Pergamon

Tetrahedron Letters 43 (2002) 2367–2370

TETRAHEDRON LETTERS

The constitution of micrococcin P1: the Bycroft–Gowland hypothesis confirmed

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Received 29 January 2002; accepted 6 February 2002

Abstract—Extensive NMR studies validate the 1978 Bycroft–Gowland hypothesis, hitherto unsupported by experimental evidence, regarding the constitution of micrococcin P1. Published synthetic work in the micrococcin area must be reinterpreted in light of these findings. © 2002 Elsevier Science Ltd. All rights reserved.

Micrococcin P1 (MP1)[1] is one of the structurally simpler thiopeptide natural products. These architecturally unique, sulfur-rich substances display antitumor, antibiotic and gene-regulating properties. This notwithstanding, synthetic activity in the thiopeptide area has been limited,[2] undoubtedly because of nagging, persistent structural uncertainties. For instance, the structure of MP1 was initially assigned as **1** by Walker, Lukacs, et al.[3a] Remarkably, the order of individual components of the macrocycle was proposed based solely on a *presumed* analogy with other thiopeptide antibiotics.[3a] Furthermore, significant uncertainty surrounded the configuration of the valine-derived thiazole (segment **a**, Fig. 1), assigned as being *probably* (*R*).[3b] Bycroft and Gowland subsequently showed that structure **2** was more consistent with the hydrolytic behavior of MP1,[4] but they left unresolved the possibility of errors in the Walker–Lukacs sequence of remaining macrocycle subunits. Even so, structure **2** was tacitly accepted.[5] More troubling is the fact that neither the Bycroft–Gowland paper nor any subsequent public-domain literature records any evidence whatsoever regarding the configuration of the threonine-derived thiazole (segment **b**), which nonetheless came to be consistently represented as having L-threonine-like stereochemistry.[2e,f,6,7] Rather than resolving already tenuous structural issues, recent synthetic work on MP1 has spawned additional confusion. Indeed, totally synthetic *epimers* of **1** and **2**,[6,7] wherein the side chain isoalaninol (segment **c**) has the (*S*)-configuration, and not the secure[8] (*R*)-configuration

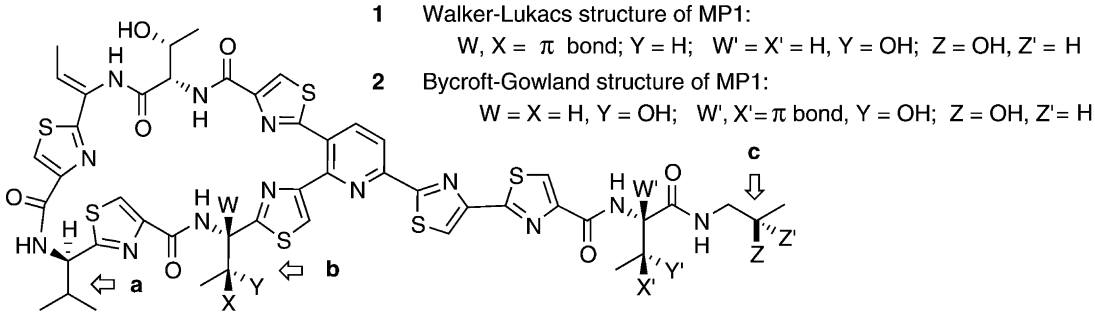

Figure 1.

Keywords: antibiotics; antitumor agents; gene regulators; micrococcin; thiopeptides.

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PII: S0040-4039(02)00266-6

shown in Fig. 1, are both described as being identical to natural material, even though structures **1** and **2** would seem to be mutually exclusive. Furthermore, we have found that synthetic **2** is *not* identical to the natural product.[9]

Massive constitutional and stereochemical uncertainties thus subsist for MP1. This has prompted us to reinvestigate its structure. Unequivocal NMR evidence now enables us to make a complete assignment of ^{1}H and ^{13}C resonances and to define the constitution of the antibiotic.

