Bioavailability of Aminosalicylic Acid and Its Various Salts in Humans III: Absorption from Tablets

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Abstract □ The relative bioavailability of commercially available preparations of aminosalicylic acid and its sodium, potassium, and calcium salts is presented. The doses administered were 4 g for aminosalicylic acid and 2.8, 2.6, and 2.6 g for the sodium, potassium, and calcium salts, respectively. Absorption from tablets of the salts was rapid and complete, but absorption of the free acid was only 77% of the dose. Dissolution of the relatively insoluble acid is a rate-limiting factor in absorption. Although the amount of free acid administered and the absolute amount absorbed were higher, the area under the plasma concentration curve of unmetabolized drug was less than for the salts. This is attributed to capacity-limited acetylation of drug, especially during the first pass. The amount of bioavailable drug is dependent on the rate of metabolism and, hence, the rate of absorption.

Keyphrases □ Aminosalicylic acid and salts—absorption from commercial tablets compared, humans □ Bioavailability—absorption of aminosalicylic acid and salts from commercial tablets, humans □ Absorption—aminosalicylic acid and salts from commercial tablets, humans

Numerous aminosalicylic acid preparations are currently available to the practitioner. Choice of product is rendered difficult by the confusing literature on the merits of different preparations (1–4). The confusion has arisen from inadequate standardization and sampling during studies and from the previous poor understanding of the significant nonlinear pharmacokinetics of this drug.

The present report concerns absorption from tablets of aminosalicylic acid and several salts. The data were obtained as part of a series of bioavailability studies on this compound. A previous report (5) showed that the absorption of its salts in solution differed with respect to rate of absorption and the peak levels achieved. Dissolution of drug in suspension was a limiting factor in absorption. The current report describes crossover studies concerning absorption of aminosalicylic acid and the sodium, potassium, and calcium salts from commercially available tablets.

EXPERIMENTAL

Drugs—Tablets of aminosalicylic acid (I) (0.5 g), its sodium salt (II) (0.5 g), and its calcium salt (III) (0.5 g) were supplied by the Food and Drug Administration (FDA) from a single manufacturer[1]. The potassium salt (IV) (0.5 g) was purchased on the open market from a second manufacturer[2]. All tablets complied with USP specifications[3].

Subjects—Twelve healthy volunteers (nine men and three women) with no history of liver or kidney disease participated in the study. Prospective participants were screened by physical examination and laboratory tests including complete blood count, serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, urinalysis, and serum alkaline phosphatase and creatinine. Written informed consent was obtained from each volunteer.

[1] Parasal, Lot 721789; Parasal sodium, Lot 732043; and Parasal calcium, Lot 736132; Panray Division of Ormont, Englewood, N. J.
[2] Paskallium, Lot k448, Glenwood Laboratories, Tenafly, N. J.
[3] Analyses performed by FDA.

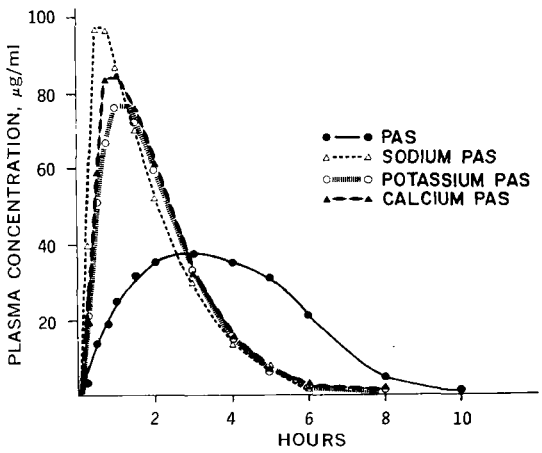

Figure 1—*Mean plasma concentrations of unchanged drug from 12 subjects following administration of four different preparations of aminosalicylic acid (PAS). Data were corrected to 70 kg body weight.*

Dose and Administration—Subjects abstained from all drugs for 2 weeks and from alcoholic beverages for 3 days prior to the study. After an overnight fast, the designated form of 4 g I, 4 g II (equivalent to 2.80 g I), 4 g III (equivalent to 2.60 g I), or 4 g IV (equivalent to 2.60 g I) was ingested with 250 ml water. The fast was continued for 5 hr, with water allowed *ad libitum*. Serial blood samples were taken from a peripheral vein at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 10 hr. The blood was collected in tubes containing sodium heparin and plasma was obtained by centrifugation. All urine was collected at 2-hr intervals for 10 hr and a cumulated sample was collected from 10 to 24 hr. Subjects were ambulatory during the study period. The four preparations were randomly administered in one of four sequences at intervals of at least 1 week.

