IVb which had been prepared from IIIb. The infrared spectra of the two samples of IVb were identical.

Methyl 3α,20α-Diacetoxy-11-oxo-5β-pregnan-21-oate (IVa) from XVa.—Treatment of 92.8 mg. (0.20 mmole) of methyl 3α,20α-diacetoxy-11β-hydroxy-5β-pregnan-21-oate with chromic acid as described in the previous paragraph (except that the reaction period was 45 min.) gave 64 mg. (m.p. 194–195°) of product. Its infrared spectrum was identical with that of IVa which had been prepared from IIIa.

Acknowledgment.—We wish to acknowledge several helpful suggestions from Dr. H. L. Mason during the course of this investigation.

The Muconomycins. I. Studies on the Structure of Muconomycin A, a New Biologically Active Compound[1]

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The chemistry of Muconomycin A has been investigated. This compound can be reduced catalytically to what appears to be a hexahydro derivative and may be acetylated to a diacetate. Basic hydrolysis yields three products. The structure of two of these is discussed.

During a search for new fungicides from natural sources, a crystalline compound was isolated from the cultures of the mold *Myrothecium verrucaria*.[2] This substance, which has been designated as Muconomycin A, proved to be quite interesting because of its high antifungal activity, its extreme toxicity (0.5–0.75 mg./kg.) to albino mice,[3] and the fact that it possesses allergic properties which result in skin irritation on contact. Recent studies by Guarino showed that some of the toxic properties are manifested by severe creatinurea in vitamin E deficient albino rats, a fact which is indicative of a possible interference with oxidative phosphorylation.[3]

This paper describes some studies on the chemistry of this biologically active compound.

Muconomycin A (I), when purified either by repeated recrystallization from acetone–water or by chromatography on alumina, yielded clear, colorless plates which turned yellow at 240° and slowly decomposed over a wide temperature range. An infrared spectrum of the antibiotic shows a main carbonyl peak at 1725 cm.$^{-1}$ with shoulders at 1710 and 1740 cm.$^{-1}$, a hydroxyl peak at 3557 cm.$^{-1}$, and double bond bands at 1637 and 1591 cm.$^{-1}$. The antibiotic is characterized by a single peak in the ultraviolet spectrum at 258.5 mμ (ε 21,200). A molecular weight determination by boiling point elevation in acetone showed the molecular weight to be 496 ± 15. A formula of $C_{27}H_{34}O_9$ (mol. wt., 502.5) was assigned based on this molecular weight and its elemental analysis. A methyl determination was found to be 8.34% methyl, which corresponds to at least three methyl groups for a compound with a molecular weight of 502.5. The molecule contains one active hydrogen and no methoxyl or ethoxyl groups. No derivative could be obtained with carbonyl reagents; thus it was concluded that the main carbonyl peak of I is that of an ester group, a conclusion which was supported by titration with base. The saponification equivalent of Muconomycin A was found to be 168 as compared with an expected value of 167 based on three ester groups.

When I was hydrogenated over Adam's catalyst, a compound was isolated which recrystallized from ether as colorless crystalline clusters, m.p. 145–146.5°. Elemental analysis suggested that the reduced material is most likely a hexahydro derivative, though the possibility that it is a tetrahydro derivative could not be eliminated on the basis of this analysis alone. In one experiment in which I was reduced with hydrogen at atmospheric pressure over palladium on charcoal, 2.80 moles of hydrogen were absorbed per mole of I.

An infrared spectrum of the reduced material shows a single carbonyl peak at 1742 cm.$^{-1}$ and no evidence of unsaturation. It was concluded from these observations that I contains at least one double bond in conjugation with an ester carbonyl.

Muconomycin A formed an acetate readily when treated with acetic anhydride and pyridine at steam bath temperatures. It is interesting to note that though I contains only one active hydrogen, it forms a diacetate.

When the antibiotic was subjected to hydrolysis with dilute base, three principal fragments were isolated from the reaction mixture. One of these was a dicarboxylic acid which was identified as one of the geometrical isomers of muconic acid by analysis, the infrared and ultraviolet spectra, and the fact that it consumed two moles of hydrogen on catalytic reduction with the formation of adipic acid. The preparation of the benzhydryl ester showed it to be the *cis–trans* isomer (II) (m.p. 142.5–143° as reported[4]), a conclusion which was confirmed by synthesis by conventional methods.[4,5]

In addition to *cis,trans*-muconic acid, two alcohols were isolated from the hydrolysis reaction of I. These were designated as alcohol A (III) and alcohol C (IV).

