TABLE III.—EFFECT OF THE THIAZOLE MOIETY OF THIAMINE HYDROCHLORIDE ON THE STABILITY OF B_{12} AT 45°

Test Soln.	Original Assay[a]	1 Wk.	2 Wk.	3 Mo.	6 Mo.	1 Yr.
Vitamin B_{12} control	97	93	97	92	80	113
Vitamin B_{12} + thiazole moiety	97	78	91	82	82	97

[a] All assays expressed as per cent of label claim, 25 mcg./ml.

suggest that during storage the thiazole ring may rupture, giving rise to a degradation product which does adversely affect cyanocobalamin stability.

SUMMARY

Data are presented to show that the thiazole moiety of thiamine hydrochloride, the 3-benzyl derivative of the thiazole moiety, the 3-(4-nitrobenzyl) derivative of the thiazole moiety, or dimethylformamide, a structurally related possible breakdown product of the thiazole moiety, had no adverse effect on the stability of cyanocobalamin in aqueous solution at pH 4.0. Cysteine hydrochloride, on the other hand, caused significant breakdown of cyanocobalamin, thus suggesting that a thiol-containing degradation product of thiamine hydrochloride may be responsible for losses in B_{12} potency during storage.

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Synthesis and Pharmacological Screening of 3-Aminoalkyl-Sydnones

By TIBERIO BRUZZESE, SILVANO CASADIO, ERNESTA MARAZZI-UBERTI, and CARLA TURBA

Fourteen 3-aminoalkyl-sydnones have been synthesized and submitted to comprehensive pharmacological screening. Some of the compounds show an analgesic, hypoglycemic, and anti-inflammatory activity.

COMPOUNDS containing the sydnone *meso*-ionic ring have for many years been studied for their synthesis and structure (1–4). However, the pharmacological aspect of such compounds has been investigated only recently. In particular, Daeniker and Druey (5) have found that some polymethylene-bis-sydnones show a certain degree of antitumoral activity, while Greco *et al.* (6) have observed a similar action for 3-(*p*-methoxybenzyl)-sydnone. It has been reported that other sydnones stimulate the central nervous system (7, 8) or display a saluretic activity (9).

This paper reports the synthesis of a series of 3-aminoalkyl-sydnones and their comprehensive pharmacological screening. The compounds have been prepared by the classical technique (3), *i.e.*, nitrosation of the appropriate *N*-aminoalkyl-glycine and treatment of the *N*-nitroso derivative with acetic anhydride. The *N*-nitroso derivatives have been isolated as the hydrochlorides and are difficult to crystallize. (See Table I. Other compounds required have not been characterized.) Cyclization necessitates a very short initial heating, otherwise a resinous product which cannnot be purified is obtained.

3-Aminoalkyl-sydnone hydrochlorides are

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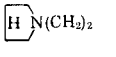
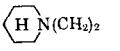
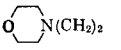
TABLE I.—*N*-NITROSO-*N*-AMINOALKYL-GLYCINE HYDROCHLORIDES

$$R_1—N(N{=}O)—CH(COOH)—R_2 \cdot HCl$$

R_1	R_2	Yield,[a] %	M.p.,[b] °C.	Formula	Anal., % Calcd.	Found
$(C_2H_5)_2N(CH_2)_2$	H	72	144–145	$C_8H_{18}ClN_3O_3$	C, 40.08 H, 7.56 Cl, 14.80 N, 17.53	C, 40.20 H, 7.72 Cl, 14.81 N, 17.31
	H	96	148–149	$C_8H_{16}ClN_3O_3$	C, 40.42 H, 6.79 Cl, 14.92 N, 17.68	C, 39.94 H, 6.79 Cl, 15.15 N, 17.81
	H	96	152–153	$C_9H_{18}ClN_3O_3$	C, 42.94 H, 7.21 Cl, 14.09 N, 16.70	C, 42.98 H, 7.33 Cl, 14.34 N, 16.75
	H	77	173–174	$C_8H_{16}ClN_3O_4$	C, 37.88 H, 6.36 Cl, 13.98 N, 16.57	C, 37.65 H, 6.43 Cl, 13.88 N, 16.87
$(CH_3)_2N(CH_2)_3$	H	97	130–131	$C_7H_{16}ClN_3O_3$	C, 37.26 H, 7.15 Cl, 15.72 N, 18.63	C, 37.55 H, 7.29 Cl, 15.58 N, 18.44
$(CH_3)_2N(CH_2)_3$	CH_3	85	147–148	$C_8H_{18}ClN_3O_3$	C, 40.08 H, 7.56 Cl, 14.80 N, 17.53	C, 40.68 H, 7.59 Cl, 14.59 N, 17.33

