

The Stepwise Mammalian Oxidation of the Hydantoin 1-Methylimidazolidine-2,4-dione into Methylimidazolidinetrione *via* 5-Hydroxy-1-methylimidazolidine-2,4-dione^{1,2}

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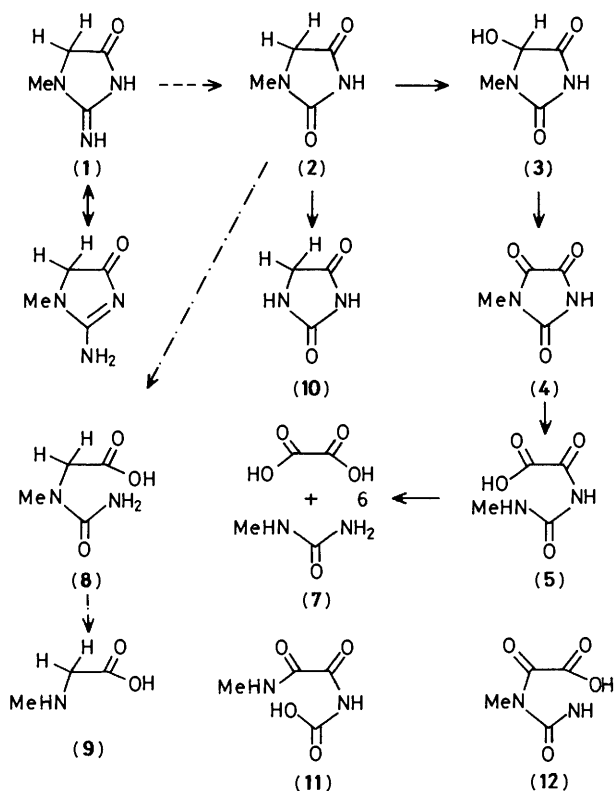
The metabolism of 1-methylhydantoin (**2**) is described. The major and general metabolic route in mammals, represented by formulae (**2**)→(**7**), includes two consecutive stepwise oxidations giving 5-hydroxy-1-methylhydantoin (**3**) and thence 1-methylparabanic acid (**4**). Since the first oxidation proved to be stereoselective, the step was thought to be enzymatic. Although enantiomeric products (**3a**) and (**3b**) (*ca.* 3:1) could not be separated directly, the mixture was converted into (*S*)- and (*R*)-5-(*N*-benzyloxycarbonyl-*L*-prolyloxy)-1-methylhydantoin (**13a**) and (**13b**) which proved separable and were identified by *X*-ray analysis of the (*R*)-diastereoisomer. The regioselective ring-fission of the second product (**4**) into the methylloxaluric acid (**5**) is discussed. Two minor oxidative routes from the substrate (**2**) into sarcosine (**9**) and parabanic acid (**10**) were also shown to exist.

During our research on plant growth regulators isolated from animals,³⁻⁵ we reported that formation of the two hydantoin, 1-methyl- (**2**) and 5-hydroxy-1-methyl-hydantoin (**3**), was induced in rabbit skin by vaccination.³ We have also proved that the mammalian metabolism of (**2**) represented by formulae (**2**)→(**7**) (see Scheme 1) is a general and major one which

desimidase,^{6,7} a known abnormal process^{8,9} induced in the host. The present paper describes in further detail the bio-organic chemistry involved in the metabolism of (**2**), a process differing fundamentally from that of hydantoin itself.¹⁰

Preparations of Metabolite Specimens.—Authentic specimens of five materials (**2**), (**6**), (**7**), (**9**), and (**10**) were purchased (Aldrich) and three others (**3**)–(**5**) were synthesized: the racemic hydroxyhydantoin (**3**) was prepared in 62% yield by oxidation of (**2**) as reported in the communication on its isolation;³ methylparabanic acid (**4**) was synthesized in 88% yield by modification of a known method,¹¹ and the methylloxaluric acid (**5**) was obtained in 55% yield by hydrolysis of (**4**) with aqueous ammonia. Since the analytically pure ammonium salt of (**5**) had m.p. 194 °C, instead of the literature value¹² of 215 °C, the structure (**5**) required confirmation. Hydrolysis in 1*M*-alkali at room temperature overnight gave the products (**6**) (73%) and (**7**) (identified but not isolated pure), thereby precluding the isomeric structure *N*-carboxy-*N'*-methylloxamide (**11**); and the doublets at 2.69 and 8.04 p.p.m. in its ¹H n.m.r. spectrum in [²H₆]dimethyl sulphoxide (DMSO) supported structure (**5**) and precluded the isomeric structure (**12**).

