bands. The data in the last two columns of Table III resemble those which Lewis and Bigeleisen[12d] obtained on a series of eight dyes except that $\bar{\nu}'/\bar{\nu}$, the ratio of the frequencies of the second- and first-order $x$-bands, is always greater than 2. The ratio $\bar{\nu}'/\bar{\nu}$ increases with increasing wave length of the $x$-band, as found by Lewis and Bigeleisen, but only within a given series. Furthermore, $\bar{\nu}'/\bar{\nu}$ is much larger for the trimethine dyes than for the higher vinylogs, and is in the range found for several triphenylmethane-type dyes. The values of $\bar{\nu}'/\bar{\nu}$ for the higher vinylogs are intermediate between those for the triphenylmethanes and several cyanines. The trimethine dye III again is anomalous, although $\bar{\nu}'/\bar{\nu}$ fits into the general pattern.

TABLE III

FIRST- AND SECOND-ORDER $x$-BANDS IN POLYMETHINE DYES

| Dye no.[a] | First-order $x$-band $\bar{\nu} \times 10^{-2}$[b] | First-order $x$-band $\epsilon \times 10^{-3}$ | Second-order $x$-band $\bar{\nu}' \times 10^{-2}$[b] | Second-order $x$-band $\epsilon \times 10^{-3}$ | $\bar{\nu}'/\bar{\nu}$ | $\epsilon'/\epsilon$ |
|---|---|---|---|---|---|---|
| VII | 135.1 | 36.1 | 289 | 21.3 | 2.14 | 0.59 |
| VIII | 123.5[c] | 183 | 276 | 20.1 | 2.24 | .110 |
| IX | 109.8 | 186 | 255 | 15.5 | 2.32 | .083 |
| X | 132.5 | 100 | 274 | 18.2 | 2.07 | .182 |
| XI | 120.0[d] | 208 | 249 | 17.3 | 2.08 | .083 |
| XII | 107.0 | 195 | 224 | 13.8 | 2.09 | .071 |
| XIII | 129.8 | 100 | 270 | 18.2 | 2.08 | .182 |
| XIV | 118.4 | 187 | 247 | 16.4 | 2.08 | .088 |
| XV | 106.2 | 151 | 222 | 13.8 | 2.09 | .091 |
| XVI | 116.1 | 214 | 247 | 18.6 | 2.12 | .087 |
| XVII | 103.0 | 178 | 221 | 17.8 | 2.15 | .100 |

[a] See Table II for structures. [b] Frequency of absorption maxima in cm.$^{-1}$. [c] Wizinger (ref. 6) reported the absorption maximum at 11390 cm.$^{-1}$ (*i. e.*, 878 m$\mu$) in acetic acid. Essentially no difference was detected in our curves in chloroform and in acetic acid. The value of 12350 cm.$^{-1}$ (810 m$\mu$) was closely checked on a Beckman model DU spectrophotometer. Furthermore, the curve obtained on the Cary appeared to be rapidly approaching a maximum at 800 m$\mu$. [d] Wizinger (ref. 6) reported $\lambda_{max}^{AcOH}$ 876 m$\mu$ (11410 cm.$^{-1}$); see footnote *c*.

Series B, C and D differ only in the progressive introduction of chlorine into the rings not bearing dimethylamino groups and the spectra of comparable vinylogs are similar, as would be expected from the weak auxochromic effect of chlorine. However, increasing the electron-withdrawing ability of the phenyl group by mono- and dichlorination results with one exception, in a slight bathochromic shift of the short wave length bands and a much larger shift of the long wave length band. The bathochromic effect of increasing the electron-withdrawing power of a substituent on the essential part of the chromophore finds a close analogy in the triphenylmethane dyes and has been attributed by Lewis[12e] to an increase in the fraction of the unit positive charge distributed between the auxochromic amino groups. The bathochromic shift of the $y$-bands as conjugation is extended requires further study. Possibly a quantum mechanical treatment of these dyes such as has been done by Dewar[14] on some triphenylmethane and related dyes would be helpful in arriving at a theoretical understanding of this effect.

**Acknowledgment.**—We express our thanks to Drs. J. M. Edwards and W. T. Cave, Mr. R. L. Van Asselt and the members of the Spectroscopy Laboratory for determining the spectra. For the microanalyses, we are indebted to Messrs. E. M. Hubbard and D. F. Stolz and Mrs. W. Harden.

(14) M. J. S. Dewar, *J. Chem. Soc.*, 2329 (1950).

DAYTON, OHIO

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER & CO., INC.]

# Oleandomycin (PA-105). II.[1,2] Chemical Characterization (I)

BY HANS ELS, WALTER D. CELMER AND KOTARO MURAI

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The characterization and properties of the antibiotic oleandomycin, $C_{35}H_{61}NO_{12}$,[3] are described in detail. Degradation studies led to the characterization of oleandomycin as a polyhydroxy, epoxy, polymethyl ketolactone of the macrolide type,[4] containing glycosidically bound desosamine and L-oleandrose. Degradation products and derivatives are described and a partial structural formula is presented.

**Molecular Formula and Characterization.**—Oleandomycin (I) is a crystalline, colorless, basic compound ($pK_a$ 8.5) with the molecular formula $C_{35}H_{61}NO_{12}$.[3] It is slightly soluble in water and ligroin, but dissolves readily in most of the other common organic solvents. The compound crystallizes from aqueous methanol or aqueous acetone solutions without solvent retention, melting at 110° with decomposition. The antibiotic forms characteristic solvated crystals with chlorinated solvents like methylene chloride or chloroform. The chloroform solvate, m.p. 120–121°, contains one equivalent of tenaciously bound solvent. Such preparations have been shown to be essentially pure by countercurrent distribution and paper chromatographic analysis utilizing several solvent–pair systems.[5]

Oleandomycin is readily soluble in acidic media and forms crystalline salts with a variety of mineral and organic acids. The specific optical rotation of

(1) (a) Oleandomycin is the generic name proposed for PA-105; Matromycin is the trademark of Chas. Pfizer & Co., Inc., for this antibiotic. (b) B. A. Sobin, A. R. English and W. D. Celmer, "Antibiotics Annual 1954-1955," Medical Encyclopedia, Inc., New York, N. Y., p. 827.

