Table I—Protocol, Mean Animal Weight, and Food and Fluid Daily Intake

Group	Protocol	Animal Weight, g (LSD = ±86.9)	Food Intake, g/animal/day (LSD = ±4.72)	Fluid Intake, ml/animal/day (LSD = ±26.1)
I	Scorbutic, no galactose	363.1	53.44[a]	190.8[a]
II	Scorbutic with galactose	395.0	47.36	67.6[a]
III	Ascorbic acid with galactose	462.7[a]	48.16	111.8[a]

[a] Significant at $p = 0.05$.

I. All animals were fed a diet[1] lacking ascorbic acid. Group II animals received galactose as a 10% solution as their drinking supply, while the drinking supply of Group III animals contained both galactose (10%) and ascorbic acid (1%). Group I animals received tap water. Fresh solutions were prepared each day, and daily food and fluid consumption were recorded.

Throughout the 25 days, the animals were weighed and examined with an ophthalmoscope every other day. One drop of 0.25% atropine sulfate ophthalmic solution was applied to the ocular conjunctiva as a mydriatic. The presence or absence of cataract formation was recorded. When cataract formation was present, appropriate drawings were made to depict their extent and rate of change. Food and fluid consumption and animal weight data were subjected to least significant difference analysis of variance.

RESULTS AND DISCUSSION

By Day 7 of the study, two animals from Group II had faint rings on the periphery of both lenses. By Day 9, all animals in Group II had rings on the periphery of the lens, indicative of marginal cataracts (Fig. 1A). At the end of 25 days, these peripheral rings had progressed slightly to a more coarse and thicker opacity (Fig. 1B). At Day 25, none of the animals from Group I or III showed any signs of cataract formation (Fig. 1C). The daily food and fluid intake of Group II guinea pigs was less than that of the other two groups, yet their mean weight was not significantly different.

The appearance of cataracts in both eyes of all animals fed galactose and placed on a scorbutic diet and the absence of cataracts in any of the control groups indicate that ascorbic acid has a retarding or delaying effect on the development of galactose cataracts in the guinea pig.

[1] Charles River rabbit diet.

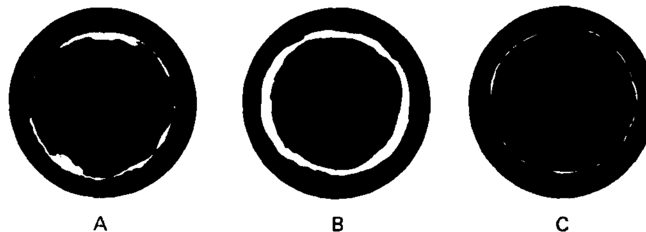

Figure 1—*Drawings of lenses illustrating the effect of ascorbic acid on the formation of galactose cataracts in the guinea pig.*

While it has been shown that rats have ample galactose 1-phosphate uridyl transferase (11), there have been no reports on the level of galactose 1-phosphate uridyl transferase in the guinea pig. Moreover, lens aldol reductase activity has been studied in rats (12, 13) but not guinea pigs.

Further investigations are being conducted in these laboratories on guinea pig galactose 1-phosphate uridyl transferase and lens aldol reductase activity in relation to galactosemic cataract formation. The present study demonstrates that the guinea pig is a suitable model for galactosemic cataract investigations, which is desirable considering the similarity between humans and the guinea pig with regard to the dietary requirement for ascorbic acid.

REFERENCES

(1) H. S. Mitchell and W. M. Dodge, *J. Nutr.*, **9**, 37 (1935).
(2) J. W. Patterson, *Am. J. Ophthalmol.*, **36** (6)II, 143 (1953).
(3) Z. Dische and E. Borenfreund, *ibid.*, **38**, 165 (1954).
(4) R. van Heyningen, *Nature (London)*, **184**, 194 (1959).
(5) J. H. Kinoshita, *Invest. Ophthalmol.*, **13**, 713 (1974).
(6) S. D. Varma and J. H. Kinoshita, *Exp. Eye Res.*, **19**, 577 (1974).
(7) N. K. Monjukowa and M. J. Fradkin, *Arch. Ophthalmol.*, **133**, 328 (1935).
(8) H. Tojo, *Acta Soc. Ophthalmol. Jpn.*, **58**, 556 (1954).
(9) S. D. Varma, E. Mikuni, and J. H. Kinoshita, *Science*, **188**, 1215 (1975).
(10) J. D. Heffley and R. J. Williams, *Proc. Natl. Acad. Sci. USA*, **71**, 4164 (1974).
(11) S. Kelly and L. DesJardins, *Proc. Soc. Exp. Biol. Med.*, **138**, 545 (1971).
(12) S. D. Varma and J. H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976).
(13) A. B. Segelman, F. P. Segelman, S. D. Varma, H. Wagner, and O. Seligman, *J. Pharm. Sci.*, **66**, 1358 (1977).

