2-Benzyl-5-(N,N-dimethylamino)-γ-valerolactone, an Inhibitor of Plasma Cholinesterase[1a]

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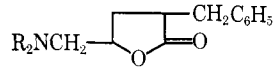
2-Benzyl-5-amino-γ-valerolactone (I) was prepared as a possible intermediate for the synthesis of 2-benzyl-5-aminolevulinic acid.[2] It was recognized that the N,N-dimethyl derivative of I might possess acetylcholine-like activity;[3] therefore, 2-benzyl-5-(N,N-dimethylamino)-γ-valerolactone (II) was prepared and tested against isolated human plasma pseudocholinesterase system. II was found to have an I_{50} of 2.14×10^{-5} M in a previously described assay procedure.[4]



I, R = H
II, R = CH_3

Experimental Section[5]

2-Benzyl-2-carboxy-5-bromo-γ-valerolactone.—A suspension of 34.3 g (0.193 mole) of N-bromosuccinimide in 200 ml of H_2O was added to a solution of 45 g (0.192 mole) of allylbenzylmalonic acid[6] in 200 ml of Et_2O. After being stirred for 12 hr the Et_2O layer was removed and the H_2O layer was extracted twice with 25 ml of Et_2O. The combined ethereal extract was concentrated to a heavy oil; this was dissolved in 75 ml of Et_2O, and petroleum ether (bp 37–60°) was slowly added until precipitation was complete. The crystals were removed by filtration and recrystallized twice from Et_2O–petroleum ether (bp 37–60°); yield 27.3 g. Concentration of the mother liquor produced additional product which weighed 12.5 g after three recrystallizations. The total yield was 40.8 g (67.8%), mp 135° dec. *Anal.* ($C_{13}H_{13}BrO_4$) C, H.

2-Benzyl-5-phthalimido-γ-valerolactone.—2-Benzyl-2-carboxy-5-bromo-γ-valerolactone (20 g, 0.064 mole) was heated at 130° for 48 hr to effect decarboxylation. The resulting liquid showed ir absorption bands as expected. It was used in a modified Gabriel synthesis,[7] conducted at 90° for 24 hr. Isolation by the reported procedure and crystallization from C_6H_6–petroleum ether yielded 14.7 g (69%) of product, mp 130–135°. Five recrystallizations gave a product with mp 135–137°. *Anal.* ($C_{20}H_{16}NO_4$) H, N; C: calcd, 71.85; found, 71.42.

2-Benzyl-5-amino-γ-valerolactone Hydrochloride (I).—A 3.65-g sample of 2-benzyl-5-phthalimido-γ-valerolactone was added to 200 ml of 6 N HCl. The mixture was alternately refluxed and cooled to room temperature four times. H_2O and HCl were removed in a rotary evaporator. Absolute EtOH was repeatedly added in 20-ml portions and evaporated to remove excess HCl. The residue was taken up in absolute EtOH, and Et_2O was added until precipitation was complete; yield 2.2 g (83.3%), mp 196–199° dec.

Compound I gave a purple color with ninhydrin. R_f values were, respectively, 0.38 in BuOH saturated with H_2O, 0.77 in MeOH–NH_3 (19:1), and 0.89 in pyridine–H_2O (65:35). *Anal.* ($C_{12}H_{16}ClNO_2$) C, H, N.

2-Benzyl-5-(N,N-dimethylamino)-γ-valerolactone Hydrochloride (II).—A solution of 2.0 g of I, 9.0 ml of 1 N NaOH, 5 ml of H_2O, 2 ml of 88% formic acid, and 2 ml of 40% CH_2O was heated for 6 hr at 100°. The mixture was concentrated to a heavy oil under reduced pressure. Absolute EtOH in 20-ml portions was added and was then evaporated four times. The oil was dissolved in 20 ml of anhydrous EtOH, and the NaCl was filtered off. Concentrated HCl (40 ml) was added to the filtrate and the solution was then concentrated to an oil. Absolute EtOH was added and evaporated four times. The dry crystals were thoroughly triturated with anhydrous Et_2O and filtered; yield 1.9 g (85.0%), mp 175–177°. *Anal.* ($C_{14}H_{20}ClNO_2$) C, H, N.

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$N^4,N^{4'}$-Decamethylenebis-4-aminopyridine and $N^9,N^{9'}$-Decamethylenebis-9-aminoacridine

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The title compounds (see Table I) are essentially the "ring removal" and "ring addition" compounds of $N^4,N^{4'}$-decamethylenebis-4-aminoquinaldine (**1**), a known antimicrobial agent.[1] The bisdecamethylene derivatives of 2- and 3-aminopyridine were also prepared. The compounds were tested *in vitro* according to methods previously described.[2] Table II contains the screening data for the amines, compound **1**, and 9-aminoacridine (**9**), a compound whose antimicrobial activity is well documented.[3]

Experimental Section

$N^9,N^{9'}$-Decamethylenebis-9-aminoacridine (2) was prepared from 9-chloroacridine (25 g, 0.11 mol) and 1,10-diaminodecane (8.6 g, 0.05 mol) according to the method of Strauss and Rosenstock.[1]

N,N'-Bis(pyridyl)sebacylamides (6–8).—Sebacyl chloride (30 g, 0.125 mol) was added dropwise to a stirred mixture of the appropriate aminopyridine (23.5 g, 0.25 mol) and Et_3N (25 ml) in THF (250 ml). After about 10 ml of the chloride had been added, the mixture began to boil, and addition was continued at such a rate that reflux was maintained. After the addition was complete (about 10 min) stirring was continued for an additional 15 min. The solvent was then removed by evaporation *in vacuo*. The residue was triturated with 500 ml of 10% K_2CO_3 solution, filtered, washed with H_2O, and purified.

