Summary

The preparation of eleven substituted derivatives of 2-hydroxy-3-phenyl-1,4-naphthoquinone is described in connection with antimalarial and bacteriostatic studies.

NORTH CHICAGO, ILL. RECEIVED MAY 13, 1947

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Naphthoquinone Antimalarials. XV. Distribution between Organic Solvents and Aqueous Buffers[1,2]

BY LOUIS F. FIESER, MARTIN G. ETTLINGER AND GEORGE FAWAZ[3,4]

In the investigation of drug metabolism (Paper XVIII) use was made of fractional extraction from ether with a series of buffers of increasing alkalinity for the separation of metabolites from the less hydrophilic starting materials. Approximate estimations of the *p*H at which appreciable extraction begins to occur revealed marked differences among the naphthoquinones examined and suggested that the precise determination of distribution ratios might be of value for purposes of characterization and separation. The present quantitative study was undertaken with this objective in view and also to see whether a correlation could be established between distribution and some biological factor involved in drug action.

The hydroxyalkylnaphthoquinones are all acids (type HA) of about the same acidity (*pKa* 5–5.5). When such a substance is distributed between ether and an alkaline buffer at a total concentration in the order of 5×10^{-4} molar it may be assumed that the ratio of the concentrations of unionized HA in the ether and water phases is a constant, *K*. Since the material found in the aqueous phase is present almost exclusively as the anion, the measurable distribution ratio *C* is given by the expression

$$C = \frac{[\text{Total quinone}]^{\text{ether}}}{[\text{Total quinone}]^{\text{water}}} = \frac{[\text{HA}]^{\text{ether}}}{[\text{A}^-]}$$

Since

$$K = [\text{HA}]^{\text{ether}}/[\text{HA}]^{\text{water}} \text{ and } Ka = [\text{H}^+][\text{A}^-]/[\text{HA}]$$

it follows that

$$C = [\text{H}^+](K/Ka)$$

An **extraction constant E** may be defined as the hydrogen ion concentration corresponding to the ratio $C = 100$ (whence $K/Ka = 100/\text{E}$). The constant is conveniently employed as its negative logarithm, *p*E, which can be calculated from measurement of the distribution ratio at an appropriate *p*H by means of the equation

$$p\text{E} = \log C + p\text{H} - 2$$

The value 100 for *C* is chosen as a basis for comparison because *p*E then defines accurately the roughly determinable *p*H at which, at a total concentration of 20 mg./100 cc., visible color appears in the aqueous phase; *p*E also indicates the upper limit of *p*H at which oxidation products can be safely separated without extracting any more than 1/101 part of starting material (equal volumes of solvents).[5] The theory was tested by calculation of *p*E for hydrolapachol from distribution determinations conducted over wide ranges of hydrogen ion and quinone concentrations (Table I); the results validate the theory with an accuracy of ±0.05 *p*H unit.

TABLE I

DETERMINATION OF *p*E FOR HYDROLAPACHOL

Buffer *p*H	*p*E found at an initial concentration in ether of (mg./100 cc.) 10	20	50
8.65		7.76	7.73
8.95	7.82	7.75	7.72
9.50	7.78	7.77	7.80
10.10		7.82	

*p*E (av.) = 7.77

Extraction Procedure.—A standard solution of the quinone was prepared by dissolving 50 ± 0.2 mg. of material in ether that had been saturated with water and diluting the solution to a volume of 250 cc. with the same solvent. Such a solution is susceptible to autoxidation unless stored in darkness. The buffers were suitable mixtures of 0.2 *N* primary and secondary phosphate or of 0.2 *N* sodium hydroxide and 0.1 *N* glycine–0.1 *N* sodium chloride solutions. The *p*H values were determined with a *p*H meter. The *p*H values of standard solutions of sodium hydroxide were either assumed from the concentrations or determined by the use of a special electrode suitable for high alkalinity.

A dry 125-cc. separatory funnel was shaken with more than enough ether to saturate the vapor space, the excess ether was drained off, and 25 cc. of the standard quinone solution (5 mg.) was transferred to the funnel by pipet. A 30-cc. portion of buffer was saturated with ether in a separatory funnel and 25 cc. of the aqueous layer was shaken with the quinone solution. When fully clear, the aqueous extract was separated and the concentra-

(1) For acknowledgments to CMR and the Rockefeller Foundation, see Paper I.

(2) Preliminary experiments were conducted by C. Heidelberger.

