Potential Naphthoquinone Antimalarials. 2-Acylhydrazino-1,4-naphthoquinones and Related Compounds[1]

KENNETH H. DUDLEY,[2-5] H. WAYNE MILLER,[5] PETER W. SCHNEIDER,[4] AND ROBERT L. MCKEE[4]

Venable Chemical Laboratory, University of North Carolina, Chapel Hill, North Carolina 27514, and Chemistry and Life Sciences Laboratory, Research Triangle Institute, Durham, North Carolina 27709

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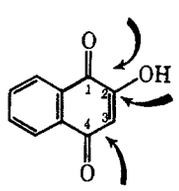
The course of condensation of a carboxylic acid hydrazide with 2-hydroxy-1,4-naphthoquinone (1) is dependent upon the solvent employed. In 80% acetic acid, condensation occurred at C_2 of the naphthoquinone nucleus; in weakly alkaline solution, condensation occurred at C_1. These reactions are discussed in context with earlier work regarding the condensations of phenylhydrazine and hydroxylamine with 1 in neutral and alkaline solution. Reaction of hydrazide with ammonium 1,2-naphthoquinone-4-sulfonate constituted a method for obtaining a condensation product at C_4 of the 2-hydroxy-1,4-naphthoquinone (1) nucleus. Ir, nmr, uv, and visible spectra favored assignment of naphthoquinone structures to the three classes of condensation products, rather than an assignment of potential tautomeric naphthalene structures.

This Article describes reactions of a carboxylic acid hydrazide with some naphthoquinones, reactions which permit preparation of the three possible, positional condensation products of the 2-hydroxy-1,4-naphthoquinone system (*e.g.*, as denoted by arrows in 1). Attention was directed especially to an understanding of the physical properties of the 2-acylhydrazino-1,4-naphthoquinone system (2, R = alkyl), for this system showed an unusual acidic character and was amenable to distribution (between aqueous buffers and ether). A knowledge of certain physical parameters of 2 was of particular interest because of bearing to the *critical extraction value* (p*E*), a measurement which had been correlated with, and related to, the optimal antirespiratory (antimalarial) activities of certain 2-hydroxy-3-alkyl-1,4-naphthoquinones,[6] and which, thus could be determined as a guideline in the synthesis of analogs of 2 for biological evaluation (antimalarial activity). A more extensive list of 2-acylhydrazino-1,4-naphthoquinone (2, R = alkyl or aryl), their p*E* data, and their biological data, will be submitted elsewhere.



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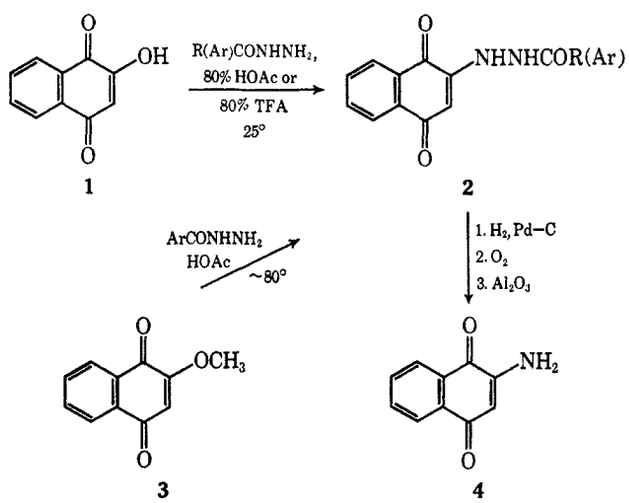
2-Acylhydrazino-1,4-naphthoquinones.—2-Acylhydrazino-1,4-naphthoquinones (2) were prepared by reaction of a carboxylic acid hydrazide with 1 in 80% acetic acid, this type of reaction having precedent in the classical preparation of 2-anilino-1,4-naphthoquinone.[7] A simple, unambiguous structure proof of 2 lay in the reaction of hydrazide with 2-methoxy-1,4-naphthoquinone (3) in glacial acetic acid. In addition, the point of condensation in 2 was identified by catalytic hydrogenolysis of the –NHNH– bond, which, when followed by aerobic oxidation, led to isolation of 2-amino-1,4-naphthoquinone (4).



Infrared (ir) and nuclear magnetic resonance (nmr) spectra of the condensation products (*e.g.*, 2) were consistent with an assignment of a naphthoquinone structure. For example, the ir spectrum of 2-acetylhydrazino-1,4-naphthoquinone (5) contained two bands at 3330 and 3230 cm^{-1}, assignable to –NH– stretching frequencies of the acylhydrazino group (–CONHNH–), and two bands at 1675 and 1640 cm^{-1} (overlapping hydrazide carbonyl at 1675 cm^{-1}), typical of 1,4-naphthoquinone bands. The nmr spectrum of 5 (in dimethyl-d_6 sulfoxide, DMSO-d_6) contained a sharp one-proton singlet at δ 5.80, the positioning of which correlated with the assignment of the quinone-ring proton resonance (δ 5.96, DMSO-d_6) in the spectrum of 4.

Ultraviolet and visible absorption spectra of the 2-acylhydrazino-1,4-naphthoquinones (2, R = alkyl, undissociated species) were similar to curves measured of 2-amino-1,4-naphthoquinone (4) (note Figure 1). However, a 2-acylhydrazino-1,4-naphthoquinone (2) proved uniquely different from 4,[8] in that the acylhy-

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(2) Union Carbide Research Fellow, University of North Carolina, 1961–1963.

(3) To whom correspondence should be addressed at the Center for Research in Pharmacology and Toxicology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.

(4) University of North Carolina.

(5) Research Triangle Institute.

(6) (a) L. F. Fieser, "The Scientific Method," Reinhold Publishing Corp., New York, N. Y., 1964, pp 163–191; (b) L. F. Fieser, M. T. Leffler, and coworkers, *J. Amer. Chem. Soc.*, **70**, 3151 (1948); (c) L. F. Fieser, S. Archer, and coworkers, *J. Med. Chem.*, **10**, 513 (1967), and references therein; (d) L. F. Fieser, M. G. Ettlinger, and G. Fawaz, *J. Amer. Chem. Soc.*, **70**, 3228 (1948). See also C. M. Moser and M. Paulshock, *ibid.*, **72**, 5419 (1950).

