

chloropropiono-2',6'-xylylidide (7.0 g, 0.033 mol), *cis*-octahydro-1*H*-pyridine HCl (3.75 g, 0.03 mol), Na₂CO₃ (7.5 g, 0.06 mol), and KI (0.1 g) in 4-methyl-2-pentanone (250 ml) was stirred and refluxed for 72 hr. After cooling, H₂O was added. The organic layer was separated and dried (MgSO₄), and the solvent was removed *in vacuo*. The oily residue was converted into its HCl salt in *i*-Pr₂O-HCl. Crystallization from *i*-Pr₂O-*i*-PrOH gave pure 11 (7.27 g, 72%), mp 202–203°. *Anal.* (C₁₈H₂₈N₂O·HCl) C, H, N.

Method B. *cis*-3,4,4a,5,6,7,8a-Octahydro-1(2*H*)-quinoline-propiono-2',6'-xylylidide Hydrochloride (21). A suspension of 3-chloropropiono-2',6'-xylylidide (7.0 g, 0.033 mol), *cis*-decahydroquinoline HCl (5.27 g, 0.03 mol), and NaHCO₃ (8.4 g, 0.1 mol) in EtOH (200 ml) was stirred and refluxed overnight. After cooling the mixture was concentrated *in vacuo* and 2*N* aqueous HCl was added. The mixture was extracted with *i*-Pr₂O; the aqueous layer was alkalinized with 50% NaOH and extracted with *i*-Pr₂O. The organic layer was separated and dried (MgSO₄), and the solvent was removed *in vacuo*. Conversion of the residue to its HCl salt and crystallization from *i*-PrOH afforded pure 21 (6.5 g, 62%), mp 227–228°. *Anal.* (C₂₀H₃₀N₂O·HCl) C, H, N.

Resolution of *trans*-2,3,4,4a,5,6,7,7a-Octahydro-1*H*-pyridine (4). A mixture of 4 (78 g, 0.624 mol) and (+)-camphor-10-sulfonic acid monohydrate (144.7 g, 0.624 mol) was boiled in Me₂CO (1250 ml) and allowed to cool. The crystalline precipitate was collected by filtration, which afforded crude (+)-base *d*-camphorsulfonate (94 g). Several recrystallizations from MeCN gave 36 g of pure (+)-*trans*-2,3,4,4a,5,6,7,7a-octahydro-1*H*-pyridine *d*-camphorsulfonate (4a): mp 155–156°; [α]²⁴_D +27.7° (MeOH). *Anal.* (C₈H₁₃N·C₁₀H₁₆O₄S) C, H, N.

The resolution liquor was allowed to stand at room temperature for another 3 days. The precipitate was removed by filtration and filtrate was concentrated *in vacuo*. The residual crude (–)-base *d*-camphorsulfonate was resistant to several attempts of crystallization and was consequently converted to base in the usual way. Conversion of the latter base to 14 according to method B gave 14b.

Conversion of the base, corresponding to 4a, to 14 according to method B afforded 14c.

Resolution of *trans*-6'-Chloro-2,3,4,4a,5,6,7,7a-Octahydro-1*H*-1-pyridine-1-propiono-*o*-toluidide (14a). A mixture of 14a (9.6 g, 0.03 mol) and (+)-camphorsulfonic acid monohydrate (6.96 g, 0.03 mol) was boiled in EtCOMe (40 ml). The solvent was removed *in vacuo* and the residue dissolved and boiled in *i*-Pr₂O until crystallization started. The crystalline precipitate was collected by filtration yielding 9 g of crude 14b as *d*-camphorsulfonate: mp 137–139°; [α]²⁴_D +19.5° (MeOH). Several crystallizations from EtCOMe (until constant rotation), followed by conversion to base in the usual way, and recrystallization from *i*-Pr₂O afforded 0.5 g of 14b: mp 121–122°; [α]²⁴_D +40° (MeOH). *Anal.* (C₁₈H₂₃ClN₂O) C, H, N.

The resolution liquor was evaporated *in vacuo*. The residual *d*-camphorsulfonate of 14c was triturated with EtOAc (10 ml), the precipitate removed by filtration, and the filtrate concentrated to dryness yielding 2 g of 14c as *d*-camphorsulfonate salt: [α]²⁴_D –8° (MeOH). Conversion to base in the usual way and crystallization from *i*-Pr₂O afforded a pure 0.1 g of 14c: mp 120–121°; [α]²⁴_D –41.5° (MeOH). *Anal.* (C₁₈H₂₃ClN₂O) C, H, N.

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Chemical Transformations of Antibiotic X-537A and Their Effect on Antibacterial Activity†

John W. Westley,* Eugene P. Oliveto, Julius Berger, Ralph H. Evans, Jr., Richard Glass, Arthur Stempel, Voldemar Toome, and Thomas Williams

Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. Received September 21, 1972

A number of derivatives of the polyether antibiotic X-537A have been tested *in vitro* vs. *Bacillus* E and *Bacillus* TA and the results clearly indicate that all the oxygen functions involved in ligand formation with cations and intramolecular hydrogen bonding (as revealed by X-ray analysis) contribute to the biological activity of the antibiotic. In addition, a number of derivatives at the C-5 position of the aromatic chromophore exhibited a qualitative correlation between their partition coefficients (octanol–water) and *in vitro* activity.

The isolation of antibiotic X-537A (1) was first reported¹ in 1951. The structure,^{2,3} biosynthesis,^{4,5} and nitration⁶ of the antibiotic have been discussed in earlier communications.

†Microanalytical and spectral data will appear immediately following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D. C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JMED-73-397.

The studies reported here involve a number of chemical modifications of the antibiotic and the resulting effects that were observed in the *in vitro* antibacterial activity against *Bacillus* E and *Bacillus* TA.

The X-ray crystallographic analysis of the barium salt of the antibiotic^{3,7} revealed an unsymmetrical complex (Figure 1) in which the cation was bound by nine ligands, six of which involved oxygens from one antibiotic molecule, two were from a second antibiotic molecule, and the ninth ligand

was associated with a water molecule. The conformation of this complex is proposed as a model to explain the mode of action of the antibiotic in biological systems as well as providing a reason for the high solubility of the antibiotic salts in nonpolar solvents (e.g., >50% in chloroform, methylene chloride). Antibiotic X-537A belongs to the polyether group of antibiotics which includes monensin,⁸ nigericin⁹ (X-464¹⁰), X-206,¹¹ dianemycin,¹² and grisorixin.¹³ Because of their ability to solubilize inorganic cations of the alkali and alkaline earth groups in lipid-like solvents, the polyether antibiotics are believed¹⁴⁻¹⁶ to owe their biological activity

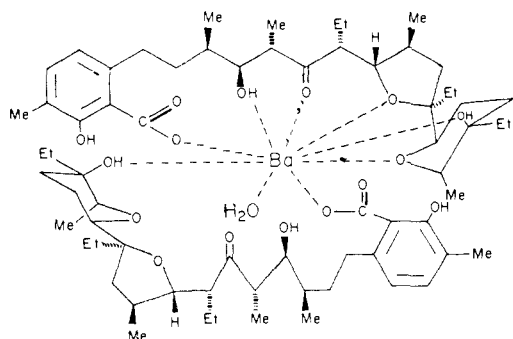
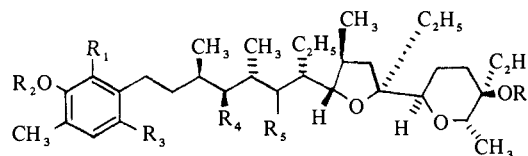


Figure 1. Schematic drawing of the (X-537A)₂ · Ba · H₂O complex.

to interfere with the transport of essential cations across membranes.

