Methyl $\Delta^{9,11}$-3α-acetoxy-12-ketocholenate (III) upon admixture with an authentic sample gave no depression, and upon ultraviolet absorption analysis showed a maximum at 240 mμ, log ϵ 4.04.

Methyl 3α-Acetoxy-11β-hydroxycholanate (IV).—A solution of 315 mg. of methyl 3α-acetoxy-9β,11β-oxidocholanate in 30 ml. of glacial acetic acid was treated with 0.1 g. of platinum oxide and hydrogen at room temperature and shaken for 16 hours, at which time 0.75 mole equivalent had been taken up. The catalyst was filtered, the filtrate diluted with water and extracted thoroughly with ether. Evaporation of the washed ether layer gave 295 mg. of a white crystalline residue which after chromatographic purification afforded 80 mg. of crystalline IV. Recrystallization from methanol gave fine needles, m.p. 149.5–151°. Infrared analysis showed hydroxyl absorption at 2.82 μ, and ester carbonyl absorptions at 5.77 and 5.83 μ.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.07; H, 10.02.

Treatment of IV with acetic anhydride and pyridine for three hours on the steam-bath afforded only unchanged starting material.

Methyl 3α-Acetoxy-11-ketocholanate (V).—To a solution of 20.5 mg. of IV in 0.5 ml. of glacial acetic acid and 0.16 ml. of water was added 0.26 ml. of a solution of chromium trioxide in 95% aqueous acetic acid (containing 50 mg. CrO_3/ml.). The reaction was allowed to stand at room temperature for one hour. Careful dilution with water afforded crystalline V which upon recrystallization from methanol melted at 132–134°; $[\alpha]^{24}D$ +66° (*c* 0.99 in acetone). Upon admixture with an authentic sample of methyl 3α-acetoxy-11-ketocholanate, the melting point was not depressed.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.40; H, 9.60.

9α,11α-Oxido-5α,22α-spirostan-3β-ol Acetate (9α,11α-Oxidotigogenin Acetate) (VII).—A solution of 570 mg. of 5a;22a-spirost-9,11-en-3β-ol acetate ($\Delta^{9,11}$-tigogenin acetate) (VI) in 70 ml. of glacial acetic acid was cooled to 15° and treated dropwise with 40 ml. of a 5% aqueous solution of potassium permanganate over a period of one hour. The cooled solution was stirred an additional hour then treated with a saturated aqueous solution of sodium bisulfite until colorless. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extracts were washed thoroughly and dried over anhydrous magnesium sulfate. After evaporation *in vacuo*, the crystalline residue (500 mg.) was chromatographed over acid-washed alumina and yielded 215 mg. of 9α,11α-oxidotigogenin acetate (VII), m.p. 263–265°. Recrystallization from chloroform–methanol gave an analytical sample melting at 264–265°, $[\alpha]^{24}D$ −78° (*c* 1.0 in chloroform). Infrared absorption analysis showed only ester carbonyl and side-chain absorption.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.91; H, 9.61.

Oxidation of $\Delta^{9,11}$-tigogenin acetate with perbenzoic acid essentially according to the procedure of Djerassi, ref. 11, gave, after chromatography, pure 9α,11α-oxidotigogenin acetate, m.p. 263.5–265.5°. The infrared spectrum of this compound was identical with that of the product obtained by permanganate oxidation.

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

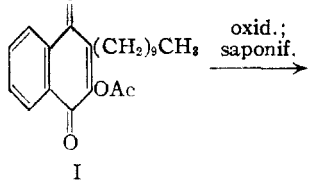
Chromic Anhydride Oxidation of the *n*-Undecyl Side Chain of an Acetoxynaphthoquinone

BY KOJI NAKANISHI[1] AND LOUIS F. FIESER

RECEIVED APRIL 4, 1952

A previous study of the oxidation of alkyl side chains has been extended by identification of the acidic products by paper chromatography and by fractionation of the neutral products in a Craig countercurrent machine. Oxidation of the *n*-undecyl side chain gives the acids $-(CH_2)_{1-8}CO_2H$ and the 7′ and 9′-keto derivatives. Oxidation of a synthetic acid with the side chain $-(CH_2)_9CO_2H$ gave, in addition to a series of lower homologous acids, the ketone $-(CH_2)_7COCH_3$, which must have arisen by β-oxidation and decarboxylation.

This investigation is an extension of a research[2] in which it was found that the side chains of 2-alkyl-3-acetoxy-1,4-naphthoquinones are oxidized rapidly at room temperature by a suspension of chromic anhydride in anhydrous acetic acid. Alkyl groups of various types were found to be oxidized to alcoholic, ketonic and acidic derivatives, isolated by differential extraction from ether, before and after hydrolysis of the acetyl group, with aqueous buffers of increasing alkalinity; in one instance separation of a series of homologous acids was effected by countercurrent distribution conducted manually in a series of separatory funnels. Quinone I, with an *n*-decyl side chain, yielded a neutral oxidation fraction, from which the 7′-keto derivative II was isolated, and the series of acids III, n = 2–7. An acetoxy quinone with the side chain $-CH(CH_3)C_6H_{13}$-*n* was likewise found to yield the 7′-keto derivative, $-CH_2CH(CH_3)(CH_2)_4COCH_3$, and the acids $-CH_2CH(CH_3)(CH_2)_nCO_2H$, n = 4,3,1; the 7′-acid $-CH_2CH(CH_3)(CH_2)_4CO_2H$



