one component was detected upon paper electrophoresis (Whatman 3MM, 1000 V, 60 min) with the following buffer systems: pH 2.8 (1 M HOAc); pH 4.9 (0.1 M pyridine acetate); pH 9.9 (0.2 M sodium carbonate–bicarbonate). The peptides were located with a hypochlorite spray and, in the case of 4 and 7, also with ninhydrin. The properties of the three new peptides are summarized on Table II.

Amino acid analyses were made on a Beckman 120C instrument, after hydrolysis with 2 mL of 6 N HCl, containing 0.5% (v/v) mercaptoethanol and 0.2% (v/v) phenol, in a nitrogen atmosphere for 72 h at 110 °C. For estimation of tryptophan content the hydrolysis was also done with *p*-toluenesulfonic acid, containing 1% indole, for 48 h at 110 °C. The following amino acid ratios were found: **3**, Lys (1.03), Glu (1.01), Pro (5.0), Gly (1.99), Ile (1.02), Leu (0.93); **4**, Lys (1.01), Glu (1.92), Pro (5.13), Gly (0.95), Ile (0.99), Leu (1.02); **7**, Lys (0.98), Arg (1.04), Trp (0.98), Glu (1.04), Pro (4.12), Ile (1.04).

Acknowledgment. This work was done with the technical assistance of Nelson A. Mora, José M. Pontinha, and J. Roque Santos and was supported by grants from Financiadora de Estudos e Projetos (FINEP), the Brazilian National Council for Scientific and Technological Development (CNPq), and the São Paulo State Research Foundation (FAPESP).

References and Notes

(1) S. H. Ferreira, D. C. Bartelt, and L. J. Greene, *Biochemistry*, **9**, 2583 (1970).
(2) M. A. Ondetti, N. J. Williams, E. F. Sabo, J. Pluscec, E. R. Weaver, and O. Kocy, *Biochemistry*, **10**, 4033 (1971).
(3) H. Kato and T. Suzuki, *Biochemistry*, **10**, 972 (1971).
(4) S. H. Ferreira, *Br. J. Pharmacol.*, **24**, 163 (1965).
(5) A. C. M. Camargo and S. H. Ferreira, *Br. J. Pharmacol.*, **42**, 305 (1971).
(6) M. Tominaga, J. M. Stewart, T. B. Paiva, and A. C. M. Paiva, *J. Med. Chem.*, **18**, 130 (1975).
(7) The nonapeptide BPP9a was synthesized by the Squibb Institute for Medical Research, named SQ 20 881, and has been extensively used in basic and clinical studies of the kinin and angiotensin systems.
(8) (a) D. W. Cushman and H. S. Cheung in "Hypertension 72", J. Genest and E. Koiw, Ed., Springer, Berlin, 1972, p 532; (b) R. L. Soffer, *Annu. Rev. Biochem.*, **45**, 73 (1976).
(9) H. Y. T. Yang, E. G. Erdös, and Y. Levin, *J. Pharmacol. Exp. Ther.*, **177**, 291 (1971).
(10) T. B. Paiva, M. E. Miyamoto, L. Juliano, and A. C. M. Paiva, *J. Pharmacol. Exp. Ther.*, **202**, 294 (1977).
(11) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(12) B. W. Erickson and R. B. Merrifield in "The Proteins", 3rd ed, Vol. II, H. Neurath and R. Hill, Ed., Academic Press, New York, N.Y., 1976, p 255.
(13) R. B. Merrifield, J. M. Stewart, and N. Jernberg, *Anal. Chem.*, **38**, 1905 (1966).
(14) D. Yamashiro and C. H. Li, *J. Org. Chem.*, **38**, 2594 (1973).
(15) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(16) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 3102 (1972).
(17) K. Suzuki, K. Nitta, and N. Endo, *Chem. Pharm. Bull.*, **23**, 222 (1975).
(18) C. H. W. Hirs, *Methods Enzymol.*, 11, 328 (1967).

