Mesoionic Xanthine Analogs as Inhibitors of Cyclic AMP Phosphodiesterase

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Abstract □ Several derivatives of two mesoionic xanthine analogs, mesoionic thiazolo[3,2-*a*]pyrimidine-5,7-diones and mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine-5,7-diones, were synthesized and evaluated as inhibitors of cyclic AMP phosphodiesterase. A significant number of these compounds demonstrated theophylline-like activity.

Keyphrases □ Xanthine analogs, mesoionic—synthesized, evaluated for effect on cyclic AMP phosphodiesterase activity *in vitro* □ Thiazolo[3,2-*a*]pyrimidines, various—synthesized, evaluated for effect on cyclic AMP phosphodiesterase activity *in vitro* □ Thiadiazolo[3,2-*a*]-pyrimidines, various—synthesized, evaluated for effect on cyclic AMP phosphodiesterase activity *in vitro* □ Cyclic AMP phosphodiesterase—effect of various thiazolo- and thiadiazolo[3,2-*a*]pyrimidines *in vitro* □ Enzyme activity—cyclic AMP phosphodiesterase, effect of various thiazolo- and thiadiazolo[3,2-*a*]pyrimidines *in vitro* □ Structure–activity relationships—various thiazolo- and thiadiazolo[3,2-*a*]pyrimidines evaluated for effect on cyclic AMP phosphodiesterase activity *in vitro*

Methylated xanthines, such as theophylline, inhibit cyclic nucleotide phosphodiesterases, an effect believed to be responsible for their clinical utility (1). Structural modification of the xanthines has led to more potent and more selective inhibitors (2, 3). Although molecular modification of purines and pyrimidines continues to be extensively studied, mesoionic purinone analogs represent a virtually unknown class of heterocyclic compounds.

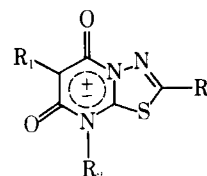
Mesoionic purinones are divided into several subclasses, *i.e.*, xanthine, hypoxanthine, and purin-2-one analogs, based on either five-membered ring (Class I) or six-membered ring (Class II) mesoionic systems (4). Mesoionic thiazolo[3,2-*a*]pyrimidine-5,7-diones (8-substituted anhydro-5-hydroxy-7-oxothiazolo[3,2-*a*]pyrimidinium hydroxides, I*a*–I*i*, Table I) and mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine-5,7-diones (8-substituted anhydro-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium hydroxides, II*a*–II*g*) are examples of Class II mesoionic xanthine analogs (5, 6).

Because of their close structural similarity with methylated xanthines, it was suggested previously (7) that such derivatives be evaluated as inhibitors of adenosine 3′,5′-monophosphate (cyclic AMP) phosphodiesterase. Coburn[1] found that several derivatives similar to II*a*–II*g* possess such activity.

The synthesis of 8-alkyl derivatives I*a*–I*i* is reported here, along with preliminary findings on their ability to inhibit cyclic AMP phosphodiesterase. For comparative purposes, the activities of II*a*–II*g* also were examined.

DISCUSSION

Compounds I*a*–I*i* (Table I) were prepared conveniently according to Scheme I. 2-Aminothiazole (III) was acylated using either an acid anhydride or an acyl chloride to give the 2-acylaminothiazoles IV*a*–IV*g*



