The linear free energy relationships applicable to the relative rate terms in eq 1′ and 2′ have been determined by Charton[7b,c] to be

$$\log (k_x/k_{CH_3})_A = \rho_A\sigma_R \quad (4')$$

$$\log (k_x/k_{CH_3})_B = \rho_B\sigma_I \quad (5')$$

Upon substituting eq 3′, 4′, and 5′ into eq 1′, the value of σ_0* becomes

$$\sigma_0^* = [1/(\rho_B^* - \rho_A^*)](\rho_B\sigma_I - \rho_A\sigma_R) \quad (6')$$

Rearrangement of eq 1′ yields

$$\log (k_x/k_{CH_3})_B = 2.48\sigma_0^* + \log (k_x/k_{CH_3})_A \quad (7')$$

Further, the linear steric energy relationship applicable to eq 2′ has been given by Taft[6h] as

$$\log (k_x/k_{CH_3})_A = \delta E_s^0 \quad (8')$$

Substituting eq 3′, 5′, and 8′ into eq 7′ yields

$$\rho_B\sigma_I = (\rho_B^* - \rho_A^*)\sigma_0^* + \delta E_s^0 \quad (9')$$

Therefore

$$\sigma_I = [(\rho_B^* - \rho_A^*)/\rho_B]\sigma_0^* + (\delta/\rho_B)E_s^0 \quad (10')$$

The Metabolism of Pyrovalerone Hydrochloride

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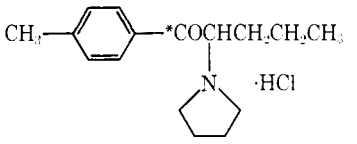
The absorption, distribution, and excretion of ^{14}C-labeled pyrovalerone hydrochloride (1,4′-methyl-2-(1-pyrrolidinyl)valerophenone hydrochloride) were investigated after both oral and intravenous administration of a single dose of 20 mg/kg and 10 mg/kg, respectively, to the mouse. After oral administration, the substance was rapidly and completely absorbed and after both intravenous and oral administration, the radioactivity was excreted rapidly in the urine. Regardless of the mode of administration, within 24 hr over 90% reappeared in the urine whereas less than 10% was detected in the feces. The radioactivity found in the body was concentrated in the liver, bile, and kidneys. The brain contained only traces of radioactivity; this consisting of unchanged pyrovalerone. An examination was also made of human, rabbit, and mouse urine after administration of single doses of 60 mg for the human, 40 mg/kg po. for the rabbit, and 10 mg/kg iv. for the mouse. The substance was excreted very rapidly by all three species and mainly as metabolite **8**. In no instance could unchanged pyrovalerone be detected.

Pyrovalerone hydrochloride (**1**)[1] is a psychostimulant synthesized by Heffe.[2] Both in pharmacological[3] and in clinical[4] experiments, it differs markedly from amphetamine.



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An account is given of the absorption, distribution, and excretion of pyrovalerone·HCl (**1**) in the mouse after both oral and intravenous administration of a single dose of the ^{14}C-labeled substance (*C = ^{14}C). Attempts to elucidate the chemical nature of the radioactivity detected in the brain and experiments to identify pyrovalerone and its metabolites in the urine of mice, rabbits, and humans are also described.

Results and Discussion

Absorption, Distribution, and Excretion in the Mouse.—In a preliminary trial with mice housed in a closed metabolite cage, no $^{14}CO_2$ was expired during 24 hr. The results[5] presented were obtained from animals which, during the experiment, were housed in open metabolite cages.

Using the methods described in the experimental section, the excretion curves shown in Figure 1 were obtained for urine and feces. Figure 2 is a graphical presentation of the distribution of activity in the gastrointestinal tract.

After oral administration, pyrovalerone·HCl was absorbed rapidly. Thirty minutes after administration, only 29% of the dose remained in the gastrointestinal tract. Radioactivity was excreted in the urine rapidly, and, only 4 hr after administration, 70% had been excreted by this route. In all, over 90% of the administered radioactivity was excreted in the urine and 6–8% in the feces. The fractions of radioactivity in the stomach and intestine correspond with the excretion patterns (see Figure 2).

After intravenous administration, the onset of excretion of radioactivity in the urine was even more rapid. Radioactivity was detected in the urine only 5 min after injection and 15 min after administration 20% of the dose had already been excreted by this route. From Figure 1 it is evident that the pattern of excretion in the urine and feces is about the same with both methods of administration. This confirms that pyrovalerone·HCl is absorbed rapidly and completely after oral administration.

The concentrations in the most important organs further confirm the rapid absorption. After oral administration (Table I), all investigated organs except the bile attained their highest concentrations during the

(1) 4′-Methyl-2-(1-pyrrolidinyl)valerophenone hydrochloride (F-1983) (Dr. A. Wander S.A., 3001 Berne, Switzerland).

(2) W. Heffe, *Helv. Chim. Acta*, **47**, 1289 (1964).

(3) G. Stille, H. Ackermann, E. Eichenberger, and H. Lauener, *Arzneim. Forsch.*, **13**, 871 (1963).

(4) (a) H. Heimann and K. Vetter, *Schweiz. Med. Wochenschr.*, **95**, 306 (1965); (b) A. R. Holliday, R. B. Morris, and R. P. Sharpley, *Psychopharmacologia*, **6**, 192 (1964); H. Heimann and G. Lukacs, *ibid.*, **8**, 79 (1965).

(5) The concentrations of radioactivity in the organs are presented as micrograms of radioactive substance (calculated as unchanged pyrovalerone·HCl) per gram of fresh tissue. The listed fractions of radioactivity (as a percentage of the dose) and the concentrations represent the average of the single pools of both animals. The points on the excretion curve (Figure 1) for the times listed are the arithmetic averages of all estimations.

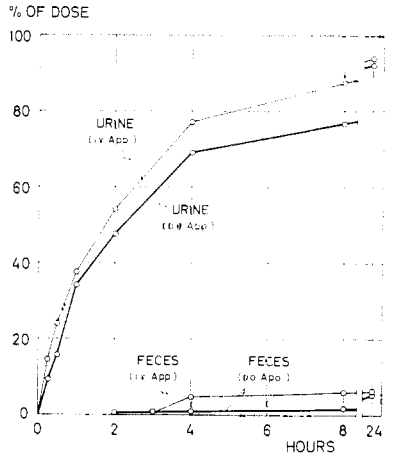


Figure 1.—Excreted fractions of radioactivity in the feces after administration of pyrovalerone·HCl to the mouse (i.v.: 10 mg/kg; orally: 20 mg/kg).[5]

