• TECHNICAL

The Reactions of 1,2-Dioctylcyclopropene with Silver Nitrate[1, 2]

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Abstract

Sterculene (1,2-di-n-octylcyclopropene) reacts slowly with dilute solutions of silver nitrate in acetonitrile with formation of a silver mirror and production of 9-methylene-10-octadecanone. The reaction is rapid in alcohols; finely-divided silver is formed and sterculene is transformed into 9-alkoxymethyl-9-octadecenes together with smaller amounts of the unsaturated ketone. In neither case is the silver formed in stoichiometric quantities. The reaction of *Sterculia foetida* oil methyl esters with alcoholic silver nitrate was used to demonstrate the possible application of this reaction to the determination of cyclopropene fatty acids.

Introduction

THE FORMATION of a dark color or precipitate with alcoholic silver nitrate has been used for the detection of cotton, kapok, and baobab seed oils (2,4,16, 17,24), all of which also give the Halphen test (17) and are now known to contain cyclopropene fatty acids as triglycerides (5,20).

It has also been observed (25) that cyclopropene is quantitatively removed from a nitrogen gas stream by aqueous silver nitrate. The black precipitate that formed was partially soluble in 30% perchloric acid to leave a light-colored residue insoluble in all common solvents. Wiberg (25) reasoned that the black material was not metallic silver because the metal is not soluble in 30% perchloric acid.

The reaction first came to our attention when a papergram spotted with sterculic acid gave a brown spot at room temperature when it was sprayed with alcoholic silver nitrate (13). Subsequently, it was found that all sterculic acid derivatives having an intact cyclopropene ring gave an immediate black precipitate with this reagent at room temperature. After reaction the isolated lipid no longer gave the Halphen test and spectral and chromatographic analysis showed the cyclopropene ring had been destroyed. When methyl oleate, linoleate and stearolate were refluxed with alcoholic silver nitrate, the esters could be recovered unchanged from the mixture.

There was no reaction between silver nitrate and methyl sterculate in pyridine. In acetonitrile, a powerful solvent for silver nitrate, the reaction was slow with formation of a silver mirror on the side of the flask and the product obtained was different from that of the reaction in ethanol. The product obtained in acetonitrile was also observed in a number of other nonhydroxylic solvents (acetone, ethyl acetate, tetrahydrofuran) but because of the very low solubility of silver nitrate in them, they were not further investigated. Sterculene was chosen as a model compound to study the reactions of silver nitrate with cyclopropenes because of its molecular simplicity.

Experimental

Methyl sterculate and sterculene were prepared as described previously (13). Ag^+ was titrated with CNS^-(Fe^{+++} indicator), microanalyses were performed by Schwartzkopf Microanalytical Laboratory, Woodside 77, N.Y., and GLC done with the Aerograph A-90-C, 20% EGS 10 ft. columns.

Initial Studies. Sterculene (0.5 g) was mixed with 5–20 ml of methyl, ethyl, n-propyl, n-butyl and n-amyl alcohols saturated with $AgNO_3$ and with 20 ml 1% $AgNO_3$ in 1:1 acetonitrile-ether. After 2 days water was added to each mixture and the products isolated by extraction with petroleum ether (p.e.) and evaporation of solvent. The results of the GLC analysis of each product are shown in Fig. 1; they suggest that the alcohol used as solvent enters into the products of the reaction. The IR spectrum of the product from the reaction in methanol had a large band at 9.05 μ indicative of an aliphatic ether (3). A single product was formed in acetonitrile, its spectrum exhibited bands indicative of a methylene group α to a ketone; the same compound (B, Fig. 1) was observed as a minor component in all of the alcohol reactions.

