Heterocyclic Mesoionic Structures, a Novel Class of Monoamine Oxidase Inhibitors. I. Arylsydnones.

DONALD P. CAMERON AND EDWARD H. WISEMAN[1]

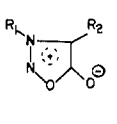
Medical Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Connecticut

Received January 8, 1968

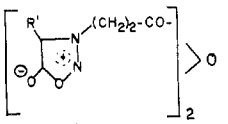
A series of N-arylsydnones (I) have been found to be noncompetitive inhibitors of the enzyme, monoamine oxidase. A reinterpretation of the mechanism of sydnone formation is discussed, as well as the mechanism of their noncompetitive inhibition of monoamine oxidase. It is believed that the sydnones fulfill many of the spatial and electronic requirements ascribed to the classical inhibitors although they are structurally unique to that family.

The sydnones I, a class of heterocyclic compounds possessing unique aromatic character, have been the subject of considerable chemical[2a] and pharmacological[2b] study. This report is an account of the structure–activity relationships for inhibition of the enzyme, monoamine oxidase (MAO), in a series of N-alkyl- and N-arylsydnones.

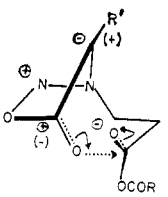
N-Arylsydnones and precursor N-nitrosoamino acids were prepared using established procedures, trifluoroacetic anhydride (TFA) in diethyl ether or tetrahydrofuran being the cyclization agent of choice. 3-Carboxethylsydnones were prepared[3] from the appropriate amino acids. However, N-(2-carboxyethyl)-N-nitroso-DL-isoleucine and N-(2-carboxyethyl)-N-nitroso-DL-phenylglycine were atypical, yielding not the expected sydnones, but the anhydrides IIa,b. Furthermore, at pH 7, IIa hydrolyzed to **19**, whereas IIb suffered ring cleavage.



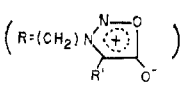
I: R_1 = alkyl or aryl



IIa: R' = C_6H_5
b: R' = $CH_2CH(CH_2)_2$

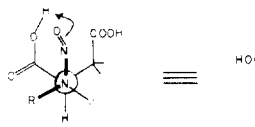


IIc

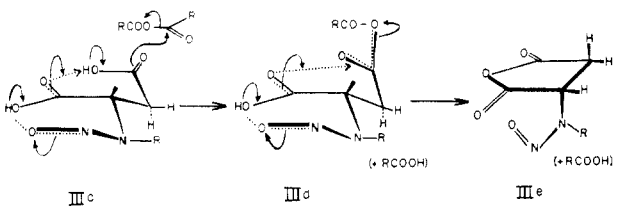


The divergent hydrolytic paths taken by IIa and IIb could reflect the relative stability of the derived sydnone acids. Alternatively, intramolecular carboxyl group–sydnone ring interaction could give the intermediate IIc which in effect provides assistance to nucleophilic attack, and whose stability and mode of cleavage are clearly a function of R'; relative to alkyl, a phenyl group should favor retention of the mesoionic system. The formation of IIc (which resembles the intermediate invoked to explain carboxyl group activation by imidazoles)[4] would proceed by a sterically and energetically feasible process, a contention supported by the nucleophilic character of N-2, by mo calculations, and by 1,3 addition of carbonyl compounds to sydnone; acid-catalyzed hydrolysis of sydnones has been pictured to involve protonation at N-2.[2]

4-Carboxymethylsydnones were prepared by cyclization of N-nitrosoaspartic acids. In our hands, the intermediate anhydride always contained up to 10% of sydnone.[5] It is felt, therefore, that postulation of neighboring-group interaction in sydnone formation during hydrolysis of the anhydride is unnecessary.[6] A simpler accommodation of the observation is that of two competitive dehydration routes; the one leading to anhydride formation is favored, but in a subsequent slow reaction the thermodynamically stable sydnone is formed. Comparison of nonbonded interactions in Dreiding models of N-nitroso-N-benzylaspartic acid does not allow a choice to be made between configurations IIIa and IIIb which are in equilibrium due to nitrogen inversion. The former is capable of sydnone formation, if the α-mixed anhydride is first formed.



IIIc IIIb



IIIc IIId IIIe

However, β-carboxyl mixed anhydride formation should predominate for steric reasons,[7] and hydrogen bonding between the nitroso oxygen and the α-carboxyl groups (IIIc) should enhance β-mixed anhydride formation (IIIc → IIId) (in addition to favoring IIIa). The net result would be formation of the cyclic anhydride IIIe. Similar interactions in IIIb require a seven-membered hydrogen-bond ring.

Although IIIe is conformationally compatible with formation of the intermediate or transition state IVa, the resulting increase in nonbonded interactions will decrease the rate of sydnone formation. In contrast, six-membered anhydrides (IVb) possess fewer and less

(1) To whom reprint requests should be addressed.

