Table II. Comparison of the Chemical Shifts (τ Values) for Nucleosides VIIa and VIIIa with Those for 9-(4,6-Di-*O*-acetyl-2,3-dideoxy-α- and -β-D-*erythro*-hex-2-enopyranosyl)-6-chloropurine (XIII and XII),[a] 60 MHz, $CDCl_3$

Compd	Anomeric confign	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-6′	H-2	H-8	SCH_3	AcO	
VIIa	β	3.29	3.86	3.74	4.46	———	5.74	———	1.29	1.87	7.29	7.86	7.96
VIIIa	α	3.37	3.73	3.64	4.52	6.07	5.77	5.91	1.2	1.85	7.24	7.84	8.00
XII	β	3.24	3.89	3.70	4.44	5.78	5.72	5.74	1.16	1.68		7.82	7.92
XIII	α	3.35	3.76	3.60	4.52	6.05	5.75	5.90	1.18	1.67		7.85	8.00

[a] See ref 5.

tion coefficients were in the range of those reported for the model compound 9-methyl-6-methylthiopurine.[6]

Nucleosides IIIb and Vb and the acetylated derivatives IIIa, IVa, Va, VIIa, and VIIIa were evaluated for their *in vitro* cytotoxic activity against HeLa tumoral cells according to the criteria established by the CCNSC.[7] Compounds IIIa, IIIb, Va, and Vb were found to be active at a 50% inhibiting dose (ID_{50}) of 62, 23, 54, and 19 μg/ml, respectively. The ID_{50} of all the other compounds screened was higher than 100 μg/ml.

Experimental Section

Melting points are uncorrected. Nmr spectra were recorded on a Perkin-Elmer R-12 spectrometer with TMS as the internal standard. Uv spectra were recorded on a Perkin-Elmer 350 spectrophotometer. Optical rotations were obtained with a Perkin-Elmer 141 polarimeter. Preparative layer chromatography was performed on glass plates (20 × 20 cm) coated (2 mm thickness) with silica gel PF_{254} (Merck). Silica gel GF_{254} (Merck) was used for tlc (0.25 mm thickness). Spots were visualized with uv light (254 nm).

Reaction of 6-Methylthiopurine with 3,4-Di-*O*-acetyl-D-xylal. A mixture of 1.66 g (0.01 mol) of 6-methylthiopurine (I) and 4 g (0.02 mol) of 3,4-di-*O*-acetyl-D-xylal (II) in 50 ml of EtOAc containing a few drops of trifluoroacetic acid was heated at 95° in a sealed tube under continuous agitation for 96 hr. After this time a few additional drops of trifluoroacetic acid were added and the reaction was continued for 24 hr. The solvent was evaporated under reduced pressure to a thick syrup which was dissolved in chloroform and the solution applied to 22 preparative plates. The plates were developed ten times with a mixture of Et_2O-petroleum ether (3:1) resulting in the separation of three major bands.

9-(4-*O*-Acetyl-1,2,3-trideoxy-D-*threo*-pent-1-enopyranos-3-yl)-6-methylthiopurine (IIIa). The fastest band gave 1.22 g of a syrup which was further purified by preparative tlc (five plates, EtOAc–petroleum ether 1:2) to give solid IIIa: mp 112–114° (from EtOAc–petroleum ether); $[\alpha]D$ −86.4° (*c* ~0.75, $CHCl_3$); uv λ_{max} (EtOH) 284 nm (ε 17,800), 291 (sh, 16,800); yield 40%. *Anal.* ($C_{13}H_{14}N_4O_3S$) C, H, N.

9-(4-*O*-Acetyl-2,3-dideoxy-α-D-*glycero*-pent-2-enopyranosyl)-6-methylthiopurine (IVa). Extraction of the second band followed by rechromatography using EtOAc-petroleum ether (1:2) afforded 0.25 g of IVa as a syrup: $[\alpha]D$ +50.4° (*c* ~0.5, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 18,190), 290 (sh, 17,300); yield 8.2%. *Anal.* ($C_{13}H_{14}N_4O_3S$) C, H, N.

9-(4-*O*-Acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enopyranosyl)-6-methylthiopurine (Va). The slowest band afforded 0.5 g of a syrup which was further chromatographed (tlc, EtOAc–petroleum ether 1:2) to give Va as an amorphous solid: $[\alpha]D$ +145° (*c* ~0.5, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 19,100), 290 (sh, 17,950); yield 16.5%. *Anal.* ($C_{13}H_{14}N_4O_3S$) C, H, N.