Kinetic Runs and Silver Balance. Sterculene [10 g, 31 meq, 81% pure by HBr titration (10)] was mixed with $AgNO_3$ in 500 ml 1:1 methanol-acetone, methanol, and 1:1 acetonitrile-acetone. Acetone was used because of the limited solubility of sterculene in methanol and acetonitrile. Samples (5 ml) were removed periodically, titrated for Ag^+, then extracted with p.e. to yield a mixture of starting material and products that was analyzed by GLC. At the end of the reaction the metallic silver formed was removed by filtration (from the reactions in methanol) and weighed. It was then dissolved in nitric acid (as was the silver mirror

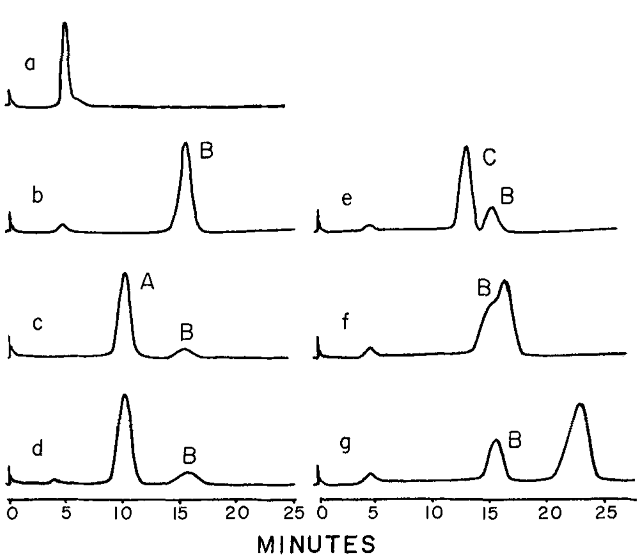

FIG. 1. GLC of (a) sterculene, and the products of the reactions of sterculene with silver nitrate in (b) acetonitrile, (c) methanol, (d) ethanol, (e) 1-propanol, (f) 1-butanol, (g) 1-pentanol. 200°/5# He.

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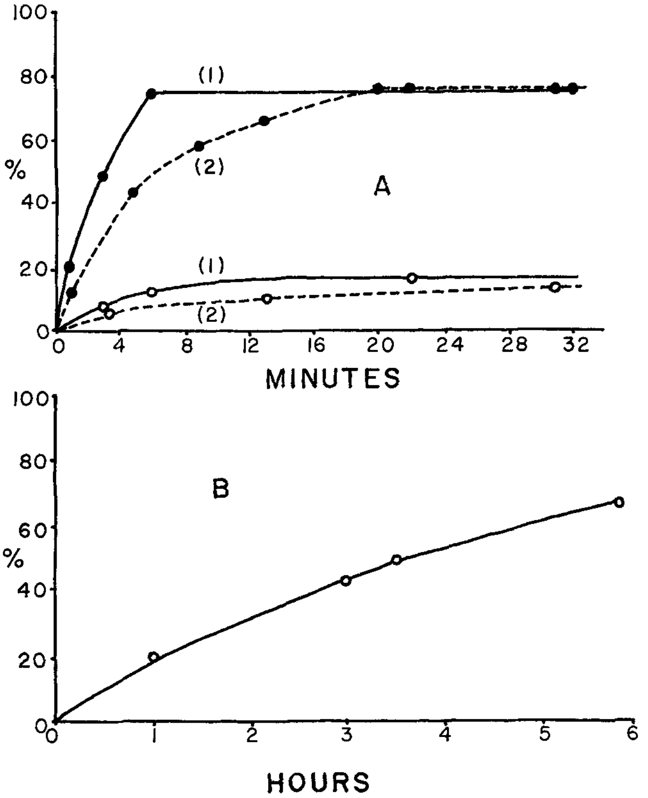


FIG. 2. Yields of products, ● 9-methoxymethyl-9-octadecene, ○ 9-methylene-10-octadecanone, estimate by GLC of volatile constituents. A-1, 0.093 N $AgNO_3$ in 1:1 methanol-acetone; A-2, 0.052 N $AgNO_3$ in methanol; B, 0.103 N $AgNO_3$ in 1:1 acetonitrile-acetone.

formed in acetonitrile) and titrated. The results are shown in Table I and Fig. 2.

