9.15 g (91%); bp 110–125 °C (0.1 mm); IR 3400 cm^{-1}.

2-Methyl-*trans*-decahydroisoquinolin-5-one Oxime (3). The diastereoisomeric mixture of alcohols 1 (20.8 g, 0.12 mol) was dissolved in 300 mL of benzene and refluxed in a flask equipped with a Dean–Stark trap for 30 min to ensure dryness. To this solution were added 34.6 g (0.31 mol) of potassium *tert*-butoxide and 112.3 g (0.62 mol) of benzophenone. The mixture was purged with dry nitrogen and refluxed for 6 h with a continuous stream of dry nitrogen slowly passing through the reaction flask. After cooling, the reaction medium was extracted with a 10% HCl solution. The acidic extract was poured over ice and then made strongly alkaline with NaOH solution. The resulting basic solution was extracted with ether. The ethereal extract was washed twice with a saturated aqueous NaCl solution, dried over anhydrous Na_2SO_4, and concentrated. The crude 2-methyl-*trans*-decahydroisoquinolin-5-one (2), 20 g (97%), was obtained as a dark brown oil. Purification was achieved by distillation at 55–65 °C (0.1 mm) [lit.[11] 85 °C (0.3 mm)]: IR 1710 cm^{-1}; picrate (recrystallized from methanol) mp 213 °C (lit.[11] 211–213 °C. Conversion of the ketone 2 to its oxime was achieved according to the method of Fryer et al.[12] Compound 2, 1 g (0.006 mol), and hydroxylamine hydrochloride, 1.6 g (0.023 mol), were dissolved together with 3.19 g (0.023 mol) of hydrated sodium acetate in 10 mL of H_2O and 20 mL of 95% EtOH. The mixture was heated under reflux for 30 min. The volume of the mixture was then concentrated under reduced pressure by rotary evaporation until a precipitate formed. The precipitate was collected by vacuum filtration. A further product crop was obtained by concentration of the filtrate. The combined fractions of precipitated oxime and salts were added to water, which conveniently separated the aqueous soluble salts from the H_2O-insoluble oxime. The oxime was collected by vacuum filtration and washed with H_2O, yielding 0.78 g (72%) of the desired 2-methyl-*trans*-decahydroisoquinolin-5-one oxime (3) as a white solid: mp 182–183 °C; IR (C=NOH) 1667 cm^{-1}. Anal. ($C_{10}H_{18}N_2O$) C, H, N.

5-Amino-2-methyl-*trans*-decahydroisoquinoline (4). 2-Methyl-*trans*-decahydroisoquinolin-5-one oxime (3) was reduced to the desired amine 4 according to the procedure of Burger and Bennet.[13] Compound 3, 1 g (0.005 mol), was suspended in 65 mL of hot, dried ($LiAlH_4$) tetrahydrofuran and added dropwise to a stirring suspension of 0.67 g (0.02 mol) of $LiAlH_4$ in 50 mL of dried tetrahydrofuran. The mixture was heated to reflux and maintained for 24 h. After cooling, the reaction complex was decomposed by the careful dropwise addition of H_2O. The decomposed reaction mixture was treated with 50 mL of a 10% HCl solution and then poured into a separatory funnel. The tetrahydrofuran layer was washed three times with 10% HCl solution. The acid extracts were combined, made strongly alkaline with NaOH, and extracted with ether. The ethereal extract was dried over anhydrous Na_2SO_4 and concentrated. The crude 5-amino-2-methyl-*trans*-decahydroisoquinoline, 0.82 g (89%), was obtained as a dark yellow oil. Purification was achieved by distillation at 50–60 °C (0.1 mm): IR (NH) 3300 cm^{-1}.

Conversion of this amine to its 3,4,5-trimethoxybenzamide according to the procedure described by Mathison[2a] yielded a solid, mp 217–219 °C. Mixture melting point with an authentic sample[2a] of 5-(3,4,5-trimethoxybenzamido)-2-methyl-*trans*-decahydroisoquinoline produced no depression of the melting point.

General Preparation of 5-Benzamido-2-methyl-*trans*-decahydroisoquinolines 5–15. The appropriate acid chloride (0.0065 mol) was dissolved in dried benzene and added to a stirring solution of 5-amino-2-methyl-*trans*-decahydroisoquinoline (4; 0.0057 mol) and triethylamine (0.015 mol) dissolved in dry benzene. The mixture was allowed to reflux with stirring for 48 h. Removal of the benzene under reduced pressure by rotary evaporation yielded a solid residue, which was dissolved in chloroform and washed three times with a 10% Na_2CO_3 solution followed by H_2O. The chloroform extract was dried over anhydrous Na_2SO_4 and concentrated to yield the crude product. The structures of the compounds, recrystallization solvents, melting points, and percentage yields are listed in Table I.

