some of the structures that are hard to explain on the basis of the known oxidation mechanisms of the common unsaturated acyl groups.

To test this possibility, U-^{14}C-stearic acid was esterified into soybean oil, the soybean oil was oxidized and the volatiles were isolated by high vacuum distillation. No oxidation of the stearic acid could be detected.

The U-^{14}C-stearic acid was purchased from Amersham Searle of Des Plaines, Illinois and had a specific activity of 92 m Ci/mM. The purity of the stearic acid was established by paper chromatography (2) and silver ion chromatography (3). No chain length other than 18 and no unsaturation could be detected. The stearic acid was converted to methyl stearate with 1% sulfuric acid in methanol. Soybean oil was deodorized at 180 C for 5 hr in an all glass apparatus (4). Next, 50 μCi of the methyl stearate were interesterified with 1000 ml of deodorized soybean oil with a sodium methoxide catalyst at 150 C and 0.2 mm Hg for 1.5 hr. The oil was cooled, washed once with 1% acetic acid and twice with water. A control flask of soybean oil was given the same interesterification treatment except no methyl stearate was added.

Peroxide values were determined by the method of Hamm et al. (5). The oil was allowed to oxidize at approximately 25 C with air bubbling through it to maintain oxygen level. When the peroxide value reached 10 and 137, 50 ml aliquots of oil were passed through a Kontes falling film molecular still at 8 drops/min, 2 μ of Hg and 25 C. Volatiles distilled from the oil were trapped in liquid nitrogen. Next, the entire lot of oil that had been oxidized to a peroxide value of 137 was deodorized and reoxidized at 125 C for eight days. About 50 ml of the viscous, partly polymerized oil was distilled as before.

To detect C^{14} in the volatiles, the distillation trap was filled with a counting solution containing 5 g PPO and 0.1 POPOP/liter of toluene. The trap contents were transferred to counting vials and counted on a Packard 32 scintillation counter for 100 min.

At 25 C, the peroxide value of the control and labeled soybean oil both reached 10 at 17 days. The peroxide value of the two day samples stayed together up to peroxide value 50, after which the peroxide value of the labeled sample pulled ahead of the control, reaching a peroxide value of 137 in 153 days. These results indicate that the radioactivity did not speed up autoxidation appreciably and the results obtained with the labeled samples should be representative of those obtained in normal samples.

The high vacuum distillation yielded volatiles with counts not significantly above background for both the oils oxidized at 25 C and 125 C. From the specific activity of the stearic acid and the composition of soybean oil, one may calculate that if as little as 1 μg (or $4 \cdot 10^{-5}$%) of the stearic acid in the 50 ml of oil used as a sample had been oxidized to volatile materials, the count would have been double that of background. These results indicate that stearic acid does not make a significant contribution to the scission products produced by oxidation below 125 C.

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ACKNOWLEDGMENT

Supported in part by a PHS research grant UI 00090 from the National Center for Urban and Industrial Health, Public Health Service.

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[Received September 11, 1970]

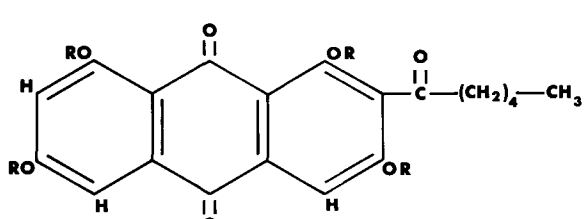
Norsolorinic Acid From a Mutant Strain of *Aspergillus Parasiticus*[1]

ABSTRACT

A mutant formed after UV irradiation of a potent aflatoxin producing strain of *Aspergillus parasiticus* elaborated 80% less aflatoxin than did the parent strain and produced an orange-red pigment. This new metabolite which represents 1% of the mycelial mass has been identified as 2-hexanoyl-1,3,6,8-tetrahydroxyanthraquinone (norsolorinic acid), mol wt 370, mp 256-257 C, and molecular formula $C_{20}H_{18}O_7$.

Certain strains of the mold *Aspergillus parasiticus* are known to produce a family of secondary metabolites known as aflatoxins which have become the subject of intense investigations. In this laboratory, one aspect of research has been the study of genetic inheritance of aflatoxin production by mutation of toxic mold strains (1). One mutant produced by UV irradiation of a potent strain had a much lowered ability to elaborate aflatoxins, and concomitantly produced an orange-red pigment. The isolation and identification of this pigment is the subject of this communication.

The mutant strain (NRRL A-17,996) was cultured at 30 C for seven days on a chemically defined media (2). As all of the orange-red pigment was contained in the nonsporulating mycelia, the liquid media was discarded and the washed mycelia extracted with acetone until colorless in a Waring Blendor. Each Fernbach flask contained approximately 50 g of wet mycelia which required 2000 ml of acetone for extraction. The acetone extracts were



I, R=H

II, R=CH

FIG. 1. Structures of norsolorinic acid, I, and the tetramethyl ether derivative norsolorinic acid, II.