Isolation of the Ketone (B, Fig. 1). Sterculene, *1* (27 g 0.1 mole) was stirred for 24 hr with 400 ml 1:1 acetonitrile-acetone containing 0.1 mole $AgNO_3$. Water was added and the product taken up in p.e. and washed with 5% HCl, bicarbonate and water and dried. Evaporation of solvent left 27 g product, bp 110–134 C/0.15 mm, to yield 18 g distillate. This was fractionally distilled to yield 10.5 g pale yellow liquid, bp 118 C/0.05, that still contained small amounts of impurities by GLC. The large residue left after each distillation suggested that decomposition or polymerization accompanied this process. The compound was finally purified by chromatography on 580 g silica gel and elution with 3% ether in p.e.

Anal. calc for $C_{19}H_{36}O$, 81.4 C, 12.94 H; found 81.29 C, 12.90 H. U.V. max 221 mμ, ε 10,000 [α, β-unsat ketone (8)], IR bands 5.96 μ [α, β-unsat ketone (3)], 6.15 and 10.72 μ [conjugated *unsym*-disubstituted olefin (3)], NMR spectrum showed protons at 9.13 (CH_3-), 8.74 ($-CH_2$-), 7.78 and 7.42 ($-CH_2-CO-$, $-CH_2-C=CH_2$), and at 4.35 and 4.04τ ($CH_2 = C<$).

Identification of 9-Methylene-10-octadecanone, 2. The compound absorbed 0.99 mole H_2 over Pd (5% on charcoal) and was completely oxidized to pelargonic acid, *3*, by periodate-permanganate (23), mp and mmp of pelargonic acid p-toluidide 81.5–83C [lit. 84 (22)].

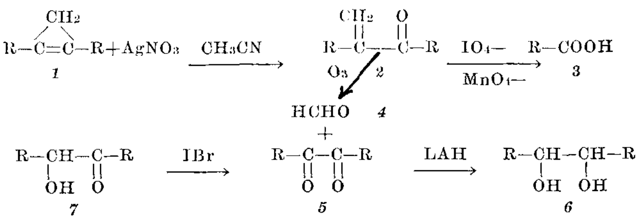
The ketone (2.62 g) in 100 ml methylene chloride was ozonized at −40C. Excess ozone was removed with nitrogen after which the solution was vigorously shaken in a separatory funnel with 1 g zinc dust and 50 ml 50% aqueous acetic acid. The aqueous phase was added to 3 g methone in 20 ml alcohol to yield 1.26 g of the methone derivative of formaldehyde (*4*), mp and mmp 189.5–190C [lit. 189 (11)].

The methylene chloride was evaporated and the residue crystallized from 50 ml methanol to yield 0.61 g pale yellow plates of 9,10-octadecadione, *5*, mp and mmp 56.5–58C. Its 2,4-dinitrophenylosazone was prepared, mp and mmp 171–2C. The diketone, *5*, was reduced with LiAlH4 in tetrahydrofuran to *meso*-9,10-octadecadiol, *6*, mp 127–129C [lit. 127.5–128 (6)].

9-Methylene-10-octadecanone 2,4-Dinitrophenylhydrazone. The derivative was prepared (22) and recrystallized from ethanol, mp 47–48C, U.V. max 372 mμ, ε23,900 (ethanol).

Anal. calc for $C_{25}H_{40}N_4O_4$ 65.2 C; 8.75 H, 12.16 N; found 65.43 C, 8.95 H, 12.17 N.

9,10-Octadecadione, 5. Nonyloin, *7*, prepared by the method of Hansley (9) was oxidized with IBr (9) to 9,10-octadecadione, *5*, pale yellow plates from methanol, mp 56.5–58.5C. A 2,4-dinitrophenylosazone was prepared from the diketone in ethanol-benzene, mp 171–172C.



