Pharmacokinetic Study of an Iridoid Glucoside: Aucubin

Nan-Joo Suh,[1] Chang-Koo Shim,[1,2] Min Hwa Lee,[1] Shin Keun Kim,[1] and Il-Moo Chang[3]

Received September 10, 1990; accepted March 13, 1991

Aucubin, a promising hepatoprotecting iridoid glucoside, was given intravenously (*iv*), orally (*po*), intraperitoneally (*ip*), and hepatoportally (*pv*) to rats. A linear pharmacokinetic behavior was obtained after *iv* administration of 40–400 mg/kg of aucubin. The half-life of aucubin in the postdistributive phase ($t_{1/2,\beta}$), total-body plasma clearance (CL_t), and volume of distribution (Vd_{ss}) were 42.5 min, 7.2 ml/min/kg, and 346.9 ml/kg, respectively, for a 40 mg/kg dose. There was no significant difference in the parameters as a result of increasing dose. The partition coefficients of aucubin between *n*-octanol and buffers of pH 3.0–10.0 were low, while 18.5 ± 1.3% of aucubin in whole blood partitioned into the blood cells. Plasma protein binding of aucubin was only 9%. The bioavailabilities of aucubin after administration at a dose of 100 mg/kg through *pv*, *ip*, and *po* routes were 83.5, 76.8, and 19.3%, respectively. The pH-stability profile indicated rapid degradation of aucubin at pH 1.2, 1.6, and 2.0, with degradation half-lives of 5.1, 5.8, and 14.8 hr, respectively, at 37°C. Therefore, the low oral bioavailability of aucubin may be attributed to pH-instability in the gastric fluid, poor GI absorption due to low lipophilicity, and the possible metabolism in the GI mucosa and liver (so called first-pass effect).

KEY WORDS: aucubin; preformulation study; pharmacokinetics; bioavailability; pH-stability; partition coefficient; iridoid glucoside.

INTRODUCTION

Aucubin [1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl) cyclopenta (c) pyran-1-yl-β-D-glucopyranoside] is a common iridoid glucoside (Fig. 1) isolated from the leaves of *Aucuba japonica* (Cornaceae) (1), *Eucommia ulmoides* (Eucommiaceae) (2), and *Plantago asiatica* (Plantaginaceae) (3) etc., which are used in folk medicine and traditional Chinese medicine.

Aucubin was found to protect against liver damage induced by CCl_4 or α-amanitin in mice and rats when 80 mg/kg was dosed intraperitoneally (4–7) and to inhibit the synthesis of RNA and proteins in the liver of mice (8). It was known as a synthetic intermediate of prostaglandin in the body (9–12). Choleretic action was also reported (13). The aim of this study was to characterize the pharmacokinetic behavior of this compound. Plasma concentration profiles of aucubin after oral (*po*), hepatoportal (*pv*), intraperitoneal (*ip*), and intravenous (*iv*) administration were examined, and their absolute bioavailabilities were compared. Plasma protein binding and plasma-to-blood partition of aucubin were examined *in vitro*. Important physicochemical properties such as pH-stability and *n*-octanol/water partition coefficient of aucubin were also assessed in this preformulation study of aucubin.

MATERIALS AND METHODS

Aucubin and Chemicals

Aucubin was isolated as white crystalline powder from the leaves of *Aucuba japonica* (Cornaceae), which were collected in a southern area of Korea, according to the method of Trim and Hill (14) after minor modification as previously described (15,16). The isolated aucubin was authenticated by Natural Products Research Institute of Seoul National University. Methanol (HPLC grade) was purchased from Merck (Darmstadt, FRG) and water (HPLC grade) was purchased from Fischer Co. Solvents for HPLC were filtered through a 0.45-μm filter and throughly degassed in an ultrasonic bath before use. All other reagents were analytical grade and used without further purification.

Administration of Aucubin via Various Routes

Male Wistar rats weighing 230–280 g were used in all experiments. The rats were fixed at supine position during the experiment. Under light ether anesthesia, femoral arteries of the rats were cannulated with polyethylene tubings (PE-50, Intramedic, Clay Adams, USA) for blood sampling. Femoral veins were also cannulated with PE-50 for *iv* administration of aucubin. For *pv* administration of aucubin, the pyloric vein was cannulated as follows: the abdomen was opened through a midline incision and the tip of an injection needle (25 gauge) attached to a PE-50 was inserted into the hepatic portal vein and was fixed with surgical glue (Aron Alpha, Sankyo Co., Tokyo). The needle was bent 120° for the convenience of insertion. This catheter was connected to a 1-ml syringe and the dose of aucubin was given through the syringe into the portal vein. Oral administration was performed by insertion a round-tip needle which was connected to a 1-ml syringe. Intraperitoneal administration was performed with a 26-gauge needle.

