under these conditions. Together, these two amino acids constitute almost 30% of casein, and very likely account for the action of hydrolyzed casein. (2) Those listed from methionine to glycine, which appear to effect an incomplete conversion in the same direction under these conditions. In some cases (*e. g.*, tyrosine, arginine, glycine) assay with *S. fecalis* indicates more complete conversion to pyridoxamine than does assay with *L. casei*. Some of these discrepancies can be attributed to variations in the assays, which are somewhat less accurate than most microbiological methods. It cannot be concluded, however, that the reaction in these cases takes place in the same manner demonstrated earlier for glutamic acid, although this seems most likely. (3) With some amino acids (*e. g.*, proline, serine) no detectable reaction occurs. (4) Autoclaving with tryptophan and histidine under these conditions destroys the activity of pyridoxal almost completely for all organisms. Only in these cases (and possibly with serine) is a significant destructive action for yeast evident. With tryptophan, the reaction probably represents the well-known condensation of this amino acid with aldehydes. Indole displays a similar destructive effect on pyridoxal.

Reaction of Pyridoxamine with Other Ketoacids.—This subject has not been sufficiently investigated. Under proper conditions pyruvic acid acts in the same way as α-ketoglutaric acid, but the reaction is not as fast, and cannot be forced to completion so readily.

Summary

Pyridoxal reacts with glutamic acid at elevated temperatures to produce pyridoxamine and α-ketoglutaric acid. The reaction is reversible, and can be driven to completion in either direction if sufficient glutamic acid or α-ketoglutaric acid is employed. The reaction is partially inhibited by strong acids and alkalies. Microbiological assays indicate occurrence of a similar reaction when pyridoxamine is heated with certain other ketoacids, or when pyridoxal is heated with other amino acids. The extent of reaction varies markedly with the amino acid or ketoacid employed.

The reaction represents a new example of the transamination reaction, and its possible significance is briefly discussed.

Autoclaving with tryptophan or histidine destroys the growth-promoting activity of pyridoxal for all organisms tested.

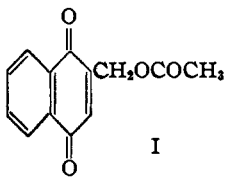
AUSTIN, TEXAS RECEIVED NOVEMBER 6, 1944

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLLEGE OF ARTS AND SCIENCES, AND THE DEPARTMENT OF BIOCHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF ROCHESTER]

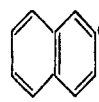
The Synthesis of Some Derivatives of 2-Methyl-1,4-naphthoquinone

BY D. S. TARBELL, DAVID K. FUKUSHIMA AND H. DAM

In connection with some studies on the mechanism of vitamin K activity, it was desirable to study 2-acetoxymethyl-1,4-naphthoquinone (I), which has been reported to have slight activity.[1] If the activity of this substance is due to the substance itself and not to a partial conversion in the organism to 2-methyl-1,4-naphthoquinone (or some other active quinone), the presence of a large amount of I might inhibit the activity of simultaneously ingested 2-methyl-1,4-naphthoquinone, if vitamin K activity of a given substance involves its reversible combination with some substrate.



I

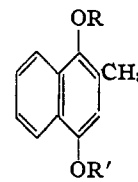


II, R = Cl
III, R = $OCOCH_3$

Experiment showed that this did not occur; a ten-fold excess of I did not inhibit the normal activity of 2-methyl-1,4-naphthoquinone when administered simultaneously.[2] This result agrees with the idea that vitamin K activity depends on conversion of the substance into 2-methyl-1,4-naphthoquinone (or other active substance) in the organism.[3]

2-Acetoxymethyl-1,4-naphthoquinone has been prepared previously, but no details were reported.[1] In the present work, an improved method for the preparation of 2-chloromethylnaphthalene (II) was developed, and compounds I and III were completely characterized.