In order to test the X-ray structure (Figure 1) as a model for the biological action of the antibiotic, a number of oxygen functions involved in ligand formation with the barium cation were either modified or removed (Table I).

All sixteen derivatives listed in Table I exhibited diminished



1, R₁ = COOH; R₂ = H; R₃ = H; R₄ = OH; R₅ = O; R₆ = H

activity at the levels tested against *Bacillus* TA and *Bacillus* E. These results were in agreement with the hypothesis that all the ligand-binding sites in the antibiotic X-537A molecule contribute to its biological activity. Another group of derivatives which gave similar results was the anhydro derivatives 18-22 and the epoxide 23 (Table II).

The only oxygen function in the barium salt of antibiotic X-537A that was not involved in cation binding as revealed by X-ray analysis⁷ was the phenolic group of the salicylic acid chromophore (see Figure 1). A number of derivatives of this hydroxyl group were therefore prepared. In addition, the reactivity of the aromatic carbon C-5 toward electro-

Table I. Derivatives of Antibiotic X-537A

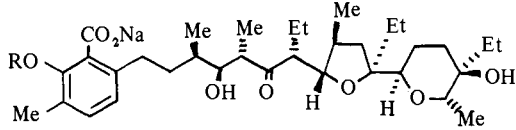
No.	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	Formula	[α] _D (MeOH), deg	Mp, °C
2	CO ₂ CH ₃	H	H	OH	=O	H	C ₃₅ H ₅₆ O ₈	-7.2	
3	CO ₂ CH ₃	H	H	OH	=O	CH ₃	C ₃₆ H ₅₈ O ₈	-7.9	
4	CO ₂ CH ₃	CH ₃	H	OH	=O	H	C ₃₆ H ₅₈ O ₈	-10.8	
5	CO ₂ CH ₂ C ₆ H ₅	H	H	OH	=O	H	C ₄₁ H ₆₀ O ₈		42-44
6	CO ₂ CH ₂ C ₆ H ₅	H	H	OH	=O	CH ₂ C ₆ H ₅	C ₄₈ H ₆₆ O ₈	-3.9 ^a	
7	CO ₂ CH ₃	CH ₃ CO	H	OH	=O	H	C ₃₇ H ₅₈ O ₉	+1	
8	CO ₂ CH ₃	H	H	OH	=NOH	H	C ₃₅ H ₅₇ NO ₈	+5.8	
9	CO ₂ CH ₃	H	H	OH	HON=	H	C ₃₅ H ₅₇ NO ₈	+32.6	
10	CO ₂ Na	H	H	OH	=NOH	H	C ₃₄ H ₅₄ NNaO ₈	-12.0	173-174
11	CO ₂ Na	H	H	OH	HON=	H	C ₃₄ H ₅₄ NNaO ₈	+27.2	220 dec
12	CO ₂ H	H	H	OH	OH	H	C ₃₄ H ₅₆ O ₈	+1	151-153
13	CO ₂ Na	H	H	H	=O	H	C ₃₄ H ₅₃ O ₇ Na	+50.6 ^a	119-121
14	CO ₂ H	CF ₃ CO	H	CF ₃ CO · O	=O	H	C ₃₈ H ₅₂ F ₃ O ₁₀	-42.4 ^a	66-68
15	H	H	H	OH	=O	H	(C ₃₃ H ₅₄ O ₆) ₂ KBr	-7.5 ^a	151-154
16	Br	H	Br	OH	=O	H	C ₃₃ H ₅₂ Br ₂ O ₆	-8.0 ^a	54-57
17	NO ₂	Na	NO ₂	OH	=O	H	C ₃₃ H ₅₁ N ₂ NaO ₁₀	+19.6 ^b	165-170

^a[α]_D measured in ethanol. ^b[α]_D measured in dimethyl sulfoxide.

Table II. Anhydro and Epoxide Derivatives of Antibiotic X-537A

No.	R ₁	R ₂	R ₃	Formula	[α] _D (MeOH), deg	Mp, °C
18	CO ₂ Na	H		C ₃₄ H ₅₁ NaO ₇	+27.6	125
19	CO ₂ Na	H		C ₃₄ H ₅₁ NaO ₇	-20.0	220
20	CO ₂ Na	Br		C ₃₄ H ₅₀ BrNaO ₇	+9.6	235
21	CO ₂ Na	NO ₂		C ₃₄ H ₅₀ NNaO ₉	+75.5	219
22	NO ₂	NO ₂		C ₃₃ H ₄₉ NNaO ₉	+66.2	222
23	CO ₂ · C ₁₂ H ₂₁ NO ₅ ^a	H		C ₄₆ H ₇₂ NO ₁₃	+34.4	170-172

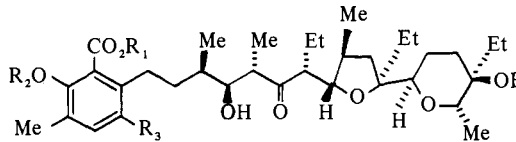
^a1-Amino-1-deoxy-2,3:4,6-di-O-isopropylidene-α-L-sorbofuranose salt.

Table III. Comparison of Partition Coefficient and *in Vitro* Activity of Some Acyl and Alkyl Derivatives of Antibiotic X-537A


Compd ^a	R	Partition coeff ^b	Rel <i>in vitro</i> activity ^c		Molecular formula	Mp, °C	[α] _D (MeOH), deg
			<i>Bacillus</i> TA	<i>Bacillus</i> E			
1	H	705	100	100	C ₃₄ H ₅₃ NaO ₈	172-174	-85.1 ^d
24	CH ₃	349	1	2	C ₄₇ H ₇₇ NO ₁₃ ^e	192-193	-28.1 ^f
25	CH ₃ CO	9	1	2	C ₃₆ H ₅₅ NaO ₉	186-187	-18.8
26	CH ₃ CH ₂ CO			2	C ₃₇ H ₅₇ NaO ₉	191-192	-20.3
27	CH ₃ (CH ₂) ₂ CO		1	4	C ₃₈ H ₅₉ NaO ₉	191-193	-20.1
28	CH ₃ (CH ₂) ₃ CO	120	1	9	C ₃₉ H ₆₁ NaO ₉	191-192	-20.1
29	CH ₃ (CH ₂) ₄ CO			23	C ₄₀ H ₆₃ NaO ₉	191	-19.5
30	CH ₃ (CH ₂) ₅ CO			19	C ₄₁ H ₆₅ NaO ₉	191-192	-20.5
31	CH ₃ (CH ₂) ₆ CO	163	35	14	C ₄₂ H ₆₇ NaO ₉	170-171	-20.1
32	CH ₃ (CH ₂) ₈ CO	557	16	4	C ₄₄ H ₇₁ NaO ₉	130-132	-18.7
33	<i>p</i> -BrC ₆ H ₄ CO	3031	28	10	C ₄₁ H ₅₆ BrNaO ₉	170-174	-19.6

^aCompounds listed in order of increasing molecular weight. ^bPartition coefficient = concentration in 1-octanol/concentration in water.