(2) Presented in part at the 130th National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1956, p. 15-N.

(3) Present analytical data favor the $C_{35}H_{61}NO_{12}$ empirical formula over previously reported tentative formulations; ref. 1b and 2.

(4) R. B. Woodward, *Angew. Chem.*, **69**, 50 (1957).

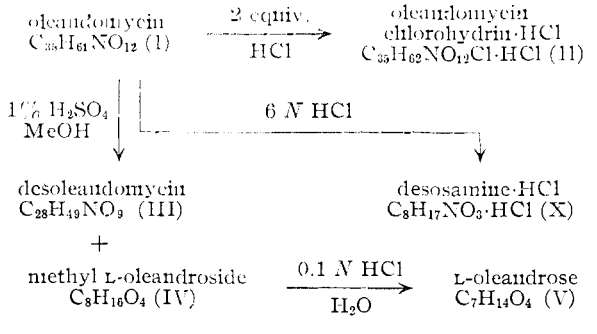
(5) W. D. Celmer, Hans Els and K. Murai, Oleandomycin Derivatives—Preparation and Characterization, "Antibiotics Annual, 1957-1958," in press.

the free base in methanol is $-65 \pm 1°$. The compound absorbs weakly in the ultraviolet region near 290 mμ, ε 50, attributed to an unconjugated ketone grouping. Its infrared spectrum, determined in chloroform solution, shows strong absorption at 2.85 μ (–OH), 3.43, 3.46 μ (–$CH_2$–), 5.84 μ with a shoulder at 5.82 μ (>C=O), 6.84, 7.22, 7.81, 8.47, 8.62 μ, followed by a series of bands in the 9.0–10.42 μ region. The band at 5.82–5.84 μ suggests the presence of two unresolved carbonyl peaks. These two bands were resolved when the crystalline base or the chloroform solvate was subjected to infrared analysis in a potassium bromide pellet. Carbonyl absorption of approximately the same intensity was found at 5.78 and 5.9 μ attributed to an ester linkage and an unconjugated carbonyl group (supported by ultraviolet absorption data).

The empirical formula of oleandomycin and its molecular weight were determined in the usual manner by elemental analysis and equivalent titrations of the base and several of its derivatives. The results are consistent with the empirical formula $C_{35}H_{61}NO_{12}$ for the free base.[5] Group analyses indicate the presence of eight C-methyl groups, one methoxyl group, two N-methyl groups, three active hydrogens and one ester or lactone group (determined by hydrolysis experiments). The oleandomycin triacetate ester was prepared and characterized.[5] Analysis showed the absence of active hydrogen in the molecule.

The antibiotic did not absorb hydrogen on attempted hydrogenation with Adams catalyst in ethanol, indicating the absence of double bonds in the molecule. Sodium borohydride reduction of oleandomycin gave a mixture of reduction products from which a biologically inactive dihydro-derivative $C_{35}H_{63}NO_{12}$ was isolated, which no longer showed the characteristic carbonyl absorption in the ultraviolet region. Treatment of the antibiotic with two equivalents of hydrochloric acid under carefully controlled conditions yielded the biologically active oleandomycin chlorohydrin, isolated as the hydrochloride salt, $C_{35}H_{62}NO_{12}Cl \cdot HCl$ (II), m.p. 152–153°. This compound was reconverted readily to oleandomycin by the addition of two equivalents of sodium hydroxide to a solution of II. The reaction sequence is characteristic for the behavior of an epoxide function.

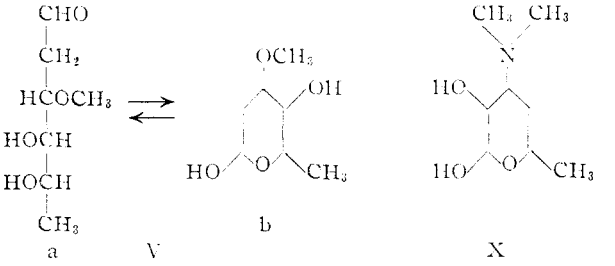
**Degradation Studies.**—Methanolysis of oleandomycin (I) under very mild acidic conditions yielded a basic and a neutral fraction. **Desoleandomycin** (III), $C_{28}H_{49}NO_9$, was isolated from the crude basic mixture in poor yields by chromatography on aluminum oxide.




Other attempts to hydrolyze oleandomycin or its sodium borohydride reduction product under varying conditions failed to produce the desired basic degradation product in reasonable yields. In all attempts mixtures of hydrolysis and/or dehydration products were obtained. Further efforts to effect a direct hydrolysis of the antibiotic were temporarily abandoned, in favor of the degradation of a modified oleandomycin.

The neutral compound isolated from the methanolysis of I was a colorless liquid which analyzed for the empirical formula $C_8H_{16}O_4$. This compound was identified as the methyl glycoside of L-oleandrose by the sequence of reactions: Hydrolysis of the methyl glycoside IV with dilute aqueous hydrochloric acid caused formation of the free sugar V in excellent yields. Compound V consumed one mole of periodate on oxidation[6] in aqueous solution within 18 hours. Acetaldehyde was isolated and identified as the 2,4-dinitrophenylhydrazone. Oxidation of V with 50% nitric acid gave a hydroxymethoxyglutaric acid VI, m.p. 174–175°. Compound VI was further characterized as the *p*-bromophenacyl ester of the lactonized hydroxy acid VII.

Oxidation of the sugar V with bromine in aqueous solution yielded the sugar lactone VIII which was converted to the S-benzylthiouronium salt[7] IX of the sugar acid by way of the barium salt.