In order to test the activity of a water-soluble derivative of 2-methyl-1,4-naphthoquinone containing ether linkages, 2-methyl-1,4-naphthoxydiacetic acid IV was prepared by the action of chloroacetic acid on 2-methyl-1,4-naphthohydroquinone diacetate. This ether IV, when administered either as the free acid or the dipotassium salt, showed less than 1% of the vitamin K activity of 2-methyl-1,4-naphthoquinone. It is known that the rate of cleavage of ethers is greatly increased by the presence of a carbon–carbon double bond in the β,γ-position to the oxygen,[4]



IV, R = R′ = $-CH_2COOH$
V, R = R′ = $-CH_2CH{=}CHCOOCH_3$
VI, R′ = $-CH_2CH{=}CHCOOH$, R = H
VII, R = R′ = $-CH_2CH{=}CH_2$

(1) Dam, Glavind and Karrer, *Helv. Chim. Acta*, **23**, 224 (1940).

(2) Assays were carried out by a method previously described (Dam and Glavind, *Biochem. J.*, **32**, 1018 (1938)), except that the procedure was shortened by giving only one dose and determining the effect on the clotting power about twenty hours later.

(3) Evidence in support of this idea has been presented recently by Richert, *J. Biol. Chem.*, **154**, 1 (1944).

(4) *Cf.* Tronow and Ladigina, *Ber.*, **62**, 2844 (1929), for measurements of rates of cleavage of different ethers.

and since the vitamin K activity of compounds such as IV probably depends on formation of the hydroquinone, followed by oxidation to the quinone, the synthesis of V was undertaken. Compound V, because of the unsaturation, should cleave to the hydroquinone much more rapidly than IV.

Treatment of 2-methyl-1,4-naphthohydroquinone with methyl γ-bromocrotonate yielded the corresponding crystalline diester V, in one run. In other runs the monobasic acid VI (or isomer, with the crotonic acid group on the 4-hydroxyl) was obtained; this product was unstable, and showed little vitamin K activity. The dibasic acid corresponding to V could not be obtained from V by hydrolysis, in spite of attempts by a variety of methods; apparently the ether linkage was cleaved under hydrolytic conditions, and complex decomposition ensued. Compounds V and VI showed activity of the same order of magnitude as IV.

The diallyl ether VII of 2-methyl-1,4-naphthohydroquinone was prepared, and found to have about 0.1% of the activity of 2-methyl-1,4-naphthoquinone. It was expected that this ether would have activity comparable to that of the corresponding dibenzyl ether,[5] since it should hydrolyze at a comparable rate.

Experimental[6]

2-Chloromethylnaphthalene (II).—The procedure of Schulze[7] was modified as follows. A mixture of 50 g. of 2-methylnaphthalene and a few grams of phosphorus trichloride was maintained at 230–260° and illuminated with a 200-watt lamp; chlorine gas was passed in until there was an increase in weight of 10 g., corresponding to 80% conversion to the monochloro derivative. After removing the hydrogen chloride by bubbling air through the mixture, fractional distillation yielded 32 g. (53%) of crude 2-chloromethylnaphthalene, b. p. 163–170° (22 mm.), m. p. 46.5–48.5°, which, upon recrystallization from ethanol, gave white crystals, m. p. 48–49° Schulze[7] gives the m. p. as 47°.

2-Acetoxymethylnaphthalene (III).—A solution of 15 g. of crude 2-chloromethylnaphthalene, m. p. 44–46°, and 30 g. of freshly fused potassium acetate in 75 cc. of glacial acetic acid was refluxed for one-half hour. The potassium chloride was filtered off and approximately 40 cc. of acetic acid removed by distillation. The solution was cooled, water was added to dissolve potassium chloride and excess potassium acetate, and the solid which precipitated was recrystallized to give 8.9 g. (52%) of 2-acetoxymethylnaphthalene (II), m. p. 54–56°. Recrystallizations from methanol and petroleum ether (60–70°) gave white crystals, whose m. p. 57.5–58.5° was slightly lower than that (61°) reported by Dam, *et al.*,[1] and higher than that of other workers.[8]

Anal. Calcd. for $C_{13}H_{12}O_2$: C, 77.96; H, 6.04. Found: C, 78.22; H, 6.18.

