HN_3, indicating that the sequence of reactions 6–8 is not an important path for VN_3^{2+} formation under these conditions.[12]

Finding direct evidence for inner-sphere reaction in the case of V^{2+} and *cis*-$Co(en)_2(N_3)_2^+$ should not be regarded as proof that V(II) reductions in general, or even other V(II)–Co(III) reactions, proceed by inner-sphere mechanisms. Halpern and co-workers[13] have shown that $Co(CN)_5^{3-}$ can act by both mechanisms and that the balance in that instance can be tipped in favor of one or the other pathway by seemingly minor changes.

V(II) and Cr(III) are isoelectronic; substitution reactions of $V(OH_2)_6^{2+}$ are expected to be slow relative to other divalent metal ions, as Cr(III) substitutions are relative to trivalent ions. A barrier to the inner-sphere reaction 2 is the replacement of H_2O by X in the primary coordination sphere of V(II). In the case of the relatively slow cobalt(III) reactions this does not seem likely to provide a barrier to an inner-sphere process, but faster reactions could be forced outer sphere.

(12) At much higher $[HN_3]$, >0.01 *M*, there is a noticeable effect of added HN_3. This may correspond to eq 6–8, or it may be a consequence of substances generated in the reduction of V(II) by HN_3 which is important at the higher concentrations.

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Cyclosemigramicidin S[1]

Sir:

Schwyzer and Sieber synthesized *cyclo*-[L-Val-L-Orn-(δ-Tos)-L-Leu-D-Phe-L-Pro-]$_2$, the ditosyl derivative of gramicidin S, by dimerization of H-Val-Orn(δ-Tos)-Leu-D-Phe-Pro-ONp in the presence of large amount of pyridine; their preparation showed no production of *cyclo*-[Val-Orn(δ-Tos)-Leu-D-Phe-Pro-], the monotosyl derivative of cyclosemigramicidin S.[2,3] We wish to report the synthesis of cyclosemigramicidin S monohydrochloride tetrahydrate and its antibacterial properties.

$$\left[\begin{matrix}\text{L-Val-L-Orn-L-Leu-D-Phe-L-Pro}\\ \text{L-Pro-D-Phe-L-Leu-L-Orn-L-Val}\end{matrix}\right]$$

gramicidin S

$$\left[\text{L-Val-L-Orn-L-Leu-D-Phe-L-Pro}\right]$$

cyclosemigramicidin S

H-Leu-D-Phe-Pro-OEt·HCl (I), mp 225–228° dec, $[\alpha]^{18}D$ −30.9° (*c* 0.5, AcOH) (*Anal.* Calcd for $C_{22}H_{33}O_4N_3 \cdot HCl \cdot 0.25H_2O$: C, 59.44; H, 7.82; N, 9.46. Found: C, 59.47; H, 7.83; N; 9.57), was prepared in 93% yield by hydrogenation of Z-Leu-D-Phe-Pro-OEt.[4] Condensation of the azide derived from Z(OMe)-Val-Orn(δ-Z)-$NHNH_2$[5] with I gave Z(OMe)-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OEt (II), 81%, mp 149–150°, $[\alpha]^{24}D$ −26.8° (*c* 2, DMF). *Anal.* Calcd for $C_{49}H_{66}O_{11}N_6 \cdot H_2O$: C, 63.07; H, 7.35; N, 9.01. Found: C, 63.04; H, 7.18; N, 9.29. II was saponified with alkali to give Z(OMe)-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH (III), 84%, mp 143–145°, $[\alpha]^{20}D$ −18.2° (*c* 2, DMF). *Anal.* Calcd for $C_{47}H_{62}O_{11}N_6 \cdot 1.5H_2O$: C, 61.75; H, 7.17; N, 9.20. Found: C, 61.85; H, 6.87; N, 9.42. Treatment of III with 5 equiv of di-*p*-nitrophenyl sulfite[6] gave amorphous acyl pentapeptide *p*-nitrophenyl ester (IV); the active ester content was estimated spectrophotometrically[7] to be 106%. The *p*-methoxybenzyloxycarbonyl group of IV was removed by the action of trifluoroacetic acid and the pentapeptide *p*-nitrophenyl ester trifluoroacetate (V) so obtained was treated with a large amount of pyridine at 60° for the cyclization reaction. The concentration of V in pyridine was 3×10^{-4} *M*. The residue obtained after cyclization was dissolved in aqueous methanol, and the solution was passed through columns of Dowex 50 (H^+ form) and Dowex 1 (OH^- form). It was observed that the effluent contains two compounds with the character of benzyloxycarbonyl-substituted cyclic peptide. The product less soluble in aqueous methanol was isolated by fractional crystallization (the mother liquor was set aside for the isolation of the more soluble product), and it was assigned as *cyclo*-[Val-Orn(δ-Z)-Leu-D-Phe-Pro-]$_2$ (VI), 12% from III, mp 250–252° dec, $[\alpha]^{20}D$ −275° (*c* 0.3, AcOH). *Anal.* Calcd for $C_{76}H_{104}O_{14}N_{12} \cdot 2H_2O$: C, 63.13; H, 7.53; N, 11.62. Found: C, 62.98; H, 7.72; N, 11.46.[8] The molecular weight of VI was determined on a Hitachi Type 115 osmometer (solvent, methanol): calcd, 1446; found, 1420. Catalytic hydrogenation of VI in the presence of 2 equiv of hydrogen chloride in methanol yielded gramicidin S dihydrochloride octahydrate, 91%, mp 274–276° dec, $[\alpha]^{20}D$ −269° (*c* 0.1, EtOH) (*Anal.* Calcd for $C_{60}H_{92}O_{10}N_{12} \cdot 2HCl \cdot 8H_2O$: C, 53.04; H, 8.16; N, 12.37. Found: C, 52.80; H, 8.03; N, 12.05), R_f 0.95[9] and 0.76.[10] The more soluble product was isolated as follows. The mother liquor was evaporated, and the residue was fractionated into two compounds with a column of Sephadex LH-20 using methanol as a developing solvent. Two peaks were observed on the chromatogram. The faster eluting fraction yielded VI, 8% from III, mp 250–252° dec. Total yield of VI was 20% from III. The slower elution fraction yielded the product more soluble in the pure state, assigned as *cyclo*-[Val-Orn(δ-Z)-Leu-D-Phe-Pro-] (VII),[11] 16% from III, dec pt above 240°, $[\alpha]^{20}D$ −49.7° (*c* 0.1, AcOH). *Anal.* Calcd for $C_{38}H_{52}O_7N_6 \cdot H_2O$: C, 63.13; H, 7.53; N, 11.62; mol wt, 723. Found: C, 62.83; H, 7.49; N, 11.23; mol wt, 710. Hydrogenation of VII yielded cyclosemigramicidin S monohydrochloride tetrahydrate, 88%, mp 223–225° dec, $[\alpha]^{20}D$ −76.1° (*c* 0.06,

