reference standards were identical with those reported previously.[5-9]

Some of the substances exerted a general CNS depression, this effect having however only slight pharmacological significance. A number of the compounds displayed a marked antipyretic activity not accompanied by hypothermic action, the activity of **10** and **11** being also superior to that of phenylbutazone. Compounds **6**, **7**, and **13** markedly increased the pain threshold of rats, even though less than phenylbutazone. In anti-inflammatory action, a number of the substances inhibited the carrageenin-induced edema, and the effect of **4** was also superior to that of phenylbutazone. Some of the compounds displayed a noteworthy uricosuric activity, even though inferior to that of phenylbutazone. The diuretic action of **1**, **4**, **5**, and **11** was significant but clearly inferior to that of dihydrochlorothiazide. A number of the substances displayed hypoglycemic action, but in this case, too, the potency was inferior to that of the standard (chlorpropamide). None of the compounds showed significant antibacterial, antifungal, and antiparasitic activities.

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Synthesis and Pharmacological Properties of N-Derivatives of 5,7,12,13-Tetrahydro-6*H*-dibenz[*c*,*g*]azonine

Silvano Casadio,* Gianfranco Pala, Antonio Mantegani, Ernesta Marazzi-Uberti, Germano Coppi, and Carla Turba

Research Laboratories of Istituto De Angeli, 20159 Milan, Italy

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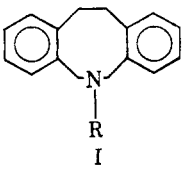
Twenty N-substituted 5,7,12,13-tetrahydro-6*H*-dibenz[*c*,*g*]azonines were prepared for pharmacological screening. Some of the substances displayed considerable antitussive activity, and of these 5,7,12,13-tetrahydro-6-(2-pyrrolidinoethyl)dibenz[*c*,*g*]azonine (**15**) was found to be the most promising.

We have recently reported on the pharmacological properties of N-substituted 5,6-dihydro-7*H*,12*H*-dibenz[*c*,*f*]azocines.[1] This paper deals with the preparation and pharmacological screening of the corresponding derivatives of the homolog, 5,7,12,13-tetrahydro-6*H*-dibenz[*c*,*g*]azonine (I).



I

Alkyl, hydroxyalkyl, and two aminoalkyl derivatives (**12** and **13**) were prepared by reaction of 2,2′-bis(bromomethyl)diphenylethane with the proper amines. Chloroalkyl derivatives were obtained by reaction of $SOCl_2$ with corresponding hydroxyalkyl compounds, while the other aminoalkyl derivatives were synthesized by treating the chloroalkyl compounds with the proper amines.

Pharmacological screening included studies of acute toxicity, behavioral effects, action on CNS and on arterial pressure, and analgetic, antiinflammatory, diuretic, antitussive, hypoglycemic, antispasmodic, local anesthetic, peripheral vasodilator, anthelmintic, antibacterial, and antifungal actions.

Experimental Section[2]

The intermediate amines, were prepared as previously described.[1] **N-Substituted 5,7,12,13-tetrahydro-6*H*-dibenz[*c*,*g*]azonines** are listed in Table I, and their preparation is illustrated by the following methods.

Method A. 5,7,12,13-Tetrahydro-6-(2-hydroxyethyl)dibenz[*c*,*g*]azonine (6).—A solution of 1-amino-2-hydroxyethane (10.6 g, 0.17 mole) in MeCN (10 ml) was dropped during 30 min into a boiling solution of 2,2′-bis(bromomethyl)diphenylethane[3] (20 g, 0.054 mole) in MeCN (450 ml). The mixture was refluxed for 2 hr with stirring, the solvent was evapd, and the residue was poured into H_2O. The solid which sepd was filtered, dried, and recrystallized from dil EtOH to give colorless crystals, mp 137–138°.

Method B. 5,7,12,13-Tetrahydro-6-(2-chloroethyl)dibenz[*c*,*g*]azonine (9).—$SOCl_2$ (20.7 g, 0.174 mole) was dropped during 30 min into a boiling solution of **6** (13 g, 0.048 mole) in dry C_6H_6 (350 ml). The suspension was refluxed for 1 hr and then the solvent and excess $SOCl_2$ were distd off. The residue was taken up with EtOH, filtered, suspended in H_2O, and made alkaline with 10% NaOH. The product was then extracted with $CHCl_3$ and the extract was washed (H_2O) and dried (Na_2SO_4). Evaporation of the solvent yielded a solid which, on recrystn from petroleum ether (bp 40–70°), gave colorless crystals, mp 116–117°.