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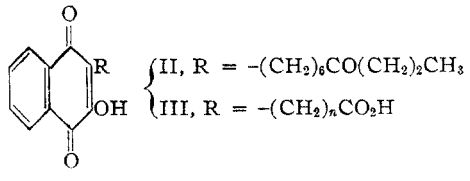


is a product of human metabolic oxidation of the hydroxyalkylnaphthoquinone.[3]

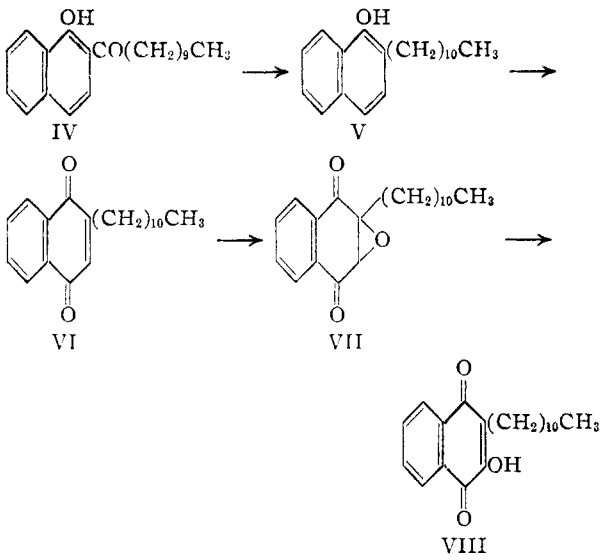
The present work was undertaken with the thought that application of newer techniques of separation and analysis might afford further information on the course and mechanism of the interesting side chain oxidation. The substance

(1) Research Fellow studying under the sponsorship of the Institute of International Education as participant in the Japanese Student Program of the Department of the Army and SCAP.

(2) L. F. Fieser, THIS JOURNAL, **70**, 3237 (1948).

(3) L. F. Fieser, F. C. Chang, W. G. Dauben, C. Heidelberger, H. Heymann and A. M. Seligman, *J. Pharmacol. Exp. Therap.*, **94**, 85 (1948).

selected for study was the acetate of 2-undecyl-3-hydroxy-1,4-naphthoquinone (M-1926) (VIII)[4] prepared more efficiently than previously by application of the general synthesis of Fawaz and Fieser,[5] starting with the condensation of undecylic acid with α-naphthol in the presence of boron fluoride etherate to give IV. Clemmensen reduction (V), chromic acid oxidation to the quinone (VI), formation of the oxide (VII) and acid hydrolysis gave the hydroxyquinone VIII in good over-all yield.



Oxidation of the acetate of M-1926 (VIII) was conducted as before with a suspension of chromic anhydride in acetic acid at room temperature. A sharp separation of unchanged starting material, a fraction containing neutral oxidation products of the type of ketone II, and an acidic (carboxylic) fraction was effected by a slightly modified technique described in the Experimental part. The acidic fraction was analyzed by paper chromatography, conducted with a solvent system made by shaking a mixture of *n*-butanol, ethanol and aqueous ammonia; the upper layer served as the mobile phase, the lower (aqueous) as the stationary phase. The easily assembled chamber shown in Fig. 1 was employed for application of the ascending-flow method. A sheet of filter paper impregnated at the bottom with a series of spots of known and unknown mixtures was formed into a cylinder (C) and stood up in an evaporating dish containing the mobile phase (A). Twelve or more chromatograms can be run simultaneously. Descending-flow chromatography was conducted in the apparatus shown in Fig. 2, which is useful also in some applications of the ascending-flow method.

The homologous ω-(3-hydroxy-1,4-naphthoquinonyl-2)-acids with the side chains $-CH_2CO_2H$ to $-(CH_2)_9CO_2H$ are well suited to paper chromatography since, in an atmosphere containing ammonia, their migration is observable from the red color of the anions; in a dried paper the spots can be brought out by exposure to ammonia. Concordant R_f values were found by the ascending- and descending-flow methods for the whole series of synthetically prepared homologs (Table I, Experi-

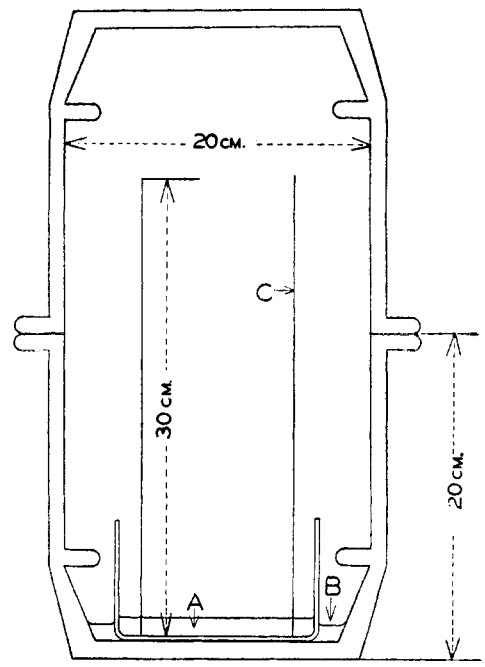


Fig. 1.—Ascending flow chromatography in a chamber made by placing the inverted bottom member of one desiccator over that of another: A, mobile phase; B, stationary phase; C, cylinder of filter paper.

