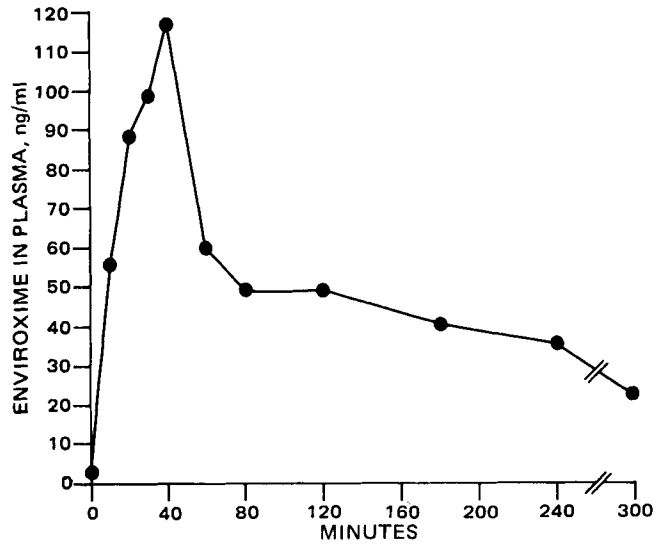


Figure 5—*Enviroxime concentration in dog plasma (ng/ml) after oral administration of an 8-mg/kg dose.*

terminable as early as 10 min after oral dosing to as long as 5–8 hr post-dose. A second example illustrates the value of a chemical assay, as compared with the original plaque reduction assay. The amount of II found in the plasma of dogs following oral dosing with I was usually very small, between 5 and 10% of I. However, when II was administered orally to dogs, I was still the major peak found in plasma. Thus, either by metabolism or by acid-catalyzed isomerization in the GI tract, the two isomers tend to form an equilibrium mixture in the plasma, in which I predominates.

The proven versatility of the methodology here points out the power of the coupling of liquid chromatography with electrochemical detection for determining oxidizable drugs in biological matrixes.

REFERENCES

(1) J. H. Wikel, *et al.*, *J. Med. Chem.*, **23**, 368 (1980).

(2) D. C. DeLong and S. E. Reed, *J. Infect. Dis.*, **141**, 87 (1980).

(3) C. J. Parli, R. J. Bopp, and J. F. Quay, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **39**, 307 (1980).

(4) J. F. Quay, C. J. Parli, J. F. Stuckey II, K. S. E. Su, D. C. DeLong, J. D. Nelson, and J. H. Wikel, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **39**, 850 (1980).

(5) R. J. Phillpotts, R. W. Jones, D. C. Delong, S. E. Reed, J. Wallace, and D. A. J. Tyrrell, *Lancet*, **i**, 1342 (1981).

(6) J. A. Bogan and S. Marriner, *J. Pharm. Sci.*, **69**, 422 (1980).

(7) D. J. Miner and P. T. Kissinger, *Biochem. Pharmacol.*, **28**, 3285 (1979).

(8) P. T. Kissinger, *Anal. Chem.*, **49**, 447A (1977).

(9) R. C. Koile and D. C. Johnson, *ibid.*, **51**, 741 (1979).

(10) D. J. Miner, *Anal. Chim. Acta*, **134**, 101 (1982).

(11) L. R. Snyder and J. J. Kirkland, in "Introduction to Modern Liquid Chromatography," 2nd ed., Wiley, New York, N.Y., 1979, pp. 698–717.

ACKNOWLEDGMENTS

This paper was presented in part at the First International Symposium on the Neurochemical and Clinical Applications of LCEC, Indianapolis, Ind., May 12, 1980, Abstract #15.

The authors thank J. H. Fouts, P. R. Page, and R. J. Pierson for running the assays, Drs. C. L. Gries, K. S. Israel, and J. F. Quay for providing the samples, and E. Reed for help with the manuscript.

NOTES

Antihypertensive Agents: Pyridazino(4,5-b)indole Derivatives

A. MONGE VEGA *x, I. ALDANA *, P. PARRADO *, M. FONT *, and E. FERNANDEZ ALVAREZ ‡

Received December 3, 1981, from the **Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain* and the ‡*Instituto de Química Orgánica del CSIC, Juan de la Cierva, 3 Madrid-6 Spain.* Accepted for publication January 27, 1982.