(7) (a) C. Liebermann, *Ber.*, **14**, 1664 (1881); (b) C. Liebermann and P. Jacobson, *Ann.*, **211**, 82 (1881).

(8) L. F. and M. Fieser, *J. Amer. Chem. Soc.*, **56**, 1565 (1934).

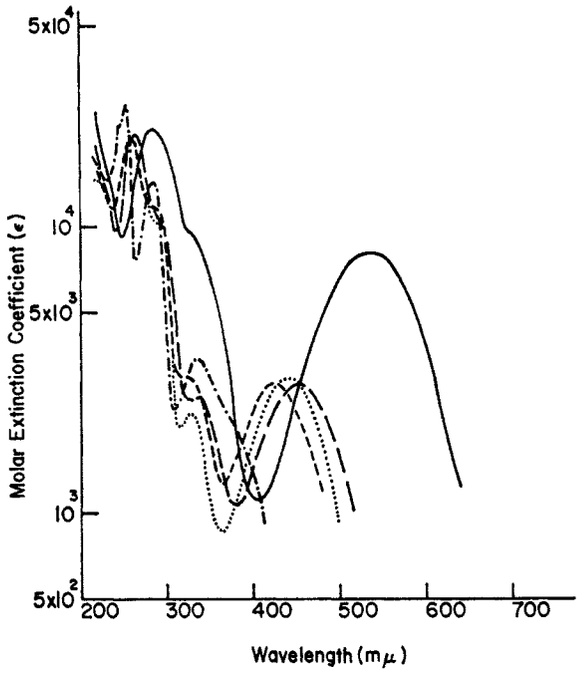
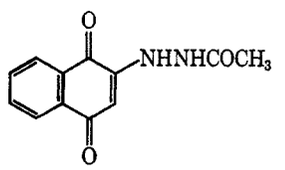


Figure 1.—The ultraviolet and visible light absorption curves of 2-acetylhydrazino-1,4-napthoquinone in methanol (-------) and alkaline (———) solutions; 2-acetamido-1,4-naphthoquinone in methanol (·—·—·) and alkaline (— —) solutions; and 2-amino-1,4-naphthoquinone in methanol (·····) solution.

drazino moiety (-CONHNH-) exhibited an acidity comparable with a phenol. Proton release in **2** was marked by a striking, reversible color change. Acid solutions (pH 1–6) were bright yellow, while alkaline solutions (pH 8–13) were royal purple (alkyl series, *i.e.*, **2**, R = alkyl) or deep blue (aromatic series, *i.e.*, **2**, R = *para*-substituted C_6H_5). Such properties, say of **5**, were contrasted furthermore by the character of 2-acetamido-1,4-naphthoquinone (**6**). The acetamido derivative **6** may be regarded as a vinylog of an imide and, in analogy,[8] ought to be "imidelike". It was therefore surprising that **6** was cleaved readily by alkali to 2-amino-1,4-naphthoquinone (**4**) during spectral measurement (note Figure 1) and potentiometric titration (note Experimental Section).



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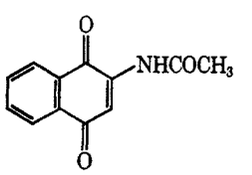

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Potentiometric titration of the 2-acylhydrazino-1,4-naphthoquinones (**2**, R = alkyl) showed curves typical of monobasic acids; the ionization exponents (pK_a') lay in the range 8–9 (note Table I).[9] The reversible yellow-to-purple color change exhibited by the 2-acylhydrazino-1,4-naphthoquinones (**2**, alkyl series) was shown by potentiometry and spectrophotometry to be associated with the one-proton release commensurate with the acidic and monobasic forms. The variation of the molar extinction coefficients (ϵ) of various light absorption with $-\log [H^+]$ is shown in Figure 2. The in-

(9) These exponents are 1.5–2.0 pK_a units above values reported for 2-hydroxy-3-alkyl-1,4-naphthoquinones (pK_a' 6.3–6.7) representative of the antimalarial drugs, *cf. ref 6b*.

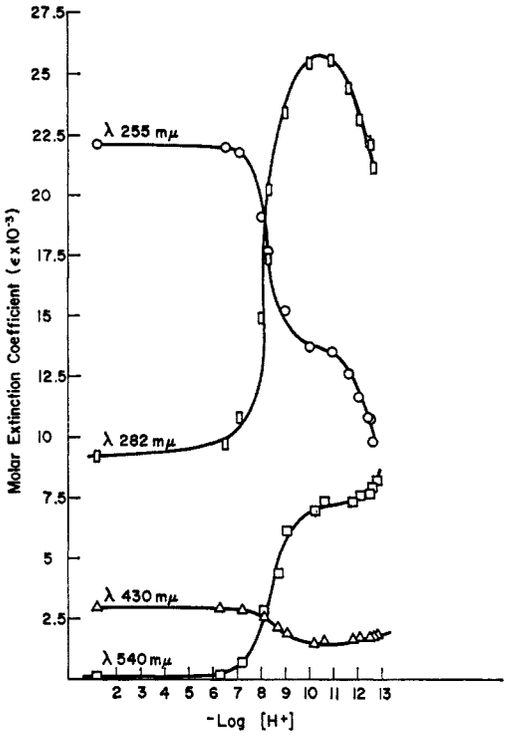


Figure 2.—The variation of the molar extinction coefficient of 2-hexanoylhydrazino-1,4-naphthoquinone [**2**, R = $-(CH_2)_4CH_3$] for various light absorption bands with $-\log [H^+]$. At pH $\lesssim$9, isosbestic points were indicated at 265 mμ (log ϵ 4.25) and at 447 (3.45). At pH $\gtrsim$12, isobestic points were indicated at 290 mμ (log ϵ 4.33) and 620 (3.49).

