would also be able to reduce the nitro group of this compound, since Fouts and Brodie (*4*) have shown that nitro reductase activity is a property of several tissues in other species. Further, it is probable that such a reduction could be effected by bacteria of the intestinal flora. The high level of nitro reductase activity shown by both turkey liver and kidney suggests that this pathway may represent a major route of metabolism of this compound.

Data obtained in experiments relative to separation of metabolites resulting from incubation of carbon-14–labeled 2-acetamido-5-nitrothiazole with turkey liver or kidney slices are shown in Figures 2 and 3. Figure 4 represents a blank containing labeled compound carried through the incubation and separation procedure. The small amount of radioactive material remaining at the origin in this chromatogram was also found in freshly prepared solutions of the compound in acetone, and thus was not produced during incubation and subsequent separation steps. In the liver incubation mixture, six radioactive spots are evident with spot 5 corresponding to unchanged 2-acetamido-5-nitrothiazole. In the kidney incubation mixture, three spots are apparent with spot 3 corresponding to unchanged compound. Values obtained for the analysis of incubation media for unchanged compound by polarography correspond well with the concentrations of unchanged compound present on chromatograms. This suggests that the majority of the metabolites produced under these in vitro conditions no longer have the nitro group intact. Further work is required to substantiate this point.

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FAT COMPONENTS

Unsaponifiable Fraction of Pork Fat as Related to Boar Odor

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The unsaponifiable matter obtained from pork fat was fractionated and found to contain carbonyls, cholesterol, squalene, vitamin A, and four saturated hydrocarbons. In addition, cholesterol esters, a 7-ketosterol, a triterpene alcohol, and two oxidation products of squalene were indicated. No evidence of primary or secondary alcohols or of sulfur- or nitrogen-containing compounds could be found. No important differences were observed in the components from fat containing boar odor as compared to fat free from boar odor.

Although meat from the uncastrated male pig (boar) has been known for many years to produce an undesirable permeating odor on heating, attempts to establish the frequency and to identify the responsible components have been relatively recent (*5, 17*). Craig *et al.* (*5*) reported that the component(s) responsible for boar odor was (were) located in the unsaponifiable fraction of pork fat. The presence of cholesterol in lard and whole pig fat was noted by Lange (*12*). Fitelson (*8*) reported the presence of squalene in pork lard and beef tallow. The presence of cholesterol and squalene in the unsaponifiable material from both boar and barrow fat was also reported by Craig *et al.* (*5*). However, these authors were unable to associate the presence of either cholesterol or squalene with boar odor in pork. The presence of vitamin A in pork lard was indicated by others (*2, 10*).

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The present study was undertaken to ascertain the chemical composition of the unsaponifiable fraction from pork fat and to determine relationship to boar odor.

Experimental Methods

Saponification. Both boar fat containing boar odor and barrow (castrated male pig) fat free from boar odor were saponified in the cold as suggested by Deuel (*6*). The cold saponification method has been described in some detail by Craig *et al.* (*5*). In brief, the procedure consisted of saponifying the fat dissolved in diethyl ether at room temperature by adding a concentrated solution of sodium ethylate. The unsaponifiable material was isolated and purified by filtering off the soap and washing repeatedly with water. The solution was then dried with anhydrous sodium sulfate, filtered, and reduced to a few milliliters under vacuum. The unsaponifiable residue was stored in the refrigerator under nitrogen.

Qualitative Tests. The nitrochromic acid test for alcohols, the 2,4-dinitrophenylhydrazine test for carbonyls, and the LeRosen test for aromatic nucleus and aliphatic unsaturation were carried out utilizing the reagent solutions described by Walsh and Merritt (*16*). The Lieberman-Burchard test for cholesterol as described by Litwack (*13*) and the hydroxylamine test for esters as described by Hall and Shaefer (*9*) were employed. Sulfur-containing compounds were determined by the Feigl method (*7*), and the micro-Kjeldahl procedure was used to measure the nitrogen content of the unsaponifiable material (*1*).

Removal of Free Fatty Acids. The free fatty acids remaining in the unsaponifiable material were removed by the basic copper carbonate method described by Capellas *et al.* (*4*).

Column Chromatography. A 100-mg. sample of unsaponifiable material was subjected to silicic acid chromatography (*4*). The unsaponifiable matter was dissolved in a minimum of hexane and added to the top of a chromatographic column 11 mm. in diameter fitted with a stopcock and containing 3 grams of Mallinckrodt's 100-mesh silicic acid. The type and

volume of eluents were those recommended by Capellas *et al.* (*4*).

