second phenyl group nearly doubles the shift, but the effect of the further introduction of oxygen in aromatic ether linkage is negligible. The most profound shift in pE observed is that resulting from the introduction of hydroxyl or carbalkoxyl groups into the side chain. A tertiary alcoholic group produces a shift of no less than −4.0 units. The effects of primary and secondary hydroxyls are equal and somewhat less than that of a tertiary group; a second alcoholic group has an effect just half that of the first. The effect of the two oxygen atoms of an ester group approximates that of one hydroxyl group.

The results of the distribution studies are of particular significance to the problem in chemotherapy because they explain the shifts in the peak of *in vivo* activity from series to series (Paper II). That maximum potency is found in a C_9 *n*- or *i*-alkyl side chain, a C_{10}-C_{11} ω-cyclohexylalkyl group, a C_{13}-decalylalkyl group, and a C_{15}-ω-phenylalkyl group can be correlated with the observation that these structural changes produce a progressive increase in hydrophilic character. The still greater effect of a hydroxyl group explains why satisfactory antimalarial activity in such compounds is attained only in members of very high molecular weight. Although the bioassay data do not always permit a sharp definition of the peak of activity, and although some series of compounds have been only partially explored, the figures given in the last column of Table V for the values of pE (found or calculated) of the most active members of eight different series of naphthoquinones are all in the range pE = 10–12. Evidently a balance between lipophilic and hydrophilic characteristics defined by a pE value in the range indicated is required for optimum antimalarial activity. This finding indicates a further criterion for the laboratory evaluation of new candidate compounds. It suggests, further, that effective drug action is dependent upon a proper balance between lipophilic and hydrophilic characteristics, perhaps both for absorption from the intestines and for favorable distribution between the cell membrane and body fluids.

That the above criterion is limited to compounds possessing a common structural unit essential to activity is exemplified by the fact that the 5,6,7,8-tetrahydro-1,4-naphthoquinone listed at the end of Table II has an extraction constant in the favorable range but is devoid of activity.

Summary

The evidence presented in this paper to the effect that chemotherapeutic activity is dependent in part upon the distribution characteristics of the compounds concerned is summarized in Paper I. Practical use, for purposes of identification, of the method here defined for the determination of distribution constants is illustrated in Paper XVII.

CAMBRIDGE, MASSACHUSETTS RECEIVED MAY 13, 1947

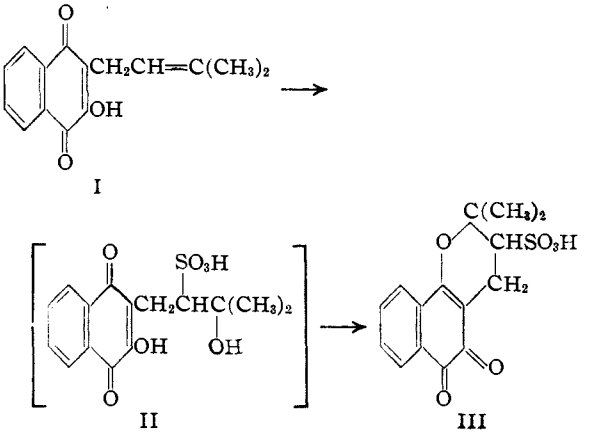
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Naphthoquinone Antimalarials. XVI. Water-Soluble Derivatives of Alcoholic and Unsaturated Compounds

BY LOUIS F. FIESER

For a considerable period in the study of drug degradation the alcoholic metabolites of M-1916 were mistakenly thought to be just as devoid of biological activity as the carboxylic acid derivatives of other members, and it seemed possible that the residual activity of administered M-1916 is due to a product of desaturation. The object of this work was to develop methods for the quantitative extraction of alcoholic metabolites from mixtures containing saturated and unsaturated quinones and for the subsequent analysis of the residual mixtures.

Lapachol derivatives from the Hooker collection having saturated and unsaturated side chains with and without primary, secondary and tertiary alcoholic groups served as convenient models, and the method of study was by the formation of water-soluble derivatives and the colorimetric determination of the pigments distributed between an organic solvent and an aqueous buffer. The alcoholic quinones are all readily convertible into sulfate esters in pyridine solution and are stable in this solution. The tertiary sulfates are very labile in the presence of water, but the decomposition is sufficiently slow at pH 1.5 to permit separation from water-insoluble products. Techniques were found for the recovery of the free terti-



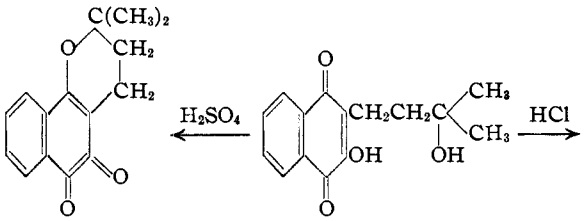
ary alcohol in high yield or for effecting the alternate process of HX-cleavage of the ester.

