was evaporated, leaving a white solid. Recrystallization from acetone–MeOH gave an HCl salt of **2**: yield 1.58 g (81%); mp 217–221 °C. Anal. ($C_{12}H_{19}N_2I$·2HCl) C, H, N.

4-Iodo-α,α′-dipiperidino-*m*-xylene (3). To a solution of **6** (2.65 g, 6.8 mmol) in 60 mL of benzene was added piperidine (2.38 g, 28 mmol) in 40 mL of benzene. The mixture was stirred at room temperature overnight. The product was isolated as in **2**. Recrystallization from benzene–MeOH gave an HCl salt of **3**: yield 1.91 g (59%); mp 213–216 °C. Anal. ($C_{18}H_{27}N_2I$·2HCl) C, H, N.

4-Iodo-*N*,*N*′-isopropyl-*m*-xylylenediamine (4). To a solution of **6** (1.00 g, 2,56 mmol) in 30 mL of benzene was added isopropylamine (0.62 g, 10.5 mmol) in 25 mL of benzene. The reaction was stirred at room temperature overnight. Workup as in the preparation of **2**. Recrystallization from acetone–hexane gave an HCl salt of **4**: yield 0.68 g (63%); mp 202–205 °C. Anal. ($C_{14}H_{21}N_2I$·2HCl·0.5H_2O) C, H, N.

2-Iodo-*N*,*N*-dimethylbenzylamine (5). To a solution of 2-iodobenzyl chloride (2.05 g, 8.1 mmol) was added 3.5 mL of anhydrous dimethylamine. The reaction mixture was sealed and stirred at room temperature overnight. The salt that formed was filtered, and the filtrate was washed twice with saturated NaCl. The organic layer was dried over Na_2SO_4 and evaporated to give a brown oil. The oil was dissolved in hexane–ether and passed through a short pad of silica gel. The solvent was evaporated, and the clear oil was transformed into its dihydrochloride salt in the usual way. Crystallization from acetone–MeOH gave an HCl salt of **5**: yield 0.95 g (39%; mp 195–197 °C. Anal. ($C_9H_{12}NI$·HCl) C, H, N.

α,α′-Dibromo-4-iodo-*m*-xylene (6). 4-Iodo-*m*-xylene (8.7 g, 37.5 mmol) and *N*-bromosuccinimide (15 g, 84.3 mmol) were suspended in 100 mL of CCl_4. Benzoyl peroxide (0.3 g) was added in portions, and the reaction mixture was heated at gentle reflux for 18 h. The succinimide was filtered off, and the CCl_4 filtrate was evaporated in vacuo to leave a brown oil. The oil was chromatographed on silica gel and eluted with petroleum ether (bp 30–60 °C) to give 3.51 g (24%) of dibromo compound **6**. A small sample was crystallized from hexane, mp 102–105 °C. Anal. ($C_8H_7IBr_2$) C, H.

General Radiolabeling Procedure. The ^{125}I-labeled compounds were prepared by exchange reaction with $Na^{125}I$ (Amersham, 17 Ci/mg). To 5–10 mg of the amine salt (**2–5**) in 0.5 mL of water was added 200–500 μCi of $Na^{125}I$ (no carrier added in NaOH, pH 7–11). The mixture was transferred to a glass tube, sealed, and placed in a water-filled bomb. The bomb was heated at 200 °C for 18–24 h. The mixture was allowed to cool and was made basic by the addition of 0.1 N NaOH. The aqueous phase was extracted twice with 1.5-mL portions of $CHCl_3$. The organics were combined, dried over Na_2SO_4, and evaporated under a stream of nitrogen. The residue was dissolved in acidic normal saline (0.6 N HCl) to give a solution of the radioiodinated amine. The purity was determined to be greater than 95% by TLC in two systems: (1) Gelman ITLC eluted with $CHCl_3$–hexane–diethylamine (4:6:0.1) and (2) silica gel 60 F-254 (Merck), $CHCl_3$–EtOH–NH_4OH (8.5:1:0.5). In all cases, the radioactivity was coincident with the R_f values of the authentic cold compound run side by side. The isolated radiochemical yields on typical runs for the compound were **2**, 32%; **3**, 30%; **4**, 39%; **5**, 61%.