TABLE I

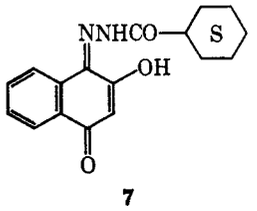
SOME IONIZATION EXPONENTS (pK_a') OF 2-ACYLHYDRAZINO-1,4-NAPHTHOQUINONES DETERMINED BY POTENTIOMETRIC TITRATION

Side chain	50% acetone	50% ethanol
$-CH_3$	8.23	8.16
$-CH_2CH_3$	8.42	
$-(CH_2)_4CH_3$	8.41	
$-CH_2$-*c*-C_5H_9	8.50	
-Cyclohexyl	8.83[a]	8.90[a]

[a] The cyclohexyl compound was titrated as a slight suspension.

flections, particularly those of the 430- and 540-mμ bands in the range pH 6–10, agreed with data obtained potentiometrically. In addition, there was evidence for a second ionization occurring at higher alkalinity (pH 10–13).

2-Hydroxy-1,4-naphthoquinone-1-acylhydrazones.[10] —In weakly alkaline solution, reaction of cyclohexane carboxylic acid hydrazide with **1** resulted in formation of the 2-hydroxy-1,4-naphthoquinone-1-acylhydrazone **7**. The yellow compound titrated as a monobasic acid, $pK_a' = 4.3$; its ir spectrum (KBr) had two bands in the carbonyl region at 1660 and 1618 cm^{-1}; and its nmr



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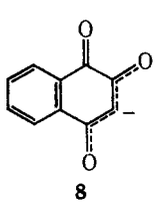

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(10) Observations regarding a similar variance observed in the condensation reactions of phenylhydrazine with **1** in 80% acetic acid and aquous ethanolic solutions are noted in the Experimental Section.

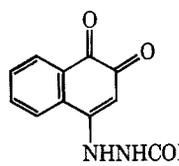
spectrum (DMSO-d_6) showed a one-proton singlet at δ 5.95 (quinone-ring hydrogen).

It would seem that the variance observed in the course of condensation of hydrazide with **1** upon changing from 80% acetic acid to alkaline solution would be related to the concentrations of the conjugate forms of **1** present under the specific conditions. 2-Hydroxy-1,4-naphthoquinone (**1**, pK_a[8] = 3.98) in weakly alkaline solution would be present largely as its conjugate base **8**, and the fact that condensation occurred largely at position C_1 would seem consistent with the electronic disposition of **8**. No significant condensation at position C_1 occurred in 80% acetic acid solution, and no significant condensation at position C_2 occurred in alkaline solution (as evidenced by tlc). It would thus seem that the formation of **2** in 80% acetic acid involved the acid form of **1**; the acid form (*i.e.*, **1**) would be required to exist only to the measure at which hydrazide "condensation" at position C_2 would be exclusively rate favored. Plausible mechanisms would include[11] (1) addition of hydrazide at position C_2 of **1** followed by loss of water, and/or (2) addition of hydrazide at position C_3 of **1** followed by loss of water.

2-Hydroxy-1,4-naphthoquinone-4-acylhydrazones.—The reaction of aniline with ammonium 1,2-naphthoquinone-4-sulfonate (**9**) is well established,[12] and an analogous displacement of bisulfite by hydrazide has been reported.[13] Reaction of either cyclohexanecarboxylic acid hydrazide or isobutyric acid hydrazide with **9** provided products which, after inspection of their spectral data, were assigned structures as 2-hydroxy-1,4-naphthoquinone-4-acylhydrazones (*e.g.*, **10**). This

9 **10**

structural assignment was based principally upon correlations of ultraviolet and visible light absorption data. That is, as spectra of **2** (R = alkyl, undissociated form) and **4** were nearly identical with respect to band positions and molar absorptivities (note Figure 1), it would thus seem that the displacement products obtained here, if actually possessing basic structures as 4-acylhydrazino-1,2-naphthoquinones (*e.g.*, **11**), ought to correlate spectrally with 4-amino-1,2-naphthoquinone (**12**). However, the displacement products (*i.e.*, **10**), like the 2-hydroxy-1,4-naphthoquinone-1-acylhydrazone **7**, were bright yellow in the crystalline state and bore no visual resemblance to the deeply colored, red-brown **12**. Spectrographically, the longer wavelength absorption maximum of **10** (R = isopropyl) was positioned at $\lambda_{max}^{pH\ 1.3}$ 394 mμ (log ϵ 4.22), which proved different in position and molar absorptivity from the principal visible band of **12**, $\lambda_{max}^{pH\ 2}$ 462 mμ (log ϵ 3.47).



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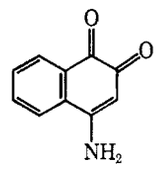

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Infrared and nmr data were consistent with the naphthoquinone structure **10**. Infrared spectra of **10** contained a broad absorption in the 3600–2900-cm^{-1} region (ν_{max} 3260 cm^{-1}), typical of the hydroxyl stretching frequency shown by **1** itself; in the carbonyl region, maxima of equal intensity were positioned at 1670 and 1640 cm^{-1}. In the nmr spectra (DMSO-d_6), a one-proton singlet was observed at δ 7.50 for each of the two examples (*i.e.*, **10**, R = cyclohexyl or isopropyl) and was attributed to the quinone-ring hydrogen in **10**. Although the chemical shift of the quinone-ring hydrogen in **10** was displaced from the region (δ 5.5–6.5) where such resonances commonly occur, this chemical shift was paralleled in the spectrum (DMSO-d_6) of 2-acetamido-1,4-naphthoquinone (**6**) by the positioning of the quinone-ring hydrogen resonance at δ 7.70.

Experimental Section[14]

Carboxylic Acid Hydrazides.—Except for one compound employed here, the hydrazides are known compounds.

