Synthesis and Pharmacological Activity of Thiohexital Enantiomers

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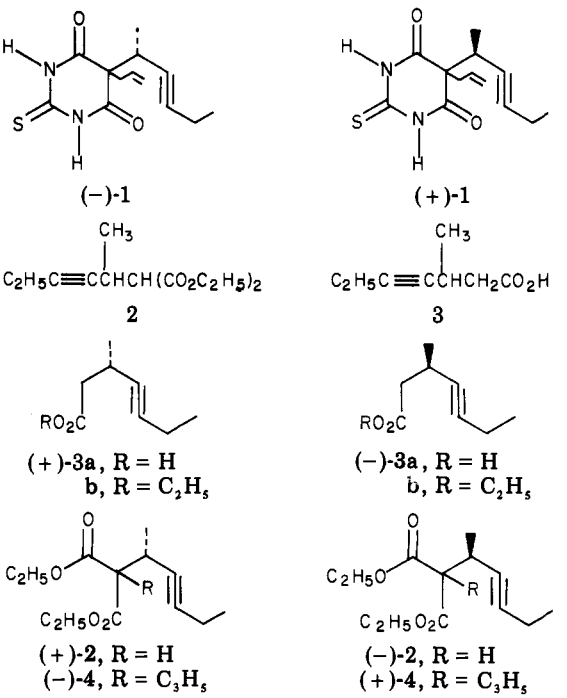
(*S*)-(+)- and (*R*)-(−)-5-Allyl-5-(1-methyl-2-pentynyl)-2-thiobarbituric acid (1, thiohexital) were prepared. The anesthetic activity (loss of righting reflex) and acute toxicity of the optically pure enantiomers of 1 were compared to the racemic isomer in mice. The *S*(+) isomer was found to be more potent as an anesthetic agent than the *R*(−) or *RS*(±) isomers. The therapeutic index was 2.5, 2.4, and 3.2 for the *RS*(±), *R*(−), and *S*(+) isomers, respectively. There were no significant differences in onset and duration of anesthesia when administered at the respective AD_{50} values. The prominant side effect is tremor, which is less for the *S*(+) isomer than for the *R*(−) or *RS*(±) isomers.

Reports from our laboratory demonstrated differences in the potency of the enantiomers of thiopental and thiamylal in mice.[1] The *S* isomer in both cases showed the highest potency, as expressed by the lowest LD_{50} and AD_{50}. Clinically the *S* isomer of thiopental was more potent than the *R* isomer; however, the thiamylal isomers were practically indistinguishable.[2] The *S* isomer of both barbiturates was more slowly metabolized with significantly longer plasma half-life. An ideal intravenous anesthetic agent should rapidly penetrate into the brain to produce anesthesia, and upon termination the agent should redistribute rapidly without extensive accumulation in body fat depots. This would be aided by rapid biotransformation. However, biotransformation of thiopental or thiamylal is too slow to contribute importantly. Mark and co-workers[3] reported that 5-allyl-5-(1-methyl-2-pentynyl)-2-thiobarbituric acid (1, thiohexital) was the most rapidly metabolized intravenous barbiturate ever studied in man. In clinical studies with 1, human subjects awake earlier with a shorter period of drowsiness than with other barbiturates.[3] Thus, 1 approaches the requirements of an ideal intravenous anesthetic agent. Unfortunately, undesirable motor side effects of hiccoughs and twitching of extremities occurred with 1 in 35–40% of cases. Thiohexital (1) possesses an asymmetric center similar to that present in thiopental and thiamylal. Separation of 1 into its enantiomers might allow separation of therapeutic activity and rapid metabolism from undesirable effects. In addition, the rate of metabolism of one of the enantiomers might be even more rapid than that of racemic 1. In this paper we report the preparation of the *R* and *S* isomers of 1 and describe their pharmacological activity in mice.