Major and Minor Metabolic Pathways of (2).—The main metabolic pathway of (**2**) has been clearly represented by formulae (**2**)→(**7**) (Scheme 1).¹ However, more careful sampling of urine under cooling conditions, after administration of 1-methylhydantoin (**2**) to the rabbits and more detailed analyses of metabolites of (**2**) disclosed new facts: two minor pathways were proposed and the key metabolite (**3**) was obtained as an optically active enantiomeric mixture. Similarly to the previous paper, the compounds (**2**)–(**4**) and (**7**) were isolated in 43, 28, 0.7 and 4.4% yield, respectively, and the analytical values for compounds (**2**)–(**5**) by h.p.l.c. were 45, 29, 0.6 and 4.1%, respectively. A comparison of the ¹H n.m.r. spectra of solids derived from the urine of untreated rabbits with those from rabbits, to whom substrate (**2**) had been administered, showed crucial differences: the latter included the signals assigned to methyl and/or methylene group in the five metabolites (**2**)–(**5**) and (**7**);¹ in addition, three small peaks in D₂O were newly assigned: two as the methyl and methylene group of sarcosine (**9**) (2.77 and 3.64 p.p.m.) and one as the



can be observed even in normal animals.^{1,2} Hence the production of (**3**) in vaccination-inflamed skin appears to depend on the enzymatic formation of (**2**) from creatinine (**1**) by

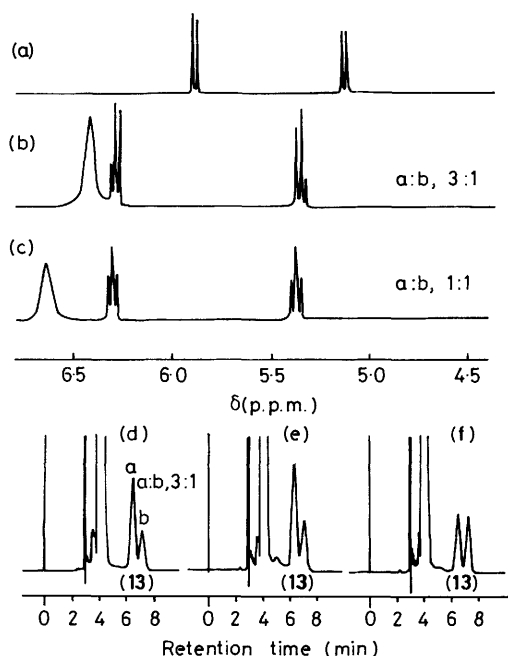


Figure 1. Analyses of enantiomers of (3) and diastereoisomers of (13). (a), (b), and (c) ^1H N.m.r. spectra of (3) in $[\text{}^2\text{H}_6]\text{acetone}$, (b) and (c) with and (a) without $\text{Eu}(\text{tfc})_3$ shift reagent. (d), (e), and (f) Chromatograms of reaction mixtures of the urinary (3) (**a:b**, 3:1) and *Z*-Pro-containing product (13) with reverse phase h.p.l.c. Reaction time; (d), 30 min; (e), 2 h; (f), 24 h

