= 1.6 Hz, CH_2). Trans isomer: mp 206.5–207.5° dec; colorless crystals; 130 mg (1.9%); ir (KBr) 3000–2500 (CO_2H), 1670 (CO_2H), 1640 cm^{-1} (CO); nmr (DMSO-d_6) δ 8.20 (1 H, s, chromone H_2), 8.08 (1 H, dd, chromone H_5), 7.0–8.0 (4 H, m, OH and chromone $H_{6,7,8}$), 7.68 (1 H, H_β of acrylic acid), 7.20 (5 H, s, Ph), 3.87 (2 H, s, CH_2). *Anal.* ($C_{19}H_{14}O_4$) C, H.

3-(4-Oxo-4*H*-1-benzopyran-2)propionic Acid (15). A mixture of 3-(4-oxo-4*H*-1-benzopyran-2)acrylic acid (14, 500 mg), zinc powder (2.5 g), and AcOH (17 ml) was heated to mild reflux for 1 hr. After cooling the mixture, the separated salt was removed by filtration and washed with a small amount of AcOH. The combined filtrate and washings were concentrated *in vacuo* and trituration of the residue with Me_2CO gave a solid. Recrystallization of the crude solid from AcOH afforded 350 mg of colorless plates, mp 206–208°.

3-(4-Oxo-4*H*-1-benzopyran-3)propionic Acid (16). Compound **5a** (1 g, 4.63 mmol) was dissolved in AcOH (30 ml) at 90°. To this solution was added a small amount of Pd black and the mixture was hydrogenated under atmospheric pressure at 90°. After 1 equiv of hydrogen was absorbed by the mixture, the catalyst was removed by filtration. The filtrate was concentrated *in vacuo* and the residual solid was recrystallized from MeOH to afford 715 mg (71%) of colorless crystals, mp 168–169°.

2-Methyl-4-oxo-4*H*-1-benzopyran-3-acetonitrile (17b). To a solution of NaCN (2.0 g, 40.8 mmol) in dried DMSO (50 ml) maintained at 60–63° was added 2-methyl-3-chloromethylchromone (**17a**,[13] 8.00 g, 38.4 mmol) over a 50-min period. The mixture was stirred at 60–65° for 1 hr, cooled, and poured into 300 ml of ice-water. The resulting precipitate was collected by filtration and dried. Chromatography of the resulting solid on silica gel with benzene–Et_2O (2:1) as the eluent afforded 5.9 g (77%) of colorless crystals, mp 118–121°. An analytical sample was recrystallized from benzene to afford colorless needles, mp 122–124°. *Anal.* ($C_{12}H_9NO_2$) C, H, N.

2-Methyl-4-oxo-4*H*-1-benzopyran-3-acetic Acid (17c). A solution of **17b** (100 mg, 0.50 mmol) in AcOH (1 ml) and 6 *N* H_2SO_4 (1 ml) was heated to reflux for 36 hr. After cooling the mixture, the separated crystals were collected by filtration, washed with water, and dried to afford 65 mg (59%) of colorless needles, mp 169–173°.

Biological Assay. Male Sprague-Dawley rats, 7 weeks old and weighing 250 g, were used. Rat antiserum containing homocytotropic antibody was prepared according to the method of Mota.[7] In brief, the animals were sensitized by intramuscular injections of 1 mg of egg albumin in 1 ml of saline solution concomitantly with an intraperitoneal injection of 20 billion of *B. pertussis* vaccine. Serum collected from each animal 12 days after sensitization was pooled and frozen until use. The biological properties of the skin sensitizing antibody contained in these sera satisfy the requirements for a homocytotropic antibody, *i.e.*, it fixes homologous skin tissue for a long time and is heat labile. The antisera showed passive cutaneous anaphylaxis (PCA, 72-hr latent period) titers of 1:32–1:64. Homologous rat PCA response was elicited as follows. Four 0.05-ml aliquots of serum diluted fourfold with physiological saline solution were injected intradermally into the shaved dorsal skin of the rat. After 72 hr the rat was challenged with an intravenous injection of 1 ml of saline solution containing 5 mg of egg albumin and 10 mg of Evans Blue. Drugs to be tested or vehicles (saline or polyethylene glycol 400) were administered intravenously immediately before antigen challenge. Rats were sacrificed by bleeding 30 min later, and the area of the dye leakage was measured in square millimeters. The dose giving 50% inhibition (ID_{50}) for each drug was calculated graphically from the dose–inhibition relationship expressed in inhibition per cent of the bluing areas against doses on a logarithmic scale. At least three doses and three animals for each dose (*i.e.*, 12 spots) were used for obtaining the dose–inhibition relationship.

