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Tissue Distribution of ^{3}H-Canrenoate Potassium in Rabbits

A. MICHAELS FINN, R. BROWN, and W. SADÉE ˣ

Abstract ☐ Plasma and various organ concentrations of canrenone, canrenoate, and total ^{3}H-activity were measured following single doses of 20 mg of ^{3}H-canrenoate/kg iv to rabbits. Organs studied included heart, lungs, brain, kidneys, liver, adrenal glands, and spleen. Canrenoate was shown to be in rapid equilibrium with canrenone. Both were eliminated from plasma and other tissues with a half-life of about 1 hr. Plasma concentrations of both drugs were equal as early as 10 min after intravenous drug administration. Canrenone was concentrated about 10-fold in organ tissues when compared to plasma, while no such preferential uptake was found with canrenoate. Total ^{3}H-activity declined slowly in all tissues with a half-life of approximately 15 hr, indicating extensive metabolism and metabolite retention in the rabbit.

Keyphrases ☐ Canrenoate potassium—tissue distribution and metabolism, radiochemical study, rabbits ☐ Distribution, tissue—canrenoate potassium, radiochemical study, rabbits ☐ Metabolism—canrenoate potassium, radiochemical study, rabbits ☐ Radiochemistry—study of tissue distribution and metabolism, canrenoate potassium, rabbits ☐ Aldosterone antagonists—canrenoate potassium, tissue distribution and metabolism, radiochemical study, rabbits

The antimineralocorticoid compounds spironolactone[1] (I) and canrenoate potassium (III) are useful diuretic agents (1). Spironolactone is rapidly metabolized *via* a 7α-thiol derivative (2) to canrenone (II), a major metabolite, which exists in enzymatic equilibrium with III (Scheme I). The pharmacokinetics of I–III were investigated in rats (3), dogs (4), and humans (5, 6). Data on the kinetic disposition and further metabolism of II and III are scant (2, 7). Large differences between the tissue distribution of the lipophilic II and the water-soluble III can be expected and should be considered in the interpretation of plasma II and III levels. In this study, the tissue distribution of III potassium was investigated in rabbits.

EXPERIMENTAL

Chemicals—Pure crystalline samples of I and III were used[2], and II was prepared from III as previously described (8). ^{3}H-Canrenoate potassium[3], with a specific activity of 860 μCi/mg, was purified by solvent extraction (8) and TLC[4] in dichloromethane–methanol (8:2) prior to use. All reagents and chemicals were spectroquality.

Analytical Procedures—The fluorometric determination of II and III was performed as described previously (9). This method was specific for II and III in the presence of all other metabolites of III (8). Tritium measurements were performed by liquid scintillation counting as described previously (8). Efficiency was measured by the channels ratio method.

Rabbit Protocols—*Serial Blood Sampling*—Female New Zealand White rabbits, 2–2.5 kg, were used. Each rabbit was prepared for blood sampling by insertion of a polyethylene 50 catheter about 4 cm into an ear artery. Doses of 5, 10, and 20 mg of III/kg in aqueous solution were given by venous injections into the opposite ear. Blood samples of 2 ml each were taken with heparinized syringes at appropriate time intervals and centrifuged, and the plasma was frozen for subsequent analysis.

Tissue Analysis—Doses of 20 mg of ^{3}H-III/kg, with a specific activity of 0.2–0.5 μCi/mg, were given intravenously into an ear vein. Rabbits were sacrificed by decapitation at the following times after drug administration: 5, 10, 20, 30, 60, and 90 min and 2, 4, 8, 16, and 32 hr. One animal was used per time interval, with the exception of the 2-hr experiments where three animals were used.