Acknowledgment. The authors acknowledge the valuable assistance and interest of Drs. Robert L. Stanley and Ben R. Durian, Ferris State College, School of Pharmacy, at several stages of this investigation.

(11) S. Kimoto and M. Okamoto, *J. Chem. Pharm. Bull.*, **9**, 480 (1961).
(12) R. I. Fryer, J. V. Earley, E. Evans, J. Schneider, and L. H. Sternbach, *J. Org. Chem.*, **35**, 2455 (1970).
(13) A Burger and W. Bennett, *J. Am. Chem. Soc.*, **72**, 5414 (1950).

Synthesis and Biological Activity of Flavipucine Analogues

N. N. Girotra, A. A. Patchett, S. B. Zimmerman, D. L. Achimov, and N. L. Wendler*

Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, New Jersey 07065.
Received July 20, 1979

A series of analogues of flavipucine was prepared possessing side chain as well as nuclear variants. The analogue with an octyl side chain (**5d**) was found to exhibit enhanced activity against several bacteria and fungi as compared with the natural product itself. The separation and characterization of the individual diastereoisomeric pairs both spectroscopically and with respect to chromatographic mobility have been effected.

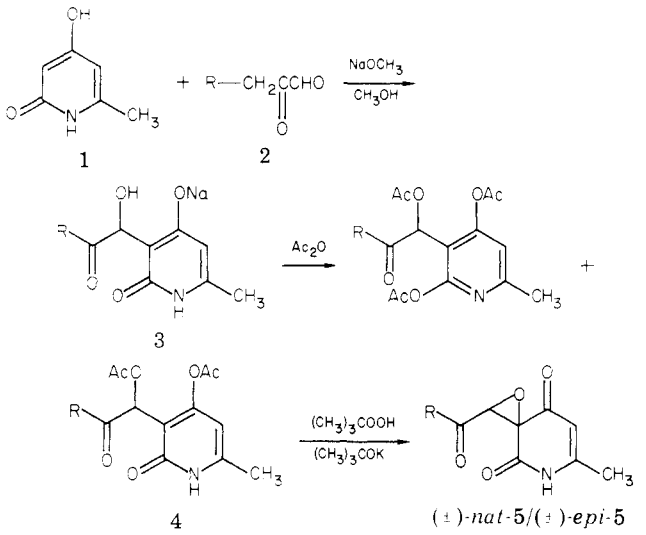
In 1968 Casinovi et al.[1] reported the moderate activity of flavipucine (isolated from *Aspergillus flavipes*) against certain Gram-positive and Gram-negative organisms. The total synthesis of flavipucine in 1976[2] made possible the selective synthesis of nuclear as well as side-chain variants of this antibiotic. In the process of preparing these analogues, the original synthesis has been materially improved, especially in the epoxidation sequence, thereby permitting yields in several instances in excess of 70% to be realized. The mechanism of this reaction, as well as other chemistry related to the flavipucine structure, has already been published elsewhere.[3,4]

The single most remarkable aspect regarding the biological activity of flavipucine is the finding that both synthetic (±)-flavipucine and its (±) diastereoisomer exhibit activity against a variety of organisms equal to that of natural (−)-flavipucine itself.[5] A degree of differenti-

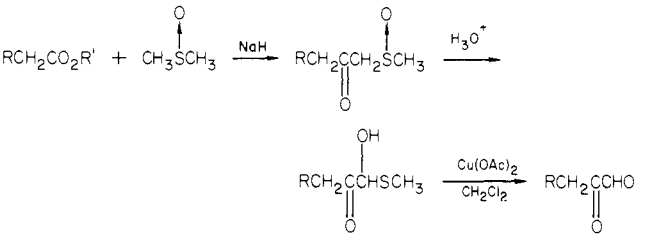
(1) C. G. Casinovi, G. Grandolini, R. Mercantini, N. Oddo, R. Olivieri, and N. Tonolo, *Tetrahedron Lett.*, 3175 (1968).
(2) N. N. Girotra, Z. S. Zelawski, and N. L. Wendler, *J. Chem. Soc., Chem. Commun.*, 566 (1976). See also J. A. Findlay, J. W. H. Tam, and J. Krepinsky, *Synth. Commun.*, 149 (1977).
(3) (a) N. N. Girotra, A. A. Patchett, and N. L. Wendler, *Heterocycles*, **6**, 1299 (1977); (b) J. A. Findlay, J. Krepinsky, A. Shum, C. G. Casinovi, and L. Radics, *Can. J. Chem.*, **55**, 600 (1977).
(4) N. N. Girotra and N. L. Wendler, *Heterocycles*, **9**, 417 (1978); J. A. Findlay, *ibid.*, **12**, 389 (1979).
(5) Similar observations were made with racemic natural and unnatural dihydroquinine with respect to activity and toxicity in mice infected with *Plasmodium berghei*. Cf. A. Brossi, *Heterocycles*, **5**, 631 (1976), and references therein.

