in the synthesis indicated the presence of only 90% of the unsaturated acid (R. H. B.).

Summary

Hydroxynaphthoquinones having alcoholic groups in the side chain can be separated from hydroxyl-free derivatives through the water-soluble sulfate esters; the sulfates of tertiary alcohols are very labile, but methods have been found for effecting partition, hydrolysis or HX-cleavage.

Mixtures of saturated and unsaturated members of the series can be separated by formation of C-sulfonic acids by the action of acetylsulfuric acid.

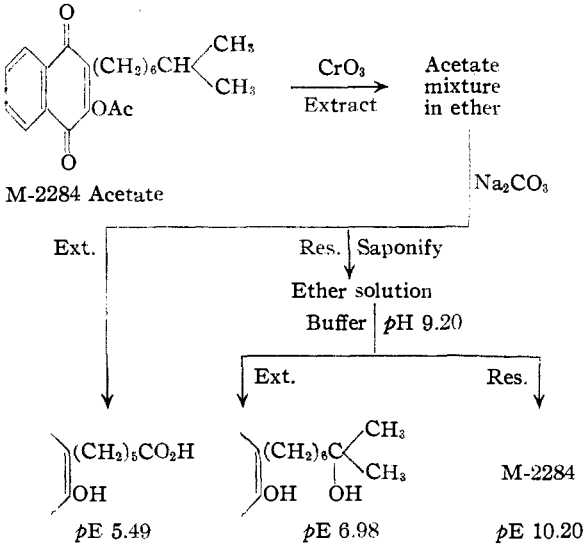
CAMBRIDGE, MASSACHUSETTS RECEIVED MAY 13, 1947

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Naphthoquinone Antimalarials. XVII. Chromic Anhydride Oxidation[1]

BY LOUIS F. FIESER

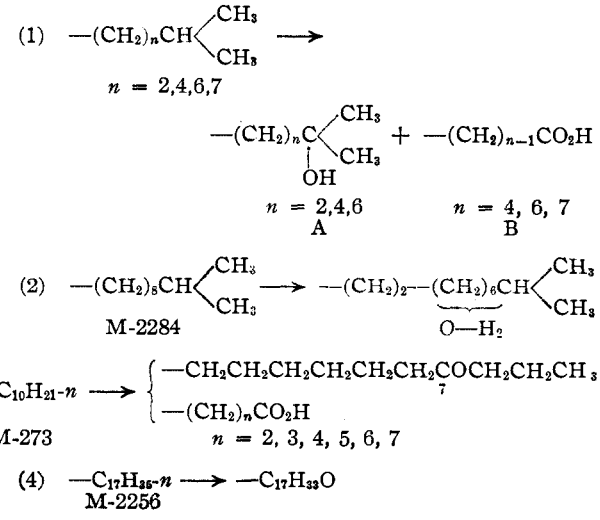
Although the chromophoric nucleus of the hydroxyalkylnaphthoquinones is highly sensitive to attack by permanganate,[2] hydrogen peroxide[3] or hypochlorite,[3] the first point of attack in metabolic oxidation in the liver is the hydrocarbon side chain and not the nucleus (Paper XVIII). It has now been found that similar side-chain degradation can be accomplished by oxidation of the acetates with chromic anhydride. The reactions usually afford a mixture of several quinone acids and a more nearly homogeneous neutral fraction found to contain either a tertiary alcohol or a ketone. The separation of the mixture of these two fractions and unchanged starting material is easily accomplished (see chart). Extraction from ether



with soda solution removes the quinone acids and effects hydrolysis of the 3-acetoxyl group of extracted material (red solution). The residual material is saponified and the more hydrophilic oxidation products separated from starting material by extraction from ether with a buffer of *p*H intermediate between the extraction constants (*p*E, Paper XV) of the products to be separated.

Colorimetric determination of the amounts and *p*E of the fractions provides a useful indication of their nature and homogeneity. With use of these convenient analytical methods, time–yield curves were determined with millimole quantities of the acetates for guidance of the isolation work. An initial study of the effect of varying the solvent in oxidations conducted at 25° (Fig. 1) revealed the striking fact that oxidation is much faster and more efficient when done with a suspension of chromic anhydride in glacial acetic acid (lower chart) than by the classical procedure (upper chart). The oxide begins to dissolve within a few minutes with darkening of the solution and temperature rise. In the example cited the starting material was all consumed in forty-five minutes and the total yield of products was 85%. With a homogeneous solution containing 10% water, starting material was still present after one day and the maximum total yield was 57%.

The products isolated in oxidations conducted by the anhydrous procedure at 20–25° are indi-



(1) I am greatly indebted to Research Corporation for a grant that materially assisted the investigation.

(2) Hooker, THIS JOURNAL, **58**, 1168 (1936).

(3) Paper XII, *ibid.*, **70**, 3215 (1948).

(5) $-CH_2CH(CH_3)C_6H_{13-n}$ (M-285) → (A) $-CH_2CH(CH_3)CH_2CH_2CH_2CH_2COCH_3$ (7); (B) $-CH_2CH(CH_3)CH_2CH_2CH_2CH_2CO_2H$ (7); (C) $-CH_2CH(CH_3)CH_2CH_2CH_2CO_2H$ (6); (D) $-CH_2CH(CH_3)CH_2CO_2H$ (4)

(6) $-(CH_2)_8CO_2CH_3$ (M-1917) → (A) $-(CH_2)_4CO_2H$; (B) $-(CH_2)_3CO_2H$; (C) $-(CH_2)_2CO_2H$

(7) $-CH_2CH_2CH_2CH_2CH_2C_6H_5$ (M-2276) → $-CH_2CH_2CH_2CH_2COC_6H_5$

(8) $-CH_2CH_2CH_2C_6H_4CH_3$-*p* (M-1952) → (A) $-CH_2CH_2COC_6H_4CH_3$-*p*; (B) $-CH_2CH_2CH_2C_6H_4CO_2H$-*p*

(9) $-CH_2CH_2CH_2$-Cyclohexyl (M-1916) → $-CH_2CH_2CO_2H$

ketone or ketone mixture. The ketone from M-273 (3) was obtained pure only after several crystallizations of a mixture of the same analysis. The structure was tentatively inferred by rearrangement of the oxime and hydrolysis (poor yield), and determination of the extraction constant of the quinone acid; the η-ketone was then synthesized and found identical with the oxidation product. The structure of the ketone from M-285 (5) was established by oxidation of the ketone acetate and isolation of the companion acids, A, B and C, of the initial oxidation. The isolation of acid B in small amounts proves that the carbonyl group is in the η position; B and C probably arise by cleavage on the two sides of the keto group