(+)- and (−)-3-Methoxycyproheptadine. A Comparative Evaluation of the Antiserotonin, Antihistaminic, Anticholinergic, and Orexigenic Properties with Cyproheptadine

David C. Remy,* Kenneth E. Rittle, Cecilia A. Hunt, Paul S. Anderson, Edward L. Engelhardt,

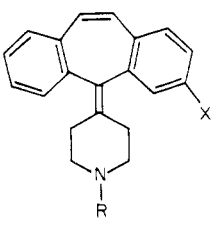
Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486

Bradley V. Clineschmidt, and Alexander Scriabine

Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486. *Received June 22, 1977*

The synthesis and resolution of (±)-3-methoxycyproheptadine [(±)-4] are described. As a peripheral serotonin antagonist, (±)-4 was found to be one-half as potent as cyproheptadine (1b). The peripheral anticholinergic and antihistaminic activities as well as the orexigenic property of (±)-4 are less than those of 1b. A further comparison of the enantiomers (+)-4 and (−)-4 shows that all of the anticholinergic activity of (±)-4 resides solely in the dextrorotatory enantiomer, (+)-4, while the antiserotonin activity, which is similar to that of 1b, resides in the levorotatory enantiomer, (−)-4. Antihistaminic and orexigenic activity also resides in (−)-4 but these properties are reduced compared to those of 1b.

A previous report[1] from this laboratory described the stereoselective antipsychotic and central anticholinergic activities that were found to reside in the levo- and dextrorotatory enantiomers of the cyproheptadine analogue, 1-cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cycloheptene-5-ylidene)piperidine (**1a**), respectively. During an investigation into the stereostructure–activity relationships of other enantiomeric 3-substituted cyproheptadine analogues and derivatives, (±)-3-methoxycyproheptadine [(±)-4] was prepared and resolved. In a preliminary pharmacological evaluation, the racemate (±)-4 was found to have a biological profile similar to that observed with cyproheptadine (**1b**), a clinically useful serotonin and histamine antagonist with anticholinergic and orexigenic properties.[2,3] This finding prompted a comparative evaluation of the enantiomers (+)- and (−)-4 with **1b** to determine whether enantiomeric differences in biological activities existed.



1a, X = SCF_3; R = CH_2-c-C_3H_5
b, X = H; R = CH_3
c, X = CN; R = CH_3

Chemistry. (±)-3-Methoxycyproheptadine [(±)-4] was prepared by the addition of 1-methyl-4-piperidyl-

Table I. In Vivo Antiserotonin, Antihistamine, and Peripheral Anticholinergic Activity[a]

Compd	Antiserotonin ED_{50}, mg/kg po (95% confidence limits)	Antihistamine ED_{50}, mg/kg ip (95% confidence limits)	Anticholinergic $ED_{1.5}$,[b] mg/kg po (95% confidence limits)
(±)-4	0.43 (0.32-0.53)	1.16 (0.19-1.99)	41.2 (30.1-56.5)
(+)-4	23.9 (13.9-51.3)	4.45 (2.31-20.13)	11.45 (5.75-22.79)
(−)-4	0.38 (0.16-0.61)	0.51 (0.38-0.68)	>80
1b	0.24 (0.02-0.50)	0.077 (0.044-0.13)	6.0 (4.6-8.1)

[a] See Experimental Section for a description of the pharmacological testing. [b] Dose required to increase pupil diameter to 1.5 micrometer units.

magnesium chloride to ketone **2**[4] followed by a subsequent dehydration. Resolution of (±)-4 was effected using both di-*p*-toluoyl-*d*- and *l*-tartaric acids to give, after recrystallization of the salts to constant optical rotations and conversion to the free amines, (−)-4 and (+)-4, respectively.

The levorotatory isomer, (−)-4, was also prepared from (−)-3-iodocyproheptadine [(−)-5].[1] Reaction of this chiral iodo compound with sodium methoxide in DMF on a steam bath for 2.5 h gave, after crystallization, a 35% yield of (−)-4 that was identical with the levorotatory enantiomer obtained by the direct resolution of (±)-4.

Pharmacology and Discussion

The racemate (±)-4 and the enantiomers (+)-4 and (−)-4 were compared to the known antiserotonin, antihistaminic, and anticholinergic properties (Table I) and orexigenic properties (Table II) of **1b**.

As seen from the data of Table I, there is a distinct separation of anticholinergic and antiserotonin activities between the enantiomers (+)-4 and (−)-4, respectively. The peripheral anticholinergic activity, as measured by mydriatic potency in mice, was found to reside in the dextrorotatory enantiomer (+)-4, this isomer being one-half as potent an anticholinergic agent as is **1b**. In contrast, (+)-4 was a relatively poor antagonist of serotonin-elicited edema in the rat paw as compared to its enantiomer (−)-4. The levorotatory isomer (−)-4 is not statistically different, after oral administration, from **1b** in antiserotonin potency. With respect to anticholinergic activity, these findings parallel the recent report of Robinson[5] in that all of the anticholinergic activity of 3-cyanocyproheptadine (**1c**) resides exclusively in the dextrorotatory enantiomer.