Thin-Layer Chromatography. This technique was employed in an attempt to identify compounds in total and fractionated unsaponifiable material. Chromatography was carried out on 20- × 20-cm. glass plates coated with a 300-micron layer of silica gel. The plates were activated at 100° C. for 30 minutes and stored in a desiccator until used. Chromatoplates were spotted with a 10-μl. sample using a Hamilton microsyringe and developed in an equilibrated chromatographic tank utilizing 100 ml. of solvent. After trying various solvent systems, hexane–diethyl ether (4 to 1, v./v.) was found most successful and was used throughout the remainder of these studies. In addition, 1 to 2 ml. of concentrated acetic acid were added to the solvent to prevent excessive tailing. After development, the chromatoplates were allowed to dry at room temperature. The developed plates were visualized by utilizing an ultraviolet lamp or charring the spots with a 50% solution of aqueous sulfuric acid. After spraying, the spots were traced on acetate paper for a permanent record.

Gas Chromatography. The total unsaponifiable material from pork fat was analyzed using a Barber-Colman Model 20 gas chromatograph with a $7^1/_2$-foot × $^1/_4$-inch copper column packed with 2% silicone SE-30 on 60- to 80-mesh Chromosorb W. Unknowns were identified by comparing retention times with those of known compounds suspected of being present. Because of difficulties in resolving this material by gas-liquid chromatography (GLC), identifications were substantiated with thin-layer chromatography (TLC) rather than by using a different GLC column. When the retention time of a known compound indicated that it was present in the unsaponifiable material, this compound was subjected to conditions which enhance autoxidation and then rechromatographed. This technique was designed to show the presence or absence of oxidation products in the unsaponifiable material.

Solubility properties of compounds in the unsaponifiable material were also studied by extracting the unsaponifiable material twice with cold concentrated H_2SO_4 and then rechromatographing the insoluble residue.

Results and Discussion

Results of qualitative tests on unsaponifiable material in which boar odor was present were compared with identical tests on unsaponifiable material from which boar odor was absent (Table I). Tests for primary and secondary alcohols, sulfur compounds, and nitrogen compounds were negative in the unsaponifiable material, whether it contained boar odor or not.

Traces of carbonyls were found in unsaponifiable material from both material containing boar odor and identically treated material from which boar odor was absent. These results were not unexpected, since Hornstein and Crowe (*11*) had reported pork fat to contain a small amount of volatile, steam-distillable carbonyls. The occurrence of carbonyls also verified the results of O'Daniels and Parsons (*15*), who indicated their presence in the unsaponifiable fraction of pork fat and attributed the increased yellow color occurring during saponification to the aldol condensation of carbonyl compounds.

The presence of Δ-5 sterols in the unsaponifiable fraction of the samples both with and without boar odor was expected, since Craig *et al.* (*5*) had identified cholesterol in the unsaponifiable material from both boars and barrows (Table I). The positive LeRosen test reported for both boar odor–free and boar odor–containing unsaponifiable material does not verify the presence or absence of a particular compound, but the unsaturated nature of the unsaponifiables helps to account for their instability during and following extraction.

Results of silicic acid chromatography of the unsaponifiable material both with and without boar odor showed no differences in any fractions (Table II). However, boar odor could be detected organoleptically in some fractions from the original boar odor–positive material. Unfortunately, it was not possible to trace boar odor completely in all fractions because of the strong odor of several solvents, even when the undesirable odor was known to be present initially.

The amount of material collected in fractions 1, 3, and 6 was extremely small. No differences could be detected between the material containing boar odor and that which was free. The material eluted in fraction 2 (Table II) on silicic acid chromatography was yellow and viscous. Figure 1 indicates that squalene is the major component of this fraction. Spot 2 (observed by TLC) of this fraction turned blue when sprayed with sulfuric acid, indicating the presence of vitamin A or its esters (*14*). Qualitative testing indicated that this material was vitamin A. Figure 2 also shows that spot 6 from the total unsaponifiable material on the thin-layer chromatogram was identical to a standard vitamin A sample. As before, there was no difference in any of the components observed between the material containing boar odor and that containing none.