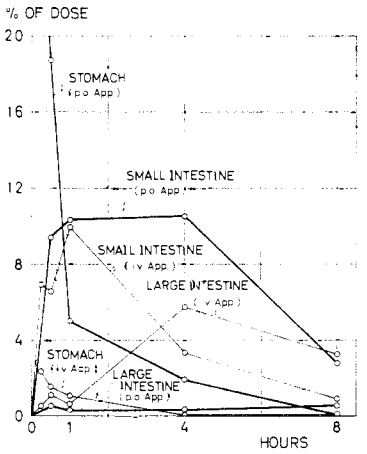


Figure 2.—Distribution of radioactivity in the gastrointestinal tract after administration of pyrovalerone·HCl to the mouse (iv.: 10 mg/kg; orally: 20 mg/kg).[5]

first half hour. During the first hour, the liver had the greatest fraction of radioactivity with 10%; the fraction in the kidneys was distinctly less, its maximum value of 2.1% being attained 30 min after administration. Owing to the rapid excretion of radioactivity, the remaining organs (thymus gland, heart, adrenal glands, spleen, pancreas, seminal vesicles, and testicles) also contained only small fractions of several thousandths of the administered dose within the initial period of the experiment. Only small fractions were determined in the brain; 0.17% 30 min after administration and 0.11% 1 hr after administration. The highest concentrations were registered in the liver, bile, and kidneys with the bile reaching the highest single value. Pyrovalerone·HCl and its metabolites appear to cross the blood brain barrier only with difficulty for the concentrations determined in the brain are distinctly less than those for the blood. The large concentrations in the liver and bile indicate a pronounced biliary excretion. Since, however, over 90% of the radioactivity administered is excreted in the urine that which is excreted through the bile must then be reabsorbed. The total-body residual concentrations also confirm the rapid elimination of the administered dose. These values are 17% 4 hr after administration and 0.65% 24 hr after administration; in the last value, the carcass—contaminated possibly with urine—alone contributes 0.4%.

TABLE I

Organ[a]	Hours after administration: 0.5	1	4	8	24
Blood	8.7	6.1	1.0	0.4	
Lungs	7.0	4.1	0.8	0.3	
Liver	35	30	11	4.6	0.4
Bile	87	5	170	70	
Kidneys	30	16	3.1	1.5	0.2
Brain	1.5	1.0	0.2		
Carcass	5.7	4.1	0.7		

[a] Concentrations (μg of radioactive substance/g of fresh tissue) in several organs after oral administration of 20 mg/kg of pyrovalerone·HCl to the mouse.[5]

TABLE II

Organ[a]	Hours after administration: 0.25	0.5	1	4	8	24
Blood	5.2	3.0	1.5	0.2		
Lungs	8.6	4.2	1.6	0.2	0.1	
Liver	24	15	11	3.5	2.4	0.6
Bile	60	200	85	65	45	
Kidneys	20	13	5.4	0.7	0.5	0.2
Brain	3.1	1.4	0.7	0.1		
Carcass	2.3	4.1	4.5	1.1	0.9	0.2

[a] Concentrations (μg of radioactive substance/g of fresh tissue) of several organs after i.v. administration of 10 mg/kg of pyrovalerone·HCl to the mouse.[5]

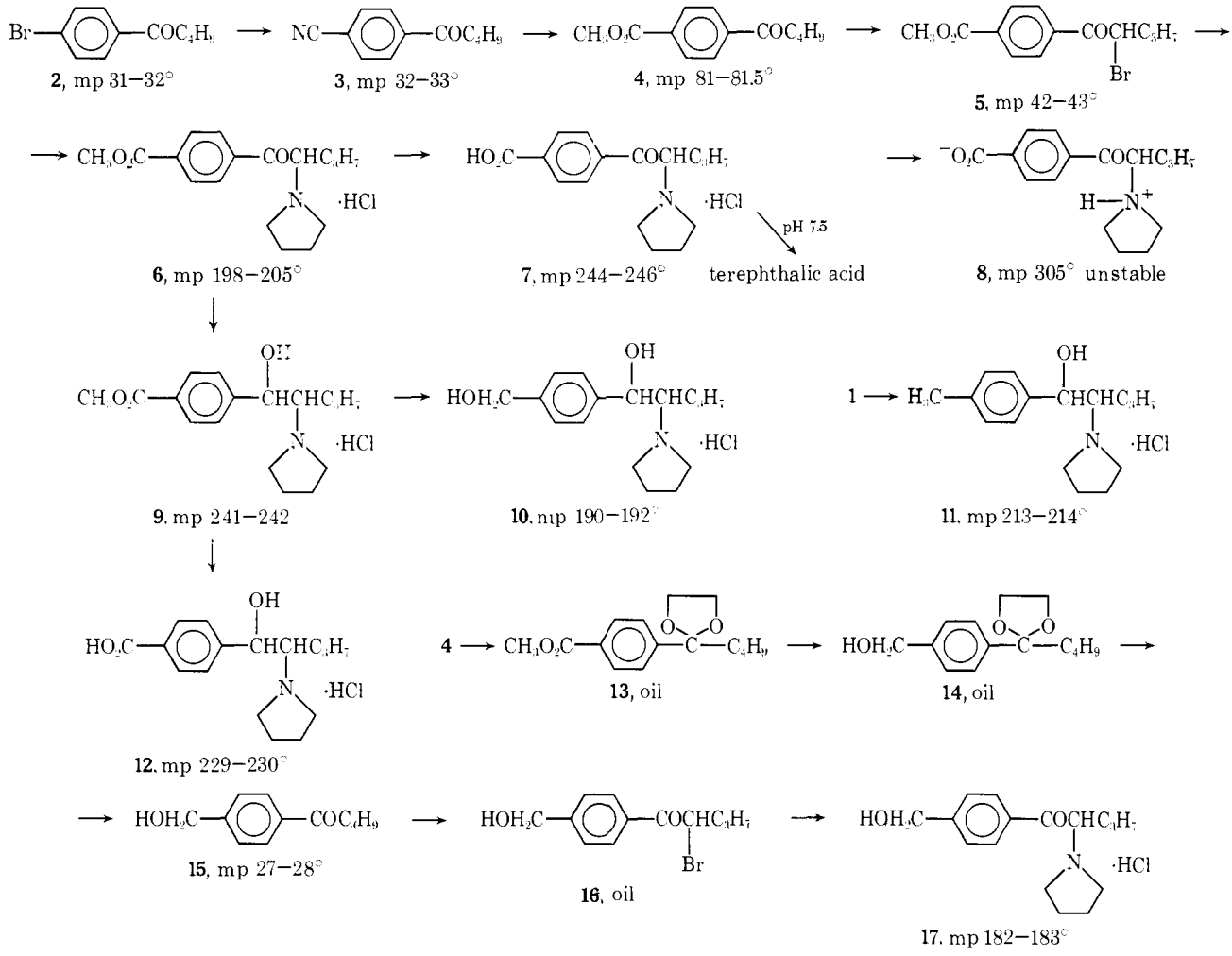
The level of radioactivity in the blood after intravenous administration falls rapidly (Table II) and the distribution in the various organs closely resembles that after oral administration. Once again the liver contains the largest fraction, 12–15% of the radioactivity administered being registered during the first 30 min. Again too, the fraction in the kidneys is relatively small, the highest value determined being 2.9% after 15 min. Larger fractions of radioactivity are registered in the gastrointestinal tract while those of 0.7% measured in the brain 15 min after administration and 0.35% 30 min after administration are also relatively low. Even at the start of the experiment, the fractions of radioactivity in the remaining organs investigated (thymus gland, heart, adrenal glands, spleen, pancreas, seminal vesicles, and testicles) never exceeded 1%. Their concentrations resemble those of the blood; an accumulation of radioactivity cannot be detected.

