*-acetylpiperazine, 13889-98-0; pyrrolidine, 123-75-1; piperazine, 110-85-0; ethylene bromohydrin, 540-51-2; 2-fluorethyl tosylate, 383-50-6; 2-oxopiperazine, 5625-67-2; propionaldehyde, 123-38-6; butyraldehyde, 123-72-8; sodium chlorodifluoroacetate, 1895-39-2; ethylenediamine, 107-15-3; piperidine, 110-89-4; morpholine, 110-91-8; thiomorpholine, 123-90-0; homopiperazine, 505-66-8; azepine, 12764-48-6; azocine, 1121-92-2; 1-phenylpiperazine, 92-54-6; 1-benzylpiperazine, 2759-28-6; 1-ethylpiperazine, 5308-25-8.

# Structure-Activity Relationships of Sparsomycin and Its Analogues. Octylsparsomycin: The First Analogue More Active than Sparsomycin

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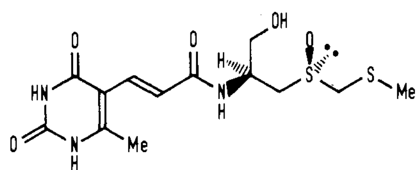
Nine analogues of sparsomycin (1) were synthesized, and their cytostatic activity was studied in an in vitro clonogenic L1210 assay by measuring the inhibition of colony formation. The activity of an analogue, expressed as an $ID_{50}$ value, was compared to that of sparsomycin (Table I). Each analogue possesses not more than two structural modifications of the sparsomycin molecule 1. Comparison of the activity of 1 with that of the stereomers 2-4, having $R_CS_S$, $S_CS_S$, and $R_CR_S$ chirality, respectively, shows that the *S* configuration of the chiral carbon atom is essential for an optimal activity, whereas the *R* chirality of the sulfoxide sulfur atom of sparsomycin is of importance. Study of the $ID_{50}$ values of the *S*-deoxo analogues 10 and 11, as well as the compounds 14 and 15 having the $\beta$-sulfoxide function, indicate that the presence of an oxygen atom on the $\alpha$-sulfur atom is essential. Isomerization of the trans double bond into the cis double bond yields isosparsomycin (16, Scheme II), which has a low activity. The cytostatic activity of sparsomycin seems to be related to its lipophilicity: octylsparsomycin (19) was shown to be three times as effective as sparsomycin.

The development of a flexible synthesis is a prerequisite for thorough studies on the biological activity and/or biochemical mechanism of the interaction of a molecule. An example, which underlines this view, is sparsomycin (1).[1]




1: Sparsomycin ($S_C$-$R_S$)

This antibiotic has been synthesized only recently.[2-4] Consequently, the structure-activity relationship studies of 1 that have appeared so far[5-7] concern analogues in which several structural parameters have been varied simultaneously, thus allowing only a limited interpretation of the results with regard to the role of the various structural fragments.

The interpretaton and comparison of the available information on structure-activity relationships of sparsomycin encounters a second difficulty; the biological activity of the analogues has been determined in different systems (in vitro: KB cell culture[5] and cell-free ribosomal systems[6]; in vivo: P-388 system[6] and Walker 256 system[7]).

Sparsomycin (1) is a strong inhibitor of protein biosynthesis and has therefore attracted widespread attention. There is ample evidence[8] that sparsomycin has its site of interaction in the large ribosomal subunit, where it prevents peptide transfer by interfering with the peptidyltransferase center. Sparsomycin manifests its action in intact prokaryotic cells,[9] eukaryotic cells[10] (including transformed[5,11] and/or virus infected cells[12]), and various cell-free systems.[13] The behavior of sparsomycin with

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(1) Sparsomycin is a metabolite of *Streptomyces sparsogenes* (Argoudelis, A. D.; Herr, R. R. *Antimicrob. Agents Chemother.* 1962, 780) and *Streptomyces cuspidosporus* (Higashide, E.; Hasegawa, T.; Mizuno, K.; Akaide, H. *Takeda Kenkyusho Nempo.* 1966, *25*, 1; *Chem. Abstr.* 1967, *66*, 54328).
(2) Ottenheijm, H. C. J.; Liskamp, R. M. J.; Tijhuis, M. W. *Tetrahedron Lett.* 1979, 387. Ottenheijm, H. C. J.; Liskamp, R. M. J.; van Nispen, S. P. J. M.; Boots, H. A.; Tijhuis, M. W. *J. Org. Chem.* 1981, *46*, 3273.
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(4) Helquist, P.; Shekhani, M. S. *J. Am. Chem. Soc.* 1979, *101*, 1057.
(5) Lin, C.-C. L.; Dubois, R. J. *J. Med. Chem.* 1977, *20*, 337.
(6) Vince, R.; Brownell, Lee, C. K. *Biochem. Biophys. Res. Commun.* 1977, *75*, 563. Lee, C. K.; Vince, R. *J. Med. Chem.* 1978, *21*, 176.
(7) Dubois, R. J.; Lin, C.-C. L.; Michel, B. L. *J. Pharm. Sci.* 1975, *64*, 825.
(8) Pestka, S. *Annu. Rev. Microbiol.* 1971, *25*, 488. Vazquez, D. *FEBS Lett.* 1974, *40*, S63. Vazquez, D. *Mol. Biol. Biochem. Biophys.* 1979, 30.
(9) Slechta, L. In "Antibiotics I"; Gottlieb, D.; Shaw, P. D., Eds.; Springer Verlag: New York, 1967; p 410. Bannister, R. E.; Hunt, D. E.; Pitillo, R. F. *Can. J. Microbiol.* 1967, 595.
(10) Goldberg, I. H.; Mitsugi, K. *Biochem. Biophys. Res. Commun.* 1966, *23*, 453. Contreras, A.; Vazquez, D.; Carrasco, L. *J. Antibiot.* 1978, *31*, 598. Gupta, R. S.; Siminovitch, L. *Biochemistry* 1977, *16*, 3209.
(11) (a) Owen, S. P.; Dietz, A.; Camiener, G. W. *Antimicrob. Agents Chemother.* 1962, 772. (b) Kuwano, M.; Takenaka, K.; Ono, M. *Biochim. Biophys. Acta* 1979, *563*, 479. (c) Bhuyan, B. K.; Scheidt, L. G.; Fraser, I. J. *Cancer Res.* 1972, *32*, 398.
(12) Contreras, A.; Carrasco, L. *J. Virol.* 1979, *29*, 114. Thiry, L. *J. Gen. Virol.* 1968, *2*, 143.
(13) Baglioni, C. *Biochim. Biophys. Acta* 1966, *129*, 642. Emmerich, B.; Hoffmann, H.; Erben, V.; Rastetter, J. *Biochim. Biophys. Acta* 1976, *44*, 460. Pestka, S. *Proc. Natl. Acad. Sci. U.S.A.* 1968, *61*, 726. Carrasco, L. Fresno, M.; Vazquez, D. *FEBS Lett.* 1975, *52*, 236.