Abstract □ A series of 4-hydrazino-5H-pyridazino(4,5-b)indoles (VIII) and their potential metabolites (3,4-dihydro-4-oxo-5H-pyridazino(4,5-b)indoles (V) and 11H-1,2,4-triazolo(4,3-b)pyridazino-(4,5-b)indoles (IX) were investigated for antihypertensive activity in spontaneously hypertensive rats. All compounds showed antihypertensive activity at 25 mg/kg ip. Compound VIII was the most active, and the most toxic.

Keyphrases □ Antihypertensive agents—synthesis of pyridazino-(4,5-b)indole derivatives, rats, metabolites □ Metabolites—synthesis of antihypertensive agents, pyridazino(4,5-b)indole derivatives, rats □ Pyridazino(4,5-b)indole—derivatives, synthesis of antihypertensive agents, hypertensive rats

In connection with work related to the preparation and study of new products as potential antihypertensive agents, a series of pyridazino(4,5-b)indole derivatives (1–3) related to the well-known antihypertensive agent hydralazine[1] (I), as well as its metabolites (4, 5) were synthesized. The metabolism of these compounds takes place essentially (5, 6) through N^2-acylation (formyl, acetyl) of the hydrazino group and further cyclization to triazole derivatives (II), as well as the hydrolysis of the hydrazino to give the oxo derivatives (III).

The results of a preliminary evaluation in spontaneously hypertensive rats of Compounds V, VIII, and IX (see Scheme) are reported. All of these compounds are structurally related to hydralazine (I)[1] and its metabolites.

Some preliminary data for Compound VII*a* in normotensive and hypertensive (renal fistula), anesthetized

[1] Apresoline (1-hydrazinophtalazine).

0022-3549/82/1200-1406$01.00/0

Table I—Antihypertensive Activity in Spontaneously Hypertensive Rats for Pyridazino (4,5-b)indole Derivatives V, VIII, and IX

	V Compounds					VIII Compounds					IX Compounds				
			Falls[d]					Falls[d]					Falls[d]		
Compounds	Dose	LD_{50}	3 hr	5 hr	25 hr	Dose	LD_{50}	3 hr	5 hr	25 hr	Dose	LD_{50}	3 hr	5 hr	25 hr
a	25[a]	1000	16	19	—	5[a]	225	92	84	32	25[a]	794	1	—	—
a						2,5[a]		66	61	36					
a						1,0[a]		47	44	38					
a						0,5[a]		48	3	1					
a						5[b]		50	92	65					
a						2,5[b]		33	28	4					
a						5[c]		80	60	20					
a						2,5[c]		61	30	10					
b	25[a]	100	18	20	6	5[c]	105	21	16	8	25[a]	200	14	4	1
b						2,5[c]		19	16	8					
b						5[a]		22	17	9					
b						2,5[a]		14	10	6					
c	25[a]	758	28	30	19										
d						25[a]	224	67	44	15	25[a]	1000	8	13	2
						19[b]		16	12	3					
e	10[a]	1000	1	19	15	10[a]	970	12	31	1	10[a]	1000	3	18	4

[a] Dose, mg/kg, ip (spontaneously hypertensive). [b] Dose; mg/kg, oral (spontaneously hypertensive). [c] Dose, mg/kg, ip (desoxycorticosterone-saline rats). [d] Falls in systolic blood pressure at the indicated times after the indicated doses (average for four rats 190 ± 5 mmHg).

(thiobarbital), and nonanesthetized dogs have been reported (3).

EXPERIMENTAL

Chemistry[2]—Compounds V–IX have been prepared according to Scheme I. The following compounds have been previously reported: V*a* and VIII*a* (3); V*b* (mp 283–285°, and erroneously reported mp 218°) and V*e* (7); IX*a* and IX*b* (2); V*c* (8, 9) and V*d*; VIII*d* and IX*d* (9). Compounds VIII*b*, VIII*e*, and IX*e* were prepared by methods similar to those previously reported (2, 3, 9) but these compounds had not been reported previously.