Our sample of natural MP1[10] contained 16% of a congener, micrococcin P2, wherein segment **c** is an aminoacetone unit (cf. Fig. 1; *Z*,*Z*′=O). The sample was used directly for NMR studies without separation of the components, so as to avoid any possibility of isomerization during chromatography.[11] Spectroscopic studies (500 MHz ^{1}H/125 MHz ^{13}C) were conducted using a solution of 5 mg of the mixture in 0.5 mL of DMSO-d_6, and the total assignment was based on a combination of 2D methods. First, isolated spin systems were identified by a COSY experiment. The standard techniques for sequential attribution utilized in the study of peptide structures were unsuitable in the present case, because of the thiazole and pyridine segments separating individual spin systems. Accordingly, the complete C–H and C–C connectivity was determined through gradient HSQC and HMBC experiments. The mixing time was critical for the simultaneous observation of all cross-peaks in HMBC. Best results were obtained by adding the transformed matrix of two HMBC spectra acquired with mixing times of 50 and 120 ms, respectively. In order to resolve each carbon resonance in the aromatic–olefinic region of the spectrum, the resolution in F1 was enhanced by acquiring 1024 increments with a regioselective HMBC experiment between 100 and 180 ppm, and by applying linear prediction in F1 prior to Fourier transform. Observed ^{1}J, ^{2}J, and ^{3}J C–H correlations permitted definition of atomic connectivity and complete assignment of ^{1}H and ^{13}C chemical shifts. The correlation diagram that emerged from NMR experiments (Fig. 2) is fully consistent with the Bycroft–Gowland hypothesis, and it sets this hitherto unproven structural proposal on firm experimental foundations. Fig. 3 shows the numbering system adopted herein for MP1 and the

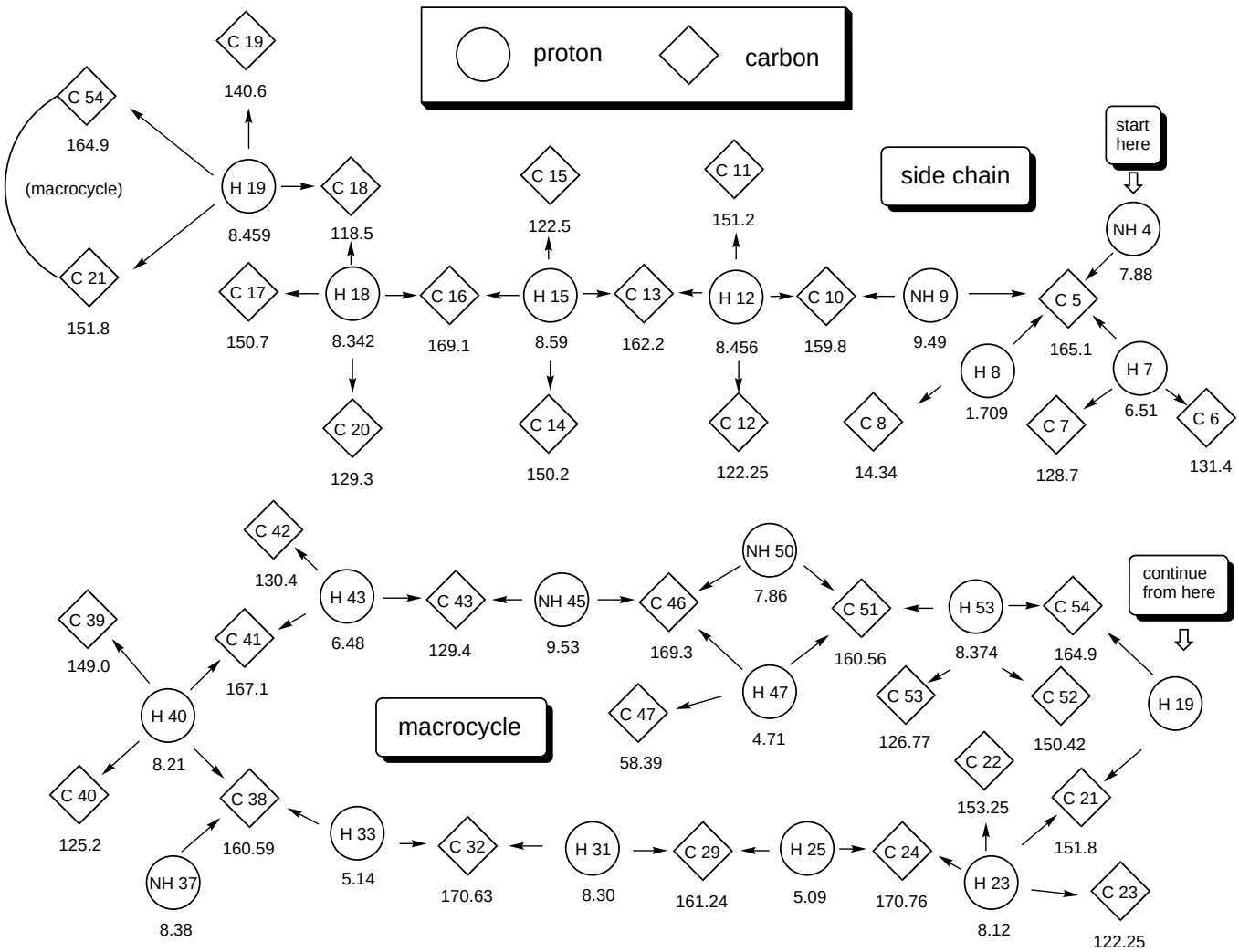
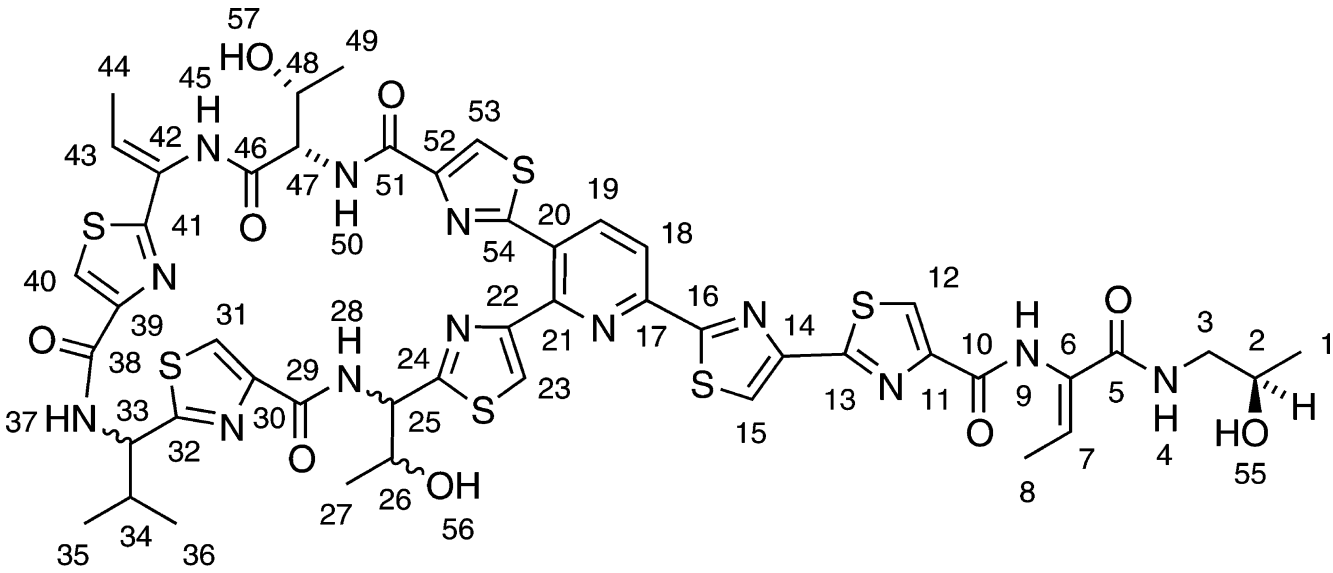