Cyclopentylacetic acid hydrazide was prepared as follows. Cyclopentylacetic acid (7.0 g)[15] was treated with an excess of etheral diazomethane (0–5°, 15–20 min), the excess diazomethane was destroyed by dropwise addition of glacial acetic acid, and the ethereal solution was washed with two 100-ml portions of saturated

(11) Either of these mechanisms could be applied to rationalize the formation of **2** from the reaction of hydrazide with **3** in glacial acetic acid. It is interesting to note that neither 2-hydroxy-3-methyl-1,4-naphthoquinone (phthicol) nor 2-hydroxy-3-bromo-1,4-naphthoquinone would react with a carboxylic acid in 80% acetic acid (25°), or even under more forcing conditions (refluxing glacial acetic acid). If these reactions were granted to be not wholly retarded by steric or electronic effects of the 3-methyl and 3-bromo groups, it would appear that the mechanism would actually involve an addition of hydrazide at position C_3 followed by loss of the hydroxyl group from position C_2. The "normal" (C_2 addition) and "abnormal" (C_3 addition) displacement mechanisms have been considered previously in connection with the nucleophilic displacement of halogen by thiol in 2-halogeno-1,4-naphthoquinones: F. G. Rothman, *J. Org. Chem.*, **23**, 1049 (1958), and J. W. McLeod and R. H. Thomson, *ibid.*, **25**, 36 (1960).

(12) L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1961, p 859.

(13) W. L. Mosby and M. L. Silva, *J. Chem. Soc.*, 3990 (1964).

(14) Thin layer plates were prepared by coating microscope slides with silica gel H. The following abbreviations represent the solvent systems employed for elution of chromatograms: (A) the organic phase of 1-butanol–pyridine–saturated sodium chloride (1:1:2); (B) the organic phase of benzene–acetic acid–water (2:2:1); (C) the organic phase of acetic acid–1-butanol–water (2:2:1); (D) the organic phase of 1-butanol–pyridine–water (3:2:1); (E) benzene–chloroform (2:1); (F) benzene–ethyl acetate–acetic acid (90:10:1); (G) benzene–ethyl acetate–acetic acid (9:1:1). Potentiometric titrations were carried out by dissolving an accurately weighed sample (0.800–0.850 mmol) in 50 ml of acetone, diluting a 20-ml aliquot with an equal volume of water, and titrating with 0.100 *N* sodium hydroxide. Values reported in Table I are averaged from at least two determinations (±0.06 accuracy). The set of curves used for construction of Figure 2 was measured from buffered solutions (pH 2.0–11.0) prepared with tablets of Coleman Instruments, Inc. (Maywood, Ill.), and sodium hydroxide solutions (pH 11.0–12.7) of known concentration. The pH's of final solutions were measured. To test a possible salt effect on the negative variation of absorbance (*A*) at 255 and 283 mμ, spectra were measured of **2** in 0.0125 *N* sodium hydroxide with added salt (so that final solutions were 0.00–0.10 *N* in sodium chloride). The 282-mμ band showed a positive 0.02 *A* unit change at NaCl = 0.10 *N*, and the 255-mμ band showed a positive 0.045 *A* unit change at NaCl 0.10 *N*. The stability of **2** in alkaline solution was indicated by the following spectral changes: the visible curves of **2** [R = $-(CH_2)_4CH_3$] at $\lambda^{0.1\ N\ HCl}$ 430 mμ and $\lambda^{0.1\ N\ NaOH}$ 535 mμ were measured at identical concentration; when the alkaline solution was acidified with 1 small drop of concentrated sulfuric acid, the resultant curve was identical with the standard curve and varied by 0.005 *A* unit. Melting points were taken in capillaries (copper block) and are uncorrected. Infrared spectra were measured on Perkin-Elmer Models 421 and 237 spectrophotometers; samples were prepared in the form of pressed KBr disks. Uv and visible spectra were measured on Cary Models 14 and 15 spectrophotometers. Nmr spectra were measured on a Varian A-60 instrument using dimethyl sulfoxide-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. Microanalyses were carried out by one of us (K. H. D), Mr. A. Bernhardt (Mülheim, Germany), and Micro-Tech Laboratories (Skokie, Ill.).

(15) Aldrich Chemical Co., Inc.

bicarbonate solution and dried (anhydrous Na_2SO_4). The crude methyl ester, obtained by removal of the ether, was treated with 95%+ anhydrous hydrazine (3.0 g), the mixture was heated (steam bath) for 16 hr, and volatiles were removed at reduced pressure (aspirator, 50°). The solid residue was recrystallized from benzene (15 ml) to give the product as a white cake; this material was suitable for preparation of **2** (R = $-CH_2$-*c*-C_5H_9) but was not pure. A small sample would not dissolve completely in water, even upon heating; the slight suspension of insoluble product was presumably a diacylhydrazine as judged by its insolubility in dilute acid.

For purification the sample (6.5 g) was added to water (100 ml) and the solution was filtered free of the slight suspension. The hydrazide solution was diluted with 50 ml of saturated sodium chloride solution and was extracted with six 50-ml portions of ether. Another portion (50 ml) of saturated salt solution was added and an ether extraction (three 100-ml portions) was repeated. The ether solution was dried (anhydrous Na_2SO_4) and stripped, and the residue was redissolved in benzene and further dried (anhydrous Na_2SO_4). Evaporation of the solvent provided a white crystalline residue which was recrystallized from *dry* ether to give **cyclopentylacetic acid hydrazide** (3.4 g), mp 89–91° (sharp), and characterized by formation of a sublimate (slender needles) at ~70°. Sublimation, 60–65° (0.05–0.10 mm), raised the melting point to 94–95°.

Anal. Calcd for $C_7H_{14}N_2O$ (142.2): C, 59.12; H, 9.92; N, 19.70. Found: C, 58.98; H, 10.08; N, 20.00.

Condensations of Hydrazide with 1 in 80% Acetic Acid. A. 2-Benzoylhydrazino-1,4-naphthoquinone (2, R = C_6H_5) and Related 2-Aroylhydrazino-1,4-naphthoquinones. 1—A suspension of equimolar quantities (0.056 mol) of finely pulverized **1**[16] and benzhydrazide in 80% acetic acid (200 ml) was stirred at 25° for 24 hr, whereafter the original suspension of quinone had given place to a thick mass of crystalline product (suspension checked by microscopic examination prior to work-up). The product was filtered off and was washed successively with 80% acetic acid, ethanol, and ether. Recrystallization of the product from absolute ethanol gave **2** (R = C_6H_5) as a matt of long silky, orange threads (37%): mp 210–212° dec, gas; ir 3340, 3300, 1685, 1635, 1614, and 1578 cm^{-1}; visible spectrum $\lambda_{max}^{0.1\ N\ HCl}$ 435 mμ (ε 3030); $\lambda_{max}^{0.1\ N\ NaOH}$ 555 mμ (11,500).

