[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Methymycin. Reduction and Oxidation Studies

BY CARL DJERASSI, A. BOWERS[1a] AND H. N. KHASTGIR[1b]

RECEIVED SEPTEMBER 12, 1955

Oxidation and reduction experiments with the antibiotic methymycin ($C_{25}H_{43}NO_7$) are described which lead to a tentative definition of the various functional groups.

Methymycin is a crystalline antibiotic produced by a *Streptomyces* strain and its isolation and preliminary characterization have been reported by Donin and co-workers.[2] The empirical formula $C_{25}H_{43}NO_7$ was established by analysis of the base and of several salts; evidence for an α,β-unsaturated carbonyl group was presented by ultraviolet, infrared and polarographic means. Catalytic hydrogenation was shown to result in the reduction of a double bond or the loss of an oxygen function, depending upon the experimental conditions. Through the kind coöperation of the Squibb group[2] a sample of methymycin was placed at our disposal and this has permitted a more detailed study of its structure. The present report is concerned with a description of pertinent reduction and oxidation studies.

Aside from the earlier reported[2] form (m.p. 195.5–197°) methymycin appears to exist in at least one other polymorphic modification (m.p. 203–205°), which possesses the same ultraviolet and infrared spectrum and the same rotation. The presence of at least six C-methyl groups is indicated by Kuhn–Roth oxidation and under acid-catalyzed conditions, methymycin affords an amorphous diacetate. As demonstrated below, the two acylable hydroxyl groups are not readily oxidized and may be tertiary in nature; the extremely slow uptake of periodic acid would suggest that they are not on adjacent carbon atoms.

The pertinent ultraviolet (λ_{max}^{EtOH} 225 mμ, log ϵ 4.06) and infrared ($\lambda_{max}^{CHCl_3}$ 5.95 and 6.14 μ of nearly equal intensity in addition to 5.82 μ) bands suggest either a cyclopentenone or open chain (*e.g.*, mesityl oxide)[3] unsaturated carbonyl grouping and this is confirmed by the ultraviolet absorption maximum[4] ($\lambda_{max}^{CHCl_3}$ 371 mμ) of its derived 2,4-dinitrophenylhydrazone. The presence of a dimethylamino grouping, indicated by the analytical results,[2] has been confirmed by the isolation of dimethylamine from the alkali fusion of methymycin.

Catalytic hydrogenation of methymycin with palladium or platinum catalysts followed by careful chromatography yielded at least three products. The initially eluted material, which could not be obtained crystalline, clearly represented a hydrogenolysis product because of its relatively non-polar character (ease of elution). The chief fraction, approximately of the same polarity as methymycin, was represented by dihydromethymycin, which had already been obtained earlier[2] by Donin, *et al.* A considerably more polar substance, which by analysis appeared to contain one additional oxygen function, was also formed but it has not been investigated further.

Dihydromethymycin shows only a single carbonyl band in the infrared (5.79 μ) due to the ester or lactone (see below) originally ($\lambda_{max}^{CHCl_3}$ 5.82 μ) present in methymycin and the saturated carbonyl group produced by reduction of the double bond. The infrared bands (5.95 and 6.14 μ) and the ultraviolet absorption maximum (at 225 mμ) associated with the α,β-unsaturated ketone are now absent. The extinction in the 200–220 mμ region is typical[5] of a saturated tertiary amine and dihydromethymycin as well as methymycin form only N-oxides[6] with perbenzoic acid. From the absence of additional unsaturation it follows that methymycin contains two rings.

Hydrogenation of methymycin in glacial acetic acid afforded two well-defined products, tetrahydromethymycin and tetrahydrodesoxymethymycin.[7] The former could also be obtained by sodium borohydride reduction of dihydromethymycin, thus demonstrating that the formation of tetrahydromethymycin involved the hydrogenation of the double bond and of the carbonyl group. Dihydromethymycin could be transformed to tetrahydrodesoxymethymycin by hydrogenation in glacial acetic acid and since the latter is unaffected by sodium borohydride and hence no longer contains the carbonyl group, this would indicate that the oxygen atom removed by hydrogenolysis is not the one present originally as a carbonyl group.[8]

Reduction of methymycin with sodium borohydride is complicated and leads to at least two isomers, which are most likely not epimeric at the newly formed hydroxyl group since the two substances differ enormously in their polarity on an alumina column. It would appear that reduction elsewhere in the molecule may also have occurred[9]

(1) (a) Squibb Postdoctorate Research Fellow, 1954–1955; (b) Squibb Postdoctorate Research Fellow, 1952–1954.

