dried over sodium sulfate, filtered and evaporated to dryness, to give xanthomycin A as a deep orange-red amorphous solid.

Crystalline Hydrochloride of Xanthomycin A.—Xanthomycin A was dissolved in absolute ethanol and treated with a solution of dry hydrogen chloride in the same solvent. The deep orange-red solution turned bright yellow. The solution was evaporated to dryness *in vacuo* and the orange-yellow crystalline residue recrystallized from a mixture of absolute ethanol and ethyl acetate (3:1). It separated out in the form of bright orange-yellow rectangular plates.

Anal. Calcd. for $C_{23}H_{29-31}N_3O_7 \cdot 2HCl$: C, 51.76; H, 6.08; N, 7.88; Cl, 13.32; $-OCH_3$ for 1, 8.44; $-NCH_3$ for 1, 2.81; $-CCH_3$ for 2, 5.62. Found: C, 51.76, 51.94; H, 6.25, 6.71; N, 7.94, 7.82; Cl, 13.71, 12.57; $-OCH_3$, 9.15; $-NCH_3$, 2.62; $-CCH_3$, 5.26. An aqueous solution of the hydrochloride showed a specific rotation, $\alpha^{25}D$ +115° (*c* 0.4 in water). The absorption spectrum in 0.1 *N* hydrochloric acid (Fig. 2) showed maxima at 265 mμ ($E^{1\%}_{1\,cm.}$ 196) and at 345 mμ ($E^{1\%}_{1\,cm.}$ 19.8).

The free base of xanthomycin A was purified by precipitating twice from a chloroform solution with ether. The product was a deep orange-red amorphous solid.

Anal. Calcd. for $C_{23}H_{29-31}N_3O_7$: C, 60.00; H, 6.52; N, 9.01; O, 24.35; $-NCH_3$ for 1, 3.26; mol. wt., 460. Found: C, 60.15, 60.40; H, 6.32, 6.56; N, 8.75, 9.00; O by direct analysis, 24.73; $-NCH_3$, 3.16; mol. wt. by ebullioscopic method in methanol, 455, 512, 520. The sum of the averages for the elementary composition, 100.32%, shows that xanthomycin A contains no other elements than C, H, N and O. The absorption spectrum in absolute ethanol (Fig. 2) has two maxima, one at 288 mμ ($E^{1\%}_{1\,cm.}$ 148) and the other at 460 mμ ($E^{1\%}_{1\,cm.}$ 118), and points of inflection at 225 mμ ($E^{1\%}_{1\,cm.}$ 253) and 315 mμ ($E^{1\%}_{1\,cm.}$ 116).

Paper Chromatography.—One to two micrograms of xanthomycin A hydrochloride was spotted on filter paper strips (0.5 × 60 inches, Eaton Dikeman 613) and developed with 1-butanol containing 1% by volume of 1 *N* hydrochloric acid. After the downflow development at 37° the strips were dried and analyzed by plating them on agar seeded with spores of *Bacillus subtilis* according to the method of Karnovsky and Johnson.[6]

(6) M. L. Karnovsky and M. J. Johnson, *Anal. Chem.*, **21**, 1125 (1949).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Xanthomycin A. Quinonoid Behavior[1]

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Xanthomycin A ($C_{23}H_{29-31}N_3O_7$) has quinonoid properties. Sulfur dioxide, stannous chloride and titanous chloride reduced it to dihydroxanthomycin which was isolated as the sulfate $C_{23}H_{31-33}N_3O_7 \cdot H_2SO_4$. In the presence of Adams catalyst xanthomycin A takes up two moles of hydrogen to form tetrahydroxanthomycin A which is readily reoxidized by ceric sulfate to isodihydroxanthomycin A. In this oxidation only one mole of oxygen is consumed. Because of this behavior it appears that xanthomycin A has two centers of unsaturation. One of these is irreversibly reduced by catalytic hydrogenation. The other is a quinonoid system which may be reversibly reduced and oxidized. The acetyl derivative of tetrahydroxanthomycin A forms an unstable crystalline chloroplatinate having the composition $C_{23}H_{33-35}N_3O_7(CH_3CO)_4 \cdot H_2PtCl_6$. The corresponding methyltetrahydroxanthomycin A has been prepared and isolated as the stable crystalline chloroplatinate, $C_{25}H_{37-39}N_3O_7 \cdot H_2PtCl_6$. Oxidation–reduction potentials of xanthomycin A and isodihydroxanthomycin A indicate that a benzoquinone type of structure exists in xanthomycin A.