A comparison of the distribution of radioactivity with the two routes of administration shows that within a short period, both the distribution in the body and also the excretion in the feces and urine are very similar.

Investigation of the Radioactivity in the Mouse Brain.—Considering that pyrovalerone·HCl has a central action, it was necessary to determine in what form the radioactivity detected in the brain exists. In the chromatographic investigation of brain extracts, it was found that the radioactivity was concentrated at the same site on the chromatograms as the concurrently chromatographed unlabeled pyrovalerone·HCl. It could be confirmed that the radioactivity constitutes pyrovalerone by the procedure whereby unlabeled pyrovalerone·HCl was recrystallized 5 times together with radioactive brain extract, and the specific activity of the fractions was determined. The constancy of the specific activity of the last three fractions proves that the radioactivity in the brain constituted unchanged pyrovalerone. Since no additional radioactive substances were detected in the brain, it may be accepted

SCHEME I

SYNTHESES OF THE REFERENCE SUBSTANCES **7**, **8**, **10**, **11**, **12**, AND **17**



that the effect of pyrovalerone·HCl on the central nervous system is due to the unchanged substance.

Investigation of the Urine of Mice and Rabbits.— The initial experiments were conducted in rabbits in an attempt to obtain excretion products in quantities sufficient to determine their constitution. The experimental material was a mixture mostly of unlabeled pyrovalerone·HCl with a small amount of ^{14}C-labeled substance, whose specific activity was sufficient to permit supervision of the processing of the urine. The urine collected over 8 hr contained 61% of the administered dose.

Informative preliminary experiments showed that most of the radioactivity could be extracted from the urine only with a markedly polar solvent mixture, thereby indicating that the substances must be highly polar. From analogies, the following metabolic changes can be considered that increase the polarity of the pyrovalerone molecule; (1) oxidation of the aromatic Me to $HOCH_2$[6] or to CO_2H[7]; (2) Reduction of carbonyl to CH_2OH.[8] Hence, compounds **7**, **8**, **10**, **11**, **12**, and **17** (Scheme I) were synthesized as reference substances (see Experimental Section). Compound **8** could be identified as a metabolite in the urine of rabbits and was isolated as the HCl salt **7**. As it constituted at least 25% of the administered dose it can be designated as the main metabolite, since the residual radioactivity was divided among several substances whose individual fractions were all less than 8%. As the properties of the reference substances were unknown at the start of the isolation experiments, the instability of metabolite **8** in aqueous solution could not be recognized. Subsequently it was realized that with the isolation methods used an appreciable fraction of this metabolite had been degraded to terephthalic acid which could then be obtained as the crystallized dimethyl ester. The cited value of 25% for the fraction of radioactivity excreted in the urine for this metabolite is therefore a minimum value. Neither unchanged pyrovalerone, *p*-toluic acid, nor any of the other synthesized reference compounds (**10**, **11**, **12**, and **17**) could be detected in the urine of rabbits.

Metabolite **8** was also detected in the urine of mice after intravenous administration. The fraction in the urine of mice constituted about two-thirds of the administered dose. Hence, **8** is also the major excretion product in the mouse. Unchanged pyrovalerone could not be detected with certainty, and constituted, if at all, less than 5% of the total.

The experiments conducted in mice and rabbits show that in both species pyrovalerone·HCl is metabolized rapidly and that metabolism is virtually complete. As

(6) R. C. Thomas and G. J. Ikeda, *J. Med. Chem.*, **9**, 507 (1966).
(7) R. T. Williams, "Detoxication Mechanisms," Chapman and Hall Ltd., London, 1959, p 194.
(8) E. C. Schreiber, *et al.*, *J. Pharmacol. Exp. Ther.*, **159**, 372 (1968).

8 occurs as the highly water-soluble zwitterion in aqueous solutions of pH 4–8 it is understandable that this highly hydrophilic substance will be largely excreted in the urine.

Investigation of Human Urine.—The intention of these experiments was to elucidate whether pyrovalerone·HCl is metabolized rapidly and virtually completely in humans and whether the main metabolite **8** detected in animals is also excreted in the urine. Both expectations were verified. In human urine, no unchanged pyrovalerone could be detected over 48 hr,[9] whereas in the first few hours after administration and in all additional fractions collected up to 24 hr **8** could be detected. There was no indication of other metabolites. Although these investigations were conducted with only one trial subject and no quantitative statements are possible, these results indicate that the metabolism and excretion of pyrovalerone·HCl in the human resembles that in animals (mice and rabbits).

Pharmacological Activity.—Substance **7** was tested pharmacologically for motor activity and found to be inactive.

Experimental Section[10]

Synthesis of ^{14}C-Labeled Pyrovalerone·HCl.—Sodium valerate-1-^{14}C (53.5 mg, 0.23 mmol, approximately 1 mCi, obtained from Radiochemical Centre, Amersham, England) was dissolved in 1.5 ml of PhMe and 0.3 ml of $SOCl_2$, and the mixture was refluxed for 6 hr with stirring. After cooling to 20°, the mixture was filtered through charcoal and the clear filtrate added dropwise, within 8 min, to a suspension of $AlCl_3$ (60 mg, 0.22 mmol) in 0.8 ml of PhMe while stirring continually, the dropping funnel being rinsed with two 0.5-ml portions of PhMe. The acylation was completed by stirring for 45 min at 20° and afterwards for 45 min at 90°. The solution was diluted with Et_2O and after washing neutral with 2 *N* H_2SO_4, 2 *N* Na_2CO_3, H_2O, and brine, yielded 114.6 mg of neutral crude product. Of this crude product 105.5 mg was soluble in Et_2O. The residue, after evaporation of the ether, was distilled in a molecular distillation flask, bath temperature 120–123° (10 mm); yield 77.4 mg

For bromination, the 77.4 mg was added to 0.24 ml of a mixture of Br_2 (1 ml) and 9 ml of $CHCl_3$ and kept at 20° for 75 min. The reaction mixture was diluted with Et_2O and after washing neutral (10% $KHCO_3$, H_2O, brine) gave a yellow oil; yield 117.8 mg. This oil was refluxed for 1 hr with 0.2 ml of pyrrolidine in 3 ml of PhH. After diluting with Et_2O, the basic fraction was extracted by shaking out with 2 *N* HCl. The acid extract was basified with concentrated KOH and shaken out with $CHCl_3$ which, on evaporation, gave a yellow-brown oil; yield 29 mg. This oil was distilled in a molecular distillation flask, bath temperature 120° (0.1 mm); yield 27 mg of colorless oil. The hydrochloride of this oil was crystallized (Me_2CO–Et_2O); yield 26 mg of colorless platelets, mp 162–168°, specific activity 4.4 × 10^6 cpm/mg. Physicochemical data for the labeled substance were the same as those of the unlabeled reference substance. No radioactive impurities were detected on chromatographic investigation. The ^{14}C-labeled substance was diluted with unlabeled pyrovalerone·HCl according to the degree of radioactivity required.