(2) M. N. Donin, J. Pagano, J. D. Dutcher and C. M. McKee, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, N. Y., pp. 179–185.

(3) The extremely strong double bond band at 6.14 μ is typical of cyclopentenones (*cf.* C. F. Hiskey, R. Hirschmann and N. L. Wendler, THIS JOURNAL, **75**, 5135 (1953)) or open chain ketones such as mesityl oxide (private communication from Prof. R. B. Woodward, Harvard University).

(4) *Cf.* J. D. Roberts and C. Green, THIS JOURNAL, **68**, 214 (1946); E. A. Braude and E. R. H. Jones, *J. Chem. Soc.*, 498 (1945).

(5) N. J. Nelson and D. M. Locke, THIS JOURNAL, **77**, 437 (1955).

(6) *Cf.* C. C. J. Culvenor, *Revs. Pure Appl. Chem.*, **3**, 83 (1953).

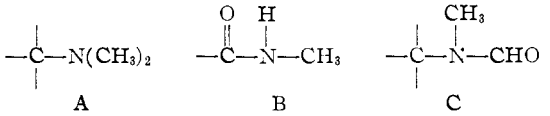
(7) Apparently the same substance has already been obtained by Donin, *et al.* (ref. 2), who formulated it as a dihydrodesoxy derivative ($C_{25}H_{45}NO_6$). Their as well as our analytical results are in good agreement with the H_{45} formula, but the evidence presented in the present paper would require H_{47}.

(8) Tetrahydromethymycin, which is stable to further reduction in an acid medium, must still possess the original carbonyl oxygen (in the form of the derived alcohol) since it is also produced by sodium borohydride reduction of dihydromethymycin. Consequently, the hydrogenolysis must have occurred in some other portion of the molecule, which did not require allylic activation since dihydromethymycin also leads to the hydrogenolysis product.

(9) In which case one of the products should possess at least two more hydrogen atoms.

but the important fact to be deduced from this experiment is that the infrared band at 5.82 μ, found in methymycin, cannot be due to another keto group, but rather must be a lactone or ester. Unambiguous proof for this is presented below in the amide series by re-oxidation of the newly formed alcohol function to the original carbonyl group. As is to be anticipated, all infrared bands in the carbonyl region disappear upon reduction with lithium aluminum hydride of methymycin or of its sodium borohydride reduction product. Neither the water-soluble derivative nor its ozonization products could be characterized, but the treatment with lithium aluminum hydride did not seem to have resulted in the loss of any appreciable fragment. This observation, coupled with the fact that methymycin gives a nearly negative Zeisel value[2] and cannot be recovered after warming with 5% methanolic potassium hydroxide, might suggest that the carbonyl band in the 5.8 μ region is due to a lactone rather than an ester.

Of the various oxidizing agents examined, the action of the chromium trioxide–pyridine complex[10] on methymycin appeared most promising and was studied in detail. The crystalline, high-melting product isolated in *ca.* 35% yield proved to be neutral and in addition to the usual carbonyl bands of methymycin, there was noticeable now a strong band at 6.0 μ attributable to an amide linkage. Consequently, one of the methylene groups connected to the nitrogen atom must have suffered oxidation and analysis indicated the presence of only one N-methyl group in the product. Since methymycin is known to possess the dimethylamino moiety (A), two possibilities (B or C) present themselves.



