Total Structure of the Polythiazole-containing Antibiotic Micrococcin P. A ^{13}C Nuclear Magnetic Resonance Study

By JAMES WALKER

(*National Institute for Medical Research, Mill Hill, London* NW7 1AA)

and ALAIN OLESKER, LYDIA VALENTE, ROSA RABANAL, and GABOR LUKACS*

(*Institut de Chimie des Substances Naturelles du C.N.R.S.* 91190 *Gif-sur-Yvette, France*)

Summary The structure of the antibiotic micrococcin P has been determined by ^{13}C n.m.r. spectroscopy.

IN earlier reports,[1-4] the structure of subunits A, B, C, D, and E (Figure) of the antibiotic micrococcin P[1] was established. We now demonstrate, on the basis of ^{13}C n.m.r. spectroscopy, the presence of one molecule of subunits A, C, D, and E and two molecules of subunit B per molecule of the antibiotic and revise the constitution of subunit E. Furthermore, we show that these fragments account for the total composition of micrococcin P (**1**) and establish its complete structure.

The noise-decoupled ^{13}C n.m.r. spectrum of micrococcin P (**1**), recorded in $(CD_3)_2SO$ solution on a Bruker HX-360 spectrometer operating at 90·52 MHz, exhibits 48 signals. Their assignment, based on the single frequency decoupled spectrum, from chemical shift rules[5] and the spectra of model fragments deriving from the micrococcin P-related thiostrepton (**3**)[6,7] and nosiheptide,[8-12] is indicated in the Table. Taking into account the presence of six carbonyl carbon atoms and assuming that the propionyl side chain of fragments A and C[1,3,4] corresponds, as in the case of thiostrepton (**3**)[6,7] and nosiheptide,[9,12] to enamido ethylidene units in the antibiotic, tentative assignments for all the 33 sp^2 carbon signals can be made.

The chemical shift values in the Table show the presence of 3 $>C-O-$, 3 $>C-NH-$ and 1 $-CH_2-NH-$ type signals. However the previously proposed structure[2] for subunit E,