Sample Analysis—Plasma and urine were stored at 0° until analyzed by the modified Marshall method described previously (5). All samples were assayed for acetylated and nonacetylated drug. The data, normalized to a 4-g dose and 70 kg body weight, were subjected to an analysis of variance for crossover experiments. Where significant differences were observed, further multiple-range analyses (6) were performed to identify the source of difference. Pharmacokinetic parameters were obtained by fitting the data to a single-compartment open model using the BMDX85 program.

RESULTS

Plasma—Mean peak plasma concentrations of nonmetabolized drug were highest with the sodium salt (II) followed by the calcium salt (III), potassium salt (IV), and free acid (I), respectively (Figs. 1 and 2). Peak concentrations after administration of I were 3.5 times lower than levels achieved for the sodium salt. Differences in peak concentration were statistically significant (Table I). Formulation I had high significant lower peak levels than II, III, or IV (Table I). High peak levels were associated with rapid absorption rates and, consequently, with short peak time values. Only peak times following I were significantly different from the other compounds (Table II). Areas under the plasma concentra-

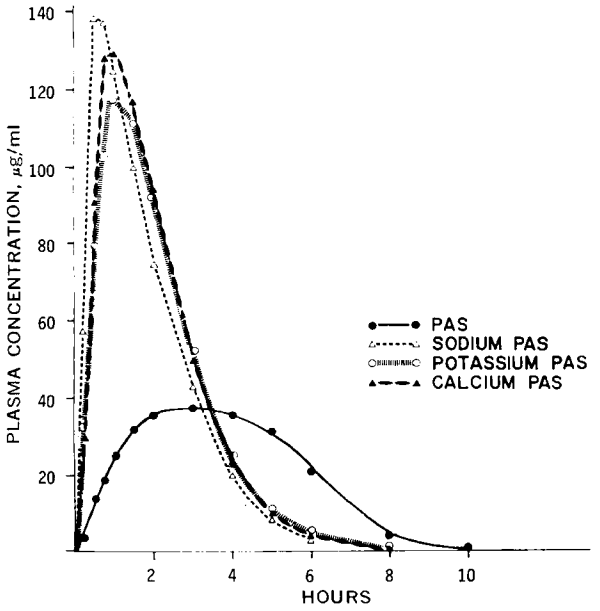

Figure 2—*Mean plasma concentrations of unchanged drug from 12 subjects following administration of four different preparations of aminosalicylic acid (PAS). Data were corrected to 70 kg body weight and to a dose equivalent to 4 g free acid.*

tion–time curve *(AUC)* were calculated using the trapezoidal rule and relative bioavailability ascertained. The *AUC* for I was significantly lower than for II, III, and IV, both with the uncorrected data and the data normalized to a 4-g dose. The analysis of the corrected data is given in Table III. Although absorption of I was markedly slower, half-lives of elimination in all cases were very similar once the absorption phase was complete. Mean half-lives were 0.94, 0.96, 0.91, and 0.91 hr for I, IV, III, and II, respectively.

Urine—Excretion of drug and metabolites was very rapid, with 50% of the dose being excreted in 2–3 hr for II, III, and IV and in 5–6 hr for I. Total recovery in urine amounted to 77% for I and was almost complete for II, III, and IV (Fig. 3 and Table IV). Drug I was the only preparation significantly different in terms of total absorption. The percentage absorption of I was somewhat lower than the absorption of the drug from suspension reported earlier (5). In that study it was found that aminosalicylic acid given in suspension was almost completely absorbed. No differences were observed in the percent of dose excreted as acetylated drug. The means for the four preparations ranged from 52 to 57% of the dose (Table V). However, significant differences were observed for the percent of dose excreted as nonacetylated drug (Table VI). Administration of I resulted in a smaller percentage of dose excreted as unchanged drug and *p*-aminosalicyluric acid (Figs. 4 and 5). The apparent discrepancy between Tables V and VI can be resolved by comparing cumulative excretion as percent of absorbed drug. Since the absolute amounts absorbed for the four preparations were different, the cumulative excretions of acetylated and nonacetylated drug were calculated as percentages of absorbed drug. When this was done, the percentages of the absorbed dose excreted as acetylated metabolite were 73.1, 54.5, 57.7 and 56.4 for I, IV, III, and II, respectively. Drug I was significant-