Tissue Distribution Studies. Sprague–Dawley male rats (220–300 g) under light ether anesthesia were injected intravenously with 0.2 mL of a saline solution containing 0.5–2.0 μCi of test compound. At different time periods after injection the animals were put under ether anesthesia and killed by cardiectomy. The organs of interest were excised, weighed, and counted in a Beckman automatic γ counter (Model 4000).

The percent dose per organ was determined by comparison of tissue radioactivity levels to suitably diluted aliquots of the injected dose. The approximate percent dose per gram of wet tissue or organ can be calculated by dividing the percent dose per organ by the mean organ weight (mean weights: heart, 0.85 g; brain, 1.65 g; blood, 18 g; liver, 9 g; kidneys, 1.9 g; lungs, 1.6 g). The brain to blood concentration ratios were calculated from the percent dose per gram of wet tissue.

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Hydrogen Bonding and Anesthetic Potency†

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Hydrogen bond strengths in terms of the proton chemical shifts of five potent inhalation anesthetics containing acidic hydrogen were measured in cyclohexane and in methanol using the proton magnetic resonance spectroscopic method. The purpose of this study is to quantitatively compare the relative polar character of potent anesthetics. The hydrogen bond shift (Δppm) of each anesthetic is the difference in the chemical shifts of the infinitely diluted unassociated anesthetic in cyclohexane and that of the infinitely diluted hydrogen bonded anesthetic in methanol. It was found that the hydrogen bond shifts (in Δppm) are as follows: methoxyflurane, 0.72; chloroform, 0.75; halothane, 1.06; isoflurane, 1.38; enflurane, 1.44. There is a good correlation between the hydrogen bond shifts and the clinical potencies (minimum alveolar concentration in man). The conclusion from this study is that the acidic halogenated inhalation anesthetics are more potent if they form weaker hydrogen bonds.

In tabulating and comparing the thermodynamic activities (as suggested by Ferguson), Suckling,[1] one of the halothane inventors, found that the presence of acidic hydrogen on the halocarbons tends to increase anesthetic potency. This discovery was later confirmed by Poznak and Artusio, Jr.,[2] when they tested the halocarbons and halo ethers synthesized by Larsen.[3] Terrell et al.,[4] in reporting their synthesis of 36 halogenated methyl ethyl ethers as anesthetic agents, also confirmed that effective anesthetics, in general, contain at least one acidic hydrogen. Davies et al.[5] examined 45 halocarbons containing from 1 to 4 carbon atoms and concluded that anesthetics are more potent if they contain more acidic hydrogens. Two other groups of investigators[6,7] also found that a polar

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(1) C. W. Suckling, *Br. J. Anaesth.*, **29**, 466 (1957).
(2) A. van Poznak and J. F. Artusio, Jr., *Toxicol. Appl. Pharmacol.*, **2**, 363 (1960).
(3) E. R. Larsen, *Fluorine Chem. Rev.*, **3**, 1 (1969).
(4) R. C. Terrell, L. Speers, A. J. Szur, J. Treadwell, and T. R. Ucciardi, *J. Med. Chem.*, **14**, 517 (1971).
(5) R. H. Davies, R. D. Bagnall, and W. G. M. Jones, *Int. J. Quantum. Chem., Quantum Biol. Symp.*, **1**, 201 (1974).
(6) C. Hansch, A. Vittoria, C. Silipo, and P. Y. C. Jow, *J. Med. Chem.*, **18**, 546 (1975).
(7) T. DiPaolo, L. B. Kier, and L. H. Hall, *Mol. Pharmacol.*, **13**, 31 (1977).

0022-2623/82/1825-0873$01.25/0

parameter has to be assigned to some polar anesthetics in addition to hydrophobicity in order to improve the correlation with anesthetic potency. Hansch et al.[6] assigned the same polar value for nitrous oxide, diethyl ether, and all of the halocarbons and halo ethers containing acidic hydrogens. Di Paolo, Kier, and Hall[7] also used the same polar value for chloroform, halothane, and methoxyflurane in their correlation.

Trudeau et al.,[8] using the infrared spectroscopic method, studied the hydrogen bond breaking property of halocarbon and halo ether anesthetics. They concluded that those anesthetics that contain acidic hydrogen, such as chloroform, halothane, and methoxyflurane, are more powerful hydrogen bond breakers than those halocarbons and halo ethers containing no acidic hydrogen.