A method for the analysis of mixtures of saturated and unsaturated quinones is based on the observation that 2-hydroxy-3-allyl-1,4-naphthoquinone is converted by acetic anhydride and sulfuric acid into a water-soluble product.[1] The product from lapachol has been characterized as a C-sulfonic acid probably formed by the addition of $HO\text{-}SO_2OAc$ to the double bond (II) and subsequent cyclization and hydrolysis (III).

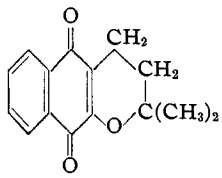
Experimental Part

Sulfate Esters of Alcoholic Derivatives

1. Hydroxyhydrolapachol.—One of the isolated products of metabolic oxidation is a tertiary alcohol identified as Hooker's hydroxyhydrolapachol, a particularly appropriate case for the study of the formation and hydrolysis of water-soluble sulfate esters because its ready conversion into cyclic derivatives under the influence of mineral acids (Hooker) afforded a convenient index of one type of side reaction encountered.



β-Lapachone Hydroxyhydrolapachol



α-Lapachone

Tertiary and other alcoholic derivatives are converted quantitatively into sulfate esters by interaction with pyridine–sulfur trioxide in pyridine solution, employed in the form of the suspension resulting from the addition of chlorosulfonic acid to pyridine (the chlorosulfonic acid should be added before and not after the quinone). The procedures were designed for small quantities and for following the reactions by colorimetry.[2]

Procedure A.—A 100.0-mg. sample of the quinone is weighed into a tared centrifuge tube, and 1-cc. portions of pyridine are pipetted into two other tubes. One of these is held in a slanting position in an ice-bath while 0.1 cc. of chlorosulfonic acid is introduced by small drops (16) delivered from a dropping tube with a capillary tip. The suspension of pyridine–sulfur trioxide is homogenized with a stirring rod and thoroughly cooled. The quinone is dissolved by warming in about half of the second 1-cc. portion of pyridine and the solution is transferred by capillary dropping tube to the suspension of reagent and the remainder of the pyridine is used for rinsing the sample tube. The reaction mixture is stirred and warmed on the steam-bath for a minute or two until an orange-yellow solution results. This is completely stable; when cooled in ice it becomes gelatinous.

The sulfate esters of the tertiary alcohols undergo rapid decomposition in aqueous solution in the presence of either bases or acids. For exploration of the decomposition of hydroxyhydrolapachol sulfate, successive portions of the pyridine solution of approximately 0.1-cc. each were added to mixtures of 8 cc. of a given buffer and 5 cc. of ether in a separatory funnel. Each mixture was shaken for one-half minute, let stand for a total of five minutes, shaken again and the layers separated. The washed ether layer contained both alkali-soluble and alkali-insoluble pigment, and the former was extracted with dilute alkali and determined colorimetrically. The ethereal solution (containing lapachone) was evaporated without being dried, the residue was saponified with alkali, and the resulting hydroxyhydrolapachol was determined. The buffer solution of the sulfate ester was extracted at intervals with further 5-cc. portions of ether and the amount of ester taken in each experiment was determined as the summation of all alkali-soluble and alkali-insoluble fractions (the samples varied from 4.6–7.3 mg. equivalents). The results in Table I are expressed as the initially observed per cent. decomposition per minute. Decomposition of the water-soluble sulfate to ether-extractable products occurs very rapidly in strongly acidic solutions and also in the range *p*H 4–10. However, the rate of decomposition is less in 0.5 *N* hydrochloric acid than in 1 *N* acid and passes through a minimum at about *p*H 1.5, which is thus the most favorable acidity for the manipulation of the labile ester. The distribution of a reaction mixture between a citric acid buffer and ether can be accomplished in periods under one minute and the tertiary ester separated from water-insoluble products with losses due to decomposition of less than 1%.

