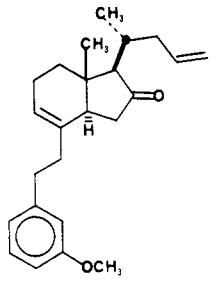
11 (68%, 4:1 trans/cis). The conversion of 11 into fully



11

elaborated steroids is currently in progress.

**Acknowledgment.** NMR spectra were obtained with the 7 T spectrometer at the Rockefeller University (purchased in part with funds from the NSF and the Camille and Henry Dreyfus Foundation. We also thank Dr. Brian Willis (Fritsche, Dodge and Olcott, NY) for NMR spectra.

**Registry No.** **2**, 83845-86-7; **3**, 83845-87-8; **4**, 83845-88-9; **5**, 83845-89-0; **6a**, 83845-90-3; **6b**, 83915-74-6; **6c**, 83845-91-4; **6d**, 83845-92-5; **6e** (isomer 1), 83845-93-6; **6e** (isomer 2), 83915-75-7; **7a**, 83845-94-7; **7c**, 83845-95-8; **7d**, 83845-96-9; **8a**, 83845-97-0; **8c**, 83845-98-1; **8d**, 83845-99-2; **9**, 83846-00-8; **10**, 83846-01-9; **11** (isomer 1), 83861-70-5; **11** (isomer 2), 83861-71-6; 3-MPL, 51852-87-0; 3-methoxybenzeneacetic acid, 1798-09-0.

**Stephen R. Wilson,* M. Serajul Haque**

*Department of Chemistry*
*New York University*
*New York, New York 10003*

*Received September 8, 1982*

## Structure of a New Antifungal Antibiotic, Irumamycin

*Summary:* The structure of irumamycin, a new antifungal antibiotic, was determined by chemical degradation, NMR spectroscopy, and $^{13}C$-enriched irumamycin.

*Sir:* Recently, novel macrocyclic lactone antibiotics possessing various biological activities have been isolated from the metabolites of *Streptomyces*. In the course of screening for antifungal substances, a new macrolide antibiotic, irumamycin[1] (**1**), mp 95–97 °C, $[\alpha]^{25}_D$ +12° (*c* 1, $CHCl_3$), $C_{41}H_{65}NO_{12}$, was found in the culture broth of *Streptomyces subflavus* subsp. *irumaensis* nov. subsp. AM-3603.

We now report complete structural analysis of **1** by means of feeding experiments using $^{13}C$-labeled precursors, chemical degradation, and 400-MHz $^1H$ NMR spectroscopy. Irumamycin belongs to a group of macrolide antibiotics that include venturicidin,[2] concanamycin,[3] oligomycin,[4] cytovaricin,[5] etc. and appears to be the first practical agricultural antifungal drug.

The $^{13}C$ NMR spectral data [ketone carbonyl (δ 211.5), lactone carbonyl (δ 173.8), carbamoyl (δ 158.1) and six olefinic carbons, an anomeric (δ 98.7) and a ketal carbon (δ 94.4), nine carbons bonded to oxygen, two of which (δ 66.4 and 64.6) are due to an epoxide, four methines, eight methylenes, and nine methyls] suggested that the antibiotic possesses a polyketide skeleton derived biosynthetically from malonate, methylmalonate, and a sugar. Acetylation of **1** with $Ac_2O$ in pyridine afforded a diacetate **2**, mp 105–106 °C, $[\alpha]^{20}_D$ +59.4° (*c* 0.6, $CH_3OH$), IR ($CCl_4$) $\nu_{OH}$ 3450 $cm^{-1}$ and $\nu_{CO}$ 1710–1730 $cm^{-1}$, $^1H$ NMR ($CDCl_3$) δ 2.05 and 2.10 ($OCOCH_3$), suggesting the presence of two secondary hydroxyl groups and a ketalic hydroxyl in **1**. Hydrogenation of **1** over Pd–C afforded the hexahydro derivative; this indicated the presence of three double bonds. Methanolysis of **1** gave a crystalline sugar **3**, mp 108–109 °C, $[\alpha]^{22}_D$ +92° (*c* 0.6, $CH_3OH$), $^{13}C$ NMR ($CDCl_3$) δ 158.2 ($OCONH_2$) and 98.9 ($J_{CH}$ = 166.8 Hz, assignable to an α-anomeric carbon).[6] The $^1H$ NMR spectral data [δ 5.10, H-3′ ($J_{2'a,3'}$ = 11.7 and $J_{2'e,3'}$ = 5.4 Hz), δ 4.70, H-4′ ($J_{3',4'}$ = 9.2 nd $J_{4',5'}$ = 9.5 Hz)] of the monoacetate **4**, mp 107–108 °C, $C_8H_{15}NO_5$ ($M^+$ *m/z* 247), established the structure of **3** as methyl 3-*O*-carbamoyl-2-deoxy-α-D-rhamnoside by comparison with the NMR spectrum of the corresponding 4-*O*-carbamoyl derivative from concanamycin.[3]

