unsuccessful. During reaction with sodium methoxide in methanol, rearrangement occurred to a compound tentatively identified as 2,4-dimethyl-2-hydroxymethylcyclohex-3- and -4-enecarboxylic acid lactone: ir 1775 (s), 1136 (s), 1018 cm^{-1} (s); nmr δ 5.43 (broad) and 5.20 (broad s, 1 H, vinylic H), 3.95 and 3.98 (2 H, two absorptions of AB q, H α to oxygen), 1.66 (3 H, broad s, vinylic methyl), 1.15 and 1.17 (3 H, two s, methyl); mol wt 166 (mass spectrum).

Chrysanthenone (2) from Irradiation of Verbenone (1) and Reduction to Chrysanthenol (8). Dilute solutions of 1 (2.5 g of 1 in 150 ml of solvent, 0.11 *M*) in either cyclohexane or glacial acetic acid were irradiated as described above. Chrysanthenone (2) was isolated from the irradiation mixture by chromatography on silica gel (2 was eluted with 3:1 hexane–ether and then the purest fractions of 2 were rechromatographed with 9:1 hexane–ether) and distilled under vacuum on a Kugelrohr apparatus.

After 1.5 hr of irradiation in acetic acid, glc showed 26% remaining 1; 2 isolated from this irradiation had optical rotation $[\alpha]^{25}D$ −91.5° (*c* 1.0). After 25 min of irradiation in cyclohexane, glc showed 26% remaining 1; 2 isolated from this irradiation had optical rotation $[\alpha]^{25}D$ −81.2° (*c* 0.42). After 1.5 hr of irradiation in cyclohexane, glc showed essentially no remaining 1; 2 isolated from this irradiation had optical rotation $[\alpha]^{25}D$ −62.2° (*c* 0.43).

Chrysanthenone (2) with rotation −91.5° was reduced with lithium aluminum hydride to chrysanthenol (8) with $[\alpha]^{25}D$ +38.7° (*c* 0.91).

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Registry No.—**4,** 51897-93-9; **5,** 51849-16-2; **6,** 51897-95-1; **7,** 51897-96-2; **9,** 51849-17-3; **10,** 51897-97-3; **11,** 51849-18-4; 9-trideuterio-α-pinene, 51897-94-0; 9-acetoxy-α-pinene, 23971-93-9; 9-trideuterio-β-pinene, 51849-19-5.

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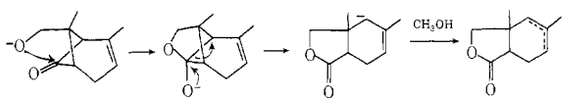

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Configuration of 9-Imino Derivatives of Erythromycin

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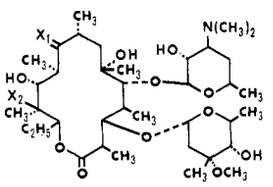
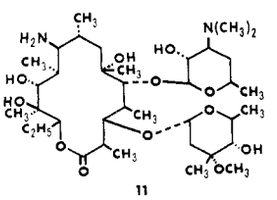
Both isomers of erythromycin B oxime have been isolated and configurationally identified. The major stable isomer has been shown to be (*E*)-erythromycin B oxime by ^1H nmr and ir. The *E* isomer has been suggested to predominate in the oxime, hydrazone, and isopropylidene hydrazone of both erythromycin A and B.

Several imino derivatives of the ketone of erythromycin A (1) have been prepared, including the oxime (**2**),[1,2] hydrazone (**3**),[1,3] isopropylidene hydrazone (**4**),[1] and the imine (**5**).[4,5] Interest in these derivatives centers on their utility as antibiotics and as substrates for further modification such as reduction to erythromycyclamine.[1,2,4,5]We wish now to report our results in this area which concerns the preparation, isolation, and configurational assignments of the two erythromycin B oxime isomers (**7a** and **7b**) and the configurational analysis of **2, 3, 4, 8,** and **9.**



X_1	X_2 = OH	X_2 = H
O	1	6
N–OH	2	7
$N–NH_2$	3	8
$N–N=C(CH_3)_2$	4	9
NH	5	10



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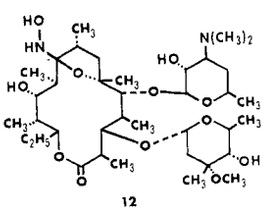

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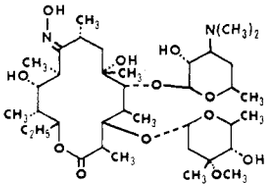
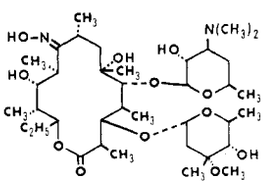


