in structure **11**. These chemical data are in full agreement with structure **1** for nocardicin A.

The absolute configuration of the acylamino group on the β-lactam ring and the carboxyl group of the p-hydroxyphenylglycine moiety were established to be L and D, respectively, from optical data of **13**, $[\alpha]D +20.3°$ (1 N HCl),[8] and **14**, $[\alpha]D -80.0°$ (0.1 N HCl) (54% optical purity).[9] With regard to the stereochemistry of the remaining homoserine unit, the benzoic acid derivative **15**, obtained by treatment of **10** with H_2O_2, was hydrogenated over Pt in 3 N HCl to generate D-α-aminobutyrolactone (HCl salt), $[\alpha]D +29.0$ (0.1 N HCl);[10] the absolute configuration of the homoserine part is thus D.

The oxime configuration was established to be syn to the acylamino group on the following grounds. Nocardicin B (**2**), $C_{23}H_{24}O_9N_4$, mp 262-264° dec, $[\alpha]D -162.0°(H_2O)$,[3] isolated as a minor product from the same culture, was shown to be a stereoisomer of **1** at the oxime function; on treatment with $NaHSO_3$, **2** was also converted to the keto derivative **6**. The ^1H NMR spectrum of **1** (Me_2SO-d_6) shows the amide proton at 9.12 ppm (as described above), while in **2** it is at 8.81 ppm (d, $J = 8$ Hz). This difference in the chemical shift of the amide protons suggests the presence of an internal hydrogen bonding between the oxime O and amide H in **1**. This is possible only when the oxime OH is syn to the amide group.[11,12]

The structures of nocardicin A and B are hence established as being **1** and **2**, respectively. Nocardicin A is active against a variety of gram-negative bacteria and shows an especially high antimicrobial activity against *Pseudomonas*, while the activity of nocardicin B is weaker.[13] These antibiotics are unique in several respects: (1) they are the first examples of monocyclic β-lactam antibiotics[14] possessing relatively high potency; (2) they have an oxime function[15] whose syn relation to the acylamino group is favored for antimicrobial activity; (3) they contain p-hydroxyphenylglycine (two such units) which is found rarely in nature;[16] (4) their structures are stereochemically related to the penicillin molecule (carboxyl, α; acylamino, β); and (5) similarly to penicillins and cephalosporins, they are enzyme inhibitors in the cell wall biosynthesis of bacteria.[17]

Chemical modification of nocardicins and preparation of new 3-acyl derivatives of 3-aminonocardicinic acid (3-ANA) **16**[18] are in progress.

References and Notes

(1) For recent reviews on the chemistry and biology of the β-lactam antibiotics, see, e.g., J. H. C. Nayler, *Adv. Drug Res.*, **7**, 1 (1973); D. N. McGregor, *Fortschr. Chem. Org. Naturst.*, **31**, 1 (1974); A. K. Mukerjee and A. K. Singh, *Synthesis*, 547 (1975); R. J. Stoodley, *Tetrahedron*, **31**, 2321 (1975); J. C. Jaszberenyi and T. E. Gunda, *Prog. Med. Chem.*, **12**, 395 (1975).

(2) The fermentation, isolation, and characterization of nocardicins have been carried out by H. Aoki, H. Sakai, M. Kohsaka, T. Konomi, J. Hosoda, T. Kubochi, E. Iguchi, and H. Imanaka, *J. Antibiot.*, in press.

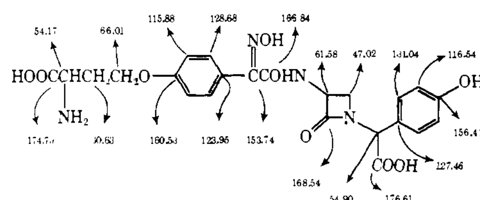
(3) The $[\alpha]D$ measurements were performed on the sodium salts: 1, $C_{23}H_{23}O_9N_4Na$, mp 234-235° dec; **2**, $C_{23}H_{23}O_9N_4Na$, mp 257-260° dec.

