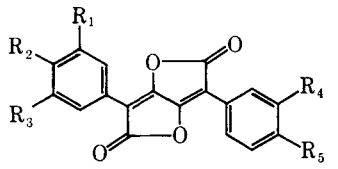
Isolation of Diphenyl-Substituted Tetronic Acids from Cultures of *Paxillus atrotomentosus*

M. C. GAYLORD, R. G. BENEDICT, G. M. HATFIELD, and L. R. BRADY

Abstract □ Pigments which accumulated in surface cultures of *Paxillus atrotomentosus* (Batsch) Fr. were studied. The nutrient broth and mycelium of 2-month-old cultures were blended, and the pigments were partitioned into ether. Two pigments were isolated by dry-column chromatography using a silica gel adsorbent and ether saturated with concentrated HCl as a chromatographic solvent. The major pigment was identified as xerocomic acid, and the minor pigment was shown to be atromentic acid. Identification of these acids was based on UV and IR spectra, mass spectral fragmentation patterns, high-resolution mass spectroscopy, and properties of their acetyl derivatives. Examination of fresh carpophores of *P. atrotomentosus* failed to demonstrate detectable amounts of either of these tetronic acids. These pigments have been reported to occur in fresh carpophores of some species in the Boletaceae and Gomphidiaceae, but this is the first reported presence of any diphenyl-substituted tetronic acid in the saprophytic culture of a fungus.

Keyphrases □ Tetronic acids, diphenyl substituted—*Paxillus atrotomentosus* cultures □ Pigment formation—*P. atrotomentosus* cultures □ Column chromatography—separation □ Paper chromatography—identification □ Electrophoresis—separation monitoring

Diphenyl-substituted tetronic acids (pulvinic acid derivatives) have long been known to be characteristic constituents of certain lichens (1, 2). Experimental evidence (3) suggests that polyporic acid, a terphenylquinone present in basidiomycetes as well as in lichens, is a precursor of pulvinic acid lactone (I) and calycin in the thalli of the lichen *Pseudocyphellaria crocata*. A number of terphenylquinones are known to occur in basidiomycetes (4), especially in the Hydnaceae, but information on the occurrence of pulvinic acid derivatives in basidiomycetes is restricted essentially to recent reports on the presence of atromentic acid (II), gomphidic acid (III), variegatic acid (IV), and xerocomic acid (V) in carpophores of some species in the Boletaceae and Gomphidiaceae (Table I).



	R_1	R_2	R_3	R_4	R_5
I, pulvinic acid lactone	H	H	H	H	H
II, atromentic acid lactone	H	OH	H	H	OH
III, gomphidic acid lactone	OH	OH	OH	H	OH
IV, variegatic acid lactone	OH	OH	H	OH	OH
V, xerocomic acid lactone	OH	OH	H	H	OH

Pigmentation was noted in surface cultures of *Paxillus atrotomentosus* (Batsch) Fr., and studies were initiated to determine the identity of the pigments produced by vegetative mycelia of this agaric. Carpophores of *P. atrotomentosus* are known to contain the terphenylquinone, atromentin (9), but no information is available in the literature on the pigments formed by vegetative growth of this species or on the occurrence of tetronic acids in carpophores of the fungus.

Table I—Occurrence of Diphenyl-Substituted Tetronic Acids in Carpophores of Basidiomycetes

Species	Reported Tetronic Acid
Boletaceae	
Boletus appendiculatus Fr.	Variegatic acid (5)
B. calopus Fr.	Variegatic acid (6)
B. erythropus (Fr.) Secr.	Variegatic acid (5, 6)
Suillus bovinus (Fr.) Kuntze	Variegatic acid (5)
S. variegatus (Fr.) Kuntze	Variegatic acid (5, 7)
Xerocomus chrysenteron (St. Amans) Quél.	Atromentic acid (6), variegatic acid (6), xerocomic acid (6)
Gomphidiaceae	
Gomphidius glutinosus (Fr.) Fr.	Atromentic acid (8), gomphidic acid (8), xerocomic acid (8)

EXPERIMENTAL

Origin of Culture and Carpophores—A culture of *P. atrotomentosus* was used in this study.[1] Stock cultures were maintained on a recommended cherry agar and were transferred monthly.