Reaction of 6-Methylthiopurine with 3,4,6-Tri-*O*-acetyl-D-glucal. A mixture of 1.66 g (0.01 mol) of 6-methylthiopurine and 5.44 g (0.02 mol) of 3,4,6-tri-*O*-acetyl-D-glucal in 70 ml of EtOAc containing trifluoroacetic acid as a catalyst was heated for 40 hr as above. After this time trifluoroacetic acid was added (4 drops) and the heating was continued for 30 hr. The crude reaction product was separated into two major fractions by preparative layer chromatography (40 plates) after 15 consecutive developments using Et_2O-petroleum ether (3:1).

9-(4,6-Di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIa). The fastest moving band afforded 1.46 g of a syrup which was rechromatographed using Et_2O-petroleum ether (3:1) to give 1.25 g of VIIa as a chromatographically homogenous syrup: $[\alpha]D$ +93.9° (*c* ~0.75, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 18,500), 290 (sh, 17,800); yield 33%. *Anal.* ($C_{16}H_{18}N_4O_5S$) C, H, N.

9-(4,6-Di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIIa). The slowest moving band afforded 2.26 g of a solid material which was further purified by preparative tlc (Et_2O-petroleum ether 3:1) to give 1.69 g of solid VIIIa: mp 119–120° (from EtOAc–petroleum ether); $[\alpha]D$ +35.3° (*c* ~0.6, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 18,350), 290 (sh, 17,400); yield 44%. *Anal.* ($C_{16}H_{18}N_4O_5S$) C, H, N.

9-(1,2,3-Trideoxy-D-*threo*-pent-1-enopyranos-3-yl)-6-methylthiopurine (IIIb). Treatment of IIIa (0.5 g) with 40 ml of methanolic ammonia (methanol saturated with ammonia at 0°) at room temperature for 24 hr caused the formation of IIIb which was purified by preparative tlc (EtOAc) to give solid IIIb: mp 176–177° (from EtOAc–Et_2O); $[\alpha]D$ −142.4° (*c* ~0.5, EtOH). *Anal.* ($C_{11}H_{12}N_4O_2S$) C, H, N, S.

9-(2,3-Dideoxy-β-D-*glycero*-pent-2-enopyranosyl)-6-methylthiopurine (Vb). Treatment of 0.16 g of Va as above afforded 40 mg of Vb: mp 170–171° (from EtOAc–petroleum ether); $[\alpha]D$ +145° (*c* ~0.5, EtOH). *Anal.* ($C_{11}H_{12}N_4O_2S$) C, H, N.

9-(2,3-Dideoxy-β-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIb). Treatment of VIIa (0.47 g) as in the preceding cases afforded a crude compound which was purified by preparative tlc (EtOAc) to give VIIb: mp 131° (from EtOAc–petroleum ether); $[\alpha]D$ +130.7° (*c* 0.25, EtOH). *Anal.* ($C_{12}H_{14}N_4O_3S$) C, H, N.

9-(2,3-Dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIIb). Deacetylation of VIIIa (0.34 g) as above afforded 0.21 g of solid VIIIb: mp 91–92° (from H_2O); $[\alpha]D$ −2° (*c* ~1, MeOH). *Anal.* ($C_{12}H_{14}N_4O_3S$) C, H, N, S.

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β-Chloroethylamines Related to Mescaline

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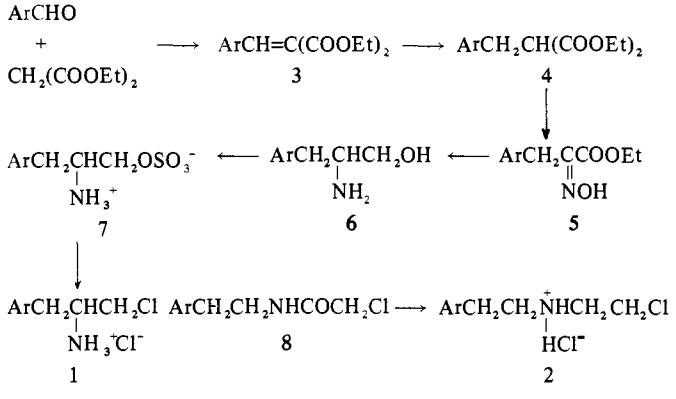
β-Haloethylamines are precursors of a variety of aziridines of interest as irreversible blocking agents. Current evidence indicates that a drug can be transformed into an alkylating analog without disturbing its receptor specificity. Examples

are the β-haloethylamine derivatives of such diverse types as adrenergic blocking agents and local anesthetics.[1] Brewster and Pinder[2] have recently reported the pharmacological properties of some aziridine analogs of amphetamines. At the time of that report, I had completed the synthesis of some related β-chloroethylamines. These compounds, α-chloromethylmescaline hydrochloride (**1**) and *N*-(β-chloroethyl)mescaline hydrochloride (**2**), are aziridine precursors which have structural features resembling mescaline and its analogs. Consequently, they were expected to have either psychotomimetic or antipsychotomimetic activity of long duration, depending on the as yet unknown mechanism of action of mescaline. In this context the compounds would have been useful for receptor-labeling experiments, for studying the mechanism of action of mescaline, and perhaps for therapeutic applications. I therefore wish to describe my work.