0022-2623/80/1823-0209$01.00/0

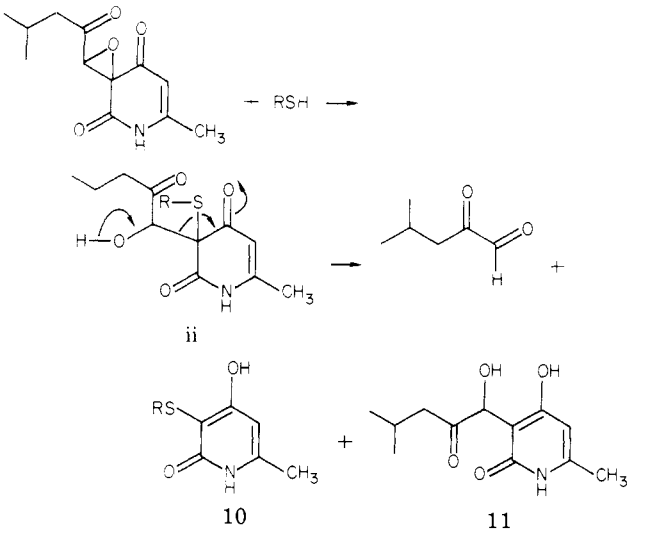
Scheme I



Scheme II

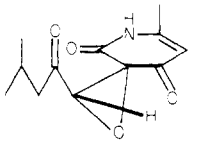


Scheme III



tiation in activity in the analogues, however, does develop both between the diastereoisomers themselves as well as with respect to natural flavipucine.

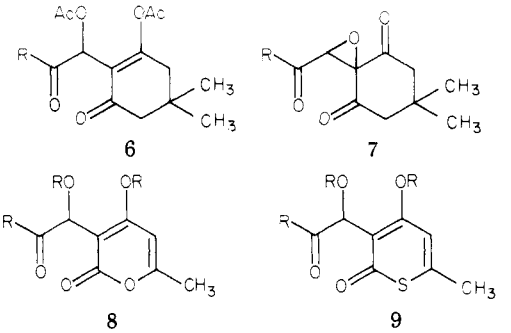
Recently, White et al.[6a] determined the relative configuration of (±)-flavipucine by X-ray determination. These authors found the acyl side chain to be cis with respect to the amide segment of the pyridone ring and designated this as the *Z* configuration (cf. i). We recently arrived at



i

the same configurational conclusion based on chemical findings to be published elsewhere.[6b] We, furthermore, have been able by means of relative TLC mobility and NMR correspondence to classify the diastereoisomeric pairs of the various analogues as belonging to either the natural ("nat") or unnatural ("epi")[7] series, respectively. In this connection, *nat*-flavipucine and analogues are more mobile on silica gel than the epi isomers. The nat compounds also exhibit a higher field epoxide proton and lower field pyridinone proton than their epi counterparts (see Table II).

The synthetic procedure employed in preparing the flavipucine analogues followed essentially that originally reported for flavipucine itself.[2] In each instance, the appropriate pyridinone 1 or its counterpart was permitted to condense with the corresponding α-ketoaldehyde 2 (Scheme I) in methanolic sodium methoxide solution to afford the coupled enolate 3 as its sodium salt (for exceptions, see Experimental Section). Acetylation of 3 with acetic anhydride yielded the corresponding diacetate 4 (see Table I), together with a minor amount of triacetate convertible to 4. The latter on treatment in *tert*-butyl hydroperoxide solution with 2 equiv of potassium *tert*-butoxide at 0 °C, followed by appropriate workup and purification, gave the corresponding mixture of (±)-*nat*- and (±)-*epi*-flavipucine analogues. The yields varied from 25 to 77%, depending on the specific structure. Only in the case of flavipucine itself, however, was the yield optimized (77%). Pertinent data on these compounds are recorded in Table II. Compound 7 contains the dimedone instead



of the pyridinone nucleus. The nuclear oxygen and sulfur systems 8 and 9 underwent apparent oxidative degeneration under the epoxidation conditions, thereby contravening our attempts to prepare the corresponding flavipucine analogues.

