Chemical Structure and Pharmacological Activity of Glycine Derivatives. Influence of Disubstitution of the Amine Function of Glycine Esters and Hydrazides[1a]

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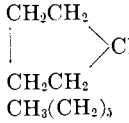
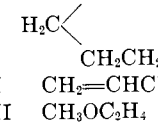
The substitution of the amine function of ethyl glycinate by aliphatic or cyclic radicals leads to the formation of compounds of interesting biological activity, in particular, spasmolytic, antihistaminic, and hypotensive activities. Study of the replacement of the ester function by a hydrazide group has been made. Contrary to expectation, the hydrazides are not convulsants.

Glycine and its salts have formed the subject of numerous studies of therapeutic interest, particularly with regard to myopathy, though it seems that no investigations have as yet been undertaken to find out if the nature of the disubstitution of the amine function causes any variations in its biological effects. These derivatives structurally resemble dimethylaminoethanol (deanol) that is used as an analgesic and sedative and has been recommended for certain states of mental collapse.[2] We have attempted to study further the variations in pharmacological activity brought about by the lengthening, branching, and cyclization of the substituents of the amine function of glycine esters and hydrazides. The hydrazide function was chosen because it is implicated in a number of pharmacological activities. Thus, tuberculostatic,[3] antiamine oxidase,[4] anthelmintic,[5] glucodepressor,[6] and convulsant activity[7] has been reported for related hydrazides. Besides monoamine oxidase (MAO) inhibitory activity of hydrazides, it is also of interest that glycine, although devoid of hypnotic activity, potentiates barbiturate sleep.[8] Thus, it seemed likely that glycine hydrazides might have effects on the central nervous system. Furthermore, these derivatives are useful intermediates for the preparation of heterocyclic compounds that will be the subject of a later publication.

TABLE I

N,N-DISUBSTITUTED ETHYL GLYCINATES

$$\begin{matrix}R\\R\end{matrix}\!>\!NCH_2CO_2C_2H_5$$

No.	R	Yield, %	B.p., °C. (mm.)	n_D (°C.)	Formula	Calcd., % C	H	N	O	Found, % C	H	N	O
I	C_2H_5	80	76 (13)[a]	1.4215 (25)	$C_8H_{17}NO_2$	60.34	10.76	8.79	20.09	60.1	11.0	8.7	
II	$CH_3(CH_2)_2$	94	99–100 (20)	1.4275 (18)	$C_{10}H_{21}NO_2$	64.13	11.30	7.48	17.09	63.9	11.1	7.7	17.5
III	$(CH_3)_2CH$	71.5	91–92 (16)[b]	1.433 (18)	$C_{10}H_{21}NO_2$	64.13	11.30	7.48	17.09	63.6	11.4	7.6	17.8
IV	$CH_3(CH_2)_3$	78.4	135–136 (25)[c]	1.430 (24)	$C_{12}H_{25}NO_2$	66.93	11.70	6.50	14.86	67.3	11.7	6.7	15.0
V	$(CH_3)_2CHCH_2$	64	117 (23)	1.4275 (18)	$C_{12}H_{25}NO_2$	66.93	11.70	6.50	14.86	66.9	11.7	6.6	14.8
VI	$CH_3(CH_2)_4$	85.3	145 (16)	1.4378 (18)	$C_{14}H_{29}NO_2$	69.08	12.01	5.75	13.15	69.7	12.0	5.8	12.8
VII	$(CH_3)_2CH(CH_2)_2$	76	127–128 (13)	1.4345 (19.5)	$C_{14}H_{29}NO_2$	69.08	12.01	5.75	13.15	69.3	11.5	6.1	13.3
VIII		61	110–111 (0.5)	1.478 (24)	$C_{14}H_{25}NO_2$	70.25	10.53	5.85	13.37	70.1	10.4	6.1	13.4
IX	$CH_3(CH_2)_5$	75	163 (14)	1.4418 (18)	$C_{16}H_{33}NO_2$	70.79	12.25	5.16	11.79	70.7	12.4	5.8	11.4
X		75	132 (0.4)[d]	1.1481 (25)	$C_{16}H_{29}NO_2$	71.86	10.93	5.24	11.97	71.5	10.7	5.2	11.7
XI	$CH_2{=}CHCH_2$	87.5	189 (12.5)[e]	1.4475 (21)	$C_{10}H_{17}NO_2$	65.54	9.34	7.64	17.46	65.2	8.9	8.0	17.8
XII	$CH_3OC_2H_4$	91.7	78 (0.4)	1.4358 (24)	$C_{10}H_{21}NO_4$	54.77	9.65	6.39	29.18	54.8	9.1	6.8	29.5
XIII	$C_2H_5OC_2H_4$	91	145 (14)	1.4812 (25)	$C_{12}H_{25}NO_4$	58.27	10.19	5.66	25.87	58.2	9.9	5.8	26.3