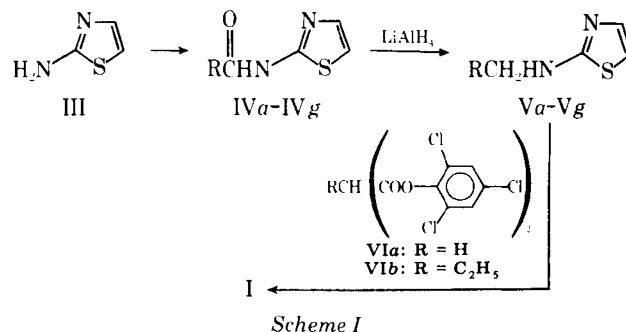
II*a*: $R_1 = R_2 = R_3 = CH_3$
II*b*: $R_1 = CH_3$, $R_2 = C_2H_5$, $R_3 = H$
II*c*: $R_1 = R_2 = C_2H_5$, $R_3 = H$
II*d*: $R_1 = CH_3$, $R_2 = C_3H_7$, $R_3 = H$
II*e*: $R_1 = CH_3$, $R_2 = CH(CH_3)_2$, $R_3 = H$
II*f*: $R_1 = CH_3$, $R_2 = CH(CH_3)CH_2CH_3$, $R_3 = H$
II*g*: $R_1 = CH_3$, $R_2 = C_5H_{11}$, $R_3 = H$

(Table II). Lithium aluminum hydride reduction of IV*a*–IV*g* afforded the 2-alkylaminothiazoles V*a*–V*g* (Table III), which were condensed with bis(2,4,6-trichlorophenyl) malonate ester VI*a* or VI*b* to yield the desired mesoionic thiazolopyrimidines (Table I).

Most phosphodiesterase preparations, including bovine heart phosphodiesterase, contain both high and low affinity enzymes or enzyme forms (2, 3). The preparations can be assayed at a low cyclic AMP concentration to observe effects on the high affinity enzyme and at a high concentration to observe inhibition of the low affinity enzyme (actually this high concentration reflects total enzyme activity). Initial results of inhibitor studies for I*a*–I*i*, II*a*–II*g*, and theophylline are given in Table IV.

Compounds I*c*, I*i*, II*c*, and II*e*–II*g* are theophylline like in inhibitor potency against both forms of the enzyme. Generally, I_{50} values obtained at the higher substrate concentration were two to three times greater than those obtained at the lower substrate concentration. Ethanol (2.5% final assay mixture concentration) was required to solubilize some inhibitors. The I_{50} values for theophylline in the absence and presence of ethanol were essentially the same, although the amount of ethanol utilized diminished enzyme activity by 15–25%.

For I*a*–I*i*, an alkyl substituent on the 6-position appears beneficial. The low order of activity of I*g* indicates that increasing the chain length of the 8-substituent does not necessarily increase activity; however, branching of the chain may be important (I*i*). Compound II*a*, the only compound with a 2-substituent other than hydrogen, was inactive. Increasing the chain length of the 8-substituent appears beneficial (II*g* > II*d* ≥ II*b*) as does branching (II*e* > II*d*). Interestingly, increasing the chain length of the 6-substituent from methyl (II*b*) to ethyl (II*c*) improved activity fourfold. These results parallel, somewhat, the results with the alkylated xanthines where increased chain length and branching at N-1 and N-3 were favorable, although alkyl chains at C-8 also increased activity; none of the mesoionic compounds thus far examined is as potent as the alkylated xanthines (2).



Scheme I

[1] R. A. Coburn, State University of New York at Buffalo, Amherst, N.Y., personal communication.