After complete recovery (1 hr) from the anesthesia, aucubin was administered at the dose of 40, 50, 100, 200, and 400 mg/kg for intravenous (*iv*) study and 100 mg/kg for oral (*po*), hepatoportal venous (*pv*), and intraperitoneal (*ip*) study. Aucubin was dissolved in sterilized physiological saline to yield appropriate concentrations. The administered volume of aucubin solutions was 2 ml/kg. Blood samples (0.15 ml) were collected into heparinized tubes from the femoral artery via PE-50 catheter. The heparinization was conducted by treating the tubes with 10 μl of heparinized saline (150 IU/ml) and successive drying of the saline. Blood samples were withdrawn prior to administration and at 2, 5, 10, 20, 40, 60, 90, 120, 180, and 240 min postadministration for *iv* and *pv* administration, and prior to administration and at 5, 15, 30, 45, 60, 90, 120, 180, and 240 min postadministration for *po* and *ip* administration. Plasma samples were obtained by centrifuging immediately the blood samples at 4000*g* for 10 min and stored at −20°C until analysis.

[1] College of Pharmacy, Seoul National University, Seoul 151-742, Korea.

[2] To whom correspondence should be addressed.

[3] Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea.

0724-8741/91/0800-1059$06.50/0

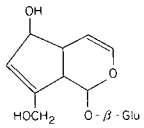

Fig. 1. The chemical structure of aucubin.

HPLC Assay

A HPLC assay for aucubin in biological fluids was developed by modifying the reported HPLC methods (17–19) for aucubin in nonbiological solutions. To 100 μl of plasma samples, 30 μl of zinc sulfate solution (10%, w/v) and 70 μl of methanol were added and mixed successively. Then the mixture was centrifuged at 4000*g* for 10 min and 20 μl of the supernatant was directly injected onto the guard column (Direct Connect, Alltech-Applied Science) connected to a reversed-phase C18 column (10 μm, μ-Bondapak, 30 cm × 0.39-cm i.d., stainless steel, Waters, P/N 27324). The temperature of the column was maintained at 45 ± 1°C. The pump and variable detector were from Spectraphysics (SP 8810, Precision Isocratic Pump) and Applied Biosystems (757, Absorbance Detector), respectively. Aucubin was monitored at 210 nm and the detection limit was 1.0 μg/ml. The mobile phase was a 97:3 (v/v) mixture of deionized water and methanol. The flow rate of the mobile phase was adjusted to 1.0 ml/min. A typical chromatogram of aucubin extracted from rat plasma after *iv* administration of 100 mg/kg dose is shown in Fig. 2. Aucubin was appropriately separated from the other substances in the plasma with a retention time of 15 min. The total run time per injection was 30 min to eliminate any possible interfering peaks. Recovery of aucubin from the plasma samples was 86.0% in the concentration range of 10–500 μg/ml, and calibration curves were linear in this concentration range. Intraday and interday variations of the assay were below 4.2 and 6.0%, respectively.

Pharmacokinetic Analysis

Total-body plasma clearance (CL_t), distribution volume at steady-state (Vd_{ss}), and pharmacokinetic half-life ($t_{1/2\beta}$) were calculated using *iv* data as follows:

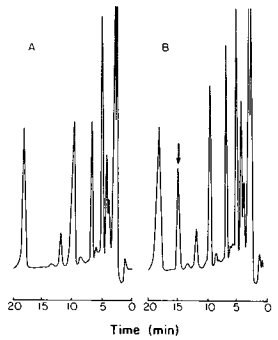

Fig. 2. Typical chromatogram of the blank plasma (A) and aucubin in the plasma after *iv* administration of 100 mg/kg (B). Arrow indicates the peak of aucubin.

$$CL_t = \text{dose/AUC} \quad (1)$$

$$Vd_{ss} = \text{dose AUMC/AUC}^2 \quad (2)$$

$$t_{1/2}\beta = 0.693/\beta \quad (3)$$

where *AUC*, *AUMC*, and β are the area under the plasma concentration–time curve, area under the moment of the plasma concentration–time curve, and slope of the natural log concentration–time curve at postdistributive phase (β-phase), respectively. *AUC* and *AUMC* of all administration routes were calculated by trapezoidal rule after fitting the plasma concentration data to the conventional two-compartment model using MULTI program (20). β was also obtained by the fitting.