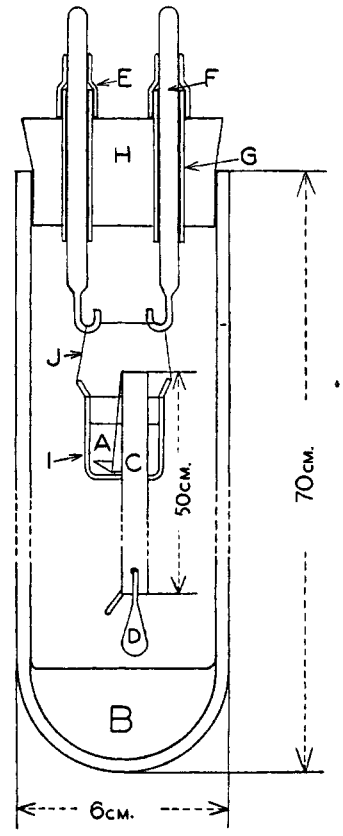


Fig. 2.—Descending-flow chromatography: A, mobile phase; B, stationary phase, contained in a beaker suspended from glass hooks; C, paper strip; D, glass weight.

(4) L. F. Fieser, M. T. Leffler and co-workers, THIS JOURNAL, **70**, 3175 (1948).

(5) G. Fawaz and L. F. Fieser, *ibid.*, **72**, 996 (1950).

mental part). The constituents of an acidic oxidation fraction can be identified unambiguously by comparing the chromatogram with that of a simultaneously run known mixture of synthetic acids. Quantitative determination of the component acids was done by cutting out the spots, extracting the pigment, and measuring the extinction coefficient of the solution.

Oxidation of 2-undecyl-3-acetoxy-1,4-naphthoquinone (M-1926 acetate) was found to afford homologous acids of side chains $-(CH_2)_nCO_2H$, n = 1–8, as reported in the time–yield curves of Fig. 3. All the acids, excepting n = 1, appeared within ten minutes after oxidation. The lower homologs, n = 2,3,4, appeared in relatively high proportion, even initially, and the proportion increased with increasing time of oxidation at the expense of the higher homologs. The acid with the side chain $-CH_2CO_2H$ was an exception, since it was invariably produced in only very low relative yield.

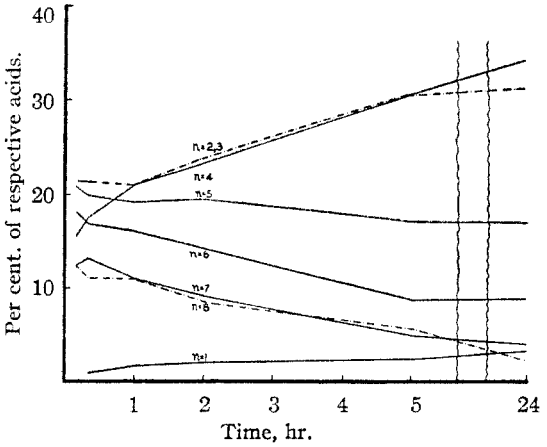


Fig. 3.—Relative yields of hydroxynaphthoquinone acids with the side chains $-(CH_2)_nCO_2H$ formed on oxidation of M-1926 acetate, of side chain $-(CH_2)_{10}CH_3$.

The yield of neutral fraction containing non-acidic products of oxidation reached a maximum of 20% in 30 minutes and then fell off. The total neutral fraction from oxidation of 500 mg. of M-1926 acetate was heated briefly with aqueous sodium carbonate solution, which completely deacetylates the ketonic oxidation products but leaves M-1916 acetate almost completely untouched, and fractionated by distribution between ether and a series of buffers of pH ranging from 11.2 to 6.9 in a 30-tube Craig countercurrent machine[6]; the course of the distribution was followed by plotting tube number against concentration of naphthoquinone pigment in the aqueous phase, as determined colorimetrically. Unchanged M-1916 acetate remained in the ether phase on extraction at pH 10.5 and 11.2; the total amount of unoxidized material amounted to 44%. Distribution between buffers of increasing acidity, with recycling where required (Table II, Experimental part), afforded two crystalline neutral oxidation products that appeared, from the symmetry of the distribution curves, to be homogenous: Product A (40 mg.), and Product D (5 mg., extracted at a lower pH). Two other crystalline products, B and C appeared in trace quantities, together with a red oil, an evident mixture. Products A and D, as well as the red oil, corresponded closely in infrared spectrum with a series of substances with ketonic side chains available by synthesis, for example 2-(11′-ketononadecyl)-3-hydroxy-1,4-naphthoquinone.[7] Three of the synthetic substances were oxidized, in the form of the acetates, to see if paper chromatographic analysis of the acid fraction would afford a reliable indication of the position of the keto group in the side chain. The quinone with the side chain $-(CH_2)_{10}CO(CH_2)_7CH_3$ afforded all the homologous acids in the series $-(CH_2)_{2\text{-}10}CO_2H$, even in a 10-minute period of oxidation (Fig. 4).

