[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY]

# The Structure of the Antibiotic Methymycin[1]

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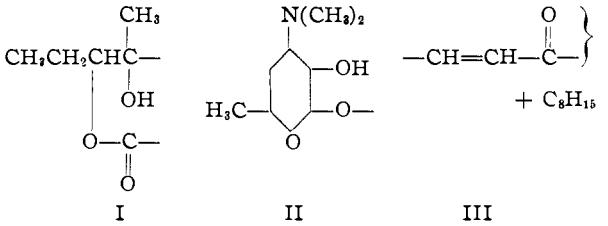
Methymycin ($C_{25}H_{43}NO_7$) is shown to contain a twelve-membered lactone ring and evidence for the complete structure XIV of the antibiotic is presented.

Methymycin[3] belongs to a group of antibiotics which during the past few years has been shown to contain a great number of representatives,[4] at least two of which, erythromycin[5] and carbomycin (magnamycin),[6] have found clinical application. Partial structures have been reported for erythromycin[5] and for pikromycin[7] but a complete constitution has not yet been presented for any antibiotic of this class. We should now like to describe salient experiments which lead to structure XIV[1] as the complete expression for methymycin.

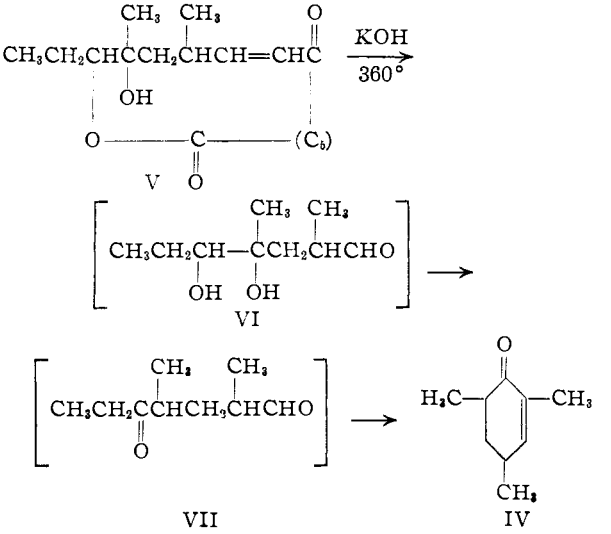
The past structural information[8,9] concerning methymycin can be summarized as



I II III

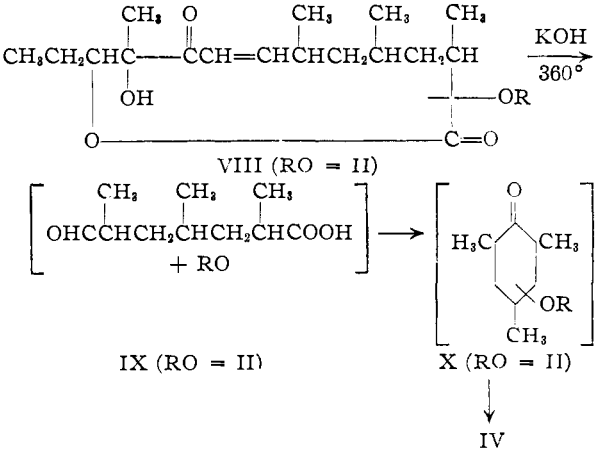
The unknown $C_8H_{15}$ fragment must contain the termination point of the lactone ring (I), the site of attachment of the desosamine (II) moiety and three C-methyl groups since a total of six are present in methymycin. One of the most important degradation products of methymycin is 2,4,6-trimethylcyclohex-2-en-1-one (IV), formed[9] in the alkali fusion of methymycin, since its nine carbon atoms must overlap either in part or wholly with the unknown $C_8H_{15}$ fragment. Trimethylcyclohexenone (IV) is not present[8,9] *per se* in methymycin, but rather must have been formed as a result of cleavage and cyclization processes in the alkaline medium. It seems profitable to consider these reactions in more detail since they have a direct bearing on the structural argument developed in this paper.

One conceivable route to trimethylcyclohexenone (IV) could have originated from partial structure V and proceeded *via* VI and VII, the potential aldehyde function being produced by a reverse aldol condensation involving the $\alpha,\beta$-unsaturated ketone grouping III known to be present in methymycin. Fragment V coupled with desosamine (II), would account for 20 out of the 25 carbon atoms of methymycin.



V VI VII IV

Alternatively, trimethylcyclohexenone (IV) could have arisen from a type of Claisen or Dieckmann cyclization involving IX[10] and X, the potential aldehyde[10] again being formed by a reverse aldol reaction involving the unsaturated ketone III. In that event, attachment of IX to fragment I leads to VIII, which would represent a complete structure for methymycin except for the precise location of the desosamine (II) portion (RO in VIII). This in



VIII (RO = II) IX (RO = II) X (RO = II) IV

(1) For preliminary communication see C. Djerassi and J. A. Zderic, THIS JOURNAL, **78**, 2907 (1956).

(2) Squibb Postdoctorate Research Fellow, 1955–1956.

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

(4) Leading references are given by R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, A. Wettstein and H. Zähner, *Helv. Chim. Acta*, **39**, 304 (1956).

(5) *Cf.* P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal and U. C. Quarck, THIS JOURNAL, **77**, 3677 (1955).

(6) R. L. Wagner, F. A. Hochstein, K. Murai, N. Messia and P. P. Regna, *ibid.*, **75**, 4684 (1953).