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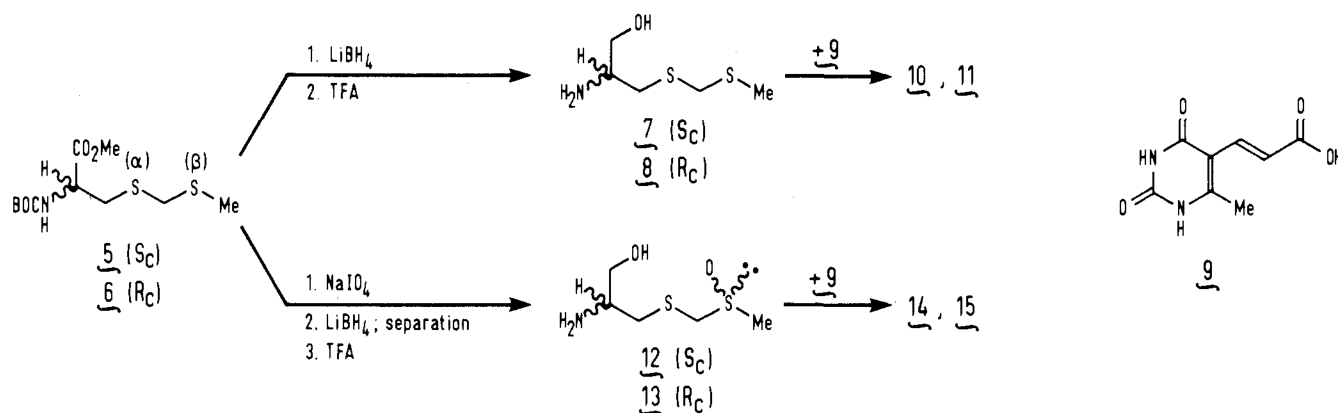
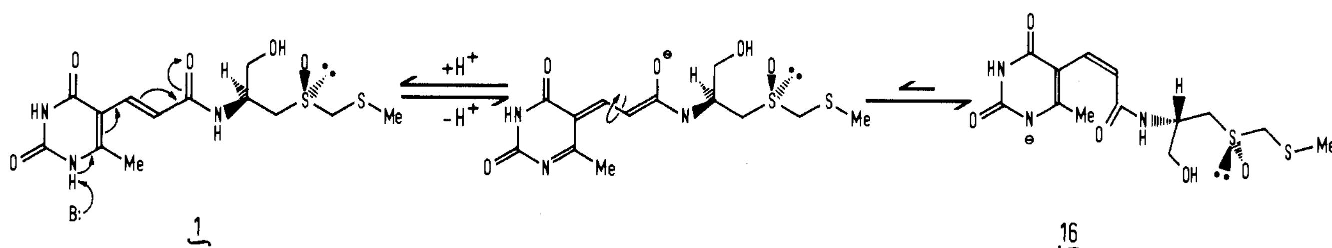
Scheme I




Scheme II




regard to its inhibitory action and its influence on the polyribosomes has also been investigated in vivo.[14] Since sparsomycin has been shown to be active against transformed cells (vide supra) and various tumors[11a] it has been investigated as a potential antineoplastic compound. A clinical trial of sparsomycin, however, revealed eye toxicity.[15]

Recently, both we[2,3] and Helquist[4] succeeded in developing a total synthesis of sparsomycin. In addition, we were able to synthesize some analogues.[2] It is our aim to obtain insight into the relationship between the structure and activity of sparsomycin. In addition, we are attempting to develop a compound with more selective biochemical and pharmalogical properties, while determining the minimum structural and stereochemical requirements for the antitumor activity.

We report here[16] the synthesis of a number of relevant analogues and the investigation of their activity against lymphocytic leukemia L1210 cells in vitro. The synthesis of analogues also seems promising for studies on the molecular mechanism of action of sparsomycin.

