(1) T. S. Chen, "Pharmacology Encyclopedia of Chinese Drugs," (in Chinese) Eastern Books, Taipei, Taiwan, Republic of China, 1958, p. 250.
(2) S. K. Chandrasekaran, W. Bayne, and J. E. Shaw, *J. Pharm. Sci.*, **67**, 1370 (1978).
(3) A. Karim, *Drug Dev. Ind. Pharm.*, **9**, 671 (1983).
(4) W. R. Good, *Drug Dev. Ind. Pharm.*, **9**, 647 (1983).
(5) Y. W. Chien, *Drug Dev. Ind. Pharm.*, **9**, 497 (1983).
(6) A. Gerardin, J. Hirtz, P. Frankhauser, and J. Moppert, "Abstracts," *APhA/APS 31st National Meeting Abstracts*, 11(2), 84 (1981).
(7) P. W. Armstrong, J. A. Armstrong, G. S. Marks, J. McKinven, and S. Slaughter, *Am. J. Cardiol.*, **46**, 670 (1980).
(8) G. A. Portmann, in "Current Concepts in Pharmaceutical Sciences—Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1970, chap. 1.
(9) Y. W. Chien, *J. Pharm. Sci.*, in press.

Yie W. Chien
Controlled Drug Delivery Research Center
Rutgers University
College of Pharmacy
Busch Campus
Piscataway, NJ 08854

Received on July 7, 1982.
Accepted for publication December 1, 1983.
The author wishes to extend his great appreciation to Dr. B. Nicolova, who believed in the idea and has worked diligently to make it possible. He also wishes to thank Mrs. E. Skurkay for manuscript preparation.

Variability in the Determination of Fraction Metabolized in a Triangular Metabolic Problem and Its Resolution with Stable Isotope Methodology

Keyphrases □ Cinromide—anticonvulsant, fraction metabolized □ Isotope methodology—administration of labeled metabolites □ Triangular metabolic pathway—fraction metabolized-cinromide, rhesus monkey

To the Editor:

A particular case of precursor–product relationship arises when one metabolite (metabolite II) of a parent drug is formed from two precursors, the parent drug as well as one of its proximal metabolites (metabolite I). The resolution of this metabolic problem is complicated by the fact that the fraction of parent drug metabolized directly to metabolite II can only be obtained indirectly from the following difference:

$$f_m(P \rightarrow MII, \text{direct}) = f_m(P \rightarrow MII, \text{total}) - f_m(P \rightarrow MI) \times f_m(MI \rightarrow MII) \quad \text{(Eq. 1)}$$

where f_m represents fraction metabolized, P refers to parent drug, and MI and MII refer to metabolites I and II, respectively. The classical approach to the determination of the fractions metabolized (Eq. 1) using blood data requires three separate studies: administration of parent drug, metabolite I, and metabolite II to the same subject at different times. This approach involves several assumptions of constancy of metabolite clearances among the three studies. As a result, estimates of the various fractions of Eq. 1 are subject to significant error. This type of metabolic problem arose during the elucidation of the pharmacokinetic profile of cinromide (3-bromo-*N*-ethylcinnamamide)(P), a new antiepileptic drug. This drug is metabolized by *N*-deethylation to 3-bromocinnamamide (I) and by amide hydrolysis to 3-bromocinnamic acid (II). However, I is also metabolized to II by amide hydrolysis. The resolution of this metabolic scheme prior to the efficacy evaluation of cinromide in the primate model (1, 2) was of particular significance because I was found to have anticonvulsant properties (3, 4) and II reached steady-state levels 10–20-fold higher than those of the parent drug (2). In an earlier study where the classical approach (administration of cinromide, I, and II separately) was used in a group of six rhesus monkeys, several unrealistic findings were obtained (5). The fraction of dose of cinromide metabolized directly to II ranged from 5 to 100% (mean ± *SD* = 48 ± 32%). Also, the sum of the two fractions (f_m(P → I) + f_m(P → II, direct)) ranged from 64 to 140% and was larger than 100% in three of six monkeys. This large variability was attributed to the intrasubject variability in clearances of I and II among the three studies. To test this hypothesis, a new approach using stable isotope methodology was devised. This approach involved the simultaneous administration of cinromide, and different stable isotope-labeled variants of I and II, in which case the clearances of I and II were obtained while these metabolites were formed *in situ*.