Carpophores of this mushroom were obtained from the Quinault River area of western Washington during September 1969.[2]

Vegetative Growth of *P. atrotomentosus*—Liquid nutrient medium for surface cultivation of the fungus was prepared by steaming 1 kg. of pitted fresh, or unsweetened frozen, sour cherries in 1 l. of distilled water for 2 hr. Twenty milliliters of strained cherry decoction and 30 ml. of distilled water were added to each 500-ml. Roux bottle, and the nutrient solutions were autoclaved for 15 min. at 15 lb. pressure. Sterile nutrient solutions were inoculated immediately after preparation, using a homogenate obtained by removing the mycelium of *P. atrotomentosus* from 10–14-day-old agar-slant cultures and blending for 5 sec. with a small volume of sterile water.

Preliminary observations suggested that the best results were obtained when the cultures were incubated at 20°, that a pigment with chromatographic properties of atromentic acid was present in the cultures after 35 days, and that maximum pigment accumulation occurred between 50 and 60 days. Shortly after this optimal period, the cultures tended to undergo degradative changes, and pigments were no longer extractable with ether. Similar deteriorations occurred very rapidly in cultures incubated at 30° or exposed to intense light for a significant period. Thus, the cultures employed in this study were incubated routinely in the dark at 20° for 50–60 days.

Very little growth was evident in the cultures at the end of the 1st week. Fine vegetative mycelia gradually radiated from the submerged fragments of inoculum. Brown spherical aggregations began to appear in zonations around each growing point; after 21 days, scattered buff-colored tufts of aerial mycelium were developing from the dark-brown aggregations. In 38 days a yellowish-beige mycelial growth covered approximately one-half of the yellow-colored nutrient broth in the Roux bottles. Between 50 and 60 days, aerial mycelia became yellow-orange at the edges of the mycelial mats and fluoresced a bright orange under UV light. Microscopic examination

[1] Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

[2] Identification was provided by Dr. D. E. Stuntz, Department of Botany, University of Washington, Seattle, Wash.

Table II—Chromatographic Separation of Selected Terphenylquinones and Pulvinic Acid Derivatives

Compound	Chromatographic System[a] and R_f Values					
	A	B	C	D	E	F
Aurantiacin	0.85	0.64	0.99	0.99	—	—
Thelephoric acid	0.63	0	0.05	0	—	—
Polyporic acid	0.15	0.98	0.70	0.72	—	—
Pulvinic acid lactone	0.84	0.94	0.25	0.95	—	—
Pulvinic acid	0.63	0.90	0.54	0.92	—	—
Atromentin	0.21	0.74	0.52	0.35	0.71	0.93
Atromentic acid lactone	0.83	0.81	0	0.99	0.97	0.87
Atromentic acid	0.53	0.64	0.07–0.17	0.72	0.90	0.82
Xerocomic acid	0.50	0.58	0.04–0.12	0.50	0.85	0.93

[a] System A: silica gel G thin layer, methyl ethyl ketone–H_2O–formic acid (250:25:1); System B: silica gel G thin layer, ether saturated with concentrated HCl; System C: polyamide thin layer, methyl ethyl ketone–H_2O–formic acid (250:25:1); System D: kieselguhr G thin layer, benzene–methyl formate–formic acid (13:5:4); System E: Whatman No. 1 paper, *n*-butanol–acetone–H_2O (2:5:2); and System F: Whatman No. 1 paper, H_2O–methyl ethyl ketone–diethylamine (921:77:2).

of the aerial mycelia at this stage revealed the presence of specialized strand formations; these strands resembled the dark velvety hyphal structures found with numerous brown incrustations on the stipe of the mature carpophore of *P. atrotomentosus*.