(3) On leave of absence from the American University, Beirut.

(4) With the technical assistance of Eva Fawaz.

(5) The constant *p*E closely resembles the hydrochloric acid number employed by Willstätter in the characterization and separation of porphyrins; see Zeile and Rau, *Z. physiol. Chem.*, **250**, 197 (1937); Keys and Bruegsch, THIS JOURNAL, **60**, 2135 (1938).

tion of quinone pigment determined by colorimetry.

Determinations; Procedure A (M. G. E.).—The determinations were made with a Fisher Electrophotometer (525-mμ filter) or with a Klett–Summerson Photoelectric Colorimeter (green filter). In the experiments with quinones of pE below 11 the quinone remaining in the ether phase was extracted with alkali and determined. Since the sum of the concentrations found in the alkali extract of the ethereal phase (x) and in the aqueous phase (y) deviated somewhat from the total concentration taken (generally in excess), the distribution constant C was calculated from the following empirical equation derived from the method of least squares (a and b are the dilutions, relative to 25 cc., at which concentrations in the alkaline extract of the ether phase and in the buffer extract are measured, and d is the initial concentration in the ether)

$$C = [ad/b + (bx - ay)]/[bd/a - (bx - ay)]$$

Example: When 25 cc. of an ethereal solution containing 20 mg./100 cc. of 2-hydroxy-3-isobutyl-1,4-naphthoquinone was extracted with 25 cc. of a buffer of pH 7.97, the concentration found in the buffer was 1.6 mg./100 cc. (y; $b = 1$). The ethereal solution on extraction with 50 cc. of alkali afforded 10.0 mg./100 cc. of pigment ($x = 10.0$; $a = 2$), whence $C = 46.8/3.2 = 14.6$ and $p\text{E} = 5.97 + 1.16 = 7.13$.

Procedure B (G. and E. F.).—The colorimetric determinations were made with a Coleman Junior Spectrophotometer set to a wave length of 490 mμ (one exception). The calibration curves for the different quinones showed slight deviations from molecular equivalence in color density but invariably exhibited a linear relationship between color density and concentration. Determination was made only of the pigment present in the aqueous extract and that in the ether phase was calculated by difference.

Salt Correction.—When a quinone of $p\text{E} > 10$ is distributed between ether and a buffer containing sodium as the sole metallic ion the ether is colored red and contains both the un-ionized quinone HA and its salt NaA. The observed gross distribution ratio C' is then a sum of two terms

$$C' = [\text{HA}]^{\text{ether}}/[\text{A}^-] + [\text{NaA}]^{\text{ether}}/[\text{A}^-] = C + C_{\text{Na}}$$

Distributions conducted in different buffers yield a series of values of $[\text{H}^+]$, $[\text{Na}^+]$ and the over-all ratio C'. A resolution of C' into C and C_{Na} is accomplished by conducting one extraction with 0.13 N sodium hydroxide, for which C_{Na} exceeds C by a factor of some tenfold for all compounds studied. As an initial approximation, C_{Na} for 0.13 N NaOH is taken equal to $0.9C'$, and proportional values of C_{Na} are calculated for the other solutions on the assumption that C_{Na} is proportional to $[\text{Na}^+]$. From the resulting values of $C = C' - C_{\text{Na}}$ an average value of pE is obtained that suffices for the computation of C and an accurate value of $C_{\text{Na}} = C' - C$ for 0.13 N NaOH. Repetition of the calculations gives the final value of pE. The calculated value of C_{Na} for $[\text{Na}^+] = 0.1$ is denoted by K_{Na}, and is recorded in order to describe completely the distribution of the quinone and its sodium salt.

Example: 2-Hydroxy-3-(*trans*-4′-cyclohexylcyclohexyl)-1,4-naphthoquinone; the experimental data are

NaOH, N	pH	$[\text{Na}^+]$	C'
0.13	13.1	0.13	5.05
.01	12.0	.01	8.5
.05	12.7	.05	3.45

On the assumption that for 0.13 N NaOH $C_{\text{Na}} = 0.9C' = 4.55$, for 0.01 N NaOH $C_{\text{Na}} = 4.55 \times 0.01/0.13 = 0.35$, $C = 8.15$, $p\text{E} = 10.91$; for 0.05 N NaOH $C_{\text{Na}} = 4.55 \times 0.05/0.13 = 1.75$, $C = 1.7$, $p\text{E} = 10.93$. If $p\text{E} = 10.9$, then for 0.13 N NaOH $C = 0.65$, $C_{\text{Na}} = 4.4$. Recalculation gives for 0.01 N NaOH $p\text{E} = 10.91$; for 0.05 N NaOH $p\text{E} = 10.94$. $K_{\text{Na}} = 4.4 \times 0.10/0.13 = 3.4$.