7a (E) - oxime



7b (Z) - oxime

Discussion

Preparation, Isolation, and Characterization of Oxime Isomers. Two new compounds analyzing for the oxime structure were obtained on reaction of erythromycin B with hydroxylamine. The major product was readily identified as an oxime in the infrared.[6] However, the minor product, which could be obtained only 80% pure, failed to show a significant band in the 1600-cm^{-1} region in both the infrared and the Raman.[7]

Positive evidence for an oxime was considered necessary, as an alternate hemiacetal type structure **12** was possible for the minor isomer. The latter structure was suggested by a band in the hydroxyl region at 3240 cm^{-1} ($\Delta\nu_{1/2}$ 100 cm^{-1}) in CCl_4 which could be interpreted as a hydrogen-bonded NH absorption of a hydroxylamine.[8] Such a structure is also consistent with the known chemistry of the erythromycins.[9]

Conclusive evidence for the oxime structure of the minor product was derived from both ^1H nmr spectra in dimethyl sulfoxide-d_6 solution and ^{13}C nmr spectra. In DMSO-d_6,

Table I
Chemical Shifts of Aglycone Ring Protons in $CDCl_3$ Solution

	Major 7a	Minor 7b
H-2	2.88	~2.9
H-3	4.05	4.09
H-4	~2.2	~2.1
H-5	3.60	3.60
H-10	2.59	2.68
H-11	3.65	3.83
H-13	5.42	5.19

the major isomer showed a one-proton absorption at 10.62 ppm, which was exchangeable with D_2O. The minor isomer showed corresponding signals at 10.43 and 10.62 ppm (*ca.* 20% impurity of major isomer) also exchangeable with D_2O. These chemical shifts are clearly consistent with an oxime hydroxyl.[10] The ^{13}C nmr spectrum of the major isomer showed peaks at 177.7 and 171.1 ppm downfield from TMS for the lactone[11] and the oxime carbons[12] while the minor isomer showed peaks at 178.0 and 169.4 ppm. With the compounds firmly established as isomeric oximes, configurational assignments were made on the basis of infrared and nmr data.

Configurational Analysis of Oxime Isomers. Nuclear Magnetic Resonance. The most significant differences between the spectra of the two oxime isomers which could be observed at 100 MHz are the changes in the chemical shifts of H-11 and H-13 (Table I). Although H-11 is not α to C-9, it is significantly deshielded in the minor isomer compared to the major product. The deshielding of H-11 can be attributed to a cis hydroxyl of the oxime group and establishes that the minor isomer is **7b**, the *Z* isomer.[13] The magnitude of the deshielding is greater than that observed for β-CH protons in simple oximes and hydrazones,[14-18] which may be a reflection of the rigid conformation of the aglycone ring (Figure 1).[19-21]

The chemical shift changes of H-10 are considerably smaller and less diagnostic, which is consistent with its unfavorable orientation with respect to the OH group. Nevertheless, the minor isomer shows the expected deshielding of H-10 consistent with its syn orientation.

The shielding of H-13 in the spectrum of the minor component is unusual in view of the distance between C-9 and H-13, suggesting that the difference is not due entirely to the C-9 substituent but rather may be accentuated by a secondary effect. For example, the change in the chemical shifts may result in part from a decrease in the deshielding arising from the 11-hydroxyl group. This decrease may be caused by hydrogen bonding of the 11-hydroxyl proton to the oxygen of the lactone carbonyl. This interaction causes changes in the electronic character and therefore anisotropy of the carbonyl which is reflected in a decrease in the deshielding of H-13. The required hydrogen bonding is supported by infrared evidence which follows.

Attempts to determine the configuration of the erythromycin A oxime in a similar manner were hindered by the insolubility of the major isomer in $CDCl_3$ and the unavailability of the as yet unisolated minor isomer. Comparison of the nmr spectra of both major isomers in pyridine-d_5 shows no differences not attributable to introduction of the 12-OH in the erythromycin A isomer and which are not exactly mirrored in the comparison of the patent antibiotics (*cf.* Table II). Therefore both major isomers are assigned the same stereochemistry in pyridine-d_5 solution which is presumed to be *E*.

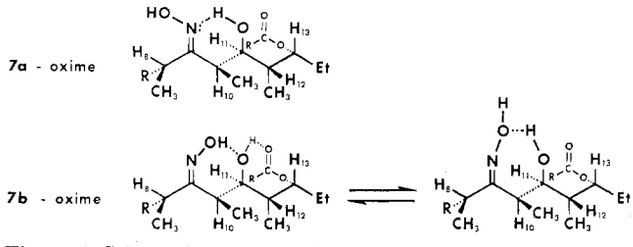


Figure 1. Schematic representation of (*E*)- and (*Z*)-oxime isomers (R = remainder of ring).

