Frankiamide: A Structural Revision to Demethyl (C-11) Cezomycin

Karel D. Klika[a], J. Pasi Haansuu[b], Vladimir V. Ovcharenko[a], Kielo K. Haahtela[b], Pia M. Vuorela[c], Reijo Sillanpää[d], and Kalevi Pihlaja[a]

[a] Structural Chemistry Group, Department of Chemistry, University of Turku, FIN-20014 Turku, Finland
[b] Department of Biosciences, Division of General Microbiology, University of Helsinki, FIN-00014 Helsinki, Finland
[c] Department of Pharmacy, Viikki Drug Discovery Technology Center, University of Helsinki, FIN-00014 Helsinki, Finland
[d] Department of Chemistry, University of Jyväskylä, FIN-40500 Jyväskylä, Finland

Reprint requests to Dr. Karel D. Klika. Fax: 358-(2)-3336700. E-mail: karel.klika@utu.fi

Z. Naturforsch. **58b,** 1210 – 1215 (2003); received September 3, 2003

A compound (née frankiamide) isolated from *Frankia* was initially structurally elucidated on the basis of NMR and MS. However, X-ray analysis provided a structure that is in discord with this structure and in light of this the structure has now been revised and the compound renamed as demethyl (C-11) cezomycin.

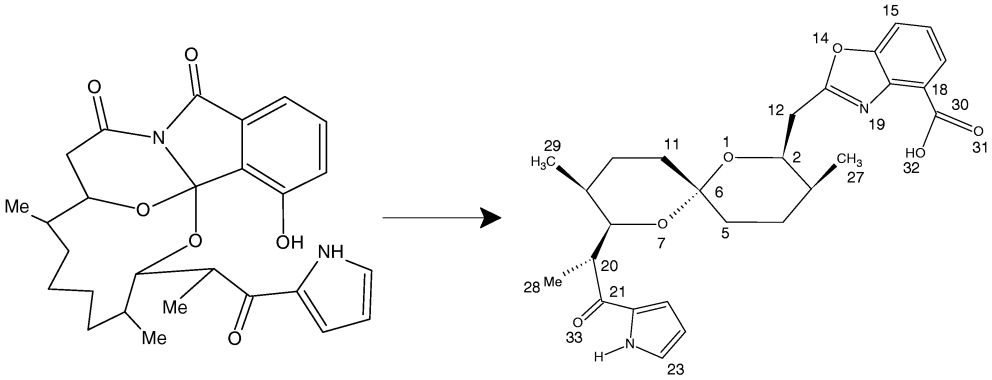
Key words: Frankiamide, Cezomycin, Frankia

Introduction

Frankia is a symbiotic actinomycete that forms nitrogen-fixing root nodules in actinorhizal plants like alder (*Alnus sp*.) and *Casuarina sp*., but it also commonly resides in soils lacking host plants [1−4]. The antimicrobial activity of the natural products produced by *Frankiae* have been well studied [5 – 9] as these compounds may serve a defensive role in assisting the slowly-growing *Frankia* to survive in non-symbiotic conditions [10 – 14]. Other physiological aspects of *Frankia*-culture broth extracts or compounds isolated from these extracts have also been examined, *e.g.* calcium channel antagonistic effects [5, 8, 9]. Recently, a compound that inhibited the growth of several pathogenic fungi and Gram-positive bacteria was isolated [8, 15] from a particular *Frankia* culture (strain AiPs1) [2] originating from a stand of Finnish Scots pine (*Pinus sylvestris* L.). The isolation procedure of this compound, together with the cultivation of the *Frankia* strain and assessment of the bioactivity of the isolated compound, have been well described [8, 9]. The structural elucidation of the compound, dubbed *frankiamide* (**1**, Fig. 1), was originally based [15] on NMR and MS studies but subsequent single-crystal X-ray crystallographic analysis, reported herein, of the material, however, provided a structure (**2**, Fig. 1) that was in discord with the original structure. Re-evaluation of the NMR data revealed that it is in fact consistent with the X-ray structure and therefore a structural revision of this compound is warranted. Given that the compound belongs to the well-known class of polyether ionophores and the calcimycins in particular and can be simply regarded as a derivative of cezomycin [16 – 20], the compound has been renamed as demethyl (C-11) cezomycin.