As an appetite stimulant in the cat, (±)-4 consistently caused an increase in food consumption at 0.25 mg/kg po but not at 0.0312 mg/kg po, whereas a significant increase in food consumption was observed for **1b** at both these doses. That the orexigenic activity of (±)-4 resides in (−)-4 and not (+)-4 may be seen from the data of Table II where a consistent increase in food consumption at 0.25 mg/kg po is seen to occur with (−)-4 but not with (+)-4.

Although (+)-4 retains some antihistaminic activity, its ED_{50} is 15 times greater than that of **1b**. As with the antiserotonin and orexigenic activities, the antihistaminic activity resides principally in (−)-4. A possible relationship between antihistaminic and orexigenic properties has been discussed recently by Clineschmidt.[3] In this regard, it is interesting to note that (−)-4 possesses both moderate antihistaminic and orexigenic activity, while (+)-4 has very weak antihistaminic activity and shows no apparent orexigenic activity at a dose where (−)-4 is active.

These results demonstrate that (−)-4 has antiserotonin potency similar to that of **1b** while its ancillary pharmacologic properties are less than those of **1b**. The enantiomer (−)-4 thus appears to be a more selective antiserotonin agent. These results also suggest not only that cholinergic, serotonergic, and histamine H_1 receptor sites in the periphery may have rigid, stereoselective requirements but also that the cholinergic receptor site may have an opposite stereochemical geometry from the serotonin and histamine H_1 receptor sites. Clearly, further work is necessary to establish definitive conclusions regarding these stereostructure–receptor site relationships.

Table II. Food Consumption[a]

Compd	Dose, mg/kg po	Av g consumed ± SD, control day/test day
(±)-4	0.0312	155 ± 33/180 ± 39[b,d]
		126 ± 26/110 ± 42
		139 ± 30/142 ± 42
	0.25	143 ± 37/188 ± 50[b]
		118 ± 52/143 ± 38[b]
		142 ± 24/155 ± 33[b]
(+)-4	0.0312	146 ± 24/143 ± 20
		135 ± 39/115 ± 33[c]
		107 ± 29/116 ± 28
	0.25	155 ± 34/172 ± 43
		119 ± 39/160 ± 42[b]
		121 ± 42/140 ± 35
(−)-4	0.0312	150 ± 52/179 ± 29
		126 ± 34/138 ± 32
		136 ± 35/131 ± 30
	0.25	147 ± 42/196 ± 57[b]
		132 ± 30/181 ± 49[b]
		114 ± 25/134 ± 31[b]
1b	0.0312	133 ± 31/196 ± 56[b]
	0.25	179 ± 31/220 ± 52[b]
Methylcellulose		181 ± 40/195 ± 33
		210 ± 39/198 ± 41
		195 ± 55/184 ± 52

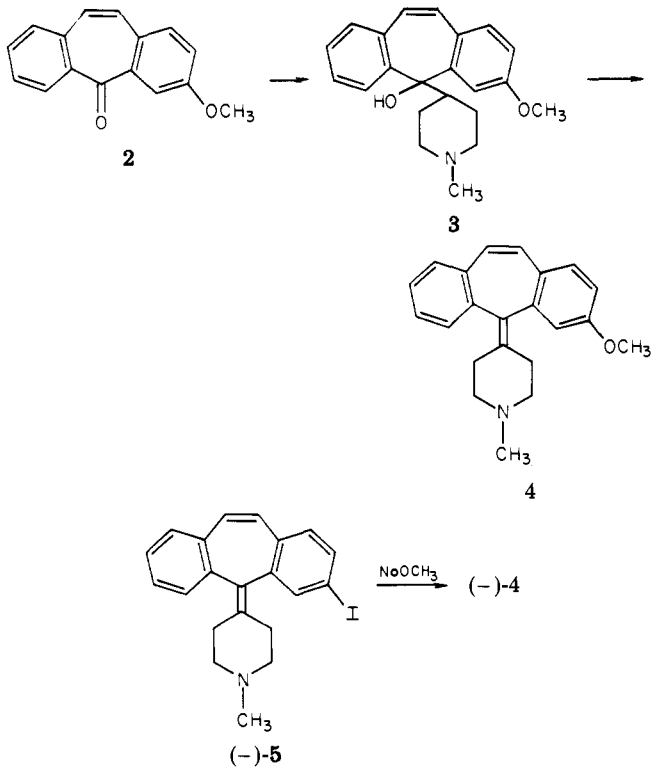
[a] See Experimental Section for a description of pharmacological testing. [b] Represents a significant increase in food consumption, $p < 0.05$ (two-tailed paired "t"). [c] Represents a significant decrease in food consumption, $p < 0.05$ (two-tailed paired "t"). [d] Each compound, except 1b, was tested at two dose levels on three separate days with ten cats/dose/day.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter, using the solvents and concentrations specified. At least two readings were recorded at each wavelength and showed a deviation of 0.005°. NMR spectra were recorded on a Varian T-60 spectrometer in $CDCl_3$ and all shifts are relative to tetramethylsilane as an internal standard. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.3% of the theoretical values.