The 3,4,5-trimethoxyphenylalaninol (**6**) required for the synthesis of **1** was prepared according to Scheme I. Total

Scheme I



Ar = 3,4,5-trimethoxyphenyl

reduction of the oximino ester **5** proved difficult and was best effected using $Na(CH_3OCH_2CH_2O)_2AlH$. Compound **6** was converted to **1** by a modified Wenker reaction[3] without isolating the intermediate aziridine.

Compound **2** was prepared by the selective reduction of *N*-chloroacetylmescaline **8** with AlH_3, followed by reaction of the unstable intermediate aziridine with HCl.

α-Chloromethylphenethylamine hydrochloride (**9**), required for pharmacological comparison, was prepared by treating benzylaziridine[3] with dilute aqueous HCl.

Compound **1** was administered intraperitoneally to 13 mice in graded doses of 5-320 mg/kg (16.7-1070 μmol/kg). All doses in excess of 40 mg/kg (134 μmol/kg) provoked a twitch syndrome of 80-100-min duration, characteristic of mescaline action.[4] For example, an animal which had received 80 mg/kg (238 μmol/kg) of **1** showed 216 twitch responses over the 100-min duration, with peak activity in the first 20 min. By comparison, an animal which had received 40 mg/kg (163 μmol/kg) of mescaline hydrochloride showed 353 responses of this kind during 100 min. Two of the animals which had received 238 μmol/kg of **1** were not protected against a challenge dose of 163 μmol/kg of mescaline hydrochloride administered 30 min later.

Compound **2** was likewise tested on six mice in graded doses of 10-320 mg/kg (32-1040 μmol/kg). The response (periodic spasticity) was qualititatively different from that due to **1** or to mescaline, and only a few mescaline-like twitches were observed. Ten animals which had been pretreated with 256 μmol/kg of **2** were not protected against 163 μmol/kg of mescaline hydrochloride administered 20 min later.

Compound **6** HCl, the potential hydrolysis product of **1**, did not produce the mescaline syndrome (three mice, 275 μmol/kg), nor did compound **9** (five mice, 238 μmol/kg). Compound **9** showed amphetamine-like action of brief duration (60-90 min) which would be expected if it is transformed *in vivo* to 2-benzylaziridine.[2]

The following tentative conclusions are drawn. If **1** is transformed into an aziridine *in vivo*, which would sterically resemble the psychotomimetic α-methylmescaline, it interacts reversibly with the receptor and is incable of alkylating it. The inactivity of **6**, which is unable to cyclize, either supports this conclusion or simply indicates that if **1** does not cyclize *in vivo*, an α-chloromethyl substituent on mescaline is less detrimental to psychotomimetic activity than an α-hydroxymethyl group. If **2** cyclizes *in vivo*, the resultant aziridine resembles the inactive *N*,*N*-dimethylmescaline and is therefore relatively incapable of interacting with the receptor. Finally, the negative response to **9**, which should have alkylating ability about equal to that of **1**, confirms that the mescaline-like effect of **1** is due to its trimethoxyphenyl substituent and not to its potential as an alkylating agent.

Experimental Section

Corrected capillary melting points are reported. Satisfactory analyses (within ±0.4% of the theoretical values) were obtained except as otherwise indicated. Compounds were also identified by ir spectroscopy.

Diethyl (3,4,5-Trimethoxybenzal)malonate (3). A benzene solution of 3,4,5-trimethoxybenzaldehyde and diethyl malonate containing a trace of piperidine was refluxed for 5.5 hr with continuous removal of water *via* a Dean-Stark trap to obtain 83% of **3**, mp 69.5-71°. *Anal.* ($C_{17}H_{22}O_7$) C, H.

Diethyl (3,4,5-Trimethoxybenzyl)malonate (4). A solution of compound **3** in EtOH was hydrogenated over 10% Pd/C at 2 atm of H_2 to give 91% of **4**, mp 78.5-80°. *Anal.* ($C_{17}H_{24}O_7$) C, H.