(1) The nomenclature of cyclosemigramicidin S has been introduced by E. Schröder and K. Lübke in their monograph (*Peptides*, **2**, 429 (1966)) for *cyclo*-(L-Val-L-Orn-L-Leu-D-Phe-L-Pro-).

(2) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 2186 (1958).

(3) The abbreviations followed are from *Biochemistry*, **5**, 2485 (1966); Z-, benzyloxycarbonyl; Z(OMe)-, *p*-methoxybenzyloxycarbonyl; Mz-, *p*-methoxyphenylazobenzyloxycarbonyl; -ONp, *p*-nitrophenoxy; Dbu, L-α,γ-diaminobutyric acid residue.

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(5) T. Kato, M. Kondo, M. Ohno, and N. Izumiya, *Bull. Chem. Soc. Japan*, **38**, 1202 (1965).

(6) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **43**, 1760 (1960).

(7) R. Schwyzer and P. Sieber, *ibid.*, **41**, 1582 (1958).

(8) The same product was obtained by benzyloxycarbonylation of the native gramicidin S, 80%, mp 250–251° dec, $[\alpha]^{20}D$ −273° (*c* 0.3, AcOH).

(9) The R_f on paper chromatography refers to the system 1-butanol–acetic acid–pyridine–water (4:1:1:2, v/v).

(10) The R_f on thin layer chromatography with Merck silica gel refers to the same solvent system described.[9]

(11) It would be of interest to note that the solubility of *cyclo*-[Val-Orn(δ-Z)-Leu-D-Phe-Sar- or -Gly-] in any of the solvents tested is smaller than that of *cyclo*-[Val-Orn(δ-Z)-Leu-D-Phe-Sar- or -Gly-]$_2$.

Table I. Ratio of Protected Cyclic Pentapeptide and Decapeptide after Cyclization of Various Linear Pentapeptide Active Esters

p-Nitrophenyl ester of [l]	Ratio of compd in product[a] Cyclic monomer[b]	Cyclic dimer[c]
H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH	32	68
-Gly-OH[d,e]	79	21
-Sar-OH[f]	85	15
-Gly-Pro-OH[g]	25	75
-D-Ala-[h]	0	100
-D-Leu-[h]	+	++
-Lys(ε-Z)- -D-Phe-[h]	29	71
-Dbu(γ-Z)-[h]	30	70
H-Gly-Orn(δ-Z)-[i]	100	0
H-Ala-[i]	91	9
H-Leu-[h]	78	22
H-Gly-Lys(ε-Z)-[h,i]	100	0
H-Gly-Orn(δ-Z)- -Gly-OH[k]	100	0
H- Orn(δ-Z)-Leu-D-Phe-Gly-Gly-OH[k]	100	0