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Synthesis and Absolute Stereochemistry of 5-Alkyl-5-(3′-hydroxy-1′-methylbutyl)barbituric Acid and 5-Alkyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric Acids[1]

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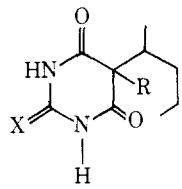
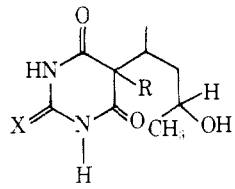
5-Alkyl-5-(3′-hydroxy-1′-methylbutyl)barbituric acid (**2**) and 5-alkyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric acids (**3**) are metabolites of 5-alkyl-5-(2′-pentyl)barbituric acid and 5-alkyl-5-(2′-pentyl)-2-thiobarbituric acid, respectively. We have synthesized the four possible optical isomers of **2** and **3** by a procedure which established the absolute stereochemistry of each isomer. The two racemic pairs in each case were also prepared. The properties of these synthetic samples of **2** and **3** of known stereochemistry are compared to the properties of **2** and **3** which have been isolated from metabolism studies.

5-Ethyl-5-(2′-pentyl)barbituric acid (**1a**)† and 5-allyl-5-(2′-pentyl)barbituric acid (**1b**)† are metabolized *in vivo* to give 5-ethyl- and 5-allyl-5-(3′hydroxy-1′-methylbutyl)-barbituric acids (**2**)[1,2] as their major metabolites. In addition, 5-ethyl-5-(2′-pentyl)-2-thiobarbituric acid (**1c**)† and 5-allyl-5-(2′-pentyl)-2-thiobarbituric acid (**1d**)† give 5-ethyl- and 5-allyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric acids (**3**) as minor metabolites.[3,4] The metabo-

†The generic names of compounds **1a**, **1b**, **1c**, and **1d** are pentobarbital, secobarbital, thiopental, and thiamylal, respectively.



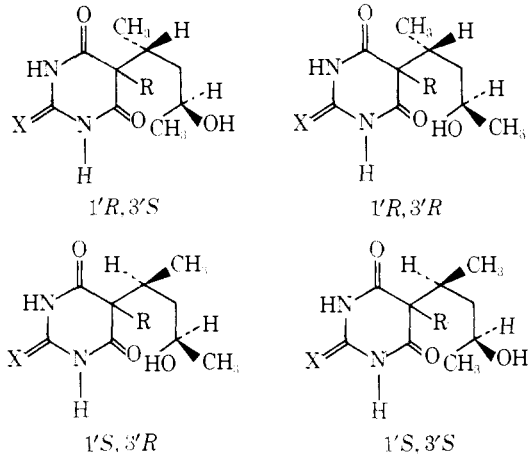
1a, R = C_2H_5; X = O
b, R = C_3H_5; X = O
c, R = C_2H_5; X = S
d, R = C_3H_5; X = S



2a, R = C_2H_5; X = O
b, R = C_3H_5; X = O
3a, R = C_2H_5; X = S
b, R = C_3H_5; X = S

lites 2 and 3 contain two asymmetric centers and, thus, the four optical isomers shown in Chart I are possible in each case. The four optical isomers of **2a** have been isolated from the metabolism studies of optically pure (*R*)-**1a** and (*S*)-**1a**.‡[5] In other cases, mixtures of diastereoisomeric pairs have been obtained from metabolism studies. However, except for preliminary reports[6] of the present work, the stereochemical assignments of these metabolites have not been determined. In this paper we report the synthesis of all four optical isomers as well as the racemic pairs of **2a**, **2b**, **3a**, and **3b**. The synthetic method used allows the absolute stereochemistry of the optical isomers to be determined.