Table I—Peak Plasma Drug Concentrations from Tablets of Aminosalicylic Acid and Three of Its Salts

Drug[a]	I	IV	III	II
Mean Peak Concentration[b], μg/ml	49.98	121.09	139.51	155.44

Analysis of Variance

Source of Variation	*df*	*SS*	*MS*	*F*
Between individuals	11	12,273	1,115	2.14
Between drugs	3	77,903	25,967	49.76
Between periods	3	768	256	0.49
Error	30	15,654	521	
Total	47	106,598		

SE of means = 6.59
$F_{0.05}$ (3,30) = 2.92
$F_{0.05}$ (11,30) = 2.12

Multiple-Range Analysis

p	2	3	4
Shortest significant ranges	19.05	20.03	20.56

II–I = 105.46 > 20.56; significant
II–IV = 34.35 > 20.03; significant
II–III = 15.93 < 19.05; not significant
III–I = 89.53 > 20.03; significant
III–IV = 18.42 < 19.05; not significant
IV–I = 71.11 > 19.05; significant

[a] I = aminosalicylic acid, II = sodium salt, III = calcium salt, and IV = potassium salt. [b] Any two or more means not statistically different are underscored by the same straight line.

Table II—Times for Plasma Drug Concentration to Peak from Tablets of Aminosalicylic Acid and Three of Its Salts

Drug[a]	II	III	IV	I
Mean Peak Times[b], hr	0.83	1.02	1.10	3.54

Analysis of Variance

Source of Variation	*df*	*SS*	*MS*	*F*
Between individuals	11	13.14	1.19	1.89
Between drugs	3	59.18	19.73	31.20
Between periods	3	1.72	0.57	0.91
Error	30	18.97	0.63	
Total	47	93.01		

SE of means = 0.23
$F_{0.05}$ (3,30) = 2.92
$F_{0.05}$ (11,30) = 2.12

Multiple-Range Analysis

p	2	3	4
Shortest significant ranges	0.66	0.70	0.72

I–II = 2.71 > 0.72; significant
I–III = 2.52 > 0.70; significant
I–IV = 2.44 > 0.66; significant
IV–II = 0.27 < 0.70; not significant; hence,
IV–III = not significant
III–II = 0.19 < 0.66; not significant

[a] I = aminosalicylic acid, II = sodium salt, III = calcium salt, and IV = potassium salt. [b] Any two or more means not statistically different are underscored by the same straight line.

Table III—Areas under the Plasma Concentration–Time Curve *(AUC)* following Administration of Tablets of Aminosalicylic Acid and Three of Its Salts

Drug[a]	I	IV	II	III
Mean *AUC*[b], μg hr/ml	209.07	313.22	313.22	326.83

Analysis of Variance

Source of Variation	*df*	*SS*	*MS*	*F*
Between patients	11	99,499	9,045	4.75
Between drugs	3	107,807	35,935	18.87
Between periods	3	5,901	1,967	1.03
Error	30	57,124	1,904	
Total	47	270,333		

SE of means = 12.60
$F_{0.05}$ (3,30) = 2.92
$F_{0.05}$ (11,30) = 2.12

Multiple-Range Analysis

p	2	3	4
Shortest significant range	36.41	38.30	39.30

III–I = 117.76 > 39.30; significant
III–IV = 13.61 < 38.30; not significant; hence,
III–II = not significant
II–I = 104.15 > 38.30; significant
II–IV = 0 < 36.40; not significant
IV–I = 104.15 > 36.40; significant

[a] I = aminosalicylic acid, II = sodium salt, III = calcium salt, and IV = potassium salt. [b] Any two or more means not underscored by the same straight line are significantly different.