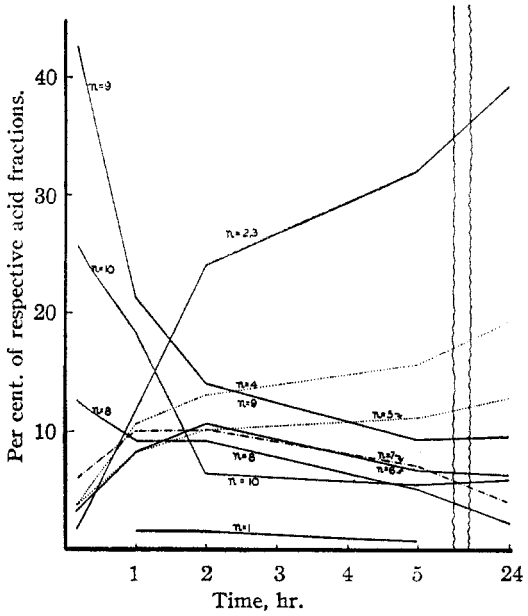


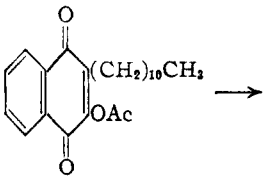
Fig. 4.—Yield of acids of side chain $-(CH_2)_nCO_2H$ resulting from oxidation of a synthetic 2-acetoxy-1,4-naphthoquinone with the 3-substituent: $-(CH_2)_{10}CO(CH_2)_7CH_3$.

The results showed that cleavage occurs on both sides of the carbonyl group, predominantly on the side nearest the quinone ring. Acetoxyquinones with the side chains $-(CH_2)_8CO(CH_2)_7CH_3$ and $-(CH_2)_9COCH_3$ afforded the acids $-(CH_2)_{1\text{-}8}CO_2H$ and $-(CH_2)_{1\text{-}9}CO_2H$, respectively. With this evidence of its validity, the method was applied to the neutral oxidation products isolated by countercurrent distribution. Product A gave all the acids in the series $-(CH_2)_{1\text{-}8}CO_2H$, and product D gave the acids $-(CH_2)_{1\text{-}6}CO_2H$; hence the substances must have the side chains $-(CH_2)_8COCH_2CH_3$ and $-(CH_2)_6COCH_2CH_2CH_2CH_3$. The combined results of chromic anhydride oxidation of the undecyl side chain are summarized in formulas IX–XII. Thus oxidation of the n-alkyl side chain affords the 7′-keto derivative, as in the previously studied case,[2] but gives even more of the 9′-keto derivative.

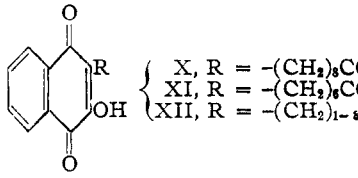
The isolation of 7′- and 9′-keto and acidic derivatives indicates some preferential attack at a certain distance from the quinonoid nucleus, but it is also possible that oxidation yields initial products with

(6) L. C. Craig and O. Post, *Ind. Eng. Chem., Anal. Ed.*, **21**, 500 (1949); L. C. Craig, *ibid.*, **22**, 1346 (1950).

(7) D. J. Cram, THIS JOURNAL, **71**, 3950 (1949).

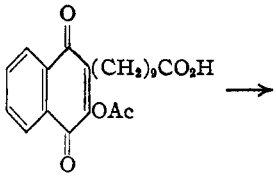


IX

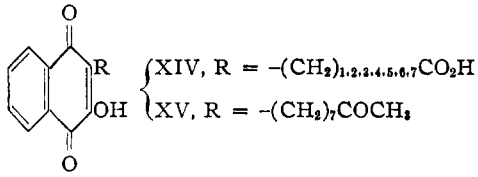


X, R = $-(CH_2)_8COCH_2CH_3$
XI, R = $-(CH_2)_6COCH_2CH_2CH_2CH_3$
XII, R = $-(CH_2)_{1-8}CO_2H$

keto groups nearer to the quinone ring but that these substances suffer oxidative cleavage to acids so readily that they have escaped isolation. Oxidation of synthetic acetoxynaphthoquinones with acid side chains proceeded more slowly and in lower yield than oxidation of the ketones. Degradation of the side chain $-(CH_2)_8CO_2H$ gave, in the early stages, predominantly the product $-(CH_2)_5CO_2H$, with smaller amounts of the n = 2, 3 acids; the other homologs appeared only later. In a 10-minute period of oxidation, the substance XIII with $-(CH_2)_9CO_2H$ similarly afforded chiefly $-(CH_2)_6CO_2H$, along with the n = 2,3 acids; later the acids n = 1, 4, 5, 7 (not 8) appeared. In the oxidation of each of these acidic model sub-



XIII



XIV, R = $-(CH_2)_{1,2,3,4,5,6,7}CO_2H$
XV, R = $-(CH_2)_7COCH_3$

stances the reaction mixture was found to contain a small amount of a neutral fraction that gave a positive iodoform test. In the case of XIII sufficient crystalline material was isolated for characterization. Analysis and the infrared spectrum indicated that the substance has a C_9-ketonic side chain. The positive iodoform test identifies the group as 8′-ketononyl (XV), and this structure was confirmed by oxidation of a sample of acetylated material and identification of the acids $-(CH_2)_{1-7}CO_2H$.

The observations strongly suggest that degradation of an acid side chain (a) proceeds by an initial β-oxidation (b) and decarboxylation of the β-keto acid (c). The lower homologous acids (d) may well arise largely by similar processes. Hence the phenomenon of β-oxidation is not limited to biological oxidations.

$$-(CH_2)_9CO_2H \xrightarrow{(a)} [-(CH_2)_7COCH_2CO_2H] \xrightarrow{(b)} -(CH_2)_7COCH_3 \xrightarrow{(c)} -(CH_2)_{1-7}CO_2H \quad (d)$$

Experimental

2-Undecyloyl-1-naphthol.—A mixture of 186 g. of undecylic acid, 148 g. (1.03 equiv.) of α-naphthol and 750 cc. of boron fluoride etherate was heated on the steam-bath for 4 hr., and then water was added and the mixture heated on the steam-bath to remove the ether and then cooled. The yellow-brown solid product was triturated with water, washed by decantation, and crystallized from alcohol to give light yellow leaflets, m.p. 72–73°; yield 271 g. (87%).