(±)-1-Methyl-4-(3-methoxy-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine [(±)-4]. To an ice-cooled solution of 20.35 g (0.0858 mol) of **2**[4] in 100 mL of dry THF was added dropwise a THF solution of 1-methyl-4-piperidylmagnesium chloride[6] until the ketone had been completely consumed. The THF was removed on a rotary evaporator. The remaining red oily residue was dissolved in benzene and H_2O was added dropwise until a clear benzene supernatant and a gelatinous aqueous phase were obtained. The benzene phase was decanted and the aqueous phase was extracted with three 100-mL portions of boiling benzene. The combined benzene extracts were concentrated. The residue that remained was triturated with CH_3CN, and the product that crystallized was collected by filtration, washed with cold CH_3CN, and dried to give 11.20 g (60%) of **3**. A mixture of 17.20 g of **3** and 300 mL of 6 N HCl was stirred and heated

Scheme I



at reflux for 0.5 h. After the solvent was removed by evaporation under reduced pressure, the residue was made basic by the addition of a saturated solution of sodium carbonate. The oil that precipitated was extracted into Et_2O, this Et_2O phase was washed with water, dried ($MgSO_4$), and filtered, and the Et_2O was removed on a rotary evaporator. The residue was triturated with CH_3CN and the crystalline product was collected by filtration and dried to give 12.0 g (74%) of (±)-4, mp 132–135 °C. An analytical sample was prepared by recrystallization from CH_3CN: mp 134–135.5 °C; NMR ($CDCl_3$) δ 1.8–2.5 (11 H, m, with a peak at 2.18 (N-CH_3), aliphatic CH), 3.70 (3 H, s, OCH_3), 6.6–7.2 (9 H, m, aromatic and vinyl CH). Anal. ($C_{22}H_{23}NO$) C, H, N.

Resolution of (±)-1-Methyl-4-(3-methoxy-5*H*-dibenzo[*a*,*d*]cycloheptен-5-ylidene)piperidine [(±)-4]. A. Levorotatory Isomer (−)-4. To a solution of 10.0 g (0.0315 mol) of (±)-4 in 1.8 L of boiling EtOH was added 12.17 g (0.0315 mol) of di-*p*-toluoyl-*d*-tartaric acid. The solution was stirred and concentrated by boiling to 600 mL. The crystalline precipitate that formed on cooling was removed by filtration, washed with EtOH, collected, and dried to give 9.0 g of material, designated A. The clear EtOH filtrate and washings were combined and the solvent was removed to give a residue, B.

The 9.0 g of A was recrystallized from EtOH three times to give 3.77 g of a product with a constant rotation: $[\alpha]^{25}_{589}$ −149°, $[\alpha]^{25}_{578}$ −157°, $[\alpha]^{25}_{546}$ −188°, $[\alpha]^{25}_{436}$ −436° (*c* 0.68, pyridine). This salt was converted to free base (−)-4 using sodium bicarbonate solution and extracting it into Et_2O. The Et_2O extract was washed with H_2O, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure. The product, (−)-4, weighed 1.63 g and was TLC homogeneous (fl. alumina–$CHCl_3$): mp 115–116 °C; $[\alpha]^{25}_{589}$ −153°, $[\alpha]^{25}_{578}$ −161°, $[\alpha]^{25}_{546}$ −195°, $[\alpha]^{25}_{436}$ −504° (c 0.39, $CHCl_3$); NMR ($CDCl_3$) δ 2.0–2.6 [11 H, m, with a peak at 2.20 (NCH_3), aliphatic CH], 3.93 (3 H, s, OCH_3), 6.8–7.3 (9 H, m, aromatic and vinyl CH). Anal. ($C_{22}H_{23}NO$) C, H, N.