GLC was used as an additional means for identifying fraction 2 (Table III). Injection of a standard vitamin A sample into the chromatograph resulted in elution of two peaks with retention times of 4 and 5 minutes. Since TLC analysis of the same standard sample of vitamin A resulted in only one spot (Figure 2), it is suggested that the high temperature used during GLC altered vitamin A to

Table I. Chemical Components in Unsaponifiable Material Free of Boar Odor and Containing Boar Odor

Component	Material Containing Boar Odor	Material Free of Boar Odor
Primary and secondary alcohols	Negative	Negative
Carbonyl compds.	Positive	Positive
Unsaturated ring structure or aliphatic unsaturation	Positive	Positive
Δ-5 sterols compds.	Positive	Positive
Sulfur-containing compds.	Negative	Negative
Nitrogen-containing compds.	Negative	Negative

Table II. Results of Silicic Acid Chromatography of Unsaponifiable Material

Fraction No.	Eluent	Mg. Recovered	Compounds in Boar Odor Material	Compounds in Boar Odor–Free Material
1	Hexane	5	Unsaturated	Unsaturated
2	Carbon disulfide	19	Vitamin A, squalene	Vitamin A, squalene
3	95% hexane, 5% benzene	4	Unsaturated	Unsaturated
4 and 5[a]	90:10 hexane, 80:20 benzene	6	Esters, unsaturated	Esters, unsaturated
6	70% hexane, 30% benzene	6	Unsaturated	Unsaturated
7	98% hexane, 2% ethyl ether	11	Lanosterol	Lanosterol
8	92% hexane, 8% ethyl ether	31	Cholesterol, 7-keto-sterol	Cholesterol, 7-keto-sterol
9	5% methanol, 95% chloroform	17	Cholesterol	Cholesterol

[a] Fractions 4 and 5 combined because of the very small amount of material obtained in each.

give two compounds differing in their retention times. Five major peaks by GLC were observed in fraction 2 (Table III). Peaks with retention times identical to those of fraction 2 were also observed on GLC of the total unsaponifiable material under identical conditions (Table III, Figure 3). A comparison of the retention times of the peaks of silicic acid fraction 2 observed on GLC showed that peaks 2 and 3 corresponded to the two peaks of vitamin A observed on GLC. Peaks 1 and 4 appeared to be oxidation products of squalene and peak 5 was due to squalene itself. On testing the oxidized squalene with 2,4-dinitrophenylhydrazine, a precipitate appeared, indicating the presence of a carbonyl compound. However, no differences were observed between material containing and free from boar odor.

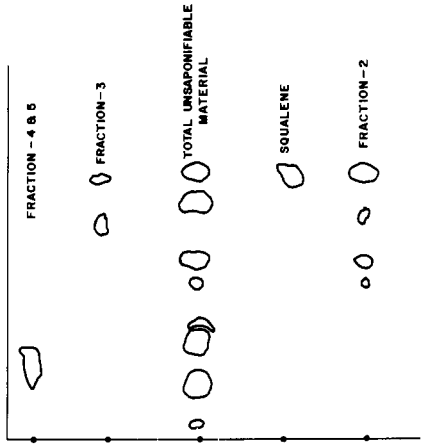

Figure 1. Thin-layer chromatogram of fractionated and unfractionated unsaponifiable material from pork fat containing boar odor

Solvent system. 80% hexane–20% diethyl ether–2 ml. of acetic acid
Chromatoplates. SiO_2 with 15% calcium sulfate binder
Spray reagent. 50% aqueous H_2SO_4