2-Acetoxymethyl-1,4-naphthoquinone (I).—Using the method of Fieser and co-workers,[9] 4 g. of crude 2-acetoxymethylnaphthalene, m. p. 55–57°, yielded, on oxidation with chromic oxide in glacial acetic acid, 1.6 g. (35%) of glistening yellow platelets of 2-acetoxymethyl-1,4-naphthoquinone, m. p. 106–109°. On crystallization from methanol, pale yellow needles, m. p. 109–110°, were obtained; Dam and co-workers[1] report a m. p. of 110°.

Anal. Calcd. for $C_{13}H_{10}O_4$: C, 67.80; H, 4.38. Found: C, 67.90; H, 4.52.

2-Methyl-1,4-naphthoxydiacetic Acid (IV).—To a refluxing solution of 4 g. of crude 2-methyl-1,4-naphthohydroquinone diacetate[10] and 10 g. of chloroacetic acid in 10 cc. of methanol, was added, in small portions, 70 cc. of 10% methanolic potassium hydroxide solution and 3 g. of solid potassium hydroxide. In order to prevent the oxidation of free 2-methyl-1,4-naphthohydroquinone, the reaction was conducted in a nitrogen atmosphere in the presence of sodium hydrosulfite. The reaction mixture was refluxed for a total period of twelve hours, and was then cooled, acidified and extracted with ether. The aqueous layer was discarded, and the ether solution extracted with sodium bicarbonate solution. Acidification of the bicarbonate extract yielded a reddish semi-crystalline solid, from which 2.8 g. (62%) of crude product, m. p. 213.5–217.5° with decomposition, was obtained on crystallization from acetone–benzene. A second crystallization from acetone–benzene with charcoal yielded white prisms of 2-methyl-1,4-naphthoxydiacetic acid (IV), m. p. 221–221.5°.[11] Numerous variations of this method of synthesis were less successful.

Anal. Calcd. for $C_{15}H_{14}O_6$: C, 62.05; H, 4.86; neut. equiv., 145.05. Found: C, 62.34; H, 5.12; neut. equiv., 144.7.

1-Hydroxy-2-methyl-4-naphthoxy-γ-crotonic Acid (VI or Isomer).—To a refluxing solution of 6 g. of 2-methyl-1,4-naphthohydroquinone and 18 g. of methyl γ-bromocrotonate[12] in 20 cc. of methanol was added 110 cc. of 10% methanolic potassium hydroxide solution over a period of three hours; the reaction was conducted in a nitrogen atmosphere in the presence of a small amount of sodium hydrosulfite. After the addition of alkali was complete, the reaction mixture was refluxed for one hour; longer periods of refluxing yielded only intractable tars. About one-half of the solvent was removed by distillation, the reaction was diluted with water and extracted with ether. The ether solution was washed repeatedly with 10% aqueous sodium hydroxide solution containing hydrosulfite, and the alkaline extracts were saturated with carbon dioxide. Ether extraction yielded 2.5 g. of the original hydroquinone. The sodium bicarbonate solution yielded, on acidification, 5 g. of reddish-brown semi-crystalline solid, from which 2 g. (22%) of pink crystals, m. p. 220–223°, was obtained by recrystallization from ethanol. Further crystallization gave soft, white needles, m. p. 222–223° with dec. (an admixture with 2-methyl-1,4-naphthoxydiacetic acid, m. p. 221–221.5° with dec., depressed the melting point to 197–207°). The product was sensitive to heat and air; attempts to acetylate the free hydroxyl group yielded no pure products.

Anal. Calcd. for $C_{15}H_{14}O_4$: C, 69.74; H, 5.43; neut. equiv., 258.1. Found: C, 69.62; H, 5.58; neut. equiv., 250.8.