Results and Discussion

The structure of frankiamide (**1**) was published without X-ray corroboration as considerable difficulties were encountered in obtaining suitable crystals of the compound amenable to X-ray analysis. Numerous subsequent attempts finally provided a solitary crystal of mediocre quality just sufficient for X-ray analysis, the result of which is depicted in Fig. 2 where it is shown that two molecules of **2** complex to a Na^+ ion. The difficulties encountered in obtaining suitable crystals were probably simply due to the fact that a mixture of species – with respect to (de)protonation and (de)complexation to various metal ions – was probably present in solution. The affinity of this class of compounds for complexation to metal ions is well established as many complexes, neutral or cationic, have been reported [17, 18, 20 – 22] in solution between a number of calcimycin-class compounds and alkali and



1 **2**

Fig. 1. The structure of frankiamide (**1**) together with the revised structure (**2**) of the isolated material, now termed demethyl (C-11) cezomycin, derived from single-crystal X-ray crystallographic analysis. The numbering system in use for the new structure is indicated together with the relative stereochemistry of the chiral centers (2*S*,3*S*,6*R*,8*R*,9*S*,20*R*) obtained from X-ray analysis. The absolute stereochemistry of the compound could not be ascertained to any degree of certainty. The pyrrole-containing sidechain and the benzoxazole-containing sidechain are each defined as α substituents for their respective pyranose rings, thus denoting the ring methylene protons as either α or β.

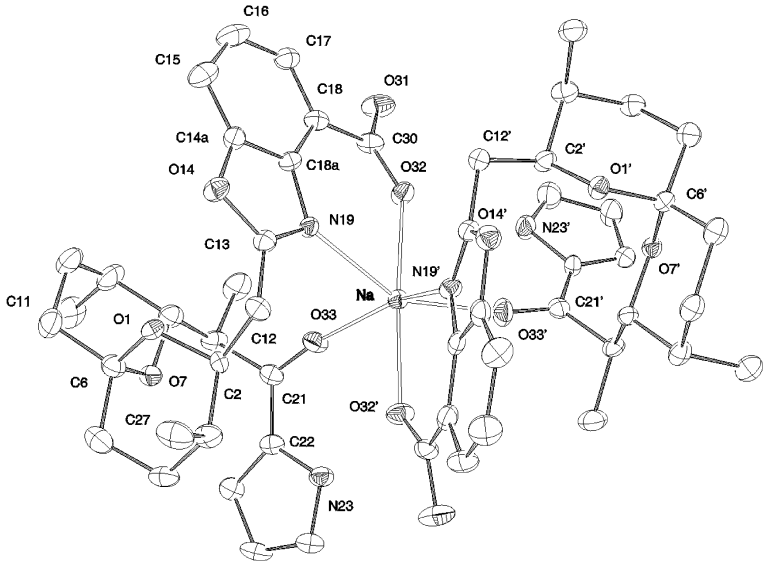


Fig. 2. The structure of **2** derived from single-crystal X-ray crystallographic analysis.

alkaline earth cations as 1:1 or 2:1 complexes. For this compound, in both methanol and chloroform solutions, the presence of a dynamic equilibrium had been reported that was shown to involve Na^+ ions [15]. Refinement of the X-ray data with Na^+ as the central metal ion provided the best convergence in comparison to K^+, Ca^{2+} or Mg^{2+} ions (from a survey of CCDC with respect to bond distances, angles, *etc*., the fit is best for Na^+ with Mg^{2+} providing only slightly higher R-values) and the structure is therefore represented as such with a Na^+ ion. However, a suitable signal from the original sample could not be detected by ^{23}Na NMR despite the high sensitivity of this nucleus and therefore the sole crystal obtained may not necessarily represent the bulk of the material. Na^+ ion adducts and salts of the material could readily be detected [15] by MS under EI^+, $ESI^{+,-}$ and FAB^+ conditions, but given the ubiquitous nature of this ion these observations are

not remarkable for the latter two techniques and are in fact quite usual. Despite the ubiquitous nature of K^+, the ready observation of K^+ ion adducts and salts under MS conditions is, however, unusual and prompted the search for a ^{39}K NMR signal, but which was also unsuccessful. Given the low receptivity or other limitations of other nuclei such as ^{43}Ca and ^{25}Mg, the identification by NMR of the central metal ion in the bulk of the sample was not pursued.