Alcohol A crystallized from ether in the form of flat needles, m.p. 156–157°. The infrared spectrum shows

(1) The research on elucidation of the structure of Muconomycin A, herein reported, was carried out for the most part at the laboratories of the Rohm and Haas Company, Bristol, Pa. Further characterization of the physical properties of degradation products and derivatives of Muconomycin A was carried out at the University of Rhode Island, supported in part by P.H.S. research grant E-4352 from the National Institutes of Health, Public Health Service.

(2) Patent application allowed, Smythe-Kraskin, assigned to the Rohm and Haas Co. The organism has been deposited with the American Type Culture Collection, Washington, D. C., and has been assigned the number ATCC 13667.

(3) A. Guarino, Chemistry Department, University of Rhode Island, unpublished data. This value is in confirmation of the LD_{50} previously determined under Rohn and Haas sponsorship.

(4) J. A. Elvidge, R. P. Linstead, P. Sims, and B. A. Orkin, *J. Chem. Soc.*, 2235 (1950).

(5) J. Pospishil and V. Ettel, *Chem. Prumysl*, **7**, 244 (1957).

hydroxyl bands at 3550 and 3300 cm.$^{-1}$, a weak double bond band at 1690 cm.$^{-1}$, and a band at 1381 cm.$^{-1}$ assignable to the vibrations of a C–CH_3 group. No significant ultraviolet absorption was noted in the range 220–350 mμ. A molecular formula of $C_{15}H_{22}O_4$ was assigned based on its elemental analysis and molecular weight determination. The molecule contains two active hydrogens and three or four methyl groups as determined by analysis.

Alcohol A when treated with acetic anhydride and pyridine formed an acetate, m.p. 147–148°. Assuming a molecular weight of about 270 for the alcohol, a molecular formula of $C_{19}H_{26}O_6$ was calculated for the acetate based on its elemental analysis. The per cent acetate calculated as CH_3CO– was found to be 24.20 which is in good agreement with the expected value of 24.56% for a diacetate. The infrared spectrum of the acetate showed no hydroxyl bands and led to the conclusion that the remaining oxygen atoms are in the form of ethers.

Alcohol C is soluble in water and most organic solvents. It crystallizes from ether in the form of long needles, though an analytical sample is best prepared by sublimation, m.p. 102.5–103°. In the alkaline hydrolysis of Muconomycin A, IV is isolated by extraction with chloroform only after the reaction mixture has been made strongly acidic, a fact which indicates the presence of a lactone ring. The most prominent features of the infrared spectrum of alcohol C are strong bands at 3350 cm.$^{-1}$ (potassium bromide) or 3460 cm.$^{-1}$ (bromoform) (hydroxyl band) and at 1730 cm.$^{-1}$ (potassium bromide or bromoform) (ester). Only end absorption was observed in the ultraviolet spectrum.

No carbonyl derivative was obtained with either *p*-nitrophenylhydrazine or semicarbazide hydrochloride, and ammoniacal silver was not reduced in the Tollens test.

The titration curve of IV is similar to that reported[6] for mevalonic acid lactone and indicates an equivalent weight of 128 which was used with the elemental analysis to calculate a formula of $C_6H_{10}O_3$. Following the titration, the starting material was isolated unchanged providing further evidence that the alcohol and carboxyl groups are present in the same molecule. The infrared spectrum of alcohol C in morpholine was taken at intervals over a period of two hours and changes in the carbonyl region noted. During this time the band at 1742 cm.$^{-1}$ (lactone) disappeared and a new band simultaneously formed at 1640 cm.$^{-1}$ (tertiary amide) and became intensified. These observations clearly indicate the presence of a lactone ring system. A δ-lactone rather than a γ-lactone is more consistent with the infrared spectrum.

A similar morpholinolysis experiment has been described for the isomeric mevalonic acid lactone, and it was reported that at least forty-eight hours was required to open this lactone ring.[7] We found that approximately the same length of time was necessary for the morpholinolysis of δ-valerolactone.

