Compounds III and IV.—To a paste of 30 mg. of I in chloroform was added excess hydriodic acid (sp. gr. = 1.7). After refluxing for 1 hr., the dark red crystalline material had changed to a fine emerald green solution. The solution was cooled, diluted with water, and extracted with chloroform. The chloroform layer was washed with a saturated sodium thiosulfate solution and water, dried over anhydrous sodium sulfate, and concentrated to dryness; 25 mg. of the crude product was obtained. Recrystallization from chloroform afforded III as dark red needles, m.p. >300°. Further purification of III was carried out by acetylation with a mixture of acetic anhydride and pyridine. The acetate (IV) of I was recrystallized from benzene and dried at 160° for 20 hr. under high vacuum; m.p. 295–297°.

Anal. Calcd. for $C_{46}H_{34}O_{20}$: C, 60.91; H, 3.77. Found: C, 61.00; H, 3.48.

Erythroglaucin (V).—To a suspension of 10 mg. of finely powdered I in 50 ml. of saturated sodium carbonate solution was added 500 mg. of sodium dithionite (under nitrogen). The solution was warmed on a boiling water bath for 10 min. After removal of unchanged I by filtration, the filtrate was acidified with hydrochloric acid and the acidic solution was extracted with benzene. The benzene layer was washed several times with water, dried over anhydrous sodium sulfate, and concentrated to dryness. The product was purified by paper chromatography (30 × 30 cm.), using the upper layer of a mixture of acetone–petroleum ether (boiling range 50–90°)–water (5:5:3.5 by volume). A yellowish orange zone corresponding to an R_f value of 0.95–0.97 was extracted with benzene and concentrated to dryness; V was obtained by sublimation of the residue under high vacuum. It had m.p. 202° and showed no melting point depression with authentic erythroglaucin. V was also obtained from I by means of refluxing with excess lithium aluminum hydride in tetrahydrofuran solution for 2 hr.

Catenarin (VI).—VI was obtained from the 10 mg. of III by means of the same procedure as above for erythroglaucin; the R_f value of VI was 0.80 in the same solvent system.

Acknowledgment.—The author is grateful to Drs. S. Fujise, S. Mitsui, S. Hishida, M. Shibata, and S. Shibata for their encouragement, and to Drs. A. B. Anderson and L. Jurd for their advice and aid during this research.

The Muconomycins. II. Muconomycin B, a New Biologically Active Compound[1]

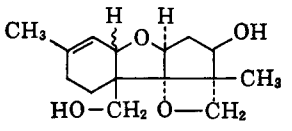
JACQUELINE S. VITTIMBERGA[2] AND BRUNO M. VITTIMBERGA

Department of Chemistry, University of Rhode Island, Kingston, Rhode Island, and The Rohm and Haas Company, Bristol, Pennsylvania

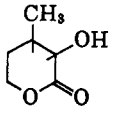
Received February 24, 1964

The chemistry of muconomycin B has been investigated. This antibiotic appears to be similar to muconomycin A but lacks one water molecule. A partial structure is proposed.

The chemistry of muconomycin A (I), an antibiotic obtained from cultures of the mold *Myrothecium verrucaria*[3] was recently reported in the literature.[4] We have since learned that this antibiotic is in fact identical with verrucarin A, a compound isolated by E. Härri, *et al.*,[5] and studied in detail by Tamm and Gutzwiller.[6,7] Hydrolysis of verrucarin A gave verrucarol to which formula II was assigned, verrucarinic acid lactone (III), and muconic acid which are, respectively, identical with alcohol A, alcohol C, and muconic acid isolated from muconomycin A.[4] Modification of the structure of verrucarol to IV was, however, recently proposed by Godtfredsen and Vangedal based on work done on the related compounds, trichothecin and trichodermin.[8]

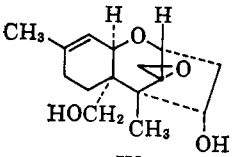


verrucarol (II)



verrucarinic acid lactone (III)

This paper describes another antibiotic obtained from *Myrothecium verrucaria*.[3] The new compound, termed muconomycin B (V), is active against the same



IV

microorganisms as I but was found to be approximately twice as potent against most organisms tested.[9] The mammalian toxicity of IV is comparable with that of I.[10]