4-Hydrazino-5-methyl-5H-pyridazino(4,5-b)indole (VIII*b*)—Compound VIII*b* was prepared from Compound V*b* (7) in a similar way to that reported previously (3) for Compound VIII*a*. Yield: 90%; mp = 178–180°; (ethanol), white crystals IR (potassium bromide): ν (cm^{-1}) = 3200 (bs, NH); 1625 (s, C=N); 765 (s, 1,2-aromatic disubstituted) ^{1}H-NMR (dimethyl sulfoxide-d_6): δ = 3.40 (sb, 2H, NH_2); 4.25 (s, 3H, CH_3); 7.10–7.90 (m, 3H, H_{6-8}); 7.85–8.30 (dd, H_9); 8.50 (sb, 1H, NH); 9.95 (s, H_1).

Anal.—Calc. for $C_{10}H_{12}N_5$: C, 61.7; H, 5.62; N, 32.7. Found: C, 61.9; H, 5.61; N, 32.7.

4-Hydrazino-8-benzyloxy-5H-pyridazino(4,5-b)indole (VIII*e*)—Compound VIII*e* was prepared from Compound V*e* (7) in a similar way to that reported previously (3) for Compound VIII*a*. Yield: 80%; mp 340° (*N*,*N*-dimethylformamide–water). Light brown crystals formed. IR (potassium bromide): ν (cm^{-1}) = 3000–3200 (m) and 3280–3399 (m) (NH); 1645 (s, C=N); 830 (m, 1,2,4-aromatic trisubstituted); 700 (s, aromatic monosubstituted) ^{1}H-NMR (dimethyl sulfoxide-d_6): δ = 5.30 (s, 2H, CH_2—O); 6.00–7.00 (bs, 3H, NH_2—NH—); 7.20–7.65 (m, 7H, $H_{6-7}C_6H_5$—); 7.85 (dd, H_9); 9.20 (s, H_1).

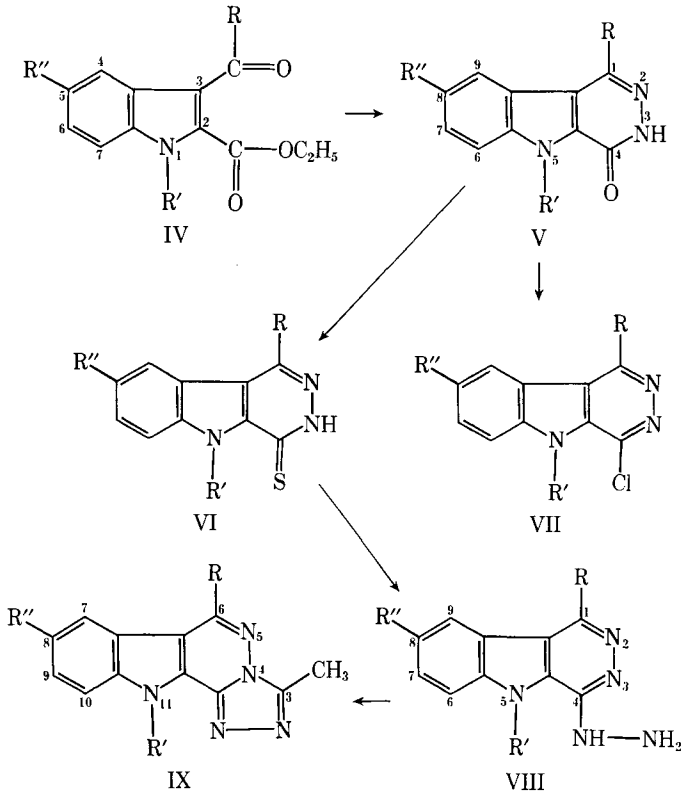
Anal.—Calc. for $C_{17}H_{15}N_5O$: C, 66.9; H, 4.91; N, 22.9. Found: C, 66.5; H, 4.75; N, 22.5.