Five chaired normal male rhesus monkeys (chronically catheterized) were used in this study. Dideuterated I (I–d_2) and monodeuterated II (II–d_1) were administered along with the unlabeled parent drug (d_0). These were synthesized using the methods described for the corresponding unlabeled species (6–8). The deuterium labels were placed on the α, β ethylene carbons of I–d_2, and the β carbon of II–d_1 to minimize any possible isotope effect. Any secondary kinetic deuterium isotope effect on deethylation and amide hydrolysis or on epoxide formation and ring opening of the ethylene carbons would generally not be significant (9). Single intravenous doses of cinromide–d_0 (75 mg) and II–d_1 (80 mg) were administered consecutively to each monkey. I–d_2 (70 mg) was administered intravenously 2 h later such that the concentrations of II–d_2 would not be too far apart from those of II–d_0 and II–d_1 and could be analyzed at the same time. Solutions of cinromide–d_0, I–d_2, and II–d_1 were prepared in 60% polyethylene glycol 400 to concentrations of 25, 25, and 10 mg/mL, respectively. Blood samples (2 mL) were taken at 0.2, 0.5, 0.75, 1, 1.5, 2, 2.25, 2.5, 3, 4, 5, 6, 8, 10, 12, and 16 h after administration of II–d_1. EDTA was used as an anticoagulant in each blood sample. After the administration of cinromide–d_0, I–d_2 and II–d_1, six species were monitored in each blood sample: the three administered species as well as I–d_0 and II–d_0 formed from cinromide (d_0) and II–d_2 formed from I–d_2. Aliquots of 0.2 mL of each sample were used to analyze cinromide by HPLC (10). Aliquots of 0.5 to 0.8 mL of blood were used to analyze I and II by direct probe insertion chemical ionization mass spectrometry. Compounds I and II were separated by successive extractions at different pH. Blood samples were extracted with benzene under neutral conditions to extract I; the aqueous phases were acidified and extracted with benzene to recover II and benzene extracts containing I were washed with 0.1 M NaOH. Benzene extracts containing II were shaken with 0.1 M NaOH, then reacidified and extracted back into benzene. Chlorinated analogues of I and II were used as internal standards for

0022-3549/84/0200-0285$01.00/0

Table I—Fraction Metabolized for the Various Pathways of the System Cinromide 3-Bromocinnamamide (I) 4-3-Bromocinnamic Acid (II)

Method	Monkey	Cinromide → II, Total	Cinromide → I	I → II	Cinromide → II, Direct	Cinromide (I + II
Stable isotope (1 study)	203	0.48	0.29	0.23	0.41	0.70
	514A	0.49	0.45	0.34	0.34	0.79
	712A	0.37	0.39	0.34	0.24	0.63
	79321	0.79	0.40	0.58	0.52	0.92
	79325	0.52	0.40	0.23	0.43	0.83
	Mean	0.53	0.39	0.34	0.39	0.77
	SD	0.16	0.06	0.14	0.10	0.11
Classical (3 studies)	Mean	0.73	0.53	0.53	0.48	1.01
	SD	0.35	0.24	0.21	0.32	0.34

Table II—Assumptions of Constancy Involved in Solving a Triangular Metabolite System Using the Classical Approach

	Studies		
	1	2	3
Species administered	Parent Drug	MI	MII
Species formed	MI, MII	MII	
Assumptions	ClMI (Study 1) = ClMI (Study 2)	ClMII (Study 2) = ClMII (Study 3)	
	ClMII (Study 1) = ClMII (Study 3)		
	Cl[MI → MII] (Study 1) = Cl[MI → MII] (Study 2)		

the assays. The MS (VG-7070)[1] was interfaced with a data system (VG-2035F/B)[1] and operated in the selected ion monitoring mode. Methane was used as reagent gas for chemical ionization. The source temperature was 190°C. The protonated molecular ions of internal standard, d_0, d_1, and d_2 were monitored (m/z 182, 226, 227, and 230 for the analysis of I; 183, 227, 228, and 231 for the analysis of II). To avoid the interference of ^{81}Br–d_0 and ^{79}Br–d_2, the ion of ^{81}Br–d_2 was monitored in each dideuterated metabolite. The natural abundance of carbon-13 in each species was taken into consideration in calculating concentrations.

The kinetic analysis of the data was aimed at the determination of the various fractions metabolized required in Eq. 1 and was based on area calculations (11). The fraction metabolized[2] was calculated by the following equation:

$$f_m = \frac{(AUC_{met})p/mol.\ wt._{met} \times CL_{met}}{Dose\ p/mol.\ wt.\ p} \quad \text{(Eq. 2)}$$

where $(AUC_{met})p$ represents the area under the metabolite concentration curve after a dose of the precursor, CL_{met} is the elimination clearance of the metabolite, and mol. wt. represents the molecular weight. The area under the blood concentration–time curve for each compound was measured by the trapezoidal rule with extrapolation to infinity using the last several points of each curve. On the average, the area obtained without extrapolation accounted for 83% of the total area. Clearance was calculated from dose–area ratio. In this study, both metabolites had low extraction ratios and, therefore, are not subject to hepatic sequential single pass metabolism (13).