Anal. Calcd. for $C_{21}H_{28}O_2$ (312.43): C, 80.73; H, 9.03. Found: C, 81.11; H, 8.97.

2-Undecyl-1-naphthol.—A mixture of 60 g. of 2-undecyloyl-1-naphthol, 1260 cc. of 95% ethanol, 315 cc. of 36% hydrochloric acid and 63 g. of zinc that had been freshly poured and then amalgamated was refluxed for 10 hr., diluted with 4 l. of water, and shaken with ether. The organic layer, containing ether and ethanol, was evaporated in vacuum at 50° until all the ether was removed and then poured into a large volume of water. The resulting solid product crystallized from 40–60° petroleum ether in flocculent white crystals, m.p. 54–55°; yield 55 g. (95%).

Anal. Calcd. for $C_{21}H_{30}O$ (298.45): C, 84.51; H, 10.13. Found: C, 84.61; H, 10.27.

2-Undecyl-1,4-naphthoquinone.—A solution of 55 g. of 2-undecyl-1-naphthol in 500 cc. of acetic acid was cooled to 50° and treated with a solution of 75.6 g. of chromic anhydride in 50 cc. each of water and acetic acid and the solution was kept at 67 ± 2° for 5 hr. The green solution was then diluted with 2.5 volumes of water and let stand overnight at 5°. The resulting crop of fine yellow needles was crystallized from acetic acid–water (5:1) and finally from methanol to give 32 g. (55%) of needles, m.p. 63–64°.

Anal. Calcd. for $C_{21}H_{28}O_2$ (312.43): C, 80.73; H, 9.03. Found: C, 80.95; H, 9.22.

2-Undecyl-1,4-naphthoquinone-2,3-oxide.—A solution of 16 g. of 2-undecyl-1,4-naphthoquinone in 90 cc. of dioxane was treated with a solution of 5.4 g. of sodium carbonate in 30 cc. of water and 13 cc. of 30% hydrogen peroxide and the mixture kept at 70° for 10 min., when the solids had dissolved, evolution of gas had ceased, and a three-phase system had resulted. On addition of an equal volume of water the product separated as a granular solid. Crystallization from methanol gave yellowish needles, m.p. 72–73°, yield 16 g. (96%).

Anal. Calcd. for $C_{21}H_{28}O_3$ (328.43): C, 76.79; H, 8.59. Found: C, 76.60; H, 8.83.

2-Undecyl-3-hydroxy-1,4-naphthoquinone (M-1926).[4]—The oxide (16 g.) was stirred into 70 cc. of 96% sulfuric acid and the red solution stirred at room temperature for 20 min. and poured into water. The yellow-brown precipitate was crystallized first by adding water to a solution in acetic acid and finally from 40–60° petroleum ether. The product formed yellow needles, m.p. 81.4–82.4°; yield 14.5 g. (90%).

Anal. Calcd. for $C_{21}H_{28}O_3$ (328.43): C, 76.79; H, 8.59. Found: C, 76.60; H, 8.83.

The acetate was prepared by treating a suspension of 10 g. of the hydroxyquinone in 50 cc. of acetic anhydride at room temperature with 10 cc. of boron fluoride etherate, when the quinone promptly dissolved. After 10 min., dilution with water precipitated the acetate (10.5 g.), which after several crystallizations from methanol formed pale yellow needles, m.p. 53°.

Anal. Calcd. for $C_{23}H_{30}O_4$ (370.47): C, 74.56; H, 8.16. Found: C, 74.38; H, 8.35.

Synthesis of ω-(3-Hydroxy-1,4-naphthoquinonyl-2) Acids.—The homologous acids with the side-chains $-(CH_2)_8CO_2H$ to $-CH_2CO_2H$ were all prepared from ω-(3-hydroxy-1,4-naphthoquinonyl-2)-decanoic acid[8] by successive two-step Hooker oxidations[9] of 0.01-mole portions (yields 70–90%). The melting points were as follows: n = 9, 104–105°; 8, 124–125°; 7, 127–128°; 6, 129–130°; 5, 120–121°; 4, 159–160°; 3, 145–146°; 2, 194–195°; 1, 176–177°.

Oxidation Procedure.—Oxidations were carried out by occasionally shaking a suspension of excess chromic anhydride in a solution of hydroxynaphthoquinone acetate

(8) G. Fawaz and L. F. Fieser, THIS JOURNAL, **72**, 996 (1950).
(9) L. F. Fieser and M. Fieser, *ibid.*, **70**, 3215 (1948).

equivalent to 200 mg. of free hydroxynaphthoquinone in 10 cc. of acetic acid at room temperature. Continuous shaking or stirring was abandoned because then oxidation proceeded too fast for satisfactory analysis. Oxidation with a solution of sodium dichromate in acetic acid proceeded extremely slowly.