^cCup-plate (12 mm) agar diffusion assay. ^dIn chloroform. ^e1-Amino-1-deoxy-2,3,4,6-di-*O*-isopropylidene-α-L-sorbofuranose salt. ^fIn ethanol.

Table IV. Comparison of Partition Coefficient and Apparent pK_a' with the *in Vitro* Activity of X-537A and Some 5-Substituted Aromatic Derivatives


Compd ^a	R ₁	R ₂	R ₃	Partition coeff ^b	pK _a ' in 60% aqueous methanol ^c	Rel <i>in vitro</i> activity ^b vs.		Molecular formula	Mp, °C	[α] _D , deg
						<i>Bacillus</i> TA	<i>Bacillus</i> E			
34	H	H	NH ₂	1.9	6.0	<1	<2	C ₃₄ H ₅₅ NO ₈	223-225	+17.9 ^d
35	Na	H	N=CHC ₆ H ₅	7.3		1	2	C ₄₁ H ₅₈ NNaO ₈	208-209	-131.8 ^d
36	Na	H	NH·COCH ₃	11	4.3	1	2	C ₃₆ H ₅₆ NNaO ₉	189-190	-17.5 ^d
37	Na	CH ₃ CO	Br	31	4.25	27	3	C ₃₆ H ₅₄ BrNaO ₉	213-215	-3.5 ^d
38	Na	H	NO ₂	291	2.4	24	16	C ₃₄ H ₅₂ NNaO ₁₀	214-215	-98.1 ^e
1	Na	H	H	705	4.4	100	100	C ₃₄ H ₅₃ NaO ₈	172-174	-85.1 ^f
39	Na	H	Cl	1330	4.0	140	73	C ₃₄ H ₅₂ ClNaO ₈	183-185	-44.3 ^f
40	Na	H	I	1556	3.9	112	55	C ₃₄ H ₅₂ I NaO ₈	223	-48.7 ^f
41	Na	H	Br	1775	3.9	124	67	C ₃₄ H ₅₂ BrNaO ₈	185	-49.7 ^f

^aCompounds listed in order of increasing partition coefficient. ^bSee Table III. ^cCalculated from uv spectral data at various pH's according to A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, 1962. ^dIn methanol. ^eDMSO. ^fIn chloroform.

philic reagents made it possible to synthesize a number of derivatives at that position. The aim was that these transformations would not affect the availability of the cation-binding sites of the antibiotic but that esterification of the phenolic group and substitution meta to the carboxylic acid would result in two major effects: (a) changes in pK_a of the carboxylic acid which should in turn affect the stability of the salt complexes; (b) changes in the organic solvent solubility which should result in changes in permeability of the salt complexes across lipid membranes.

The results of acylating the phenolic group of antibiotic X-537A are presented in Table III. As expected, increasing the chain length of the acyl group resulted in higher partition coefficients with a value of 9 for the acetyl derivative 25 and 557 for the decanoyl ester 32. However, there were only slight changes in the *in vitro* activity with increased chain length from negligible activity for 25 to about one quarter the activity of the parent compound vs. *Bacillus* E for the hexanoyl ester 29 and 35% relative activity vs. *Bacillus* TA for the octanoyl ester 31. The two esters with the highest partition coefficients, 32 and the *p*-bromobenzoyl ester 33 (partition coefficient 3031), were in fact less active than 29 vs. *Bacillus* E and 31 vs. both organisms. The methyl

ether 24 exhibited minimal activity and our conclusion from the results presented in Table III is that although the phenolic hydroxyl is not directly involved in ligand formation with the cation, the hydrogen bond shown by X-ray⁷ to exist between the phenol and carboxyl group appears to play an important role in the activity of the antibiotic.

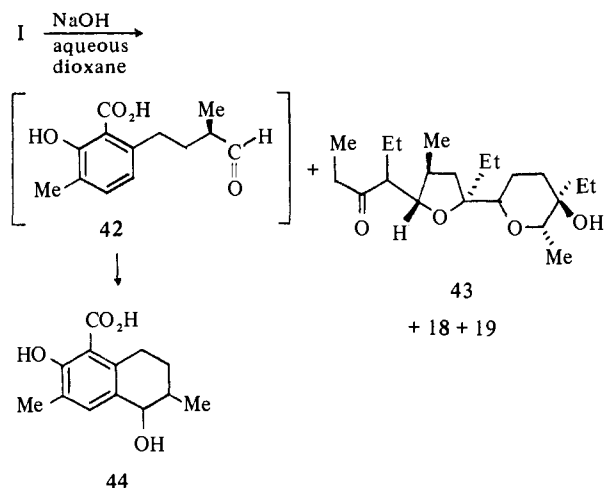
From the results presented in Table IV, there is a much closer correlation between partition coefficients and *in vitro* activity for the 5-substituted derivatives of X-537A than is the case for the esters discussed earlier. The 5-amino (34), 5-benzylideneamino (35), 5-*N*-acetylamino (36), 5-bromo-*O*-acetyl (37), and 5-nitro (38) derivatives all have lower partition coefficients (1.9, 7.3, 11, 31, and 291, respectively) than the parent antibiotic 1 (partition coefficient 705) and are considerably less active, whereas the 5-chloro (39), 5-iodo (40), and 5-bromo (41) derivatives have higher partition coefficients (1330, 1556, and 1775, respectively) than antibiotic X-537A and are of the same order of magnitude in activity as the parent antibiotic *in vitro* vs. both *Bacillus* TA and *Bacillus* E. No simple correlation was apparent between the pK_a' values of the derivatives and their *in vitro* antibacterial activity.

Chemistry. The major factors which determine the chem-

istry of antibiotic X-537A are the labile nature of the β -ketol system in the center of the molecule, the reactivity of the aromatic carbon at C-5 toward electrophilic reagents, the ease of decarboxylation, and finally the cyclic conformation of the molecule due to hydrogen bonding between the tertiary alcohol and the carboxylic acid functions.