Method C. 5,7,12,13-Tetrahydro-6-(1-methyl-3-piperidylmethyl)dibenz[*c*,*g*]azonine·HCl (12).—A solution of 1-methyl-3-aminomethylpiperidine (26.75 g, 0.208 mole) and 2,2′-bis(bromomethyl)diphenylethane (24 g, 0.065 mole) in 1-hexanol (300 ml) was refluxed for 15 hr. The solvent was evapd and the residue was dissolved in dil HCl. The acid solution was filtered with charcoal and made neutral with 10% NaOH. Upon standing

* To whom correspondence should be addressed.
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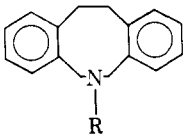
Table I

N-Substituted 5,7,12,13-Tetrahydro-6*H*-dibenz[*c*,*g*]azonines



Compd	R	Method	Yield, %	Mp, °C	Recrystn solvent[a]	Formula[b]
1	CH_3	A	69[c]	67–69	A	$C_{17}H_{19}N$
				184–186	B	$\cdot C_4H_4O_4$[d]
2	C_2H_5	A	75[c]	60–61	C	$C_{18}H_{21}N$
				185–186	B	$\cdot C_4H_4O_4$[d]
3	n-C_3H_7	A	76[c]	89–90	A	$C_{19}H_{23}N$
				259–260	D	$\cdot HCl \cdot H_2O$
4	i-C_3H_7	A	45[e]	41.5–42	C	$C_{19}H_{23}N$
				191–192	B	$\cdot C_4H_4O_4$[d]
5	$CH_2{=}CHCH_2$	A	60[e]	91–92	A	$C_{19}H_{21}N$
6	$HO(CH_2)_2$	A	84[e]	137–138	E	$C_{18}H_{21}NO$
7	$HO(CH_2)_3$	A	70[e]	126–127	E	$C_{19}H_{23}NO$
8	$CH_3CHOHCH_2$	A	75[e]	134.5–136	E	$C_{19}H_{23}NO$
9	$Cl(CH_2)_2$	B	92[c]	118–119	A	$C_{18}H_{20}ClN$
10	$Cl(CH_2)_3$	B	88[c]	94–95.5	A	$C_{19}H_{22}ClN$
11	$CH_3CHClCH_2$	B	96[c]	125–126	A	$C_{19}H_{22}ClN$
12	*f*	C	46[e]	248–251	F	$C_{28}H_{30}N_2 \cdot HCl$
13	*g*	C	38[e]	198–199	G	$C_{24}H_{32}N_2 \cdot HCl$
14	$(C_2H_5)_2N(CH_2)_2$	D	77[e]	236–237	B	$C_{22}H_{30}N_2 \cdot HCl$
15	*h*	E	86[e]	82–83.5	A	$C_{22}H_{28}N_2$
		D	83[e]	240–241	B	$\cdot HCl$
				235–237	B	$\cdot 2HCl$
16	$(CH_3)_2N(CH_2)_3$	E	81[c]	36.5–38		$C_{21}H_{28}N_2$
				192–194	B	$\cdot (C_4H_4O_4)_2$[d]
17	$CH_3CH[N(CH_3)_2]CH_2$	D	81[e]	235–236	F	$C_{21}H_{28}N_2 \cdot HCl$
18	$CH_3NH(CH_2)_3$	D	80[c]	204–205.5	F	$C_{20}H_{26}N_2 \cdot HCl$
				175–177	B	$\cdot (C_4H_4O_4)_2$[d]
19	*i*	E	94[c]	55–56	A	$C_{24}H_{33}N_3$
				257–258.5	B	$\cdot 3HCl$
20	*j*	E	89[c]	96–97	C	$C_{25}H_{35}N_3O$
				185–186	B	$\cdot (C_4H_4O_4)_3$[d]