(10) M-266 → CO_2H, CO_2H (A); H, CO_2H, H (B)

(11) M-2293 → A′ (CO_2H, CO) + A (HO_2C, CO)

(12) $-CH_2C(CH_3)_3$ (M-1934); no side-chain oxidation, 85% recovery.

cated in formulations 1–12 (by side chain). The isoalkyl compounds yielded mixtures of quinone acids, and the chief component was isolated by fractional crystallization. The three acids derived from M-285 (5) were separated by fractional extraction from ether with buffers of increasing *p*H. The more complex acid mixture from the *n*-decyl compound (6) was resolved by Fischer esterification (carboxyl only) and countercurrent distribution of the esters between ether and a series of buffers in separatory funnels,[4] from a total of fifty-two fractions, each of the six homologous acids was isolated in pure form. The three acids produced in oxidation (6) were easily isolated by the same method from 150 mg. of mixture. For comparison with the straight-chain acids isolated, the complete series was prepared by Hooker oxidations of synthetic $-(CH_2)_8CO_2H$ and $-(CH_2)_4CO_2H$ (Paper X). The branched-chain acids are fully identified by the analyses and *p*E values.

The first of the three tertiary alcohols isolated (1) is known and the third was synthesized. The neutral product from the next higher isoalkyl derivative (2) is not identical with the synthetic tertiary alcohol expected and the analysis indicates that it is a ketone. Oxidations (3) and (5) definitely yielded ketones, and the *n*-C_{17} derivative (4) gave a substance having the composition of a (chiefly on the side adjacent to the quinone ring) and D must come from B or C. The ease with which an acid side chain is shortened is further demonstrated by (6). In the oxidation of M-273 (3), the η-ketone probably is the immediate precursor of the acids having five and six methylene groups; the formation of the acid $-(CH_2)_7CO_2H$ suggests that the ketone mixture probably contains the isomer $-(CH_2)_8COCH_3$.

In the case of the aralkyl derivatives (7) and (8) the point of attack is at the activated methylene and methyl groups. The oxidations proceed rapidly at 20° and give the ketones and the acid indicated in 90–94% total yield. The cyclohexyl derivative M-266 (10) gave a C_{16}-diacid identified as resulting from the opening of the ring at the 3,4-position by the isolation of an identical acid as one product of the oxidation of M-2293 acetate (11) for a brief period. The other product of brief oxidation is a C_{22}-keto acid corresponding to structure A or A′. When the oxidation period was extended the keto acid was absent and a high melting C_{17}-acid (B) was formed. This acid B was then obtained by oxidation of the keto acid methyl ester acetate, and hence the keto acid has the structure A. The isomer A′ is the probable precursor of the C_{16}-diacid but was not isolated.

The oxidations at the η-position observed with a normal C_{10}-chain and a β-branched C_8-chain indicate preferential attack at a certain distance from the quinone nucleus rather than in any orientation with respect to the end of the chain. A possible explanation is that the oxidant and the quinone

(4) I wish to thank Drs. J. M. Chermerda and N. R. Trenner of the Merck Research Laboratories for advice on application of this method.

form a complex in which the metallic oxide or ion is bound to either the 4-keto or 2-acetoxy group of the quinone. Stuart models show that the first few methylene groups of the side chain would be out of reach of oxidant so bound but that the zig-zag chain can become oriented to bring the eta or neighboring carbon atom into a vulnerable position. No instances of attack at the activated position adjacent to the quinone ring or at the β-position have been observed, even in the degradation of acid side chains.

That the tertiary alcohols of the first oxidations (1) are the precursors of the acids isolated is indicated by the yield curves (Fig. 1) and in one case was established by oxidation of the 2-acetate of the alcohol (A) and isolation of the acid (B). The ready oxidative fission of a tertiary alcoholic group to an acidic and probably a ketonic fragment may be a step in the opening of alicyclic rings with formation of keto acids (11). In the extensive investigations of the chromic acid degradation of the sterol-bile acid side chain, a tertiary alcohol has been isolated in only one instance (as a lactone[9]), but the present results suggest that all of the many ketones and acids isolated arise by the formation and fission of tertiary alcohols. Observations to be reported later indicate that the oxidation reactions here described are not dependent upon the presence of a quinone ring.