B. Dextrorotatory Isomer (+)-4. Residue B was treated with 5% NaOH solution. The free base that precipitated was extracted into Et_2O. Evaporation of the Et_2O gave 5.87 g of solid that was dissolved in 400 mL of hot EtOH and was treated with 7.48 g of di-*p*-toluoyl-*l*-tartaric acid monohydrate dissolved in 100 mL of hot EtOH. The solution was stirred, concentrated by boiling to 400 mL, and allowed to cool to room temperature. The crystalline precipitate that formed was removed by filtration, washed with EtOH, collected, and dried to give 8.03 g of material. Three recrystallizations of this material from EtOH gave 4.05 g of salt having a constant rotation: $[\alpha]^{25}_{589}$ +148°, $[\alpha]^{25}_{578}$ +157°, $[\alpha]^{25}_{546}$ +187°; $[\alpha]^{25}_{436}$ +434° (*c* 0.68, pyridine). This salt was converted to free base (+)-4 using sodium carbonate solution and extracting it into Et_2O. The Et_2O extract was washed with H_2O, dried ($MgSO_4$), and filtered, and the solvent was removed. The product, (+)-4, weighed 1.40 g and was homogeneous by TLC; the NMR spectrum was identical with the NMR spectrum of (−)-4: $[\alpha]^{25}_{589}$ +152°, $[\alpha]^{25}_{578}$ +161°, $[\alpha]^{25}_{546}$ +195°, $[\alpha]^{25}_{436}$ +498° (*c* 0.41, $CHCl_3$); mp 115–116 °C. Anal. ($C_{22}H_{23}NO$) C, H, N.

(−)-1-Methyl-4-(3-methoxy-5*H*-dibenzo[*a*,*d*]cycloheptен-5-ylidene)piperidine [(−)-4] from (−)-1-Methyl-4-(3-iodo-5*H*-dibenzo[*a*,*d*]cycloheptен-5-ylidene)piperidine [(−)-5]. A mixture of 3.74 g (0.00905 mol) of (−)-5,[1] $[\alpha]_{589}$ −142°, 9.77 g (0.181 mol) of $NaOCH_3$, 5.56 g of electrolytic Cu dust, and 87 mL of DMF was stirred and heated on a steam bath for 2.5 h. The mixture was cooled, 150 mL of H_2O and 150 mL of Et_2O were added, and, after stirring, the mixture was filtered through a pad of Celite. The Et_2O phase was separated, washed with H_2O, dried ($MgSO_4$), and filtered, and the Et_2O was evaporated. The residue, 2.67 g, was dissolved in 50 mL of warm CH_3CN. On standing overnight, the solution deposited crystals. The supernatant, containing the desired product, was decanted from the crystals. This supernatant was concentrated to give 2.0 g of solid which was recrystallized from 40 mL of hexane. The product that crystallized was collected and recrystallized from CH_3CN to give 1.0 g (35%) of (−)-4: $[\alpha]_{589}$ −153°, $[\alpha]_{578}$ −163°, $[\alpha]_{546}$ −198°, $[\alpha]_{436}$ −515° (*c* 0.49, $CHCl_3$); mp 115–116 °C.

Pharmacology. Antiserotonin activity was studied by the ability of compounds to antagonize the edema resulting from the local injection of serotonin into the right hind paw of rats. Sprague–Dawley male rats from 140 to 210 g body weight were used. The compounds were administered orally 1 h prior to the injection of 5 μg of serotonin (base) into the right hind paw of the rat. The left hind paw was injected with the same volume of saline (0.05 mL) and served as a basis for comparison. Thirty minutes after the serotonin and saline injections, the animals were sacrificed and both feet were removed and weighed. The results are expressed as percent inhibition of the weight gain due to the serotonin as compared to that obtained in the saline-treated foot. Four to 16 rats were used at each dose level of each drug. Compounds (±)-4 and (−)-4 were tested each at five dose levels ranging from 0.156 to 40 mg/kg po. Cyproheptadine (**1b**) was tested at 0.175, 0.7, and 2.8 mg/kg po, whereas (+)-4 was tested at six dose levels ranging from 2.5 to 80 mg/kg po.