TABLE I

DECOMPOSITION OF HYDROXYHYDROLAPACHOL SULFATE IN AQUEOUS BUFFERS (25°)

Buffer	*p*H	Decomposition in first 5 min., % per min. Alk.-sol.	Alk.-insol.	Total
1 *N* HCl	0.05	5.94	0.87	6.81
0.5 *N* HCl	0.35	1.61	.41	2.02
0.5 *M* Citric acid	1.49	0.96	.30	1.26
0.2 *M* Citrate	2.25	1.48	1.66	3.14
0.2 *N* Acetate	3.60	1.97	2.00	3.97
0.2 *N* Acetate	4.40	2.78	5.72	8.50
0.07 *M* Phosphate	7.07	2.14	8.38	10.52
0.1 *N* Glycine	10.05	0.75	8.76	9.51

Products Derived from Hydroxyhydrolapachol.—The alkali-insoluble material resulting from the decomposition of the sulfate ester in 1 *N* hydrochloric acid was crystallized from ligroin and obtained as bright orange needles of β-lapachone, m. p. 154–155°. A mixture of α- and β-lapachone can be separated readily by treating an alcoholic solution with saturated sodium bisulfite solution followed by water; α-lapachone is precipitated in a pure condition, and pure β-lapachone is recovered by the addition of sodium carbonate to the colorless filtrate. The neutral fractions resulting from the decomposition of the sulfate ester at all regions of *p*H were examined and in all cases the sole cyclic product found was β-lapachone.

The total alkali-soluble material (119 mg.) derived from the alkaline decomposition of the sulfate from 500 mg. of hydroxyhydrolapachol was separated by fractional extraction from ether with a *p*H 7.1 buffer into an early fraction (65 mg.), several intermediate fractions, and a late fraction (48 mg.). The early fraction, on recovery of the product and crystallization from ligroin, afforded

(1) Fieser, THIS JOURNAL, **48**, 3208 (1926).

(2) Determinations were made with a Fisher Electrophotometer with a 525-mμ filter. A separate calibration curve was used for each compound studied because the molecular color density was not always the same.

fine lemon-yellow needles that melted at 126–127° both alone and when mixed with hydroxyhydrolapachol. The late fraction gave an initial crystallizate from ligroin melting over the range 88–130°. Three recrystallizations afforded a small crop of lustrous yellow plates melting at 139–140° and identified as lapachol (mixed m. p. 140–141°). The total mother liquor pigment (34.4 mg.) was submitted to Procedure A and the pyridine solution distributed between citric acid buffer and ether for the removal of any residual hydroxyhydrolapachol (trace). The recovered ether-soluble acidic product (33.6 mg.) was then treated with 96% sulfuric acid, and thereby converted into alkali-insoluble material that was completely soluble in bisulfite but melted at about 128–135° and afforded only a small crop of β-lapachone on crystallization; the next crops appeared to be mixtures, and finally a few massive crystals melting at about 110° were obtained. Thus lapachol is one product of the decomposition and is accompanied by another olefinic product capable of undergoing cyclization to an ortho-quinone other than β-lapachone. When 100.0 mg. of pure lapachol was submitted to Procedure A no ether-insoluble material was found and the ether layer on extraction with alkali (97.3 mg. of pigment) and recovery afforded unchanged lapachol (m. p. 140.5–141.5°).

Hydrolysis, Cyclization and Olefin-Formation.—The following experiments were made with the object of defining conditions for the conversion of a tertiary alcohol sulfate into either the product of hydrolysis, HX-cleavage to a cyclic derivative, or cleavage to an olefinic derivative. Hydroxyhydrolapachol sulfate was submitted to decomposition under various conditions and the β-lapachone in the neutral fraction determined by saponification (maximum color density after one to three hours). The acidic fraction extracted by alkali was recovered by ether extraction, submitted to Procedure A, and the pyridine solution was distributed between ether and 0.5 *M* citric acid in order to separate hydroxyhydrolapachol (water layer) from the olefin fraction (ether layer). The aqueous extract was made alkaline, heated for ten minutes on the steam-bath, and assayed colorimetrically; the ether layer was extracted with alkali and the pigment concentration determined.

The results of the first few static decomposition experiments (Table II) suggested that further information concerning the primary reactions might be gained by providing for the prompt removal of the products of decomposition from the reaction mixture as follows.

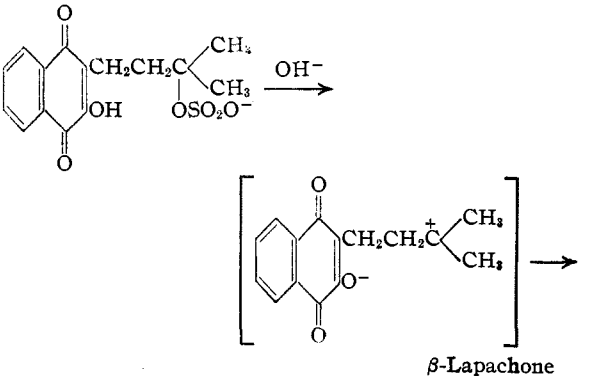
TABLE II

DECOMPOSITION OF THE SULFATE ESTER OF HYDROXYHYDROLAPACHOL (100.0 MG.)

