cis- and *trans*-2-Mercaptocyclobutylamines. Synthesis and Antilipolytic Properties *in Vitro*[1,†]

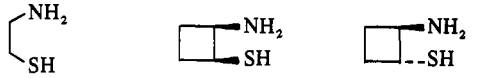
Donald T. Witiak,* Birandra K. Sinha, Ock Soon Lee, and Dennis R. Feller

Divisions of Medicinal Chemistry and Biochemical Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210. *Received February 14, 1972*

Cysteamine, an effective radioprotective agent, has a number of other biological properties which may be related to its ability to form mixed disulfides with SH-containing enzymes. For purposes of exploring stereostructure–activity relationships in a number of biological systems we synthesized the geometrically pure *cis-* and *trans*-2-mercaptocyclobutylamine analogs. The effect of these compounds on drug-stimulated glycerol release from rat adipose tissue is described in this article.

Cysteamine (2-mercaptoethylamine, **1**) is regarded as one of the most potent radioprotective agents.[2] Debated mechanisms which have been proposed to account for the activity of **1** include radical trapping,[3] anoxia or hypoxia,[4] and reaction with cellular components.[5] The latter mode of action involves the mixed-disulfide hypothesis advanced by Eldjarn and Pihl.[5] In addition, mercaptoethylamines have other actions; for example, they either potentiate the activity of bradykinin on smooth muscle, or, if appropriately substituted, exhibit noncompetitive antagonism.[6] Thiol compounds such as cysteine also potentiate the antilipolytic effects of digitoxin, 2,4-dinitrophenol, and dicoumarol.[7]

Since many enzymes contain SH groups which could form mixed disulfides, stereostructure-activity relationships could be investigated for many biological systems using appropriate constructed analogs.[8] Correlations of structure and activity *in vivo* and *in vitro* with such compounds may provide insight concerning modes of action of these drugs as radioprotective agents and/or aid in a study of differential actions relating to other enzymatic effects. For these reasons we desired samples of *cis-* and *trans*-2-mercaptocyclobutylamine (**2**). These analogs are related to **1**. In this paper we describe our synthetic efforts and the action of **1**, *cis*-**2**, and *trans*-**2** on drug-stimulated glycerol release from adipose tissue *in vitro*.



1 *cis*-**2** *trans*-**2**

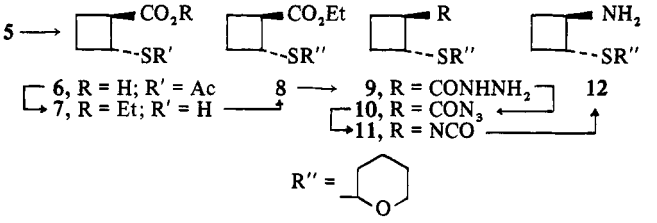
Synthetic Aspects. Ethyl cyclobutanecarboxylate (**3**) served as starting material and was readily converted to the known cyclobutene-1-carboxylic acid (**5**) by way of the α-Br derivative **4**. α-Bromination was accomplished using NBS; this method is superior to the one reported by Combell and Rydon[9] and affords **4** in 95% yields.

Our first approach to the synthesis of *cis-* and *trans*-**2** involved a study of the light-catalyzed (75-W lamp) addition of thiolacetic acid to **5**.[10] Under these conditions adduct **6** was obtained; only the trans isomer could be isolated. The stereochemical assignment for *trans*-**6** was confirmed by a reaction sequence described later in this section and its purity was determined by gas–liquid phase chromatography (glpc). The cis isomer was not isolated, but its presence in trace amounts in the reaction mixture was detected by glpc.

†We are grateful to the Department of the Army and U. S. Army Medical Research and Development Command for support of this work through Contract No. DADA17-72-C-2073. We are also grateful to the National Heart and Lung Institute, National Institute of Health, which supported part of this work through Grant No. HE-12740.

Concommitant hydrolysis of the thiolacetate function and esterification of the carboxyl group of *trans*-**6** using 4 *N* HCl in EtOH afforded ethyl *trans*-2-mercaptocyclobutanecarboxylate (**7**) in 97% yield. The SH group of *trans*-**7** was protected by reaction with dihydropyran.[11] The resulting tetrahydropyranyl derivative **8** was converted[12] to the trans acid hydrazide **9**.

Curtius degradation of acid hydrazide **9** was accomplished under reverse addition conditions.[13] Reverse addition was necessary to minimize cleavage of the tetrahydropyranyl group under acidic conditions. The intermediate acid azide **10** and isocyanate **11** were not isolated but were detected by their characteristic ir absorption bands (see Experimental Section). By this route *trans*-2-(2′-tetrahydropyranylmercapto)cyclobutylamine (**12**) was obtained from **9** in 25% yield. However, at this stage the reaction sequence failed owing to the inability in our hands to obtain analytically pure *trans*-**2** after hydrolysis of the tetrahydropyranyl group of **12**. A similar experience was observed in the cyclopropane series.[8]

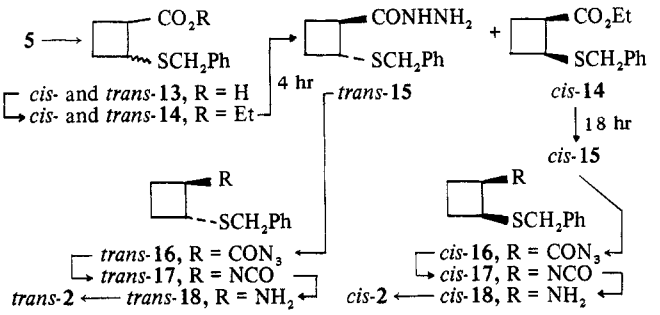


Since we were not able to obtain analytically pure *trans*-**2** or various salts of **2** when SH was protected with a tetrahydropyranyl group and we also desired a sample of *cis*-**2** we investigated a second synthetic route. This involved studies of both base- (piperidine)[14] and light- (75-W lamp) catalyzed addition of $PhCH_2SH$ to **5**. Piperidine-catalyzed addition afforded a mixture of *trans-* and *cis*-**13** (1.2:1.0) in 40% yield; however, many other impurities, detected by glpc, could not be separated from the desired isomers by physical methods. On the other hand, light-catalyzed addition afforded an isomeric mixture of *cis-* and *trans*-**13** (1.0:1.0) in 66% yield. Under these conditions the isomeric mixture was easily obtained free of impurities (glpc), but the isomers could not be separated by physical methods. When the mixture was maintained at 45° in the absence of light no reaction took place. The isomeric mixture of esters **14**, prepared from **13**, also could not be separated by physical methods; their presence was determined by glpc.