In the experiment recorded in Fig. 3, 226 mg. of 2-undecyl-3-hydroxy-1,4-naphthoquinone acetate (equivalent to 200 mg. of free M-1926) was dissolved in acetic acid in a 10-cc. volumetric flask, 0.8 g. of chromic anhydride was added, the mixture was diluted with acetic acid to the mark, and the flask shaken occasionally. After a given interval of time, a 1-cc. aliquot was removed and shaken with 20 cc. each of water and ether, and the aqueous layer was extracted with a 10-cc. portion of ether. The combined ether extract was washed twice with water and then shaken with 5% sodium carbonate solution, whereupon the acetoxy-carboxylic acid oxidation products are extracted and undergo immediate hydrolysis to give a red solution of hydroxynaphthoquinone acids. Since all the homologous acids of the series have the same color density at the same molar concentration, the yield of acidic products in terms of millimole equivalents of starting material could be determined with use of a Beckman spectrophotometer. The yields at respective time intervals were as follows: 10 min., 5%; 20 min., 7%; 1 hr., 21%; 2 hr., 49%; 5 hr., 40%; 24 hr., 47%. Each carboxylic acid fraction was then analyzed by the method of paper chromatography described below.

The non-acidic residue is a mixture of starting material and more hydrophilic neutral oxidation products (alcoholic and ketonic derivatives). In place of the previously applied buffer extraction procedure,[2] the following method of separation was used. The ethereal solution of the neutral fraction was evaporated and the residue heated with 5% sodium carbonate solution for 10 min. on the steam-bath. This treatment hydrolyzes the acetate of the neutral oxidation products completely but cleaves the starting acetate to only a slight extent. The red mixture was acidified (yellow), extracted with ether, and the neutral oxidation products separated by repeated extraction with a buffer of *p*H 10.50, which leaves unhydrolyzed M-1926 acetate in the ether phase along with the minute amount of hydrolyzed M-1926 (*p*E 11.15). The total neutral fraction in the buffer extract was determined with reference to the calibration curve for M-1926. Finally the ether was evaporated, the residue was heated with 5% sodium hydroxide for 30 min. on the steam-bath and the unoxidized starting material determined colorimetrically; the yields are based upon starting material consumed. Under the conditions used the yield of neutral oxidation products (not shown in Fig. 1) reached a maximum of 20% in a 30-minute period of oxidation; the starting material was exhausted after two hours.

Techniques of Paper Chromatography.—A suitable pair of solvents was prepared by shaking a mixture of 96 cc. of *n*-butanol, 24 cc. of absolute ethanol, 40 cc. of 28% ammonia and 200 cc. of water and letting the mixture stand at room temperature for 24 hr. The upper layer served as the mobile phase (A) and the lower layer as the stationary phase (B).

A convenient chamber for application of the ascending-flow method of paper chromatography was made by fitting together the bottom members of two 20-cm. desiccators, as shown in Fig. 1. An evaporating dish containing a 2-cm. layer of the mobile phase (A) was placed in the bottom member and surrounded by the stationary phase (B). A 30 × 40-cm. sheet of Whatman No. 1 filter paper, impregnated as described below, was formed into a 30-cm. cylinder and the overlapping ends secured at top and bottom with paper clips; the cylinder (C) was then set in the evaporating dish containing the mobile (aqueous) phase, the second, inverted desiccator bottom was put in place, and the chromatogram allowed to develop for about 15 hr. The paper, prior to being formed into a cylinder, was impregnated with test materials placed in a series of spots 2.5 cm. apart on a pencilled line 5 cm. from the bottom of the sheet. A spot was made by allowing an ethereal solution to flow into a melting-point capillary, open at both ends, and touching the tube onto the paper. The spots of unknown oxidation mixtures to be analyzed were alternated with spots of known mixtures of synthetic acids. The cylinder accommodates about 12 simultaneous chromatograms. The number of simultaneous runs can be increased by introducing a second and third concentric paper cylinder of progressively smaller diameter.

Descending-flow chromatography was carried out in a 6 × 70-cm. glass tube as shown in Fig. 2. A layer of stationary phase (B) was introduced into the bottom of the large tube, and the mobile phase (A) was contained in a 10-cm. beaker (I) suspended by a wire (J) from the hooked ends of two glass rods (F) inserted through glycerol-moistened sections of rubber tubing (E) capping glass tubes (G) passing through a rubber stopper (H). A 55-cm. strip of 1-cm. filter paper (C) was folded at one end in the manner shown and hung into the beaker; if desired, the horizontal fold of the paper strip can be weighted down with a glass stopper introduced into the small beaker. A small glass weight (D) hooked into a hole at the bottom of the strip holds the strip extended and vertical. Four strips can be suspended from the beaker at the same time.

The apparatus shown in Fig. 3 can be used for ascending-flow chromatography in instances where it is necessary that the gas space of the vessel be saturated with the stationary phase prior to commencement of chromatography without exposure to the atmosphere. In this case the glass dish containing the mobile phase is placed at the bottom of the large glass tube and surrounded by the stationary phase. A weighted paper strip, impregnated with a spot of test material at the bottom, is hung by a wire from the pair of glass hooks, which initially are raised to a position such that the bottom of the paper strip is above the level of the mobile-phase liquid. Saturation of the vapor-space can be assumed to be complete after 4 hr.; the rods (F) are then lowered until the paper strip dips into the solvent (A).