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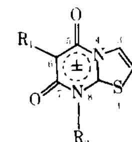
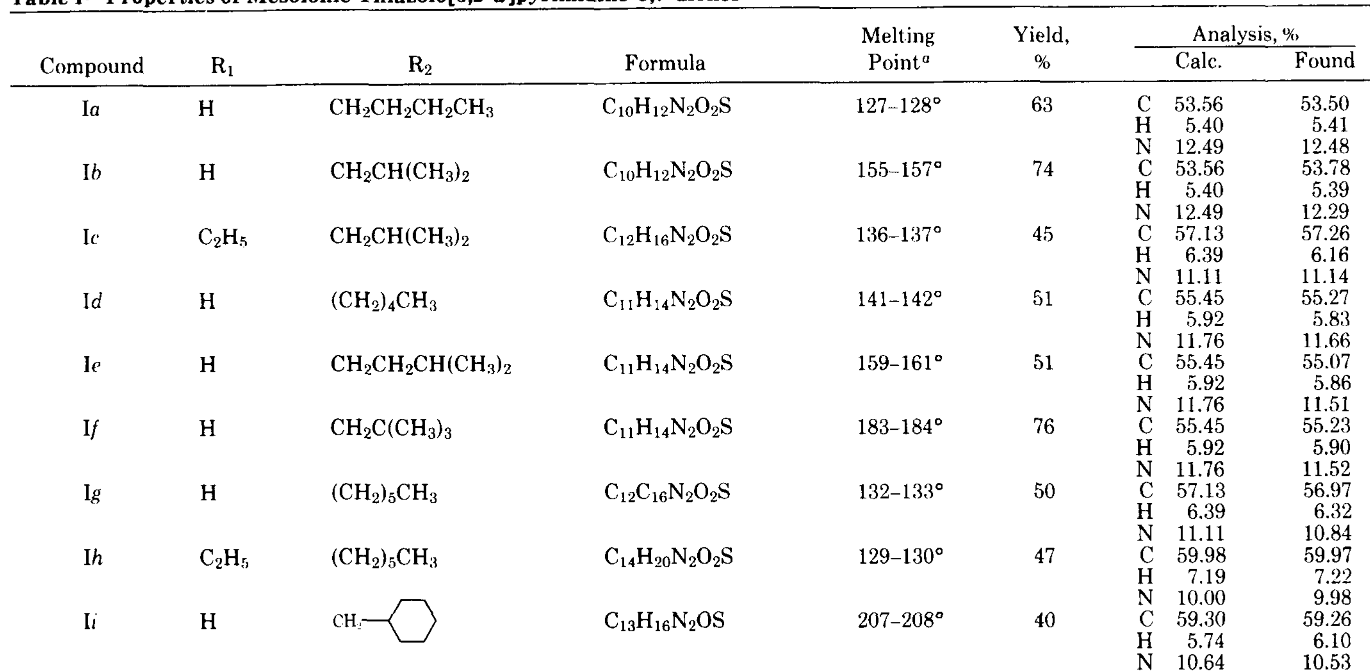


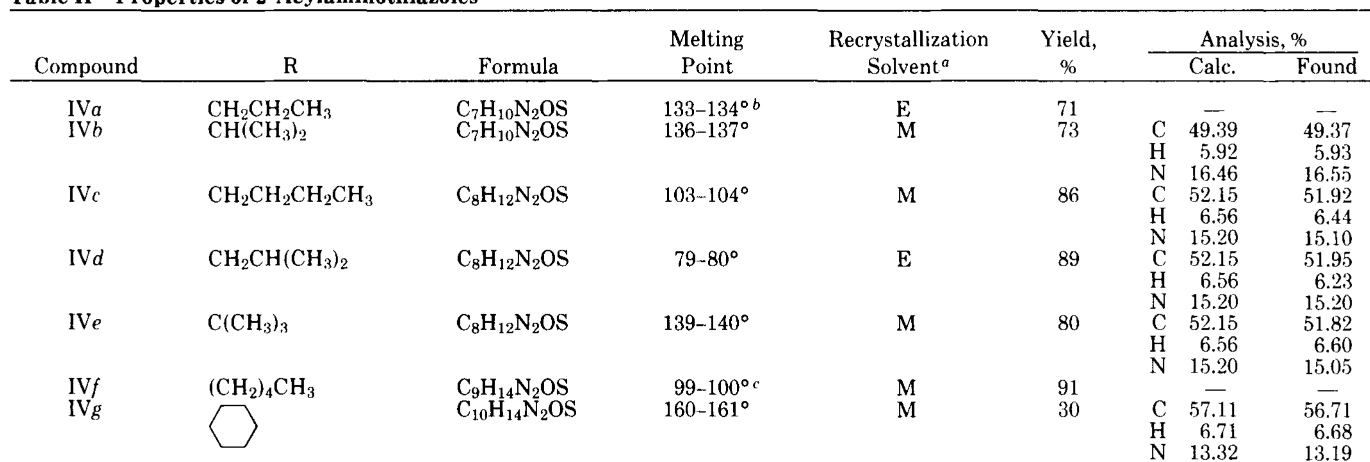
Table I—Properties of Mesoionic Thiazolo[3,2-*a*]pyrimidine-5,7-diones



