The consistently low analytical values are attributed to difficulties in handling the material due to its high volatility. No impurities could be detected by glpc, ir, and nmr analysis.

Registry No.—Ia, 3620-16-4; IIa, 14011-22-4; IIb, 14011-20-2; IIIa, 2375-33-9; V, Z = CN, hal = F, 17659-21-1; V, Z = $CONH_2$, hal = F, 17659-22-2; fluoronitromalonamide, 17659-24-4; chloronitromalonamide, 5514-96-5; chlorofluoronitroacetamide, 17659-23-3; ethyl chlorofluoronitroacetate, 1683-93-8.

Isolation and Structure of Norjavanicin

W. S. CHILTON

Department of Chemistry, The University of Washington, Seattle, Washington 98105

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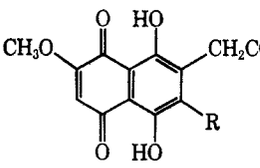
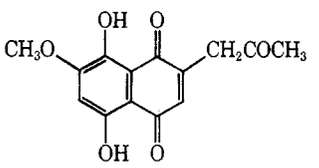
Substituted naphthazarins have been found in sea urchins,[1] microorganisms,[2] higher plants,[3] and frequently in species of *Fusaria*.[4,5] Structures **1a** and **4** proposed for javanicin[6] and fusarubin[7,8] have been verified by syntheses.[9,10] We have isolated fusarubin and a new quinone, norjavanicin, from a mold obtained as an aerial contaminant and identified as a *Fusarium* species.

An examination of the culture filtrate of the *Fusarium* species grown on a defined medium showed the presence of a colorless, optically active, aliphatic ketone, the triglyceride of oleic acid, and at least 11 pigments. A minor component was obtained from the pigment complex by preparative thin layer chromatography (tlc) followed by crystallization. It had the electronic spectrum of a 2-methoxynapthazarin,[6,7] and since its elemental analysis, $C_{14}H_{12}O_6$, differed from that of javanicin by a methyl group, it was named norjavanicin. The lack of infrared (ir) absorption in the normal hydroxyl region, 3300–3600 cm^{-1}, and the presence of a single type of quinone carbonyl (1610 cm^{-1}), strongly shifted by chelation,[11] supplied confirmatory evidence of the presence of the naphthazarin nucleus. In addition to the chelated quinone carbonyls, the presence of an aliphatic ketone or aliphatic ester was indicated by a 1725-cm^{-1} absorption band. The nmr spectrum of norjavanicin is composed of six singlet signals: a methoxyl resonance at δ 3.91, a quinone ring hydrogen at 6.15, a C-methyl resonance at 2.28, and a methylene resonance at 3.78. Since there is one phenolic and one quinone hydrogen,[12] each ring must bear one substituent. Structure **1b**, 6-desmethyljavanicin, is shown to be the correct structure by conversion of norjavanicin into its anhydromonoacetate and by comparison of the mass spectra of javanicin[10] (*m/e* 43, 205, 219, 230, 248, and 290) and norjavanicin (*m/e* 43, 191, 206, 216, 234, and 276). As required by the desmethyl structure, all of the principal mass spectral peaks of norjavanicin are also present in the mass spectrum of javanicin shifted +14 mass units with the exception of a common peak at *m/e* 43. Cracking of the acetonyl side chain appears to be the major reaction in the mass spectral decomposition of both of these compounds. One mode of cleavage is loss of the acetylium ion to produce a very strong *m/e* 43 peak in both spectra and another is the loss of ketene from the molecular ion radical to produce an intense M − 42 peak in the spectra of both compounds.



1a, R = CH_3
b, R = H



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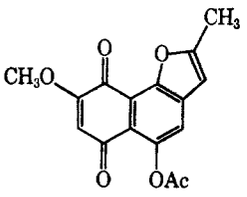

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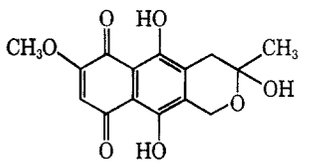


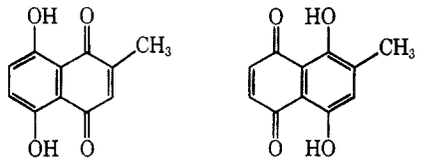
4

Acetylation of norjavanicin with acetic anhydride and sulfuric acid converts it into monoacetylanhydronorjavanicin (**3**). Javanicin itself undergoes similar dehydration on acetylation.[13] The anhydromonoacetate structure is confirmed by the loss of the 1725-cm^{-1} carbonyl band of norjavanicin following acetylation, appearance of an aryl acetate band at 1765 cm^{-1}, and shift of the quinone carbonyl band from the chelated (1610 cm^{-1}) to unchelated position (1685 cm^{-1}). In the nmr spectrum of **3** a doublet furano methyl signal (δ 2.44, J = 1 cps) has replaced the acetonyl methyl signal of norjavanicin.

