Alkaline oxidation of diastereoisomeric 4a,10a-dihydrofusarubins to norjavanicin, fusarubin, and a new antibiotic isofusarubin: nonenzymic formation of products in *Fusarium solani* cultures[1]

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Conversion of two diastereoisomeric 4a,10a-dihydrofusarubins, produced by *Fusarium solani*, to fusarubin, norjavanicin, and a new compound, isofusarubin ((*E*)-6,7-dihydro-5,8-dihydroxy-2-methoxy-6-methylene-7-(2-oxopropylidene)-1,4-naphthalenedione), has been studied under a variety of culture conditions and in alkaline solution. Antibiotic and phototoxic activities of these compounds were determined and the structures of isofusarubin and of anhydroisofusarubin mono- and diacetates were elucidated by ^1H and ^{13}C nmr.

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Utilisant plusieurs conditions différentes de culture en solution basique, on a étudié la transformation de deux dihydrofusarubines diastéréoisomères 4a,10a, produites par la *Fusarium solani* en fusarubine, en nojavanicine et en un nouveau composé la (*E*) dihydro-6,7 dihydroxy-5,8 méthoxy-2 méthylène-6 (oxo-2 propylidène)-7 naphtalènedione-1,4) isofusarubine. On a déterminé les activités antibiotique et phototoxique de ces composés et on a établi les structures des mono et diacétates de l'isofusarubine et de l'anhydroisofusarubine en faisant appel à la rmn du ^1H et du ^{13}C.

[Traduit par le journal]

Introduction

We have recently reported (1) that fusarubin **2** (2–8) is formed by nonenzymic oxidation of the diastereoisomeric 4a,10a-dihydrofusarubins **1***a* and **1***b* in alkaline cultures of *Fusarium solani*. Fusarubin has been dehydrated to anhydrofusarubin **3** by heating in glacial acetic acid (9); hydration of **3** under acidic conditions (4), or on standing in contact with the atmosphere (9), yielded **2**. The related metabolites javanicin **4** (2, 3), norjavanicin **5** (9), and bostrycoidin **6** (10) have also been obtained from *Fusarium* species. In this communication we report the isolation and structure of a new antibiotic and phototoxic compound **7** which was obtained from **1** by alkaline oxidation and to which we have given the trivial name isofusarubin. We provide evidence that **5** is also formed by nonenzymic oxidation of **1** in alkaline solutions and cultures.

Materials and methods

Organisms

Fusarium solani strain 199 was obtained from Professor P. E. Nelson, Department of Plant Physiology, Pennsylvania State University, University Park, PA 16802, U.S.A.

Culture medium

One litre of medium had the following composition: maltose, 20–60 g; ammonium tartrate, 4.6–9.2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NaCl, 0.1 g; $CaCl_2$, 0.1 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $ZnSO_4 \cdot 7H_2O$, 8.8 mg; $CuSO_4 \cdot 5H_2O$, 0.4 mg; $MnSO_4$, 0.06 mg; H_3BO_3, 0.06 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.04 mg.

Cultivation

Spores from cultures grown on potato-dextrose agar for 2 weeks at 25°C were suspended in sterile distilled water and filtered through a cotton plug. The optical density of the filtrate at 640 nm was adjusted to 0.25. Each 50 mL aliquot of culture medium in a 250 mL Erlenmeyer flask was inoculated with 2.5 mL of the spore suspension and then incubated at 25°C on a rotary shaker (3.8 cm eccentricity, 220 rpm) for 2 to 6 days.

Isolation of metabolites

The mycelium was removed by filtration and washed with water until free of pigment. The filtrate, adjusted to about pH 3 with 1 *N* HCl, was extracted with an equal volume of ethyl acetate. The extract was washed with water, concentrated *in vacuo*, and chromatographed on a column of silicic acid using benzene – ethyl acetate mixtures for elution. Compound **3** was eluted with a 12:1, **4**, **5**, and **6** with a 6:1, **1***a* and **2** with a 3:1, and **1***b* with a 2:1 mixture. The physical constants as well as the ir, uv, and ^1H nmr spectral data of these compounds were indistinguishable from those reported in the literature (1, 9, 10, 11).

