MHz, $CDCl_3$) δ 9.60 (bs, 1 H, OH), 2.06–2.17 (m, 1 H), 1.92–2.05 (m, 1 H), 1.77–1.89 (m, 2 H, 2PCH), 1.20–1.41 (m, 2 H), 1.19 (dd, 6 H, $2PCH(CH_3)$, J_{HH} = 7.0, J_{PH} = 15.6); ^{31}P NMR (121.4 MHz, $CDCl_3$) δ 77.7; ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.7 (d, 2PC, J_{PC} = 91.6), 30.5 (d, $2CH_2$, J_{PC} = 13.5), 12.9 (d, $2PCH(CH_3)$, J_{PC} = 3.1); MS (CI, NH_3/CH_4) *m/e* 149 (MH^+, base peak). Anal. Calcd for $C_6H_{13}O_2P$: C, 48.65; H, 8.84. Found: C, 48.42; H, 8.68.

(2S*,5S*)-2,5-Dibenzylphospholanic acid (12b):[1] 70%.

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Pseudorotational, Conformational, and NOE Studies of Pentacovalent Spirophospholenes Derived from Ephedrine and α-Diketones

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Variable-temperature NMR studies showed the chiral pentacovalent dioxaphospholenes **1–3** to be pseudorotationally stable below 60 °C. $\Delta G^{\ddagger}_{PSDRTN}$ for **1** and **2** were determined to be 22.0 ± 2.1 kcal/mol and 33.0 ± 2.6 kcal/mol, respectively. The $\Delta G^{\ddagger}_{PSDRTN}$ for **2** is the largest value reported to date. ^1H NMR nuclear Overhauser effect studies on the major isomer of **1** confirmed that it was **1a** and not **1b**. Conformational analysis of the ^1H NMR data indicated a twist-envelope conformation for the ephedrine-derived five-membered ring.

Introduction

Phosphorus-containing compounds (generally phosphates and phosphonates) are of biological interest as enzyme modulators, inhibitors, and active-site probes.[3] Many of these compounds also have medicinal value as antivirals,[4] antibiotics,[5] and antiacidosis agents,[6] and for the treatment of calcification diseases.[7] Proposed modes of action of these compounds generally include attack on the phosphorus by a nucleophile (e.g., an enzyme or water) to form a trigonal bipyramidal pentacovalent phosphorus transition state or intermediate, which can then either trigger the enzyme into action or short-circuit it by failure of the appropriate ligand on phosphorus to cleave (generally a P–C bond).[3]

In order to adequately model these transition states and predict reactivities of the organophosphorus compounds, pseudorotational and conformational studies of trigonal bipyramidal pentacovalent organophosphorus compounds have seen renewed interest.[8] Berry pseudorotation is the accepted mechanism whereby the apical ligands on pentacovalent organophosphorus compounds in a trigonal bipyramidal geometry are exchanged for the equatorial ones.[9] Recent emphasis has been on cAMP models,[8f–h] where it has been proposed that activated cAMP involves a pentacovalent phosphorus species in a trigonal bipyramidal geometry. The studies of these systems have extensively involved X-ray crystallographic, and more recently, solution NMR spectroscopic investigations of these models. The conformations of the six-membered rings in these pentacovalent phosphorus-containing models were also determined.

In connection with our interest in the utilization of pentacovalent (P(V)) organophospholenes as synthetic reagents,[10] we are investigating the production of chiral, and therefore configurationally stable, P(V) phospholenes. In order to produce configurationally defined P(V) compounds, pseudorotation must be prohibited at reaction

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temperatures. The formation of a *spiro* pentacovalent organophosphorus compound is well-known slow and, in some cases, completely inhibit pseudorotation.[11] The use of several ligands of low apicophilicity (electronegativity) also restricts pseudorotation.[12]

Our choice of ligands on the pentacovalent organophosphorus compounds in this current study was based on the results of Burgada and Bernard.[13] While ephedrine-derived 1,3,2-oxazaphospholanes formed suitable spirophospholenes with α-diketones, they found it was the fifth, exocyclic ligand that determined the pseudorotational behavior of their compounds. When this fifth ligand was methoxy, pseudorotation of the spirophospholene was rapid at room temperature. With an exocyclic dimethylamino ligand, pseudorotation did not occur below 60 °C. The use of the dimethylamino ligand, however, produced a fairly unstable pentacovalent spirophospholene, as this ligand is easily exchanged.[14] The relative configuration was not determined for any of these P(V) compounds.

In order to avoid the instability problem of the dimethylamino ligand and still inhibit pseudorotation, we employed an aryl group as the fifth ligand on phosphorus. The aryl group was expected to prefer an equatorial position due to its relatively low electronegativity and high steric bulk.[15] Since we ultimately play to use these compounds in electrophilic condensation reactions, we needed to know which diastereomer was the major one in solution, not in the crystalline state.[16] We reasoned that the solution structure of the major isomer could be determined via nuclear Overhauser effect (NOE) ^{1}H NMR studies. The ortho protons on the aryl ligand would be different enough in chemical shift from the other aromatic protons to allow for accurate integration. Proton NMR data should also supply us with information regarding the conformation(s) of the ephedrine-derived saturated five-membered rings in our spirophospholenes. Little data is available regarding the conformational preferences of these saturated five-membered rings in a *pentacovalent* system.[17]

We now report our successful results on the production of chiral P(V) phospholenes, one of which exhibits the highest $\Delta G^{\ddagger}_{PSDRTN}$ to date, and, to our knowledge, the first determination of the relative configuration of a chiral P(V) compound via ^{1}H NMR nuclear Overhauser effect studies. Conformational analysis of the ephedrine-derived five-membered ring via ^{1}H NMR indicates a preference for a twist-envelope conformation.