[a] A = petroleum ether (bp 40–70°), B = EtOH, C = hexane, D = H_2O, E = EtOH-H_2O, F = *i*-PrOH, G = Me_2CO. [b] All compounds were analyzed for C, H, N and the analytical results were within ±0.4% of the theoretical values. [c] Crude product. [d] Maleate. [e] Recrystallized product. [f] 1-Methyl-3-piperidylmethyl. [g] 2-(1-Methyl-2-piperidyl)ethyl. [h] 2-1-Pyrrolidinoethyl. [i] 3-(4-Methyl 1-piperazino)propyl. [j] 3-[4-(2-Hydroxyethyl)-1-piperazino]propyl.

and cooling a colorless solid sepd which was recrystd from *i*-PrOH, mp 248°.

Method D. 5,7,12,13-Tetrahydro-6-(2-pyrrolidinoethyl)-dibenz[*c*,*g*]azonine·HCl (15).—A solution of **9** (39.5 g, 0.138 mole) and pyrrolidine (39.3 g, 0.553 mole) in 1-hexanol (800 ml) was refluxed for 5 hr. The solvent was removed and the residue was taken up with H_2O and made acid (pH 2.5) with 10% HCl. The solution was washed (Et_2O), filtered with charcoal, and adjusted to pH 6 with 10% NaOH. The solid which sepd was filtered, dried, and recrystd from EtOH to give colorless crystals, mp 240–241°.

Method E.—The same as method D, except that the product was isolated as the base instead of the hydrochloride.

Results and Discussion

The most interesting results of the pharmacological screening are given in Table II. Test procedures and reference standards were identical with those reported previously.[1]

Nearly all of the substances displayed a marked CNS depressant action, the activity of **7**, **8**, **19**, and **20** being also superior to that of meprobamate. Some of the compounds showed a peripheral vasodilator action, and this effect was pronounced for **9**, even though it was inferior to that of azapetine. Compounds **12**–**15** and **17**–**19** caused a fall of the arterial pressure in rabbits. Most of the substances markedly increased the pain threshold of mice, especially **3** and **13**, and the activity of these was also superior to that of phenylbutazone. A number of the compounds inhibited the experimental cough more than isoaminil, the highest activity being shown by **9** and **15**. Regarding local anesthetic action, **14** and **17** were as active as cocaine, but irritant. Only **12** displayed an antispasmodic activity *in vitro* worthy of note. Many of the substances inhibited the formalin-induced edema more than phenylbutazone. The anthelmintic action *in vivo* of **5** and **9** was close to that of piperazine. None of the compounds showed significant antihistaminic *in vivo*, hypoglycemic, diuretic, antibacterial, and antifungal activities. Due to lack of material, **10** and **11** were not screened.

From the point of view of the general pharmacological picture, 5,7,12,13-tetrahydrodibenz[*c*,*g*]azonines appear to be qualitatively very similar to the previous 5,6-dihydro-7*H*,12*H*-dibenz[*c*,*f*]azocines.[1] Regarding CNS depressant and antitussive activities, dibenzazonines displayed higher potency. Due to the promising results shown in the preliminary antitussive testing of **15** [5,7,12,13-tetrahydro-6-(2-pyrrolidinoethyl)-dibenz[*c*,*g*]azonine], this compound is now undergoing

