Sleep-Inducing *N*-Alkyl-5-[*m*-(trifluoromethyl)phenyl]-5-hydroxy-2-pyrrolidinones and *N*-Alkyl-3-(trifluoromethyl)cinnamamides[1]

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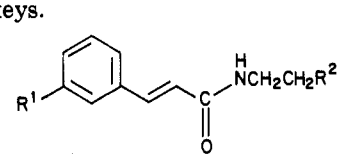
A series of *N*-alkyl-3-[*m*-(trifluoromethyl)phenyl]-5-hydroxy-2-pyrrolidinones and *N*-alkyl-3-(trifluoromethyl)-cinnamamides were prepared and screened in a series of tests designed to detect potential sleep inducers. The more active members of the series were evaluated for their ability to induce sleep in *Cebus* monkeys. The most active compound, *N*-methyl-5-[*m*-(trifluoromethyl)phenyl]-5-hydroxy-2-pyrrolidinone, was equal to methaqualone.

A number of cinnamic acid amides have been reported to possess CNS activity as sedatives,[2] muscle relaxants,[3] anticonvulsants,[4] and antidepressants.[4c,5] Two members of this class of compounds that have received advanced studies are cinromide (I) as an anticonvulsant agent[4a] and drocilamide (II; Brolitene), a substance that is clinically active in man as a muscle relaxant.[3] In this paper we describe the synthesis of the *m*-CF_3 analogues (IV) of I and II, the related 5-aryl-5-hydroxy-2-pyrrolidinones (V), and their tautomeric open-chain analogues (VII). These substances have been evaluated for CNS depressant activity in mice and several of them as potential sleep inducers in *Cebus* monkeys.

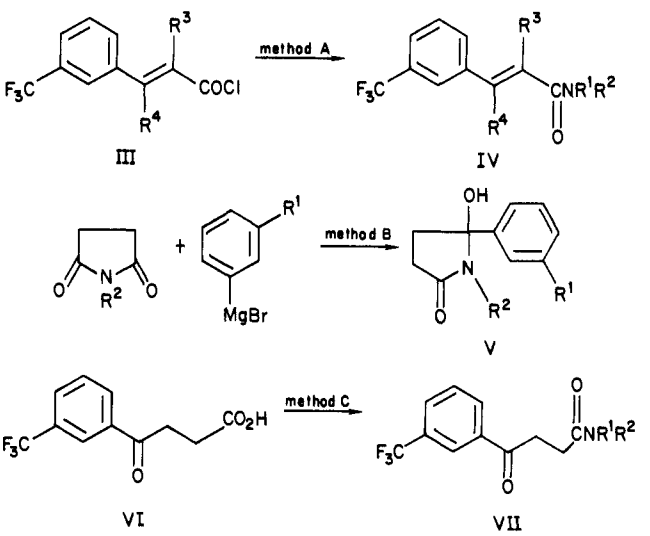


I, R^1 = Br; R^2 = H
II, R^1 = H; R^2 = OH

Chemistry. The synthesis of the compounds used in the present work are depicted in Scheme I and listed in Table III. Cinnamamides IV were prepared by reacting the acid chloride III with an excess of a primary or amine (method A). Preparation of the required 5-aryl-5-hydroxy-2-pyrrolidinones V was accomplished by treating a *N*-alkylsuccinimide with the appropriate aryl-Grignard reagent in THF (method B). The 3-aroylpropionamides VII were obtained from the mixed anhydride of the 3-aroylpropionic acid VI and ethyl chloroformate followed by treatment with a bulky primary or secondary amine (method C).

Since it has been demonstrated[6] that N-monosubstituted γ-keto amides such as VII (R^1 = H, $R^2 \neq$ H) and the 5-hydroxy-2-pyrrolidone V can exist in either the open or cyclic form, the ^{13}C NMR spectra was determined for these substances. A typical carbinolamine ^{13}C signal was observed at 90 ± 2 ppm for compounds assigned structure V (Table II, **10–15, 22–24**) while the open-chain analogues VII (Table II, **16–21**) gave an aryl CO signal at 190 ± 5 ppm.