The free sugar V was purified by vacuum distillation and crystallization from ether–Skellysolve B. Comparison of the physical properties of V and of the thiouronium salt IX with authentic samples of L-oleandrose, D-sarmentose and their derivatives showed that V was identical in all respects with L-oleandrose, 2-desoxy-L-glucomethylose-3-methyl ether.[8,9] The infrared spectrum indicates that the crystalline sugar exists primarily in the hemiacetal form Vb (negligible infrared carbonyl absorption). Similar observations have been made[10] on related, branched-chain 2-desoxy sugars (cladinose and mycarose, configuration unknown).

The properties of four 2-desoxy-3-methoxymethylhexoses are presented in Table I.[11]

The hydrolysis of oleandomycin (I) with 6 *N* hydrochloric acid resulted in extensive decomposi-

(6) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **25**, 1622 (1942).

(7) C. W. Shoppee and T. Reichstein, *ibid.*, **25**, 1617 (1942).

(8) F. Blindenbacher and T. Reichstein, *ibid.*, **31**, 2061 (1948).

(9) We are indebted to Prof. T. Reichstein, Basel, Switzerland, for samples of crystalline L-oleandrose and D-sarmentose.

(10) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, THIS JOURNAL, **76**, 3121 (1954).

(11) For original literature see: E. Vischer and T. Reichstein, *Helv. Chim. Acta*, **27**, 1332 (1944)

TABLE I

| Compound | Free sugar M.p., °C. | Free sugar $[\alpha]_D$, $H_2O$ | S-Benzylthiouronium salt of acid M.p., °C. | S-Benzylthiouronium salt of acid $[\alpha]_D$ (MeOH) |
|---|---|---|---|---|
| Cymarose | 93 | +52° | 130 | 0 ± 2° |
| Diginose | 90–92 | +55 | 137 | −9.2 ± 2 |
| D-Sarmentose | 78–79 | +15.8 | 146 | +6.5 ± 2 |
| L-Oleandrose | 68–70[a] | +11.9 | 130 | +5.8 ± 2 |
| PA-105 sugar | 59–60 | +11.7 | 132 | +6.5 ± 2 |

[a] Reichstein's sample melted at 60°.

tion of the compound. From the acidic aqueous layer a crystalline basic compound, $C_8H_{17}NO_3$, was isolated as the hydrochloride salt, which was identical in all respects with desosamine hydrochloride X.[12] This amino sugar also has been found as a degradation product of picromycin,[13,14] narbomycin,[14] griseomycin,[14] erythromycin[15] and methymycin.[16]

That the L-oleandrose was glycosidically linked to the oleandolide nucleus and not to the desosamine hydroxyl group was concluded for the following reasons: (a) In analogy to carbomycin[4] a definite shift in the $pK_a$ value could be expected on hydrolysis of such a glycosidic bond. (b) No shift or only a minor shift in $pK_a$ values should be observed on esterification of the hydroxyl groups in oleandomycin if the amino-sugar hydroxyl is already protected by a glycosidic link.

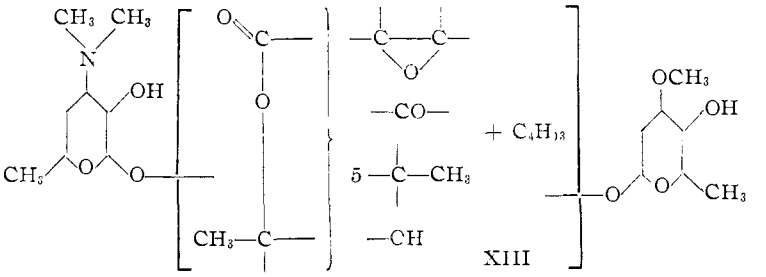
Measurements of the $pK_a$'s for oleandomycin and desoleandomycin give identical values, namely, 8.5. Furthermore, esterification of the hydroxyl groups in oleandomycin and desoleandomycin brings about a sharp drop in the $pK_a$'s to 6.6, excluding, therefore, a glycosidic link between L-oleandrose and desosamine. A similar arrangement has been found in erythromycin.[10]

Treatment of oleandomycin with excess alkali followed by immediate back titration with standard acid showed negligible consumption of alkali. Mild alkaline treatment of oleandomycin for a prolonged time resulted in the utilization of approximately one equivalent of base without the formation of volatile acids, indicating the saponification of a lactone grouping. This hydrolysis product was isolated as a "zwitterionic" substance (XI) easily recognized by its characteristic infrared absorption at 6.3 $\mu$ (carboxylate ion) and a weaker band at 5.9–6.1 $\mu$. Treatment of compound XI with diazomethane gave the methyl ester XII ($pK_a$ 8.2). Infrared analysis showed the complete disappearance of the 6.3 $\mu$ band and the appearance of a strong absorption peak at 5.81 $\mu$, with a shoulder at 5.84 $\mu$, assigned to the ester and ketone carbonyl groups, respectively.

Oleandomycin (I) reacted sluggishly with sodium periodate, but no volatile carbonyl fragment was detected over a period of 72 hours. The saponification product XI, however, reacted rapidly with nearly one equivalent of sodium periodate and acetaldehyde was recovered from the volatile fraction. These data suggest that the saponification reaction opens a *lactone* which was bound to a hydroxyl group of a glycol (or potential glycol) having the structure >COHCHOHCH$_3$. It is of interest that the well characterized antibiotics methymycin[17] and erythromycin[18] contain large ring lactone groupings which are bound to a *propionaldehyde* precursor grouping, *i. e.*, >COHCHOHCH$_2$CH$_3$.

The fact that oleandomycin does not form iodoform on oxidation with sodium hypoiodite allowed us to write the preliminary partial structural formual XIII for oleandomycin.

Further degradation reactions will be discussed in a forthcoming paper.



XIII

**Acknowledgments.**—The authors are indebted to Mr. W. Boegemann and Dr. T. M. Lees for paper chromatographic analysis. We should like to thank Dr. R. L. Wagner, Dr. J. A. Means and Mr. T. J. Toolan and staff for physical measurements, and Mr. R. C. Kersey and Dr. A. R. English for biological data. We wish to acknowledge the capable assistance of Mr. F. Rajeckas and Mr. A. Adriansen.

