1-Benzyl-3-pyrrolidinol (XVIII).—1-Benzyl-3-pyrrolidone (8.75 g., 0.05 mole) was dissolved in 100 ml. of methanol and 5.7 g. (0.15 mole) of sodium borohydride was added with stirring. The mixture was stirred for 5 hr. at room temperature. The solvent was removed *in vacuo*, the residue was dissolved in water, and the product was extracted with chloroform and dried over anhydrous magnesium sulfate. After the solvent was removed the residual oil was distilled *in vacuo* to yield 8.2 g. (92.5%) of product, b.p. 110–112° (0.2 mm.), $n^{20}D$ 1.5475. The hydrochloride was recrystallized from *n*-butanol; m.p. 123–124°.

The product was identical with an authentic sample[4] prepared from 1,4-dibromo-2-butanol and benzylamine. The infrared spectra were superimposable and the hydrochlorides showed no melting point depression; reported b.p. 120–121° (0.4 mm.); $n^{25}D$ 1.5473.

1-Benzyl-3-hydroxymethyl-4-hydroxypiperidine (XX).—1-Benzyl-3-carboethoxy-4-piperidone (XIX) was prepared freshly from its commercially available hydrochloride.[17] The free keto ester showed absorption in the infrared spectrum at 1730 and 1710 cm.$^{-1}$ (keto tautomer) and two strong bands at 1648 and 1610 cm.$^{-1}$ (enol tautomer). Using a procedure similar to the one described for the reaction of V, 13.05 g. (0.05 mole) of XIX was reduced with 11.4 g. (0.3 mole) of sodium borohydride in 100 ml. of methanol. Compound XX (4.6 g., 41.7%) could be distilled as a highly viscous white oil, b.p. 142–146° (0.03 mm.). The product solidified on standing: m.p. 115–120°; infrared absorption 3305 (hydroxyl groups), 743, and 696 cm.$^{-1}$ (monosubstituted benzene), no carbonyl absorption.

Anal. Calcd. for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.65; N, 6.33; O, 14.46. Found: C, 70.45; H, 8.55; N, 6.10; O, 14.44.

(17) Aldrich Chemical Co., Milwaukee, Wis.

Acknowledgment.—The authors wish to thank Dr. Martin Ettlinger for helpful discussions during the course of this work, and Professor E. E. Smissman and Dr. J. B. Carr, University of Kansas, for the n.m.r. spectra and their interpretation.

Isolation and Structure of Fusaroskyrin[1]

SUMU MATSUEDA

Department of Chemistry, Faculty of Literature and Science, Hirosaki University, Hirosaki, Aomori, Japan

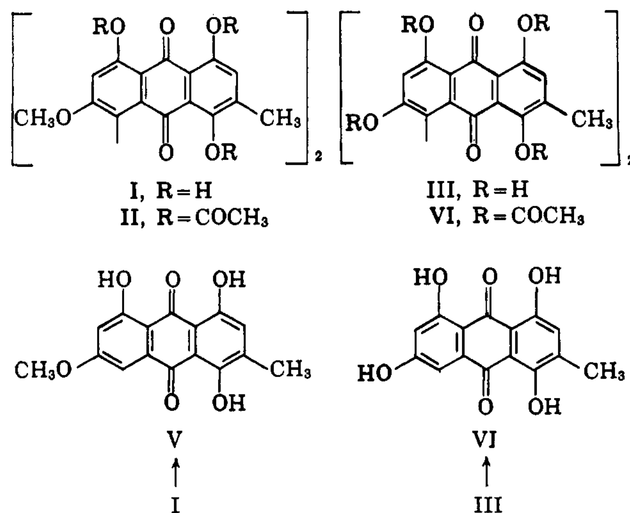
Received June 22, 1964

Fusaroskyrin, the main metabolic pigment of *Fusarium* species which is one of the organisms responsible for Purple Speck disease of soy beans, is obtained by acetone extraction of the dried mycelium, and has been purified by chromatography of its acetyl derivative on alumina. Fusaroskyrin gives erythroglaucin on sodium dithionite reduction. The structure of fusaroskyrin has been established as 1,1′-bi(4,5,8-trihydroxy-2-methoxy-7-methylanthraquinone).