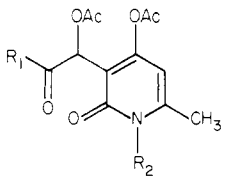
Synthesis of the α-ketoaldehyde (glyoxal) component 2 (Scheme II) proceeded by adaptation of the elegant method of Russell et al.[8] This method proved particularly fruitful, since yields were generally good (50–75%) and isolation of the free glyoxal was unnecessary. Thus, assay of the pertinent glyoxal content of the final product solution was simply effected by quantitative disemicarbazone formation and/or use testing in the condensation with pyridinone 1.

(6) (a) P. S. White, J. A. Findlay, and W. H. J. Tam, *Can. J. Chem.*, 1904 (1978). (b) N. N. Girotra and N. L. Wendler, *Tetrahedron Lett.*, 4793 (1979).

(7) In deference to White et al. (ref 6), we adopt their designation of "epi" for the unnatural diastereoisomer and abandon our earlier use of the term "iso" to avoid confusion with prior usage of the latter term as applied to the rearrangement product of flavipucine (see ref 3b).

(8) H.-D. Becker, G. J. Mikal, and G. A. Russell, *J. Am. Chem. Soc.*, 85, 3410 (1963); "Organic Syntheses", Collect. Vol V, Wiley, New York, 1973, p 937.

Table I

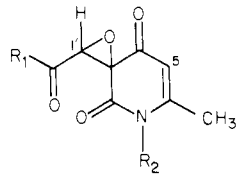


4

no.	compd	mp, °C	solvent	formula[a]
4a	$R_1 = (CH_3)_2CHCH_2$; $R_2 = H$	129-130	acetone-hexane	$C_{16}H_{21}O_6N$
4b	$R_1 = C_6H_5$; $R_2 = H$	206-208	CH_2Cl_2-ether	$C_{18}H_{17}O_6N$
4c	$R_1 = (CH_3)_3C$; $R_2 = H$	162-165	ether-hexane	$C_{16}H_{21}O_6N$
4d	$R_1 = CH_3$; $R_2 = H$	148-150	CH_2Cl_2-ether	$C_{13}H_{15}O_6N$
4e	$R_1 = CH_3(CH_3)_7$; $R_2 = H$	amorphous[b]		$C_{20}H_{29}O_6N$
4f	$R_1 = (CH_3)_2CHCH_2$; $R_2 = CH_3$	130-132 143-146	CH_2Cl_2-ether	$C_{17}H_{23}O_6N$
6	$R = (CH_3)_2CHCH_2$	amorphous		$C_{18}H_{26}O_6$
6a	$R = C_6H_5$	amorphous		$C_{20}H_{22}O_6$
8	$R_1 = C_6H_5$; $R_2 = OAc$	164-167	CH_2Cl_2-ether	$C_{18}H_{16}O_7$
9	$R_1 = (CH_3)_2CHCH_2$; $R_2 = OAc$	84-86	ether-hexane	$C_{16}H_{20}O_6S$

[a] C, H, and N (where applicable) for all crystalline compounds were within ±0.4% of the theoretical values. [b] $M^+ = 379$.