N,N'-Bis(pyridyl)decamethyleneamines (3–5).—A slurry of the appropriate diamide **6–8** (12.5 g, 0.035 mol) in warm THF (200 ml) was added, with stirring, to LAH (2.3 g, 0.06 mol) in THF (50 ml). The resulting mixture was heated under reflux for 1.5 hr. The reaction mixture was cooled in an ice bath and stirred during the successive dropwise additions of H_2O (3 ml), 15% NaOH solution (3 ml), and H_2O (20 ml). The mixture was

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TABLE I

No.	R	Yield,[a] %	Crystn solvent	Mp, °C	Formula
		Amines:	$RNH(CH_2)_{10}NHR$		
2	9-Acridyl	52.7	C_6H_6–C_6H_{14}	139–145	$C_{36}H_{38}N_4$
3	2-Pyridyl	24.4	DMF–toluene	161–163	$C_{30}H_{30}N_4$[c,d]
4	3-Pyridyl	37.7	C_6H_6	139–142	$C_{30}H_{30}N_4$
5	4-Pyridyl	28.1	C_6H_6–C_7H_{16}	107–113	$C_{30}H_{30}N_4$
		Amides:	$RNHCO(CH_2)_8CONHR$		
6	2-Pyridyl	24.2	MEK	132–133	$C_{30}H_{36}N_4O_2$
7	3-Pyridyl	70.0	MEK	160–161	$C_{30}H_{36}N_4O_2$
8	4-Pyridyl	24.4	MEK	149–151	$C_{30}H_{36}N_4O_2$

[a] Yields are for analytically pure materials. [b] All compounds were analyzed for C, H, and N. Values are within ±0.4% of theoretical except where indicated. [c] Dihydrochloride. [d] C: calcd, 60.14; found, 59.72.

TABLE II

	Min inhib conen, μg/ml[a]					
No.	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Trichomonas vaginalis*	*Trichophyton mentagrophytes*	*Candida albicans*
1	125	500	250	10	100	10
9	25	PI 100	25	100	N	6
2	N	N	N	100	10	100
6	N	N	N	N	PI 250	N
7	N	N	N	N	PI 500–100	N
8	100	500	500	500	PI 100	500

[a] PI = partial inhibition, N = no activity at 500 μg/ml.

filtered and worked up in the usual manner. The resulting compounds (free base or 2HCl salt) were purified by crystallization.

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N^1-Sulfato-N^2-isonicotinylhydrazine. A Potential Metabolite of Isoniazid[1]

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During studies on the metabolism of the antituberculous isoniazid (INH), in animals[3] and man,[4] we considered the possibility that one of the group of acid-labile urinary metabolites was an N-sulfate conjugate of INH. This was suggested by earlier *in vivo*[5,6] and *in vitro*[7] studies, using various laboratory animals, in which arylamines such as aniline and 1- and 2-naphthylamine were conjugated with sulfate to form highly acid-labile sulfamates. Since INH is acetylated by the same pigeon liver enzyme that acetylates arylamines,[8] a similar parallel activity seemed possible for sulfate conjugation. If highly acid labile, an INH-sulfate conjugate would contribute to the incompletely identified acid-labile fraction of urinary INH metabolites.

Therefore, we synthesized the INH analog of aryl sulfamates, N^1-sulfato-N^2-isonicotinylhydrazine, by procedures employed previously.[5] It was found to be stable to the mild acidic conditions known to hydrolyze the pyruvic and α-ketoglutaric acid hydrazones of INH[3] and the aryl sulfamates[5] (pH 1.0, room temperature). However, it could be split quantitatively to INH by heating at 45° in 1 *N* HCl for 24 hr, conditions developed for the hydrolysis of acetylisoniazid to INH without the decomposition of INH.[4a] When this hydrolysis procedure was applied to urine collected from dogs receiving INH in studies reported previously,[3] no increase of INH content could be detected. These results indicated that the sulfate conjugate was not a metabolite of INH in the dog. Therefore we tested the *in vivo* stability of the sulfate compound in subsequent experiments.

For preliminary tests on the toxicity of this compound, we administered single doses of 48.1 mg/kg iv (equivalent to 25 mg of INH/kg) to six 26-day-old Holtzman female rats. Similar groups of untreated rats and rats receiving 25 mg of INH/kg served as controls. No untoward reactions were noted in the rats receiving either INH or the INH-sulfate conjugate. The rats were weighed immediately before injection and daily for the next 9 days. Necropsies were performed after killing the animals with $CHCl_3$, with special attention being given to the liver, kidneys, and spleen, which were examined internally as well as externally. No gross changes from the untreated rats were noted in the treated groups. In addition, no toxic effects of the compounds were noted in the growth of the animals, as evidenced by nearly identical weight gains in the treated and untreated groups during the study period.

To determine the *in vivo* stability of the INH-sulfate conjugate, we injected four young adult female beagle dogs (weight range, 5.2–9.7 kg) with doses of 9.6

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