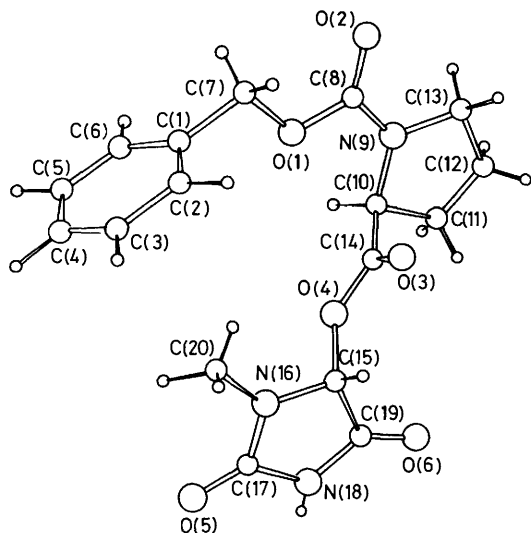


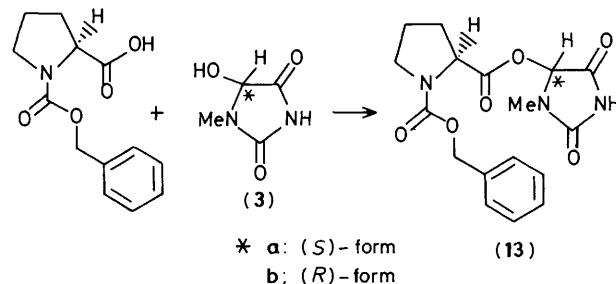
Figure 2. Structure of compound (13b) with crystallographic numbering

methylene group of hydantoin (10) (4.12 p.p.m.). The detection of (9) and (10) suggested two possible minor metabolic pathways for the substrate (2) (see Scheme 1). The formation of product (10) was subsequently proved by its isolation (5%) from the urine; however, the product (9) could not be isolated, nor could its precursor (8) even be detected.

Oxidation of Compound (2) in Rabbit.—The oxidation of (2) at the 5-position, to give (4), is not a one-step reaction but proceeded *via* the 5-hydroxy derivative (3).¹ The carefully isolated product (3), whose melting point (147–150 °C) was higher than that (138 °C) of the synthetic specimen, was shown to be optically active, $[\alpha]_D^{25} = -5.1^\circ$ (*c* 1.0, MeOH), and its enantiomeric excess was 56% by ^1H n.m.r. analysis using a shift

reagent¹³ (see Figure 1), *i.e.* the ratio of the enantiomers (3a) and (3b) was *ca.* 3:1 [on the basis of conventional h.p.l.c. and ^1H n.m.r. analyses, the optically active (3) could not be distinguished from synthetic racemate (3)]. Although the product (3) isolated from inflamed rabbit skin was first reported³ as optically inactive and the urine-derived (3) which was obtained previously¹ was also inactive, these were probably caused by racemization under the conditions used. The formation of optically active (3) suggests that the oxidative process should be enzymatic.

Absolute Structure of Compounds (3a) and (3b).—All direct attempts to resolve the racemate (3) failed but the optically pure derivatives (13a) and (13b) were isolated without difficulty. The mixture (3) was allowed to react with a chiral amino acid derivative, benzyloxycarbonyl-L-proline, in the presence of a water-soluble carbodi-imide to give the optical mixture (13) (see Scheme 2). The diastereoisomeric excess of this mixture, as



Scheme 2.

calculated by h.p.l.c. analysis (Figure 1), corresponded with the original value for enantiomeric excess in the substrate mixture (3), providing the minimum reaction time was employed to avoid racemization: in this way, each of the enantiomers in the urinary mixture (3) (**a:b**, 3:1) could be correlated with the corresponding diastereoisomer in the mixture (13) (**a:b**, 3:1). The derivatives (13a) and (13b) were separated by fractional recrystallization: the synthetic racemate (13), synthesized from the synthetic substrate (3) (*R:S*, 1:1) in 56% yield, was repeatedly crystallized from acetone to give pure prismatic crystals (13b) (30%); the mother liquor of the first crystallization was evaporated and the residue was recrystallized from ethyl acetate to give a different prismatic product (13a) (8%). The absolute structure of (13b) was determined by *X*-ray crystallographic analysis, which showed that the chiral centre of the hydantoin was in the (*R*)-form (see Figure 2). Hence, the chiralities of (13a), (3a), and (3b) were (*S*), (*S*), and (*R*), respectively. Direct hydrolysis of the esters (13a) and (13b) to the corresponding (3a) and (3b) has not yet been achieved without racemization.

Regioselective Ring Fission of (5).—The highly strained *N*(1)–C(5) bond of (4) led to its selective cleavage by ammonolysis to give only (5); neither of the isomeric products (11) and (12) could be detected. This is an important fact whether the natural fission is enzymatic or non-enzymatic.