Fraction of aucubin transported (availability = 1 − extraction ratio) across the liver (F_3), GI mucosa (F_4), and GI lumen (F_5) were calculated from Eqs. (4)–(6) (21,22) using *AUC* data after various routes of administration. In this model, aucubin was presumed not to be cleared in the lung and not to be subject to enterohepatic recirculation, although these assumptions were not tested.

$$AUC_{po}/AUC_{iv} = F_3F_4F_5 \quad (4)$$

$$AUC_{pv}/AUC_{iv} = F_3 \quad (5)$$

$$AUC_{ip}/AUC_{iv} = F_3F_4 \quad (6)$$

Partition Coefficient

Buffers of pH 3.0, 4.0, 6.8, 8.4, and 10.0 were prepared as described in USP XXII. The buffers and *n*-octanol were presaturated with each other in amber-colored bottles for 24 hr. Ten milliliters of the *n*-octanol and the buffer solution was mixed with 10 mg of aucubin in a screw-capped tube on a shaking water bath at 25°C. Samples were taken at 2 hr after the start of the shaking. Two hours was confirmed to be enough to reach equilibrium of the partition. The water layers of the samples were analyzed for aucubin by HPLC.

Plasma-to-Blood Partition

All procedures were carried out immediately after blood collection from the carotid artery. To 1.0 ml of whole blood of rats, 10 μl of aucubin solution was added and mixed in the heparinized Vacuject tubes (Green Cross Med. Co., Seoul, Korea). The resultant concentrations of aucubin in the blood were 25, 50, 100, and 250 μg/ml. The mixtures were incubated at 37°C for 30 min. Plasma was separated by centrifuging the blood samples at 4000*g* for 10 min and analyzed for aucubin by HPLC.

Plasma Protein Binding

Plasma was separated from blood, obtained through the carotid artery, by centrifugation for 10 min at 4000*g* immediately after the blood collection. The plasma free fraction of aucubin was determined *in vitro* by equilibrium dialysis at 37°C for 8 hr using a Spectrapor-2 dialysis membrane (Spectrum Medical Industries, Inc., Los Angeles, CA: molecular cutoff, 12,000–14,000) against Tris–HCl buffer (pH 7.4), containing 10–1000 μg/ml of aucubin. Twenty microliters of the buffer was sampled and analyzed by HPLC. The protein

binding of aucubin to plasma was unchanged between 8 and 12 hr of dialysis at 37°C.

Stability of Aucubin

Buffers of pH 1.2, 1.6, 2.0, 3.0, 4.0, 6.8, 8.4, and 10.0 were prepared as described in USP XXII. Aucubin solutions (1 mg/ml) prepared in the respective buffers were maintained at 37°C in a water bath. Samples were taken at various time intervals for 6 hr and analyzed by HPLC. The log concentration of aucubin versus time profile was plotted to determine the degradation rate constants at all pH values. The pH-stability profile was constructed by plotting rate constant versus pH. The stability of aucubin in fresh plasma (100 and 1000 μg/ml) was also examined at 37°C for 6 hr.

Statistical Analysis

The differences between the pharmacokinetic parameters of aucubin after *iv* administration of various doses were compared by ANOVA test at the $P = 0.05$ level.

RESULTS

Stability of Aucubin

The log aucubin concentration versus time profiles were linear at all pH's, indicating first-order degradation. Degradation rate constants obtained from the slopes of the curves were used to determine the half-lives at various pH's (Table I). The pH-stability profile of aucubin at 37°C is shown in Fig. 3. The degradations were highly acid specific, and the degradation half-lives were 5.02, 5.78, and 14.84 hr at pH 1.2, 1.6, and 2.0, respectively. At pH above 3.0, the degradation of aucubin was negligible (e.g., the degradation half-lives were more than several days). Aucubin was stable in plasma for at least 6 hr at 37°C.

Partition Coefficient

Aucubin was readily dissolved in all buffers of various pH's (3.0–10.0). The concentration of aucubin in *n*-octanol after partitioning from the respective buffers was below the sensitivity of the assay (1 μg/ml), indicating extremely low *n*-octanol/water partition coefficients. No degradation products of aucubin were observed by HPLC during the partition study.