Anal. Calcd for $C_{17}H_{12}N_2O_3$ (292.3): N, 9.59. Found: N, 9.73.

The above procedure was adequate for the preparation of six other *para*-substituted benzoylhydrazino-1,4-naphthoquinones, using a *para*-substituted benzhydrazide and **1**; yields of recrystallized product ranged from 40 to 55%.

2.—A solution of 544 mg (2.9 mmol) of 2-methoxy-1,4-naphthoquinone (**3**)[16] and 390 mg (2.9 mmol) of benzhydrazide in glacial acetic acid (15 ml) was heated on the steam bath for 1 hr, allowed to stand at 25° for 30 hr, and the clear, deep red solution was seeded with a crystal of 2-benzoylhydrazino-1,4-naphthoquinone. After 20 hr, the product was filtered off and was recrystallized (absolute ethanol) to give **2** (R = C_6H_5) (100 mg, 12%), mp 210–212°.

Method 2 was applied to the preparation of three of the compounds prepared under method 1; the yields in all examples of **2** ranged from 10 to 15%.

B. 2-Acylhydrazino-1,4-naphthoquinones (2, R = Alkyl or Cycloalkyl).—Representatives of **2** (R = alkyl or cycloalkyl) are included in Table II. A suspension of 0.029 mol of **1**[16] and 0.056 mol of hydrazide in 200 ml of 80% acetic acid was stirred at 25° for 12–24 hr. If product precipitated, the reaction period was extended at least 2 hr after microscopic examination indicated complete solution of **1**, whereafter the product was filtered off and was recrystallized. In cases where the product was soluble, the reaction was permitted to run for 24 hr, and the solution was freed by filtration from any small amount of insoluble material. The solvent was stripped *in vacuo* (temperature of bath did not exceed 50°), first at the aspirator and then at the vacuum pump. The orange-red residue was washed with an appropriate solvent to remove unreacted hydrazide, and the residual solid afforded a chromatographically pure (or nearly so) product upon one recrystallization.

Catalytic Hydrogenolysis of 2-Benzoylhydrazino-1,4-naphthoquinone.—A suspension of 630 mg of naphthoquinone and 100 mg of 10% palladium on charcoal in 100 ml of absolute ethanol was shaken for 24 hr in a Paar apparatus under hydrogen (initial pressure: 60 psi). The catalyst was filtered off, and a stream of air was drawn through the solution for several hours, after which the solvent was stripped (orange crystalline residue contained starting material, as evidenced by tlc and color imparted to an alkaline solution). The residue was extracted with 100 ml of 2% ethanol–chloroform, this extract then being filtered through a column of 25 g of Merck alumina (basic) and worked up to give 130 mg of crude **4**. The sample was chromatographed through 50 g of Merck alumina (basic) to give **4** (100 mg), mp 206–208°, identified by mixture melting point and comparison of ir spectra.

TABLE II

Side chain[a,b]	Mp,[c] °C	Calcd,[d] % C	H	N
$-CH_3$	225–227			12.35[e]
$-C_2H_5$	201–205	63.82	5.09	11.49
$-(CH_2)_4CH_3$	157–159.5	66.90	6.13	9.92
$-CH_2$-*c*-C_5H_9	187–190	68.69	6.18	9.68
-Cyclohexyl	202–205	68.41	6.11	9.49

[a] Compounds in Table II were recrystallized from acetone for purification and analysis. [b] Yields of recrystallized products ranged from 30 to 65%. [c] Fusion was accompanied by decomposition and gas evolution. [d] Elemental analyses (C, H, N) agreed with theoretical values within ±0.3%. [e] Analysis performed by K. H. D.

2-Acetamido-1,4-naphthoquinone (6).—A solution of 2.0 g of **4** in a mixture of 25 ml of acetic anhydride and 2 ml of pyridine was heated under reflux for 1.5 hr. The crude solid (1.95 g), obtained by hydrolysis of excess acetic anhydride, was washed with small portions of cold acetone, thus giving 1.35 g of deep yellow material. Chromatography of this product through alumina,[17] followed by recrystallization of the main band from acetone, gave **6** (0.86 g), mp 205–207°, as bright yellow plates (lit.[17] mp 206°).

During potentiometric titration (50% acetone) of **6**, in which a total of 1.67 molar equiv of base was added, the solution of **6** developed a red-orange color which quickly passed to olive green. The olive color was also unstable, passing after 30 min to a murky brown. The similarity of the uv and visible spectra measured from alkaline solutions of **6** to those of **4** suggested deacetylation of **6**. In a separate experiment, 1 mmol of **6** in 10 ml of acetone was diluted with 5 ml of 1% sodium hydroxide and the resultant solution was kept at 25° until a final decomposition color was obtained (~1 hr). Dilution of an aliquot with water and tlc (*e.g.*, solvent system G) of an ether extract confirmed the presence of **6** and **4**.

2-Hydroxy-1,4-naphthoquinone-1-cyclohexanecarbonylhydrazone (7).—A suspension of 4.0 g (23.0 mmol) of **1**[16] in 320 ml of 50% aqueous ethanol containing 12.0 ml of freshly prepared 1% sodium hydroxide solution was warmed to 65° to effect solution and a very small amount of insoluble material was removed by filtration. Cyclohexane carboxylic acid hydrazide (3.4 g, 23.6 mmol) was added in one portion, and the resulting solution was stirred at room temperature for 2.5 days. The thick mass of microcrystalline needles was filtered off, washed with water, and most of the water was removed by pressing during suction filtration. The solid crystallized from absolute methanol (~350 ml) as soft, bright yellow needles (2.88 g), mp 205–208° dec, and was chromatographically (tlc) uniform in solvent system G: ir 1660 (s), 1610 (s), 1595 (s), and 1562 cm^{-1}; uv $\lambda_{max}^{pH\ 1.3}$ 370 mμ (ε 16,400), 304 (19,300), 269 (10,900), 262 (11,100); $\lambda_{max}^{pH\ 12.2}$ 355 mμ (16,400), 311 (16,700), 266 (12,600); nmr δ 5.95, s, one proton (quinone-ring H); pK_a' (50% acetone, potentiometrically) = 4.30.