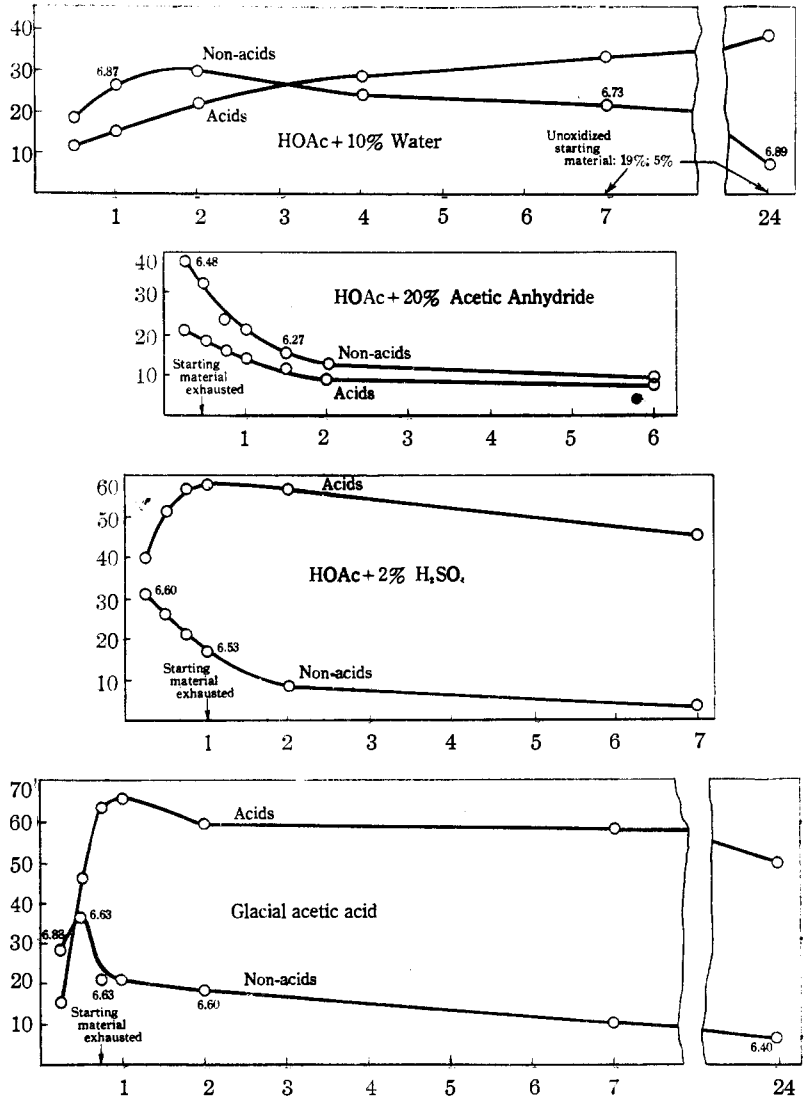


Fig. 1.—Oxidation of M-2284 acetate: per cent. yield (by colorimetry) plotted against time in hours. The figures are negative logarithms (pE) of extraction constants of the fractions.

They also are probably not specific to the non-aqueous procedure, but the fact that they have remained unknown or obscure points to a greater efficiency of the anhydrous procedure over the classical one.

Three of the products of chromic anhydride oxidation are identical with products of metabolic oxidation in man: 1A, 5B and 8B. In the metabolic reaction oxidation always seems to occur at or near the end of the chain, and several of the isoalkyl derivatives investigated suffer ω-methyl oxidation. Chromic anhydride, on the other hand, has in no instance been found to attack methyl groups. Methylene and methine groups seem to be of comparable vulnerability, but methyl groups are fully resistant to attack. Thus the neopentyl compound (12) yielded no products of side chain oxidation. The general success of the Kuhn–Roth method for the determination of C-methyl groups shows that methyl groups are not subject to attack by chromic anhydride in sulfuric acid solution.

Westheimer and Novick[6] interpreted the kinetics of the chromic acid oxidation of isopropyl alcohol in dilute aqueous solution in terms of an ionic mechanism. In a qualitative study of oxidations effected by chromic anhydride in dilute solution in glacial acetic acid, Waters[7] found that some oxygen was absorbed in those instances where a reaction occurred and suggested that the

(5) Miescher and W. H. Fischer, *Helv. Chim. Acta*, **22**, 155 (1938); Billeter and Miescher, *ibid.*, **30**, 1409 (1947).

(6) Westheimer and Novick, *J. Chem. Phys.*, **11**, 506 (1943).

(7) Waters, *J. Chem. Soc.*, 1151 (1946).

reaction proceeds by an oxygen-propagated free-radical mechanism. When a typical naphthoquinone oxidation (M-266 acetate) was conducted in the Warburg apparatus, about 0.03 mole of oxygen per mole of naphthoquinone was consumed in one hour, about half of it in the first fifteen minutes. A large-scale oxidation conducted in a nitrogen atmosphere gave the same product and yield as one conducted in a vigorously stirred mixture exposed to air. Oxygen absorption in this instance thus appears to be a minor side reaction not essential to the main oxidation.

Experimental Part[8]

Acetylation.—A suspension of 1 g. of hydroxyalkylnaphthoquinone in 5 cc. of acetic anhydride was treated at room temperature with 1 cc. of boron fluoride etherate, when the solid soon dissolved. In a test case water was added one minute after the solid had dissolved and acetylation was found to be complete. Quinones with α-branched side chains react more slowly, and a reaction period of two to three hours (25°) is required before the addition of water. After several hours the acetates often solidified and were crystallized once or twice from methanol; otherwise the product was collected in ether and the solution washed neutral with soda solution, dried and evaporated. Quinones having carboxyl groups in the side chains give polymeric products by the above procedure and are best acetylated with acetic anhydride and sodium acetate.[9]

Oxidation Procedures.—In the analytical experiments an acetic acid solution of acetate equivalent to 200 mg. of the hydroxyquinone was prepared in a 10-cc. volumetric flask, 0.8 g. of chromic anhydride was added, solvent was added to the mark, and the stoppered flask was rotated mechanically along the axis at an angle of 45°. When a rise in temperature was noted the flask was cooled under the tap. Large-scale oxidations were conducted under turbulant agitation with a Hershberg stirrer and the temperature was controlled by intermittent cooling; the reactions proceeded notably faster.

Analytical Procedure.—After a given interval a 1-cc. aliquot was shaken with water–ether (20 cc. each) and the aqueous layer extracted with a second portion of ether. The combined extract was washed twice with water and an acid fraction was removed by two or three extractions with 5% soda solution. Except with α- or β-branched compounds, hydrolysis of the acetylated acids is so fast that the soda extract acquires maximum color density almost immediately. The yield of the acidic fraction in milligram equivalents of starting material was determined colorimetrically with use of the calibration curve for the starting material. A determination of pE was made by extracting the pigment from a suitable volume of acidified extract into about 10 cc. of ether, reducing the volume to 8 cc. by washing with water in a calibrated separatory funnel with a constriction in the middle, adding 8 cc. of ether-saturated buffer and determining the amount of pigment distributed into the aqueous phase.

The ethereal solution of the total neutral material was evaporated and the residue heated with aqueous alkali for ten minutes on the steam-bath to effect hydrolysis; this procedure is quantitative, whereas hydrolysis with mixtures of aqueous alkali with either methanol or dioxane gave low and variable yields.[10] When the aqueous hydrolysis resulted in the separation of an oily red salt, this was brought into solution with methanol prior to acidification and extraction with ether. The ethereal solution was washed until neutral and extracted repeatedly with a buffer that just fails to acquire appreciable color when shaken with an ethereal solution of the starting material. The extracts were drained into a funnel through a layer of back-wash ether, and determinations were made of the quantity and pE of the neutral oxidation product. The residual pigment was recovered by evaporation of the ether and determined in methanol–alkali; in several instances the pigment was isolated and identified as pure starting material. The separation from starting material is accurate to 2–3%; the separation of acidic material is quantitative.

The time-yield data recorded in Fig. 1 are typical of results obtained with a number of quinones having normal, isoalkyl and cyclohexylalkyl side chains. The inhibitory effect of 10% of water was observed in each of four instances investigated.

Isolation.—The results are summarized in Table I. The yields given for the total acidic and neutral fractions are based upon starting material employed and take no account of material recovered. The seventh column gives the pH of a buffer found suitable for the extraction of the neutral fraction from unoxidized product or of a methyl ester from the esterified total acidic fraction.