Quinazolinylformamidines and Quinazolinediylbisformamidines as Antihypertensive Agents

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Received October 12, 1977, from the *Chemical Research Department, Lederle Laboratories, American Cyanamid Co., Pearl River, NY 10965.* Accepted for publication February 9, 1978.

Abstract □ Eleven quinazolinylformamidines and quinazolinediylbisformamidines were synthesized and investigated for antihypertensive activity in spontaneous hypertensive rats. Several compounds showed moderate antihypertensive activity at 100 mg/kg po. The same compounds were not hypotensive in the normotensive dog.

Keyphrases □ Quinazolinylformamidines, substituted—synthesized, evaluated for antihypertensive activity in rats □ Antihypertensive activity—various substituted quinazolinylformamidines evaluated in rats □ Structure–activity relationships—various substituted quinazolinylformamidines evaluated for antihypertensive activity in rats

In an investigation of the potential antihypertensive activity of 2,4-diaminoquinazoline derivatives, the quinazolinediylbisformamidine (III) was observed to be a moderately active (25 mg/kg) antihypertensive agent when orally administered to spontaneously hypertensive rats (1). Further evaluation of III revealed it to be free of

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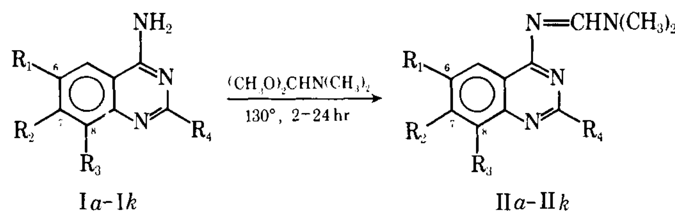
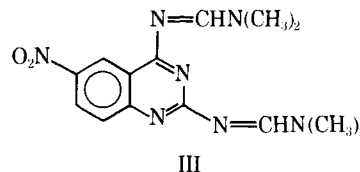
Table I—Substituted 2,4-Diaminoquinazolines

Compound	Substituents	Preparation Reference Number	Melting Point
I*a*	$R_1 = R_2 = R_3 = H, R_4 = NH_2$	3	249–251°
I*b*	$R_1 = NO_2, R_2 = R_3 = H, R_4 = NH_2$	3	>300°
Ic	$R_1 = Br, R_2 = R_3 = H, R_4 = NH_2$	4	188–190°
I*d*	$R_1 = R_4 = NH_2, R_2 = R_3 = H$	3	256–258°
I*e*	$R_1 = R_2 = CH_3O, R_3 = H, R_4 = NH_2$	5	238–240°
I*f*	$R_1 = Cl, R_2 = NO_2, R_3 = H, R_4 = NH_2$	4	—
I*g*	$R_1 = H, R_2 = Cl, R_3 = CH_3, R_4 = NH_2$	4	227–227.5°
I*h*	$R_1 = Cl, R_2 = R_3 = H, R_4 = NH_2$	4	265–267°
I*i*	$R_1 = NC, R_2 = R_3 = H, R_4 = NH_2$	3	>300°
I*j*	$R_1 = R_2 = R_3 = R_4 = H$	6	267–268°
I*k*	$R_1 = NO_2, R_2 = R_3 = R_4 = H$	6	319–320°



Scheme I



adverse side effects such as ataxia, sedation, agitation, or tachycardia at 25 mg/kg in the spontaneously hypertensive rat.

The only published biological activity for the quinazolinylformamidines or the quinazolinediylbisformamidines was the antiparasitic activity of a series of *N'*-(6-benzylamino-4-quinazolinyl) -*N,N*- dimethylformamidines (2).

These observations prompted the synthesis of 10 related formamidines and the investigation of their antihypertensive activity.

RESULTS AND DISCUSSION

Chemistry—The required starting materials, including the unsubstituted as well as the 10 substituted 2,4-diaminoquinazolines, were synthesized by routes analogous to published procedures. The substituents were either electron donating or withdrawing and attached to the benzene ring of the quinazoline nucleus at position 6, 7, or 8 (Table I).

The desired formamidines, II*a*–II*k* (Table II), were readily synthesized by the general procedure shown in Scheme I. Reaction of diaminoquinazolines I*a*–I*k* with excess dimethylformamide dimethyl acetal at 130°, either neat or in a dimethylformamide or dimethyl sulfoxide solution, yielded, after workup, II*a*–II*k* in 50–85% yield.