In 1951, Shibata and Masumura demonstrated that *Fusarium* sp. is one of the pathogens which cause Purple Speck disease in Japanese soy beans.[2] *Cercosporina Kikuchii* Matsumoto et Tomoyasu, another pathogen of the same disease of soy beans, had been well known at that time.[3] However, Shibata and Masumura tried unsuccessfully to isolate this organism from soy beans infected with Purple Speck disease, but obtained only the *Fusarium* sp.. From the dried mycelium of the latter, they isolated palmitic, stearic, linoleic, and linolenic acids by ether extraction, while the crude pigments were obtained by extracting the residue with acetone.[4]

These mixed pigments have now been acetylated and the acetyl derivatives chromatographed on alumina. A pale yellow crystalline hexaacetate II, $C_{44}H_{34}O_{18}$, mol. wt. 830, and other acetates were thereby obtained. Alkaline deacetylation of II then gave the pure pigment, fusaroskyrin (I), $C_{32}H_{22}O_{12}$, as dark red crystals, m.p. >300°, mol. wt. 598. Fusaroskyrin gave a blue color with magnesium acetate[5]; it contained two C-methyl and two methoxyl groups as shown by Kuhn–Roth and Zeisel analyses, respectively. Demethylation of fusaroskyrin with hydroiodic acid yielded a red crystalline product III, m.p. >300°, $C_{28}H_4O_4(OH)_8(Me)_2$, which formed a yellow octaacetate IV, m.p. 295–297°.

Reduction of I, by lithium aluminum hydride or with sodium dithionite and alkali by the method of Raistrick and Howard,[6] gave erythroglaucin (V) (1,4,5-trihydroxy-7-methoxy-2-methylanthraquinone). Similarly, reduction of III gave catenarin (VI) (1,4,5,7-tetrahydroxy-2-methylanthraquinone). V and VI are the only products formed on reduction of



I and III, respectively. Ready cleavage by alkaline dithionite, yielding simple anthraquinones, is a typical property of 1,1′-bianthraquinones having hydroxyl or methoxyl groups in the positions *ortho* to the bond connecting the two moieties. Our findings thus suggest that I belongs to this class of compounds, a conclusion supported by the high melting point of I, and the molecular weight of 830 found for II. This in-

(1) A short communication of this work has been published by S. Fujise, S. Hishida, M. Shibata, and S. Matsueda [*Chem. Ind.* (London), 1754 (1961)].

(2) M. Shibata and M. Masumura, *Tohoku Seibutsu kenkyu*, **II**, 16 (1951).

(3) S. Kuyama and T. Tamura, *J. Am. Chem. Soc.*, **79**, 5725, 5726 (1951).

(4) Shibata and Masumura reported[2] that the yield of mixed pigments was 0.01% of dry fungus, and the yields of fatty acids were unknown.

(5) S. Shibata, *J. Pharm. Soc. Japan*, **61**, 320 (1941).

(6) B. H. Howard and H. Raistrick, *Biochem. J.*, **56**, 56 (1954).

terpretation is further corroborated by spectroscopic findings.

The infrared spectrum of fusaroskyrin hexaacetate shows the presence of phenolic acetate (1770 cm.$^{-1}$) and quinone carbonyl groups (1663 cm.$^{-1}$) and the absence of alcoholic acetate groupings. The infrared spectrum of fusaroskyrin itself shows a chelated quinone carbonyl band at 1615 cm.$^{-1}$ and C-methyl bands at 1452 and 1348 cm.$^{-1}$. Nonchelated quinone carbonyl bands are absent. All six hydroxyls of fusaroskyrin are therefore phenolic and all quinone carbonyl groups are chelated with these phenolic groups. The spectra of I and III do resemble those of polyhydroxyanthraquinones[7] and 1,1′-bipolyhydroxyanthraquinones.[8] The spectra of the acetates of 1,1′-bianthraquinones,[8] furthermore, are closely similar to those of the acetates II and IV, suggesting the presence of a 1,1′-bianthraquinone structure in I.