## Experimental

**Crystalline Oleandomycin Base (I).**—Twenty grams of amorphous oleandomycin base was dissolved in 20 ml. of methanol. To the clear solution water was added slowly until a faint turbidity pertained. Prolonged scratching of the walls of the crystallization flask with a glass rod initiated crystallization. The solution was now stirred and more water was added at a slow rate, causing the material to crystallize rapidly. The addition of water was stopped at a ratio of water to methanol of 9:1. The crystalline material was filtered by suction after 3 hours and dried in a conventional oven at 60° for 8 hours. Fifteen grams of colorless prismatic crystals was obtained, m.p. 110° dec., $[\alpha]_D$ −65 ± 1° (*c*, 2 in MeOH), $\lambda_{max}$ 290–295 m$\mu$, $\epsilon$ 50, $pK_a$ 8.5 in 50% ethanol; neut. equiv. 688, calcd. 687.84. *Anal.* Calcd. for $C_{35}H_{61}NO_{12}$: C, 61.11; H, 8.94; N, 2.04; C–$CH_3$ (8), 17.48; O–$CH_3$ (1), 4.5. Found: C, 61.20, 61.02; H, 8.88, 9.07; N, 2.27, 2.02; C-$CH_3$, 17.01, 14.08; $OCH_3$, 4.60, 4.81.

**Oleandomycin Chloroform Adduct.**—Oleandomycin phosphate salt (100 g.) was dissolved in 500 ml. of water. Chloroform (200 ml.) was added and the *p*H of the aqueous phase was slowly adjusted to 9 by the addition of 20% sodium hydroxide solution. The precipitating base was extracted into the chloroform layer as fast as it formed. The two layers were separated and the chloroform solution was filtered through Super-Cel in order to remove suspended water. To the clear filtrate was added 200 ml. of hexane with continuous mechanical stirring. After approximately 20 minutes, crystallization started and an additional volume of 600 ml. of hexane was added within the next 45 minutes. The dense crystalline material was filtered by suction and dried in a conventional oven for 6 hours at 60°, giving 89 g. of colorless crystals, m.p. 120–122°, $[\alpha]_D$ −55.5° (*c* 2 in

(12) We are grateful to Dr. R. K. Clark, Jr., Abbott Laboratories, for a sample of desosamine hydrochloride.

(13) H. Brockman, *Angew. Chem.*, **69**, 237 (1957).

(14) R. Anliker, *et al.*, *Helv. Chim. Acta*, **40**, 119 (1957).

(15) E. H. Flynn, *et al.*, THIS JOURNAL, **76**, 3121 (1954).

(16) C. Djerassi and J. A. Zderic, *ibid.*, **78**, 6390 (1956).

(17) C. Djerassi and J. A. Zderic, *ibid.*, **78**, 2907 (1956).

(18) P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., and U. C. Quarck, *ibid.*, **77**, 3676 (1955); **77**, 3677 (1955).

MeOH), ultraviolet absorption $\lambda_{max}$ 295 mμ, ε 50. *Anal.* Calcd. for $C_{35}H_{61}NO_{12}\cdot CHCl_3$: C, 53.56; H, 7.78; N, 1.73; Cl, 13.17; $H_2O$, 0.0; $CHCl_3$, 14.78. Found: C, 53.68, 53.96; H, 7.64, 7.89; N, 1.89, 1.79; Cl, 12.80; $H_2O$, 0.09 (Karl Fischer); $CHCl_3$, 15.36.

**Oleandomycin Hydrochloride.**—Amorphous oleandomycin base (2 g.) was dissolved in 50 ml. of water, the *p*H was adjusted to 7.4 with hydrochloric acid and the clear solution was freeze-dried. The amorphous, colorless material was dissolved in 80 ml. of ethyl acetate, the solution was filtered through Super-Cel and the hydrochloride was crystallized by the addition of 0.3 ml. of 30% ethanol. The crystals were filtered by suction and dried for 2 days at 25° (0.01 mm.) over phosphorus pentoxide, giving 2.2 g. of colorless, crystalline material, m.p. 134–135°, $[\alpha]_D$ −56.8° (*c* 2 in MeOH), neut. equiv. 722 (in $H_2O$, calcd. 724), ultraviolet absorption $\lambda_{max}$ 290 mμ, ε 45. *Anal.* Calcd. for $C_{35}H_{61}NO_{12}\cdot HCl$: C, 58.04; H, 8.63; N, 1.93; Cl, 4.89. Found: C, 57.81, 58.06; H, 8.63, 8.66; N, 1.83, 1.84; Cl, 4.75, 4.58.

**Oleandomycin Chlorohydrin Hydrochloride.**—To a solution of 131 g. of oleandomycin base in 1.5 liters of ethyl acetate was added slowly 200 ml. (1.8 equiv.) of 1.33 *N* anhydrous hydrochloric acid in ethyl acetate with continuous stirring. After standing for 2 hours at room temperature, another 35 ml. of the acid was added (totaling 2 equiv.). After 30 minutes at room temperature 10 ml. of 50% ethanol was added in 5 portions over a period of 20 minutes. After 30 minutes the crystals were filtered and dried at 60° (1.0 mm.) for 3 hours. *Anal.* Calcd. for $C_{35}H_{62}NO_{12}Cl.HCl$: Cl, 9.32. Found: Cl, 8.77.

Above product (71 g.) was added with stirring to 190 ml. of isopropyl alcohol at 50–60° over a period of 20 minutes. A solution of 1.33 *N* anhydrous hydrochloric acid (10.5 ml.) was added and, after standing for 15 minutes at 50°, 4 liters of cold ethyl acetate was added. The hydrochloride was crystallized by the slow addition of 10 ml. of water. The crystals were filtered by suction, washed with 200 ml. of cold ethyl acetate and dried at 60° (1.0 mm.). Oleandomycin chlorohydrin hydrochloride salt (52 g.) was obtained as colorless, felt-like crystals, m.p. 150–153°, $[\alpha]_D$ −79.2° (*c* 2 in MeOH). *Anal.* Calcd. for $C_{35}H_{62}NO_{12}Cl\cdot HCl$: total Cl, 9.32; ionic Cl, 4.66. Found: total Cl, 9.42; ionic Cl, 4.72.