## Results and Discussion

**Choice and Synthesis of the Analogues.** The synthesis of sparsomycin (1) and the analogues 2–4 (Table I) has been reported before.[2] The analogues 2–4 allow us to study the dependence of the biological activity on the absolute configuration of the chiral carbon atom, as well as the sulfoxide sulfur atom.[17]

Compounds 10, 11, 14, and 15 (Table I) were included in order to evaluate the role of the oxidation state of the sulfur atom, as well as the influence of the position of the sulfoxide moiety ($\alpha$ or $\beta$ S). To circumvent partial racemization of the chiral carbon atom during the synthesis of the $S$-deoxo analogues 10 and 11, we first reduced 5 and 6[2] to the corresponding N-protected amino alcohols with lithium borohydride (Scheme I). This conversion decreases the acidity of the proton at the chiral carbon atom, so that the chiral carbon atom is less prone to racemization. Subsequently, the Boc group was removed by TFA, and the resulting amino alcohols 7 and 8 were coupled to the uracil acrylic acid fragment 9[2] to give 10 and 11 in yields of 31 and 28%, respectively.[18]

We prepared 14 and 15 starting from 5 and 6 as follows (Scheme I). Oxidation with sodium metaperiodate resulted in formation of the isomer containing a $\beta$-sulfoxide in excess over the $\alpha$-sulfoxide isomer ($\beta/\alpha \approx 4{:}1$). These regioisomers were separated by HPLC after reduction of the ester function. Removal of the N-protecting group gave 12 and 13, respectively, which were coupled by a mixed anhydride procedure with 9 to give 14 (pseudosparsomycin, 54%) and 15 (47%), respectively. We invariably noticed the formation of a byproduct after the last step of sparsomycin's total synthesis—i.e., the coupling procedure.[2] This byproduct, albeit present in a small amount, is hard to remove from sparsomycin by chromatography or gel filtration. This product was shown to be the cis isomer of sparsomycin [i.e., isosparsomycin (16), Scheme II] by $^1$H NMR. Since perusal of the NMR spectrum of an authentic sample of sparsomycin[19] also showed the presence of a significant amount of the cis isomer, we decided to investigate this phenomenon more extensively. It has been shown before by Wiley and MacKellar[20] that irradiation of sparsomycin with a

(14) Trakastellis, A. C. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *59*, 854. Sidransky, H.; Verney, E. *J. Natl. Cancer Inst.* **1979**, *63*, 81. Sarma, D. S.; Murty, C. N.; Sidransky, H. *Biochem. Pharmacol.* **1974**, *23*, 857.

(15) Close, H. P.; McFarlane, J. R. *Cancer. Chemother. Rep.* **1969**, *43*, 29.

(16) Investigation of the activity in a cell-free, ribosomal system will be the subject of a future report.

(17) The chiral carbon atom in sparsomycin possesses the $S$ configuration, denoted as $S_c$, whereas the absolute configuration of the sulfoxide sulfur atom is $R$, denoted as $R_s$. See also: Ottenheijm, H. C. J.; Liskamp, R. M. J.; Helquist, P.; Lauher, J. W.; Shekhani, M. *J. Am. Chem. Soc.* **1981**, *103*, 1720.

(18) Slightly different routes to 11 (ref 2) and the racemic mixtures of 10 and 11 (ref 5 and 6) have been described before.

(19) We are grateful to Dr. P. F. Wiley (Upjohn Co.) for supplying us with a sample of authentic sparsomycin.

(20) Wiley, P. F.; MacKellar, F. A. *J. Org. Chem.* **1976**, *41*, 1858.

Scheme III

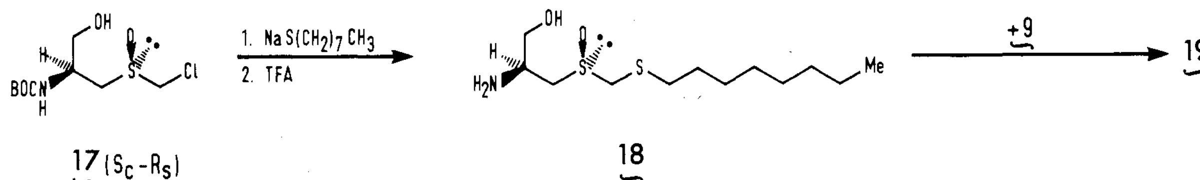



Table I. Inhibition of L1210 Colony Formation (Expressed as $ID_{50}$) by Sparsomycin (1) and Its Analogues

| R | | chirality | $ID_{50}$ (μg/mL)[a] |
|---|---|---|---|
| | 1 | $S_C$ - $R_S$ | 0.15[c] |
| | 2 | $R_C$ - $S_S$ | — |
| | 3 | $S_C$ - $S_S$ | 2.1 |
| | 4 | $R_C$ - $R_S$ | — |
| | 10 | $S_C$ | 4.4 |
| | 11 | $R_C$ | — |
| | 14 | $S_C$ - $R_S$ / $S_C$ - $S_S$ | 66 |
| | 15 | $R_C$ - $R_S$ / $R_C$ - $S_S$ | — |
| | 16[b] | $S_C$ - $R_S$ | 0.3 |
| | 19 | $S_C$ - $R_S$ | 0.05 |

[a] The highest dose tested was 100 μg/mL; a dash indicates that the compound showed no activity at this dose. [b] A mixture of 1 and 16 (ratio 2:3) was used. [c] The hydrolytically unstable *O*-acetyl derivative had identical activity.

fluorescent desk lamp for a long period (7 days) results in the formation of 16 in 20% yield. In repeating the irradiation experiment with some modification of the experimental conditions, we found, monotoring the cis–trans conversion by NMR, that irradiation for 20 min with a 300-nm lamp (Rayonet RPR 200) is sufficient to produce a mixture of isosparsomycin (16) and sparsomycin (1) in a ratio of 4:1. Longer periods of irradiation did not result in complete conversion of 1 into 16.