Muconomycin B was obtained from broth cultures by benzene extraction and was purified by chromatography on alumina followed by recrystallization from ether. A microcrystalline solid was obtained which decomposed over a wide range above 235°. The infrared spectrum of V (potassium bromide) is similar to that of I though not identical. Bands are present at 1705 cm.$^{-1}$ due to vibrations of a conjugated unsaturated ester and at 1595 and 1650 cm.$^{-1}$ for a conjugated diene system. The ultraviolet spectrum is characterized by two maxima, one at 261 mμ (ε 22,000) and the other at 220.5 mμ (ε 21,600). Elemental analysis showed only carbon, hydrogen, and oxygen to be present in the molecule. Muconomycin B was found to contain one active hydrogen and at least three C–CH_3 groups, determined by analysis. No ethoxyl or methoxyl groups were found in the molecule. When V was subjected to catalytic reduction with hydrogen

(1) The work described in this paper was carried out largely at the University of Rhode Island and supported through P.H.S. Research Grant AI-04352-02 from the National Institutes of Health, Public Health Service.

(2) Postdoctoral Fellow, Jan. 1, 1962–Nov. 30, 1963.

(3) C. V. Smyth and K. S. Kraskin (to the Rohm and Haas Co.), U. S. Patent 3,087,859(1953). The organism has been deposited with the American Type Culture Collection, Washington, D. C., and has been assigned the number ATCC 13667.

(4) B. M. Vittimberga, *J. Org. Chem.*, **28**, 1786 (1963). This work was described previously in a patent application submitted by the Rohm and Haas Co. in March 1961 (U. S. Patent 3,087,859).

(5) E. Härri, W. Loeffler, H. P. Sigg, H. Stähelin, C. Stoll, C. Tamm, and D. Wiesinger, *Helv. Chim. Acta*, **45**, 840 (1962).

(6) C. Tamm and J. Gutzwiller, *ibid.*, **45**, 1726 (1962).

(7) J. Gutzwiller and C. Tamm, *ibid.*, **46**, 1786 (1963).

(8) W. O. Godtfredsen and S. Vangedal, *Proc. Chem. Soc.*, 188 (1964).

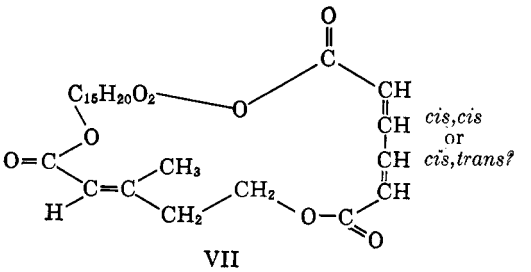
(9) Patent is applied for by Rohm and Haas Co., Philadelphia, Pa.

(10) A. Guarino, College of Pharmacy, University of Rhode Island, unpublished data.

over palladium on charcoal, 4 moles of hydrogen were absorbed per mole of compound. Hydrolysis of V with 3% sodium hydroxide in aqueous ethanol followed by extraction with chloroform yielded a crystalline compound which proved to be identical with alcohol A obtained from the hydrolysis of I.[4,11] Acidification of the reaction mixture and extraction with ether yielded *cis,trans*-muconic acid.[11] Further extraction of the acidified mixture with chloroform resulted in the isolation of a minute quantity of colorless oil which was shown by infrared analysis to be a mixture of unsaturated carboxylic acids. The ultraviolet spectrum of this oil showed one main area of absorption at 214 mμ characteristic of α,β-unsaturated carboxylic acids. A weak shoulder was present at about 260 mμ. Hydrolysis of V following catalytic reduction over Adams catalyst yielded three fragments. Two of these were found to be alcohol A and adipic acid, respectively.[11] The third fragment (VI) had an infrared spectrum which is superimposable on that of β-methyl δ-valerolactone. No significant absorption appeared in the ultraviolet spectrum. In order to establish the identity of VI the preparation of the benzhydryl amides of authentic β-methyl δ-valerolactone and VI was undertaken. The benzhydrylamide of VI, purified by chromatography on alumina followed by recrystallization from benzene–hexane, melted at 126–128°. The benzhydrylamide of β-methyl δ-valerolactone melted at 109–110.5°. The infrared spectra of both these amides are superimposable as are also the n.m.r. spectra. Furthermore, mass spectrometry showed identical fragmentation patterns for the two amides with the last peak at *m/e* 279 (P − H_2O).[12] From these observations it was concluded that the two benzhydrylamides are identical. The difference in melting points can be explained on the basis of the difference in the optical purity of the two compounds. It is expected that the racemic synthetic sample would melt at a lower point[13] than that derived from an optically active lactone obtained as a result of a stereospecific, catalytic reduction of V. Further attempts were made to isolate the lactone of 5-hydroxy-3-methyl-2-pentenoic acid from the hydrolysis of V, but these met with no success. It seems logical to assume, therefore, that the hydroxymethyl and carboxyl groups are situated *trans* to one another along the double bond of the unsaturated acid.