R = n—$C8H_{17}$— throughout this work

Isolation of the Ether from the Reaction in Methanol (A, Fig. 1). The products from the two kinetic reactions in methanol were combined (16.15 g) and chromatographed on 1 kg silica gel. Elution with 3% ether in p.e. gave no separation between A and B (Fig. 1), nor did fractional distillation of the mixture, although both procedures removed other impurities. The ether distilled without decomposition. The IR spectrum of the ~90% pure ether showed a strong band at 9.05 μ with shoulders at 8.95 and 9.17 μ [aliphatic ether (3)] and a small sharp band at 5.96 μ coming from the 9-methylene-10-octadecanone, *2*, impurity. Hydrogenation of the material consumed 1.08 moles H_2 per MW 296 (sterculene + CH_3OH) to yield a dihydroderivative with a single sharp band at 8.95 μ

TABLE I

Reaction of Sterculene (31 meq, 0.062 M) with $AgNO_3$ in Several Solvents

			Ag⁺ consumed						
				From Ag° formed		Products formed [a]			
Solvent	Initial conc Ag⁺ moles/1	Reaction ½ complete time [a]	Titration of rx mixt meq	weighed meq	titrated meq	Ether [c]	Ketone [d]	Impurities	Moles Ag⁺ consumed per mole sterculene
Methanol-acetone 1:1	0.093	3 min	12.2	10.4	10.7	65%	15.6%	19.4%	~0.35
Methanol	0.052	5 min	12.5	11.9	12.0	72%	15%	12%	~0.4
Acetonitrile-acetone 1:1	0.103	3.5 hr	13.5	—(b)	14.7	0	80%	20%	~0.45

[a] Estimated by GLC (of volatile constituents).
[b] Silver mirror formed, not weighed.
[c] A, Fig. 1.
[d] B, Fig. 1.

(aliphatic ether) and a small saturated ketone band at 5.85 μ. The NMR spectra showed protons at 9.12 (CH_3-), 8.74 (–CH_2-), 8.0 (–CH_2–C = C–), 6.85 (CH_3–O–), 6.29 (–CH_2–O–) and 4.63 τ (–C = CH–).

Identification of 9-Methoxymethyl-9-octadecene, 8. The compound (16 g) in 200 ml CH_2Cl_2 was ozonized as above and the products separated into acid and neutral fractions. The former (2.7 g) consisted of only pelargonic acid, *3,* by GLC of the methyl ester. The latter (12.21 g) was distilled to yield pelargonaldehyde, *9,* bp 92–105 C/30 mm (4.63 g) and 1-methoxy-2-decanone, *10,* bp 130–140 C/30 mm (4.59 g). Pelargonaldehyde was identified as its 2,4-dinitrophenylhydrazone, mp and mmp 104–105C [lit. 105–6 (12)]. 1-Methoxy-2-decanone, *10,* was purified by fractional distillation, bp 70 C/0.53 mm, n$^{20}_{D}$ 1.4328.

Anal. calc for $C_{11}H_{22}O_2$ 70.93 C, 11.90 H; found 71.05 C, 11.86 H.

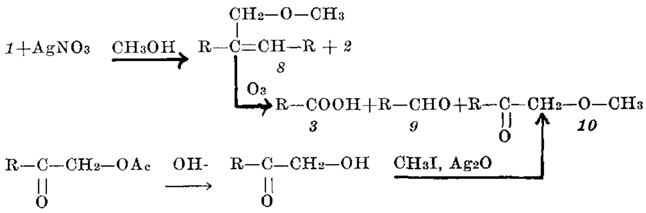
1-Methoxy-2-decanone 2,4-dinitrophenylhydrazone, mp and mmp 84.5–85C.

Anal. calc for $C_{17}H_{26}N_4O_5$ 55.72 C, 7.15 H, 15.29 N; found 55.95C, 7.20 H, 15.21 N.

1-Hydroxy-2-decanone, 12. The compound was prepared by the cold alkali saponification of 1-acetoxy-2-decanone, *11,* (14, 19) and recrystallization from p.e., mp 39.5–41C.

Anal. calc for $C_{10}H_{20}O_2$ 69.72 C, 11.70 H; found 69.99 C, 11.69 H.

