Tetrahedron Letters,Vol.26,No.39,pp 4731-4734,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

CHETRACIN A, A NEW EPIPOLYTHIODIOXOPIPERAZINE HAVING A TETRASULFIDE BRIDGE FROM *Chaetomium abuense* AND *C. retardatum*

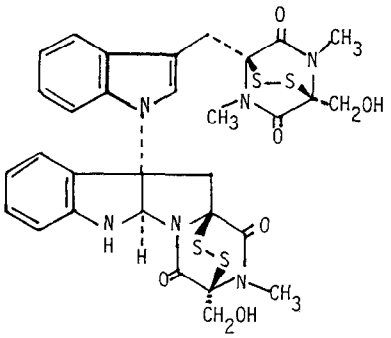
Takao SAITO,[a)] Kiyotaka KOYAMA,[a)] Shinsaku NATORI,[a,*)] and Yoichi IITAKA[b,*)]

a) Meiji College of Pharmacy, Yato-cho, Tanashi-shi, Tokyo 188, Japan
and b) Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo 113, Japan

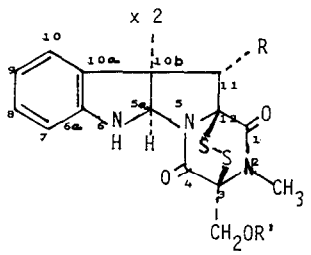
Summary: A new epipolythiodioxopiperazine, designated chetracin A, exhibiting remarkable cytotoxicity, was isolated from *Chaetomium* spp. The compound was proved to have the 11α, 11'α-dihydroxychaetocin nucleus by NMR and correlation reactions and found to have the tetrasulfide bridge by X-ray analysis.

The epipolythiodioxopiperazines are the mold secondary metabolites exhibiting antimicrobial and cytotoxic properties. Production of the group of compounds such as chetomin (I) and chaetocin (II) by the genus *Chaetomium* was reported.[1,2)] In our course of studies on mycotoxin production by the fungi belonging to the genus and related fungi,[3)] the extracts of the culture on rice of three species, *C. abuense* Lodha (a), *C. retardatum* Carter & Khan (b), and *C. tenuissimum* Sergejeva (c), attracted our attention by the production of the mycotoxins detected by the remarkable cytotoxicity to HeLa cells and by the positive spots on TLC by Ehrlich's and silver nitrate reagents.

Now the causative agents of the three (a - c) were isolated and identified respectively as (a) a new tetrasulfide type epipolythiodioxopiperazine named chetracin A (IVa), (b) 11α,11'α-dihydroxychaetocin[4)] (melinacidin-IV)[5)]) (IIIa) and IVa, and (c) I.[6)] All these compounds showed nearly the same order of strong cytotoxicity to HeLa cells (IC_{50} ca 0.03 μg/ml). IIIa had been isolated from *Verticillium tene-*



chetomin (I)



R : R' : H chaetocin (II)
R : OH, R': H 11α, 11'α-dihydroxychaetcin (IIIa)
R : OH and OAc, R': Ac (IIIb)

rum[4] and *Acrostalagmus cinnabarinus* var. *melinacidinus*.[5]

Colorless precipitate formed by concentration of the ethyl acetate extract of molded rice of *C. retardatum* was mainly composed of chetracin A (IVa). Due to its small solubility, it was converted to the acetate with acetic anhydride and pyridine and purified by HPLC using Develosil 60-3. The main fraction was hydrolyzed with NH_4OH-MeOH to the original compound, chetracin A (IVa)(identified by TLC and HPLC), amorphous powder, mp 248-251° (decomp), $[\alpha]_D^{20}$ +723.5° ($CHCl_3$), UV λ_{max}^{EtOH} nm (log ε): 242, 306, (4.33, 3.70), IR_{max}^{KBr} cm^{-1}: 3410, 1660, 1635, 1480, 1415, 1380, 1310, 1205, 1140, 1095, 1065, 750.