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

(8) C. Djerassi, A. Bowers and H. N. Khastgir, THIS JOURNAL, **78**, 1729 (1956).

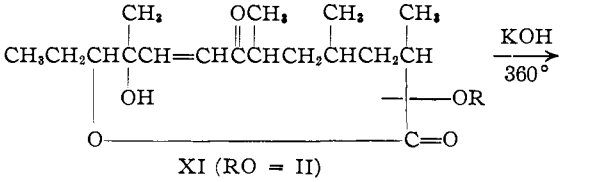
(9) C. Djerassi, A. Bowers, R. Hodges and B. Riniker, *ibid.*, **78**, 1733 (1956).

(10) The corresponding acid, produced by oxidation of the aldehyde (*e.g.*, *via* Cannizarro reaction) in the 360° alkali fusion, could be considered its equivalent.

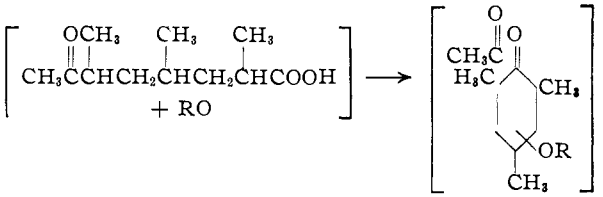
turn would imply that all of the hitherto unidentified carbon atoms ($C_8H_{15}$) of methymycin are represented in trimethylcyclohexenone (IV).

Structure VIII for methymycin is excluded, however, by the observation[9] that the antibiotic[11] after reduction with lithium aluminum hydride followed by treatment with periodic acid consumes only one mole of reagent and yields propionaldehyde. If VIII were correct, then two equivalents of periodic acid would have reacted and both propionaldehyde and acetic acid would have been formed.

A slight variation of the above cleavage and cyclization scheme leads to a third structural alternative for methymycin which in the sequel is shown to be the correct one. If the $\alpha,\beta$-unsaturated ketone III is attached to fragment I in a reverse manner to that shown in VIII, then structure XI results for methymycin. Reverse aldol condensation and lactone opening would produce XII which could cyclize to XIII; $\beta$-diketone cleavage and loss of the desosamine fragment (RO in XIII) would then furnish trimethylcyclohexenone (IV).



XI (RO = II)

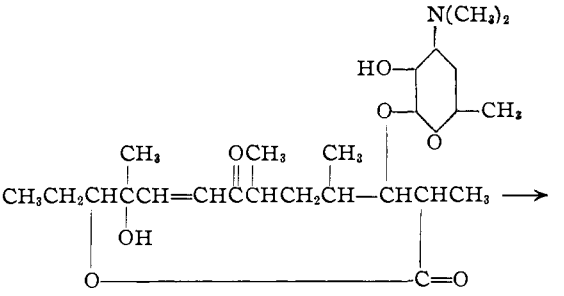


XII (RO = II) XIII (RO = II)

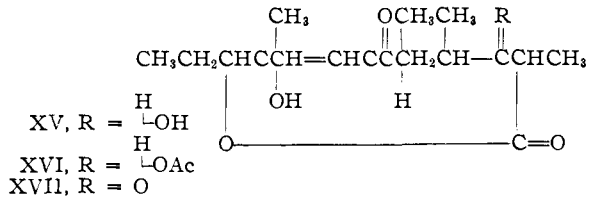
IV

It was felt that an experimental verification of the two most likely structural alternatives V and XI could best be accomplished on a methymycin cleavage product—methynolide—which would still have intact all of the pertinent structural features of the antibiotic except for the glycosidic linkage and consequent loss of desosamine (II). The presence of desosamine (II) had been demonstrated[9] earlier by hydrolysis of methymycin with hydrochloric acid but this reaction was accompanied by a more deep-seated alteration in the rest of the molecule since the characteristic ultraviolet absorption maximum near 225 m$\mu$, due to the $\alpha,\beta$-unsaturated ketone grouping III, had disappeared. This also applied to methanol–sulfuric acid, but when the cleavage of methymycin was carried out under carefully controlled conditions with aqueous sulfuric acid it was possible to isolate the unchanged, desosamine-free fragment "methynolide." For the sake of convenience, all subsequent discussion will be based on the correct structure XV of this key degradation product of methymycin (XIV).

(11) This statement applies also to methynolide (XV) as shown in the Experimental section of this paper.



XIV



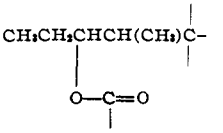
XV, R = $\begin{matrix}H\\ \llcorner OH\end{matrix}$
XVI, R = $\begin{matrix}H\\ \llcorner OAc\end{matrix}$
XVII, R = O

That the acid cleavage had not caused any structural changes in methynolide, other than removing desosamine (II), was demonstrated by the analytical results and by the presence of all of the relevant ultraviolet and infrared maxima associated with the various functional groups of methymycin (XIV). Lithium aluminum hydride reduction followed by oxidation with periodic acid yielded—just as with methymycin[9]—propionaldehyde. Mild acetylation with pyridine–acetic anhydride led to methynolide monoacetate (XVI) while chromium trioxide oxidation (under conditions where the hydroxyl groups of methymycin (XIV) were not attacked[8]) furnished the corresponding ketone[12] dehydromethynolide (XVII). It is clear, therefore, that this reactive hydroxyl group of methynolide XV represents the point of attachment[13] of the desosamine fragment (II) and this was shown to be $\beta$ to the latent carboxyl group since saponification of dehydromethynolide (XVII) followed by acidification yielded nearly 60% of carbon dioxide, thus requiring a $\beta$-keto ester fragment in XVII.