Results.—Table II records the averages of pE determinations conducted at three pH levels each for those quinones that exhibited the normal behavior typified by the example of Table I; more detailed results for quinones showing an abnormal behavior are given in Table III. Independent determinations by Procedures A and B on the same or different samples gave results that seldom differed by more than 0.1 unit and never by more than 0.2 unit.

A few comparative determinations were made with the use of cyclohexane as the organic solvent (superscript a). Two of the compounds contain alkyl or hydroxyalkyl side chains, and the pE values coincide with the ether values. The other two quinones are aralkyl derivatives and the pE values for cyclohexane are both about 0.5 unit lower than those for ether. Even with quinones of pE as high as 11 the cyclohexane shows no coloration from dissolved sodium salt.

The six quinones listed in Table III were the only compounds of molecular weight greater than 380 whose salts are sufficiently soluble in water to permit determinations; they all have oxygenated or aralkyl side chains. In each instance the apparent values of pE increase markedly with the alkalinity of the aqueous phase. Since, in the best case for comparison (second compound), the deviation from constancy is as great with cyclohexane as with ether, the deviation can hardly be dependent upon the presence of sodium salt in the organic phase. The direction and magnitude of the effect are not compatible with association of the quinone in the organic phase. It therefore appears that even very dilute aqueous solutions of the sodium salts of these quinones of high molecular weight possess some abnormality, perhaps comparable with phenomena associated with the soaps of higher fatty acids.

TABLE II

AVERAGE VALUES OF pE

Side chain	Procedure	pE	K_{Na}
Alkyl Series			
n-C_4	A	7.18	
i-C_4	A	7.10	
i-C_5	A	7.77	
n-C_6	B	8.40	
i-C_7	B	9.15	0.05
n-C_9	B	10.20	.76
n-C_{10}	B	10.78	2.7
	B[a]	10.74[a]	
i-C_{10}	A	10.63	2.3
i-C_{11}	A	11.19	8.5
	B	11.23	6.1
n-C_{12}	B	11.74	23
ω-Cyclohexylalkyl Series			
C_7	B	8.43	0.01
C_8	B	9.14	.04
C_9	A	9.43	.19
	B	9.47	
C_{10}	A	10.20	.63
	B	10.14	.62
C_{11}	B	10.64	2.0
ω-Phenylalkyl Series			
C_7[b]	A	6.66[c]	
	B	6.70[c]	
C_8	A	7.54	
C_9	A	7.93	
	B	8.11	
	B[a]	7.45[a]	
C_{11}	A	9.11	0.14
C_{12}	B	9.66	.27
C_{13}	B	10.15	.79
C_{14}	B	10.77	3.3
C_{15}	B	11.30	9.0
Esters			
$-(CH_2)_4CO_2CH_3$	B	6.03	
$-(CH_2)_4CH(CH_3)CO_2CH_3$	B	7.32	
$-(CH_2)_8CO_2CH_3$	B	8.47	
Tertiary Alcohols			
$-CH_2C(OH)(CH_3)_2$	B	4.18	
$-(CH_2)_2C(OH)(CH_3)_2$	B	4.74	
$-(CH_2)_8C(OH)(CH_3)_2$	A	7.86	
Miscellaneous			
-Cyclohexyl	A	8.26	0.01
-*trans*-4′-Cyclohexylcyclohexyl	A	10.92	3.4
$-(CH_2)_3$-β-Tetralyl	A	9.27	0.26
$-(CH_2)_3$-*trans*-β-Decalyl	A	10.87	4.5
$-CH_2CH{=}C(CH_3)_2$	A	7.31	
$-(CH_2)_3$-Δ^3-Cyclohexenyl	A	8.95	0.05
$-(CH_2)_3$-α-Thienyl	A	7.85	
$-(CH_2)_2CH(CH_3)CH_2OH$	B	5.08	
$-CH_2CH{=}C(CH_3)CH_2OH$	B	4.83	
$-CH_2CH(OH)CH(CH_3)_2$	B	5.15	
$-CH_2COCH(CH_3)_2$[b]	A	4.80	
$-(CH_2)_8CH(OH)CH_2OH$	B	6.17	
$-(CH_2)_2C_6H_{10}OH(4')$ (identical with Metabolite, m. p. 156°)	A	6.44	
$-(CH_2)_3C_6H_{10}OH$ (Metabolite, m. p. 112°)	B	6.35	
$-(CH_2)_3C_6H_5Cl$-*p*	A	8.58	0.13
2-Hydroxy-3-cyclohexylpropyl-5,6,7,8-tetrahydro-1,4-naphthoquinone	B[c]	10.35	.30