Chart I



1′*R*,3′*S* 1′*R*,3′*R*

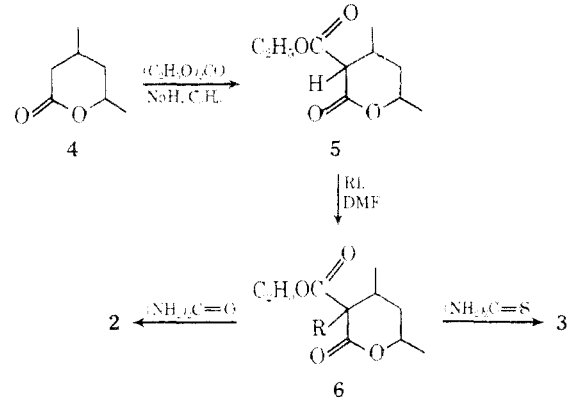
1′*S*,3′*R* 1′*S*,3′*S*

Synthesis. In previous reports we have described the synthesis of all four optical isomers of 3,5-dimethylvalerolactone (**4**).[7] Starting with these lactones, we have prepared **2** and **3** as outlined in Scheme I. A benzene solution of the appropriate lactone isomer **4** was condensed with diethyl carbonate in the presence of sodium hydride to give the 2-ethoxycarbonyl derivative **5**. Treatment of a dimethylformamide solution of **5** with the proper alkyl iodide in the presence of sodium hydride afforded 2-ethoxycarbonyl-2-alkyl-3,5-dimethylvalerolactone (**6**). The condensation of **6** with urea or thiourea gave the desired 5-alkyl-5-(3′-hydroxy-1′-methylbutyl)barbituric acid (**2**) and 5-alkyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric acid (**3**), respectively. The compounds prepared along with their physical properties are listed in Table I. Since the absolute stereochemistry of the starting lactones **4** has been established, the absolute stereochemistry of the products **2** and **3** is also established and is as represented in Table I.

Structure and Stereochemistry of Products Isolated from Metabolism Studies of 1a–d. By comparing the physical constants of the synthetic products **2a** and **2b** of

The Cahn, Ingold, and Prelog *R* and *S* designation of configuration is used in this paper.[5]

Scheme I



4 5 6 2 3

known absolute stereochemistry listed in Table I to the metabolites isolated from metabolism studies of **1a** and **1b**, it is now possible to determine the structure and stereochemistry of the metabolite isomers which were isolated. Those studies which have involved isolation of chemically pure metabolites, **2a** and **2b**, are listed in Table II. No pure 3′-hydroxy metabolites of **1c** and **1d** have been isolated. However, studies where the presence of these metabolites has been implicated are also listed in Table II. Each of the four compounds will be discussed in turn.

5-Ethyl-5-(2′-pentyl)barbituric Acid.† The title compound **1a** is the only one of the four compounds for which metabolism studies of each of the individual optical isomers have been studied separately. Even in this case, the study has only been conducted in one species, dog. Maynert and Dawson[1c] isolated two optically active fractions of **2a** from urine of dogs that had been dosed with (*R*,*S*)-**1a**. One fraction [(+) diastereoisomer] had a (+) rotation, and the second fraction [(−) diastereoisomer] had a (−) rotation. In a more recent report Maynert[1d] described the isolation of a third metabolite fraction (racemic metabolite) which did not show optical activity. The metabolism of (*R*,*S*)-**1a** has recently been repeated, and in addition the metabolism of pure (*S*)-**1a** and (*R*)-**1a** has been reported.[1a,b] These studies showed that (*R*)-**1a** is metabolized in the dog to give almost equal amounts of (1′*R*,3′*S*)-**2a** and (1′*R*,3′*R*)-**2a**.§ In contrast, (*S*)-**1a** gave primarily (1′*S*,3′*S*)-**2a** with a little of (1′*S*,3′*R*)-**2a** formed.§ Taking these studies into account and comparing the melting points and $[\alpha]_D$'s of the fractions isolated by Maynert and coworkers to the **2a** isomers listed in Table I, it is now clear that Maynert's (+) diastereoisomer was a mixture of (1′*R*,3′*S*)- and (1′*S*,3′*R*)-**2a** that contained a preponderance of the 1′*R*,3′*S* isomer, thus giving this fraction a (+) rotation. The (−) diastereoisomer was a mixture of (1′*S*,3′*S*)- and (1′*R*,3′*R*)-**2a** which contained an excess of 1′*S*,3′*S* isomer, thus giving this fraction a (−) rotation. The racemic metabolite isolated by Maynert is (1′*RS*,3′*SR*)-**2a** and contains equal amounts of (1′*R*,3′*S*)- and (1′*S*,3′*R*)-**2a**.