The same compound was obtained from *C. abuense* by the same procedure.

Reacetylation of IVa gave the triacetate (IVb), mp 260° (decomp), $[\alpha]_D^{20}$ +830° ($CHCl_3$). M^+ ions were not detectable in IVa and IVb by any modes of mass spectrometry. However the elemental analyses suggested that the molecular formula, $C_{36}H_{34}N_6O_{11}S_8$, is most probable for IVb. ^1H- and ^{13}C-NMR spectra of IVb showed similarity to those of the triacetate (IIIb) of IIIa as shown in Table 1. All the data suggested that IVa might be a tetrathio homolog of IIIa. In order to confirm the assumption, IVa was treated with boiling potassium hydroxide to give bi-indol-3-yl,[7] while the treatment with triphenylphosphin-NH_4OH-MeOH (a modified method of that reported for sporidesmins[8]) of IVa gave IIIa.

Table 1 ^1H- and ^{13}C-NMR Data for Dihydroxychaetocin Triacetate (IIIb) and Chetracin A Triacetate (IVb)

δ (ppm) in $CDCl_3$ (*J* in Hz)

	^1H (400 MHz)		^{13}C (100 MHz)	
	IIIb	IVb	IIIb	IVb
1, 1'	--------	--------	162.64, 165.29 (s)	166.11, 167.24 (s)
3, 3'	--------	--------	74.59* (s)	76.77 (s)
4, 4'	--------	--------	160.13 160.27 (s)	164.98 (s)
5a, 5'a	5.168, 5.179	5.434, 5.450	82.37, 82.97 (d)	83.17 (d)
6, 6'	5.246, 5.276	5.012, 5.047	----------	----------
6a, 6'a	--------	--------	148.29, 148.77 (s)	149.19, 149.50 (s)
7, 7'	6.623-7.914	6.527-7.796	110.45, 110.98 (d)	109.45, 109.97 (d)
8, 8'			130.41, 130.50 (d)	130.67, 130.77 (d)
9, 9'			120.30, 120.44 (d)	119.62, 120.12 (d)
10, 10'			125.76 (d)	125.22 (d)
10a, 10'a	--------	--------	128.17, 128.19 (s)	128.57, 128.67 (s)
10b, 10'b	--------	--------	64.98, 65.84 (s)	64.39, 64.10 (s)
11	6.970	6.692	81.46 (d)	82.57 (d)
11'	5.056	4.822	79.69 (d)	77.20 (d)
11-OAc CO	--------	--------	169.37*(s)	169.22*(s)
CH_3	2.467	2.328	21.98 (q)	21.95 (q)
11'-OH	5.514	4.087	----------	----------
12, 12'	--------	--------	74.99*, 75.17*(s)	79.52, 80.63 (s)
2, 2'-N-CH_3	2.984, 3.047	2.981, 2.995	27.71, 28.02 (q)	28.93, 29.08 (q)
3, 3'-CH_2OAc	4.603, 4.625 4.850, 4.862 (d, 12.7)	4.546, 4.616 (d, 11.5)	59.23, 59.29 (t)	61.67, 61.98 (t)
3, 3'-CH_2OAc CO	--------	--------	164.24*, 169.34*(s)	168.32*, 168.80*(s)
CH_3	2.113	2.113, 2.142	20.47 (q)	20.42, 20.51 (q)

* Assignments may be interchanged.

The CD spectra of the acetates (IIIb and IVb) also showed the same sign to indicate the same absolute configurations.

In order to confirm the number of S atoms, the absolute configuration, and the conformation, X-ray analysis of IVb was performed using a single crystal obtained by recrystallization by vapor diffusion method from benzene saturated with water and xylene. A single crystal of approximate dimensions 0.1 x 0.08 x 0.12 mm was cut out from the aggregate and sealed in a thin walled glass capillary tube to prevent the

loss of water of crystallization. The diffraction data were obtained using CuKα radiation monochromated by a graphite plate. The crystal data are shown in Table 2.