Xanthomycin A is a basic antibiotic with a molecular formula $C_{23}H_{29-31}N_3O_7$ and contains two salt-forming groups, one methoxyl, one methylimide and two terminal methyl groups.[2]

Xanthomycin A exhibits several properties typical of quinonoid substances. It is deep orange-red in color, dissolves in aqueous alkali to give a bright reddish pink solution. In alcoholic sodium methoxide it forms an intense purple color. It releases iodine from acidified potassium iodide solutions and is reduced by sulfur dioxide.

In their preliminary investigation on xanthomycin A, Mold and Bartz[3] showed that the antibiotic could be reduced catalytically to a colorless, microbiologically inactive product which regained its color but not its activity on shaking with air. This reoxidized material could again be reduced to a colorless compound but less hydrogen was consumed. Thiele acetylation or chemical reductive acetylation of xanthomycin A gave no loss of color. However, no quantitative studies were reported on the reduction of xanthomycin A in their work. Our paper deals with studies on the reduction of xanthomycin A, made with a view to obtaining information regarding the nature of the quinonoid system in the molecule.

Stannous chloride, titanous chloride and sulfur dioxide reduce xanthomycin A to a light yellow, unstable dihydro derivative. In the presence of air this product readily undergoes reoxidation. Attempts to stabilize dihydroxanthomycin A by acetylation under various conditions resulted invariably in the formation of dark-colored resinous products.

Xanthomycin A can be reduced in the presence of Adams catalyst to the tetrahydro derivative. If tetrahydroxanthomycin A is exposed to air while in solution, it rapidly undergoes reoxidation to the corresponding quinone, isodihydroxanthomycin A. Conventional methods of acetylation of tetrahydroxanthomycin led to considerable decomposition. However, when hydrogenation and acetylation were carried out simultaneously, a colorless, stable derivative was obtained. The acetyltetrahydroxanthomycin A was purified as the crystalline chloroplatinate and analyzed. It contained four acetyl and two basic nitrogen groups. Although stable to atmospheric oxidation, it was unsuitable for degradation work because it was readily deacetylated in acid or alkaline solutions and then gave rise to tarry decomposition products.

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(2) K. V. Rao and W. H. Peterson, THIS JOURNAL, **76**, 1335 (1954).

(3) J. D. Mold and Q. R. Bartz, *ibid.*, **72**, 1846 (1950).

A stable derivative suitable for degradation purposes was finally obtained by the simultaneous reduction and methylation of xanthomycin A. The product, designated methyltetrahydroxanthomycin A, was colorless and stable under both acid and alkaline conditions. It was isolated as the chloroplatinate and hydrochloride salts which on analyses showed that two additional methoxyl groups had been formed by the reduction–methylation procedure. Degradation studies on methyltetrahydroxanthomycin A will be reported in a later paper.

The absorption spectrum of xanthomycin A resembles that of a typical quinone.[4] It exhibits two maxima, one at 288 mμ and the other at 460 mμ, and two points of inflection at 225 and 315 mμ. Similar behavior is also shown by isodihydroxanthomycin A (Fig. 1). The change in the absorption spectrum of xanthomycin A on reduction (Fig. 2) reflects the conversion of a quinone to a hydroquinone.[4] Likewise, the spectra of the acetylation and methylation products of tetrahydroxanthomycin A (Fig. 3) show marked changes similar to those observed for corresponding derivatives of other hydroquinones.[5]