(4) *Nocardia uniformis* var. *tsuyamanensis* ATCC 21806.

(5) For data of geminal and vicinal coupling constants of substituted β-lactams, see K. D. Barrow and T. M. Spotswood, *Tetrahedron Lett.*, 3325 (1965); P. V. Demarco and R. Nagarajan, "Cephalosporins and Penicillins, Chemistry and Biology". H. Flynn, Ed., Academic Press, New York, N.Y., 1972, pp 330-340.

(6) Upon acetylation, this 1 H triplet underwent a downfield shift to 4.70 ppm (**3** in $CDCl_3$).

(7) Each signal in the ^{13}C NMR spectrum was assigned as follows by comparison with the spectra of degradation products. Details will be discussed in a forthcoming full paper.

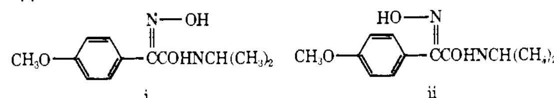


(8) Lit. $[\alpha]D +25.2°$ (1 N HCl): S. M. Birnbaum, R. J. Koegel, S.-C. J. Fu, and J. P. Greenstein, *J. Biol. Chem.*, **198**, 335 (1952).

(9) During the reaction, partial racemization took place in **14**; prolonged heating led to complete racemization of **14**. An optically pure sample (HCl salt) showed $[\alpha]D -149°$ (0.1 N HCl) (lit. $[\alpha]D -108°$ (H_2O)): A. A. W. Long, J. H. C. Naylor, H. Smith, T. Taylor, and N. Ward, *J. Chem. Soc. C*, 1920 (1971).

(10) L-α-Aminobutyrolactone (HCl salt) prepared from L-homoserine for comparison showed $[\alpha]D -29.0°$ (0.1 N HCl) (lit. $[\alpha]D +26.7°(H_2O)$ for the D isomer): S. M. Birnbaum and J. P. Greenstein, *Arch. Biochem. Biophys.*, **42**, 212 (1953).

(11) This behavior was also observed in model compounds i and ii: i (syn to the amide), 8.30 ppm (d, $J = 8$ Hz); ii (anti to the amide), 8.00 ppm; difference, 0.30 ppm.



(12) The uv absorptions due to partial structure b of nocardicin A and B were calculated by substraction of the absorbance of p-hydroxyphenylglycine from those of nocardicins: 1, $\lambda_{max}^{EtOH-H_2O}$ 270 nm (ϵ, 14 900) and $\lambda_{max}^{EtOH-0.1\,N\,NaOH}$ 283 nm (ϵ, 9500); **2**, $\lambda_{max}^{EtOH-H_2O}$ 267 nm (ϵ, 8900) and $\lambda_{max}^{EtOH-0.1\,N\,NaOH}$ 275 nm (ϵ, 9400). In comparison to **2**, the uv absorption band of 1 was longer and stronger in both neutral and basic media. This is in agreement with the data of models i and ii: i, $\lambda_{max}^{EtOH-H_2O}$ 270 nm (ϵ, 15 800) and $\lambda_{max}^{EtOH-0.1\,N\,NaOH}$ 283 nm (ϵ, 11 400); ii, $\lambda_{max}^{EtOH-H_2O}$ 267 nm (ϵ, 9800) and $\lambda_{max}^{EtOH-0.1\,N\,NaOH}$ 275 nm (ϵ, 9500). These data also confirmed that the oxime function is syn to the amide group in **1** and anti in **2**.

(13) Y. Mine, S. Nonoyama, H. Kojo, S. Fukada, M. Nishida, S. Goto, and S. Kuwahara, to be submitted for publication.

