Table I—Dissolution of Cyproheptadine Hydrochloride

Dissolution Medium	Sample Identification	Peak[a] Area ($\times 10^6$)	Cyproheptadine Hydrochloride Available, %
Distilled water	USP reference standard	6.859	100
Distilled water	Cyproheptadine hydrochloride (used in tablet formulation)	6.410	93.4
Distilled water	Blank powder	None detected	
Simulated gastric fluid	USP reference standard	2.973	43.3
Simulated gastric fluid	Tablets	2.932	42.7
Simulated intestinal fluid	USP reference standard	3.369	49.1
Simulated intestinal fluid	Tablets	0.784	11.4

[a] One determination each.

Analytical Procedure—Five tablets (each containing approximately 0.7 mg cyproheptadine hydrochloride) were placed in a 50-ml volumetric flask. Twenty-five ml 50:50 (v/v) acetonitrile–water was added and swirled until the tablets were completely dissolved and then brought to volume with acetonitrile–water. The amount of cyproheptadine in the tablets was calculated from the linear regression of the standard curve.

A solution of powdered excipients without cyproheptadine hydrochloride was prepared at a concentration equal to five tablets in a 50-ml solution. No interferences from other ingredients or contaminants in the analysis were detected in the blank solution.

Three samples of the powdered excipient spiked with cyproheptadine gave recoveries of 100.4, 100.0, and 99.8% (mean = 100.1 ± 0.3%).

RESULTS AND DISCUSSION

Cyproheptadine (a weak aliphatic base) pairs with octanesulfonate in a weakly acidic mobile phase. A mobile phase of acetonitrile–aqueous solution (85:15) (0.01 *M* octanesulfonic acid, 1% acetic acid, 0.5% triethylamine) gave the best separation (Fig. 1) with cyproheptadine resolved from other ingredients (Fig. 2) with no interferences from contaminants or excipients.

Table II—Assays of Three Production Lots

No. Lot	Theoretical Concentration, mg/tablet	Obtained Concentration, mg/tablet Replicate Analyses 1	2	3	Mean	*SD*
1	0.667	0.639	0.635	0.637	0.637	0.002
2	0.667	0.649	0.628	0.667	0.648	0.020
3	0.667	0.616	0.653	0.627	0.632	0.019

A comparison of analyses of cyproheptadine dissolved in distilled, deionized water, simulated gastric fluid [USP XX (2)], and simulated intestinal fluid [USP XX (2)] showed irregular results (Table I). Preliminary results indicate that dissolution is slow in the simulated fluids with total release times of several hours.

Since water appeared to be the best dissolution solvent, a mixture of acetonitrile–water was used, which made the resultant solution more compatible with the mobile phase. The assay of three production lots of tablets was measured to be 95.5, 97.2, and 94.8% of theoretical content (Table II). The method precision by triplicate assays was 0.3, 3.1, and 3.0%, respectively.

REFERENCES

(1) "Physicians Desk Reference," 35th ed. Medical Economics Co., Oradell, N.J.

(2) "The United States Pharmacopeia XX/The National Formulary XV," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 193–194.

(3) A. W. Missen, Rep.—*N.Z. Dep. Sci. Ind. Res., Chem Div. Rep.*, ISS C. D. 2282, 36 (1979).

(4) E. C. G. Clarke, "Isolation and Identification of Drugs," Vol. I, Pharmaceutical Press, London, 1974, p. 278.

(5) B. B. Wheals, *J. Chromatogr.*, **187**, 65 (1980).

(6) D. L. Massaret and M. R. Detavernier, *J. Chromatogr.*, **187**, 139 (1980).

(7) D. L. Massaret and G. Hoogewijis, *Anal. Lett.*, **13**, 389 (1980).

ACKNOWLEDGMENTS

Acknowledgment is given to the sponsor for funding of this work and giving permission to publish. Details of the formulation have been omitted to protect client confidentiality.

Synthesis and Anticonvulsant Testing of 4-Phenylsemicarbazides

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Abstract □ A series of compounds based on the semicarbazide structure have been synthesized. Anticonvulsant activity was found in a majority of the compounds using both the maximal electroshock seizure and the subcutaneous pentylenetetrazol seizure threshold tests. Activity of the compounds was weaker than the 1,1,2-trisubstituted semicarbazides previously reported.

Keyphrases □ 4-Phenylsemicarbazides—synthesis and anticonvulsant activity □ Anticonvulsants—synthesis of 4-phenylsemicarbazides

Earlier work (1–5) on the synthesis and anticonvulsant activity of 4-phenylsemicarbazides was concerned primarily with compounds in which N-1 and N-2 are fully substituted by alkyl or aryl residues. To obtain additional information regarding structure–activity relationships for these types of compounds, it was desirable to prepare the series of compounds represented by III. This series differs from all of the previous series in that the compounds contain a hydrogen atom at N-2. This series includes 2-methyl, 2,6-dimethyl, and 2-chloro-6-methyl substituents in the aromatic ring since such substitution generally proved to be optimal in the previous series of 4-phenylsemicarbazides studied (1, 3).