Syntheses of Reference Substances. 4-Bromovalerophenone (2).—Valeroyl chloride (65.1 g, 540 mmol) was added dropwise, while stirring at 10°, to $AlCl_3$ (78.2 g, 586 mmol) in PhBr (406 g, 2.6 mols). The reaction mixture was held for 1 hr at 20° and then for 1 hr at 120°, cooled, and poured onto ice. After adjusting the pH to 2.0 with concentrated HCl, the mixture was extracted three times, each with 150 ml of Et_2O. The combined extracts were washed twice with 2 *N* HCl and twice with H_2O, dried (Na_2SO_4), and evaporated to dryness; yield 76 g (58%) of a colorless oil; bp 145–151° (15 mm); colorless needles from hexane, mp 31–32°. *Anal.* ($C_{11}H_{13}BrO$) C, H, Br, O.

4-Cyanovalerophenone (3). A mixture of **2** (76 g, 315 mmol) and CuCN (23 g, 256 mmol) was heated under reflux for 5 hr in 100 ml of *N*-methyl-2-pyrrolidone. After cooling, the reaction mixture was taken up in 600 ml of $CHCl_3$, filtered, washed twice (H_2O), dried (Na_2SO_4), and evaporated to dryness; yield 55.6 g (94%) of an almost colorless oil that crystallized after standing for a short time; colorless needles from MeOH, mp 32–33°. *Anal.* ($C_{12}H_{13}NO$) C, H, N, O.

Methyl 4-Valeroylbenzoate (4).—A solution of **3** (55 g) in 1.5 l. of absolute MeOH was heated under reflux for 4 hr with continual passage of HCl gas. After elimination of the excess HCl gas by a current of N_2, the reaction mixture was concentrated under vacuum to one-third of its volume; yield after cooling 57.1 g (88%) of colorless flakes, mp 81–81.5°. *Anal.* ($C_{13}H_{16}O_3$) C, H, O.

Methyl 4-(1-Bromovaleroyl)benzoate (5).—To **4** (28.6 g, 130 mmol) in 250 ml of $CHCl_3$ was added dropwise, while stirring, Br_2 (22.8 g, 143 mmol) in 75 ml of $CHCl_3$. Two minutes after starting the addition, several drops of HBr in glacial acetic acid were added. After stirring for 3 hr, the reaction mixture was washed twice, each with 120 ml of H_2O, and 150 ml of 10% $NaHCO_3$ solution, and once with 50 ml of H_2O. After drying (Na_2SO_4), the $CHCl_3$ was evaporated to dryness; yield 38.2 g (99%), colorless needles from MeOH, mp 42–43°. *Anal.* ($C_{13}H_{15}BrO_3$) C, H, Br, O.

Methyl 4-(1-Pyrrolidinylvaleroyl)benzoate·HCl (6).—A mixture of **5** (17.5 g, 58.5 mmol) in 100 ml of absolute PhH and pyrrolidine (9.0 g, 126.5 mmol) was heated under reflux for 4 hr. The reaction mixture was extracted twice, each with 30 ml of H_2O, and four times, each with 30 ml of 2 *N* HCl. The combined acidic extracts were shaken out once with Et_2O and adjusted to pH 10 with 30% KOH. After extracting the alkaline solution three times, each with 60 ml of Et_2O, the organic extracts were washed once (H_2O), dried (Na_2SO_4), and evaporated to dryness; yield 15.7 g (93%) of an orange oil; hydrochloride, colorless needles from MeOH–Me_2CO, mp 198–205°. *Anal.* ($C_{17}H_{23}NO_3$·HCl) C, H, Cl, N, O.

4-(1-Pyrrolidinylvaleroyl)benzoic Acid Hydrochloride (7).—A solution of **6** (650 mg) in 40 ml of 5 *N* HCl was heated under reflux for 12 hr. The reaction mixture was cooled to 0°, after which crystals separated. After recrystallizing twice from MeOH–Me_2CO 580 mg of colorless needles were obtained, mp 244–246°. The aqueous mother liquor was evaporated to dryness under vacuum; the residue yielded 30 mg of colorless needles from MeOH–Et_2O, mp 230–245°. (Total yield 87%.) *Anal.* ($C_{16}H_{21}NO_3$·HCl) C, H, Cl, N, O. pK_a^I 3.33 ± 0.02, pK_a^{II} 8.86 ± 0.05.[11]

4-(1-Pyrrolidinylvaleroyl)benzoic Acid (8).—A solution of **7** (50 mg) in 5 ml of absolute EtOH was treated with 20 mg of $KHCO_3$. After 10 min at room temperature the mixture was warmed to 40° till gas evolution ceased, then cooled, filtered, and evaporated to dryness. The residue was taken up in a few drops of H_2O and cooled. A few milligrams of brownish crystals separated from the saturated solution. After careful drying the melting point was 305°. The compound slowly decomposed in the air.

1-(4-Carbomethoxyphenyl)-2-pyrrolidinyl-1-pentanol·HCl (9).—To a solution of the free base of **6** (10.8 g, 37.3 mmol) in 50 ml of MeOH and 5 ml of 0.02 *N* NaOH was added, while cooling with ice and stirring, $NaBH_4$ (1.25 g, 33 mmol) in portions, and the mixture was stirred for 90 min at 20–22°. The reaction mixture was acidified to pH 2 by dropwise addition of 2 *N* HCl, diluted to twice the volume with H_2O, and freed from MeOH under vacuum at 40°. After adjusting to pH 10 with 30% KOH, the mixture was extracted three times, each with 80 ml of Et_2O.

(9) A. Beckett [*J. Pharm. Pharmacol.*, **19**, 273 (1967)] described a method to detect stimulant in urine, which also includes pyrovalerone. Since, however, in humans pyrovalerone·HCl is virtually completely metabolized and the main metabolite which is strongly polar is not detected with Beckett's extraction procedure, the negative result in his test for pyrovalerone·HCl may not be accepted as proof that none has been ingested.