Alkaline oxidation of **1**a *and* **1**b

In preliminary experiments solutions of **1***a* and **1***b* in 0.1 *N* NaOH were sampled periodically and the formation of products was monitored by tlc. This established the following optimum conditions for producing **7**. Samples of **1***a* (708 mg) and **1***b*

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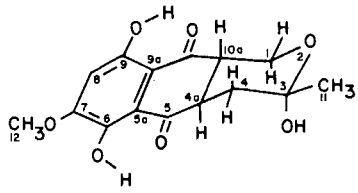
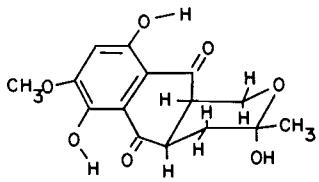


1a



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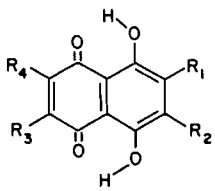
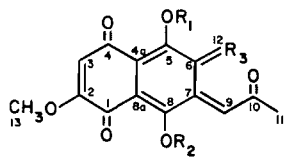


2 $R_1+R_2=CH_2OC(CH_3)(OH)CH_2$; $R_3=OCH_3$; $R_4=H$
3 $R_1+R_2=CH_2OC(CH_3)=CH$; $R_3=OCH_3$; $R_4=H$
4 $R_1=CH_3$; $R_2=CH_2COCH_3$; $R_3=OCH_3$; $R_4=H$
5 $R_1=H$; $R_2=CH_2COCH_3$; $R_3=OCH_3$; $R_4=H$
6 $R_1=H$; $R_2=OCH_3$; $R_3+R_4=CH=C(CH_3)N=CH$



7 $R_1=R_2=H$; $R_3=H_1, CH_2OH$
8 $R_1=COCH_3$; $R_2=H$; $R_3=CH_2$
9 $R_1=R_2=COCH_3$; $R_3=CH_2$

(168 mg) dissolved in 100 and 25 mL, respectively, of 0.1 *N* NaOH were aerated by stirring for 5 minutes at room temperature. After acidification with 0.2 *N* HCl the solutions were extracted with ethyl acetate. The extracts were washed with water, concentrated *in vacuo*, and chromatographed on columns of silicic acid. Reaction products **2** and **5** were eluted with the benzene – ethyl acetate mixtures already described above, whereas a 3:2 mixture gave isofusarubin **7**, mp 157–158°C (dark red prisms from ethanol); $[\alpha]_D^{20}$ +224 (*c* 0.051, acetone); λ_{max} (95% ethanol): 224, 296, 475, 500, and 536 nm (log ε: 4.41, 3.93, 3.83, 3.85, and 3.64); ν_{max} (KBr): 3485, 3400, 1708, and 1595 cm^{-1}; M^+ at *m/e* 306.0752 (calcd. for $C_{15}H_{14}O_7$ 306.07396).

Acetylation of **7** with acetic anhydride in pyridine for 3 h at room temperature, and evaporation under reduced pressure, gave a brown gummy residue. Preparative tlc of this material on 2 mm thick Merck silica gel F254 precoated plates using ethyl acetate as developer gave a major band which on extraction with $CHCl_3$ gave anhydroisofusarubin monoacetate **8** in 47% yield, mp 168–172°C (bright yellow rosettes from ethyl acetate – petroleum ether or ethanol); ν_{max} (KBr): 3450, 1770, 1685, 1644, and 1613 cm^{-1}; M^+ at *m/e* 330.0725 (calcd. for $C_{17}H_{14}O_7$: 330.07396). In contrast, direct extraction of the gummy residue with $CHCl_3$ gave anhydroisofusarubin diacetate **9** in 80% yield, mp 133–134°C (yellow needles from 3:1 $CHCl_3$–hexane); λ_{max} (95% ethanol): 220 and 226 nm (log ε: 4.37 and 4.36); ν_{max} (KBr): 1760 and 1618 cm^{-1}; M^+ at *m/e* 372.