Degradative changes in the cultures appeared to commence shortly after a complete mycelial mat was developed. These changes were associated with a rapid transition of mycelial color from yellow-orange to brown. The color of the nutrient broth also changed from yellow to brown.

Chromatographic Examination—Various chromatographic procedures were examined for their utility in detecting pigments in the cultures and in monitoring the isolation and purification manipulations. The four TLC and two paper chromatographic systems which offered the greatest value are listed in Table II. The silica gel G[3] and kieselguhr G[3] TLC plates were activated at 110° for 30 min. The polyamide[4] system had been developed to examine fungal terphenylquinones (10), and the kieselguhr system had been used successfully with certain diphenyl-substituted tetronic acids (6, 8). The paper chromatographic systems were particularly useful for distinguishing atromentic and xerocomic acids.

The various pigments could be detected on the chromatograms without the use of any visualization reactions. However, observations of the chromatograms under longwave UV light provided some information which aided in distinguishing the various pigments. Atromentin, aurantiacin, polyporic acid, and thelephoric acid absorb UV light without fluorescing. The fluorescent colors noted with the other compounds were: atromentic acid, dull yellow; atromentic acid lactone and pulvinic acid, orange; pulvinic acid lactone, bright yellow; and xerocomic acid, dull orange.

Electrophoretic Examination—A paper electrophoretic procedure was also found to be useful in monitoring the isolation and purification manipulations. Samples were applied 20 cm. from the positive edge of 46 × 57-cm. sheets of Whatman No. 3 paper. The paper was then carefully dampened with a pH 2 buffer (formic acid–acetic acid–H_2O, 1:4:28). The dampened sheet was placed on a rack, covered, and run at 2500 v. and 100 ma. for 2 hr.; the sheet was covered with a crude fraction of nonane (b.p. approx. 100°), since the high voltage required the use of a cooling system.

Atromentic and xerocomic acids moved distances of 6.5 and 5.8 cm., respectively, toward the positive pole under the experimental conditions. Atromentic acid lactone and atromentin showed no migration in this electrophoretic system, and polyporic acid and pulvinic acid apparently were either eluted or ran off the sheet in the 2-hr. time interval.

Isolation and Purification of Pigments from *P. atrotomentosus* Cultures—Stability problems with some terphenylquinones and hydroxylated diphenyl-substituted tetronic acids and the suspected distribution of pigments in both the mycelium and culture broth suggested the desirability of extracting the total culture mixture without drying. The pooled mycelium and nutrient broth from 56-day-old cultures were blended in a large blender, and the mixture was extracted by shaking repeatedly with ether. Approximately 50 l. of ether was required to extract exhaustively the pigments from 50 Roux bottle cultures. The initial ether extract was dried over anhydrous Na_2SO_4. The ether solution was separated, and the solvent was removed under reduced pressure at 32°. Most of the pigmented material in the resulting red residue was soluble in water; the pigments were dissolved in approximately 60 ml. of cold water and separated from less polar impurities by filtration. Pigments in the aqueous filtrate were partitioned into ether, and the ether was removed to give a crude pigment fraction. Precautions were taken during all of the purification manipulations to minimize exposure of the pigments to light.

The chromatographic and electrophoretic monitoring systems indicated the presence of one major pigment and one minor component in the crude pigment fraction. Dry-column chromatography (11) was selected for the separation of these substances. Silica gel was washed successively with water, methanol, and ether. The washed silica gel was dried at 140° for 24 hr. and then was equilibrated with 10% w/v water for 3 hr. in a ball mill. The crude pigment fraction was adsorbed on five times its weight of silica gel, and aliquots of this material were added to the top of approximately 1.8 × 30-cm. silica gel columns. Ether saturated with concentrated HCl was used as the chromatographic solvent system. The major and minor pigments, which were subsequently established to be xerocomic and atromentic acids, respectively, migrated at approximately R_f 0.51 and 0.60. These bands were removed from the columns, the pigments were eluted from the adsorbent with the HCl-saturated ether, and the pigment materials were rechromatographed individually on a second series of columns for ultimate separation. Each 100 mg. of the crude pigment fraction yielded 75 mg. of xerocomic acid and 2 mg. of atromentic acid. Some pigmented material could not be eluted from the adsorbent and remained at the top of the columns. No evidence of a pigment band was noted at R_f 0.80, the approximate migration of atromentin in the dry-column system.