Recently, the hydrogen bond association constant (K), the enthalpy ($\Delta H°$), and the entropy ($\Delta S°$) of a 1:1 hydrogen bond complex of chloroform[9] and halothane[10] were reported. Brown and Chaloner[10] also observed some correlation between the acidity (pK_a) of the C–H bond in the anesthetics and their potency.

Further quantitative investigation of the polar character of a wider variety of anesthetics is needed to understand the role of the polar property of anesthetics in anesthesia. In this paper, a nuclear magnetic resonance (NMR) study of the polar hydrogens, in terms of their proton chemical shifts, of five potent anesthetics were carried out. The hydrogen bond shifts, which are the difference of the chemical shifts of the infinitely diluted unassociated anesthetic in cyclohexane and that of the infinitely diluted hydrogen bonded anesthetic in methanol, are compared with their clinical potencies.

Experimental Section

All of the ^1H NMR spectra were accumulated for 10 scans at a probe temperature of 37 ± 0.5 °C in the JEOL FX 100 NMR spectrometer, equipped with variable temperature and operating at 100 MHz in the pulsed Fourier transformed (FT) mode. The temperature was calibrated with a Telethermometer, Model 42, YSI.

The inert solvent, cyclohexane, was used to dilute the anesthetics for determining their degree of self-association through hydrogen bonding. Methanol was used to simulate the hydroxyl group in water as a proton acceptor for the anesthetics. Various concentrations of the binary mixture of the anesthetic and the solvent were prepared. Tetramethylsilane was added to the mixture as an internal reference for the measurement of proton chemical shift. Chemical shifts are expressed as part per million (ppm) downfield from Me_4Si, which appears at 0 ppm (δ scale). The hydrogen bond shift (Δppm)[11] of each anesthetic is the difference in the chemical shifts of the infinitely diluted unassociated anesthetic in cyclohexane and that of the infinitely diluted hydrogen bonded anesthetic in methanol (extrapolated values). Taking the chemical shift of the pure liquid anesthetic as zero, the dilution shift in cyclohexane is upfield and positive, while the hydrogen bonded chemical shift in methanol is downfield and negative.

The five inhalation anesthetics are chloroform ($CHCl_3$), halothane ($CF_3CHBrCl$), enflurane (CHF_2OCF_2CHClF), isoflurane ($CHF_2OCHClCF_3$), and methoxyflurane ($CH_3OCF_2CHCl_2$).

Chloroform and methanol are spectroscopic and chromatographic grade Omni Solv from MCB. Cyclohexane is an Eastman spectrograde solvent. Halothane, without inhibitor, was a gift from Halocarbon Laboratories, Inc. Enflurane was obtained from Ohio Medical Products. Isoflurane was a gift from Ohio Medical Products. Methoxyflurane was purchased from Abbott Laboratories. Me_4Si was obtained from J. T. Baker. The purity of all chemicals was checked by accumulating the proton FT NMR for 10 scans. No measurable impurities were found.

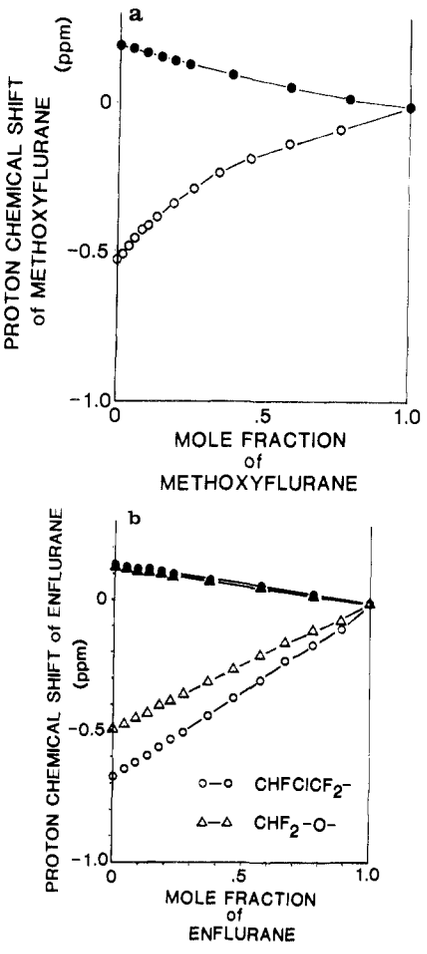

Figure 1. Proton chemical shifts (δ, ppm) of methoxyflurane (**1a**) and enflurane (**1b**), respectively, are plotted with respect to their mole fraction concentrations in cyclohexane (upper curve) and in methanol (lower curve).