Ozonolysis of **2** followed by treatment with 30% $H_2O_2$–concentrated HCl and then with diazomethane afforded the trichloro compound **5** as the main product, $[\alpha]^{22}_D$ +0.3° (*c* 0.6, $CH_3OH$). Anal. Calcd for $C_{23}H_{37}O_8Cl_3$: C, 50.41; H, 6.76; Cl, 19.45. Found: C, 50.24; H, 6.79; Cl, 19.21. The $^{13}C$ NMR spectrum revealed that **5** is a methyl ester of a $C_{13}$ fatty acid possessing a ketone carbonyl ($\delta_C$ 208.9), two acetoxyl ($\delta_C$ 170.5 and 165.0), a secondary hydroxyl methylene ($\delta_C$ 74.7), and five other methyl groups. The $^{13}C$ chemical shift of the ester carbonyl ($\delta_C$ 165.0) as well as the proton signal at $\delta_H$ 5.90 (1 H, s) due to the hydrogen bonded to a chlorinated carbon ($\delta_C$ 65.3) indicates the presence of a dichloroacetoxy group which, as in the case of venturicidin A,[2] was produced by chlorination ($HCl$–$H_2O_2$) and decarboxylation of a malonate moiety formed by ozonolysis and $H_2O_2$ oxidation of **2**. Acetylation of **5** with $Ac_2O$ in pyridine afforded the diacetate **6**, $C_{25}H_{39}O_9Cl_3$, $^1H$ NMR ($CDCl_3$) δ 2.05 and 2.12 ($OCOCH_3$). This indicated that **5** contains a newly generated hydroxyl group. Introduction of this hydroxyl group and the third chlorine atom of **5** resulted from epoxide ring opening with concentrated HCl after ozonolysis. In the $^{13}C$ NMR spectrum of **6**, a high-field shift of the signals for a methylene ($\delta_C$ 35.7, Δ 1.4 ppm) and a ketone carbonyl ($\delta_C$ 207.8, Δ 2.1 ppm) compared with those in **5** suggested that both of these are located γ to the new acetate in **6**. Treatment of **5** with zinc in acetic acid gave an oily monochloro compound **7**: $[\alpha]^{22}_D$ +2.5° (*c* 0.6, $CH_3OH$); $C_{23}H_{37}O_7Cl$ ($M^+$ *m/z* 460); UV (EtOH) $\lambda_{max}$ 235 nm (log ε 4.01) (α,β-unsaturated ketone); IR ($CCl_4$) $\nu_{CO}$ 1670 $cm^{-1}$; $^1H$ NMR δ 6.36 (1 H, d, *J* = 9.0 Hz, olefinic proton), 1.77 ($CH_3C$=). Consequently the epoxide ring in **1** must be located adjacent to the ketone carbonyl. The structure of **5** was clearly shown to be 3-(acetyloxy)-10-chloro-5-[(dichloroacetyl)oxy]-9-hydroxy-11-oxo-2,4,6,8,10-pentamethyltridecanoic acid methyl ester, by careful proton spin decoupling experiments on compounds **5** and **6**, as shown in Chart I.