Nuclear magnetic resonance spectra

Spectra were recorded in $CDCl_3$ solution (5 mm tubes) with TMS as internal reference. The ^1H nmr spectra of **7** and **9** at 100 MHz were obtained on a Varian HA-100D continuous-wave spectrometer, using the TMS signal for internal lock. The ^{13}C nmr spectra of **7** and **9** were recorded at 25.16 MHz with a Varian XL-100/15 Fourier transform spectrometer under the following conditions: sweep width (SW) 6016 Hz, acquisition time (AT) 1.6 s (data accuracy ±0.3 Hz), flip angle (FA) 35°, internal ^2H lock to solvent, temperature 29°C, ^1H irradiation at 100 MHz, (a) $\gamma H_2/2\pi \sim 3800$ Hz, broadband by 0 to 180° phase modulation at 150 Hz, or (b), as for (a), but irradiating only for 1.6 s between data acquisition periods to provide high resolution (HR) spectra with retained nOe.

The ^1H (80 MHz) and ^{13}C (20 MHz) nmr spectra of **7** and **8** were recorded on a Varian FT-80A spectrometer with internal ^2H lock to the solvent and a temperature of 35°C. Conditions for the ^1H spectra were SW 1000 Hz, AT 4.1 s (data accuracy ±0.1 Hz), FA 90°. The solution of **8** was degassed prior to nOe measurements. The ^{13}C spectra were acquired with SW 4132 Hz (**8**), 4505 Hz (**7**), AT 0.99, 0.91 or 1.98, 1.82 (data accuracy ±0.5 or ±0.3 Hz), FA 23° or 45°, ^1H irradiation at 80 Hz, (a) $\gamma H_2/2\pi \sim$ 4000 Hz, modulation bandwidth 2000 Hz (broadband decoupling), (b) as for (a) but irradiating for 2.0 s between data acquisitions only to retain nOe (HR spectra), and (c) as for (b) but with additional constant ^1H irradiation $\gamma H_2/2\pi$ 196 Hz at frequencies of individual ^1H resonances (single frequency decoupling with nOe retained at all resonances).

Biological activity

The method of Daniels (12) was used for the general assays with bacteria and yeasts, as described previously (13, 14). Duplicate cultures were incubated overnight after the discs containing 20 μg of compound were placed on them.

Results

Production of 2, 5, and 7 from 1a and 1b

Monitoring the reactions by tlc indicated that conversion of **1***a* and **1***b* to **2**, **5**, and **7** in 0.1 *N* NaOH at room temperature began immediately and

TABLE 1. Yields of **2**, **5**, and **7** produced from dihydrofusarubin in alkaline solution*

	1*a*		**1***b*	
Compound	mg	%	mg	%
2	331	46.8	89	53.0
5	70	9.8	47	28.6
7	274	38.7	21	12.5
Total	675	95.3	158	94.0

*Samples of **1***a* (708 mg) and **1***b* (168 mg) were dissolved in 0.1 *N* NaOH and the stirred solutions kept at room temperature for 5 min. Products were isolated as described in Materials and methods and crystallized once. Yields were calculated as a percentage by weight of the starting material. Value for **2** has been corrected for small amount of **3** produced during purification process.

TABLE 2. Antibiotic and phototoxic activities*

	1*a*	1*b*	2	3	4	5	6	7
Yeast								
Candida albicans	anti	anti	—	—	—	anti	anti	+
Saccharomyces cerevisiae	—	(anti)	anti	anti	—	(+)	NT	+
Gram positive bacteria								
Bacillus subtilis	anti	anti	anti	anti	anti	anti	NT	anti
Streptococcus faecalis	anti	anti	anti	anti	anti	anti	NT	anti
Streptomyces albus	anti	anti	—	+	—	—	NT	—
Gram negative bacteria								
Escherichia coli	—	—	—	—	—	—	NT	(anti)
Proteus vulgaris	—	(+)	—	—	—	—	NT	(+)
Pseudomonas fluorescens	—	—	—	—	—	—	NT	—

*Assays were scored by the method of Towers *et al.* (13): + = phototoxic, — = inactive, ( ) = slightly active, anti = antibiotic, NT = not tested.