Synthesis. (+)- and (−)-5-Allyl-5-(1-methyl-2-pentynyl)-2-thiobarbituric acid, (+)-1 and (−)-1 respectively, were prepared from diethyl 1-methyl-2-pentynylmalonate (**2**).[4] Alkaline hydrolysis of **2**, followed by acidification and thermal decarboxylation, gave 3-methyl-4-heptynoic acid (**3**). Optical resolution of **3** with (+)- and (−)-ephedrine gave (+)- and (−)-**3a**, respectively. Esterification of the acids using ethanol gave the esters (+)- and (−)-**3b**. Base-catalyzed condensation of (+)- and (−)-**3b** with diethyl oxalate using sodium hydride as the base, followed by thermal decarbonylation, gave the malonate derivatives (+)- and (−)-**2**, respectively. Conversion of (+)- and (−)-**2** to their anions using sodium hydride in DMF, followed by treatment with allyl bromide, afforded (−)- and (+)-**4**, respectively. Condensation of (−)- and (+)-**4** with thiourea gave the desired thiobarbiturates (−)- and (+)-**1**, respectively.



Absolute Configurational and Optical Purity Studies. The determination of the chirality of optically active **3** was achieved by catalytic reduction of (+)-**3** to (+)-3-methylheptanoic acid [(+)-**5**] using 5% palladium on carbon as catalyst. Since (+)-**5** has been shown to possess the *R* configuration,[5-8] this establishes the chirality of optically active **3** as (*S*)-(+)-**3** and (*R*)-(−)-**3**. Moreover, since (+)- and (−)-**3** were converted to (−)- and (+)-**1**, respectively, by a route which did not effect the chiral center,

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(4) Doran, W. J. *J. Org. Chem.* **1960**, *25*, 1737.
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(6) The observed $[\alpha]_D$ for (+)-**5** indicated that 15% hydrogen scrambling had occurred at the 1′ position. Reduction of **3** using tris(triphenylphosphene)chlororhodium(I) as catalyst gave a chemically impure sample of **5**.
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Table I. Comparison of AD_{50} Values, LD_{50} Values, and Therapeutic Indexes of Thiohexital Stereoisomers after Intraperitoneal Administration

stereoisomer	AD_{50},[a] mg/kg	slope	potency ratio	therapeutic index: LD_{50}/AD_{50}
		AD_{50}		
racemic	25.8 (24.5-27.2)[b]	1.14 (1.06-1.22)		2.5
$S(+)$	18.8 (17.4-20.3)	1.25 (1.06-1.48)	1.37[c]	3.2
$R(-)$	27.3 (25.9-28.8)	1.16 (1.09-1.24)	1.06	2.4
		LD_{50}		
racemic	63.5 (58.0-69.5)	1.23 (1.02-1.49)		
$S(+)$	59.8 (56.0-63.9)	1.14 (1.05-1.23)	1.06	
$R(-)$	66.1 (59.9-72.9)	1.21 (1.06-1.40)	1.04	

[a] The AD_{50} was determined by the loss of the righting reflex. [b] 95% confidence limits. [c] More potent than the racemate, $p < 0.001$.

Table II. Onset and Duration of Action of AD_{50} Doses of Stereoisomers of Thiohexital Given Intraperitoneally

stereoisomer	onset, min	duration, min
racemic	3.69 ± 0.45	12.36 ± 2.07
$S(+)$	3.49 ± 0.34	12.15 ± 1.94
$R(-)$	3.85 ± 0.42	11.81 ± 1.76

this established their structures as (*R*)-(−)-1 and (*S*)-(+)-1, respectively.

The NMR spectrum ($CDCl_3$) of (±)-1 showed a triplet at 1.04 (5′-CH_3), a doublet at 1.29 (1′-CH_3), a multiplet at 2.08 (4′-CH_2), and a multiplet at 3.09 ppm (1′-H). Double irradiation at 2.08 ppm reduces the triplet at 1.04 to a singlet, whereas double irradiation at 3.09 ppm collapses the 1.29 doublet to a singlet. The NMR spectrum of (±)-1 containing 0.17 mol of tris[3-(heptafluorobutyryl)-*d*-camphorato]praseodymium(III) [Pr(hfbc)] per mole of (±)-1 showed two triplets for the 5′-CH_3 resonance and two doublets for the 1′-CH_3 resonance. Double irradiation at the 4′-CH_2 frequency gave two singlets of equal intensity separated by 20.6 Hz for the 5-CH_3 group, and double resonance at the 1′-H frequency gave two singlets of equal intensity separated by 10.6 Hz for the 1′-CH_3 resonance. These results established that this method could be used to determine the optical purity of (+)- and (−)-1. When (+)-1 ($[\alpha]_D$ +64.1°) or (−)-1 ($[\alpha]_D$ −65.6°) was analyzed as described above in the presence of Pr(hfbc), double irradiation at the resonance frequency of the 4′-CH_2 and 1′-H gave single peaks for the 5′-CH_3 and 1′-methyl, respectively. Since a contamination of either optical isomer with the other of 2% could be distinguished by this method, (+)- and (−)-1 are at least 98% optically pure.