Compound	R_1	R_2	Formula	Melting Point[a]	Yield, %		Analysis, % Calc.	Found
I*a*	H	$CH_2CH_2CH_2CH_3$	$C_{10}H_{12}N_2O_2S$	127–128°	63	C	53.56	53.50
						H	5.40	5.41
						N	12.49	12.48
I*b*	H	$CH_2CH(CH_3)_2$	$C_{10}H_{12}N_2O_2S$	155–157°	74	C	53.56	53.78
						H	5.40	5.39
						N	12.49	12.29
I*c*	C_2H_5	$CH_2CH(CH_3)_2$	$C_{12}H_{16}N_2O_2S$	136–137°	45	C	57.13	57.26
						H	6.39	6.16
						N	11.11	11.14
I*d*	H	$(CH_2)_4CH_3$	$C_{11}H_{14}N_2O_2S$	141–142°	51	C	55.45	55.27
						H	5.92	5.83
						N	11.76	11.66
I*e*	H	$CH_2CH_2CH(CH_3)_2$	$C_{11}H_{14}N_2O_2S$	159–161°	51	C	55.45	55.07
						H	5.92	5.86
						N	11.76	11.51
I*f*	H	$CH_2C(CH_3)_3$	$C_{11}H_{14}N_2O_2S$	183–184°	76	C	55.45	55.23
						H	5.92	5.90
						N	11.76	11.52
I*g*	H	$(CH_2)_5CH_3$	$C_{12}C_{16}N_2O_2S$	132–133°	50	C	57.13	56.97
						H	6.39	6.32
						N	11.11	10.84
I*h*	C_2H_5	$(CH_2)_5CH_3$	$C_{14}H_{20}N_2O_2S$	129–130°	47	C	59.98	59.97
						H	7.19	7.22
						N	10.00	9.98
I*i*	H	CH_2–⬡	$C_{13}H_{16}N_2OS$	207–208°	40	C	59.30	59.26
						H	5.74	6.10
						N	10.64	10.53

[a] Ethyl acetate was the recrystallization solvent.

Table II—Properties of 2-Acylaminothiazoles



Compound	R	Formula	Melting Point	Recrystallization Solvent[a]	Yield, %		Analysis, % Calc.	Found
IV*a*	$CH_2CH_2CH_3$	$C_7H_{10}N_2OS$	133–134°[b]	E	71		—	—
IV*b*	$CH(CH_3)_2$	$C_7H_{10}N_2OS$	136–137°	M	73	C	49.39	49.37
						H	5.92	5.93
						N	16.46	16.55
IV*c*	$CH_2CH_2CH_2CH_3$	$C_8H_{12}N_2OS$	103–104°	M	86	C	52.15	51.92
						H	6.56	6.44
						N	15.20	15.10
IV*d*	$CH_2CH(CH_3)_2$	$C_8H_{12}N_2OS$	79–80°	E	89	C	52.15	51.95
						H	6.56	6.23
						N	15.20	15.20
IV*e*	$C(CH_3)_3$	$C_8H_{12}N_2OS$	139–140°	M	80	C	52.15	51.82
						H	6.56	6.60
						N	15.20	15.05
IV*f*	$(CH_2)_4CH_3$	$C_9H_{14}N_2OS$	99–100°[c]	M	91		—	—
IV*g*	⬡	$C_{10}H_{14}N_2OS$	160–161°	M	30	C	57.11	56.71
						H	6.71	6.68
						N	13.32	13.19

[a] The recrystallization solvent was either 95% ethanol (E) or aqueous methanol (M). [b] Lit. (8) mp 128–130°. [c] Lit. (8) mp 102–103°.