When, however, the isomeric esters **14** were treated with hydrazine hydrate under short reaction conditions (4 hr) only the trans acid hydrazide **15** was obtained. This compound was easily separated by crystallization from unreacted

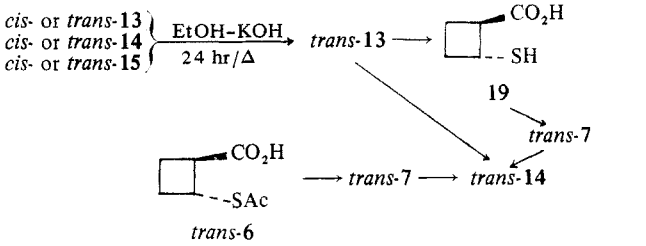
cis ester **14**. The more sterically hindered cis ester, which was isolated from the reaction mixture, was converted in nearly quantitative yields to the cis acid hydrazide **15** by carrying out the reaction for 18 hr.

Curtius degradation of pure cis and trans acid hydrazides **15** afforded pure *cis*- and *trans*-2-benzylmercaptocyclobutylamines (**18**) by way of their respective intermediate acid azides **16** and isocyanates **17**. Intermediates **16** and **17** were not isolated, but were detected by their characteristic ir absorption spectra. Debenzylation of the pure stereoisomeric benzylamines **18** with Na-liquid NH_3[14b,15] afforded the desired *cis*- and *trans*-2-mercaptocyclobutylamines (**2**). These isomers were shown to be isomerically pure by glpc and were converted to analytically pure HCl salts for biological studies.



The stereochemical assignment for these cyclobutane analogs was based on several observations. In all cases those isomers to which we have assigned the trans configuration have a shorter retention time (glpc) than the corresponding cis compound. All reactions were followed by glpc analysis and it could be determined whether we were working with an isomerically pure sample, a mixture of isomers, or whether isomerization occurred during the reaction. The selective short reaction (4 hr) between hydrazine hydrate and the sterically less hindered trans isomer and long reaction (18 hr) to afford quantitative yields of *cis*-**15** are in agreement with the stereochemical assignment for esters **14** and acid hydrazides **15**. Glpc analysis of a reaction run for 8 hr shows the presence of approximately 30–35% cis hydrazide, 100% trans hydrazide, and 70–65% unreacted cis ester. These results are consistent with those obtained by Gelin and coworkers; the rate of base-catalyzed hydrolysis of *trans*-dicarbethoxycyclobutane (k_1 and k_2) is more rapid than hydrolysis of the cis isomer.[16] Their results suggest that the carbethoxy groups have a diaxial conformation in the trans compound and an axial-equatorial conformation in the cis compound. Hence, the ester functions of the trans isomer are more exposed to attack by ^-OH.[16b] Similarly, the carbethoxy and benzymercapto groups in *trans*-**14** likely exist preferentially in the diaxial conformation and the carbethoxy group is more exposed to attack by hydrazine. The cis isomer reacts more slowly because a greater population of conformer having an equatorial carbethoxy group is present. Further, even in that conformer having an axial carbethoxy group the equatorial benzylmercapto function likely provides steric hindrance to attack by hydrazine.

Additional support for the stereochemical assignments is derived from base-catalyzed isomerization of cis and trans acids **13**, esters **14**, and acid hydrazides **15**. Refluxing these mixtures or the pure cis or trans isomers with EtOH-KOH for 24 hr afforded the expected thermodynamically more stable trans acid **13** in quantitative yields. Pure *trans*-**13** was debenzylated under conditions of Na-liquid NH_3 affording *trans*-2-mercaptocyclobutane-1-carboxylic acid (**19**). Esterification of *trans*-**19** afforded *trans*-**7** identical in every respect with the compound obtained by esterification of **6**. These results establish the trans geometry for compounds **6–12**. In addition, *trans*-**7** was converted to *trans*-**14** by benzylation and was identical in all respects with the trans ester **14** obtained from pure trans acid **13**. If the cis-trans mixture **13** is debenzylated, a mixture of *cis*- and *trans*-**19** is obtained without isomerization. This cis-trans mixture **19** was esterified under the same conditions discussed previously affording a cis-trans mixture **7** without isomerization. These experimental results, coupled with the observation that pure *cis*- and *trans*-2-benzylmercaptocyclobutylamines (**18**) are obtained by Curtius degradation of the respective pure cis and trans acid hydrazides **15**, establish the configurational assignments for compounds **15–18**. Since *cis*- and *trans*-**2** are obtained from the respective *cis*- and *trans*-**18** analogs without any apparent contamination by the other isomer their configuration also is established.



Biological Aspects. The effect *in vitro* of 2-mercaptoethylamine (**1**) and *cis*- and *trans*-2-mercaptocyclobutylamine (**2**) analogs on the release of glycerol from rat epididymal adipose tissue in the absence of exogenous stimulation is shown in Table I. At concentrations of 5×10^{-4} and $5 \times 10^{-3} M$ these compounds exhibited a small stimulatory action. The basal glycerol release rate was increased 0.8–3.0-fold at $5 \times 10^{-3} M$ mercaptoalkylamine concentrations. At the latter concentration, *cis*- and *trans*-**2** and, to a lesser extent, **1** exhibited agonist activities which range from 11 to 31% of the maximum lipolytic activity observed with norepinephrine (NE). In these concentration ranges, the trans isomer appeared to show the greatest effect. The nature of the lipolytic action obtained for these analogs was not examined further.

Table I. Effect of 2-Mercaptoethylamine (**1**) and Cyclobutane Analogs *cis*- and *trans*-**2** on the Release of Glycerol from Rat Epididymal Adipose Tissue in the Absence of Exogenous Stimulation *in Vitro*

Compound	Concentration, *M*	% stimulation of glycerol release in the absence of exogenous agonist[a]	Intrinsic activity[b]
1	5×10^{-5}	0	0
	5×10^{-4}	+49 (57, 41)	0.07
	5×10^{-3}	+80 (85, 75)	0.11
cis-**2**	5×10^{-5}	0	0
	5×10^{-4}	+33 (34, 32)	0.05
	5×10^{-3}	+270 (266, 273)	0.24
trans-**2**	5×10^{-5}	0	0
	5×10^{-4}	+194 (179, 208)	0.12
	5×10^{-3}	+315 (307, 323)	0.31

[a] Each value represents the average of two determinations. Values in parentheses represent the individual values expressed as a per cent increase in the basal release rate of 0.49 μmole of glycerol released g^{-1} hr^{-1}. [b] Results are expressed as a ratio of the net glycerol release rate observed for each compound to the maximal release rate observed with NE.