The straight-chain acids isolated from the oxidations were all compared (as acids, esters, or both) with synthetic acids (Table III) and found identical. Although homologous acids and esters frequently melt at nearly the same temperature, mixtures show marked depressions. In the oxidation of the isoalkyl derivatives the specific acid listed was isolated in a pure form only after many slow crystallizations. The lower acids are only sparingly soluble in benzene and very soluble in water containing a little methanol; the higher ones crystallize well from ligroin containing a little benzene or from aqueous methanol. The acids often separate well from acetic acid but the crystals are solvated and must be dried for a few hours at 90° to remove acetic acid.

Extraction Constants of Hydroxyquinone Acids.—For the characterization of a quinone having two acidity constants, probably both in the range pK 5–6, use has been made of the following approximate formula based upon the assumption that the quinone exists in the buffer entirely as the doubly charged anion.

$$p\mathrm{E} = (\log_{10} C)/2 + p\mathrm{H} - 1$$

Typical data are recorded in Table II. The wide drift in pE from buffer to buffer observed with the first compound is probably because pE, pK_1, and pK_2 are of the same magnitude. That in all instances C increases with increasing concentration of quinone in the ether phase probably is indicative of association of the quinone acid in ether. Because of the deviations from constancy and the small increment in pE from homolog to homolog, free acids are less advantageous for characterization or for separation than their esters.

Specific Oxidations. M-1523.—The neutral product gave no depression when mixed with hydroxyhydrolapachol.[11]

M-1929.—The same acid (cryst. toluene) was isolated from oxidations conducted for one-half and for two hours. Another oxidation was conducted for one hour, when the amount of starting material remaining was estimated from an analytical run to be only about 3%; the acidic material was removed and the residual acetylated neutral fraction was oxidized for one hour. The resulting acidic material was processed by buffer extraction and crystallization of the methyl ester mixture from aqueous methanol, and the acid $-(CH_2)_3CO_2H$ was identified. The neutral oxidation product was obtained crystalline only after repeated processing with ligroin; the solution at first deposited oily material, and when the mixture was heated to boiling a part of the oil failed to dissolve readily and was discarded. After several portions of tarry residue had been removed the product crystallized in spongy masses.

(8) With the exceptions noted, the microanalyses were done by Shirley R. Katz. The melting points are uncorrected. Peroxide-free ether was employed in all extractions. The designation "ligroin" refers to material of b. p. 60–90°. Colorimetric determinations were made with a Coleman junior spectrophotometer.

(9) Fieser and Turner, THIS JOURNAL, **69**, 2338 (1947).

(10) Esters of quinone acids are hydrolyzed quantitatively by this method and the reductive procedure[9] is unnecessary.

(11) Hooker, *J. Chem. Soc.*, **61**, 611 (1892).

TABLE I

OXIDATION OF 2-HYDROXY-3-ALKYL-1,4-NAPHTHOQUINONES (AS ACETATES) AT 25°

Code no. and side chain	Time, hr.	% Yield[a] Acidic ract.	Neut. prod.	Product, side chain	M. p., °C.	Extract buffer, *p*H	*p*E	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen found
M-1523	0.5	2	18	$-(CH_2)_2C(OH)(CH_3)_2$	125–126	6.81		$C_{15}H_{16}O_4$	69.21	69.48	6.20	6.36
$-(CH_2)_2CH(CH_3)_2$												
M-1929	0.5	24	38	$-(CH_2)_4C(OH)(CH_3)_2$	85–86	8.00	5.74	$C_{17}H_{20}O_4$	70.81	70.77	6.99	6.88
$-(CH_2)_4CH(CH_3)_2$	1	40	34	$-(CH_2)_3CO_2H$	144–145							
	9	46	9	Methyl ester	131–132			$C_{15}H_{14}O_5$	65.71	65.93	5.27	5.39
M-2284	0.5	46	36	$-(CH_2)_6C(OH)(CH_3)_2$	84–85	9.20	6.88	$C_{19}H_{24}O_4$	72.12	72.10	7.65	7.72
$-(CH_2)_6CH(CH_3)_2$	1	66	21	$-(CH_2)_5CO_2H$	119.5–120.5			$C_{16}H_{16}O_5$	66.42	66.49	5.58	5.77
M-300	24	?	?	$-(CH_2)_6CO_2H$	127–129			$C_{17}H_{18}O_5$	67.64	67.65[b]	6.00	6.18[b]
$-(CH_2)_7CH(CH_3)_2$												
M-2287	0.5	23	38	$-C_{11}H_{21}O$	90.5–92	10.22	7.82	$C_{21}H_{28}O_4$	73.68	73.17	7.66	7.94
$-(CH_2)_8CH(CH_3)_2$	1	72	22	$-(CH_2)_7CO_2H$	127–128			$C_{18}H_{20}O_5$	68.34	68.45[b]	6.37	6.31[b]
M-273	0.75	34	22	$-(CH_2)_6COCH_2CH_2CH_3$	94.5–96	9.52	7.77	$C_{20}H_{24}O_4$	73.15	72.99	7.54	7.36
$-C_{10}H_{21}$-*n*	0.75[c]	40	24[d]	$-(CH_2)_{2\text{-}7}CO_2H$, see text								
M-2256	0.5	21	?	$-C_{17}H_{33}O$	76.5–78			$C_{27}H_{38}O_4$	76.02	75.74	8.98	9.22
$-C_{17}H_{35}$-*n*	1	53	?									
M-1916	0.5	42	21	$-(CH_2)_2CO_2H$	194–196			$C_{13}H_{10}O_5$	63.41	63.89[b]	4.09	4.39[b]
$-(CH_2)_3$-Cyclohexyl	1	61	11	Methyl ester	133–134			$C_{14}H_{12}O_5$	64.61	64.41[b]	4.65	4.79[b]
				CH_2CO_2H								
M-266	0.75[c]	4[d]	1[d]	$-CHCH_2CH_2CO_2H$	218–219 dec.			$C_{16}H_{14}O_7$	60.38	60.27	4.43	4.48
-Cyclohexyl	1.75	11[d]	Trace	Dimethyl ester	103–104			$C_{18}H_{18}O_7$	62.42	62.42	5.24	5.01
				$CH_2CH_2CHCO(CH_2)_4CO_2H$								
M-2293	1[c]	37[d]	Trace	$-CHCH_2CH_2$	160–161			$C_{22}H_{24}O_6$	68.74	68.97	6.29	6.17
-4′-Cyclohexyl-cyclohexyl				Methyl ester	119–120			$C_{23}H_{26}O_6$	69.33	69.23	6.58	6.63
				Methyl ester acetate	122–123			$C_{25}H_{28}O_7$	68.16	68.05	6.40	6.19
				CH_2CO_2H								
				$-CHCH_2CH_2CO_2H$	219–220			$C_{16}H_{14}O_7$	60.38	60.85	4.43	4.51
				Dimethyl ester	103.5–104.5			$C_{18}H_{18}O_7$	62.42	62.64	5.24	5.08
				$CH_2CH_2CHCO_2H$								
				$-CHCH_2CH_2$	277 dec.			$C_{17}H_{16}O_5$	67.99	67.38	5.37	5.60
M-1952	0.5	20	57	$-(CH_2)_2COC_6H_4CH_3$-*p*	169.5–170	8.25	6.4	$C_{20}H_{16}O_4$	74.98	75.07	5.04	5.06
$-(CH_2)_3C_6H_4CH_3$-*p*	1	41	37	$-(CH_2)_3C_6H_4CO_2H$-*p*	205–206			$C_{20}H_{16}O_5$	71.46	71.54	4.80	4.78
M-2276	0.25	14	20	$-(CH_2)_4COC_6H_5$	119–120	9.08	7.4	$C_{21}H_{18}O_4$	75.43	75.56	5.43	5.39
$-(CH_2)_5C_6H_5$	0.75[c]	Trace	90[d]	Acetate	137–138			$C_{23}H_{20}O_5$	73.40	73.47	5.36	5.68
M-285	1.5	22[d]	7[d]	$-CH_2CH(CH_3)(CH_2)_4COCH_3$	97.5–99	8.25	7.42	$C_{19}H_{22}O_4$	72.59	72.50	7.06	6.95
$-CH_2CH(CH_3)C_6H_{11}$-*n*				$-CH_2CH(CH_3)CH_2CO_2H$	191–192.5			$C_{15}H_{14}O_5$	65.69	65.94	5.14	4.92
				Methyl ester	97–98	7.21	5.9	$C_{16}H_{16}O_5$	66.66	66.62	5.60	5.71
				$-CH_2CH(CH_3)(CH_2)_3CO_2CH_3$	66.5–68	8.00	6.9	$C_{18}H_{20}O_5$	68.34	68.37	6.37	6.59
				$-CH_2CH(CH_3)(CH_2)_4CO_2CH_3$	79–80	9.08	7.6	$C_{19}H_{22}O_5$	69.07	69.15	6.71	6.80
M-1934	1	0	0									
$-CH_2C(CH_3)_3$												
M-1917	0.25	10[d]		$-(CH_2)_2CO_2CH_3$	133–134		5.2	$C_{14}H_{12}O_5$	64.61	64.36	4.65	4.81
$-(CH_2)_8CO_2CH_3$	1	27[d]		$-(CH_2)_3CO_2CH_3$	130–131		5.6	$C_{15}H_{14}O_5$	65.71	65.45	5.27	5.58
				$-(CH_2)_4CO_2CH_3$	98–99		6.2					