The average clearances of cinromide, I–d_2 and II–d_1 were 8.04 ± 3.32, 0.91 ± 0.29, and 0.77 ± 0.3 L/h, respectively. Table I lists individual values of the fraction metabolized *via* the various pathways of the cinromide–metabolite I–metabolite II system. The fraction of dose of cinromide metabolized to II by all routes (directly and indirectly) was 53 ± 16%. The fraction of dose of cinromide metabolized to I was 39 ± 6%, while the fraction of dose of I metabolized to II was 34 ± 14%. The fraction of dose of cinromide metabolized directly to II calculated according to Eq. 1 was 39 ± 10%. Thus, as primary metabolites of cinromide, I and II together account for 77 ± 11% of a dose. The corresponding fractions metabolized obtained in a previous study by the classical approach are also given in Table I. Although the values obtained in the present study were generally smaller, comparison of the values obtained by both methods showed no statistically significant difference for any of the pathways (Mann–Whitney test). However, the variability in the various fractions metabolized determined in the present study was much smaller. There were significant decreases in variance in the fraction of cinromide metabolized to I, in the fraction of cinromide metabolized to II directly, and in the sum of these two fractions (variance ratio test, $p < 0.05$). The superiority of the stable isotope method is also supported by the fact that no unrealistic value, (larger than 100%) was obtained for the sum of the two fractions of cinromide metabolized to I and II (directly). The reduction in the coefficient of variation of the fraction cinromide → I (45% to 15%) is of particular interest. In the previous study, it was found that the half life of I was significantly longer after a dose of cinromide (5.8 ± 1.2 h) than after administration of I (4.4 ± 0.8 h) which suggested that the clearance of I measured when it is administered would be an overestimate of its clearance when formed *in situ*. In such a case, the stable isotope approach offers unique advantages since it allows an estimate of the clearance of the metabolite formed *in situ*.

The findings of the present study can be put in broader perspective if an analysis of the limitations inherent to the classical approach is considered. This approach requires three studies (1, 2, and 3) in which three species (cinromide, I, and II) are administered separately (Table II). To solve Eq. 1, four assumptions of constancy of clearance are

[1] VG-Analytical, Altrincham, Cheshire, U.K.

[2] In light of the experimental design (intravenous administration of parent drug and metabolites), this fraction refers to the fraction of total body clearances as well as the fraction of dose of parent drug (12).

required. Three clearances operating in study 1 are estimated in studies 2 and 3. Study 2 estimates the elimination clearance of I while study 3 estimates the elimination clearance of II. Studies 2 and 3 together estimate the formation clearance of II from I. Also, the elimination clearance of II operating in study 2 is measured in study 3. Intrasubject variability in any of these clearances leads to unrealistic values for the fraction of parent drug metabolized directly to metabolite II. This variability can be minimized by the simultaneous administration of metabolite(s) and precursor(s). This is possible with the use of stable isotope methodology.

(1) J. S. Lockard, R. H. Levy, L. L. DuCharme, and W. C. Congdon, *Epilepsia*, **20**, 339 (1979).

(2) A. J. Wilensky, E. A. Lane, R. H. Levy, L. M. Ojemann, and P. N. Friel, *Eur. J. Clin. Pharmacol.*, **21**, 149 (1981).

(3) J. A. Cramer and R. H. Mattson, American Epilepsy Society Meeting, San Diego, Calif., 1980.

(4) J. S. Lockard, R. H. Levy, L. L. DuCharme, and W. C. Congdon, *Epilepsia*, **21**, 177 (1980).

(5) E. A. Lane, "Study on Parent-Metabolite Relationships for Cinromide and Two Metabolites," Ph.D. thesis, University of Washington, 1981.

(6) R. P. Hatch, J. Shringapure, and S. M. Weinreb, *J. Org. Chem.*, **43**, 4175 (1978).

(7) E. J. Corey and J. W. Suggs, *Tetrahedron Lett.*, **31**, 2647 (1975).

(8) E. C. Horning, "Org. Syn., Coll." Vol. III, Wiley, New York, N.Y., 1955, p. 172.

(9) R. B. Westkaemper and R. P. Hanzlik, *Arch. Biochem. Biophys.*, **208**, 195 (1981).

(10) E. A. Lane and R. H. Levy, *J. Pharm. Sci.*, **72**, 493 (1983).