Experimental

M.p.s are uncorrected. ^1H N.m.r. spectra were obtained using *t*-butyl alcohol (1.23 p.p.m.) in D_2O or tetramethylsilane in $[\text{}^2\text{H}_6]\text{DMSO}$ and $[\text{}^2\text{H}_6]\text{acetone}$ as an internal standard with a Bruker AM-400 spectrometer. Tris[3-trifluoromethylhydroxymethylene]-(+)-camphorato]europium(III), abbreviated as $\text{Eu}(\text{tfc})_3$,¹³ was used as a shift reagent: into a solution of (3) (5 mg) in $[\text{}^2\text{H}_6]\text{acetone}$ (0.65 ml) 25 mg of $\text{Eu}(\text{tfc})_3$ was added.

Table 1. Atomic parameters for non-hydrogen atoms in hydantoin derivative (**13a**). Estimated standard deviations are given in parentheses

Atom	x	y	z
C(1)	0.127 2(4)	0.023 9(9)	0.144 5(15)
C(2)	0.119 0(5)	0.126 1(14)	-0.042 3(18)
C(3)	0.057 4(7)	0.218 6(18)	-0.054 9(26)
C(4)	0.009 1(6)	0.208 9(20)	0.108 0(27)
C(5)	0.016 4(6)	0.107 1(9)	0.289 9(30)
C(6)	0.075 3(6)	0.017 8(15)	0.305 7(21)
C(7)	0.192 0(4)	-0.075 7(11)	0.141 3(21)
C(8)	0.313 0(3)	-0.096 8(9)	0.340 7(13)
N(9)	0.364 9(2)	-0.041 5(7)	0.499 1(9)
C(10)	0.356 9(3)	0.084 9(8)	0.649 5(9)
C(11)	0.431 9(4)	0.094 3(10)	0.807 6(14)
C(12)	0.474 0(7)	-0.005 7(25)	0.709 4(36)
C(13)	0.438 5(4)	-0.102 0(9)	0.538 0(13)
C(14)	0.343 0(3)	0.215 2(8)	0.486 4(10)
C(15)	0.304 2(4)	0.456 5(8)	0.510 4(12)
N(16)	0.231 5(3)	0.505 9(7)	0.486 9(11)
C(17)	0.222 7(3)	0.616 4(9)	0.648 9(13)
N(18)	0.291 3(3)	-0.639 5(8)	0.782 2(12)
C(19)	0.343 3(4)	0.551 7(9)	0.710 2(13)
C(20)	0.172 6(6)	0.456 9(14)	0.306 2(24)
O(1)	0.249 4(2)	-0.022 1(0)	0.327 9(10)
O(2)	0.319 9(3)	-0.204 3(8)	0.215 8(10)
O(3)	0.359 6(4)	0.230 8(11)	0.282 4(9)
O(4)	0.309 0(3)	0.313 7(7)	0.613 5(7)
O(5)	0.166 6(3)	0.681 0(8)	0.670 4(13)
O(6)	0.407 2(3)	0.547 8(8)	0.787 0(12)

Optical rotations were measured with a JASCO DIP-140 spectrometer. For h.p.l.c. analysis the JASCO-TRIROTAR-IV system was used.

Collection of the Rabbit Urine.—1-Methylimidazolidine-2,4-dione (**2**) (1.0 g/kg) in water was administered orally to a male rabbit (New Zealand White; 2.4 kg), and urine was collected during the following 24 h with immediate cooling. Control urine was collected similarly after oral administration of the same volume of water.

Identification of Metabolites of (2) from Rabbit Urine using ¹H N.m.r. and H.p.l.c.—According to the procedure reported previously, ¹H n.m.r. and h.p.l.c. analyses of the rabbit urine were carried out, using the corresponding synthetic samples for calibration. ¹H N.m.r. signals shown by the urinary solids at 2.92, 3.12, 2.84, 2.68, 2.77, 4.09, 4.12, and 3.64 p.p.m. in D₂O were assigned to the methyl groups of metabolites (**3**)–(**5**), (**7**), and (**9**), and to the methylene groups of (**10**), (**2**), and (**9**), respectively. For h.p.l.c., a Develosil ODS-5μ column (4.6 × 250 mm) was used. Elution was carried out with 50 mM phosphate buffer (pH 7.0) at a flow rate of 0.8 ml/min at 25 °C. The eluant was monitored by absorbance at 225 nm. The recovery values for (**2**)–(**5**) were 45, 29, 0.6, and 4.1%, respectively.