Table II

Pharmacological Screening Results

Compd	Approx LD_{50} (mouse), mg/kg ip	Act. on spontaneous motility (mouse)		Analgetic act. (mouse)		Surface local anesthetic act. (guinea pig), %[b]	Antispasmodic act. *in vitro*, % inhib of spasm produced by[c]				Antiinflammatory act. (rat)		Antitussive act. (guinea pig), inhibn %[e]	Hypotensive act. (rabbit)[f]	Act. on the binder part of the rat increase of flow, %[g]	Anthelmintic act. cleared mice %[h]
		Decrease %	mg/kg ip	Increase of reaction time, %[a]	mg/kg ip		Acetylcholine 1×10^{-5} g/ml	Histamine 1×10^{-6} g/ml	Nicotine 2×10^{-6} g/ml	5-HT 1×10^{-6} g/ml	Inhibn of edema, %[d]	mg/kg ip				
1	147	24	25	Inact	25	Inact	15	35	Inact	37	35	25	22	+	60	Inact
2	220	27	50	47	25	Inact	Inact	41	34	25	37	100	Inact	+	57	Inact
3	233	42	25	101	25	Inact					Inact	25	Inact	Inact	50	60
4	150	36	12.5	36	12.5	Inact	37	39	33	95	Inact	12.5	Inact	Inact	28	Inact
5	72	54	50	59	50	Inact	25	59	Inact	69	48	50	Inact	Inact	40	100
6	300	54	100	87	100	Inact	Inact	33	31	23	24	100	41	Inact		Inact
7	775	55	100	41	100	Inact	Inact	42	33	29	24	100	Inact	Inact		Inact
8	315	74	50	94	50	Inact	Inact	40	18	31	21	50	Inact	Inact		20
9	77	54	50	71	50	31	Inact	15	33	50	65	50	76	Inact	84	100
12	102	77	50	78	50	Inact	44	100	97	83	42	50	31	++	Inact	20
13	157	87	100	228	100	34	45	87	45	48	52	100	Inact	++	Inact	40
14	98	63	25	40	25	88	43	61	95	57	Inact	25	31	++	Inact	Inact
15	156	36	25	45	25	22	29	89	93	56	Inact	25	57	++	Inact	Inact
16	305	33	100	51	100	Inact	30	31	27	50	37	100	33	Inact	28	40
17	152	63	25	65	25	76	32	18	32	45	21	25	Inact	++	Inact	Inact
18	270	58	50	37	50	Inact	36	39	Inact	35	33	50	Inact	++	Inact	Inact
19	280	70	50	37	50	51	Inact	31	14	58	20	50	Inact	++	Inact	20
20	575	85	100	51	100	Inact	33	40	57	36	54	100	Inact	Inact	Inact	20
Meprobamate		50	200													
Isoaminil													23			
Azapetine·H_3PO_4														++	234	
Piperazine·$6H_2O$																100

[a] Hot plate test. [b] The compounds were tested at a concentration of 1 mg/ml. The ED_{50} value for the standard cocaine hydrochloride is 0.70 mg/ml. [c] The compounds were tested at a concentration of 1 μg/ml. The ED_{50} values for the standards are: atropine sulfate 0.0035 μg/ml; diphenhydramine·HCl 0.0074 μg/ml; hexamethonium bitartrate 0.88 μg/ml; chlorpromazine·HCl 0.055 μg/ml. [d] Formalin-induced edema. [e] Acrolein vapors. The compounds were tested at 5 mg/kg ip. [f] The compounds were tested at 5 mg/kg iv. Azapetine at 1 mg/kg, + moderate hypotension, ++ marked hypotension. [g] Perfusion with epinephrine, 10^{-5} g/ml. The compounds were tested at 0.125 mg/rat. [h] The compounds were tested orally for 4 days at 300 mg/kg per day *vs. Syphacia obvelata*. Piperazine at 100 mg/kg per day for 4 days.

a more detailed pharmacological and toxicological study.

Acknowledgments.—The authors wish to thank Dr. G. Sekules for performing microanalyses, Mr. E. Zugna for assistance in preparing the compounds, Miss L. Tomasi and Miss A. Franchi for carrying out the pharmacological tests, and Mrs. C. Ciani-Bonardi and Mr. E. Pavesi for help in biological investigation.

Specific Competitive Inhibition of L-Asparaginyl Transfer Ribonucleic Acid Lygase by β-Aminoamides

M. R. Harnden and T. O. Yellin[1]

Experimental Therapy Division, Abbott Laboratories, North Chicago, Illinois 60064

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A series of β-aminoamides and closely related derivatives have been synthesized. The structural and stereochemical requirements for specific competitive inhibition of L-asparaginyl-tRNA lygase by these compounds have been investigated.

