Chemical Modifications of Erythromycin Antibiotics. I. 3′-De(dimethylamino)erythromycin A and B

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The pyrolytic elimination of N,N-dimethylhydroxylamine from the amine oxides of the macrolide antibiotics, erythromycin A and B, led to the corresponding allylic alcohols, 3′-de(dimethylamino)-3′,4′-dehydroerythromycin A and B, which were converted into 3′-de(dimethylamino)erythromycin A and B by catalytic reduction. The structures were verified by spectral and degradative studies.

The structures[1] and stereochemistry [1c,2] of the macrolide antibiotics, erythromycin A (1) and B (2), are well established. Several reports of structural modifications of the intact antibiotic have been published such as the syntheses of desosamine[3] esters,[4] the demethylation studies with carbobenzyloxy chloride[5] and subsequent syntheses of new N-alkylated derivatives,[6] preparation of the erythromycin A oxime, and reduction of the latter to the corresponding amine.[7] This report describes our initial studies of the structural modification of the erythromycins.

Cope has shown that the pyrolysis of tertiary amine oxides in the absence of solvent at 120–150° usually produces the corresponding olefin and N,N-dimethylhydroxylamine.[8] Cram, Sahyun, and Knox found that the Cope elimination proceeded smoothly at room temperature in tetrahydrofuran and dimethyl sulfoxide, but that the rate was retarded by the presence of water.[9] The instability of the macrolide ring of erythromycin suggested the application of the low-temperature conditions. Although no study of this elimination with an unmodified macrolide antibiotic has been described, Celmer has used the reaction in his studies of the absolute configuration of the macrolide antibiotic, oleandomycin.[10] In this manner he removed the dimethylamino group from the desosamine moiety of a lactone-opened oleandomycin derivative and subsequently cleaved the resulting neutral sugar under conditions much milder than those needed to cleave a basic sugar.

The amine oxide **3** of erythromycin A, prepared by the method of Flynn and coworkers,[1a] was recrystallized from methanol–ether to yield a crystalline solid (mp 221–223°) identical with the reported product. When this material was recrystallized from hot methanol–water, a crystalline dihydrate (mp 165–170°) was isolated. Since both crystalline forms were readily interconvertible by recrystallization from the appropriate solvent, it was evident that they were different crystalline forms derived from the same amine oxide.

The Cram modification[9] of the Cope elimination was attempted on both forms of **3**. When the low-melting form was dissolved in dry tetrahydrofuran, the high-melting form crystallized from the solution. When dimethyl sulfoxide was used, the amine oxide was recovered unchanged after 10 days, even when molecular sieve was used to remove water. Celmer, in his studies with the oleandomycin derivatives, used this method successfully.[10]

The amine oxide dihydrate (mp 165–170°) (**3**), upon heating without solvent for 6 hr at 150°, was converted into 3′-de(dimethylamino)-3′,4′-dehydroerythromycin A (**5**) (Scheme I). The high-melting form remained unchanged under these conditions, but, when the temperature was raised to 220°, it was converted into a single product which was shown to be different from **5** by thin layer chromatography. The loss of the ketone band in the infrared spectrum indicated that a secondary reaction involving the 9-ketone had occurred in the aglycone ring. Preliminary chemical degradation studies indicate that the allylic alcohol had been formed but that water had been eliminated from the macrolide ring. Upon heating at 210°, **5** was also converted into this high-temperature pyrolysis product.

An explanation of the large differences in the reactivities of the two crystalline forms of the amine oxide was obtained by examination of their solid phase infrared spectra. The solid phase infrared spectrum (Nujol) of the higher melting amine oxide showed a broad chelated hydroxyl band[11] at 3165 cm^{-1}, which was absent in the infrared spectrum (Nujol) of the lower melting dihydrate. In the higher melting chelate the electronegative oxygen of the amine oxide function is probably strongly hydrogen bonded to the neighboring 2′-hydroxyl group (**3a**). It has been demonstrated that the thermal decomposition of alkyldimethylamine oxides to yield olefins, *e.g.*, **3b** → **5**, proceeds by an intramolecular *cis* elimination of N,N-dimethylhydroxylamine.[12] The amine oxide group of the chelate **3a**

(1) (a) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, and K. Gerzon, *J. Am. Chem. Soc.*, **76**, 3121 (1954); (b) M. V. Sigal, Jr., P. F. Wiley, K. Gerzon, E. H. Flynn, U. C. Quarck, and O. Weaver, *ibid.*, **78**, 388 (1956); (c) K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monahan, and U. C. Quarck, *ibid.*, **78**, 6396 (1956); (d) P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., O. Weaver, U. C. Quarck, R. R. Chauvette, and R. Monahan, *ibid.*, **79**, 6062 (1957); (e) P. F. Wiley, M. V. Sigal, Jr., O. Weaver, R. Monahan, and K. Gerzon, *ibid.*, **79**, 6070 (1957).

(2) (a) C. Djerassi, O. Halpern, D. I. Wilkinson, and E. J. Eisenbraun, *Tetrahedron*, **4**, 369 (1958); (b) W. Hofheinz and H. Grisebach, *Tetrahedron Letters*, 377 (1962); (c) W. Hofheinz, H. Grisebach, and H. Friebolin, *Tetrahedron*, **18**, 1265 (1962); (d) W. Hofheinz and H. Grisebach, *Ber.*, **96**, 2867 (1963); (e) D. R. Harris, S. G. McGeachin, and H. H. Mills, *Tetrahedron Letters*, 679 (1965); (f) W. D. Celmer, *J. Am. Chem. Soc.*, **87**, 1799 (1965); (g) W. D. Celmer in "Biogenesis of Antibiotic Substances," Z. Vanek and Z. Hostalek, Ed., Academic Press Inc., New York, N. Y., 1965, Chapter 10.