As mentioned previously, the carbonyl absorption band of I occurs at 1615 cm.$^{-1}$. The infrared spectra of a number of 1,1′-bianthraquinones[9] containing both nonchelated and chelated quinone carbonyl groups, *e.g.*, 1,1′-bichrysazine [1,1′-bi(4,5-dihydroxyanthraquinone)] and 1,1′-bichrysophanol [1,1′-bi(4,5-dihydroxy-7-methylanthraquinone)], showed the nonchelated carbonyl band at 1668 and chelated carbonyl at 1630–1629 cm.$^{-1}$. Compounds with only chelated quinone carbonyl, *e.g.*, 1,1′-biislandicine [1,1′-bi(4,5,8-trihydroxy-7-methylanthraquinone)], showed only one band at 1605 cm.$^{-1}$. On the basis of these comparisons I is a derivative of 1,1′-bi(4,5,8-trihydroxyanthraquinone).

TABLE I

ABSORPTION SPECTRA IN CHLOROFORM

Compd.	λ_{max}, mμ				
I	256	277	305–310[a]		505
II		275		370	
III	254	275	310–320[a]		510
IV		275		365	

[a] Inflection.

Compound I dissolved in concentrated sulfuric acid with reddish violet color, while III gave a fine greenish solution. This fact indicates that in compound III free hydroxyl groups at positions 2 and 2′ were generated by demethylation of I, and that III in concentrated sulfuric acid formed a pseudo-compound analogous to that obtained[10] from skyrin [1,1′-bi(2,4,5-trihydroxy-7-methylanthraquinone)] under the same conditions.

Comparison of the ultraviolet spectra of V and VI with those of I and III supported the formulation of I and III as 1,1′-bierythroglaucin and 1,1′-bicatenarin respectively (see Figure 1).

On the basis of these observations, fusaroskyrin is 1,1′-bi(4,5,8-trihydroxy-2-methoxy-7-methylanthraquinone).

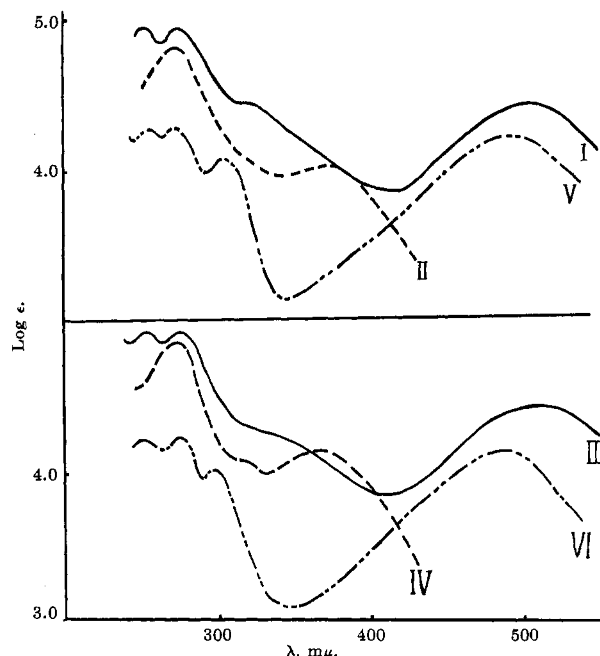


Figure 1.—Ultraviolet spectra in chloroform solution of I, fusaroskyrin; II, hexaacetate of I; III, demethylfusaroskyrin; IV, octaacetate of III; V, erythroglaucin; and VI, catenarin.