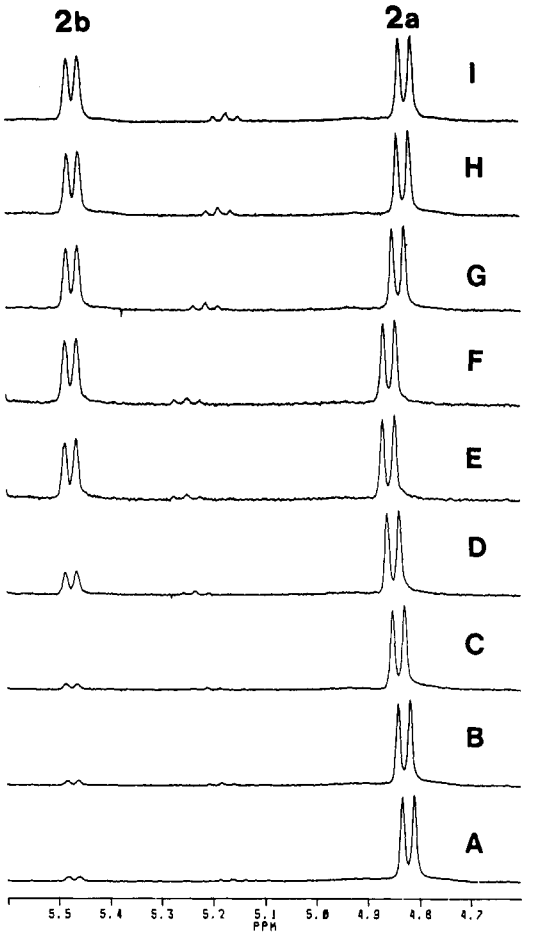

Figure 1. ^{1}H NMR spectra of the changes seen in the resonance of the methine proton at C10 in **2a** and **2b** upon heating the sample to the temperatures indicated and cooling to 21 °C. **A** = 21 °C, **B** = 35 °C, **C** = 60 °C, **D** = 79 °C, **E** = 100 °C, **F** = 100 °C (after 1 h), **G** = 60 °C, **H** = 35 °C, I = 21 °C.

Table I. High-Temperature ^{1}H NMR Ratios[a] of the Diastereomers of 1, 2 and 3

compd 1		compd 2		compd 3	
temp (°C)	ratio	temp (°C)	ratio	temp (°C)	ratio
17	88/1	21	14.5/1	20	110/1
30	88/1	35	14.5/1	40	110/1
47	88/1	60	14.5/1	60	22/1
60	8/1	79	3/1	80	4.6/1
79	1.5/1	100	1.2/1	100	1.6/1
100	1.3/1	60	1.2/1	80	1.6/1
79	1.3/1	35	1.2/1	60	1.6/1
60	1.3/1	21	1.2/1	40	1.6/1
35	1.3/1			20	1.6/1
17	1.3/1				

[a] Determined by integration of the methine proton at C10.

Results and Discussion

Syntheses of Compounds 1–3. The spirophospholenes, **1–3**, were readily prepared from the chiral oxazaphospholidines **5**[18] and α-diketones. The methyl vinyl ketone adduct, **4**, was also prepared, but could not be purified sufficiently for the variable-temperature studies. While the compounds **1–3** were recrystallized to purity before the variable-temperature studies, we note that the purified products exhibited the same diastereomeric mixture as the crude reaction mixtures (NMR).[19]

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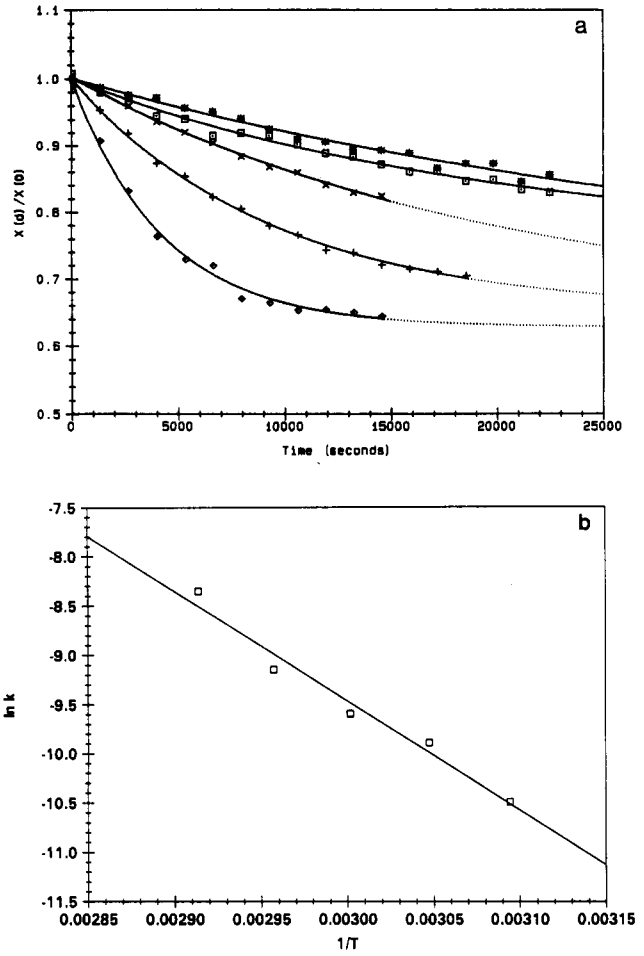


Figure 2. (a) Plot of the mole fraction (normalized) of the major/minor diastereomer of **1** vs time at five temperatures to yield the rate constant, k; 50 °C (*), 55 °C (□), 60 °C (×), 65 °C (+), and 70 °C (♦). (b) Plot of ln k (rate constant) vs $1/T$ (K).