Expt.	Buffer or solvent, cc.	Time, hr.	Products, as mg. equiv. of hydroxy compound: Residual ester	β-Lapachone	Olefin fraction	Hydroxy compound
	Static decomposition (25°)					
1	0.5 *N* HCl, 75	18	0.0	61.3	12.7	21.3
2	0.5 *M* Cit., ac., 75	20	.0	30.5	20.2	45.0
3	3.75 *M* Cit. ac., 10	20	.0	30.0	25.7	40.0
4	HOAc(3)-H_2O (2), 5	72	8.7	42.5	25.4	18.8
5	0.2 *N* NaOH, 75	24	0.0	0.0	10.3	84.5
	With continuous ether extraction					
6	1 *N* HCl, 75	1¼	0.0	12.6	7.5	73.5
7	0.5 *N* HCl, 75	1½	.0	8.2	7.2	81.3
8	0.5 *M* Cit. ac., 75	1¼	.0	9.5	8.2	74.0
9	*p*H 7.07 Phosph., 75	1½	.0	26.9	7.5	65.0
10	0.2 *N* NaOH, 75	¼	.0	40.0	7.5	50.0
	Cleavage in non-aqueous solution (91°)					
11	HOAc, 7.5	1	4.0	46.3	47.2	0.0

Procedure B.—A suspension of pyridine–sulfur trioxide is prepared as in A and the solid is scraped from the walls, washed by centrifugation with two 5-cc. portions of chloroform, and dried *in vacuo* (weight 156 mg.). The powder is scraped into the extraction chamber of an apparatus for continuous extraction with ether (sintered glass delivery tube extending to the bottom of the chamber) and mixed with a 100.0-mg. sample of hydroxyhydrolapachol transferred with the use of 2 cc. of pyridine. The mixture was warmed until a clear solution of the sulfate resulted, the condenser was put in place and the ether in the boiler brought to reflux; 75 cc. of buffer was then added quickly to the reaction mixture and the extractor was fully assembled. Usually a rapid flow of ether through the buffer could be attained in thirty to forty-five seconds from the time of first contact of the buffer with the sulfate ester. The reaction times recorded are those at which an acidic solution lost its yellow color or, in case of an alkaline reaction, when the ethereal extract became colorless.

A comparison of the results of the static and dynamic decomposition experiments shows that the former do not reflect the true course of the reaction because the hydrolysis product undergoes cyclization in contact with acids and β-lapachone suffers hydrolysis in the alkaline buffers. When the reaction products are removed nearly as fast as they are formed the relative rate of HX-cleavage to olefinic material is not influenced by the acidity of the medium but HX-cleavage to β-lapachone increases in speed with increasing alkalinity. A likely explanation is that the severance of the C—O bond of the sulfate ester affords a carbonium ion group that is attracted by the anionic group that appears on the quinone nucleus as the alkalinity is increased. The extent of ionization of the



β-Lapachone

nuclear hydroxyl group in this case does not seem to influence olefin formation.

The most efficient process for the recovery of a tertiary alcohol from its sulfate ester is by hydrolysis in an acid solution with continuous ether extraction (Expts. 6, 7). Some olefin formation cannot be avoided, but the alternate mode of HX-cleavage can be kept at a minimum by working in the acidic range where the hydroxyquinone is unionized.

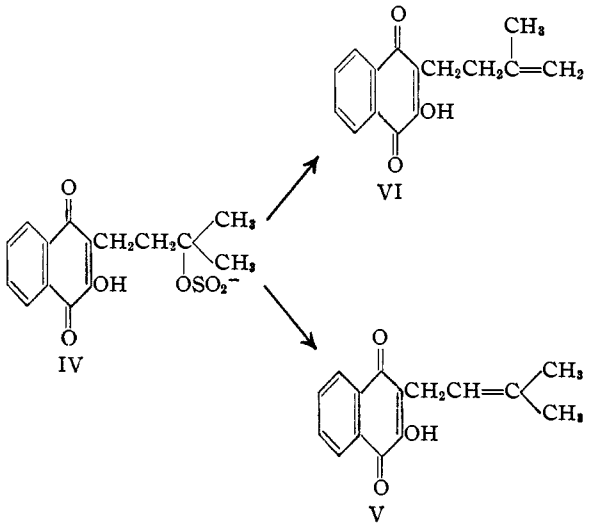
A means for substantially increasing the proportion of olefin was suggested by a comparison of the results of experiments 1 and 2; more olefin was produced in the solution of higher *p*H where the competitive reversible reaction of hydrolysis should be slower. Progressive reductions in the amount of water (Expts. 3 and 4) seemed to further suppress hydrolysis and give higher proportions of olefin. Finally decomposition was tried in the complete absence of water and found to proceed readily in hot glacial acetic acid solution with the formation of about equal parts of β-lapachone and olefin, and the hydroxy compound was absent (Expt. 11). By this expedient about half of the sulfate ester can be cleaved to olefinic material.