Scheme I



Pharmacology. All derivatives of IV, V, and VII were submitted to a battery of behavioral and drug-interaction tests[7,8] in mice that have been used in our laboratories to detect compounds with potential CNS activity. The results of these tests are shown in Tables I and II. Anticonvulsant activity was measured by determining the antagonism to *N*-sulfamoylhexahydroazepine[9] (N-SA). General CNS-depressant activity was defined by the ability of substances to produce neurologic deficit on the rotarod[10] and by their abilities to reinduce "anesthesia" following recovery of loss of righting reflex obtained with hexobarbital.[11]

Table I contains a list of the cinnamamides prepared for this study. In behavioral tests the compounds as a group showed a weak to moderate central nervous system depressant profile with the most active member **1** being in the range of methaqualone. Protection against N-SA was demonstrated by several members of the series with compound **1** active in the range of methaqualone. Reinduction of hexobarbital anesthesia was found to be most potent in compounds **1** and **9** with activity similar to methaqualone, whereas compounds **2**, **4**, and **5** were equal to brolitene. Four analogues (**1**, **2**, **5**, and **9**) were selected for evaluation to induce sleep in *Cebus* monkeys on the basis of on their activity in the hexobarbital test and overall CNS profile. No correlation was found between the ability of these substances to induce sleep in *Cebus* monkeys and

(1) Presented in part at the 184th National Meeting of the American Chemical Society, Sept 1982, Kansas City, MO.
(2) Van Heyningen, E.; Brown, C. N.; José, F.; Henderson, J. K.; Stark, P. *J. Med. Chem.* **1966**, *9*, 675.
(3) (a) Bayssat, M.; Boucherle, A.; Coeur, A.; Grand, M. *Chim. Ther.* **1973**, *8*, 202. (b) Grand, M.; Depin, J. C.; Fontaine, L.; Bayssat, M. Réunion de l'Association des Pharmacologistes, Lyon, France, Apr 22, 1972.
(4) (a) Lockard, J. S.; Levy, R. H.; DuCharme, L. L.; Congdon, W. C. *Epilepsia* **1979**, *30*, 339. (b) Grivsky, E. M. U.S. Patent 4 107 320, Aug 15, 1978; *Chem. Abstr.* **1979**, *90*, 76576. (c) Smith, Kline and French Laboratories, Brit. Pat. 1 131 727, Oct 23, 1968; *Chem. Abstr.* **1969**, *70*, 37497.
(5) Horrom, B. W. U.S. Patent 3 801 636, Apr 2, 1974; *Chem. Abstr.* **1974**, *81*, 3634.
(6) Flitsch, W. *Chem. Ber.* **1970**, *103*, 3205.
(7) Irwin, S. "Animal and Clinical Pharmacologic Techniques in Drug Evaluation"; Nodine, J. H., Siegler, P. E., Ed.; Year Book Publishers: Chicago, IL, 1964; pp 36–54.
(8) Chen, G. *Symp. Sedat. Hypn. Drugs* **1954**, 1953, 45.
(9) Iorio, L. C.; Ryan, E. A.; Gogerty, J. H. *Arch. Int. Pharmacodyn. Ther.* **1973**, *206*, 282.
(10) Dunham, N. W.; Miya, T. S. *J. Pharm. Sci.* **1957**, *46*, 208.
(11) Winter, C. F. *J. Pharmacol. Exp. Ther.* **1948**, 94, 7.