In an attempt to prepare a solid derivative, IV was treated with various amines. A crystalline amide, however, was obtained only with benzhydrylamine.

(6) D. E. Wolf, C. H. Hoffman, P. E. Aldrich, H. R. Skeggs, L. D. Wright, and K. F. Folkers, *J. Am. Chem. Soc.*, **79**, 1486 (1957).

(7) D. E. Wolf, C. H. Hoffman, P. E. Aldrich, H. R. Skeggs, L. D. Wright, and K. Folkers, *ibid.*, **78**, 4499 (1956).

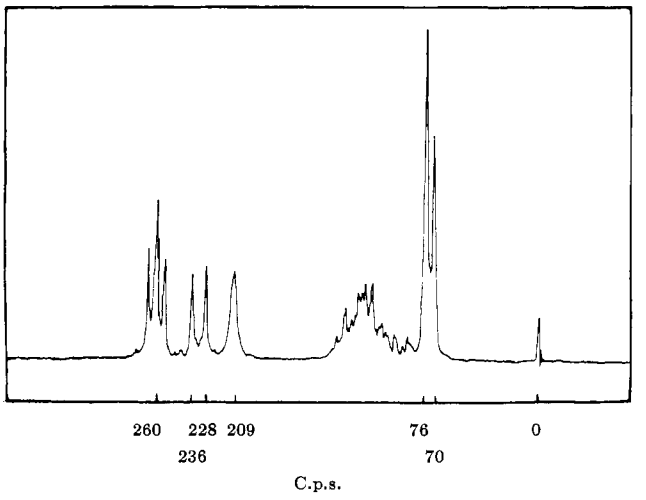
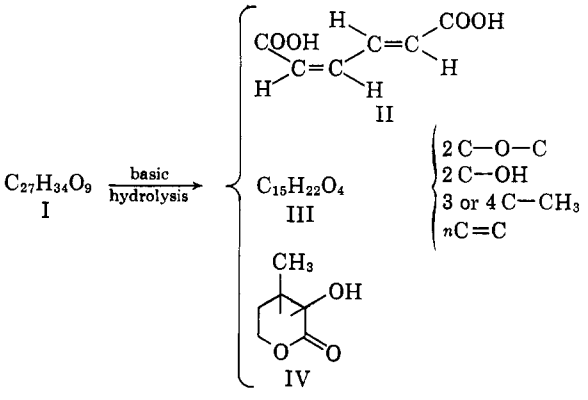


Fig. 1.—Nuclear magnetic resonance spectrum of alcohol C at 60 Mc. with tetramethylsilane as internal standard.

Methyl analysis of IV was indicative of the presence of at least one methyl group. The position of the methyl and hydroxyl groups on the δ-lactone ring of alcohol C remained to be determined. IV gave a negative iodoform reaction and hence the methyl group would not be expected to be in the δ-position. The periodate reaction was inconclusive.

Based on the reactivity of the carbonyl group of alcohol C, the hydroxyl group was tentatively assigned to the α-position. The final assignment for the positions of the methyl and hydroxyl groups was based on the nuclear magnetic resonance (n.m.r.) spectrum of the compound.



The 60-Mc. n.m.r. spectrum of IV (Fig. 1) shows signals from the methyl group at 76 and 70 c.p.s. relative to tetramethylsilane as internal standard. The doublet signal is produced by spin coupling of the methyl group with a single proton on the adjacent carbon atom and confirms the presence of the grouping CH_3–CH in the molecule. Since the methyl and hydroxyl groups must be on different carbon atoms there would be statistically twelve different position isomers of hydroxymethyl-δ-valerolactone. An examination of the expected low field pattern for each isomer reveals that the pattern for β-methyl-α-hydroxy-δ-valerolactone seems to fit the spectrum of IV the best. The signal at 209 c.p.s. is from the hydroxyl group. The doublet signal at 228 and 236 c.p.s. is from the proton on the α-carbon containing the hydroxyl group and is split into bands by spin coupling with the single proton on the β-carbon atom. The triplet signal cen-

tered around 260 c.p.s. is from the $-CH_2-$ group in the δ-position α to the ring oxygen. The remaining three protons are found in the multiplet between 209 and 76 c.p.s. A 40-Mc. spectrum taken before and after adding benzenesulfonic acid to the solution facilitated the location of the hydroxyl proton band and showed that this proton exchanges sufficiently fast so that it does not spin couple with any neighboring protons. Furthermore, the triplet–doublet pattern assumed for the α-hydroxyl-β-methyl-δ-valerolactone structure was confirmed by comparison of the coupling constants from the 40- and 60-Mc. spectra.