(3) The common name for the basic sugar attached to erythromycin A and B is desosamine and for the neutral sugar, cladinose.[1a] The numbering system shown in structures **1** and **2** was chosen for use in this study.

(4) (a) H. W. Murphy, *Antibiot. Ann.*, 500 (1954); (b) V. C. Stephens, *ibid.*, 514 (1954); (c) V. C. Stephens and J. W. Conine, *ibid.*, 346 (1959); (d) R. K. Clark and E. L. Varner, *Antibiot. Chemotherapy*, **7**, 487 (1957).

(5) E. H. Flynn, H. W. Murphy, and R. E. McMahon, *J. Am. Chem. Soc.*, **77**, 3104 (1955).

(6) R. K. Clark, Jr., and M. Freifelder, *Antibiot. Chemotherapy*, **7**, 483 (1957).

(7) S. Djokic and Z. Tamburasev, *Tetrahedron Letters*, 1645 (1967).

(8) For most recent reference, see A. C. Cope, E. Ciganek, and J. Lazar, *J. Am. Chem. Soc.*, **84**, 2591 (1962).

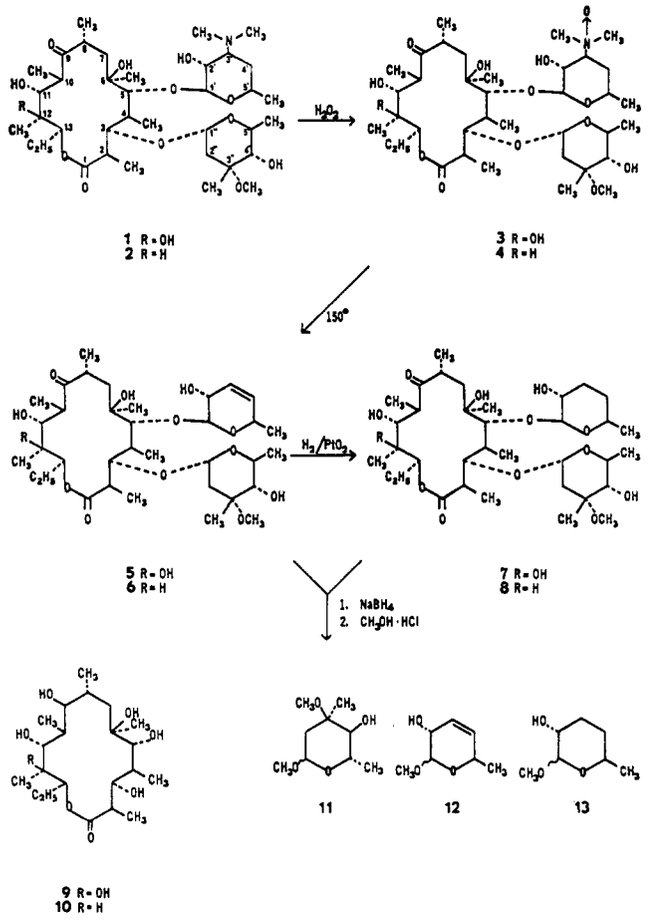
(9) D. J. Cram, M. R. V. Sahyun, and G. R. Knox, *ibid.*, **84**, 1734 (1962).

(10) (a) W. D. Celmer, *ibid.*, **87**, 1797 (1965); (b) ref 2g, pp 103–105.

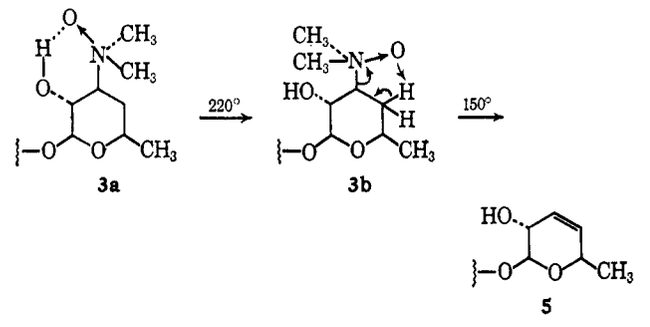
(11) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co. Ltd., London, 1956, p 84.

(12) (a) A. C. Cope, T. T. Foster, and P. H. Towle, *J. Am. Chem. Soc.*, **71**, 3929 (1949); (b) D. J. Cram and J. E. McCarty, *ibid.*, **76**, 5740 (1954).

SCHEME I



would not be favorably situated to remove the hydrogen from the 4′ position. The cyclic mechanism of the Cope elimination dictates that rotamer **3a** must first be converted into rotamer **3b** before reacting, a process which breaks the chelate bond. The strength of this bond coupled with the stability of the crystal lattice (as indicated by the high melting point) stabilizes the



chelate as rotamer **3a**. At the melting point the chelate bond breaks and the N-oxide group is free to rotate in the melt. Since the temperature is 60° above the normal Cope elimination temperature, this elimination as well as the elimination of water from the aglycone occur very rapidly. The two water molecules of the dihydrate solvate the electronegative oxygen of the amine oxide, thereby reducing its tendency to form a chelate bond. This permits the amine oxide group of the dihydrate to rotate more freely so that rotamer **3b** is more populated in the crystalline state. From this evidence one would predict that the dihydrate would undergo the elimination below its melting point and at a lower temperature than the chelate.