The irradiation-induced isomerization was also observed with the stereoisomers 2–4 of sparsomycin, the *S*-deoxy analogues 10 and 11, and the pseudosparsomycin analogues 14 and 15. Although the *Z* isomer 16 is likely to be thermodynamically less stable than the *E* isomer 1, in the photoequilibrium state the *Z* isomer 16 predominates. In order to avoid *E* to *Z* isomerization, sparasomycin should be preferentially stored as a solid in the dark, because we noticed that the compound in solution isomerizes slowly when exposed to daylight or laboratory TL-light.[21,22,24] In the dark, isomerization occurs only in alkaline (pH >9) solution. We suppose that this base-catalyzed isomerization involves an intermediate as depicted in Scheme II. To study the biological activity of isosparsomycin, we used a sample containing 60% isosparsomycin (16) and 40% sparsomycin (1).

Beside the aforementioned modifications of sparsomycin, variations leading to a less polar molecule are important. It has been shown[23] that sparsomycin displays no activity against intact reticulocytes. This has been ascribed to the inability of sparsomycin to penetrate into these cells, which might be due to sparsomycin's polar character. We thought that a lipophilic analogue of sparsomycin might penetrate cells more easily. Furthermore, we observed[24] in pharmokinetic experiments in the dog that sparsomycin has a short half-life of elimination (70 min). A more lipophilic compound might have a longer half-life and, thus, may be used in smaller quantities to reach a certain plasma level. Therefore, the octyl analogue of sparsomycin (19, Table I) has been chosen to study whether an increased lipophilicity will result in an increase of the biological activity. The octyl analogue 19 was prepared by using the α-chloro sulfoxide 17,[2] which was converted into the cysteinol oxodithioacetal with sodium octyl mercaptide (Scheme III). Deprotection with trifluoroacetic acid at 0 °C and subsequent deprotonation with an ion-exchange resin gave the amino alcohol 18 in 85% yield. The latter was coupled to the uracil acrylic acid fragment 9 to give 19 in 37% yield.

**Biological Activity.** Colony assays are widely used to measure the response of established lines of animal and human cells treated with cytotoxic agents. Recently, for example, the Raji cell culture line of Burkitt's lymphoma[25] has been used to determine the effect of several anticancer drugs on the ability of these cells to form colonies in soft

(21) The UV detector of the HPLC or gel filtration equipment may also cause contamination of the trans isomer with the cis isomer.

(22) Our findings suggest that several of the reported biological and biochemical studies, in which sparsomycin's photolability has not been mentioned, have been carried out with samples containing variable amounts of isosparsomycin (16).

(23) Colombo, B.; Felicetti, L.; Baglioni, C. *Biochim. Biophys. Acta* **1966**, *119*, 109.

(24) Winograd, B.; Oosterbaan, M.; Liskamp, R.; van der Kleijn, E.; Ottenheijm, H.; Wagener, Th. *J. Chromatogr.* **1981**, *275*, 145.

(25) Wu, P.-C.; Ozols, R. F.; Hatanaka, M.; Boone, C. W. *J. Natl. Cancer Inst.* **1982**, *68*, 115.

agar. The results also suggest that established human tumor cell lines may be useful for the screening of new anticancer drugs. The growth of tumor colonies in soft agar from primary human tumor explants[26] is even more promising. Preliminary results indicate that the assay is 90–95% accurate in predicting clinical resistance and 60–65% in predicting a clinical response.[27,28] Furthermore, this assay is of potential importance as a screening test for new antitumor agents.[29]

For a first evaluation of the relationship between the structure and antitumor activity of sparsomycin and its analogues, we used an in vitro clonogenic assay of leukemia L1210 cells. A study on the activity of sparsomycin and relevant analogues against tumor cells derived from primary human tumors is presently under investigation.[30] The leukemia L1210 in vivo system (in the mouse) is generally used in standard screening of compounds of potential interest.[29,31] However, for certain (semi)quantative studies, the L1210 in vitro system is more sensitive and more practical. This system has been used in suspension culture[32] and in soft agar medium.[33,34]

In this study we used leukemia L1210 cells in soft agar medium (0.3%) in an in vitro clonogenic assay. We have observed before (unpublished observations) that there is a good correlation between the in vitro and in vivo activity of the drugs tested. Thus, the in vitro system is of predictive value for the in vivo system. Inhibition of L1210 colony formation by sparsomycin and its analogues was determined for several concentrations, and the dose causing 50% inhibition of colony formation ($ID_{50}$) relative to untreated control cells was calculated. The results are collected in Table I.

## Discussion

Comparison of the $ID_{50}$ value of **1** with that of **4** demonstrates unequivocally the necessity of an *S* configuration of the chiral carbon atom for an optimal biological activity. This has been proposed earlier by Lin and Dubois[5] and by Vince et al.[6] Confirmation of this conclusion is found in the $ID_{50}$ values of the *S*-deoxy analogues **10** and **11** and the pseudo analogues **14** and **15**: compounds having an *S* configuration of the chiral carbon atom have a significantly lower $ID_{50}$ value.

The higher $ID_{50}$ value of the analogue **3**, which only differs from sparsomycin in having the opposite configuration at the sulfoxide sulfur atom, clearly demonstrates the importance of an *R* configuration of the sulfoxide sulfur atom. A similar difference in biological activity between molecules that only differ in configuration of the sulfoxide sulfur atom has been observed, although evaluated in vivo, with Amanita toxins.[35] 6-Methoxy-$\alpha$-amanitin, having a sulfoxide sulfur atom with a *R* configuration, is at least ten times more toxic than the corresponding compound with a *S* configuration.

