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TOTAL SYNTHESIS AND ABSOLUTE CONFIGURATION OF RHIZOBACTIN, A STRUCTURALLY NOVEL SIDEROPHORE

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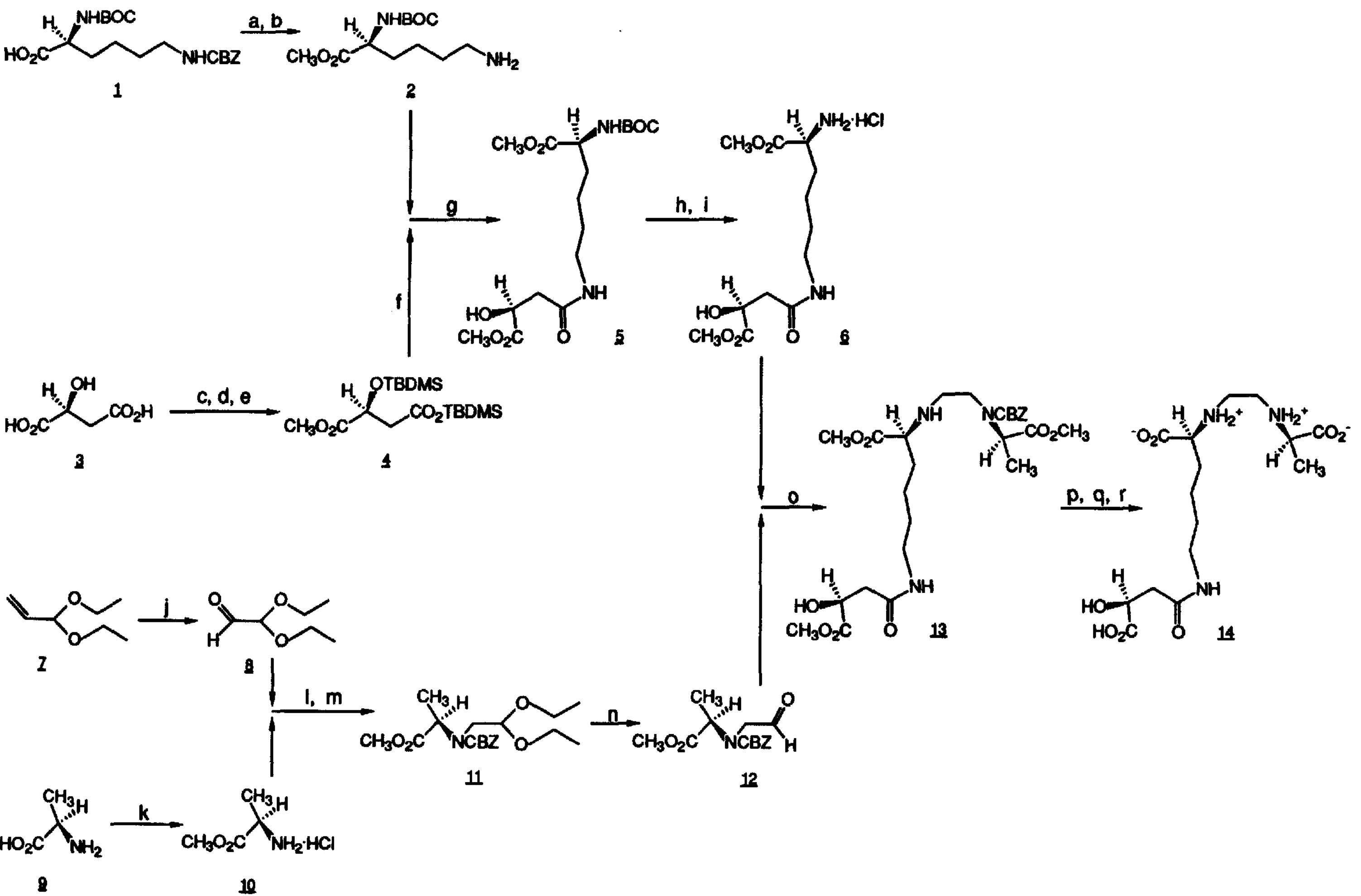
Summary: The actual stereoisomer of rhizobactin, N^2-[2-[(R)-(1-carboxyethyl)amino]-ethyl]-N^6-(S)-(3-carboxy-3-)hydroxy-1-oxopropyl)-(S)-lysine **14**, *has been synthesized and substantiates the conclusion that this siderophore is biochemically related to the pyruvic acid derived opines.*