Evidence for the structure of **5** was provided by its nmr spectrum and chemical degradation. The nmr spectrum of **5** in deuteriochloroform showed a sharp singlet at 339 Hz which was assigned to the two vinyl protons H-3′ and H-4′. This assignment requires that both protons of **5** in chloroform solution are coincidentally magnetically equivalent and that neither is strongly coupled to the adjacent protons H-2′ or H-5′. When the spectrum was run in dimethyl-d_6 sulfoxide, this peak broadened to a multiplet centered at 332 Hz which indicates a different solvent dependency of the chemical shifts of these protons and demonstrates that they are not magnetically equivalent. The dihedral angles, between H-2′ and H-3′ and between H-4′ and H-5′, are 82 and 90°, respectively. The Karplus relationship between the coupling constants and the dihedral angles of vicinal protons predicts that $J_{2'3'}$ and $J_{4'5'}$ should be very small.[13] Finally the nmr spectrum of **5** showed no peaks which could be attributed to a methyl group attached to a double bond (100–140 Hz).[14] In the entire erythromycin A molecule the only pairs of adjacent carbon atoms which do not have a methyl substituent are the C-2′, C-3′, and the C-3′, C-4′ in the desosamine moiety. Therefore, in the absence of any major skeletal rearrangement, the double bond must be located between one of these pairs of carbon atoms.

The ketone in the aglycone portion of **5** was reduced with sodium borohydride to the corresponding dihydro compound. Subsequent cleavage of the glycosides by methanolic hydrogen chloride produced 9-dihydroerythronolide A (**9**) identical in all respects with a sample prepared by the method of Sigal and coworkers.[1b] Methyl cladinoside (**11**), shown identical by glpc, tlc, and mixture melting point of its 3,5-dinitrobenzoate with a sample prepared by the method of Flynn and coworkers,[1a] and a new methyl glycoside (**12**) were isolated from the mother liquors and separated by glpc into mixtures of their α and β anomers.[15]

The nmr spectrum (Figure 1) of a mixture of the α and β anomers of the unsaturated methyl glycoside **12** in deuteriochloroform again showed the absorptions of the vinyl protons as a sharp singlet at 341 Hz. The similarity of the nmr of the vinyl protons of **5** and **12** indicates that no allylic rearrangement or epimerization of the 2′-hydroxyl occurred during the acid cleavage.

Conclusive proof of the structure of **12** was provided by the 100-MHz nmr spectrum (deuteriochloroform) of its 3,5-dinitrobenzoate (Figure 2). The absorption of the vinyl protons appears as an AB quartet at 601 and 577 Hz (J_{AB} = 10 Hz) in good agreement with the coupling constants expected for *cis*-1,2-vinyl protons.[16] Each peak of the AB quartet was additionally split by H-2 and H-5 into a triplet which was verified by decoupling experiments. Irradiation of H-5 (450 Hz) collapsed each triplet of the AB quartet into a doublet as well as each pair of doublets of the 5-methyl (135 and 142 Hz, J = 7 Hz) into a singlet. Irradiation of H-2

(13) R. H. Bible, Jr., "Interpretation of NMR Spectra," Plenum Press, New York, N. Y., 1965, pp 35–36.

(14) See ref 13, p 16.

(15) Throughout this study no attempt was made to separate the α- and β-methyl glycosides. All spectra and analyses were carried out on the equilibrium mixtures obtained from the acidic methanol cleavage.

(16) See ref 13, p 38.

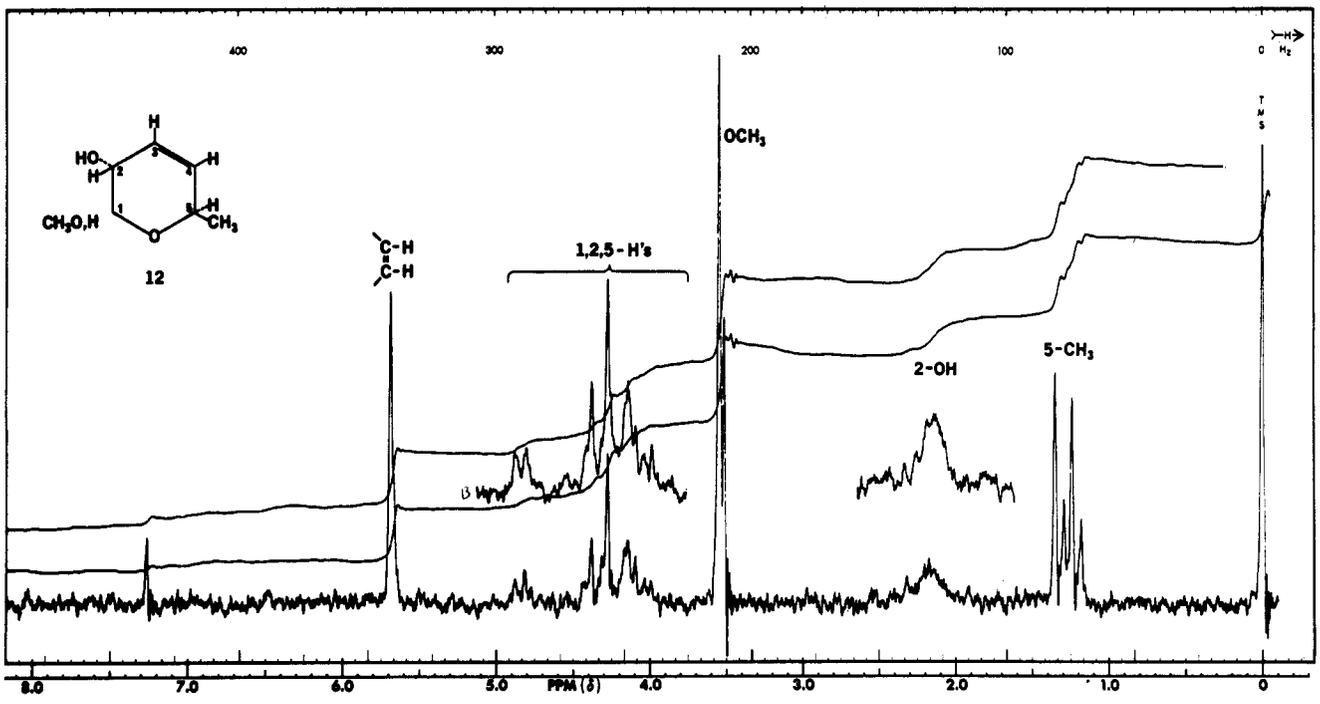


Figure 1.