5-Allyl-5-(2′-pentyl)barbituric Acid.† The metabolism of (*R*,*S*)-**1b** has been studied in man and two 5-allyl-5-(3′-hydroxy-1′-methylbutyl)barbituric acid metabolites (metabolite-S and metabolite-F) were isolated.[2a] A comparison of the properties of these metabolites to the synthetic **2b** isomers shows that metabolite-S is (1′*RS*,3′*RS*)-**2b** and metabolite-F is (1′*RS*,3′*SR*)-**2b**. The

§ A comparison of the nmr spectra of the synthetic products of known structure to the spectra of the two 3′-hydroxy metabolites isolated from the metabolism studies of (*R*)-**1a** showed that the original stereochemical assignments should be reversed.[1a,b]

Table I. Properties of 5-Alkyl-5-(3′-hydroxy-1′-methylbutyl)barbituric Acids and 5-Alkyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric Acids[a]

Compound[b]	Stereochemical assignment	R	X	Recrystn solvents	Yield, %[c]	Mp, °C	$[\alpha]^{24-26}D$, deg (c)[d]	Analyses
2a	1′*S*, 3′*R*[e]	C_2H_5	O	H_2O	39	208–210	−31.5 (0.95)	C, H, N
	1′*R*, 3′*S*	C_2H_5	O	H_2O[f]	32	202–206	+29.2 (0.95)	C, H, N
	1′*S*, 3′*S*[e]	C_2H_5	O	C_6H_{14}–EtOAc	52	181–183	−15.3 (0.85)	C, H, N
	1′*R*, 3′*R*	C_2H_5	O	C_6H_{14}–EtOAc	50	177–179	+14.6 (0.86)	C, H, N
	1′*RS*, 3′*SR*[f]	C_2H_5	O	H_2O	39	191–192	0	C, H, N
	1′*RS*, 3′*RS*	C_2H_5	O	C_6H_{14}–EtOAc	40	145–147	0	
2b	1′*S*, 3′*R*	C_3H_5	O	H_2O	41	193–196	−25.1 (0.71)	C, H, N
	1′*R*, 3′*S*	C_3H_5	O	H_2O	29	193–196.5	+23.3 (0.67)	C, H, N
	1′*S*, 3′*S*	C_3H_5	O	H_2O	37	175.5–179	−10.2 (0.92)	C, H, N
	1′*R*, 3′*R*	C_3H_5	O	H_2O	31	174–178	+9.9 (0.64)	C, H, N
	1′*RS*, 3′*SR*	C_3H_5	O	H_2O	53	179–180	0	
	1′*RS*, 3′*RS*	C_3H_5	O	C_6H_{14}–EtOAc	55	163–166	0	
3a	1′*S*, 3′*R*	C_2H_5	S	C_6H_{14}–EtOAc	39	140–142	−25.3 (0.79)	C, H, N
	1′*R*, 3′*S*	C_2H_5	S	H_2O	35	139–142	+25.6 (0.83)	C, H, N
	1′*S*, 3′*S*	C_2H_5	S	H_2O	33	147–148	−12.5 (0.82)	C, H, N, S
	1′*R*, 3′*R*	C_2H_5	S	H_2O	26	144–147	+11.6 (0.83)	C, H, N, S
	1′*RS*, 3′*SR*	C_2H_5	S	H_2O	50	170–172	0	
	1′*RS*, 3′*RS*	C_2H_5	S	H_2O	47	168–168.5	0	
3b	1′*S*, 3′*R*	C_3H_5	S	C_6H_{14}–CH_2Cl_2	25	63–65	−19.4 (0.81)	C, H, N
	1′*R*, 3′*S*	C_3H_5	S	C_6H_{14}–CH_2Cl_2	25	63–66	+19.0 (0.80)	C, H, N
	1′*S*, 3′*S*	C_3H_5	S	MeCN	22	162–165	−8.7 (0.82)	C, H, N, S
	1′*R*, 3′*R*	C_3H_5	S	MeCN	22	163–166	+8.0 (0.79)	C, H, N, S
	1′*RS*, 3′*SR*	C_3H_5	S	MeOH–H_2O	46	144–147	0	C, H, N, S
	1′*RS*, 3′*RS*	C_3H_5	S	C_6H_{14}–EtOAc	57	186–187	0	C, H, N, S