Blood was collected by exsanguination from the abdominal aorta. The heart, lungs, brain, kidneys, liver, adrenal glands, and spleen were re-

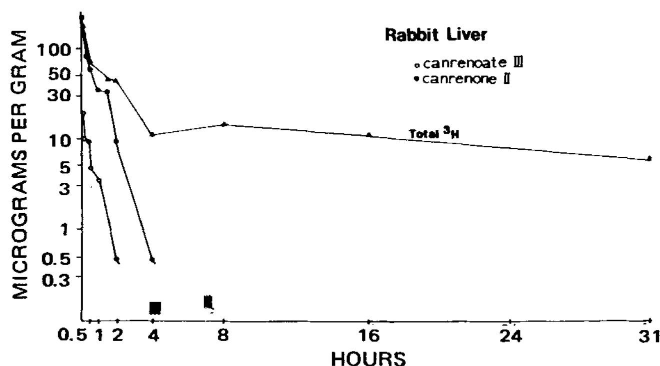


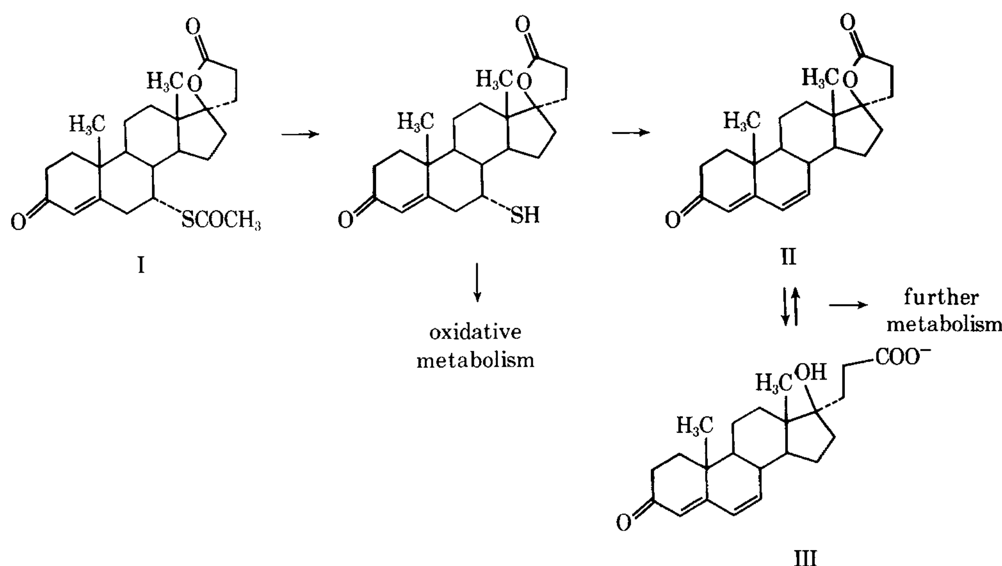
Figure 1—*Concentrations of total ^{3}H-radioactivity measured by liquid scintillation counting and concentrations of II and III measured by a specific fluorescence assay in rabbit liver.*

[1] Aldactone, Searle and Co.

[2] Boehringer Mannheim G.M.b.H.

[3] G. D. Searle and Co.

[4] Silica gel K C F254 Merck plates were used.



Scheme I—Metabolic pathways of the spirolactones

moved. Tissue samples were homogenized using a tissue homogenizer in a fivefold volume of methanol. After centrifugation at 2000 rpm, the supernate was removed and the tissue pellet was washed with methanol and kept at 20° for further analysis. The supernate (0.3 ml) or 0.1 ml of plasma was placed in a counting vial, and ^{3}H-counts were determined by scintillation counting or measured fluorometrically for II and III.

Tissue pellets were analyzed to measure the extraction yield by methanol homogenization. Liquefaction of the methanol and water-washed pellet was achieved using a tissue solubilizer[5], and an aliquot of the total volume was counted in 10 ml of counting solution[6]. Pellet extractibility was quantitative in all tissues, since all counts were found in the supernate. ^{3}H-Activity was not significantly accounted for by ^{3}H-water following possible ^{3}H-exchange from ^{3}H-III or its metabolites, since the ^{3}H-activity of the supernates was the same when measured directly and following evaporation to dryness.