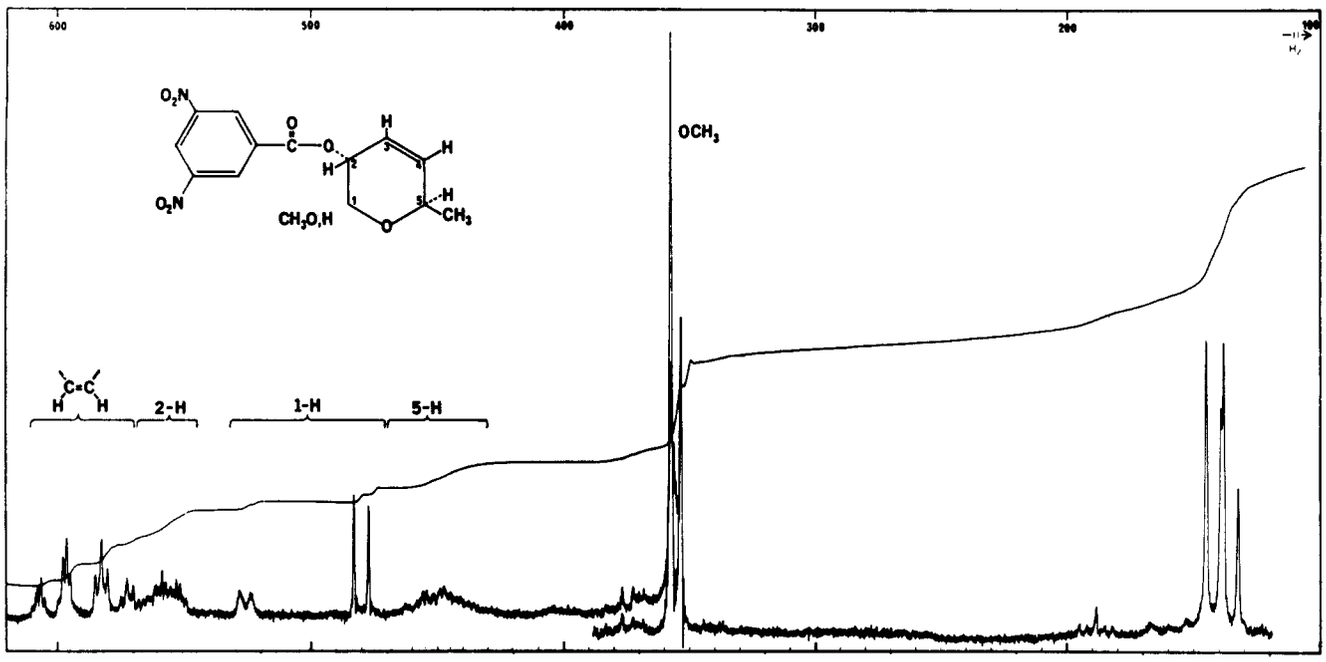


Figure 2.

(558 Hz) again collapsed each triplet of the AB quartet to a doublet and the pair of doublets of H-1 (480 Hz, J = 6 Hz; 526 Hz, J = 4 Hz) into a pair of singlets. These decoupling experiments confirmed the assignments of H-2 and H-5 and demonstrated that each is coupled to the vinyl protons of the AB quartet. Thus the double bond of **12** must be in the 3–4 position.

The stereochemistry at C-2 and C-5 of desosamine (3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexose) is well established.[2b,17] Since the elimination of N,N-dimethylhydroxylamine from the amine oxide **3** should not alter the configurations of these centers,[8,12] methyl 3,4,6-trideoxy-3,4-anhydro-D-*threo*-hexopyranoside (**12**) is assigned as the structure of the unsaturated glycoside. Thus **5** may be identified as 3′-de(dimethylamino)-3′,4′-dehydroerythromycin A based on its mild chemical degradation to **9**, **11**, and **12**.

The double bond of **5** was hydrogenated to form 3′-de(dimethylamino)erythromycin A (**7**). The absence of the vinyl protons was shown by nmr spectroscopy. From the sodium borohydride reduction and subsequent glycolysis of **7**, there were isolated **9** and **11** as well as the new methyl trideoxyglycoside **13**. The structural assignment of **13** as a mixture of the α and β anomers of methyl 3,4,6-trideoxy-D-*threo*-hexopyranoside was confirmed by nmr spectroscopy (Figure 3). The spectrum clearly distinguished the two anomers.

(17) (a) C. H. Bolton, A. B. Foster, M. Stacey, and J. M. Webber, *J. Chem. Soc.*, 4831 (1961); (b) P. W. K. Woo, H. W. Dion, L. Durham, and H. S. Mosher, *Tetrahedron Letters*, 735 (1962); (c) C. H. Bolton, A. B. Foster, M. Stacey, and J. M. Webber, *Chem. Ind.* (London), 1945 (1962).