In an early experiment in which Muconomycin A was subjected to basic hydrolysis with 20% sodium hydroxide in aqueous ethanol, two compounds were isolated. One of these was *cis,trans*-muconic acid. The other was a white, crystalline solid melting at 151–151.5° and was designated as alcohol B. Infrared analysis indicates the presence of a primary alcohol and a *gem*-dimethyl group. No carbonyl bands are present in the spectrum. Alcohol B was obtained from only one reaction and attempts to isolate this compound from subsequent reaction mixtures were not successful.

Further efforts are being made to elucidate the structure of Muconomycin A and other closely related compounds and additional reports are forthcoming.

Experimental[8-10]

Muconomycin A.[11]—Muconomycin A was obtained as a semipure crystalline solid which was purified further by chromatography on alumina. Recrystallization from acetone–water yielded small colorless plates which decomposed slowly over a wide range above 240° with $[\alpha]^{18}D$ +184°, λ_{max} 258.5 mμ (ϵ 21,200).

The infrared spectrum of the antibiotic shows a main carbonyl peak at 1725 cm.$^{-1}$ with shoulders at 1710 and 1740 cm.$^{-1}$, a hydroxyl peak at 3557 cm.$^{-1}$, and double bond absorption at 1637 and 1591 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{34}O_9$: C, 64.53; H, 6.82; O, 28.65. Found: C, 64.40; H, 6.77; O, 28.79.

Reduction of Muconomycin A.—A solution of 993.9 mg. (1.98×10^{-3} mole) of Muconomycin A in 150 ml. of absolute ethanol was placed in a hydrogenation flask. A small quantity of Adam's catalyst was added and the mixture reduced in a Parr apparatus at room temperature and 35-lb. pressure. The reduction was stopped at the end of 1 hr. The catalyst was removed by filtration and the solvent by evaporation at reduced pressure at about 45°. A colorless amorphous solid remained as residue. The residue was taken up in acetone and induced to crystallize by the addition of water. Small plates were obtained, yield 420.1 mg., m.p. 106–108.5°. After many recrystallizations from acetone–water the melting point was not improved. The solid was then recrystallized from ether. Crystalline clusters were obtained which after two further recrystallizations melted at 145–146.5°, $[\alpha]^{18}D$ +19°, wt., 100 mg.

Anal. Calcd. for $C_{27}H_{40}O_9$: C, 63.76; H, 7.93. Found: C, 63.80; H, 7.76.

In one experiment in which I was reduced quantitatively with hydrogen over palladium on charcoal at atmospheric pressure, 2.80 moles of hydrogen were absorbed per mole of I.

The infrared spectrum of this reduced material shows a single carbonyl peak at 1742 cm.$^{-1}$. No double bond bands appear in the 1600-cm.$^{-1}$ region. Only end absorption appeared in the ultraviolet spectrum.

Acetylation of Muconomycin A.—Muconomycin A (243.7 mg., 4.85×10^{-4} mole) was placed in a 50-ml. round-bottomed flask with 7 ml. of anhydrous pyridine and 5.0 ml. of acetic anhydride. The solution was heated on a steam bath for 15 min. and then evaporated at reduced pressure at about 50°. The viscous oil that remained as residue was taken up in chloroform and the solution washed with dilute (3 *N*) sulfuric acid and then with water. The chloroform layer was separated and dried over anhydrous magnesium sulfate. After removal of the drying agent and solvent, a colorless amorphous solid remained as residue. This material was purified by chromatography on alumina followed by recrystallization from ethanol–water. The acetate was obtained in low yield as small needles which decomposed slowly on heating above 230°.

Acetate analysis showed 13.78% acetate to be present indicating that a diacetate had formed (calculated for two acetates, 14.67%).