The recent isolation[1] and structure elucidation[2] of rhizobactin, N^2-[2-[(1-carboxy-ethyl)amino]-ethyl]-N^6-(S)-(3-carboxy-3-)hydroxy-1-oxopropyl)lysine, from low-iron cultures of the Gram-negative, dinitrogen-fixing, phytosymbiotic bacterium *Rhizobium meliloti* DM4, disclosed a third general category for microbial siderophores (Gr. *sidero* = iron; *phore* = bearer[3]). The compound's ethylenediamine group is novel as a natural product and unprecedented as a ligand in the siderophore series, which characteristically contain catechol or hydroxamate groups.[4] It was previously demonstrated that natural rhizobactin contains an L-malic acid constituent using a malic dehydrogenase assay on a hydrolyzed specimen.[2] However, determination of the absolute configuration of the two remaining chiral centers and proof of the entire structure awaited total synthesis, which is reported in the following.

It is pertinent that rhizobactin is biochemically related to other unusual N^2-substituted amino acids collectively referred to as opines, insofar as its strain-specific synthesis and utilization is concerned.[1,5] A general class of imino acid dehydrogenases generate a wide variety of such compounds via reductive amination of an α-keto acid with an L-amino acid.[6] By analogy, rhizobactin can be regarded as being biosynthesized from reductive amination between pyruvic acid / glycine, giving rise to the "alanine" moiety, a second reductive amination between glycine / lysine, and amidation between lysine / L-malic acid. As the stereochemistry of opines derived from pyruvic acid is $D^{ala},L^{amino\ acid}$ [7] the present synthesis employed D-alanine, L-lysine and L-malic acid as the starting chiral materials.

The complete synthetic scheme is depicted below. The four subsections of the molecule (**1**, **3**, **7**, and **9**) were coupled together via one amidation and two reductive aminations. The first half of the molecule, comprised of malic acid and lysine, was prepared in the following way. Malic acid **3** was treated with trifluoroacetic anhydride to generate the trifluoroacetyl ester, cyclic anhydride,[9] and the residue was dissolved in MeOH to produce the desired C-1 monomethyl ester.[9] This compound was treated sequentially with TBDMS-Cl / imidazole / DMF, followed by oxalyl chloride / DMF to provide the TBDMS-protected acid chloride. The acid chloride was treated *in situ* with N^2-BOC-Lys-OMe **2** to provide the lysyl-malate couple **5**, which was deprotected (CF_3CO_2H / CH_2Cl_2; HCl / MeOH) to afford intermediate **6**.

The second half of the molecule was prepared from acrolein diethyl acetal **7** and alanine **9**. Ozonolysis of **7**, followed by vacuum distillation, yielded diethoxyacetaldehyde **8**.[11] Reductive amination[12] of **8** [Ala-OMe·HCl **10** / $NaBH_3CN$] provided the imino ester in good yield, which was protected with a CBZ group **11**. Deacetalation of **11** to the corresponding aldehyde **12** was achieved by stirring with Amberlyst-15 in acetone. Finally, **12** and **6** were coupled via reductive amination ($NaBH_3CN$) to afford protected rhizobactin **13**.



__Reagents__: **a**, CH_2N_2 / $(CH_3CH_2)_2O$; **b**, H_2 / Pd-C / MeOH (A,B 91% overall); **c**, $(CF_3CO)_2O$; **d**, MeOH (C,D 26% overall);[9] **e**, TBDMS-Cl / imidazole / DMF (31%); **f**, ClCOCOCl / DMF / CH_2Cl_2;[10] **g**, pyridine(pyr) / CH_2Cl_2 (F,G 26 to 90% overall, variable); **h**, 20% CF_3CO_2H / CH_2Cl_2; **i**, 4 eq HCl / MeOH; **j**; O_3 / EtOH (48%);[11] **k**, $SOCl_2$ / MeOH (95%); **l**, $NaBH_3CN$ / MeOH (85%); **m**, CBZ-Cl / aq. Na_2CO_3 (84%); **n**, Amberlyst-15 / $(CH_3)_2CO$ (quant.); **o**, $NaBH_3CN$ / MeOH (H,I,O 67% overall); **p**, KOD / D_2O; **q**, H_2 / Pd-C / 50mM pyr-CH_3CO_2H (HOAc), pH 5.8 : MeOH (1:1); **r**, DEAE-Sephadex A-25 (OAc^-) / 50mM pyr-HOAc, pH 5.8, to H_2O, to 50mM HOAc / 5mM pyr.