(2) (a) F. H. C. Stewart, *Chem. Rev.*, **64**, 129 (1964), and references therein; (b) L. B. Kier and E. B. Roche, *J. Pharm. Sci.*, **56**, 149 (1967), and references therein.

(3) (a) L. L. McKinney, E. H. Uhing, E. A. Setzhorn, and J. C. Corwan, *J. Am. Chem. Soc.*, **72**, 2599 (1950); (b) L. L. McKinney, E. A. Setzhorn, and E. H. Uhing *ibid.*, **74**, 1942 (1952).

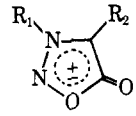
(4) M. Bender, *Chem. Rev.*, **60**, 53 (1960).

(5) P. Brooks and J. Walter, *J. Chem. Soc.*, 4409 (1957).

(6) F. H. C. Stewart, *ibid.*, 3158 (1963).

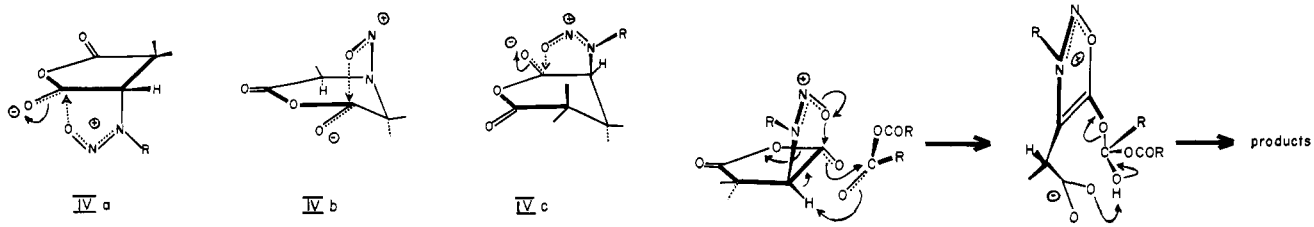
(7) B. R. Baker, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **17**, 116 (1952).

TABLE I
ARYL- AND ALKYLSYDNONES



No.	R_1	R_2	Yield, %	Mp, °C[a]	Absorption Infrared (Carbonyl) μ	Absorption Ultraviolet mμ (ε × 10^3)	Formula	Analyses	MAO inhib *In vitro*[e]	MAO inhib *In vivo*[f]
1	C_6H_5	CH_2COOH	57	156–156.5 (A)	5.80	312 (6.98)	$C_{10}H_8N_2O_4$	C, H, N	0	0
2	4-HOC_6H_4	H	50	247–248 (B)	5.73	295 (7.40)	$C_8H_6N_2O_3$	C, H, N	1	0
3	4-$CH_3C_6H_4$	H	74	144.5–146 (C)[b]	5.70	310 (5.84)	$C_9H_8N_2O_2$	C, H, N	1	1
4	2,4-$(CH_3)_2C_6H_3$	H	68	92.5–94 (D)	5.69	298 (6.99)	$C_{10}H_{10}N_2O_2$	C, H, N	1	1
5	2,4-$(CH_3O)_2$-5-ClC_6H_2	H	55	185.5–186 (C)	5.87	305 (9.54)	$C_{10}H_9ClN_2O_4$	C, H, N, Cl	2	2
6	2,4-$(CH_3O)_2$-5-ClC_6H_2	CH_3	50	173–173.5 (C)	5.80	302 (10.8)	$C_{11}H_{11}ClN_2O_4$	C, H, N, Cl	1	2
7	2,4-$(CH_3O)_2$-5-ClC_6H_2	*n*-C_4H_9	17	112.5–114 (E)	5.70		$C_{14}H_{17}ClN_2O_4$	C, H, N, Cl	0	
8	2,4-$(CH_3O)_2$-5-ClC_6H_2	Cl	84	161.5–162 (D)	5.65	310 (9.36)	$C_{10}H_8Cl_2N_2O_4$	C, H, N, Cl		
9	2,5-$(CH_3O)_2$-4-ClC_6H_2	H	71	151–151.5 (F)	5.82	309 (9.43)	$C_{10}H_9ClN_2O_4$	C, H, N, Cl	2	2
10	2,5-$(CH_3O)_2$-4-ClC_6H_2	CH_3	52	193.5–194.5 (F)	5.75	307 (13.2)	$C_{11}H_{11}ClN_2O_4$	C, H, N, Cl	0	0
11	2,5-$(CH_3O)_2$-4-ClC_6H_2	C_6H_5	18	230–230.5 (C)	5.70	326 (11.5)	$C_{16}H_{13}ClN_2O_4$	C, H, N, Cl	0	0
12	CH_3	3-$CH_3OC_6H_4$	42	124–125 (D)	5.66	316 (10.5)	$C_{10}H_{10}N_2O_3$	C, H, N		
13	$NCCH_2CH_2$	C_6H_5	66	110–111 (G)	5.85	316 (11.1)	$C_{11}H_9N_3O_2$	C, H, N	0	0
14	$HOOCCH_2CH_2$	C_6H_5	92	141.5–142 (B)	5.75	313 (10.1)	$C_{11}H_{10}N_2O_4$	C, H, N		
15	$NCCH_2CH_2$	CH_3	52	65–66 (B)	5.81	300 (7.20)	$C_6H_7N_3O_2$	C, H, N	0	
16	$NCCH_2CH_2$	$CH_2CH(CH_3)_2$	63	74.5–75 (H)	5.80		$C_9H_{13}N_3O_2$	C, H, N		
17	$NCCH_2CH_2$	$CH_2C_6H_5$	50	71.5–72 (I)	5.89	305 (8.70)	$C_{12}H_{11}N_3O_2$	C, H, N		
18	$COOHCH_2$	H	70	130–131 (A)[c]	5.80	293 (9.38)	$C_4H_4N_2O_4$	C, H, N	0	
19	$C_6H_5CH_2$	CH_2COOH	43	131–132 (B)[d]	5.80	304 (7.32)	$C_{11}H_{10}N_2O_4$	C, H, N		
20	$-CH(COOH)(CH_2)_3-$		82	170 (dec) (J)	5.79	299 (5.47)	$C_7H_8N_2O_4$	C, H, N	0	0