Table II



nat 1' H = α
epi 1' H = β

5

no.	compd	yield, %[a]	mp, °C	solvent	formula[b]	^{1}H NMR: chem shifts in δ[c] 1' H	5 H	solvent
5a	$R_1 = (CH_3)_2CHCH_2$; $R_2 = H$	77						
	nat		154-155	benzene	$C_{12}H_{15}O_4N$	3.83 (s)	5.70 (br s)	$CDCl_3$
	epi		136-138	benzene	$C_{12}H_{15}O_4N$	3.92 (s)	5.65 (br s)	$CDCl_3$
5b	$R_1 = (CH_3)_3C$; $R_2 = H$	47						
	nat		162-167[g]	CH_2Cl_2-ether	$C_{12}H_{15}O_4N$	4.09 (s)	5.57 (br s)	CD_3COCD_3
	epi		149-153	CH_2Cl_2-ether	$C_{12}H_{15}O_4N$	4.17 (s)	5.53 (br s)	CD_3COCD_3
5c	$R_1 = C_6H_5$; $R_2 = H$	71						
	nat		192-195[g]	acetone	$C_{14}H_{11}O_4N$	4.59 (s)	5.57 (br s)	CD_3SOCD_3
	epi		222-224[g]	acetone	$C_{14}H_{11}O_4N$	4.65 (s)	5.42 (br s)	CD_3SOCD_3
5d	$R_1 = CH_3(CH_2)_7CH_2$; $R_2 = H$	70[d]						
	nat		138-141	CH_2Cl_2	$C_{16}H_{23}O_4N$	3.89 (s)	5.65 (br s)	$CDCl_3$
	epi		135-137	CH_2Cl_2	$C_{16}H_{23}O_4N$	3.92 (s)	5.57 (br s)	$CDCl_3$
5e	$R_1 = CH_3$; $R_2 = H$	29						
	nat		185-188[g]	methanol	$C_9H_9O_4N$	3.83 (s)	5.60 (br s)	CD_3COCD_3
	epi		191-193[g]	methanol	$C_9H_9O_4N$	3.90 (s)	5.55 (br s)	CD_3COCD_3
5f	$R_1 = (CH_3)_2CHCH_2$; $R_2 = CH_3$[e]	25						
	nat		101-103	ether-hexane	$C_{13}H_{17}O_4N$	3.77 (s)	5.64 (br s)	$CDCl_3$
	epi		128-131	ether-hexane	$C_{13}H_{17}O_4N$	3.82 (s)	5.59 (br s)	$CDCl_3$
7	$R_1 = (CH_3)_2CHCH_2$	46[f]	53-55	hexane	$C_{14}H_{20}O_4$	3.60 (s)		$CDCl_3$
7a	$R_1 = C_6H_5$	53[f]	136-137	CH_2Cl_2-ether	$C_{16}H_{16}O_4$	4.20 (s)		$CDCl_3$

[a] Combined yield of both the isomers except in the last two cases; only in the case of 5a was the yield optimized. [b] C, H, and N (where applicable) for all compounds were within ±0.4% of theoretical values. [c] $(Me)_4Si$ used as internal standard. [d] A mixture of diacetate and triacetate was used for the last step. [e] The two isomers were indistinguishable by TLC [$CHCl_3$-acetone (85:15) and $CHCl_3$-CH_3OH (95:5)] on silica gel; they were, however, characterized by NMR. [f] Crude diacetate was used for the epoxidation step. [g] Decomposition.

Suggestive of a possible mode of biological action of the flavipucines is the very facile reaction of these substances with sulfhydryl groupings. Thus, under neutral to mildly alkaline conditions at ambient temperatures a rapid fission of side chain and nucleus occurs with, for example, thiocyanate or thiophenol. This cleavage appears to proceed via nucleophilic attack by RSH at the spirocyclic oxide center, yielding isobutylglyoxal and the corresponding thio-substituted pyridinone derivative **10** in high yield (Scheme III), together with a minor amount of **11** (cf. Experimental Section). A related cleavage of a spirooxide system with heterocyclic bases to give betaines was reported recently.[9]

Antibacterial Data. Testing data on ten bacteria and fungi are summarized in Table III. The modest potency but rather broad antibacterial spectrum of flavipucine (**5**) was described by Casinovi et al.[1] in their announcement of this antibiotic's isolation and characterization. It was a matter of considerable surprise to us to find that our

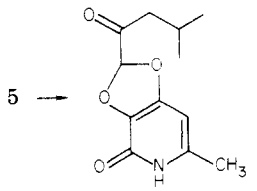
(9) I. K. Raiskuma, G. G. Pukitis, P. Y. Neiland, *Khim. Geterotsikl. Soedin.*, 889, 92 (1978).

Table III. In Vitro Antimicrobial Effects of (-)-Flavipucine and Its Analogues

	agar dilution: min inhibitory concn, μg/mL[a]									
	bacteria[b]							fungi[c]		
compd	*S.a.*	*B.b.*	*K.p.*	*A.a.*	*E.c.*	*P.a.*	*P.v.*	*P.p.*	*A.n.*	*A.s.*
( )-*nat*-flavipucine	4.7	33.9	4.7	100	50	>100	4.7	>100	>100	100
nat-5a	6.3	50	6.3	100	50	>100	6.3		>100	50
epi-5a	6.3	50	6.3	>100	50	>100	3.1		>100	25
epi-5f	12.5	100	50	>100	100	>100	6.3		50	6.3
nat-5f	25	100	100	>100	>100	>100	12.5		>100	12.5
epi-5e	100	>100	>100	>100	50	50	>100	>100	>100	>100
nat-5e	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
epi-5d	0.4	3.1	>100		>100		12.5	0.2	0.2	0.2
nat-5d	0.8	0.8	>100		>100			0.2	1.6	0.2
7	25	50	50	25	>100	50	50	6.3	25	3.1