Homonuclear proton spin decoupling of **1** at 400 MHz indicated that a methylene group (C-12, δ 1.49, 1.68) and

(1) Ōmura, S.; Tanaka, Y.; Nakagawa, A.; Iwai, Y.; Takahashi, Y.; Tanaka, H. *J. Antibiot.* **1982**, *35*, 256.
(2) (a) Brufani, M.; Keller-Schierlein, W.; Löffler, W.; Mansperger, I.; Zähner, H. *Helv. Chim. Acta* **1968**, *51*, 1293. (b) Brufani, M.; Cellai, L.; Musu, C.; Keller-Schierlein, W. *Helv. Chim. Acta* **1972**, 55, 2329.
(3) (a) Kinashi, H.; Someno, K.; Sakagucni, K.; Higashijima, T.; Miyazawa, T. *Tetrahedron Lett.* **1981**, *22*, 3857. (b) *Ibid.* **1981**, *22*, 3861.
(4) (a) Von Glehn, M.; Norrestam, R.; Kierkegaard, P.; Maron, L.; Ernster, L. *FEBS Lett.* **1982**, *20*, 267. (b) Arnoux, B.; Garcia-Alvarez, M. C.; Marazano, C.; Das, B. C.; Pascard, C.; Merienne, C.; Staron, T. *J. Chem. Soc. Chem. Commun.* **1978**, 318.
(5) Kihara, T.; Kusakabe, H.; Nakamura, g.; Sakurai, T.; Isono, K. *J. Antibiot.* **1981**, *34*, 1074.
(6) (a) Feeney, J.; Shaw, D. *Chem. Commun.* **1970**, 554. (b) Bock, K.; Lundt, I.; Pederson, C. *Tetrahedron Lett.* **1973**, 1037.

0022-3263/82/1947-5413$01.25/0 

Chart I

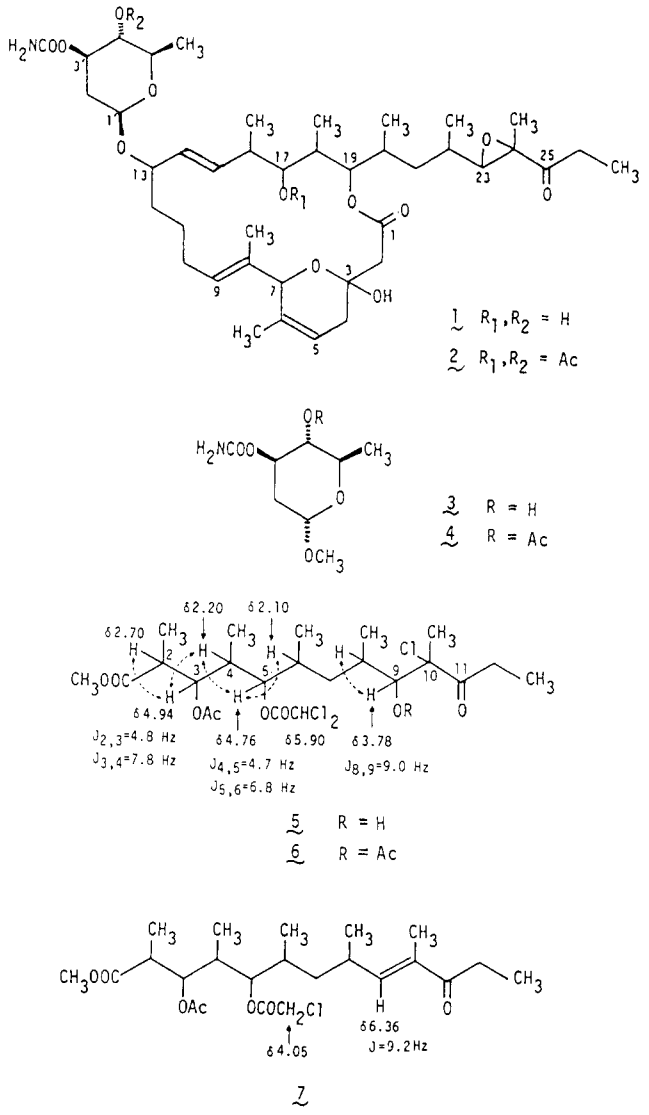
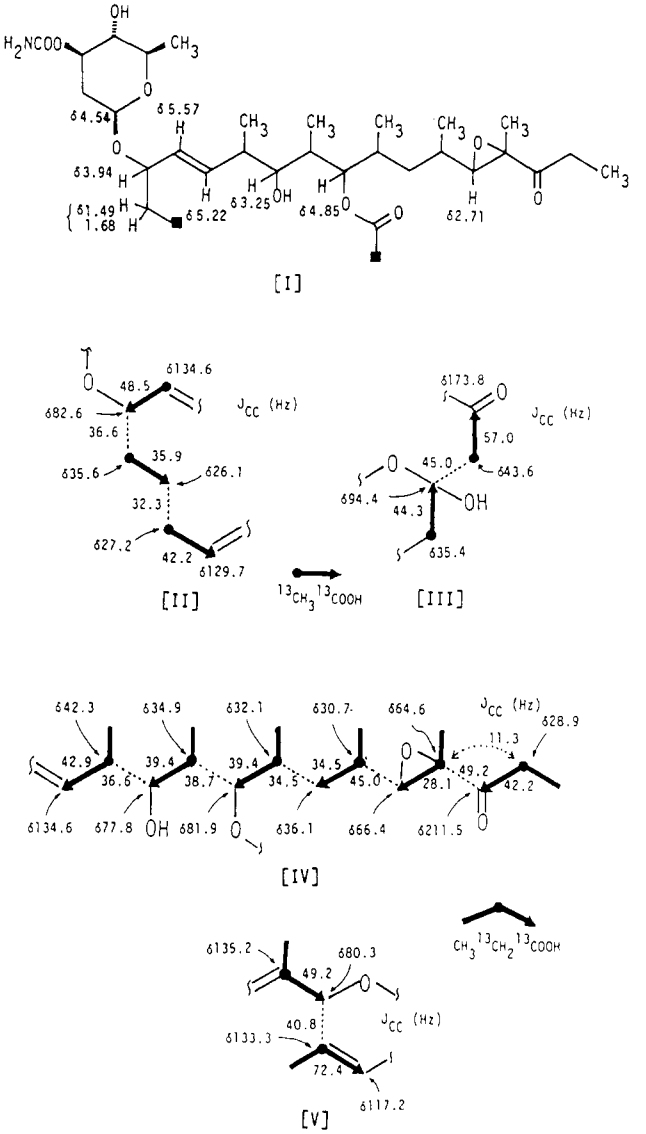