[a] Recrystallization solvents: (A) EtOAc, (B) Me_2CO, (C) CH_2Cl_2–Et_2O, (D) CH_2Cl_2–*i*-Pr_2O, (E) *i*-Pr_2O, (F) EtOH, (G) Me_2CO–C_6H_6, (H) EtOAc–Et_2O, (I) C_6H_6–Et_2O, (J) CH_2Cl_2. [b] Lit.[2a] mp 142–144°. [c] Lit.[2a] mp 132.5–133.5. [d] Lit.[6] mp 143°. [e] Relative potency of inhibitor at 17 μg/ml: 0, no inhibition; 1, partial inhibition; 2, total inhibition. [f] Relative potency of inhibitor at oral dose of 100 mg/kg: 0, no inhibition; 1, partial inhibition; 2, total inhibition.



IVa IVb IVc Va Vb

serious eclipsed interactions than does the essentially planar IIIa, and sydnone formation is favored. In acetic anhydride, all nitrosoamino acids that can give six- or seven-membered cyclic anhydrides capable of forming the bicyclic intermediate IVb, yield exclusively the sydnone; those forming a fused-ring intermediate (IVa or IVc) yield the N-nitrosoamino acid anhydride as the major product.

Formation and collapse of IVa, b, or c to sydnone should be acid catalyzed,[8] explaining the relative effectiveness of TFA and acetic anhydride as sydnone-forming agents. In refluxing benzene containing one drop of trifluoroacetic acid, the amount of sydnone **1** in N-nitroso-N-phenyl-DL-aspartic anhydride increased from 1–2 to 25% (isolated), but was unchanged in the absence of acid (precluding a thermal interconversion of anhydride and sydnone). Finally, IIIe (R = phenyl or benzyl) gave **1** and **19** in good yield in TFA–benzene at 50° for 2 hr. Procedures utilizing trifluoroacetic acid and TFA may be equivalent, assuming the presence of traces of moisture. Alternatively, the conversion may involve concerted activation (Va) of the cyclic anhydride by interaction with TFA to give sydnone *via* the intermediate Vb, or simply stabilization of IVa.

Inhibition of Monoamine Oxidase (MAO) Activity (Table I). *In Vitro.*—Guinea pig liver homogenates were prepared as described by Weissbach, *et al.*,[9] MAO activity, using kynuramine as substrate, being determined spectrophotometrically. Measurements of optical density at 360 mμ were made immediately after adding substrate, and subsequently at 2-min intervals for a total period of 10 min. Reaction rate was then determined by plotting optical density against time.

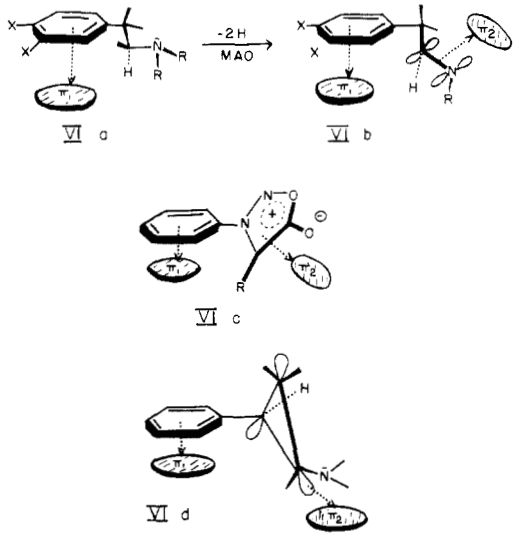
In Vivo.—Male albino rats, 150–200 g, were dosed orally (1–100 mg of inhibitor/kg). After 2 hr the animals were stunned, and the livers were quickly removed, weighed, and homogenized in 5 vol of cold water. The homogenate was strained through cheesecloth and refrigerated until assayed. MAO activity was determined as in the preceding section.