[a] Crude product. [b] The compounds were recrystallized from ethanol and melt with decomposition.

colorless solids very soluble in water. Their properties and ultraviolet absorption data are given in Table II. The free bases are oily products which can be purified by distillation under high vacuum. They are not very stable on prolonged exposure to the air.

EXPERIMENTAL

Melting points were taken on a Townson-Mercer melting point apparatus and are corrected. Ultraviolet spectra were determined with a Beckman model DB spectrophotometer.

The method for preparing the *N*-aminoalkyl-glycine dihydrochlorides required for this work will be reported later.

Preparation of N-Nitroso-N-aminoalkyl-glycine Hydrochlorides.—A 0.12-mole quantity of sodium nitrite and 16 ml. of water was added dropwise, over 1.5 hr., to a solution of 0.1 mole of *N*-aminoalkyl-glycine dihydrochloride in 75 ml. of water at −5°. The reaction mixture was stirred for 2 hr. at 10°, and the temperature then was reduced again to −5°, adjusting the pH to 2 by cautious addition of concentrated hydrochloric acid. The solution was evaporated to dryness *in vacuo* at approximately 40°, and the residue was extracted with 200 ml. of boiling ethanol in portions. The combined alcoholic extracts then were distilled, leaving a residue consisting of the required *N*-nitroso-*N*-aminoalkyl-glycine hydrochloride.

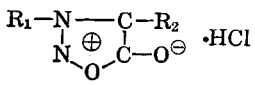
3-(2-Piperidinoethyl)-4-phenyl-sydnone Hydrochloride (IX).—*Method A.*—A mixture of 32.8 Gm. of *N*-nitroso-*N*-(2-piperidinoethyl)-glycine hydrochloride and 150 ml. of acetic anhydride was heated cautiously at 55–60°, and the resulting solution was allowed to stand overnight at room temperature. The excess acetic anhydride was removed *in vacuo* at 50° and the partially oily residue was triturated with ether. The product (19.8 Gm.) was recrystallized from ethanol, giving colorless crystals, m.p. 180–181° dec.

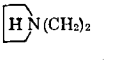
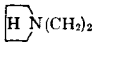
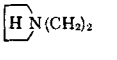
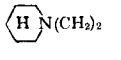
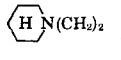
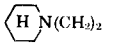
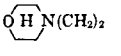
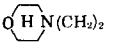
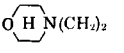
3-(2-Pyrrolidinylethyl)-sydnone Hydrochloride (IV).—*Method B.*—A mixture of 47.5 Gm. of *N*-nitroso-*N*-(2-pyrrolidinylethyl)-glycine hydrochloride and 300 ml. of acetic anhydride was heated at 85° to give a colorless solution. After cooling, a solid precipitated which, filtered and dried at 80° *in vacuo*, weighed 33.4 Gm. After recrystallization from ethanol, the product melted at 174–175° dec.

SCREENING RESULTS

After an approximate evaluation of the acute toxicity, in order to obtain some indications as to the dosage to use, the compounds were submitted to screening. This included the action on the CNS (10), and the analgesic (11), anti-inflammatory (12), hypoglycemic (13), IMAO (14), antidepressive (15), anticonvulsant (16), diuretic (17), antipyretic (18), *in vitro* antispasmodic (19), antiulcer (20), and hypotensive actions and that on the heart (21) and isolated vessels (22), besides the *in vitro* antifibrillar (23), antibacterial and antifungal (24), antiamebic (25), and *in vitro* antitrichomonas actions. The compounds were administered by intraperitoneal injection, in the form of aqueous solution, except for the hypoglycemic and diuretic tests where they were given orally.

