Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 2, pp. 159–164, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00
© 1990 Pergamon Press plc

Analysis of anticancer drugs in biological fluids: determination of taxol with application to clinical pharmacokinetics*

JINEE RIZZO,†§ CHRISTOPHER RILEY‡§ DANIEL VON HOFF,|| JOHN KUHN,|| JERRY PHILLIPS and THOMAS BROWN

§ *Department of Pharmaceutical Chemistry, Malott Hall, University of Kansas, Lawrence, KS 66045-2504, USA*
|| *University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA*

Abstract: Taxol, a novel antimitotic, antitumor agent is currently undergoing Phase 1 clinical trials for the treatment of various tumors. An isocratic HPLC method has been developed for the determination of taxol in human plasma and urine. The method was then applied to the clinical pharmacokinetics of taxol following 6-h intravenous (i.v.) infusions at doses of 175 and 225 mg m^{-2}. A mobile phase of methanol–acetate buffer (0.02 M, pH 4.5) (65:35, v/v) was used to elute a C_8 column with detection at 227 nm. The sample preparation involved extraction with t-butyl methyl ether followed by further clean-up of the sample by solid-phase extraction. The method was linear from 0.10–10 μM injected, with a chromatographic run time of 6 min. The results obtained from the clinical study indicate that the plasma pharmacokinetics of taxol are best characterized by a two compartment open body model. Additionally, the present study resulted in the detection of a previously unreported peak which may be a metabolite of taxol.

Keywords: *HPLC; taxol; method development; plasma; urine; metabolite; clinical pharmacokinetics.*

Introduction

Taxol (Fig. 1) is an experimental antineoplastic agent of novel chemical structure and unusual mechanism of action. The drug itself is a diterpene isolated from the bark of the western or Japanese yew, *Taxus brevifolia* [1]. *In vitro*, taxol has been shown to promote the assembly of calf brain microtubules [2] and to stabilize tubulin polymers against depolymerization [3]. Cell culture studies have indicated that replication is inhibited at the G_2/M phase and immunofluorescence studies have demonstrated the formation of abnormal arrays of tubulin [4, 5]. Taxol has been tested in a variety of tumor models [6], showing good activity against B16 melanoma and MX-1 breast cancer and somewhat lower activity against L1210 and P388 leukaemias as well as CX-1 colon and LX-1 lung cancers. The promising outcome of these pre-clinical investigations has led to the present testing of taxol in multi-centre, Phase I clinical trials [7–10].

Early clinical investigations of taxol have

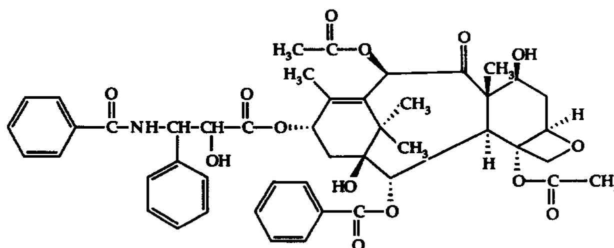

Figure 1
Structure of taxol (M_w = 853.9).

*Presented at a Symposium at the 40th Pittsburgh Conference and Exposition, Atlanta, Georgia, March 1989.
†Present address: Radian Corporation, Division of Pharmaceutical Sciences, P.O. Box 201088, Austin, TX 78720-1088, USA.
‡Author to whom correspondence should be addressed.

suggested that the drug may have activity against non-small cell lung and ovarian cancers [7]. The major clinical toxicity of taxol appears to be myelosuppression which is dose dependent and reversible [7–10]. In addition, there is a significant incidence of anaphylactoid reactions associated with the use of taxol. However, it appears that these reactions are due to the polyoxyethylated castor oil in which the taxol is formulated. Due to its poor solubility in most pharmaceutically acceptable solvents, this vehicle (Cremaphor) is required as a solvent for taxol.