The analytical results favor C, the net result being the oxidation of one of the methyl groups without loss of carbon. Structure C is supported by the infrared spectrum, which shows no band in the 6.5 μ region where the N–H deformation of secondary amides is usually found[11] and alkali fusion produced methylamine (compatible with either B or C). That none of the other oxygen functions in the methymycin molecule are implicated in the change A → C was demonstrated as follows. The amide still shows the relevant infrared and ultraviolet maxima associated with the unsaturated ketone and lactone (or ester) moieties. Chromium trioxide–pyridine oxidation of methymycin diacetate produced an amide, identical with the acid-catalyzed acetylation product[12] of the free amide and the latter was also formed in the mild basic hydrolysis of the amide diacetate. This cycle demonstrates that the acylable hydroxyl groups are resistant to chromium trioxide–pyridine oxidation, which led to the earlier mentioned, tentative suggestion that they are tertiary; further support is presented by the fact that the amide is recovered unchanged after treatment with chromium trioxide in acetone–sulfuric acid.[13] The possibility exists, however, that the hydroxyl group(s) may be secondary and that its resistance toward oxidizing agents is caused by other substituents. The amide, together with unchanged methymycin, were the only identifiable products of ozonolysis and together accounted for over 70% of the starting material. Traces of formaldehyde, isolated as the 2,4-dinitrophenylhydrazone, were obtained in a more drastic ozonolysis but it is not certain whether this is of any structural significance.

The presence of the unaltered α,β-unsaturated carbonyl function in the amide was already demonstrated by the infrared and ultraviolet data but additional chemical evidence could be adduced. Sodium borohydride reduction of the amide yielded a dihydro derivative, which lacked the ultraviolet absorption maximum at 225 mμ and showed only two infrared bands at 5.80 μ (lactone or ester) and 6.0 μ (amide), but which could be re-oxidized in excellent yield to the original amide of methymycin.

Methymycin appears to resemble in many of its properties a group of antibiotics such as pikromycin ($C_{25}H_{43}NO_7$),[14] narbomycin ($C_{28}H_{47}NO_7$),[15] magnamycin[16] and erythromycin,[17] (the latter two yielding hydrolysis products ($C_{29}H_{47}NO_{12}$ and $C_{29}H_{49}NO_8$) which also place them into this series). The presence of a lactone grouping in pikromycin[14] and erythromycin[18] already has been demonstrated and if methymycin falls into the same general pattern, then this would afford additional presumptive evidence in favor of a lactone rather than an ester function in methymycin. Two of the seven oxygen atoms in methymycin, for which no evidence has been presented so far, form part of the same type of acetal linkage[19] as has been found in some of the above mentioned antibiotics.

Acknowledgment.—We are greatly indebted to the Squibb Institute for Medical Research for generous assistance in the form of fellowships and material. We should also like to acknowledge the benefit of stimulating discussions with Drs. M. N. Donin, J. D. Dutcher, J. Vandeputte, O. Wintersteiner and F. Y. Wiselogle.

(10) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(11) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley & Sons, Inc., New York, N. Y., 1954, chapter 12.

(12) Since acid-catalyzed acetylation seemed to be the method of choice in the case of methymycin and its transformation products, the possibility exists that one of the two acetylable hydroxyl groups was not present as such in methymycin, but rather was liberated during the acetylation reaction. This appears unlikely in view of the fact that methymycin amide is regenerated in the mild alkaline hydrolysis of its diacetate.

(13) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(14) *Cf.* H. Brockmann and R. Oster, *Naturwiss.*, **42**, 155 (1955).

(15) R. Corbaz, L. Ettlinger, E. Gaumann, W. Keller, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, R. Eusser and H. Zahner, *Helv. Chim. Acta*, **38**, 935 (1955).

(16) R. L. Wagner, F. A. Hochstein, K. Murai, N. Messina and P. P. Regna, THIS JOURNAL, **75**, 4684 (1953).

(17) E. H. Flynn, M. V. Sigal, P. F. Wiley and K. Gerzon, *ibid.*, **67**, 3121 (1954).

(18) P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal and U. C. Quarck, *ibid.*, **77**, 3677 (1955).

(19) C. Djerassi, A. Bowers, R. Hodges and B. Riniker, *ibid.*, **78**, 1733 (1955).