The ease with which *cis,cis*-muconic acid isomerizes to its more stable *cis,trans* isomer has been well established.[14] Hence, the failure to isolate the former from the hydrolysis of V does not preclude its presence in the intact molecule. Utilizing the structure of verrucarin proposed by Tamm and Gutzwiller[6] and assuming either structure II or IV for verrucarol, there are four basic structures that may be assigned to muconomycin B if *cis,trans*-muconic acid is present and only two structures if the *cis,cis* isomer is present.

Structure VII shows one of these possibilities. It is noteworthy that, although the molecular formulas of



VII

muconomycins A and B differ only by one molecule of water, the arrangement of fragments in the two molecules need not be the same. An attempt is now being made in this laboratory to relate the two compounds chemically and additional reports are forthcoming.

During the past several years, considerable activity has been directed toward the isolation of the many compounds produced by the various species of *Myrothecium*. Consequently some confusion has resulted from the assignment of different names to structurally similar compounds and in some instances to identical compounds. Glutinosin,[15] myrothecin,[16] compounds 379-X and 379-Y,[17] the muconomycins,[3,4] the verrucarins,[5-7] and the roridins[5] are all names that have been assigned to compounds produced by various species of *Myrothecium*. Of these muconomycin A and verrucarin A are identical, as discussed above, and it appears that compound 379-X and roridin A are also identical, as is evidenced by the similarity of the published physical properties. Several other compounds have been isolated from the same sources but these have not been named yet.[18]

Experimental[19-20]

Muconomycin B.[21]—Muconomycin B was obtained as a wettable powder from the Rohm and Haas Co. The antibiotic was extracted with acetone and partially purified by chromatography on alumina. Final purification by recrystallization from ether yielded a microcrystalline solid which decomposed over a wide range above 235°: $[\alpha]^{19}D$ +54° (benzene), λ_{max} 261 mμ (ε 22,000) and 220.5 mμ (ε 21,600). The infrared spectrum (KBr) showed a strong carbonyl peak at 1705 $cm.^{-1}$ and double-bond bands at 1595 and 1650 $cm.^{-1}$.

Anal. Calcd. for $C_{27}H_{32}O_8$: C, 66.92; H, 6.66; mol. wt., 484.5. Found: C, 67.01; H, 6.75; mol. wt., 512 ± 17 (ebullioscopic in acetone).

Hydrolysis of Muconomycin B.—Muconomycin B (403.6 mg., 0.833 × 10^{-3} moles) was placed in a small flask with 2 ml.

(11) This was shown by mixture melting point determination and infrared analysis.

(12) The mass spectra were determined by Mr. Maurice Bazinet, Analytical Laboratory, U. S. Army Natick Laboratories, Natick, Mass.

(13) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 43–47.

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

(15) P. W. Brian and J. C. McGowan, *Nature*, **157**, 334 (1946).

(16) A. Nespiak, M. Kocor, and A. Sieurnski, *ibid.*, **192**, 138 (1961).

(17) A. N. Kishaba, D. L. Shankland, R. W. Curtis, and M. C. Wilson, *J. Econ. Entomol.*, **55**, 211 (1962).

(18) J. P. Bowden and E. I. Schantz, *J. Biol. Chem.*, **214**, 365 (1955).

(19) All melting points are corrected. The infrared spectra were determined in part by Walter Smith and Vincent Pierro of the Rohm and Haas Co., Bristol, Pa. The microanalyses were performed by Clyde Nash, Rohm and Haas, Co., Bristol, Pa., Pascher and Pascher Microanalytical Laboratory, Bonn, Germany, and Micro-Analysis, Inc., Wilmington, Del. The n.m.r. spectra were determined Nuclear Magnetic Resonance Specialties, New Kensington, Pa. The molecular weight of Muconomycin B was determined by Harry Mason, Rohm and Haas Co., Philadelphia, Pa.