Table III shows the relative weights of the various components found in 50 Roux-bottle cultures which were harvested at 56 days. After the blended cultures were extracted with ether, the mycelium and nutrient broth were separated and freeze-dried.

Identification of Xerocomic Acid[5]—The UV spectra of the major pigment, $\lambda_{max.}^{methanol}$ 408 and 260 mμ, $\lambda_{max.}^{water}$ 378 and 256 mμ, and $\lambda_{max.}^{0.01\ M\ NaHCO_3}$ 620, 362, 320 (sh), and 230 mμ, agreed with the published absorption properties of xerocomic acid (6) and a related tetronic acid (5). The IR spectrum (KBr pellet), with absorption peaks at 3190, 2882, 2558, 1739, 1675, 1600, and 1513 $cm.^{-1}$, was also in general agreement with the reported spectrum for xerocomic acid (6).

The mass spectral fragmentation of diphenyl-substituted tetronic acids has been studied (12), and the fragmentation patterns of some hydroxylated pulvinic acid derivatives have been confirmed (6, 8). Water is readily lost from these tetronic acids, and the molecular ions in the mass spectra correspond to the respective lactones. The tetronic acid lactones exhibit two basic fragmentation series. The first series involves successive elimination of small fragments; this established fragmentation series for xerocomic acid lactone is *m/e* 338 → 310 → 282 →226. The second series involves cleaving the lactone into two equal or approximately equal ions and subsequent elimination of small fragments. Xerocomic acid lactone, an unsym-

[3] Obtained from Brinkmann Instruments Inc., Westbury, N. Y.
[4] Obtained from Alupharm Chemicals, New Orleans, La.

[5] UV and IR spectra were obtained with a Beckman UV spectrophotometer, model DB, and a Beckman IR spectrophotometer, model IR5A, Beckman Instruments, Inc., Fullerton, Calif. Mass spectra were determined with a Picker-AEI MS9 mass spectrometer, Picker Nuclear Division, White Plains, N. Y.

Table III—Weight of Culture Components and Pigment Fractions from 50 Roux Bottle Cultures of *P. atrotomentosus* Harvested at 56 Days

	Dry Weight, g.	Yield Based on Total Solids, %
Extracted mycelia	20.03	43.7
Solids from extracted nutrient broth	19.55	42.7
Initial ether extract	6.17	13.5
Partitioned crude pigment fraction	2.76	6.0
Xerocomic acid	2.07	4.5
Atromentic acid	0.055	0.001

metrical molecule, fragments initially into ions *m/e* 177 and 161, and these undergo the following fragmentations: *m/e* 177 → 149 → 121 → 75 and *m/e* 161 → 133 → 105 → 77.

The mass spectrum of the isolated pigment revealed ions at *m/e* 338, 310, 282, 226, 177, 161, 149, 133, 121, 105, 77, and 75. These observations agree completely with the known fragmentation patterns of xerocomic acid lactone. The high-resolution parent ion peak at *m/e* 338.0426, both observed and calculated for $C_{18}H_{10}O_7$, and other ionic fragments in the high-resolution mass spectrum further supported the identity of xerocomic acid lactone.