Experimental[20]

Methymycin.—A sample of the antibiotic[2] which had been purified by chromatography crystallized from ethyl acetate as needles, m.p. 203–205°, $[\alpha]_D$ +79°, λ_{max}^{EtOH} 225 mμ, log ε 4.06, $\lambda_{max}^{CHCl_3}$ 2.93, 5.82, 5.95 and 6.14 μ. The infrared spectrum was identical with that of the earlier described[2] form (prisms from ethanol, m.p. 195.5–197°).

Anal. Calcd. for $C_{25}H_{43}NO_7$: C, 63.94; H, 9.23; N, 2.98; 2 N-CH_3, 6.4; 6 C-CH_3, 19.2. Found: C, 63.93; H, 9.28; N, 2.84; N-CH_3, 5.83; C-CH_3, 18.3.

The 2,4-dinitrophenylhydrazone had to be prepared under carefully standardized conditions since longer heating caused decomposition. A solution of 200 mg. of methymycin in 25 cc. of ethanol was refluxed for 10 minutes with a solution of 100 mg. of 2,4-dinitrophenylhydrazine in 10 cc. of ethanol containing a few drops of concd. hydrochloric acid. The cooled reaction mixture was made alkaline, the product extracted with ether and chromatographed in benzene solution on 20 g. of deactivated alumina. Removal of excess reagent with benzene followed by elution with 300 cc. of 4:1 benzene–ether yielded a fraction (176 mg.) which crystallized as orange needles from ethyl acetate–hexane; m.p. 205–207°, $\lambda_{max}^{CHCl_3}$ 371 mμ, log ε 4.34.

Anal. Calcd. for $C_{31}H_{47}N_5O_{10}$: C, 57.30; H, 7.29; N, 10.78. Found: C, 57.45; H, 6.93; N, 11.02.

The N-oxide was formed when 56 mg. of methymycin in 15 cc. of chloroform was treated at room temperature for 4 hours with 1.2 equivalents of perbenzoic acid. After washing successively with sodium iodide, sodium thiosulfate and finally potassium hydroxide solution, the solvent was removed and the residue crystallized from acetone; m.p. 206–207°, $[\alpha]_D$ +65°, λ_{max}^{EtOH} 224 mμ, log ε 4.06, λ_{max}^{nujol} 5.78, 5.93 and 6.13 μ.

Anal. Calcd. for $C_{25}H_{43}NO_8$: C, 61.83; H, 8.93; N, 2.88. Found: C, 61.90; H, 9.26; N, 3.03.

In a quantitative experiment, methymycin consumed one equivalent of peracid in less than 30 minutes at 0°.

Acetylation of Methymycin.—A solution of 200 mg. of methymycin in 5 cc. of acetic anhydride was stirred at room temperature for 26 hours with 80 mg. of *p*-toluenesulfonic acid monohydrate and then diluted with water. After making alkaline with bicarbonate solution, the amorphous precipitate was extracted with ether. Since all attempts at crystallization failed, even after chromatography, a sample was evaporatively distilled at 165° and 0.01 mm.; $\lambda_{max}^{CHCl_3}$ 5.80, 5.95, 6.15 and 8.05 μ.

Anal. Calcd. for $C_{29}H_{47}NO_9$: C, 62.90; H, 8.55; acetyl, 15.6. Found: C, 62.77; H, 8.46; acetyl, 16.1.

Dihydromethymycin.—A solution of 500 mg. of methymycin was hydrogenated in 50 cc. of absolute ethanol with 100 mg. of 5% palladized charcoal catalyst for 5 hours at which time the hydrogen uptake had ceased. The total product was chromatographed in benzene solution on 50 g. of deactivated alumina. Elution with 9:1 benzene–ether yielded 190 mg. of an oil, which could not be crystallized even after repeated chromatography and which may represent one or more hydrogenolysis products. Further elution with 4:1 benzene–ether followed by recrystallization from ethyl acetate furnished 207 mg. of needles of dihydromethymycin, m.p. 193–195°, $[\alpha]_D$ +28°, $\lambda_{max}^{CHCl_3}$ 5.79 mμ, no selective ultraviolet absorption but at 204, 210 and 216 mμ, the substance exhibited[21] log ε 3.38, 3.17 and 2.94 which indicates[5] the absence of any additional double bonds.