[1]Presented at the AOCS Meeting, New Orleans, April 1970.

TABLE I

Comparison of UV and NMR Spectra of Methyl Ethers of Norsolorinic Acid From *A. Parasiticus* and *A. Versicolor*

Source of pigment	UV λ mμ	UV Log ε	Aromatic proton position	NMR Shielding values	NMR Side chain position	NMR Shielding values[a]
A. parasiticus	223	4.52	4	2.46	O Me	6.02, 6.03
	279	4.54	5	2.65 (d, J = 2.2 H_z)	(1,3,6,8)	6.04, 6.08
	335	3.73	7	3.21 (d, J = 2.2 H_z)		
	400	3.67			Methylenes	
					α	7.24 (t, J = 7 H_z)
					β	8.3 (broad)
					λ, Δ	8.65 (broad)
					ε	9.10 (t, J = 6 H_z)
A. versicolor[c]	223	4.49	4	2.45		
	280	4.53	5	2.66 (d, J = 2.5 H_z)		
	335	3.68	7	3.21 (d, J = 2.5 H_z)		
	400	3.66				

[a]Data reported in tau units, solvent *d*-chloroform.
[b]Spectrum run on internally locked Varian HR-100.
[c]Reference 5.

filtered through sodium sulfate to remove water and evaporated to dryness. The residues were combined and 500 mg portions were placed on a Mallinckrodt CG-7, 100-200 mesh silicic acid column (3.5 x 15 cm). Impurities were removed by a diethyl ether wash (500 ml) followed by a methanol wash (500 ml). The pigment was then eluted with 9:1 (v/v) benzene-acetic acid (1000 ml/500 mg sample) and recrystallized as needles from acetone. The yield was approximately 2 g of combined pigment from the mycelia grown in 20 Fernbach flasks. Calculated composition on a dry weight basis indicates that the pigment represented 1% of the mycelia mass. It melts at 256-257 C; UV λ_{max}^{EtOH}; mμ (ε): 234 (23,667), 265 (16,650), 283 (17,352), 297 sh (19,872), 313 (23,763), 465 (7,336). Analysis C, 64.40; H, 4.41; O, 30.56; OH, 20.7; CH_3, 4.1, mol wt 370 (mass spectroscopy). Calculated for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90; O, 30.24, OH(4), 18.37; CH_3(1), 4.06; mol wt 370. The pigment was sparingly soluble in acetone, ethanol and methanol; it was insoluble in water, hexane, ether and in sodium bicarbonate; it dissolved in sodium carbonate and in sodium hydroxide to give a purple color.

The methyl ether derivative was formed by refluxing the pigment (70 mg) with dimethyl sulfate (0.7 ml) in dry acetone and anhydrous potassium carbonate (3.5 g) for 8 hr (3). The reaction mixture was poured into water and the yellow methyl derivative extracted from this with chloroform. Recrystallization from methanol yielded fine yellow needles, mp 130-131 C, UV λ_{max}^{EtOH}; mμ (ε): 223 (36,610), 279 (34,190), 335 (6,170), 400 (5,290). Analysis C, 67.64; H, 6.12; mol wt 426 (mass spectroscopy). Calculated for $C_{24}H_{26}O_7$; C, 67.59; H, 6.15, mol wt 426. The NMR spectrum of the methyl derivative was entirely consistent with the presence of an anthraquinone nucleus with four methoxyl groups in the 1, 3, 6 and 8 positions and a 2-hexanoyl side chain (Fig. 1, II).

Based on the NMR analysis the structure, 2-hexanoyl-1,3,6,8-tetrahydroxyanthraquinone, was assigned to the original pigment (Fig. 1, I). This pigment, norsolorinic acid, has been identified previously in the lichens *Solorina crocea* (3) and *Lecidea piperis* (4) and the mold *A. versicolor* (5). A comparison of the UV and NMR spectra of the methyl ether derivatives of the pigments from the two mold sources is listed in Table I. These data show good agreement and the melting points of both the derivatives and of the original pigments are identical. Moreover, the UV and IR spectra of an authentic sample of norsolorinic acid from the lichen *Solorina crocea* are superimposable on the spectra of norsolorinic acid isolated in this study.

There is no report in the literature of aflatoxin production by the lichens or by the mold *A. versicolor*. These toxins are produced almost exclusively by the *A. flavus-parasiticus* group (6). Yellow pigmentation is associated with aflatoxin production by toxic strains (7) and anthraquinone pigments have been isolated from a wild toxic strain of *A. flavus* (8), presumably in low yield. However, norsolorinic acid was a major secondary metabolite of this mutant and was not detected in the parent toxin-producing strain. Since the mutant strain produced 80% less aflatoxin than did the parent strain, it is suggested that aflatoxins and norsolorinic acid are biogenetically linked.

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ACKNOWLEDGMENTS

R.H. Thomson contributed an authentic sample of norsolorinic acid; A.O. Franz, laboratory assistance, W.A. Pons, Jr., and Alva F. Cucullu, advice.

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[Received November 23, 1970]