TABLE II.—3-AMINOALKYL-SYDNONE HYDROCHLORIDES



Compd.	R_1	R_2	Method	Yield,[a] %	M.p., °C.	Recrystn. Solvent	Formula	Anal., % Calcd.	Found	U.V. C_2H_5OH mμ max.	log ε
I	$(C_2H_5)_2N(CH_2)_2$	H	*A*	67	134–135 dec.	Ethanol	$C_8H_{16}ClN_3O_2$	C, 43.34 H, 7.28 Cl, 15.99 N, 18.95	C, 42.90 C, 7.25 Cl, 16.02 N, 19.17	292	3.84
II	$(C_2H_5)_2N(CH_2)_2$	CH_3	*A*	61	116–117 dec.	Ethanol	$C_9H_{18}ClN_3O_2$	C, 45.89 H, 7.70 Cl, 15.05 N, 17.83	C, 45.96 H, 7.88 Cl, 15.11 N, 17.53	298	3.84
III	$(C_2H_5)_2N(CH_2)_2$	C_6H_5	*A*	54	154–155 dec.	Ethanol	$C_{14}H_{20}ClN_3O_2$	C, 56.49 H, 6.77 Cl, 11.90 N, 14.11	C, 56.42 H, 6.74 Cl, 12.03 N, 14.18	254 320	3.53 4.00
IV		H	*B*	76	174–175 dec.	Ethanol	$C_8H_{14}ClN_3O_2$	C, 43.74 H, 6.42 Cl, 16.14 N, 19.13	C, 43.37 H, 6.52 Cl, 15.97 N, 19.33	294	3.97
V		CH_3	*A*	53	173–174 dec.	Ethanol	$C_9H_{16}ClN_3O_2$	C, 46.25 H, 6.90 Cl, 15.17 N, 17.98	C, 45.95 H, 7.06 Cl, 15.02 N, 17.75	299	3.87
VI		C_6H_5	*A*	48	187–188 dec.	Isopropanol	$C_{14}H_{18}ClN_3O_2$	C, 56.85 H, 6.13 Cl, 11.99 N, 14.21	C, 56.48 H, 6.33 Cl, 11.72 N, 14.26	244 318	3.82 4.00
VII		H	*B*	76	175–176 dec.	Ethanol	$C_9H_{16}ClN_3O_2$	C, 46.25 H, 6.90 Cl, 15.17 N, 17.98	C, 46.71 H, 6.95 Cl, 15.24 N, 18.14	291	3.81
VIII		CH_3	*A*	52	170–171 dec.	Isopropanol	$C_{10}H_{18}ClN_3O_2$	C, 48.44 H, 7.32 Cl, 14.31 N, 16.96	C, 48.19 H, 7.47 Cl, 14.16 N, 16.93	298	3.88
IX		C_6H_5	*A*	64	180–181 dec.	Ethanol	$C_{15}H_{20}ClN_3O_2$	C, 58.15 H, 6.51 Cl, 11.45 N, 13.57	C, 58.19 H, 6.62 Cl, 11.52 N, 13.67	254 320	3.48 3.97
X		H	*A*	75	173–174 dec.	Ethanol	$C_8H_{14}ClN_3O_3$	C, 40.78 H, 5.99 Cl, 15.05 N, 17.83	C, 40.77 H, 6.03 Cl, 15.11 N, 17.94	291	3.83
XI		CH_3	*A*	82	186–187 dec.	Methanol	$C_9H_{16}ClN_3O_3$	C, 43.30 H, 6.46 Cl, 14.20 N, 16.83	C, 43.44 H, 6.58 Cl, 14.08 N, 16.80	296	3.90
XII		C_6H_5	*A*	59	185–186 dec.	Ethanol	$C_{14}H_{18}ClN_3O_3$	C, 53.97 H, 5.82 Cl, 11.37 N, 13.48	C, 53.90 H, 5.72 Cl, 11.42 N, 13.64	242 315	3.77 3.94
XIII	$(CH_3)_2N(CH_2)_3$	H	*A*	65	153–154 dec.	Ethanol	$C_7H_{14}ClN_3O_2$	C, 40.50 H, 6.80 Cl, 17.08 N, 20.24	C, 40.60 H, 6.89 Cl, 17.24 N, 19.95	292	3.82
XIV	$(CH_3)_2N(CH_2)_3$	CH_3	*B*	70	218–219 dec.	Ethanol	$C_8H_{16}ClN_3O_2$	C, 43.34 H, 7.28 Cl, 15.99 N, 18.95	C, 43.88 H, 7.38 Cl, 16.18 N, 18.75	298	3.86