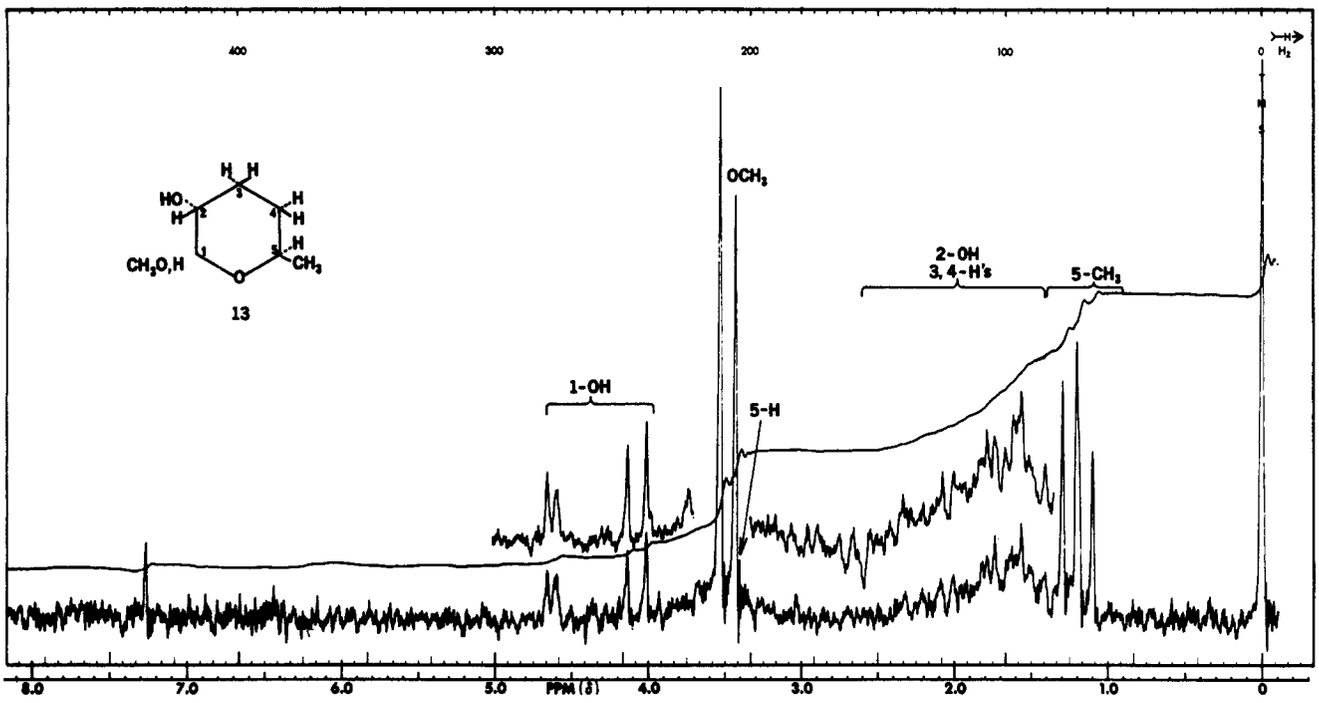


Figure 3.

A similar series of experiments were carried out on erythromycin B (2). The crystalline amine oxide **4**, mp 190–192°, prepared by the published procedure,[1e] was also shown by its infrared spectrum (Nujol) to be a chelate. Attempts to form a crystalline, nonchelated hydrate failed, but an amorphous, nonchelated form was obtained by rapidly removing the solvent from a chloroform solution of the chelate. The pyrolytic elimination of N,N-dimethylhydroxylamine at 150° from **4** as an amorphous solid produced 3′-de(dimethylamino)-3′,4′-dehydroerythromycin B (**6**). The chelate (mp 190–192°) was inert at this temperature but decomposed at 185° to **6**. As in the erythromycin A series the amine oxide group was locked as the wrong rotamer (**3a**) by the chelation bond and the crystal lattice.

The spectral properties (infrared and nmr) of **6** were practically identical with those of the allylic alcohol **5**. The absorption of both vinyl protons again appeared as a sharp singlet at 340 Hz (deuteriochloroform). Catalytic reduction of the double bond led smoothly to 3′-de(dimethylamino)erythromycin B (**8**). From the sodium borohydride reduction and acid cleavage of **6** and **8** were isolated methyl cladinoside (**11**) and 9-dihydroerythronolide B (**10**) identical by tlc, infrared, and nmr spectra to a sample prepared by the method of Wiley and coworkers.[1e] In addition to **10** and **11**, the methyl glycosides **12** and **13** were isolated from **6** and **8**, respectively, and shown by chromatographic and spectral techniques to be identical with the glycosides isolated from **5** and **7**. As in the erythromycin A series, the isolation of **10** showed that the aglycone was not degraded during the amine oxide elimination. This, coupled with the similarity of the nmr spectra of the vinyl protons of **5**, **6**, and **12** and the isolation of the expected glycosides, is conclusive evidence for the structural assignments to the allylic alcohol **6** and its reduction product **8**.

Experimental Section[18]

Erythromycin A N-Oxide Dihydrate (3).—To a solution of erythromycin A (80 g) dissolved in 2880 ml of methanol was added 1920 ml of 3% hydrogen peroxide. After 48 hr at room temperature, the excess peroxide was destroyed with Adams catalyst. After filtering, the volume was reduced to about one-third of its original volume at which time crystals of the amine oxide were formed. After cooling to 0°, filtering, and washing with warm water, 72 g (88%) of erythromycin A N-oxide dihydrate was collected as white needles: mp 165–170°; infrared absorption (Nujol) at 3580, 3530, and 3370 (OH), 1735 (lactone C=O), and 1700 cm^{-1} (ketone C=O).

Anal. Calcd for $C_{37}H_{67}NO_{14}\cdot 2H_2O$: C, 56.54; H, 9.11; O, 32.57. Found: C, 56.53; H, 9.38; O, 32.52.

When a small sample of the dihydrate was dissolved in anhydrous tetrahydrofuran, a new crystal form precipitated. Filtration of the solution after 1 hr gave a quantitative yield of the higher melting chelate of erythromycin A N-oxide: mp 222–224° (lit.[1a] mp 220–223°); infrared absorption (Nujol) at 3510 and 3470 (OH) and 3165 cm^{-1} (chelated OH). This amine oxide (mp 222–224°) was converted into the other crystal form (mp 165–170°) by crystallization from hot methanol–water.