[a] By colorimetry, except as noted. [b] Analysis kindly carried out by E. F. Shelberg and Jane Morris of the Abbott Laboratories. [c] With vigorous mechanical stirring and careful control of the temperature to 20°. [d] By weight.

TABLE II

DISTRIBUTION BETWEEN ETHER AND BUFFER (8 CC. EACH)

Buffer, *p*H	Total quinone, mg.	*C*	*p*E
	Side chain $-(CH_2)_4CO_2H$		
5.54	4	11.5	5.07
	10	13.9	5.11
6.81	4	0.24	5.49
	10	.28	5.54
	12	.30	5.55
	Side chain $-(CH_2)_8CO_2H$		
6.81	4.8	9.0	6.76
	10	10.5	6.83
	20	11.8	6.88
8.00	4.8	0.24	6.38
	10	.39	6.59
	20	.59	6.77

M-2284.—The neutral product when processed with ligroin as above separated as an oil that slowly crystallized. Three recrystallizations gave clusters of irregular short needles, m. p. 69–71°, but three further slow recrystallizations gave well formed prismatic needles of the constant m. p. 84–85°, identical with the synthetic alcohol No. 16; the synthetic material likewise tended to oil out, solidified slowly (three days), and gave well-formed crystals only after repeated crystallization.

M-2287.—The neutral product was obtained as a solid by prolonged processing with ligroin and was then crystallized four times from dilute methanol. The resulting small sample may not have been fully homogeneous, but the analysis is close to that of a keto derivative and the material melted higher than the synthetic tertiary alcohol (M-2231, Paper X) and depressed the m. p. of this substance to 55–64°.

M-273.[12]—When an alkaline solution of the neutral fraction was acidified and allowed to stand overnight the

(12) I am indebted to Eva Kjelland-Mørdre for the preparation of starting material.

product separated as a granular solid, m. p. 70–76°. Three crystallizations from slightly diluted methanol afforded heavy prisms of material that melted at 77–79°, appeared homogeneous, and had the analysis of a keto derivative (C, 73.24; H, 7.47). The substance did not reduce Fehling solution, with or without dioxane. The hydroquinone triacetate likewise gave a satisfactory analysis (calcd. for $C_{26}H_{32}O_7$: C, 68.39; H, 7.07. Found: C, 68.32; H, 6.77) but formed poor microcrystals and melted over the range 85–92°. A large sample of the ketonic product (0.66 g., 77–79°) was then recrystallized twice from methanol-water (81–83°) and four times from ether (very slow separation), when ball-like clusters of short needles were obtained that gave no depression when mixed with the synthetic ketone No. 18. The substance depressed the m. p. of the isomeric ketone No. 17 to 81–86°.

The structure of the ketonic product was investigated by oximation of the hydroquinone triacetate mixture (in pyridine under nitrogen), rearrangement (Ac_2O, HOAc, HCl), and hydrolysis (HOAc, HCl, H_2O). The reactions proceeded poorly but afforded a few milligrams of pigment recognized by buffer fractionation as a quinone acid and having a *p*E indicative of the homolog with the chain $-(CH_2)_6CO_2H$ (6.11). Chromic anhydride oxidation of the acetylated neutral product (m. p. 77–79°) for two hours resulted in 60% conversion to acids.