Pseudorotational NMR Studies. We have studied the spirophosphoranes **1**, **2**, and **3** via variable-temperature proton and phosphorus NMR. Upon cooling the samples to −75 °C, little change was indicated by ^{1}H and ^{31}P NMR spectroscopy. Slowly heating the samples to 100 °C produced another set of ^{1}H and ^{31}P NMR signals, indicating conversion to the other diastereomer. See Figure 1. Cooling to room temperature again did not appreciably change this new ratio. These results suggest that pseudorotation was negligibly slow on the NMR time scale at 20 °C and epimerization did not occur until temperatures greater than 60–80 °C were reached. See Table I. Since variable-temperature ^{31}P NMR showed only a small change in the chemical shift of the phosphorus, we presumed that this epimerization occurred via pseudorotation and not bond rupture.[20] Assignment of the diastereomer configuration is discussed below.

Kinetic Studies. Kinetic studies have allowed us to calculate the free energy $\Delta G^{\ddagger}_{\text{PSDRTN}}$ of 22.0 ± 2.1 kcal/mol for the biacetyl adduct **1** (five points from 50 to 70 °C) and 33.0 ± 2.6 kcal/mol for the benzil adduct **2** (five points from 70 to 90 °C). See Figures 2 and 3 and the Experimental Section for details.

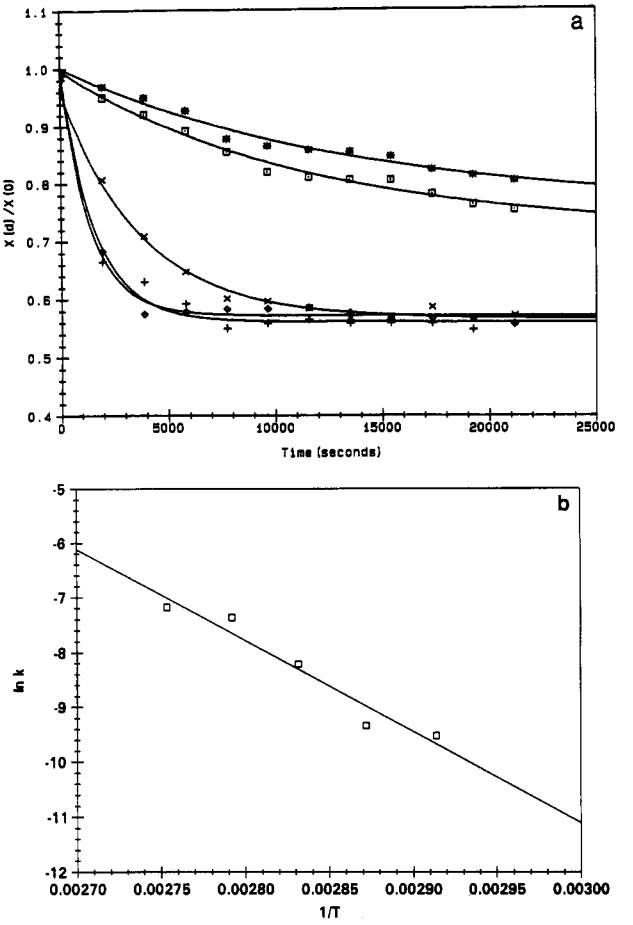
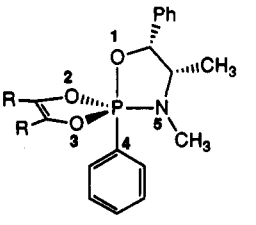


Figure 3. (a) Plot of the mole fraction (normalized) of the major/minor diastereomer of **2** vs time at five temperatures to yield the rate constant, k: 70 °C (*), 75 °C (□), 80 °C (×), 85 °C (+), 90 °C (♦). (b) Plot of ln k (rate constant) vs $1/T$ (K).



"41"

Figure 4. Highest energy intermediate in the pseudorotation pathway of 13 (=12) ⇄ 25 (=35) ⇄ 41 (=14) ⇄ 53 (=52) ⇄ 21 (=31) for **1** (R = Me) and **2** (R = Ph).

The $\Delta G^{\ddagger}_{\text{PSDRTN}}$ calculated for **1** is less than the 28.3 kcal/mol reported for the corresponding compound possessing an exocyclic amino ligand.[13] Several factors affect the barrier to pseudorotation. Muetterties and others have shown that pπ–dπ back-donation by sulfur or nitrogen ligands reduces the barrier to pseudorotation and restricts rotation about the S–P or N–P bond.[21] There is no π donor interaction between the exocyclic phenyl ligand and the phosphorus atom, and **1**, therefore, has a lower pseudorotational energy barrier.

The very large $\Delta G^{\ddagger}_{\text{PSDRTN}}$ seen for **2** is most probably due to the large steric interactions between the phenyls in the various pseudorotomers and, to our knowledge, is the largest value reported to date.[8a,m] The highest energy

(19) Bernard and Burgada (ref 13) also observed that the crude reaction mixture for their P(V) spiro compound containing a dimethylamino fifth ligand exhibited a diastereomer mixture similar to that of the purified product.