3′-De(dimethylamino)-3′,4′-dehydroerythromycin A (5).—Erythromycin A N-oxide dihydrate (**5**, mp 165–170°, 50 g) was heated without solvent at 150° and under vacuum (0.3 mm) for 6 hr. A tlc analysis (system A) of the resulting tan solid indicated a trace of the amine oxide as well as a single fast-moving compo-

(18) Infrared spectra were obtained with a Perkin-Elmer Model 421 grating spectrophotometer. Unless otherwise stated the nmr spectra were recorded on a Varian A-60 instrument using 10% deuteriochloroform solutions. Values are measured in hertz downfield from tetramethylsilane as an internal standard. Gas–liquid partition chromatographies (glpc) were carried out on a Barber–Coleman Model 10 unit fitted with a 8-ft SAIB on Gas-Chrom P (60–80 mesh) column operated at 125° and 20 psig of helium. Since all runs were in sequence, the retention times (minutes) and per cents for the various experiments can be compared. Thin layer chromatographies (tlc) were carried out on silica gel G plates activated at 100° for 2 hr. The plates were developed using the following solvent systems: A, chloroform–methanol 95:5; B, benzene–methanol 80:20; C, benzene–methanol 99.5:0.5. The spots were detected by spraying with arsenomolybdate reagent[19] and heating for several minutes at 100°. Since R_f values varied widely from plate to plate, they were not recorded. Optical rotations were determined with a Hilger and Watts polarimeter on 1% solutions in methanol. The X-ray powder patterns were determined with a General Electric XRD-5F unit. The petroleum ether used for crystallization was the fraction boiling at 66–70°.

(19) N. Nelson, *J. Biol. Chem.*, **153**, 375 (1944).

nent. Upon dissolving in anhydrous tetrahydrofuran (350 ml), the starting material crystallized and was removed by filtration to yield 3 g (6%) of erythromycin A N-oxide (mp 220–223°) undepressed upon admixture with an authentic sample. The filtrate was evaporated to dryness and the resulting tan solid (42 g) was dissolved in methanol (200 ml). After decolorizing with Darco the methanol was removed until crystals of **5** appeared (~100 ml). The solution was warmed to dissolve the crystals and 5 ml of water added. Upon cooling to 0° and filtering, 28 g (61%) of white crystals was obtained, mp 216–221°. A second crop yielded an additional 10 g (21%). The combined crops recrystallized from methanol yielded 32 g (78%) of white needles: mp 220–221°; $[\alpha]^{26}D$ −113°; infrared absorption (Nujol) at 3614, 3519, and 3440 (OH), 1738 (lactone C=O), and 1680 cm^{-1} (ketone C=O); nmr absorption ($CDCl_3$) at 339 Hz (singlet, 2 H, CH=CH), (DMSO) 332 Hz (multiplet, 2 H, CH=CH).

Anal. Calcd for $C_{35}H_{60}O_{13}$: C, 61.03; H, 8.78; O, 30.19. Found: C, 61.09; H, 8.70; O, 30.16.

Pyrolysis of Erythromycin A N-Oxide Chelate.—Erythromycin A N-oxide (**3**, mp 222–224°, 1.0 g) was heated under vacuum without solvent at 165°. After 6 hr a small sample was removed and shown by tlc (system A) to contain only unreacted starting material. When the temperature was raised to 220°, the sample melted to clear glass. After several hours the glass became dark brown and was shown to contain neither **3** nor **5** (tlc, system A). The product was decolorized with Darco and crystallized from acetone to yield 0.6 g of white, fine needles: mp 105–115°; infrared absorption ($CHCl_3$) at 3590 and 3540 (OH), 1700 cm^{-1} (lactone C=O); nmr absorption at 339 Hz (singlet, 2 H, CH=CH); $[\alpha]^{26}D$ −66°.

Anal. Calcd for $C_{35}H_{59}O_{12}$: C, 62.66; H, 8.71; O, 28.62. Found: C, 62.59; H, 8.71; O, 28.33.

High-Temperature Pyrolysis of 3′-De(dimethylamino)-3′,4′-dehydroerythromycin A (5).—A small sample of **5** (1.0 g) was heated under vacuum (0.3 mm) without solvent at 210°. After 2 hr a small sample was removed and shown by tlc (system A) to contain a single new component identical in mobility with the product of high-temperature pyrolysis of the chelate. After decolorizing with Darco and crystallizing from acetone, 0.6 g of white needles (mp 106–115°) was obtained. This product was shown to be identical with the pyrolysis product of the chelate by infrared and nmr spectra.

Sodium Borohydride Reduction and Glycoside Cleavage of 5.—3′-De(dimethylamino)-3′,4′-dehydroerythromycin A (**5**, 1.0 g, 1.5 mmoles) was dissolved in 50 ml of methanol and cooled to −12° with a salt–ice bath. Sodium borohydride (500 mg, 13 mmoles) was added slowly (0.5 hr) as a solid. After stirring at −12° for 1 additional hr, methanol (50 ml) containing 3% hydrogen chloride was added. After 0.5 hr at −12°, the solution was stirred at room temperature for 2 days. Solid sodium bicarbonate was added to neutralize the acid and the resulting mixture poured into water and extracted three times with chloroform. The combined chloroform layers were washed with 2% sodium bicarbonate and finally with water. Removal of the chloroform yielded 930 mg of a pale yellow oil which was shown by tlc (system A) to contain three major components of which two had the same R_f's as 9-dihydroerythronolide A (**9**) and methyl cladinoside (**11**) which were prepared by published procedures.[1a,b] The yellow oil was crystallized from chloroform–petroleum ether to yield 337 mg (55%) of 9-dihydroerythronolide A (**9**): mp 199–200°; $[\alpha]^{25}D$ +9.5° (lit.[1b] mp 185–187°; $[\alpha]^{27}D$ +9.5° (*c* 2 in methanol)); infrared absorptions at 3614 and 3480 (OH) and 1701 cm^{-1} (lactone C=O).

Anal. Calcd for $C_{21}H_{40}O_8$: C, 59.97; H, 9.59; O, 30.44. Found: C, 60.12; H, 9.58; O, 30.08.