Mild permanganate oxidation of methynolide (XV) yielded a complex mixture from which three crystalline products (XVIII, XIX, XXa) could be

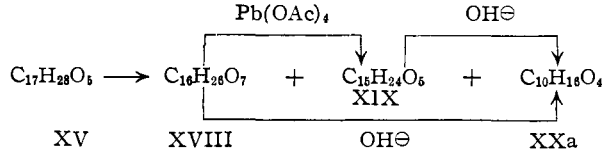
(12) *A priori*, the newly formed carbonyl group could also have been an aldehyde and it was not a simple matter to differentiate between these two possibilities. The infrared spectrum was complicated because of the other carbonyl bands and the nuclear magnetic resonance spectrum (kindly determined by Dr. Aksel A. Bothner-By, Harvard University) suggested an aldehyde rather than a ketone. While a Schiff test was negative, positive Fehling and Tollens reactions were observed even with the precursor methynolide (XV), possibly due to generation of an aldehyde by a reverse aldol condensation in the alkaline medium or to the presence of a *trans*-annular ketol. An unambiguous decision in favor of a ketone was obtained by the isolation of the lactonic acid XXa and to a certain extent also by the formation of the spiroketal XXII. In the latter case an aldehyde beta to the carboxyl group would have implied the formation of an eight-membered oxide in the reaction XV → XXII, which would have been rather unlikely.

(13) The alternative—attachment of desosamine *via* the tertiary hydroxyl group of methynolide (XV)—is excluded by the oxidation evidence (*cf.* also ref. 1) and by the fact that desoxytetrahydromethymycin (ref. 8) must contain (ref. 9) the fragment

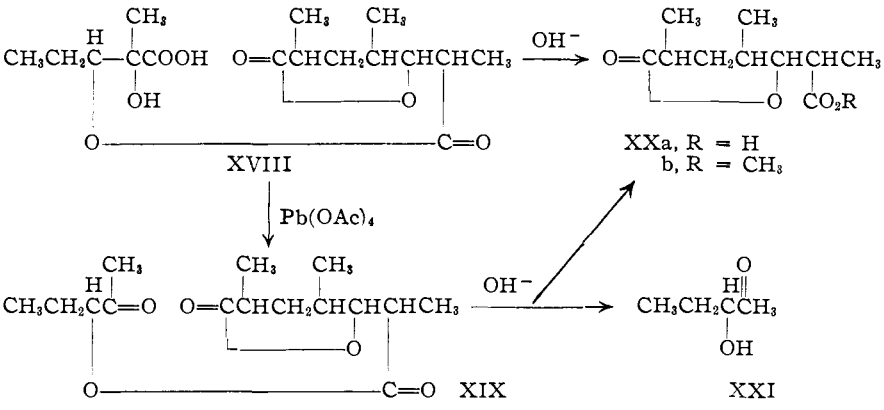


as well as desosamine (II).

separated in pure form and the relationship of these substances in terms of analytical composition[14] is




None of the three substances showed the high ultraviolet absorption maximum at 225 mμ present in methymycin (XIV) or methynolide (XV) and since the formation of two of them—XVIII and XIX—involved only the loss of one or two carbon atoms, the site of oxidative attack must have been the α,β-unsaturated ketone grouping III of the starting material XV. Although XVIII ($C_{16}H_{26}O_7$) was acidic and XIX ($C_{15}H_{24}O_5$) neutral, their close structural relationship was established when it was found that XVIII could be transformed into XIX (positive iodoform reaction) by lead tetraacetate oxidation. Furthermore, both XVIII and XIX upon alkaline hydrolysis led to a lactonic acid XXa[15] ($C_{10}H_{16}O_4$), which could also be isolated directly in the permanganate oxidation of methynolide (XV). In addition to XXa, there was formed from XIX by alkaline saponification 3-hydroxy-2-pentanone (XXI), which was isolated and identified[16] as the bis-2,4-dinitrophenylhydrazone of pentane-2,3-dione. On the basis of the earlier proposed expression XV for methynolide, the structures XVIII, XIX and XXa follow automatically for the three oxidation products and they afford an adequate explanation for the observed chemical reactions.




The structure of the lactonic acid XXa, according to the argument presented earlier for methynolide and methymycin, is derived from the same carbon sequence as is trimethylcyclohexenone (IV). Independent and welcome evidence of a degradative nature has been secured by Prelog and collaborators[17] who have isolated the same acid XXa from some related antibiotics (narbomycin and pikromycin) and have shown that the pyrolysis product of XXa after ozonolysis yields pyruvic acid and meso-α,α′-dimethylglutaric acid.

It was mentioned earlier that when the sulfuric acid cleavage of methymycin was conducted in methanolic rather than aqueous solution, the presence of the α,β-unsaturated ketone could not anymore be demonstrated by spectroscopic means. When this reaction was carried out on a larger scale, there was isolated a crystalline substance, $C_{18}H_{30}O_5$, which could also be obtained from methynolide (XV) ($C_{17}H_{28}O_5$) on similar treatment. In contrast to methynolide (XV), the new cleavage product exhibits no infrared bands corresponding to either free hydroxyl or conjugated carbonyl groups, contains no active hydrogen atoms but does possess one methoxyl group which is not present in methynolide (XV). This methoxyl group is not present as an ester since lithium aluminum hydride reduction leads without loss of carbon to a diol which still retains the methoxyl group. These observations are only consistent with structure XXIIa arising from the acid-catalyzed addition of methanol to the α,β-unsaturated ketone and concomitant spiroketal formation of the suitably situated hydroxyl groups with the ketone function.[18] The lithium aluminum hydride reduction product can now be assigned structure XXIII and it may be pertinent to mention that hydrochloric acid cleavage of methymycin appears to give an analogous spiroketal which is best represented as the chloro-spiroketal XXIIb.