**Oleandomycin from Oleandomycin Chlorohydrin Hydrochloride (II).**—Two grams of the chlorohydrin was dissolved in 100 ml. of 50% ethanol–water and the *p*H was adjusted to 10. The conversion of chlorohydrin was conveniently followed by optical rotation. After approximately 30 minutes, the specific rotation reached −65 ± 2°. The solution was neutralized with dilute sulfuric acid and the ethanol was evaporated. The aqueous phase was adjusted to *p*H 9 with sodium hydroxide and the compound was extracted into 100 ml. of chloroform. The chloroform solution was dried over sodium sulfate and concentrated *in vacuo* to 5 ml. Hexane (5 ml.) was added with stirring and after the crystallization of the chloroform adduct had started an additional 30 ml. of hexane was added. The compound (1.2 g.) was filtered after 1 hour and dried at 60° (0.1 mm.) for 30 minutes, m.p. 121–122°, identical in mixed melting point, paper chromatographic migration and infrared spectrum with an authentic sample of oleandomycin chloroform solvate.

**Dihydroöleandomycin.**—Oleandomycin (10 g.) was dissolved in 50 ml. of isopropyl alcohol and added dropwise to a solution of 3 g. of sodium borohydride in 200 ml. of isopropyl alcohol. The reaction solution was stirred for 20 hours at room temperature (25°). The excess reagent was destroyed with dilute hydrochloric acid, the *p*H was adjusted to 7 and the solvent was evaporated. The residue was extracted with a total of 300 ml. of methylene chloride. The solvent layer was washed with two 100-ml. portions of sodium bicarbonate solution, two 50-ml. portions of water and finally was dried over sodium sulfate. After evaporation of the solvent, an amorphous colorless material was obtained (8.5 gr.) which was dried for 3 hours at 80° (0.1 mm.). Paper chromatography indicated the presence of three components. A minor component was identified with starting material.

The mixture (1.1 g.) was chromatographed on 20 g. of neutral aluminum oxide, giving the following main fractions.

| Solvent | Total, ml. | Total, mg. | Remarks |
|---|---|---|---|
| $CH_2Cl_2$ | 100 | 50 | Oleandomycin |
| EtOAc | 100 | 67 | |
| 2% MeOH–EtOAc | 200 | 480 | M.p. 127–129°, $[\alpha]_D$ −58.2° (*c* 2, in MeOH) |
| 5% MeOH–EtOAc | 160 | 189 | M.p. 211–216°, $[\alpha]_D$ +5.1° (*c* 2, in MeOH) |
| 10% MeOH–EtOAc | 200 | 54 | |
| 50% MeOH–EtOAc | 180 | 26 | |

The material with m.p. 127–129°, $[\alpha]_D$ −58.2° (*c* 2 in MeOH) was analyzed. *Anal.* Calcd. for $C_{35}H_{63}NO_{12}$: C, 60.93; H, 9.20; N, 2.03. Found: C, 60.44; H, 9.03; N, 2.17; ultraviolet absorption, end absorption only.

The higher melting material, m.p. 211–216°, was identified by paper chromatography with a reduction product of a dehydrated oleandomycin.

The mixture was also separated by a 600 transfer countercurrent distribution in a benzene–80% ethanol system to yield, after crystallization from benzene, material whose infrared absorption spectrum and chromatographic behavior was identical with the m.p. 127–129° material above. It melted (open capillary) at 125–127°, resolidified around 135° and melted at 217–220°.

**Desoleandomycin (III).**—Oleandomycin (10 g.) was dissolved in 300 ml. of 2% sulfuric acid. The solution was kept at 25° for two days. The acid was neutralized with barium hydroxide to *p*H 7. The precipitate was filtered by suction through Super-Cel and the clear filtrate was extracted with three 200-ml. portions of ethyl acetate. The solvent layer was washed twice with 200 ml. of water, dried over sodium sulfate and the solvent was evaporated leaving 1.3 g. of an oily residue. Paper chromatography revealed that the residue consisted mostly of L-oleandrose, containing small amounts of desoleandomycin.

The *p*H of the aqueous mother liquors was adjusted to 9.5 with dilute sodium hydroxide and the liberated base was extracted twice with 300 ml. of ethyl acetate. The extract was washed twice with 100 ml. of water, dried over sodium sulfate and the solvent was evaporated, leaving 7.2 g. of an amorphous material.

The hydrolysis product (500 mg.) was chromatographed on 20 g. of neutral aluminum oxide. These major fractions were obtained:

| Solvent | Total, ml. | Total, mg. | Remarks |
|---|---|---|---|
| $CH_2Cl_2$ | 100 | 9 | |
| EtOAc | 100 | 3 | |
| 2% MeOH–EtOAc | 140 | 126 | Mixture |
| 5% MeOH–EtOAc | 200 | 95 | M.p. 116–118° |
| 10% MeOH–EtOAc | 140 | 63 | Mixture |
| 50% MeOH–EtOAc | 200 | 31 | |

The fraction obtained from 5% MeOH–EtOAc was dissolved in 50 ml. of water, treated with carbon and filtered. The clear filtrate was freeze-dried giving 85 mg. of colorless material, m.p. 116–118°, $[\alpha]_D$ −25.3° (*c* 2 in MeOH). *Anal.* Calcd. for $C_{28}H_{49}NO_9$: C, 61.85; H, 9.08; N, 2.57. Found: C, 61.69; H, 9.18; N, 2.35; ultraviolet $\lambda_{max}$ 293–296 mμ ε 40.