Biology—Formamidines II*a*–II*k* were administered orally in two doses of 100 mg/kg each to spontaneously hypertensive rats. The second dose was given 24 hr after the first. The mean arterial blood pressure was measured at 4 hr after the second dosing. All determinations were made in restrained, conscious animals by direct femoral puncture. The antihypertensive activity of the active formamidines in this series, together with the values for known reference agents, is listed in Table III.

Of the formamidines reported in Table II, II*a*, II*b*, II*e*, and II*i* decreased the mean arterial pressure of the spontaneously hypertensive rats at 100 mg/kg by 12–29%. The congeners II*a* and II*b* were the most

Table II—Quinazolinylformamidines and Quinazolinediylbisformamidines

Compound	Substituents	Melting Point	Yield, %	Formula	Analysis, %	Calc.	Found
II*a*	$R_1 = R_2 = R_3 = H$, $R_4 = N{=}CHN(CH_3)_2$	142°	80	$C_{14}H_{18}N_6$	C	61.03	61.20
					H	6.70	6.71
					N	30.88	31.09
II*b*	$R_1 = NO_2, R_2 = R_3 = H$, $R_4 = N{=}CHN(CH_3)_2$	228°	60	$C_{14}H_{17}N_7O_2$	C	52.94	53.32
					H	5.34	5.44
					N	31.00	31.09
IIc	$R_1 = Br, R_1 = H_3 = H$, $R_4 = N{=}CHN(CH_3)_2$	204°	50	$C_{14}H_{17}BrN_6$	C	48.15	47.95
					H	4.91	4.79
					Br	22.88	23.26
					N	24.06	24.25
II*d*	$R_1 = R_4 = N{=}CHN(CH_3)_2$, $R_2 = R_3 = H$	173°	70	$C_{17}H_{18}N_8$	C	61.06	60.95
					H	5.43	5.35
					N	33.51	33.21
II*e*	$R_1 = R_2 = CH_3O, R_3 = H$, $R_4 = N{=}CHN(CH_3)_2$	216°	50	$C_{16}H_{22}N_6O_2$	C	56.62	56.72
					H	6.83	6.94
					N	24.76	24.52
II*f*	$R_1 = Cl, R_2 = NO_2, R_3 = H$, $R_4 = N{=}CHN(CH_3)_2 \cdot \frac{1}{2}H_2O$	209°	56	$C_{14}H_{16}ClN_7O_2 \cdot \frac{1}{2}H_2O$	C	46.47	47.09
					H	4.78	4.47
					Cl	9.88	10.26
					N	27.33	27.17
II*g*	$R_1 = H, R_2 = Cl, R_3 = CH_3$, $R_4 = N{=}CHN(CH_3)_2$	184°	69	$C_{15}H_{19}ClN_6$	C	56.51	56.51
					H	6.01	5.99
					Cl	11.12	11.10
					N	26.36	26.14
II*h*	$R_1 = Cl, R_2 = R_3 = H$, $R_4 = N{=}CHN(CH_3)_2$	218°	61	$C_{14}H_{17}ClN_6$	C	55.17	55.09
					H	5.62	5.62
					Cl	11.60	11.55
					N	27.60	27.93
II*i*	$R_1 = NC, R_2 = R_3 = H$, $R_4 = N{=}CHN(CH_3)_2$	270°	85	$C_{15}H_{17}N \cdot \frac{1}{2}H_2O$	C	59.20	59.54
					H	5.96	5.62
					N	32.22	32.20
II*j*	$R_1 = R_2 = R_3 = R_4 = H$	69°	75	$C_{11}H_{12}N_4$	C	66.00	66.20
					H	6.04	6.17
					N	28.00	28.20
II*k*	$R_1 = NO_2, R_2 = R_3 = R_4 = H$	153°	70	$C_{11}H_{11}N_5O_2$	C	53.90	53.80
					H	4.52	4.37
					N	28.60	28.90

Table III—Antihypertensive Quinazolinylformamides and Quinazolinediylbisformamidines

Compound	n	Mean Arterial Blood Pressure	Dose, mg/kg	Percent Decrease from Control
II*a*	2	131	100	21
II*b*	3	118	100	29
II*b*	3	129	25	22
II*e*	2	146	100	12
II*i*	2	139	100	16
II*i*	4	144	25	13
Guanabenz	20	123	25	26
Clonidine	6	96	0.5	43
Control	60	166	—	—

active of this series at the 100-mg/kg dose level. The most active congener was the 6-nitro derivative II*b*, while the least active congener was the 6,7-dimethoxy derivative II*e*. Of the active formamides, only II*b* (6-nitro) and II*i* (6-cyano) were active at the 25-mg/kg dose level in the spontaneously hypertensive rat. Congener II*b* was approximately twice as active as II*i*, a 29 *versus* 13% decrease in mean arterial blood pressure. Thus, quinazolinediylbisformamidines bearing an electron-withdrawing group at C-6 of the quinazoline nucleus apparently are more active than the corresponding quinazolinediylbisformamidines bearing electron-donating substituents.