Results and Discussion

N-Arylsydnones do not conform to any structural types previously found to be active inhibitors of MAO. In general, structural requirements for MAO inhibitors have been a flat π system (aromatic ring) and an amine head, both moieties being spatially related in a fashion similar to either the natural enzyme substrates (DOPA, epinephrine, norepinephrine, tyramine, etc., VIa), or to

(8) (a) W. G. Finnegan and R. A. Henry, *J. Org. Chem.* **30**, 576 (1965); (b) T. Yvermault, *Compt. Rend.*, **235**, 177 (1952).

(9) H. Weissbach, T. E. Smith, J. W. Daly, B. Witkop, and S. Udenfriend, *J. Biol. Chem.*, **235**, 1160 (1960).

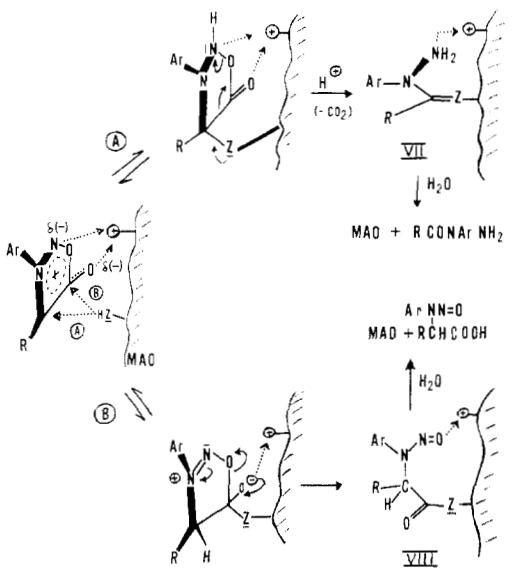


the proposed imine intermediate (or transition state VIb) formed on oxidation of the amine.[10]

Enzyme inhibitors are classically divided into two types. Noncompetitive inhibitors combine irreversibly with the enzyme, destroying its ability to act upon suitable substrate. Duration of action is dependent, not on the biological half-life of the inhibitor, but rather on the time of resynthesis of additional enzyme. Hydrazine derivatives are excellent examples of this class. It has been shown that the inhibition of MAO produced by iproniazid persists for many days after the drug has disappeared from the body.[11] The duration of action of a competitive inhibitor will be limited by its biological half-life, in that the latter will determine the period over which effective concentrations of inhibitor, capable of competing with natural substrate, will be present at the active site. MAO inhibition of this nature has been described for harmaline.[12] It is conceivable, of course, that a competitive inhibitor, with a high binding affinity for an enzyme for which it is not a substrate, might approach a duration of action similar to that of a noncompetitive inhibitor. Such is believed to be the case for tranylcypromine,[13] which appears to have a duration of action intermediate between that of the noncompetitive hydrazine derivatives and the competitive harmala alkaloids.[13,14] Although tranylcypromine is difficult to remove by dialysis from MAO,[15] characteristic of noncompetitive reaction, its inhibition can be reversed readily by some competitive substrates.[16]

N-Arylsydnones (Table I) are moderate inhibitors of MAO both *in vivo* and *in vitro*. MAO inhibition is peculiar to this class, since N-alkylsydnones and the isomeric 4-arylsydnones were inactive both *in vitro* and *in vivo*. The modest level of inhibitory activity, coupled with a less sensitive assay, could explain the failure of earlier workers to show the inhibitory activity of N-

SCHEME I



arylsydnones.[17] Inhibition of MAO has been depicted as involving competitive or noncompetitive binding of either the saturated amine VIa, or the oxidized inhibitor (intermediate or transition state) VIb, to the enzyme.[10] Comparison with N-arylsydnone (VIc) shows that each structure possesses, in approximately similar spatial relationships, the aromatic binding potential thought necessary for interaction with the enzyme, as well as a basic function, supplied in the sydnone ring by the carbonyl oxygen. N-Arylsydnones may inhibit MAO by direct interaction at either the 4-carbon or the carbonyl carbon with a nucleophilic function of the enzyme. The former, by a process which is essentially an acid-catalyzed cleavage of the mesoinic ring (Scheme I, route A), can give rise to the enzyme-bound N-aminoamidine (Z = N) VII; attack at the carbonyl carbon of the sydnone (Scheme I, route B) would lead to the enzyme-bound nitrosoamino acid ester (Z = O or S) or amide (Z = N) VIII. These enzyme–substrate interactions are compatible with the known[2,18] cleavage reactions of sydnones at low and high pH, respectively. Depending on the stability of the enzyme-bound fragment VII or VIII, and possible ancillary binding of the remainder of the molecule to the enzyme, either noncompetitive (slow dissociation of the complex) or competitive (rapid dissociation) inhibition of the enzyme would occur.