[a] These compounds were prepared from 3,5-dimethylvalerolactone (**4**) which was prepared from the optical isomers of 3-methyl-5-oxohexanoic acid. The optical isomers of this acid were shown to be >95% optically pure. Since the lactones (**4**) used were pure samples of the cis and trans isomers, the compounds **2** and **3** must be at least 95% optically pure (see ref 7). [b] General procedures for the synthesis are given in the Experimental Section. [c] The overall yield of pure product obtained from lactone **4** is given. The (3*RS*,5*SR*)-**4** and (3*RS*,5*RS*)-**4** samples were distilled before use. The optical isomers of **4** were shown to contain only one isomer by glc analysis and were used without distillation in the preparation of the **2b**, **3a**, and **3b** isomers. These undistilled samples of lactone **4** may have contained some nonvolatile impurities and thus account for the lower yields obtained in the case of the optical isomers of **2b**, **3a**, and **3b**. [d] Rotations were measured using 95% EtOH, c given in g/100 ml. [e] The (*S*)-3-methyl-5-oxohexanoic acid used to prepare (1′*S*,3′*R*)-**2a** and (1′*S*,3′*S*)-**2a** was prepared from the antibiotic 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide (commonly called cycloheximide) and is presumed to be optically pure. [f] This isomer is identical with a sample of this isomer prepared by the method of T. J. Dickert, P. J. Shea, and L. P. McCarty [*J. Med. Chem.*, **9**, 249 (1966)], which used totally racemic lactone **6**. This probably results from the fact that the crude sample of this isomer isolated by these authors was purified by recrystallization from water. The (1*RS*,3*SR*)-**2a** is considerably more insoluble in water than the (1′*RS*,3′*RS*)-**2a** isomer.

metabolism of **1b** in rabbit has been reported and an optically active 5-allyl-5-(3′-hydroxy-1′-methylbutyl)barbituric acid metabolite (metabolite 2) was isolated.[2b] However, it is not possible to ascertain the composition of this metabolite from the physical properties reported by the authors.

5-Ethyl- and 5-Allyl-5-(2′-pentyl)-2-thiobarbituric Acid.† 5-Ethyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric acid and 5-allyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric acid are obtained as minor biotransformation products from metabolism studies of **1c** and **1d** in rabbits. In the study of **1c** the presence of the metabolite was established only by physical methods. In the case of **1d** a hydroxy metabolite (metabolite-4) was obtained which had mp 175° and $[\alpha]D$ +119.3°. The optical rotation of metabolite-4 is an order of magnitude larger than any of the synthetic isomers of **3b**. It is highly probable that this metabolite was contaminated or is an entirely different compound than **3b**.

Summary of Metabolism Studies. The physical properties of the synthetic samples of **2** and **3** of known stereochemistry were compared to the 3′-hydroxy metabolites isolated from metabolism studies of **1a–d**. The comparison was used to establish the correct stereochemical assignments to these metabolites. Metabolism studies which involve the isolation of chemically pure 3′-hydroxy metabolites of **1a–d** are limited. The metabolism of **1a** in dogs is the only study which has been completely resolved. The availability of optically pure samples of both optical isomers of **1a–d**[8,9] along with the correct stereochemical assignment to all potential 3′-hydroxy metabolites of these compounds reported in this paper will greatly simplify the interpretation of results obtained from future metabolism studies involving **1a–d**.