The formation of the spiroketals XXII (a, b, c) represents further support[8] for the structure of methynolide (XV) insofar as the relation of the hydroxyl groups with respect to the carbonyl function is concerned. Since it has been pointed out above[13] that the glycosidic linkage with desosamine (II) must involve the secondary alcoholic function of methynolide the antibiotic methymycin can now be represented by the complete expression XIV.

Spiroketal formation is accompanied by a large levorotatory change and it was attempted to follow the course of this reaction (XV → XXIIa) in a polarimeter tube in methanol solution containing some acid. Unfortunately, the reaction did not proceed under such mild conditions (the mixture was heated in the preparative experiment), but polarimetric examination of dihydromethynolide (XXIVa) under similar conditions proceeded ex-

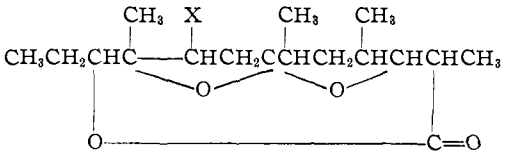
(14) It should be noted that the isolation of a $C_{10}$-fragment from the oxidation eliminated (on arithmetic grounds) partial structure V from further consideration since oxidative attack at the double bond would divide the methynolide molecule ($C_{17}$) into $C_8$ and $C_9$-portions.

(15) The presence of both lactone and carboxyl groups was demonstrated by the infrared spectrum and by titration data—immediate titration showing only one acid function while back titration from alkaline solution corresponded to two such groupings.

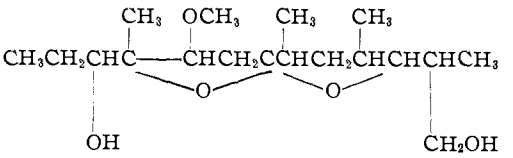
(16) We are grateful to Dr. K. Gerzon (Eli Lilly and Co.) for carrying out a comparison by means of X-ray diffraction with an authentic specimen (P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal and U. C. Quarck, THIS JOURNAL, **77**, 3676 (1955)).

(17) We should like to express our indebtedness to Prof. V. Prelog (E. T. H., Zurich) for informing us of his results prior to publication (see R. Anliker, D. Dvornik, K. Gubler, H. Heusser and V. Prelog, *Helv. Chim. Acta*, **39**, in press (1956)) and for carrying out the direct comparison.

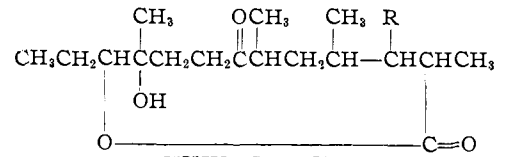
(18) A close structural analogy is provided by the steroidal sapogenins with the spiroketal side chain.



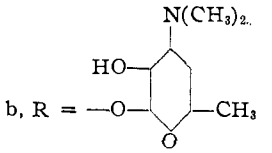
XXIIa, X = $CH_3O$
b, X = Cl
c, X = H



XXIII



XXIVa, R = OH



b, R = —O—

tremely rapidly (complete after 4 minutes at room temperature) to yield the corresponding spiroketal XXIIc. Apparently spiroketal formation is inhibited for steric reasons in the presence of the double bond and saturation (either by hydrogenation (*cf.* XXIVa) or by prior addition of methanol or hydrogen chloride) is required before this is possible. Evidently, the initial acid-catalyzed addition of methanol to methynolide (XV) does not proceed at room temperature thus accounting for the much milder conditions under which dihydromethynolide (XXIVa) undergoes cyclization.

The large rotation changes have also prompted us to examine the rotatory dispersion curves[19] of some of these compounds. Earlier work[20] has shown that even simple saturated alicyclic ketones show anomalous rotatory dispersion characterized by "maxima" and "minima" while unsaturated cyclic ketones[21] show typical fine structure in the 300–400 mμ region. As shown in Fig. 1, methymycin (XIV) and methynolide (XV) do show some fine structure although this is only indicated by inflections rather than by sharp resolution as in the earlier reported[21] alicyclic ketones with 5- or 6-membered rings. The rotatory dispersion curve of the spiroketal XXIIa is perfectly smooth with a large negative drift and is thus quite similar to the curves exhibited by steroidal sapogenins possessing the spiroketal side chain.[22] However, it was quite surprising to note that dihydromethynolide (XXIVa) or dihydromethymycin (XXIVb)[8] do not show the typical[20] anomalous dispersion of

(19) For experimental technique and introduction to subject see C. Djerassi, E. W. Foltz and A. E. Lippman, THIS JOURNAL, **77**, 4354 (1955).

(20) C. Djerassi, R. Riniker and B. Riniker, *ibid.*, **78**, 6362 (1955), and earlier papers.

(21) C. Djerassi, R. Riniker and B. Riniker, *ibid.*, **78**, 6377 (1956).