The choice of Na^+ as the central ion actually presents a quandary regarding the acidic protons due to the poor quality of the data, as either both carboxylate groups could be deprotonated and both ligands are equivalent (and to maintain charge neutrality one of the solvent molecules is protonated); both acidic protons could be present (although the evidence is only weak for this solution); or only one acidic proton is present and the ligands are therefore not equivalent. This last alternative was the solution that provided the best fit. The solvent molecules present in the lattice were also not well described and the formula was approximated as $[Na(C_{27}H_{32}N_2O_6)(C_{27}H_{31}N_2O_6)]\cdot 3H_2O$ with possibly one other solvent molecule such as acetonitrile being incorporated. The structure provided by the X-ray analysis is quite interesting as two molecules of **2** coordinate in a tridentate manner *via* the O-33 carbonyl oxygens, the N-19 nitrogens of the benzoxazoles and one of the oxygens of each of the carboxylate groups to the Na^+ to form an octahedral complex. A similar octahedral complex of Na^+, with analogous binding of the same coordinate atoms, has been reported previously [19]. Both six-membered rings of the spiro ring structure are in chair forms with the large sidechains equatorially orientated and with both of the methyl groups in axial dispositions, and this conformation appears to be maintained in solution. Nothing can be inferred regarding the absolute stereochemistry given the poor value obtained for the Flack's parameter, but the relative stereochemistry of **2** concurs with that given for other naturally-occurring, calcimycin-class compounds for the six, in-common asymmetric centers [17, 19, 22, 23].

The spectra of the samples obtained throughout the course of these studies [8, 9, 15] were often very different in appearance depending on the particular conditions under which they had been isolated (resulting in (de)protonation and (de)complexation to various metal ions) and the various samples often only bore scant resemblance to one another and were only proven to be the same compound after admixture. A sample was also subjected to treatment with dilute aqueous acid resulting in the equilibrium being pushed very much to one side (and away from the major species originally present) and revealing the presence of three species in the ratio of 100:10:5 which were slow to exchange on the NMR-timescale. The component in lowest concentration, on the basis of chemical shifts, bore close resemblance to the major species present prior to treatment with acid. Evidently, depending on the complexed metal, deprotonation of the acid group is required for the formation of a stable complex and protonation can successfully lead to the obtainment of the free ligand. The full assignment of the ^{13}C NMR spectrum for both the complexed original sample and the free ligand, together with a full spin analysis of the ^{1}H NMR spectrum using Perch iteration software [24] for the free ligand (spectral overlap of the considerable amount of the minor species in the complexed sample precluded full spin analysis) are now reported, including some re-assignment of the $J_{H,H}$s of the pyrrole ring.

Although upon initial inspection the two structures **1** and **2** appear to be very distinct, in actuality only the movement of a few atoms is required to transform the two structures and clearly there is considerable consistency with regards to the gross structure. Re-evaluation of the NMR data that originally provided the structure of the isolate as **1** led to the realization that the data is in fact entirely consistent with the structure **2**. Evidently only the lack of a few critical long-range ^{1}H-^{13}C correlations allowed **1** to remain as a plausible candidate structure, coupled with the false positive provided by the phenol test together with some unusual chemical shifts. For the modified Folin – Ciocalteu phenol test [25] used in this work, although the result was "weak", one cannot discount the fact that a positive was obtained due to an enol structure rather than attributing it to the presence of an impurity. The unusual shifts included the benzoxazole nitrogen appearing at -161 ppm instead of the more expected -138 ppm [26] due to complexation (found at -148 ppm in the free ligand) and the carboxylic acid carbon appearing at 168 ppm instead of within the expected range of 150 – 160 ppm [27] due to deprotonation/complexation (although still found outside this range at 165 ppm in the free ligand). Even the pyrrole nitrogen, despite not being directly bound to the metal, displayed considerable chemical shift differences between the bound (-216 ppm) and free states (-231 ppm), thus shifting to a greater extent than the benzoxazole nitrogen but in an opposite direction.