R_f-Values of Synthetic Hydroxynaphthoquinone Acids.—Ethereal solutions of synthetic acids were transferred to a paper strip by use of a melting-point capillary open at both ends. The chromatogram was then allowed to run at room temperature (25°) for approximately 15 hr. Since the acids acquire a red tinting when exposed to the ammonia-containing vapor, the development of the chromatogram can be followed visually. Subsequent to the run, the strips or sheets were dried and then exposed to ammonia vapor to bring the spots to maximum color intensity. The positions were then measured and the R_f values calculated, with the results shown in Table I. The acids with the side chain $-(CH_2)_2CO_2H$ and $-(CH_2)_3CO_2H$ proved to be inseparable and were treated as a single substance. The methods of ascending and descending flow gave results in good agreement with each other.

TABLE I

R_f-VALUES OF SYNTHETIC HYDROXYNAPHTHOQUINONE ACIDS

	Number of methylene groups								
Method	1	2 or 3	4	5	6	7	8	8	10[a]
Descending	0.33	0.44	0.49	0.54	0.60	0.67	0.74	0.80	0.86
Ascending	0.30	0.40	0.44	0.49	0.56	0.65	0.74	0.82	0.89

[a] Product of oxidation of the neutral product with the side chain $-(CH_2)_{10}CO(CH_2)_7CH_3$.

Quantitative Paper Chromatography.—The soda extract from a 1-cc. aliquot of the oxidation mixture, subsequent to measurement of the color density, was acidified, extracted with ether, and the dried solution was evaporated. The residue was dissolved in about 0.2 cc. of ether and the solution was transferred to the chromatogram paper until an adequate concentration had been achieved. After the paper had been impregnated with a series of unknown mixtures and known reference standards, the chromatograms were developed and the components of the unknowns identified by the position of the spots. The quantity of naphthoquinone acid in a given spot was determined by cutting out the spot, extracting the pigment with 3 cc. of 5% sodium carbonate solution, and measuring the extinction coefficient in a 1-cm. cell with a Beckman spectrophotometer (490 mμ). Since equivalent quantities of all the acids have the same color density, the ratio of molar concentration of the acids produced in an oxidation is directly proportional to the ratio of measured extinction coefficients. Since the total amounts of acids in the 1-cc. aliquots were known, the absolute quantity of the respective acids could be calculated.

Countercurrent Fractionation.—The total neutral fraction resulting from a 30-minute oxidation of 500 mg. of M-1926 acetate was heated on the steam-bath for 10 minutes with 5% sodium carbonate solution and the resulting mixture

distributed between ether and a series of aqueous buffers in a 30-tube Craig countercurrent machine.[6] The buffers were suitable mixtures of primary and secondary alkali phosphates or mixtures of 0.2 *N* sodium hydroxide and 0.1 *N* glycine–0.1 *N* sodium chloride solutions; the *p*H of each buffer was determined with a *p*H meter. The extinction coefficient of each slightly red-colored buffer extract was measured with a Coleman Junior Spectrophotometer (490 mμ); a plot of extinction *vs.* tube number afforded a series of distribution curves indicating the presence of various component substances. Table II summarizes the results of the fractionation. In the third and fourth cycle, the distribution curves corresponded well with the theoretical curves for a series of pure components. Unchanged M-1926 acetate accounted for about 50% of the total neutral fraction; a crystalline Product A amounted to 8% of the total; another, Product D, amounted to 1%; traces of two other crystallizates, B and C, were encountered, as well as a red oil (2%).

TABLE II

COUNTERCURRENT DISTRIBUTION OF THE TOTAL NEUTRAL FRACTION FROM OXIDATION OF 500 MG. OF M-1926 ACETATE

Distribution	Material distributed	Buffer *p*H	Fractions separated Tube No.	Fractions separated Designation	Identity; disposition
I	Total neut. fraction	10.50	0–4	I-1	Combined with II-1
			5–22	I-2	Further distributed
			23–29	I-3	M-1926 Acetate
II	I-2	11.20	0–3	II-1	Red oil; combined with I-1
			4–15	II-2	Product A (bulk of neut. prod.)
			16–23	II-3	Product B (trace)
			24–29	II-4	M-1926 acetate
III	I-1 + II-1	8.58	0–1	III-1	Red oil
			2–21	III-2	Further distributed
			22–29	III-3	Product A (same as II-2)
IV	III-2	6.88	0–3	IV-1	Red oil
			4–16	IV-2	Product C (trace)
			17–29	IV-3	Product D

Final yields: M-1926 Acetate (+ free M-1926), 220 mg.; Product A (II-2, III-3), 40 mg.; Product B, trace; Product C, trace; Product D, 5 mg.; red oil, 10 mg.

Oxidation of Synthetic Model Compounds. (a) **2-(11′-Ketononadecyl)-3-hydroxy-1,4-naphthoquinone,[7] Side Chain** $-(CH_2)_{10}CO(CH_2)_2CH_7$.—Oxidation of 0.43-millimole quantities of acetate, equivalent to 200 mg. of free hydroxyquinone, were carried out at the time intervals: 10 min. (14), 20 min. (20), 1 hr. (46), 2 hr. (49), 5 hr. (36), 24 hr. (34); the figures in parentheses showing the yield (%) of the total acidic products. The acidic products were identified and determined by the method of paper chromatography described above, with the results recorded in Fig. 4. Acids with the side chains $-(CH_2)_nCO_2H$, n = 2 to 10, were produced in all instances, even in the 10-minute oxidation. As the time of oxidation increased, the proportion of higher homologs dropped and the proportion of lower acids correspondingly increased. The results show that cleavage occurs on both sides of the carbonyl group, predominantly on the side adjacent to the quinone ring, since the yield of n = 9 acid surpasses that of the n = 10 acid.