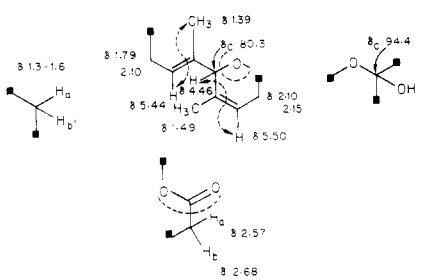

Chart II




a CH=CH moiety (C-14, $\delta_H$ 5.57, dd, $J$ = 8.8, 15.4 Hz; C-15, $\delta_H$ 5.22, dd, $J$ = 9.5, 15.4 Hz) must be located on the same carbon (C-13, $\delta_C$ 82.6, $\delta$ 3.94 (m) for its methine proton) that is bonded to the sugar moiety. The appearance of the anomeric proton at $\delta_H$ 4.54 ($J_{1'a,2'a}$ = 9.7 Hz, $J_{1',2'e}$ = 1.7 Hz) indicated the presence of $\beta$-glycosidic linkage; this leads to partial structure I ($C_{29}H_{47}NO_{10}$; Chart II). As for the remaining portion ($C_{12}H_{18}O_2$) of 1, $^{13}C$ and $^1H$ NMR data suggested that the two olefinic segments[7] contain a methyl, methylene groups attached to a double bond, a hemiketal (CHOC(OH)) and two other methylenes [$\delta$ 2.57 and 2.68 (AB ($J$ = 17.1 Hz), 2 H) and $\delta$ 1.3–1.6 (2 H].

In order to elucidate the connectivity pattern of these partial structures in 1, biosynthetic studies were performed with $^{13}C$-labeled precursors. Two feeding experiments[8] using $^{13}C$-labeled acetate and propionate clearly indicated an alternating labeling pattern typical of polyketides. The $^{13}C$ assignments of 1 were based on chemical shift considerations, selective $^{13}C\{^1H\}$ decoupling experiments, the $^{13}C$ spectra of the degradation products, and the results obtained from incorporation of $^{13}C$-enriched precursors. The $^{13}C$ spectrum of irumamycin labeled with [1-$^{13}C$]-acetate showed strong enrichment for five carbon signals at $\delta$ 173.8 (C-1), 129.7 (C-9), 94.4 (C-3), 82.6 (C-13), and 26.1 (C-11) and weak enrichment[9] for eight signals corresponding to the carbons arising from the carboxyl carbon of the propionate. The $^{13}C$ spectrum of [1,2-$^{13}C$]acetate-

(7) The following functional groups and partial structures for $C_{12}H_{18}O_2$ were inferred from $^{13}C$ and $^1H$ NMR spectral data.