The mass spectra of the isolated material under EI^+ conditions is also consistent with either of the structures **1** or **2** as the gross features remain in effect such as the presence of the pyrrole-containing side chain, confirmed by the formation of the stable ion $C_7H_9NO^{+\bullet}(m/z\ 123)$ and the characteristic $C_5H_4NO^+(m/z\ 94)$ ion for acyl pyrroles [28]. The sequential loss of two water molecules and one water molecule + carbon monoxide (equivalent to formic acid for the combined loss of HCO_2H) as observed can also be rationalized for both structures. Of course, the elemental composition of the molecular ion and the various adducts/salt-adducts which it formed all remain correct. The propensity for the material to form adducts with metal ions under MS conditions is also now much more readily appreciated given the exhaustive studies on the complexation behavior of this class of compounds (*vide supra*).

Although originally an incorrect structure for the isolate was reported, the significant biological activities of this nonetheless, still new compound remain in place and form the basis for the continuing biological evaluations of this material with respect to its effect on various *Frankia* plant hosts and other bacteria with which *Frankia* is in competition. It is also notable that this is the first reported [9] instance of a calcimycin-class compound occurring in *Frankiae*.

Experimental Section

General

X-Ray crystallographic data were collected on an area-detector diffractometer using graphite-monochromatized Mo-K_α radiation ($\lambda = 0.71073$ Å). Lattice parameters were determined from 10 images recorded with 1° φ scans and subsequently refined on all data. The data collection was performed using φ and ω scans with 1° steps using an exposure time of 150 s per frame. The crystal-to-detector distance was 30 mm. The data were processed using DENZO-SMN v0.93.0 [29]. The structure was solved by direct methods using the SHELX-97 program [30] and full-matrix least-squares refinements on F^2 were also performed using the same program [30]. All heavy atoms were refined anisotropically whilst hydrogen atoms were included at calculated distances from their host atoms with fixed displacement parameters. The figure was drawn using ORTEP-3 for *Windows* [31].

NMR spectra were acquired at 500.16 MHz for ^{1}H, 125.78 MHz for ^{13}C, 50.69 MHz for ^{15}N, 132.3 MHz for ^{23}Na and 18.66 MHz for ^{39}K at 25 °C in $CDCl_3$. Both ^{1}H and ^{13}C spectra were referenced internally to tetramethylsilane (0 ppm for both). ^{15}N, ^{23}Na and ^{39}K spectra were referenced externally to 90% nitromethane in CD_3NO_2 (at 0 ppm), a saturated solution of NaBr in D_2O (at 0 ppm) and a saturated solution of KI in D_2O (at 0 ppm), respectively. 1D spectra consisted of normal proton, carbon, sodium, potassium and nitrogen (acquired with single-pulse excitation and inverse-gated decoupling or with INEPT optimized on a $^1J_{HN}$ of 90 Hz), DEPT 135°, selective INEPT (optimized on a $^nJ_{HC}$ of 8, 4, or 2 Hz) and NOE difference measurements. 2D spectra comprised of both phase-sensitive {DQF COSY, NOESY/EXSY and CHSHF (^{1}H-{^{13}C} with partial homonuclear decoupling in f1 and optimized on a $^1J_{HC}$ coupling of 145 Hz)} and absolute-value mode variants {HSQC (^{1}H-{^{15}N}, optimized on a $^1J_{HN}$ coupling of 95 Hz) and HMBC (^{1}H-{^{13}C}, ^{1}H-{^{15}N}, both optimized on a long-range coupling of 8 Hz for both $^nJ_{HC}$ and $^nJ_{HN}$)}, mostly with field gradients. Spectral widths and resolution were appropriately optimized from the 1D spectra and generally processed with zero-filling (×2, ×4) and exponential weighting (plus a $2\pi/3$-shifted sinebell function for the absolute-value mode spectra) applied in both dimensions prior to Fourier transformation. For the extraction of δ_H and $J_{H,H}$, spin analysis was performed using PERCH iteration software [24]. Legend for abbreviations: s, singlet; d, doublet; t, triplet; qt, quartet; m, multiplet; h o, higher order; v, very; sl, slightly; AB, pertaining to part of an AB system; o'ld, overlapped.