[a] K. Maurer and E. H. Woltersdorf [*Z. Physiol. Chem.*, **254**, 18 (1938)] report b.p. 72–74° (18 mm.). [b] H. Niwa [*Tohoku Yakka Daigaku Kiyo*, **4**, 1 (1957); *Chem. Abstr.*, **52**, 7236e (1958)] reports b.p. 82° (7 mm.). [c] Wander [British Patent 728,767 (April 27, 1955); *Chem. Abstr.*, **50**, 7876g (1956)] reports b.p. 113–115° (13 mm.). [d] H. H. Fox and W. Wenner [*J. Org. Chem.*, **16**, 225 (1951)] report b.p. 113–116° (0.3 mm.), $n^{27}D$ 1.4779. [e] Lit.[10] b.p. 112–114° (0.35 mm.), $n^{25}D$ 1.4448.

Synthesis.—The esters described in Table I were synthesized in excellent yield by Gault's method[9] using ethyl chloroacetate and a secondary amine, usually at

(1) (a) This work was supported by grants given by the Institut National de la Santé et de la Recherche Médicale. (b) Director of the Institute, Professor J. Baumann.

(2) (a) C. C. Pfeiffer, *Science*, **124**, 29 (1956); (b) C. C. Pfeiffer, *Intern. Rev. Neurobiol.*, **1**, 196 (1959).

(3) N. P. Buu Hoï, M. Welsh, G. Dechamps, H. Le Bihan, F. Binon, and C. Mentzer, *Compt. Rend.*, **234**, 1925 (1952).

(4) (a) P. Zeller, A. Pletscher, K. F. Gey, H. Gutmann, B. Hegedus, and O. Straub, *Ann. N. Y. Acad. Sci.*, **80**, 555 (1959); (b) A. Horita and W. R. McGrath, *Proc. Soc. Exptl. Biol. Med.*, **103**, 753 (1960); (c) G. Marinier, French Patent M1609 (Jan. 7, 1963); *Chem. Abstr.*, **58**, 11174*b* (1963).

(5) J. K. Valley, *Vet. Record*, **69**, 815 (1957).

(6) S. Izume and H. B. Lewis, *J. Pharmacol. Exptl. Therap.*, **30**, 87 (1927).

(7) (a) H. L. Williams and J. A. Bain, *Intern. Rev. Neurobiol.*, **3**, 319 (1961); (b) E. H. Jenney and C. C. Pfeiffer, *J. Pharmacol. Exptl. Therap.*, **122**, 110 (1958).

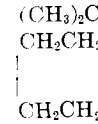
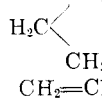
(8) A. Goldin, D. Dennis, J. M. Venditti, and S. R. Humphreys, *Science*, **121**, 364 (1955).

(9) H. Gault, *Bull. soc. chim. France*, [4] **3**, 368 (1908).

