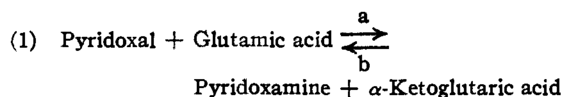
[CONTRIBUTION FROM THE UNIVERSITY OF TEXAS, BIOCHEMICAL INSTITUTE, AND THE CLAYTON FOUNDATION FOR RESEARCH]

The Vitamin B_6 Group. V. The Reversible Interconversion of Pyridoxal and Pyridoxamine by Transamination Reactions

BY ESMOND E. SNELL

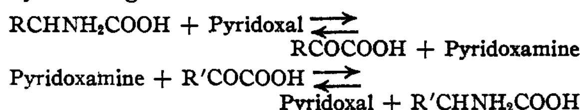
In a previous paper[1] it was suggested that pyridoxal and pyridoxamine might be interconvertible by transamination reactions. This suggestion was based upon the observation that heating pyridoxal with a casein hydrolyzate greatly decreased its growth-promoting activity for *Lactobacillus casei*, while its activity for *Streptococcus fecalis* was increased and that for *Saccharomyces carlsbergensis* remained unchanged.[2] Each of these effects on physiological activity would be brought about by the conversion of pyridoxal to pyridoxamine which might result from this treatment. Somewhat later, it was found that heating pyridoxamine with α-ketoglutaric acid greatly increased its growth-promoting activity for *L. casei*,[3] a change which would be explicable if pyridoxamine were converted to pyridoxal by this treatment (transamination). Since pyridoxamine and pyridoxal appear to occur naturally,[3] and since such behavior may be connected with their catalytic role *in vivo*, the reactions were further investigated. By isolation of the products of the reaction, or of suitable derivatives of them, it was established that interconversion of pyridoxamine and pyridoxal does occur according to the equation:



(1) Pyridoxal + Glutamic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ Pyridoxamine + α-Ketoglutaric acid

The data indicate that a similar reaction may occur between pyridoxal and certain other amino acids, and between pyridoxamine and certain other ketoacids. The reaction is a new example of the transamination reaction, which in the narrow sense has been defined as "the intermolecular transfer of an amino group from an α-amino acid to an α-ketoacid,"[4] but which is recognized as a special case of oxidative deamination of amino acids which may be brought about by a variety of organic compounds containing the carbonyl grouping.[4] In previously reported instances of the reaction produced by substances other than α-ketoacids (*e. g.*, alloxan, ninhydrin, isatin, quinones and α-dicarbonyl compounds[4]) the α-amino group was liberated as ammonia, or reacted further with the oxidizing agent, as in the case of ninhydrin. With α-ketoacids, and with pyridoxal, the amino group is transferred intact to the oxidizing compound.

Further striking differences between reaction (1) and previously noted non-enzymatic examples of the transamination reaction lie in the reversibility of the former, and in the failure of the oxidatively deaminated amino acid to undergo decarboxylation. In both respects, the reaction resembles enzymatic transaminations, as described by Braunstein[5] and by Cohen.[6] A further similarity lies in the especial effectiveness of glutamic and α-ketoglutaric acids in producing the change. If the non-enzymatic reaction between α-ketoacids and α-amino acids could be made to proceed by the stages



$RCHNH_2COOH$ + Pyridoxal $\rightleftharpoons$ $RCOCOOH$ + Pyridoxamine

Pyridoxamine + $R'COCOOH$ $\rightleftharpoons$ Pyridoxal + $R'CHNH_2COOH$

the summation would result in a reversible transamination catalyzed by pyridoxal (or pyridoxamine), unaccompanied by decarboxylation, and analogous to the enzymatic reaction.[7]

This reaction of pyridoxal and pyridoxamine has been fully investigated only with glutamic and α-ketoglutaric acids. It is possible that decarboxylation of the amino acid may occur in some other cases. In this connection, it is suggestive that pyridoxal[8] or a phosphorylated pyridoxal[9] has been shown to serve as a coenzyme for tyrosine decarboxylation by cell preparations of *S. fecalis* R.

The ease with which reaction (1) occurs, and the occurrence of both amino acids and ketoacids in extracts of natural origin should serve to emphasize the difficulties involved in obtaining accurate differential assays of pyridoxamine and pyridoxal in natural materials.

Experimental

Assay Methods.—Pyridoxamine and pyridoxal were determined separately by utilizing their differential growth effects on *L. casei* and *S. fecalis* R, as previously described.[3] Total vitamin B_6 activity was determined by a minor modification[2] of the yeast-growth method of Atkin, *et al.*,[10] utilizing *S. carlsbergensis*.

Reaction of Pyridoxal with Glutamic Acid.—The effect of heating with glutamic acid on the growth-promoting

(1) E. E. Snell, *J. Biol. Chem.*, **154**, 313 (1944).