(20) The P(V) ⇄ zwitterionic phosphonium salt equilibrium can be detected via ^{31}P NMR spectroscopy in compounds with several ligands of low electronegativity. See ref 11a, Chapter 2 and Ramirez, F. *Acc. Chem. Res.* **1968**, *1*, 168.

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Chart I

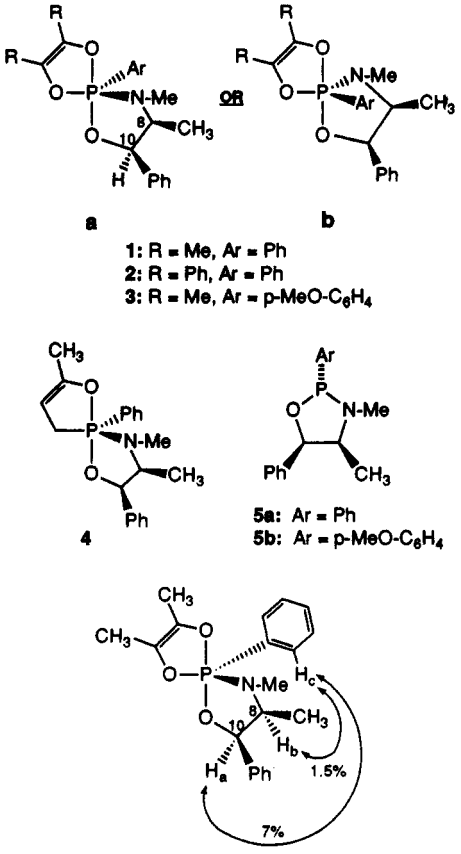


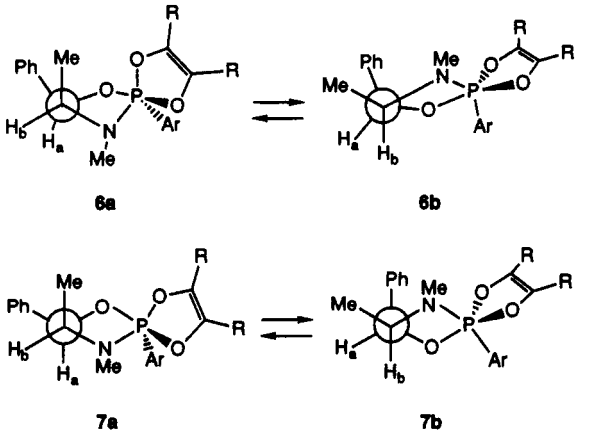
Figure 5. Nuclear Overhauser effects seen in 1a.

intermediate (41) in the pseudorotation pathway for both 1 and 2 would put the phenyl ligand in the more sterically sensitive apical position (Figure 4).[22] While 1 (R = Me) would experience methyl–phenyl interactions, 2 (R = Ph) would suffer from the relatively worse phenyl–phenyl interactions.

Isomer Determination via Nuclear Overhauser Effect Studies. ^{1}H NMR NOE investigations were performed on the major isomer (before heating) of the biacetyl derivative 1 in order to determine whether the exocyclic phenyl is trans (1a) or cis (1b) to the methyl and phenyl groups on the ephedrine bidentate ligand. If the reactions between 5 and the α-diketones proceeded with retention of configuration as has been seen with 1,3,2-dioxaphosphorinanes,[8f] then we would expect 1a to be the kinetically produced diastereomer.

The very large coupling constant ($^1J_{P-C}$) seen between the phosphorus and the ipso carbon of the exocyclic aryl group confirms the equatorial placement of this aryl group.[23] For diastereomer 1a (Chart I), molecular modeling[24] suggests that the ortho protons on the exocyclic phenyl are in close spacial proximity to the methine protons at C8 and C10 of the ephedrine derived ligand, while in 1b these protons are very far apart. Irradiation of the methine proton H_a on C10 produced an NOE enhancement of 7.0% at the aromatic ortho protons H_c, while irradiation of the methine proton H_b on C8 produced a smaller NOE

Scheme I



enhancement of 1.5% at H_c. Irradiation of the methyl group on C8 produced no observable changes in any of the ^{1}H NMR resonances. The only diastereomer that could produce these results is the sterically less hindered trans isomer 1a.

Conformational Analysis of the Ephedrine-Derived Ring. Confirmation of the NOE results came from the analysis of the conformation of the ephedrine-derived five-membered ring via the ^{1}H NMR data of compounds 1–3. Five-membered rings can adopt half-chair, envelope, the twist-envelope conformations.[25] Assuming that a classical Karplus-like correlation exists in these cases, the three-bond coupling constants ($^3J_{P-H}$) observed between the phosphorus atom and H_a or H_b in compounds 1–3 are evidence that the saturated five-membered ring exists predominantly in the twist-envelope conformation 6a.[17]

The 0 Hz coupling constant ($^3J_{P-H}$) between H_a and P corresponds to a dihedral angle P–O–C–H_a near 90°, while the large $^3J_{P-H}$ coupling constants of 24–26 Hz are evidence for an antiperiplanar relationship between H_b and P (P–N–C–H_b dihedral angle near 180°). These relationships are seen in the twist-envelope conformation 6a (Scheme I). The observed vicinal coupling constants ($^3J_{H-H}$) between H_a and H_b of 5.1–5.8 Hz are also predicted by this conformation, with dihedral angles H_a–C–C–H_b of 35–40°. The twist-envelope conformation 6b would have a larger $^3J_{P-H}$ value between H_a and P than between H_b and P, opposite of what was observed. The half-chairs (7a,b) would exhibit non-zero values for $^3J_{P-H}$ between H_a and P and much smaller $^3J_{P-H}$ values between H_b and P. Similar Karplus-like correlations have been seen in the six-membered rings of spirophospholenes derived from 1,3,2-dioxaphosphorinanes[8f,l] and also in five-membered 1,3,2-oxazaphospholanes.[17]

The twist-envelope conformation 6a also puts H_a closer in space to the ortho aromatic protons H_c than it does H_b. Thus, irradiation of H_a would produce a larger nuclear Overhauser effect on H_c than H_b would, as was observed (vide infra). In 6b, H_b is closer in space than H_a to the ortho aromatic protons H_c and would have produced a larger NOE on H_c than would H_a.