**Methyl L-Oleandroside (IV).**—Oleandromycin (60 g.) was dissolved in 1.5 liters of 1% methanolic hydrogen chloride solution. After three days at room temperature the *p*H was adjusted to 8 by the addition of sodium carbonate solution. The solution was concentrated to 200 ml. *in vacuo* and the *p*H was adjusted to 6 with dilute hydrochloric acid. Concentration was continued until a heavy flowing sirup resulted. The residue was thoroughly digested with five 400-ml. portions of ether. The extracts were combined, dried over sodium sulfate and the ether was evaporated, leaving 11.5 g. of a light brown sirup. This material was distilled in a claisen column (30 mm. vacuum). Fraction i, b.p. 117–118°, 4.57 g., $n^{25}D$ 1.4479; ii, b.p. 119–124°, 3.95 g., $n^{25}D$ 1.4478; iii, b.p. 125°, 0.86 g., $n^{25}D$ 1.4528. Fraction ii was distilled for analysis in a micro-Craig column.

| Fract. | Bath, $t$, °C. | Jacket, $t$, °C. | Mm. | Ml. | $n^{25}D$ |
|---|---|---|---|---|---|
| 1 | 135 | 75 | 3.5 | 0.2 | 1.4474 |
| 2 | 116 | 85 | 3.5 | .3 | 1.4474 |
| 3 | 117 | 86 | 3.5 | .3 | 1.4474 |
| 4 | 117 | 87 | 3.5 | .3 | 1.4475 |
| 5 | 117 | 87 | 3.5 | .2 | 1.4474 |

Fraction 3 was analyzed. *Anal.* Calcd. for $C_8H_{16}O_4$: C, 54.52; H, 9.15. Found: C, 54.64; H, 9.36.

**L-Oleandrose (V).**—Methyl L-oleandroside (3 g.) was suspended in 40 ml. of 0.01 *N* hydrochloric acid. The reaction solution was heated for 45 minutes on the steam-bath. The hydrochloric acid was neutralized with dilute sodium hydroxide and the solvent was evaporated *in vacuo*. The residue was extracted with ether, the extract was treated with Darco G-60 and the ether was evaporated. The sirupy residue (2.3 g.) was distilled in a micro-Craig column.

| Fract. | Bath, $t$, °C. | Jacket, $t$, °C. | Mm. | Mg. | $[\alpha]^{25}D$ |
|---|---|---|---|---|---|
| 1 | 157 | 110 | 0.2 | 219.8 | |
| 2 | 158 | 122 | .2 | 102.0 | −20.8° (EtOH) |
| 3 | 160 | 130 | .2 | 396.8 | −20.3 (EtOH) |
| | | | | | −11.7 ($H_2O$) |

Fraction 3 was crystallized from ether–hexane giving 215 mg. of colorless crystalline material which was dried in a vacuum tube over $P_2O_5$ for 2 weeks (0.1 mm.), m.p. 59–60°, mixed melting point with authentic L-oleandrose 59–60°, L-oleandrose m.p. 60°, $[\alpha]D$ −11.7° (*c* 1.5 in water). *Anal.* Calcd. for $C_7H_{14}O_4$: C, 51.75; H, 8.70; $-OCH_3$, 19.13. Found: C, 51.47; H, 8.82; $-OCH_3$, 18.68.

**Oxidation of L-Oleandrose with $H_5IO_6$.**—L-Oleandrose (487.1 mg.) was dissolved in 100 ml. of water containing 2.543 g. of paraperiodic acid. At intervals, 1 ml. of the solution was diluted with 20 ml. of water, 5 ml. of saturated sodium bicarbonate solution and 2 ml. of 20% potassium iodide solution was added and the liberated iodine was titrated after 3 minutes with 0.01 molar sodium arsenite solution.

| Time | Blank | Ox. soln. | Moles $IO_4^-$/mole cpd. |
|---|---|---|---|
| 5 min. | 11.0 | 10.2 | 0.26 |
| 30 min. | 10.9 | 10.0 | .31 |
| 90 min. | 10.8 | 9.55 | .45 |
| 2 hr. | 10.9 | 9.5 | .47 |
| 18.5 hr. | 10.8 | 7.6 | 1.03 |

For the isolation of the aldehyde, 0.3 g. of L-oleandrose was dissolved in 50 ml. of water containing 2 g. of paraperiodic acid. The reaction solution was heated to 50–60° and a stream of nitrogen was blown through the solution. The outgoing gases were bubbled through a solution of 2,4-dinitrophenylhydrazine. After a few minutes a heavy precipitate formed which was filtered after 30 minutes, and after washing with water was crystallized from ethanol–water giving 56 mg. of yellow needles, m.p. 166–167°. The compound was identified as acetaldehyde 2,4-dinitrophenylhydrazone by mixed melting point, paper chromatogram and infrared spectrum.

**Oxidation of L-Oleandrose with 50% Nitric Acid.**—Five grams of L-oleandrose was dissolved in 80 ml. of 50% nitric acid. After three days at room temperature, the nitric acid was removed *in vacuo* on the steam-bath. The residue was evaporated twice with 100 ml. of water and twice with 100 ml. of ethanol–benzene (1:1). The residue crystallized from methyl isobutyl ketone giving 750 mg. of a dibasic acid, m.p. 174–175°, $[\alpha]D$ +56.9° (*c* 1.05 in EtOH), neut. equiv. 180. The acid forms a γ-lactone, of which the *p*-bromophenacyl ester was prepared, m.p. 155–156°. *Anal.* Calcd. for $C_{14}H_{13}O_6Br$: C, 47.08; H, 3.67; Br, 22.36. Found: C, 47.26; H, 3.99; Br, 22.28. This calculates for an empirical formula of $C_6H_{10}O_6$, mol. wt. 178.14 for the α-hydroxy-β-methoxyglutaric acid.