The β -ketol system is cleaved² to **42** and **43** by a retrograde aldol reaction (Scheme I) in base. It also dehydrates

Scheme I



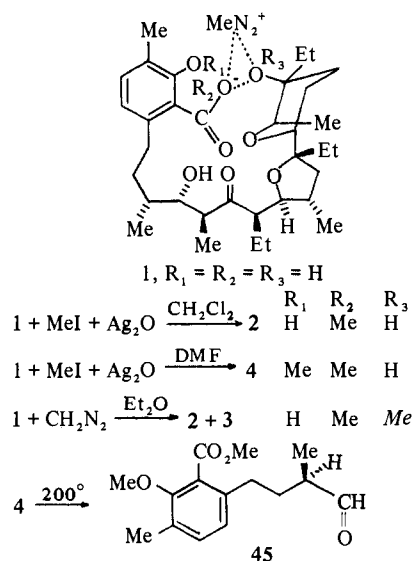
under both basic and acidic conditions to give **18** and **19** (Table II).

Aldehyde **42** spontaneously cyclizes to **44** and this was the first illustration of the ease of electrophilic substitution at C-5. Further examples occurred on bromination to **41**, chlorination to **39**, iodination to **40**, and nitration⁶ to **38**. When bromination was carried out with *N*-bromosuccinimide in acetone rather than bromine in CH_2Cl_2 , the product was the dibromodecarboxy compound **16**. The analogous dinitro compound **17** was the major product of the nitration reaction.⁶ Hydrogenolysis of the dibromophenol **16** gave the decarboxy form of the antibiotic **15**.

Methylation of **1**, using methyl iodide and silver oxide in DMF, gave the dimethoxy derivative **4**, which was subsequently pyrolyzed to yield aldehyde **45**. The CD spectrum of **45** resulted in the assignment of absolute configuration² to the antibiotic.

The cyclic conformation of the molecule is a property common to all the polyether antibiotics and appears essential for their biological action. In addition, we believe this characteristic property is responsible for the unexpected product **3** (Scheme II) from methylation of the antibiotic with diazomethane. The diazomethane reaction gave, in addition to the expected methyl ester **2**, a second dimethoxy derivative **3**. Structure **3** was assigned by nmr which indicated two methoxyl groups in the molecule and retroaldol cleavage which gave a derivative of ketone **43**, in which the tertiary alcohol was shown by nmr and mass spectrometry to be methylated. Neither diazomethane treatment of the methyl ester **2** nor ketone **43** resulted in methylation of the tertiary alcohol. These results suggest a mechanism by which the diazomethane cation intermediate¹⁷ is partially trapped by the tertiary alcohol prior to esterification of the carboxyl group as suggested in Scheme II. Similarly, a dibenzyl derivative **6** was formed on treatment of the antibiotic with phenyldiazomethane.

Methylation of a tertiary alcohol has been reported only when an acid catalyst such as boron trifluoride etherate¹⁸ or fluoroboric acid¹⁹ has been employed. This supports our

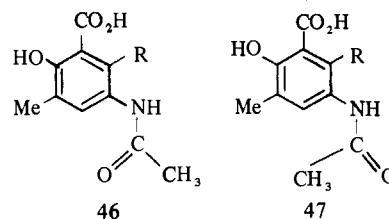
Scheme II. Methylation of Antibiotic X-537A and Cleavage of the Dimethoxy Derivative **4**

hypothesis that intramolecular acid catalysis is involved and arises from the proximity of the carboxyl and hydroxyl groups as shown⁷ by X-ray crystallography.

Heating the antibiotic in its free acid form with pyridine and hydroxylamine hydrochloride in ethanolic solution gave the hydroxylammonium salt of an oxime. This salt could be converted to its sodium form **10** by acidification and treatment with sodium carbonate. When the conversion to the sodium salt was carried out prior to isolation, two isomeric oximes **10** and **11** were isolated and separated by fractional crystallization. The two oxime salts had quite distinct physical chemical properties (see Table I).

The amine **34** was prepared by catalytic hydrogenation of the 5-nitro derivative **38** using Raney nickel. Acetylation of the amine was carried out using acetic anhydride in glacial acetic acid. The ir spectrum of **36** exhibited two equal peaks in the *N*-acetyl region at 1640 and 1660 cm^{-1} . The nmr spectrum also suggested a mixture with two types of aromatic protons at δ (CDCl_3) 7.10 and 6.85 and three methyl peaks at δ 1.86 (s, 1.5, CH_3CONH), 2.08 (s, 1.5, CH_3CONH), and 2.17 (s, 3, aromatic CH_3). These results suggest that the molecule may exist as an equal mixture of *endo*-**46** and *exo*-**47** conformers.

A similar case has been reported for *N*-acetyl-1,2,3,4-tetrahydroquinoline²⁰ and may arise in the case of **46** and **47** because of restricted rotation (large ortho substituent, R = remainder of antibiotic X-537A molecule).



Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained were within $\pm 0.3\%$ of the theoretical values. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All spectral data were compatible with the assigned structures. The ultraviolet spectra were measured with a Cary recording spectrophotometer Model 14M. Nuclear magnetic resonance spectra were obtained with a Varian Associates Model A-60 or HA-100 spectrophotometer. Chemical

shifts are reported in δ with the following abbreviation: s, singlet. Optical rotations were measured with a Perkin-Elmer polarimeter Model 141 using a 1% solution at 25°. The mass spectra were taken with a CEC-21-110 mass spectrometer at 70 V. The isolation of antibiotic X-537A (1), 3-methyl-6-[7(R)-ethyl-4(S)-hydroxy-3(R),5(S)-dimethyl-6-oxo-7-[5(S)-ethyl-3(S)-methyl-5-(5(R)-ethyl-5-hydroxy-6(S)-methyl-2(R)-tetrahydropyran-2(S)-tetrahydrofuryl)heptyl]-salicylic acid, has been described in an earlier communication.¹

Preparation of Antibiotic X-537A Methyl Ester 2. To a solution of 1 (10 mM) in 125 ml of CH_2Cl_2 was added 4.6 g of Ag_2O and 14 g of MeI. After stirring for 18 hr, the reaction mixture was filtered and decolorized with Darco, and the filtrate was concentrated to 6.09 g of a colorless foam. A portion (4 g) of the foam was chromatographed on a Florisil column eluting with a gradient between CH_2Cl_2 - Et_2O and then Et_2O - Me_2CO . Rechromatography of the slightly yellow oil, recovered from the Florisil column on silica gel and eluting with a gradient between CH_2Cl_2 and 15% Et_2O - CH_2Cl_2 , gave 200 mg (5%) of a clear viscous oil, bp 170° dec (0.05 mm). *Anal.* ($\text{C}_{35}\text{H}_{56}\text{O}_8 \cdot 0.5\text{H}_2\text{O}$) C, H, OCH₃.