Ethyl [2-Oximino-3-(3,4,5-trimethoxyphenyl)]propionate (5). Compound **4** was condensed with $EtNO_2$ in anhydrous EtOH containing EtONa to give 96% of **5**, mp 95-98°. *Anal.* ($C_{14}H_{17}NO_6$) C, H, N.

3,4,5-Trimethoxyphenylalaninol (6). A benzene solution of **5** was added to 5 mol of $Na(CH_3OCH_2CH_2O)_2AlH$ in benzene over 40 min at 45-50°. The mixture was acidified with H_2SO_4 and the aqueous phase extracted with CH_2Cl_2. The aqueous phase was then basified with NaOH and again extracted with CH_2Cl_2. This CH_2Cl_2 extract was dried over Na_2SO_4 and treated with HCl gas to obtain 44% of 6 HCl, mp 206-207°. *Anal.* ($C_{12}H_{20}NO_4Cl$) C, H, N.

3,4,5-Trimethoxyphenylalaninol Sulfate Ester (7). To a rapidly stirred suspension of 6.95 g (0.025 mol) of **6** HCl in 100 ml of dry EtOH-free $CHCl_3$ at 0° under N_2 was added, over 1 hr, a solution of 3.17 g (0.0127 mol) of $ClSO_3H$ in 20 ml of $CHCl_3$. The mixture was stirred a further 1 hr at 25° and the solid collected and recrystallized from boiling H_2O to give 4.89 g (61%) of **7**, mp 293-296° *Anal.* ($C_{12}H_{19}NO_7S$) C, H, N, S.

α-Chloromethylmescaline Hydrochloride (1). To a solution of 17 g of NaOH in 40 ml of H_2O covered with 20 ml of toluene and heated on an oil bath at 135° was added, over 1 hr, a solution of 3.89 g (0.0126 mol) of **7** in 20 ml of 2.5% aqueous NaOH. The hot mixture was extracted with 70-ml portions of toluene at 15-min intervals during the addition and again upon its termination. The toluene extracts were concentrated and extracted with dilute aqueous HCl. The H_2O phase was evaporated to dryness and the residue recrystallized from MeOH to give 2.53 g (68%) of **1**, mp 226-228° dec. *Anal.* ($C_{12}H_{19}NO_3Cl_2$) C, H, N.

***N*-(β-Chloroethyl)mescaline Hydrochloride (2).** To a stirred solution of 0.236 g (0.007 mol) of AlH_3[5] in 21 ml of THF was added, over 15 min, a solution of 1.44 g (0.005 mol) of *N*-chloroacetylmescaline[6] in 15 ml of THF. After a further 20 min the mixture was decomposed with aqueous NaOH. The organic phase was evaporated to dryness at 25° and the residue was immediately taken up in benzene and treated with HCl gas. The benzene was removed, the residue triturated with acetone, and the solid collected and recrystal-

lized from boiling acetone to give 0.31 g (20%) of **2**, mp 189.5–191° *Anal.* ($C_{13}H_{21}NO_3Cl_2$) C, H, N.

α-Chloromethylphenethylamine Hydrochloride (9). To 0.350 g (0.00264 mol) of benzylaziridine,[3] cooled to −10°, was added 1.5 ml of cold 18% aqueous HCl. The solution was evaporated to dryness at 60° (10 mm) to give 0.415 g (76.5%) of **9**, mp 175–180°. *Anal.* Calcd for $C_9H_{13}NCl_2$: Cl (ionic), 17.21; Cl (total), 34.42. Found: Cl (ionic), 16.98; Cl (total), 35.14.

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N-Substituted Derivatives of Erythromycylamine

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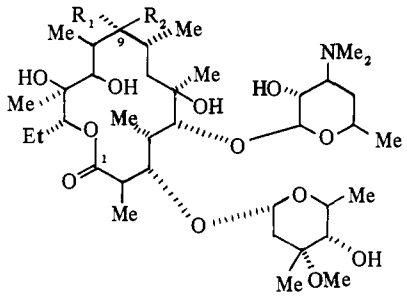
The antibacterial activities of derivatives of erythromycin **A** (**1**, $R_1R_2 = O$) are dependent on the nature of the substituent at C_9. Thus erythromycin itself,[1] the oxime,[2] and the hydrazone[3] are highly active against gram-positive bacteria, whereas the alcohol,[3] obtained by borohydride reduction of erythromycin, has much lower activity.