11-H-1,2,4-Triazolo(4,3-b)pyridazino(4,5-b)-8-benzyloxyindole (IX*e*)—Compound IX*e* was synthesized from Compound VIII*e* in a similar way to that previously reported (2, 9) for Compounds IX*a*,*b*,*d*. Yield: 90%; mp 340° (*N*,*N*-dimethylformamide). White crystals formed. IR (potassium bromide) ν (cm^{-1}) = 3080–3120 (m, NH), 1640 (s) and 1625 (s) (C=N): 820 (s, 1,2,4-aromatic trisubstituted) ^{1}H-NMR (dimethyl sulfoxide-d_6): δ = 3.20 (s, 3H, CH_3); 5.40 (s, 2H, CH_2—O); 7.40–7.55 (m, 7H, $H_{6-7}C_6H_5$); 7.85 (dd, H_9); 9.60 (s, H_1).

Anal.—Calc. for $C_{19}H_{15}N_5O$: C, 69.3; H, 4.59; N, 21.3. Found: C, 69.5; H, 4.82; N, 21.4.

Pharmacology—Compounds were evaluated for antihypertensive activity in unanesthetized spontaneously hypertensive, 8-week-old male rats (spontaneously hypertensive)[3], of the Okamoto strain weighing 300–350 g and with systolic blood pressure levels >180 mmHg. Changes in the arterial pressure were measured by mechanical transduction[4] and registered on paper. A dose was given to each of four animals and blood pressures were measured at 3, 5, and 24 hr. The tested compounds were administered (2.5 ml/kg ip) either dissolved or suspended in normal saline containing 0.2% carboxymethylcellulose and 1% polysorbate 80.

The initial dose was 25 mg/kg. The more active compounds were tested at lower concentrations, as shown in Table I. The most active compound



		R	R′	R″
IV–IX:	a)	H	H	H
IV–IX:	b)	H	CH_3	H
IV–V:	c)	OH	H	H
IV–IX:	d)	Cl	H	H
IV–IX:	e)	H	H	C_6H_5—CH_2—O—

[2] All melting points were determined in a Gallenkamp apparatus in glass capillary tubes and were uncorrected. Analysis included microanalysis of samples dried in vacuum on phosphorus pentoxide (2–3 hr, 60–70°). Where a molecular formula is given, analytical results are within 0.4% of the theoretical values. IR spectra were recorded in a Perkin-Elmer model 681 apparatus. The ^{1}H-NMR spectra were recorded in a Perkin-Elmer model R-32 spectrometer (90 MHz) at room temperature, with tetramethylsilane as an internal reference: s, d, t, for singlet, doublet, triplet; bs = broad signal; dd = deformed doublet.

[3] Navarra University Farms.

[4] W+W, BP Recorder 8005.

(VIII*a*) was also similarly tested in desoxycorticosterone–saline rats (intraperitoneally) (10).

Acute toxicities were determined in male mice weighing 20 ±2 g. A dose was given orally to each of five animals and mortalities were recorded 1 week later. The LD_{50} was calculated according to a previous procedure (11).

RESULTS AND DISCUSSION

Table I summarizes the results of the pharmacological assays. All the compounds studied showed appreciable activity in the spontaneously hypertensive rat assay. In decreasing order of activity they are: VIII*a* > VIII*d* > VIII*e* > VIII*b* > V*c* > V*a* > V*b* > V*e* > IX*e* > IX*b* > IX*d* > IX*a*.

The hydrazino group is not essential for activity although it does considerably increase antihypertensive activity and toxicity. Group VIII compounds are thus the most active and the most toxic. Furthermore, the antihypertensive activity of V compounds may be related to that of other non hydrazine antihypertensive compounds (12, 13). It seems that this is the first report of antihypertensive activity in the oxo and triazole metabolites of pyridazino–hydrazine antihypertensives.

REFERENCES

(1) A. Monge Vega, J. A. Palop, M. T. Martínez, and E. Fernández Alvarez, *J. Heterocycl. Chem.*, **17**, 249 (1980).

(2) A. Monge Vega, I. Aldana, M. M. Rabbani, and E. Fernandez Alvarez, *ibid.*, **17**, 77 (1980).

(3) A. Monge Vega, I. Aldana, and E. Fernández Alvarez, *Eur. J. Med. Chem.*, **13**, 573 (1978).

(4) J. Druey and J. Triod, in "Antihypertensive Agents," E. Schilitter, Ed., *Academic*, New York, N.Y., 1967, pp. 223–262.