Table II
Chemical Shifts of Aglycone Ring Protons in C_5D_5N Solution

	1	6	2	7a
H-2	3.02	3.04	3.08	3.08
H-3	4.43	4.43	4.42	4.39
H-5	3.95	3.96	3.99	3.97
H-10	3.26	3.05	2.95	2.80
H-11	4.29	4.27	4.14	4.06
H-13	5.41	5.65	5.51	5.78

Table III
Chemical Shifts of Aglycone Ring Protons in $CDCl_3$ Solution

	3	4	8	9
H-2	2.80		2.80	2.90
H-3	4.00	4.04	4.01	4.02
H-4	~2.0		2.06	2.16
H-5	3.56	3.66	3.58	3.62
H-10	2.66		2.62	2.65
H-11	3.51	3.72	3.49	3.66
H-13	5.06	5.14	5.41	5.48

Infrared. At 2×10^{-3} *M* the major isomer showed peaks at 3592, 3560, and 3475 cm^{-1} and a broad complex peak at about 3330 cm^{-1}. The lactone was observed as a single strong band at 1730 cm^{-1}. When diluted to 6×10^{-5} *M* the free oxime hydroxyl at 3592 cm^{-1} increased at the expense of peaks at 3560 and 3330 cm^{-1}. The observed spectral changes indicate the oxime hydroxyl is not intramolecularly hydrogen bonded[22] and is directed away from the 11-hydroxyl group as shown for the *E* isomer **7a** in Figure 1.

The minor isomer at 2×10^{-3} *M* showed bands at 3593, 3560, 3465, and 3240 cm^{-1} in the hydroxyl region. The latter band is unusually sharp ($\Delta\nu_{1/2}$ 100 cm^{-1}) and is assigned to an intramolecularly hydrogen bonded oxime hydroxyl.[23] The carbonyl region showed two bands at 1730 and 1712 cm^{-1}, each about half as intense as that of the major isomer. The appearance of the band at 1712 cm^{-1} along with weakening of the 1730-cm^{-1} band is evidence of a mixture of hydrogen-bonded forms in which one form exists with a hydrogen bond to the lactone carbonyl.[24] On further dilution to 6×10^{-5} M the hydroxyl region showed no change, confirming the assignment of the 3240-cm^{-1} band to an intramolecular hydrogen bond. This compound is assigned as the *Z* isomer with the hydrogen-bonding arrangement as shown in **7b** (Figure 1).

Configurational Analysis of Other 9-Imino Derivatives. The same trends observed in the nmr spectra of the oxime isomers are seen in the spectra of **3**, **4**, **8**, and **9** (Table III), although the magnitudes of the changes vary depending on the nature of the 9-imino substituent. Identical correlations can be made although they suffer from lack of data for the unisolated *Z* isomers.

The largest chemical shift differences should be associated with H-8. Unfortunately, this resonance cannot be directly observed in the 100-MHz spectra of these compounds; however, the 220-MHz spectra of **8** and **9** reveal complex multiplets at ca. 3.5 ppm which can be attributed

to H-8. The comparable chemical shift in the erythromycins is 2.7 ppm. The large deshielding of H-8 indicates that the *E* isomer is present in both cases, even without the spectra of the second isomers for comparison.

In summary, the data clearly reveal that the major stable isomers of the oxime and hydrazone of the erythromycins are configurationally homogeneous *E* isomers. The isolation of the minor unstable oxime isomer represents the first and only example of a *Z* isomer.

Experimental Section

General. ^1H nmr spectra were obtained on Varian Associates HA-100 and HR-220 spectrometers. Chemical shifts were measured from internal TMS in $CDCl_3$ solution at 55°. ^{13}C nmr spectra were obtained on a Bucher HFX-90 spectrometer. Chemical shifts were determined in methanol and methanol-d_4 solutions and measured from an external capillary containing CS_2. Chemical shifts were converted to the TMS scale by the formula $\delta_{TMS} = 193.7 - \delta_{CS_2}$ external.[25]

Infrared data were obtained in both 3.0-mm NaCl cells and 100-mm quartz cells using a Perkin-Elmer Model 521 infrared grating spectrophotometer. Raman data were obtained on solid samples using a Cary Model 83 Raman spectrophotometer which employs an Argon-Ion laser. Melting points are uncorrected.