Table II. Inhibition of NE-, Theo-, and DBc-AMP-Stimulated Glycerol Release from Rat Adipose Tissue by 2-Mercaptoethylamine (**1**) and Cyclobutane Analogs *cis*- and *trans*-**2**.

Agonist concn, *M*	Inhibitor concn, *M*[a]	% response observed in the presence of mercaptoalkylamines[b]		
		1	*cis*-**2**	*trans*-**2**
NE (2×10^{-6})	5×10^{-3}	7.4 ± 1.8	15.7 ± 2.0	34.9 ± 0.7
Theo (10^{-2})	5×10^{-4}	30.0 ± 1.0	58.3 ± 1.4	76.5 ± 0.6
DBc-AMP (10^{-3})	3×10^{-4}	52.8 ± 0.5	60.1 ± 0.5	71.2 ± 0.4

[a]Concentrations of inhibitors were chosen on the basis of preliminary experiments designed to determine the range of inhibitory concentrations in the presence of each agonist. [b]Values are calculated as a per cent of the maximal response observed in the absence of inhibitors. Values represent the mean of 3–4 determinations ± SE. Data are representative of results obtained in two experiments.

While these compounds do exhibit a stimulatory effect, the data presented in Table II illustrate that these mercaptoalkylamines are able to block NE, theophylline (Theo), and $N^6,O^{2'}$-dibutyryl adenosine 3',5'-cyclic monophosphoric acid (DBc-AMP) stimulated lipolysis. At concentrations of the agonists which elicited near maximal lipolytic responses, the mercaptoalkylamines (**1**, *cis*- or *trans*-**2**) were observed to differ slightly in their relative ability to inhibit the release of glycerol. The rank order of blocking activity for these analogs was **1** > *cis*-**2** > *trans*-**2** in the presence of the three agonists while in the absence of any agonist *trans*-**2** > *cis*-**2** > **1** as a stimulator of the lipolytic system. Further, against DBc-AMP-stimulated lipolysis, the mercaptoalkylamine antagonists possessed the same efficacy (Figure 1). Although the data is not included in this report, these analogs also possessed the same efficacy against NE. The dual action of *trans*-**2**, which might be considered to be a partial agonist, is best illustrated in Figures 2 and 3. In the presence of a constant concentration of each mercaptoalkylamine ($2.5 \times 10^{-3} M$) the trans isomer was found to stimulate glycerol release at $10^{-7} M$ concentrations of NE while *cis*-**2** and **1** exhibited only slight (or no) blocking action under these conditions. At the higher concentrations of NE employed these compounds blocked lipolysis in a noncompetitive manner. Similarly, at lower concentrations of DBc-AMP used, the trans isomer ($3 \times 10^{-4} M$) potentiated glycerol release whereas *cis*-**2** and **1** showed little stimulatory activity. At a concentration of $10^{-3} M$ DBc-AMP these mercapto analogs exhibited an inhibition; the relative blocking activity observed was **1** > *cis*-**2** > *trans*-**2**.

Discussion

A number of SH reagents (iodoacetate, *p*-chloromercuribenzoate, *N*-ethylmaleimide, and iodoacetamide) have been shown to inhibit hormone-induced lipolysis and this antilipolytic effect can be reversed by prior incubation with dimercaptopropanol (BAL) and, in part, with mercaptoethanol.[17] Based on their observations, Calvert and Lech have have suggested that the release of FFA induced by NE, Theo, and DBc-AMP requires the activation of SH-containing enzymes which are mediated at an intracellular site, possibly the triglyceride lipase system.[17] More recently, Yu and coworkers[18] found that NE-, Theo-, and DBc-AMP-stimulated glycerol release was inhibited by the presence of cardiac glycosides (CG), SH reagents, or oxidative phosphorylation inhibitors. These investigators examined the nature of the CG antagonism by a comparison with the antilipolytic actions observed with SH and oxidative phosphorylation inhibitors in conjunction with anti-SH reagents [glutathione (GSH), cysteine, BAL]. In these studies the influence of

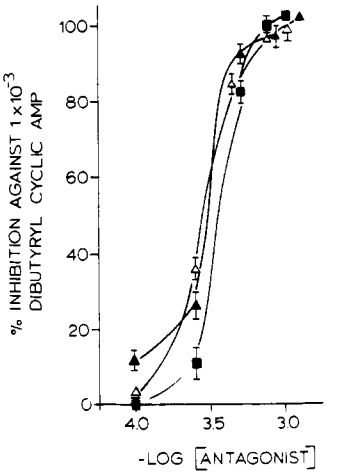


Figure 1. Per cent inhibition of DBc-AMP-induced glycerol release by mercaptoalkylamine analogs. ■–■, 2-mercaptoethylamine (**1**); △–△, *cis*-**2**; ▲–▲, *trans*-**2**. Abscissa: negative log molar concentrations of antagonist. Ordinate: per cent inhibition of glycerol release. Values plotted represent the mean of three to four determinations ±SE.

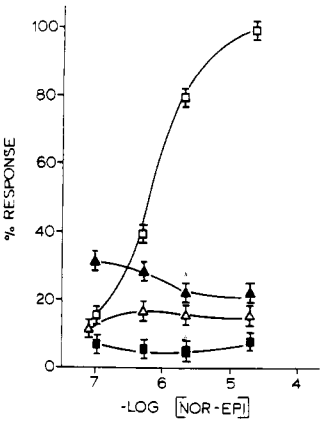


Figure 2. Dose-response curves for NE on the release of glycerol in the presence of mercaptoalkylamine analogs ($2.5 \times 10^{-3} M$). □–□, NE alone; ■–■, 2-mercaptoethylamine (**1**) + NE; △–△, *cis*-**2** + NE; ▲–▲, *trans*-**2** + NE. Abscissa: negative log molar concentrations of NE. Ordinate: per cent of maximal glycerol release. Values plotted represent the mean of three to four determinations ±SE.