It is considered that because of the weak basicity of the sydnone carbonyl oxygen, inhibition of MAO by competitive (harmaline-type) binding with the enzyme is unlikely. However, the oxidized intermediate (VIb), whose carbon–nitrogen double-bond π system has been suggested[10] to play an important role in the oxidation step *via* binding to a second π area on the enzyme, resembles tranylcypromine (VId); the mesoionic π-electron system mimics that of the cyclopropyl ring and the imine intermediate formed from the natural substrates.

With one exception, substitution at the 4 position of the sydnone ring led to a loss of MAO inhibitory

(10) B. Belleau and J. Moran, *Ann. N. Y. Acad. Sci.*, **107**, 822 (1963).
(11) S. Hess, H. Weissbach, B. G. Redfield, and S. Udenfriend, *J. Pharmacol. Exptl. Therap.*, **124**, 189 (1958).
(12) S. Udenfriend, B. Witkop, B. G. Redfield, and H. Weissbach, *Biochem. Pharmacol.*, **1**, 160 (1958).
(13) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. E. Tedeschi, and A. Burger, *J. Med. Pharm. Chem.*, **5**, 1265 (1962).
(14) R. E. Tedeschi, D. H. Tedeschi, L. Cook, P. A. Mattis, and E. J. Fellows, *Fed. Proc.*, **18** 451 (1959).
(15) S. Sarkar, R. Banerjee, M. S. Ise, and E. A. Zeller, *Helv. Chim. Acta*, **43**, 439 (1960).
(16) E. A. Zeller and S. Sarkar, *J. Biol. Chem.*, **237**, 2333 (1962).
(17) P. Oehme, E. Görs, K. Schwarz, G. Petsch, H. D. Faulhaber, and P. Lange, *Acta Biol. Med. Ger.*, **14**, 369 (1965).
(18) (a) J. C. Earl and A. W. Mackney, *J. Chem. Soc.*, 899 (1935); (b) R. A. Eade and J. C. Earl, *ibid.*, 591 (1946).

activity. The single exception to this generalization is **6**, an active inhibitor both *in vitro* and *in vivo*. The noncompetitive mechanism (outlined in Scheme I, route A), requires that the inhibitor be sterically compatible with the enzyme group Z. It is possible that, despite a sterically induced lower binding affinity of **6** for the enzyme, once an intermediate of type VII is formed it has (due to steric factors) an appreciable half-life. Such steric considerations cannot operate in the alternative (competitive, with high binding affinity) mechanisms, and the activity displayed by **6** is considered further support for the conclusion that the N-arylsydnones are noncompetitive inhibitors of MAO.

The limited aqueous solubility of N-arylsydnones prevented a classical investigation of the kinetics of their interactions with MAO. Since, in general, competitive enzyme inhibitors only display *in vivo* activity if they possess an extended biological half-life, the experimental observation that those sydnones active as inhibitors *in vitro* retain activity *in vivo* would indicate that the inhibition is of the noncompetitive type.[19] The single exception to this correlation between *in vitro* and *in vivo* activity is 3-(*p*-hydroxyphenyl)sydnone (**2**). Since phenols are known to be subject to rapid conjugation and excretion,[20] it is possible that the lack of *in vivo* activity is due to a short biological half-life and hence low concentration at the site of action.

Experimental Section[21]

Intermediates were prepared using standard technology and were generally characterized spectroscopically, purity being assessed by paper or thin layer chromatographic techniques. With the exception of the sydnones derived from nitrosoaspartic acids, the conversion of nitrosoamino acids to the sydnones was smoothly accomplished using trifluoroacetic anhydride in THF or Et_2O.

N-β-Cyanoethylamino Acids and N-β-Carboxyethylamino Acids (prepared by the method of McKinney, *et al.*[3]).—The previously unreported N-(2-cyanoethyl)-DL-phenylglycine was prepared from DL-α-aminophenylacetic acid (88% yield), mp 252.5° dec (EtOH–H_2O). *Anal.* ($C_{11}H_{11}N_2O_2$) C, H, N. Hydrolysis afforded N-(2-carboxyethyl)-DL-phenylglycine (40%), mp 255–256° dec.

N-Nitrosoamino acids were prepared by the reported procedure[1] from N-arylamino acids, or by hydrolysis (methanolic 10 *N* NaOH at 50°) of the N-nitrosoamino acid ethyl esters which were obtained by nitrosation of the amino acid ester. The latter route is the method of choice for N-*p*-tolyl-, N-(2,4-xylyl)-, and N-(5-chloro-2,4-dimethoxyphenyl)-N-nitrosoglycine. An exception to the general procedure, N-nitroso-N-(5-chloro-2,4-dimethoxyphenyl)-DL-α-aminohexanoic acid was prepared (96% yield) by addition of 12 *N* HCl to a solution of the amino acid sodium salt in 0.1 *N* $NaNO_2$.