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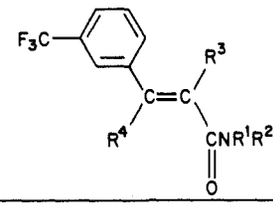
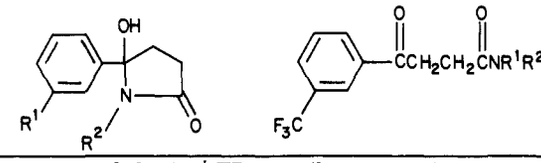
Table I. Neuropharmacological Data on 3-(Trifluoromethyl)cinnamamides

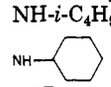
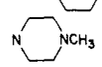


no.	NR^1R^2	R^3	R^4	LD_{50},[a] mg/kg ip	behavior,[b] ED_{50} mg/kg ip: ataxia	docility	LRR	N-SA:[c] ED_{50}, mg/kg ip	rotarod:[d] ND_{50}, mg/kg ip	barbiturate[e] reinduction: RD_{50}, mg/kg ip	sleep[f] monkey ratio
1	NH_2	H	H	259.6	42.9	37.5	54.5	49.5	46.9	17.2	NA
2	$NHCH_3$	H	H	275	120	92.9	200	84.1	149	76.3	0.5
3	NHC_2H_5	H	H	333.3				90	>75	>75	
4	$NHCH_2CH_2OH$	H	H	400	116.7	78.6	185.7	100	115.8	66.7	
5	$NHCH_2CH(C_2H_5)OH$	H	H	333.3	90	87.5	92.9	64.4	57.9	68.8	NA
6	$N(CH_3)_2$	H	H	300				>75	>75	>75	
7		H	H	300	>100	>100	>100	>75	>75	>75	
8	$NHCH_3$	H	CH_3	>400	87.5	87.5	>100	>75	>75	56.3	
9	$NHCH_3$	CH_3	H	300	75.0	75.0	>100	68.8	>75	24.7	0.3
11	broliterene			>400	183.3	183.3	>200	116.7	>200	75	
	methaqualone			565	34.4	35.6	39.8	31.5	31.5	21.9	1.0

[a] Acute toxicity studies were carried out with paired male Royal Hart Wistar rats, 136–160 g, placed in 7 × 7 × 14 wire cages. The LD_{50} values were obtained 2-h postadministration of compounds with use of four rats per substance and estimated by probit analysis. [b] Analyses of behavior used a modification of the method of ref 7; LRR = loss of righting reflex; 10 mice per dose. [c] N-SA = *N*-sulfamoylhexahydroazepine; methods of ref 9; 10 mice were used per dose. [d] Method of ref 10; 10 mice per dose, ND = neurological deficit. [e] Modified anesthesia (70 mg/kg iv) and reinduction of "anesthesia" (loss of righting) was measured from that time. [f] Ratio of the oral dose of the test substance relative to that of methaqualone (15 mg/kg po) to induce sleep in the same *Cebus* monkey. NA = not active at any dose as regards induction of sleep in the *Cebus* monkey. See Experimental Section for details.

Table II. Neuropharmacological Data on *N*-Alkyl-5-aryl-5-hydroxy-2-pyrrolidinones and 3-Aroylpropionamides



no.	R^1	R^2	NR^1R^2	LD_{50},[a] mg/kg ip	behavior,[b] ED_{50}, mg/kg ip: ataxia	docility	LRR	N-SA:[c] ED_{50} mg/kg ip	rotarod:[d] ND_{50}, mg/kg ip	barbiturate[e] reinduction: RD_{50}, mg/kg ip	sleep[f] monkey ratio
10	CF_3	H		>400	>200	>200	>200	>200	>200	150	0.1
11	CF_3	CH_3		366.7	128.6	93.8	133.3	51.6	106.5	45.1	1.0
12	*p*-CF_3	CH_3		>400	>200	>200	>200	131.9	140.5	>200	
13	CF_3	C_2H_5		>400	>100	>100	>100	>75	>75	68.8	NA
14	CF_3	*n*-C_3H_7		>400	>200	>200	200	150	>200	131.9	
15	CF_3	*n*-C_4H_9		>400	>200	>200	>200	183.3	>200	150	NA
16	CF_3		$N(CH_3)_2$	300	>50	>50	>50	>37.5	>37.5	127.6	
17	CF_3		NH-*i*-C_3H_7	>400	>200	>200	>200	>150	>150	126.9	
18	CF_3		NH-sec-C_4H_9	300	>200	>200	>200	>200	>150	>50	
19	CF_3		NH-*i*-C_4H_9	162.5	>200	>200	>200	>200	>200	>200	
20	CF_3			>400	>200	>200	>200	>200	>200	>200	
21	CF_3			>400	>50	>50	>50	>37.5	>37.5	>37.5	
22	H	CH_3		300	>200	>200	>200	137.5	>200	150	
23	Cl	CH_3		>400	186	158	>200	>150	>150	>150	
24	CH_3	CH_3		300	93	81.5	138	60.9	111.5	49.5	NA