Anal. Calcd. for $C_{31}H_{38}O_{11}$: C, 63.47; H, 6.53; mol. wt., 586.6. Found: C, 63.24; H, 6.68; mol. wt., 593, 581.

The infrared spectrum of Muconomycin A diacetate had carbonyl peaks at 1752 cm.$^{-1}$ due to the acetate and at 1726 cm.$^{-1}$ assignable to a conjugated ester.

Infrared and elemental analysis of other fractions from the chromatogram indicated that other acetates were formed in the reaction, though as yet none of these have been obtained in pure form.

Hydrolysis of Muconomycin A with Sodium Hydroxide.—Muconomycin A (959.9 mg., 1.91×10^{-3} moles) was placed in a 100-ml. flask with 15 ml. of 3% aqueous sodium hydroxide and 3 ml. of ethanol. A reflux condenser was attached and the mixture heated on a steam bath gently for 30 min. The reaction mixture was then cooled to room temperature and was extracted thoroughly with chloroform. The combined extracts were dried over anhydrous magnesium sulfate and the drying agent and solvent removed as usual. A colorless, clear amorphous solid remained as residue which crystallized immediately on standing at room temperature. The residue was dissolved in chloroform and recrystallized from ether–chloroform solution, yield 401.2 mg. of flat needles, m.p. 155.5–156°. A highly purified sample melted at 158–158.5°, $[\alpha]^{19}D$ −55°.

The infrared spectrum (potassium bromide) shows hydroxyl bands at 3550 and 3300 cm.$^{-1}$, a weak double bond band at 1690 cm.$^{-1}$, and a band at 1381 cm.$^{-1}$ assignable to the vibrations of a C–CH_3 group. No significant absorption appeared in the ultraviolet spectrum.

The molecular weight (Rast) of III was found to be 270 and 280 in two determinations.

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.67; H, 8.33; O, 24.03; mol. wt., 266.3. Found: C, 67.38; H, 8.38; O, 23.93.

The basic reaction mixture was made strongly acidic (about pH 2) and extracted with ether several times. The combined extracts were dried over anhydrous magnesium sulfate. After removal of the drying agent and solvent, a powder with a yellow tint remained as residue. The yellow tint was removed by washing with chloroform, yield 184 mg., m.p. 187–188° dec. A highly purified sample was obtained by dissolving the powder in 0.1 *N* sodium hydroxide and reisolating the free acid from the

(8) All melting points are corrected. The infrared spectra were determined in part by Walter Smith and Vincent Pierro of the Rohm and Haas Company, Bristol, Pa. The microanalyses were performed by Clyde Nash, Rohm and Haas Company, Bristol, Pa., Clark Microanalytical Laboratory, Urbana, Ill., Pascher and Pascher Microanalytical Laboratory, Bonn, Germany, and Micro-analysis, Inc., Wilmington, Del. The molecular weight of Muconomycin A was determined by Harry Mason, Rohm and Haas Co., Philadelphia, Pa.

(9) The infrared spectra were obtained on either a Perkin-Elmer Model 21 spectrophotometer or on a Baird-Atomic Model KM-1 recording spectrophotometer and were taken in carbon tetrachloride unless otherwise indicated; the ultraviolet spectra were obtained on a Beckman DK-2 recording spectrophotometer. Rotations were taken on a Rudolph Precision polarimeter.

(10) All rotations and ultraviolet spectra were taken in methanol solution.

(11) The following procedure for obtaining Muconomycin A was described in a personal communication from C. Smythe, Rohm and Haas Co., Bristol, Pa. The organism was grown in a medium containing 1.0% glucose, 0.5% rolled oats, 0.1% Bacto-peptone, 0.1% Difco yeast extract, 0.05% K_2HPO_4, and 0.02% $MgSO_4 \cdot 7H_2O$. The medium was adjusted to pH 7.0 with sodium hydroxide, dispensed in 100-ml. portions into 1-l. wide-mouthed erlenmeyer flasks and sterilized at 15 p.s.i. for 30 min. After inoculation with 1.0% of aqueous spore suspension derived from a well sporulated potato-dextrose-agar slant of ATCC 13667, incubation was carried out on a shaker rotating at 260 r.p.m. at 26° for 72 hr. The concentration of Muconomycin was about 100 μg./ml.