From another run, **dimethyl 2-methyl-1,4-naphthoxydi-γ-crotonate** (V), m. p. from ethanol 124–125°, was isolated from the ether extract containing the neutral compounds.

Anal. Calcd. for $C_{21}H_{22}O_6$: C, 68.07; H, 5.99. Found: C, 67.94; H, 6.41.

(5) Fieser, Tishler and Sampson, *J. Biol. Chem.*, **137**, 659 (1941), reported the benzyl compound had appreciable activity.

(6) Melting points corrected; carbon and hydrogen analyses by R. Baumann, C. Claus and W. Gleich.

(7) Schulze, *Ber.*, **17**, 1527 (1884); *cf.* Wahl, Goedkoop and Heberlein, *Bull. soc. chim.*, [5] **6**, 533 (1939).

(8) Szperl, *Rocsniki Chemji*, II, 291 (1923); (*C. A.*, **18**, 1290 (1924)), reported a m. p. of 51–53° for this compound, but no particulars or analysis are available.

(9) Fieser, Campbell, Fry and Gates, THIS JOURNAL, **61**, 3216 (1939).

(10) Anderson and Newman, *J. Biol. Chem.*, **103**, 405 (1933).

(11) This product has been described in a recent patent as melting at 204–210° (Fernholz, U. S. Patent 2,352,189; *C. A.*, **38**, 5507 (1944)).

(12) Ziegler, Späth, Schaaf, Schumann and Winkelmann, *Ann.*, **551**, 80 (1942).

2-Methyl-1,4-naphthohydroquinone Diallyl Ether (VII).—2-Methyl-1,4-naphthohydroquinone (11 g.) was refluxed for six hours with 22.8 g. of allyl bromide, 22 g. of potassium carbonate, and 50 ml. of acetone in a nitrogen atmosphere and in the presence of sodium hydrosulfite. The solvent was evaporated and the oily product extracted with ether. The ether solution was washed repeatedly with 10% sodium hydroxide solution containing sodium hydrosulfite to remove all traces of unreacted 2-methyl-1,4-naphthohydroquinone. The ether solution was dried over magnesium sulfate and the ether evaporated. The residual oil was distilled under reduced pressure and 14 g. (87% yield based on 2-methyl-1,4-naphthohydroquinone) of amber-colored liquid which boiled at 164–166° (5 mm.) was collected. This liquid gave a positive Craven test[13] for quinones. The product was taken up in ether, the solution again washed with 10% sodium hydroxide solution containing sodium hydrosulfite, dried, and the ether evaporated. The oil was then distilled under reduced pressure in a nitrogen atmosphere and in the presence of a small amount of sodium hydrosulfite. 2-Methyl-1,4-naphthohydroquinone diallyl ether boiled at 155–155.5° (3 mm.); $n^{25}D$ 1.5580; negative Craven test.

(13) Craven, *J. Chem. Soc.*, 1605 (1931).

Anal. Calcd. for $C_{17}H_{18}O_2$: C, 80.27; H, 7.12. Found: C, 79.98; H, 7.28.

Summary

1. 2-Acetoxymethyl-1,4-naphthoquinone does not inhibit the vitamin K activity of 2-methyl-1,4-naphthoquinone, when administered simultaneously in ten times the amount of the methylnaphthoquinone. The synthesis of the former compound has been described in detail, and an improved procedure for the preparation of 2-chloromethylnaphthalene is given.

2. 2-Methyl-1,4-naphthoxydiacetic acid, its dipotassium salt, 1-hydroxy-2-methyl-4-naphthoxy-γ-crotonic acid, dimethyl 2-methyl-1,4-naphthoxydi-γ-crotonate and the diallyl ether of 2-methyl-1,4-naphthohydroquinone have been synthesized, and have been found to have less than 1% of the activity of 2-methyl-1,4-naphthoquinone.