(14) Several compounds containing monocyclic β-lactam rings have been isolated from microorganisms: see, e.g., W. W. Stewart, *Nature (London)*, **229**, 174 (1971); H. K. Schnoes and R. D. Durbin, *Biochem. Biophys. Acta*, **286**, 107 (1972); T. Takita, Y. Muraoka, T. Yoshioka, A. Fujii, K. Maeda, and H. Umezawa, *J. Antibiot.*, **25**, 755 (1972); J. P. Scannell, D. L. Pruess, J. F. Blount, H. A. Ax, M. Kellett, F. Weiss, T. C. Demny, T. H. Williams, and A. Stempel, *J. Antibiot.*, **28**, 1 (1975).

(15) Aside from semisynthetic compounds, only few microbial products bearing an oxime function are known; see P. H. Wiley, R. R. Herr, F. A. Mackellar, and A. O. Argondelis, *J. Org. Chem.*, **30**, 2330 (1965); H. Sakakibara, H. Naganawa, M. Ohno, K. Maeda, and H. Umezawa, *J. Antibiot.*, **27**, 897 (1974); B. W. Bycroft and R. Pinchin, *J. Chem. Soc., Chem. Commun.*, 121 (1975).

(16) See, e.g., K. A. Smith, D. H. Williams, and G. A. Smith, *J. Chem. Soc., Perkin Trans. 1*, 2369 (1974).

(17) A study on the inhibitory mechanism of nocardicins will be reported by H. Aoki et al.; cf. ref 2.

(18) Preparation of **16** from nocardicin A will be reported elsewhere.

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Spectinomycin Biosynthesis Studied by Carbon Magnetic Resonance Spectroscopy[1]

Sir:

We have recently reported[2] that the biosynthesis of deoxystreptamine, the aminocyclitol moiety of neomycin, proceeds from glucose by a pathway in which [6-^{13}C]D-glucose labels C-2 of deoxystreptamine and [1-^{13}C]D-glucosamine[3] labels C-1 of deoxystreptamine (Figure 1, path b).[4] More recently we showed that [6-^{13}C]D-glucose labels C-6 of streptidine, the substituted aminocyclitol moiety of streptomycin,[1b] which would agree with earlier reports that [1-^{14}C]D-glucose labels C-5 of streptidine.[5]

Thus, the two aminocyclitols deoxystreptamine and streptidine are biosynthesized by different pathways. A third aminocyclitol antibiotic,[6] spectinomycin (Figure 2),[7,8] which is used clinically in the treatment of gonorrhea, contains a different aminocyclitol unit, actinamine, with similarities to both deoxystreptamine and streptidine. Actinamine does not contain the highly basic guanido groups of streptidine, but, unlike deoxystreptamine, it contains a hydroxyl group at C-2. A priori, then, either (or neither) of the two biosynthetic pathways might be followed.

No report exists of the precise location of label in the am-

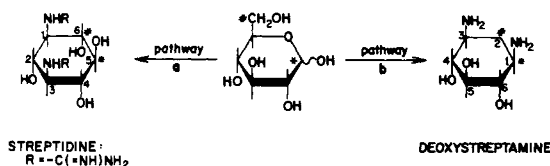

Figure 1. Location of labeled carbons in deoxystreptamine and streptidine from C-1 (*) and C-6 (#) labeled D-glucose or D-glucosamine.[3]

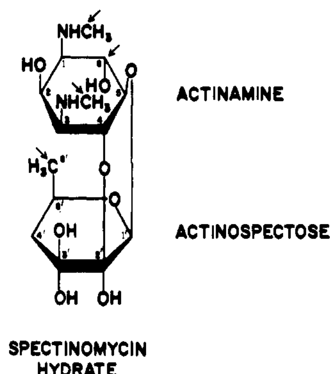

Figure 2. Structure of spectinomycin and carbons labeled (arrows) by [6-^{13}C]D-glucose.