TABLE 3. ^{1}H nuclear magnetic resonance data for isofusarubin (**7**), and the mono- and diacetate derivatives (**8**, **9**)*

Compound	H-12 (t)	H-12 (g)	H-3	H-6	H-9	H-13	H-11 CH_3CO_2	5-OH	8-OH	12-OH
7†	4.12	3.89	6.19(s)	4.37	7.19(s)	3.95(s)	2.20(s)	12.72(s),	12.54(s)	2.45(bs)
8‡	6.03(s),	6.32(s)	6.01(s)		7.21(d)	3.86(s)	2.38(s), 2.43(s)		10.71(d)	
9	6.03(s),	6.36(s)	5.96(s)		7.28(s)	3.85(s)	2.32(s), 2.38(s), 2.42(s)			

*Spectra were recorded (Varian HA-100-D (**7**, **9**) and FT-80A (**8**) spectrometers) in C^2HCl_3 with TMS as internal standard.
†H-12(t), H-12(g), and H-6 formed an ABC system in **7** (t = trans, g = gauche). Analysis of the system, with the Varian spin simulation program on a 620L computer, gave J_{AB} 11.0, J_{AC} 7.0, J_{BC} 4.5 Hz. Signals for 5, 8, and 12-OH disappeared on addition of ^2H_2O.
‡Signal for 8-OH in **8** disappeared on addition of ^2H_2O and that for H-9 (d, $^5J \sim 0.4$ Hz) became a singlet. The following nuclear Overhauser enhancements were observed (irradiated proton in parentheses): H-12, δ6.03 (H-12, δ6.32) 16%; H-3, H-9 (H-12, δ6.32) 0%; H-12's, H-3 (H-9) 0%.

was complete after 5 minutes. Yields at this time are given in Table 1. Over a 30 minute period **7** changed into many unidentified compounds and finally disappeared, whereas **5** was gradually degraded over 24 hours. On the other hand, **2** was relatively stable, with only a small amount being dehydrated to **3** over a 24 hour period.

Production of 1a, *1*b, *2, 4, 5, and 6 in cultures of* F. solani

Only **1***a*, **1***b*, and **4** were produced when the culture medium was rich in maltose (40 gL^{-1} or above), irrespective of the ammonium tartrate concentration used (4.6–9.2 gL^{-1}). When maltose and ammonium tartrate were both low (20 and 4.6 gL^{-1}, respectively), **1***a*, **1***b*, and **4** could be isolated from a 2 day harvest and **2**, **4**, and **5** after 6 days. The production of metabolites was strongly inhibited when the medium was low in maltose (20 gL^{-1}) and high in ammonium tartrate (6.9–9.2 gL^{-1}), although some **2**, **4**, and **6** could be isolated after a 2 day interval.

Antibiotic and phototoxic activities

The results for **1***a* and **1***b*, their alkaline oxidation products, and related compounds isolated from *F. solani* are reported in Table 2. Compounds with phototoxic activity inhibit growth on exposure to uv irradiation only, whereas antibiotic activity is indicated if growth is inhibited in the dark also. The bioassay for **6** is incomplete because of lack of material.

Discussion

Structures of 7, 8, and 9

The molecular formula $C_{15}H_{14}O_7$ obtained for **7** by accurate mass measurement is the same as that of **2** and the mass fragmentation patterns for the two compounds were similar. Thus **7** is formed, like **2** (1), by an autoxidation process involving the removal of a pair of hydrogens from **1***a* and **1***b*, the molecular skeleton probably remaining intact. Mass spectral measurements also indicated molecular formulae of $C_{17}H_{14}O_7$ for **8** and $C_{19}H_{16}O_8$ for **9**.