GSH reversed the digitoxin inhibition of NE-, Theo-, and DBc-AMP-stimulated lipolysis while cysteine and BAL potentiated the antagonistic effect. Moreover, unlike the results observed for the mercaptoalkylamine analogs (**1**, *cis*- and *trans*-**2**) in the present study, GSH ($10^{-3} M$), cysteine ($10^{-2} M$), and BAL ($10^{-3} M$) alone did not alter the basal- or NE-, Theo-, and DBc-AMP-induced release of glycerol. It seems that the observed antagonism of **1** and *cis*- and *trans*-**2** may be related to the antagonism by SH reagents[17,18] on NE-, Theo-, and DBc-AMP-stimulated lipolysis; *i.e.*, both types of compounds are able to react with SH groups on enzymes.

On the basis of the evidence presented, it would appear that the major blocking action of **1** and *cis*- and *trans*-**2** is mediated at a terminal site of the hormone-sensitive lipase system. The inhibition may be directed at the tri-, di-, or monoglyceride lipase enzymes. The same site likely is involved for the mercaptoalkylamine antagonists since their relative inhibitory potency follows the same order irrespective of the agonists employed (NE, Theo, or DBc-AMP, Table II). In addition to the SH reagents, these mercaptoalkylamines behave in a qualitative manner similar to results obtained in previous studies with salicylate[19] and various analogs of α-

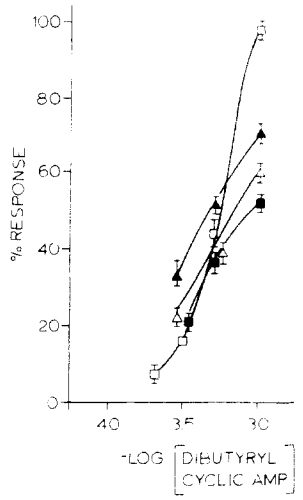


Figure 3. Dose–response curves for DBc-AMP on the release of glycerol in the presence of mercaptoalkylamine analogs ($3 \times 10^{-4} M$). □–□, DBc-AMP alone; ■–■, 2-mercaptoethylamine (**1**) + DBc-AMP; △–△, *cis*-**2** + DBc-AMP; ▲–▲, *trans*-**2** + DBc-AMP. Abscissa: negative log molar concentration of DBc-AMP. Ordinate: per cent of maximal glycerol release. Values plotted represent the mean of three to four determinations ±SE.

(*p*-chlorophenoxy)-α-methylpropionic acid,[20] but the actual mechanism on a molecular basis may be quite different. It is evident that any elucidation of the ultimate mechanism(s) of antilipolytic action possessed by these compounds must await isolation of the entire system for lipase activation. It is possible that the overall inhibitory action possessed by the mercaptoalkylamines may include an effect on the SH-containing enzyme (Na^+-K^+ATPase),[21] on uncoupling oxidative phosphorylation,[18,22] and/or on cyclic 3′,5′-AMP phosphodiesterase.

The stereoselective difference in inhibitory potency may be attributed to differences in affinity for enzyme-active (or allosteric) sites or ability to penetrate tissue barriers by various analogs. On the basis of chemical evidence discussed in this paper, it might be anticipated that the sterically less hindered trans isomer would more readily form mixed disulfides with enzymes. If disulfide formation is involved, the stronger stimulatory (and weaker blocking) action of the trans analog may be a reflection of more facile disulfide formation with stimulatory sites on enzymes involved in the lipolytic sequence.

Experimental Section‡

1-Bromo-1-carbethoxycyclobutane (4). A soln of 50.0 g (0.39 mole) of ethyl cyclobutanecarboxylate (**1**) in 50 ml of dry CCl_4 was added rapidly to a suspension of 100 g (0.56 mole) of NBS in 500 ml of dry refluxing CCl_4. During the addn the flask was irradiated by a 75-W spot light. After addn, the red-brown reaction mixt was refluxed for an addnl 6–8 hr or until the soln became colorless. The mixt was cooled and filtered, and the filtrate concd under reduced pressure and distd, affording 77 g (95%) of a colorless liquid, bp 85–88° (12 mm), identical in all respects with the compd reported prepd in 3 steps from cyclobutane-1-carboxylic acid.[9]

1-Cyclobutanecarboxylic acid (5) was prepd by the method of Combell and Rydon,[9] affording a white crystn solid, mp 70–71°, lit.[9] mp 72°. This compd was used immediately in subsequent reactns because of its instability in air.

***trans*-2-Acetylmercaptocyclobutanecarboxylic Acid (6).** The acid **5** (25 g, 0.255 mole) was dissolved in 150 ml of dry CCl_4 and added to 38.0 g (0.50 mole) of commercial grade thiolacetic acid. The reaction flask was irradiated with a 75-W spot light while stirring for 24 hr at room temp. After 24 hr the solvent and excess thiolacetic acid were removed under reduced pressure. The semisolid residue was refluxed with approx 1 l. of petr ether (bp 30–60°). On cooling, 18.0–26.0 g (42–60%) of white crystals, mp 83–84°, identified as *trans*-**6** was obtained. Glpc on 3.8% silicon gum rubber on chromosorb W (80–100 mesh), 4 ft × 0.25 in. glass column with column temp 150°, inject port temp 265°, detector temp 250°, inlet pressure 40 psi, and carrier gas (He) flow rate of 45 ml/min showed one peak at 2.66 min. *Anal.* ($C_7H_{10}O_3S$) C, H, S: calcd, 18.4; found, 19.1.

The mother liquor obtained after filtration of *trans*-**6** was concd under reduced pressure affording 10.0 g of a viscous liquid. This liquid decomp during distn (0.13 mm) and was not further purified. Glpc analysis showed the presence of 6 compounds. *Trans*- and *cis*-**6**, present in a ratio of 4:6, respectively, represent 35% of this liquid. The presence of *trans*- and *cis*-**6** and their stereochemical assignment was confirmed as described for pure *trans*-**6** by conversion of *cis*- and *trans*-**6** to *cis*- and *trans*-**7** which in turn was converted to *cis*- and *trans*-**14** and analyzed by glpc.