The acidic fraction (butyric odor) from a one-hour oxidation of M-273 acetate was characterized by esterification, fractional extraction with five buffers (*p*H 5.8 to 11.0), hydrolysis, crystallization of the acid mixtures from ligroin (m. p. 138–149° to m. p. 60–70°), esterification, and determination of the extraction constants of the methyl ester mixtures (*p*E 5.3, 5.6, 6.5, 7.5, 7.9). The results indicated that the mixture contained acids having from two to seven methylene groups (see below).

M-2256.—Separation of the neutral oxidation product from the starting material was accomplished by extraction from ether–ligroin (1:1) with a few portions of a mixture of 25 cc. of 0.1 *N* glycine buffer of *p*H 9.52, 75 cc. of water, and 100 cc. of methanol (exhaustive extraction removes M-2256). The extract was back-washed with ether–ligroin and the product recovered and crystallized three times from ligroin (m. p. 70–72°), once from aqueous methanol and once from methanol. The analysis is indicative of a ketonic derivative or mixture.

The acidic fraction (butyric odor) remaining after prolonged heating at 80° and 15 mm. pressure was esterified and fractionally extracted from ether with six buffers of *p*H from 7.2 to 12.0. The extraction constants of the fractions (*p*E 5.8, 6.5, 7.5, 8.0, 9.0; 7.3 as acid) suggest that the mixture contains acids having from four to about twelve methylene groups.

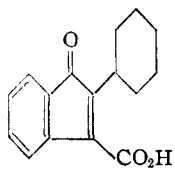
M-266.—Acidification of the soda extract gave a clear yellow solution from which the acidic product began to separate after a delay of a few minutes. The melting point (215–216°, dec.) was raised only slightly by crystallization from a boiling acetic acid solution after extensive dilution with water. The once crystallized dimethyl ester had the correct analysis, but the melting point was raised from 101.5–102.5° by three recrystallizations to 103–104°, corresponding to the sample from M-2293; probably the initial product contains a small amount of the isomer arising from opening of the ring at C_2–C_3.

In the 1.75-hour oxidation 30.5% of M-266 was recovered. The hydrolysis of the total neutral fraction proceeded slowly by the usual procedure and was best conducted under nitrogen with the addition of hydrosulfite, followed by air oxidation. No neutral product was found.

An isomer of M-266 was encountered in an early attempt to hydrolyze the acetate mixture with aqueous alkali. The same compound was then obtained from pure M-266 acetate and finally from the free compound. A mixture of 1 g. of M-266, 100 cc. of water and 20 cc. of 25% sodium hydroxide was heated on the steam-bath under nitrogen until the solid had dissolved and the flask was then stoppered and the heating continued. After twenty-seven hours the red color had changed to yellow and the solution was allowed to stand at room temperature overnight. The clear yellow solution was decanted from a small sludge of oily material that could be washed with brine; it dissolves in water to give a greenish blue solution, and in the presence of alkali the salt is extracted by ether to given an intense blue color. The yellow alkaline solution was extracted three times with ether to remove more of the blue salt and then acidified and the main product collected in ether and crystallized once from methanol (very soluble)-water to give 0.66 g. of yellow needles, m. p. 169–170°. The isomer also crystallizes well from ligroin (moderately soluble); the pure substance melts at 171–172°.

Anal. Calcd. for $C_{16}H_{16}O_3$: C, 74.97; H, 6.29. Found: C, 75.13, 75.23; H, 6.12, 6.25.

An experiment conducted as above but under a reflux condenser without exclusion of air gave only 0.4 g. of satisfactory product and no ether-extractable blue salt was observed. In experiments conducted in air for shorter periods the isomer was separated efficiently from starting material by extraction from ether with *p*H 7.2 buffer; acidification of the extract gave nearly pure product as a granular solid, m. p. 170–171°. The substance is not a quinone, for it dissolves in aqueous alkali or sodium carbonate solution with a yellow color that is not discharged by hydrosulfite. The structure may be that shown in the formula.



The oxidation of 10 mg. of M-266 acetate in 2 cc. of acetic acid with 30 mg. of chromic acid at 40° was conducted in the Warburg apparatus in an atmosphere of air.[13]

In a typical experiment the oxygen uptake at fifteen-minute intervals was: 11.7, 16.2, 22.0, 23.1, 24.5 cmm. An oxidation of 4.64 g. of M-266 acetate conducted for one and three-quarters hours exactly as in the experiment of Table I except that it was done under nitrogen afforded 0.45 g. (9.5%) of acid, m. p. 216–217° and 2.0 g. of total neutral residue.

M-2293.—Because of the sparing solubility of the acetate, the amount of acetic acid was increased to 150 cc./g. Oxidations conducted for one hour gave mixtures of the C_{22}-keto acid (28%) and the C_{16}-diacid (9%) that were easily separated. When the soda extract of total acids was acidified the keto acid began to deposit at once and was collected after ten minutes and crystallized from dilute acetic acid, methanol–water (fine needles), or ether (dissolves slowly, separates after concentration in excellent needles). The yellow aqueous filtrate remained clear for several hours but on standing overnight deposited the diacid in nearly pure form, m. p. 218–219°. The diacid is very sparingly soluble in glacial acetic acid and separates very slowly as microcrystals. Analyses of the diacid were irregular, but the dimethyl ester was satisfactorily characterized. This ester is hydrolyzed completely by 1% sodium hydroxide in twenty minutes at 25°. No depressions in m. p. were observed with mixtures of the acid and ester with the samples derived from M-266.

Another oxidation (0.5 g. acetate) was conducted for three hours and the acid separating from the acidified soda extract was collected after ten minutes. The crude solid (0.11 g.) decomposed at 210–230° (from toluene, 230–250°) and probably contained the diacid and the C_{17}-monoacid. The latter was obtained by oxidation of 107 mg. of the methyl ester acetate of the keto acid for two hours (11 mg. unchanged); the sparingly soluble monoacid precipitated at once from the acidified soda extract (dec. 270°). Two crystallizations from acetic acid gave small prisms. In combustion analyses the sample underwent sudden decomposition and the results were irregular.

M-1952.—A sample of the acidic oxidation product crystallized from toluene and then from acetic acid when mixed with the product of metabolic oxidation of M-1952 melted at 205–206°.

(13) Experiments by Grace Nahm.