Table I. Distribution of Label within Spectinomycin from [6-^{13}C]D-Glucose

Atom	δ, ppm[a]	Enrichment[b]
C-1	62.5	0.84
C-2	60.7	0.92
C-3	59.5	0.88
C-4	66.5	1.28
C-5	70.7	1.06
C-6	66.9	3.14
1-N-CH_3	31.8	1.67
3-N-CH_3	31.3	1.66
C-1′	94.4	1.00[c]
C-2′	94.4	1.00[c]
C-3′	92.6	1.00
C-4′	42.3	0.87
C-5′	69.2	0.96
C-6′	20.5	3.40

[a] Measured in deuterium oxide at pD 4.6, with dioxane as internal reference ($\delta_{dioxane}$ 67.4 ppm downfield from Me_4Si). [b] Calculated by comparing peak ratios in spectrum of labeled spectinomycin to those in spectrum of unenriched compounds, using 94.4 ppm as standard. [c] By definition.

inocyclitol unit of spectinomycin from specifically labeled glucose, although Mitscher et al.[9] noted that [6-^{3}H]D-glucose was incorporated into both subunits of spectinomycin and suggested a biosynthetic pathway which would apparently require C-6 of glucose to label C-1 and/or C-3 of actinamine. Glucose C-6 labeled actinospectose at C-6′ (the C-methyl group).[9] The incorporation of *myo*-inositol into actinamine[9] would argue tentatively for the streptidine pathway (path a), since *myo*-inositol is well incorporated into streptidine[10] but not into deoxystreptamine.[11]

In the present study, 1.75 g of [6-^{13}C]D-glucose[2] (63% ^{13}C) was administered in three increments at 12-h intervals beginning after 72 h growth to a growing culture of *Streptomyces spectabilis* in a fermentation medium based on that described previously.[12a,b] The fermentation was halted after 6 days. Spectinomycin (487 mg) was isolated,[12a,c] purified by extraction with methanol, and recrystallized from aqueous acetone. The incorporation of carbon-13 was 2.4% and the dilution was 1:6.4.

Examination of the carbon magnetic resonance spectrum[13] of the labeled spectinomycin at pH 4.6 (Table I) indicated that two atoms (C-6 of actinamine and C-6′ of actinospectose) were labeled to the extent of approximately 3.3 times natural abundance and the two *N*-methyl groups to the extent of about 1.7 times natural abundance. Methionine has been shown[9] to be the source of the *N*-methyl groups of actinamine. Labeling of the *N*-methyl groups by C-6 of glucose presumably follows the accepted (abbreviated) conversions[16] [6-^{13}C]glucose → 3-phospho[3-^{13}C]glyceric acid → [3-^{13}C]serine → [methylene-^{13}C]tetrahydrofolic acid → [methyl-^{13}C]methionine.

Labeling of C-6′ of actinospectose by [6-^{13}C]glucose argues for the direct conversion of glucose to this neutral fragment and confirms the earlier report.[9] More importantly, the present demonstration that the label from [6-^{13}C]glucose is found at C-6 of actinamine argues for a biosynthetic pathway to actinamine related to that for streptidine rather than that for deoxystreptamine. Thus, guanidinylation of an amino group is not a prerequisite for path a of Figure 1.

Acknowledgment. This investigation was supported by NIH Research Grant No. AI 1278 from the National Institute of Allergy and Infectious Diseases. Carbon magnetic resonance spectra were obtained on a spectrometer purchased with an instrumentation grant from the National Science Foundation.