(22) C. Djerassi and R. Ehrlich, *ibid.*, **78**, 440 (1956).

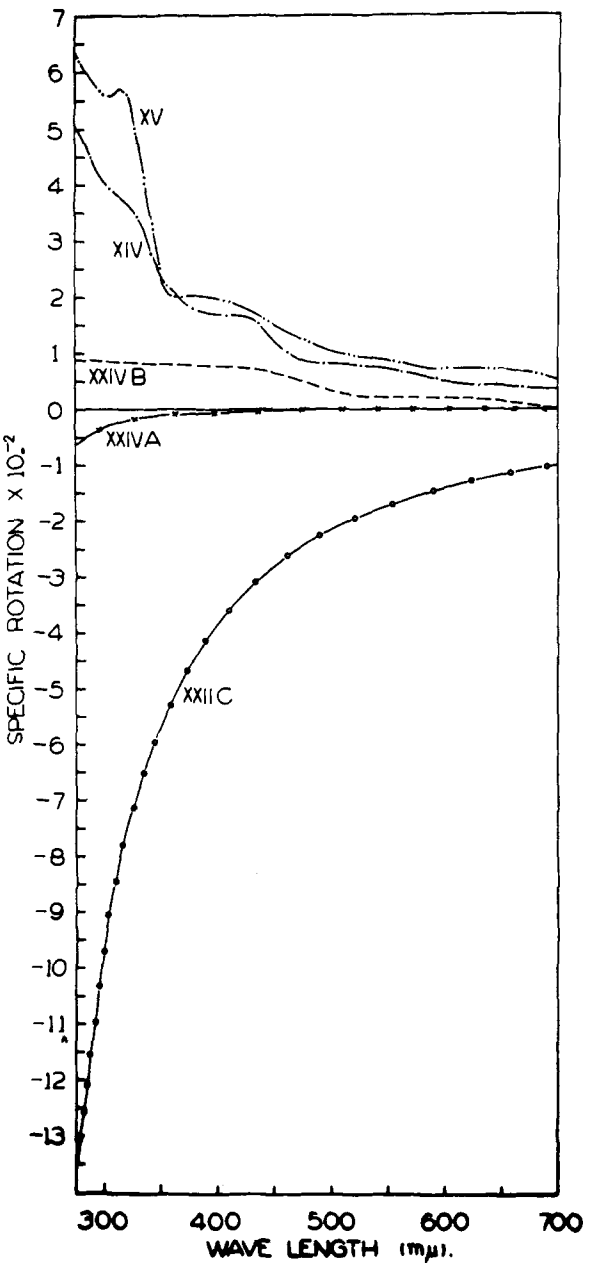



Fig. 1.—Rotatory dispersion curves (dioxane solution) of: methymycin (XIV); methynolide (XV); dihydromethynolide spiroketal (XXIIc); dihydromethynolide (XXIVa); dihydromethymycin (XXIVb).

saturated alicyclic ketones[23] and it is conceivable that these two substances (XXIVa and b) exist largely in the hemiketal[24] form for which the steric conditions are very favorable.

**Acknowledgment.**—We are indebted to the Squibb Institute for Medical Research for fellow-

(23) Admittedly no medium-size ring ketones have been studied earlier and this may be a peculiarity associated with such ring ketones (cycloheptanones still show the typical anomalous dispersion characteristics; unpublished observation from this Laboratory).

(24) The infrared bands of the saturated ketone and of the lactone occur at essentially the same position so that qualitative infrared spectroscopy will be of little value in deciding this point. No definite ultraviolet absorption maximum corresponding to a saturated ketone could be observed.

ship support and for generous supplies of methymycin. We should also like to express our grateful thanks to Mr. Joseph F. Alicino (Squibb Institute) for his outstanding microanalytical contributions.

## Experimental[25]

**Methynolide (XV).**—The cleavage of methymycin was accomplished by heating 12.0 g. of the antibiotic with 600 cc. of 5 *N* sulfuric acid under reflux for four minutes. The resulting heterogeneous mixture was immediately cooled, diluted with water, extracted with chloroform and washed with water until neutral. Drying and evaporation of the solvent left 6.45 g. of viscous oil which crystallized from ether–hexane. After trituration with hexane and one recrystallization from ether there remained 1.91 g. of nearly colorless crystals, m.p. 161–165°; these were used in all subsequent experiments. Further recrystallization afforded the analytical sample which exhibited the following properties: m.p. 163–165°, $[\alpha]_D$ +79° (chloroform), +63° (methanol); $\lambda_{max}^{EtOH}$ 225 mμ, log ε 4.03; $\lambda_{max}^{CHCl_3}$ 2.80, 2.95, 5.76, 5.91 and 6.08 μ; negative ferric chloride reaction but positive Tollens and Fehling tests on heating.[12]

*Anal.* Calcd. for $C_{17}H_{28}O_5$: C, 65.36; H, 9.03; 5 C-$CH_3$, 24.03; mol. wt., 312. Found: C, 65.17; H, 9.11; C-$CH_3$, 19.34; Rast mol. wt., 279.