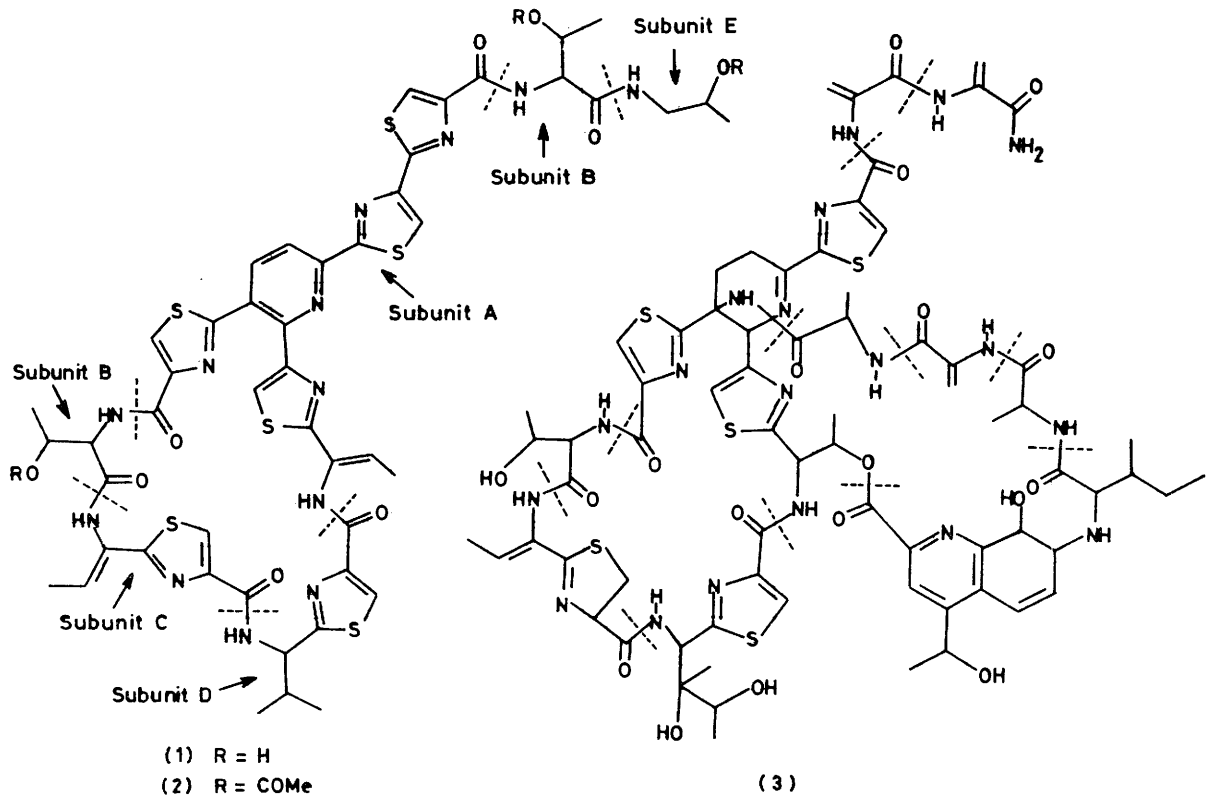

FIGURE. Subunits A and C were isolated after acid hydrolysis with propionyl instead of enamino side chains (refs. 1, 3, and 4).

$-NHCHMeCH_2OH$, would require 1 $-CH_2-O-$ type signal which is absent from the observed spectrum. Two of the >C–O– and two of the >C–NH– signals are characteristic of threonine β- and α-carbon atoms.[7,10] The remaining >C–NH– signal and the >CH– signal at 32·4 p.p.m. arise from subunit D.[1] Thus, besides the Me signals only 1 >C–O– signal and 1 $-CH_2-NH$ signal remain to be assigned. These results can be most easily interpreted in terms of a revision of the structure of subunit E to $-NHCH_2CH(OH)Me$ (Figure) instead of the previously proposed $-NHCHMe-CH_2OH$.[2] In view of the identification of aminoacetone[2] from acid hydrolysis of the antibiotic, the presence of the $-NHCH_2CH(OH)Me$ unit in micrococcin P (**1**) is also reasonable. Subunit E originates biogenetically from decarboxylation of threonine and its occurrence is consistent with two intact threonine units in (**1**).

The high-field part of the ^{13}C n.m.r. spectrum of micrococcin P (**1**) indicates 7 C–*Me* signals in agreement with expectations. The similarity in shifts of the vinylic methyl carbon atoms in (**1**) and in (**3**)[7] and nosiheptide[10,12] suggests that the stereochemistry of the ethylidene double bond is identical in all the three compounds. Further proof for the presence of 2 threonine and 1 $-NHCH_2CH-(OH)Me$ units in the antibiotic is provided by the ^{13}C n.m.r. spectrum of the triacetate (**2**) of micrococcin.† Compared with the spectrum of (**1**), this spectrum shows 3 additional C=O and 3 –O–CO*Me* signals.[5] The acetylation influences markedly the chemical shift of only 9 signals: the 3 >CH–O– carbon atoms are deshielded[5] while 2 >CH–NH– (2 × threonine-α), the $-CH_2-NH-$ (subunit E), and 3 Me signals (2 × threonine-γ + subunit E) are shielded by a few p.p.m. as expected.

TABLE. ^{13}C Chemical shifts[a] in $(CD_3)_2SO$ solution from internal Me_4Si (δ 0) for micrococcin P (1) and tentative assignments.