Table 2 Crystal Data of IVb

chetracin A triacetate, $C_{36}H_{34}N_6O_{11}S_8 \cdot 2H_2O$
FW = 1019.2, crystal system, tetragonal
space group $P4_3$, Z = 4
unit cell demensions, a = b = 15.337(8), c = 19.922(10) Å
V = 4683 Å^3, D_{calcd} = 1.445 g.cm^{-3}, D_m = 1.414 g.cm^{-3}
μ for CuK_α = 40.4 cm^{-1}

Intensities of 3062 reflections were measured in 2θ range of 6° through 178°. The structure was solved by the direct method using MULTAN [9] and refined by the block-diagonal least-squares method to an R value of 0.084. The difference map indicated three water oxygen atoms. The occupancy factors of these oxygen atoms were estimated by the difference electron-density map. Absolute configuration was determined by the anomalous dispersion method. $|F|^2$ values were calculated for 392 Friedel pairs introducing the dispersion corrections for atomic scattering factors of C, O, N and S atoms for CuK_α radiation and compared with the observed values. Of the total of 64 pairs for which the difference of structure factors between h, k, l and $\bar{h}$, $\bar{k}$, $\bar{l}$ exceeds 2σ (F_o), 55 pairs showed clearly the absolute configuration shown in Fig.1.

The final refinement was carried out introducing the dispersion correction for C, N, O and S stoms and anisotropic thermal parameters for all atoms. Hydrogen atoms were not included. The R values was reduced to 0.081 for 3062 observed structure factors. Fig. 1 shows the molecular structure of chetracin A triacetate and the chemical structure of chetracin A has now been established as shown in the formula IVa.

The absolute configuration of the compound was proved to be the same as IIIa. The dioxopiperazine rings are in the boat conformations but the ring is more planar than in the case of a disulfide bridge.[10] The four terminal S-S bonds (1.996, 2.005, 2.002, 2.011 Å) are shorter than the two central S-S bonds (2.066 and 2.092 Å) as in the case of sporidesmin G having the same tetrasulfide bridge.[11]

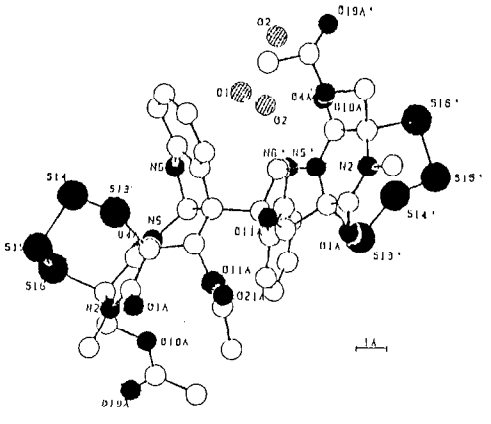
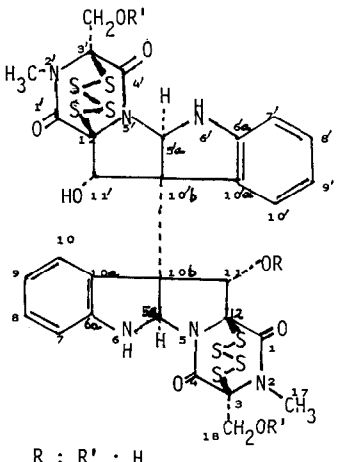


Fig. 1. Absolute Configuration of Chetracin A Triacetate (IVb) by X-Ray Crystallography



R : R' : H chetracin A (IVa)

R : R' : Ac chetracin A triacetate (IVb)