Experimental Section

Melting points were determined on a Kofler hot-stage microscope using a calibrated thermometer. Ir spectra were measured with a Perkin-Elmer Model 467 grating infrared spectrophotometer. Nmr spectra were recorded on a Varian Model HA-100 spectrometer with tetramethylsilane as an internal standard. All observed rotations at the sodium D line were determined with a Perkin-Elmer Model 141 polarimeter (1-dm cell). Mass spectra were

Table II. 5-Alkyl-5-(3'-hydroxy-1'-methylbutyl)barbituric Acids and 5-Alkyl-5-(3'-hydroxy-1'-methylbutyl)-2-thiobarbituric Acids Isolated from *in Vivo* or *in Vitro* Metabolism Studies

Compd studied	Name of metabolite[a]	Type of study	Species	Mp, °C	$[\alpha]_D$, deg	(solvent)	Stereochemical assignment	Ref
			5-Ethyl-5-(2'-pentyl)barbituric Acid (Pentobarbital)					
(S)-1a	Metabolite-1-(S)-II	*In vivo*	Dog	209–211	−32.6	(95% EtOH)	(1'S, 3'R)-2a[b]	1a
(S)-1a	Metabolite-2-(S)-II	*In vivo*	Dog	176.5–178	−15.5	(95% EtOH)	(1'S, 3'S)-2a[b]	1a
(R)-1a	Metabolite-1-(R)-II	*In vivo*	Dog	210–211	−30.8	(95% EtOH)	(1'R, 3'S)-2a[b]	1b
(R)-1a	Metabolite-2-(R)-II	*In vivo*	Dog	175–178	−13.2	(95% EtOH)	(1'R, 3'R)-2a[b]	1b
(R,S)-1a	Metabolite-1-(RS)-II	*In vivo*	Dog	195–200	−25.0	(95% EtOH)	*c*	1b
(R,S)-1a	Metabolite-2-(RS)-II	*In vivo*	Dog	149–154	−5.2	(95% EtOH)	*d*	1b
(R,S)-1a	(+) diastereoisomer	*In vivo*	Dog	209–210	+26.6	(HOAc)	*e*	1c, d
(R,S)-1a	(−) diastereoisomer	*In vivo*	Dog	152–153	−5.6	(HOAc)	*d*	1c, d
(R,S)-1a	Racemic metabolite	*In vivo*	Dog	188–189	0		(1'RS, 3'SR)-2a	1d
(R,S)-1a	Major metabolite	*In vivo*	Man	205–206			*f*	1f
			5-Allyl-5-(2'-pentyl)barbituric Acid (Secobarbital)					
(R,S)-1b	Metabolite S	*In vivo*	Man	162–163	0		(1'RS, 3'RS)-2b	2a
(R,S)-1b	Metabolite F	*In vivo*	Man	176–178	0		(1'RS, 3'SR)-2b	2a
(R,S)-1b	Metabolite 2	*In vivo*	Rabbit	167	−19.3[c]		*h*	2b
			5-Ethyl-5-(2'-pentyl)-2-thiobarbituric Acid (Thiopental)					
(R,S)-1c		*In vitro*	Rabbit					3[c,i]
			5-Allyl-5-(2'-pentyl)-2-thiobarbituric Acid (Thiamylal)					
(R,S)-1d	Metabolite 4	*In vivo*	Rabbit	175	+119.3	(EtOH)	*h*	4

[a] The names refer to the designation used by the authors in the referenced studies. [b] A comparison of the nmr spectra of the synthetic products of known structure to the spectra of the two 3'-hydroxy metabolites isolated from the metabolism studies of (R)-1a showed that the original stereochemical assignments should be reversed.[1a,b] [c] This metabolite is a mixture of (1'S,3'R)- and (1'R,3'S)-2a which contains a greater amount of the latter isomer. [d] This metabolite is a mixture of (1'S,3'S)- and (1'R,3'R)-2a which contains a greater amount of the 1'S,3'S isomer. [e] The rotation was not reported. [f] The authors report that a mixture of this metabolite with that of the (+) diastereoisomer isolated by Maynert and coworkers[1c] showed no depression of the melting point. [g] The solvent was not given. [h] Since only one isomer was isolated and since the solvent used to determine the $[\alpha]_D$ was not given, it is not possible to suggest a structure for this metabolite. [i] A 3'-hydroxy metabolite of 1c has not been isolated. Paper chromatograms indicated that it might be present.[3] [j] We have established by gas chromatography–mass spectral studies that the urine of humans given 1c contains small amounts of a metabolite whose mass spectrum is identical with that of authentic samples. [k] Since the mp 175° of this metabolite is higher than any of the authentic synthetic isomers of 2d (Table I) except the racemic (1RS,3RS)-2d, it seems likely that the metabolite is largely this compound but possibly contaminated with the other isomers. However, the unusually large rotation, $[\alpha]_D$ +119.3°, if correct would indicate that the metabolite isolated is a metabolite other than the 3'-hydroxy derivative.

determined on an AEI-MS 902 spectrometer. Microanalyses were carried out by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.5% of theoretical values.