Anal. Calcd. for $C_{25}H_{45}NO_7$: C, 63.66; H, 9.62; N, 2.97. Found: C, 64.03; H, 9.42; N, 2.93.

Elution with ether yielded 92 mg. which after rechromatography and crystallization from methanol–ethyl acetate was obtained as needles, m.p. 223–226°, $[\alpha]_D$ +33°, λ_{max}^{CHCl} 5.80 μ. The product was not investigated further.

Anal. Found: C, 62.31; H, 9.99; N, 2.85.

Substantially the same results were obtained when platinum oxide was substituted for the palladium catalyst.

Dihydromethymycin N-oxide, prepared with perbenzoic acid, was recrystallized from ethyl acetate, m.p. 193–195° dec., $[\alpha]_D$ +13°.

Anal. Calcd. for $C_{25}H_{45}NO_8$: C, 61.57; H, 9.30. Found: C, 61.18; H, 9.02.

Sodium Borohydride Reduction of Dihydromethymycin.—Sodium borohydride (25 mg.) in 3 cc. of dil. dioxane (1:1) was added to 158 mg. of dihydromethymycin in 20 cc. of the same solvent and the mixture was kept at 20° for 18 hours. Addition of water and isolation with chloroform gave a product which was adsorbed from benzene solution on 10 g. of deactivated alumina and eluted with ether–methanol (9:1).

Tetrahydromethymycin separated from acetone–petroleum ether as either long needles, m.p. 169–174°, or as microcrystals, m.p. 90–92°, $[\alpha]_D$ +32°, $\lambda_{max}^{CHCl_3}$ 5.80 μ.

Anal. Calcd. for $C_{25}H_{47}NO_7$: C, 63.35; H, 10.00; N, 2.95. Found: C, 63.10; H, 9.85; N, 3.03.

The substance was recovered unchanged after attempted hydrogenolysis with platinum oxide in acetic acid.

Tetrahydrodesoxymethymycin and Tetrahydromethymycin.—When the hydrogenation of 460 mg. of methymycin was carried out in glacial acetic acid with 100 mg. of platinum oxide for 6 hours, there was obtained a gum which was chromatographed on 50 g. of deactivated alumina. Elution with benzene followed by recrystallization from hexane produced 250 mg. of **tetrahydrodesoxymethymycin**, m.p. 170–173°, raised by further recrystallization to 173–175°, $[\alpha]_D$ +35°, $\lambda_{max}^{CHCl_3}$ 5.78 μ, no selective ultraviolet absorption maximum (204, 210 and 216 mμ, log ε 3.36, 3.17 and 2.96.[20,21] Perbenzoic acid consumption, equivalent to 0.98 equivalent (N-oxide formation) was complete in 25 min.

Anal. Calcd. for $C_{25}H_{47}NO_6$: C, 65.61; H, 10.35. Calcd.[7] for $C_{25}H_{45}NO_6$: C, 65.90; H, 9.96. Found: C, 66.05, 66.01; H, 9.75, 9.60.

Tetrahydrodesoxymethymycin was obtained in nearly 90% yield when the hydrogenation was carried out as above starting with dihydromethymycin. In contrast to the latter, tetrahydrodesoxymethymycin is recovered unchanged after treatment for 48 hours with sodium borohydride.

Further elution of the original (methymycin) hydrogenation product with ether–methanol (4:1) afforded 125 mg. of **tetrahydromethymycin**, m.p. 90–93°, $[\alpha]_D$ +30°, which proved to be identical in all respects with the lower melting modification of the specimen described above from the sodium borohydride reduction of dihydromethymycin.

Sodium Borohydride Reduction of Methymycin.—The reduction of 500 mg. of methymycin was carried out as described above for dihydromethymycin and the product was chromatographed on 50 g. of deactivated alumina. Elution with benzene–ether (1:1) led to 302 mg., m.p. 163–180°, raised by repeated recrystallization from acetone–hexane to m.p. 194–197°: the substance also exists in a lower melting, microcrystalline form, m.p. 98–101°, $[\alpha]_D$ + 68°, $\lambda_{max}^{CHCl_3}$ 5.79 μ.