Table I. First-Order Degradation Half-life ($t_{1/2}$) of Aucubin at 37°C in Buffers of Various pH's

pH[a]	$t_{1/2}$ (hr), mean ± SD[b]
1.2	5.0 ± 0.2
1.6	5.8 ± 0.5
2.0	14.8 ± 1.2
3.0	162.3 ± 13.7
4.0	358.1 ± 23.1
6.8	157.7 ± 14.0
8.4	148.8 ± 12.3
10.0	136.0 ± 12.0

[a] All buffers were prepared as described in USP XXII.
[b] N = 3.

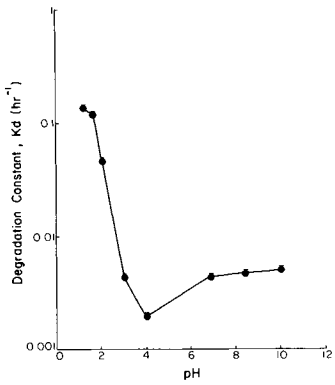

Fig. 3. The pH-stability profile of aucubin at 37°C. Each point represents the mean value of three observations with standard deviation bar.

Plasma-to-Blood Partition

Aucubin partitioning reached equilibrium within 30 min with negligible hemolysis during the incubation. Mean (±SD) partition of aucubin to red blood cells was constant over the blood concentration range of 25–250 μg/ml, *i.e.*, 18.4 (±1.1), 20.3 (±1.0), 17.1 (±1.3), and 18.2 (±1.2)% of aucubin were found in the blood cells when determined three times at the blood concentrations of 25, 50, 100, and 250 μg/ml, respectively. The average ratio of aucubin present in the blood cells among total aucubin was calculated to be 18.5 (±1.3)%.

Plasma Protein Binding

Plasma protein binding of aucubin was determined by *in vitro* equilibrium dialysis. The unbound fractions of aucubin were calculated to be 83.2 (±6.5), 92.0 (±5.0), 97.8 (±7.7), 88.6 (±4.3), and 94.7 (±4.7)% for aucubin concentrations of 10, 50, 200, 500, and 1000 μg/ml, respectively, when expressed as mean (±SD) of three experiments. There was no concentration dependency in the binding. In conclusion, 91% of aucubin was found to exist in the unbound form.

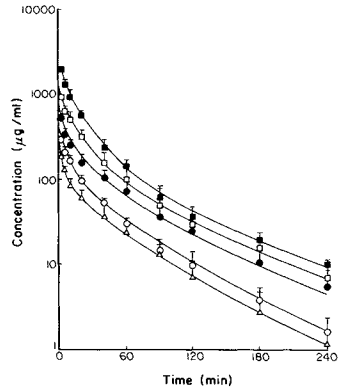

Fig. 4. Plasma profiles of aucubin after *iv* administration of 40 (△), 50 (○), 100 (●), 200 (□), and 400 (■) mg/kg doses to the rats. Each point represents the mean ± SD of four experiments. The lines were generated by two-compartment model using MULTI program (20).

Table II. Pharmacokinetic Parameters of Aucubin After iv Administration of Various Doses to Rats[a]

Parameters	Dose (mg/kg)				
	40	50	100	200	400
$t_{1/2,\beta}$ (min)	42.5 (8.0)	48.7 (8.1)	50.9 (10.2)	58.1 (19.4)	43.5 (12.9)
Vd_{ss} (ml/kg)	347 (37)	296 (73)	383 (46)	362 (57)	366 (77)
CL_t (ml/min/kg)	7.2 (2.6)	7.0 (0.8)	7.4 (2.0)	9.3 (2.7)	10.7 (1.8)
AUC (μg min/ml)	6,110 (2,430)	7,040 (727)	13,550 (3,220)	22,160 (6,390)	37,070 (5,810)

[a] Each value represents the mean (±SD) of four experiments. All parameters are not significantly different from each other among doses.

Table III. Bioavailability Parameters of Aucubin after pv, ip, and po Administration at a Dose of 100 mg/kg[a]

Parameters	po	ip	pv
C_{max} (μg/ml)	17.4 (6.6)	111.2 (6.4)	—
T_{max} (min)	54.5 (10.7)	19.6 (3.3)	—
AUC (μg min/ml)	2,610 (794)	10,400 (2,450)	11,320 (1,330)
Bioavailability (%)	19.3 (5.9)	76.8 (18.1)	83.5 (9.8)

[a] Each value represents the mean (±SD) of four experiments.