TABLE II

N,N-DISUBSTITUTED GLYCINE HYDRAZIDES

$$\begin{matrix} R \\ R \end{matrix}\!>\!NCH_2CONHNH_2$$

No.	R	Yield, %	B.p., °C. (mm.)	n_D (°C.)	Formula	Calcd., % C	H	N	O	Found, % C	H	N	O
XIV	C_2H_5	68.9	125–130 (13)[a]	1.4783 (25)	$C_6H_{15}N_3O$	49.65	10.34	28.96	11.03	49.5	10.3	28.9	11.1
XV	$CH_3(CH_2)_2$	79.4	157–159 (18)	1.472 (20)	$C_7H_{17}N_3O$	55.46	11.05	24.25	9.23	55.3	11.0	24.3	9.6
XVI	$(CH_3)_2CH$	50	155–156 (18)	1.4775 (21)	$C_8H_{19}N_3O$	55.46	11.05	24.25	9.23	55.6	11.1	24.3	
XVII	$CH_3(CH_2)_3$	82.6	176 (17)	1.471 (21.8)	$C_{16}H_{23}N_3O$	59.66	11.51	20.87	7.95	59.5	11.4	20.8	8.0
XVIII	$(CH_3)_2CHCH_2$	75	162–163 (16)	1.4675 (18)	$C_{16}H_{23}N_3O$	59.66	11.51	20.87	7.95	59.7	11.4	21.0	
XIX	$CH_3(CH_2)_4$	71.3	131 (0.5)	1.4691 (25)	$C_{12}H_{27}N_3O$	62.84	11.86	18.32	6.98	62.8	11.5	18.6	
XX	$(CH_3)_2CH(CH_2)_2$	69.8	141 (0.3)	1.4699 (17)	$C_{12}H_{27}N_3O$	62.84	11.86	18.32	6.98	62.6	11.4	18.6	7.1
XXI		78.5	215 (15)	[b]	$C_{12}H_{23}N_3O$	63.96	10.29	18.65	7.64	63.7	10.4	18.8	7.1
XXII	$CH_3(CH_2)_5$	87.5	159–160 (0.5)	1.4722 (16)	$C_{14}H_{31}N_3O$	65.32	12.14	16.32	6.22	64.9	12.4	16.4	7.0
XXIII		54	176 (0.3)	[c]	$C_{14}H_{27}N_3O$	66.39	10.75	16.59	6.32	66.5	10.8	16.7	6.5
XXIV	$CH_2{=}CHCH_2$	47.3	123 (0.5)	1.5005 (25)	$C_8H_{15}N_3O$	56.78	8.93	24.83	9.45	56.5	9.0	24.6	
XXV	$CH_3OC_2H_4$	61.5	130–131 (0.3)	1.4755 (25)	$C_8H_{19}N_3O_3$	46.81	9.33	20.47	24.39	47.0	9.5	20.4	23.6
XXVI	$C_2H_5OC_2H_4$	65.3	159 (0.7)	1.468 (25)	$C_{10}H_{23}N_3O_3$	51.48	9.94	18.01	20.57	51.9	10.0	17.9	20.8

[a] T. Momose and H. Tanaka [*J. Pharm. Soc. Japan*, **73**, 105 (1953); *Chem. Abstr.*, **47**, 4250*g* (1953)] report b.p. 105° (2 mm.). [b] M.p. 72° (hexane). [c] M.p. 143° (hexane).

TABLE III

In Vivo RESULTS

No.	Approx. LD_{50}, mg./kg.	Respiration[a]	Heart rhythm[b]	Blood pressure (dose, mg./kg.)[c]	Hemoreflect.[d]
I	>1000	0	0	0	0
II	>1000	0	0	TH (5)	VD, s.d.
III	>1000	0	0	TH (3)	VD, s.d.
IV	500	0	0	TH (2)	VD, s.d.
V	500[e]	D	0	TH (7)	VD, s.d.
VI	70[e]	P	TF	TH (1)	VD, s.d.
VII	150[e]	P	TF	TH (1)	VD, s.d.
VIII	>1000[f]	B	B	TH (5)	0
IX	500	P	F	TH (5)	VC
X	100[e]	P	F	IH (0.5)	VC
XI	600[e]	D	0	TH (10)	VD
XII	>1000	0	0	0	0
XIII	>1000	0	0	0	0
XIV	>1000[f]	0	0	TH (8)	0
XV	500	P	0	TH (4)	0
XVI	300	D	T	TH (1)	VD, s.d.
XVII	100	P	T	TH (1)	VD, s.d.
XVIII	100	P	F	TH (8)	VD, s.d.
XIX	40	D	T	TH (1)	VD, s.d.
XX	150	P	T	TH (1)	VD
XXII	100	0	T	TH (1)	0

[a] D = dyspnea, P = polypnea, B = bradypnea. [b] TF = tachycardia fibrillation, B = bradycardia, F = fibrillation, T = tachycardia. [c] TH = transient hypotension, IH = intense hypotension with collapse. [d] VD, s.d. = vasodilation of short duration (less than 3 min.), VC = vasoconstriction, VD = vasodilation of more than 3 min. [e] Convulsant. [f] Depressant.

room temperature and without solvent. It should be noted that sterically hindered amines such as diisopropyl-, dicyclopentyl-, or dicyclohexylamine react only slightly with ethyl chloroacetate but easily with bromoacetate. However, once the condensation is started with the bromine derivative, it can be successfully completed with the chloroacetate. Differences of reactivity of this type have already been described in the case of ethyl monoalkylaminoacetate by Speziale and Jaworski.[10] The hydrazides (Table II) were easily prepared according to Curtius's technique,[11] by refluxing for 24 hr. a mixture of 1 mole of the esters described above with 1.5 moles of hydrazine hydrate and a minimum amount of ethanol to homogenize the mixture.