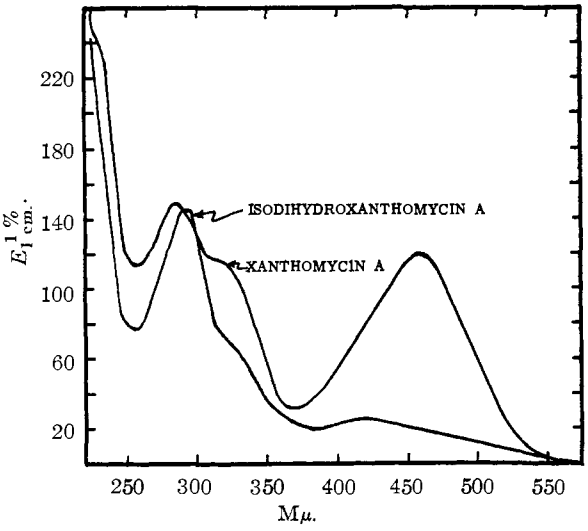

Fig. 1.—Absorption spectra of xanthomycin A and isodihydroxanthomycin A.

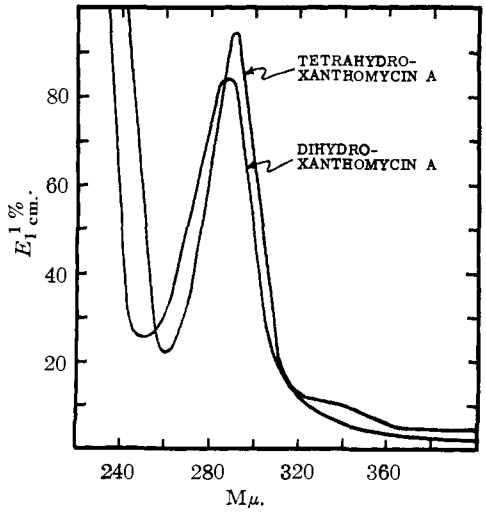

Fig. 2.—Absorption spectra of dihydro- and tetrahydroxanthomycin A.

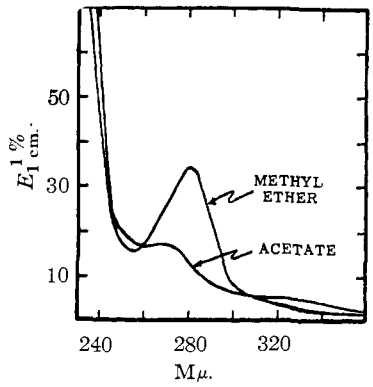

Fig. 3.—Absorption spectra of an acetylated and methylated tetrahydroxanthomycin A.

In order to obtain information regarding the type of quinonoid system present in xanthomycin A, reduction potentials were determined. When xanthomycin A was reduced with titanous chloride, an amount of reagent corresponding to one mole of hydrogen was consumed. On reoxidation with ceric sulfate an equivalent amount of oxidizing agent was required. The reduction and reoxidation could be carried out reversibly several times on the same sample. The oxidation–reduction potential of the system xanthomycin A–dihydroxanthomycin A is 643 mv.

When xanthomycin A was reduced to the tetrahydro derivative by catalytic hydrogenation and then oxidized with ceric sulfate an amount of oxidizing agent equivalent to only one mole of oxygen was consumed. The product, isodihydroxanthomycin A, could again be reduced with an equivalent amount of titanous chloride. The reversible system isodihydroxanthomycin A–tetrahydroxanthomycin A has the oxidation reduction potential of 633 mv. It is thus evident that there are two centers of unsaturation in xanthomycin A. One of these centers is irreversibly reduced by catalytic hydrogenation, the other is the quinonoid system which may be reversibly reduced and oxidized.

A comparison of the value of the oxidation–reduction potential of the system, xanthomycin A–dihydroxanthomycin A, with the values known for different types of quinone systems is given in Fig. 4. From these certain conclusions may be drawn regarding the nature of the quinonoid grouping in xanthomycin A. The value of 643 mv. obtained with xanthomycin A is much higher than the potentials of naphthoquinones and anthraquinones, thus precluding the presence of such a structure in xanthomycin A. Taking into consideration the effect of substituents in the molecule on the potentials, it is probable that the parent quinone corresponding to xanthomycin A has a higher potential than that of xanthomycin A. This inter-

(4) E. Schjänberg, *Svensk Kem. Tid.*, **52**, 185 (1940).