The characteristic singlet resonances obtained for methoxyl and quinone hydrogens in the ^{1}H nmr spectra of **2**, **3** (9), and **4** (11) are also observed in the ^{1}H nmr spectra of **7**, **8**, and **9** (see Table 3), suggesting that these three compounds contain the same substituted quinone ring system. Signals were present in the spectrum of **7** for an alcoholic and two phenolic hydroxyl hydrogens (chemical shifts temperature dependent and hydrogens exchangeable with deuterium), methyl hydrogens, an olefinic or aromatic hydrogen (δ 7.19), and hydroxymethyl hydrogens which are vicinally coupled to an aliphatic methine hydrogen. It was noteworthy

TABLE 4. ^{13}C nuclear magnetic resonance data (δ_C TMS; $^nJ_{CH}$, Hz) for isofusarubin (**7**), and the mono- and diacetate derivatives (**8**, **9**)*

Compound	C-1	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8
7	182.03(d) ^3J 7.9	160.43(dq) $^2J \sim {}^3J \sim 4.0$	110.01(d) ^1J 164.4	187.02(m)	109.85(m)	156.52(ddd) $^2J \sim 5$ $^3J \sim 5, 9$	53.31(m) ^1J 130.7	135.98†	156.52(ddd) $^2J \sim 5$ $^3J \sim 5, 9$
8	185.00(d) ^3J 8.1	159.05(dq) $^2J \sim {}^3J \sim 3.9$	111.81(d) ^1J 165.0	182.23(dd) ^2J 1.2 ^5J 1.7	121.40(dd) ^3J 5.4 ^5J 4.4	144.75(m)	142.13(d) ^3J 3.8	135.26(dd) ^3J_t 10.6 ^3J_c 5.3	158.22(dd) ^2J 4.7 ^3J 9.2
9	178.16(d) ^3J 8.3	159.34(dq) $^2J \sim {}^3J \sim 4.0$	110.28(d) ^1J 165.3	182.51(dd) ^2J 1.2 ^5J 1.7	123.94(dd) ^3J 5.8 ^5J 4.7	144.05(m)	146.89(d) ^3J 4.5	139.77(dd) ^3J_t 10.5 ^3J_c 5.4	145.45(d) ^3J 9.3

	C-8a	C-9	C-10	C-11	C-12	C-13	CH_3CO_2	CH_3CO_2
7	111.35(d) ^3J 4.3	129.64(ddd) ^1J 163.4 $^3J \sim {}^5J \sim 6.0$	206.98(dq) $^2J \sim {}^2J \sim 6$	29.33(q) ^1J 128.0	62.01(dt) ^1J 146.5 ^2J 6.1	56.47(q) ^1J 147.1		
8	113.97(d) ^3J 4.2	134.21(d) ^1J 164.8	197.36†	26.67(q) ^1J 128.0	128.10(t) ^1J 161.1	56.63(q) ^1J 146.3	169.31(q) ^2J 7.1	21.02(q) ^1J 130.8
9	123.66(s)	132.31(d) ^1J 166.7	196.58(dq) $^2J \sim {}^2J \sim 6$	26.63(q) ^1J 128.2	129.13(t) ^1J 161.8	56.57(q) ^1J 146.9	168.45(q), 169.14(q) ^2J 7.1 ^2J 7.2	20.71(q), 21.02(q) ^1J 130.5 ^1J 130.4

*Pulse Fourier transform spectra recorded on Varian XL-100/15 and FT-80A spectrometers at 30°C in C^2HCl_3, with TMS as internal standard.
†Multiplicity not determined because of poor S/N.

that the resonances for the aliphatic methine hydrogens of **1***a* and **1***b* were absent from the spectrum of **7**.