Pharmacology.[12]—The pharmacological studies were made on rats, mice, guinea pigs, rabbits, and cats.[13] Acute toxicity was determined in mice (i.p.) (LD_{50}), with observation of the behavior for doses above the LD_{50}. Respiration and cardiac rhythm were measured in cats and rabbits, using an i.v. dose that had been proved to produce a constant and reproducible effect on the blood pressure. We used hemoreflectometry[14] to estimate the effects on capillary circulation (Table III). The antiacetylcholine, antihistamine, and anti-$BaCl_2$ activities were measured on isolated rat duodenum or guinea pig ileum and compared with known effective compounds (atropine, papaverine, promethazine). They are expressed by the reciprocal logarithm of the molecular concentration of the products that reduces by half the maximum effect of the spasmogen (Table IV). The action on isolated frog heart is expressed in concentration (Table V).

Comments.—Although complete pharmacologic data are not yet available, the results obtained so far suggest the following remarks. From the toxicities (i.p.) of the esters and hydrazides it can be seen (Table III) that there are two interesting maxima, one for the ester and one for the hydrazide, for the same radical, *i.e.*, amyl, and that the corresponding isoamyl derivatives

(10) A. J. Speziale and E. G. Jaworski, *J. Org. Chem.*, **25**, 728 (1960).

(11) T. Curtius, *Ber.*, **23**, 3023 (1890).

(12) Some results, especially for the hydrazides, are not yet available. They will be published later in a pharmacological study of the action of glycine derivatives, at low doses, on the CNS. This will include the MAO inhibiting activity.

(13) We thank Dr. Paulais and Miss Lepoullenec for having undertaken, with one of us (P. M.), the pharmacological testing.

(14) P. Magnin, P. Drutel, and M. Lamarche, *Therapie*, **17**, 1017 (1962).

TABLE IV

ACTION ON ISOLATED RAT DUODENUM AND GUINEA PIG ILEUM

No.[a,b]	Acetylcholine	No.[b,c]	$BaCl_2$	No.[b,d]	Histamine
Atr.[e]	8.9	Pap.[f]	8.4	Prom.[g]	8.2
VI	5	V	4	XIII	5.8
V	4.5	XX	4	VI	5.7
IX	4.2	XIX	3.5	VII	5.5
X	4	IX	3	V	4
XX	4	XVIII	3	X	3.8
II	3.75	VII	3	XI	3.7
VII	3.75	VI	2.75	II	3.7
IV	3.5	X	2.75	VIII	3.7
XIX	3	II	2.70	IV	3.5
VIII	2.8	IV	2.5	XVII	3.5
XI	2.7	XI	2	XX	3.5
III	2.6	XVII	1.8	XIX	3
XVII	2	III	1.7	XVIII	2.5

[a] Compounds I and XII–XVI are inactive. [b] Activities of other numbers are not yet available. [c] Compounds I, VIII, and XII–XVI are inactive. [d] Compounds I, III, IX, XII, and XIV–XVI are inactive. [e] Atropine. [f] Papaverine. [g] Promethazine.

show only half that toxicity or even less (VI, 70 mg., and VIII, 150 mg.; XIX, 40 mg., and XX, 150 mg.). Concerning the effects on blood pressure, Table III shows that, here also, there are two peaks, this time in the doses required for hypotensive activity, encountered for both an ester and a hydrazide with the same radical, *i.e.*, isobutyl. From Table IV, which lists the antispasmodic activities in their order of potency, a minimum of 3 carbons is required in the radical substituting the amino group for the musculotropic, neurotropic, and, in part, antihistaminic activities to appear; branching at the α-carbon atoms of the amine substituent causes a decrease in or loss of activity, while branching at the β-carbon does not. Results in the hydrazide series are as yet insufficient for similar comparisons to be made, but already they appear to follow the same lines though the degree of activities is lower; it may also be noted that the hydrazides, contrary to expectation,[7] are not convulsant (XIV is a weak depressant). This suggests that the influence of the dialkylamino group is by no means negligible.