The importance of the presence of an oxygen atom on the $\alpha$-S can be derived from a comparison of the $ID_{50}$ values of **1** and **3**, on the one hand, and **10**, on the other hand. This is in accordance with the findings of Lin and Dubois,[5] who demonstrated that the biological activity, if any, of the synthetically more accessible monosulfide analogues (R = *S*-alkyl-substituted cysteinol) is invariably lower than the biological activity of the corresponding sulfoxide analogues [R = S(O)-alkyl-substituted cysteinol).

The importance of the proper position of the sulfoxide moiety in the cysteinol side chain of **1** ($\alpha$-S vs. $\beta$-S) is demonstrated by comparing the $ID_{50}$ values of **1** and **14**; when the positions of the sulfoxide function and the sulfur atom are reversed, the biological activity is markedly reduced. In addition, the pseudosparsomycin analogue **14** possesses a diminished activity compared to the corresponding *S*-deoxy analogue **10**, suggesting an adverse effect of a sulfoxide function at the $\beta$-S.

Although a study on the biological activity of the separate diastereomers of **14** might give additional insight into the role of the stereochemistry of the $\beta$-sulfoxide function, no attempts were made to separate these diastereomers, since this would not lead to a more active antitumor agent. Comparison of the $ID_{50}$ value of **14** with the values of **1** and **3** shows that the oxodithioacetal moiety as such does not determine the biological activity. Apparently, a bivalent $\beta$-sulfur atom is preferable for an optimal activity.

The $ID_{50}$ value of the mixture of **1** and **16** (2:3) is about two times the $ID_{50}$ value of pure **1**. This suggests that isosparsomycin (**16**) has an $ID_{50}$ value of about 0.6 $\mu$g/mL. However, this result should be interpreted with caution, because of the error present in this biological assay. It will only be regarded as indicative, and future experiments will be necessary to clarify the activity of pure isosparsomycin (**16**).

The $ID_{50}$ value of the octyl analogue (**19**) of sparsomycin is three times lower than that of sparsomycin. This result seems to indicate that an increase of sparsomycin's lipophilicity facilitates cell penetration. However, **19** showed also in a cell-free system a considerably higher activity than **1**.[16] From this we conclude that the low $ID_{50}$ value of **19** is at least partially due to an increase of its affinity for the peptidyl transferase center. The high activity of **19**, demonstrated in this L1210 clonogenic assay, is comparable to the activities found for the clinically used cytostatic compounds 5-fluorouracil and adriamycin ($ID_{50}$ = 0.04 and 0.03 $\mu$g/mL, respectively) in the same assay (unpublished observations).

Under present investigation is the antitumor activity of sparsomycin (**1**) and octylsparsomycin (**19**) in mice against leukemia L1210, as well as solid tumors. The results will indicate whether the increased activity of octylsparsomycin compared to sparsomycin reflects the in vivo situation.

## Experimental Section

**Biological Activity.** The L1210 in vitro clonogenic assay used in this study is an improved variant of the method described earlier[33,36] for the growth into colonies of L1210 cells in soft agar


(26) This method was originally described: Courtenay, V. D.; Smith, I. E.; Peckham, M. J.; Steel, G. G. *Nature (London)* **1976**, *263*, 771. Hamburger, A. W.; Salmon, S. E. *Science* **1977**, *197*, 461.

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(28) Alberts, D. S.; Salmon, S. E.; Chen, H. S. *Lancet* **1980**, *2*, 340.

(29) Boldin, A.; Venditti, J. M.; MacDonald, J. S.; Muggia, F. M.; Henney, J. E.; Devita, Jr., V. T. *Eur. J. Cancer* **1981**, *17*, 129.

(30) Herman, C., personal communication.

(31) Skipper, H. E.; Schabel, Jr., F. M.; Wilcox, W. S. *Cancer Chemother. Rep.* **1964**, *35*, 1.

(32) Li, L. H.; Luentzel, S. L.; Murch, L. L.; Psichigoda, L. M.; Kruger, W. R.; Martin, H. *Cancer Res.* **1979**, *39*, 4816.

(33) Himmelfarb, P.; Thayer, P. S.; Martin, H. *Cancer Chemother. Rep.* **1967**, *51*, 451.

(34) van der Huizen, A. A.; van de Grampel, J. C.; Akkerman, W.; Lelieveld, P.; van der Meer-Kalverkamp, A.; Lamberts, H. B. *Inorg. Chim. Acta.* **1983**, *78*, 239.

(35) Faulstich, H.; Wieland, T. "Peptides 1971"; Nesvadba, H., Ed.; North-Holland Publishing Co.: Amsterdam, 1973; p 343. Faulstich, H.; Bloching, M.; Zobeley, S.; Wieland, T. *Experienta* **1973**, 343. Wieland, T.; de Vries, M. P. J.; Indes, H.; Faulstich, H.; Giren, A.; Sturm, M.; Hoppe, W. *Justus Liebigs Ann. Chem.* **1974**, 1570. Buku, A.; Altmann, R.; Wieland, T. *Ibid.* **1974**, 1580.

medium.

From a suspension culture, 100 L1210 cells were plated into 35-mm culture dishes (Falcon), containing 1 mL of soft agar growth medium and the compound to be tested in appropriate concentrations. The soft agar growth medium consisted of Dulbecco's medium supplemented with 20% horse serum, 60 μmol of 2-mercaptoethanol, 20 mg/mL of L-asparagine, 75 mg/mL of DEAE-dextran (molecular weight $2 \times 0^6$), and 0.3% bacto agar (Difco). The culture dishes were incubated at 37 °C in an atmosphere of 10% $CO_2$ in humidified air for 8 days. After this period of continuous drug exposure, colonies were counted, and dose–effect curves were made. From these curves, the drug dose causing 50% inhibition of colony formation ($ID_{50}$) relative to untreated control cells was calculated.