***trans*-2-Mercapto-1-carbethoxycyclobutane (7).** A soln of *trans*-**6** (17.5 g, 0.1 mole) in 100 ml of EtOH was refluxed with 50 ml of 4 *N* HCl for 4 hr. The reaction mixt was cooled and extd (Et_2O). The Et_2O soln was washed with H_2O, followed by satd $NaHCO_3$, and finally with H_2O, dried (Na_2SO_4), and concd under reduced pressure affording 16.0 g (99%) of a yellow liquid which was colorless after distn at 40–42° (0.05 mm), ir (neat, cm^{-1}) 2440 (SH), 1725 (CO), 1380 (C-CH_3). This compd decomposes and/or dimerizes on standing and was not subjected to elemental analysis. The nmr spectrum was in agreement with the assigned structure.

***trans*-2-(2′-Tetrahydropyranylmercapto)-1-carbethoxycyclobutane (8).** A mixt of 32.0 g (0.20 mole) of **7**, 20.2 g (0.24 mole) of distd dihydropyran, and 0.2 g of TsOH was gently heated on a steam bath for 2 hr. After standing at room temp for 2 hr the mixt was dissolved in Et_2O, washed (10% KOH followed by H_2O), and dried (Na_2SO_4), and the solvent removed under reduced pressure. The residual oil was distd affording 45.0 g (91%) of a colorless liquid, bp 105–110° (0.25 mm), ir (neat, cm^{-1}) 1740 (CO), 1380 (C-CH_3) and 1000, 1030, 1075, and 1100 (characteristic of tetrahydropyranyl ring). No band was observed at 2440 cm^{-1} (SH). The nmr spectrum is consistent with the assigned structure; the compd decomp on standing. Glpc using a column described for compd **6** with column temp 150°, injection port temp 260°, detector temp 260°, inlet pressure at 40 psi, and carrier gas (He) flow rate of 60 ml/min showed 1 peak at 6.0 min. This compd decomp slowly and only a rough analysis could be obtained. *Anal.* ($C_{12}H_{20}O_3S$) H, S, C: calcd, 58.99; found, 56.55.

***trans*-2-(2′-Tetrahydropyranylmercapto)cyclobutanecarboxhydrazide (9)** was prepd by a method similar to the one described by Buchman and coworkers.[12] Hence, to 20.0 g (0.40 mole) of 85% hydrazine hydrate, maintained at 130°, was added dropwise with stirring 29.0 g (0.12 mole) of trans ester **8**. After addn the mixt was refluxed (130°) for 6 hr and cooled (ice, 24 hr) affording 25.0 g (90%) of semisolid **9**. The excess hydrazine hydrate was removed under reduced pressure affording a semisolid residue, ir ($HCCl_3$, cm^{-1}) 3250–3300 (broad, NH_2), 1660 (CO), 1540 (NH bending), and 1000, 1030, 1075, 1100 (tetrahydropyranyl ring). This compd was not further purified and immediately was converted to the amine.

***trans*-2-(2′-Tetrahydropyranylmercapto)-1-aminocyclobutane (12).** A soln of 23.0 g (0.10 mole) of hydrazide **9** in 200 ml of Et_2O was layered with a soln of 10.0 g (0.145 mole) of $NaNO_2$ in 25 ml of H_2O. To this stirred mixt (0–5°) was added dropwise 40 ml of 6 *N* HCl. After addn the reaction mixt (0–5°) was stirred for 15 min; during this time the mixt turned red. The Et_2O layer was sepd, washed (H_2O), and dried (Na_2SO_4). Dry toluene (100 ml) was added and the Et_2O was removed under reduced pressure. Ir (toluene, cm^{-1}) 2150 (CON_3), 1700 (CO), and 1000, 1030, 1075, and 1100 (tetrahydropyranyl ring) was characteristic for compd **10**.

The toluene soln containing azide **10** was refluxed for 1 hr (N_2↑) after which time the ir absorption band at 2150 cm^{-1} (**10**) was absent and a new band at 2250 cm^{-1} (N=C=O) for **11** was present.

The toluene soln containing isocyanate **11** was refluxed for 8 hr with 12.0 g (0.21 mole) of KOH in 100 ml of abs EtOH. The mixt was cooled and continuously extd with Et_2O for 24 hr. The Et_2O layer was dried (Na_2SO_4) and concd under reduced pressure affording after distn 5.0 g (25%) of liquid, bp 79–80° (0.01 mm). This amine was further purified by dissolving in Et_2O and ppt with gaseous HCl which afforded a gummy HCl salt. The salt was treated

‡Elemental analyses were performed by Clark Microanalytical Labs., Urbana, Ill. Ir spectra were recorded on a Varian A-60A spectrophotometer. Glpc was performed using the F and M scientific Model 402 high efficiency gas chromatograph. Melting points were taken on a calibrated Thomas-Hoover mp apparatus.

with 10% NaOH soln, extd (Et_2O), dried (Na_2SO_4), concd, and distd under reduced pressure, ir (neat, cm^{-1}) 3340 and 3280 (NH_2), 1600 (NH bending), 1000, 1035, 1075, and 1100 (tetrahydropyranyl ring). Glpc using a column described for compd **6** with column temp 145°, injection port temp 250°, detector temp 225°, inlet pressure at 40 psi, and carrier gas (He) flow rate of 60 ml/min shows 1 peak at 4.6 min. *Anal.* ($C_9H_{17}ONS$) C, H, S, N: calcd 7.48; found, 6.1.

***cis*- and *trans*-2-Benzylmercaptocyclobutanecarboxylic Acid (13).** To a soln of 30.0 g (0.30 mole) of cyclobutene-1-carboxylic acid **(5)** in dry CCl_4 (150 ml) was added 40.3 g (0.32 mole) of commercial grade $PhCH_2SH$. The soln was stirred and irradiated with a 75-W spot light for 36 hr at 45°. The solvent was removed under reduced pressure and the residual liquid was dissolved in 500 ml of Et_2O. The Et_2O layer was extd with several portions of satd $NaHCO_3$ soln. The $NaHCO_3$ layer was acidified (dil HCl), extd (Et_2O), dried (Na_2SO_4), and concd under reduced pressure affording 40.0 g (66.0%) of crude acid **13**, ir (neat, cm^{-1}) 3065, 3030, 1600, 1490, 1450 (Ar), 1700 (CO). The double bond ir absorption bands (1610) for starting **5** were absent. The liquid was distd and the fraction bp 150–160° (0.1 mm) was collected. Some decomposition occurred during distillation. Glpc using a column described for **6** with column temp 200°, injection port temp 310°, detector temp 280°, inlet pressure at 40 psi, and carrier gas (He) flow rate of 60 ml/min showed two peaks at 2.0 min (trans) and 2.5 min (cis) in a ratio of 1.0:1.0. All attempts to separate these isomers failed and the mixt was used without further purification in subsequent reactions.