The nature of the olefinic fraction was investigated by fractional crystallization of material from the acetic acid cleavage of the ester from 2–4-g. lots of hydroxyhydrolapachol. The most successful procedure was to secure a

first crystallizate from slightly diluted methanol; the crude material (m. p. 100° or above) on several recrystallizations afforded characteristic thin plates of pure lapachol (m. p. and mixed m. p.). The low-melting material recovered from the first mother liquor was then crystallized several times from 60–90° ligroin and eventually afforded clusters of radiating, heavy yellow needles of an **isomeric olefin**, m. p. 89.5–90.5°.

Anal.[3] Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 73.98; H, 5.70.

This substance when cyclized with sulfuric acid gave an orange precipitate, m. p. 143–145°, that on one crystallization from dilute methanol or ligroin afforded characteristic needles of β-lapachone, m. p. and mixed m. p. 155–156°. The carbon skeleton is therefore the same as that of lapachol, and since the quinone is yellow and its sodium salt red, the double bond cannot be in the α,β-position. The substance, therefore, must have the alternate Δ^{γ}-unsaturated structure: **2-hydroxy-3-(γ-methyl-γ-butenyl)-1,4-naphthoquinone** (VI). The HX-olefinic cleavage therefore follows both alternate paths



A search for other components of the olefin fraction was fruitless. Thus the combined mother liquor material from all fractions when cyclized with acetic-hydrochloric acid according to Hooker yielded nothing but successive crops of α-lapachone, m. p. 117–118°, 116–117°, 113–115°.

2. 2-Hydroxy-3-β-hydroxyisobutyl-1,4-naphthoquinone[4]

The sulfate ester from 100.0 mg. of the quinone (B) was hydrolyzed as in Table II-6; the reaction was complete in one-half hour. The neutral fraction was of the same magnitude (13.1 mg. equiv. on saponification), but the acidic fraction (82.3 mg.) consisted solely of hydroxy compound (m. p. and mixed m. p. 121–122°) and contained no olefin. A static decomposition in 1 *N* hydrochloric acid gave the hydroxy compound and the cyclic derivative (red needles, m. p. and mixed m. p. 185–186°).

3. M-2231: —$(CH_2)_8C(OH)(CH_3)_2$

The sulfate ester of this quinone is distinctly more labile than the two esters previously investigated. A static hydrolysis experiment in dilute hydrochloric acid was complete in twenty minutes and afforded fairly pure starting material (very slow crystallization from ligroin gave material melting at 62–64°; a mixture with pure M-2231 melted at 65.5–67.5°).

(3) This analysis was kindly carried out by E. F. Shelberg and Jane Morris of the Abbott Laboratories.

(4) Hooker, THIS JOURNAL, **58**, 1168 (1936); m. p. 121–122°; cyclic derivative, m. p. 186.5–187°.

The best method found for separating the sulfate from water-insoluble substances is as follows. A separatory funnel is charged with suitable volumes of 0.5 *M* citric acid buffer and petroleum ether[5] and a few pieces of ice and the mixture shaken. A pyridine solution of the sulfate ester prepared as in (A) is washed in with ice-cold buffer, or else an aliquot of a solution prepared by diluting this with acetic acid to a volume of 5.0 cc. (with cooling), is added by pipet, and the mixture is shaken for one-half minute. The petroleum ether layer is washed with water and the pigment extracted with alkali and determined; the pigment found in this fraction (inadvertent hydrolysis) was 3% of the total.