References and Notes

(1) (a) Paper 6 in the series "Carbon-13 as a Biosynthetic Tool"; (b) Paper 5: M. H. G. Munro, M. Taniguchi, K. L. Rinehart, Jr., D. Gottlieb, T. H. Stoudt, and T. O. Rogers, *J. Am. Chem. Soc.*, **97**, 4782 (1975).
(2) K. L. Rinehart, Jr., J. M. Malik, R. F. Nystrom, R. M. Stroshane, S. G. Truitt, M. Taniguchi, J. P. Rolls, W. J. Haak, and B. A. Ruff, *J. Am. Chem. Soc.*, **96**, 2263 (1974).
(3) Labeling of deoxystreptamine by D-glucosamine actually proceeds with loss of the amino group, presumably through the reversal of the reaction by which D-glucose is converted to D-glucosamine (E. Umbarger and B. D. Davis in "The Bacteria. III. Biosynthesis", I. C. Gunsalus and R. Y. Stanier, Ed., Academic Press, New York, N.Y., 1962, pp 167–251). We have demonstrated this by administering [^{15}N]glucosamine to a growing culture of *S. fradiae*, isolating neomycin B in the usual way, and hydrolyzing it to neamine and neobiosamine B. The neamine isolated was hydrolyzed by refluxing 48% hydrobromic acid to deoxystreptamine. The four compounds named were converted first to ammonium sulfate by Kjeldahl digestion (J. M. Bremer in "Methods of Soil Analysis", Part 2, C. A. Black, Ed., American Society of Agronomy, Madison, Wisc., 1965, pp 1164–1170) and then by the Rittenberg procedure (D. Rittenberg, A. S. Keston, F. Roseburg, and R. Schoenheimer, *J. Biol. Chem.*, **127**, 291 (1939)] to nitrogen gas which was analyzed on a Varian MAT GD150 isotope ratio mass spectrometer. Neomycin B contained 0.2468 atoms of nitrogen-15 per mole; neobiosamine B, 0.1022; neamine, 0.1286; and deoxystreptamine, 0.0057; corresponding natural abundance values would be 0.0228, 0.0076, 0.0152, and 0.0076.
(4) We have assumed (K. L. Rinehart, Jr., and R. M. Stroshane, *J. Antibiot.*, in press) that the conversion of [6-^{13}C]glucose to [2-^{13}C]deoxystreptamine involves initial amination of an inosose or deoxyinosose at what will become C-3 of deoxystreptamine, followed by oxidation and amination at the future C-1 of deoxystreptamine ["clockwise" oxidation–amination, analogous to the results for streptomycin (J. B. Walker, *Lloydia*, **34**, 363 (1971) and ref 1)]. However, an initial amination at future C-1 of deoxystreptamine followed by oxidation–amination at what will become C-3 cannot be eliminated by present data.
(5) (a) R. M. Bruce, H. S. Ragheb, and H. Weiner, *Biochim. Biophys. Acta*, **158**, 499 (1968); (b) W. H. Horner and G. A. Russ, *ibid.*, **237**, 123 (1971).
(6) A review: K. L. Rinehart, Jr., and R. M. Stroshane, *J. Antibiot.*, in press.
(7) D. J. Mason, A. Dietz, and R. M. Smith, *Antibiot. Chemother.*, **11**, 118 (1961).
(8) T. D. Cochran, D. J. Abraham, and L. L. Martin, *J. Chem. Soc., Chem. Commun.*, 494 (1972).
(9) L. A. Mitscher, L. L. Martin, D. R. Feller, J. R. Martin, and A. W. Goldstein, *Chem. Commun.*, 1541 (1971).
(10) W. H. Horner, *J. Biol. Chem.*, **239**, 2256 (1964).
(11) F. C. Falkner, Ph.D. Thesis, University of Illinois, Urbana, Ill., 1969.
(12) (a) M. E. Bergy and C. De Boer, U.S. Patent 3 234 092, Feb 8, 1966; cf. British Patent 959 675, June 3, 1964 (*Chem. Abstr.*, **61**, 7664g (1964)); (b) D. J. Mason, A. Dietz, and R. M. Smith, *Antibiot. Chemother.*, 11, 118 (1961); (c) M. E. Bergy, T. E. Eble, and R. R. Herr, *ibid.*, 11, 661 (1961).
(13) The carbon magnetic resonance spectrum of spectinomycin was previously assigned by Grutzner (J. B. Grutzner, *Lloydia*, **35**, 375 (1972)). We have reinvestigated[14] the published assignments, studying the vari-

ation of chemical shift with pH and employing as model compounds actinamine, *N,N'*-diacetylactinamine, dihydrospectinomycin, and *N,N'*-diacetyldihydrospectinomycin.[15] Our assignments (Table I) agree with those of Grutzner except for C-1, C-3, C-4, and C-6. In addition, we assign the signals at 31.8 and 31.3 ppm to the 1-*N*- and 3-*N*-methyl carbons, respectively, and differentiate between C-2' and C-3' while Grutzner did not distinguish between these atoms.