Previous Phase I clinical trials [7–10] were supported by pharmacokinetic investigations [8–10] in which taxol was measured by high-performance liquid chromatography (HPLC). Longnecker *et al.* [8] have described a gradient elution method on a radial compression C_{18} column for the determination of taxol in human plasma and urine. This method had the sensitivity required for clinical studies and the recovery of taxol from biological fluids was >90%. However, the total run time was over 30 min and the taxol peak eluted with a retention time of 20 min on the front of a large endogenous component. Wiernik *et al.* [9] have published a similar gradient elution method. However, the lower limit of the linear range for this method was only 0.7 μM and the extraction efficiency of taxol from biological fluids was 76%. Recently, Grem *et al.* [10] have described an isocratic method for the determination of taxol in human plasma and urine. This method uses a C_{18} bonded phase with mobile phases of methanol–phosphoric acid (0.1 M; 75:25, v/v) for the analysis of plasma samples, and acetonitrile–perchloric acid (0.1 M; 58:42, v/v) for the analysis of urine samples.

None of the previous reports [8–10] on the determination of taxol by HPLC has provided a great deal of information on the reversed-phase chromatographic properties of taxol or how the separation of this compound from the components of biological fluids may be optimized. In addition, the question of the degradation of taxol during sample handing has not been addressed, and this may be important since taxol is known to undergo epimerization in aqueous as well as non-aqueous solutions [11, 12].

The objectives of the present study were to conduct a thorough characterization of the reversed-phase chromatographic properties of taxol and to develop a rapid isocratic method for the determination of taxol and possible metabolites in biological fluids. Ideally, the determination of *in vivo* taxol concentrations could allow for correlation between experimentally determined pharmacokinetic parameters and observed clinical toxicities. In addition, this information may be useful for the rational design of dosing protocols for future clinical studies. The methodology developed has been used to assay patient samples for pharmacologic studies during Phase 1 clinical trials.

Experimental

Chemicals and reagents

Taxol was obtained from the Drug Census and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD, USA). t-Butyl methyl ether was purchased from Aldrich Chemical (Milwaukee, WI, USA). Methanol and acetonitrile were HPLC grade and obtained from Fisher Scientific (Fair Lawn, NJ, USA). Distilled-deionized water was used throughout the study. All the other chemicals used were at least reagent grade and were obtained from various sources.

Instrumentation and chromatographic conditions

Liquid chromatography was conducted with a Shimadzu LC-6A pump (Shimadzu Co., Columbia, MO, USA), a variable wavelength (set at 227 nm, 0.02 AUFS) Shimadzu Model SPD-6AV UV detector, and a Model SIL-6A Shimadzu Autosampler. The system was controlled with a Shimadzu 6A controller and the data were reduced with a Shimadzu C-R3A reporting integrator. The injection volume was 125 μl.

Preliminary studies were carried out to examine the reversed-phase properties of taxol in various chromatographic systems. Three columns, cyanopropyl (CPS Hypersil), phenyl (Spherisorb Phenyl) and C_8 (MOS Hypersil) (each 5 μm; 150 × 4.6 mm, i.d.) were eluted with mobile phases containing various concentrations of methanol, acetonitrile or tetrahydrofuran in an acetate buffer (pH 4.5; 0.02 M). The final system chosen for the analysis of clinical samples was an MOS Hypersil column eluted with a mobile phase of methanol–sodium acetate buffer (0.02 M, pH

4.5; 35:65, v/v) at a flow rate of 2.0 ml min^{-1}. The mobile phase was filtered under vacuum prior to use. On-line UV spectra were obtained with a Perkin–Elmer Model LC-235 diode array detector (Perkin–Elmer Corp., Norwalk, CT, USA).

Standard solutions

Stock solutions of 100, 10 and 1 μg ml^{-1} of taxol were prepared in 40% acetonitrile and could be stored at −4°C for a period of 2 weeks without any perceptible degradation. Calibration curves were prepared by spiking blank donor plasma or urine with appropriate amounts of taxol to give final concentrations of 0.10–20.0 μM ($n \geq 6$). Blank extracts were also prepared and analysed at the same time.

Sample preparation

Prior to extraction, 1.0 ml of patient plasma was centrifuged for 10 min at 2000*g*. Extraction of taxol was accomplished by adding 4.0 ml of t-butyl methyl ether and vortex mixing the sample for 30 s. The mixture was then centrifuged for 10 min at 2500*g* after which 3 ml of the organic layer was removed and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 1 ml of 40% aqueous acetonitrile and applied to a C_{18} Sep-Pak (Waters Assoc., Milford, MA, USA). The sample cartridges were pre-conditioned with 2 ml of acetronitrile and 5 ml of water. The absorbed taxol was eluted with 5 ml of 70% aqueous acetonitrile [10]. The extract was evaporated to dryness at room temperature under a gentle stream of nitrogen, reconstituted in 400 μl of mobile phase and injected into the chromatograph.