[a–f] See corresponding footnotes in Table I.

their abilities to modify behavior or interact with centrally acting drugs in mice. Compounds **1** and **5** did not show any sleep-inducing activity in monkeys at a dose where methaqualone induced sleep while **2** and **9** were 0.5 and 0.3 times as active as methaqualone in inducing sleep, respectively. Additional testing with **2** at 30 mg/kg po in three *Cebus* monkeys confirmed the sleep-inducing activity with a duration of ca. 9 h. The ability to induce sleep did not appear to interfere with postsleep behavior in that no significant alteration in operant conditioned performance was noted in these monkeys 9–11 h following administration of **2**. On the other hand, compound **2** was tested at 40 mg/kg po in the rat Geller conflict test and found to cause an initial increase in the variable interval (VI) response followed by a secondary depression of the VI response that was still noted 6 days later. At doses of 80 and 160 mg/kg po, an increase in conflict responding was noted which was maintained for 24 h after drug administration. Since the increased responses found in the VI portion of the Geller conflict test have been correlated with potential drug side effects in our laboratories, it was decided not to pursue **2** or related compounds.

We then turned our attention to compounds of structure V which can be considered as hydrated and cyclized ho-

Table III. Physical Properties

no.	method,[a] % yield	mp, °C (recrystn solvent)[b]	empirical formula
1	A, 95	102–103 (C)	$C_{10}H_8F_3NO$
2	A, 92	124–125 (A)	$C_{11}H_{10}F_3NO$
3	A, 98	81–82 (A)	$C_{12}H_{12}F_3NO$
4	A, 97	110–112 (B)	$C_{12}H_{12}F_3NO_2$
5	A, 96	107–108 (D)	$C_{14}H_{16}F_3NO_2$
6	A, 97	82–84 (E)	$C_{12}H_{12}F_3NO$
7	A, 90	80–81 (E)	$C_{15}H_{17}F_3N_2O$
8	A, 82	89–90 (F)	$C_{12}H_{12}F_3NO$
9	A, 86	95–96 (G)	$C_{12}H_{12}F_3NO$
10	B, 88	130–132 (F)	$C_{11}H_{10}F_3NO_2$
11	B, 47	137–139 (H)	$C_{12}H_{12}F_3NO_2$
12	B, 36	173–175 (A)	$C_{12}H_{12}F_3NO_2$
13	B, 41	117–118 (A)	$C_{13}H_{14}F_3NO_2$
14	B, 56	113–115 (A)	$C_{14}H_{16}F_3NO_2$
15	B, 59	110–111 (A)	$C_{15}H_{18}F_3NO_2$
16	C, 47	oil	$C_{13}H_{14}F_3NO_2$
17	C, 36	119–120 (G)	$C_{14}H_{16}F_3NO_2$
18	C, 38	104–105 (I)	$C_{15}H_{18}F_3NO_2$
19	C, 69	174–175 (A)	$C_{16}H_{20}ClF_3N_2O_2$
20	C, 78	143–144 (A)	$C_{17}H_{20}F_3NO_2$
21	C, 39	123–125 (A)	$C_{15}H_{18}F_3NO_2$
22	B, 72	158–160 (A)	$C_{11}H_{13}NO_2$
23	B, 53	132–133 (A)	$C_{11}H_{12}ClNO_2$
24	B, 81	128–130 (A)	$C_{12}H_{15}NO_2$

[a] See Experimental Section. [b] A, EtOH; B, CH_2Cl_2–Et_2O; C, C_6H_6; D, CH_2Cl_2; E, Et_2O–hexane; F, CH_2Cl_2–hexane; G, hexane; H, Et_2O; I, $CHCl_3$–Et_2O. [c] Unless otherwise stated, the C, H, N analyses of 1–24 and the Cl analyses of 19 and 23 are within ±0.4% of the theoretical values.