(14) R. M. Stroshane, H. M. Rubenstein, and K. L. Rinehart, Jr., to be submitted.
(15) P. F. Wiley, A. D. Argoudelis, and H. Hoeksema, *J. Am. Chem. Soc.*, **85**, 2652 (1963).
(16) A. L. Lehninger, "Biochemistry", 2d ed, Worth Publishers, New York, N.Y., 1975, pp 422-429, 697, 713-714.
(17) National Institutes of Health Predoctoral Trainee in Biophysical Chemistry (Training Grant No. 2TI GM 00722).

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Electrochemical Reduction and Bonding in the O_2, S_2, and Se_2 Adducts of $[Ir\{(C_6H_5)_2PCH_2CH_2P(C_6H_5)_2\}_2]^+$

Sir:

We wish to report here novel electrochemical behavior and its bonding implications for the series $[Ir(dppe)_2X_2]^+$ (dppe = $Ph_2PCH_2CH_2PPh_2$; X_2 = O_2, S_2, and Se_2).[1-4] This study reveals for the first time the dissociation of $X_2\cdot^-$ consequent to the addition of one electron to the antibonding orbital of the π-component of the widely accepted Dewar-Chatt-Duncanson model[5] for MX_2 bonding; it also provides a means of assessing the π-back-bonding interaction between the metal and the X_2 group.

Cyclic voltammograms (100 mV/s) of degassed 10^{-3} M solutions of $[Ir(dppe)_2X_2]^+$ and $[Ir(dppe)_2]^+$ in CH_3CN (using 0.1 M $(n\text{-}C_4H_9)_4N^+ClO_4^-$ as supporting electrolyte, Ag/0.01 *M* $AgNO_3$ as reference electrode and a hanging mercury drop as working electrode) are shown in Figure 1. $Ir(dppe)_2^+$ has one quasi-reversible reduction wave at −2.05 V. The O_2, S_2, and Se_2 adducts each have two reduction waves: the first wave (A) is irreversible and progresses to more negative potential, viz., −1.64, −1.75, and −1.95 V, along the sequence Se_2, S_2, O_2 whereas the second wave (B) is quasi-reversible with potential (−2.05 V) and shape resembling that of the $Ir(dppe)_2^+$ species. At slow scan rates (10 mV/s) the cyclic voltammogram of $Ir(dppe)_2^+$ has no anodic peak. In the 100 mV/s scan $i_p^c/i_p^a \approx 2$ and the peak separation is 45 mV, while in a 200 mV/s scan $i_p^c/i_p^a \approx 1.5$ and the peak separation is 52 mV. A one-electron reversible charge transfer has $i_p^c/i_p^a = 1$ and a peak separation of 59 mV.