(20) The infrared spectra were obtained on either a Perkin-Elmer Model 21 spectrophotometer or on a Baird-Atomic Model KM-1 recording spectrophotometer; the ultraviolet spectra were obtained on a Beckman DK-2 recording spectrophotometer. Rotations were taken on a Rudolph Precision polarimeter.

(21) The following procedure for obtaining muconomycin B was described in a personal communication from C. Smythe, Rohm and Haas Co., Bristol, Pa. The organism was grown in a medium containing 3.0% glucose, 3.0% rolled oats, 0.1% Difco yeast extract, 0.5% Difco peptone, 0.05% K_2HPO_4, 0.02% $MgSO_4 \cdot H_2O$, and 93% H_2O. The isolation procedure for muconomycin B is similar to that described for muconomycin A.[4] The concentration of V was 500 μg./ml.

of ethanol and 8 ml. of 3% sodium hydroxide. A reflux condenser was attached and the mixture was heated at reflux on a steam bath for a period of 1 hr. The reaction mixture was then cooled to room temperature and filtered to remove any solid matter in suspension. The filtrate was extracted several times with chloroform and the extracts were combined and dried over anhydrous magnesium sulfate. After drying agent and solvent were removed, a crystalline residue remained which after one recrystallization from ether melted at 154–155° and weighed 82.8 mg. A mixture melting point determination with a sample of alcohol A from the hydrolysis of muconomycin A showed no depression.

The basic reaction mixture was then made strongly acidic with 6 *N* hydrochloric acid and extracted with ether. After the combined extracts were dried and the solvent was removed, a powder was obtained as a residue. The residue was washed with chloroform and collected on a filter: 50 mg., m.p. 189.5–190.5 dec. A mixture melting point determination with a synthetic sample of *cis,trans*-muconic acid showed no depression.

The acidified reaction mixture was then extracted with chloroform, the chloroform solution was dried, and solvent was removed as usual. A colorless oil remained as residue. The infrared spectrum of this oil was indicative of an α,β-unsaturated carboxylic acid as was also the ultraviolet spectrum: 214 (s) and 255 mμ (w, sh).

This oily residue was transferred to a hydrogenation flask in chloroform solution, the chloroform was removed, and 30 ml. of methanol was added. Adams catalyst (approximately 50 mg.) was added and the mixture was reduced in a Parr apparatus at room temperature and 30-lb. pressure. The reduction was stopped at the end of 2 hr. The catalyst was removed by filtration and the solvent by evaporation. Chloroform was added to the oil remaining, the solution was filtered free from the small amount of insoluble material present, and the solvent then was removed. An infrared spectrum (film) of this treated oil shows no evidence of unsaturation.

To the treated oil was added 10 ml. of water and then the solution was made acidic (about pH 2) with hydrochloric acid. The acidic solution was filtered and extracted 5 times with a total of 35 ml. of benzene. The extracts were combined and dried over anhydrous magnesium sulfate; the drying agent was removed. Evaporation of the solvent yielded a very small amount of oil. The infrared spectrum (film) of this material is very similar to that of β-methyl δ-valerolactone and indicates that the oil was in fact an impure sample of this lactone. Bands are present at 1731 (C=O), about 1395 (C—CH_3), 1250, and 1222 cm.$^{-1}$.

A few drops of benzhydrylamine were added to this oil and the solution was heated 4 hr. on a steam bath. After cooling to room temperature the viscous oil was dissolved in chloroform and washed several times with 0.1 *N* hydrochloric acid and then with water until the washings were neutral to litmus. The chloroform solution was dried and the solvent was removed in the usual manner. The oil remaining was subjected to chromatography on alumina in a semimicrocolumn. The infrared spectrum of the purified amide is superimposable on that of the benzhydrylamide of β-methyl δ-valerolactone described below. Since a small amount of material was obtained in this manner, the product could not be investigated further.

All attempts to isolate an unsaturated lactone from the hydrolysis of muconomycin B were unsuccessful.