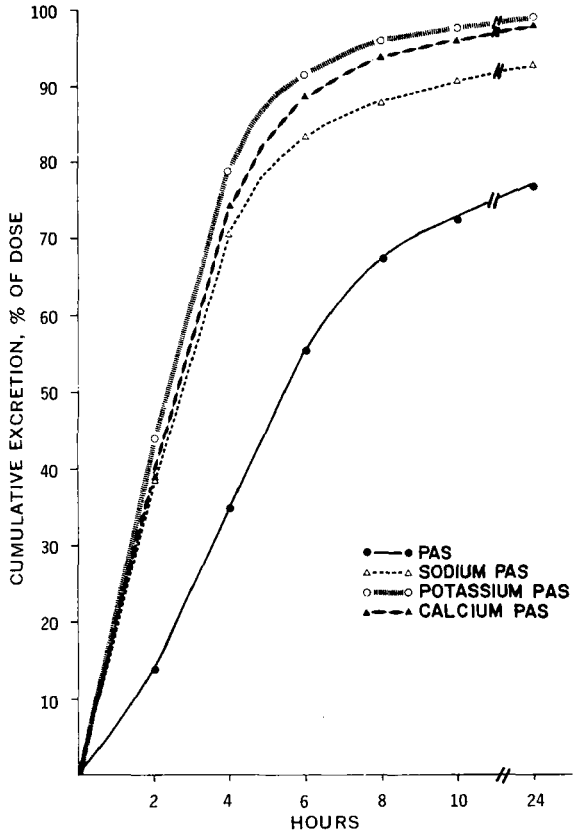

Figure 3—*Mean cumulative excretion of total drug from 12 volunteers following administration of four different preparations of aminosalicylic acid (PAS).*

ly higher. The percentages of the absorbed dose excreted as nonacetylated drug were 26.7, 45.5, 42.3, and 43.8 for the same drugs, respectively. Again, I had an inversely smaller proportion of nonacetylated drug. The ratios of acetylated to nonacetylated drug were 2.74, 1.20, 1.36, and 1.29 for I, IV, III, and II, respectively. Only I was significantly different from II, III, and IV.

DISCUSSION

Metabolism of aminosalicylic acid is mainly by conjugation. Acetylation accounts for 50–70% of the absorbed dose, and glycine conjugation to *p*-aminosalicyluric acid accounts for up to 25% of the dose. These two metabolites together constitute greater than 90% of the metabolites found in urine (7, 8). Other metabolites are of minor importance and the balance is unchanged drug. Since the analytical methods used in the present study do not differentiate between unchanged drug and the glycine conjugate, the terminology of nonacetylated drug was used in the description of drug and metabolite content in urine. Because of rapid clearance of the glycine conjugate, the nonacetylated fraction in plasma has been shown to consist of 95% unmetabolized drug, with only 5% constituting glycine-conjugated drug (9). Lauener *et al.* (10) published similar results in addition to showing that conversion of drug to the glycine conjugate at therapeutic doses is first order and independent of dose; therefore the ratio of unchanged drug to the glycine conjugate in urine is constant for an individual independent of dose. Thus, measurements of nonacetylated drug in plasma are essentially measurements of unchanged drug, and cumulative nonacetylated drug in urine will have a constant ratio of unchanged drug to the glycine conjugate.

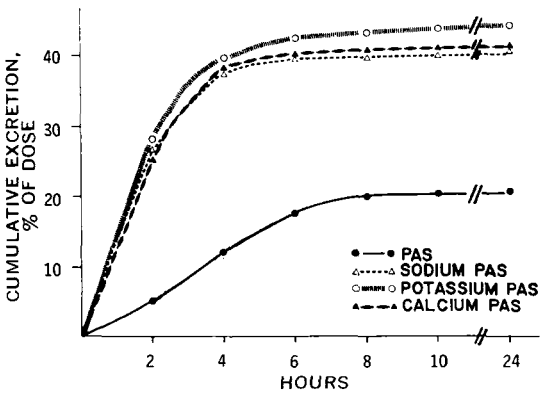

Figure 4—*Mean cumulative excretion of nonacetylated drug from 12 volunteers following administration of four different preparations of aminosalicylic acid (PAS).*

Table IV—Total Excretion of Drug and Metabolites following Administration of Tablets of Aminosalicylic Acid and Three of Its Salts

Drug[a]	I	II	III	IV
Mean Percent of Dose Excreted[b]	77.2	92.8	98.2	98.5

Analysis of Variance

Source of Variation	*df*	*SS*	*MS*	*F*
Between patients	11	1732	157	2.31
Between drugs	3	3618	1206	17.68
Between periods	3	902	300	4.41
Error	30	2047	68	
Total	47	8300		

SE of means = 2.38
$F_{0.05}$ (3,30) = 2.92
$F_{0.05}$ (11,30) = 2.12

Multiple-Range Analysis

p	2	3	4
Shortest significant range	6.88	7.24	7.43

IV–I = 21.3 > 7.43; significant
IV–II = 5.7 < 7.24; not significant; hence,
IV–III = not significant
III–I = 21.0 > 7.24; significant
III–II = 5.4 < 6.88; not significant
II–I = 15.6 > 6.88; significant

[a] I = aminosalicylic acid, II = sodium salt, III = calcium salt, and IV = potassium salt. [b] Any two or more means not underscored by the same straight line are significantly different.