N-Nitroso-N-phenylaspartic Acid.—N-Phenylanilinum aspartate (98 g, 0.32 mole, mp 146–147°)[22] was dissolved in 6 *N* HCl (300 ml) and a solution of $NaNO_2$ (27.7 g, 0.4 mole) in H_2O (60 ml) was added over 3 hr at 0°. The reaction mixture was degassed *in vacuo* at 25°, cooled, and filtered. The residue (82 g) was thoroughly washed (H_2O) and was recrystallized (Et_2O–pentane) to yield 58 g (74%) of product: mp 129–130° dec; λ_{max} 263 mμ (ε 3610); λ_{max} 5.81, 6.99 μ. *Anal.* ($C_{10}H_{10}N_2O_5$) C, H, N.

N-Nitrosopiperidine-2,6-dicarboxylic acid, mp 156–157° (from Et_2O), was prepared similarly in 57% yield. *Anal.* ($C_7H_{10}N_2O_5$) C, H, N.

3-(2-Carboxyethyl)-4-phenylsydnone (14).—N-(2-Carboxyethyl)-N-nitroso-DL-phenylglycine (0.25 mole) in Et_2O (500 ml) was treated with trifluoroacetic anhydride (125 ml). After 48 hr, filtration gave **3-(2-carboxyethyl)-4-phenylsydnone anhydride** (37 g): mp 110–112° (Me_2CO–Et_2O); λ_{max} 5.46, 5.66, 5.80 μ; λ_{max} 313, 242 mμ (ε 9900, 7200). *Anal.* ($C_{22}H_{18}N_4O_7$) C, H; N: calcd, 12.4; found, 11.9. The anhydride (5.6 g) was hydrolyzed at 25° for 1 hr to yield **14** (5.0 g).

3-(2-Carboxyethyl)-4-(isobutyl)sydnone Anhydride.—N-(2-Carboxyethyl)-N-nitroso-DL-isoleucine (0.19 mole), treated as above, gave the anhydride (11 g): mp 112–114° (Me_2CO–Et_2O); λ_{max} 5.52, 5.72, 5.85 μ; λ_{max} 300 mμ (ε 6900). *Anal.* ($C_{18}H_{26}N_4O_4$) C, H, N. On hydrolysis, the anhydride (4.0 g) gave an unidentified compound (2.5 g), mp 88–88.5° dec, λ_{max} 5.72, 5.90 μ, which decomposed on attempted recrystallization.

4-Chloro-3-(5-chloro-2,4-dimethoxyphenyl)sydnone (8).—A stirred suspension of **5** (5.12 g, 0.02 mole) and N-chlorosuccinimide (3.1 g, 0.023 mole) in CCl_4 (400 ml) was heated under reflux for 48 hr, when paper chromatographic assay indicated the absence of **5**. On cooling, **8** (6.6 g) separated from the reaction mixture.

Cyclization of N-Nitrosoaspartic Acids.—In the following experiments, using either N-phenyl- or N-benzyl-N-nitrosoaspartic acid, assay of the various fractions was achieved by two methods: uv spectra (CH_2Cl_2), using the sydnone absorbance at 305 mμ (ε 7570) (**20**) and 312 mμ (ε 6980) (**1**); and tlc on silica gel GF, using two developing systems, 1, $CHCl_3$–EtOAc–HCO_2H (5:4:1), and 2, EtOAc–AcOH (20:1).

General Procedure and Effect of H_2O on Yield.—The N-nitrosoamino acid (0.005 mole) in dry C_6H_6 (5.0 ml) and trifluoroacetic anhydride (2.5 ml) was heated under reflux for 12 hr. The reaction mixture was concentrated *in vacuo*, and the residue was shown (tlc) to contain both starting material and sydnone. Either recrystallization (Et_2O) or treatment with ice–water afforded pure sydnone. Samples prepared by either method were identical spectroscopically and chromatographically and were uncontaminated with starting material; a mixture melting point of the two samples showed no depression.

Preparation of the N-Nitrosoaspartic Anhydrides.—N-Nitrosoaspartic acid (1.5 g) suspended in Ac_2O (4.0 ml) was stirred at 25° under N_2. Sydnone was absent after 16 hr but present (tlc) after 96 hr. The reaction mixture was concentrated and recrystallized to yield (from the appropriate N-nitrosoamino acid) N-phenyl-N-nitrosoaspartic anhydride (410 mg from CH_2Cl_2), mp 115–116° [*Anal.* ($C_{10}H_8N_2O_4$) C, H, N], or N-benzyl-N-nitrosoaspartic anhydride (330 mg from EtOAc), mp 126–126.5° (lit.[6] mp 136–138°) [*Anal.* ($C_{11}H_{10}N_2O_4$) C, H, N].