Figure 2. NMR correlation diagram for natural micrococcin P1.



position	^{1}H	^{13}C	position	^{1}H	^{13}C	position	^{1}H	^{13}C	position	^{1}H	^{13}C
1	1.028	21.9	**15**	8.590	122.5	**29**	- - -	161.3	**43**	6.479	129.4
2	3.717	65.9	**16**	- - -	169.1	**30**	- - -	149.9	**44**	1.763	14.6
3	3.085	43.6	**17**	- - -	150.7	**31**	8.297	125.5	**45**	9.531 [a]	- - -
4	7.88 [a]	- - -	**18**	8.345	118.5	**32**	- - -	170.6	**46**	- - -	169.3
5	- - -	165.1	**19**	8.459	140.6	**33**	5.145	56.0	**47**	4.713	58.4
6	- - -	131.4	**20**	- - -	129.3	**34**	2.510	33.1	**48**	4.385	68.3
7	6.515	128.7	**21**	- - -	151.8	**35**	(0.872)	(20.4)	**49**	1.386	20.6
8	1.720	14.3	**22**	- - -	153.2	**36**	(0.981)	(19.2)	**50**	7.860 [a]	- - -
9	9.480 [a]	- - -	**23**	8.120	122.2	**37**	8.377[a]	- - -	**51**	- - -	160.6
10	- - -	159.8	**24**	- - -	170.8	**38**	- - -	160.6	**52**	- - -	150.4
11	- - -	151.2	**25**	5.089	56.8	**39**	- - -	149.0	**53**	8.374	126.8
12	8.456	122.2	**26**	4.030	67.8	**40**	8.210	125.2	**54**	- - -	164.9
13	- - -	162.2	**27**	1.044	21.3	**41**	- - -	167.1	**55**	4.670[b]	- - -
14	- - -	150.2	**28**	8.223[a]	- - -	**42**	- - -	130.4	**56**	4.800[b]	- - -
									57	5.421[b]	- - -