(5) D. T. Ewing, J. M. Vandenbelt and O. Kamm, *J. Biol. Chem.*, **131**, 345 (1939).

pretation makes it appear likely that a benzoquinone-like ring system exists in xanthomycin A.

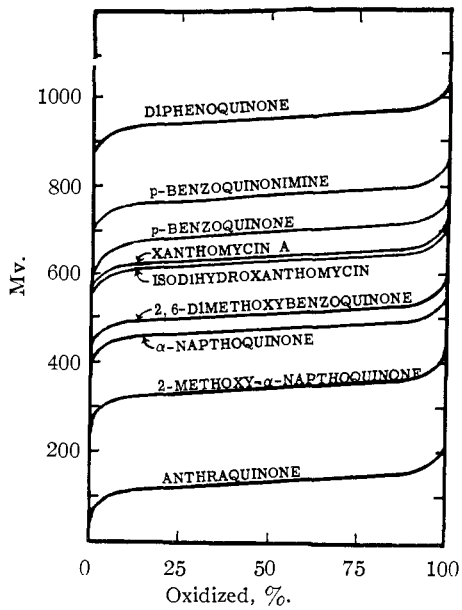

Fig. 4.—Comparison of the oxidation-reduction potentials of xanthomycin A–dihydroxanthomycin A with those of various quinone systems.

Experimental

Dihydroxanthomycin A Sulfate.—Sulfur dioxide gas was bubbled through a solution of 50 mg. of xanthomycin A in 0.5 ml. of water for 5 minutes. The initial orange-red color of the solution changed to pale yellow. The reaction mixture was evaporated to dryness out of contact with air under vacuum, the residue was triturated with absolute ethanol and filtered. The product was redissolved in methanol and precipitated with ethanol whereupon it separated as a light yellow microcrystalline powder.

Anal. Calcd. for $C_{23}H_{31-33}N_3O_7 \cdot H_2SO_4$: S, 5.72. Found: S, 5.60.

Solutions of dihydroxanthomycin A sulfate in water or alcohol turned deep yellow due to atmospheric oxidation.

Reduction of xanthomycin A could also be done with stannous chloride or titanous chloride, but isolation of the product from the reaction mixture was not feasible. The reduction product could be reoxidized with ceric sulfate. Quantitative data showed equal quantities of reducing and oxidizing agent were consumed in the two operations.

Tetrahydroxanthomycin A Hydrochloride.—Fifty mg. of xanthomycin A hydrochloride was dissolved in ethanol and to this was added 50 mg. of Adams platinum oxide catalyst. The mixture was stirred with a magnetic stirrer in the presence of hydrogen. After one hour the hydrogenation was stopped and a small piece of Dry Ice was added to the reaction mixture to provide an inert atmosphere. Anhydrous ether was added drop by drop to the ethanolic solution until there was a slight opalescence. This prevents the platinum from forming a colloidal suspension and passing through the filter. The mixture was then filtered through an asbestos mat. Pieces of Dry Ice were placed on the filter as well as in the receiving tube to exclude air. Washing the residue was done with a mixture of absolute ethanol and ether (4:1) to avoid the formation of colloidal platinum. The filtrate was then treated with excess of ether and the amorphous colorless precipitate, tetrahydroxanthomycin A hydrochloride, was filtered off, washed with ether and dried.

Anal. Calcd. for $C_{23}H_{33-35}N_3O_7 \cdot 2HCl$: Cl, 13.21. Found: Cl, 13.50.

When an inert atmosphere was not used during the processing of the reduction product, the alcoholic solution rapidly turned yellow. The hydrochloride of the tetrahydroxanthomycin A was found to be somewhat more stable to oxidation than the free base itself.

Isodihydroxanthomycin A Hydrochloride.—A solution of xanthomycin A in ethyl acetate was hydrogenated catalytically (two moles of hydrogen was taken up per mole of xanthomycin A) and the mixture filtered to remove the catalyst. The filtrate was shaken with air for 15 minutes, when the sparingly soluble isodihydroxanthomycin A separated out as an orange-red powder. It was converted into the hydrochloride by the addition of a slight excess of ethanolic hydrochloric acid and was precipitated by the addition of ethyl acetate. The hydrochloride was obtained as a yellow amorphous powder.

