Sterculic Derivatives and Pink Egg Formation 1, 2

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Abstract
Sterculyl alcohol has been methylated and reduced to form the methoxyl and hydrocarbon derivatives. These two derivatives, steruleryl alcohol and methyl sterulenate were fed to laying hens. The sterculic derivatives caused pink egg formation but the polymers did not.

The feeding of malvalic and sterulic acids to hens has been reported to cause pink discoloration in cold stored eggs (1, 2). These acids give a positive Halphen test (3, 4), which has been postulated to be specific for the cyclopropene ring (5). However, the property of derivatives of these fatty acids, such as the hydrocarbon or the alcohol, to produce these effects has not been reported.

Sterculic acid is sensitive to heat, forming a polymer the structure of which has been established (6). The loss of the characteristic infrared bands at 5.38 μ and 9.92 μ indicates that the cyclopropene ring is gone. Further evidence for this destruction is a negative Halphen test. The glyceride or methyl ester of the acid is considerably more stable to heat than the acid.

Nunn, in isolating sterulic acid (7), noted that the cyclopropene ring is resistant to lithium aluminum hydride reduction (LAH) by the method of Nystrum (8). This resistance was also observed by Smith (9) where LAH was used to differentiate cyclopropene fatty acids from oxirane oxygen-containing fatty acids. The cyclopropene ring has also been reported to be stable during saponification of Sterculia foetida oil at room temperature with 10% alcoholic KOH (1).

Very few methylation and reduction studies on fatty alcohols to form methyl ethers and hydrocarbons have been reported. Sekera and Marvel (10) reported preparation of saturated fatty acid tosylates in very good yields. Strating (11) prepared hexadecane from LAH reduction of the tosylate in 98% yield. Although methoxy derivatives of hydroxy fatty acids and dimethoxy derivatives of fatty aldehydes have been reported in the literature, 1-methoxy-9-octadecene and similar compounds have not been hitherto reported. Studies of the methylation of sterulyl alcohol and oleyl alcohol with methyl iodide and silver oxide in dimethyl formamide revealed the presence of numerous side products. Methylation with methyl sulfate in tetrahydrofuran produced a methylated product in nearly quantitative yields.

The work reported in this paper shows that the hydrocarbon, ether, and alcohol of sterulic acid produce pink discoloration in eggs and give positive Halphen reactions.

Methods
Oil was extracted at room temperature from 2 kg of ground Sterculia foetida seeds 3 with Skellysolve F (3 x 31). The extracts were dried over anhydrous Na₂SO₄ and the solvent removed by distillation at room temperature under reduced pressure. The yield was 568 g of a golden yellow oil.

Transesterification. Transesterification of 200 g of Sterculia foetida oil was carried out at room temperature. The oil was added to 1 l of dry methanol containing 1% sodium methoxide. The heterogeneous solution was stirred rapidly for 45 min at which time the solution had turned homogeneous and a dark orange in color. The mixture was transferred to a separatory funnel and 300 ml diethyl ether was added followed by 250 ml of ice water. The aqueous layer was extracted with Skellysolve B (3 x 300 ml). The extracts were combined and washed with water (3 x 300 ml), 10% sodium carbonate (2 x 300 ml), 5% sodium bicarbonate (2 x 300 ml), and water (2 x 200 ml) to pH 7-8. The ether solution was dried over anhydrous sodium sulfate and the solvent removed on a rotovane. The yield was 186.5 g of methyl esters.

Fractionation. The methyl esters (100 g) were added to urea-saturated boiling methanol (125 g urea in 365 ml dry methanol). After 3 hr of occasional shaking the adducts were filtered and washed with 100 ml of urea-saturated methanol. Filtrate and washings were combined. A second urea adduct on the combined filtrate and washings removed essen-

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Generally all the saturated esters. The second filtrate was extracted with Skellysolve F with the aid of 200 ml of ice water. The Skellysolve solution was washed free of methanol with water (3 x 200 ml), dried over anhydrous Na₂SO₄ and the solvent removed under reduced pressure. The yield was 36.6 g of methyl sterulate. The methyl sterulate was crystallized from a 10% solution of methanol at −35°C. The crystals obtained at this temperature were pure methyl sterulate as determined by gas-liquid chromatography (GLC), nD = 1.4571. Distillation of 750 mg of the ester under reduced pressure (bp 123°C/0.05 mm) revealed no appreciable polymerization or decomposition. Quantitative infrared spectral analysis on the crystallized and on the distilled ester were identical (bands at 5.38, 8.27, 8.55, 9.11, 9.92 μ). The yield of crystallized methyl sterulate was 20.8 g.

Stereulic acid was prepared from 1 g of the methyl sterulate by conventional saponification at room temperature, nD = 1.4656 [lit. (12), nD = 1.4650] I.R. bands at 5.38, 8.61, 9.15, 9.92 μ. Stereulyl Alcohol. Stereulyl alcohol was prepared according to Nunn, nD = 1.4647 [lit. (7), nD = 1.4617]. The yield was 8.5 g (93.5%) from 10 g of methyl sterulate. I.R. analysis showed bands at 2.80, 5.58, 8.59, and 9.92 μ. Stereulic Acid and Methyl Sterculate Polymers. The method followed was essentially that of Rhinehart (6). The Sterculia foetida acids were prepared from Sterculia foetida methyl esters by cold saponification with 10% KOH in ethanol. Fifteen g of Sterculia foetida fatty acid was heated for 26 hr at 130°C. The polymer was removed from the oil bath and allowed to cool to approximately 60°C. The polymer was washed free of any monomers by rinsing with warm methanol (3 x 50 ml), and the methanol decanted. The polymer was taken up in ethyl ether and dried over Na₂SO₄. The Sterculia foetida methyl ester polymer was prepared in a similar manner. The solvent-free polymers gave negative Halphen tests. Analysis. The purities of the methyl sterulate, stereulyl alcohol, and stereulyl alcohol derivatives preparations assayed at 98% or better by GLC (Aerograph No. A-100-C, 5 ft. diethylene glycol succinate column, 200°C, 30 psi). Halphen tests were run on the derivatives by the method of Deutschman and Klaus (4). For I.R. analysis concentrations of 6% sample in CCl₄ were run on a Perkin-Elmer Model 137 Infracord.

Feeding Experiments. Three feeding experiments containing 4 lots of 5 laying White Leghorn hens were conducted. The hens were individually caged in order to identify the eggs, fed a practical laying ration and given water, ad libitum. The various stereulic derivatives were administered orally by capsule once daily for 15 days. The eggs were collected daily after the 5th day of supplementary diet for 15 consecutive days. They were stored at 2°C for periods of 1 to 3 mo.

The capsules were prepared to contain an amount of cyclopropane equivalent to 25 mg of Sterculia foetida oil. In 10 of 12 trials the derivatives were diluted to 125 mg with corn oil per capsule. The capsules were stored at 7°C until administered. The supplemental diets are given in Table 1.

The eggs were examined for pink discoloration and pH change after 1 mo storage. The degree of discoloration was rated visually on a 5-point scale as used by Kemmerer et al. (17). The pH of the whites and the yolks were determined with a Beckman pH meter.

Results and Discussion

The stability of the cyclopropane ring toward neutral or alkaline reagents is illustrated in the analysis of the preparations presented in this paper. In all the steps of the preparations the most gentle treatment possible was carried out on the cyclopropane ring compounds. No attempts was made to determine the limits of these treatments. It may be noted that preparation of methyl sterulate by transesterification as described in this paper eliminates the use of heating sterulic acid with HCl or esterification with...