Prior to extraction, the urine samples were centrifuged for 5 min at 2000*g* to separate any insoluble materials. The urine samples (1 ml) were extracted in the same way as the plasma samples, with the final residue dissolved in 400 μl of mobile phase and injected. Samples from 0–6 h during and 0–6 h post-infusion were diluted 1:10 with distilled water prior to extraction.

Clinical samples

Taxol was administered as a 6-h i.v. infusion to two patients at doses of 175 and 225 mg m^{-2}. Plasma samples were collected by i.v. sampling prior to the administration of the drug, 3 h after the start of the infusion, at the end of the infusion, and at 5, 15, 30 min and 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h following termination of the infusion. Urine samples were also collected prior to administration of the drug, during the infusion, and then during the following periods after the end of the infusion: 0–6, 6–12, 12–24, 24–36 and 36–48 h.

Results and Discussion

Chromatography

Table 1 shows the retention of taxol on the three stationary phases studied decreased with increasing concentration of organic modifier (acetonitrile, methanol or tetrahydrofuran). The optimum retention (k' = 5–10) for taxol could be obtained with any of the nine combinations of column and organic modifier studied, and the final selection of chromatographic conditions was made on the basis of peak shape and the separation of taxol from endogenous substances in plasma and urine. Accordingly, the best separation of taxol from plasma and urine was achieved on a C_8 column eluted with a mobile phase of methanol–acetate buffer (0.02 M; pH 4.5; 65:35, v/v). Under these conditions, taxol had a k' value of 5.5 and was eluted with excellent peak shape ($A_s = B/A = 1.0$, at 10% height) and column

Table 1
Capacity ratios (k') of taxol in different HPLC systems

Mobile phase* modifier (%, v/v)	k'		
	C_8†	CPS‡	Phenyl§
Acetonitrile			
35	ND	12.0	27.0
40	16.5	6.0	13.0
45	7.1	ND	5.2
50	4.2	2.2	3.7
60	2.5	ND	ND
Methanol			
45	ND	18.0	ND
50	ND	7.4	16.0
55	27.0	4.7	9.0
60	11.0	3.1	5.0
65	5.5	2.0	2.5
Tetrahydroruran			
40	13.4	10.0	16.7
45	5.5	4.5	9.2
50	4.5	ND	5.2
55	ND	ND	2.7

ND, not done.
*Type and concentration of organic modifier in an acetic acid–sodium acetate buffer (0.02 M; pH 4.5).
†MOS Hypersil (5 μm; 150 × 4.6 mm, i.d.).
‡CPS Hypersil (5 μm; 150 × 4.6 mm, i.d.).
§Spherisorb Phenyl (5 μm; 150 × 4.6 mm, i.d.).

efficiency ($N = 5.54 \times (t_r/w_{0.5})^2 \approx 8000$). The absorbance maximum for taxol in this solvent system was 227 nm which was chosen as the detection wavelength.

Extraction from biological fluids

Preliminary results for the recovery of taxol from plasma using various solvents gave the following results: chloroform 62 ± 3%; ethyl acetate 85 ± 3%; t-butyl methyl ether 101 ± 3% (RSD, $n = 3$). t-Butyl methyl ether was the solvent of choice since it gave essentially complete recovery. However, as the chromatogram in Fig. 2 illustrates, the resulting plasma extract contained several late eluting peaks that led to an overall run time approaching 30 min. Consequently, a solid-phase extraction step was included after the liquid–liquid extraction step for the removal of lipophilic components of plasma and urine.