The introduction of the enzyme L-asparaginase (L-asparagine amidohydrolase) into clinical trials has been the most dramatic development in the treatment of leukemia in recent years. The ultimate usefulness of this enzyme has yet to be determined but the initial results are encouraging. In human as in animal lymphomas the effectiveness of the enzyme is correlated with a metabolic deficiency of the cancer cells, namely an inability to synthesize asparagine.[2]

Since asparaginase is relatively scarce and problems such as allergic response and development of resistance in susceptible leukemias have been encountered in asparaginase therapy,[3] we have initiated experiments designed to provide a chemotherapeutic approach to the treatment of those tumors for which L-asparagine is an essential amino acid, to be used, initially at least, with the enzyme. What we would hope for is a degree of synergism which would minimize the therapeutic dose of the enzyme, reduce its untoward effects and prevent the development of resistance and the reappearance of the disease. It is not known if a resistant form of the disease develops through a process of selection or by transformation of sensitive cells during asparaginase therapy. This question presents an intriguing theoretical as well as a practical problem of some significance but in either case some form of combination therapy should increase the likelihood of a complete cure.

Utilization of L-asparagine by the cell could be limited either by inhibition of the entry of asparagine into the cell or by interference with its essential role in cell metabolism. It is along the latter path that our efforts have been directed. The only vital function known for asparagine in mammalian cells is in protein synthesis, and so the problem defines itself as an attempt to limit the availability of asparagine in this process through specific inhibitors of L-asparaginyl-tRNA lygase. Evidence was recently obtained which indicates that such an approach might be possible.[4]

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Experimental Section

Chemistry.—Melting points were taken with a Thomas-Hoover capillary apparatus and are corrected. Elemental analyses were carried out by Mr. V. Rauschel and his associates in the analytical department of Abbott Laboratories. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Ir and nmr spectra of all compounds listed here and in Table I were consistent with the structures given.

β-Aminoamide Hydrochlorides (1a, 2–8, 10, 11).—A solution of ethyl chloroformate (10.9 g, 0.1 mole) in dry PhMe (50 ml) was added over 30 min to a stirred solution of the appropriate α,β-unsaturated acid[5] (0.1 mole) and Et_3N (10.1 g, 0.1 mole) in dry PhMe (50 ml) maintained at 0°. The mixture was stirred at 0° for an additional 2 hr and then filtered. Excess NH_3 was added to the filtrate and PhMe removed at reduced pressure. The residue was dissolved in EtOH (100 ml), liquid NH_3 (50 ml) added, and the solution heated at 100° for 24 hr in a 270-ml Hastelloy autoclave. The reaction mixture was cooled, filtered, and concd under reduced pressure. The residue was dissolved in *i*-PrOH (100 ml) and the solution concentrated again. The residue was then dissolved in *i*-PrOH (50 ml) and a solution of HCl in *i*-PrOH (2.9 *N*) carefully added until pH 7 was reached. The white crystals of the β-aminoamide·HCl that deposited were filtered, washed with cold *i*-PrOH (3 portions of 20 ml), and recrystd from *i*-PrOH.

3-Amino-4,4,4-trifluorobutyramide·HCl (9), 3-Amino-4-hydroxyvaleramide·HCl (13), and 3-Amino-4-ethylphosphonobutyramide (15).—Compounds **9**, **13**, and **15** were prepared by addition of NH_3 to ethyl-4,4,4-trifluorocrotonate,[6] α-angelicalactone,[7] and triethyl-4-phosphonocrotonate,[7] respectively. The same reaction conditions (EtOH, 100°, 24 hr) as described above were employed. Compounds **9** and **13** were isolated as their HCl salts in the usual manner. Compound **15** was obtained as a white crystalline solid on addition of *i*-PrOH to the residue obtained upon concentration of the reaction mixture, and was recrystd from EtOH-*i*-PrOH.

3-Amino-5-methylsulfonylvaleramide·HCl (12).—The methylthio derivative **11** (2.0 g, 0.01 mole) was suspended in AcOH (20 ml) and 80% *m*-chloroperbenzoic acid (4.3 g, 0.02 mole-equiv) added. A clear solution was obtained. The solution was allowed to remain at 20° for 20 hr and then EtOAc (100 ml) added. The product **12** was obtained as a white hygroscopic precipitate and was purified by dissolving in EtOH (50 ml) and reprecipitating with EtOAc (100 ml).

(5) The α,β-unsaturated acids were, with the exception of crotonic acid and cinnamic acid which were commercially available, prepared in 70–90% yield by reaction of the appropriate aldehyde with malonic acid in pyridine solution, as described by K. V. Auwers, *Justus Liebigs Ann. Chem.*, **432**, 58 (1923). No attempt to separate geometric isomers was made.

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