RESULTS AND DISCUSSION

1,1-Disubstituted hydrazines (II) readily added to aryl isocyanates (I) and afforded III in good yields (Scheme I, Table I). Several different 1,1-disubstituted hydrazines were used (Table I) including the cage-like compound, 3-amino-3-azabicyclo[3.2.2]nonane. The latter compound

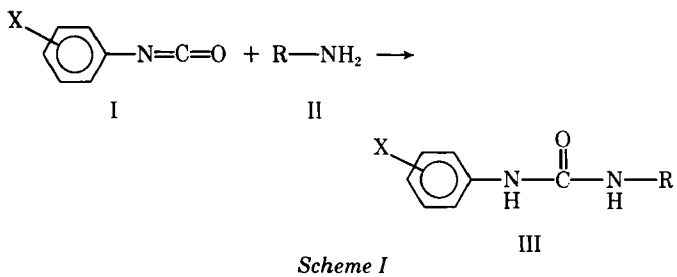
0022-3549/83/1000-1213$01.00/0

Table I—Physical Properties of 4-Phenylsemicarbazides

Compound	X	R	Melting Point	Yield, %	Recrystallization Solvent[a]	Formula	Analysis, % Calc.	Found
IIIa	o-CH_3	$N(CH_3)_2$	140–142°[b]	93	A	$C_{10}H_{15}N_3O$	C 62.15	
							H 7.82	
							N 21.74	
IIIb	2,6-$(CH_3)_2$	$N(CH_3)_2$	156–158°	91	A	$C_{11}H_{17}N_3O$	C 63.74	63.44
							H 8.27	8.28
							N 20.27	20.24
IIIc	2-Cl,6-CH_3	$N(CH_3)_2$	149.5–151°	93	A	$C_{10}H_{14}ClN_3O$	C 52.75	52.55
							H 6.20	6.31
							N 18.45	18.16
IIId	o-CH_3	$N(CH_2)_4$	147–148°	50	A	$C_{12}H_{17}N_3O$	C 65.73	65.54
							H 7.81	8.05
							N 19.16	19.43
IIIe	2,6-$(CH_3)_2$	$N(CH_2)_4$	189–190°	62	A	$C_{13}H_{19}N_3O$	C 66.92	66.80
							H 8.21	8.17
							N 18.01	18.25
IIIf	2-Cl,6-CH_3	$N(CH_2)_4$	157–158°	73	A	$C_{12}H_{16}ClN_3O$	C 56.81	56.60
							H 6.36	6.49
							N 16.56	16.20
IIIg	o-CH_3	$N(CH_2)_5$	153–155.5°	77	A	$C_{13}H_{19}N_3O$	C 66.92	66.80
							H 8.21	8.04
							N 18.01	17.89
IIIh	2,6-$(CH_3)_2$	$N(CH_2)_5$	197–199°	79	B	$C_{14}H_{21}N_3O$	C 67.98	67.70
							H 8.56	8.56
							N 16.99	16.67
IIIi	2-Cl,6-CH_3	$N(CH_2)_5$	173–175°	80	A	$C_{13}H_{18}ClN_3O$	C 58.32	58.02
							H 6.78	6.50
							N 15.69	15.39
IIIj	o-CH_3	$N(CH_2CH_2)_2O$	187–189°	86	B	$C_{12}H_{17}N_3O_2$	C 61.26	60.99
							H 7.28	7.15
							N 17.86	17.59
IIIk	2,6-$(CH_3)_2$	$N(CH_2CH_2)_2O$	222–223°	82	B	$C_{13}H_{19}N_3O_2$	C 62.63	62.35
							H 7.68	7.48
							N 16.85	16.57
IIIl	2-Cl,6-CH_3	$N(CH_2CH_2)_2O$	204–206°	78	B	$C_{12}H_{16}ClN_3O_2$	C 53.44	53.14
							H 5.98	5.90
							N 15.58	15.38
IIIm	o-CH_3	$N(CH_2)_6$	122–124°	88	A	$C_{14}H_{21}N_3O$	C 67.98	67.77
							H 8.56	8.61
							N 16.99	16.91
IIIn	2,6-$(CH_3)_2$	$N(CH_2)_6$	162–164°	92	A	$C_{15}H_{23}N_3O$	C 68.93	68.67
							H 8.87	8.67
							N 16.08	15.80
IIIo	2-Cl,6-CH_3	$N(CH_2)_6$	127.5–129.5°	92	A	$C_{14}H_{20}ClN_3O$	C 59.68	59.38
							H 7.15	7.02
							N 14.91	14.79
IIIp	2-Cl,6-CH_3	$N(CH_2CH_2)_2NCH_3$	196–198°	27	A	$C_{13}H_{19}ClN_4O$	C 55.22	54.99
							H 6.77	6.90
							N 19.81	19.65
IIIq	o-CH_3	$N[CH_2CH(CH_2CH_2)]_2$	199–201°	82	A	$C_{16}H_{23}N_3O$	C 70.30	70.04
							H 8.48	8.65
							N 15.37	15.26
IIIr	2,6-$(CH_3)_2$	$N[CH_2CH(CH_2CH_2)]_2$	218–220°	73	A	$C_{17}H_{25}N_3O$	C 71.05	71.22
							H 8.77	8.57
							N 14.62	14.48
IIIs	2-Cl,6-CH_3	$N[CH_2CH(CH_2CH_2)]_2$	244–246°	73	A	$C_{16}H_{22}ClN_3O$	C 62.43	62.17
							H 7.20	7.10
							N 13.65	13.63
IIIt	2,6-$(CH_3)_2$	$N(CH_3)CH_2CO_2C_2H_5$	112–114°	79	A	$C_{14}H_{21}N_3O_3$	C 60.20	60.38
							H 7.58	7.34
							N 15.04	15.26