Conclusions and Future Directions

We have been successful in preparing spirophospholenes that are configurationally stable below 60 °C and have determined the structure of the major diastereomer of 1 to be 1a via ^{1}H NMR NOE studies. The spirophospholene 2 exhibited the largest $\Delta G^{\ddagger}_{PSDRTN}$ reported to date. The

(22) The very unfavorable, very high energy intermediate (45=54) that puts both atoms of lowest electronegativity (carbon and nitrogen) in the apical positions is avoided here. See Chapter 2 in ref 11a.

(23) Cavell, R. G. In *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis*; Verkade, J. G.; Quin, L. D., Eds.; VCH Publishers: Deerfield Beach, FL, 1987; Chapter 7.

(24) Molecular modeling was performed on an Evans and Sutherland PS300 using Mogli/P.S. Show software package.

(25) Miles, J. A.; Benny, M. T.; Ratts, K. W. *J. Org. Chem.* **1975**, *40*, 343.

conformation of the saturated five-membered ring derived from ephedrine is a twist-envelope as deduced from the ^1H NMR data. We are currently investigating the reactivity of these chiral spirophospholenes in condensation reactions with various electrophiles and will be reporting the results in due course.

Experimental Section

General. All glassware was flame-dried and purged under a stream of N_2 or Ar directly before use. All reagents were purified directly before use and stored under an atmosphere of argon, except for (−)-ephedrine which was used without further purification. Dichloro(4-methoxyphenyl)phosphine was prepared according to the literature procedure.[25] Et_3N was distilled under Ar and stored over KOH pellets. Et_2O was freshly distilled from sodium benzophenone ketyl. Pentane was distilled from CaH_2, stored over 4-Å molecular sieves, and used within 2 weeks after distillation. $CDCl_3$ was distilled from P_2O_5 just before use as the solvent for NMR samples. Melting points were obtained on a Laboratory Devices Mel-Temp and are uncorrected. IR data were obtained on a Nicolet 5DXB FTIR using dried $CDCl_3$ or $CHCl_3$ as the solvent. Specific rotations were obtained on a Rudolf Research Automatic polarimeter. Mass spectral information was obtained on the VG 70-70F mass spectrometer.

Proton, carbon, and phosphorus NMR spectra were obtained on either a Bruker AM-250 or WM-250 spectrometer. The ^1H spectra are measured in ppm downfield from TMS, while ^{13}C spectra are referenced from $CDCl_3$. ^{31}P spectra are reported in ppm from an external reference of 85% H_3PO_4. For the variable-temperature studies, the samples were brought to the indicated temperature as quickly as possible and kept at that temperature for 30 min, except for at 100 °C where they were kept for 1 h. Nuclear Overhauser effect data were obtained on a Varian VXR 400S (400 MHz) NMR spectrometer. Samples were prepared by dissolution into freshly distilled $CDCl_3$ and degassed by three successive freeze–thaw cycles under Ar. Data were collected using the parameters provided in the Varian VNMR version 3.1 software.

Kinetic data were reduced in the following manner. Initially, the ratios of diastereomers were measured directly from the integral values on the ^1H NMR spectra and transformed into mole fractions. Mole fractions and time were subjected to Noggle's F-Curve II[26] computer program to fit information to a first-order kinetics model. Normalization of this data yielded the first-order rate constant. The values of the natural log of the rate constants were plotted vs $1/T$ (K) (Figures 2b and 3b), and the slopes of these linear lines were fitted into the first-order rate expression to yield $\Delta G^{\ddagger}_{PSDRTN}$.

(7*R*,8*S*)-2,3,8,9-Tetramethyl-5,7-diphenyl-1,4,6-trioxa-9-aza-5-phospha(P^V)spiro[4.4]non-2-ene (1a). A solution of 2,3-butadnedione (0.141 g, 1.64 mmol) in dry pentane (20 mL) was added dropwise to a solution of the 1,3,2-oxazaphospholane **5a**[18] (0.445 g, 1.64 mmol) in dry pentane (25 mL). The mixture was allowed to stir for 24 h and filtered through a sintered glass frit containing dried Celite (1.5 g) in a closed system under N_2 to remove the precipitated impurities (oxidized **5a**). The pentane was then removed under a slow argon purge. The resulting solid was recrystallized twice from pentane under argon and dried under vacuum to produce **1a** as a white crystalline solid (0.500 g, 85%): mp 115–117 °C dec; ^1H NMR ($CDCl_3$) 7.80 (2 H, m), 7.4–7.1 (8 H, m), 4.74 (1 H, d, J_{H-H} = 5.8 Hz), 3.28 (1 H, d quint, J_{P-H} = 23.8 Hz, J_{H-H} = 6.1 Hz), 3.07 (3 H, d, J = 9.1 Hz), 1.88 (3 H, d, J = 0.8 Hz), 1.70 (3 H, d, J = 1.2 Hz), 0.81 (3 H, d, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) 139.4 (d, J_{P-C} = 215.2 Hz), 139.2 (d, J_{P-C} = 9.6 Hz), 131.2, 130.8 (d, J_{P-C} = 59.5 Hz), 129.1, 128.0, 127.9, 127.6, 126.3, 126.0, 72.3 (d, J_{P-C} = 4.5 Hz), 59.4 (d, J_{P-C} = 14.6 Hz), 35.1 (d, J_{P-C} = 3.5 Hz), 14.9, 11.3 (d, J_{P-C} = 7.4 Hz), 11.0 (d, J_{P-C} = 12.3 Hz); ^{31}P NMR ($CDCl_3$) −35.81 ppm; IR ($CHCl_3$, cm^{-1}) 1452 (C=C), 1082 (PPh), 993 (PO); $[\alpha]_D$ +8.9° (c = 0.34, $CHCl_3$); exact mass calcd for $C_{20}H_{24}NO_3P$ $(M)^+$ 357.1488, found 357.1472.