Table I. ^1H NMR Hydrogen Bond Chemical Shifts of Methoxyflurane (M), Chloroform (C), Halothane (H), Isoflurane (I), and Enflurane (E) in the Solvents Cyclohexane and Methanol

	hydrogen bond chemical shift, ppm				
solvent	M	C	H	I	E
cyclohexane	0.19	0.16	0.14	0.23	0.26
methanol	0.53	0.59	0.92	1.15	1.18
total shift	0.72	0.75	1.06	1.38	1.44

Results and Discussion

Figure 1a is a plot of the acidic hydrogen chemical shift vs. the mole faction concentration of methoxyflurane in cyclohexane and in methanol. Figure 1b is a similar plot for the two acidic hydrogens of enflurane. The NMR hydrogen bond shifts (Δppm) of five anesthetics are listed in Table I. Both enflurane and isoflurane have two different acidic hydrogens. Their hydrogen bond shifts are the sum of the hydrogen bond shifts of the two acidic hydrogens in the same molecule. The self-association shifts of halo ether anesthetics are relatively larger than the halocarbon anesthetics. The ether-type oxygen probably is responsible for the larger chemical shift by acting as a

(8) G. Trudeau, K. C. Cole, R. Massuda, and C. Sandorfy, *Can. J. Chem.*, **56**, 1681 (1978).
(9) G. R. Wiley and S. I. Miller, *J. Am. Chem. Soc.*, **94**, 3287 (1972).
(10) J. M. Brown and P. A. Chaloner, *Can. J. Chem.*, **55**, 3380 (1977).
(11) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance", McGraw-Hill, New York, 1959, p 402.

Table II. Potency, Oil Solubility, and Hydrogen Bond Shift Data of Inhalation Anesthetics Studied in This Paper

agent	dog MAC,[a] % atm	man MAC,[a] % atm	H bond shift, Δppm	mouse ED_{50},[b] % atm	oil/gas partition coefficient[c]
methoxyflurane	0.24	0.16	0.72	0.22	950
chloroform	0.77	0.5[c]	0.75	0.84	400
halothane	0.87	0.74	1.06	0.77	220
isoflurane	1.28	1.15	1.38	0.57	97 (97.8)[d]
enflurane	2.06	1.68	1.44		98 (98.5)[d]

[a] Data taken from ref 13. [b] Data taken from ref 17 and 19. [c] Data taken from ref 12. [d] Data taken from the descriptions of enflurane and isoflurane by Ohio Medical Anesthetics, a division of Airco, Inc., Madison, WI.

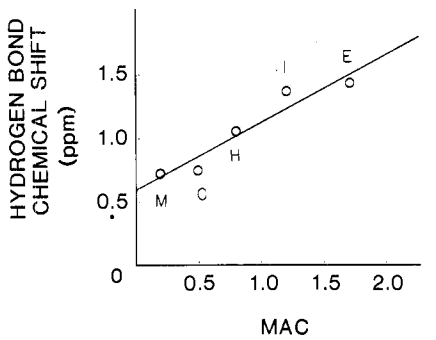

Figure 2. NMR hydrogen bond shifts (Δ ppm) of methoxyflurane (M), chloroform (C), halothane (H), isolflurane (I), and enflurane (E) and their clinical potencies (MAC in man) are correlated. A good correlation is evident (correlation coefficient, r = 0.89).

proton acceptor. The hydrogen bond shifts of these anesthetics correlate well (correlation coefficient r = 0.89) with their clinical potencies, minimum alveolar concentration in man[12,13] (MAC in man) (Table II). This is shown in Figure 2.