[a] Twofold agar dilution method in nutrient agar-0.2% yeast extract using Steers' multiple inoculator apparatus (ref 14 and 15). [b] Abbreviations used are: *S.a.*, *Staphylococcus aureus*; *B.b.*, *Bordetella bronchiseptica*; *K.p.*, *Klebsiella pneumoniae*; *A.a.*, *Aerobacter aerogenes*; *E.c.*, *Escherichia coli*; *P.a.*, *Pseudomonas aeruginosa*; *P.v.*, *Proteus volgaris*. [c] Abbreviations used are: *P.p.*, *Pullaria pullulans*; *A.n.*, *Aspergillus niger*; *A.s.*, *Alternaria solani*.

synthetic (±)-flavipucine (**5a**) and its epimer, (±)-*epi*-**5a**, had, within experimental error, identical antibacterial spectra and potency with natural (-)-flavipucine. To our knowledge, this observation is without precedent among antibiotics. This suggests the absence of an active transport system in providing access of these compounds to their site of antibacterial action. Nor, apparently, is such a site responsive to a difference between -COCH= and -CONH- functions nor to the absolute configuration of the epoxide. At first we suspected that a common rearrangement product formed in a nonstereoselective process might account for the equivalent antibacterial findings. Flavipucine and its epimer are readily transformed to isoflavipucine (**12**)[3] by a variety of means, the mildest



12

being treatment with silica gel. Isoflavipucine, however, is inactive as an antibacterial. Both flavipucine and its epimer react with mercaptans under physiological conditions, as detailed above, with qualitatively equal facility. It may be that a relatively accessible, optically inactive mercaptan or similar nucleophile remote from stereochemical influence is involved in the antibiotic action of these compounds. Clearly, the above are only speculations, but they are consistent with the retention of some activity by the N-CH_3 analogue (**5f**). More importantly, they suggest focusing on side-chain variations to probe possible biological changes dependent on passive diffusion and to determine the specificity, if any, in a side-chain recognition site. Although we have made no extensive study of these possibilities, the high activity of the octyl ketone (**5d**) is noteworthy, especially in respect to three fungi under test compared with essentially no antifungal activity with flavipucine itself. Interestingly, the activity of this compound (**5d**) against Gram-negative organisms (*Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus vulgaris*) was sacrificed. Analysis of a more extensive series of compounds might permit optimization toward selected antibacterial and antifungal targets whose potentials have been suggested but not realized by the natural product itself.

Conclusion

The natural and *epi*-octyl analogues **5d** demonstrated markedly greater antimicrobial activity than (-)-flavipucine against *S. aureus*, *B. bronchiseptica*, and all three fungi tested. The **5f** natural compound was more active than (-)-flavipucine vs. *Alternaria solani* but was less effective against all other microorganisms tested.

Experimental Section

Melting points were taken on a microscope hot-stage apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer infracord instrument. NMR spectra were determined on a Varian T-60A spectrometer.

Compounds **5b-f** were prepared by the method outlined for **5a**. Compounds **7** [R = $(CH_3)_2CHCH_2$] and **7a** (R = C_6H_5) were also prepared according to the same general procedure, except that methanol was replaced by water and sodium methoxide by sodium hydroxide during the condensation of dimedone and the glyoxals. The use of methanol led to the partial introduction of the methoxyl moiety at C_1. The corresponding unstable amorphous diacetates (**6** and **6a**) were used for epoxidation without further purification.

Isobutylglyoxal. Methyl isovaleroyl methylsulfoxide (36.5 g) was prepared by the method of Corey and Chaykovsky[10] from 32.5 g (0.25 mol) of ethyl isovalerate: NMR ($CDCl_3$) δ 3.77 (s, $COCH_2SO$), 2.68 (s, $SOCH_3$). The latter without further purification was allowed to rearrange in acid solution according to the method of Russell et al.[8] to give 33.5 g (83%) of the methyl hemithioacetal of isobutylglyoxal: NMR ($CDCl_3$) δ 5.23 [s, COC*H*(OH)S], 1.95 (s, CH_3). The hemithioacetal was further converted with copper acetate in methylene chloride[8] to **isobutylglyoxal** [21.5 g (91%) by sc analysis] **disemicarbazone** (CH_3)OH): mp 243-246 dec; overall yield 75%. Anal. ($C_8H_{16}N_6O_2$) C, H, N.

***n*-Octylglyoxal** was prepared by the above method in 51% yield (CH_3OH), semicarbazone mp 225-227 °C. Anal. ($C_{12}H_{24}N_6O_2$) N.