The *F* values for interindividual variations were significant in three out of the six parameters measured. This was to be expected since many factors contribute to interindividual variation. In addition, there were differences between periods in the amount of drug and metabolites excreted in 24 hr. The latter differences arise from the sequence in which the drugs were administered. The reason for this difference is not known. Whatever it may be, these differences were small compared to the much greater difference between drugs.

The amounts absorbed from tablets of II, III, and IV were comparable and close to 100%, negating claims of product superiority for any specific preparation based on total absorption. When

Table V—Total Excretion of Acetylated Drug following Administration of Tablets of Aminosalicylic Acid and Three of Its Salts

Drug[a]	II	IV	I	III
Mean Percent of Dose Excreted[b]	52.3	53.7	56.4	56.7

Analysis of Variance

Source of Variation	*df*	*SS*	*MS*	*F*
Between individuals	11	1206	107	1.74
Between drugs	3	165	55	0.88
Between periods	3	506	168	2.68
Error	30	1890	63	
Total	47	3768		

$F_{0.05}$ (3,30) = 2.92
$F_{0.05}$ (11,30) = 2.12

[a] I = aminosalicylic acid, II = sodium salt, III = calcium salt, and IV = potassium salt. [b] Any two or more means not underscored by the same straight line are significantly different.

Table VI—Total Excretion of Nonacetylated Drug following Administration of Tablets of Aminosalicylic Acid and Three of Its Salts

Drug[a]	I	II	III	IV
Mean Percent of Dose Excreted[b]	20.8	40.6	41.5	44.8

Analysis of Variance

Source of Variation	*df*	*SS*	*MS*	*F*
Between individuals	11	1318	120	17.92
Between drugs	3	4275	1425	213.15
Between periods	3	749	250	37.36
Error	30	200	6.7	
Total	47	6542		

SE of means = 0.75
$F_{0.05}$ (3,30) = 2.92
$F_{0.05}$ (11,30) = 2.12

Multiple-Range Analysis

p	2	3	4
Shortest significant range	2.17	2.28	2.34

IV–I = 24.0 > 2.34; significant
IV–II = 4.2 > 2.28; significant
IV–III = 3.3 > 2.17; significant
III–I = 20.7 > 2.28; significant
III–II = 0.9 < 2.17; not significant
II–I = 19.8 > 2.17; significant

[a] I = aminosalicylic acid, II = sodium salt, III = calcium salt, and IV = potassium salt. [b] Any two or more means not underscored by the same straight line are significantly different.

using blood level as a parameter, however, differences in products may be observed. Similar percentages of absorbed drug were found in two other studies comparing other preparations of this drug[4] (11). The lower amounts of drug absorbed from Formulation I can be attributed to the relative insolubility of free acid in water. Peak times, peak plasma concentrations, and absorption rates of the three salts were different. These findings can be attributed to degree of ionization and differences in tablet formulation. A previous study (5) showed that even when the salts are given in solution, the apparent rates of absorption for the three salts are not the same, indicating absorption differences arising from degree of ionization.

Areas under the plasma concentration–time curve *(AUC)* were lowest for I and were not significantly different among II, III, and IV. However, the dose of I was also highest at 4 g. In terms of absolute amount absorbed, it was 3.09, 2.56, 2.55, and 2.6 g for I, IV, III, and II, respectively, from doses of 4, 2.6, 2.6, and 2.8 g, respectively. The discrepancy between *AUC* and amounts absorbed can be explained by rate-limited metabolism of drug. Lauener *et al.* (10) showed that while the biotransformation of aminosalicylic acid to the glycine conjugate is first order, the formation of acetylated compound is capacity-limited in humans. Rate-limited acetylation of similar compounds in humans and animals is also known (12, 13). Since the absorption rate of I was slowest, the concentrations achieved were also lowest; administration of II, III, and IV gave higher blood levels which would be more likely to approach capacity-limited metabolism. This postulation is further substantiated by the ratios of acetylated to nonacetylated drug found in urine. This ratio was 2.74 for I as compared to a mean of 1.28 for II, III, and IV. The rate constant for elimination of aminosalicylic acid was the same in all cases, suggesting that the capacity-limited phenomenon was a first-pass effect in the GI and liver tissues. During the absorption phase, concentrations of drug perfusing these tissues are high, especially with rapidly absorbed preparations, and metabolism during the first pass can reconcile the data presented here and elsewhere (5). Several drugs are known to be metabolized extensively during absorption (14–16), and acetylation of aminosalicylic acid during the first pass can now be added to that list.