Preparation of Antibiotic X-537A Methyl Ester Methyl Ether 3. A solution of 1 in Et_2O was treated with an ethereal solution of CH_2N_2 . At the end of 1 hr, the solvent was removed and the slightly yellow oil was chromatographed on silica gel. The column was eluted with a gradient between CH_2Cl_2 and 15% Et_2O - CH_2Cl_2 . The column eluate was divided into three fractions (tlc). The third fraction (1.2 g of oil, 60%) was shown to be 2 by tlc, glc, ir, and nmr, while the first fraction (60 mg, 3%) was 3, bp 170° dec (0.05 mm). *Anal.* ($\text{C}_{36}\text{H}_{58}\text{O}_8$) OCH₃.

Preparation of Antibiotic X-537A Methyl Ester Methyl Ether 4. A solution of 5 g of 1 in 50 ml of DMF was stirred overnight with 6 g of Ag_2O and 50 ml of MeI. After assay (tlc silica gel) an additional 5 g of Ag_2O and 10 ml of MeI were added. After 48 hr the solids were removed by filtration and the filtrate was diluted with H_2O and CH_2Cl_2 . The solvent was separated and removed under reduced pressure, and the remaining DMF was removed by H_2O washing of an Et_2O solution. The Et_2O layer was chromatographed on Florisil eluting with a gradient between $n\text{-C}_6\text{H}_{14}$ and Me_2CO . The fractions containing the product were pooled and concentrated to a clear oil (5.25 g, 99%). *Anal.* ($\text{C}_{36}\text{H}_{58}\text{O}_9 \cdot 0.5\text{C}_3\text{H}_6\text{O}$) C, H.

Preparation of Antibiotic X-537A Benzyl Ester 5 and Benzyl Ester Benzyl Ether 6. A red solution of PhCHN_2 in Et_2O prepared according to the procedure of Sarin and Fasman²¹ was added dropwise to a solution of 1 (10 g) in Et_2O (200 ml) until the color of the solution remained orange. AcOH was then added until the color of the solution was yellow. The solution was concentrated to dryness to yield an orange oil which was chromatographed over silica gel. The sample was eluted successively with CH_2Cl_2 , a gradient formed from CH_2Cl_2 , and a 1:1 mixture of Et_2O and CH_2Cl_2 . The elution was monitored by tlc on silica gel and the fractions obtained were combined on the basis of their tlc mobility. In this way two compounds were obtained as oils on concentration. The compound of greater R_f (32%) was assigned structure 6. *Anal.* ($\text{C}_{48}\text{H}_{66}\text{O}_8$) C, H.

The compound (58%) of lesser R_f was assigned structure 5. Another sample of this compound after careful purification solidified. *Anal.* ($\text{C}_4\text{H}_{60}\text{O}_8$) C, H.

Preparation of Antibiotic X-537A Acetate Sodium Salt 25. To a solution of 6.12 g (10 mM) of 1 in 10 ml of dry pyridine was added 2 ml of Ac_2O . After 2 hr, 10 g of ice was added to the reaction solution and the resulting mixture was washed in a separatory funnel with EtOAc and 1 N HCl. The EtOAc solution was washed with 1 N HCl until the pyridine had been removed. The EtOAc solution was then washed with saturated Na_2CO_3 solution, dried (Na_2SO_4), and concentrated to dryness. The residue was dissolved in CH_2Cl_2 and the solution evaporated on the steam bath with additions of $n\text{-C}_6\text{H}_{14}$ until crystallization started. Cooling to 0° gave 5.9 g (88% yield) of 25 as white needles. *Anal.* ($\text{C}_{36}\text{H}_{55}\text{NaO}_9 \cdot \text{H}_2\text{O}$) C, H, Na.

Preparation of Antibiotic X-537A Bromobenzoate Sodium Salt 33. To 5.0 g of 1 in 100 ml of pyridine was added a solution of 1.98 g of $p\text{-BrC}_6\text{H}_4\text{COCl}$ dissolved in 100 ml of pyridine- CH_2Cl_2 (1:1). After 3 hr an additional 200 mg of $p\text{-BrC}_6\text{H}_4\text{COCl}$ was added. The following morning the reaction mixture was poured into 2 N HCl and extracted twice with CH_2Cl_2 . The combined solvent layers were washed with 2 N HCl and then with a saturated solution of Na_2CO_3 . The dried (Na_2SO_4) solvent layer was then concentrated with the addition of $n\text{-C}_6\text{H}_{14}$ until crystallization. The 3.2 g of recovered crystals was recrystallized from the same solvent to yield 2.07 g (32%) of 33. *Anal.* ($\text{C}_{41}\text{H}_{56}\text{BrNaO}_9$) C, H, Br, Na. The remaining esters 26 [*Anal.* ($\text{C}_{37}\text{H}_{55}\text{NaO}_9$) C, H], 27 [*Anal.* ($\text{C}_{38}\text{H}_{59}\text{NaO}_9$) C, H], 28 [*Anal.* ($\text{C}_{35}\text{H}_{61}\text{NaO}_9$) C, H, Na], 29 [*Anal.* ($\text{C}_{40}\text{H}_{63}\text{NaO}_9$) C, H, Na], 30 [*Anal.* ($\text{C}_{41}\text{H}_{65}\text{NaO}_9$) C, H, Na], 31 [*Anal.*

($\text{C}_{44}\text{H}_{67}\text{NaO}_9$) C, H, Na], and 32 [*Anal.* ($\text{C}_{44}\text{H}_{71}\text{NaO}_9$) C, H, Na] were prepared by either one of the two above methods.

Preparation of Antibiotic X-537A Acetate Methyl Ester 7. 25 (1.344 g, 2 mmol) was treated with Et_2O (30 ml) and 1 N HCl (30 ml). The mixture was shaken in a separatory funnel until all the starting material had dissolved. The Et_2O layer was then washed twice with H_2O and treated with a 20-ml solution of ethereal CH_2N_2 . After drying (Na_2SO_4), the solution was evaporated to give 1.167 g (90%) of 7 as an oil. *Anal.* ($\text{C}_{37}\text{H}_{58}\text{O}_9$) C, H.

Preparation of the Sodium Salt of Antibiotic X-537A Oximes 10 and 11. A mixture of 10 g of 1 and 5 g of $\text{HONH}_2 \cdot \text{HCl}$ in 50 ml of EtOH containing 5 ml of pyridine was heated under reflux for 60 hr. The solution was evaporated to dryness and treated with a mixture of 1 N HCl and EtOAc. The EtOAc layer was washed with 1 N HCl and a saturated solution of Na_2CO_3 , separated, and dried (Na_2SO_4). After evaporation to dryness, the residue was fractionally crystallized from EtOH. The first fraction (3.55 g, 34%) on recrystallization from CH_2Cl_2 - $n\text{-C}_6\text{H}_{14}$ gave white prisms of 10. *Anal.* ($\text{C}_{34}\text{H}_{54}\text{NNaO}_8$) C, H, N.