Initially, the sample pre-treatment included the elution of taxol from the solid-phase extraction cartridge with methanol–water (65:35, v/v). However, this resulted in reduced recoveries of the drug (≈50%). This was attributed to chemical degradation of the drug during the evaporation of the solvent. This was supported by the observation that the disappearance of taxol ($t_r = 3.5$ min) was accompanied by the appearance of a new peak attributed to 7-epitaxol [12] ($t_r = 4.5$ min; Fig. 3). Previous reports have indicated that 7-epitaxol has a decreased chromatographic polarity and elutes after taxol in reversed-phase systems. The reduced polarity of 7-epitaxol, compared with the parent compound, is thought to be due to hydrogen bonding between the 7-*alpha* hydroxyl group and the acetate moiety in the 4-position [12]. Due to the degradation of taxol under these conditions, an alternative method for the recovery from the solid-phase extraction cartridge was explored and the final procedure involved elution of the drug from the C_{18} Sep-Pak with 70% acetonitrile. Since acetonitrile–water mixtures are azeotropic, it was no longer necessary to heat the samples and the solvent could be evaporated at room temperature. Using this procedure, formation of the epimer was not seen.

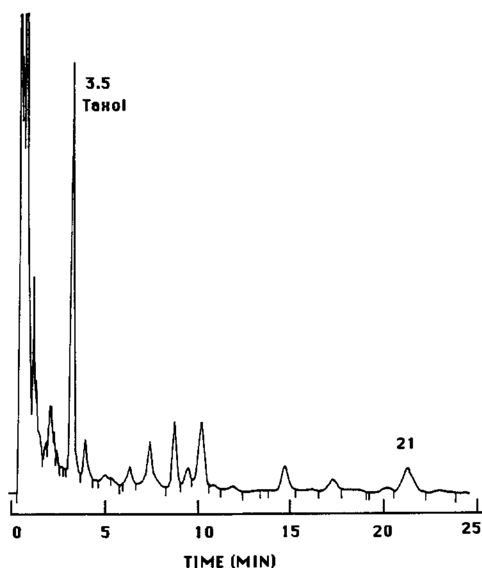

Figure 2
t-Butyl methyl ether extract of human plasma spiked with taxol. Stationary phase: MOS Hypersil (5 μm; 150 × 4.6 mm, i.d.). Mobile phase: methanol–0.02 M acetate buffer (pH 4.5) (35:65, v/v). Flow rate: 2.0 ml min^{-1}.

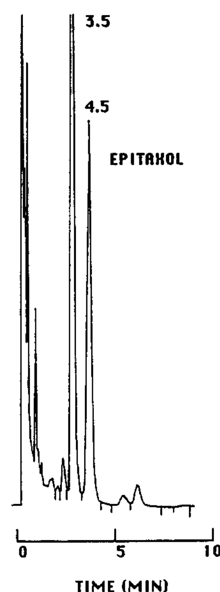

Figure 3
Chromatogram of taxol (3.5 min) and 7-epitaxol (4.5 min) after solid-phase extraction, showing the chemical instability of taxol during the evaporation of methanolic solutions. See Fig. 2 for chromatographic conditions.

Assay validation

Standard curves, in spiked plasma, were linear from 0.10–20 μM and the equation of a typical calibration curve, relating peak area, P, to the concentration of taxol, C, originally spiked into the plasma samples was:

$$P = 1.64 + 50.9C;\ r^2 = 0.998;\ n = 6$$

The between-day reproducibility of the slopes (RSD, $n = 3$) was 3.95%. The accuracy

(amount found/amount added × 100) of the procedure was determined by preparing spiked plasma samples at 0.50 and 5.0 μM (n = 2 for both concentrations), prior to the validation study. Samples at both concentrations were analysed on each day of validation study. The accuracy for 0.05 and 5.0 μM was 97.5 and 93.0%, respectively. The absolute recovery of taxol was obtained by comparing peak areas from the plasma calibration curves with those obtained from direct injection of taxol clean solutions prepared in mobile phase. The overall, absolute recovery of taxol from plasma was 88 ± 3%. Very similar results were found for the validation experiments with urine.