**Synthesis.** $^1$H NMR spectra were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer with $Me_4Si$ or $Me_3SiCD_2CD_2CO_2Na$ as an internal standard. UV spectra were measured on a Perkin-Elmer spectrophotometer, Model 555. For determination of the specific rotation, a Perkin-Elmer 241 polarimeter was used. The irradiation experiments were carried out in a Rayonet RPR 100 or RPR 200 photochemical reactor, fitted with 300-nm lamps in Pyrex tubes. Thin-layer chromatography (TLC) was carried out by using Merck precoated F-254 plates (thickness 0.25 mm). Spots were visualized with a UV lamp, ninhydrin, and TDM.[38] For column chromatography, Merck silica gel H type 60 was used. A Miniprep LC (Jobin Yvon) was used for preparative HPLC; to avoid $Z$ to $E$ isomerization, the UV detector was switched off.[21] All compounds tested for biological activity were homogeneous by HPLC and TLC.

**Sparsomycin (1), Sparsomycin Enantiomer 2, and Sparsomycin Diastereomers 3 and 4.** Compounds 1–4 were prepared as described earlier.[2] However, the reactions were carried out under the exclosure of light, and the purification of the end product was changed; the crude products were chromatographed over silica gel by HPLC (eluent $MeOH/CH_2Cl_2/NH_4OH$, 80:20:0.2, v/v), followed by gel filtration over Sephadex LH 20 (eluent $H_2O/MeOH$, 15:85, v/v).

***S*-[(Methylthio)methyl]-D- and -L-cysteinol (7 and 8).** The ester functions of 5 and 6[2] (885 mg, 3 mmol) were reduced with lithium borohydride as described earlier.[2] The N-protected cysteinol derivative was purified by HPLC (eluent $MeOH/CH_2Cl_2$, 5:95, v/v). Subsequently, the BOC group was removed by trifluoroacetic acid as described for the preparation of 18 (vide infra) to give 7 (40%) and 8 (50%), respectively: $R_f$ 0.56 (eluent *s*-$BuOH/NH_4OH$, 5:2, v/v); NMR ($CD_2Cl_2$) δ 2.18 (s, 3 H, $SCH_3$), 2.73–2.93 (m, 2 H, $CHCH_2S$), 3.67 (s, 2 H, $SCH_2S$), 3.69–3.89 (m, 3 H, $CHCH_2OH$).

**($S_C$)- and ($R_C$)-*S*-Deoxosparsomycin (10 and 11).** Compounds 10 and 11 were obtained by coupling (*E*)-3-(2,4-dioxo-6-methyl-5-pyrimidinyl)acrylic acid (9)[2] with 7 and 8, respectively (each 250 mg, 1.5 mmol), in a mixed anhydride procedure as has been described earlier[2] for the preparation of 1–4. The yields were 31 and 28%, respectively, after HPLC (eluent $MeOH/CH_2Cl_2$, 9:91, v/v). Compound 10: $R_f$ 0.51 (eluent $MeOH/CHCl_3$, 1:4, v/v); $[\alpha]^{25}_D$ +82° (*c* 0.205, water); NMR ($D_2O$) δ 2.18 (s, 3 H, $SCH_3$), 2.42 [s, 3 H, C(6) $CH_3$], 2.76 and 3.03 [AB part of ABX spectrum, 8 lines, $J_{AX}$ = 8 Hz, $J_{BX}$ = 5 Hz, $J_{AB}$ = 14 Hz, 2 H, $CHCH_2S(O)$], 3.59–3.88 (m, 2 H, $CH_2OH$), 3.78 (s, 2 H, $SCH_2S$), 4.07–4.37 (br m, 1 H, $CHCH_2OH$), 7.08 and 7.41 (AB spectrum, $J$ = 15.5 Hz, 2 H, HC═CH). Compound 11 was identical in every aspect with 10, except for $[\alpha]^{25}_D$, which had a value of −76° (*c* 0.225, water). Anal. ($C_{13}H_{19}N_3O_4S_2$) C, H, N.

***S*-[(Methylsulfinyl)methyl]-D- and -L-cysteinol (12 and 13).** Compounds 5 and 6[2] (932 mg, 3 mmol) were oxidized with sodium metaperiodate, followed by reduction with lithium borohydride according to procedures described earlier.[2]

The regio isomers containing a β-sulfoxide was separated from those with an α-sulfoxide function by HPLC (eluent $MeOH/CH_2Cl_2$, 5:95, v/v). The desired compounds had a lower $R_f$ value on TLC than the corresponding regio isomers. No attempts were made to separate the N-protected diastereomers of 12 ($S_CR_S$–$S_CS_S$) or 13 ($R_CR_S$–$R_CS_S$). Deprotection of the amino function was achieved by treatment with trifluoroacetic acid, as described for the preparation of 18 (vide infra). The products were obtained in 85% yield. Compounds 12 and 13: $R_f$ 0.42 (eluent *s*-$BuOH/NH_4OH$, 5:2, v/v); NMR ($CD_2Cl_2$) δ 2.6 (s, 3 H, $SCH_3$), 2.67–3.20 [m, 2 H, $CHCH_2S(O)$], 3.20–3.62 (m, 3 H, $CHCH_2OH$), 3.20–3.98 [AB spectrum partly covered by $CHCH_2OH$ signals, 2 H, $S(O)CH_2S$].