Reaction with Trifluoroacetic Anhydride in Et_2O at 25°.—The N-nitrosoamino acid (2.5 g), suspended in Et_2O (10 ml) and trifluoroacetic anhydride (2.4 ml), dissolved in 0.5 hr. After 3 hr, concentration *in vacuo* gave a solid containing equal amounts of sydnone and the corresponding anhydride.

Reaction with Ac_2O in Et_2O at 32°.—The N-nitrosoamino acid (0.02 mole) and Ac_2O (10 g) in Et_2O (15 ml) were heated under reflux for 3.5 hr. The reaction mixture was either quenched on ice or concentrated *in vacuo* and gave crude N-nitrosoamino acid anhydride containing traces of starting acid and sydnone.

Conversion of Crude Aspartic Anhydrides to Sydnone.—The crude anhydride was heated at 50° for 2 hr in C_6H_6 containing trifluoroacetic anhydride. Concentration and recrystallization gave essentially pure sydnone.

Effect of H_2O on Conversion of Crude Aspartic Anhydrides to Sydnone.—The anhydride was boiled with water for 5 min. Cooling and extraction yielded mixtures containing the N-nitrosoamino acid and anhydride and sydnone. Sydnones were unaffected by boiling in H_2O for 5 min.

Acid-Catalyzed Conversion of Anhydride to Sydnone.—A solution of N-nitroso-N-phenylaspartic anhydride (400 mg) in C_6H_6 (10 ml) containing 1 drop of trifluoroacetic acid was heated

(19) This is supported by observations in the related 1,2,3-thiadiazolium series. By analysis of enzyme kinetics it was shown that only analogs exhibiting noncompetitive interaction *in vitro* demonstrated MAO inhibitory activity *in vivo* (E. H. Wiseman and D. P. Cameron, in preparation).

(20) R. T. Williams, "Detoxication Mechanisms," John Wiley and Sons, Inc., New York, N. Y., 1959.

(21) Melting points are uncorrected and were determined using a Thomas–Hoover capillary melting point apparatus. Ir spectra were measured in KBr with a Perkin-Elmer Model 21 spectrometer and uv spectra were measured in MeOH with a Cary spectrophotometer using a 2-cm cell. Analyses were carried out by the Physical Measurements Laboratory of Chas. Pfizer & Co., Inc. Where analyses are indicated by the symbols of the elements, the analytical results for those elements were within 0.4% of the theoretical values.

(22) R. Anschütz and Q. Wirtz, *Ann.*, **239**, 151 (1887).

under reflux for 2 hr. After 12 hr at 25° the supernatant was decanted and concentrated *in vacuo* at 25° to give **1** (100 mg).

Stability of N-Nitroso-N-phenylaspartic Anhydride.—The compound, refluxed in C_6H_6 alone (2 hr), was essentially unchanged with respect to sydnone.

Acknowledgment.—The authors express their appreciation to Miss Josephine Chiaini and Messrs. Andrew Popson and Louis J. Navarro for their technical assistance.

N-Substituted Derivatives of 2-Aminoethanethiol and 2-Hydrazinoethanethiol[1]

ROGER D. WESTLAND, JOYCE L. HOLMES, BILLIE GREEN, AND JOHN R. DICE

Research Laboratories, Parke, Davis & Co., Ann Arbor, Michigan 48106

Received January 5, 1968

A study was made of the effect on radioprotective action of many variations in nitrogen substitution of 2-aminoethanethiol. Direct alkylation of primary amines with ethylene sulfide (generated *in situ*) provided many of the analogs. Other derivatives were obtained by debenzylation of N-[2-(benzylthio)ethyl]alkylamines. These benzylthio ethers were prepared by (1) reduction ($LiAlH_4$) of amides obtained from either (benzylthio)-acetyl chloride or 2-(benzylthio)ethylamine, and (2) alkylation of 2,2,2-trifluoroacetamides with benzyl 2-chloroethyl sulfide. Alkylation of 1,2-bis(trifluoroacetyl)-1-alkylhydrazines using benzyl 2-chloroethyl sulfide afforded substituted 2-hydrazinoethanethiols. None of the compounds was superior to 2-aminoethanethiol in protecting against radiation damage. Antibacterial activity was found for some compounds against *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis*.

Derivatives and analogs of 2-aminoethanethiol are still the most promising antiradiation agents available. Many structural variations incorporating a variety of synthetic methods have been reported.[2] Considering the mechanisms of protective action postulated[3] for active agents, it seemed likely that increased activity could result from changes in drug transport properties and/or selective absorption by tissues most vulnerable to radiation damage. Accordingly, mercaptoethyl analogs of drugs which are known to be transported and selectively absorbed *in vivo* were synthesized (Table I). Analogs were prepared from norephedrine, amphetamine, 1-phenylcyclohexylamine, some *o*-alkoxyphenoxyalkylamines, *trans*-2-phenylcyclopropylamine, norepinephrine, and (α-methylphenethyl)hydrazine. Additionally, mercaptoethylamines possessing cyclopropyl and cyclobutyl groups and derivatives of hydrazine were prepared.