The mycelia was then removed by filtration with the aid of diatomaceous earth and the filtrate treated with about 0.3% of Darco G60. The Darco adsorbate was collected on a filter, dried in air, and the activity eluted with benzene in a Soxhlet extractor. After evaporation of the benzene, a yellow oil remained which was taken up in acetone and induced to crystallize by the addition of water.

acidified solution. In this way a sample melting at 190.5–191°, λ_{max} 259 (ϵ 24,600) was obtained.

The neutralization equivalent was found to be 72.5 as determined by titration with 0.1 *N* sodium hydroxide. Assuming two equivalents to be present in the molecule, a molecular formula of $C_6H_6O_4$ was calculated on the basis of its elemental analysis.

Anal. Calcd. for $C_6H_6O_4$: C, 50.71; H, 4.26. Found: C, 50.59; H, 4.35.

The infrared spectrum (potassium bromide) of this material shows broad absorption in the 3000-cm.$^{-1}$ region, a carbonyl band at 1680 cm.$^{-1}$, double bond bands at 1625 and 1600 cm.$^{-1}$, and is superimposable on a spectrum of a synthetic sample of *cis,trans*-muconic acid.

The acidified reaction mixture was then extracted many times with chloroform until no more material could be removed. The extracts were combined and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent by distillation at reduced pressure. A colorless, clear oil remained which crystallized immediately on standing at room temperature. This residue was purified by sublimation at 65° at 15-mm. pressure, yield 98.8 mg., m.p. 98–101°. A highly purified sample melts at 102.5–103°, $[\alpha]^{18}D$ −17.2°.

The infrared spectrum of this material shows strong bands at 3350 cm.$^{-1}$ (potassium bromide) or 3460 cm.$^{-1}$ (bromoform) (hydroxyl band) and at 1730 cm.$^{-1}$ (potassium bromide or bromoform) (ester). No evidence of unsaturation is present in the 1600–1700-cm.$^{-1}$ region of the spectrum.

The equivalent weight of this material was found to be 128 by potentiometric titration.

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75; O, 36.88. Found: C, 55.12; H, 7.78; O, 36.82.

Catalytic Reduction of the Dicarboxylic Acid (II).—The dicarboxylic acid (57.4 mg.) was reduced over palladium on charcoal in ethanol. Two moles of hydrogen were absorbed per mole of acid. The product was a white solid (35.4 mg.) which melted at 152–153° after one recrystallization from ether.

The infrared spectrum of the reduced material is superimposable on that of adipic acid. A mixture melting point determination with an authentic sample of adipic acid showed no depression.

Synthesis of *cis,trans*-Muconic acid.—*cis,cis*-Muconic acid was prepared by a procedure adapted from that of Pospishil and Ettel.[5] Acetic anhydride (50 g.) and glacial acetic acid (51 g.) were placed in a 1-l., four-necked flask fitted with reflux condenser and drying tube, mechanical stirrer, dropping funnel, and thermometer. Then while the temperature was kept at 30°, 40 g. of 90% hydrogen peroxide was added rapidly, and the solution stirred for 10 min. Pyrocatechol (50 g.) was dissolved in acetic anhydride (100 g.) and the resulting solution was added to the reaction mixture dropwise over a period of 60 min. The temperature was maintained at 30° with an ice bath. After 1 hr. 10 ml. of a concentrated solution of manganous acetate and cupric acetate (1:1 ratio) in acetic anhydride was added. Stirring at 30° was continued for another 3 hr. The mixture was stored in the dark for 4 days and the *cis,cis*-muconic acid collected by filtration, yield 24.8 g. of product, m.p. 184–185° (reported[12] m.p. 184°). Recrystallization from methanol did not improve the melting point.

cis, trans-Muconic acid was prepared from *cis,cis*-muconic acid by recrystallization from water. *cis,cis*-Muconic acid (15 mg.) was placed in a small flask with 5 ml. of distilled water. The mixture was heated under reflux on a steam bath for 1 hr. The acid dissolved rapidly on heating. When the reaction mixture was cooled, a white granular solid separated, m.p. 181–182.5° (reported[12,13] m.p. 184 or 190–191°). The infrared spectrum of this material is consistent with that expected for *cis,trans*-muconic acid.