(5) R. Mori, J. Ono, T. Saito, T. Aximoto, and M. Sano, *Chem. Pharm. Bull.*, **25**, 830 (1977).

(6) H. Zimmer, J. Kokosca, and D. R. Garteiz, *Arzneim.-Forsch.*, **23**, 1028 (1973).

(7) A. Monge Vega, J. A. Palop, M. T. Martínez, and E. Fernández Alvarez, *An. Quin.*, **27**, 889 (1979).

(8) E. H. Huntress and W. H. Haarom, *J. Am. Chem. Soc.*, **63**, 2762 (1941).

(9) A. Monge Vega, I. Aldana, and E. Fernández Alvarez, *J. Heterocycl. Chem.*, **18**, 889 (1981).

(10) J. De Champlain and M. R. Van Ameriger, *Circ. Res.*, **31**, 617 (1972).

(11) J. T. Lithfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

(12) L. R. Bennet, C. J. Blankey, R. W. Fleming, R. D. Smith, and D. K. Tessmann, *J. Med. Chem.*, **24**, 382 (1981).

(13) J. D. Warren, S. A. Lang, Jr., P. S. Chan, and J. W. Marsico, *J. Pharm. Sci.*, **67**, 1479 (1978).

ACKNOWLEDGMENTS

Supported by a grant from the Comision Asesora de Investigación Científica y Técnica (No. 4421, *1980*), Spain.

Simple Methods for Estimating Percent Disintegrated–Time Data

ADNAN EL-YAZIGI

Received March 9, 1981, from the *Department of Pharmaceutics, College of Pharmacy, University of Riyadh, Riyadh, Saudi Arabia.* Accepted for publication of February 3, 1982. Present Address: Cancer and Radiation Biology Research, Cancer Therapy Institute, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

Abstract □ Two techniques are described for the treatment of dissolution rates to estimate the percent disintegrated–time data for tablets and capsules. The first is an extension of an equation derived previously with the assumption of first-order disintegration and dissolution processes; whereas, the second involves the determination of the rate constant from the terminal segment of the curve and the use of numerical derivatives according to a disintegration kinetics-independent approach. The dissolution data of six commercial tablet and capsule formulations were treated according to the described techniques. Good agreement was found between the percent disintegrated–time data estimated by the second approach for an acetaminophen tablet and those obtained by a well-established model where a Weibull function was employed.

Keyphrases □ Dissolution—tablets, simple methods for estimating percent disintegrated–time data □ Disintegration—tablets, simple methods for estimating percent disintegrated–time data, dissolution

There are few methods available for utilizing dissolution rate data to estimate the fraction disintegrated as a function of time for tablets (1, 2). These techniques, however, lack simplicity and require computer programs which may not be available.

A simple method was described recently for the disintegration–dissolution analysis of percent dissolved–time data (3). The technique is based on a biexponential equation with the assumption of first-order disintegration and dissolution according to a simple dissolution model.

The present report describes two approachs that can be used to estimate the percent disintegrated–time data for tablets and capsules. The first is an extension to the above report (3), whereas the other is based on disintegration kinetics—independent approach. Both techniques are simple and rapid and allow computations to be carried out manually.

THEORETICAL

Estimation of Percent Disintegrated–Time Data (Approach I) —Assuming first-order disintegration and dissolution processes, if A, A_p, and A_s represent the amounts of drug in dosage form, small particles, and solution, respectively, at any given time, and it is assumed that the disintegration and dissolution are first-order processes whose apparent rate constants are k_d and k_s, respectively, or:

$$A \xrightarrow[\text{first-order disintegration}]{k_d} A_p \xrightarrow[\text{first-order dissolution}]{k_s} A_s$$

Scheme I

the following equation can be derived (3):

$$100 - f_s = \frac{100\,k_d}{k_d - k_s} e^{-k_s t} - \frac{100\,k_s}{k_d - k_s} e^{-k_d t} \qquad \text{(Eq. 1)}$$

where f_s is the cumulative percent dissolved at time t.

Based on Scheme I, the following also can be derived:

0022-3549/82/1200-1408$01.00/0