For comparison, a sample of **9** was prepared from erythromycin A by the published procedure:[1b] mp 188–190° (lit.[1b] mp 185–187°); mixture melting point with **9** obtained from **5**, 193–197°. The infrared and nmr spectra, optical rotation, X-ray powder pattern, and tlc mobilities (system A) of both samples were identical.

The filtrate remaining from the crystallization of **9** was distilled under reduced pressure to give 200 mg of a mixture of the methyl glycosides **11** and **12** (bp 55–70° at 0.2 mm). The distillate was analyzed by glpc and found to contain five components: 3.1 min (11.2%); 5.6 min (31.5%); 6.9 min (7.3%); 10.9 min (44.2%); 13.9 min (5.8%). When methyl cladinoside (**11**)[1a] was analyzed under the same experimental conditions, two components were found which correspond to the α and β anomers: 6.7 min (17.1%); 10.9 min (82.9%). Several milligrams of a mixture of peaks 3 and 4 was collected by repeated glpc runs and shown by nmr ($CDCl_3$) to be a mixture of the α and β anomers of methyl cladinoside. In the same manner several milligrams of a mixture of peaks 1 and 2 was collected and the nmr spectrum ($CDCl_3$) (Figure 1) measured: 341 Hz (singlet, 2 H, CH=CH).

3,5-Dinitrobenzoate Esters of 11 and 12.—To a solution of 100 mg of the mixture of methyl glycosides **11** and **12** dissolved in anhydrous pyridine (3 ml) was added 3,5-dinitrobenzoyl chloride. The solution was warmed for 20 min on a steam bath and poured into 10% sodium bicarbonate. The yellow solid (150 mg) was collected and shown by tlc (system C) to contain four components which moved in pairs of two. The pair of spots nearest the origin had the same mobility as a sample of the 3,5-dinitrobenzoate ester of methyl cladinoside prepared by the published procedure.[1a]

The esters were separated on a silica gel column (2.5 × 30 cm) eluted with benzene–ether (95:5) (20-ml fractions). Progress of the chromatography was followed by tlc. Fractions 25–29 were combined and crystallized from 95% ethanol to yield 54 mg of the 3,5-dinitrobenzoate ester of **12** as white plates: mp 148–150°, $[\alpha]^{26}D$ −128° (*c* 0.5, CH_3OH).

Anal. Calcd for $C_{14}H_{14}N_2O_8$: C, 49.71; H, 4.17; N, 8.28. Found: C, 49.63; H, 4.33; N, 8.16.

The tlc (system C) of the analytical sample showed the presence of the α and β anomers which could not be separated even by repeated recrystallizations. The 100-MHz nmr spectrum ($CDCl_3$) of this mixture is shown in Figure 2.

The column was then eluted with benzene–ether (1:1). Fractions 35–40 were combined and crystallized from 95% ethanol to yield 63 mg of the 3,5-dinitrobenzoate ester of methyl cladinoside: mp 155–162° (lit.[1a] mp 159–161 and 195–196° (α and β anomers)). An analysis of the ester (tlc, system C) showed both anomers. For comparison, a sample was prepared from erythromycin A by the published procedure[1a] (separation of the anomers was not attempted): mp 155–160°; mmp 155–160°. The infrared and nmr spectra and tlc mobilities (system C) of both samples were identical.

3′-De(dimethylamino)erythromycin A (7).—3′-De(dimethylamino)-3′,4′-dehydroerythromycin A (**5**, 2.8 g, 4.0 mmoles) dissolved in 75 ml of absolute ethanol was stirred in a hydrogen atmosphere in the presence of 80 mg of Adams catalyst. After 30 min 4.0 mmoles of hydrogen was absorbed and the solution filtered. A tlc analysis (system B) of the filtrate showed a single component which was not starting material. The ethanol was removed under reduced pressure to yield 2.8 g of a white solid, mp 120–130°. After several recrystallizations from ethanol–water, 2.1 g (75%) of 3′-de(dimethylamino)erythromycin A (**7**) was obtained as a white solid: mp 134–140° resolidifying and melting at 209–210°; $[\alpha]^{26}D$ −89°; infrared absorption ($CHCl_3$) at 3605 and 3480 (OH), 1727 (lactone C=O), and 1685 cm^{-1} (ketone C=O); no nmr absorption ($CDCl_3$) at 320–360 Hz (CH=CH).

Anal. Calcd for $C_{35}H_{62}O_{13}$: C, 60.84; H, 9.06; O, 30.10. Found: C, 60.77; H, 8.79; O, 29.97.

Sodium Borohydride Reduction and Glycoside Cleavage of 7.—3′-De(dimethylamino)erythromycin A (**7**, 1.0 g) treated with sodium borohydride and methanolic hydrogen chloride by a procedure identical with the chemical degradation of **5** yielded 320 mg (52%) of 9-dihydroerythronolide A (**9**) melting at 199–200°, undepressed upon admixture with an authentic sample of **9**. The infrared and nmr spectra of this material were identical with those obtained with the previous sample of **9**.

Distillation of the filtrate from the crystallization of **9** yielded a mixture of the methyl glycosides **11** and **13**: bp 55–70° (0.2 mm); yield 195 mg. The distillate was analyzed by glpc and found to contain four components: 3.1 min (21.7%); 4.0 min (16.7%); 6.8 min (10.6%); 10.7 min (51.0%). An nmr spectrum ($CDCl_3$) of several milligrams of a mixture of peaks 3 and 4 collected from the column was identical with the spectrum of methyl cladinoside (**11**). In the same manner several milligrams of a mixture of peaks 1 and 2 was collected and the nmr spectrum ($CDCl_3$) recorded (Figure 3).