2-Alkyl-2-carbethoxy-3,5-dimethylvalerolactones (5). To a stirred refluxing mixture of 0.20 g of a 50% sodium hydride dispersion in white oil (washed free of oil with dry C_6H_6) and 1.89 g (0.016 mol) of diethyl carbonate in 10 ml of dry C_6H_6 was added dropwise 0.513 g (0.004 mol) of the appropriate 3,5-dimethylvalerolactone isomer 4[7] in 5 ml of dry C_6H_6, and the mixture was refluxed an additional 1 hr after the addition. The cooled reaction mixture was acidified with HOAc and diluted with 10 ml of C_6H_6 and 20 ml of H_2O. The layers were separated, and the aqueous layer was extracted with C_6H_6. The organic layers were combined, washed with 0.4% Na_2CO_3 solution and H_2O, dried (Na_2SO_4), concentrated on a rotary evaporator, and dried under high vacuum to give the 2-carbethoxy-3,5-dimethylvalerolactone as a light tan liquid. These products were used to prepare 2-alkyl-2-carbethoxy-3,5-dimethylvalerolactone (6) without further purification.

To a stirred suspension of 0.20 g of 50% sodium hydride dispersion in oil (washed free of oil with dry C_6H_6) in 1 ml of dry DMF was added the lactone 6 prepared above in 1 ml of DMF. The addition was added dropwise at a rate to control hydrogen evolution. After the addition, the mixture was stirred until hydrogen evolution ceased. A total of 0.016 mol of the appropriate alkyl iodide was added in small portions over a 1-hr period, and the resulting mixture was stirred at 25° for 16 hr. The reaction mixture was diluted with 2 vol of H_2O and extracted with Et_2O. The Et_2O extracts were dried (Na_2SO_4), concentrated on a rotary evaporator, and dried under high vacuum at 50° to give the desired 2-alkyl-2-carbethoxy-3,5-dimethylvalerolactone (6) as a light tan liquid. These liquids without further purification were used to prepare the 5-alkyl-(3'-hydroxy-1'-methylbutyl)barbituric acids and 5-alkyl-(3'-hydroxy-1-methylbutyl)-2-thiobarbituric acids.

5-Alkyl-5-(3'-hydroxy-1'-methylbutyl)barbituric Acid (2). A solution of the lactone 6 from the previous experiment in 2 ml of EtOH was added to a solution of 0.72 g (0.012 mol) of urea and 0.28 g (0.012 g-atom) of sodium in 5 ml of EtOH, and the resulting solution was refluxed 40 hr. The solution was concentrated on a rotary evaporator. The residue was dissolved in 9 ml of water and extracted with 3 × 5 ml portions of Et_2O. The cooled aqueous solution was acidified to pH 5 with 5 *N* HCl. In some cases the desired product 2 precipitated from the acid solution. In other cases the aqueous layer was extracted with EtOAc in a liquid–liq-

uid extractor. The residue obtained on concentration of the extracts was chromatographed on silica gel (25 g) using ethyl acetate as the eluent. The combined fractions of product were concentrated on a rotary evaporator. The residue obtained was recrystallized from the appropriate solvent. The recrystallization solvent, yield (from lactone 4), and physical constants for each individual isomers of 2 are given in Table I.

5-Alkyl-5-(3′-hydroxy-1′-methylbutyl)-2-thiobarbituric Acids (3). A solution of sodium methoxide was prepared from 0.17 g (0.0075 g-atom) of sodium and 3 ml of MeOH. To this cooled solution was added 0.27 g (0.0035 mol) of dried thiourea, followed by lactone 6 (amount prepared from 0.004 g of lactone 4). The mixture was heated at 55–58° for 21 hr. The reaction mixture was cooled, diluted with 4 ml of H_2O, and extracted with 2 × 5 ml of Et_2O. The aqueous layer was cooled in an ice bath, acidified to pH 5 with concentrated HCl, and extracted with 3 × 5 ml portions of Et_2O. Concentration of the dried (Na_2SO_4) Et_2O extracts gave an oil. This oil was chromatographed on silica gel (150 g) using first $CHCl_3$, followed by $CHCl_3$–EtOAc (1:1), and then EtOAc as the eluent. Fractions containing pure product (by tlc) were combined and recrystallized from the appropriate solvent. The recrystallization solvent, yield (from lactone 4), and physical constants for each of the individual isomers of 3 are given in Table I.