Pharmacology. The results of both intravenous (iv) and intraperitoneal (ip) administration of the barbiturates are shown in Tables I–III. The acute toxicity of the *S* isomer and *R* isomer based on lethality was similar to that of the racemate and included arching of the back and excitation prior to death. The *S* isomer was more potent as an anesthetic agent, and the *R* isomer was equipotent or less potent than the racemate by both routes of administration. Therapeutic indexes were slightly better for the *S* isomer than for the *R* isomer or racemate. Since the onset and, particularly, the duration of the sleeping time were dose dependent, these parameters were measured at the AD_{50} for each antipode and its racemate (see Table II). There were no significant differences in the onset (3.68 ± 0.40 min) and duration of anesthesia (12.11 ± 1.92 min). A comparison of iv and ip routes of administration indicates, as expected, that the AD_{50} is lower after iv administration. In contrast to other barbiturates, the AD_{50} after iv administration is very dependent upon the rate of administration.[1] A potency difference of 2 occurs if the rate is decreased from 3.0 to 0.5 s (Table IV).

Qualitatively, the isomers produce the same side effects of which tremor is the most prominent. When *RS*(±) or

Table III. Comparison of AD_{50} Values, LD_{50} Values, and Therapeutic Indexes of Thiohexital Stereoisomers after Intravenous Administration

stereoisomer	AD_{50},[a] mg/kg	slope	potency ratio	therapeutic index: LD_{50}/AD_{50}
		AD_{50}		
racemic	19.8 (18.3-21.4)[b]	1.17 (1.09-1.25)		2.7
$S(+)$	12.3 (11.1-13.6)	1.25 (1.09-1.43)	1.61[c]	
$R(-)$	24.1 (22.1-26.3)	1.18 (1.10-1.26)	1.22	
		LD_{50}		
racemic	53.5 (48.6-58.9)	1.25 (1.14-1.38)		
$S(+)$	not determined			
$R(-)$	not determined			

[a] The AD_{50} was determined by the loss of the righting reflex. [b] 95% confidence limits. [c] More potent than racemate, $p < 0.001$.

Table IV. Comparison of AD_{50} Values, LD_{50} Values, and Therapeutic Indexes of Racemic Thiohexital for Different Rates of Intravenous Injection

stereoisomer	AD_{50},[a] mg/kg	slope	potency ratio	therapeutic index: LD_{50}/AD_{50}
		AD_{50}		
fast[b]	9.2 (8.2-10.2)[c]	1.21 (1.05-1.39)	2.15	3.4
slower[d]	19.8 (18.3-21.4)	1.17 (1.09-1.25)		2.7
		LD_{50}		
fast[b]	31.5 (28.3-35.1)	1.24 (1.04-1.48)	1.70	
slower[d]	53.5 (48.6-58.9)	1.25 (1.14-1.38)		

[a] The AD_{50} was determined by the loss of the righting reflex. [b] Fast injection, approximately 0.5 s. [c] 95% confidence limits. [d] Slower injection, approximately 3.0 s.