An Alternative Synthesis for 13. To a mixt of 10.0 g (0.10 mole) of **5** and 14.0 g (0.113 mole) of commercial $PhCH_2SH$ in 50 ml of dry C_6H_6 was added 13.0 g (0.15 mole) of piperidine. The soln was refluxed for 18 hr with constant stirring, cooled, and concd under reduced pressure. The residual oil was distd and the fraction bp 150–160° (0.1 mm) was collected affording 10.0 g (45%) of colorless **11**. Glpc under conditions described previously show a trans to cis ratio of 5.5:4.5. Impurities totaling 20% were uncharacterized. This product was considerably less pure than the product obtained when a 75-W spot light was employed.

***cis*- and *trans*-2-Benzylmercapto-1-carbethoxycyclobutane (14).** A soln of 40.0 g (0.18 mole) of the acid **13** (obtained from the photochemical reaction) dissolved in 200 ml of abs EtOH was refluxed with stirring in the presence of 5 ml of concd HCl for 4 hr. The soln was cooled, poured into ice-H_2O, and extd (Et_2O). The Et_2O soln was washed with H_2O and $NaHCO_3$ soln, dried (Na_2SO_4), and concd under reduced pressure. The residual oil was distd and the fraction bp 112–120° (0.05 mm) was collected affording 35.0 g (77%) of colorless liquid. Glpc under conditions described for compd **13** showed two peaks at 1.9 min (trans) and 2.2 min (cis) in a 1.0:1.0 ratio. All attempts to separate these isomers employing physical methods failed. Chemical methods resulting in the prepn of pure isomers are outlined in this section.

Synthesis of Pure *trans*-2-Benzylmercapto-1-carboxylic Acid Hydrazide (15) and *cis*-2-Benzylmercapto-1-carbethoxycyclobutane (14). The cis–trans mixt of esters **14** (45.0 g, 0.18 mole) was added dropwise to 12 g (0.28 mole) of hydrazine hydrate which was maintained at 130–135°. The mixt was refluxed for an additional 4 hr. Upon cooling trans hydrazide **15** crystd. The compd was filtd, washed with hot petr ether, and recrystd from aqueous EtOH (1:1) affording 20.0 g (94%) of pure trans hydrazide **15**, mp 89–90°, ir (KBr, cm^{-1}) 3275 (NH_2), 1630 (CO), and 1520 (NH bending). Glpc under conditions described for compd **13** showed 1 peak at 4.7 min. *Anal.* (*trans*-$C_{12}H_{16}ON_2S$, **15**) C, H, S; N: calcd, 11.9; found, 12.6.

The combined filtrates resulting from filtration and washing of crude *trans*-**15** were acidified with dil HCl and extd (Et_2O). The Et_2O extract was washed (H_2O), dried (Na_2SO_4), and concd under reduced pressure affording 15.0 g of cis ester **14** which contained by glpc analysis 1% trans isomer. This liquid distd at 118–120° (0.025 mm). *Anal.* (*cis*-$C_{14}H_{18}O_2S$) H; C: calcd, 67.2; found, 66.7; S: calcd, 12.8; found, 13.5.

The cis ester **14** (15.0 g, 0.06 mole) was added dropwise to 10.0 g (0.20 mole) of hydrazine hydrate kept at 130°. After the addn was complete, the reaction mixt was further heated at 130° for 18 hr, cooled, and stored at 0–5° overnight. The resulting semisolid could not be crystd. However, this compd was purified by dissolving in $HCCl_3$ followed by ppt with petr ether. White crystals of cis hydrazide **15**, mp 80–81°, were obtained from Et_2O–petr ether after the above purification procedure. Glpc under conditions described for the trans compd showed 1 peak at 6.0 min. The crystalline sample gas chromatographed with a retention time identical with that of the liquid material; the liquid also showed one peak by glpc.

***trans*-2-Benzylmercapto-1-carbethoxycyclobutane (14).** A pure sample of this compd was obtained utilizing 3 different routes.

1. Base-Catalyzed Isomerization of the Cis–Trans Mixture 13. A soln of 2.0 g (0.009 mole) of *cis*- and *trans*-**13** (1:1) in 50 ml of abs EtOH was treated with 4.0 g (0.071 mole) of KOH and refluxed for 18 hr. The reaction flask was cooled, diluted (H_2O), and acidified (HCl). The aqueous EtOH soln was extd (Et_2O) and the Et_2O layer washed with H_2O, dried (Na_2SO_4), and concd under reduced pressure affording 2.0 g (100%) of a liquid, bp 155–156° (0.2 mm). Glpc under conditions previously described for cis–trans acid **13** showed 1 peak at 2.0 min for *trans*-**13**.

Treatment of 2.0 g (0.008 mole) of *trans*-**13** by refluxing in 25 ml of abs EtOH containing a few drops of concd HCl afforded, after the usual work-up, 1.5 g (67%) of trans ester **14**, bp 130–132° (0.3 mm). Glpc under conditions previously described for cis–trans ester **14** showed 1 peak at 1.9 min.

2. Reaction of *trans*-2-Mercapto-1-carbethoxycyclobutane (7) with Benzyl Chloride. Trans ester **7** (2.0 g, 0.0125 mole) was dissolved in 20 ml of dry Me_2CO ($CaCl_2$ overnight) and K_2CO_3 (1.75 g, 0.013 mole) was added. The mixt was stirred for 10 min at room temp; 1.6 g (0.013 mole) of $PhCH_2Cl$ was added dropwise. The mixt was heated at gentle reflux for 6 hr, cooled, filtd, and extd (Et_2O). The Et_2O layer repeatedly was washed with H_2O and aqueous 10% NaOH, dried (Na_2SO_4), and concd under reduced pressure affording 3.0 g (96%) of a colorless liquid, bp 130–132° (0.3 mm) identical in all respects with the trans ester **14** prepared by other routes.