>C=O	170·1, 170·0, 168·6, 168·4, 166·5, 164·5
–N=C–S–	164·2, 161·6, 160·5, 159·9, 159·9, 159·0
–N=C<	152·7, 151·1, 150·5, 149·9, 149·9, 149·5, 149·2, 148·5 (6 thiazole + 2 pyridine *ortho* C atoms)
=CH–	140·5 (pyridine *para*)
=C<	130·6 (pyridine *meta*)
=C–NH–	129·7, 128·6 (ethylidene atoms of subunits A and C)
=CH–S– + =CH–	128·6, 127·7, 125·7, 125·3, 124·6, 124·2, 118·6 (1 pyridine *meta*)
=CH–	121·6, 121·3 (ethylidene C atoms of subunits A and C)
>CH–O–	67·5, 67·2 (threonine β)
>CH–O–	65·3 (subunit E)
>CH–NH–	57·8 (subunit D)
>CH–NH–	*56·2, 55·5* (threonine α)
$-CH_2-NH-$	*47·0* (subunit E)
>CH–	32·4 (subunit D)
Me	*21·0, 20·4, 19·5* (threonine γ) + (subunit E)
Me	19·7 and 18·4 (subunit D)
Me	13·7, and 13·5 (ethylidene atoms of subunits A and C)

[a] Signals in italics are shielded by 2—4 p.p.m. in the ^{13}C n.m.r. spectrum of micrococcin P tri-*O*-acetate (**2**).

Thus, subunits A, 2 × B, C, D, and E account for the total constitution of the antibiotic (48 carbon signals)

† The preparation and the physicochemical characteristics of micrococcin P tri-*O*-acetate will be described elsewhere.

indicating that no fragment was overlooked in earlier chemical degradation.[1–4] Although we have no evidence concerning the linkage of the fragments, it seems reasonable that micrococcin P (**1**), thiostrepton (**3**),[6,7] and nosiheptide[8,12] should be structurally closely related compounds and that their subunits should be present in the same sequence. This assumption is based on the structural similarity between the isolated subunits for these three antibiotics. *X*-Ray crystallographic investigations have proved that the related fragments of thiostrepton (**3**) and nosiheptide are attached to one another in the same way.[6,8] Thus the second threonine unit of (**1**) should be the starting unit of its side chain and fragment E must be terminal. It is of interest that the side chain of (**1**) and that of (**3**) are formed by two biogenetically identical amino-acids: threonine and decarboxythreonine in the case of (**1**) and two identical dehydroalanines in the case of (**3**).

Based on the arguments presented above, structure (**1**) can be assigned to micrococcin P whose empirical formula should thus be $C_{48}H_{49}N_{13}O_9S_6$ corresponding to a molecular weight of 1143·40.

(*Received*, 1*st July* 1977; *Com.* 666.)

[1] P. Brookes, A. T. Fuller, and J. Walker, *J. Chem. Soc.*, 1957, 689.
[2] M. P. V. Mijovic and J. Walker, *J. Chem. Soc.*, 1960, 909.
[3] M. N. G. James and K. J. Watson, *J. Chem. Soc.*, 1966, 1371.
[4] G. E. Hall, N. Sheppard, and J. Walker, *J. Chem. Soc.*, 1966, 1361.
[5] J. B. Stothers, 'Carbon-13 N.M.R. Spectroscopy,' Academic Press, New York, 1972.
[6] B. F. Anderson, D. C. Hodgkin, and M. A. Viswamitra, *Nature*, 1970, **225**, 233.
[7] K. Tori, K. Tokura, K. Okabe, M. Ebata, H. Otsuka, and G. Lukacs, *Tetrahedron Letters*, 1976, 185.
[8] T. Prangé, A. Ducruix, C. Pascard, and J. Lunel, *Nature*, 1977, **265**, 189.
[9] H. Depaire, J.-P. Thomas, A. Brun, and G. Lukacs, *Tetrahedron Letters*, 1977, 1395.
[10] H. Depaire, J.-P. Thomas, A. Brun, A. Olesker, and G. Lukacs, *Tetrahedron Letters*, 1977, 1397.
[11] H. Depaire, J.-P. Thomas, A. Brun, W. E. Hull, A. Olesker, and G. Lukacs, *Tetrahedron Letters*, 1977, 1401.
[12] H. Depaire, J.-P. Thomas, A. Brun, A. Olesker, and G. Lukacs, *Tetrahedron Letters*, 1977, 1403.