The neutral product was easily purified (flat needles) and was recovered unchanged from an orange solution in 96% sulfuric acid. A two-step Hooker oxidation of 0.8 g. of product gave a solid ketol but a tarry final product, from which the lower homolog (side-chain $-CH_2COC_6H_4-CH_3$) was isolated in low yield by extraction from ether with *p*H 7.21 buffer and crystallization from alcohol and then benzene: heavy prisms, m. p. 190–191°; orange-yellow solution in dilute alkali, λmax 480 mμ; color in 96% sulfuric, green changing to intense blue.[14] 2-Hydroxy-3-benzoylmethyl-1,4-naphthoquinone[15] likewise gives an orange-yellow solution in alkali, λmax. 480, and a blue-green → blue solution in sulfuric acid.

Anal. Calcd. for $C_{19}H_{14}O_4$: C, 74.52; H, 4.61. Found: C, 74.95; H, 4.96.

M-2276.—Initial experiments showed that little acidic material is formed when the vigorous reaction is controlled by careful cooling and that the acetate of the neutral product is sparingly soluble in ether. In the second experiment recorded 1.1 g. of acetate in 150 cc. of acetic acid was oxidized with 4 g. of chromic anhydride; the addition of water to the stirred solution precipitated the acetoxy ketone as a granular solid; 1.02 g., m. p. 131–133°. The acetate (flat needles, moderately soluble in alcohol) is attacked only slowly by aqueous alkali but is hydrolyzed readily by alkaline hydrosulfite under nitrogen; after air oxidation the solution is acidified (90%; crystallizes very slowly in prisms). Mixed m. p. determinations with the synthetic ketone No. 19 and its acetate established the identity of the samples. Hooker oxidation proceeded poorly and gave an unidentified product, m. p. 138.5–139.5° (prisms).

M-285.—The hydrolysis of the acetate of this β-branched quinone and its derivatives proceeds noticeably slower than usual. Thus in the soda extraction about ten minutes must be allowed with each portion for the development of full color intensity. The precipitated acidic material was a solid, m. p. range 120–135°. It was esterified and the methyl esters separated into three main fractions by successive extractions with the buffers indicated; weakly pigmented initial, intermediate and tail fractions were discarded. The yields of fractions containing the C_{15}-, C_{17}- and C_{18}-acids were in the ratio 7:3:1. The early fraction is best processed for isolation of the C_{15}-acid by hydrolysis and crystallization of the crude free acid (m. p. 150–165°) about three times from toluene. The other two fractions can be purified by a few crystallizations of the esters. A sample of the C_{18}-acid obtained from partially purified methyl ester melted at 160–163° and a mixture with the M-285 metabolite (m. p. 164–170°) melted at 163–167°. A mixture of the pure ester with the metabolite ester showed no depression: m. p. 78.5–80°.

The neutral product was extracted at *p*H 8.25, after a few washings at *p*H 7.21 had been discarded, and was obtained as an oil that solidified readily, m. p. 80–84° (19% of M-285 was recovered). Three or four crystallizations gave clusters of prismatic needles of constant m. p. Mother liquor material was treated with 96% sulfuric acid to see if it contained any of the tertiary alcohol (Paper X), but only a trace of alkali-insoluble pigment was produced and most of the material was re-extractable at *p*H 8.25 and yielded some of the above ketone. For establishment of structure, samples of the pure ketone were acetylated and oxidized. Acetylation in a suspension of acetic anhydride in the presence of boron fluoride etherate at 25° was allowed to proceed for just one minute after the solid had dissolved in order to avoid condensations, and the acetate was collected in ether and washed with soda solution. The acidic product from 64 mg. of ketone when processed as above gave ester fractions of *p*E 5.8, 6.7 and 7.5. The material from 217 mg. of ketone afforded enough of the three products for full identification by m. p. and mixed m. p. as follows: C_{15}-acid, 190–191°; C_{18}-ester, 68–69°; C_{19}-ester, 78–79°. The ratio of C_{15}-, C_{17}- and C_{18}-fractions was 10:2.5:1.

Countercurrent Distribution: (a) **M-1917.**—The acidic fraction from a one-hour oxidation (300 mg.) was removed by soda extraction, esterified, and the methyl ester mixture processed by countercurrent extraction in ten separatory funnels. In each bulb distribution was made between 100 cc. of ether and 100 cc. of ether-saturated *p*H 8.0 buffer; at the end, determinations were made of the concentration and *p*E of the pigment in each aqueous phase. The total pigment in each bulb was then recovered with ether and dissolved in ligroin; crystallizates were obtained from bulbs 3–10 inclusive. Pure esters were isolated from bulbs 9, 7 and 4 and identified in all cases by m. p., mixed m. p., and *p*E, and in the first two instances by analyses (see Table); the first and third esters gave depressions when mixed with synthetic samples of the next higher homologs. Only traces of homologs with more than four methylene groups appeared to be present; the pigment from bulb 2 (3.3 mg. in aqueous phase) had the *p*E 6.5. Another oxidation conducted for just fifteen minutes gave nearly identical results (150 mg. of acid mixture); the same three esters were isolated from bulbs 9, 7 and 4 and identified by m. p. and mixed m. p., and the highest *p*E (bulb 3) was 6.3.

(b) **M-273.**—The total acidic material (3.0 g.) from a one-hour oxidation of 8.4 g. of M-273 acetate at 20° was esterified and the esters distributed between ether and *p*H 8.0 buffer in ten bulbs as in (a). Six bulbs at the more hydrophilic end were removed and the fractions collected and the remaining bulbs were employed as the first units of a new series. A total of twenty fractions was obtained in three series of distributions at *p*H 8.0. Further fractions were then collected by distributions at *p*H 9.1 and 10.2. Each fraction was collected in ether and the product taken up in ligroin; crystallizates were obtained in all but a few instances. Four of the six pure components isolated from the mixture were secured from a total of thirty-five fractions. The other two were then isolated by further subseries of distributions conducted on fractions selected with guidance from the melting point and *p*E data; seventeen more fractions afforded the missing homologs.

The esters isolated were purified by crystallization from ligroin or ligroin–benzene and compared with the synthetic esters by mixed m. p. determinations. The samples were then hydrolyzed and the acids crystallized and similarly compared. The melting points of the esters (and acids) were as follows (increasing mol. wt.): 133–134° (194–195°); 129.5–131° (143.5–145°); 98–99° (158–159°); 94–95° (118.5–120°); 90–91° (130.5–131.5°); 88–89° (128–129°).