1-Methoxy-2-decanone, 10. 1-Hydroxy-2-decanone, *12,* was stirred 4 hr with 10 ml methyl iodide and 3 g silver oxide. Filtration and evaporation of the reaction mixture left a liquid, bp 140 C/30 mm, n$^{20}_{D}$ 1.4330 having the same IR and r.t. on GLC as the compound obtained from the ozonolysis of 9-methoxymethyl-9-octadecene, *8.* Its 2,4-dinitrophenylhydrazone was prepared, mp 84–85.5C.



Isolation of the Ether from the Reaction in 1-Propanol (C, Fig. 1). Sterculene, *1* [20.5 g, 76% pure by HBr titration (10)], was stirred for 18 hr under N_2 with 500 ml 0.05 N $AgNO_3$ in 1-propanol after which time the mixture was filtered and added to water. The precipitated silver was dissolved in nitric acid and titrated (21.5 meq). The reaction mixture was extracted with p.e. and the aqueous phase titrated (3.1 meq Ag^+). About ⅓ mole Ag^+ was reduced to Ag° per mole of sterculene used.

Evaporation of the p.e. left a liquid that was purified by chromatography on 1 kg silica gel and elution with 3% ether in p.e. followed by two fractional distillations. A chromatographically-pure sample was obtained, bp 116 C/0.05 mm.

Anal. calc for $C_{22}H_{44}O$ (sterculene plus C_3H_7OH) 81.40 C, 13.66 H; found 81.17 C, 13.51 H.

The IR spectrum showed a strong band at 9.1 μ with a shoulder at 8.95 μ [aliphatic ether (3)] and a weaker band at 9.6 μ. After hydrogenation (1.03 mole H_2) the saturated derivative had only a single band with a max at 8.95 μ.

Identification of 9-n-Propoxymethyl-9-octadecene, 13. The compound (15 g) was ozonized in 200 ml methylene chloride as above. The acid fraction contained only pelargonic acid, *3,* identified by GLC of its methyl ester. The neutral fraction was distilled to yield pelargonaldehyde, *9* (identified by GLC), and 1-propoxy-2-decanone, *14.* The latter was purified by preparative GLC and distilled, bp 68 C/0.05 mm.

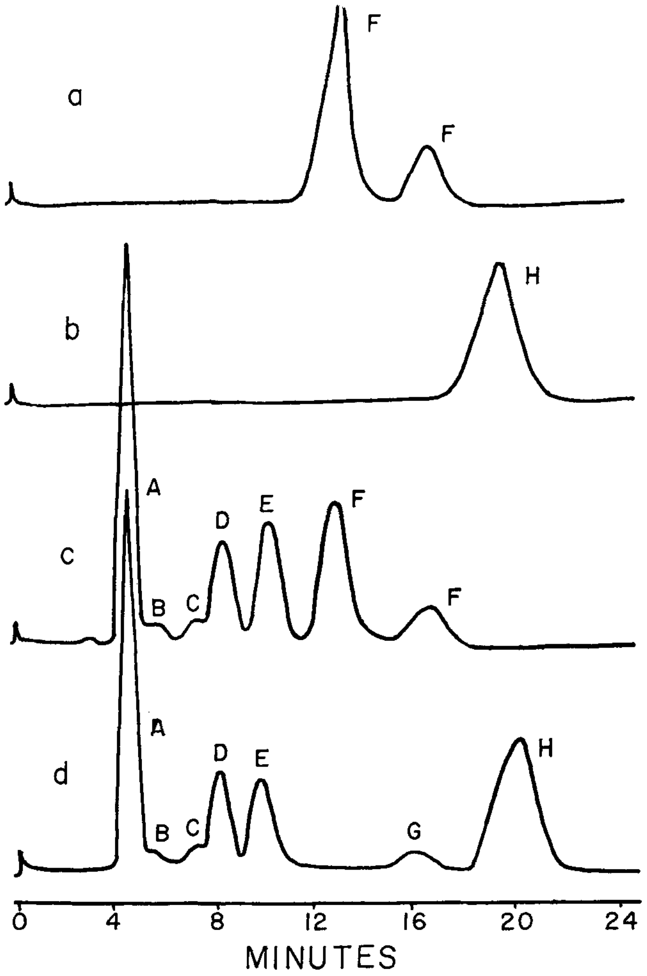


FIG. 3. GLC of methyl sterculate before (a) and after (b) reaction with methanolic silver nitrate and of *Sterculia foetida* methyl esters before (c) and after (d) the reaction. A-palmitate, B-palmitoleate, C-stearate, D-oleate, E-linoleate and malvalate, F-sterculate rearranged by GLC, G-malvalate reaction product with $AgNo_3$, H-sterculate reaction product with $AgNO_3$. 225C/10# He.