In the same manner, ***n*-butylglyoxal** (58%) **disemicarbazone** [mp 235-236 °C. Anal. ($C_8H_{16}N_6O_2$) N] and ***sec*-butylglyoxal** (51%) **disemicarbazone** [mp 268-271 °C ($C_8H_{16}N_6O_2$) N] were prepared.

tert-Butylglyoxal was prepared by selenium dioxide oxidation of pinacolone.[11] Phenyl- and methylglyoxals are commercially available.

4-Hydroxy-6-methyl-3-(1′-hydroxy-2′-oxo-4′-methylpentyl)-2-pyridinone, Sodium Salt (3). To a stirred suspension of pyridinone 1[12] (3.128 g, 0.025 mol) in 300 mL of methanol was added sodium methoxide (1.350 g, 0.025 mol), followed by a solution of 3.542 g (0.031 mol) of freshly distilled isobutylglyoxal [bp 35 °C (25 mm), bath 80 °C; reported[13] 46 °C (12 mm)] in 25

(10) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **87**, 1345 (1965).
(11) R. C. Fuson, H. Gray, and J. J. Gonza, *J. Am. Chem. Soc.*, **61**, 1938 (1939).
(12) S. Seto, H. Sasaki, and K. Ogura, *Bull. Chem. Soc. Jpn.*, **39**, 281 (1966).

mL of methanol. After 16 h, at 25 °C the clear solution was concentrated to about 30 mL and diluted with 800 mL of ether to precipitate the product. The solid was collected by filtration, washed with ether, and dried under high vacuum to give colorless material (7.10 g, 109% containing some ether and methanol as indicated by NMR): IR (Nujol) 5.84, 6.08–6.29 μm; NMR (D_2O) δ 5.82 (br s, C_5H), 5.35 (s, $C_{1'}H$), 2.15 (s, C_6CH_3), 0.85, 0.82 [each d, J = 6 Hz, $-CH(CH_3)_2$].

4-Acetoxy-6-methyl-3-(1'-acetoxy-2'-oxo-4'-methylpentyl)-2-pyridinone (4a). Acetic anhydride (50 mL, 0.529 mol) was added to a stirred sample of **3** (6.76 g, 95.2% of the above sodium salt); the latter dissolved with a slight exotherm, followed by the appearance of crystalline sodium acetate. After 16 h, the reaction mixture was evaporated at 25 °C and flushed with toluene three times to remove acetic anhydride. The residue was dissolved in methylene chloride, extracted with 10% aqueous potassium bicarbonate, dried (Na_2SO_4), and evaporated to give 7.840 g of a mixture (80:20) of **4a** and the corresponding triacetate (see text) as shown by NMR. Recrystallization from ether provided crystalline diacetate (5.175 g; mp 129–130 °C). The triacetate present in the mother liquor underwent partial hydrolysis to **4a** when allowed to stand in ether at 25 °C for several weeks. An additional 1.873 g of **4a** was thus obtained: total weight 7.048 g (92%); IR ($CHCl_3$) 5.63, 5.73, 5.79, 6.08 μm; NMR ($CDCl_3$) δ 6.43 (s, $C_{1'}H$), 6.05 (br s, C_5H), 2.37 (s, C_6CH_3), 2.28 and 2.12 (each s, 2 OAc), 0.93 and 0.90 [each d, J = 6 Hz, $-CH(CH_3)_2$]. Anal. ($C_{16}H_{21}O_6N$) C, H, N.

(±)-Flavipucine and (±)-*epi*-Flavipucine (5a). To a stirred solution of **4a** (3.233 g, 0.01 mol) in *t*-BuOOH (40 mL, Pennwalt product dried over $MgSO_4$) cooled in a dry ice–acetone bath was added 1 M KO-*t*-Bu-*t*-BuOH (20 mL, 0.02 mol) in 7 min at 0–5 °C. After an additional 5 min at 0–5 °C, the bright yellow reaction mixture was diluted with methylene chloride, washed with 10% $KHCO_3$, dried (Na_2SO_4), and evaporated to a thick paste first on a water aspirator then high vacuum. The last traces of *t*-BuOOH were removed by flushing with toluene a number of times. The semicrystalline product (1.963 g) was slurried in cold ether to provide 1.746 g of **5a** (nat and epi in about equal amounts). The ether layer was evaporated and the residue further purified via preparative TLC (silica gel; $CHCl_3$–acetone, 65:35) to provide additional **5a** (0.076 g, total weight 1.822 g, 77%). Fractional crystallization from benzene provided (±)-flavipucine (mp 154–155 °C, identical with natural (−)-flavipucine in TLC, UV, IR, NMR, and mass spectrum) and (±)-*epi*-flavipucine: mp 136–138 °C; IR ($CHCl_3$) 2.92, 3.06, 3.14, 5.82, 6.00, 6.12 μm; NMR ($CDCl_3$) δ 5.65 (br s, C_5H), 3.92 (s, $C_{1'}H$), 2.20 (s, C_6CH_3), 0.98 [d, J = 6.5 Hz, $-CH(CH_3)_2$]. Anal. ($C_{12}H_{15}O_4N$) C, H, N.