The optical rotation is expressed as $[\alpha]_D$ values in 10^{-1} deg cm^2 g^{-1} units.

*Frankia strain and culturing and isolation of demethyl (C-11) cezomycin (**2**)*

The *Frankia* AiPs1 strain used [8, 15], its identification [2, 8] and culturing [8, 9, 15, 32, 33] and the isolation [8, 9, 15] by HPLC of demethyl (C-11) cezomycin (**2**) have been previously described. The sample of **2** so obtained was examined by NMR as received and then subsequently after acid treatment: the sample in 2 ml of chloroform was washed (×2) with dilute HCl (pH 1) and the aqueous phase back washed with 1 ml of chloroform (×2). The combined organic phases were taken to dryness and the resulting residue dried in a dessicator over silica gel.

*Demethyl (C-11) cezomycin (**2**, sample obtained from HPLC)*

$[\alpha]_D^{22} + 192.4° \pm 2.6°$ (c 0.0078 in $CHCl_3$). – ^{1}H NMR (500.16 MHz, $CDCl_3$, partial Perch analysis, major species): $\delta = 0.734$ (d, $J_{H9} = 6.9$ Hz, H-29), 0.735 (d, $J_{H20} = 6.6$ Hz, H-28), 0.843 (d, $J_{H3} = 7.01$ Hz, H-27), 1.50 – 0.65 {9H overlapped, approximate δs: 1.35 and 1.25 (H-11s); 1.35 and 1.05 (H-5s); 2 × 1.13 (H-10s); 1.08 (H-9); 0.97 and 0.83 (H-4s)}, 1.923 (m, $J_{H27} = 7.12$, $J_{H4\beta} = 4.12$, $J_{H2} = 2.27$, $J_{H4\alpha} = 2.19$, Hz, H-3), 2.511 (dd, $J_{H20} = 10.0$, $J_{H9} = 2.1$ Hz, H-8), 2.804 (d(sl AB)d, $J_{H12} = -12.99$, $J_{H2} = 11.74$ Hz,

H-12'), 2.905 (d(sl AB)d, $J_{H12'} = -12.99$, $J_{H2} = 2.58$ Hz, H-12), 3.010 (dqt, $J_{H8} = 10.0$, $J_{H28} = 6.7$ Hz, H-20), 4.049 (dt, $J_{H12'} = 11.74$, $J_{H12} = 2.58$, $J_{H3} = 2.27$ Hz, H-2), 6.239 (dt, $J_{H26} = 3.86$, $J_{H24} = 2.28$, $J_{H23} = 2.18$ Hz, H-25), 6.969 (ddd, $J_{H25} = 3.86$, $J_{H23} = 2.17$, $J_{H24} = 1.30$ Hz, H-26), 7.410 (ddd, $J_{H23} = 3.10$, $J_{H25} = 2.28$, $J_{H26} = 1.30$ Hz, H-24), 7.474 (t, $J_{H15} = 8.13$, $J_{H17} = 7.77$ Hz, H-16), 7.649 (dd, $J_{H16} = 8.13$, $J_{H17} = 1.09$ Hz, H-15), 8.297 (dd, $J_{H16} = 7.77$, $J_{H15} = 1.09$ Hz, H-17), 13.533 (br. s, $\nu_{1/2} \approx 9$ Hz, H-23). – $^{13}C\{^1H\}$ NMR (125.78 MHz, $CDCl_3$, major species): $\delta = 9.72$ (qt, C-29), 11.18 (qt, C-27), 11.61 (qt, C-28), 25.05 (t, C-4), 26.17 (t, C-10), 26.68 (d, C-9), 28.95 (d, C-3), 29.11 (t, C-11), 29.55 (t, C-5), 32.20 (t, C-12), 43.00 (d, C-20), 72.25 (d, C-2), 75.28 (d, C-8), 96.39 (s, C-6), 110.80 (d, C-25), 112.87 (d, C-15), 121.10 (d, C-26), 124.95 (d, C-16), 126.86 (s, C-18), 127.53 (d, C-17), 129.18 (d, C-24), 133.88 (s, C-22), 138.81 (s, C-18a), 150.11 (s, C-14a), 168.22 (s, C-30), 170.32 (s, C-13), 196.01 (s, C-21). – $^{15}N\{^1H\}$ NMR (50.69 MHz, $CDCl_3$, major species): $\delta = -161.45$ (s, N-19); -215.68 (d, N-23).