**($S_C$)- and ($R_C$)-Pseudosparsomycin (14 and 15).** Compounds 14 and 15 were obtained by coupling 9 with 12 and 13, respectively (each 275 mg, 1.5 mmol), in a mixed anhydride procedure as described earlier.[2] The yields of 14 and 15 were 54 and 47%, respectively. Compound 14: $R_f$ 0.14 (eluent ($MeOH/CHCl_3$, 1:4, v/v), $[\alpha]^{25}_D$ +89° (*c* 0.218, water); NMR ($D_2O$) δ 2.42 [s, 3 H, C(6) $CH_3$], 2.77 [s, 3 H, $S(O)CH_3$], 2.77–3.27 [m, 2 H, $CHCH_2S(O)$], 3.60–3.90 (AB part of ABX spectrum, 2 H, $CH_2OH$), 3.91–4.16 [AB spectrum of two doublets, 2 H, $SCH_2S(O)$], 7.09 and 7.40 (AB spectrum, $J_{AB}$ = 15.5 Hz, HC═CH). Anal. ($C_{13}H_{19}N_3O_5S_2$) C, H, N. Compound 15 was identical with 14 in every aspect, except for $[\alpha]^{25}_D$, which had a value of −82° (*c* 0.174, water).

**Isosparsomycin (16)–sparsomycin (1) Mixture.** A solution of sparsomycin (1; 30 mg, 0.08 mmol) in 20 mL of water was irradiated (Rayonet RPR 100) at 300 nm in a Pyrex tube for 50 min. Subsequently, the solvent was removed by freeze–drying. The isosparsomycin (16)/sparsomycin (1) ratio was shown to be 3:2, as determined by NMR from the ratio of integration of the signals due to the cis HC═CH protons (δ 6.26 and 6.55), on the one hand, and the signals due to the trans HC═CH protons (δ 7.07 and 7.41), on the other hand. This ratio corresponds to the ratio of integration of the C(6) $CH_3$ (δ 2.17) signal in isosparsomycin and the C(6) $CH_3$ (δ 2.40) signal in sparsomycin. The remaining signals in the $^1$H NMR spectrum of 16 and 1 coincide. Base-catalyzed isomerization was studied as follows: the pH of a $D_2O$ solution (1 mL) of 1 (2 mg) was adjusted to 9 with LiOD and stored in the dark. After 12 h 5% of the *Z* isomer 16 had been formed: $R_f$ 0.17 [for comparison, $R_f$ of 1 0.21 (eluent $MeOH/CHCl_3$, 1:4, v/v)], 0.22 [for comparison, $R_f$ of 1 0.35 (eluent *n*-$BuOH/EtOH/H_2O$, 70:27:3, v/v)].

***S*-Oxo-*S*-[(octylthio)methyl]-D-cysteinol (18).** A solution of sodium octyl mercaptide[37] (353 mg, 2.1 mmol), of which the purity was checked as described earlier,[2] in 10 mL of dry ethanol was added at once to a stirred solution of the chloro sulfoxide 17[2] (542 mg, 2 mmol) in 10 mL of dry ethanol. Argon was passed through both solutions for 15 min. The reaction mixture was stirred overnight at room temperature. After completion of the reaction, as monitored by TLC (eluent $MeOH/CH_2Cl_2$, 1:9, v/v), the solvent was evaporated, and water (5 mL) and dichloromethane (30 mL) were added. Removal of the turbidness, due to finely divided sodium chloride, could be achieved by stirring with $Na_2SO_4$ for about 1 h. Filtration and removal of the solvent afforded the N-protected *S*-oxo-*S*-[(octylthio)methyl]-D-cysteinol in 85% yield: $R_f$ 0.45 (eluent $MeOH/CH_2Cl_2$, 1:9, v/v); NMR ($CDCl_3$) δ 0.47–2.16 [m, 15 H, $(CH_2)_6CH_3$], 1.44 (s, 9 H, *t*-Bu), 2.56–2.82 [m, 2 H, $SCH_2(CH_2)_6CH_3$], 2.96 and 3.22 [AB part of ABX spectrum, 8 lines, $J_{AX} = J_{BX}$ = 6 Hz, $J_{AB}$ = 14 Hz, 2 H, $CHCH_2S(O)$], 3.71–3.97 [m, 4 H, $CH_2OH$, $S(O)CH_2S$], 3.97–4.28 (br, 1 H, $CHCH_2OH$), 5.22–5.33 (br, 1 H, NH). Anal. ($C_{17}H_{35}NO_4S_2$) C, H, N.

For removal of the BOC group, the above compound (190 mg, 0.5 mmol) was dissolved in 10 mL of trifluoroacetic acid. The solution was stirred for 30 min at 0 °C, after which the trifluoroacetic acid was evaporated in vacuo at room temperature. The residue was dried in vacuo over KOH for 1 h and then dissolved in a minimal amount of water. The solution was placed on an ion-excange column (Amberlite IRA-410, 20–50 mesh $OH^-$ form).

Elution with water and removal of the solvent by freeze–drying gave 18 in 90% yield: $R_f$ 0.40 (eluent $MeOH/CHCl_3$, 1:4, v/v); NMR ($CD_2Cl_2$) δ 0.70–2.20 [m, 15 H, $(CH_2)_6CH_3$], 2.60–2.85 [m, 2H, $SCH_2(CH_2)_6CH_3$], 2.95 and 3.23 [AB part of ABX spectrum, 8 lines, $J_{AX} = J_{BX}$ = 6 Hz, $J_{AB}$ = 14 Hz, 2 H, $CHCH_2S(O)$], 3.70–3.98 [m, 4 H, $CH_2OH$, $S(O)CH_2S$], 3.98–4.35 (br, 1 H, $CHCH_2OH$).