Anal. Calcd for $C_{17}H_{18}N_2O_3$ (298.3): C, 68.44; H, 6.08; N, 9.39. Found: C, 68.72; H, 6.08; N, 9.52.

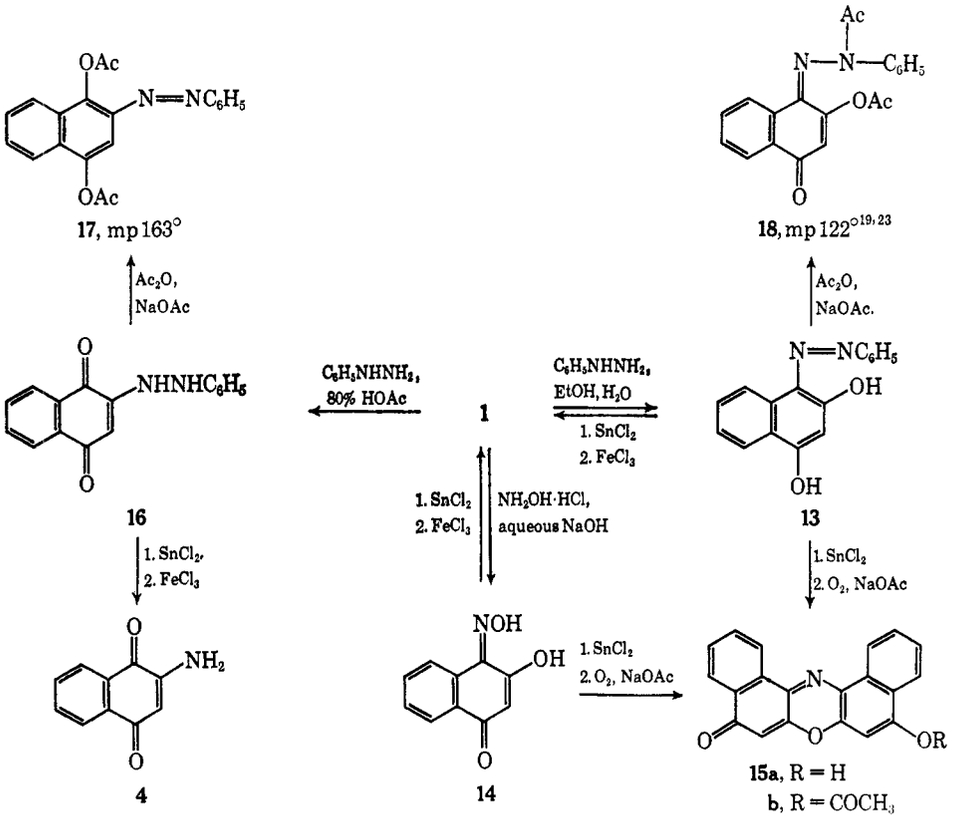
2-Hydroxy-1,4-naphthoquinone-4-acylhydrazone (10).—To a filtered solution of 2.0 g (7.8 mmol) of **9**[16] in 40 ml of 50% aqueous ethanol was added a solution of 7.8 mmol of hydrazide in 30 ml of 50% aqueous ethanol. The yellow precipitate was collected after 24–48 hr and was recrystallized from boiling acetic acid.

2-Hydroxy-1,4-naphthoquinone-4-cyclohexanecarbonylhydrazone (10, R = cyclohexyl) had a 0.70-g yield: mp 272–278° dec; ir 3255, 1675, and 1643 cm^{-1}; uv (50% methanol) $\lambda_{max}^{pH\ 1.5}$

(16) L. F. Fieser and E. L. Martin, "Organic Syntheses," Coll. Vol. III, John Wiley & Sons Inc., New York, N. Y., 1955, p 465.

(17) G. B. Barlin, K. G. Pausacker, and N. V. Riggs, *J. Chem. Soc.*, 3122 (1954).

SCHEME I



395 mμ (19,800); $\lambda_{max}^{pH\ 12.1}$ 450 mμ (12,600); pK_a' (50% MeOH) ~ 6.7; nmr δ 7.50, s, one proton (quinone-ring proton).

Anal. Calcd for $C_{17}H_{18}N_2O_3$ (298.3): C, 68.44; H, 6.08; N, 9.39. Found: C, 68.41; H, 5.99; N, 9.17.

2-Hydroxy-1,4-naphthoquinone-4-isobutyroylhydrazone [**10**, R = $CH(CH_3)_2$] had a 0.57 g yield: mp 279–284° dec; ir 3260, 1670, and 1638 cm^{-1}; uv (30% methanol) $\lambda_{max}^{pH\ 1.2}$ 394 mμ (16,800), 305 (10,800), 257 (7400), 224 (19,800), $\lambda_{max}^{pH\ 12.2}$ 450 mμ (11,000); 294 (9500); pK_a' (30% methanol) ~ 6.5; nmr δ 7.50, s, one proton (quinone ring proton).

Anal. Calcd for $C_{14}H_{14}N_2O_3$ (258.3): C, 65.10; H, 5.46; N, 10.85. Found: C, 65.13; H, 5.53; N, 11.11.

The pK_a' values for **10** (R = cyclohexyl or isopropyl) were determined spectrophotometrically (visible spectra), employing acid (0.1 *N*), base (0.1 *N*), and buffer solutions (pH 4–6). However, these values remain questionable even as a crude approximation, for the ultraviolet curves measured in each case from 0.1 *N* base solution did not pass through apparent isosbestic points seen at 309 and 229 mμ in the acid and buffer solutions spectra. It would thus seem that the values are an over-all pK_a' of two ionizations, the second of which has very little effect, if any, in altering the position of the visible bands; the isosbestic point seen at 415 mμ must then be fortuitous. The extreme insolubility of **10** (R = cyclohexyl and isopropyl) in aqueous ethanol or aqueous acetone prohibited potentiometric titration.