(11) S. A. Kaplan, M. L. Jack, S. Cotler, and K. Alexander, *J. Pharmacokinet. Biopharm.*, **1**, 201 (1973).

(12) K. S. Pang and K. C. Kwan, *Drug Metab. Dispos.*, **11**, 79 (1983).

(13) K. S. Pang and J. R. Gillette, *J. Pharmacokinet. Biopharm.*, **7**, 275 (1979).

Huey-shin Lin
Biochemicals Department
Stine Laboratories
E.I. Dupont de Nemours and Company
Newark, DE 19711

René H. Levy[x]
Departments of Pharmaceutics
and Neurological Surgery, BG-20
Schools of Pharmacy and Medicine
University of Washington
Seattle, WA 98195

Elizabeth A. Lane
Clinical Studies
National Institute of Alcohol Abuse and Alcoholism
Alcohol, Drug Abuse and Mental Health Administration
Bethesda, MD 20205

W. Perry Gordon
National Institute of Public Health
Geitmyrsvegen, 75-Oslo- Norway

Received June 6, 1983.
Accepted for publication November 28, 1983.
Supported by National Institutes of Health Contract N01-NS-1-2282, Grant NS-04053, and Biomedical Research Development Grant 1 S08 RR 09082.

BOOKS

Natural Product Chemistry—A Mechanistic and Biosynthetic Approach to Secondary Metabolism. By KURT B. G. TORSSELL. John Wiley & Sons, Inc., One Wiley Drive, Somerset, NJ 08873. 1983. 401 pp. 16 × 23 cm. Price $54.95.

This book presents an overview of the current known and postulated biosynthetic pathways of the secondary metabolites, those natural products whose precursors are the common α-amino acids, sugars, and low-molecular weight carboxylic acids of the Krebs cycle. Although the physiological functions of many of these natural products have not yet been established, some have been found to play important roles in the regulation of animal and insect social behavior and development, and plant protection mechanisms.

The contents of this volume are organized into eight chapters, each of which is subdivided into various topics. An overview of the natural products field and the importance of these metabolites are presented in the first chapter. The remaining chapters present the biosynthetic pathways for seven different classes of natural products in addition to a brief introduction about the general chemistry, nomenclature, and background of each class. In the second chapter on carbohydrates, photosynthesis, the Krebs or citric acid cycle, monosaccharides, and polysaccharides are among the topics discussed. The biosynthesis of shikimic acid; its conversion to aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine; biological hydroxylation mechanisms, and the biosynthesis of aromatic carboxylic acids, coumarins, quinones, and lignins are included in the third chapter. Chapter 4 details the biosyntheses of compounds (such as saturated and unsaturated fatty acids and the aromatic anthraquinones, flavonoids, and tropolones) that originate from the polyketide pathway by condensation of acetyl CoA. The mevalonic acid pathway to the terpenes, squalene, steroids, and carotenes is the subject of the fifth chapter. In the sixth chapter, the biosyntheses of amino acids, peptides, and proteins are presented. The alkaloids derived from the amino acids ornithine, lysine, tyrosine, and tryptophan, from anthranilic acid, and from the amination of terpenes are found in the seventh chapter. In the last chapter, the *N*-heterocyclic aromatic compounds, including pyrimidines, purines, pteridines, pyrroles, porphyrins, and corrin ring-containing compounds, are discussed. Each chapter also includes historical and biological information about some of the metabolites. The inclusion of this material appreciably enhances reader interest. A set of biosynthetic mechanistic problems and a bibliography are presented at the end of each chapter. Answers to the problems and author and subject indices are included at the end of the volume.

On the basis of the table of contents, this work appears to be an informative and valuable text on natural product biosynthesis which emphasizes the biosynthetic pathways in an organic mechanistic approach. This book is recommended by the publishers as a text for undergraduates and graduate students and a reference work for researchers in the field. However, many mechanistic discussions are vague and not very clear. The internal organization of paragraphs is poor. For example, the section on the NIH shift is very confusing for a reader not familiar with this topic. This lack of clarity is found in many other areas of this book. The absence of numbered structures forces the reader to search each figure or reaction sequence for a particular compound that is being discussed in the text. The absence of carbon atom numbering on some of the structures is not helpful. In conclusion, only those scientists well-versed in biosynthetic pathways will find this an easily readable text. The literature cited is very current, and the notable accomplishments of workers in the area of metabolic biosynthetic pathways have been included. The mechanistic approach to biosynthetic processes used by the author is a valuable and informative one.

Reviewed by Marcia Dawson
Life Sciences Division
SRI International
Menlo Park, CA 94025