mologues of IV and VII and open-chain tautomers of V (Table II). In addition to the N-substituted *m*-CF_3 compounds, a *p*-CF_3 derivative (12) and the *m*-Cl and *m*-CH_3 analogues 23 and 24 were prepared and evaluated. As a class, compounds related to structure V (10–15, 22–24) were considerably less toxic and gave a weaker CNS-depressant profile while compounds related to structure VII (16–21) were essentially devoid of any activity at the doses used for testing. Five compounds (10, 11, 13, 15, and 24) were selected for evaluation in the monkey sleep model on the basis of their overall CNS profile. As with compounds related to IV, no correlation could be found between any of the test systems and their ability to induce sleep. Although compounds 11 and 24 have almost equal activity in their ability to reinduce sleep in the hexobarbital test, the *m*-CH_3 analogue 24 showed no activity in the sleep monkey model while 11, the *m*-CF_3 isomer, was equipotent to methaqualone. Further testing in the sleep monkey model with 11 indicated that it induced sleep at 15–30 mg/kg po for a duration of ca. 6 h with minimal disruption of the normal physiological sleep pattern. It also failed to cause any significant alteration in performance postdrug, and at doses up to 60 mg/kg po in rats it did not disrupt the conflict or VI response in the Geller conflict test. Compound 11 has been recommended for clinical evaluations as a sleep inducer.

Experimental Section

Chemical Synthesis. Melting points were determined in a Thomas-Hoover capillary melting point apparatus and have not been corrected. For compounds listed in Table III, ^1H NMR ($CDCl_3$ or Me_2SO-d_6) spectra were obtained on a Varian Associates A-60 spectrometer with $SiMe_4$ as an internal standard and the ^{13}C NMR were taken at 25.2 MHz on a Varian XL-100-15 spectrometer equipped with a pulse Fourier transform package. Analytical thin-layer chromatography was conducted on precoated 40 × 80 mm plastic sheets of silica G with fluorescent indicator for all compounds reported in this paper.

Method A. General Procedure. A stirred solution of *m*-(trifluoromethyl)cinnamoyl chloride (0.02 mol) in 100 mL of anhydrous THF was cooled with an ice bath and then treated dropwise with a solution of amine (0.10 mol) in 50 mL of THF. The mixture was stirred overnight at room temperature, and the resultant salts were removed by filtration and then washed with THF. The filtrate and washes were combined and concentrated in vacuo. The residue was crystallized from the appropriate solvent to give compounds 1–9 listed in Table III.

***N*-Alkylsuccinimides. General Procedure.** A stirred solution of succinic acid (11.8 g, 0.10 mol) in 200 mL of THF was treated dropwise (exotherm) with 7.6 g (0.105 mol) of *n*-butylamine and then allowed to stand overnight at room temperature. The solid was removed by filtration and then distilled in vacuo on a water aspirator to give 8.8 g (56%) of *N*-*n*-butylsuccinimide, bp 137–139 °C (15 mm). In a similar manner there was obtained the *N*-ethyl [62%, bp 125–127 °C (15 mm)], *N*-*n*-propyl [59%, bp 139–142 °C (20 mm)], and *N*-isobutyl [43%, bp 159 °C (18 mm)] analogues.