Table V. Recovery Tremors after AD_{50} Doses of Stereoisomers of Thiohexital Given Intraperitoneally

stereoisomer	incidence	duration, min	severity
racemic	14/40	5.8 ± 1.0	++
$S(+)$	10/40	5.4 ± 0.8	+
$R(-)$	22/40	6.2 ± 0.6	++

$R(-)$ isomers were administered ip at their AD_{50} value, the induction consisted of a constant time pattern of tremor or excitability at 2 min lasting about 30 s and anesthesia at 3.5 min. The same pattern occurred with the $S(+)$ isomer, but the incidence was much less. Tremors also occurred during recovery (Table V). One to two minutes following awakening there is a 5- to 6-min interval of tremor. The incidence was from 50% of those animals that lost the righting reflex, $S(+)$, to 100% for the $R(-)$. The intensity or severity of the tremors was also much less for the $S(+)$ than the $R(-)$ animals.

Discussion

The AD_{50}, LD_{50}, and therapeutic index for intravenously administered racemic thiohexital were 23.7 mg/kg, 42.0 mg/kg, and 1.8 in the rat[3] compared to 19.8 mg/kg, 53.5 mg/kg, and 2.7 in the mouse (see Table III). The AD_{50} was 6.5 mg/kg in the cat and 10.8 mg/kg in the dog, while 2–3 mg/kg is an effective maintained dose in man.[3] The side effects have an apparent species variation: in mice and rats, tremors marred both induction and recovery; in cats, excitability was common during recovery; in dogs, there was no effect on either induction or recovery; and in man, hiccoughs and twitching of extremities was common.[3] The other major species difference was the duration of anesthesia at the AD_{50} value from 211 min in the cat to 12 min in the mouse.

Comparison in the mouse of thiohexital with thiopental and thiamylal indicates a similar onset, duration, and therapeutic index but more excitation during induction, plus tremors on both induction and recovery.[1] The S isomer of both thiopental and thiamylal, each of which possesses a 1-methylbutyl side chain, was more potent than the R isomer.[1,9,10] Thus, it is interesting that the S isomer of thiohexital which contains a 1-methyl-2-pentynyl side chain is also more potent than its R enantiomer. Since similar asymmetric centers are involved in each of these barbiturates, the results indicate that these actions which are a function of distribution, metabolism, and/or receptor interaction may be determined by the absolute configuration.

Conclusions

The following conclusions can be drawn from the present study: (1) The $S(+)$ isomer was more potent as an anesthetic agent than the racemic barbiturate ($p < 0.001$). The $R(-)$ isomer was equipotent to the racemate. (2) The $S(+)$ isomer and the $R(-)$-isomer acute toxicity based on lethality was similar to that of the racemate. (3) Therapeutic indexes were slightly better for the $S(+)$ isomer than for the $R(-)$ isomer or racemate; its therapeutic index is still similar to other barbiturates. (4) A comparison of routes of administration indicates that the AD_{50} is slightly lower after iv administration than after ip administration. (5) The onset and duration of anesthetic action as measured by the loss of the righting reflex at the AD_{50} concentration is the same for the three derivatives.

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 267 or 467 Grating infrared spectrophotometer. NMR spectra were recorded on a Varian Model HA-100 or Bruker WM-250 spectrometer using 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). Microanalyses were carried out by Micro-Tech Laboratories, Skokie, IL, or Integral Microanalytical Laboratories, Inc., Raleigh, NC. Where analyses are indicated by the symbols of the elements, the analytical results were within ±0.4% of the theoretical values.

The IR and NMR spectra of all compounds reported were in agreement with the assigned structures. The purity of the compounds was checked by GLC and/or TLC analysis.

3-Methyl-4-heptynoic Acid (3). A solution of 10 g (0.18 mol) of KOH in 10 mL of water was brought to reflux and the heating source removed. Diethyl 1-methyl-2-pentynlmalonate (2; 12 g, 0.05 mol)[4] was added dropwise to the hot solution at a rate so as to maintain a gentle reflux. The reaction mixture was refluxed for an additional 4 h. The cooled reaction mixture was adjusted to pH 1 with concentrated hydrochloric acid and refluxed for 16 h. The cooled reaction mixture was diluted with 50 mL of water and extracted with ether (3 × 100 mL). The ethereal solution was washed with saturated NaCl solution and dried (Na_2SO_4). The liquid remaining after removal of the ether was distilled to give 5.1 g (73%) of **3**, bp 93–95 °C (2–3 mm). Anal. ($C_8H_{12}O_2$) C, H.