Mercaptoethylamine derivatives which could be distilled using ordinary techniques were obtained by the use of ethyl 2-mercaptoethyl carbonate, which was introduced for this purpose by Reynolds and coworkers.[4,5] Although aldehydes are incompatible with mercaptans, the mercaptoethyl derivative of aminoacetaldehyde diethyl acetal was isolated. This provided a 2-alkylaminoethanethiol bearing a potential aldehyde function.

Other compounds were obtained from 2-amino-1-alkanols which were prepared conveniently by reduction of esters of DL-α-amino acids using lithium aluminum hydride.[2f,6] Metal hydride reductions of the methyl esters of glutamic acid and tyrosine on a preparative scale afforded very low yields of products. Such reductions have given some amino alcohol on a small scale,[6b-d] although the preparation of tyrosinol from tyrosine apparently is not reproducible.[6f] Catalytic hydrogenation of tyrosine methyl ester using a rhodium catalyst effected dehydration and reduction of the aromatic ring to give a derivative of cyclohexane. An attempt to prepare 2-amino-1,5-pentanediol from DL-glutamic acid by high-pressure catalytic hydrogenation using a rhenium catalyst resulted in isolation of only the lactam, 5-(hydroxymethyl)-2-pyrrolidinone, in about 48% yield. In a few instances in which the product was difficult to distil satisfactorily, the excess amine was distilled using an oil diffusion pump and the product was isolated from the undistilled residue. In two cases the mercaptan was separated from excess amine by precipitating the lead mercaptide. Recrystallization from aqueous alcohol effected purification of the lead salts.

Some of the pharmacologically active amines we wished to use were either in short supply or could not be distilled, and it was necessary to develop other procedures for these examples. In one variation used to prepare substituted 2-(benzylthio)ethylamines (Table II), amines were acylated with (benzylthio)acetyl chloride to give simple amides. Reduction of the amides using $LiAlH_4$ in ether or tetrahydrofuran as illustrated in Scheme I, method A, provided secondary amines with no detectable cleavage of the thio ether. The substituted 2-(benzylthio)ethylamines generally were purified as hydrochloride salts. Sodium–liquid

(1) This investigation was supported by the U. S. Army Medical Research and Development Command, Contract No. DA-49-193-MD-2306. We appreciate the interest and support of Drs. D. P. Jacobus and T. R. Sweeney of Walter Reed Army Institute of Research.

(2) (a) For a summary of earlier syntheses see R. J. Wineman, M. H. Gollis, J. C. James, and A. M. Pomponi, *J. Org. Chem.*, **27**, 4222 (1962), and D. Rosenthal, G. Brandrup, K. H. Davis, Jr., and M. E. Wall, *ibid.*, **30**, 3689 (1965); (b) L. Bauer and B. K. Ghosh, *ibid.*, **30**, 4298 (1965); (c) A. F. Ferris, O. L. Salerni, and B. A. Schutz, *J. Med. Chem.*, **9**, 391 (1966); (d) G. R. Handrick and E. R. Atkinson, *ibid.*, **9**, 558 (1966); (e) O. L. Salerni and R. N. Clark, *ibid.*, **9**, 778 (1966); (f) J. R. Piper, C. R. Stringfellow, Jr., and T. P. Johnston, *ibid.*, **9**, 911 (1966).

(3) Z. M. Bacq, "Chemical Protection Against Ionizing Radiation," Charles C Thomas, Publisher, Springfield, Ill., 1965, Chapter 19.

(4) D. D. Reynolds, D. L. Fields, and D. L. Johnson, *J. Org. Chem.*, **26**, 5125 (1961).

(5) Ethylene sulfide is now available from Aldrich Chemical Co. and can be handled easily.

(6) (a) K. S. Topchiev, *Dokl. Akad. Nauk SSSR*, **63**, 147 (1948); *Chem. Abstr.*, **43**, 2579 (1949); (b) P. Karrer, P. Portmann, and M. Suter, *Helv. Chim. Acta*, **31**, 1617 (1948); (c) P. Karrer and P. Portmann, *ibid.*, **31**, 2088 (1948); (d) A. Dornow, G. Messwarb, and H. Frey, *Chem. Ber.*, **83**, 445 (1950); (e) G. R. Handrick, E. R. Atkinson, F. E. Granchelli, and R. J. Bruni, *J. Med. Chem.*, **8**, 762 (1965); (f) H. Gershon and R. Rodin, *ibid.*, **8**, 864 (1965).