Deprotection of 13 was accomplished in two steps: (1) hydrolysis of the methyl esters using KOD in D_2O (monitoring by ^{1}H-NMR spectroscopy); (2) hydrogenolysis of the CBZ group using H_2 / Pd-C. Synthetic rhizobactin (14) was obtained as its electrically-neutral, inner salt complex[8] by passage over DEAE-Sephadex. However, the ease by which fully protonated rhizobactin,[8] or its trimethyl ester rearranges into six-membered lactams, has complicated the complete removal of pyridinium salts. Remedies to this difficulty are under study. The ^{1}H-NMR spectra for synthetic and natural rhizobactin are virtually superimposable; the minor δ discrepancies of ca. 0.1 ppm for the three α-carboxy protons are either due to slightly different sample pH's, pyridinium salts in synthetic rhizobactin, or concentration differences. More importantly, mixing equivalent amounts of natural and synthetic rhizobactin yielded a single set of proton resonances.

The relative stereochemistry of the two unknown chiral centers thus must *either be* D^{ala},L^{lys} *or* L^{ala},D^{lys}, thereby creating a "pseudo" C_2 axis of symmetry and conferring chemical equivalence on the four methylene protons of the ethylenediamine constituent. In order to facilitate comparison of absolute configurations of the synthetic and natural specimen, it was necessary to introduce a chromophore close to the chiral center. *N*-Benzoates of secondary amines are not suited since they lead to rotational isomers, i.e., in the present case possibly to a total of 4 isomers. Rhizobactin was therefore converted into its readily accessible (but relatively unstable) bis-N^2,N^6-2,4-dinitrophenyl derivative: λmax (1:1 MeCN / H_2O + 0.1% TFA) 368 nm (ε $2.09 \cdot 10^4$).[13] The CD curves of the synthetic and natural derivatives were virtually superimposable; CD (1:1 MeCN / H_2O + 0.1% TFA): 292(Δε-27), 325(Δε+45), 380(Δε-12); the fact that the CD extrema do not coincide with the UV maximum is due to coupling between the two proximal chromophores. The absolute configuration of natural rhizobactin is thus established as being $D^{ala},L^{lys},L^{malate}$. This result further substantiates the conclusion[5] that rhizobactin is biochemically related to the opines, for it conforms to the $D^{ala},L^{amino\ acid}$ rule for pyruvic acid derived opines. By analogy, the evident chelating activity of many opines suggests that they possess a hitherto unrecognized biological function, namely iron (metal ion) assimilation. Finally, the design of the above synthetic scheme should facilitate scale-up procedures, as well as the preparation of rhizobactin analogs, both essential to clarify the biological mode of action of this important siderophore.

Experimental: NMR data was acquired on a Bruker WM-250 spectrometer operating at 250.13 MHz. NMR chemical shifts were calibrated against $CDCl_3$ (7.24ppm) or external 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS, 0ppm), as indicated. FAB mass spectra were recorded on a VG analytical 7070 EQ using Xe gas and m-nitrobenzyl alcohol (NBA) as matrix. UV measurements were performed on a Perkin Elmer 320 UV spectrophotometer. CD spectra were recorded on a JASCO 500A spectropolarimeter driven by a JASCO DP 500N data processor. Pertinent chemical data on selected compounds are provided below.

Compound 4. Oil, ^{1}H-NMR ($CDCl_3$): 4.6 (dd, J=6,7Hz, 1H, α-H), 3.7 (s, 3H, OMe), 2.7 (m, J=6,7Hz, 2H, β-CH_2), 1.9 (2s, 18H, Si-t-Bu), 0.2 (s, 6H, Si-CH_3).

Compound 5. Oil, ^{1}H-NMR ($CDCl_3$): 6.1 (m, 1H, NH), 5.1 (d, 1H, NH), 4.6 (dd, J=5,7Hz, 1H, α-H), 4.3 (m, 1H, α-H), 3.7 (2s, 6H, OMe's), 3.2 (m, 2H, ε-CH_2), 2.6 (m, J=5,7Hz, 2H, β-CH_2), 1.8 to 1.2 (fold, 15H, β-, γ-, and δ-CH_2's, t-Bu), 0.9 (s, 9H, Si-t-Bu), 0.1 (2s, 6H, Si-CH_3).

Compound 11. Oil, ^{1}H-NMR ($CDCl_3$): (two conformers) 7.3 (m, 5H, phenyl), 5.1 (m, 2H, CH_2), 4.6+4.5 (2t, J=5Hz, 1H, OCHO), 4.4+4.3 (2q, J=7Hz, 1H, α-H), 3.8 to 3.3 (fold, 9H, OCH_2's, NCH_2, OMe), 1.4 (t, 3H, CH_3), 1.2 to 1.0 (fold, 6H, CH_3's).