Experimental[11]

Isolation and Purification of the Pigments of *Fusarium* sp.—To a solution of 300 mg. of the crude pigments and 30 ml. of pyridine was added 60 ml. of acetic anhydride. After keeping at room temperature for 6 days and at 40–60° for 6 hr., the precipitated yellow solid was collected by filtration; yield 250 mg. The mother liquors were concentrated to 30 ml. *in vacuo* and on addition of 100 ml. of ice–water a brownish yellow solid which contains more than four pigments was precipitated; yield 92 mg. The former solid (250 mg.) was purified by chromatography in chloroform on alumina (200 mesh, 1.5 × 5 cm.) which is activated by Reichstein and Shoppe's method.[12] The first eluted product (II, 160 mg.) was recrystallized from benzene, and was dried at 140–160° for 10 hr. under high vacuum; m.p. 264–266°.

Anal. Calcd. for $C_{44}H_{34}O_{18}$: C, 62.12; H, 4.03; mol. wt., 850. Found: C, 62.19; H, 4.05; mol. wt., 830 (by the cryoscopic method in benzene).

II was soluble in ether, ethyl acetate, chloroform, and benzene. A solution of II in 0.1 *N* sodium hydroxide was heated on a boiling water bath for 3 hr. under nitrogen. After cooling, the solution was acidified with 0.1 *N* sulfuric acid and the precipitated I was collected by filtration. I was further purified by sublimation at 230–240° under high vacuum (0.007 mm.). I had m.p. >300° and formed dark red needles, scarcely soluble in alcohols, ether, benzene, sulfur dioxide, and ethyl acetate, and only slightly soluble in hot acetone and acetic acid.

Anal. Calcd. for $C_{32}H_{22}O_{12}$: C, 64.21; H, 3.68; OMe, 10.1; Me, 9.1. Found: C, 63.98; H, 4.24; OMe, 9.7; Me, 6.8.

Acetyl Group Determination in Compound II.—A solution of 100 ml. of 0.1 *N* sodium hydroxide containing 100 mg. of II was heated on a boiling water bath for 3 hr. under nitrogen. After cooling, 140 ml. of 0.1 *N* sulfuric acid was added to the solution. The hydrolyzed product, fusaroskyrin (I, 69.5 mg., 98.9% of theoretical amount), was collected by filtration. The mother liquor was titrated with 0.1 *N* sodium hydroxide.

Anal. Calcd. for $C_{32}H_{16}O_{12}(COMe)_6$: COMe, 30.35. Found: COMe, 30.42.

Acetylation of 69.5 mg. of I gave 90 mg. of II (theoretical, 99 mg.).

(7) J. H. Birkinshaw, *Biochem. J.*, **59**, 486 (1955).

(8) S. Shibata, T. Murakami, I. Kitagawa, and T. Kishi, *Pharm. Bull.* (Tokyo), **4**, 111 (1956).

(9) S. Shibata and O. Tanaka, *Kagaku no Ryoiki*, Suppl. Issue **II**, 173 (1961).

(10) S. Shibata, O. Tanaka, and I. Kitagawa, *Pharm. Bull.* (Tokyo), **3**, 278 (1955).

(11) Melting points are uncorrected. Microanalyses were performed by Miss M. Matsuura, Tohoku University, Sendai. The infrared spectra were measured on a Perkin-Elmer infrared spectrophotometer, Model 21. The Ultraviolet spectra were determined with a Beckman, Model DU, quartz spectrophotometer.

(12) T. Reichstein and C. W. Shoppe, *Discussions Faraday Soc.*, **7**, 305 (1949).

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]

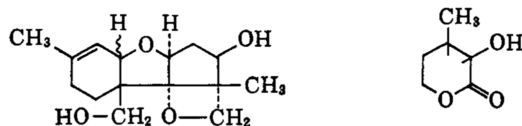
JACQUELINE S. VITTIMBERGA[2] AND BRUNO M. VITTIMBERGA

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)

(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).

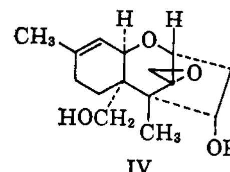
(5) E. Härri, W. Loeffler, H. P. Sigg, H. Stähelin, C. Stoll, C. Tamm, and D. Wiesinger, *Helv. Chim. Acta*, **45**, 840 (1962).

(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).

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



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

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

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