After variable-temperature NMR study, **1a**: ^1H NMR (toluene-d_8) 7.98 (2 H, m), 7.37–6.95 (8 H, m), 4.74 (1 H, d, J_{H-H} = 5.6 Hz), 3.00 (3 H, d, J = 9.4 Hz), 2.90 (1 H, dm, J_{P-H} = 25.6 Hz), 1.72 (3 H, bs), 1.58 (3 H, bs), 0.73 (3 H, d, J = 6.4 Hz); ^{31}P NMR (toluene-d_8) −35.87 ppm. **1b**: ^1H NMR (toluene-d_8) 7.97 (2 H, m), 7.37–6.94 (8 H, m), 5.39 (1 H, d, J_{H-H} = 5.1 Hz), 3.18–3.04 (1 H, m), 2.92 (3 H, d, J = 9.4 Hz), 1.78 (3 H, bs), 1.63 (3 H, bs), 0.56 (3 H, d, J = 6.4 Hz); ^{31}P NMR (toluene-d_8) −36.81 ppm.

(7*R*,8*S*)-8,9-Dimethyl-2,3,5,7-tetraphenyl-1,4,6-trioxa-9-aza-5-phospha(P^V)spiro[4.4]non-2-ene (2a). A solution of benzil (0.501 g, 2.40 mmol) in dry pentane (20 mL) was added dropwise to a solution of the 1,3,2-oxazaphospholene **5a**[18] (0.647 g, 2.40 mmol) in dry pentane (30 mL). The mixture was allowed to stir at room temperature for 48 h and filtered through a sintered glass frit containing 2 g of Celite in a closed system under N_2 to remove the precipitated impurities (oxidized **5a**). The pentane was removed under an argon purge, producing an off-white crude solid product. Recrystallization twice from pentane resulted in a white crystalline solid (0.751 g, 65%): mp 70–72 °C dec; ^1H NMR ($CDCl_3$) 7.84 (2 H, m), 7.70–7.16 (18 H, m), 4.87 (1 H, d, J = 5.8 Hz), 3.38 (1 H, d quint, J_{P-H} = 24.8 Hz, J_{H-H} = 6.1 Hz), 3.20 (3 H, d, J = 9.4 Hz), 0.86 (3 H, d, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) 139.0 (d, J_{P-C} = 9.4 Hz), 138.6 (d, J_{P-C} = 216.6 Hz), 132.0 (d, J_{P-C} = 7.0 Hz), 131.4, 131.2 (d, J_{P-C} = 6.2 Hz), 130.2 (d, J_{P-C} = 10.5 Hz), 129.4, 128.2, 128.1, 128.05, 128.0, 127.7, 127.6, 127.1, 126.8, 126.7, 126.6, 125.6, 72.6 (d, J_{P-C} = 4.1 Hz), 59.7 (d, J_{P-C} = 16.0 Hz), 35.3 (d, J_{P-C} = 2.3 Hz), 15.0; ^{31}P NMR ($CDCl_3$) −35.32 ppm; IR ($CHCl_3$, cm^{-1}) 1478 (C=C), 1065 (PPh), 971 (PO); $[\alpha]_D$ +12.0° (c = 0.27, $CHCl_3$); exact mass calcd for $C_{30}H_{28}NO_3P$ $(M)^+$ 481.1805, found 481.1827.

After variable-temperature NMR study, **2a**: ^1H NMR (toluene-d_8): 8.05 (2 H, m), 7.66–6.90 (18 H, m), 4.82 (1 H, d, J = 5.6 Hz), 3.05 (3 H, d, J = 9.4 Hz), 2.93 (1 H, m), 0.76 (3 H, d, J = 6.4 Hz); ^{31}P NMR (toluene-d_8) −35.04 ppm. **2b**: ^1H NMR (toluene-d_8) 8.07 (2 H, m), 7.62–6.90 (18 H, m), 5.47 (1 H, d, J = 5.6 Hz), 3.09 (1 H, m), 2.94 (3 H, d, J = 9.4 Hz), 0.60 (3 H, d, J = 6.4 Hz); ^{31}P NMR (toluene-d_8) −36.41 ppm.