Anal. calc for $C_{13}H_{26}O_2$ 72.89 C, 12.23 H; found 72.98 C, 12.22 H.

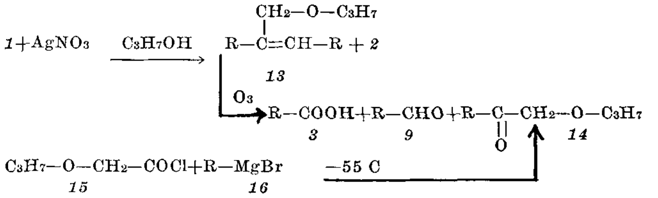
Its 2,4- dinitrophenylhydrazone was prepared mp and mmp 51–51.5C after recrystallizations from ethanol and methanol.

Anal. calc for $C_{19}H_{30}N_4O_5$ 57.85, 7.66 H, 14.20 N; found 56.81 C, 7.61 H, 14.08 N.

1-Propoxy-2-decanone, 14. The compound could not be readily prepared from 1-hydroxy-2-decanone, *12*; an alternate synthesis was devised. 2-Propoxyacetyl chloride, *15,* was prepared from sodium propoxide and iodoacetic acid in propanol followed by thionyl chloride, bp 55C/30 mm. The acyl chloride (7.82 g, 0.057 mole) was dissolved in 100 ml dry ether and cooled under N_2 to –55C. Octyl magnesium bromide, *16* (100 ml 0.57 M soln) was added slowly to the acyl chloride with stirring at a rate so that the temperature never rose above –55C. After the addition of wet ether, water and dilute acid, the ether layer was separated, washed with bicarbonate solution and water and dried. Evaporation of solvent left 11.6 g crude product that was fractionally distilled to yield 6.4 g 1-propoxy-2-

decanone, *14*, bp 73C/0.15 mm, GLC and IR same as that obtained from the ozonolysis above.

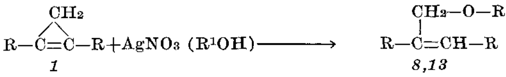
The 2,4-dinitrophenylhydrazone was prepared and melted 51–52C after recrystallization from ethanol and methanol.



Reaction of Methyl Esters and Oils with Alcoholic Silver Nitrate. About 1 ml *Sterculia foetida* oil, cottonseed oil, and the methyl esters of the fatty acids from the two oils were mixed with 25 ml methanol saturated with silver nitrate. Ether was added to the oils to enhance their solubility in the reagent. Dark precipitates were formed in all cases and the Halphen tests on the isolated lipids were negative. GLC separation diagrams of methyl sterculate before and after reaction and of *Sterculia foetida* methyl esters before and after reaction are shown in Fig. 3. No change could be detected in the cottonseed methyl esters by GLC; the concentration of cyclopropene fatty acids in this oil (~0.5%) is too low to be detectable by reaction with silver nitrate and GLC on the Aerograph A-90-C. When one drop of methyl sterculate was added to 20 drops cottonseed oil methyl esters and the mixture treated with alcoholic silver nitrate, a very small peak was observed for the rearranged cyclopropene.

Discussion

Sterculic acid derivatives that contain an intact cyclopropene ring react with silver nitrate in homogeneous solution. The reaction is rapid in alcohols to form alkoxymethyl olefins plus smaller amounts of an α, β-unsaturated ketone.