3. Conversion of *trans*-2-Benzylmercaptocyclobutanecarboxhydrazide (15) to *trans*-2-Benzylmercapto-1-carbethoxycyclobutane (14). The trans hydrazide **15** (10.0 g, 0.042 mole) was treated with 25 ml of 4 *N* aqueous HCl in 50 ml of EtOH. The soln was refluxed for 24 hr, cooled, diluted with H_2O, and extd (Et_2O). The Et_2O layer repeatedly was washed with H_2O and aqueous $NaHCO_3$, dried (Na_2SO_4), and concd under reduced pressure affording after distn 9.5 g (90%) of a colorless liquid, bp 130–132° (0.3 mm), identical in all respects with trans ester **14** prepared by other methods. *Anal.* (*trans*-$C_{14}H_{18}O_2S$) C, H, S.

***trans*-2-Benzylmercapto-1-aminocyclobutane (18).** A soln of 7.0 g (0.1 mole) of $NaNO_2$ in 25 ml of H_2O was added with stirring to a soln of 10.0 g (0.042 mole) of trans hydrazide **15** in 100 ml of Et_2O–$HCCl_3$ (1:1). The mixt was cooled (ice bath) to 0–5° and 20 ml of 6 *N* HCl was added dropwise with vigorous stirring. After the addn the mixt was allowed to stand at 0–5° for 10 min. The Et_2O layer was sepd and the aqueous phase was washed with Et_2O. The combined Et_2O layers were washed (cold H_2O), dried (Na_2SO_4), and concd under reduced pressure to 50 ml. Dry toluene (100 ml) was added to the Et_2O soln and the Et_2O was removed under reduced pressure, ir (toluene, cm^{-1}) 2150 for azide **16.**

The toluene soln was refluxed for 1 hr. After this time the ir absorption band at 2150 cm^{-1} was absent and a new band at 2250 cm^{-1} (isocyanate **17**) formed. The toluene was removed under reduced pressure and abs EtOH was added to the remaining brown oil. The resulting urethane was hydrolyzed by addition of 9.0 g (0.16 mole) of KOH in 100 ml of H_2O followed by refluxing with stirring for 24 hr. The soln was cooled, dild (H_2O), and extd (Et_2O). The Et_2O layers were dried (Na_2SO_4), and the amine purified as the HCl salt. The free base was obtained and distd, affording 5.2 g (69%) of colorless liquid, bp 125–130° (1.5 mm), ir (neat, cm^{-1}) 3360 (NH), 1600 (NH bending), 1490, 1450 (Ar). Glpc using a column described for compd **6** with column temp 160°, injection port temp 270°, detector temp 260°, inlet pressure of 40 psi, and carrier gas (He) flow rate of 60 ml/min showed one peak at 3.12 min; HCl salt mp 136–137°. The trans amine was analyzed as the acetamido derivative, which was prepared by conventional methods. Crystn from MeOH afforded white needles, mp 94–95°. *Anal.* (*trans*-$C_{13}H_{17}ONS$) C, H, N, S.

***cis*-2-Benzylmercapto-1-aminocyclobutane (18)** was prepared from *cis*-**15** by the method described for the trans isomer affording 3.0 g (40%) colorless oil, bp 103–105° (0.04 mm). In this case the cis isocyanate **17** absorbs at 2260 cm^{-1}. Glpc under conditions identical with those previously described for the trans isomer afforded 1 peak at 3.5 min; HCl salt mp 178–179°. This cis amine was similarly analyzed as the acetamido derivative. *Anal.* ($C_{13}H_{17}ONS$) C, H, N, S.

***cis*-2-Mercaptocyclobutylamine (2) Hydrochloride.** Debenzylation of *cis*-**18**·HCl was achieved by a method similar to the one described by Carroll and coworkers.[14b]

In a 250-ml, three-neck flask equipped with a stirrer, a gas inlet tube, and a Dry Ice condenser was placed 3.0 g (0.013 mole) of *cis*-**18**·HCl. Dry liquid NH_3 (100 ml) was introduced and the system was protected against moisture by providing a $CaCl_2$ tube. Na metal was added in small pieces until a permanent blue color persisted for 45 min. The Na (1.10 g, 0.0465 g-atom), in small pieces, was added while dry N_2 was blown over the soln. The reaction was contd for another 2 hr. The excess Na was decompd by adding a small

portion of NH_4Cl and the NH_3 was evapd after adding 100 ml of dry Et_2O and heating the mixt gently over a hot water bath. The stirred Et_2O suspension was cooled, 100 ml of dry Et_2O satd with gaseous HCl was added, and the contents were stirred for 1 hr. The solids were filtered, washed with dry Et_2O, and treated with dry *i*-PrOH. The alcohol soln was concd to 25 ml under reduced pressure, dry Et_2O added, and crystn induced in an ice bath (0–5°). The crude product was filtd and 3 crystn from *i*-PrOH–Et_2O afforded 1.6 g (84%) of analytically pure *cis*-**2**·HCl as a white crystalline solid, mp 105–106°. *Anal.* ($C_4H_{10}NSCl$) C, H, S, N: calcd, 10.03; found, 10.72.

trans-**2-Mercaptocyclobutylamine (2) Hydrochloride.** *trans*-**2** was prepd from *trans*-**18**·HCl by the method described for the cis isomer. This afforded 1.7 g (90%) of analytically pure *trans*-**2**·HCl as a white crystalline solid, mp 126–128°. *Anal.* ($C_4H_{10}NSCl$) C, H, N, S.

Biological studies were carried out using adipose tissue from non-fasted, white male Harlan Wistar rats according to methods previously published.[21] All drug concentrations were added in 0.1 ml of aqueous solution. β-Mercaptoethylamine hydrochloride was purchased from Aldrich Chemical Co., Milwaukee, Wis., and used in these studies without further purification.