Pharmacokinetics and Bioavailability of Aucubin

Plasma levels of aucubin after *iv* administration of aucubin at the dose of 40–400 mg/kg are plotted as a function of time in Fig. 4. The profiles showed bioexponential decay. Pharmacokinetic parameters were calculated by moment analysis and are summarized in Table II. The parameters did not differ significantly among doses of 40–400 mg/kg, indicating that the pharmacokinetics of aucubin are linear in the dose range examined. Mean values of $t_{1/2,\beta}$, Vd_{ss}, and CL_t of aucubin in the dose range were 48.7 (±6.3) min, 350.8 (±33.4) ml/kg, and 8.3 (±1.6) ml/min/kg, respectively. The mean plasma concentration profiles of aucubin after *iv*, *po*, *pv* and *ip* administration of 100 mg/kg are shown in Fig. 5, and the relevant bioavailability parameters are summarized in Table III. Bioavailabilities were calculated by comparing the *AUC* ratio of each route to that of the *iv* route.

The plasma profile after *pv* administration was nearly parallel to that of *iv* administration. The terminal slopes of the curves following *pv*, *po*, and *ip* administration were identical to that of *iv* administration. The peak plasma concentrations (C_{max}) of aucubin after *ip* and *po* administration were much lower than those of *iv* administrations. The C_{max} of oral aucubin was much lower than that of *ip* aucubin by a factor of 5 or 6, and the time to reach the peak (T_{max}) of oral aucubin was twofold longer than that of *ip* aucubin.

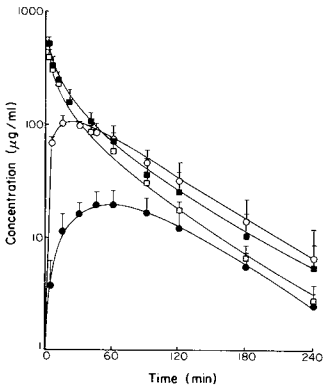

Fig. 5. Plasma profiles of aucubin after *iv* (■), *pv* (□), *ip* (○), and *po* (●) administration of 100 mg/kg doses to the rats. Each point represents the mean ± SD of four experiments. The lines were generated by two-compartment model using MULTI program (20).

From the relationships shown by Eqs. (4)–(6), the availability of aucubin across the liver (F_3), GI mucosa (F_4), and GI lumen (F_5) were 0.92 ± 0.22, 0.84 ± 0.10, and 0.25 ± 0.08, respectively.

DISCUSSION

The volume of distribution (Vd_{ss}) of aucubin was far below body weight, which indicates poor distribution of aucubin to body tissues. *In vitro* plasma-to-blood partition of aucubin was also rather small. The small Vd_{ss} and plasma-to-blood partition, in spite of very low plasma protein binding (approximately 9%) of aucubin, could be attributed to the low partition coefficient of aucubin.

The F_3 (0.84) indicates that 16% of aucubin administered through the *pv* route is extracted by the liver. On the other hand, the fraction of aucubin transported into the GI mucosa across the gut wall (F_5) was only 0.25, possibly because of rapid degradation in the acidic condition of the stomach fluid (Table I) and incomplete absorption from the GI lumen, expected from the low partition coefficient of aucubin. The small fraction of aucubin transported across the GI mucosa (F_4) could have been caused by enzymatic hydrolysis by β-glucosidase (23) in the GI mucosa.

Bioavailability of *pv* aucubin (84%) reflected extraction (16%) by the liver. The slightly lower bioavailability after *ip* administration (77%) than after *pv* administration may be attributed to the loss of the drug during absorption through the abdominal cavity. The low oral bioavailability of aucubin (19%) was a result of low availability across the GI lumen (F_5). Further, presystemic metabolism could also contribute to the low bioavailability. The contribution of presystemic metabolism, however, seems to be small, since F_3 and F_4 are fairly high. pH-instability and incomplete absorption largely account for the difference in bioavailability seen between *ip* and *po* administration.

REFERENCES

1. R. Bernini, C. Iavarone, and C. Trogolo. 1-O-β-D-Glucopyranosyleucommiol, an iridoid glucoside from *Aucuba japonica*. *Phytochemistry* 23:1431–1433 (1984).
2. A. Bianco, C. Iavarone, and C. Trogolo. Structure of eucommiol, a new cyclopentenoid-tetrol from *Eucommia ulmoides*. *Tetrahedron* 30:4117–4121 (1974).