Confirmation of the identity of the major pigment as xerocomic acid was obtained by preparing an acetyl derivative, using an established procedure (6). The tetronic acid (50 mg.) was refluxed for 5 min. with 1 ml. of acetic anhydride and a trace of concentrated H_2SO_4. The yellow needles of triacetylxerocomic acid lactone, which separated upon cooling, were removed by filtration and recrystallized from glacial acetic acid. The properties of this acetyl derivative, m.p. 225°[6] (lit. 221–223°), UV spectrum $\lambda_{max.}^{acetone}$ 379 mμ and IR spectrum (KBr pellet) 1821, 1764, 1658, 1499, 1364, and 1205 cm.$^{-1}$, were generally consistent with those reported previously (6). The mass spectrum of the acetyl derivative revealed a parent ion of *m/e* 464, an initial loss in mass equivalent to three acetyl groups to give fragments *m/e* 338 and 43, and fragmentation patterns of *m/e* 338 which were identical to those observed for xerocomic acid lactone.

Identification of Atromentic Acid—The UV spectra $\lambda_{max.}^{ethanol}$ 391 and 257 mμ, $\lambda_{max.}^{water}$ 375 and 258 mμ, and $\lambda_{max.}^{0.01\ M\ NaHCO_3}$ 360 (sh), 320, and 238 mμ agreed with those of a reference sample of atromentic acid, the identity suggested for the minor pigment by the chromatographic and electrophoretic studies.

Atromentic acid lactone is symmetrical, and the mass spectral fragmentation patterns would be represented by *m/e* 322 → 294 → 266 → 238 → 210 and *m/e* 322→ 161 → 133 → 105 → 77. The mass spectrum of the isolated material was in complete agreement with these fragmentation patterns with ion peaks at *m/e* 322, 294, 266, 238, 210, 161, 133, 105, and 77. The high-resolution parent ion peak at *m/e* 322.1474, both observed and calculated for $C_{18}H_{10}O_6$, and other ionic fragments in the high-resolution mass spectrum provided additional evidence for the identity of atromentic acid lactone.

A crystalline acetyl derivative was not obtained due to the limited availability of the minor pigment, but a small quantity of the acetylated product was prepared and purified on a silica gel column using the ether–HCl solvent. The acetyl derivative was eluted from the chromatographic adsorbent, and this solution was used to obtain a mass spectrum. A parent ion was observed at *m/e* 406, as anticipated for diacetylatromentic acid lactone. Fragments equivalent to two acetyl substituents were lost to give an ion peak at *m/e* 322, and the fragmentation patterns of this product were identical to those observed for atromentic acid lactone.

Examination of Carpophores of *P. atrotomentosus* for Tetronic Acids—Fresh carpophores (5 kg.) of the mushroom were placed in a blender and reduced to a pulpy slurry. Sufficient water was added to maintain the fluidity of the slurry. The blended mixture was extracted exhaustively by shaking with several portions of ether and then with cold methanol. The ether and methanol extracts were studied using the various chromatographic and electrophoretic procedures previously described. Atromentin was readily detected in the ether extract and was observed to be present in the methanol extract. Using these experimental procedures, no evidence for the presence of pulvinic acid derivatives was noted in the limited quantity of mushrooms available.

[6] Determined with a Kofler micro hot-stage apparatus.

RESULTS AND DISCUSSION

A temperature of 20° and an incubation period of approximately 2 months were selected as standard conditions for growing the vegetative mycelium of *P. atrotomentosus* in surface cultures. The cultures were grown in the dark to prevent any light-induced destruction or transformation of the pigments, and precautions were employed during all experimental manipulations to minimize exposure to light. Concern for stability also prompted extraction of the pigments from a blended mixture of the fresh mycelium and nutrient broth. A number of fungal terphenylquinones (10) and pulvinic acid derivatives (5–7) are known to present stability problems under certain conditions, and avoidance of a drying process would lessen the probability of artifact formation of the type that has recently been suggested for amitenone in carpophores of *Suillus bovinus* (13). Bovinone was obtained as the major pigment when fresh carpophores of *S. bovinus* were extracted without the application of heat, and the amitenone present in the dried mushroom may be of chemical rather than biologic origin.