Patient samples

Figure 4 shows two chromatograms of plasma obtained from a patient, prior to and 30 min after a 6-h i.v. infusion of taxol (225 mg m^{-2}). The overall chromatographic analysis time was 6.0 min with taxol eluting as a symmetrical peak with a retention time of 3.5 min. Figure 4 also shows an additional peak (t_r = 2.9 min) in the plasma extract which was not present in the sample obtained prior to treatment. This peak was present in most of the patient samples and since the subjects in this study did not receive any pre-medication, the unknown peak was probably a metabolite of taxol. Chromatograms of urine extracts from the same patient (Fig. 5) show that this unknown compound was not present prior to treatment but was apparently excreted by the kidney. On-line UV spectra obtained from the unknown peak in the urine were identical to spectra obtained from the parent compound (Fig. 6), strongly suggesting that this compound arises as a result of metabolism of taxol at a site remote from the chromaphore. Further work is being conducted in these laboratories to isolate this compound and to elucidate its structure. Following termination of the infusion, plasma disappearance of taxol was biphasic, indicating both a distribution and elimination phase (Fig. 7). These pharmacokinetics investigations are continuing and the results will be presented elsewhere.

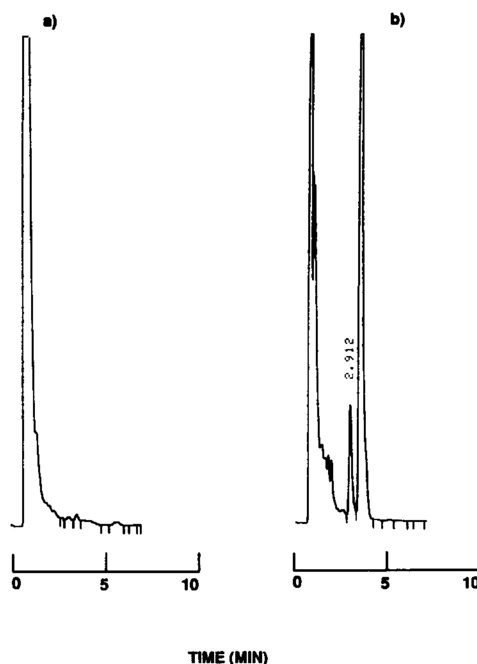

Figure 5
Chromatograms of extracts of urine from a cancer patient, obtained (a) prior to and (b) during the 6 h after a 6-h i.v. infusion of taxol (225 mg m^{-2}). See Fig. 2 for chromatographic conditions. The concentration of taxol in this sample was 43.6 μM (37.2 μg ml^{-1}).

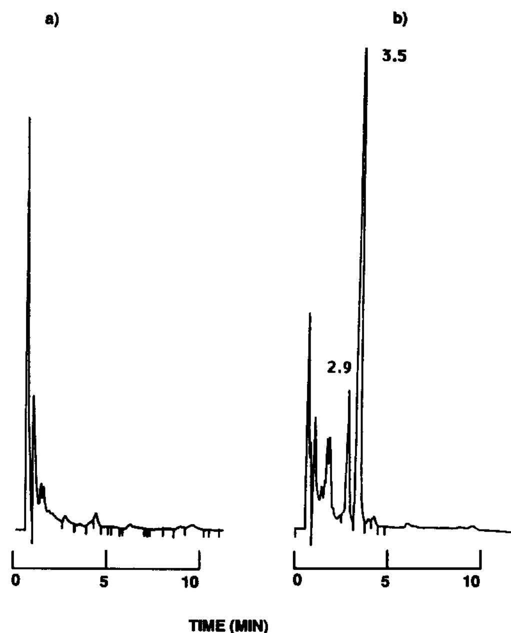

Figure 4
Chromatograms of extracts of plasma from a cancer patient, obtained (a) prior to and (b) 30 min after a 6-h i.v. infusion of taxol (225 mg m^{-2}). The peak eluting at 2.9 min was present in most patient samples and was never present in the pre-dose samples. The concentration of taxol in this sample was 2.97 μM (2.54 μg ml^{-1}). See Fig. 2 for chromatographic conditions.

Conclusions

Taxol is a very promising new anticancer agent currently undergoing clinical trials. To facilitate the accumulation of relevant phar-

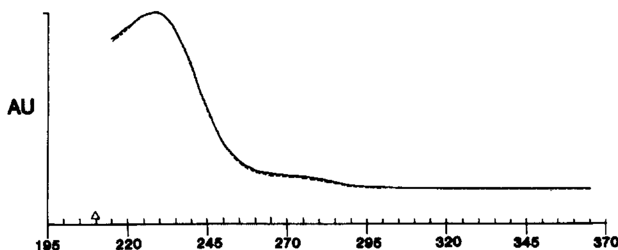

Figure 6
On-line UV spectra of taxol (———) and the unknown compound (----) having a retention time of 2.9 min (Fig. 5). The spectra were obtained at the respective peak maxima and their absorbances normalized.

