# Biological Half-Life of Chlordiazepoxide and Its Metabolite, Demoxepam, in Man

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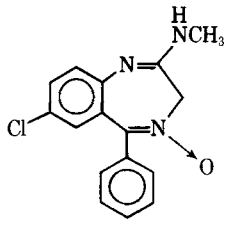
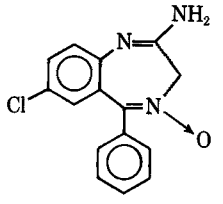
**Abstract** □ In a crossover study, single 20-mg. oral doses of chlordiazepoxide and one of its metabolites, demoxepam, were administered to six subjects. After chlordiazepoxide administration, the maximum plasma levels of intact drug were approximately 1 mcg./ml. and the half-life of plasma chlordiazepoxide ranged from 6.6 to 28 hr. In general, these half-lives were shorter and more variable than the range previously reported. In addition, a second metabolite, desmethylchlordiazepoxide, was found to reach maximum plasma levels of 0.14–0.46 mcg./ml. Demoxepam was not detected in the plasma of any subject after the single dose of chlordiazepoxide. The absorption of chlordiazepoxide was estimated to be 81% in the one subject given both an oral and an intravenous dose of the drug. After demoxepam administration, a redistribution of drug was apparent from the discontinuous fall-off curves. The eventual elimination of demoxepam from the plasma was relatively slow, with a range in half-life of 14–95 hr. In each subject, the half-life of demoxepam was markedly longer than that of chlordiazepoxide.

**Keyphrases** □ Chlordiazepoxide, metabolites—plasma levels, half-life, man □ Demoxepam—chlordiazepoxide metabolite, half-life, man □ Desmethylchlordiazepoxide—chlordiazepoxide metabolite, half-life, man □ Plasma levels, half-life—chlordiazepoxide, metabolites, man

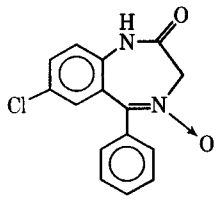
In 1963, Koechlin and D'Arconte (1) published a fluorometric assay for plasma chlordiazepoxide[1] and demoxepam[2]. They reported that the half-life of chlordiazepoxide in three human subjects ranged from 20 to 24 hr. and that demoxepam was a plasma metabolite of chlordiazepoxide in both man and dog. This biotransformation of chlordiazepoxide was confirmed in subsequent studies in which labeled demoxepam was shown to be a urinary metabolite of $^{14}$C-chlordiazepoxide in both species (2). A subsequent investigation (3) of the *in vitro* metabolism of chlordiazepoxide resulted in the identification of another metabolite, the *N*-desmethyl derivative of chlordiazepoxide (desmethylchlordiazepoxide)[3], which could not be distinguished from chlordiazepoxide by the fluorometric assay. A separate specific fluorometric assay for desmethylchlordiazepoxide was incorporated into the Koechlin and D'Arconte assay to yield a method that provided for the direct measurement of plasma demoxepam and desmethylchlordiazepoxide and the differential assay of plasma chlordiazepoxide. Using this procedure, it was found (3) that desmethylchlordiazepoxide, in addition to demoxepam, was present in plasma following the intravenous administration of chlordiazepoxide hydrochloride to man.



chlordiazepoxide



desmethylchlordiazepoxide



demoxepam

One purpose of the present study was to redetermine the rate of chlordiazepoxide elimination in man, using the method that differentiates between chlordiazepoxide and desmethylchlordiazepoxide. In addition, since demoxepam is excreted in the urine in appreciable amounts after chlordiazepoxide administration (2), it was of interest to compare the rates of elimination in man of orally administered chlordiazepoxide and demoxepam. A crossover design was used to obtain a valid comparison of both rates.

## EXPERIMENTAL

**Design**—Six healthy volunteers, normal with respect to hemoglobin, hematocrit, red blood cell count, white blood cell count, differential, serum glutamic-pyruvic transaminase, alkaline phosphatase, and urinalysis, participated in this study. They reported that they had taken no medication at all for 2 weeks prior to the study and that no chronic drug administration had occurred for the preceding 2 months. Each subject was given a 20-mg. oral dose of chlordiazepoxide and demoxepam, with a 1-week interval between doses. Information pertinent to the subjects, drugs, and the crossover design of treatment is presented in Table I.