(b) **2-(9′-Ketoheptadecyl)-3-hydroxy-1,4-naphthoquinone,[7] Side Chain** $-(CH_2)_8CO(CH_2)_7CH_3$.—Oxidation of 20 mg. of material for 24 hr. afforded all of the acids of the series n = 1 to 8.

(c) **2-(10′-Ketoundecyl)-3-hydroxy-1,4-naphthoquinone,[7] Side Chain** $-(CH_2)_9COCH_3$.—Oxidations conducted for 20 min. and for 1 hr. of 0.058-millimole samples of acetate, equivalent to 20 mg. of free hydroxy compound, afforded all the homologs in the range n = 1 to 9, are reported in Table III. The acid n = 8 predominated.

(d) **ω-(3-Hydroxy-1,4-naphthoquinonyl-2)-nononoic Acid, Side Chain** $-(CH_2)_8CO_2H$.[5]—Oxidation of 0.61 millimole of the acetate (equivalent to 200 mg. of hydroxy compound) gave the results recorded in Table IV.

In the early stages of the oxidation the acids with 2 and 3, and particularly with 5-, methylene groups predominated; later all the other homologs appeared. A small amount of a non-acidic oxidation product was isolated and found to give a positive iodoform test.

(e) **ω-(3-Hydroxy-1,4-naphthoquinonyl-2)-decanoic Acid, Side Chain** $-(CH_2)_9CO_2H$.[5]—Oxidation was conducted as in (d). After 10 minutes, acids with 2, 3 and 6 methylene groups were found present; after 5 hr., acids with 1, 4, 5 and 7 methylene groups also appeared. The acid n = 8 was not found. The combined neutral fractions afforded 12 mg. of a crystalline product, m.p. 73–78° (from methanol–water) of properties and analysis consistent with the structure **2-(8′-ketononyl)-3-hydroxy-1,4-naphthoquinone.**

TABLE III

ACIDS OF THE SIDE CHAIN $-(CH_2)_nCO_2H$ FORMED ON OXIDATION OF 0.029 MILLIMOLE OF 2-ACETOXY-1,4-NAPHTHOQUINONE-3-$(CH_2)_9COCH_3$

Oxidation time	Millimoles × 10^{-3} of acids and % of total acid fraction (parentheses); Acids, number of methylene groups (n) 1	2, 3	4	5	6	7	8	9	Yield Total	Yield %
20 min.	0.07	0.33	0.20	0.25	0.29	0.67	1.50	0.25	3.56	12.3
	(1.9)	(9.3)	(5.6)	(7.0)	(8.0)	(18.7)	(42)	(7)		
1 hr.	0.04	0.64	0.35	0.32	0.45	0.72	1.46	0.33	4.31	15.0
	(1.1)	(14.8)	(8.2)	(7.4)	(10.4)	(16.6)	(33.8)	(7.6)		

Product A.—The predominating neutral oxidation product (Table II) was acetylated, the acetate was oxidized, and the acidic fraction was analyzed by paper chromatography. All acids of side chains n = 1 to 8, $-(CH_2)_nCO_2H$, were found present, which indicates that the product is **2-(9′-ketoundecyl)-2-hydroxy-1,4-naphthoquinone**, with the side chain $-(CH_2)_8COCH_2CH_3$. Analysis of a sample of material crystallized once from methanol–water, m.p. 66–68°, was in approximate agreement with the theoretical value.

Anal. Calcd. for $C_{21}H_{26}O_4$ (342.42): C, 73.66; H, 7.65. Found: C, 72.92; H, 7.64.

The infrared spectrum was identical with that of the synthetic material of side chain $-(CH_2)_{10}CO(CH_2)_7CH_3$.

Product D. (**Table II**).—Oxidation of a sample of acetylated material afforded all the acids having from one to six methylene groups. Since the infrared spectrum of D corresponded exactly to that of A, and of the synthetic model, Product D must have the side chain $-(CH_2)_8CO(CH_2)_3CH_3$.

Other Neutral Products.—The minor crystalline products B and C were obtained in amounts too minute for characterization. The red oil (Table I) appeared to be a mixture; the infrared spectrum suggested that the components have ketonic side chains.

TABLE IV

ACIDIC PRODUCTS, $-(CH_2)_nCO_2H$, RESULTING FROM OXIDATION OF 0.061 MILLIMOLE OF 2-ACETOXY-1,4-NAPHTHOQUINONE-3-$(CH_2)_8CO_2H$

Oxidation time	Millimoles × 10^{-3} of acids 1	2,3	4	5	6	7	Yield Total	Yield %
10 min.		1.06		2.60			3.66	6
20 min.		1.23		2.73			3.96	6.5
30 min.	0.10	1.69	0.55	2.35			4.70	7.2
1 hr.	0.34	4.80	1.34	5.20			11.65	17.5
2 hr.	0.22	6.38	1.32	3.89			11.80	19.4
5 hr.		6.48	2.67	1.77	0.41	0.37	11.70	19.2
24 hr.	1.97	4.70	2.24	0.80	0.29	0.22	8.23	13.5

Anal. Calcd. for $C_{19}H_{22}O_4$ (326.38): C, 72.60; H, 6.80. Found: C, 72.52; H, 7.07.

The substance gave a positive iodoform test and the infrared spectrum corresponded to spectra of the ketonic model compounds (a), (b), (c). A sample of acetylated material on oxidation for 10 minutes gave predominantly acids with 2, 3, and 6 methylene groups and smaller amounts of the n = 1, 4, 5, 7 acids.

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