Isolation and Determination of Metabolites of (2) from Rabbit Urine.—The rabbit urine containing (**2**) and its metabolites was diluted with a two-fold volume of methanol and evaporated to give a residue, to which methanol-ethyl acetate (1:6) was added. The resulting insoluble material was filtered off, and the filtrate was concentrated and submitted to silica-gel column chromatography (chloroform-methanol, 9:1). From the column separation were obtained pure crystalline samples of (**3**), (**4**), (**7**), and (**10**), as well as unmetabolized (**2**) in 28, 0.7, 4.4, 5.0, and 43% yield, respectively. The isolated powder (**3**), m.p. 147–150 °C, $[\alpha]_D^{25} = -5.1^\circ$ (c 1.0, MeOH), was analyzed by ¹H n.m.r. in [²H₆]acetone containing the shift reagent Eu(tfc)₃

Table 2. Bond lengths (Å) and valence angles (°) in hydantoin derivative (**13b**)

Bond	Length	Bond	Length
C(1)–C(2)	1.381(14)	C(1)–C(6)	1.337(14)
C(1)–C(7)	1.507(12)	C(2)–C(3)	1.415(18)
C(3)–C(4)	1.296(19)	C(4)–C(5)	1.359(24)
C(5)–C(6)	1.357(19)	C(7)–O(1)	1.458(10)
C(8)–N(9)	1.309(8)	C(8)–O(1)	1.349(7)
C(8)–O(2)	1.224(11)	N(9)–C(10)	1.449(9)
N(9)–C(13)	1.450(9)	C(10)–C(11)	1.532(9)
C(10)–C(14)	1.509(10)	C(11)–C(12)	1.350(22)
C(12)–C(13)	1.398(21)	C(14)–O(3)	1.164(8)
C(14)–O(4)	1.334(9)	C(15)–N(16)	1.395(9)
C(15)–C(19)	1.513(10)	C(15)–O(4)	1.451(10)
N(16)–C(17)	1.370(10)	N(16)–C(20)	1.442(13)
C(17)–N(18)	1.390(8)	C(17)–O(5)	1.202(9)
N(18)–C(19)	1.339(10)	C(19)–O(6)	1.193(9)
Bond	Angle	Bond	Angle
C(2)–C(1)–C(6)	117.3(9)	C(2)–C(1)–C(7)	117.1(8)
C(6)–C(1)–C(7)	125.6(9)	C(1)–C(2)–C(3)	119.5(10)
C(2)–C(3)–C(4)	120.6(14)	C(3)–C(4)–C(5)	120.0(14)
C(4)–C(5)–C(6)	120.1(13)	C(1)–C(6)–C(5)	122.4(12)
C(1)–C(7)–O(1)	106.5(7)	N(9)–C(8)–O(1)	112.6(6)
N(9)–C(8)–O(2)	124.9(6)	O(1)–C(8)–O(2)	122.5(6)
C(8)–N(9)–C(10)	125.2(5)	C(8)–N(9)–C(13)	122.1(6)
C(10)–N(9)–C(13)	112.6(5)	N(9)–C(10)–C(11)	102.6(5)
C(9)–C(10)–C(14)	111.7(4)	C(11)–C(10)–C(14)	111.0(6)
C(10)–C(11)–C(12)	104.8(9)	C(11)–C(12)–C(13)	117.5(10)
N(9)–C(13)–C(12)	101.5(9)	C(10)–C(14)–O(3)	126.5(8)
C(10)–C(14)–O(4)	109.5(5)	O(3)–C(14)–O(4)	124.1(8)
N(16)–C(15)–C(19)	103.9(6)	N(16)–C(15)–O(4)	111.3(6)
C(19)–C(15)–O(4)	106.0(5)	C(15)–N(16)–C(17)	111.4(5)
C(15)–N(16)–C(20)	126.0(7)	C(17)–N(16)–C(20)	122.5(7)
N(16)–C(17)–N(18)	106.6(6)	N(16)–C(17)–O(5)	126.6(6)
N(18)–C(17)–O(5)	126.8(8)	C(17)–N(18)–C(19)	112.4(7)
C(15)–C(19)–N(18)	105.7(6)	C(15)–C(19)–O(6)	126.6(8)
N(18)–C(19)–O(6)	127.7(7)	C(7)–O(1)–C(8)	114.0(5)
C(14)–O(4)–C(15)	117.8(5)		

(Figure 1), and its enantiomeric excess was determined as 56%, i.e. a 78:22 mixture.