3-De(dimethylamino)-3′,4′-dehydroerythromycin B (6).—Erythromycin B N-oxide[1c] (**4**, 7.5 g, mp 188–190°, lit.[1c] mp 191–193°), which showed a chelated hydroxyl band at 3160 cm^{-1}, was dissolved in chloroform (100 ml). Removal of the solvent at reduced pressure produced a clear glass which was no longer chelated. After heating for 3 hr at 150° (0.2 mm) an analysis (tlc, system B) showed one major spot as well as a trace of the N-oxide. The tan solid was dissolved in methanol and decolorized

(Darco). Removal of the methanol and crystallization from acetone–petroleum ether yielded 4.5 g (67%) of white prisms: mp 189–190°; $[\alpha]^{26}D$ −128°; infrared absorption ($CHCl_3$) at 3605 and 3500 (OH), 1723 (lactone C=O), and 1695 cm^{-1} (ketone C=O); nmr absorption ($CDCl_3$) at 340 Hz (singlet, 2 H, CH=CH).

Anal. Calcd for $C_{35}H_{60}O_{12}$: C, 62.48; H, 8.99; O, 28.54. Found: C, 62.41; H, 9.02; O, 28.47.

3′-De(dimethylamino)erythromycin B (8).—3′-De(dimethylamino)-3′,4′-dehydroerythromycin B (**6**, 930 mg) dissolved in 75 ml of absolute ethanol was stirred in a hydrogen atmosphere in the presence of 40 mg of Adams catalyst. After 30 min the theoretical amount of hydrogen was taken up and the solution was filtered. A tlc analysis (system A) of the filtrate showed a single new component. The volume was reduced to 7 ml and, when several drops of water were added, crystallization began. Filtration yielded 770 mg (83%) of 3′-de(dimethylamino)-erythromycin B (**8**): mp 194–198°; $[\alpha]^{26}D$ −113°; infrared absorption ($CHCl_3$) at 3604 and 3500 (OH), 1724 (lactone C=O), and 1696 cm^{-1} (ketone C=O).

Anal. Calcd for $C_{35}H_{62}O_{12}$: C, 62.29; H, 9.26; O, 28.45. Found: C, 62.35; H, 9.18; O, 28.46.

Sodium Borohydride Reduction and Glycoside Cleavage of 6 and 8.—The reduction and acid cleavage of **6** and **8** were carried out according to the procedure described for compounds **5** and **7**. From 1 g each of **6** and **8** was obtained 354 (59%) and 410 mg (68%), respectively, of 9-dihydroerythronolide B (**10**): mp 179–182° (from acetonitrile) (lit.[1e] mp 182°); $[\alpha]^{25}D$ +5.9° (lit.[1e] $[\alpha]^{25}D$ +6.0°); infrared absorption at 3614 and 3460 (OH), and 1699 cm^{-1} (lactone C=O).

For comparison, a sample of **10** was prepared from erythromycin B by the published procedure:[1e] mp 178–180° (lit.[1e] mp 182°); mixture melting point with **10** obtained from **6** and **8**, 178–180°. The infrared and nmr spectra, X-ray powder pattern, and tlc mobilities (systems A and B) of all three samples were identical.

The methyl glycosides were recovered from the filtrates of the crystallization of **10** by distillation (bp 55–70° at 0.2 mm) and compared by glpc to methyl glycosides obtained from **5** and **7**. The retention times of the methyl glycosides **11**, **12**, and **13** from **6** and **8** agreed within experimental error with the methyl glycosides from **5** and **7**, respectively. The nmr spectra of **12** and **13** isolated from **6** and **8** were identical with those shown in Figures 1 and 3.

The 3,5-dinitrobenzoate esters of the methyl glycosides **11** and **12** from **6** were prepared and separated by the previously described procedure. The ester of **13** (mp 148–150°, mmp 148–150°) and the ester of **11** (mp 157–162°, mmp 155–162°) were isolated and shown to be identical with the esters obtained from **5** by infrared and nmr spectra and tlc (system C).

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The Synthesis of 4,5-Dihydro-3H-1,3-benzodiazepines and 4,5-Dihydro-1H-2,4-benzodiazepines

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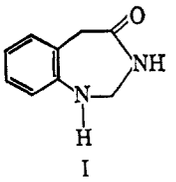
A general synthesis of dihydro-1,3- and -2,4-benzodiazepines has been devised. The method consists of the condensation of the appropriate diamine, *i.e.*, an *o*-aminophenethylamine or α,α'-diamino-*o*-xylene, respectively, with a wide variety of imidate hydrohalides. The effect of the structure of both the imidate hydrohalides and the diamines on the course of the reaction has been evaluated. The order of reactivity of the imidate hydrohalides toward condensation is haloalkyl ≫ alkyl > *p*-NO_2-aryl ≫ *p*-CH_3O-aryl. Steric crowding and decreased basicity of the diamine seriously retard the reaction. Solvent effects have also been explored. Alkylation experiments for the dihydro-1,3-benzodiazepine system indicate attack on the nitrogen, N-3, proven by independent synthesis. Additional chemistry of individual derivatives has been explored. Spectral data for both heterocyclic systems are reported.

Considerable attention has been directed in recent years to the synthesis of seven-membered ring compounds. Our particular interest in this area centered about the benzodiazepines. Syntheses of 1,2-, 1,4- and 1,5-benzodiazepines have been well worked out,[1-5] but 1,3- and 2,4-benzodiazepines have been only briefly explored. Plieninger[6] and Nogradi published a report in which a derivative form of a 1,3-benzodiazepine was synthesized. Later, deStevens[7] and Dughi reported on the synthesis of tetrahydro-1,3-benzodiazepines (I), but there has been no systematic study of the preparation of dihydro-1,3- and -2,4-benzodiazepines.



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Dihydro-3H-1,3-benzodiazepines.—In accordance with procedures which proved successful for the synthesis of benzimidazoles[8] and quinazolines,[9] there exist for the synthesis of 1,3-benzodiazepines pathways *via* Scheme A, internal condensation of *o*-amino-N-acylphenethylamines or activated derivatives thereof,

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