Preparation of Erythromycin B Oxime (7). To a solution of 15 g (20.9 mmol) of erythromycin B in 225 ml of methanol was added 9.60 ml of triethylamine and 6.30 g (90.6 mmol) of $H_2NOH \cdot HCl$. The mixture was refluxed for 65 hr and then cooled and diluted with 2.6 l. of water containing 260 ml of concentrated NH_4OH. The product was extracted with $CHCl_3$ and the combined extracts were washed with dilute NH_4OH. The $CHCl_3$ was dried over anhydrous Na_2SO_4 and the solvent was evaporated to give 17.9 g of crude product. The sample was crystallized from $CHCl_3$–hexane to give 12.3 g of oxime, mp 176–195°, obtained as a chloroform solvate. The tlc of this product (silica gel G, benzene–methanol, 80:20, atmosphere saturated with ammonia, arsenomolybdate reagent) showed two compounds, a major product with R_f value 0.4 (*ca.* 90%) and a minor product with R_f value 0.3 (*ca.* 10%). The isomers were separated by chromatography of *ca.* 2.0-g samples on a 200 g silica gel column (4 × 30 cm^2) using 98:1:1 EtoAc–H_2O–Et_3N as the solvent system.

(*E*)-Erythromycin B Oxime (7a). Fractions obtained by chromatography of 2.2 g of the isomeric mixture were combined and the solvent was evaporated to give 1.9 g of the pure *E* isomer. Crystallization from methanol–water and drying at 75° in a vacuum oven gave 1.0 g of **7a:** mp 171–174°; $[\alpha]^{24}D$ −81.4° (*c* 1.00, MeOH). *Anal.* Calcd for $C_{37}H_{68}N_2O_{12}$ (732.96): C, 60.63; H, 9.35; N, 3.82; O, 26.19. Found: C, 60.95; H, 9.40; N, 3.84; O, 26.49. Nmr (see Table I for aglycone ring proton chemical shifts): $J_{2,3}$ = 9.0; $J_{3,4} \simeq 1$; $J_{4,5}$ = 7.0; $J_{10,11}$ = 1.0; $J_{11,12}$ = 10.5; $J_{12,13} \simeq 1$; $J_{13,14}$ = 9.0, 5.0 Hz; H-1′, δ 4.43, $J_{1',2'}$ = 7.0 Hz; H-2′, δ 3.22, $J_{2',3'}$ = 10.0 Hz; H-1″, δ 4.92, $J_{1'',2''}$ = 4.5, ~ 1 Hz; H-4″, δ 2.97; H-5″, δ 4.05, $J_{4'',5''}$ = 9.0 Hz; CH_3-6, δ 1.49; OCH_3, δ 3.30; $N(CH_3)_2$, δ 2.28.

(*Z*)-Erythromycin B Oxime (7b). The minor isomer could not be obtained in pure form because of isomerization during chromatography and work-up. The purest sample contained 20–25% of the *E* isomer and was obtained by concentrating the eluent at 25° to a small volume, diluting with benzene, washing with water, drying over Na_2SO_4, and finally evaporating the benzene solution to dryness at 25° to give 0.2 g. Analytical and spectral data were collected on the glass obtained in this way: $[\alpha]^{24}D$ −84.9° (*c* 1.00, MeOH). *Anal.* Calcd for $C_{37}H_{68}N_2O_{12}$ (732.96). Found: C, 60.89; H, 9.58; N, 3.80; O, 25.90. Nmr (see Table I for aglycone ring proton chemical shifts): $J_{2,3}$ = 9.0; $J_{3,4} \simeq 1$; $J_{4,5}$ = 7.5; $J_{10,11}$ = 1.0; $J_{11,12}$ = 10.5; $J_{12,13} \simeq 1$; $J_{13,14}$ = 9.0, 5.0 Hz; H-1′, δ 4.42, $J_{1',2'}$ = 7.5 Hz; H-2′, 3.21, $J_{2',3'}$ = 10.0 Hz; H-1″, δ 4.91, $J_{1'',2''}$ = 4.5, ~ 1 Hz; H-4″, δ 2.99; H-5″, δ 4.04, $J_{4'',5''}$ = 9.5 Hz; CH_3-6, δ 1.45; OCH_3, δ 3.30; $N(CH_3)_2$, δ 2.28.

Thermal Isomerization of the *Z* Isomer. The *Z* isomer was heated at 136° for 16 hr under vacuum. The sample was identified as the *E* isomer by tlc and infrared spectrum (CCl_4, 4000–1650 cm^{-1}). The crystalline *E* isomer was unchanged by the same treatment.

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Registry No.—**7a,** 51820-81-6; **7b,** 51830-04-7; erythromycin B, 527-75-3; $H_2NOH \cdot HCl$, 5470-11-1.

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