Anal. Calcd. for $C_{25}H_{45}NO_7$: C, 63.66; H, 9.62; N, 2.97. Found: C, 64.01; H, 9.50; N, 2.92.

Further elution with ether–methanol (9:1) yielded 150 mg. which after several crystallizations from acetone–hexane gave microcrystals with m.p. 93–100°, $[\alpha]_D$ +40°, $\lambda_{max}^{CHCl_3}$ 5.79 μ, no selective ultraviolet absorption.

Anal. Calcd. for $C_{25}H_{45}NO_7$: C, 63.66; H, 9.62; N, 2.97. Found: C, 63.88; H, 9.37; N, 3.34.

Chromium Trioxide–Pyridine Oxidation of Methymycin.—Methymycin (1.0 g.) in 15 cc. of pyridine was added to a mixture of 1.5 g. of chromium trioxide in 15 cc. of pyridine and kept at room temperature overnight. Chloroform was added and the brown precipitate was washed several times with the same solvent. The combined chloroform solutions were washed with dilute hydrochloric acid and dilute alkali, dried, concentrated and filtered through a small column (7.0 g.) of deactivated alumina. Evaporation of the chloroform and trituration with ethyl acetate furnished 335 mg. of **methymycin amide**, m.p. 246–251°, raised by recrystalli-

(20) Melting points were determined on the Kofler block. Unless noted otherwise, ultraviolet absorption spectra were measured in absolute ethanol and rotations and infrared spectra in chloroform solution. We are indebted to Mrs. Dolores Phillips for the spectral determinations and to Mr. Joseph F. Alicino (Squibb Institute for Medical Research) for the microanalyses. "Deactivated Alumina" refers to Alcoa alumina which has been shaken (until homogeneous) with petroleum ether containing 10% of its weight of 10% acetic acid.

(21) We are indebted to Dr. A. Fletcher, University of Manchester, for these measurements which were carried out with a Unicam SP 50 spectrophotometer.

zation from ether–hexane to 255–256°, $[\alpha]D$ +65°, λ_{max}^{EtOH} 222 mμ, log ε 3.97, $\lambda_{max}^{CHCl_3}$ 5.79, 5.95 (inflection), 6.01 and 6.12 μ.

Anal. Calcd. for $C_{25}H_{41}NO_8$: C, 62.09; H, 8.55; N, 2.90; N-CH_3, 3.12; 6 C-CH_3, 18.62. Found: C, 61.47; H, 8.32; N, 3.10; N-CH_3, 3.40; C-CH_3, 18.14.

The resistance of the hydroxyl groups to further oxidation was demonstrated as follows. The amide (100 mg.) in 20 cc. of acetone was treated with a large excess of 8 *N* chromium trioxide reagent.[13] After the first few drops of reagent had been added, the solution remained orange. Water was added after 5 minutes at room temperature and the amide was recovered in quantitative yield by extraction with chloroform.

A sample (230 mg.) of the amide was mixed with 2 g. of powdered potassium hydroxide and heated in a Wood metal-bath, raising the temperature over a period of 15 minutes from 140–240°. The liberated gas was passed into 10 cc. of 0.1 *N* hydrochloric acid and after an additional 5 minutes at 240°, the acid solution was washed with ether, evaporated to dryness and crystallized from methanol–ethyl acetate. The methylamine hydrochloride (22.5 mg., 65%) showed m.p. 228–232°, undepressed upon admixture with an authentic specimen.

Anal. Calcd. for CH_6ClN: C, 17.76; H, 8.95. Found: C, 18.03; H, 9.22.

Chromium Trioxide–Pyridine Oxidation of Methymycin Diacetate.—The oxidation of 150 mg. of methymycin diacetate was carried out as above and furnished 80 mg. of amide diacetate, m.p. 120–125°. The analytical sample exhibited m.p. 127–129° (after recrystallization from ether–hexane), $[\alpha]D$ +119°, λ_{max}^{EtOH} 223 mμ, log ε 3.96. The identical product was obtained when a sample of the amide was acetylated by the acetic anhydride–*p*-toluenesulfonic acid procedure employed for methymycin itself.