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Aminobenzoic Acid Diuretics. 7.[1] 3-Substituted 4-Phenyl-, 4-Arylcarbonyl-, and 4-Arylmethyl-5-sulfamoylbenzoic Acids and Related Compounds

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Various 4-substituted 3-alkylamino-, 3-alkoxy-, 3-alkylthio-, and 3-alkyl-5-sulfamoylbenzoic acids related to known aminobenzoic acid diuretics were synthesized and screened for their diuretic properties in dogs. The tabulated results from a 3-hr test period revealed that generally the diuretic profile and potency could be retained when 3-alkoxy, 3-alkylthio, and 3-phenethyl were substituted for the 3-alkylamino moiety. The high potency of several 3-alkoxy-, 3-alkylthio-, and 3-phenethyl-4-benzoyl-5-sulfamoylbenzoic acids confirmed previous suggestions that the apparent diuretic effect of 4- and 5-alkylamino-6-carboxy-3-phenyl-1,2-benzisothiazole 1,1-dioxides originates from the corresponding 4-benzoyl-5-sulfamoylbenzoic acid derivatives due to an existing equilibrium in plasma. 4-Benzoyl-5-sulfamoyl-3-(3-thenyloxy)benzoic acid (118) is among the most potent benzoic acid diuretics hitherto synthesized and shows significant diuretic activity in dogs at 1 μg/kg. The results obtained with different 3-substituted 4-phenyl-5-sulfamoylbenzoic acids supported the earlier concept regarding the steric influence of the 4-substituent on the diuretic potency of sulfamoylbenzoic acid diuretics.

We concluded previously[2] that substituted or unsubstituted phenyl attached by NH, O, S, SO, SO_2, CO, or CH_2 to the 4 position of 2- or 3-alkylamino-5-sulfamoylbenzoic acids contributed to high-ceiling diuretic activity of high potency. We suggested that the influence of the 4-substituent is of steric rather than physicochemical nature. In order to obtain more precise information on the steric requirements for high-ceiling diuretic activity, we decided to investigate some 3-alkylamino-4-phenyl-5-sulfamoylbenzoic acids (7–11).

In the preceding paper of this series[1] it was shown that certain 3-alkylthio-4-phenoxy- and 3-alkylthio-4-phenylthio-5-sulfamoylbenzoic acids possess diuretic activity of the same level of potency as previously reported[3] for the corresponding 3-alkylaminobenzoic acids. This observation prompted us to extend our synthetic program in order to elucidate extensively the effect on the diuretic activity of a departure from the amino function in different 4-substituted 3-alkylamino-5-sulfamoylbenzoic acids. Consequently, we synthesized the title compounds having various 3-alkoxy, 3-alkylthio, and 3-alkyl side chains.

Chemistry. The preparation of the 3-alkylamino-4-phenyl-5-sulfamoylbenzoic acids 7–11 (Table I) was based on the Ullman reaction and is given in Scheme I. The synthesis of the 4-substituted 3-alkoxy-, 3-alkylthio-, and 3-alkyl-5-sulfamoylbenzoic acids 103–150 (Table I) is outlined in Scheme II. The introduction of the oxygen and sulfur function in the 3 position was performed by means of generally known reactions with the diazonium salts of 4 and 12–15. The Meerwein alkylation reaction[4] with the diazonium chloride of 12 and cinnamic acid followed by hydrogenation of the resulting styrene intermediate 28 to the 3-phenethyl compound 65 was the key reaction in the preparation of the 3-phenethylbenzoic acids 139 and 150. The influence of the 4-substituents on the chemical behavior resulted in the different sequences.

It is remarkable that the different 3-substituted 4-benzoyl-5-sulfamoylbenzoic acids prepared in this study could be isolated as such while under similar conditions the corresponding 3-alkylaminobenzoic acids as well as the 4-benzoyl-5-sulfamoylanthranilic acid derivatives underwent spontaneous cyclodehydration to the corresponding benz-