TABLE I

Concentration[a] of Total Carbonyl and Acetone Derivatives Present in Male and Female Poultry Skin at Three Ages

Age at slaughter, weeks	Total carbonyl		Acetone	
	Male	Female	Male	Female
Chickens				
8	46.5	65.3	2.8	1.2
10	49.2	55.2	1.1	0.9
12	38.8	33.1	0.6	1.1
Turkeys				
20	263.4	30.4	110.1	2.0
24	397.5	48.6	182.2	4.3
30	50.0	69.3	2.9	2.7

[a]Mean of triplicate samples in μmoles/10 g lipid.

with 2,4dinitrophenyl (DNP) hydrazine, phosphoric acid and water to convert the carbonyls to their 2,4-DNP hydrazone derivatives (3). The monocarbonyl derivatives were eluted from the total carbonyls on a Celite 545-Sea Sorb 43 (Fisher) column and freed of ketoglycerides on a neutral alumina column (4). The monocarbonyl derivatives were further fractionated into classes by column chromatography (5). Quantificiation of the 2,4-DNP hydrazones were determined spectrophotometrically using a Beckman spectrophotometer, model DB-G.

The only class of carbonyl present in the monocarbonyl fraction was methyl ketone derivatives with an absorbance maximum of 365 mμ in chloroform. Further characterization of this fraction utilizing thin layer chromatography to separate the methyl ketone 2,4-DNP-hydrazones into their carbon lengths (6) revealed only the three carbon methyl ketone (acetone) to be present. To confirm that the only derivative present was the DNP hydrazone of acetone, melting points were determined on recrystallized samples of the methyl ketone fraction. The melting point of 126 C confirmed the exclusive presence of acetone. Continuous monitoring showed no significant acetone contamination in the solvents.

Mean concentrations of the total carbonyls and acetone are shown in Table I. Age produced significant variations in the acetone concentration in turkey skin. An increased acetone concentration from 20 to 24 weeks was seen in both sexes; by 30 weeks of age both males and females had dropped to low levels, being similar to each other. The sex differences were particularly striking in the turkeys. While female turkey skin showed an increase in acetone content at 24 weeks, the level at any of the three ages was not high. The greater acetone level in the male turkeys was statistically significant. The interesting finding is the decrease which occurred in the quantity of lipid in the skin of the 24-week-old turkeys. Normally birds are thought to be

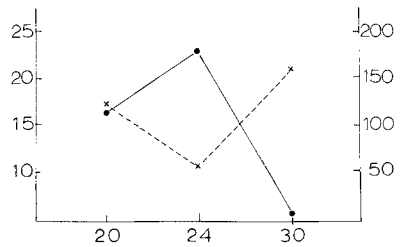

FIG. 1. Relationship between lipid and acetone concentrations in raw male turkey skin. The solid line is acetone, the broken line is lipid. left ordinate, gram lipid/50 g skin; abscissa, age in weeks; right ordinate, μ moles of acetone/10 g lipid.

continually laying down skin lipids during this stage of maturation. This period where a decrease in lipid deposition occurs was marked by increased acetone concentrations in the tissue (Fig. 1). Whether this unusually high level of acetone in the male turkey's skin is also reflected in other tissue is unknown at present. The source of the acetone, however bears consideration. Lipogenesis in avian species occurs primarily in the liver (7), unlike other animals in which extrahepatic tissue also may be an active site of lipid synthesis. In the liver acetyl CoA is formed directly from glucose or from pyruvate via the tricarboxylic acid cycle. This acetyl CoA, independent of its source, is then available for hepatic synthesis of fatty acids for glyceride formation. The triglycerides are transported via low density or β-lipoproteins in the plasma to the adipose tissue. The decreased lipid accumulation in the skin of the 24-week-old male turkeys therefore may be a reflection of depressed hepatic lipogenesis and result in the diversion of acetyl CoA to acetoacetate and thus acetone. However, one cannot discount the possibility of depletion of the lipid stores in the skin by oxidation yielding C-2 units which, because of limited lipogenic capacity at this site, may be diverted into ketone bodies.

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The Contribution of Saturated Acyl Groups to the Volatiles of Oxidized Soybean Oil[1]

ABSTRACT

U-^{14}C-Stearic acid was interesterified into soybean oil and the resulting oil was oxidized to high peroxide values at 25 C and at 125 C. Volatile oxidation products were isolated and found to have a count that was not significantly above background.

Pure saturated fatty acids and esters are quite resistant to oxidation at temperatures below 100 C, but the presence of unsaturated fatty acids decreases their stability. In reviewing the oxidation of saturated fatty acids, Brodnitz (1) made the interesting suggestion that, in the oxidation of natural fats and oils, oxidation of saturated acyl groups may be enhanced by concurrent oxidation of unsaturated acyl groups. He suggested that the oxidation products of saturated acyl groups might make a significant contribution to volatile products that are produced and account for

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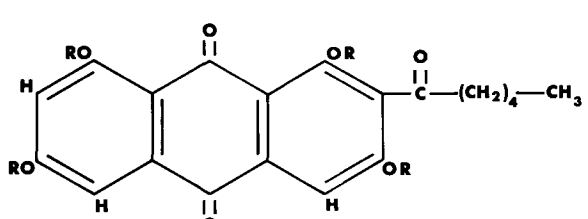
Norsolorinic Acid From a Mutant Strain of *Aspergillus Parasiticus*[1]

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.