(7*R*,8*S*)-2,3,8,9-Tetramethyl-5-(4-methoxyphenyl)-7-phenyl-1,4,6-trioxa-9-aza-5-phospha(P^V)spiro[4.4]non-2-ene (3a). The preparation was carried as for the preparation of **1a**, using the following quantities of reagents: 1,3,2-oxazaphospholane **5b** (0.181 g, 0.60 mmol) and 2,3-butanedione (51.6 mg, 0.60 mmol): yield 91% (0.275 g); mp 111 °C dec; ^1H NMR ($CDCl_3$) 7.80 (2 H, dd, J_{P-H} = 13.9 Hz, J_{H-H} = 8.5 Hz), 7.4–7.2 (5 H, m), 6.86 (2 H, dd, J_{H-H} = 8.8 Hz, J_{P-H} = 3.9 Hz), 4.78 (1 H, d, J = 5.8 Hz), 3.80 (3 H, s), 3.25 (1 H, d quint, J_{P-H} = 23.6 Hz, J_{H-H} = 6.1 Hz), 3.04 (3 H, d, J_{P-H} = 9.0 Hz), 1.85 (3 H, s), 1.74 (3 H, s), 0.80 (3 H, d, J_{H-H} = 4.3 Hz); ^{13}C NMR ($CDCl_3$) 160.5 (d, J_{P-C} = 3.8 Hz), 139.4 (d, J_{P-C} = 9.4 Hz), 133.4 (d, J_{P-C} = 11.9 Hz), 131.7 (d, J_{P-C} = 88.7 Hz), 128.9 (d, J_{P-C} = 2.7 Hz), 127.9, 127.0, 126.4, 126.0, 113.2 (d, J_{P-C} = 17.5 Hz), 72.4 (d, J_{P-C} = 4.7 Hz), 59.4 (d, J_{P-C} = 14.5 Hz), 55.1, 35.1 (d, J_{P-C} = 3.3 Hz), 15.0, 11.3 (d, J_{P-C} = 7.6 Hz), 11.1 (d, J_{P-C} = 3.7 Hz); ^{31}P NMR ($CDCl_3$) −36.83; IR ($CHCl_3$, cm^{-1}) 1450 (C=C), 1085, (PPh), 945 (PO); $[\alpha]_D$ +7.88 (c = 0.37, $CHCl_3$); exact mass calcd for $C_{21}H_{26}NO_4P$ $(M)^+$ 387.1598, found 387.1594.

After variable-temperature NMR study, **3a**: ^1H NMR (toluene-d_8) 7.98 (2 H, dd, J_{P-H} = 14.0 Hz, J_{H-H} = 8.9 Hz), 7.36–6.98 (5 H, m), 6.70 (2 H, dd, J_{H-H} = 8.8 Hz, J_{P-H} = 3.9 Hz), 4.81 (1 H, d, J = 5.6 Hz), 3.26 (3 H, s), 2.99 (3 H, d, J_{P-H} = 9.0 Hz), 2.92 (1 H, d quint, J_{P-H} = 24.1 Hz, J_{H-H} = 6.0 Hz), 1.73 (3 H, bs), 1.66 (3 H, bs), 0.76 (3 H, d, J_{H-H} = 6.2 Hz); ^{31}P NMR (toluene-d_8): −36.83 ppm. **3b**: ^1H NMR (toluene-d_8) 8.05 (2 H, dd, J_{P-H} = 14.5 Hz, J_{H-H} = 9.0 Hz), 7.39–6.92 (5 H, m), 6.72 (2 H, m), 5.52 (1 H, d, J = 5.6 Hz), 3.28 (3 H, s), 2.90 (3 H, d, J_{P-H} = 9.0 Hz), 3.22–3.02 (1 H, m), 1.77 (3 H, bs), 1.72 (3 H, bs), 0.58 (3 H, d, J_{H-H} = 6.2 Hz); ^{31}P NMR (toluene-d_8) −38.25 ppm.

(7*R*,8*S*)-2,8,9-Trimethyl-5,7-diphenyl-1,6-dioxa-9-aza-5-phospha(P^V)spiro[4.4]non-2-ene (4). Methyl vinyl ketone (0.125 mL, 1.51 mmol) was added via syringe to a refluxing mixture of the 1,3,2-oxazaphospholane **5a**[18] (0.410 g, 1.51 mmol) in dry pentane (50 mL). The mixture was allowed to reflux for a period of 3 days and cooled to rt and then to −30 °C for an additional 24 h. The reaction mixture was filtered through a sintered glass frit filter containing 2 g of Celite in a closed system under Ar. Pentane was initially removed under an argon purge and then under vacuum. The residue was an off-white oily solid (0.358 g, 75%) that was stable neat for only a few hours and then decomposed rapidly: ^1H NMR ($CDCl_3$) 7.82 (2 H, m), 7.4–7.1 (8 H, m),

(26) Noggle, J. H. *Practical Curve Fitting*, Prentice-Hall: Englewood Cliffs, NJ, 1992.

4.70 (1 H, d, J = 5.5 Hz), 4.57 (1 H, dm, J_{P-H} = 41.8 Hz), 3.27 (1 H, d quint, J_{P-H} = 20.9 Hz, J_{H-H} = 6.0 Hz), 3.08 (3 H, d, J = 8.5 Hz), 2.76 (2 H, dm, J_{P-H} = 23.8 Hz), 1.70 (3 H, d, J = 1.2 Hz), 0.70 (3 H, d, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) 152.0 (d, J_{P-C} = 13.3 Hz), 141.3 (d, J_{P-C} = 168.2 Hz), 140.0 (d, J_{P-C} = 6.2 Hz), 130.3 (d, J_{P-C} = 10.5 Hz), 128.0, 127.9, 127.7, 127.5, 126.1 (d, J_{P-C} = 74.9 Hz), 125.7, 72.7, 59.8 (d, J_{P-C} = 13.8 Hz), 34.9 (d, J_{P-C} = 4.9 Hz), 30.2 (d, J_{P-C} = 124.6 Hz), 17.2 (d, J_{P-C} = 2.6 Hz), 14.6; ^{31}P NMR ($CDCl_3$) –27.23 ppm; IR ($CHCl_3$, cm^{-1}) 1461 (C=C), 1097 (PPh), 1014 (PO); $[\alpha]_D$ +6.1° (c = 0.41, $CHCl_3$); exact mass calcd for $C_{20}H_{24}NO_4P$ $(M)^+$ 341.1543, found 341.1532.