RESULTS AND DISCUSSION

Plasma concentration *versus* time curves of II and III were obtained by serial blood sampling after 5, 10, and 20 mg of III/kg iv. Equilibrium between II and III was obtained as early as 10 min following administration of III. The plasma elimination half-life was about 1 hr, which is much faster than that observed in other species (2, 4, 5), and was unchanged at all dose levels studied. Following increased doses, proportionally increased plasma levels with the same elimination half-life demonstrated linear first-order disposition kinetics. A dose of 20 mg of III/kg was selected for all subsequent studies.

Figure 1 shows measurements of total radioactivity and of II and III

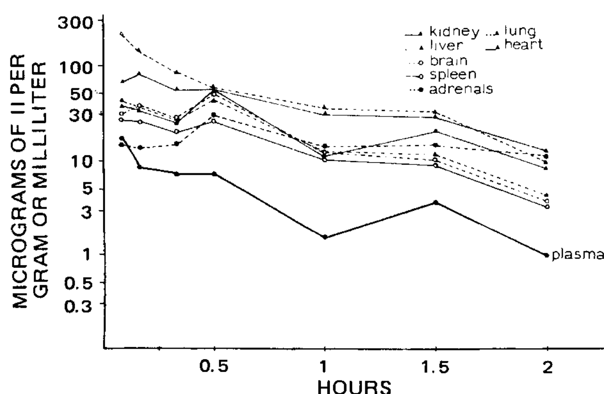


Figure 2—*Plasma and organ concentrations of II.*

[5] NCS, Amersham/Searle Corp.
[6] Aquasol, New England Nuclear.

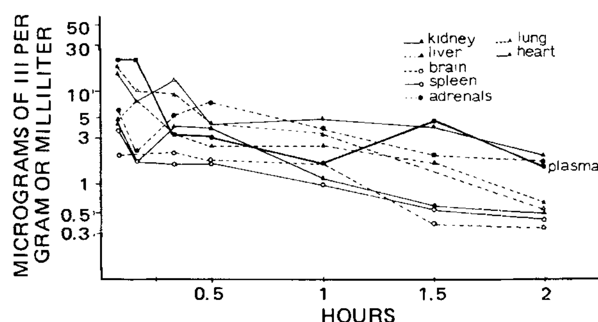


Figure 3—*Plasma and organ concentrations of III.*

measured by a specific fluorescence assay in rabbit liver tissue. Liver appears to show a high uptake of II and not III, even at the earliest time point of 10 min after a dose of III potassium where II accounts for most of the radioactivity. This result indicates a rapid conversion of III to II. The high concentration and slow elimination of ^{3}H-activity suggest extensive metabolism.

Figure 2 shows II levels in plasma and all tissues analyzed. Canrenone is rapidly taken up and concentrated in tissues, by a factor of 10 compared to plasma. The large differences in II concentration after 10 min in various tissues may be attributed in part to perfusion differences of the various organs. Levels of III in plasma and tissues (Fig. 3) are similar in order of magnitude, indicating a lower tissue affinity of III when compared to II.

To examine the variability between animals, three animals were analyzed at the 2-hr time point. All other points are represented by single-animal experiments. Table I shows the averaged values and ranges for the 2-hr experiments in rabbit plasma and liver.

Plasma concentrations of II represent a larger amount of this metabolite in the body when compared to plasma levels of III. Therefore, it seems justified to consider II as a major metabolite upon which pharmacokinetic studies such as bioavailability should be based. This result

Table I—Levels of Canrenone (II) and Canrenoate (III) in Plasma and Liver at 2 hr following Administration of ^{3}H-III to Three Rabbits

	Mean (Range)		
	Total ^{3}H-Activity	II	III
Plasma, µg/ml	18 (12–25)	1.16 (0.6–1.48)	1.3 (0.36–1.76)
Liver, g	68 (35–119)	10 (4–16)	1 (0.6–1.2)

can probably also be applied to species other than the rabbit. However, extensive transformation to metabolites of unknown structures and pharmacological activity appears to occur, which will be discussed in a separate report.