No atromentin was detected in the cultures of *P. atrotomentosus*. The water-insoluble portion of the initial ether extract and the ether-exhausted slurry were treated with a $NaHCO_3$ solution and reextracted to exclude the possibility that atromentin was present as its leuco form, which is known to account for a significant percentage of this terphenylquinone in carpophores of this species (9).

Chromatographic, electrophoretic, and UV and IR spectral data all suggested and supported the conclusion that the pigments isolated from the cultures were diphenyl-substituted tetronic acids. The mass spectra and mass spectral fragmentation patterns of the acids and their acetyl derivatives established the number and distribution of hydroxyl substituents. The major pigment in the cultures was xerocomic acid, and atromentic acid was isolated in a considerably lower concentration. These observations provide the first established formation of pulvinic acid derivatives by the vegetative growth phase of a basidiomycete.

Several hydroxylated pulvinic acid derivatives are known to occur in the fresh carpophores of some mushrooms (5–8), especially members of the Boletaceae. The Boletaceae and the Paxillaceae are recognized as being closely related (14), and the possibility that pigments represent chemotaxonomic links between the two families has been noted (5). The recent report of atromentin in carpophores of *S. bovinus* (13) and the discovery of pulvinic acid derivatives in cultures of *P. atrotomentosus* provide further experimental support for such chemotaxonomic relationships and for the close relationship between terphenylquinones and pulvinic acid derivatives.

The distinctive metabolic capabilities of carpophores and vegetative growth of *P. atrotomentosus* raise a very interesting question of comparative biochemistry. The extremely limited information on the biosynthetic relationships of terphenylquinones and tetronic acids (3) suggests that atromentin is a precursor of atromentic acid; this sequence would imply that the vegetative phase of this species has a more complete biosynthetic capability than the specialized fruiting body. Experimentally controlled carpophore development of this species in the laboratory has never been achieved, but vegetative cultures may offer a tool for clarification of biosynthetic relationships and some biologic control mechanisms.

REFERENCES

(1) M. E. Hale, Jr., "The Biology of Lichens," Edward Arnold Ltd., London, England, 1967, pp. 105–107.

(2) W. Karrer, "Konstitution und Vorkommen der organischen Pflanzenstoffe," Birkhäuser Verlag, Basel, Switzerland, 1958, pp. 444–448.

(3) W. S. G. Maass and A. C. Neish, *Can. J. Bot.*, **45,** 59(1967).

(4) R. G. Benedict and L. R. Brady, in "Fermentation Advances," Academic, New York, N. Y., 1969, pp. 63–88.

(5) P. C. Beaumont, R. L. Edwards, and G. C. Elsworthy, *J. Chem. Soc., C,* **1968,** 2968.

(6) W. Steglich, W. Furtner, and A. Prox, *Z. Naturforsch.*, **23B,** 1044(1968).

(7) R. L. Edwards and G. C. Elsworthy, *Chem. Commun.*, **1967,** 373.

(8) W. Steglich, W. Furtner, and A. Prox, *Z. Naturforsch.*, **24B**, 941(1969).

(9) F. Kögl, H. Becker, A. Detzel, and G. de Voss, *Ann. Chem.*, **465**, 211(1928).

(10) M. C. Gaylord, J. R. DeBoer, and L. R. Brady, *J. Pharm. Sci.*, **56**, 1069(1967).

(11) B. Loev and M. M. Goodman, *Chem. Ind. (London)*, **1967**, 2026.

(12) R. M. Letcher, *Org. Mass Spectrom.*, **1**, 805(1968).

(13) P. C. Beaumont and R. L. Edwards, *J. Chem. Soc., C*, **1969**, 2398.

(14) R. Singer, "The Agaricales in Modern Taxonomy," 2nd ed., J. Cramer, Weinheim, West Germany, 1962, p. 694.