[a] After cyclization of *p*-nitrophenyl ester with pyridine. The concentrations of linear pentapeptide *p*-nitrophenyl esters in pyridine were ~3 × 10^{-3} *M*. [b] Mono-Z-substituted; the figures were derived by calculation on a molar basis. [c] Di-Z-substituted; the figures were derived by calculation in which 0.5 mole is used as a unit. [d] Isolation of cyclic decapeptide: see H. Aoyagi, *et al.*, *J. Am. Chem. Soc.*, **86**, 5700 (1964); *Bull. Chem. Soc. Japan*, **38**, 2138 (1965). [e] Isolation of cyclic pentapeptide: see H. Aoyagi, M. Kondo, T. Kato, S. Makisumi, and N. Izumiya, *ibid.*, in press. [f] H. Aoyagi and N. Izumiya, *ibid.*, **39**, 1747 (1966). [g] R. Nagata, M. Waki, M. Kondo, H. Aoyagi, T. Kato, S. Makisumi, and N. Izumiya, *ibid.*, in press. [h] N. Izumiya, *et al.*, to be published. [i] M. Kondo and N. Izumiya, *Bull. Chem. Soc. Japan*, in press. [j] R. Schwyzer reported that the cyclization reaction of H-Gly-Lys(ε-Mz)-Leu-D-Phe-Pro-ONp with pyridine yields exclusively the dimer, *cyclo*-[Gly-Lys(ε-Mz)-Leu-D-Phe-Pro]$_2$; see *Chem. Abstr.*, **57**, 949 (1962). [k] M. Kondo, H. Aoyagi, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Japan*, **39**, 2234 (1966). [l] After the first compound listed, only variations of residue will be shown.

EtOH) (*Anal.* Calcd for $C_{30}H_{46}O_5N_6 \cdot HCl \cdot 4H_2O$: C, 53.04; H, 8.16; N, 12.37. Found: C, 52.73; H, 7.90; N, 11.97), R_f 0.93[9] and 0.85;[10] amino acid ratios in the acid hydrolysate, $Val_{1.0}Orn_{0.9}Leu_{1.0}Phe_{1.0}Pro_{1.0}$. Gramicidin S and cyclosemigramicidin S were distinguishable by paper electrophoresis (solvent, formic acid–acetic acid–methanol–water, 1:3:6:10, pH 1.8) and on a carboxymethylcellulose column (solvent, 0.2 *M* pyridinium acetate with 30% methanol).

The proportion of monomer VII in the crude product after cyclization is increased with decrease in the concentration of the pentapeptide active ester V in pyridine. Weight ratios of monomer VII and dimer VI in the crude product were found to be 29:71 at 30 × 10^{-3} *M*, 32:68 at 3 × 10^{-3} *M*, and 45:55 at 0.3 × 10^{-3} *M* V in pyridine. Table I shows the results obtained in this laboratory which are ratios of the protected cyclic monomer and the protected cyclic dimer in the crude products after the cyclization reaction of various linear pentapeptide active esters.

The antibacterial activity of cyclosemigramicidin S toward several microorganisms, *E. coli*, *P. vulgaris*, *S. aureus*, *B. subtilis*, and *M. avium*, was examined. The cyclic pentapeptide had no retarding effect on the growth of any of the microorganisms, even at as high a concentration as 100 μg/ml of the assay medium. Synthetic or natural gramicidin S, however, showed substantial activity under the same conditions toward *S. aureus* and *B. subtilis*.

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Absolute Signs of Indirect Nuclear Spin–Spin Coupling Constants[1]

Sir:

There has been a large amount of recent interest in the absolute signs of indirect nuclear spin–spin coupling constants. One reason this interest developed was because of the apparent discrepancy between the experimental determination of the opposite relative signs of $J_{vic\text{-}HH}$ and $J_{gem\text{-}HH}$ in various ethane derivatives[2] and the theoretical prediction that both are positive.[3] It was subsequently proposed[4] that "absolute" signs of J may be found if it is assumed that $J_{^{13}CH}$ is positive for a proton directly bonded to carbon. In this way $J_{gem\text{-}HH}$ is shown to be negative.[5] Despite the fact that the positive sign of $J_{^{13}CH}$ rests on fairly firm theoretical ground,[6] it remains desirable to determine its *absolute* sign experimentally.[7] This communication describes such a determination.

If the molecules being studied could be partially oriented, a nonzero direct nuclear magnetic dipole–dipole interaction, D_{ij}, between nuclei i and j, would appear in the nmr spectrum and would either add to or subtract from the splitting due to J_{ij} depending upon the details of the orientation and the sign of J_{ij}. Such an experiment has been proposed[8] and attempted[9] for molecules with an electric dipole moment in an electrostatic field, but recent work[10] has shown that at the present time such orientational effects cannot be detected. An alternative method of producing oriented molecules involves the use of liquid-crystal solvents. It has been shown that nematic liquid crystals become oriented in strong magnetic fields, and, moreover, that molecules dissolved in the nematic liquid crystals are

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