1-Methylimidazolidine-2,4-dione (2).—The synthetic compound (**3**) (300 mg) in concentrated hydriodic acid (3 ml) was heated at 60 °C for 30 min, and the solvent was evaporated under reduced pressure. The residue was purified by silica-gel t.l.c. [methanol-chloroform (1:9)] to yield 1-methylimidazolidine-2,4-dione (**2**) (92%), m.p. 158 °C, identical with an authentic specimen (Aldrich).

5-Hydroxy-1-methylimidazolidine-2,4-dione (3).—Commercial 1-methylimidazolidine-2,4-dione (**2**) (200 mg) and lead tetra-acetate (780 mg) in benzene (50 ml) were heated at 60 °C for 24 h, and the mixture was evaporated under reduced pressure. The residue was applied to a short silica-gel column and chromatography (benzene-hexane) gave 5-acetoxy-1-methylimidazolidine-2,4-dione (202 mg). This was hydrolysed by stirring it in aqueous 0.3M-sulphuric acid at room temperature for 40 min. After neutralization and evaporation of the mixture, the residue was submitted to silica-gel t.l.c. (ethyl acetate) to give the pure *hydantoin* (**3**) (64%) (*R:S* = 1:1), m.p. 138 °C, $[\alpha]_D^{25} = 0.0^\circ$ (c 1.0, H₂O) (Found: C, 36.8; H, 4.6; N, 21.4. C₄H₆N₂O₃ requires C, 36.9; H, 4.7; N, 21.5%).

5-(N-Benzylxycarbonyl-L-prolyloxy)-1-methylimidazolidine-2,4-dione (13).—(a) The hydroxyhydantoin (**3**) (*R:S*, 1:1; 10.0 g), *Z*-L-Pro-OH (19.2 g), 1-(3-dimethylaminopropyl)-3-

ethylcarbodi-imide hydrochloride (14.7 g), and dimethylamino-pyridine (0.94 g) in acetonitrile were stirred at 0 °C for 2 h. After being stirred at room temperature for an additional 3 h, the solution was evaporated to dryness under reduced pressure, and the residue submitted to silica-gel column chromatography [ethyl acetate-hexane, (7:3)] to give the crystalline ester (**13**) (56%) (*R*:*S*, 1:1). Fractional crystallization of the solid (**13**) (10.0 g) from acetone gave the pure *diastereoisomer* (**13b**) (30%), m.p. 189–192 °C, $[\alpha]_D^{25} = -2.1^\circ$ (*c* 1.0, MeOH); $\delta_H([^2H_6]DMSO)$ 1.8–2.0 (3 H, m), 2.2–2.4 (1 H, m), [2.52 (s) + 2.75 (s) (3 H)], 3.4–3.55 (2 H, m), [4.35 (dd, *J* 4, 9 Hz) + 4.46 (dd, *J* 4, 9 Hz) (1 H)], [5.03 (s) + 5.10 (s) (2 H)], [6.09 (s) + 6.10 (s) (1 H)], 7.25–7.45 (5 H, m), and 11.21 (1 H, br s) (Found: C, 56.7; H, 5.5; N, 11.7. $C_{17}H_{19}N_3O_6$ requires C, 56.5; H, 5.3; N, 11.6%). After evaporation of the initial mother liquor, the residue was crystallized twice from ethyl acetate to give a second pure *diastereoisomer* (**13a**) (8%), m.p. 147–150 °C, $[\alpha]_D^{25} = -68.9^\circ$ (*c* 1.0, MeOH); $\delta_H([^2H_6]DMSO)$ 1.7–2.0 (3 H, m), 2.2–2.4 (1 H, m), [2.63 (s) + 2.73 (s) (3 H)], 3.35–3.55 (2 H, m), [4.36 (dd, *J* 4, 9 Hz) + 4.47 (dd, *J* 4, 9 Hz) (1 H)], [5.04 (d, *J* 13 Hz) + 5.07 (d, *J* 13 Hz) (1 H)], [5.07 (d, *J* 13 Hz) + 5.13 (d, *J* 13 Hz) (1 H)], [6.05 (s) + 6.12 (s) (1 H)], 7.25–7.45 (5 H, m), and 11.25 (1 H, br s) (Found: C, 56.3; H, 5.5; N, 11.7. $C_{17}H_{19}N_3O_6$ requires C, 56.5; H, 5.3; N, 11.6%).