**S-Benzylthiouronium Salt of L-Oleandronic Acid (IX).**—Five grams of L-oleandrose was dissolved in 100 ml. of water. Bromine (3 ml.) was added and the flask was shaken gently until all the bromine had dissolved. After standing overnight at room temperature, the excess bromine was removed by blowing a stream of nitrogen through the solution. The hydrobromic acid was neutralized with silver carbonate and after filtration the dissolved silver was removed by precipitation with hydrogen sulfide at 80°. The precipitate was filtered by suction and the filtrate was treated with Darco G-60. The clear filtrate gave on evaporation 4.05 g. of a faintly yellow, viscous material.

One gram of the lactone was dissolved in 49 ml. of 0.1 *N* barium hydroxide solution and heated on the steam-bath for 10 minutes at 70°, and then for 10 minutes at 90°. The cooled solution was neutralized with sulfuric acid and the precipitated barium sulfate was removed by filtration. The filtrate was concentrated *in vacuo*. The residue dissolved in 5 ml. of hot methanol and the barium salt was precipitated by the slow addition of 40 ml. of acetone. The barium salt was filtered and washed with dry ether. After drying for 30 minutes (25°, 0.1 mm.), 900 mg. of colorless barium salt was obtained.

The barium salt (500 mg.) was dissolved in 5 ml. of methanol and 500 mg. of S-benzylthiouronium sulfate was added in 15 ml. of methanol. The solution was heated on the steam-bath for 15 minutes and the precipitated barium sulfate was removed by centrifugation and decanting of the clear supernatant. After evaporation of the methanol and addition of 2 ml. of acetone to the residue, rapid crystallization of the S-benzylthiouronium salt occurred, m.p. 130°, $[\alpha]D$ +7.5° (*c* 2 MeOH). After two recrystallizations the sample melted at 132–133°, $[\alpha]D$ +6.7 ± 2° (*c* 2, MeOH). *Anal.* Calcd. for $C_{15}H_{24}O_5N_2S$: C, 52.31; H, 7.02; N, 8.13. Found: C, 52.08; H, 7.19; N, 8.18.

**Desosamine (X).**—Thirty grams of crude, hydrolyzed oleandomycin was dissolved in 150 ml. of ethanol and added to 450 ml. of 6 *N* hydrochloric acid. The reaction solution was refluxed for 4 hours. The solution was cooled in an ice-bath and the brown liquid was decanted from some tarry material. The aqueous solution was extracted with eight 100-ml. portions of chloroform and five 100-ml. portions of wet butanol. The butanol extract was washed with 100 ml. of water. The aqueous layers were combined and concentrated *in vacuo*. The remaining viscous oil was dissolved in 40 ml. of 85% ethanol and ether was added until the solution became turbid. On scratching the walls of the vessel with a glass rod, crystallization started immediately. After 20 hours in the ice-box 3.7 g. of pink crystals were obtained. The compound was recrystallized three times from the same solvent pair giving 2.1 g. of colorless crystals, m.p. 189–192°, $[\alpha]D$ +18.9° (*c* 2 in MeOH), neut. equiv. 220 (50% ethanol), mixed melting point with desosamine hydrochloride 189–192°, infrared spectrum identical with that of desosamine hydrochloride. *Anal.* Calcd. for $C_8H_{18}NO_3Cl$: N, 6.61; Cl, 16.78. Found: N, 6.50; Cl, 16.61.

**Hydrolysis of Oleandomycin with 0.01 *N* Sodium Hydroxide.**—Oleandomycin (8 g.) was dissolved in 20 ml. of methanol. This solution was added with mechanical stirring to 1.2 liters of 0.01 *N* sodium hydroxide at room temperature. In the course of two hours, a turbidity appeared and after 24 hours 1.87 g. of colorless material was removed by filtration. The compound crystallized from methanol–water, m.p. 198–199°, $[\alpha]D$ +64 ± 2°, identical with anhydro oleandomycin in mixed melting point, paper chromatographic migration and infrared spectrum.

The aqueous filtrate was extracted with three 150-ml. portions of chloroform, the *p*H was adjusted to 7 with hydrochloric acid and the water was evaporated (50°, 25 mm.). The residue was extracted with 200 ml. of dry ethanol, the extract was treated hot with carbon and the carbon was removed by filtration. The solvent was evaporated, leaving an amorphous residue. The infrared spectrum showed absorption peaks at 5.89 and 6.3 μ assigned to a keto carbonyl and a carboxylate ion, respectively. The compound on burning left no inorganic ash, so that a "zwitterionic" structure was proposed for the hydrolysis product.

**Methyl Ester of Hydrolysis Product XII.**—Above hydrolysis product (2.5 g.) was dissolved in 10 ml. of ethanol and 50 ml. of ether was added. An ethereal solution of diazomethane was added until a faint yellow color remained after three hours; the solvent was removed *in vacuo*, the residue was taken up in ethyl acetate (200 ml.) and washed with three 50-ml. portions of sodium bicarbonate and two 100-ml. portions of water. The solvent was dried and evaporated, giving 2.1 g. of amorphous, colorless, basic materials.

Infrared analysis showed the complete disappearance of the 6.3 peak and the appearance of a strong peak at 6.84 to 6.89 $\mu$, $[\alpha]_D$ −54.7° (*c* 2 in MeOH), neut. equiv. 692 (theory 720), $pK_a$ 8.2 (in 50% ethanol).

**Sodium Periodate Oxidation of the Hydrolysis Product XI.**—One gram of compound XI was oxidized with 1.3 g. of sodium periodate in 100 ml. of water at 50°. The volatile aldehydes were recovered by blowing a nitrogen stream through the solution and bubbling the outgoing gases through a solution of 2,4-dinitrophenylhydrazine. The precipitating aldehyde derivative was filtered and crystallized from ethanol–water, m.p. 157–159°, identical with acetaldehyde 2,4-dinitro-phenylhydrazone in mixed melting point, paper chromatographic migration and infrared spectrum.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO.]