TABLE V

ACTION ON ISOLATED FROG HEART

No.	Concn.	Inotropic act.[a]	Toxic concn.	No.	Concn.	Inotropic act.[a]	Toxic concn.
I	10^{-7}	+	0	IX	10^{-4}	−	10^{-3}
II	10^{-7}	+	10^{-2}	X	10^{-8}	+	10^{-3}
II	10^{-3}	−		X	10^{-4}	−	
III	10^{-4}	−	0	XI	10^{-9}	+	10^{-3}
IV	10^{-8}	+	10^{-2}	XI	10^{-6}	−	
V	10^{-8}	+	10^{-3}	XII	10^{-14}	+	10^{-2}
V	10^{-4}	−		XVI	10^{-9}	+	10^{-7}
VI	10^{-12}	−	10^{-9}	XVII	10^{-5}	+	10^{-3}
VII	10^{-9}	+	10^{-3}	XVIII	10^{-9}	+	10^{-3}
VIII	10^{-14}	+	0				

[a] No chronotropic action noticed.

Experimental[15]

Preparation of Esters (Table I). General Procedure.—One mole of ethyl chloroacetate was added all at once, at room temperature, to 2 moles of a secondary amine, with stirring; the mixture may be warmed to start the reaction, but cooling was required when the hydrochloride of the secondary amine began to deposit. When the mixture had cooled completely and set solid, which required at least 4 hr., it was filtered through sintered glass, washed four times with isopropyl ether, the solvent was evaporated, and the residue was distilled on a spinning-band fractionating column under vacuum. For the less stable amines, in particular the dialkoxyethylamine, it is preferable to add a saturated solution of sodium carbonate instead of filtering and extracting and to flash-distil in a rotary evaporator under vacuum. The liquid obtained by this means is then distilled without any special precautions: infrared absorption, 3.4–3.5, 5.75, 6.85, 8.35–8.55 μ.

Preparation of Hydrazides (Table II). General Procedure.—The hydrazides were prepared by refluxing for 24 hr. a mixture of 1 mole of ester and 1.5 moles of hydrazine hydrate to which an adequate quantity of ethanol was added to homogenize. The alcohol and excess hydrazine hydrate were then evaporated in a rotary evaporator under vacuum and the residue was distilled: infrared absorption, 3, 3.4, 5.9–6.2, 6.6–6.8, 9.15, 9.9 μ.

(15) Melting points are uncorrected and were determined on a Reichert microscope with heating stage. Boiling points were taken during separation on a spinning-band fractionating column. Infrared spectra were recorded on an Infracord 137 Perkin-Elmer. Analyses were carried out by the Department of Microanalyses of the Centre National de la Recherche Scientifique.

Notes

3-Piperonylsydnone. A New Type of Antimalarial Agent[1]

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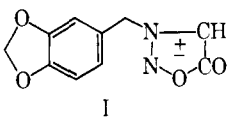
In connection with our continuing investigation on the structural variation of 3-(*p*-methoxybenzyl)sydnone,[2] an analog, 3-piperonylsydnone (I), has been synthesized in our laboratory. Compound I has now been shown to be an active agent in preliminary antimalarial evaluation. At a dose of 10 mg./kg., I was



I

found to be active against *Plasmodium berghei* in mice. The compound, which is comparable to chloroquine, is active when administered either orally or subcutaneously. No toxicity was observed at a dose of 500 mg./kg.[3]

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service, Contract SA-43-ph-3025.

(2) The confirmed antitumor activity of 3-(*p*-methoxybenzyl)sydnone in preliminary screening has been reported in our previous paper: see C. V. Greco, W. H. Nyberg, and C. C. Cheng, *J. Med. Pharm. Chem.*, **5**, 861 (1962).

(3) Information kindly provided by an antimalarial screening contractor of Walter Reed Army Institute of Research. We thank Dr. David P. Jacobus of the Walter Reed Army Medical Center for his permission to release this information. All compounds tested in the malaria program were furnished to WRAIR under the auspices of the CCNSC.