[a] Recrystallized once.

The results of the activity tests considered most interesting are reported in Table III. This shows that the action on the CNS varies within the series, Some sydnones (II, III, V, VI, VII, IX, XII, XIII, XIV) act as excitants, others (I, IV, VIII, X) as depressants. The activity was slight in every case, also taking into account the doses administered. All the compounds show an analgesic action, in particular II and X. V and IX display a certain hypoglycemic effect, while II and XII appear to be somewhat effective in inhibiting formalin-induced edema. Morphine, phenylbutazone, and tolbutamide were used as standards for comparison of the analgesic, anti-inflammatory, and hypo-

TABLE III.—PHARMACOLOGICAL SCREENING RESULTS

Compd.	LD_{50} (Approx.) Mouse mmole/Kg., i.p.	mmole/Kg., i.p.	Action on the CNS, Mouse	Analgesic Activity, Mouse mmole/Kg., i.p.	Analgesic Activity, Mouse Increase of Reaction Time, %	Anti-inflammatory Activity, Rat mmole/Kg., i.p.	Anti-inflammatory Activity, Rat Inhibition of Edema, %	Hypoglycemic Action, Rat mmole/Kg., p.o.	Hypoglycemic Action, Rat Blood Sugar Decrease, %
I	2.93–3.38	0.23	Moderate spontaneous motility and irritability decrease, moderate motor incoordination, moderate ipsilateral flexor and pinna reflexes decrease	0.23	71	0.23	Inact.	0.23	10
II	1.15–1.44	0.42	Moderate behavior excitement	0.42	114	0.42	27	0.21	14
III	1.75–2.22	0.67	Moderate CNS excitement, muscle hypertonia	0.67	82	0.67	13	0.17	13
IV	2.46–2.96	0.46	Moderate CNS depression, moderate motor incoordination	0.46	48	0.46	21	0.23	15
V	2.05–2.57	0.86	Moderate CNS excitement	0.86	50	0.86	14	0.21	34
VI	0.57–0.78	0.34	Moderate behavior excitement	0.34	57	0.34	Inact.	0.17	Inact.
VII	1.54–1.80	0.43	Moderate behavior excitement	0.43	92	0.43	Inact.	0.21	Inact.
VIII	2.34–2.70	0.81	Moderate motor incoordination, muscle hypotonia, moderate palpebral ptosis	0.81	26	0.81	18	0.20	Inact.
IX	0.84–1.13	0.16	Moderate behavior excitement	0.16	52	0.16	Inact.	0.16	37
X	>6.79	0.85	Moderate CNS depression, moderate motor incoordination, moderate palpebral ptosis	0.85	129	0.85	Inact.	0.21	Inact.
XI	>12.82	1.60	Nothing noticeable	1.60	73	1.60	Inact.	0.20	Inact.
XII	1.19–1.38	0.32	Moderate behavior excitement, moderate motor incoordination, moderate muscle hypertonia, moderate palpebral ptosis	0.32	24	0.32	27	0.16	Inact.
XIII	7.23–10.60	0.96	Moderate CNS excitement	0.96	28	0.96	19	0.24	Inact.
XIV	7.67–10.38	0.90	Moderate behavior excitement	0.90	67	0.90	Inact.	0.22	Inact.
Morphine[a]				0.0133	67				
Phenylbutazone						0.32	18		
Tolbutamide								0.18	48

[a] Hydrochloride.

glycemic activities. The compounds have been found to be inactive in respect to the other activities studied.

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