Antihistaminic activity was evaluated in guinea pigs of the Duncan–Harley strain of either sex and 200–300 g body weight. The test compounds, suspended in 1% methylcellulose, were administered intraperitoneally. Thirty minutes later the animals were placed in individual chambers and exposed to histamine aerosol spray (0.5% base) for a 3-min period. After 3 min, the animals were taken out of the chambers and observed for 10 min. The dose necessary to protect 50% of the animals from death caused by histamine aerosol induced bronchoconstriction was determined. Racemate (±)-4 was tested at 0.78, 1.56, and 3.12 mg/kg ip; (+)-4 was tested at 1.56, 3.12, and 6.25 mg/kg ip; (−)-4 was tested at 0.39, 0.78, and 1.56 mg/kg ip; and **1b** at 0.031, 0.062, 0.125, and 0.25 mg/kg ip. Five animals were used at each dose level of **1b** and ten animals were used at each dose level of each of the three other compounds.

Anticholinergic activity was evaluated by the ability of the test compound to dilate the pupil of the mouse. Compounds were administered orally to female Carworth Farms (CF-1) mice. The diameter of the pupil was measured with the aid of an ocular micrometer 1 h after administration of the test compound. The test compounds were used at five dose levels with ten mice per dose. The $ED_{1.5}$ is defined as the dose required to increase pupil diameter to 1.5 micrometer units.

Orexigenic activity was evaluated by an increase in food consumption in cats. Adult male cats, individually caged with water available ad libitum, were allowed to eat for only 3 h daily (at the same time each day). The animals were maintained on this feeding schedule for at least 4 weeks prior to testing. A preweighed test meal (Science Diet Feline Ration, Riviana Foods, Inc.) was presented to each cat, and after 3 h, the amount consumed was determined by reweighing the remaining food. An amount of food was presented ensuring a surplus, thus allowing

unrestricted intake during the 3-h feeding period. Food eaten on the test (drug) day was compared with the amount consumed on the immediately preceding (control) day. Test compounds or a placebo dose of 1% methylcellulose were administered by gavage 30 min preceding presentation of the test meal. No cat was given a test compound more often than once weekly. The compounds were suspended in 1% methylcellulose prior to administration. Each compound was tested at two dose levels (0.0312 and 0.25 mg/kg) using ten cats per dose level. The compounds (±)-4, (+)-4, and (−)-4 were tested, at both doses, on three separate days.

The determination of ED_{50} and $ED_{1.5}$ was based on the dose–response relationships calculated by the regression of response on log dose in accordance with the method of Finney.[7]

Acknowledgment. Excellent technical assistance was provided by Audrey A. Morris, Terry A. Welch, Jodie C. McGuffin, Ruby Ballantine, Patricia Bunting, and Lars Flataker. Dr. W. Randall and his associates provided the analytical data, and Dr. N. Bohidar performed statistical analyses.

References and Notes

(1) D. C. Remy, K. E. Rittle, C. A. Hunt, P. S. Anderson, B. H. Arison, E. L. Engelhardt, R. Hirschmann, B. V. Clineschmidt, V. J. Lotti, P. R. Bunting, R. J. Ballentine, N. L. Papp, L. Flataker, J. J. Witoslawski, and C. A. Stone, *J. Med. Chem.*, **20**, 1013 (1977).
(2) C. A. Stone, H. C. Wenger, C. T. Ludden, J. M. Stavorski, and C. A. Ross, *J. Pharmacol. Exp. Ther.*, **131**, 73 (1961).
(3) B. V. Clineschmidt, H. M. Hanson, J. C. McGuffin, V. J. Lotti, A. Scriabine, and C. A. Stone, *Arch. Int. Pharmacodyn. Ther.*, **223**, 287 (1976).
(4) G. Berti, A. Da Settimo, G. Gregori, and F. Mancini, *Ann. Chim. (Rome)*, **52**, 514 (1962).
(5) S. E. Robinson, V. J. Lotti, and F. Sulser, *J. Pharm. Pharmacol.*, submitted for publication.
(6) E. L. Engelhardt, H. C. Zell, W. S. Saari, M. E. Christy, C. D. Colton, C. A. Stone, J. M. Stavorski, H. C. Wenger, and C. T. Ludden, *J. Med. Chem.*, 8, 829 (1965).
(7) D. J. Finney, "Statistical Method in Biological Assay", 2nd ed, Hafner Publishing Co., New York, N.Y., 1964, Chapters 2–4.