3. M. Pailer and E. Haschke-Hofmeister. Inhaltsttoffe aus *Plantago major*. *Planta Med.* 17:139–141 (1969).
4. I. M. Chang and H. S. Yun. Plants with liver protective activities (II). Potential hepatotonic activities of *Plantago asiatica* seed. *Kor. J. Pharmacog.* 9:139–144 (1978).
5. H. S. Yun, I. M. Chang, H. J. Choi, and S. Y. Lee. Plants with liver protective activities (IV). Chemistry and pharmacology of *Plantaginis Semen et Folium*. *Kor. J. Pharmacog.* 11:57–60 (1980).
6. I. M. Chang, J. C. Ryu, Y. C. Park, H. S. Yun (Choi), and K. H. Yang. Protective activities of aucubin against carbon tetrachloride-induced liver damage in mice. *Drug Chem. Toxicol.* 6:443–453 (1983).
7. K. H. Yang, T. J. Kwon, S. Y. Choe, H. S. Yun, and I. M. Chang. Protective effect of *Aucuba japonica* against carbontetrachloride-induced liver damage in rat. *Drug Chem. Toxicol.* 6:429–441 (1983).
8. I. M. Chang, H. S. Yun, Y. S. Kim, and J. W. Ahn. Aucubin: Potential antidote for alpha-amanitin poisoning. *Clin. Tox.* 22:77–85 (1984).
9. M. Naruto, K. Ohno, N. Naruse, and H. Takeuchi. Synthesis of prostaglandins and their congeners I. (+)-11-Deoxy-11α-hydroxymethyl prostaglandin F2α from aucubin. *Tetrahedron Lett.* 3:251–254 (1979).
10. W. F. Berkowitz, I. Sasson, P. S. Sampathkumar, J. Hrabie, S. Choudhry, and D. Pierce. Chiral prostanoid intermediates from aucubin. *Tetrahedron Lett.* 19:1641–1644 (1979).
11. T. Arlette, R. Yves, and G. Janine. Aucubin, a source of prostanoid synthons—new hemisynthetic pathways. *Heterocycles* 19:253–256 (1982).
12. C. Bonini and R. D. Fabio. Assignment of correct structure to two tetrahydrodideoxy aucubins. *J. Org. Chem.* 47:1343–1345 (1982).
13. S. Takeda, K. Yuasa, T. Endo, and M. Aburada. Pharmacological studies on iridoid compound II. *J. Pharm. Dyn.* 3:485–492 (1980).
14. A. R. Trim and R. Hill. The preparation and properties of aucubin, asperulosides and some related glucosides. *Biochem. J.* 50:310–319 (1952).
15. O. Sticher. The structural characterization of iridoid glucoside. In A. Sofourora (eds.), *Proceedings of a Conference on African Medicinal Plants*, 1979, pp. 140–168.
16. I. M. Chang, Y. C. Park, and H. S. Yun (Choi). Inhibition of ribonucleic acid biosynthesis in leukemia SN36 in vitro by aucubin. *Kor. Biochem. J.* 15:200–204 (1982).
17. A. Lenherr, B. Meier, and O. Sticher. Iridoid glucosides and acetylated flavonoids in the group of *Stachys recta*. *Planta Med.* 50:403–409 (1984).
18. K. Gorler, D. Oehlke, and H. Soicke. Iridoidfuhrung von Vitex Agnus-Castus. *Planta Med.* 51:530–531 (1985).
19. A. Franke, H. Rimpler, and D. Schneider. Iridoid glycosides in the butterfly *Euphydryas cynthia* (Lepidoptera, Nymphalidae). *Phytochemistry* 26:103–106 (1987).
20. K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno. A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharm. Dyn.* 4:879–885 (1981).
21. J. R. Gillette and K. S. Pang. Theoretical aspects of pharmacokinetic drug interactions. *Clin. Pharmacol. Ther.* 22:623–639 (1977).
22. Y. Sugiyama. Physiological pharmacokinetics. In M. Hanano, K. Umemura, and T. Iga (eds.), *Applied Pharmacokinetics—Theory and Experiments*, Soft Science, Tokyo, 1985, pp. 408–409.
23. P. Karrer and H. Schmid. Uber die Konstitution des Aucubins. *Helv. Chim. Acta* 29:525–552 (1946).