[a] Organic solvent: cyclohexane. [b] The salt solutions are orange. [c] The result is not included in the plot of Fig. 1; this first member differs from the other members of the series in color[b] and relative pE. [d] The salt solutions are violet; colorimetric determinations were made at wave length 535 mμ.

TABLE III

DISTRIBUTION OF QUINONES OF HIGH MOLECULAR WEIGHT

Side chain	Procedure	Buffer pH	C′	pE
$-(CH_2)_8C(OH)(C_6H_{13-n})_2$	B	12.0	19.0	11.27
(mol. wt. 485)		12.7	16.4	11.75
		13.1	16.4	
$-(CH_2)_8C(OH)(C_5H_{11-n})_2$	B	12.0	10.1	10.98
(mol. wt. 457)		12.7	8.52	11.49
		13.1	8.42	
	B[a]	12.0	9.5	10.98[a]
		12.3	7.7	11.18[a]
		12.7	6.6	11.48[a]
$-(CH_2)_6C(OH)(C_6H_{13-n})_2$	B	12.0	8.21	10.88
(mol. wt. 457)		12.3	7.5	11.09
		13.1	9.0	
$-(CH_2)_8C(OH)(C_4H_{9-n})_2$	B	12.0	5.64	10.73
(mol. wt. 429)		12.7	3.92	11.08
		13.1	4.42	
$-(CH_2)_3C_6H_4OC_6H_{5-p}$	A	10.52	15.7	9.69
(mol. wt. 384)		12.0	9.74	9.83
		12.3	0.54	9.91
		13.1	.89	
	B	12.0	.81	(9.88)
		12.3	.46	9.78
		12.7	.60	10.27
		13.1	.66	
	B[a]	12.0	.21	9.33[a]
		12.3	.08	9.20[a]
$-(CH_2)_3C_6H_4{\cdot}CH_2C_6H_{5-p}$	B	12.0	.95	9.98
(mol. wt. 382)		12.3	.56	10.04
		12.7	.51	10.51
		13.1		

[a] Organic solvent: cyclohexane.

Discussion.—The values of pE found for quinones having normal and isoalkyl side chains are indistinguishable, and a plot of the combined results (Fig. 1) establishes a linear relationship between pE and molecular weight. Curves for four other series of naphthoquinones show a similar proportionality, at least to a region of molecular weight of about 400, and are roughly parallel to the first curve but displaced in the direction of increased hydrophilic character. The terminal portion of the curve for the alcoholic derivatives, containing compounds for which pE drifts with pH, deviates from the linear relationship. The experimental results in the region of linearity of relationship can be expressed by the