Conversion of **7** to **8** and **9** resulted in one (**8**) or both (**9**) of the phenolic hydroxyl resonances in the ^{1}H nmr spectrum being replaced by signals for acetoxyl methyl groups. Furthermore, the hydroxymethyl and aliphatic methine signals were absent from the spectra of **8** and **9**, singlet resonances for two olefinic hydrogens being observed instead. Nuclear Overhauser measurements (see footnote to Table 3) combined with the absence of an observable coupling (15) indicated that these olefinic hydrogens were geminal. Thus elimination of a molecule of water at C-6 and C-12 and acetylation of the phenolic hydroxyl groups appeared to be the only structural changes that had occurred during the formation of **8** and **9** from **7**. The phenolic hydrogen of **8** was long-range coupled to the unsaturated methine hydrogen designated H-9 and the coupling was removed when the hydroxyl hydrogen was exchanged for deuterium. This evidence suggests that the phenolic hydroxyl and unsaturated methine groups are on adjacent carbons.

Structures for **7**, **8**, and **9** were finally confirmed by ^{13}C nmr studies including single ^{1}H frequency decoupling experiments to correlate the ^{1}H and ^{13}C resonances.

The ^{13}C chemical shifts for **7**, **8**, and **9** (Table 4) were, in some respects, reminiscent of our previously reported (16) and rigorously assigned data for anhydrofusarubin and its diacetate derivative, which possess 5,8-dihydroxy-2-methoxy-1,4-naphthalenedione ring systems. The close agreement between chemical shifts of corresponding carbons in these compounds is strong evidence for the presence of a structural fragment containing the quinone ring system plus C-5 and C-8 depicted in structures **7**, **8**, and **9**. Deuterium isotope chemical shifts in the spectrum of **7**-d_3 and one, two, and three bond ^{13}C—H coupling data for **7**, **8**, and **9** provide further evidence for this fragment. The signs and magnitudes of the isotope chemical shifts for C-1 ($\Delta\delta_{2H}$ +0.09), C-4 ($\Delta\delta_{2H}$ +0.14), C-5 ($\Delta\delta_{2H}$ −0.40), and C-8 ($\Delta\delta_{2H}$ −0.40) indicated that the carbonyl and phenolic carbinol carbons were geminal[4] in **7** and therefore in **8** and **9**. Also H-3 was coupled to C-1, C-2, and C-3 in all three compounds and also to C-4 and C-4a of **8** and **9**, 5-OH was coupled to C-5 of **7**, and 8-OH to C-8 and C-8a of **7** and **8**. Thus the combined evidence for the common structural unit in **7**, **8**, and **9** is overwhelming and at the same time places the acetoxyl group in **8** at C-5.

Chemical shift considerations and multiplicities due to characteristic one bond ^{13}C—H couplings indicated that the remaining resonances in the spectrum of **7** arose from the methyl (C-11) and carbonyl (C-10) carbons of an acetyl group, quaternary olefinic (C-7) and olefinic methine (C-9) carbons (which have to be adjacent from structural considerations), and the carbons of hydroxymethyl (C-12) and aliphatic methine (C-5) groups which also have to be adjacent on the basis of the ^{1}H nmr evidence and the two bond coupling of H-6 to C-12. In addition, H-9 was two bond coupled to C-10 and three bond coupled to C-8, H-11 was two bond coupled to C-10, H-6 was three bond coupled to both C-9 and C-8, and H-12 was three bond coupled to C-5 (17). These observations established the molecular relationships depicted in structure **7**, except for the geometry of the C-7,C-9 double bond. Isotope chemical shifts in the spectrum of **7**-d_3 provided information on the latter. The sign of the isotope chemical shift for C-10 ($\Delta\delta_{2H}$ +0.17) indicates that this carbonyl group is hydrogen bonded to a hydroxyl group,[4] which must be 12-OH because the two phenolic hydroxyl groups are already known to be similarly bonded to the quinone carbonyl groups. This indicates that the C-7,C-9 double bond has an *E*-configuration. Molecular model studies also suggest that a *Z*-configuration would be difficult to attain because of severe steric interactions, whereas the *E*-configuration provides ample opportunity for the formation of a hydrogen bond between the C-10 carbonyl group and 12-OH. The isotope chemical shifts of $\Delta\delta_{2H}$ −0.20 observed for C-7 and C-9 are also consistent with the presence of a hydroxyl group at C-8 and hydrogen bonding of the C-10 carbonyl group to 12-OH although no attempt was made to determine their relative contributions.