(10) Melting points were determined on a Kofler block and are corrected. Where analyses are indicated only by symbol of the element, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(11) pK_a^I: acidic function; pK_a^{II}: basic function. Determined by potentiometric titration at 25° in aqueous solution of constant ionic strength (I = 0.10). A combined glass electrode [Model U; standardization with 0.05 *M* KH-phthalate buffer (pH 4.00) and 0.05 *M* borate buffer (pH 9.18)] and a compensator E 321 of the firm Metrohm were used for measuring the pH. Evaluation of the titrations according to Albert and Serjeant.[12]

(12) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases", Methuen & Co. Ltd., London, 1962.

The extracts were washed (H_2O), dried (Na_2SO_4), and evaporated to dryness: yield 10.2 g (94%) of light yellow oil; colorless needles from Et_2O–hexane; mp 48–49°; hydrochloride: colorless needles from MeOH–Et_2O, mp 241–242°. *Anal.* ($C_{17}H_{25}NO_3 \cdot HCl$) C, H, Cl, N, O.

1-(4-Hydroxymethylphenyl)-2-pyrrolidinyl-1-pentanol·HCl (10).—To a suspension of LAH (1.0 g, 26 mmol) in 100 ml of absolute Et_2O was added dropwise with stirring, a solution of **9** (6.8 g, 24 mmol) in 80 ml of absolute Et_2O. After 2 hr at room temperature the mixture was refluxed for 1 hr, cooled, the excess LAH destroyed with 2 *N* NaOH, and diluted with 100 ml of H_2O. The reaction mixture was extracted four times, each with 100 ml of Et_2O and the combined ether solutions were extracted five times, each with 50 ml of 2 *N* HCl. The combined acid fractions were made alkaline with 30% NaOH to pH 9 and exhaustively extracted (Et_2O). The ether extracts were washed (H_2O), dried (Na_2SO_4), and evaporated to dryness; yield 6.0 g (98%); colorless oil; hydrochloride: colorless platelets from MeOH–Et_2O; mp 190–192°. *Anal.* ($C_{16}H_{25}NO_2 \cdot HCl$) C, H, Cl, N, O.

1-(4-Methylphenyl)-2-pyrrolidinyl-1-pentanol·HCl (11).—To a solution of free base of **1** (7.6 g, 31 mmol) in 40 ml of MeOH and 4 ml of 0.02 *N* NaOH was added, while cooling with ice and stirring, $NaBH_4$ (1.1 g, 29 mmol) in portions, and the mixture was stirred for 85 min at 20–22°. The reaction mixture was worked up as for **9**; yield 7.0 g (91%) yellow oil; hydrochloride: colorless needles from MeOH–Et_2O; mp 213–214°. *Anal.* ($C_{16}H_{25}NO \cdot HCl$) C, H, Cl, N, O.

1-(4-Carboxyphenyl)-2-pyrrolidinyl-1-pentanol Hydrochloride (12).—A solution of **9** (800 mg) was heated under reflux for 14 hr in 30 ml of 5 *N* HCl, the reaction mixture was cooled on ice, and the precipitated crystals filtered off: yield, 690 mg of colorless needles from MeOH–Et_2O; mp 229–230°; pK_a^I 3.94 ± 0.02, pK_a^{II} 10.33 ± 0.05.[11] *Anal.* ($C_{16}H_{23}NO_3 \cdot HCl$) C, H, Cl, N, O.

4-Valeroylbenzyl Alcohol (15).—A solution of **4** (7.3 g, 32.8 mmol) and *p*-toluenesulfonic acid monohydrate (570 mg, 3.0 mmol) in ethylene glycol (15 g, 242 mmol) and 150 ml of PhH was refluxed for 40 hr in a Soxhlet apparatus containing CaC_2 in the thimble. The reaction mixture was cooled, treated with 2 ml of pyridine and washed four times, each with 30 ml of 5% $NaHCO_3$, then once with H_2O. The organic phase was dried (Na_2SO_4) and evaporated to dryness; yield 8.5 g (97%) colorless oil (**13**). This oil was dissolved in 150 ml of abs Et_2O and added dropwise to a stirred suspension of LAH (1.25 g, 33 mmol) in 180 ml of abs Et_2O. The reaction mixture was then refluxed for 90 min, cooled, and the excess LAH destroyed with H_2O. The Et_2O solution was washed twice with a little H_2O, dried (Na_2SO_4), and evaporated to dryness; yield 6.5 g (86%) colorless oil (**14**). A solution of **14** (6.5 g) and 400 mg of *p*-toluenesulfonic acid in 150 ml of Me_2CO was refluxed for 90 min, then evaporated to dryness. The residue was taken up in 60 ml of $CHCl_3$, washed three times, each with 20 ml of 5% $NaHCO_3$ and once with H_2O, dried (Na_2SO_4), and evaporated to dryness; yield 4.8 g (91%) of colorless oil which gave colorless flakes from Et_2O–petroleum ether, mp 27–28°. *Anal.* ($C_{12}H_{16}O_2$) C, H, O.

4-(1-Bromovaleroyl)benzyl Alcohol (16).—A solution of Br_2 (2.6 g, 16.25 mmol) in 30 ml of $CHCl_3$ was added dropwise with stirring to a solution of **15** (3.1 g, 16.1 mmol) in 20 ml of $CHCl_3$ at room temperature. After stirring for 6 hr at ambient temperature the reaction mixture was washed twice, each with 15 ml of H_2O, three times each with 15 ml of 5% $NaHCO_3$, and once with H_2O. The organic phase was dried (Na_2SO_4), and evaporated to dryness; yield 4.15 g (94%) of pale yellow oil which was used for the next step without further purification.

4-(1-Pyrrolidinylvaleroyl)benzyl Alcohol·HCl (17).—A solution of **16** (4.15 g, 15.3 mmol) and pyrrolidine (2.35 g, 33 mmol) in 60 ml of PhH was refluxed for 2 hr. The cooled reaction mixture was washed four times, each with 20 ml of H_2O, then four times each with 20 ml of 2 *N* HCl. The combined HCl extracts were made alkaline with 2 *N* NH_4OH and shaken out four times with $CHCl_3$. The extracts were washed (H_2O), dried (Na_2SO_4), and evaporated to dryness; yield 3.4 g (85%) reddish oil. A chloroform solution of this oil was filtered through Alox and evaporated to dryness. The residual oil was converted into the HCl salt; colorless needles, mp 182–183° from Me_2CO. *Anal.* ($C_{16}H_{23}NO_2 \cdot HCl$) C, H, Cl, N, O.