(8) The $^{13}C$ precursor (0.1–0.2%, w/v), 90% enriched [1-$^{13}C$]acetate, [1,2-$^{13}C$]acetate, [1-$^{13}C$]propionate, [1,2-$^{13}C$]propionate were added to a 7-h fermentation broth (media: glycerol 1.0%, soybean meal 1.0%, glucose 0.2%, NaCl 0.3%, pH 7.0) and the cultivations were continued at 27 °C for 48 h. $^{13}C$-labeled irumamycins were isolated by solvent extraction, followed by silica gel column chromatography from the broth filtrate. [1,2-$^{13}C$]Sodium propionate was prepared according to Vederas, J. C.; Graf, W.; David, L.; Tamm, C. *Helv. Chim. Acta.* **1975**, *58*, 1886. Proton noise decoupled $^{13}C$ NMR spectra were measured with a JEOL FX-400 spectrometer. $^{13}C$–$^{13}C$ coupling constant values were obtained by $^{13}C\{^{13}C\}$ homonuclear spin decoupler.

(9) Ōmura, S.; Takeshima, H.; Nakagawa, A.; Kanemoto, N.; Lukacs, G. *Bioorg. Chem.* **1976**, *5*, 451.

labeled irumamycin exhibited additional satellites for all carbon signals each of which appeared as a doublet. These satellite peaks suggested that two intact $^{13}CH_3{}^{13}COOH$ units were incorporated at adjacent sites. Measurement of intra- and intermolecular $^{13}C$–$^{13}C$ coupling constants of acetate unit permitted derivation of partial structures II and III. The feeding experiment using [1-$^{13}C$]propionate indicated very strong enrichment for eight carbon signals at $\delta$ 211.5 (C-25), 134.6 (C-15), 117.2 (C-5), 81.9 (C-19), 80.3 (C-7), 77.8 (C-17), 66.4 (C-23), and 36.1 (C-21). Examination of the $^{13}C$–$^{13}C$-coupled signals arising from the incorporation of [1,2-$^{13}C$1propionate established partial structures IV and V. The biosynthetic evidence for structures IV and V is in harmony with the structures of **5** and with the two olefinic segments proposed on the basis of 400-MHz $^1H$ NMR spectral analysis of **1**, respectively. The presence of the $\alpha,\beta$-epoxy $\alpha',\beta'$-ethyl ketone moiety in **1** was also supported by the long-range $^{13}C$–$^{13}C$ coupling ($^3J_{CC}$ = 11.3 Hz) between the epoxy carbon (C-24, $\delta_C$ 64.6) and the methylene carbon of ethyl ketone (C-26, $\delta_C$ 28.9) because of high incorporation of propionate. It is noteworthy that both epoxide carbons ($\delta_C$ 66.4 and 64.6, $J_{CC}$ = 28.1 Hz) arise from one propionate molecule. Combination of structure I derived from chemical degradation with segments II, III, IV, and V deduced from biosynthetic studies leads to the 20-membered macrolide structure **1** containing a six-membered ring hemiketal for irumamycin.

**Acknowledgment.** We are grateful to Dr. H. Seto, University of Tokyo, Mr. H. Toyama, JEOL Co., Ltd., and Mrs. M. Yoshida, Kyowa Hakko Kogyo Co., Ltd. for NMR spectroscopic analyses and helpful discussions during this work. We also thank Misses N. Ohtani and K. Furuta for their excellent technical assistance.

**Registry No.** **1**, 81604-73-1; **2**, 83845-28-7; **3**, 21630-96-6; **4**, 36051-78-2; **5**, 83861-68-1; **6**, 83845-29-8; **7**, 83845-30-1.

**Satoshi Ōmura,* Akira Nakagawa**
**Yoshitake Tanaka**
*School of Pharmaceutical Sciences*
*Kitasato University and The Kitasato Institute*
*Minato-ku, Tokyo 108, Japan*
*Received July 13, 1982*

## Generation of Trialkylcyclopropenyl Radicals by Pulse Radiolysis and Radical–Ion Complex Formation[1]

*Summary:* Trimethylcyclopropenyl radical, generated by pulse radiolysis from the corresponding cation, complexes with the cation.