When II*a*–II*k* were administered in gelatin capsules to normotensive dogs, no effect on blood pressure was observed with a single dose of 25 mg/kg. Bradycardia was observed at doses greater than 25 mg/kg, which prevented further evaluation at higher doses.

The lack of hypotensive activity of II*a*–II*k* in the normotensive dog precludes further development of the quinazolinediylbisformamidines.

EXPERIMENTAL

All melting points are uncorrected. Samples for elemental analysis were dried at 55° under high vacuum for 5–24 hr. The general procedure employed in the synthesis of the quinazolinylformamidines is as follows for N^1,N^3-dimethyl -N^2,N^4- (7-chloro-8-methyl-2,4-quinazolinediyl)bisformamidine (II*g*).

A solution of 2.1 g (10.0 mmoles) of 7-chloro-8-methyl-2,4-diaminoquinazoline in 20 ml of dimethylformamide dimethyl acetal and 20 ml of dimethylformamide was refluxed for 16 hr and then cooled to room temperature. Excess solvent was removed under aspirator pressure, and the solid residue was recrystallized twice from ethyl acetate to yield 2.2 g (69%) of light-yellow crystals, mp 182–184°.

Anal.—Calc. for $C_{15}H_{19}ClN_6$: C, 56.51; H, 6.01; Cl, 11.12; N, 26.36. Found: C, 56.51; H, 5.99; Cl, 11.10; N, 26.14.

Compounds II*a*–II*k* (Table II) were prepared by a similar procedure by condensing dimethylformamide dimethyl acetal with the appropriate I*a*–I*k*.

These quinazolinediylbisformamidines were characterized by elemental analyses and the presence of characteristic resonances at 3.0–3.3 ppm (methyl group protons at the terminal nitrogen atom of the formamidine moiety) in their PMR spectra.

REFERENCES

(1) P. S. Chan, D. Poorvin, M. A. Ronsberg, G. Vice, F. Beyer, and P. Scully, *Pharmacologist*, **17**, 253 (1975).

(2) E. F. Eislager and D. F. Worth (Parke-Davis), U.S. pat. 3,668,205 (1972).

(3) J. Davoll and A. M. Johnson, *J. Chem. Soc. C*, **1970**, 997.

(4) K. Schofield, *ibid.*, **1952**, 1927.

(5) F. H. S. Curd, J. K. Landquist, and F. L. Rose, *ibid.*, **1948**, 1759.

(6) J. S. Morley and J. Simpson, *J. Chem. Soc.*, **1949**, 1354.

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Quantitative Determination of Cephalexin in Cephradine by NMR Spectroscopy

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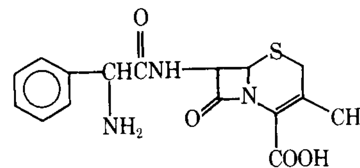
Abstract □ An NMR method to determine quantitatively the presence of cephalexin in cephradine was developed. The method is applicable to the chemical itself as well as to capsules and oral suspension formulations. The determination is based on the NMR signal arising from the five aromatic protons of the cephalexin molecule. Integration of this signal relative to a signal from cephradine provides the data necessary to determine the percentage of cephalexin present. The precision at the 2% cephalexin level is ±0.18%. The time required to carry out a single analysis is about 10 min, and five analyses can be done in about 0.5 hr.

Keyphrases □ Cephalexin—NMR spectroscopic analysis in cephradine bulk drug and dosage forms □ NMR spectroscopy—analysis, cephalexin in cephradine bulk drug and dosage forms □ Antibacterials—cephalexin, NMR spectroscopic analysis in cephradine bulk drug and dosage forms

Cephalexin (I) may be present in a given lot of cephradine (II) as an impurity from the synthesis of cephradine or as a decomposition product of cephradine. A rapid, quantitative method was required to determine the cephalexin content of cephradine chemical as well as capsule and oral suspension formulations. Present methods (1, 2) were too slow for large numbers of samples.



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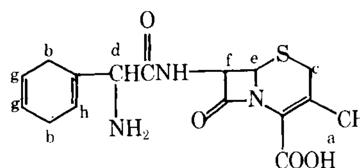

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