A 310-mg. sample of methynolide was heated overnight in 50 cc. of tetrahydrofuran with 300 mg. of lithium aluminum hydride and then treated dropwise with a saturated aqueous solution of sodium sulfate. The inorganic salts were filtered, the solvent was removed and the residual oil was dried by azeotropic distillation with benzene. The total oil (300 mg., no infrared carbonyl bands) was treated in ethanol solution (distilled from 2,4-dinitrophenylhydrazine) with 25 cc. of 0.21 *N* periodic acid solution and the course of the reaction was followed by titration. One-half equivalent of reagent was consumed during the first hour and the uptake then became progressively slower. After 20 hours 0.89 equivalents had been consumed and after 44 hours 1.07. The reaction mixture was diluted with water and distilled into a solution of 400 mg. of 2,4-dinitrophenylhydrazine in 50 cc. of purified ethanol containing a few drops of sulfuric acid. Chromatography on 60 g. of alumina and elution with benzene afforded 140 mg. of 2,4-dinitrophenylhydrazone, the infrared spectrum of which was nearly superimposable upon that of authentic propionaldehyde dinitrophenylhydrazone. Complete purification was accomplished by repeated chromatography on bentonite–kieselguhr.[26]

**Methynolide Acetate (XVI).**—Methynolide (105 mg.) was acetylated with 1 cc. each of acetic anhydride and pyridine overnight at 0°. The crude acetate (102 mg., m.p. 194–198°) was recrystallized from ether whereupon it exhibited m.p. 198–200° (change of crystal form above 180° from needles to irregular plates), $[\alpha]_D$ +93°; $\lambda_{max}^{EtOH}$ 224 mμ, log ε 4.00.

*Anal.* Calcd. for $C_{19}H_{30}O_6$: C, 64.38; H, 8.53; acetyl, 12.13. Found: C, 64.39; H, 8.29; acetyl, 12.11.

**Dehydromethynolide (XVII).**—An ice-cold solution of 400 mg. of methynolide (XV) in 10 cc. of acetone (distilled from permanganate) was treated dropwise with 0.3 cc. of a solution of 2.67 g. of chromium trioxide in 2.3 cc. of concd. sulfuric acid diluted to 10 cc. with water. After 5 minutes, the solution was diluted with 100 cc. of water, extracted with chloroform and washed until neutral. Evaporation of the solvent left 360 mg. of crystalline solid which was chromatographed on Merck acid-washed alumina to furnish 300 mg. of crystals (m.p. *ca.* 160–170°) satisfactory for further work (infrared spectrum identical with that of analytical sample). The melting point of this compound was not very reproducible and the rotation was found to be a better criterion for characterization purposes. The analytical sample was recrystallized several times from ether–hexane; m.p. 173–179°, $[\alpha]_D$ +177°; $\lambda_{max}^{EtOH}$ 224 mμ, log ε 3.96.

*Anal.* Calcd. for $C_{17}H_{26}O_5$: C, 65.78; H, 8.44; 5 C-$CH_3$, 24.19. Found: C, 65.87; H, 8.47; C-$CH_3$, 19.95.

The ketone[12] gave negative Schiff and ferric chloride tests but positive Fehling and Tollens reactions. The presence of a β-keto ester grouping was demonstrated as follows[27]: A 50-mg. sample of dehydromethynolide was heated under reflux for one hour with excess 0.2 *N* alkali. The solution was acidified and the carbon dioxide was driven into standard base with a stream of nitrogen. Back titration of the standard base indicated the presence of 3.96 mg. of carbon dioxide corresponding to a 58% yield. Blank reagents or methynolide (XV) produced only a trace of carbon dioxide while model experiment with sodium carbonate yielded over 95% of the calculated amount of carbon dioxide.

**Permanganate Oxidation of Methynolide (XV).**—A solution of 7.5 g. of potassium permanganate in 150 cc. of water was added dropwise over a period of 1.5 hours (initial cooling to avoid rise in temperature) to 2.0 g. of methynolide dissolved in 50 cc. of acetone (distilled from permanganate). After stirring at room temperature overnight (excess reagent present), 10 g. of sodium sulfite, 7.5 cc. of concd. sulfuric acid and a large amount of sodium chloride were added and the clear solution was extracted thoroughly with ether.

The ether solution was washed with 5% sodium hydroxide, water, dried and evaporated to yield 415 mg. of viscous, yellow oil. Chromatography on 10 g. of Merck acid washed alumina, followed by elution with benzene and repeated recrystallization from hexane yielded 80 mg. of the pure **ketone XIX**, m.p. 55–56°, $[\alpha]_D$ +69°, $\lambda_{max}^{CHCl_3}$ 5.68–5.76 μ (broad), positive iodoform reaction.

*Anal.* Calcd. for $C_{15}H_{24}O_5$: C, 63.36; H, 8.51; 5 C-$CH_3$, 26.41. Found: C, 63.40; H, 8.39; C-$CH_3$, 22.51.

A sample (60 mg.) of XIX was heated under reflux for 30 minutes with 6.5 cc. of 5% sodium hydroxide solution and the mixture then was slowly steam distilled into 10 cc. of methanol containing 80 mg. of 2,4-dinitrophenylhydrazine and a few drops of concd. sulfuric acid. After standing overnight, the solution was heated for 5 hours and chilled, whereupon 18 mg. of deep red crystals of the bis-2,4-dinitrophenylhydrazone of pentane-2,3-dione crystallized. Identity with an authentic specimen[16] was established by mixture melting point determination and comparison of the X-ray diffraction patterns. The alkaline solution remaining after the steam distillation was acidified strongly with sulfuric acid, salted and then extracted exhaustively with ether. The dry ether extract was evaporated and the resulting 46 mg. of amber oil was chromatographed on 4 g. of silica gel yielding 21 mg. of straw-colored crystals after chloroform elution. Sublimation at 110° and 0.005 mm. followed by recrystallization from ether–hexane led to 15 mg. of the lactonic acid XXa, m.p. and mixture m.p. 124–127°; identity was also established by infrared comparison.