The second crop (1.63 g, 16%) isolated by fractional crystallization of the crude Na salt of the oxime was found to be an isomer of 10. Recrystallization from CH_2Cl_2 - $n\text{-C}_6\text{H}_{14}$ gave white needles of 11. *Anal.* ($\text{C}_{34}\text{H}_{54}\text{NNaO}_8$) C, H, N.

Preparation of the Methyl Ester of Antibiotic X-537A Oxime 8. A solution of 3.55 g of 10 in 100 ml of CH_2Cl_2 was treated with 1 N HCl. The solvent layer was separated, washed with H_2O , and treated with an ethereal solution of CH_2N_2 . At the end of 1 hr the solvent was removed leaving an oil (8) which upon standing under a high vacuum solidified (92% yield). *Anal.* ($\text{C}_{35}\text{H}_{55}\text{NO}_8$) C, H, N. The isomeric oxime methyl ester 9 was prepared in the same manner as above (yield 86%).

Preparation of the Dihydro Derivative of Antibiotic X-537A 12. To a solution of 10 g of 1 in 500 ml of absolute EtOH was added 2.36 g of NaBH_4 . The following morning the solvent was removed and the heavy oil diluted with CH_2Cl_2 , washed with dilute HCl, dried (Na_2SO_4), and concentrated to a solid foam (11.4 g). The slightly pink solid was chromatographed on silica gel eluting with $n\text{-C}_6\text{H}_{14}$ - CH_2Cl_2 (1:1), CH_2Cl_2 , and CH_2Cl_2 - Et_2O (1:1). The fractions were assayed by tlc and pooled, concentrated to a yellow solid (4.96 g), and rechromatographed on Florisil eluting with a gradient between $n\text{-C}_6\text{H}_{14}$ and Me_2CO . The fractions containing 12 were pooled and concentrated to a small volume from which 973 mg (10%) of crystals was recovered. *Anal.* ($\text{C}_{34}\text{H}_{56}\text{O}_8$) C, H.

Preparation of the Desoxy Derivative of Antibiotic X-537A Sodium Salt 13. A mixture consisting of trans- α,β -unsaturated ketone 19 (1.20 g, 2.0 mmol), EtOH (30 ml), and 10% Pd on charcoal (339 mg) was shaken under a H_2 atmosphere (room temperature, 1 atm) for 1.4 hr. A total of 50 ml of H_2 was absorbed. The mixture was filtered, concentrated to dryness, and crystallized from $n\text{-C}_6\text{H}_{14}$ giving 0.21 g (17%) of colorless crystals of 13. *Anal.* ($\text{C}_{34}\text{H}_{52}\text{NaO}_7$) C, H.

Preparation of Bis(trifluoroacetyl) Antibiotic X-537A 14. (F_3CO)₂O (10 ml) was added to a stirred solution of 1 (3.0 g) in pyridine (20 ml) at 0°. The reaction mixture was allowed to warm to room temperature and stirred for 2 hr. Ice was then added, and the resulting mixture was extracted with EtOAc. The extract was washed with 1 N HCl, dried (Na_2SO_4), and concentrated to dryness giving a foam (3.9 g, 98%). *Anal.* ($\text{C}_{38}\text{H}_{52}\text{F}_6\text{O}_{10}$) C, H, F. Another sample prepared as above but washed last with aqueous Na_2CO_3 solidified.

Preparation of Dibromodescarboxy Antibiotic X-537A 16. A mixture consisting of 1 (5.90 g), N -bromosuccinimide (3.56 g), Me_2CO (50 ml), and H_2O (5 ml) was stirred at room temperature for 24 hr. The mixture was diluted with EtOAc and washed with saturated aqueous NaHSO_4 solution, then aqueous Na_2CO_3 , and finally H_2O . The organic phase was dried (MgSO_4), concentrated to dryness, diluted with CCl_4 (50 ml), and allowed to stand overnight at -15°. The mixture was filtered and the filtrate was concentrated to dryness giving a colorless solid (6.2 g, 88%). A portion of this material was purified by chromatography over silica gel. *Anal.* ($\text{C}_{33}\text{H}_{52}\text{Br}_2\text{O}_8$) C, H, Br.

Preparation of Descarboxy Antibiotic X-537A 15. To a solution of 16 (352 mg) and KBr (85%, 66 mg) in EtOH (10 ml) was added 10% Pd on charcoal (117 mg). The mixture was shaken in a H_2 atmosphere (room temperature, 1 atm) for 3 hr. A total of 22.4 ml of H_2 was consumed. The mixture was filtered and concentrated to dryness yielding a solid (217 mg, 80%).

Another sample of 15 (1.4 g, 2.6 mmol) prepared as above was dissolved in MeOH (40 ml) and stirred with anhydrous KBr (357 mg, 3.0 mmol) at room temperature overnight. The mixture was filtered and the filtrate was concentrated and recrystallized from Et_2O to give colorless crystals. *Anal.* ($\text{C}_{33}\text{H}_{54}\text{O}_6 \cdot 0.5\text{KBr}$) C, H, ionic Br.

Preparation of Cis- (18) and Trans- α,β -unsaturated Ketone 19, Ketone 43, and 2,5-Dihydroxy-3,6-dimethyl-5,6,7,8-tetrahydro-1-naphthoic Acid (44). To a solution of 10 g of 1 in 100 ml of *p*-dioxane was added 200 ml of 10% aqueous NaOH and the mixture stirred at room temperature. After 7 hr the mixture, which had separated into two phases, was extracted twice with an equal volume of Et₂O. The Et₂O extracts were dried (Na₂SO₄) and concentrated to a viscous oil (7.75 g) which on standing partially crystallized. The crystals (1.3 g, 13%) were removed by the addition of Et₂O and filtration. Recrystallization of a sample from Et₂O gave colorless needles of 19. *Anal.* (C₃₄H₅₁NaO₇) C, H.

The Et₂O filtrate obtained above was evaporated to dryness, dissolved in *n*-C₆H₁₄, and chromatographed on Florisil using gradient elution from *n*-C₆H₁₄-Et₂O (1:1) to Et₂O-Me₂CO (1:1). The first fraction eluted was concentrated to an oil (6.2 g) which on distillation gave 4 g (69%) of 43, bp 170° (0.05 mm). *Anal.* (C₂₁H₃₆O₄) C, H.

The second fraction was a mixture which on concentration and treatment with EtOAc gave a further 400 mg (4%) of 19. Chromatography of the filtrate on silica gel using gradient elution from CH₂Cl₂ to Me₂CO gave the second component of the mixture as an oil, which crystallized on standing (220 mg, 2%). This product, 18 [*Anal.* (C₃₄H₅₁NaO₇) C, H], was shown by nmr to be the cis isomer of 19.

The aqueous phase, which had been twice extracted with Et₂O, was acidified with 1 *N* HCl to give an amorphous white precipitate which was extracted with Et₂O. The Et₂O extract was concentrated to give 2.71 g (53%) of 44 as colorless needles. *Anal.* (C₁₃H₁₆O₄) C, H.