*Sir:* In spite of their theoretical interest, cyclopropenyl radicals have not been extensively characterized by physical methods, and their chemistry has been little explored. This can be attributed to the difficulty encountered in their preparation and to their high reactivity rendering them short-lived except in matrix isolation.[2] An obvious way to prepare a cyclopropenyl radical is to add an electron to the aromatic cation as has been done in the electrochemical reduction of several cyclopropenium ions.[3]

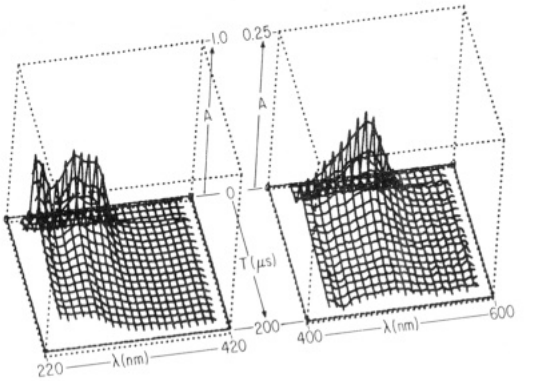



**Figure 1.** Time dependent UV and visible spectra of pulse-radiolyzed, He-saturated, aqueous solutions containing 0.001 M $Cy^+$ and 0.01 M *tert*-butyl alcohol. At 303 nm $\epsilon$ = 6600, and at 500 nm $\epsilon$ = 2800.

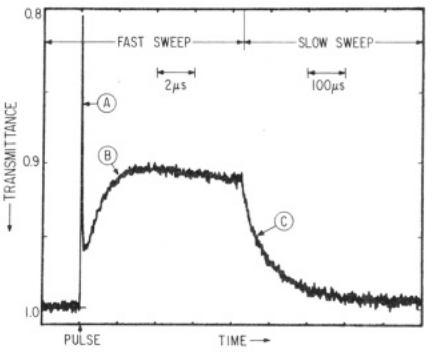



**Figure 2.** Photomultiplier response vs. time of a pulse-radiolyzed, He-saturated, aqueous solution of 0.001 M $Cy^+$ and 0.1 M *tert*-butyl alcohol (ionic strength = 0.1, pH 5.1, 25 °C, 500 nm, 10-ns pulse width): A, formation and disappearance of the hydrated electron; B, formation of $Cy^{2+}$; C, decay of $Cy_2{}^+$.

Unfortunately, the electrochemical method is not very suitable for making spectroscopic observations on the highly reactive radical produced on the electrode surface. We report here the formation of trimethylcyclopropenium radical in homogeneous solution via pulse radiolysis from the trimethylcyclopropenium cation.[4] Unexpectedly, a remarkable complex formation between the radical and the cation was found to occur.

When an aqueous solution of trimethylcyclopropenyl fluoroborate, containing 0.1 M *tert*-butyl alcohol as an OH scavenger, was subjected to a submicrosecond pulse from the ANL 20-MeV Linac, a transient absorption spectrum was recorded on a streak camera system.[5] The spectrum and its time evolution are shown in Figure 1. By measuring the rate of disappearance of the hydrated electron at 600 nm and the rate of appearance of the transient peak at 500 nm it became apparent that the carrier of the spectrum is not the primary reduction product of the ion. This is graphically shown (Figure 2) in the photomultiplier output measured at 500 nm where the sharp spike corresponds to the production and disappearance of the hydrated electron. The two rates are sufficiently different that it was possible to separate them and measure the apparent rate constant for the buildup of the 500-nm absorption, which was identical with the buildup of the absorption in the 300-nm region. By varying the concentration of the cation and by changing the radiolysis dose, it was shown that the formation of the carrier of the

(1) Work performed under contract with the Offices of Basic Energy Sciences, Division of Chemical Sciences, U.S. Department of Energy, Contract No. W-31-109-ENG-38.
(2) Closs, G. L.; Evanochko, T.; Norris, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 350.
(3) Wasielewski, M. R.; Breslow, R. *J. Am. Chem. Soc.* **1976**, *98*, 4222.
(4) Closs, G. L.; Böll, W. A.; Heyn, H.; Dev, V. *J. Am. Chem. Soc.* **1968**, *90*, 173.
(5) Schmidt, K. H.; Gordon, S.; Mulac, W. A. *Rev. Sci. Instrum.* **1976**, *47*, 356.

0022-3263/82/1947-5415$01.25/0 