The alkaline extracts from the original permanganate oxidation mixture were acidified, heavily salted and then extracted several times with ether. After washing with salt solution, drying and evaporation, there was obtained 936 mg. of acidic oil which crystallized from ether–hexane to furnish 260 mg. of the crude **acid XVIII**. The analytical sample was recrystallized three times yielding 110 mg. of acid, m.p. 164–172°, $[\alpha]_D$ +52° (acetone); $\lambda_{max}^{KBr}$ 2.90, 5.66 and 5.78 μ.

*Anal.* Calcd. for $C_{16}H_{26}O_7$: C, 58.17; H, 7.93; 5 C-$CH_3$, 22.73; neut. equiv., 330. Found: C, 58.35; H, 7.79; C-$CH_3$, 21.01; neut. equiv. (rapid titration), 318.

The above acid XVIII (50 mg.) was left at room temperature for 20 hours with 73 mg. of lead tetraacetate and 4 cc. of glacial acetic acid.[28] The neutral product was isolated by means of ether extraction and washing with sodium hydroxide solution and was recrystallized from pentane to afford 8 mg. of the ketone XIX, m.p. 52–54°. Identity was established by mixture melting point and infrared determination. Alkaline saponification of XVIII in the manner described above for XIX yielded XXa in about 15% yield.

The mother liquors from the isolation of XVIII were combined and chromatographed on 30 g. of silica gel. Chloroform elution yielded 110 mg. of oily crystals which after several recrystallizations led to 20 mg. of the **lactonic acid XXa**, m.p. 125–127°. This acid was obtained in some-

(25) All melting points were determined on the Kofler block. We are indebted to Mrs. Dolores Phillips for the ultraviolet and infrared spectral measurements. Unless noted otherwise, rotations were measured in chloroform solution.

(26) J. A. Elvidge and M. Whalley, *Chemistry & Industry*, 589 (1955).

(27) This reaction was carried out by Mr. Joseph F. Alicino, Squibb Institute for Medical Research, New Brunswick, N. J.

(28) For typical procedure for lead tetraacetate oxidation of α-hydroxy acids see E. Rohrmann, R. G. Jones and H. A. Shonle, THIS JOURNAL, **66**, 1856 (1944).

what better yield (80 mg.) when the permanganate oxidation mixture was worked up by making initially alkaline rather than acidic. The analytical sample was prepared by recrystallization from acetone–hexane followed by sublimation at 100° and 0.005 mm., m.p. 126–128°, $[\alpha]_D$ +38°; $\lambda_{max}^{KBr}$ 3.05 (broad), 5.68 and 5.76 $\mu$.

*Anal.* Calcd. for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05; mol. wt., 200. Found: C, 59.91; H, 8.05; neut. equiv., 199 (immediate titration), 100 (back titration after standing for 2 hours in excess base).

Methylation of XXa with ethereal diazomethane for 5 minutes followed by recrystallization from pentane afforded the corresponding **methyl ester XXb**, m.p. 79–81°.

*Anal.* Calcd. for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47; $OCH_3$, 14.40; 2 C-$CH_3$, 14.01; 3 C-$CH_3$, 21.02; mol. wt., 214. Found: C, 61.89; H, 8.29; $OCH_3$, 14.39; C-$CH_3$, 17.81; mol. wt. (Rast), 211.

**Methoxydihydromethynolide Spiroketal (XXIIa).** (a) **From Methymycin.**—A solution of 20 g. of methymycin (XIV) and 60 cc. of concd. sulfuric acid in 1 l. of methanol was heated under reflux for 5 hours and then left at room temperature overnight. Dilution with water, extraction with ether and washing with dilute acid, dilute base and water followed by drying and evaporation gave 6.91 g. of neutral oil. This material was first chromatographed on 210 g. of deactivated (10% by weight of 10% acetic acid[3]) alumina and the viscous oil (5.6 g.) eluted with hexane–benzene (1:1) was rechromatographed three times on Merck acid-washed alumina. The hexane and hexane-benzene (9:1) eluates crystallized spontaneously and amounted to a total of 2.25 g. of spiroketal XXIIa. The substance is very soluble in all common organic solvents and the analytical sample was obtained by slow crystallization from dilute methanol; m.p. 79–81°, $[\alpha]_D$ −68°; $\lambda_{max}^{CHCl_3}$ 5.77 $\mu$ but no free hydroxyl band and no high selective ultraviolet absorption.

*Anal.* Calcd. for $C_{18}H_{30}O_5$: C, 66.23; H, 9.26; $OCH_3$, 9.50; mol. wt., 326. Found: C, 66.51; H, 9.25; $OCH_3$, 9.45; active hydrogen, 0.0; mol. wt. (Rast), 296.

The resistance toward alkali of the lactone ring in the spiroketal XXIIa is noteworthy,[29] nearly 90% of it being recovered in the neutral fraction after heating on the steam-bath for 2 hours with 6% methanolic potassium hydroxide.

(b) **From Methynolide (XV).**—A solution of 250 mg. of methynolide (XV), 2.5 cc. of concd. sulfuric acid and 33 cc. of methanol was heated under reflux for 5 hours and was then processed exactly as described under (a). Chromatography led to 140 mg. of crude, crystalline spiroketal which upon repeated chromatography and crystallization gave 75 mg. of pure material, m.p. 77–80°.