Ten milliliters of oxalated blood was drawn just prior to the administration of each dose and 1, 2, 4, 6, 8, 12, 24, 48, and 72 hr. later. Plasma obtained on centrifugation was immediately frozen and stored at −7° until analyzed.

One additional experiment was performed approximately 6 months after the completion of the crossover study. Subject 6 (whose weight was unchanged from that shown in Table I) was given 20 mg. of chlordiazepoxide intravenously; 22.4 mg. of chlordiazepoxide hydrochloride sterile powder, Lot No. 206-11228, dissolved in 2.2 ml. of physiological saline was injected. Heparinized blood (10 ml.) was drawn at 0, 3, 6, 10, 20, and 30 min. and at 1, 2, 3, 5, 8, 24, 48, and 72 hr. Plasma was separated, frozen, and stored prior to analysis.

**Plasma Assays**—Duplicate 2-ml. aliquots of each plasma obtained after demoxepam administration were analyzed for dem-

[1] Chlordiazepoxide, 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide, is the active ingredient in the trademarked product Librium of Hoffmann–La Roche Inc., Nutley, N. J.

[2] Demoxepam, which was previously designated Ro 5-2092 or "lactam," is 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.

[3] Desmethylchlordiazepoxide, which was previously designated Ro 5-0883/1 or metabolite D-M, is 2-amino-7-chloro-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide.

**Table I**—Data Concerning the Subjects and Administration of Chlordiazepoxide and Demoxepam

| Subject[a] | | | Administration of Drugs[b] | |
|---|---|---|---|---|
| Number | Sex | Weight, kg. | First Drug | Second Drug |
| 1 | M | 85.5 | Chlordiazepoxide | Demoxepam |
| 2 | F | 67.7 | Demoxepam | Chlordiazepoxide |
| 3 | F | 59.1 | Demoxepam | Chlordiazepoxide |
| 4 | F | 61.8 | Chlordiazepoxide | Demoxepam |
| 5 | F | 61.8 | Chlordiazepoxide | Demoxepam |
| 6 | M | 68.6 | Demoxepam | Chlordiazepoxide |

[a] The subjects ranged in age from 25 to 43 years. Subjects 1–5 were Caucasians; Subject 6 was a Negro. [b] Chlordiazepoxide was given as two Libritabs (Lot Item 7603), each of which contained 10 mg. of drug (as the free base). Demoxepam was administered in two tablets, Lot No. C-7187 (C-7189)-01, each of which contained 10 mg. of the compound.

oxepam by the fluorometric assay of Koechlin and D'Arconte (1). The specificity of this method was tested by determining the fluorescence produced by two phenolic metabolites of demoxepam recently found in the dog (4). Neither of these metabolites, the 9-hydroxy derivative of demoxepam nor the 5-(4-hydroxyphenyl) derivative, produced any fluorescence. Since these two close analogs did not interfere, the assay was considered specific for demoxepam.

Following chlordiazepoxide administration, duplicate 2-ml. plasma samples were analyzed for chlordiazepoxide, desmethylchlordiazepoxide, and demoxepam by the combined fluorometric procedures already described (3). The fluorescence was measured in a spectrofluorometer (Farrand) containing a new lamp (Xenon) which was over twice as sensitive as the lamp previously used. This resulted in a two- to threefold increase in sensitivity and a new sensitivity limit of approximately 0.1 mcg./ml. of plasma for each of the three benzodiazepines. The difficulties formerly encountered (3) in measuring low levels of these compounds were thereby considerably reduced.

## RESULTS AND DISCUSSION

The plasma level data obtained after the oral administration of chlordiazepoxide and demoxepam to the six subjects are shown in Fig. 1 as a semilog plot of plasma levels *versus* time. The administered chlordiazepoxide appeared rapidly in the plasma of each subject and rose quickly to peak levels which then declined at an apparent first-order rate. The chlordiazepoxide metabolite, desmethylchlordiazepoxide, was readily determined in each subject's plasma and reached maximum levels between 8 and 24 hr. The other metabolite, demoxepam, did not reach measurable levels in any chlordiazepoxide-treated subject.