# γ-Ketophosphonic Acid Derivatives in the Indole Series[1]

BY J. SZMUSZKOVICZ

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The Mannich base methiodide (I) in the 3-acylindole series was shown to give rise to the diethyl γ-keto-propylphosphonate derivative (V) on nucleophilic substitution with triethyl phosphite. During alkaline hydrolysis of V, monosaponification and N-ethylation occurred. The resulting monobasic acid XII afforded a γ-keto-propylphosphonic acid which in the solid state was stable in the lactol form XVII. The behavior of 1-alkyl (II and III) and 5-benzyloxy (IV) Mannich bases was examined and the reactions extended also to trimethyl and triisopropyl phosphite.

γ-Ketophosphonic acid derivatives have been prepared recently employing the methiodide or hydrochloride of a β-diethylamino ketone and triethyl phosphite.[2] This reaction constitutes an extension of the Michaelis–Arbuzov reaction,[3] for which we propose the name "the Jensen reaction."

Another general method involves the treatment of α,β-unsaturated ketones with dialkyl phosphites and sodium methoxide in methanol.[4]

This paper describes the application of the "Jensen reaction" to Mannich bases of certain indolyl ketones. On refluxing with triethyl phosphite, 1-(3′-indolyl)-3-dimethylaminopropan-1-one methiodide (I)[10b] afforded in good yield diethyl γ-(3-indolyl)-γ-ketopropyl phosphonate (V)[5] which showed NH absorption in the infrared spectrum and a band of the vinylogous amide grouping common to the 3-acylindole derivatives (1634–1645 cm.$^{-1}$).

As attempted acid hydrolysis of V led to considerable decomposition, our attention was turned to the alkaline treatment.[6]

When heated with aqueous potassium hydroxide, V was converted to a monobasic acid, ethyl γ-[3-(1-ethyl)-indolyl]-propylphosphonate (XII) (along with about 8% of the N-unethylated material; see Experimental), which exhibited amide but no NH absorption in the infrared spectrum. It became evident that, during the alkaline hydrolysis, ethylation of the indole nitrogen occurred, the diethyl phosphonate acting as the alkylating agent.[7] Although the question of the molecularity of this O → N alkyl transfer was not investigated, molecular models appear not to exclude the interesting possibility of this being an intramolecular reaction.

An alternative method for the preparation of XII starts with 1-[3′-(1′-ethyl)-indolyl]-3-dimethylaminopropan-1-one methiodide (II).[10b] When II was subjected to the reaction with triethyl phosphite, diethyl γ-[3-(1-ethyl)-indolyl]-γ-keto-propylphosphonate (VI) was obtained which on alkaline hydrolysis afforded XII identical with the compound obtained starting with I. This method is superior to the first if a purer form of XII is desired.

On recrystallization from water, XII was converted to a new compound (XVII), which was soluble only in polar solvents. Compound XVII was a dibasic acid, showed typical 3-acylindole absorption in the ultraviolet spectrum in ethanolic solution, but no amidic band in the infrared (Nujol). The monosodium salt of XVII, on the other hand, exhibited an amidic band in infrared at 1637 cm.$^{-1}$. Acid XVII, therefore, *exists in the solid state* in the lactol form of the corresponding γ-[3-(1-ethyl)-indolyl]-γ-keto-propylphosphonic acid and is, to our knowledge, the first compound of its class in the γ-ketophosphonic acid series.[8]

(1) Presented at the 131st Meeting of the American Chemical Society, Miami, Fla., April, 1957; Abstracts p. 55-O.

(2) T. C. Myers, R. G. Harvey and E. V. Jensen, THIS JOURNAL, **77**, 3101 (1955).

(3) For a general review of the Michaelis–Arbuzov reaction see G. M. Kosolapoff, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 276; G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 197.

(4) A. N. Pudovik, *J. Gen. Chem.*, **22**, 525 (1952), in English translation; *C. A.*, **47**, 2686 (1953), and previous references.

(5) The structures of the phosphonate derivatives are formulated with a P=O bond on the basis of Raman data in the literature and the tentative assignment of the infrared band in the 1250 cm.$^{-1}$ region to the P=O stretching vibrations. For leading references see L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co., London, 1954, p. 258. It is the hope of the organic chemist that a more definite answer to this problem will evolve from nuclear magnetic resonance studies. For a recent paper in this field see W. Muller, P. C. Lauterbur and J. Goldenson, THIS JOURNAL, **78**, 3557 (1956).

(6) *Cf.* J. Kennedy and R. V. Davies, *Chemistry & Industry*, 378 (1956); B. S. Griffin and A. Burger, THIS JOURNAL, **78**, 2336 (1956); N. Mikhailova, *C. A.*, **40**, 555 (1946); M. Janczak, *Rocz. Chem.*, **6**, 774 (1926); *C. A.*, **21**, 3599 (1927); A. F. Torralba and T. C. Myers, *J. Org. Chem.*, **22**, 972 (1957).

(7) It is well known that 3-acylindoles undergo N-alkylation on treatment with dialkyl sulfates and alkali; *e.g.*, 1-methyl- and 1-ethyl-3-acetylindole are obtained in 95 and 98% yield, respectively, according to Y. A. Baskakov and N. N. Mel'nikov, *C. A.*, **49**, 1006 (1955). The alkylating properties of esters in the phosphorus acid series have been demonstrated in many instances in the case of esters of phosphoric acid; for references see F. R. Atherton, *Quart. Rev.*, **3**, 151 (1949). The alkylation of 2-aminofluorene with diethyl ethanephosphonate has been described by T. L. Fletcher, M. E. Taylor and A. W. Dahl [*J. Org. Chem.*, **20**, 1021 (1955)] and esterification of carboxylic acids with dialkyl phosphonates by F. W. Hoffmann and H. D. Weiss, THIS JOURNAL, **79**, 4759 (1957).

(8) Pseudo esters of γ-oxocarboxylic acids are well known [see ref. 6 in the paper by M. S. Newman and C. D. McCleary, *ibid.*, **63**, 1537 (1941)]. A method of determining proportion of free acid