Cleavage of 7 in Aqueous Solution to Terephthalic Acid.—A solution of **7** (65 mg) in 50 ml of H_2O was adjusted to pH 7.5 with 2 *N* Na_2CO_3. After evaporating the solution to dryness under vacuum at 40°, the oily residue was taken up in approximately 20 ml of $CHCl_3$ and shaken out four times, each with 5 ml of 2 *N* KOH. The KOH extracts were further extracted twice with $CHCl_3$, then adjusted to pH 3 with concentrated HCl. The crystals which precipitated on cooling to 0° were filtered, washed (cold H_2O), and dried; yield 19.5 mg of yellow granules. These did not melt below 295°, but sublimed from this temperature upwards; ir spectrum was identical with that of terephthalic acid.

Determination of Radioactivity.—The Kalberer–Rutschmann method[13] was used to process the biological samples for determination of radioactivity. A Tricarb liquid scintillation spectrometer Model 314 EX of Packard Instrument Corp. (La Grange, Ill.) was used for counting the radioactive solutions. Any quenching was corrected by the dual-channel ratio method. A "Precision-Ratemeter," serial number 260 of Packard Instrument Corp., connected to a recorder was used to evaluate the radiochromatograms. The paper chromatograms were measured using a "4 π Actigraph II chromatogram scanner, Model 1032" of the Nuclear-Chicago Corp. (Des Plaines, Ill.).

Paper Chromatography.—Descending paper chromatography was used with Schleicher and Schuell 2043 b paper and the following solvent systems: (1) *n*-BuOH saturated with 2 *N* NH_4OH; (2) *n*-BuOH–glacial AcOH–H_2O (4:1:1). Detection was accomplished by uv 254 nm, Dragendorff's reagent,[14] and activity determination. The R_f values of pyrovalerone·HCl, **7**, **10**, **11**, **12**, and **17** in system 1 were 0.89, 0.63, 0.92, 0.90, 0.49, and 0.89; and in system 2 in the same sequence were 0.82, 0.79, 0.62, 0.81, 0.75, and 0.71.

Tlc.—Tlc employed the following two systems: system 3: Alox HF; $CHCl_3$–glacial AcOH (9:1); system 4: silica gel HF 254; heptane–Et_2NH (95:5). The R_f values of pyrovalerone·HCl, **7**, and **12** in system 3 were 0.88, 0.57, and 0.46; the R_f values of pyrovalerone·HCl, **6**, and **9** in system 4 were 0.39, 0.32, and 0.10.

Animal Experiments. Absorption, Distribution, and Excretion in the Mouse.—In one experimental group, male mice of approximately 20 g weight (Charles River CD 1) each received 0.4 ml of an aqueous solution of pyrovalerone·HCl at 20 mg/kg, administered by stomach tube. In another experimental group, each animal received 0.2 ml of an isotonic glucose solution of pyrovalerone·HCl at a dosage of 10 mg/kg, injected into the tail vein. The animals were housed singly in open glass metabolite cages and the feces and urine were collected separately. From the 8th hr onwards, the animals were allowed to consume H_2O and food freely. At 0.25,[15] 0.5, 1, 4, 8, and 24 hr after administration, two animals under ether anesthesia were decapitated and dissected.

Investigation of the Radioactivity in the Mouse Brain.—Each of four mice received 0.2 ml of an isotonic glucose solution of pyrovalerone·HCl at a dosage of 10 mg/kg, injected into the tail vein; 10 min after administration the animals under ether anesthesia were decapitated, and the brains were removed immediately and frozen. The brains were suspended in 20 ml of Me_2CO–EtOAc (1:1) with a homogenizer and centrifuged off. The precipitate was extracted once more with 20 ml of the solvent mixture and then the combined organic phases were vacuum dried. The residue was distributed between 15 ml of Et_2O and 3 ml of 0.2 *N* HCl, and the Et_2O extracted a second time with 3 ml of 0.2 *N* HCl. The combined HCl solutions were adjusted to pH 11 with concentrated NH_4OH and shaken out with two 15-ml portions of $CHCl_3$, dried (Na_2SO_4), and evaporated to dryness; residue 4 mg. The work-up of the brains was controlled continually by determining the radioactivity of the extracts and residues. A residual radioactivity of less than 4% was found in the extracted brains.

The residue (1 mg) was dissolved in a little MeOH and examined together with 50 μg of unlabeled pyrovalerone·HCl in the chromatography systems 1 and 2. Residue (3 mg) together with 495 mg of unlabeled pyrovalerone·HCl was dissolved in 20 ml of $CHCl_3$, 2 drops of 15% alcoholic HCl were added and the mixture was evaporated to dryness. The residue was taken up in 10 ml of H_2O, shaken out with five 40-ml portions of Et_2O, and adjusted to pH 10 with 2 *N* KOH. After extracting this alkaline

(13) F. Kalberer and J. Rutschmann, *Helv. Chim. Acta*, **44**, 1956 (1961).

(14) E. Merck AG, Anfärbereagentien für Dünnschicht- und Papierchromatographie, Darmstadt, 1964, No. 92.

(15) Only after intravenous administration.

solution with ten 40-ml portions of Et_2O, the combined extracts were washed once with a little H_2O, dried (Na_2SO_4), and evaporated. The oily residue was converted into the hydrochloride, taken up in 50 ml of Me_2CO, treated dropwise with Et_2O till slightly turbid, and left to crystallize slowly at room temperature. An aliquot of crystalline product was retained for the estimation of radioactivity and the remainder was recrystallized. Altogether 5 fractions were obtained. Their specific activities were estimated after drying to constant weight over $CaCl_2$: fraction 1, 18.5 cpm/mg; fraction 2, 18.6 cpm/mg; fraction 3, 19.1 cpm/mg; fraction 4, 19.1 cpm/mg; fraction 5, 19.1 cpm/mg.

Chromatographic Investigations with the Urine of Mice.—Four male mice (Charles River CD 1 strain) of approximately 20 g weight, each received 0.20 ml of an isotonic glucose solution of ^{14}C-labeled pyrovalerone·HCl (10 mg/kg) injected into the tail vein. By pressing on the bladder the mice were induced to urinate, and the urine was collected with a capillary tube. The following fractions were taken: 0–0.5 hr, 0.5–2 hr, 2–4 hr, and 4–8 hr. The four fractions obtained from three animals were streaked onto paper and chromatographed in systems 1 and 2. The chromatograms of fractions 1–3 gave good results; the chromatograms of the 4th urine fraction could not be used owing to the low radioactivity and to the relatively large amounts of nonradioactive concomitant material. Since, however, after intravenous administration almost 80% of the dose given was excreted in the urine within the first 4 hr, the investigations with the first 3 urine fractions may be considered as representative of the total urine. In addition to various small peaks (in each case, the amount of activity not exceeding 5%), the chromatograms of fractions 1–3 showed a major peak whose R_f value agreed with that of reference substance **7**. This major peak constituted over 65% of the total radioactivity chromatographed; in some chromatograms virtually no other radioactive zones could be detected in addition to this major peak. There was no indication of the presence of unchanged pyrovalerone.