[a]NH proton. [b]OH proton

Figure 3. Assignment of ^{1}H/^{13}C chemical shifts for natural MP1 in DMSO-d_6.

complete tabulation of chemical shifts. Notice that the configurations of valine- and threonine-derived thiazoles are shown as undefined. Chemical shifts (^{1}H/^{13}C) for the methyl groups of the valine-derived isopropyl segment (C-35 and C-36) were firmly correlated (HSQC), but of course we are unable to assign individual resonances to the respective diastereotopic protons and carbons.

Our results rule conclusively against structure **1** for MP1 and imply the non-identity of synthetic *epi*-**1**[6] to the natural product, regardless of the configuration of the side chain and contrary to published work. Finally, they indicate that the difference between our synthetic **2**[9] and natural MP1 must be purely stereochemical. Research directed toward the resolution of this second structural issue is currently underway and will be the subject of future reports.

Acknowledgments

We thank the MENRT, the CNRS, and the Région-Rhône-Alpes, for support of our research program. M.A.C. is the recipient of a Merck Academic Development Award (2000 and 2001).

References

1. Review: Pestka, S. In *Antibiotics*; Gottlieb, D.; Shaw, P. D.; Corcoran, J. W., Eds.; Springer-Verlag: New York, NY, 1975; Vol. 3, p. 480 ff. See also Refs. 6–9 and literature cited therein.
2. E.g.: (a) Bagley, M. C.; Bashford, K. E.; Hesketh, C. L.; Moody, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 3301; (b) Okamura, K.; Saito, H.; Shin, C.-G.; Umemura, K.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1863; (c) Ciufolini, M. A.; Shen, Y.-C. *J. Org. Chem.* **1997**, *62*, 3804 and references cited therein. For pioneering work in this area, see, e.g.: (d) Kelly, T. R.; Jagoe, C. T.; Gu, Z. *Tetrahedron Lett.* **1991**, *32*, 4263; (e) Nakamura, Y.; Shin, C.-G.; Umemura, K.; Yoshimura, J. *Chem. Lett.* **1992**, 1005; (f) Okumura, K.; Shigekuni, M.; Nakamura, Y.; Shin, C.-G. *Chem. Lett.* **1996**, 1025.
3. (a) Walker, J.; Olesker, A.; Valente, L.; Rabanal, R.; Lukacs, G. *J. Chem. Soc., Chem. Commun.* **1977**, 706; (b) Dean, B. M.; Mijovic, M. P. V.; Walker, J. *J. Chem. Soc.* **1961**, 3394.
4. Bycroft, B. W.; Gowland, M. S. *J. Chem. Soc., Chem. Commun.* **1978**, 256.
5. E.g.: *The Merck Index*, 12th ed.; Budavari, S., Ed.; Merck: Rahway, NJ, 1996; p. 1056.
6. (a) Shin, C.-G.; Okamura, K.; Shigekuni, M.; Nakamura, Y. *Chem. Lett.* **1998**, 139; (b) Okamura, K.; Nakamura, Y.; Shin, C.-G. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1561.
7. Okamura, K.; Ito, A.; Yoshioka, D.; Shin, C.-G. *Heterocycles* **1998**, *48*, 1319.
8. Brookes, P.; Fuller, A. T.; Walker, J. *J. Chem. Soc.* **1957**, 689.
9. Ciufolini, M. A.; Shen, Y.-C. *Org. Lett.* **1999**, *1*, 1843.
10. Provided by one of us (Professor E. Cundliffe).
11. Prudence advocated avoidance of exposure of natural MP1 to acidic surfaces such as that of silica gel, in light of the stereochemical lability of 2-aminoalkyl oxazoles in the presence of acidic agent (cf. Refs. 2, 8–9, as well as Aguilar, E.; Meyers, A. I. *Tetrahedron Lett.* **1994**, *35*, 2473).