Following administration of demoxepam, the plasma levels of intact drug did not follow the pattern of an uninterrupted rise and decline as was observed with chlordiazepoxide. In every subject except Subject 3, an obvious plateau or trough in the fall-off curve was evident near the time at which the maximum plasma level of demoxepam was seen. This finding suggested that, in addition to the standard processes of absorption, distribution, and elimination, a redistribution of drug occurred. Apparently, the initial relatively rapid disappearance of plasma demoxepam was stopped or reversed by the reentrance of demoxepam into the plasma from some endogenous depot (such as adipose tissue) or from the GI tract (enterohepatic circulation). In Subject 3, the plateau in plasma levels of drug was not obvious because it was masked by the relatively slow disappearance of demoxepam from the plasma.

The curves of Fig. 1 also allow for a comparison of the elimination of the administered chlordiazepoxide and demoxepam. It is evident that in each subject, regardless of the order in which the drugs were given, chlordiazepoxide was eliminated much faster than was demoxepam.

In Table II, the elimination rates, determined by the method of least squares, are expressed in terms of both the rate constant ($\beta$) and the half-life. The peak plasma levels of approximately 1 mcg./ml. of chlordiazepoxide declined with rate constants which ranged from 0.025 to 0.105 $hr.^{-1}$; *i.e.*, the half-lives of plasma chlordiazepoxide ranged from a high value of 28 hr. to a low of 6.6 hr. If Subject 3 is excluded, this range is considerably shortened, to half-lives of 6.6–15 hr. The levels of the metabolite, desmethylchlordiazepoxide, reached 0.3–0.5 mcg./ml. in all subjects except Subject 3. Both the slower elimination of plasma chlordiazepoxide (half-life of 28 hr.) and the lower levels of desmethylchlordiazepoxide (0.14 mcg./ml.) in Subject 3 are indicative of an appreciably slower rate of chlordiazepoxide metabolism than that shown by the other five subjects.

It is evident that the previously reported (1) half-life of 20–24 hr. for chlordiazepoxide in man was an overestimation, resulting from the combination of two factors: (*a*) the assay used was not specific

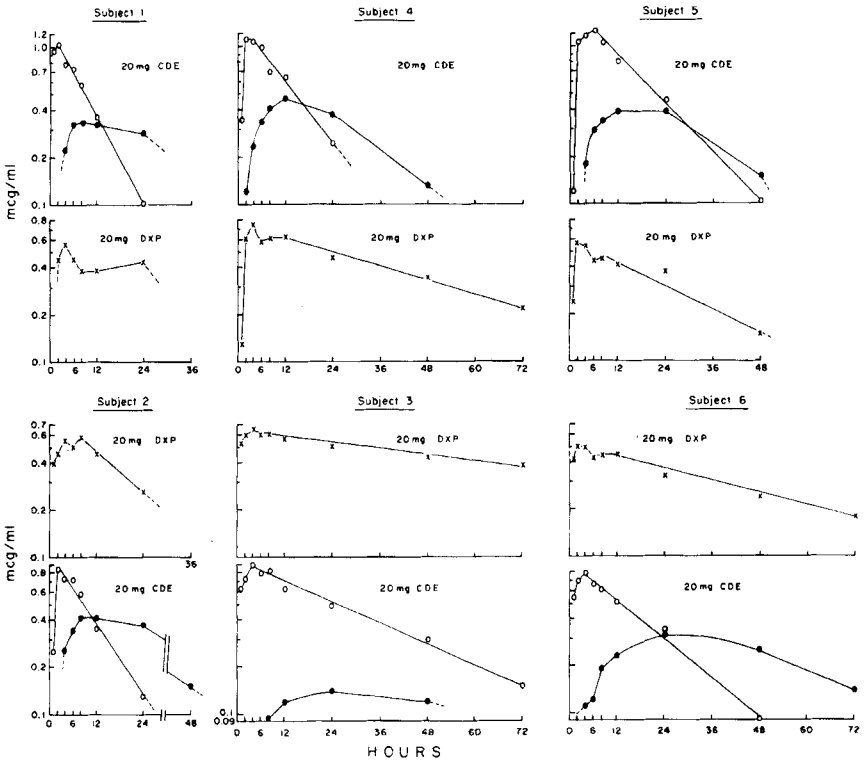



**Figure 1**—*Plasma levels of chlordiazepoxide and its metabolite, desmethylchlordiazepoxide, after a 20-mg. oral dose of chlordiazepoxide, and plasma levels of demoxepam after a 20-mg. oral dose of demoxepam. Subjects 1, 4, and 5 (top row) received chlordiazepoxide (designated CDE) first; Subjects 2, 3, and 6 (bottom row) received demoxepam (designated DXP) first. Key:* ○, *chlordiazepoxide level;* ●, *desmethylchlordiazepoxide level; and* ×, *demoxepam level. Broken line indicates that plasma concentrations were rising from or dropping to nonmeasurable levels.*