Reduction of Muconomycin B.—A solution of 1.5 g. (3.09×10^{-3} moles) of muconomycin B in 200 ml. of methanol was placed in a hydrogenation flask. A small amount of Adams catalyst was added and the mixture was reduced in a Parr apparatus at room temperature and 32-lb. pressure. The reduction was discontinued after 4.5 hr. The catalyst was removed by filtration and the solvent by evaporation on a steam bath. A light yellow oil remained as residue.

The infrared spectrum of this material shows a single carbonyl peak at 1725 cm.$^{-1}$. No double-bond bands appear in the 1600-cm.$^{-1}$ region.

Hydrolysis of Reduced Muconomycin B.—The oil obtained from the catalytic reduction of muconomycin B was placed in a 100-ml. flask with 10 ml. of ethanol. A reflux condenser was attached and the mixture was gently heated on a steam bath. When the material began to reflux, 40 ml. of 10% sodium hydroxide was added through the condenser. The light yellow ethanol solution immediately became reddish brown and turbid in appearance. The mixture was heated to reflux and became clear. After 2 hr., heating was discontinued and the solution was cooled to room temperature and extracted thoroughly with chloroform. The combined extracts were dried over anhydrous sodium sulfate and the drying agent and solvent were removed. The colorless oil (740 mg.) which remained as residue crystallized immediately at room temperature. A purified sample of this material proved to be identical with a sample of alcohol A from the hydrolysis of muconomycin A.[4]

The basic reaction mixture was made strongly acidic (about pH 2) with hydrochloric acid and extracted with benzene. The combined extracts were dried over anhydrous sodium sulfate. Upon removal of the drying agent and solvent, 170 mg. of light yellow oil (VI) remained as residue.

The infrared spectrum of this oil contains bands at 1733 (C=O) and 1400 cm.$^{-1}$ (C—CH_3) and is superimposable on that of β-methyl δ-valerolactone. Strong bands are also present at 1246 and 1223 cm.$^{-1}$.

The acidified reaction mixture was then extracted exhaustively with ether. The combined extracts were dried over anhydrous sodium sulfate and the drying agent and solvent was removed. The residue, 240 mg., consisted of a mixture of crystals in oil. The oil was removed by washing with benzene and the remaining solid was recrystallized from ethyl acetate. After one recrystallization, the melting point of the sample was 147–150°. A mixture melting point determination with a sample of adipic acid showed no depression.

The benzene wash of the crude adipic acid fraction yielded an oil which, from its infrared spectrum, appeared to be a mixture containing some lactone. Attempts to isolate lactone from this mixture, however, were unsuccessful.

Benzhydrylamide of VI.—The benzhydrylamide of VI was prepared from the lactone and benzhydrylamine[22] according to a procedure similar to that described for alcohol C.[4] The product, an oil, was purified by chromatography on silicic acid. The white solid obtained was further purified by several recrystallizations from benzene–hexane to give a product with m.p. 125.5–127°.

Anal. Calcd. for $C_{19}H_{23}NO_2$: C, 76.74; H, 7.79; N, 4.71. Found: C, 76.60; H, 7.61; N, 4.63.

The infrared spectrum (KBr) shows amide bands at 1525 and 1625 and a hydroxyl band at 3260 cm.$^{-1}$. The spectrum is identical with that of a synthetic sample of the benzhydrylamide of β-methyl δ-valerolactone described below. The n.m.r. spectrum is also identical with that of the synthetic amide.

Mass spectrometry showed,[12] in addition, that the fragmentation pattern of this compound was identical with that of the synthetic benzhydrylamide of β-methyl δ-valerolactone with the last peak at m/e 279 (P − H_2O).

Benzhydrylamide of β-Methyl δ-Valerolactone.—The procedure used to make the benzhydrylamide of β-methyl δ-valerolactone was the same as that described for VI (above). The product was a crystalline solid which was purified by recrystallization from benzene–hexane solution; the yield from 1 g. of lactone and 1.8 g. of amine was 2 g. of white crystals, m.p. 109–110.5°.

Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.74; H, 7.79; N, 4.71. Found: C, 76.50; H, 7.55; N, 4.75.

The infrared and n.m.r. spectra were both characteristic of the desired amide. Mass spectrometry showed the P − H_2O peak to be at m/e 279 indicating a molecular weight of 297 (expected 297).

(22) L. W. Jones and C. D. Hurd, *J. Am. Chem. Soc.*, **43**, 2438 (1921).