**Lithium Aluminum Hydride Reduction of Methoxydihydromethynolide Spiroketal (XXIIa).**—Reduction of 390 mg. of the spiroketal XXIIa was accomplished in ether solution at room temperature for 2 hours with 300 mg. of lithium aluminum hydride. The total product crystallized after passage through a short column of alumina but great difficulty was encountered in suitable recrystallization. Benzene–hexane afforded 60 mg., m.p. 157–161° (with crystal change at 125°), while the analytical sample of the **diol XXIII** was prepared by sublimation at 120° and 0.001 mm.; m.p. 159–161° with partial crystal change above 120°, $[\alpha]_D$ +118°, no infrared carbonyl absorption.

*Anal.* Calcd. for $C_{18}H_{34}O_5$: C, 65.42; H, 10.37; $OCH_3$, 9.38. Found: C, 65.77; H, 10.17; $OCH_3$, 9.40.

**Chlorodihydromethynolide Spiroketal (XXIIb).**—Slow crystallization from dilute methanol of 960 mg. of crude neutral material derived from a 10-minute hydrochloric acid hydrolysis[9] of 2.00 g. of methymycin (XIV) afforded *ca.* 100 mg. of crude, crystalline spiroketal, m.p. 90–100°. Six recrystallizations from the same solvent and sublimation at 80° and 0.005 mm., led to the analytical sample, m.p. 106.5–108.5°, $[\alpha]_D$ −84°, $\lambda_{max}^{CHCl_3}$ 5.74 $\mu$ but no hydroxyl absorption.

*Anal.* Calcd. for $C_{17}H_{27}ClO_4$: C, 61.69; H, 8.23; Cl, 10.72. Found: C, 61.66; H, 8.19; Cl, 10.47; $OCH_3$, 0.0.

**Dihydromethynolide (XXIVa).**—A solution of 500 mg. of methynolide (XV) in 25 cc. of ethanol containing 100 mg. of 5% palladized charcoal catalyst was hydrogenated at room temperature and atmospheric pressure for 10 minutes whereupon one equivalent of hydrogen was absorbed. The crude residue (480 mg., no high selective ultraviolet absorption) was recrystallized several times from benzene–pentane whereupon the colorless crystals exhibited m.p. 136–143°; $[\alpha]_D$ +2° (dioxane), ±0° (methanol); $\lambda_{max}^{Nujol}$ 2.96, 3.05 and 5.78 $\mu$.

*Anal.* Calcd. for $C_{17}H_{30}O_5$: C, 64.94; H, 9.62. Found: C, 65.05; H, 9.58.

**Dihydromethynolide Spiroketal (XXIIc).**—A solution of 70 mg. of dihydromethynolide (XXIVa) in 3 cc. of methanol and 3 drops of concd. sulfuric acid was allowed to stand at room temperature for 2 hours and was then diluted with 10 cc. of water. On cooling to Dry Ice temperature, there was obtained in two crops 42 mg. of the spiroketal, m.p. 48–52°. The analytical sample was recrystallized from dilute methanol, m.p. 51–52°, $[\alpha]_D$ −142° (dioxane), $\lambda_{max}^{CHCl_3}$ 5.74 $\mu$ and no free hydroxyl band.

*Anal.* Calcd. for $C_{17}H_{28}O_4$: C, 68.89; H, 9.52. Found: C, 69.36; H, 9.37.

When 17.3 mg. of dihydromethynolide (XXIVa) was dissolved in 2 cc. of dioxane, the specific rotation was +1° but dropped to −140° in 4 minutes after addition of 2 drops of concd. sulfuric acid. Under the same conditions (or in methanol solution), the specific rotation of methynolide (XV) remained unchanged for 8 hours.

**Rotatory Dispersion Results.**—All rotatory dispersions were measured in dioxane solution by the method described earlier[19] and the results are depicted in Fig. 1.

**Methymycin (XIV):** $[\alpha]_{700}$ +40°, $[\alpha]_{589}$ +60°, $[\alpha]_{275}$ +520°; inflections, $[\alpha]_{550}$ +70°, $[\alpha]_{462}$ +100°, $[\alpha]_{362}$ +210°, $[\alpha]_{300}$ +410°; *c* 0.10.

**Methynolide (XV):** $[\alpha]_{700}$ +54°, $[\alpha]_{589}$ +73°, $[\alpha]_{275}$ +645°; "max." $[\alpha]_{304}$ +572°, "min." $[\alpha]_{302}$ +555°; inflections $[\alpha]_{550}$ +90°, $[\alpha]_{350}$ +240°, $[\alpha]_{325}$ + 526°, *c* 0.11.

**Dihydromethynolide Spiroketal (XXIIc):** $[\alpha]_{700}$ − 100°, $[\alpha]_{589}$ − 142°, $[\alpha]_{275}$ − 1350°; *c* 0.19.

**Dihydromethynolide (XXIVa):** $[\alpha]_{700}$ +2°, $[\alpha]_{589}$ +2°, $[\alpha]_{500}$ 0°, $[\alpha]_{275}$ −68°, *c* 0.50.

**Dihydromethymycin (XXIVb):** $[\alpha]_{700}$ 0°, $[\alpha]_{589}$ +23°, $[\alpha]_{280}$ +85°; *c* 0.13.

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(29). A similar observation was made in the erythromycin series (private communication from Dr. K. Gerzon of the Lilly Research Laboratories).