(2) E. E. Snell and A. N. Rannefeld, *ibid.*, in press.

(3) E. E. Snell, *ibid.*, in press.

(4) R. M. Herbst, "Advances in Enzymology," Interscience Publishers, New York, 1944, Vol. 4, p. 75.

(5) A. E. Braunstein, *Enzymologia*, **7**, 25 (1939).

(6) P. P. Cohen, *Biochem. J.*, **33**, 1478 (1939).

(7) Preliminary experiments (F. Schlenk and E. E. Snell, unpublished data) to determine whether these compounds are concerned with transamination *in vivo* have shown that tissues from rats deficient in vitamin B_6 have a lowered capacity to carry out transamination reactions.

(8) I. C. Gunsalus and W. D. Bellamy, *J. Biol. Chem.*, **155**, 357 (1944).

(9) I. C. Gunsalus, W. D. Bellamy and W. W. Umbreit, *ibid.*, **155**, 685 (1944).

(10) L. Atkin, A. S. Schultz, W. L. Williams and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, **15**, 141 (1943).

activity of pyridoxal for various organisms is shown in Table I. For each organism, the activity of pyridoxal is altered in a manner suggesting its conversion to pyridoxamine. The transformation is largely inhibited by strong acids and alkali, but not by acetic acid. A very large excess of glutamic acid is required to drive the reaction to completion.

Isolation of the Reaction Products.—To establish the products definitely, the reaction was carried out on a larger scale. Twenty grams of *l*-(+)-glutamic acid and 200 mg. of pyridoxal hydrochloride (≈164 mg. of pyridoxal) were dissolved in 150 cc. of water by heating and adding sodium hydroxide to *p*H 6.8. The deep-yellow solution was diluted to 200 cc. and autoclaved at 15 lb. steam pressure for thirty minutes. Assay indicated conversion of 85% of the pyridoxal to the substance having the physiological properties of pyridoxamine. The reaction solution was adjusted to *p*H 3.0 with concentrated hydrochloric acid and allowed to stand overnight at 5°. The copious precipitate of glutamic acid (13.5 g.) was filtered out, and the filtrate concentrated *in vacuo* to 100 cc. Two hundred milligrams of 2,4-dinitrophenylhydrazine dissolved in 20 cc. of 2 *N* hydrochloric acid was now added. Separation of a lemon-yellow hydrazone began within one minute. After thirty minutes at room temperature, the suspension was extracted twice with ether. The aqueous residue was reserved for later use.

The combined ether extracts were concentrated to dryness. The 2,4-dinitrophenylhydrazones were treated with 20 cc. of a 10% solution of sodium bicarbonate; the insoluble material was filtered out and discarded. Hydrochloric acid was added to the bicarbonate solution until complete precipitation of the hydrazone was obtained. The crude product weighed 224 mg., and melted at 219°. After recrystallization from ethyl acetate, it melted at 224° (dec.). An authentic sample of the 2,4-dinitrophenylhydrazone of α-ketoglutaric acid melted at 224° (dec.) in the same bath. Weil-Malherbe and Krebs[11] give 222° (uncor.) as the melting point of this compound. A mixed melting point showed no depression. The isolated sample was further characterized by oxidation to succinic acid, as recommended by Krebs[12]; succinic acid was determined by a slight modification of the method of Moyle.[13] A sample yielded 35.8% of succinic acid (99%). Thus, the isolated product is the 2,4-dinitrophenylhydrazone of α-ketoglutaric acid, and was obtained in 81% of the yield demanded by equation (1).

The aqueous residue from the ether extraction above was adjusted to *p*H 3.0 with sodium hydroxide, diluted to 2 liters, 100 g. of "superfiltrol" added, and the mixture stirred for thirty minutes. The superfiltrol was removed by filtration, washed thoroughly with water, and the filtrate and washings discarded. The earth was eluted by stirring for fifteen minutes three successive times with 500 cc. of 0.5% sodium hydroxide in ethanol[14] each time. The combined eluates were adjusted to *p*H 6.5 with sulfuric acid, concentrated *in vacuo* to 100 cc., filtered, then evaporated to dryness. The residue was triturated thoroughly with two 10-cc. portions of boiling ethanol. The ethanol extracts were boiled briefly with 100 mg. of Darco G-60, filtered and concentrated to 5 cc. After cooling and filtering, 10 cc. of a saturated alcoholic solution of picric acid was added. After five hours in the ice box, a copious precipitate (154 mg.) was obtained. After two recrystallizations from ethanol the picrate melted at 201° (dec.). An authentic sample of pyridoxamine picrate, prepared by addition of excess alcoholic picric acid to an alcoholic solution of pyridoxamine[15] and recrystallization, melted at 201° (dec.), and there was no depression in melting points. The product is a dipicrate of pyridoxamine. Calculated for $C_{20}H_{18}O_{16}N_8$: C, 38.34; H, 2.86; N, 17.87. Found: C, 38.33, 38.34; H, 2.66, 2.72; N, 17.80, 17.95. The authentic picrate and the isolated sample were both 0.29 times as active as pyridoxamine in promoting growth of *S. fecalis* R. Theory requires 0.27.