Isolation Experiments by Crystallization.—The first urine fraction (16,300 cpm) of the fourth animal was mixed with 149 mg of reference substance **7** in 5 ml of H_2O. On warming to 40° a clear solution was obtained, which, after diluting with H_2O to 10 ml, was shaken out three times, each with 2 ml of petroleum ether and four times, each with 3 ml of Et_2O. The aqueous solutions were concentrated under vacuum at 30° to 3 ml and allowed to stand at 5°. After 12 hr the precipitate was filtered and recrystallized from MeOH–Et_2O. An aliquot of the first crystal fraction was retained for the determination of radioactivity, and the remainder was again recrystallized from MeOH–Et_2O. The process was repeated until 5 fractions were obtained, which, after drying to constant weight showed the following specific activities: fraction 1, 105.7 cpm/mg; fraction 2, 105.1 cpm/mg; fraction 3, 102.2 cpm/mg; fraction 4, 101.3 cpm/mg; fraction 5, 101.2 cpm/mg. The very slight reduction in the specific activity shows that the radioactive substance present in the urine is identical with the added reference substance **7**. If, using the specific activity of the fifth fraction (101.2 cpm/mg), the corresponding total radioactivity is calculated for the added 149 mg, the result obtained is 15,000 cpm. This constitutes over 90% of the radioactivity determined initially in the volume of urine investigated (16,300 cpm).

Investigations with the Urine of Rabbits.—Female rabbits (2–3 kg, hare colored, from our own breed) received doses of 40 mg/kg of a mixture of ^{14}C-labeled pyrovalerone·HCl diluted with unlabeled substance in aqueous solution with a stomach tube. The urine was collected over 8 hr.

Acidic and Neutral Metabolites.—The urine from one animal (200 ml, containing 95% of the administered radioactivity) was saturated with NaCl and adjusted to pH 1.5 with concentrated HCl then shaken out five times, each with 400 ml of Et_2O. The combined organic phases were dried (Na_2SO_4), and concentrated under vacuum to approximately 100 ml. The radioactivity of this solution was equivalent to 16% of the administered dose. The total radioactivity was removed by shaking out the solution three times, each with 200 ml of 5% $NaHCO_3$. The aqueous phases were combined, adjusted to pH 1 with HCl, and exhaustively extracted ($CHCl_3$). The combined extracts were dried (Na_2SO_4), concentrated, and examined by chromatography. The chromatogram in system 1 showed a main peak (R_f 0.55, 65% of the radioactivity) and a minor peak (R_f 0.42, 25% of the radioactivity). The chromatogram in system 2 showed the total radioactivity at R_f approx. 0.80. Based on the R_f values, the substance could be *p*-toluic acid. In order to check this the $CHCl_3$ was evaporated to dryness and taken up in 80 ml of MeOH. Unlabeled *p*-toluic acid (3.00 g) was dissolved with warming in this solution then cooled to 0°. The crystals which formed were washed with a little cold MeOH and dried in a vacuum drying oven. An aliquot (approximately 70 mg) was taken for the determination of radioactivity and the rest redissolved in 80 ml of MeOH and recrystallized. This procedure was repeated until six fractions had been obtained. Their specific activities (cpm/mg) were: 42.1, 30.4, 31.5, 29.7, 33.6, and 29.4. Based on an average specific activity of 31 cpm/mg, the radioactivity calculated for the 3.00 g of unlabeled acid is 93,000 cpm which is equivalent to less than 10% of the radioactivity excreted with Et_2O. The rest of the radioactivity was in the first mother liquor. This experiment shows that the main component of the Et_2O extract is not *p*-toluic acid.

Basic Metabolites.—The urine of 17 rabbits (3.6 l.) containing 1.38×10^7 cpm, equivalent to 61% of the administered dose, was adjusted to pH 3.5 with concentrated HCl and concentrated to approximately 100 ml under vacuum at 30–40°. The precipitate which formed was filtered by suction and washed (H_2O); it contained no activity. After adjusting the filtrate to pH 1 with concentrated HCl, it was shaken out three times, each with 500 ml of Et_2O. These extracts were washed three times, each with 10 ml of H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue (1.95 g, 3.0×10^6 cpm) was not further investigated.

The aqueous extracts and the wash water were concentrated to 70 ml under vacuum and the dark brown concentrate was extracted six times, each with 150 ml of $CHCl_3$–EtOH 4:1. The organic extracts were washed three times, each with 10 ml of H_2O which had been used previously for washing the Et_2O extracts, dried (Na_2SO_4), and concentrated to dryness under vacuum; yield 4.90 g of a thick brown residue containing 10^7 cpm, corresponding to 72% of the radioactivity excreted in the urine. Investigation by paper chromatography revealed that approximately 60% of this could be attributed to a single substance, whose R_f value in system 1 was 0.64 while the remainder was distributed among several small peaks, not one of which contained more than 15%. No unchanged pyrovalerone could be detected. Thus approximately 25% of the administered radioactivity was present in the zone about R_f 0.64 containing the main metabolites. The experiments for preparative isolation were restricted to these components.

In an initial experiment, 1.27 g of the fraction extracted with $CHCl_3$–EtOH (4:1) was taken up in 6.5 ml of MeOH and separated chromatographically in system 3. The zone R_f 0.38–0.56[b] with the greatest radioactivity was removed, and extracted exhaustively with methanol (460 ml). This MeOH solution was evaporated to dryness under vacuum, taken up in 10 ml of H_2O, mixed with 2 ml of 2 *N* Na_2CO_3 solution, and extracted ten times, each with 40 ml of $CHCl_3$–EtOH (4:1). The combined organic phases were dried (Na_2SO_4), and evaporated to dryness; yield 104 mg of a yellow viscous oil. This was taken up in a little MeOH–Et_2O and after standing for 24 hr at 0°, 25.9 mg of a brown precipitate was filtered off. The mother liquor yielded 2 mg of a brown precipitate from MeOH–Et_2O at −5° which was removed by centrifugation. The supernatant solution was evaporated to dryness under vacuum; yield 56.3 mg. From MeOH–Et_2O at −5°, there were obtained 11 mg of orange crystals, mp 305°. The ir spectrum of this substance was identical with that of **8**.