To clarify the nature of the reduction waves, we carried out extensive controlled potential coulometry studies on these complexes. Except for the S_2 adduct, wave A corresponds to a one (0.9-1.1) electron reduction whereas wave B corresponds to a 1.7-1.8 electron reduction. The coulometric *n* values for $Ir(dppe)_2S_2^+$ are 1.3 and 0.4 for waves A and B, respectively. We also observed that controlled potential electrolysis at potentials intermediate between waves A and B of $Ir(dppe)_2X_2^+$ produced a solution with color and cyclic voltammogram characteristic of $Ir(dppe)_2^+$. In the case of the O_2 complex, the cyclic voltammogram of this solution also showed an oxidation wave at −1.03 V, analogous to the wave found in a freshly prepared solution of authentic $O_2\cdot^-$. In all cases, further reduction at a potential more negative than wave B (i.e., −2.05 V) produced an orange precipitate identical with that obtained by similar reduction of the $Ir(dppe)_2^+$ species. The orange precipitate was characterized by elemental analysis-($Ir(dppe)_2H$), ^1H NMR in $CDCl_3$ (a 1:4:6:4:1 quintet at τ 30.04 with $J_{P\text{-}H}$ = 12 Hz), and ir (a sharp Ir-H stretching mode at 2015 cm^{-1}). A single-crystal x-ray structure determination[6] revealed a trigonal-bipyramidal-like structure (I) with the hydride (not located) presumably situated at the axial position.[7]

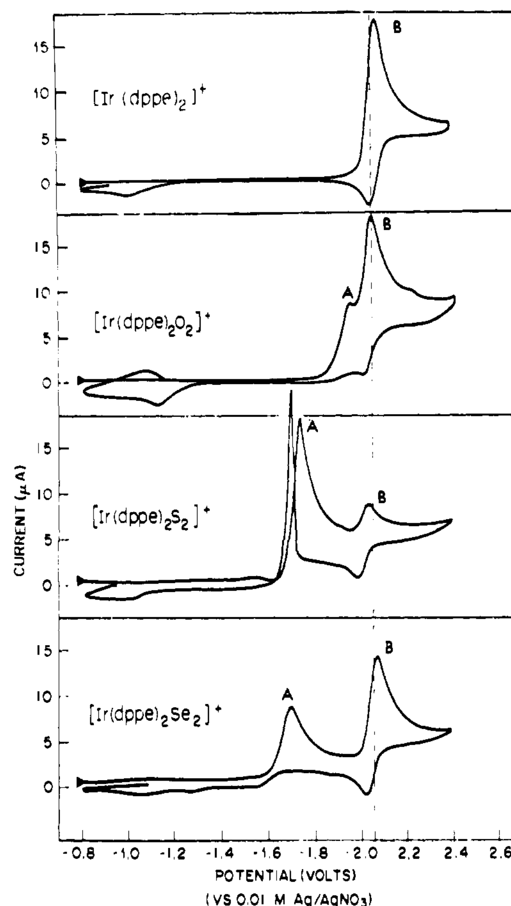
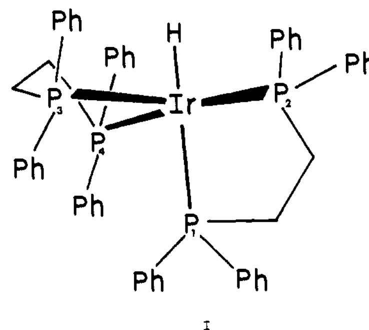


Figure 1. Cyclic voltammograms of 10^{-3} M $[Ir(dppe)_2]^+$ and $[Ir(dppe)_2X_2]^+$ (X_2 = O_2, S_2, and Se_2) in 0.10 M $(n\text{-}C_4H_9)_4N^+ClO_4^-$ in CH_3CN: scan rate, 100 mV/s; scan initiated at arrowhead. The sharp reduction peak at −1.65 V on the reverse (anodic) scan for the S_2 adduct disappears when a Pt-bead is used as the working electrode, or when the scan rate is greater than 500 mV/s with a hanging mercury drop electrode. Its origin is at present not understood.



I

To trace the source of the hydride, controlled-potential coulometry was repeated using CD_3CN as solvent. The orange precipitate now showed a loss of about 80% in the intensity of its Ir-H stretching band at 2015 cm^{-1}, and a new band appeared at 1445 cm^{-1}, as expected for the Ir-D stretch. This suggests that about 80% of the hydride comes from the solvent, with the remaining 20% most likely from the ortho-phenyl hydrogen of the dppe ligand or the supporting electrolyte.[9]