These results must be tempered by the realization that the stabilities of these ring systems under the conditions of the enzyme assay and the measures taken to solubilize the inhibitors have not been fully investigated. These studies, as well as pharmacological studies, are in progress.

In conclusion, these limited data indicate that it may be possible to prepare new mesoionic xanthine analogs that will be significantly more potent than theophylline as cyclic AMP phosphodiesterase inhibitors.

EXPERIMENTAL[2]

***N*-(2-Thiazolyl)isobutyramide (IV*b*)**—Isobutyryl chloride (5.35 g, 50 mmoles) was added dropwise, with stirring, to a solution of 2-aminothiazole (5.0 g, 50 mmoles) and triethylamine (5.0 g, 50 mmoles) in 40 ml of tetrahydrofuran at 0°. After the addition, the reaction mixture was stirred at room temperature for 2 hr and was then filtered. The filtrate was dried with anhydrous sodium sulfate and evaporated *in vacuo* to yield an oil, which crystallized upon standing.

Recrystallizaion from aqueous methanol gave 6.2 g (72%) of IV*b* as white crystals, mp 136–137°; IR (KBr): 1690 cm^{-1}; NMR ($CDCl_3$): δ 1.35 (d, 6H, methyl protons), 2.75 (m, 1H, methine proton), 7.00 (d, 1H, thiazole proton), and 7.45 (d, 1H, thiazole proton) ppm. Table II lists the analytical data.

The 2-acylaminothiazoles IV*c*–IV*g* (Table II) were prepared in the same manner as IV*b*. Compound IV*a* was prepared using *n*-butyric anhydride in place of acyl chloride.

2-(Isobutylamino)thiazole (V*b*)—A solution of IV*b* (5.1 g, 30 mmoles) in 30 ml of tetrahydrofuran was added dropwise to a stirred suspension of lithium aluminum hydride (1.2 g, 32 mmoles) in 40 ml of tetrahydrofuran at 0°. After refluxing for 3 hr, water was added dropwise at 0° until hydrogen evolution ceased. The mixture was filtered, and the

[2] PMR spectra were obtained on a Perkin-Elmer R-24 spectrometer, and chemical shifts are reported relative to tetramethylsilane. IR spectra were obtained on a Perkin-Elmer 257 spectrophotometer. Microanalyses were performed at A. H. Robins, Richmond, Va. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Compounds II*a*–II*g* were prepared following literature procedures (7).