Anal. Calcd. for $C_{29}H_{45}NO_{10}$: C, 61.36; H, 7.99; N, 2.47; N-CH_3, 2.64. Found: C, 60.71; H, 8.02; N, 2.47; N-CH_3, 2.71.

Hydrolysis of the diacetate (240 mg.) was accomplished by keeping it with 400 mg. of potassium carbonate in 5 cc. of methanol and 2 cc. of water for 48 hours at room temperature. Dilution with water, isolation with ether and recrystallization of the product (120 mg.) from chloroform–ether led to colorless needles of the amide, m.p. 255–256°, undepressed upon admixture with a specimen prepared by direct oxidation of methymycin. The infrared spectra were identical.

Sodium Borohydride Reduction of Methymycin Amide.—The amide (410 mg.) in 30 cc. of dioxane was treated for 1.5 hours at 20° with 50 mg. of sodium borohydride dissolved in 5 cc. of dilute dioxane. Dilution with water and isolation with chloroform afforded a product (400 mg.) which separated as an amorphous solid from benzene–hexane, m.p. 134–138°, $[\alpha]D$ +60°, $\lambda_{max}^{CHCl_3}$ 5.80 and 6.00 μ, no selective absorption in the ultraviolet.

Anal. Calcd. for $C_{25}H_{43}NO_8$: C, 61.83; H, 8.93; N, 2.88. Found: C, 61.86; H, 9.06; N, 3.11.

The above reduction product (100 mg.) in 100 cc. of acetone was treated with an excess of the 8 *N* chromium trioxide reagent[13] at room temperature for 3 minutes. The crude product, 93 mg., m.p. 241–247°, after recrystallization from ethyl acetate melted at 251–253°. Identity with methymycin amide was established by mixture melting point determination and infrared comparison.

Miscellaneous Reactions of Methymycin. (a) **Ozonolysis.**—A slow stream of ozonized oxygen was passed for 1 hour at room temperature through a solution of 750 mg. of methymycin in 50 cc. of ethyl acetate. The solution was washed successively with water, dilute hydrochloric acid, dilute alkali and finally water. Evaporation of the dried ethyl acetate solution furnished 100 mg. of methymycin amide (m.p. 242–250°) raised to m.p. 252–254° after further recrystallization, $[\alpha]D$ +65°. The combined acid washings were made alkaline, whereupon 435 mg. of methymycin (m.p. 190–195°) could be recovered by extraction with chloroform.

Evaporation of the initial water washings of the ethyl acetate extract afforded 40 mg. of a light brown, amorphous powder (m.p. *ca.* 140–150°) which was only soluble in water or alcohol. This substance has not been obtained pure and no further work has been carried out with it.

In an attempt to isolate any volatile carbonyl compounds which may be formed, the ozonolysis was repeated by allowing it to proceed for 3 hours and after the addition of ferrous sulfate solution, the reaction mixture was steam distilled into a solution of 2,4-dinitrophenylhydrazine. A total of 20 mg. of formaldehyde 2,4-dinitrophenylhydrazone (m.p. 158–166°) could be obtained after chromatography, but it is not certain whether this is of any structural significance.

(b) **Alkali Fusion of Methymycin.**—Methymycin (200 mg.) was fused with potassium hydroxide in the manner indicated above for the derived amide. A total of 32.5 mg. (85%) of dimethylamine hydrochloride, m.p. 168–170°, picrate m.p. 159–161°, was isolated.

(c) **Periodic Acid Oxidation.**—Over 50% of methymycin was recovered when the antibiotic was treated with an excess of periodic acid in aqueous solution for 8 days and no volatile carbonyl fragments were encountered (distillation into 2,4-dinitrophenylhydrazine solution followed by chromatography). In a quantitative experiment, a slow uptake (*ca.* 2 equivalents in 2 weeks) was observed which is probably due to side reactions involving the basic function. Practically no periodic acid was consumed during the first two days.

DETROIT, MICHIGAN