Reactions of Phenylhydrazine with 2-Hydroxy-1,4-naphthoquinone in Neutral and Acidic Media.—In 1884, Zincke and Thelen[18] described a reaction of phenylhydrazine with **1**. This reaction, which was conducted in aqueous ethanol, gave a condensation product regarded as 4-phenylazonaphthalene-1,3-diol (**13**). In 1889, Kostanecki[19] reported a related reaction, the derivation of **1** with hydroxylamine in alkaline solution, to give oxime **14**. The proposed structure of **14**, *i.e.*, the basic structure with respect to the point of condensation,[20] later accorded with conclusions drawn by Hooker,[21] but until Kehrman (1895) demonstrated conversion of **14** into "dinaphthoresorufin" (**15a**), no chemical evidence supported C_1 positioning of the oxime group.[22]

On the other hand, the final evidence[18,19,23] surrounding the phenylhydrazine **13**, though seemingly limiting the point of condensation to C_1 or C_4, was less convincing. The issue of structure **13** was regarded with additional reserve in view of Zincke and Thelen's comment[18] that phenylhydrazine underwent reaction with **1** in aqueous, or in alcoholic, or in acetic acid solution. It was not apparent in the original article,[18] nor was it clarified in the subsequent literature, whether the phenylhydrazine derivative of **1** formed in acetic acid solution was **13** as well or a positional counterpart. It was therefore of interest to relate derivatives **13** and **14**, *i.e.*, those prepared in neutral and weakly alkaline solution, and to clarify the reaction course of phenylhydrazine with **1** in acetic acid medium.

The basic structure of the phenylhydrazine derivative **13** was more firmly established by its conversion to Kehrman's dinaphthoresorufin (**15a**), a compound which was best converted into acetate **15b** for characterization purposes (Scheme I).[24] Dinaphthoresorufin (**15a**) was the principal product when the tin(II) reduction product[25] of either **13** or **14** was subjected to aerobic oxidation in acetate buffer. It is noteworthy here that iron(III) oxidation of this reduction product[25] led to **1** (note **13** → **1**, and **14** → **1**), for this set of reactions [*i.e.*, tin(II) reduction followed by iron(III) oxidation] was useful for characterizing the condensation product, presumably 2-phenylhydrazino-1,4-naphthoquinone (**16**, or a tautomer thereof), which we found to result from reaction of phenylhydrazine with **1** in 80% acetic acid. Unlike its counterpart **13**, compound **16** underwent extensive decomposition during attempted purification through its sodium salt and remained nonhomogeneous (contained a trace impurity) after several trials at purification. However, tin(II) reduction of **16** followed by iron(III) oxidation led to production of 2-amino-1,4-

(18) Th. Zincke and H. Thelen, *Ber.*, **17**, 1809 (1884).

(19) St. von Kostanecki, *ibid.*, **22**, 3163 (1889).

(20) Insofar as we are aware, the correct tautomeric representations of **13** and **14** have not been determined.

(21) S. C. Hooker and E. Wilson, *J. Chem. Soc.*, **65**, 717 (1894).

(22) (a) F. Kehrman, *Ber.*, **28**, 353 (1895). (b) In the preceding paper [*ibid.*, **28**, 345 (1895)], F. Kehrman and B. Masconi describe the use of tin(II) and iron(III) for the conversion of **14** into **1**.

(23) Th. Zincke and P. Wiegand, *Ann.*, **286**, 86 (1895).

(24) The reported melting point for **15b** is 200° (*cf.* ref 22a). This value is presumably a typographical error, for it markedly conflicts with our value of 275° for samples of **15b** prepared from either **13** or **14**. Our preparation of **15b** gave satisfactory analytical data, the reported color reactions, and was homogeneous by tlc.

(25) The reduction product, which may be isolated as its hydrochloride, is presumably 1-amino-2,4-dihydroxynaphthalene.

naphthoquinone (4) in 65% yield.[26] Mild acetylation of 16 with acetic anhydride–sodium acetate gave a homogeneous (by tlc) diacetate, assigned structure 17 on the basis of a single, intense 1760-cm^{-1} absorption contained in the carbonyl region of the ir spectrum.

4-Phenylazonaphthalene-1,3-diol (13).—Reaction of 5.0 g of phenylhydrazine and 8.0 g of 1 in aqueous ethanol by the procedure of Zincke and Thelen[18] gave crude 13 which, contrary to the opinion of Kostanecki,[19] required for purification the preparation[18] and recrystallization of the sodium salt. The yields of 13 ranged from 8.6 to 10.0 g, mp 231° dec, gas, but the samples still contained a trace impurity as evidenced by tlc in solvent systems A and B. Recrystallization of 8.6 g from absolute ethanol gave 7.1 g of chromatographically uniform, bright orange-red needles, mp 231° dec, gas.

Conversion of 13 into O-Acetyldinaphthoresorufin (15b).—A suspension of 3.0 g (11.4 mmol) of 13 in a mixture of 50 ml of water, 60 ml of ethanol, and 30 ml of concentrated hydrochloric acid containing 6.75 g (30.0 mmol) of tin(II) chloride 2-hydrate was heated under reflux for 5 hr and then concentrated by boiling to remove most of the alcohol. The hot solution was filtered by gravity and, after adding 30 ml of concentrated hydrochloric acid and cooling, the hydrochloride was isolated and recrystallized from 80 ml of 6 *N* hydrochloric acid (2.3 g of lavender needles). A solution of the hydrochloride (2.3 g) in 50 ml of water was added to a solution of 3.0 g of sodium acetate in 200 ml of water (immediate darkening), and air was drawn through the solution for 7 hr. After collection of the brown solid (1.15 g), trituration and washing with ether, the ether insoluble residue (1.07 g) appeared deep reddish purple in color. The infrared spectrum, color reactions,[22a] and thin layer chromatograms (two zones, solvent systems A, B, and D) were identical in all respects with those of 15a prepared from 14.[22a] O-Acetyldinaphthoresorufin (15b) was prepared by heating under reflux for 40 min a solution of 1.07 g of 15a in 50 ml of acetic anhydride containing 0.5 g of sodium acetate. Recrystallization of the crude acetate (1.10 g) from toluene gave 0.88 g of orange-red needles, mp 274–276° dec (lit.[22a] mp 200°), which proved identical by ir and mixture melting point with a sample (mp 275–276°) prepared from 14.[22a] The compound moved as one zone in solvent systems A, B, C, and D; chromatograms developed in A and D showed the hydrolysis of the orange acetyl derivative to a blue ion, ir 1760 and 1635 cm^{-1}.