The reaction of the sulfate ester with aqueous buffers yields mixtures of alcoholic and olefinic material. These were analyzed by recovering the total pigment, forming the sulfate ester as in (A), and distributing the mixture between petroleum ether and iced citric acid as above; the amount of olefin indicated by analysis of the petroleum ether layer is probably too high by about 3%. Thus the amount of olefin formed on hydrolysis in the extractor (Table III) is small and the recovery of the tertiary alcohol high; the yellow color of the aqueous solution was discharged in just six minutes. Alkaline decomposition resulted in a marked increase in the olefin fraction; this corresponds to the increase in the proportion of HX-cleavage with increasing *p*H noted with hydroxyhydrolapachol where a part of the tendency to form a carbonium ion is manifested in a cyclization process not possible with M-2231. Cleavage of M-2231 sulfate with acetic acid gives olefin as the predominant product.

TABLE III

DECOMPOSITION OF THE SULFATE ESTER OF M-2231 (100.0 MG.)

Treatment	Time, hr.	Products, as mg.-equiv. of M-2231 Ester	Olefin	Alcohol	Total
1 *N* HCl; cont. extr.	0.1	0.0	6.0	91.9	97.9
2% NaOH, static	1	2.2	39.0	20.4	92.2
HOAc at 91°	2	5.0	84.3	10.0	99.3

4. Hydroisolomatiol, —$CH_2CH(OH)CH(CH_3)_2$[6]

On hydrolysis as in Table II-6 (100 mg. quinone), the material extracted in the first ten minutes amounted to only 10.5 mg., and the initially bright yellow solution became colorless only after nine hours. The total pigment, recovered by extraction into alkali and then back into ether, solidified readily when the solution was evaporated in a centrifuge tube,[7] and after being dried for several hours in vacuum weighed 99.9 mg. and melted at 117–119°. One crystallization brought the m. p. to 121–122° and there was no depression on admixture with the starting material. The sulfate of this secondary alcohol is thus hydrolyzed very much more slowly than the three tertiary alcohol sulfates and is converted quantitatively to the starting alcohol without any HX-cleavage.

5. Hydrolomatiol, —$CH_2CH_2CH(CH_3)CH_2OH$[8]

In one experiment the sulfate-pyridine from 100.0 mg. of this primary alcohol was dissolved in 25 cc. of water, acidified and treated with 4 cc. of 36% hydrochloric acid. After seventeen hours at room temperature an ethereal extract gave only a trace of pink color when shaken with alkali. Hydrolysis was then accomplished by heating the

(5) Ether is a poor solvent for partitions involving substances of moderate or high molecular weight because it retains a part of the sodium salts of the products of hydrolysis and decomposition.

(6) Hooker, THIS JOURNAL, **58**, 1181 (1936); m. p. 120–120.5°.

(7) Evaporation without danger of loss by spattering can be done by removing an ethereal solution in portions with a fine-capillary dropping tube and running it dropwise into an empty centrifuge tube that rests in the rings of a steam-bath in a slanting position; solution is added as fast as the ether evaporates.

(8) Hooker,[4] m. p. 101–102°.

solution with more acid for several hours on the steam-bath; the ether-soluble product on crystallization from ligroin afforded 82 mg. of short yellow needles in three crops, m. p. 101–102°, 99–100.5°, 101–102°. The first gave no depression when mixed with starting material.

On hydrolysis as in Table II-6 the color of the aqueous solution was discharged in about seven hours, but the material carried over into the ether boiler was found to be a mixture of the alcohol (73.2%) and unchanged sulfate (23.5%).

6. Lomatiol, $-CH_2CH=C(CH_3)CH_2OH$[6,9]

The sulfate ester of this allylic primary alcohol can be partitioned between ether and citric acid solution without any noticeable hydrolysis. Hydrolysis as in Table II-6 gave the following amounts of alkali-soluble pigment: four hours, 80 mg.; overnight, 14 mg. On acidification of the total alkaline solution well-formed yellow needles of lomatiol separated (70 mg., m. p. and mixed m. p. 127.5–128.5°). The ester thus undergoes normal hydrolysis more rapidly than its saturated derivative or than a related secondary alcohol.

Sulfonic Acid Derivatives of Olefinic Quinones

Cyclolapachol Sulfonic Acid (III).—A suspension of 24.2 g. (0.1 mole) of lapachol in 100 cc. of acetic anhydride was stirred mechanically and kept at 25–40° by occasional ice cooling while 6.0 cc. of 96% sulfuric acid[10] was added by drops. The quinone soon dissolved to a red solution, and toward the end of the addition the reaction product began to separate as an orange-red paste. After thorough cooling in ice, the product was collected and washed with dry ether; the yield of orange-red powder, dried at 91°, was 29.5 g. (92%). The acid dissolves freely in water or alcohol.