Compound 12. Oil, ^{1}H-NMR ($CDCl_3$): (two conformers) 9.6 (2s, 1H, CHO), 7.3 (m, 5H, phenyl), 5.1 (2s, 2H, CH_2), 5.0+4.8 (2q, J=7Hz, 1H, α-H), 3.9 (m, 2H, NCH_2), 3.7+3.6 (2s, 3H, OMe), 1.4 (d, 7Hz, CH_3).

Compound 13. Oil, ^1H-NMR ($CDCl_3$): (two conformers) 7.3 (m, 5H, phenyl), 6.1+6.0 (2s, 1H, NH), 5.1 (m, 2H, CH_2), 4.4 (dd, J=4,7Hz, α-H), 4.4+4.3 (2q, J=7Hz, α-H), 3.8 to 3.4 (fold, 10H, OMe's, α-H), 3.2 (m, 4H, NCH_2CH_2N), 2.8+2.6 (2m, 2H, ε-CH_2), 2.6 (m, J=4,7Hz, 2H, β-CH_2), 1.7 to 1.2 (fold, 9H, β–, γ–, and δ–CH_2's, CH_3, J=7Hz). FABMS (NBA): 554 (M+H), 576 (M+Na).

Synthetic rhizobactin, Compound 14. ^1H-NMR (D_2O, DSS): (pH ca. 2.5, 5 eq. of a pyr·HCl as contaminant) 4.57 (dd, J=5,7Hz, 1H, α-H), 3.9 (q, J=7Hz, 1H, α-H), 3.8 (t, 1H, α-H), 3.5 (s, 4H, NCH_2CH_2N), 3.2 (m, 2H, ε-CH_2), 2.7 (m, J=5,7Hz, 2H, β-CH_2), 1.9 (m, 2H, β-CH_2), 1.7 to 1.2 (fold, 7H, γ– and δ–CH_2's, CH_3 J=7Hz).

Natural rhizobactin. ^1H-NMR (D_2O, DSS): (pH ca. 3.0) 4.44 (dd, J=5,7Hz, 1H, α-H), 3.8 (q, J=7Hz, 1H, α-H), 3.7 (t, 1H, α-H), 3.5 (s, 4H, NCH_2CH_2N), 3.2 (m, 2H, ε-CH_2), 2.7 (m, J=5,7Hz, 2H, β-CH_2), 1.9 (m, 2H, β-CH_2), 1.7 to 1.2 (fold, 7H, γ– and δ–CH_2's, CH_3 J=7Hz).

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1. Smith, M.J; Neilands, J.B. *J. Plant Nutri.* **1984** *7*, 449.
2. Smith, M.J; Shoolery, J.N.; Schwyn, B.; Holden, I.; Neilands, J.B. *J. Am. Chem. Soc.* **1985** *107*, 1739.
3. Neilands, J.B. *Ann. Rev. Biochem.* **1981** *50*, 715.
4. Hider, R.C. *Struct. Bonding* **1984** *58*, 25.
5. Smith, M.J; Neilands, J.B. *UCLA Symposia on Molecular and Cellular Biology, New Series, V. 48, Molecular Strategies for Crop Protection* (C.J. Arntzen and C. Ryan, eds.), Alan R. Liss, Inc., p.157, 1984.
6. Storey, K.B.; Miller, D.C.; Plaxton, W.C., Storey, J.M. *Anal. Biochem.* **1982** *125*, 50.
7. Chilton, W.S.; Hood, E.; Rinehart, K.L.; Chilton, M.-D. *Phytochemistry* **1985** *198*, 2945.
8. Smith, M.J. Ph. D. Thesis, University of California, Berkeley 1984.
9. Miller, M.J.; Bajwa, J.S.; Mattingly, P.G.; Peterson, K. *J. Org. Chem.* **1982** *47*, 4928.
10. Wissner, A.; Grudzinskas, C.V. *J. Org. Chem.* **1978** *43*, 3972.
11. Bestmann, H.J.; Ermann, P. *Chem. Ber.* **1983** *116*, 3264.
12. Borch, R.F.; Bernstein, M.D.; Durst, H.D. *J. Am. Chem. Soc.* **1971** *93*, 2897.
13. Sanger, F. *Biochem. J.* **1945** *39*, 33; excess dinitrofluorobenzene in $H_2O:CH_3CN$ (1:1), pH maintained above 8 using K_2CO_3, 40°, 6 h (30% yield).

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