Anal. Calcd for $C_{22}H_{13}NO_4$ (355.3): C, 74.37; H, 3.66; N, 3.94. Found: C, 74.33; H, 3.52; N, 4.32.

2-Phenylhydrazino-1,4-naphthoquinone (16).—A suspension of 5.0 g (28.7 mmol) of 1 (finely pulverized) in 80 ml of 80% acetic acid containing 3.4 g (31.5 mmol) of phenylhydrazine was stirred at 25° for 2 days; the deep purple precipitate was filtered and washed successively with two 10-ml portions of 80% acetic acid, two 10-ml portions of absolute ethanol, and ether: yield 4.09 g; mp 170° dec and vigorous gas. A chromatogram eluted in solvent system F contained a brilliant magenta zone (major product) and a bright yellow zone (trace product, retained near the origin). The product failed to crystallize satisfactorily from the common solvents, and attempted purification by preparation of a sodium salt (as described for 13[18]) led to immediate, extensive decomposition. A sample (0.25 g, two zones on tlc), which had been crystallized repeatedly from toluene (with considerable loss and without any apparent improvement) was suspended in chloroform and filtered through a column of silicic acid (15.0 g). Eleven fractions [(1–8 (25 ml), 9 (200 ml), 10 (100 ml), 11 (200 ml)] were collected; all showed two zones on tlc.

Because of its intense color and poorly resolved ir spectrum, the compound was considered a quinhydrone, and purification was attempted by shaking a solution of 1.0 g of 16 in 100 ml of 1:3 ethanol–chloroform with an aqueous solution containing 2.2 g of iron(III) chloride 6-hydrate. The isolated organic layer showed no less than five zones on tlc.

The uv and visible spectrum of 16 was markedly different from that of 4: λ_{max}^{MeOH} 227 mμ (18,000), 298 (13,000), 453 (10,200), and 525 (12,200). A sample was dried at 80° (0.05 mm) for analysis.

Anal. Calcd for $C_{16}H_{12}N_2O_2$ (264.3): C, 72.71; H, 4.58; N, 10.60. Found: C, 72.50; H, 4.57; N, 10.40.

Diacetyl Derivative 17.—The diacetyl derivative was prepared by heating under reflux for 15 min a solution of 1.0 g of 16 (dec pt 171°) and 0.1 g of sodium acetate in 20 ml of acetic anhydride. After hydrolysis of excess acetic anhydride by stirring with water (200 ml, 2 hr), collection, and drying of the crude product through its etheral solution (anhydrous Na_2SO_4), the product was isolated by cautious concentration of its ether–petroleum ether solution as an orange powder (0.8 g), mp 161–163°, to an orange oil. Tlc (solvent systems A, B, C, D, and E) indicated the derivative to be uniform. Chromatography of the above sample (0.8 g) through silicic acid (40 g) and several recrystallizations from petroleum ether (bp 90–100°) raised the melting point to 163–165°: ir a strong symmetrical band was seen at 1763 cm^{-1}; other bands appeared at 1602 (w), 1372 (s), 1205 (s), 1170 (s), 1064 (m), 1010 (m), 918 (w), 770 (m) and 750 cm^{-1} (m); uv λ_{max}^{MeOH} 285 mμ (ε 16,400), 295 (16,900), 331 (20,500); λ_{sh} 365 (9200).

Anal. Calcd for $C_{20}H_{16}N_2O_4$ (348.4): C, 68.94; H, 4.63; N, 8.04. Found: C, 68.87; H, 4.54; N, 8.61.

Conversion of 16 into 2-Amino-1,4-naphthoquinone (4).—A mixture of 3.15 g (12.0 mmol) of 16, 50 ml of water, 60 ml of absolute ethanol, and 30 ml of concentrated hydrochloric acid containing 6.75 g (30.0 mmol) of tin(II) chloride 2-hydrate was heated under reflux and, after 4 hr (solution contained a black suspension), the solution was concentrated by boiling to about 70 ml and was filtered (activated charcoal). The yellow filtrate was diluted with 30 ml of concentrated hydrochloric acid and chilled to 0–5° (failed to yield a precipitate of hydrochloride). To this solution was added all at once a cold solution of 13.5 g (50.0 mmol) of iron(III) chloride 6-hydrate in a mixture of 50 ml of water and 10 ml of concentrated hydrochloric acid. After 5–10 min in the cold, a copious crop of small orange-red needles separated, which was collected and washed with water until a consistent bright orange color was obtained. The vacuum dried product (1.35 g, 65%) was chromatographically uniform (solvent system B) and crystallized from benzene in the form of slender orange needles, mp 204–206°. A mixture melting point with authentic 4,[27] which was further purified by filtering its chloroform solution through Merck basic alumina and then recrystallization from benzene, was 204–206°.

Registry No.—2, R = C_6H_5, 20287-12-1; 2, R = CH_3, 20287-13-2; 2, R = C_2H_5, 20287-14-3; 2, R = $(CH_2)_4CH_3$, 20287-15-4; 2, R = CH_2-cyclopentyl, 20287-16-5; 2, R = cyclohexyl, 20287-17-6; 4, 2348-81-4; 6, 2348-74-5; 7, 20287-20-1; 10, R = cyclohexyl, 20287-21-2; 10, R = $CH(CH_3)_2$, 20287-22-3; 15b, 20287-26-7; 16, 20287-23-4; 17, 20287-24-5; cyclopentylacetic acid hydrazide, 20287-25-6.

(26) The reduction of 16 to 4 is interesting in connection with the report [D. B. Bruce and R. H. Thomson, *J. Chem. Soc.*, 1428 (1954)] that tin(II) reduction of 2-anilino-1,4-naphthoquinone, followed by oxidation, gave 1,4-naphthoquinone.

(27) L. F. Fieser and J. L. Hartwell, *J. Amer. Chem. Soc.*, **57**, 1482 (1935).