1,2,4-Triazole Amino Nucleosides. 1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamide and Related Nucleosides

A. S. Narang and Robert Vince*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
Received June 3, 1977

The synthesis of 1-β-D-3′-amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamide—the 3′-amino analogue of ribavirin—and five related nucleoside analogues is described. Each analogue exhibited LD_{50} concentrations > 100 μg/mL against P-388 mouse lymphoid leukemia cells in tissue culture. Antiviral testing indicated that none of the compounds exhibited significant activity.

The antitumor activities of 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine (puromycin aminonucleoside) and 3′-amino-3′-deoxyadenosine have generated considerable interest in the biological properties of amino nucleosides.[1] The broad-spectrum antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[2-4] (ribavirin) has stimulated a great deal of effort into the synthesis of five-membered heterocyclic nucleosides. As part of a program involving the design of chemotherapeutic nucleosides,[5] it seemed likely that the hybridization of these two nucleoside classes into one molecule should provide a new class of biologically active compounds.

Chemistry. Preparation of the appropriately blocked amino sugar was accomplished as outlined in Scheme I. The amino function of 1,2-*O*-isopropylidene-3-amino-3-deoxy-5-*O*-trityl-α-D-ribofuranose[6] was protected with the trifluoroacetyl group by treating with trifluoroacetic anhydride in pyridine. Acetolysis of **1** gave a mixture of 1,2,5-tri-*O*-acetyl-3-trifluoroacetamido-3-deoxy-β-D-ribofuranose (**2**, 51%) and the corresponding α isomer **3** (40%). The isomers were separated by crystallization of the β isomer, followed by chromatography of the filtrate to obtain the α isomer. The anomeric configurations of the sugars were determined from the anomeric proton signals in the ^1H NMR spectrum: δ 6.06 (s, β anomer), 7.16 (d, $J_{1,2}$ = 2.0 Hz, α anomer). The acid-catalyzed fusion of methyl 1,2,4-triazole-3-carboxylate[7] and **2** at 150–155 °C provided a 9:1 mixture of the two isomeric nucleosides **7** and **8** in 80% yield. The blocked nucleosides were easily separated by chromatography over silica gel. Alternatively, the condensation of the trimethylsilyl derivative **5** with 3-trifluoroacetamido-3-deoxy-2,5-di-*O*-acetylribofuranosyl bromide (**6**) in acetonitrile gave **7** and **8** in a ≃1:1 ratio. Treatment of the blocked nucleosides, **7** and **8**, with methanolic ammonia provided 1-β-D-3′-amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamide (**9**)—the 3′-amino analogue of ribavirin—and the corresponding 5-carboxamide isomer **12**. The assignments of the glycosylation sites in the 3- and 5-substituted 1,2,4-triazoles were accomplished with ^1H NMR. The aromatic proton of 1,3-disubstituted 1,2,4-triazoles occurs downfield from the aromatic proton of the 1,5-disubstituted triazoles.[8] In addition, a striking difference in the ^1H NMR spectra is a large downfield shift of the anomeric proton of the 5-substituted 1,2,4-triazole nucleosides relative to the corresponding 3-substituted isomers.[8] The observed chemical shifts of δ 8.83 and 7.93 for the aromatic protons of **9** and **12**, respectively, are consistent with the predicted values. In addition, the anomeric proton of **9** occurs at higher field (δ 5.86) than the anomeric proton of **12** (δ 6.58) as predicted.[9] The 3-carboxyhydrazide **10** was prepared by reacting **7** with hydrazine overnight, and the corresponding 3-carbohydroxamic acid **11** was readily obtained with hydroxylamine.

Condensation of 3-cyano-1,2,4-triazole[10] with **2** by the fusion method provided 1-(3′-trifluoroacetamido-3′-deoxy-2′,5′-di-*O*-acetyl-β-D-ribofuranosyl)-3-cyano-1,2,4-