From ^{13}C chemical shift and ^{13}C—H coupling considerations it was apparent that **8** and **9** had retained the $CH_3COCH{=}C\langle$ fragment present in **7** but that the hydroxymethyl and aliphatic methine groups had been replaced by a $\rangle C{=}CH_2$ group. Once again H-9 was three bond coupled to C-8. In addition it was three bond coupled to C-6 and, surprisingly, five bond coupled to C-4a. Moreover, both olefinic hydrogens bonded to C-12 were three bond coupled to C-7 and one of them, probably the one *trans* to C-5, was five bond coupled to C-4 (17). These observations place the C-12 exocyclic meth-

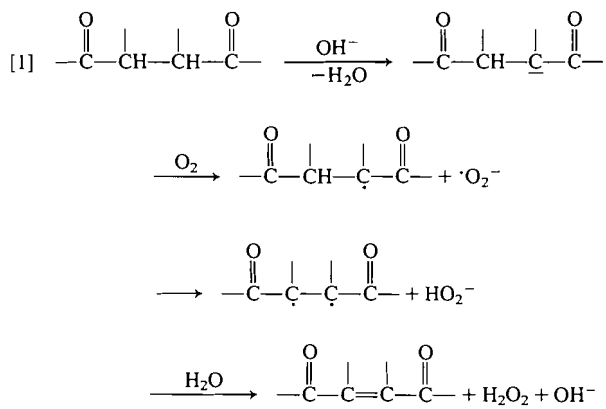
[4]Unpublished results on secalonic acids.

ylene group vicinal to the $CH_3COCH=$ and C-5 acetoxyl groups in both derivatives. The similarity of the ^{13}C chemical shifts for the $CH_3COCH=C$ carbons of **7**, **8**, and **9** strongly suggests that the double bond in all three compounds has the same *E*-configuration. This was also indicated by a careful nuclear Overhauser enhancement study which gave no enhancement of the H-12 resonances for **8** when H-9 was irradiated although a similar experiment involving the H-12 hydrogens had established that they were geminal (see footnote to Table 3). Retention of configuration at the C-7,C-9 double bond of **7** during acetylation in pyridine is not unexpected. Thus the combined evidence unequivocally established that isofusarubin and its acetate derivatives have the structures **7**, **8**, and **9**.

Nonenzymic formations of 2, 5, and 7

Although the mechanism of autoxidation in aqueous alkaline solution is not fully understood, it is probable that hydrogen loss from **1***a* and **1***b* involves the reaction of carbanions with molecular oxygen to furnish radicals and $\cdot O_2^-$ (18). Carbanion formation followed by electron abstraction at C-4a and C-10a would lead directly to **2** (Table 1) as illustrated in eq. [1]. Proton removal by base to

[1]



produce carbanions would obviously be promoted by the adjacent carbonyl groups. However, prior opening of the tetrahydropyran ring is required before **7** could be formed by removal of hydrogens from C-4 and C-4a. Moreover, production of **5** would also require prior opening of the tetrahydropyran rings of **1***a* and **1***b* and necessarily involves loss of formaldehyde via a retroaldol reaction at the resulting hydroxymethyl group to form 6,7-dihydronorjavanicin before autoxidation occurs. Differences in the rates of the retroaldol and autoxidation reactions probably account for the relative yields of **5** and **7** (Table 1). Formation of **2**, **5**, and **7** and their subsequent degradation in aqueous alkaline solution contrasts with the autoxidation of **1***a* and **1***b* which leads exclusively to **2** (1).