*Demethyl (C-11) cezomycin (**2**, sample obtained after acid extraction)*

$[\alpha]_D^{22} + 21.43^\circ \pm 0.31^\circ$ (c 0.00336 in $CHCl_3$). – ^1H NMR (500.16 MHz, $CDCl_3$, full Perch analysis): $\delta = 0.816$ (d, $J_{H20} = 6.92$ Hz, H-28), 0.920 (d, $J_{H3} = 7.02$ Hz, H-27), 0.938 (d, $J_{H9} = 6.95$ Hz, H-29), 1.119 (h o m, $J_{H4\beta} = -13.24$, $J_{H5\alpha} = 4.71$, $J_{H3} = 2.50$, $J_{H5\beta} = 2.43$ Hz, H-4α), 1.255 (o'ld with H_2O, $J_{H5\alpha} = -13.27$, $J_{H4\beta} = 4.31$, $J_{H4\alpha} = 2.43$, $J_{H3} = 0.47$ Hz, H-5β), 1.363 (o'ld m, $J_{H10\beta} = -13.40$, $J_{H11\alpha} = 4.60$, $J_{H9} = 2.65$, $J_{H11\beta} = 2.51$ Hz, H-10α), 1.399 (o'ld m, $J_{H11\alpha} = -13.68$, $J_{H10\beta} = 4.40$, $J_{H10\alpha} = 2.51$, $J_{H9} = 0.47$ Hz, H-11β), 1.435 (o'ld m, $J_{H5\alpha} = 13.87$, $J_{H4\alpha} = -13.24$, $J_{H3} = 4.33$, $J_{H5\beta} = 4.31$ Hz, H-4β), 1.465 (o'ld m, $J_{H4\beta} = 13.87$, $J_{H5\beta} = -13.27$, $J_{H4\alpha} = 4.71$ Hz, H-5α), 1.535 (o'ld m, $J_{H10\beta} = 13.97$, $J_{H11\beta} = -13.68$, $J_{H10\alpha} = 4.60$ Hz, H-11α), 1.551 (o'ld m, $J_{H27} = 7.02$, $J_{H4\beta} = 4.33$, $J_{H4\alpha} = 2.50$, $J_{H2} = 2.45$, $J_{H5\beta} = 0.47$ Hz, H-3), 1.591 (o'ld m, $J_{H29} = 6.95$, $J_{H10\beta} = 4.31$, $J_{H10\alpha} = 2.65$, $J_{H8} = 2.41$, $J_{H11\beta} = 0.47$ Hz, H-9), 1.910 (tt, $J_{H11\alpha} = 13.97$, $J_{H10\alpha} = -13.40$, $J_{H11\beta} = 4.40$, $J_{H9} = 4.31$ Hz, H-10β), 3.038 (d(AB)d, $J_{H12'} = -15.11$, $J_{H2} = 6.25$ Hz, H-12), 3.165 (d(AB)d, $J_{H12} = -15.11$, $J_{H2} = 8.09$ Hz, H-12'), 3.180 (d(sl AB)qt, $J_{H8} = 10.33$, $J_{H28} = 6.92$ Hz, H-20), 3.563 (d(sl AB)d, $J_{H20} = 10.33$, $J_{H9} = 2.41$ Hz, H-8), 4.280 (ddd, $J_{H12'} = 8.09$, $J_{H12} = 6.25$, $J_{H3} = 2.45$ Hz, H-2), 6.252 (dt, $J_{H26} = 3.80$, $J_{H23} = 2.51$, $J_{H24} = 2.49$ Hz, H-25), 6.918 (ddd, $J_{H25} = 3.80$, $J_{H23} = 2.43$, $J_{H24} = 1.33$ Hz, H-26), 7.054 (td, $J_{H23} = 2.91$, $J_{H25} = 2.49$, $J_{H26} = 1.33$ Hz, H-24), 7.476 (d(sl. AB)d, $J_{H15} = 8.15$, $J_{H17} = 7.80$ Hz, H-16), 7.816 (d(sl. AB)d, $J_{H16} = 8.15$, $J_{H17} = 0.95$ Hz, H-15), 8.116 (dd, $J_{H16} = 7.80$, $J_{H15} = 0.95$ Hz, H-17), 9.716 (br s, $\nu_{1/2} \approx 15$ Hz!, H-23). – $^{13}C\{^1H\}$ NMR (125.78 MHz, $CDCl_3$): $\delta = 10.60$ (qt, C-29), 10.89 (qt, C-27), 13.14 (qt, C-28), 25.74 (t, C-4), 26.13 (t, C-10), 26.93 (d, C-9), 29.19 (d, C-3), 29.48 (t, C-5), 29.61 (t, C-11), 32.55 (t, C-12), 42.58 (d, C-20), 68.80 (d, C-2), 73.29 (d, C-8), 96.55 (s, C-6), 110.28 (d, C-25), 115.35 (d, C-15), 116.69 (d, C-26), 119.97 (s, C-18), 124.64 (d, C-24), 124.98 (d, C-16), 126.77 (d, C-17), 133.07 (s, C-22), 140.57 (s, C-18a), 150.38 (s, C-14a), 164.87 (s, C-30), 167.54 (s, C-13), 193.88 (s, C-21). – $^{15}N\{^1H\}$ NMR (50.69 MHz, $CDCl_3$): $\delta = -148.07$ (s, N-19); -231.27 (d, N-23).