Based on the general definition of acids (A–H) and bases (B) proposed by G. N. Lewis, a hydrogen-bonded complex (A–H--B) is formed between an acid and a base. In the self-associated hydrogen bonding, the hydrogen in one molecule of the anesthetic is bonded to either the chlorine, bromine, or oxygen atom (proton acceptors) of another anesthetic molecule. In the hydrogen bonding between the anesthetics and methanol, the hydrogen in the anesthetic molecule is bonded to the oxygen atom of the methanol. Breaking hydrogen bonds in the self-associated anesthetics by cyclohexane increases the electron density around the hydrogen atom in the anesthetic molecule; the ^{1}H NMR signal of the anesthetic molecule is shifted to a higher magnetic field. On the other hand, the formation of hydrogen bonds between anesthetic and methanol molecules reduces the electron density around the hydrogen atom in the anesthetic molecule; the ^{1}H NMR signal of the anesthetic molecule is shifted to a lower magnetic field.

The accurate enthalpies measured by using a solution calorimetric technique were reported to correlate well with the NMR hydrogen bond shift for hydrogen bonding.[14] NMR hydrogen bond shift can, therefore, be used to indicate the hydrogen bond strength.

The hydrogen bond strength can be obtained spectroscopically by the measurements of thermodynamic parameters[9], such as the hydrogen bond formation constant (K), the enthalpy ($\Delta H°$), and the entropy ($\Delta S°$) of a 1:1 hydrogen-bonded complex. Wiley and Miller[9] reported the thermodynamic data for the hydrogen bonding of chloroform with 12 proton acceptors in cyclohexane solution using the NMR spectroscopic method. They estimated the reliability of their thermodynamic data according to the range of the saturation fraction (or degree of association) obtainable with all of these complexes. The above criterion was proposed by Deranleau[15] based on the fundamental binding theory that most accurate formation constants for the weak molecular complexes are acquired when the saturation fraction lies in the range of 0.2 to 0.8.

Using the standard stated above, Wiley and Miller[9] found that thermodynamic data on the self-association of chloroform and on the hydrogen bonding between chloroform and weak bases, such as methanol, are uncertain. This kind of difficulty arises when the association constant (K) is too small (<0.1 M^{-1}) and when solubility and chemical reactivity limit the concentration range accessible. Thermodynamic parameters measured for chloroform[9] and halothane[10] using N-methylpyrrolidinone as a base are $\Delta H°$ = −3.99 kcal·mol^{-1}, $\Delta S°$ = −10.9 cal·mol^{-1} deg^{-1} for chloroform and $\Delta H°$ = −4.7 kcal·mol^{-1} and $\Delta S°$ = −14 cal·mol^{-1} deg^{-1} for halothane. The hydrogen bond shifts for chloroform (0.753 ppm) and halothane (1.050 ppm) show the same trend. The saturation fraction (S) for the chloroform–N-methylpyrrolidinone hydrogen bond complex was 0.17–0.89.[9] The chloroform or halothane C–H proton is hydrogen bonded to the N-methylpyrrolidinone carbonyl group C=O.

It is generally agreed that the cell membrane is the site of anesthetic action. The good correlation between the olive oil/water partition coefficient and anesthetic potency has led Meyer-Overton to propose the lipid solubility hypothesis of anesthesia. The excellent correlation of anesthetics potencies with their olive oil/anesthetic gas partition coefficients[16–18] and the nearly as good correlation with other nonpolar solvents[17] were good evidence that the most probable site of anesthetic action is at the nonpolar part of the membrane. However, for enflurane and isoflurane, the potency predicted by the oil/gas partition coefficient and the MAC values show an opposing trend[19] (Table II). The relative potencies of anesthetics estimated from the righting reflex (rolling response) of mice (ED_{50}) and obtained by measuring MAC in the dog and in man are closely correlated for many anesthetics.[17,19] However, the anesthetic potencies based on the mouse (ED_{50}) and MAC for chloroform, halothane, and isoflurane are reversed in the trend (Table II). Our hydrogen bond shifts correlate better with MAC than with the olive oil/gas