**Table II**—Plasma Concentrations of Drug (and Metabolite) following Oral Administration of Chlordiazepoxide and Demoxepam to Each of Six Subjects

| Subject Number | Dose: 20 mg. p.o. of Chlordiazepoxide[a] — Plasma Chlordiazepoxide — Peak Level, mcg./ml. at hr.[b] | Elimination Rate[c] — $\beta$, hr.$^{-1}$ | Half-Life, hr. | Plasma Desmethylchlordiazepoxide Peak Level, mcg./ml. at hr.[b] | Dose: 20 mg. p.o. of Demoxepam — Plasma Demoxepam — Peak Level, mcg./ml. at hr.[b] | Elimination Rate — $\beta$, hr.$^{-1}$ | Half-Life, hr. | Ratio of the Rates of Elimination (Chlordiazepoxide $\beta$)/(Demoxepam $\beta$) |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.02 at 2 | 0.105 | 6.6 | 0.33 at 8 | 0.56 at 8 | n.m.[d] | n.m. | n.m. |
| 2 | 0.84 at 2 | 0.088 | 7.9 | 0.41 at 8–12 | 0.58 at 8 | 0.050 | 14 | 1.8 |
| 3 | 0.89 at 4 | 0.025 | 28 | 0.14 at 24 | 0.64 at 4 | 0.0073 | 95 | 3.4 |
| 4 | 1.10 at 2 | 0.073 | 9.5 | 0.46 at 12 | 0.74 at 4 | 0.017 | 42 | 4.3 |
| 5 | 1.24 at 6 | 0.059 | 12 | 0.38 at 12–24 | 0.56 at 2 | 0.026 | 27 | 2.3 |
| 6 | 0.78 at 4 | 0.047 | 15 | 0.31 at 24 | 0.50 at 2 | 0.015 | 46 | 3.1 |

[a] Following the chlordiazepoxide dose, plasma was analyzed for intact drug and two metabolites, desmethylchlordiazepoxide and demoxepam. Measurable levels of desmethylchlordiazepoxide, but not of demoxepam, were found in each subject. [b] The hour shown is the time at which the peak level was observed. [c] The elimination rate constant, $\beta$, and half-life are related: $\beta = 0.693/\text{half-life}$. [d] n.m. = not measurable.

but measured the combined plasma levels of chlordiazepoxide and desmethylchlordiazepoxide, and (*b*) plasma desmethylchlordiazepoxide rose to appreciable levels with time. The present study demonstrates that the half-life of chlordiazepoxide is not only shorter than previously reported but is also more variable. This variability may be genetically controlled, as was reported for antipyrine (5) and phenylbutazone (6).

The peak plasma levels of orally administered demoxepam, seen after 2–8 hr., varied within a relatively narrow range of 0.50–0.72 mcg./ml. (Table II). The half-life, however, was much more variable and ranged from 14 to 95 hr. Subject 3 was consistent in exhibiting the slowest elimination of both chlordiazepoxide and demoxepam. From the rates of elimination of chlordiazepoxide and demoxepam in the same subject (last column of Table II), it is clear that the metabolism of demoxepam was 2–4 times slower than that of chlordiazepoxide.

The intravenous administration of chlordiazepoxide to one subject provided data that allowed for a detailed pharmacokinetic analysis of its disposition. The plasma levels of chlordiazepoxide and desmethylchlordiazepoxide obtained after both routes of chlordiazepoxide administration to Subject 6 are compared in Fig. 2. The plasma levels of chlordiazepoxide found after intravenous administration apparently declined biexponentially and were fitted by means of an "N-LIN" computer program (7) to the biexponential equation:

$$C_t = Ae^{-\alpha t} + Be^{\beta - t} \qquad \text{(Eq. 1)}$$

in which $C_t$ is the plasma level at time $t$, $A$ is the zero-time intercept and $\alpha$ the rate constant of the fast disposition rate, and $B$ and $\beta$ are the corresponding parameters of the slow disposition rate.