*Crystal structure determination of demethyl (C-11) cezomycin (**2**)*

The material isolated from the HPLC was first washed with acetonitrile in which the complexed material was only slightly soluble. The insoluble residues were then taken up in $CHCl_3$ and further acetonitrile added. A single crystal of **2** amenable to X-ray analysis was obtained from the mixed solution of acetonitrile and chloroform by allowing the solution to slowly evaporate to dryness. Crystal data: molecular formula, $C_{54}H_{69}N_4NaO_{15}$; M. Wt. = 1037.15; crystal system, orthorhombic; space group, $P2_12_12_1$ (No. 19). Unit cell parameters: $a = 15.5178(3)$, $b = 18.0082(3)$ and $c = 20.7096(5)$ Å; $\alpha = \beta = \gamma = 90^\circ$; $U = 5,787.2(2)$ Å^3; $Z = 4$; $D_c = 1.190$ g/cm^3. Experimental parameters: μ(Mo-K$_\alpha$) = 0.71073 Å; $T = 173(2)$ K; crystal dimensions, $0.14 \times 0.06 \times 0.04$ mm; 31,971 reflections were measured of which 9,797 were unique ($R_{int} = 0.1251$) which were used for all calculations. The final $wR(F^2)$ was 0.2746 (all data). Determination of the absolute configuration was not possible from the data using the Flack's parameter which provided irreconcilable values. The X-ray crystallographic data (including the .cif file) for **2** and is available upon request from the authors.

The sample obtained by this method was also examined by ^1H NMR analysis but only broad lines were obtained indicating that it was possibly even less homogeneous species-wise than the original sample although the signals of the two samples roughly corresponded.

Acknowledgements

Financial support from the Academy of Finland, grant nos. 47231 (K. K. H. and J. P. H.) and 4284 (K. P.), is gratefully acknowledged.

[1] K. Huss-Danell, A. K. Frej, Plant Soil **90**, 407 (1986).
[2] L. Maunuksela, K. Zepp, T. Koivula, J. Zeyer, K. Haahtela, D. Hahn, FEMS Microbiol. Ecol. **28**, 11 (1998).