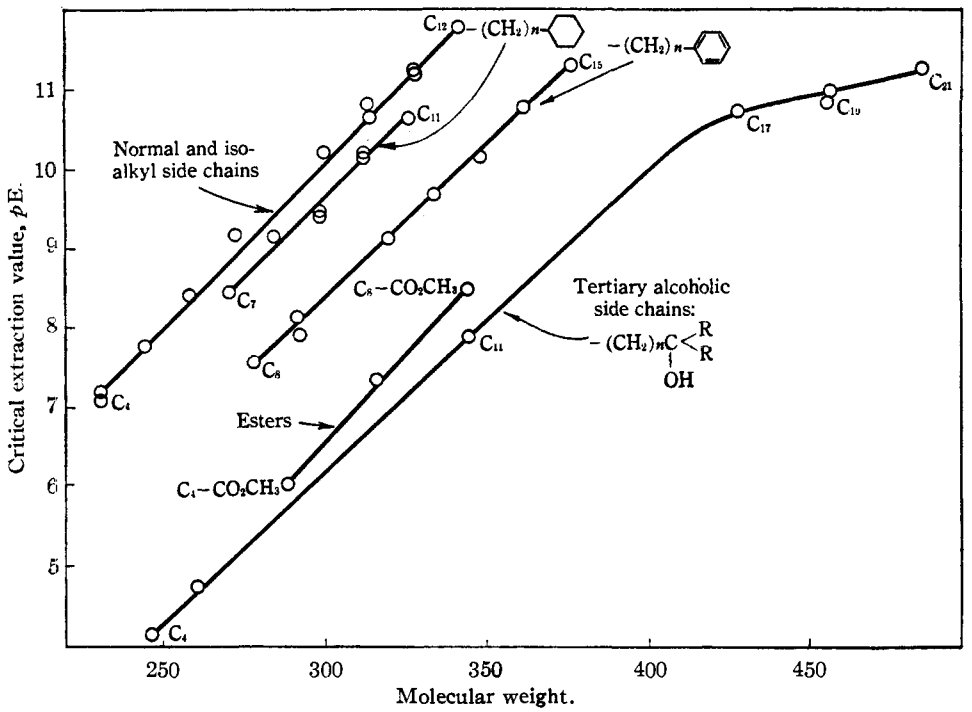

Fig. 1.—Variation of critical extraction values with molecular weight.

empirical equations given in Table IV. The data are not extensive enough to establish whether or not the slope actually varies from series to series, and a slope of 0.04 would fit all the series with an accuracy of ±0.1–0.2 unit.[6]

TABLE IV

$pE = A \times$ MOL. WT. $- B$

Series	A	B
n- and i-alkyl	0.0412	2.28
ω-Cyclohexylalkyl	.0374	1.60
ω-Phenylalkyl	.0384	3.24
Esters	.0437	6.54
t-Alcohols	.0370	4.91

The effect of substitution or other structural change in the side chain on the extraction constant is expressed in Table V in terms of the computed difference of pE from that of a hypothetical member of the n- and i-alkyl series of the median molecular weight 325. The calculations are based upon specific equations where available.

The effects of structural changes in the hydrocarbon side chain in some instances seem surprising. The introduction of one alicyclic ring at the end of an alkyl chain produces a drop in pE of 0.6 unit, and a terminal bicyclic ring has just twice the effect (−1.3 units). On the other hand, the effect of a ring joined directly to the quinone nucleus is very small. A double bond decreases pE by 0.4 unit, and an ω-phenyl substituent produces a shift of −1.9 units, roughly that calculated for three double bonds plus a six-membered ring. A

TABLE V

COMPARISON OF pE AT EQUAL MOLECULAR WEIGHT (325) AND AT MAXIMUM ANTIMALARIAL ACTIVITY

Series	pE	ΔpE	pE of most active member
n- and i-alkyl (standard)	11.11		10.2
ω-Cyclohexylalkyl	10.55	−0.56	10.4
ω-*trans*-β-Decalylalkyl	9.85	−1.26	10.9
ω-Phenylalkyl	9.24	−1.87	11.3
ω-Thienylalkyl	8.89	−2.22	
ω-p-Chlorophenylalkyl	8.51	−2.60	11.8
Diarylalkyl, —$(CH_2)_3C_6H_4CH_2C_6H_5$	7.98	−3.13	
Diaryloxyalkyl, —$(CH_2)_3C_6H_4OC_6H_5$	7.54	−3.57	9.8
Cyclo-alkyl: cyclohexyl	10.83	−0.28	
Cyclo-alkyl: *trans*-4′-cyclohexylcyclohexyl	10.38	− .73	10.9
Alkenyl	10.71	− .40	
Hydroxyalkyl: primary	7.47	−3.64	
Hydroxyalkyl: secondary	7.54	−3.57	
Hydroxyalkyl: tertiary	7.11	−4.00	11.0
Dihydroxyalkyl (*vic*-diol)	5.38	−5.37	
Carbalkoxyalkyl	7.66	−3.45	

(6) Since the quinones must be substantially identical in ionization constant (Ka), the linear relation of pE = log(K/Ka) to molecular weight shows that a linear relation of equal slope (about 0.04) exists between log K and molecular weight. It is noteworthy that a graph of the logarithm of the distribution constants of the lower fatty acids between ether and water against molecular weight is likewise a straight line [Archibald, THIS JOURNAL, **54**, 3178 (1932); Smith and Norton, *ibid.*, **54**, 3811 (1932)] of slope close to 0.04. The coincidence is the more striking because of the disparity of the two ranges of K (between 10^{-1} and 10^{2} for the carboxylic acids; between 10^{2} and 10^{9} for the quinones). The method used in the present investigation is similar to that suggested by Smith and Norton as feasible for higher fatty acids.

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-