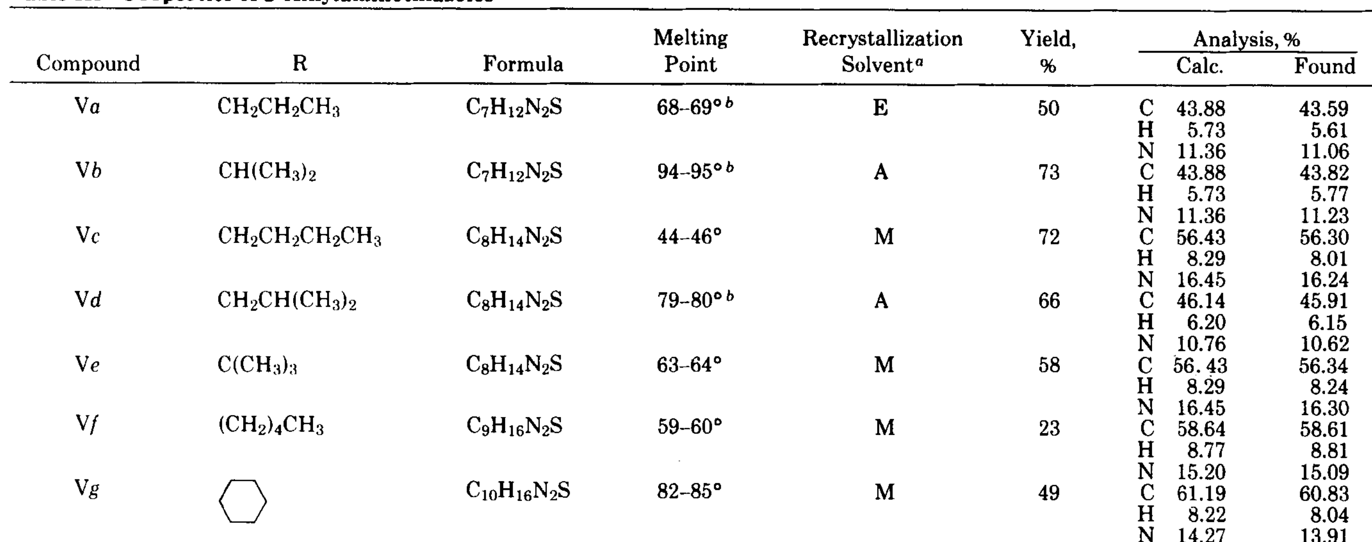


Table III—Properties of 2-Alkylaminothiazoles

Compound	R	Formula	Melting Point	Recrystallization Solvent[a]	Yield, %	Analysis, %		
							Calc.	Found
V*a*	$CH_2CH_2CH_3$	$C_7H_{12}N_2S$	68–69°[b]	E	50	C	43.88	43.59
						H	5.73	5.61
						N	11.36	11.06
V*b*	$CH(CH_3)_2$	$C_7H_{12}N_2S$	94–95°[b]	A	73	C	43.88	43.82
						H	5.73	5.77
						N	11.36	11.23
V*c*	$CH_2CH_2CH_2CH_3$	$C_8H_{14}N_2S$	44–46°	M	72	C	56.43	56.30
						H	8.29	8.01
						N	16.45	16.24
V*d*	$CH_2CH(CH_3)_2$	$C_8H_{14}N_2S$	79–80°[b]	A	66	C	46.14	45.91
						H	6.20	6.15
						N	10.76	10.62
V*e*	$C(CH_3)_3$	$C_8H_{14}N_2S$	63–64°	M	58	C	56.43	56.34
						H	8.29	8.24
						N	16.45	16.30
V*f*	$(CH_2)_4CH_3$	$C_9H_{16}N_2S$	59–60°	M	23	C	58.64	58.61
						H	8.77	8.81
						N	15.20	15.09
V*g*	(cyclohexyl ring)	$C_{10}H_{16}N_2S$	82–85°	M	49	C	61.19	60.83
						H	8.22	8.04
						N	14.27	13.91

[a] The recrystallization solvent was 95% ethanol (E), aqueous methanol (M), or absolute ethanol (A). [b] Melting points and analytical data for V*a*, V*b*, and V*d* are for the hydrogen oxalate salt.

Table IV—Inhibition of Phosphodiesterase[a]

Compound	I_{50}, μM	
	1 μM cAMP[b]	100 μM cAMP[b]
I*a*	(30)[c]	(25)[c]
I*b*	792	1888
I*c*	498	928
I*d*	(36)[c]	(20)[c]
I*e*	—	(34)[c]
I*f*	—	(14)[c]
I*g*	1139[d]	1802[d]
I*h*	—	(29)[e]
I*i*	307[d]	882[d]
II*a*	—	(1)[c]
II*b*	828	1463
II*c*	208	444
II*d*	711	1709
II*e*	245	840
II*f*	338	1131[d]
II*g*	300	571
Theophylline	306 (349[d])	917

[a] Bovine heart phosphodiesterase was used. [b] Substrate concentration. [c] Values in parentheses are the percent inhibition obtained at an inhibitor concentration of 500 μM; insufficient sample was available to determine the I_{50}. [d] Determined in the presence of 2.5% ethanol needed to solubilize inhibitors. [e] Same as c with inhibitor concentration at 250 μM.

filtrate was dried with anhydrous sodium sulfate. Removal of solvent *in vacuo* gave 3.4 g of an oil, which failed to crystallize. Dropwise addition of an ethereal solution of the oil to a solution of oxalic acid (1.96 g) in 50 ml of anhydrous ether at 0° resulted in a quantitative yield of the oxalate salt. Recrystallization from absolute ethanol gave the oxalate salt of V*b* as small white crystals, mp 94–95°.