This reaction has been repeated several times (0.05–0.25 mol), with yields varying from 60 to 73%. The ethyl ester of **3** had bp 75–78 °C (0.5–0.7 mm). Anal. ($C_{10}H_{16}O_2$) C, H.

Resolution of 3-Methyl-4-heptynoic Acid (3). To a solution of 84 g (0.6 mol) of **3** and 99.3 g (0.6 mol) of (−)-ephedrine in 1 L of THF was added 1 L of hexanes. The crystals which separated on standing at 10 °C overnight were isolated by filtration. The

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120 g of crystals were recrystallized 4 times from the same solvent mixture to give 33 g of salt. The salt was dissolved in water, neutralized with 5% hydrochloric acid, and extracted with ether. The extracts were washed with water and saturated NaCl solution and dried (Na_2SO_4). The liquid remaining after removal of the ether was distilled to give 14.5 g of (−)-**3a**: bp 95 °C (3 mm); $[\alpha]^{23}_D$ −22.7° (*c* 7.0, C_2H_5OH).

The filtrates retained from the preparation of the above salt were evaporated in vacuo and the free acid was generated. The partially resolved (+) acid was converted to its (+)-ephedrine salt and resolved in a manner analogous to the (−) isomer to give 15.7 g of (+)-**3a**: bp 96 °C (3 mm); $[\alpha]^{23}_D$ +22.1° (*c* 6.8, C_2H_5OH).

The ethyl esters (−)-**3b** and (+)-**3b** were prepared from (−)- and (+)-**3a**, respectively, in the usual manner and had bp 75 °C (0.6 mm); (−)-**3b** had $[\alpha]^{23}_D$ −28.1° (*c* 5.5, C_2H_5OH), and (+)-**3b** had $[\alpha]^{23}_D$ +28.2° (*c* 4.48, C_2H_5OH).

(*S*)-(+)-Diethyl 1-Methyl-2-pentynylmalonate [(+)-2]. To a stirred mixture of 10 g of a 50% sodium hydride dispersion in white oil (washed free of oil with dry benzene) in 20 mL of dry benzene kept at 60–70 °C was added 14.4 g (0.086 mol) of (+)-**3b** and 10 g of diethyl oxalate. The mixture was heated under vacuum (~20 mm) for an additional 2 h after the addition. During this time, the benzene and liberated ethanol were distilled from the reaction mixture. The cooled mixture was carefully neutralized with acetic acid and diluted with 100 mL of water. The organic layer was separated, and the aqueous layer was extracted with ether (3 × 100). The combined organic phases were washed with saturated $NaHCO_3$ and NaCl solutions and dried (Na_2SO_4). The ether and excess diethyl oxalate were removed under reduced pressure. The remaining liquid was heated at 160–170 °C under vacuum (20 mm) for 2 h. The reaction mixture was then distilled to give 12 g (58%) of (+)-**2**: bp 85 °C (0.2 mm); $[\alpha]^{23}_D$ +22.9° (*c* 9.65, C_2H_5OH) [lit.[1] bp 123 °C (7 mm) for racemic **2**].

(*R*)-(−)-Diethyl 1-Methyl-2-pentynylmalonate [(−)-2]. In a manner completely analogous to that described for the preparation of (+)-**2**, (−)-**3** (13 g, 0.054 mol) was converted to 8.5 g (57%) of (−)-**2**: bp 85 °C (0.2 mm); $[\alpha]^{23}_D$ −21.1° (*c* 1.48, C_2H_5OH).

(*R*)-(−)-Diethyl Allyl(1-methyl-2-pentynyl)malonate [(−)-4]. To a stirred suspension of 2.1 g (0.044 mol) of 50% sodium hydride dispersion in oil (washed free of oil with dry hexanes) in 100 mL of dry DMF was added 10 g (0.042 mol) of (+)-**2**. The ester was added dropwise at a rate to control hydrogen evolution. After the addition, the mixture was stirred until hydrogen evolution ceased. A total of 50 g of allyl bromide was added in portions over a 1-h period, and the resulting mixture was stirred at 25 °C for 16 h. The reaction mixture was diluted with 2 vol of water and extracted with ether (3 × 100 mL). The extracts were washed with saturated NaCl solution, dried (Na_2SO_4), and concentrated on a rotary evaporator. The remaining liquid was distilled to give 7.3 g (62%) of (−)-4: bp 95 (2 mm); $[\alpha]^{23}_D$ −39.3° (*c* 3.1, C_2H_5OH) [lit.[11] bp 105–107 (1 mm) for racemic 4].