The fast disposition rate constant, $\alpha$, was found to be 11.5 hr.$^{-1}$, which corresponds to a half-life of only 3.6 min. The slow disposition rate constant, $\beta$, of 0.0488 hr.$^{-1}$ corresponds to a half-life of 14 hr., which agrees with the half-life of 15 hr. obtained when chlordiazepoxide was given orally to this subject (Table II). The volume of distribution of the central compartment ($V_p$) of a two-compartment open-system model (8) was calculated from

$$V_p = \text{dose}/(A + B) \qquad \text{(Eq. 2)}$$

to be 3.3 l. (dose = 20 mg. chlordiazepoxide, $A$ = 5.0 mg./l., and $B$ = 1.1 mg./l.). This volume, which is equivalent to only 4.8% of the body weight of Subject 6, approximates the plasma volume. The total volume of distribution was 25% of the body weight, regardless of whether calculated as described by Riegelman *et al.* (8) or Gibaldi *et al.* (9).

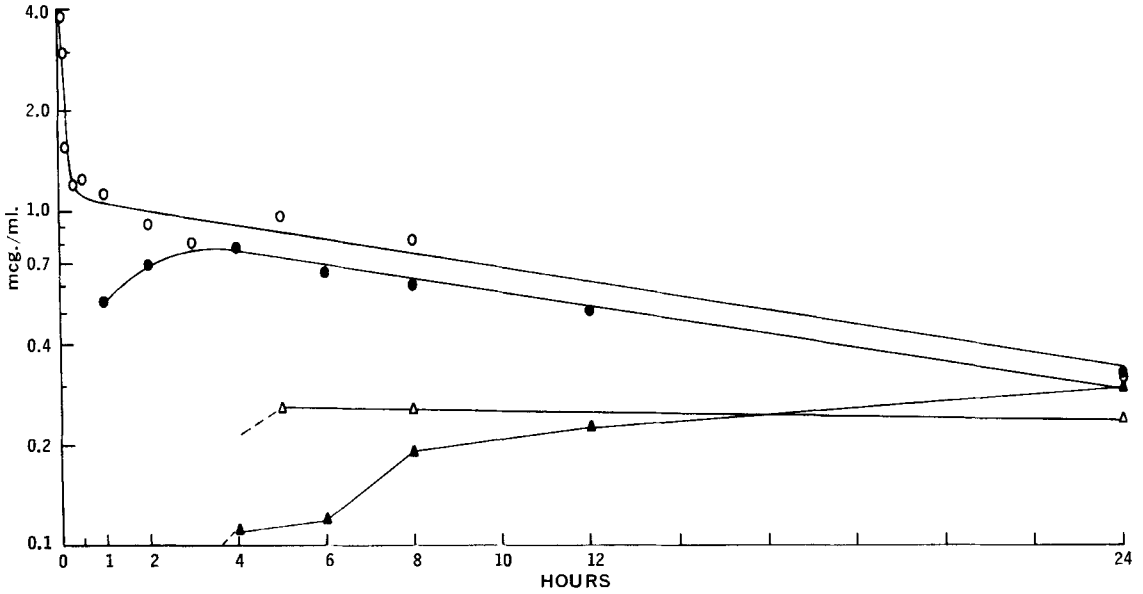



**Figure 2**—*Plasma levels of chlordiazepoxide and desmethylchlordiazepoxide after intravenous and oral administration of chlordiazepoxide to Subject 6. Key: intravenous chlordiazepoxide: plasma chlordiazepoxide* (O) *and desmethylchlordiazepoxide* (△). *Both chlordiazepoxide and desmethylchlordiazepoxide were not present in measurable amounts after 24 hr. Oral chlordiazepoxide: plasma chlordiazepoxide* (●) *and desmethylchlordiazepoxide* (▲); *these are the same curves shown in Fig. 1.*

The extent of chlordiazepoxide absorption was estimated by comparing the area subtended by the chlordiazepoxide plasma levels found after oral administration to the corresponding area seen after intravenous drug administration. This estimate indicated that 81% of the orally administered chlordiazepoxide was absorbed by Subject 6. In addition, it suggests that therapeutic doses of chlordiazepoxide are well absorbed in man.