The 2-alkylaminothiazoles V*a*–V*g* (Table III) were prepared in the same manner as V*b*.

Anhydro-5-hydroxy-7-oxo-8-isobutylthiazolo[3,2-*a*]pyrimidinium Hydroxide (I*b*)—Compound V*b* (0.55 g, 3.6 mmoles) and bis(2,4,6-trichlorophenyl) malonate (VI*a*) (9) (1.66 g, 3.6 mmoles) were heated neat at 160°, under a slow stream of nitrogen, until a clear melt resulted (~3 min). When cool, the resultant oil was triturated with 20 ml of anhydrous ether; the crude product crystallized and was collected.

Recrystallization from ethyl acetate gave 0.6 g (74%) of I*b* as off-white crystals, mp 155–157°; IR (KBr): 1630 cm^{-1}; NMR ($CDCl_3$): δ 0.80–1.28 (m, 7H), 3.84 (d, 2H, CH_2), 5.11 (s, 1H, C-6 H), 7.05 (d, 1H, thiazole proton), and 8.13 (d, 1H, thiazole proton) ppm. Table I lists the analytical data. Compounds I*a*–I*i* (Table I) were prepared in the same manner as I*b* with either VI*a* or VI*b* (9).

Enzyme Assays—The assay of Klee (10) was used, employing bovine heart phosphodiesterase[3], cyclic 8-^{3}H-adenosine monophosphate[4] (specific activity of 30 Ci/mmole), and ^{14}C-adenosine 5′-monophosphate[5] (specific activity of 570 Ci/mmole).

Ion-exchange resin[6], 200–400 mesh, H$^+$ form, was suspended in water; enough slurry was poured into disposable polypropylene columns[7] (0.7 × 4 cm) to provide a column volume of 1 ml. Prior to use, the columns were washed with 4 ml of 1 *N* NaOH, 4 ml of 1 *N* HCl, and 8 ml of water. The reaction mixtures (0.2 ml) contained routinely 3.8 μg of protein (8 × 10^{-4} enzyme unit) at the high substrate concentration or 1.3 μg of protein (2.7 × 10^{-4} enzyme unit) at the low substrate concentration along with 0.05 *M* tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 8.0), 1 m*M* disodium ethylenediaminetetraacetate, 3 m*M* $MgCl_2$, and 1 × 10^{-6}–10^{-4} *M* (150,000–200,000 cpm) ^{3}H-cyclic AMP. Incubation at 37° was started by addition of enzyme, and the reaction was stopped after 20 min by addition of 1 ml of 5% trichloroacetic acid containing 1 m*M* cyclic AMP [1 m*M* ^{14}C-5′-AMP (2000–3000 cpm)]. ^{14}C-5′-AMP was added to permit estimation of the recovery of AMP after chromatography and correction of assay values for losses.

The sample was applied to the tops of the columns, which were then washed twice with 1 ml of water and three times with 3 ml of water. The eluate from these washes was discarded. Then 5′-AMP was eluted with 4 ml of 0.4 *M* sodium citrate (adjusted to pH 7.5 with citric acid), and the 4 ml was collected directly into a scintillation vial. The fraction containing 5′-AMP was counted after addition of 12 ml of scintillation fluor[8]. The enzyme was shown to contain both high and low K_m activities comparable to those reported (11). Initial rate measurements were linear with enzyme concentration and time (to 40 min).