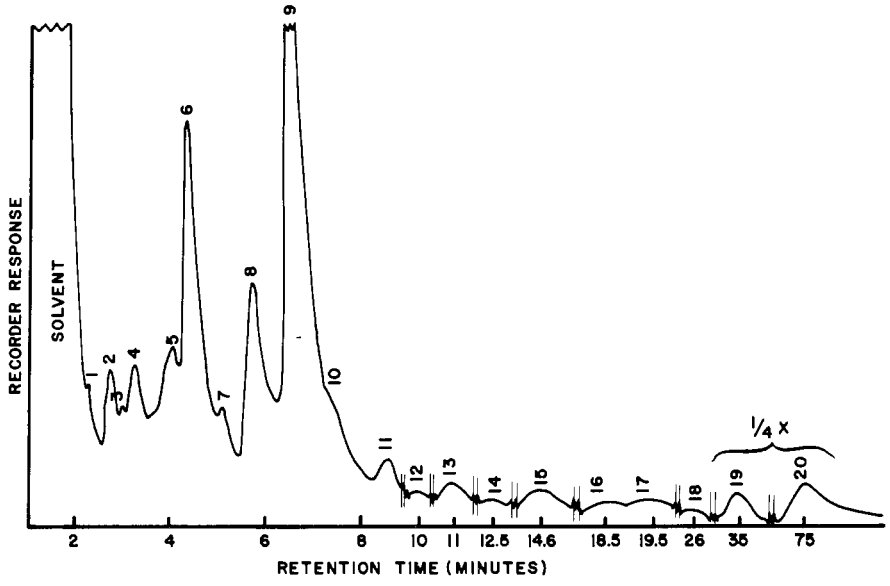

Figure 3. Gas chromatogram of total unsaponifiable material from pork fat in ether

Sample size. 1.0 μl.
Gas pressure. 20 p.s.i.
Argon flow rate. 76.9 ml./min.
Column. 7 1/2 ft. × 1/4 inch packed with 2% SE-30 on Chromosorb W
Temperature. Flosh heater 285° C. Column 225° C. Cell 270° C
Cell voltage. 1500
Relative gain. 10

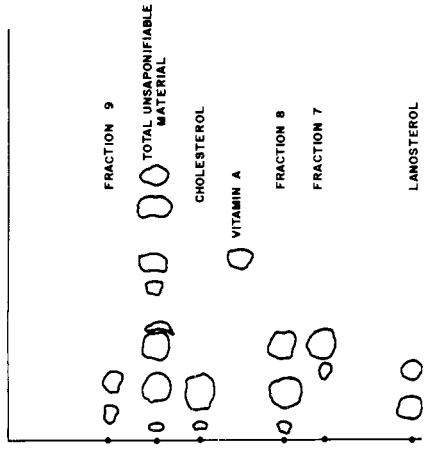

Figure 2. Thin-layer chromatogram of fractionated and unfractionated unsaponifiable material from pork fat free of boar odor

Solvent system. 80% hexane–20% diethyl ether–2 ml. of acetic acid
Chromatoplates. SiO_2 with 15% calcium sulfate binder
Spray reagent. 50% aqueous H_2SO_4

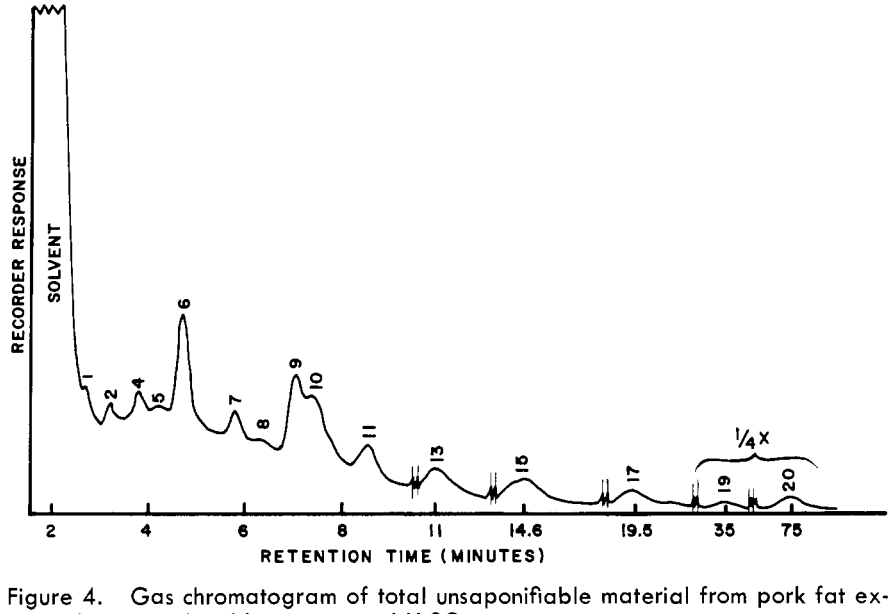

Figure 4. Gas chromatogram of total unsaponifiable material from pork fat extracted twice with cold concentrated H_2SO_4

Sample size. 1.0 μl.
Gas pressure. 20 p.s.i.
Argon flow rate. 76.9 ml./min.
Column. 7 1/2 ft. × 1/4 inch packed with 2% silicone SE-30 on Chromosorb W
Temperature. Flash heater 285° C. Column 225° C. Cell 270° C.
Cell voltage. 1500
Relative gain. 10