The product precipitated initially (25.9 mg) was taken up in 1.5 ml of MeOH and the insoluble fraction separated by centrifugation. The supernatant solution was concentrated to dryness under vacuum. The residue (19.3 mg) was dissolved in MeOH and treated with alcoholic HCl until the reaction mixture was slightly acidic and again evaporated to dryness. The residue obtained was treated with an excess of ethereal CH_2N_2 and left at room temperature for 20 min. After evaporating to dryness under vacuum, the residue was taken up in Et_2O and extracted four times with a little H_2O. The aqueous solutions were again extracted once with Et_2O, then evaporated to dryness under vacuum; yield 10 mg. The ether-soluble fraction (10.4 mg) was crystalline; for purification, it was distilled in a molecular distillation flask at 0.1 mm. Up to 100° bath temperature, 3.5 mg of distillate was obtained which yielded 1

[b] Owing to the excess quantity of concomitant material, the R_f values of the substances in the extracts do not correspond to the R_f values of the pure compounds.

mg of platelets from Et_2O–hexane at −5°, mp 125–129°. The melting point, ir spectrum, and pc (system 1) showed this to be identical with dimethyl terephthalate produced synthetically. The distillate (3.7 mg) obtained at a bath temperature of 100–120° yielded approximately 2 mg of crystals from Et_2O–hexane, mp 173–176°. According to the ir spectrum, this substance was identical with the free base of reference substance **6**.

In a second experiment, 1.28 g of the fraction extracted from urine with $CHCl_3$–EtOH (4:1) was separated preparatively in chromatographic system 3. Examination of the distribution of radioactivity in the layers revealed that about 60% was localized in a band between R_f 0.38 and 0.56.[16] This band was divided into zone A with R_f 0.38 to 0.47 and zone B with R_f 0.47 to 0.56; these zones were removed from the plate separately and extracted exhaustively with MeOH. On evaporating to dryness, zone A yielded 66.7 mg (202,000 cpm) and zone B yielded 57.1 mg (167,000 cpm). The residue from zone B was taken up in 1 ml of MeOH, acidified with HCl, then esterified by adding an excess of ethereal CH_2N_2. After concentrating to the consistency of a syrup, it was possible to separate an oil that was sparingly soluble in MeOH. The soluble fraction yielded from MeOH–Et_2O 1.5 mg of yellow crystals and on recrystallization from MeOH–Et_2O colorless platelets, mp 198–208°. According to melting point, mixture melting point, tlc, and ir spectrum, this compound was identical with **6** prepared synthetically. Similarly, 1.0 mg of colorless platelets was obtained from zone A, mp 192–199°, which, according to the same criteria, was identical with reference substance **6**.

Human Experiments. Investigations of the Urine of Humans.—A healthy male trial subject (70 kg) received 60 mg of pyrovalerone·HCl orally. In the subsequent 32 hr the urine was collected in the following fractions which were kept at −5° until used for processing: fraction 1, 0–5 hr; fraction 2, 5–11 hr; fraction 3, 11–25 hr; fraction 4, 25–32 hr.

In each case 500-ml aliquots of these fractions were adjusted with concentrated HCl to pH 2.0–2.5 and concentrated under vacuum at 50° to 40–50 ml. After cooling, a precipitate was filtered off and the filtrate extracted three times with double the volume of $CHCl_3$; the organic phases were combined, dried (Na_2SO_4), and then evaporated to dryness. The residues from each fraction were taken up in a little MeOH and chromatographed in system 4 together with pyrovalerone·HCl as reference substance. In no extract was it possible to detect unchanged pyrovalerone.

The aqueous solutions were then extracted five times with double the volume of $CHCl_3$–EtOH (4:1); the organic phases were combined, dried (Na_2SO_4), and carefully evaporated to dryness. The residues from each fraction were dissolved in a little MeOH and treated with an excess of Et_2O–CH_2N_2. After standing for 24 hr at −5° and for 2 hr at 20–25°, the reaction mixtures were partitioned between Et_2O and 0.1 *N* NaOH. The Et_2O solutions were washed three times with a little H_2O, dried (Na_2SO_4), and evaporated to dryness. In each case, a sample of the residue was chromatographed in system 4. It was evident that the material sought (**6**) was present only in traces in fraction 3 and was absent in fraction 4.

The residues of fractions 1 and 2 were dissolved in a little MeOH, and separated preparatively in chromatographic system 4. Zones around R_f 0.32 were scraped off carefully, placed in small chromatography columns, and eluted with MeOH. After evaporating to dryness, the eluates were taken up in Et_2O, washed three times with 1 *N* NH_4OH and once with H_2O, dried (Na_2SO_4), and evaporated to dryness. The residues were dissolved in a little MeOH, converted into the hydrochloride with 15% alcoholic HCl, and crystallized from MeOH–Et_2O. Fraction 1 yielded *ca.* 4 mg of colorless needles, mp 191–193°, and fraction 2 yielded *ca.* 3 mg of colorless needles, mp 193–194°. Ir spectroscopy (KBr) showed both products to be identical with reference substance **6** prepared synthetically.

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Structure-Dependent Inhibition by Synthetic Fibrinolytic Anions of Collagen-Induced Aggregation of Human Platelets[1]

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Various organic anions with a large lipophilic moiety induce fibrinolytic activity in human plasma *in vitro*. Some of the fibrinolytic compounds were found to inhibit ADP- and thrombin-induced platelet aggregation. In this study, 38 fibrinolytic congeners were investigated for inhibition of collagen-induced platelet aggregation in human plasma. The parent compounds, anthranilic, salicylic, and thiophene-3-carboxylic acid, had little fibrinolytic and aggregation inhibiting activity; however, unsymmetrical substitution increased both activities. The platelet aggregation inhibition frequently occurred at concentrations at least 10 times lower than those required for fibrinolysis. *N*-(2-Chloro-3-methylphenyl)-4-aminothiophene-3-carboxylic acid was fibrinolytic at 3×10^{-3} *M* and aggregation inhibiting at 5×10^{-5} *M*. The results suggest the theoretical possibility of designing drugs with a dual action: prevention of thrombus formation and induction of thrombolysis.

Many of the compounds which prevent collagen-induced platelet aggregation are nonsteroidal antirheumatic drugs, and it has been suggested that there may be a correlation between antirheumatic activity and aggregation prevention.[3] The antiaggregating antirheumatic drugs are unsymmetrically substituted organic anions. It is the purpose of the present study to determine if related organic anions, which induce marked fibrinolytic activity in human plasma,[4] will also prevent *in vitro* collagen-induced aggregation of human platelets, and if this is the case, to obtain information on the relationship between structure of organic fibrinolytic anions and their ability to prevent collagen-induced platelet aggregation.

Experimental Section

Glassware.—All glassware with the exception of disposable micropipettes was freshly siliconized by vaporized silicone solutions (General Electric Drifilm No. S.C.11994).[5]

Chemicals.—Buffered saline (B.S.): 4 parts of NaCl (0.85%) and 1 part of barbital acetate buffer,[6] pH 7.42. The fibrinolytic

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(2) Student Research Fellow; on leave from the University of Erlangen, Germany.

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