Plasma levels of desmethylchlordiazepoxide were first measurable 5 hr. after intravenous chlordiazepoxide was given (Fig. 2). At this time, 0.26 mcg./ml. was seen; little decline was evident for the next 19 hr. Measurable levels of demoxepam were found only after 48 and 72 hr. and were close to the sensitivity limit of 0.14 mcg./ml.

Since the oral administration of $^{14}$C-chlordiazepoxide to two human subjects resulted in the urinary excretion of only negligible amounts of intact drug (2), it is evident that chlordiazepoxide in man is eliminated almost entirely by biotransformation. The appearance of plasma desmethylchlordiazepoxide and the absence of plasma demoxepam in the present study support the contention (3) that desmethylchlordiazepoxide is an intermediate in the biotransformation of chlordiazepoxide to demoxepam. This pathway was recently demonstrated in the dog by Kaplan *et al.* (7). Their pharmacokinetic evaluation of chlordiazepoxide disposition revealed that the drug was eliminated by essentially quantitative biotransformation to desmethylchlordiazepoxide, which was then itself extensively biotransformed with approximately one-half going to demoxepam.

The accumulation of plasma levels of desmethylchlordiazepoxide and demoxepam in a human subject administered chlordiazepoxide chronically was previously reported (3). This finding is also supported by those presented here which showed that chlordiazepoxide administration led to persistent plasma levels of desmethylchlordiazepoxide and that demoxepam was eliminated at a considerably slower rate than was chlordiazepoxide. Since demoxepam was reported (10) to have antianxiety activity in man, this metabolite may play a significant role in contributing to the activity of chlordiazepoxide.

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# Comparison of Pigments in Carpophores and Saprophytic Cultures of *Paxillus panuoides* and *Paxillus atrotomentosus*


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**Abstract** □ Chromatographic studies, using several solvent systems with thin-layer polyamide and silica gel G plates, indicated that *Paxillus panuoides* (Fr.) Fr. and *P. atrotomentosus* (Batsch) Fr. contain several common pigments in both carpophores and surface cultures. Atromentin was isolated and identified from the carpophores of *P. panuoides*, and atromentic and xerocomic acids were recovered and identified from the cultures of this species. These pigments were reported previously from the corresponding growth forms of *P. atrotomentosus*. Identification of the pigments was based on comparisons of chromatographic properties and spectral data (IR, UV, and high-resolution mass spectra) with authentic materials. Preliminary interpretation of the complex mass spectrum of atromentin suggested the involvement of at least three basic fragmentation pathways. The pKa values and fluorescent spectra (before and after exposure to radiant energy) of atromentic, pulvinic, and xerocomic acids were determined, and the response of xerocomic acid to thermal energy during sublimation was clarified.



**Keyphrases** □ *Paxillus atrotomentosus*, *P. panuoides*—pigment comparison □ Atromentin, *Paxillus* carpophores—isolation, identification □ Atromentic acid, *Paxillus* cultures—isolation, identification □ Xerocomic acid, *Paxillus* cultures—isolation, identification □ Tetronic acids—*P. atrotomentosus*, *P. panuoides* □ TLC—isolation, tetronic acids


*Paxillus panuoides* (Fr.) Fr. and *P. atrotomentosus* (Batsch) Fr. are lignicolous mushrooms, frequently found around old conifer stumps (1). In addition, the smaller, sessile *P. panuoides* may be found on sawdust piles or on timbers of mines, cellars, and similar places where it is a active wood destroyer. In the latter areas, carpophore formation can occur in the semidarkness after the soft yellow mycelium causes extensive decay and a vivid yellow discoloration of the wood (2). Similarly diffusible yellow pigments, which accumulate in surface cultures of *P. atrotomentosus*, were shown to include atromentic and xerocomic acids (3), diphenyl-substituted tetronic acid derivatives. Knowledge of pigments in carpophores of *P. atrotomentosus* and *P. panuoides* is restricted to the isolation of atromentin from the former species (4).

The desirability of comparative studies of *P. atrotomentosus* and *P. panuoides* was suggested by the distinctive metabolic capabilities noted previously for carpophores and vegetative mycelium of *P. atrotomen-*