References

(1) D. T. Witiak, B. K. Sinha, O. S. Lee, and D. R. Feller, 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972, Abstract MEDI 9.
(2) W. O. Foy, "Medicinal Chemistry," A. Burger, Ed., Wiley, New York, N. Y., 1970, Chapter 66; "Radiation Protection and Sensitization," H. Moroson, Ed., Sloan-Kettering Institute for Cancer Research, Rye, N. Y., 1970; "Marcello Quintiliani," Instituto Superiore di Sanita, Rome, Barnes and Noble, Inc., New York, N. Y., 1970.
(3) (a) P. Alexander and A. Charlesby, *Nature (London)*, **173**, 578 (1954); (b) B. Smaller and C. E. Avery, *ibid.*, **183**, 539 (1959).
(4) E. Y. Graevsky, I. M. Shapiro, M. M. Konstantinova, and N. F. Barakina, *Initial Eff. Ioniz. Radiat. Cells, Proc. Int. Symp.*, **1960**, 237 (1961).
(5) L. Eldjarn and A. Pihl, *J. Biol. Chem.*, **225**, 499 (1957).
(6) D. E. Potter and E. J. Walaszek, *Pharmacology*, **5**, 359 (1971).
(7) D. H. Yu, S. Triester, and M. I. Gluckman, *Pharmacologist*, **13**, 277 (1971).
(8) D. T. Witiak and M. C. Lu, *J. Org. Chem.*, **35**, 4209 (1970).
(9) A. Combell and H. N. Rydon, *J. Chem. Soc.*, 3002 (1953).
(10) (a) E. Walton, A. F. Wagner, F. W. Bachelor, L. H. Peterson, F. W. Holly, and K. Folkers, *J. Amer. Chem. Soc.*, **77**, 5144 (1955); (b) F. G. Bordwell and W. A. Hewett, *ibid.*, **79**, 3493 (1957).
(11) W. E. Parham and E. L. Anderson, *ibid.*, **70**, 4187 (1948).
(12) E. R. Buchman, A. O. Reims, T. Skei, and M. J. Schlatter, *ibid.*, **64**, 2696 (1942).
(13) (a) T. Curtius, *Ber.*, **23**, 3023 (1890); (b) P. A. S. Smith, *Org. React.*, **3**, 337 (1946); (c) T. Curtius, *J. Prakt. Chem.*, **50**, 275 (1894).
(14) (a) L. Field and P. M. Gilles, Jr., *J. Med. Chem.*, **13**, 317 (1970); (b) F. I. Carroll, J. D. White, and M. E. Wall, *J. Org. Chem.*, **28**, 1236 (1963).
(15) J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 800 (1952).
(16) (a) R. Gelin, S. Gelin, and C. Boutin, *C. R. Acad. Sci. Ser. C*, **262**, 1084 (1966); (b) J. P. Li, J. H. Biel, D. R. Van Harken, L. T. Harmanos, C. W. Dixon, and H. D. Taylor, *J. Med. Chem.*, **13**, 858 (1970).
(17) D. N. Calvert and J. J. Lech, *J. Pharmacol. Exp. Ther.*, **171**, 135 (1970).
(18) (a) D. H. Yu, S. Triester, and M. I. Gluckman, *ibid.*, **180**, 492 (1972); (b) D. H. Yu, S. Triester, and M. I. Gluckman, *ibid.*, **177**, 284 (1971).
(19) D. B. Stone, J. D. Brown, and A. A. Steele, *Metab. Clin. Exp.*, **18**, 620 (1969).
(20) (a) D. T. Witiak, D. R. Feller, E. S. Stratford, R. E. Hackney, R. Nazareth, and G. Wagner, *J. Med. Chem.*, **14**, 754 (1971); (b) D. T. Witiak, E. S. Stratford, R. Nazareth, G. Wagner, and D. R. Feller, *ibid.*, **14**, 758 (1971).
(21) A. Schwartz and A. H. Laseter, *Biochem. Pharmacol.*, **13**, 337 (1964).
(22) G. Fassina, I. Maragno, and P. Dorigo, *Biochem. Pharmacol.*, **16**, 1439 (1967).

Antimalarials. 2. 2,6-Bis(aryl)-4-pyridinemethanols†,[1]

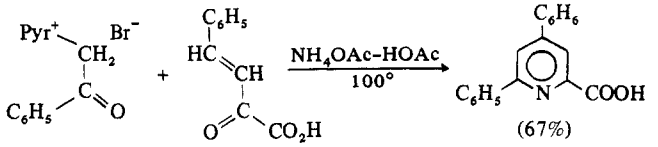
P. Blumbergs,* M. P. LaMontagne, A. Markovac, J. G. Moehring, A. B. Ash, and C. L. Stevens

Ash Stevens Inc., Detroit, Michigan 48202. *Received January 21, 1972*

A series of 2,6-bis(aryl)-4-pyridinemethanols, where aryl is substituted phenyl, were prepared and screened for antimalarial activity. Substituents in the two phenyl rings included Cl, Br, F, and OCH_3 and 11 2,6-bis(phenyl)isonicotinic acids were prepared as starting materials. The amino alcohol side chain in the 4 position of the pyridine ring was varied to include a wide spectrum of α-alkyl(and di-alkyl)aminomethyl groups. Among the 33 compounds, 26 were curative, 3 were active, and 4 were inactive at 640 mg/kg against *Plasmodium berghei*. The 3 most active compounds were curative at 40 mg/kg and active at 20 mg/kg.

The work reported here evolved from a single lead in the World War II program wherein α-di-*n*-butylaminomethyl-2,6-diphenyl-4-pyridinemethanol (SN 10760) showed quinine indices of 1.0 and 3 against two malaria strains in the duck, although only 0.3 against *Plasmodium gallinaceum* in the chick.[2] It is interesting that the same compound (as WR 135642) in the Rane mouse screen‡ is noncurative, but active, at 640 mg/kg, admittedly against a different strain, *Plasmodium berghei*. Nevertheless, as will be shown, the data served to flag a lead which, by the introduction of electronegative substituents in the two phenyl rings and by varying the alkyl groups in the amino alcohol side chain, has resulted in candidate antimalarials of a high degree of activity against *Plasmodium berghei* as measured by the Rane test.‡

This paper will report the results for 29 2,6-bis(phenyl)-4-pyridinemethanols and 4 derivatives containing various substituents other than CF_3 in the two phenyl rings. The results for compounds bearing CF_3 groups on one or both phenyl rings are reported in a following paper.



Chemistry. Zecher and Krohnke[4] reported a ring-closure reaction for the preparation of variously substituted pico-

†This work was supported by the U. S. Army Medical Research and Development Command under Contract No. DADA17-69-C-9065. This is Contribution Number 991 from the Army Research Program on Malaria.

‡The antimalarial tests were performed by Dr. Leo Rane of the University of Miami.[3] See footnote *a*, Table IV. Testing results were supplied through the courtesy of Drs. Thomas R. Sweeney and Richard E. Strube of the Walter Reed Army Institute of Research.