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Application of Clearance and Volume of Distribution to the Plateau Principle of Drugs

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Abstract □ The concentration of drug in plasma after continuous administration (plateau concentration, C_∞) has been defined as the relationship of the dosage per unit time and the half-life of elimination ($t_{1/2}$) to the volume of distribution (V_d) of a drug. The C_∞ determinants, V_d and $t_{1/2}$, have been determined in single-dose and continuous-infusion experiments. The data derived have been used to predict C_∞ for a series of substances when administered by continuous intravenous infusion. Alterations in V_d and $t_{1/2}$ of a drug may occur under clinical situations. This is reflected in changes in the plateau concentration, despite a constant dosage per unit time. An experimental example of deoxycholic acid decreasing the volume of distribution of bromsulfophthalein is given.

Keyphrases □ Plateau principle—plasma drug concentration □ Deoxycholic acid effect—volume of distribution □ Volume of distribution—plasma concentration plateau □ Drug administration rate—plasma concentration plateau

Drugs are often administered by continuous or repeated administration over long enough periods so that a relatively constant, or plateau concentration, of drug in the plasma is achieved and maintained. This plateau concentration will, in large measure, determine the effectiveness or toxicity of a drug. Bishydroxycoumarin,[1] quinidine, the anesthetics, antibiotics, and digitalis depend on a constant drug level in plasma to maintain their desired clinical response. In many cases, the drug may be regarded as distributed in a so-called volume of distribution into which the drug is administered at a constant rate and out of which the drug is removed by first-order kinetics. This model leads to a relationship of the plateau concentration of the drug to its rate of administration, its volume of distribution, and its first-order rate constant of removal. This relationship has been called the plateau principle (1). The purpose of this paper is to report on various types of kinetic experiments which demonstrate the self-consistency and utility of the plateau principle.

[1] Dicumarol.

THEORY

The basic formulas of the one-compartment model are summarized for convenience.

Drug is infused into the body at a constant rate, I (mg./min.). Upon entering the body, the drug is assumed to equilibrate "instantaneously" among the various body tissues, so the quantity $Q(t)$ (mg.) of drug in the body at time t (min.) is expressed as

$$Q(t) = V_d C_p(t) \qquad \text{(Eq. 1)}$$

where V_d (ml.) is by definition the constant volume of distribution and $C_p(t)$ (mg./ml.) is the concentration of drug in plasma at time t. The rate of removal $L(t)$ (mg./min.) of drug from the body, either as separation from the body or loss of identity within the body, is assumed expressible by the first-order expression

$$L(t) = GC_p(t) \qquad \text{(Eq. 2)}$$

where G (ml./min.) is by definition a constant clearance. The mass balance equation for the drug is, by Eqs. 1 and 2,

$$V_d(dC_p/dt) = I - GC_p(t) \qquad \text{(Eq. 3)}$$

which has the solution starting from zero drug plasma concentration (1)

$$C_p(t) = C_{p\infty}(1 - e^{-kt}) \qquad \text{(Eq. 4)}$$

In Eq. 4, the plateau concentration $C_{p\infty}$ (mg./ml.) is given by

$$C_{p\infty} = I/G \qquad \text{(Eq. 5)}$$

and the rate constant k(min.$^{-1}$) is given by

$$k = G/V_d \qquad \text{(Eq. 6)}$$

Equations 5 and 6 give

$$C_{p\infty} = I/kV_d \qquad \text{(Eq. 7)}$$

Substitution of the half-life $t_{1/2} = 0.693/k$ into Eq. 7 gives

$$C_{p\infty} = It_{1/2}/0.693V_d \qquad \text{(Eq. 8)}$$

If, after the plateau concentration is reached (to a given accuracy), the infusion is suddenly stopped, the decay of drug plasma concentration is described by Eq. 3, with $I = 0$, as

$$-V_d(dC_p/dt) = GC_p(t) \qquad \text{(Eq. 9)}$$

with solution

$$C_p(t) = C_{p\infty}e^{-kt} \qquad \text{(Eq. 10)}$$