ROCHESTER, NEW YORK RECEIVED NOVEMBER 3, 1944

[CONTRIBUTION FROM NICHOLS LABORATORY, NEW YORK UNIVERSITY]

Synthesis and Properties of 1-Cyanoethylisatin

BY FREDERICK J. DI CARLO AND H. G. LINDWALL

The reaction of acrylonitrile with isatin in the presence of trimethylbenzylammonium hydroxide as a catalyst yielded 1-cyanoethylisatin (I). Ethyl alcohol was found to be a more satisfactory solvent for this reaction than either dioxane or tertiary butyl alcohol. Upon saponification, the cyanoethylation product was converted to isatin-1-propionic acid. Catalytic hydrogenation of the β-oxime of this acid in a solution of alcoholic hydrogen chloride gave ethyl 3-amino-1-oxindolepropionate hydrochloride, which is isomeric with the ethyl ester hydrochloride of oxytryptophan. Under similar conditions, isatin-β-oxime was hydrogenated to β-amino oxindole hydrochloride which Baeyer and Knop[1] synthesized by the reduction of isatin-β-oxime with tin and hydrochloric acid. A Schiff base was prepared from the hydrochloride and *p*-dimethylaminobenzaldehyde in the presence of sodium acetate.

Cyanoethylisatin was condensed with acetone and with acetophenone under Knoevenagel conditions. Perkin conditions were employed to effect condensations with hydantoin and with rhodanine. The interaction of I and malonic acid produced normal ring expansion to yield 1-cyanoethyl-2-quinolone-4-carboxylic acid.

In the presence of iodine, cyanoethylisatin formed an anil with *p*-anisidine and 6-cyanoethylindophenazine with *o*-phenylenediamine.

Oxidations of N-methylisatin and of isatin-1-propionic acid with 3% hydrogen peroxide proceeded rapidly in alkaline solution to yield N-methylanthranilic acid and N-(2-carboxyphenyl)-β-alanine, respectively. Isatides were readily prepared by the hydrogenations of N-methylisatin and isatin-1-propionic acid in the presence of Adams catalyst. 1,1'-Dimethyl isatide was previously prepared by the condensation of equivalent amounts of 1-methylisatin and 1-methyl dioxindole in the presence of either piperidine or alcoholic hydrogen chloride.[2]

(1) Baeyer and Knop, *Ann.*, **140**, 37 (1866).

Experimental

1-Cyanoethylisatin (I).—Acrylonitrile (40 cc.) was added dropwise to a solution containing 49.0 g. (0.33 mole) of isatin and 7 cc. of "Triton B" in 2.5 liters of alcohol, with stirring. The stirring was continued for a few hours and the solution was then allowed to stand (uncovered) at room temperature for several days. Cyanoethylisatin separated as either large red prisms or large rosets of fine orange needles. After filtration and washing with methyl alcohol, the yield was 33.2 g. (50%); m. p. 130–131°. Recrystallized from acetone, the product melted at 133°.

Concentration of the mother liquor yielded a mixture of cyanoethylisatin and isatin which was difficult to separate but which could be dissolved in alcohol and treated again with acrylonitrile.

Anal. Calcd. for $C_{11}H_8O_2N_2$: N, 14.00. Found: N, 14.20.

Phenylhydrazone.—Prepared in aqueous solution, it was recrystallized from alcohol as yellow needles; m. p. 177°.

Anal. Calcd. for $C_{17}H_{14}ON_4$: N, 19.31. Found: N, 19.54.

Isatin-1-propionic Acid.—10.0 g. (0.05 mole) of cyanoethylisatin was refluxed with 120 cc. of 10% sodium hydroxide for one-half hour. The solution was acidified with concentrated hydrochloric acid. By allowing the hot solution to cool slowly and then to stand in the refrigerator for a day, 9.8 g. (90%) of long orange needles separated which

(2) Stollé and Merkle, *J. prakt. Chem.*, **139**, 334 (1934).