Ample evidence supports assignment of structure **1b** to norjavanicin rather than tautomer **2**. Introduction of an alkyl substituent into the 2 position of naphthoquinone has been found to decrease the reduction potential by about 76 mV.[14] The tautomerism of methylnaphthazarin (**5**, **6**) can be viewed as an internal redox system. The alkyl substituent effect can be used to predict that tautomer **5**, with a substituted quinone ring, will be more stable than **6**, possessing an unsubstituted quinone ring. In the nuclear magnetic resonance spectrum of methylnaphthazarin

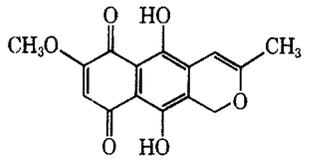
(1) Leading reference: R. E. Moore, H. Singh, and P. J. Scheuer, *J. Org. Chem.*, **31**, 3645 (1966).
(2) N. N. Gerber and B. Wieclawek, *ibid.*, **31**, 1496 (1966); K. Eckardt, *Ber.*, **98**, 24 (1965); S. Natori, Y. Kumeda, and N. Nishikawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 633 (1965); H. Brockmann, W. Muller, and K. Van der Merve, *Naturwissenshaften*, **49**, 131 (1963); H. Brockmann and E. Hieronymus, *Ber.*, **88**, 1379 (1955).
(3) I. Morimoto, T. Kishi, S. Ikegami, and Y. Hirata, *Tetrahedron Lett.*, 4737 (1965). For review of earlier literature, see R. H. Thompson, "Naturally Occurring Quinones," Academic Press, New York, N. Y., 1957.
(4) G. Kreitman, O. K. Sebek, and F. F. Nord, *Arch. Biochem.*, **28**, 77 (1950).
(5) G. P. Arsenault, *Tetrahedron Lett.*, 4033 (1965).
(6) H. R. B. Arnstein and A. H. Cook, *J. Chem. Soc.*, 1021 (1947).
(7) H. W. Ruelius and A. Gauhe, *Ann.*, **569**, 38 (1950); **570**, 121 (1951).
(8) G. P. Arsenault, *Can. J. Chem.*, **43**, 2423 (1965).
(9) E. Hardegger, E. Widmer, K. Steiner, and A. Pfiffner, *Helv. Chim. Acta*, **47**, 2031 (1964).
(10) E. Widmer, J. W. Meyer, A. Walser, and E. Hardegger, *ibid.*, **48**, 538 (1965).
(11) H. Bloom, L. H. Briggs, and B. Cleverly, *J. Chem. Soc.*, 178 (1959).
(12) R. E. Moore and P. J. Scheuer, *J. Org. Chem.*, **31**, 3272 (1966).
(13) S. Weiss and F. F. Nord, *Arch. Biochem.*, **22**, 288 (1949).
(14) L. F. Fieser and M. Fieser, *J. Amer. Chem. Soc.*, **57**, 491 (1935).

the methyl proton resonance occurs as a doublet (δ 2.23, J = 1.4 cps) coupled to the single quinone proton (δ 6.87). The presence of a doublet methyl resonance confirms methyl-substituted quinone structure **5**. Since the methoxyl substituent effect (131 mV) on quinone stabilization is considerably larger than the alkyl substituent effect,[14] norjavanicin tautomer **1b** is perferred over **2**.[15] The fact that the acetonyl methylene signal of norjavanicin is not coupled to an allylic proton indicates that the acetonyl substituent is in the phenolic ring, substantiating assignment of structure **1b** to norjavanicin and ruling out an extended quinone structure of the type proposed for the crystal structure of Cordeauxia-quinone.[16] Norjavanicin is formulated as the 2-methoxy-7-acetonylnaphthazarin rather than the 6-acetonyl isomer because of the obvious biosynthetic relationship to, and possibly even origin from, the acetogenins javanicin and fusarubin.



5

6



7

The major diffusible pigment was identified as fusarubin (**4**) by its elemental analysis and ultraviolet–visible, ir, nmr, and mass spectra. Both pairs of methylene protons in the ketal ring of fusarubin are in nonequivalent environments and give rise to broad signals at δ 5.17 and *ca.* 3.1. The proton resonance at δ 6.36 has a chemical shift suggestive of a proton on a quinone ring rather than a proton on a phenolic ring,[12] indicating that fusarubin has structure **4** rather than one of the other naphthazarin tautomeric structures.

Brief treatment of fusarubin with hot acetic acid effects dehydration to anhydrofusarubin **7** in which the protons of the remaining methylene group are in equivalent environments. The methylene proton signal now occurs as a sharp singlet at δ 5.27. In addition the C-methyl signal of anhydrofusarubin appears as a doublet (δ 1.92, J = 0.9 cps) coupled to the newly created allylic proton. The quinone proton signal occurs at δ 6.38. The electronic spectrum, nmr spectrum and tlc behavior of anhydrofusarubin are identical to those of the major purple quinone present in the *Fusarium* pigment complex. On standing in contact with the atmosphere, anhydrofusarubin slowly undergoes rehydration to fusarubin.