(*S*)-(+)-Diethyl Allyl(1-methyl-2-pentynyl)malonate [(+)-4]. In a manner completely analogous to that described for the preparation of (−)-**4**, (−)-**2** (8 g, 0.033 mol) was converted to 7.2 g (78%) of (+)-**4**: bp 89–90 °C (0.15 mm); $[\alpha]^{23}_D$ +40.3° (*c* 4.9, C_2H_5OH).

(*R*)-(−)- and (*S*)-(+)-5-Allyl-5-(1-methyl-2-pentynyl)-2-thiobarbituric Acid [(−)-1 and (+)-1]. The thiobarbiturates (−)- and (+)-1 were prepared in 25 and 40% yield from (−)- and (+)-4, respectively, by a procedure previously reported for 5-alkyl-5-(1-methylbutyl)-2-thiobarbituric acids.[12] The products were recrystallized from an ethyl acetate and hexane mixture and dried for 48 h at 78 °C: (−)-1 had mp 139–141 °C; $[\alpha]^{23}_D$ −65.6° (*c* 1.08, C_2H_5OH). Anal. ($C_{13}H_{16}N_2O_2S$) C, H, N, S. (+)-1 had mp 139–141 °C; $[\alpha]^{23}_D$ +64.1° (*c* 1.50, C_2H_5OH). Anal. ($C_{13}H_{16}N_2O_2S$) C, H, N, S. (Literature[13] mp 131–133 °C for racemic 1.)

Catalytic Reduction of (+)-3. A solution of (+)-**3** (2 g, 0.14 mmol) having $[\alpha]^{23}_D$ +9.52° in 40 mL of toluene containing 256 mg of 10% palladium on carbon was hydrogenated on an atmospheric hydrogenator at 25 °C until hydrogen ceased to be absorbed. The catalyst was separated by filtration, and the filtrate was concentrated to a clear liquid. Distillation gave 1.11 g of (+)-**5**: bp 73 °C (0.2 mm); $[\alpha]^{23}_D$ +1.47° (neat). Meyer and Whitten[14] reported $[\alpha]^{25}_D$ +3.84° (neat) for a 91% optically pure sample of (*R*)-(+)-**5**.

Pharmacological Testing. The compounds were dissolved in 0.1 M aqueous sodium hydroxide to give the monosodium salt and then diluted to the appropriate volume with saline. Weights were based on the free acid. The dose volume used, 20 mL/kg, was administered either ip or iv via the caudal vein.

Charles River male mice, CF-1 strain, weighing 29.5 ± 2.5 g (SD) were used in this study. The anesthetic activity was estimated by the number of animals that lost their righting reflex. This reflex was considered lost when the mouse, placed on its back, failed to recover from that position within 1 min. The acute toxicity was based on lethality within a 24-h observation period. The median anesthetic dose (AD_{50}) and median lethal dose (LD_{50}) with 95% confidence levels were determined from dose–response curves for each enantiomorph by the method of Litchfield and Wilcoxon.[15] Each dose–effect curve consisted of at least four drug concentrations which gave responses between 10 and 90%. Ten mice were used for each response determination. Potency comparisons were made with the racemic mixture as the standard.

An additional 120 mice were divided into groups of 40 mice to determine the onset, duration of anesthetic action, and side effects at the AD_{50} for each compound. Specifically, the mice were placed in individual observation cages, the room temperature was maintained at 24 ± 1 °C, and during the interval between the loss and recovery of the righting reflex, there was no stimulation. The onset was defined as the complete loss of the righting reflex, i.e., no attempt to move the head or body. Recovery was considered to have occurred when the animal after spontaneous righting would reright itself within 15 s when placed on its back.

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