[a] A, ethyl acetate; B, ethyl acetate–methanol. [b] M. Wilcox, *J. Med. Chem.*, 11, 171 (1968) reported mp, 142–143°.

was obtained in two steps *via* the intermediate *N*-nitroso derivative from commercially available 3-azabicyclo[3.2.2]nonane (6). Additionally, ethyl (1-methylhydrazino)acetate was prepared from methylhydrazine and ethyl bromoacetate (7).



Scheme I

Compounds IIIa–IIIt were tested in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazol seizure threshold (scMet) tests for anticonvulsant activity and in the rotorod test for neurotoxicity in male Carworth Farms No. 1 mice by reported procedures (1). In the MES test, compounds IIIb, IIId, IIIg, IIIi, IIIj, IIIk, IIIl, and IIIn exhibited activity at 300 mg/kg at 30 min. Only IIIb showed toxicity at this dosage level. Compounds IIIb, IIIc, and IIIh showed activity at the same dosage at 4 hr. Compound IIIc was the only compound active at 100 mg/kg (30 min).

Five compounds (IIIb, IIIg, IIIh, IIIi, and IIIm) were active in the scMet test at 300 mg/kg at 30 min. Compound IIIh was also active at this same dosage at 4 hr. Two compounds underwent further testing. Compounds IIIc and IIIh exhibited MES ED_{50} = 76 (61–90, 95% C.I. [confidence interval]) and 282 (244–319, 95% C.I.) mg/kg; scMet ED_{50} = 222 (146–326, 95% C.I.) and 424 (240–695, 95% C.I.) mg/kg and TD_{50} = 210 (165–272, 95% C.I.) and 528 (390–698, 95% C.I.) mg/kg, respectively.

These data indicate that 4-phenylsemicarbazides derived from 1,1-dialkylated hydrazines possess anticonvulsant activity; however, it is of a lower order than that shown by 4-phenylsemicarbazides derived from 1,1,2-trialkylated hydrazines (1, 3). The cage compounds IIIq, IIIr, and IIIs were uniformly inactive and these results are consistent with the poor activity found for other cage compounds (4, 5).

EXPERIMENTAL[1]

Ethyl (1-Methylhydrazino)acetate—A solution of 16.7 g (0.1 mole) of ethyl bromoacetate in 17 ml of benzene was added dropwise with magnetic stirring over a 90-min period to a solution of 9.2 g (0.2 mole) of methylhydrazine in 50 ml of benzene. After the reaction mixture had stirred overnight at room temperature, the benzene phase was decanted and the salt residue was washed three times with 15 ml of benzene. The benzene was distilled through a 1-ft Vigreux column at 42 mm Hg. The residue was then distilled and afforded 8.82 g (67%) of a colorless oil, bp 88° (21 mm); IR (film): 1740 cm^{-1} (C=O).

Anal.—Calc. for $C_5H_{12}N_2O$: C, 45.44; H, 9.15; N, 21.20. Found: C, 45.29; H, 9.26; N, 21.31.

[1] Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The IR spectra were taken on a Perkin-Elmer 700 spectrophotometer as either liquid films or potassium bromide pellets. NMR spectra were recorded on a Varian EM-360 or T-60 spectrometer, using tetramethylsilane as the internal reference. Mass spectra were obtained on a RMU-7 double-focusing spectrometer by Hitachi/Perkin-Elmer. Elemental analyses were performed by Baron Consulting Co., Orange, Conn. All compounds exhibited ^{1}H-NMR and mass spectra consistent with the structures shown.