Reaction of Flavipucine and *epi*-Flavipucine (5a) with Thiophenol. To a stirred solution of **5a** (0.126 g, 0.000531 mol) in $CHCl_3$–CH_3OH (1:1, 2 mL) was added pyridine (0.2 mL, 0.00248 mol), followed by thiophenol (0.2 mL, 0.00195 mol) at 25 °C. After 1 h, the pale yellow reaction mixture was evaporated and flushed with toluene, and the semicrystalline residue was slurried with ether and filtered to give 0.121 g of solid, which was purified via crystallization and preparative TLC (silica gel; $CHCl_3$–CH_3OH, 90:10) to give the diol corresponding to **4a**[2,3] [0.024 g (19%), MS M^+ = 239] and **10** [R = C_6H_5; 0.63 g (51%)]: mp 231–233 °C (acetone); NMR (CD_3OD) δ 2.25 (s, CH_3), 6.01 (br s, C_5H), 7.14 (s, 5 H). Anal. ($C_{12}H_{11}O_2NS$) C, H, N.

Treatment of the ether-soluble material with an excess of a solution of 2,4-dinitrophenylhydrazine (in H_2SO_4, H_2O, and CH_3OH) provided the di-DNPH of isobutylglyoxal (0.052 g, 21%), mp 234–238 °C. Anal. ($C_{18}H_{18}N_8O_8$) C, H, N.

(13) H. D. Dakin and H. W. Dudley, *J. Biol. Chem.*, 18, 29 (1914).
(14) E. Steers, E. L. Foltz, and B. S. Graves, *Antibiot. Chemother.*, **9**, 307 (1959).
(15) M. Misiek, D. R. Chisholm, F. Leitner, and K. E. Price, *Antimicrob. Agents Chemother.*, **225** (1970).

Notes

Oxytocin and Lysine-vasopressin with N^5,N^5-Dialkylglutamine in the 4 Position: Effect of Introducing Sterically Hindered Groups into the Hydrophilic Cluster of Neurohypophyseal Hormones[1]

Glenn L. Stahl, Clark W. Smith, Roderich Walter,*

Department of Physiology and Biophysics, University of Illinois at the Medical Center, Chicago, Illinois 60612

Theodor Tsegenidis, George Stavropoulos, Paul Cordopatis, and Dimitrios Theodoropoulos*

Laboratory of Organic Chemistry, University of Patras, Greece. *Received June 4, 1979*

The synthesis and pharmacological potencies of oxytocin and lysine-vasopressin analogues are reported in which the N^5-amide of their glutaminyl residues are dialkylated. These analogues have been studied as an ongoing exploration of the biological effects on the natural hormones of substituting individually one of the amino acid residues, which has a hydrophilic side chain and which are thought to be part of the hydrophilic surface of the hormones. [4-(N^5,N^5-Dimethylglutamine)]oxytocin (17), [4-(N^5,N^5-di-*n*-propylglutamine)]oxytocin (18), and [4-(N^5,N^5-dimethylglutamine)]lysine-vasopressin (19) were synthesized by classical solution techniques. Potencies in the in vitro rat uterotonic, avian vasodepressor, rat pressor, and rat antidiuretic assays were determined and are as follows, respectively: for compound 17 3.01 ± 0.14 units/mg, 4.55 ± 0.03 units/mg, tachyphylaxis and tachyphylaxis; for compound 18 <0.1, <0.1, <0.05, and <0.002 unit/mg; for compound 19 <0.05, <0.1, 1.27 ± 0.03, and 1.88 ± 0.04 units/mg. The potencies in all cases are significantly less than those of the parent hormone. The results are discussed in terms of the proposed biologically active conformations of the hormones at the uterotonic receptor and at the antidiuretic receptor.

In the proposed biologically active conformations of oxytocin at the uterine receptor[2,3] (Figure 1A) and of vasopressin at the antidiuretic receptor[4] (Figure 1B), one surface of the 20-membered antiparallel β-pleated sheet

0022-2623/80/1823-0213$01.00/0