The I_{50} values were determined by plotting uninhibited velocity/inhibited velocity (V_0/V) *versus* the inhibitor concentration. The I_{50} is the inhibitor concentration when $V_0/V = 2$. Five different inhibitor concentrations, giving 25–75% inhibition, were used for each inhibitor.

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GLC Determination of Tilidine, Nortilidine, and Bisnortilidine in Biological Fluids with a Nitrogen-Sensitive Detector

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Received January 30, 1978, from the *Department of Biochemistry, Gödecke Research Institute, D-7800 Freiburg, West Germany.* Accepted for publication April 25, 1978.

Abstract □ A sensitive and specific GLC method is described to determine therapeutic levels of tilidine and its two main metabolites, nortilidine and bisnortilidine, in plasma and urine. The method involves the extraction of the compounds and an internal standard with cyclohexane from alkalinized samples, followed by back-extraction into 1 *N* HCl. The hydrochloric acid solution is evaporated to dryness. After liberation of the free bases with ammonia, the residue is subjected to GLC analysis with a nitrogen-phosphorus detector and a 1.8-m (6-ft) glass column packed with 1% CRS 101 and 1.5% LAC-4-R-886 on Gas Chrom Q. Sensitivity in plasma and urine is ~1 ng/ml for a 5-ml sample.

Keyphrases □ Tilidine and metabolites—GLC analyses in biological fluids □ GLC—analyses, tilidine and metabolites in biological fluids □ Analgesics—tilidine and metabolites, GLC analyses in biological fluids

Tilidine hydrochloride[1] [ethyl DL-*trans*-2-(dimethylamino)-1-phenyl-3-cyclohexene-1-carboxylate hydrochloride] is a potent analgesic (1). Tilidine is metabolized rapidly to form the corresponding *N*-desmethyl- and *N*-bis(desmethyl) derivatives (2). A specific and sensitive analytical method was needed for the quantitative determination of tilidine and its two main metabolites, nortilidine and bisnortilidine, in biological fluids to study their kinetics in the body.

A GLC method (3) was used in previous pharmacokinetic studies, but the GLC column exhibited adsorption of bisnortilidine after a short time. A modified GLC column, well suited for the analytical control of the substances, was described recently (4).

This paper reports a new, sensitive, and selective GLC assay for tilidine, nortilidine, and bisnortilidine in plasma and urine. A thermoionic detector and a column packed with 1% CRS and 1.5% LAC-4-R-886 on Gas Chrom Q are used. The advantages of this method in comparison to the previous method are improved column stability, increased

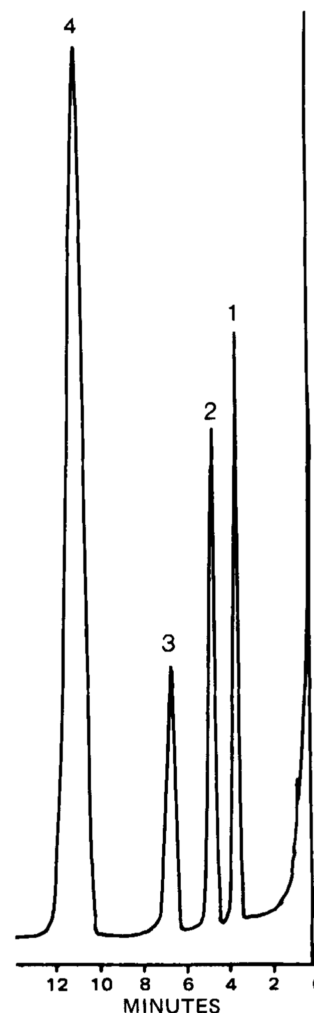

Figure 1—*Chromatogram of standards directly injected onto the columns. Key: 1, 20 ng of tilidine; 2, 20 ng of nortilidine; 3, 20 ng of bisnortilidine; and 4, 150 ng of internal standard. Attenuation was 16 × 1.*

[1] Valoron, Gödecke AG, Freiberg, West Germany (IND approved in the United States).

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