Because of the dose-dependent acetylation of aminosalicylic acid, comparisons of blood levels alone are not reliable indications of its total absorption. Urine data comparisons similarly would be misleading as to the level of nonmetabolized bioavailable drug.

[4] P. Pentikäinen, S. H. Wan, and D. L. Azarnoff, in preparation.

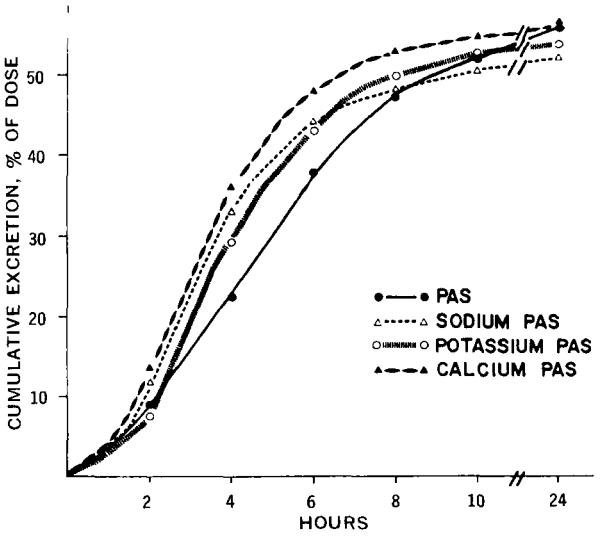

Figure 5—*Mean cumulative excretion of acetylated drug from 12 volunteers following administration of four different preparations of aminosalicylic acid (PAS).*

The confusion in past literature on aminosalicylic acid has arisen from failure to analyze blood and urine data simultaneously.

REFERENCES

(1) S. S. Cohen and W. Y. Yue, *Geriatrics*, **17**, 715(1962).

(2) W. Y. Yue and S. S. Cohen, *Dis. Chest.*, **49**, 165(1966).

(3) J. M. Schless, R. M. Inglis, J. H. Hammond, and J. M. Hale, *ibid.*, **50**, 595(1966).

(4) H. K. Fischer, R. H. Browning, R. L. Donnerberg, P. C. Pratt, and R. J. Atwell, *Amer. Rev. Tuberc. Pulm. Dis.*, **76**, 880(1957).

(5) S. H. Wan, P. Pentikäinen, and D. L. Azarnoff, *J. Pharmacokinet. Biopharm.*, in press.

(6) D. B. Duncan, *Biometrics*, **11**, 1(1955).

(7) E. L. Way, P. K. Smith, D. L. Howe, R. Weiss, and R. Swanson, *J. Pharmacol. Exp. Ther.*, **93**, 368(1948).

(8) J. Kawamata and J. Kashiwagi, *Med. J. Osaka Univ.*, **6**, 119(1955).

(9) H. Engeberg-Pedersen, P. Morch, and L. Tybring, *Brit. J. Pharmacol.*, **23**, 1(1964).

(10) H. Lauener, J. Hodler, G. Favex, E. Dettwiler, and L. Hadorn, *Klin. Wochenschr.*, **35**, 393(1957).

(11) P. Pentikäinen, S. H. Wan, and D. L. Azarnoff, *Amer. Rev. Respirat. Dis.*, **108**, 1340(1973).

(12) M. M. Drucker, S. H. Blondheim, and L. Wislicki, *Clin. Sci.*, **27**, 133(1964).

(13) K. J. W. Hartiala and T. Terho, *Nature*, **205**, 809(1965).

(14) R. N. Boyes, H. J. Adams, and B. R. Duce, *J. Pharmacol. Exp. Ther.*, **174**, 1(1970).

(15) D. G. Shand and R. E. Rangno, *Pharmacology*, **7**, 159(1972).

(16) P. A. Harris and S. Riegelman, *J. Pharm. Sci.*, **58**, 71(1969).

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