(12) J. A. Elvidge, R. P. Linstead, B. A. Orkin, P. Sims, H. Baer, and D. B. Pattison, *J. Chem. Soc.*, 2228 (1950).

(13) The melting point has been found to vary with the rate of heating.

Benzhydryl Ester of *cis,trans*-Muconic Acid (II).—The benzhydryl ester of the dicarboxylic acid (II) was prepared according to the method described by J. A. Elvidge, *et. al.*[4] Yellow mercuric oxide (2.23 g.) was placed in a vial with 2.19 g. of benzophenone hydrazone and 20 ml. of a 1:1 solution of pentane–hexane added. A wet cloth was placed around the vial and then it was shaken for 6.5 hr. After this time, the mercury and any unchanged benzophenone hydrazone was removed by filtration. To 10 ml. of the filtrate was added a solution of 0.20 g. of *cis,trans*-muconic acid in 2 ml. of methanol and the mixture was kept in the dark for 65 hr. After this time the solvent was removed by evaporation at reduced pressure and the residue washed with methanol. After one recrystallization from methanol, a white powder was obtained, yield 0.6 g., m.p. 142.5–143.5°. One further recrystallization yielded a white powder melting at 144.5–145° (reported[4] m.p. 143.5°).

Anal. Calcd. for $C_{32}H_{26}O_4$: C, 80.99; H, 5.52. Found: C, 81.02; H, 5.47.

Acetylation of Alcohol A.—In a small one-necked flask was placed 71.5 mg. of Alcohol A. To this was added 4 ml. of anhydrous pyridine and 3 ml. of acetic anhydride and the mixture heated on a steam bath for 10 min. The solution was then cooled to near room temperature and 2 ml. of water added. The solvent then was removed by distillation at reduced pressure. The oil which remained as residue was taken up in chloroform and the solution washed twice with dilute sulfuric acid and twice with water. The organic layer was separated and dried over anhydrous magnesium sulfate. When the drying agent and solvent were removed, an oil remained as residue. The residue was taken up in a benzene–hexane (4:1) solution and the acetate purified by chromatography on alumina. The fractions containing product were combined and dissolved in chloroform. The chloroform was replaced by ethanol from which the product was induced to crystallize by the addition of water. Small prisms were obtained, yield 45 mg., m.p. 145–146°. After one further recrystallization a sample melting at 147–148° was obtained.

Anal. Calcd. for $C_{19}H_{26}O_6$: C, 65.12; H, 7.48; mol. wt., 350.4. Found: C, 65.19; H, 7.27; mol. wt., 335 (Rast). The per cent acetate was found to be 24.40.

The infrared spectrum of the acetate contains strong bands at 1749 and 1241 cm.$^{-1}$ attributable to vibrations of the acetate group. No hydroxyl band is evident in the spectrum.

Benzhydryl Amide of Alcohol. C.—Alcohol C (16 mg.) was placed in a vial with 0.5-1 ml. of benzhydrylamine. The vial was stoppered and the solution was heated on a steam bath for 1 hr. The reaction mixture was then dissolved in 10 ml. of chloroform and the resulting solution washed with a 0.1 *N* hydrochloric acid solution and then with water until the washings were nearly neutral to litmus. The chloroform solution was then dried over anhydrous magnesium sulfate and the drying agent and solvent removed as usual. An oil with a yellow tint remained as residue which crystallized on standing at room temperature. The crystalline residue was dissolved in benzene and the product was induced to crystallize by the addition of hexane and cooling. Clusters of crystals formed, yield 20 mg., m.p. 117.5–118°.

Infrared analysis (chloroform) showed the presence of a monosubstituted amide (1510 and 1662 cm.$^{-1}$) and a hydroxyl group (3350 cm.$^{-1}$).

Anal. Calcd. for $C_{19}H_{23}NO_3$: C, 72.82; H, 7.40; N, 4.47. Found: C, 72.96; H, 7.43; N, 4.38. C-Methyl analysis indicated the presence of one methyl group (4.80%).

Acknowledgment.—The author is indebted to Dr. Keith McCallum of the Rohm and Haas Company for his valuable assistance in the interpretation of the nuclear magnetic resonance spectra and to the Rohm and Haas Company for the generous supply of Muconomycin A.