1 *8,13*

In nonhydroxylic solvents (CH_3CN, acetone) the reaction is slower and the ketone is the only product observed. No other

$$1+AgNO_3 \longrightarrow R-\overset{\overset{CH_2}{\|}}{C}-\overset{\overset{O}{\|}}{C}-R$$

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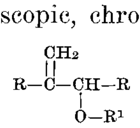
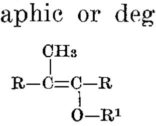
isomers such as *17*, *18*, or *19* were detected by spectroscopic, chromatographic or degradative methods.



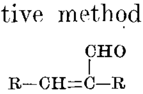
$$R-\overset{\overset{CH_2}{\|}}{C}-\underset{\underset{O-R^1}{|}}{CH}-R$$

17



$$R-\overset{\overset{CH_3}{|}}{C}=\underset{\underset{O-R^1}{|}}{C}-R$$

18



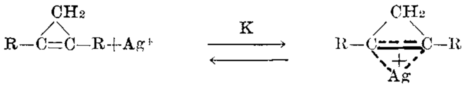
$$R-CH=\overset{\overset{CHO}{|}}{C}-R$$

19

Several pathways of ring opening have been observed during the polymerization of sterculic acid (15,19), the reaction of sterculene with acetic acid (14), the rearrangement of sterculene by activated alumina (21), and the reaction of *Sterculia foetida* oil with hydrogen halides (1); whereas no ring opening occurred during the addition of mercaptans to methyl sterculate and sterculene (13) or the polymerization of cyclopropene (25).

In pyridine, presumably because the unshared electrons on the nitrogen complex the silver ion strongly and do not allow it to interact with the cyclopropene ring, no reaction takes place. The complexing of silver ion by acetonitrile, a powerful solvent for $AgNO_3$ (138 g/100 ml), and the absence of a nucleophile other than nitrate ion explains the slow rate of reaction in this solvent. Although silver ion is reduced to the metal in both alcohols and acetonitrile and the cyclopropene ring is completely destroyed, the reaction is not stoichiometric. Only ⅓ to ½ mole of silver forms per mole of cyclopropene used.

The initial reaction is, undoubtedly, the formation of a silver ion-cyclopropene complex, *20*. The silver-olefin complex



20

has been described by Dewar (7) to arise from an overlap of the π electrons of the olefin with the 5 s orbitals of the silver ion and the interaction of the 4 d electrons of the latter with the anti-bonding π orbitals of the olefin. The stability of the complex (magnitude of K in the equation above) has been most recently studied by Muhs and Weiss (18) who reported that the formation of the silver-olefin complex is essentially instantaneous. The bonding in the complex is affected by the steric, strain and electronic properties of the system. Alkynes are midway between *cis* and *trans* olefins in their binding ability, conjugated dienes are somewhat poorer complexers than the corresponding mono-olefins, and allenes are poor complexers of silver ion. With *cis*-alkenes and strained cyclic olefins, relief of strain by slight twisting of the R groups around the double bond out of a planar configuration is used to explain their increased complexing ability over *trans*-alkenes and unstrained cycloolefins. Cyclopropenes are the most strained cycloolefins known (26) and the relief of this strain by further reaction of intermediate *20* is the driving force behind the silver nitrate-cyclopropene reaction.

The usefulness of the reaction as a quantitative method for determination of cyclopropenes is yet to be demonstrated. Certainly for qualitative work it is feasible to show the presence and approximate amount of these compounds by GLC of esters before and after silver nitrate treatment. The peaks arising from malvalate and sterculate in *Sterculia foetida* oil (20,27) are readily seen in Fig. 3. Before reaction the malvalate peak was obscured by that of methyl linoleate and the methyl sterculate gave two peaks because of decomposition on the column or in the injector.

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Relationship Between Utilization of Fat and Synthesis of Cholesterol and Total Lipid in Young Female Rats[1]

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Abstract

Young adult female rats were fed diets containing 2% of calories from corn oil plus 20, 40, 60 or 80% of calories as beef tallow or diets containing 2% corn oil and the calorie allowance restricted to 80, 60, 40 or 20% of ad libitum consumption. Incorporation of C^{14}-acetate into cholesterol and total fat was determined as an indication of rate of synthesis.