(4*S*,5*R*)-2-(4-Methoxyphenyl)-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholane (5b). The preparation was carried as for the preparation of **5a**[18] using the following quantities of reagents: (–)-ephedrine (0.610 g, 3.7 mmol), Et_3N (0.747 g, 7.38 mmol), and dichloro(4-methoxyphenyl)phosphine[25] (0.772 g, 3.7 mmol): yield 64% (0.715 g); mp 85 °C dec; ^{1}H NMR ($CDCl_3$) 7.46 (2 H, dd, J_{P-H} = 10.6 Hz, J_{H-H} = 5.4 Hz), 7.4–7.2 (5 H, m), 6.96 (2 H, d, J = 8.30 Hz), 5.53 (1 H, d, J = 6.8 Hz), 3.83 (3 H, s), 3.30 (1 H, quint d, J_{H-H} = 6.6 Hz, J_{P-H} = 2.4 Hz), 2.52 (3 H, d, J = 13.8 Hz), 0.68 (3 H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 160.6, 139.2, 133.6 (d, J_{P-C} = 49.5 Hz), 131.2 (d, J_{P-C} = 21.3 Hz), 127.9, 127.6, 127.2, 113.6 (d, J_{P-C} = 5.5 Hz), 85.9 (d, J_{P-C} = 9.3 Hz), 56.6 (d, J_{P-C} = 5.6 Hz), 55.1, 30.0 (d, J_{P-C} = 8.6 Hz), 13.7 (d, J_{P-C} = 3.6 Hz); ^{31}P NMR ($CDCl_3$) +144.23; IR ($CDCl_3$, cm^{-1}) 1094 (PPh), 975 (PO); $[\alpha]_D$ = –11.2° (c = 0.25, $CHCl_3$); exact mass calcd for $C_{17}H_{20}NO_2P$ $(M)^+$ 301.1230, found 301.1211.

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Supplementary Material Available: Experimental details and spectral data for compounds **1a**, **2a**, **3a**, **4**, and **5b** and ^{1}H NMR spectra of compounds **1a**, **2a**, **3a**, and **4** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

The Structures of A10255B, -G, and -J: New Thiopeptide Antibiotics Produced by *Streptomyces gardneri*

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The structures of the major members of a new family of important thiopeptide antibiotics, A10255B (**1**), A10255G (**15**), and A10255J (**16**), produced by *Streptomyces gardneri* (NRRL 15537), are described. Selective chemical degradation in combination with NMR, FABMS, and CID methods on the degradation products was required to solve these structures. Methanolysis of **1** resulted in the isolation of 4-carbomethoxy-2-propionyloxazole (**8**) and dimethyl sulfomycinamate (**9**) as well as *N*-((acetamidomethyl)thiazolyl)-1-(carbomethoxythiazolyl)ethanamide (**11**) after acetylation. Vigorous treatment with acid produced berninamycinic acid (**10**). Trifluoroacetolysis led to cleavage at the six dehydroalanine (deala) residues to give a complex and highly modified pentapeptide **12** which was sequenced by CIDMS and NMR techniques. Compound **12** was composed of the following: sulfomycinamic acid, threonine, 1-(4-carboxyoxazolyl)-1-aminopentene unit (dehydronorvaline masked by oxazole at its carboxyl group), 2-(aminomethyl)thiazole-4-carboxylic acid, and 2-(1-aminoethyl)-4-carboxamidothiazole. FABMS and base hydrolysis showed that **1** had a deala tetrapeptide side chain. Antibiotics **15** and **16** each had a masked dehydrobutyrine in place of the dehydronorvaline present in **1**, and **16** had a single amidated deala as a side chain.

Introduction

A10255, a novel sulfur-containing complex of antibiotics produced by *Streptomyces gardneri*, exhibits strong antimicrobial activity against Gram-positive bacteria and has potential utility as a growth promotant and as a preventative of lactic acidosis in farm animals.[1,2] The A10255 complex was extracted from the mycelia formed in submerged cultures of the producing organism and shown to be multicomponent (designated A10255B, -C, -E, -F, -G, -H, and -J) by chromatography. The major components A10255B, -J, and -G were isolated in sufficent quantity to permit determination of their structures.

Physicochemical data indicated that the A10255 antibiotics belong to the thiopeptide class. Members of this class characteristically possess a cyclic peptide core composed mostly of amino acids masked at their carboxyl groups by thiazole and/or oxazole rings as well as the presence of several dehydroamino acids. These antibiotics have presented chemists with formidable structure elucidation tasks. In the study reported here the most direct approaches were not available to the solution of the structures of the A10255 antibiotics. The noncrystalline nature of these substances precluded X-ray crystallographic work. This paper presents a chemical degradation scheme which led to the solution of this problem and the elucidation of the structures of the major components of this family of antibiotics: A10255B, A10255G, and A10255J.

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