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Synthesis and Biological Activity of a Novel Analog of Nitrofurantoin

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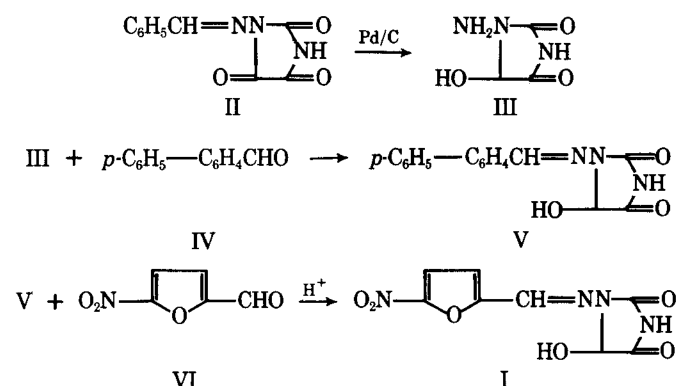
Abstract ☐ The synthesis of 5-hydroxy-1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione is described, and its antibacterial activity is reported.

Keyphrases ☐ Nitrofurantoin analog—synthesized, screened for antibacterial activity ☐ 2,4-Imidazolidinedione, substituted—synthesized, screened for antibacterial activity ☐ Antibacterial activity—substituted 2,4-imidazolidinedione evaluated

Because of the continuing interest (1–3) in the chemotherapeutic properties of 1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinediones, the 5-hydroxy analog of nitrofurantoin[1] was prepared by utilizing the general preparative route to 5-hydroxy-1-(substituted amino)-2,4-imidazolidinediones (4, 5).

DISCUSSION

The synthesis of the desired 5-hydroxy-1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione (I) (5) required catalytic reduction



Scheme I

[1] Furadantin, Eaton Laboratories, Division of Morton-Norwich Products, Inc.

Table I—Serial Dilution Method for I and Nitrofurantoin[a]

Organism	MIC, μg/ml	
	I	Nitrofurantoin
Staphylococcus aureus (Mi-12)	50	12.5
Escherichia coli (Es-90)	12.5	6.2
Salmonella typhosa (SaD-13)	25	6.2
Aerobacter aerogenes (Ae-6)	>100	100
Pseudomonas aeruginosa (Ps-44)	>100	>200
Shigella flexneri (Sh-378)	25	12.5
Proteus mirabilis (Pr-91)	>100	200
Hemophilus vaginalis (He-127)	3.1	1.5

[a] Activities are given in MIC values (minimum inhibitory concentrations).

of 1-[(phenylmethylene)amino]imidazolidinetrione (II) to yield 1-amino-5-hydroxy-2,4-imidazolidinedione (III). Compound III was not isolated but was trapped with 1,1′-biphenyl-4-carboxaldehyde (IV) to yield 1-[[[(1,1′-biphenyl)-4-yl]methylene]amino]-5-hydroxy-2,4-imidazolidinedione (V). Exchange on the 1-amino-5-hydroxyimidazolidinedione moiety of V was accomplished by stirring V and 5-nitro-2-furancarboxaldehyde (VI) in the presence of an acid catalyst, which yielded the desired product I (Scheme I).

Evidence supporting reduction of II at the 5-oxo group to give a 5-hydroxy-1-substituted 2,4-imidazolidinedione was found by comparing the NMR spectrum of I with spectra of nitrofurantoin (VII) and its isomer 3-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione (VIII) (6). The NH signal of I is at 11.5 ppm, the N_3H signal of VII occurs at 11.2 ppm, and the N_1H signal of VIII is at 8.57 ppm, indicative of a N_3H proton for I rather than an N_1H proton. These NMR data confirm that I is a 5-hydroxy-1-(substituted amino)-2,4-imidazolidinedione and not a 5-hydroxy-3-(substituted amino)-2,4-imidazolidinedione. The structure of I is thus a 5-hydroxy-substituted nitrofurantoin.

Compound I was tested for antibacterial activity against eight bacterial species in a standard broth dilution assay and had good activity against