(b) Compound (**3**) (*R*:*S*, 3:1), isolated from rabbit urine, was treated as in (a). An aliquot of the reaction mixture was analysed by h.p.l.c. at periods up to 24 h (see Figure 2), in order to detect racemization (see text) [for h.p.l.c., a Develosil ODS-5 μ column (4.6 \times 250 mm) was used. Elution was carried out with 50% aqueous MeOH at a flow rate of 1.0 ml/min at 25 °C. The eluant was monitored by absorbance at 230 nm].

1-Methylimidazolidinetrione (**4**).—To an ice-cooled solution of 1-methylurea (1.0 g) in tetrahydrofuran (50 ml), oxalyl chloride (1.3 ml) was added dropwise during 30 min. After being stirred at the same temperature for 2 h, the reaction mixture was warmed to room temperature. Water (10 ml) was added to it and the tetrahydrofuran layer was separated and mixed with an ethyl acetate extract (50 ml) of the aqueous layer. Drying (Na_2SO_4) and evaporation of the solution gave a crude solid, which was crystallized from ethyl acetate-hexane to give the pure title compound (**4**) (88%), m.p. 153–155 °C (lit.¹¹ m.p. 155–157 °C).

N-Methyl-*N'*-oxalourea (**5**).—1-Methylimidazolidinetrione (**4**) (10 g) was stirred in aqueous 1M ammonia (400 ml) at room temperature for 1 h. The solvent was evaporated under reduced pressure and the residual solid was crystallized from methanol-water to give the pure ammonium salt of *N*-methyl-*N'*-oxalourea (**5**) (55%), m.p. 194 °C (decomp.) [lit.,¹² m.p. 215 °C (decomp.)] (Found: C, 29.5; H, 5.8; N, 25.8. $C_4H_9N_3O_4$ requires C, 29.5; H, 5.6; N, 25.8%).

Hydrolysis of Compound (5).—A solution of compound (**5**) (100 mg) in 1M aqueous sodium hydroxide (5 ml) was stirred at room temperature overnight. After neutralization with hydrochloric acid, the mixture was evaporated to dryness. The residue was purified by silica-gel t.l.c. (MeOH- $CHCl_3$, 15:85) to give pure methylurea (**7**) (73%), m.p. 100 °C.

Crystal Structure Determination of Compound (13b).— $C_{17}H_{19}N_3O_6$, *M* = 361.35, monoclinic, space group $P2_1$, *a* = 18.173(4), *b* = 9.414(3), *c* = 5.320(1) Å, $\alpha = 90.00(0)$, $\beta = 95.63(2)$, $\gamma = 90.00(0)^\circ$, *U* = 905.8 Å³, *Z* = 2, *D*_c = 1.325 g cm⁻³, *R*(*R*_w) = 0.071 (0.089) for 1 335 unique reflections [*F*_o > 3 σ (*F*_o)]. A crystal with dimensions of 0.20 \times 0.20 \times 0.20 mm was used for data collection. The intensity data were collected on a Rigaku AFC-5RU diffractometer for 0 < θ < 60° using monochromated Cu-*K* α radiation ($\lambda = 1.54173$ Å), and the ω -2 θ scan method at an ω scan speed of 16° min⁻¹. Three standard reflections were measured every 56 reflections to monitor intensity fluctuations. Absorption corrections were not applied. The structure was solved by the direct method using a MULTAN program and was refined by the full-matrix least-squares method, minimizing the function $(\sum_w |F_o| - |F_c|)^2$ with $w = 1/\sigma^2$. The hydrogen atoms were located from the D-map and refined with the isotropic thermal parameters. All computations were performed on a FACOM M 382 computer in the Data Processing Center of Kyoto University, using the KPPXRAY programs. Tables of the hydrogen atomic coordinates and the anisotropic thermal parameters are available on request from the Cambridge Crystallographic Data Centre.*

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