[3] A. Smolander, Plant Soil **121**, 1 (1990).
[4] A. Smolander, R. Rönkkö, E.-L. Nurmiaho-Lassila, K. Haahtela, Can. J. Microbiol. **36**, 649 (1990).
[5] P. Haansuu, P. Vuorela, K. Haahtela, Pharm. Pharmacol. Lett. **9**, 1 (1999).
[6] L. Lang, For. Res. **12**, 47 (1999).
[7] A. G. Medentsev, B. P. Baskunov, O. S. Stupar, M. Y. Nefedova, V. K. Akimenko, Biokhimiya **54**, 926 (1989).
[8] J. P. Haansuu, K. D. Klika, P. P. Söderholm, V. V. Ovcharenko, K. Pihlaja, K. K. Haahtela, P. M. Vuorela, J. Ind. Microbiol. Biot. **27**, 62 (2001).
[9] J. P. Haansuu, in Demethyl (C-11) Cezomycin – a Novel Calcimycin Antibiotic from the Symbiotic, N_2-fixing Actinomycete *Frankia*. Ph. D. Dissertation University of Helsinki (2002).
[10] M. Arahou, H. G. Diem, A. Sasson, World J. Microbiol. Biotech. **14**, 31 (1998).
[11] D. B. Aronson, G. L. Boyer, Soil Biol. Biochem. **26**, 561 (1994).
[12] S. R. Mansour, S. A. El Melegy, Egypt. J. Microbiol. **32**, 423 (1997).
[13] S. Safo-Sampah, J. G. Torrey, Plant Soil **112**, 89 (1988).
[14] A. Séguin, M. Lalonde, Plant Soil **118**, 221 (1989).
[15] K. D. Klika, J. P. Haansuu, V. V. Ovcharenko, K. K. Haahtela, P. M. Vuorela, K. Pihlaja, J. Org. Chem. **66**, 4065 (2001).
[16] L. David, A. Kergomard, J. Antibiot. **35**, 1409 (1982).
[17] R. K. Boekman (Jr.), A. B. Charette, T. Asberom, B. H. Johnston, J. Am. Chem. Soc. **113**, 5337 (1991).
[18] A.-M. Albrecht-Gary, S. Blanc, L. David, G. Jeminet, Inorg. Chem. **33**, 518 (1994).
[19] J. W. Westley, C.-M. Liu, J. F. Blount, L. H. Sello, N. Troupe, P. A. Miller, J. Antibiot. **36**, 1275 (1983).
[20] M. Prudhomme, G. Dauphin, G. Jeminet, J. Antibiot. **39**, 922 (1986).
[21] A.-M. Albrecht-Gary, S. Blanc-Parasote, D. W. Boyd, G. Dauphin, G. Jeminet, J. Juillard, M. Prudhomme, C. Tissier, J. Am. Chem. Soc. **111**, 8598 (1989).
[22] M. O. Chaney, P. V. Demarco, N. D. Jones, J. L. Occolowitz, J. Am. Chem. Soc. **96**, 1932 (1974).
[23] D. A. Evans, C. E. Sacks, W. A. Kleschick, T. R. Taber, J. Am. Chem. Soc. **101**, 6789 (1979).
[24] R. Laatikainen, M. Niemitz, U. Weber, J.ySundelin, T. Hassinen, J. Vepsäläinen, J. Magn. Reson. **A120**, 1 (1996); or see the program website at http://www.perchsolutions.com.
[25] K. Nurmi, V. Ossipov, E. Haukioja, K. Pihlaja, J. Chem. Ecol. **22**, 2023 (1996).
[26] S. Berger, S. Braun, H. O. Kalinowski, in NMR Spectroscopy of the Non-Metallic Elements, Ch. 4, Wiley, Chichester, English Edition (1997).
[27] H. O. Kalinowski, S. Berger, S. Braun, in Carbon-13 NMR Spectroscopy, Wiley, Chichester (1988).
[28] Q. N. Porter, in Mass Spectrometry of Heterocyclic Compounds, S. 541, Wiley, New York, 2nd Ed. (1985).
[29] Z. Otwinowski, W. Minor, DENZO-SMN v0.93.0. in C. W. Carter (Jr.), R. M. Sweet, (Herausg.): Methods in Enzymology, Macromolecular Crystallography, S. 307 – 326 Academic Press, New York, Part A (1997).
[30] G. M. Sheldrick, SHELX-97, University of Göttingen, Germany (1997).
[31] L. J. Farrugia, ORTEP-3 for *Windows*, J. Appl. Cryst. **30**, 565 (1997).
[32] A. Smolander, M. L. Sarsa, Plant Soil **122**, 129 (1990).
[33] A. Weber, A. Smolander, E.-L. Nurmiaho-Lassila, V. Sundman, Symbiosis **6**, 97 (1988).