(9*S*)-Erythromycylamine[4] (**1**, $R_1 = H$; $R_2 = NH_2$) has antibacterial activity comparable to that of erythromycin



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itself, the 9*R* epimer being somewhat less active.[5] The present availability of (9*S*)-erythromycylamine in substantial quantity[6,7] has enabled us to explore the variation of antibacterial activity with N-substitution.

Simple *N*-alkyl derivatives of erythromycylamine are not easily prepared. For example, erythromycylamine reacts with aliphatic aldehydes with elimination of water to produce cyclic carbinolamine ethers[8] which are not readily reduced. Aromatic aldehydes, however, give the expected arylidene derivatives.[9,†] These Schiff bases readily revert to the aldehyde and the parent amine so that their activities cannot be determined by the usual methods. The arylidene derivatives were reduced by borohydride to a series of *N*-benzyl compounds. The mass spectra of these compounds showed good molecular ions and the presence of a benzyl group was confirmed in each case by the nmr spectrum.

Reaction of erythromycylamine with aliphatic ketones followed the pattern of the aromatic aldehydes giving imines (strong ir band at 1650 cm^{-1}) which were again readily reduced to secondary amines.

A group of *N*-alkyl derivatives bearing additional functional groups was obtained by addition of erythromycylamine to various α,β-unsaturated esters, nitriles, and ketones. In each case the nmr spectrum of the product indicated that the monoadduct only had been isolated and this was supported by elemental analysis. The adducts of erythromycylamine with ketones were reduced with borohydride to the corresponding hydroxyalkylamines which again gave good mass spectra.

The remaining compounds were prepared by reaction of erythromycylamine with various acid chlorides or anhydrides, isocyanates, and isothiocyanates as described in the Experimental Section. In each case, the nmr spectrum confirmed that reaction with only 1 equiv of the electrophile had occurred.

Antibacterial Activity. Compounds were tested *in vitro* against a range of bacteria by the agar gradient plate technique based on the method of Szybalski,[10] and the results are shown in Table I. The most striking effect on N-substitution is the marked reduction of antibacterial activity in those compounds (amides, sulfonamides ureas, and thioureas) which do not have a basic nitrogen at C_9. In most cases these compounds are virtually inactive. This is somewhat surprising in view of the high activity of erythromycin itself.

None of the new derivatives shows useful activity against either gram-negative organisms or the resistant strain of *Staphylococcus aureus* and in no case is the activity against sensitive organisms significantly greater than that of the parent compound.

Experimental Section‡

Erythromycylamine was prepared from erythromycin hydrazone[3] by nitrosation followed by borohydride reduction.[7]

***N*-(2,4,6-Trimethylbenzyl)erythromycylamine.** To a solution of *N*-(2,4,6-trimethylbenzylidene)erythromycylamine (7.75 g, 8.95 mmol) in MeOH (75 ml) at 0° was added solid $NaBH_4$ (1 g, excess). After standing 16 hr, the bulk of the MeOH was removed under reduced pressure and water added. The solution was extracted three times with CH_2Cl_2 and the dried ($MgSO_4$) extracts were evaporated to give an oil which crystallized from dry Et_2O giving 6.2 g. Dissolution in CH_2Cl_2, evaporation, and recrystallization from dry Et_2O gave 5.07 g (65%), mp 158–160°, pure by tlc. The ir spectrum showed no C=N. *Anal.* ($C_{47}H_{82}N_2O_{12}$) C, H, N.

N-Benzyl- and *N*-(*p*-nitrobenzyl)erythromycylamine were prepared similarly.

***N*-Isopropylerythromycylamine.** A solution of erythromycylamine (5.0 g) in Me_2CO (40 ml) was heated under reflux for 60 hr. Tlc showed almost complete conversion to a faster running compound. The excess Me_2CO was evaporated and to a solution of the resulting oil in MeOH (40 ml) at 0° was added $NaBH_4$ (600 mg, excess) and the solution stirred for 2 hr. The bulk of the solvent was evaporated, water added, and the solution extracted three times

†Details for the preparation of benzylideneerythromycylamine were kindly supplied by Dr. E. H. Massey, Eli Lilly & Co., Indianapolis, Ind.

‡Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Ir spectra were determined with a Perkin-Elmer 457 grating infrared spectrophotometer and nmr spectra with a Varian A-60A spectrometer. Ir and nmr spectra of all new compounds supported the proposed structures. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values. All tlc was carried out on silica gel F_{254} (Merck) using 3:1 MeOH–DMF as solvent system. In this system, erythromycylamine runs near the bottom of the plate and N-substituted derivatives have R_f's of about 0.6.