Syntheses (Table III).—The acids with eight, four and two methylene groups are described in Paper X, and the others were made by Hooker oxidation. Esters were prepared quantitatively by the action of methanol and boron fluoride etherate at 60° for twenty minutes. Acid chlorides were obtained very satisfactorily by warming the acid (0.5 g.) with oxalyl chloride (3 cc.), boiling down the solution on the steam-bath, and pumping out the residual light yellow oil at the water pump at 70°. A practically pure amide is obtained by adding 5 cc. of 29% aqueous ammonia to a cold solution of acid chloride (0.5 g. acid) in 5 cc. of dioxane and after ten minutes at 25° acidifying and diluting the solution. Ketone No. 17 was obtained by the action of methylmagnesium iodide on the hydroquinone triacetate of the corresponding nitrile (prepared by D. J. Cram). Ketone No. 18 was obtained from the amide No. 7; this was reductively acetylated and the crude product refluxed for two hours in acetic anhydride, collected, and refluxed for twelve hours in ether–benzene with *n*-propylmagnesium bromide. The fraction extracted between *p*H 8.2 and 9.5 was crystallized from methanol–water and then ligroin. The phenyl ketone No. 19 was synthesized by a Friedel–Crafts reaction as indicated.

(14) This distinctive color test for β-aroyl derivatives was suggested by Dr. M. G. Ettlinger.

(15) Hooker and Steyermark, THIS JOURNAL, **58**, 1202 (1936).

TABLE III

2-HYDROXY-3-ALKYL-1,4-NAPHTHOQUINONES BY SYNTHESIS

No.	Side chain	Method	M. p., °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
1	$-(CH_2)_8CO_2CH_3$	Acid (Paper X), CH_3OH, BF_3-etherate	86–87	$C_{20}H_{24}O_5$	69.75	69.65	7.02	6.93
2	$-(CH_2)_7CO_2H$	Hooker oxid. (71%)	128–129	$C_{18}H_{20}O_5$	68.34	68.63	6.37	6.34
3	Methyl ester		89.5–90.5	$C_{19}H_{22}O_5$	69.07	68.97	6.71	6.77
4	Hydroq. triacetate		145–146	$C_{24}H_{28}O_8$	64.85	65.01	6.35	6.49
5	$-(CH_2)_6CO_2H$	Hooker oxid. (89%)	131–132	$C_{17}H_{18}O_5$	67.64	67.48	6.00	5.86
6	Methyl ester		91–92	$C_{18}H_{20}O_5$	68.34	68.44	6.37	6.35
7	Amide	Acid + $(COCl)_2$; NH_4OH-dioxane (87%)	192–193.5	$C_{17}H_{19}O_4N$	67.76	67.60	6.36	6.70
8	$-(CH_2)_5CO_2H$	Hooker oxid. (84%)	120–121	$C_{16}H_{16}O_5$	66.66	66.81	5.60	5.79
9	Methyl ester	See Table I	94.5–95.5	$C_{17}H_{18}O_5$	67.64	67.70	6.00	6.06
10	$-(CH_2)_4CO_2CH_3$	BF_3-esterification of acid (Paper X)	100–101	$C_{16}H_{16}O_5$	66.42	66.82	5.58	5.66
11	$-(CH_2)_3CO_2H$	Hooker oxid. (87%)	145–146.5	$C_{14}H_{12}O_5$	64.61	64.92	4.65	4.76
12	Methyl ester	See Table I	131–132					
13	$-(CH_2)_2CO_2H$	Hooker oxid. (71%); see also Paper X	195–196					
14	Methyl ester		133–134					
15	$-(CH_2)_7C(OH)(CH_3)_2$	Hooker oxid. of M-2231 (70%)	84–85	$C_{20}H_{26}O_4$	72.70	72.51[a]	7.93	7.88[a]
16	$-(CH_2)_6C(OH)(CH_3)_2$	Hooker oxid. of No. 15 (diff. to cryst.)	83–84	$C_{19}H_{24}O_4$	72.12	72.39	7.65	7.98
17	$-(CH_2)_5COCH_3$	Grig. react. on nitrile	93–95	$C_{20}H_{24}O_4$	73.15	73.34	7.54	7.41
18	$-(CH_2)_6COCH_2CH_2CH_3$	Red acet. of No. 7; Grig. react.	95–96	$C_{20}H_{24}O_4$	73.15	73.35	7.54	7.41
19	$-(CH_2)_4COC_6H_5$	Acetylated acid + $SOCl_2$; C_6H_6 + $AlCl_3$	116–117					
20	Acetate	Ac_2O-BF_3 (see Table I)	136.5–137.5					

[a] Analysis kindly carried out by E. F. Shelberg and Jane Morris of the Abbott Laboratories.

Summary

Chromic anhydride oxidation of 2-acetoxy-1,4-naphthoquinones substituted in the 3-position by various alkyl, aralkyl, cycloalkyl and cycloalkyl-alkyl groups results in an attack of the side chain with the production of tertiary alcohols, ketones, acids and keto acids.

Oxidation proceeds much faster and in higher yield when a glacial acetic acid solution of the substance to be oxidized is agitated with solid chromic anhydride than when the oxide is brought into solution with the use of 10% of water.

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Dimyristo- and Eruco-stearo-cephalin

BY I. R. HUNTER, R. L. ROBERTS AND E. B. KESTER

Prior investigations at the Western Regional Research Laboratory on the storage of egg powders revealed the fact that the alcohol-insoluble portion, or cephalin-containing fraction, of the phospholipids present underwent progressive degradative changes concurrently with loss in quality of the egg material. Repeated attempts were made to obtain fresh egg cephalin in pure form to permit a study of its autoxidation in absence of non-phosphatidic matter, but without success. Attention was therefore turned to the synthesis of one or more compounds having the cephalin structure. It was particularly desired to obtain a cephalin with at least one point of unsaturation, on the assumption that oxidative deterioration is initiated at a methylene group adjacent to a double bond in one of the fatty acid groups present.

The synthesis of cephalin has been described in a number of reports,[2] but in these publications the products were not sufficiently characterized to validate the procedures used. A recent synthesis by Rose[3] which yielded a well-defined compound,

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) Grün and Limpacher, *Ber.*, **60B**, 151 (1927); Kabashima, *ibid.*, **71**, 76, 1071 (1938); Kabashima and Bunsuke, *Proc. Imp. Acad. Tokyo*, **8**, 492 (1932); *C. A.*, **27**, 1634 (1933).

(3) Rose, THIS JOURNAL, **69**, 1384 (1947).