Method B. General Procedure. A stirred mixture of magnesium shavings (0.15 mol) in 50 mL of anhydrous Et_2O (N_2 atmosphere) was treated dropwise with a solution of *m*-bromobenzotrifluoride (0.16 mol) in 50 mL of Et_2O at such a rate that reflux occurred. After the addition was complete, refluxing was continued for an additional 1 h. The mixture was allowed to come to room temperature, treated dropwise with a solution of *N*-alkylsuccinimide (0.10 mol) in 100 mL of anhydrous C_6H_6 (exothermic), refluxed for 4 h, and then allowed to stand overnight at room temperature. The reaction mixture was then treated dropwise with 50% H_2SO_4 (45 mL) at such a rate that reflux occurred and then stirred an additional 0.5 h. The aqueous layer was separated and washed with C_6H_6 (2 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ (50 mL) and H_2O (2 × 50 mL), dried with anhydrous $MgSO_4$, and then filtered. The filtrate was concentrated in vacuo and the residue crystallized from the appropriate solvent to give compounds 10, 11, 13–15, and 22–24 (Table III). In a similar manner, the Grignard reagents from 4-bromobenzotrifluoride, bromobenzene, 3-bromochlorobenzene, and 3-bromotoluene reacted with *N*-methylsuccinimide to give 12, 22, 23, and 24 respectively.

Method C. General Procedure. A stirred mixture of 3-[*m*-(trifluoromethyl)benzoyl]propionic acid (0.10 mol), triethylamine (0.10 mol), and 250 mL of anhydrous $CHCl_3$ was cooled to 0 °C and then treated dropwise with a solution of ethyl chloroformate (0.10 mol) in 100 mL of $CHCl_3$ at such a rate that the internal temperature did not exceed 10 °C. After an additional 2.5 h of stirring at 5 ± 5 °C, the mixture was treated dropwise with the appropriate amine (0.10 mol) in 25 mL of $CHCl_3$. The cooling was removed and the reaction was stirred for ca. 20 h at room temperature. The $CHCl_3$ was decanted, washed with H_2O, 1 N HCl, and saturated NaCl, respectively, and then dried with anhydrous $MgSO_4$. The solution was filtered and the solvent removed in vacuo to give a solid that was recrystallized from the appropriate solvent to give compounds 16–21 (Table III).

Effects on Sleep Patterns of *Cebus* Monkeys. To study the effects of drugs on sleep, the animals were implanted under sterile conditions with both cortical (monopolar) and subcortical (bipolar) electrodes. Stainless steel screws were inserted over the superior orbital ridges of both eyes for electrooculograms (EOG), and Nichrome wires bared at their tips were inserted in the dorsal neck muscles for electromyograms (EMG). All subjects were allowed at least 2 weeks to recover before any recordings were obtained.

For recording purposes, the monkeys were restrained via neck and waist plates in chairs, which were placed within sound-attenuated cubicles. Five channels of data per animal were recorded. Sessions began with dosing of the monkeys at 5:00 p.m. each night. EEG data were recorded for 13.5 h, Monday through Thursday, and were scored by using visual analysis. One decision (sleep epoch) was made per 50 s of data (one page), with the stage of sleep occupying the majority of the time per epoch being scored as the stage of sleep for the total epoch. The exception was deep sleep, for which 10 s per epoch was sufficient to score the epoch as deep sleep.

In order to determine the effects of disturbances in sleep patterns on behavioral performance, the monkeys were tested for their abilities to successfully complete a relatively complex operant behavior timed feeding schedule (DRL-60). Nine hours after the onset of each experimental session, an audible tone signified the

start of a 90-min operant conditioning task. The tone reoccurred every 5 min until the monkey was awakened and a lever press occurred. This lever press was immediately reinforced with a banana-flavored pellet and also initiated a DRL (differential reinforcement of low rate of responding) 60-s schedule. According to the contingencies of the DRL-60, each lever press reset a timer to zero. However, if 60 s elapsed between lever presses, the monkey was automatically reinforced with a banana-flavored pellet. The number of reinforcements were recorded, and interresponse time, i.e., the times between lever presses, were counted.

Each animal served as its own control, with each parameter of sleep and performance being statistically compared to the last 15 control days. Each control night, the subjects were dosed intragastrically with a carboxymethylcellulose placebo suspension. Drugs were administered per os. Computerized statistical readouts were obtained for all control sessions in order to determine any drug rebound and/or carry-over effects on sleep patterns and to ensure control stability between drug administration.