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**Octylsparsomycin (19)** was prepared by a mixed anhydride procedure as follows. To a stirred, cooled (0 °C) solution of the acid 9[2] (112 mg, 0.66 mmol) and triethylamine (86 mg, 0.86 mmol) in 5 mL of THF/DMF (1:1, v/v) was added ethyl chloroformate (103 mg, 0.86 mmol). Stirring was continued at 0 °C for 4 h. Subsequently, a solution of the amino alcohol 18 (140 mg, 0.5 mmol) in 5 mL of THF/DMF (1:1, v/v) was added dropwise. The reaction was stirred at room temperature for 48 h under the exclosure of light.

The solvents were removed in vacuo at room temperature. The crude product was chromatographed over silica gel 60 H by HPLC (eluent $MeOH/CH_2Cl_2$, 1:4, v/v), followed by gel filtration over Sephadex LH 20 (eluent $H_2O/MeOH$, 15:85, v/v). Compound 19 was obtained in 17% yield; no attempts were made yet to improve this yield: $R_f$ 0.43 (eluent $MeOH/CHCl_2$, 1:4, v/v), 0.60 and 0.25 (eluents $MeOH/H_2O$, 85:15, v/v, and 75:25, v/v, respectively; RPTLC Merck precoated RP8-F254 plates); $[\alpha]^{25}_D$ +77.9° (*c* 0.086, $MeOH/H_2O$, 1:1, v/v); NMR ($CD_3OD$) $\delta$ 0.94–1.76 [m, 15 H, $(CH_2)_6CH_3$], 2.35 [s, 3 H, C(6) $CH_3$], 2.55–2.84 [m, 2 H, $SCH_2(CH_2)_6CH_3$], 2.84–3.49 [m, 2 H, $CHCH_2S(O)$], 3.60–3.85 (m, 2 H, $CHCH_2OH$), 3.90–4.16 [br s, 2 H, $S(O)CH_2S$], 4.40–4.67 (m, 1 H, $CHCH_2OH$), 6.95 and 7.61 (AB spectrum, 2 H, $J_{AB}$ = 15.5 Hz, HC=CH). Anal. ($C_{20}H_{33}N_3O_5S_2$) C, H, N.

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# Structure–Activity Relationships among DNA Gyrase Inhibitors. Synthesis and Biological Evaluation of 1,2-Dihydro-4,4-dimethyl-1-oxo-2-naphthalenecarboxylic Acids as 1-Carba Bioisosteres of Oxolinic Acid[1]

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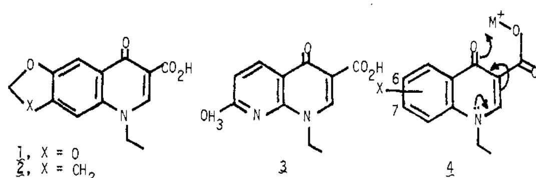
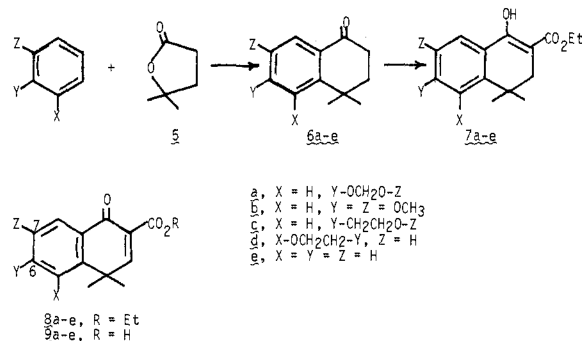
A series of oxolinic acid analogues was synthesized in an attempt to evaluate the role, if any, played by the N-1 atom in putative modes of action of antimicrobial DNA gyrase inhibitors. Carba analogues were prepared because these have no possibility of an internal resonance contribution of the nitrogen atom and yet could otherwise satisfy electronic requirements of putative modes of action. Successful routes were developed involving Friedel–Craft's cycloaddition of suitable aromatic compounds with 4,4-dimethylbutyrolactone, followed by ethoxycarbonylation, oxidation with dichlorodicyanobenzoquinone, and careful saponification. The *gem*-dimethyl group of these analogues prevents aromatization at the cost of nonplanarity. Only the unsubstituted parent compound, 1,2-dihydro-4,4-dimethyl-1-oxo-2-naphthalenecarboxylic acid (**9e**), possessed any appreciable antimicrobial activity in vitro. This may be due to a different mode of action, however, since **9e** gave no measurable inhibition of DNA gyrase in vitro. Thus, the N-1 atom plays a significant role in enzymic and bacteriological inhibition that cannot be compensated for by the presence of C-6 oxygen atoms.

Oxolinic acid (1) and nalidixic acid (3) are employed in




the treatment of urinary tract infections. Their inhibitory effect on DNA gyrase[2] has stimulated extensive structure–activity studies from this[3] and other laboratories.[4] The 1-ethyl-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid moiety is a common feature of antimicrobial agents in this class. The majority of the synthetic work has been devoted to alterations in the annelated aromatic ring.[4] Modifications of the N-1 and C-2 substituents, the C-4 oxo group, and the C-3 carboxyl group have all been made. Successful variations in this area are limited predominantly to attachment of different groups to N-1.[4] So far, little attention has been paid to the role of the N-1 atom itself as a possible contributor to the molecular mode of action of these agents. Present published speculation highlights the chelating capacity of this ring system.[5] In such a coor-

Scheme I

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