Table III. GLC Analyis of Known Standards and Unsaponifiable Material

Sample	Peak No.	Retention Time, Minutes: Boar odor–cont. material	Boar odor–free material	Standard samples
Silicic acid chrom., fraction 2	1	2.1	2.1	...
	2	4.0	4.0	...
	3	5.0	5.0	...
	4	26.0	26.0	...
	5	35.0	35.0	...
Vitamin A	1	...	...	4.0
	2	...	...	5.0
Squalene	1	...	...	35.0
Oxidized squalene	1	...	...	2.1
	2	...	...	26.0
	3	...	...	35.0
Cholesterol	1	...	...	75.0
Silicic acid chrom., fraction 8	1	75.0	75.0	...
Total unsap. material[a]	1	2.0	2.1	...
	5	4.0	4.0	...
	7	5.0	5.0	...
	18	26.0	26.0	...
	19	35.0	35.0	...
	20	75.0	75.0	...

[a] Refer to Figure 3.

Capellas *et al.* (*4*) reported the presence of esters in fractions 4 and 5 obtained on silicic acid chromatography of the unsaponifiables from olive oil. However, the quantity of material obtained from these same fractions in the present study was extremely small; so they were combined before subsequent testing. Qualitative tests indicated that sterol esters were present in the combined fractions. Attempts to identify specific esters using known standards were unsuccessful.

The material in silicic acid chromatography fraction 7 appeared to contain triterpenoid alcohols (Table II) when subjected to the Lieberman-Burchard test. This was not surprising, since the triterpenoid alcohol, lanosterol, is believed to be an intermediate in the biosynthesis of cholesterol. Figure 2 indicates a small amount of lanosterol present in fraction 7; however, this spot cannot be found in the total unsaponifiable material. GLC failed to confirm the presence of lanosterol either in this fraction or in the total unsaponifiable material. The results obtained on fraction 7 failed to contribute anything concerning the absence or presence of components responsible for boar odor.

In addition to cholesterol (Tables II and III), two other spots were observed when fraction 8 from silicic acid chromatography was subjected to TLC (Figure 2). Spot 1 of this fraction as well as spot 1 of the total unsaponifiable material had R_f (0.06) values identical to spot 1 of the cholesterol standard. Since this material was observed in the standard sample only if it was allowed to stand at room temperature and open to the atmosphere for long periods of time, it was assumed that oxidative degradation had taken place. Spot 1 in all three cases is believed to be a 7-ketosterol since Bergstrom and Wintersteiner (*3*) reported this compound to be the chief reaction product when cholesterol was subjected to oxidation in air. Spot 3 of fraction 8 seems to be the result of poor column separation.

The silicic acid column was stripped with 5% methanol in chloroform to obtain fraction 9. In Figure 2 a spot similar to cholesterol as well as two slower moving ones can be seen. The presence of cholesterol in this fraction is verified by the techniques reported in Table II. Analyses showed no differences in unsaponifiable material containing boar odor and samples free from boar odor.

The total unsaponifiable material was subjected to TLC and GLC (Figures 1, 2, and 3). A chromatograph of the unsaponifiable material containing boar odor is shown in Figure 1, whereas material free from boar odor is presented in Figure 2. Very small differences are observed in the spots resolved by this technique. The GLC analysis (Figure 3) resulted in the same findings; therefore, only the chromatogram from boar odor–free material is included. The retention times of the various peaks and standards are reported in Table III. From this chromatogram and Table III, cholesterol, squalene, vitamin A, and two degradation products of squalene are indicated.

Saturated hydrocarbons and various halogenated compounds are insoluble in cold concentrated H_2SO_4. GLC data eliminated the possibility of the presence of halogenated compounds (nonlinear results would have been obtained by using an argon ionization system of detection); therefore, any material insoluble in cold concentrated H_2SO_4 can be assumed to be a saturated hydrocarbon. After two extractions with H_2SO_4, the unsaponifiable residue was again subjected to GLC (Figure 4). After two extractions with H_2SO_4, all peaks observed in Figure 3 were reduced except 10, 11, 13, 15, and 17. It is difficult to compare the exact height for peak 10 because it is partially covered by peak 9. However, the peak height measurements appear to be about the same in both the extracted and unextracted material. Thus, results indicate that peaks 11, 13, 15, 17, and possibly 10 (Figure 3) were due to saturated hydrocarbons. Saturated hydrocarbons containing less than 10 carbon atoms were analyzed by GLC. These compounds emerged with the solvent peak and were undetectable. Therefore, it can be concluded that peaks 11, 13, 15, 17, and possibly 10 are due to saturated hydrocarbons containing more than 10 carbon atoms.