(15) Moore and Scheuer determined the structures of 34 substituted naphthazarins by nmr spectroscopy[12] and deduced from this an order for the attraction of quinonoidal properties by a substituent in the naphthazarin nucleus: OH > OCH_3 > OAc > CH_2CH_3 ≫ H ≫ $COCH_3$. This order may be compared that Fieser and Fieser's conclusion that "the groups which lower the potential of the parent quinone are those which facilitate substitution in the benzene ring; those which produce an increase in the potential have the opposite effect and retard benzene substitution."[14]

(16) M. Fehlmann and A. Niggli, *Helv. Chim. Acta*, **48**, 305 (1965).

Experimental Section

Nmr spectra were recorded on a Varian Associates A-60 instrument in chloroform with tetramethylsilane as the internal reference. Spectra of fusarubin were run in pyridine with tetramethylsilane as the internal reference. Ir spectra were determined with a Perkin-Elmer Model 37 instrument. Ultraviolet–visible spectra were determined in 95% ethanol with a Cary Model 11 instrument. Microanalyses were carried out by Dr. A. Bernhardt, Mülheim, Germany. Mass spectra were determined by Dr. K. L. Rinehart on an Atlas CH-4 mass spectrometer with an ionization energy of 70 eV.

Culture Conditions.—The *Fusarium* was grown without shaking on a medium containing 2% glucose, 1% ammonium chloride, 0.1% potassium monohydrogen phosphate, and 0.01% magnesium sulfate in tap water. Vegetative growth was maintained by transfer every 3 weeks. A complex mixture of pigments was obtained at the end of 3 weeks by extracting the culture filtrate with ether. The yield of ether-extractable solids was 50 mg/l. Ether extraction of the dried mycelia gave a 1.3% yield of an unsaturated triglyceride and virtually no pigment.

Norjavanicin (1b).—Norjavanicin and an unidentified aliphatic ketone were separated from the mixture of pigments by preparative tlc on silica gel G (Merck) developed with chloroform containing 5% methanol. The band corresponding to norjavanicin was extracted with chloroform. Removal of solvent gave a 120-mg mixture of amorphous, bright red solid and white needles. The mixture was dissolved in ether and extracted with basic aqueous magnesium acetate. Evaporation of the ether layer and two crystallizations of the residue from ethanol–chloroform gave colorless needles of an unidentified ketone, mp 244–250°.

The aqueous magnesium acetate extract was acidified and extracted with ether. Evaporation of the ether layer gave 64 mg of amorphous norjavanicin. Norjavanicin was crystallized twice from methanol–chloroform, giving red needles: mp 200–204°; ir 1725, 1610, 1580, 1440, 1360 cm^{-1} (chloroform).

Anal. Calcd for $C_{14}H_{12}O_6$: C, 60.87; H, 4.38. Found: C, 60.76; H, 4.48.

Monoacetylanhydronorjavanicin (3).—Norjavanicin (40 mg) was suspended in 1.5 ml of acetic anhydride. The suspended solid dissolved immediately on addition of a drop of concentrated sulfuric acid with attendant color change from red to yellow. Crystals began separating on standng. After 15 min the reaction mixture was poured into ice–water. The amorphous yellow-brown precipitate was purified by preparative tlc employing silica gel G and 2% methanol in chloroform as developing solvent. The material recovered from chromatography was recrystallized twice from ethanol to give 9 mg of yellow needles of monoacetylanhydronorjavanicin: mp 218° dec; ir 1765, 1685, 1642, 1627, 1590 cm^{-1} (chloroform).

Isolation of Fusarubin (4).—Crude pigment (1.2 g) was extracted with 10 ml of hot ethanol, followed by two 5-ml portions of hot acetone. The 270 mg of bright red residue was extracted with ethanol in a Soxhlet extractor. Red needles separated from the ethanol during the course of extraction. The crystals were collected and recrystallized in the same manner giving 202 mg of fusarubin: mp 210° dec; ultraviolet–visible (95% ethanol) λ_{max} 534 mμ (ϵ 3800), 498 (5800), 472 (5000), and 303 (6500).

Anal. Calcd for $C_{15}H_{14}O_7$: C, 58.83; H, 4.61. Found: C, 58.71; H, 4.55.

Anhydrofusarubin (7).—Fusarubin (67 mg) was dissolved in 3 ml of acetic acid and heated 10 min at 80°. After evaporation of the solvent, the residue was dissolved in benzene, and the resulting solution was filtered to remove a small quantity of insoluble fusarubin. Removal of solvent gave 50 mg of chromatographically pure anhydrofusarubin,[7] which decomposed above 200°.

Registry No.—**1b**, 17790-94-2; **3**, 17790-95-3; **4**, 17790-96-4.

Acknowledgments.—The author wishes to thank Dr. Lynn Brady, Dr. R. G. Benedict, and Dr. D. E. Stuntz for isolation and identification of the *Fusarium*. Financial assistance from a State of Washington research grant is gratefully acknowledged.