There exist four possible conformers in the cyclic tetrasulfide.[1] The NMR spectra of IVb indicated the presence of only one conformer. X-Ray analysis showed that the central pairs of sulfur atoms were directed towards the nitrogen atoms of the dioxopiperazine rings (Fig. 2). The interatomic distances between the central sulfur atoms and the peptide nitrogen atoms (3.14, 3.17, 3.19, 3.21 Å) were shorter than the sum of their van der Waal's radii (3.35 Å). This might arise from donar-accepter interactions and may be the reason for the stability of the conformation.[11]

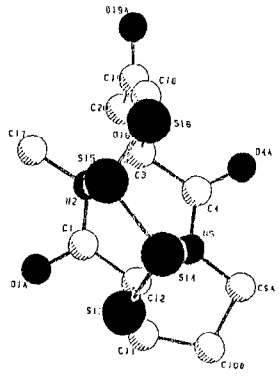


Fig. 2 Conformation of the epitetrasulfide bridge

Chetracin A (IVa) is a symmetric dimer but forms triacetate by acetic anhydride-pyridine as in the case of IIIa. Such phenomenon was also reported in the case of verticillins.[7] Examination of the crystallographic data revealed that the free hydroxyl group at $C_{11'\alpha}$ is sterically hindered and forms a hydrogen bond with the acetyl carbonyl group introduced at the 11α-hydroxyl group.

The final atomic parameters will be compiled in Cambridge Crystallographic Database. The list of F_o and F_c bond lengths and angles may be obtained from one of the authors (Y. I.) upon request.

Acknowledgements: The authors are indebted to Drs. S. Udagawa and S. Sekita, National Institute of Hygienic Sciences, Tokyo, and Prof. M. Umeda, Kihara Institute of Biological Research, Yokohama City University, for their cooperation to this work.

References

1) C. Leigh and A. Taylor, 'Mycotoxins and other Fungal Metabolites Related Food Problems' (ed. J. V. Rodricks), p. 228, Am. Chem. Soc., Washington, D. C. (1976).

2) G. W. Kirby and D. J. Robins, 'The Biosynthesis of Mycotoxins' (ed. P. S. Steyn), p. 301, Academic Press, New York (1980).

3) S. Udagawa, T. Muroi, H. Kurata, S. Sekita, K. Yoshihira, S. Natori, and M. Umeda, Canad. J. Microbiol., 25, 170 (1979); S. Sekita, K. Yoshihira, S. Natori, S. Udagawa, T. Muroi, Y. Sugiyama, H. Kurata, and M. Umeda, ibid., 27, 766 (1981).

4) D. Hauser, H. R. Loosli, and P. Niklaus, Helv. Chim. Acta, 55, 2182 (1972).

5) A. D. Argoudelis and S. A. Mizsak, J. Antibiotics, 30, 468 (1977).

6) D. Brewer, A. G. McInnes, D. G. Smith, A. Taylor, J. A. Walter, H. R. Loosli, and Z. L. Kis, J. Chem. Soc., Perkin I, 1978, 1248; T. Kikuchi, S. Kadota, K. Nakamura, A. Nishi, T. Taga, T. Kaji, K. Osaki, and K. Tubaki, Chem. Pharm. Bull., 30, 3846 (1982).

7) H. Minato, M. Matsumoto, and T. Katayama, J. Chem. Soc., Perkin I, 1973, 1919.

8) R. Rahman, S. Safe, and A. Taylor, J. Chem. Soc., (C), 1969, 1665.

9) G. Germain, P. Main, and M. M. Woolfson, Acta Cryst. A27, 368 (1971)

10) D. Hauser, W. P. Weber, and H. P. Sigg, Helv. Chim. Acta, 53, 1061 (1970).

11) M. Przybylska, E. M. Gopalakrishna, A. Taylor, and S. Safe, J. Chem. Soc., Chem. Comm., 1973, 554; M. Przybylska and E. M. Gopalakrishna, Acta Cryst., B30, 597 (1974).

(Received in Japan 29 June 1985)