Preparation of the 5-Bromo Derivative of the Trans- α,β -unsaturated Ketone 20. To 1.34 g of 1 in CS₂ (25 ml) was added dropwise a solution of Br₂ (0.12 ml) in CS₂ (10 ml). The reaction mixture was left overnight under a stream of N₂ to remove the CS₂. The residue was dissolved in EtOAc, washed successively with aqueous NaHSO₃ and saturated Na₂CO₃, dried (Na₂SO₄), and evaporated under reduced pressure. Crystallization from CH₂Cl₂-*n*-C₆H₁₄ gave 270 mg (20%) of colorless needles of 20. *Anal.* (C₃₄H₅₀BrNaO₇) C, H, Br.

Preparation of the 5-Nitro Derivative of the Trans- α,β -unsaturated Ketone 21. To 1.34 g of 38 in 35 ml of *p*-dioxane was added 30 ml of 10% aqueous NaOH. The following morning the reaction mixture was diluted with 100 ml of H₂O and 100 ml of EtOAc. The solvent layer was separated and evaporated to dryness which gave 917 mg of solid material. The addition of CH₂Cl₂ to the residue resulted in a crystalline fraction of 21 (120 mg, 10%) which was separated and recrystallized from EtOAc. *Anal.* (C₃₄H₅₀NNaO₈) C, H, N, Na.

Preparation of the Dinitrodescarboxy Derivative of the Trans- α,β -unsaturated Ketone 22. To a solution of 10 g of 17 in 325 ml of *p*-dioxane was added 375 ml of aqueous NaOH (13 g of NaOH pellets) and the mixture was stirred at room temperature for 20 hr. The solution was separated into two phases and the upper phase was diluted with 200 ml of H₂O and 200 ml of EtOAc. The organic layer was separated and concentrated to 6.13 g of oil, dissolved in CH₂Cl₂, and chromatographed on silica gel using a gradient elution from CH₂Cl₂ to CH₂Cl₂-Et₂O (1:1). The third fraction eluted was concentrated to a small volume and 501 mg (5%) of 22 was crystallized from CH₂Cl₂-*n*-C₆H₁₄. *Anal.* (C₃₃H₄₉N₂NaO₈) C, H, N, Na.

Preparation of 5-Amino Antibiotic X-537A 34. A solution of 5.0 g (7.6 mmol) of 38 in EtOH (450 ml) was hydrogenated over 2 g of Raney Ni with an uptake of 22.2 mM of H₂. The reaction was filtered (over N₂) and 17.2 ml of 1 *N* HCl was added. The solvent was removed under reduced pressure, and the residue was crystallized from aqueous Me₂CO to give 4.0 g (84%) of 34. *Anal.* (C₃₄H₅₃NO₈) C, H, N.

Preparation of the Epoxide of Antibiotic X-537A 23. A mixture consisting of the trans- α,β -unsaturated ketone 19 (2.0 g), MeOH (100 ml), 4 *N* aqueous NaOH solution (3 ml), and 30% aqueous H₂O₂ solution (6 ml) was stored at -15° for 18 days. At the end of this time an additional portion of 30% H₂O₂ solution (6.0 ml) was added. After standing at -15° for a total of 24 days the mixture was poured into H₂O and extracted with Et₂O and CHCl₃. The combined organic extracts were washed with H₂O and concentrated to dryness giving a solid (1.63 g). This material was purified by preparative tlc. One chromatography fraction could be crystallized from Et₂O-*n*-C₆H₁₄ mixtures. In another experiment this compound was isolated after preparative tlc and was crystallized from *n*-C₆H₁₄ giving a colorless solid.

The salt of 23 was prepared by washing an Et₂O solution of the above solid with 1 *N* HCl and then adding 1 molar equiv of 1-amino-1-deoxy-2,3:4,6-di-*O*-isopropylidene- α -L-sorbofuranose in Et₂O. The colorless crystals (31% yield) formed upon evaporation of the solvent. *Anal.* (C₃₄H₅₁NaO₈) C, H.

Preparation of Antibiotic X-537A Methyl Ether 24. A mixture of 5 and 6 (14 g), MeI (175 ml), DMF (175 ml), and Ag₂O (22.4 g) was stirred at room temperature for 4 days. An additional sample of MeI (70 ml) and Ag₂O (22.4 g) was then added. The mixture was stirred at room temperature for another 2 days, filtered, diluted with H₂O, and extracted with CH₂Cl₂. The organic extracts were dried (Na₂SO₄) and concentrated to dryness affording an orange oil (12.4 g) which gave a negative FeCl₃ test for phenols.

A mixture consisting of a portion (6.2 g) of the above product, EtOH (180 ml), and 10% Pd on charcoal (1.35 g) was shaken under an H₂ atmosphere (room temperature, 1 atm) for 20 hr. The mixture was then filtered and concentrated to dryness giving a golden oil (5.4 g) which was chromatographed over silica gel. The column was eluted with CH₂Cl₂, a gradient between CH₂Cl₂ and Et₂O, and a gradient between Et₂O and MeOH. The fractions containing only 24 were combined and concentrated to dryness yielding a colorless foam (4.6 g, 27%). *Anal.* (C₄₇H₇₇NO₁₃) C, H, N.

Preparation of 5-Benzylideneamino Antibiotic X-537A Sodium Salt 35. To a solution of 1.11 g of the amine 34 dissolved in 50 ml of C₆H₅CH₃ was added 0.25 g of C₆H₅CHO. The mixture was refluxed under N₂ for 1 hr and cooled, and the solvent was removed yielding a yellow solid from which 0.85 g (66%) of 35 was crystallized from CH₂Cl₂-Et₂O. *Anal.* (C₄₁H₅₈NNaO₈) C, H, N.

Preparation of 5-*N*-Acetylamino Antibiotic X-537A Sodium Salt 36. To a suspension of 1.21 g (2 mmol) of 34 in 50 ml of glacial AcOH was added 1 ml of Ac₂O. On warming the mixture to 70° on a steam bath, the amine dissolved and after a further 30 min at room temperature, the solution was poured onto crushed ice. The mixture was carefully made alkaline with Na₂CO₃ solution and extracted twice with 400 ml of Et₂O. After drying (Na₂SO₄), the Et₂O solution was evaporated to approximately 100 ml and 816 mg (64%) of 36 was recovered as a microcrystalline product. *Anal.* (C₃₆H₅₆NNaO₈) C, H, N.

Preparation of 5-Bromo Antibiotic X-537A Sodium Salt 41. To a solution of 1.53 g of 1 in 30 ml of CS₂ was added dropwise a solution of 0.14 ml of Br₂ in 10 ml of CS₂ at -5° over 10 min. The solvent was removed under a stream of N₂ at room temperature. The residue was dissolved in EtOAc and the solution washed successively with aqueous NaHSO₃ and Na₂CO₃. After drying (Na₂SO₄), the solution was evaporated under reduced pressure and the residue crystallized from EtOAc to give 1.189 g (69%) of 41 as colorless cubes. *Anal.* (C₃₄H₅₂BrNaO₈) C, H, Br.