(12) A. Steward, P. R. Allott, A. L. Cowles, and W. W. Mapleson, *Br. J. Anaesth.*, **45**, 282 (1973).
(13) A. L. Quasha, E. I. Eger II, and J. H. Tinker, *Anesthesiology* **53**, 315 (1980).
(14) A. D. Sherry in "The Hydrogen Bond, III, Dynamics, Thermodynamics and Special Systems", P. Schuster, G. Zundel, and C. Sandorfy, Eds., North Holland, Amsterdam, 1976, p 1119.
(15) D. A. Deranleua, *J. Am. Chem. Soc.*, **91**, 4004 (1969).
(16) E. I. Eger II, C. Lundgren, S. L. Miller, and W. C. Stevens, *Anesthesiology*, **30**, 129 (1969).
(17) K. W. Miller, W. D. M. Paton, E. B. Smith, and R. A. Smith, *Anesthesiology*, **36**, 339 (1972).
(18) Y. Tanifuji, E. I. Eger II, and R. C. Terrell, *Anesth. Analg. (Cleveland)*, **56**, 387 (1977).
(19) D. W. Kent, M. J. Halsey, E. I. Eger II, and B. Kent, *Anesth. Analg. (Cleveland)*, **56**, 97 (1977).

partition coefficients.

Davies et al.[5,20] attempted to quantitatively interpret the anesthetic potency by considering two kinds of molecular interactions, i.e., nonpolar (London dispersion force) and hydrogen bonding, in the solvation of halogenated hydrocarbons in olive oil. Anesthetic gas/oil partition coefficients were determined according to the additive effect of substituent groups upon a parent compound. For those compounds containing no acidic hydrogen, the nonpolar values estimated by using the additive component assumption correlated well with the observed potencies. A hydrogen bond proton donor scale was adapted to estimate the hydrogen bonding ability of the acidic hydrogen of the partially halogenated hydrocarbons but not that of the halogenated ethers. This scale was based on the Taft σ^* values, which account for the electron-attracting ability of the halogens in the aliphatic molecules. They also provided the relative liquid/gas partition coefficient data for some halogenated hydrocarbons and ethers measured in the proton acceptor solvent dinonyl phthalate and the nonpolar hydrocarbon solvent squalane. The nonpolar character of the anesthetics can thus be defined in the nonpolar solvent, and the proton donor ability of the anesthetic is proportional to the displacement of that compound from the line for the nonpolar anesthetics toward the polar solvent axis. From their figure (Figure 2 of ref 20) it is evident that isoflurane and enflurane are more potent hydrogen bond proton donors than halothane and methoxyflurane. But halothane is shown to be as equally a potent hydrogen bond proton donor as methoxyflurane, and isoflurane is a more potent hydrogen bond donor than enflurane. Qualitatively, their data and ours show the same trend; i.e., the acidic halogenated hydrocarbons and ethers are more potent as anesthetics if they form weaker hydrogen bonds. Apparently, this kind of solubility measurement cannot provide the quantitative difference of the hydrogen bond proton donor ability of the anesthetic. Their estimated hydrogen bond proton donor values for the halogenated hydrocarbons based on the polar substituent constants (Taft σ^* values) should be checked by direct measurements of hydrogen bonding.

The presence of the acidic hydrogen in the halogenated anesthetic provides a dipole to the molecule. This dipole probably is important for the anesthetic to effectively diffuse across the charged membrane surface by breaking the hydrogen bonds[8] or through dipole–dipole interaction. The fluidization of the trimethylammonium ion (choline head group) in the dipalmitoylphosphatidylcholine bilayer vesicle membrane by anesthetics[21] could also be partly related to this polar interaction. The study of the molecular mechanism of the hydrophilic and hydrophobic anesthetic interactions using model lipid bilayer membranes and multinuclear magnetic resonance techniques hopefully will help us understand the role of acidic hydrogen in these potent inhalation anesthetics.

In conclusion, the good correlation between the hydrogen bond shift and the clinical potency indicates that the hydrogen bond strength of the acidic hydrogen contained in these halogenated hydrocarbons and ether anesthetics is an important factor to be considered for designing new anesthetics. In the structure–activity correlation, different polar values should be assigned to these anesthetics containing acidic hydrogens according to their relative hydrogen bond shifts (or hydrogen bond strengths).

(20) R. H. Davies, R. D. Bagnall, W. Bell, and W. G. M. Jones, *Int. J. Quantum Chem., Quantum Biol. Symp.*, **3**, 171 (1976).

(21) S. Yokono, D. Shieh, and I. Ueda, *Biochim. Biophys. Acta*, **645**, 237 (1981).