Various other extractions failed to aid in the chemical classification of the remaining nine peaks. The possibility that these peaks are due to sterols is very minute, because the retention time of the unknown compounds was too short. Sulfur- and nitrogen-containing compounds were eliminated by qualitative tests. However, known nitrogen- and sulfur-containing compounds were not submitted to GLC, which is more sensitive than the qualitative tests. If present, obviously they occurred only in trace amounts. Infrared and qualitative analysis on unsaponifiable material revealed that alcohols were also absent. Free fatty acids were eliminated by the use of copper carbonate. After considering these findings, the remaining nine peaks were tentatively identified as unsaturated hydrocarbons and/or carbonyl compounds. However, these peaks were observed in both material containing boar odor and material free of boar odor, which makes it impossible to draw any conclusions pertaining to the component or components responsible for boar odor in pork.

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PLANT PIGMENTS

Polarographic Measurement and Thermal Decomposition of Anthocyanin Compounds

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Pelargonidin-3-monoglucoside, petunidin-3-monoglucoside, and malvidin-3-monoglucoside usually yielded two polarographic waves in HCl–sodium citrate or tartrate supporting electrolytes. The electron change was one for each of the two waves. The half-wave potentials varied according to pH. The length of time required to destroy 90% of the P-3-G upon being heated at 38°, 49°, and 71° C. followed a first-order reaction. The presence of ascorbic acid, H_2O_2, $FeCl_3$, and $NaNO_3$ significantly altered the destruction rates as compared with control solutions. At 100° C., Pt-3-G and M-3-G also decomposed according to a first-order reaction. Polarographic wave height was related to the anthocyanin content of strawberry juice. Interactions among constituents of the food, heat, and anthocyanin content may markedly affect the stability of anthocyanins in food.

CHELATION by copper of anthocyanins (*20*) and the stability of anthocyanins to heat (*6, 15*) have been studied in our laboratories as part of a program concerned with the microbiological activity of anthocyanin pigments (*19*). This report records the half-wave potentials of P-3-G, M-3-G, and Pt-3-G and the stability of P-3-G in the presence of redox compounds.

Zuman (*23*) determined the half-wave potentials of six anthocyanin compounds. He listed one half-wave potential for each compound except delphinin, which possessed two polarographic waves. Somaatmadja, Powers, and Hamdy (*20*) observed that M-3-G also yielded two waves. The effects of oxidizing and reducing agents, pH level, heat, and light on the stability of anthocyanin compounds have been extensively investigated (*2, 4–6, 9, 11–13, 16, 22*), but only some investigators (*3, 7, 8, 10, 14, 21*) have reported that anthocyanins decompose according to a first-order reaction. Pratt, Powers, and Somaatmadja (*17*) observed that strawberry and grape anthocyanins were more stable in the juice than as extracted, purified pigments.

Experimental

The P-3-G was obtained from strawberries (*10*); the Pt-3-G and M-3-G were isolated from Cabernet Sauvignon grapes by a paper chromatographic procedure (*19*). A 0.1*M* HCl solution buffered to different levels with sodium citrate and a tartrate supporting electrolyte were used for the polarographic determinations. Different pH levels were used to relate half-wave potentials to pH. After obtaining satisfactory polarograms of the isolated pigments, polarographic analyses were made using strawberry juice as the substrate to learn whether anthocyanin compounds could be measured in mixed systems.

The stability of P-3-G in the presence of $FeCl_3$, $NaNO_3$, H_2O_2, ascorbic acid, and cystine was determined at 38°, 49°, and 71° C. The concentrations used were 25 mg. per 100 ml. Quantitative measurements of the P-3-G degradation were made according to the method of Meschter (*11*), and the destruction rates were established by calculating the decimal reduction time (*1*).

For Pt-3-G and M-3-G the destruction rate curves were plotted to determine the decomposition rate.

Results

Half-Wave Potentials. Two polarographic waves resulted when P-3-G, M-3-G, and Pt-3-G, isolated from strawberries and grapes, were analyzed. Two waves also resulted when strawberry juice was used. Table I shows the half-wave potentials of P-3-G at pH 1.0 to 6.0. M-3-G yielded three waves (−0.196, −0.247, and −0.372) at pH 3.5, but only two waves above pH 3.9. Pt-3-G yielded two or three waves ac-