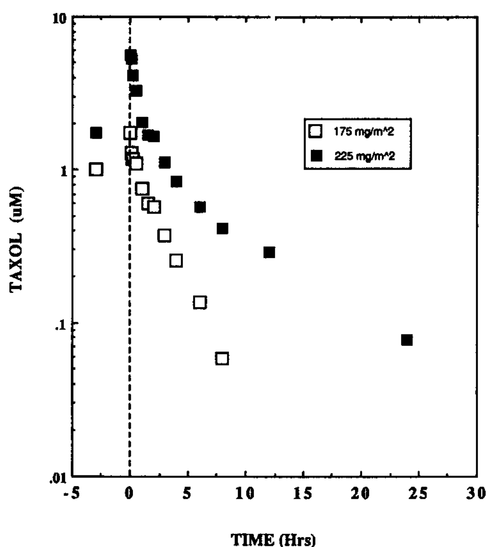

Figure 7
Representative plasma data obtained from two cancer patients receiving 6-h i.v. infusions of taxol (175 and 225 mg m^{-2}). (The dotted line represents the end of the infusion.)

macological data, a rapid isocratic HPLC method has been developed for the quantification of taxol in biological fluids. The assay requires 1 ml of plasma and has a limit of detection ($S/N = 3$) of 0.10 μM. The chromatographic run time was 6 min, and because the extracts are stable for 24 h at room temperature (<2% degradation) the method can be automated. It is expected that this assay will help to establish relationships between pharmacokinetic parameters and clinical benefits or toxicities. The rational determination of optimum dosing schedules for future clinical studies should also be facilitated.

Acknowledgements — This study was supported by National Cancer Institute Training Grant CA-09242, National Cancer Institute Contract CM-57737, National Institutes of Health Grant RR-01346, and the Veterans Research Service.

References

[1] M.C. Wani, H.L. Taylor, H.L. Wall, P. Coggon and A.T. McPhail, *J. Am. Chem. Soc.* **93**, 2325–2327 (1971).
[2] P.A. Schiff, J. Faut and S.B. Horowitz, *Nature* **227**, 655–667 (1979).
[3] P.A. Schiff and S.B. Horowitz, *Proc. Natn Acad. Sci.* **77**, 1561–1565 (1980).
[4] R.A. Bender and B.A. Chabner, In *Pharmacological Principles of Cancer Treatment* (B.A. Chabner, Ed.), pp. 256–268. Saunders, Philadelphia (1982).
[5] I.V. Sandoval and K. Weber, *J. Biol. Chem.* **255**, 6966–6974 (1980).
[6] Anon, *Clinical Brochure: Taxol (NSC-125973)*. National Cancer Institute, Bethesda (1983).
[7] R.C. Donehower, E.K. Rowinsky, L.B. Grochow, S.M. Longnecker and D.S. Ettinger, *Cancer Treat. Rep.* **71**, 1171–1177 (1987).
[8] S.M. Longnecker, R.C. Donehower, A.E. Cates, T.-L. Chen, R.B. Brundrett, L.B. Grochow, D.S. Ettinger and M. Colvin, *Cancer Treat. Rep.* **47**, 2486–2493 (1987).
[9] P.H. Wiernik, E.I. Schwartz, J.J. Strauman, J.P. Dutcher, R.B. Lipton and E. Paiette, *Cancer Res.* **47**, 2486–2493 (1987).
[10] J.L. Grem, K.D. Tutsch, K.J. Simon, D.B. Alberti, J.K.V. Wilson, D.C. Tormey, S. Swaminathan and D.L. Trump, *Cancer Treat. Rep.* **71**, 1179–1184 (1987).
[11] I. Ringel and S. Band Horwitz, *J. Pharmac. Exper. Therap.* **242**, 692–698 (1987).
[12] J.L. McLaughlin, R.W. Miller, R.G. Powell and C.R. Smith Jr, *J. Natl. Prod.* **44**, 312–319 (1981).

[*Received for review 6 March 1989; revised manuscript received 26 April 1989*]