4-Phenylsemicarbazides (III)—Compound IIIb was prepared by the dropwise addition of a solution of 2.66 g (0.0180 mole) of 2,6-dimethylphenyl isocyanate (I) in 6 ml of dry benzene to a solution of 1.20 g (0.020 mole) of 1,1-dimethylhydrazine in 10 ml of dry benzene at room temperature. After ~10 min heat was evolved. The mixture was heated for 2.5 h in an oil bath (85°). The solvent was evaporated under reduced pressure, and the residue was recrystallized from ethyl acetate and gave 3.40 g (91%) of white crystalline product, mp 156–158°.

REFERENCES

(1) M. J. Kornet, *J. Pharm. Sci.*, **67**, 1471 (1978).

(2) M. J. Kornet and R. Joyce Garrett, *J. Pharm. Sci.*, **68**, 377 (1979).

(3) M. J. Kornet and J. Chu, *J. Heterocycl. Chem.*, **18**, 293 (1981).

(4) M. J. Kornet and J. Chu, *J. Heterocycl. Chem.*, **19**, 697 (1982).

press.

(5) M. J. Kornet and J. Chu, *J. Pharm. Sci.*, **72**, 94 (1983).

(6) E. Schenker, Fr. 1,514,454; through *Chem. Abstr.*, **70**, 106529q (1969).

(7) R. B. Moffett, G. N. Evenson, and P. F. Von Voigtlander, *J. Heterocycl. Chem.*, **14**, 1231 (1977).

ACKNOWLEDGMENTS

The authors are grateful to the Antiepileptic Drug Development Program of the National Institutes of Health for the anticonvulsant activity data and to NIH for grant support for this investigation.

Investigation of the β-Cyclodextrin–Hydrocortisone Inclusion Compound

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Received March 25, 1982, from the *Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH 43210.* Accepted for publication September 8, 1982.

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Abstract □ The formation of an inclusion compound by β-cyclodextrin with hydrocortisone has been studied by proton magnetic resonance (^{1}H-NMR) and phase solubility analysis. The magnitude of the chemical shifts of the interior and exterior β-cyclodextrin protons in the presence of hydrocortisone indicated that hydrocortisone is included within the β-cyclodextrin cavity and probably interacts with protons on the edge of the torus. The overall stoichiometry of the inclusion compound was not a single, simple relationship, but was unusual in that it was variable and apparently dependent on the relative amounts of hydrocortisone and β-cyclodextrin in the system.

Keyphrases □ Inclusion complexes—β-cyclodextrin–hydrocortisone, phase solubility analyses, ^{1}H-NMR □ β-Cyclodextrin–hydrocortisone—inclusion complexes, phase solubility analyses, ^{1}H-NMR □ Phase solubility analyses—β-cyclodextrin–hydrocortisone, ^{1}H-NMR

The formation of an inclusion compound by dinoprostone (prostaglandin E_2) with β-cyclodextrin has been reported earlier (1). From phase solubility analysis and ^{1}H-NMR spectroscopy it was concluded that a 1:1 complex formed, with the dinoprostone molecule partially included within the β-cyclodextrin cavity and the remainder of the molecule extended to the exterior of the torus.

In the present study attention has been directed to the formation of an inclusion complex between hydrocortisone and β-cyclodextrin. Such an inclusion compound by itself is not necessarily unique; however, these initial studies indicated that an unusual dependence apparently existed between the stoichiometry of the interaction and the concentration of β-cyclodextrin.

EXPERIMENTAL

The experimental procedure was similar to that employed previously (1). β-Cyclodextrin[1] was recrystallized twice from distilled water and dried under vacuum at 60°; hydrocortisone USP[2], was used as received; and water was double-distilled and deionized. Samples for ^{1}H-NMR spectroscopy were prepared by saturating a 2% w/v solution of β-cyclodextrin in D_2O[3] with hydrocortisone. Excess complex was allowed to precipitate and the supernatant solution of the inclusion compound was decanted. ^{1}H-NMR spectra at 100 MHz[4] were determined on the supernatant in standard 5-mm tubes.

Samples for phase solubility analysis were prepared by placing excess quantities of hydrocortisone (0.021, 0.040, or 0.060 g) with increasing amounts of β-cyclodextrin into 20-ml culture tubes containing 10 ml of water. The samples were sealed (with screw caps[5]) and rotated end-over-end at ~41 rpm for 24 hr in a thermostated water bath at 30 ± 0.1°. Aliquots of the supernatant were filtered through a prerinsed membrane filter (0.45 μm)[6] and spectrophotometrically assayed at 248 nm.

[1] Nutritional Biochemicals, Inc.
[2] Calbiochem.
[3] Bio-Rad Laboratories (99.85 mole % D_2O).
[4] Varian XL-100 NMR spectrometer.
[5] Teflon lined.
[6] Millipore Corp., Type HA.

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