It is also noteworthy that **7** derived from both **1***a* and **1***b* had the same optical activity and therefore configuration at C-6. This can be explained if the dihydrofusarubins possess the same configuration at C-4a and **1***b* is converted through enolization to the more thermodynamically stable **1***a* (1) before conversion to **7**. The integrity of the chiral centers of **1***a* would be maintained because successive enolizations involving these sites would leave the molecule unchanged. Epimerization at C-6 of **7** would also be restricted because of the keto–enol tautomerism accompanying conversion of **1***a* to **7** and the steric interactions between the substituents at C-6 and C-7 which would hamper proton transfer from C-6 to C-4.

Cultures of *F. solani* produce only **1***a*, **1***b*, and **4** if maintained at ph $\approx$ 3 by providing a medium rich in maltose or by addition of acetic acid. When maltose and ammonium tartrate concentrations are low, **1***a*, **1***b*, and **4** are formed during the first two days when the pH is still low, but the amount of **1***a* and **1***b* decreases rapidly after 3 days. This loss is accompanied by a rapid increase in the pH of the medium (pH 8.2 after 6 days) and an increasing proportion of **2**, and to a lesser extent **5**, in the product. Addition of NaOH solution to cultures (pH $\approx$ 3) harvested after 2 days converts **1***a* and **1***b* to **2** and **5**. Thus the latter are produced nonenzymically in cultures of *F. solani* and by the same process as in 0.1 *N* NaOH. Loss of stability on prolonged exposure to basic conditions explains the low yield of **5** (5% of **2**) and the absence of **7** in the cultures.

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1. I. KUROBANE, L. C. VINING, A. G. MCINNES, and D. G. SMITH. Can. J. Chem. **56**, 1593 (1978).
2. H. R. V. ARNSTEIN, A. H. COOK, and M. S. LACEY. Nature, **156**, 333 (1946).
3. H. R. V. ARNSTEIN and A. H. COOK. J. Chem. Soc. 1021 (1947).
4. H. W. RUELIUS and A. GAUHE. Ann. **569**, 38 (1950).
5. H. W. RUELIUS and A. GAUHE. Ann. **570**, 121 (1951).
6. G. P. ARSENAULT. Can. J. Chem. **43**, 2423 (1965).
7. E. HARDEGGER, K. STEINER, E. WIDMER, and A. PFIFFNER. Helv. Chim. Acta, **47**, 2027 (1964).
8. E. HARDEGGER, E. WIDMER, K. STEINER, and A. PFIFFNER. Helv. Chim. Acta, **47**, 2031 (1964).

9. W. S. CHILTON. J. Org. Chem. **33**, 4299 (1968).
10. G. P. ARSENAULT. Tetrahedron Lett. **45**, 4033 (1965).
11. E. WIDMER, J. W. MEYER, A. WALSER, and E. HARDEGGER. Helv. Chim. Acta, **48**, 538 (1965).
12. F. DANIELS. J. Invest. Dermatol. **44**, 259 (1965).
13. G. H. N. TOWERS, C-K. WAT, E. A. GRAHAM, R. J. BANDONI, G. F. Q. CHAN, J. C. MITCHELL, and J. LAM. Lloydia, **40**, 487 (1977).
14. C-K. WAT, R. K. BISWAS, E. A. GRAHAM, L. BOHM, and G. H. N. TOWERS. J. Nat. Prod. **42**, 103 (1979).
15. L. M. JACKMAN and S. STERNHELL. Applications of nuclear magnetic resonance in organic chemistry. 2nd ed. Pergamon Press, New York. 1969. p. 279.
16. I. KUROBANE, L. C. VINING, A. G. MCINNES, and J. A. WALTER. Can. J. Chem. **58**, 1380 (1980).
17. P. E. HANSEN. Prog. Nucl. Magn. Reson. Spectrosc. **14**, 175 (1981).
18. D. H. R. BARTON and D. W. JONES. J. Chem. Soc. 3563 (1965).