Effects on Experimentally Induced Conflict in Rats. In the Geller conflict test[12] brief sessions of an approach-avoidance paradigm (conflict) were interposed upon a food-reinforced behavioral schedule (variable interval, VI). The primary activity of antianxiety drugs was demonstrable during the conflict trials as an increase in responding. Secondary activities, or side effects, were demonstrable as disruptions of the VI portion of the schedule.

(12) Geller, I.; Seifler, J. *Psychopharmacologia* **1960**, *1*, 482.

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Registry No. 1, 93040-58-5; **2**, 93040-59-6; **3**, 93040-60-9; **4**, 93040-61-0; **5**, 93040-62-1; **6**, 93040-63-2; **7**, 93040-64-3; **8**, 93040-65-4; **9**, 93040-66-5; **10**, 56948-75-5; 11, 56948-73-3; **12**, 93040-67-6; **13**, 56948-74-4; 14, 93061-24-6; **15**, 93040-68-7; **16**, 93040-69-8; **17**, 93040-70-1; **18**, 93061-41-7; **19**, 93040-71-2; **20**, 93040-72-3; **21**, 93040-73-4; **22**, 23132-30-1; **23**, 93040-74-5; **24**, 59618-42-7; *m*-(trifluoromethyl)cinnamoyl chloride, 60689-14-7; *N*-*n*-butylsuccinimide, 3470-96-0; *N*-ethylsuccinimide, 2314-78-5; *N*-propylsuccinimide, 3470-97-1; *N*-isobutylsuccinimide, 13916-45-5; *m*-bromobenzotrifluoride, 401-78-5; 4-bromobenzotrifluoride, 402-43-7; bromobenzene, 108-86-1; 3-bromochlorobenzene, 108-37-2; 3-bromotoluene, 591-17-3; 3-[*m*-(trifluoromethyl)benzoyl]-propionic acid, 56948-76-6; 2-aminoethanol, 141-43-5; 2-hydroxybutylamine, 13552-21-1; *N*-methylpiperazine, 109-01-3; succinic acid, 110-15-6.

Lack of Influence of the Carbamoyl Group on the Stereochemistry of the Acid-Catalyzed Opening of the Aziridine Ring of the Mitomycins and of Congeners

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The acid-catalyzed opening of the aziridine ring of mitomycins A and C is known to occur predominantly with cis stereochemistry. We have observed that the presence or absence of a carbamoyl group at C-10 of mitomycin C and in certain of its analogues does not have a significant influence on the stereochemistry of the opening of this ring. The trans product obtained from mitomycin C was shown to be stable when treated with acid under the conditions of its formation. Mitomycin **B** was also shown to yield predominantly the cis product when it was subjected to acid-catalyzed opening of its aziridine ring. The ^{1}H NMR spectra of acetate derivatives prepared from mitomycin **B** show two sets of signals that are due to two populations of rotamers. The analysis of these spectra has substantiated several previous spectral assignments. This paper also presents some thoughts on acid-catalyzed bifunctional DNA alkylation by mitomycins and 10-decarbamoyloxy-9-dehydromitomycins.

The mitomycins[1-3] (**1a-d**, Chart I), the 10-decarbamoyloxy-9-dehydromitomycins such as **2a-b**,[4] and several derivatives of mitomycin C[5] are powerful antitumor antibiotics among which mitomycin C (**1c**) is currently being used for the mainly palliative treatment of clinical cancers.[6] The aziridine ring of this antibiotic is known to be involved in DNA alkylation reactions after reductive activation,[7] and it has been suggested that C-10 of mitomycin C may also be activated by reduction[8,9] and then may contribute a second binding site that may be involved in the formation of DNA cross-links. There exist two related reductive chemical model reactions that support this proposal,[10,11] but there exists presently no evidence that this second site is of biological significance.[7,12] Acid-catalyzed activation of mitomycins B and C was also shown to yield cross-linked DNA in vitro,[13,14] and it is likely that C-1 of mitomycin C after opening of the aziridine ring is involved in the DNA binding; however, hardly any information is presently available on any second alkylation site that could account for cross-link formation by mitomycins activated by mild acid treatment.

The opening of the aziridine ring of mitomycin C in acid-catalyzed reactions occurs predominantly with cis

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