Anal. Calcd. for $C_{23}H_{31-33}N_3O_7 \cdot 2HCl$: Cl, 13.26. Found: Cl, 13.60.

The hydrochloride of isodihydroxanthomycin A gave a light yellow color in acidic, and bright pink color in alkaline solutions.

Chloroplatinate of Acetyl Tetrahydroxanthomycin A.—One hundred mg. of xanthomycin A hydrochloride was dissolved in a mixture of 1 ml. of glacial acetic acid and 5 ml. of acetic anhydride and hydrogenated in the presence of 50 mg. of Adams platinum oxide catalyst. After two hours of stirring at room temperature, the reaction mixture was heated to 70–80° and held at this temperature for four hours. The mixture was filtered, the catalyst washed with absolute ethanol and the filtrate evaporated to dryness. The residue was dissolved in ethanol and treated with an alcoholic solution of chloroplatinic acid. The precipitated chloroplatinate was filtered off, washed with ethanol and crystallized from aqueous ethanol. It separated as pale yellow, narrow, rectangular plates melting with decomposition above 300°.

Anal. Calcd. for $C_{23}H_{29-31}N_3O_7(CH_3CO)_4 \cdot H_2PtCl_6$: C, 35.74; H, 4.16; N, 4.04; Pt, 18.74; CH_3CO, 16.53. Found: C, 35.73; H, 4.43; N, 3.80; Pt, 19.99; CH_3CO, 18.16.

The hydrochloride of acetyltetrahydroxanthomycin A was obtained as a colorless amorphous powder readily soluble in water. On addition of alkali and shaking, the solution assumed a bright pink color.

Chloroplatinate of Methyltetrahydroxanthomycin A.—Half a gram of xanthomycin A hydrochloride was dissolved in ethanol and hydrogenated in the presence of 300 mg. of platinum oxide at room temperature. Two ml. of dimethyl sulfate and 5 g. of finely powdered potassium carbonate were added and the stirring continued in the presence of hydrogen for 12 hours. Additional quantities of platinum oxide catalyst (200 mg.), dimethyl sulfate (2 ml.) and potassium carbonate (4 g.) were added and the reaction was continued for another 12 hours in a hydrogen atmosphere. The mixture was filtered, the residue washed with ethanol and the filtrate evaporated to dryness. The methyl ether left behind was separated from the inorganic impurities by solution in chloroform, drying the solution over sodium sulfate and filtration. On evaporating the chloroform solution, the methyl ether was obtained as a light grey amorphous powder.

The methyl ether was dissolved in ethanolic hydrochloric acid and treated with an alcoholic solution of chloroplatinic acid. The chloroplatinate was filtered, washed with alcohol and crystallized from aqueous ethanol. It separated out as a light yellow microcrystalline powder.

Anal. Calcd. for $C_{25}H_{37-39}N_3O_7 \cdot H_2PtCl_6$: C, 33.25; H, 4.21; N, 4.65; Pt, 21.61. Found: C, 33.65; H, 4.48; N, 4.97; Pt, 23.19.

The hydrochloride was obtained by precipitation of an ethanolic solution of ethyl acetate and yielded a colorless amorphous powder.

Anal. Calcd. for $C_{25}H_{37-39}N_3O_7 \cdot 2HCl$: C, 53.09; H, 7.08; N, 7.43; Cl, 12.56; OCH_3 for 3, 16.46; NCH_3 for 1, 2.63. Found: C, 53.31; H, 7.49; N, 8.02; Cl, 14.27; OCH_3, 16.33; NCH_3, 3.74.

Oxidation-Reduction Potentials of Xanthomycin A.—Measurement of the reduction potentials was carried out by means of a bright platinum electrode and a silver-silver chloride half cell connected by a 1 *N* potassium chloride salt bridge. A Leeds-Northrup *p*H indicator was used for measuring the voltages. The substances were dissolved in 1 *N* hydrochloric acid and titrated with 0.01 *N* solutions of titanous chloride or ceric sulfate in 1 *N* hydrochloric acid. Nitrogen was passed through the titration vessel to prevent atmospheric oxidation and to mix the reactants. The data are given in Fig. 4.

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