The **sodium salt** was prepared by mixing filtered solutions of 3.22 g. of the acid and 1.23 g. of anhydrous sodium acetate in 50-cc. portions of alcohol. A yellow solid separated at once and was collected after cooling and found to consist of microneedles; yield (dried at 90°) 2.90 g. (84%).

Anal. Calcd. for $C_{15}H_{13}O_6SNa \cdot 0.5H_2O$: C, 50.99; H, 3.99. Found: C, 51.08; H, 3.78.

The salt crystallized from alcohol in fiery red needles, m. p. 206–207° dec. A potentiometric characterization kindly conducted by Lilli Schwenk Hornig indicates that the substance is derived from β- rather than α-lapachone. Titrations with titanous chloride in 0.1 *N* hydrochloric acid 0.2 *N* in potassium chloride against a calomel half-cell indicated the value $E_0 = 0.413$ v (uncorrected for liquid junction potential). The value found for β-lapachone under the same conditions was 0.400 v.; α-lapachone had a potential of 0.298.[11]

The **ammonium salt**, prepared similarly, is more soluble in alcohol and was obtained on recrystallization as a deep red crystalline crust consisting of microneedles.

Anal. Calcd. for $C_{15}H_{13}O_6SNH_4 \cdot H_2O$: C, 50.42; H, 5.36. Found: C, 50.83; H, 5.47.

The **acid chloride**[12] was obtained by refluxing a suspension of 1.72 g. of the sodium salt in 10 cc. of phosphorus trichloride for five and one-half hours and removing the phosphorus oxychloride by distillation in vacuum. The residual solid on one crystallization from absolute ethanol gave 1.45 g. (85%) of crystalline product, and the recrystallized material formed thick, burnt-orange needles that decomposed at about 190°.

Anal. Calcd. for $C_{15}H_{15}O_5SCl$: C, 52.87; H, 3.84; Cl, 10.41. Found: C, 52.77, 53.27; H, 4.32, 4.28; Cl, 10.89.

A solution of 0.35 g. of the acid chloride in 25 cc. of benzene when saturated with dry ammonia gas gave a dark sticky precipitate of the **amide**.[12] When a solution of this material in water was acidified the red color was largely discharged and a yellow product separated. Recrystallization from 15% aqueous dioxane gave light yellow needles, m. p. 188–189°, dec.

Anal. Calcd. for $C_{15}H_{15}O_5NS$: C, 56.06; H, 4.71. Found: C, 56.40; H, 4.73.

The **ethyl ester**[12] was isolated as a by-product in a crystallization of the acid chloride from ethanol; it formed yellow needles, m. p. 162–163°.

Anal. Calcd. for $C_{17}H_{18}O_6S$: C, 58.27; H, 5.18; S, 9.15. Found: C, 58.47, 58.39; H, 5.30, 5.49; S, 9.20.

Cyclosulfonic Acid of 2-Hydroxy-3-allyl-1,4-naphthoquinone.—The red acid[1] was prepared as above and converted into the **sodium salt** with sodium acetate in alcohol; the salt was obtained as orange yellow microplates (dried at 90°).

Anal. Calcd. for $C_{13}H_9O_6SNa \cdot 0.5H_2O$: C, 48.00; H, 3.10. Found: C, 47.86; H, 3.18.

Analytical Methods.—A mixture of 48.8 mg. of hydrolapachol and 11.1 mg. of hydroxyhydrolapachol was analyzed satisfactorily by treatment with 96% sulfuric acid in the cold, dilution with ice and water, extraction with ether, and determination of the pigment extracted by alkali (hydrolapachol, 10.5 mg.) and of the pigment obtained by evaporation of the neutral fraction (β-lapachone) and hydrolysis with 0.1 *N* alkali (hydroxyhydrolapachol, 48.6 mg.). 2-Hydroxy-3-Δ⁹-decenyl-1,4-naphthoquinone (M-289) was investigated as a model compound having a double bond too far removed from the hydroxyl group of the nucleus for easy cyclization, but the substance was found to react with cold 96% sulfuric acid to give ether-soluble material that is only partially extracted by alkali; the alkali-insoluble material is probably polymeric. Acetylation of the quinonoid hydroxyl group prevents this side reaction, and the acetylated product is best converted into a water-soluble derivative by reaction with acetylsulfuric acid as follows:

Procedure: A 20.0-mg. sample of an unsaturated quinone (or a mixture) weighed into a centrifuge tube is treated with 0.5 cc. of acetic anhydride and one drop of 96% sulfuric acid delivered from a capillary dropping tube and the mixture is heated for one minute at 70°. The pale-yellow solution of the acetate is cooled, treated with ten small drops (about 0.1 cc.) of 96% acid, heated at 70° for five minutes, cooled, diluted with 5–7 cc. of water (cooling), made alkaline (3 cc. 25% alkali), and heated for five minutes on the steam-bath to insure complete hydrolysis of the acetate. The red color is discharged with hydrochloric acid and the mixture extracted twice with petroleum ether and the extracts back-washed with water. The aqueous solution, which is rendered bright yellow by the presence of a sulfonic acid derivative, is made alkaline and the pigment determined as milligram equivalents of unsaturated derivative. The petroleum ether layer is evaporated and the residue dissolved in alcohol, made alkaline, and any saturated pigment found present is determined by colorimeter.

Trials with saturated and unsaturated quinones of various molecular weights showed that it is necessary to hydrolyze the acetylated material prior to partition between water and petroleum ether because otherwise the acetates of some saturated quinones (*e. g.*, M-1916, M-1971, M-285) form persistent emulsions in the water phase even after repeated extraction with solvent. Ether cannot be used in place of petroleum ether because it extracts appreciable amounts of the sulfonate derivatives of unsaturated quinones of molecular weight of 300 or higher.

The various synthetic unsaturated quinones available were analyzed by the above method and found to contain small amounts of saturated material. The best sample, the Δ³-cyclohexenylpropyl derivative M-2333, gave 96–97% of sulfonated pigment and 2–3% of water-insoluble pigment (analyses by R. H. Brown). Samples of the decenyl derivative M-289 were found to contain 8–10% of saturated material, and the average iodine number found for samples of the undecylenic acid employed

(9) Rennie, *J. Chem. Soc.*, 784 (1895); m. p. 127°.

(10) Chlorosulfuric acid gives the same result.

(11) Compare Fieser, THIS JOURNAL, **50**, 439 (1928).

(12) Experiment by Dr. F. C. Chang.

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.

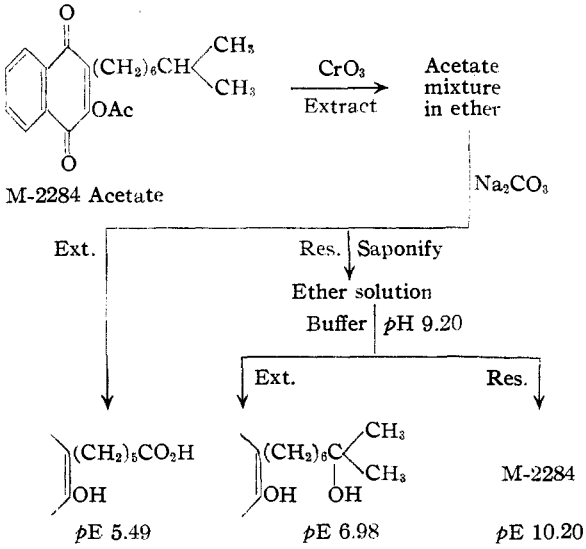
CAMBRIDGE, MASSACHUSETTS RECEIVED MAY 13, 1947

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Naphthoquinone Antimalarials. XVII. Chromic Anhydride Oxidation[1]

BY LOUIS F. FIESER

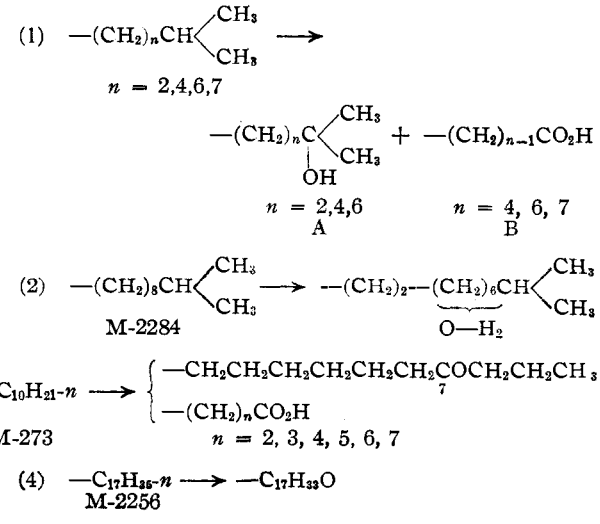
Although the chromophoric nucleus of the hydroxyalkylnaphthoquinones is highly sensitive to attack by permanganate,[2] hydrogen peroxide[3] or hypochlorite,[3] the first point of attack in metabolic oxidation in the liver is the hydrocarbon side chain and not the nucleus (Paper XVIII). It has now been 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).