Preparation of Acetyl-5-bromo Antibiotic X-537A 37. To a solution of 2.11 g of 41 in 20 ml of dry pyridine was added 0.66 ml of Ac₂O. After 4 hr at room temperature, the reaction mixture was diluted with ice H₂O containing 40 ml of HCl and EtOAc. The EtOAc was separated, washed with 1 *N* HCl and then with a saturated solution of Na₂CO₃, and dried over Na₂SO₄. The EtOAc was evaporated to 25 ml from which 585 mg (26%) of 37 was recovered by filtration. *Anal.* (C₃₆H₅₃BrNaO₈) C, H, Na, Br.

Preparation of 5-Chloro Antibiotic X-537A Sodium Salt 39. To a cold solution (~3°) of 6.12 g of 1 in 50 ml of CHCl₃ was slowly added 34 ml of CCl₄ saturated with 10 mM of Cl₂ gas. The reaction was continued for 30 min after which the solution was filtered and the filtrate treated with a saturated aqueous solution of Na₂CO₃. The solvent layer was dried over Na₂SO₄ and concentrated to a small volume from which after addition of *n*-C₆H₁₄ 4.25 g (66%) of crystalline 39 was recovered. *Anal.* (C₃₄H₅₂ClNaO₈) C, H, Cl, Na.

Preparation of 5-Iodo Antibiotic X-537A Sodium Salt 40. A solution of 1.8 g of 1 in 50 ml of glacial AcOH was treated with 980 mg of ICl (freshly distilled 97-99° fraction). The ICl was mixed with 5 ml of AcOH and added to the reaction slowly over a period of 15 min. After an additional 15 min, H₂O (100 ml) was slowly added and the mixture extracted with Et₂O. The solvent layer was separated and washed successively with aqueous solutions of NaHSO₃, NaHCO₃, and Na₂CO₃ and dried (Na₂SO₄). The solvent was concentrated to a small volume from which crystalline 40 was recovered. Recrystallization from EtOAc gave 450 mg (20%) of an analytical sample.

Alternatively, 40 could be prepared by treatment of 1 (1 mM) with 1 (1 mM) and morpholine (3 mM). The reaction was complete after 5 days and the product, purified by chromatography, was identical with the compound prepared using ICl. *Anal.* (C₃₄H₅₂I NaO₈) C, H, I.

Preparation of Ketone 43 and (*R*)-6-(3-Formylbutyl)-2-methoxy-3-methylbenzoic Acid Methyl Ester (45) by Pyrolysis of 4. Vacuum distillation of 2 g of 4 at 170° and 0.05 mm gave a clear oil (175 g) which was found by tlc on silica gel to be a mixture of 43 and 45. The mixture was separated by chromatography on silica gel using gradient elution from CH₂Cl₂ to CH₂Cl₂-Et₂O (1:1). The first

fraction eluted was concentrated to a colorless oil, **45** (730 mg, 90%). *Anal.* (C₁₅H₂₀O₄) C, H. The second fraction eluted was concentrated to give 813 mg (75%) of **43**.

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Substituted Tetrahydrofurfurylamines as Potential Antidepressants

I. Monkovic,* Y. G. Perron, R. Martel, W. J. Simpson,

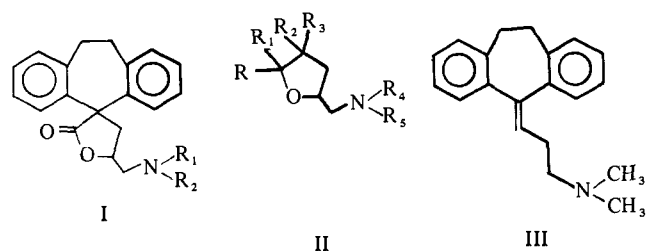
Bristol Laboratories of Canada, Candiac, Quebec, Canada

and J. A. Gyls

Pharmacology Department, Bristol Laboratories, Syracuse, New York 13201. Received October 6, 1972

A series of substituted tetrahydrofurfurylamines was prepared from the corresponding γ,δ -unsaturated alcohols by sequential bromocyclization and substitution of the tetrahydrofurfuryl bromides with methyl- or dimethylamines. Some of these compounds were found to possess potent antidepressant activity in animals.

The high antidepressant activity observed in the compounds of general formula I¹ led us to investigate synthetic approaches to and pharmacological properties of compounds of general formula II. Both I and II, when R₂ and R₃ are aryl substituents, are structurally related to the potent antidepressant amitriptyline III.

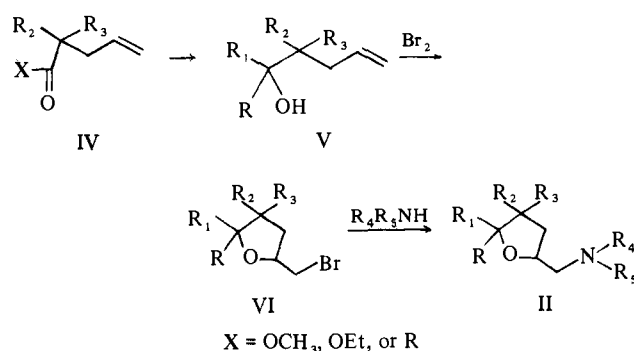


A simple general synthesis of substituted tetrahydrofurfuryl bromides from corresponding 1-en-4-ols by electrophilic addition of bromine with neighboring-group participation and, thus, an easy access to tetrahydrofurfurylamines enabled us to prepare and study the pharmacological properties of several compounds of type II.

Chemistry. A series of substituted tetrahydrofurfurylamines was prepared by the following synthetic route.

Compounds IV were prepared by allylation of the corresponding esters or ketones. The reduction of IV with NaBH₄ or LiAlH₄ afforded V. (Compounds of general structure V are listed in Table III.)

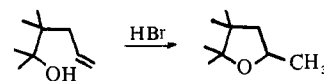
Alternatively, compounds V were prepared by the reaction of CH₃Li with IV or by Grignard reaction of benzophenone with 3-buten-1-ylmagnesium bromide. Compounds V were



converted to tetrahydrofurfuryl bromides VI by bromination.

The synthesis of heterocyclic compounds by electrophilic addition on the double bond with neighboring-group participation is well known.² However, there has not been wider application of bromocyclization of γ,δ -unsaturated alcohols which in our hands appears to be general and a useful synthetic tool.[†]

We carried out bromocyclizations in organic solvents (CCl₄, CH₂Cl₂) with 1 equiv of pyridine to neutralize the released HBr and thus prevent parallel acid-catalyzed formation of methyltetrahydrofurans. This side reaction was observed in the course of bromocyclization of alcohol **33** in the absence



[†]This facile reaction was first described by Rengevich, *et al.*³ Additional examples of bromocyclization have been reported by Levisalles and Rudler,³ Tanaka, *et al.*,⁵ and Demole and Enggist.⁶