As dietary fat was increased there was a linear increase in cholesterol radioactivity, as measured in serum, liver and carcass. As calories were decreased there were small but significant increases in cholesterol radioactivity. There was a highly significant decrease in incorporation of acetate into total fat as dietary fat increased, and a decrease in total fat radioactivity when calorie intake was restricted. The differences in rate of cholesterol biosynthesis were not accompanied by differences in total quantity of cholesterol. The conclusion reached was that utilization of fat for energy results in accelerated cholesterol biosynthesis.

Introduction

WILSON AND SIPERSTEIN (1) have reported that excretion of cholesterol in the feces of rats was greater with 20% fat in the diet than with no fat, whether the fat was corn oil or lard; 30% fat resulted in even greater excretion of cholesterol. Polyunsaturated fats seemed to enhance excretion to a greater extent than saturated fats. The enhancement of cholesterol excretion by feeding fat to humans was reported by Haust and Beveridge (2), but corn oil was the only kind of fat fed. Unsaturated fat also has been reported to enhance synthesis of cholesterol to a greater extent than does saturated fat (3,4). It has been observed many times that unsaturated fat in the diet for a prolonged period results in lowered serum cholesterol in hypercholesteremic subjects, and Wilson (5) observed that feeding linoleic acid to rats resulted in slight lowering of carcass cholesterol. Apparently cholesterol excretion is enhanced more by unsaturated fat than is synthesis. Why cholesterol is synthesized and excreted in such large quantities has not been determined.

In a report by Dupont and Lewis (6) it was suggested that increasing utilization of fat, either from the diet or from body stores as necessitated by reduction of calorie intake, caused increased biosynthesis of cholesterol by young female rats. Corn oil and a lard-butter mixture had similar effects and the total amount of cholesterol in serum and liver did not increase.

The present study is an attempt to determine whether graded increments in dietary levels of beef tallow or calorie restriction of increasing degree result in proportional increases in synthesis of cholesterol in young female rats.

Experimental

Four groups of 30, 3-month-old, Specific-Pathogen-Free, female rats were obtained from Carworth Farms at 2-week intervals. On arrival the rats were caged singly and fed pathogen-free diet biscuits (D and G Research Animal Laboratory Diet, Dietrich and Gambrill, Frederick, Maryland) for 24 hr, then weighed and distributed into 10 groups of 3, such that body weight range was roughly similar in all groups. Four replications resulted in 10 groups containing 12 rats each. Three rats were lost for reasons unrelated to the experiment.

The rats were fed ad libitum diets containing 2 to 80% of the calories as fat for 4 weeks. At the end of 4 weeks groups 7 through 10 were transferred to restricted calorie diets and groups 1 through 6 were continued ad libitum on the same diets fed them for the first 4 weeks. Calorie intake of animals in groups 1–6 was not limited to a similar level because the effect of partial starvation upon some rats would have made it more difficult to interpret the data than does variation in calorie intake.

Composition of diets formulated to contain the same amounts of all nutrients except fat and carbohydrate per 100 calories is given in Table I. Factors of 4 calories per gram of carbohydrate and protein and 9 calories per gram of fat were used. Diets for groups 5 and 6 were exactly the same, providing 2 control groups.

Every 50 calories supplied by diets 1 through 5 and the daily average food allowance for groups 7 through 10 supplied 2 g protein, 262 mg cellulose flour, and 262 mg of vitamin mix per rat. Diets 1 through 9 supplied 0.50 g of salt mix and diet 10, 0.34 g. The lower amount in diet 10 was necessary to avoid an excessive concentration of salts in that diet. All diets supplied 60–70 mg of linoleic acid (calculated) daily, on the average. The corn oil was fed only as an approximate minimum source of essential fatty acids and was assumed to have little bearing upon metabolism of beef tallow, or body fat.

Calorie intakes of groups 7 through 10 were restricted during the fifth week as indicated in Table I. The rats were fed their daily allowance in 2 portions,

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