TABLE I

THE EFFECT OF AUTOCLAVING PYRIDOXAL WITH GLUTAMIC ACID ON ITS GROWTH-PROMOTING ACTIVITY FOR VARIOUS ORGANISMS[a]

Amount of glutamic acid, mg.	Other additions[a]	*L. casei*	Activity[b] for *S. fecalis* R	Yeast
0	......	1.00	1.00	1.00
30	......	0.019	2.10	1.02
15	......	.024	2.10	1.00
7.5	......	.13	..	..
4.5	......	.30	..	..
15	NaOH[c]	.47	..	..
15	CH_3COOH[d]	.027	..	..
15	HCl[e]	.52	..	..

[a] 100 γ of pyridoxal was present in all tubes. Total volume in each case was 2.0 cc. All tubes were autoclaved for thirty minutes at 15 lb. steam pressure. *p*H was 6.8 unless otherwise indicated. [b] Unheated pyridoxal was used as standard for each organism, with activity of 1.00. The activity of pyridoxamine on the same scale was 0.006 for *L. casei*, 2.20 for *S. lactis* R and 1.00 for yeast; if pyridoxal is converted to pyridoxamine, these values should be approached as limits. [c] 0.1 cc. of 15% NaOH added. [d] 0.1 cc. of glacial acetic acid added. [e] 1.0 cc. of 6 *N* hydrochloric acid added.

Ninety-eight mg. of the picrate isolated from the reaction mixture was decomposed by extracting a suspension in dilute hydrochloric acid with benzene. The aqueous residue was concentrated to dryness (yield, 37.0 mg.) and recrystallized from an ethanol–ether mixture. Its melting point was 228° (dec.), identical with that of an authentic sample of pyridoxamine dihydrochloride, and there was no depression of the mixed melting points. Harris, *et al.*,[15] give the melting point of pyridoxamine dihydrochloride as 226–227°. Both samples had identical physiological activity for both *S. fecalis* and *L. casei*. Thus the formation of pyridoxamine from heating pyridoxal with glutamic acid is demonstrated. The isolated picrate contained 29% of the pyridoxamine which the original microbiological assay indicated was formed. Assay of the discarded fractions revealed the chief loss to have occurred at the step involving adsorption with superfiltrol.

The isolation of pyridoxamine and α-ketoglutaric acid (as derivatives) as reaction products, and their formation in approximately equimolar quantities, indicate that reaction proceeds according to equation (1a) above.

Reaction of Pyridoxamine with α-Ketoglutaric Acid.[16]—The effect of heating pyridoxamine with α-ketoglutaric acid on its growth-promoting activity for various organisms is shown in Table II. The activity of pyridoxamine is shifted in a manner suggesting its conversion to pyridoxal. The conversion is partially inhibited by alkali and by weak acids, and is completely prevented by the presence of strong acids.

Isolation of the Reaction Products.—To establish equation (1b), the reaction was carried out on a larger scale and the products isolated. Two hundred and fifty mg. of pyridoxamine and 1.25 g. of α-ketoglutaric acid were dissolved in 100 cc. of water, and the *p*H adjusted to 6.8 with sodium hydroxide. The solution was autoclaved at 15 lb. steam pressure for thirty minutes. Dilute sulfuric acid was added to *p*H 1.4, and the solution extracted continuously with ether for twenty-four hours. The ether extract, which contained the excess α-ketoglutaric acid, was

(11) H. Weil-Malherbe and H. A. Krebs, *Biochem. J.*, **29**, 2077 (1935).

(12) H. A. Krebs, *ibid.*, **32**, 108 (1938).

(13) D. M. Moyle, *ibid.*, **18**, 351 (1924).

(14) A. F. Bina, J. M. Thomas and E. B. Brown, *J. Biol. Chem.*, **148**, 111 (1943).

(15) S. A. Harris, D. Heyl and K. Folkers, THIS JOURNAL, **66**, 2088 (1944).

(16) We are indebted to Dr. F. Schlenk for generous samples of α-ketoglutaric acid.

TABLE II

THE EFFECT OF AUTOCLAVING PYRIDOXAMINE WITH α-KETOGLUTARIC ACID ON ITS GROWTH-PROMOTING ACTIVITY FOR *L. casei*[a]

Amount of α-ketoglutaric acid, mg.	Other additions[a]	Activity[b] for *L. casei*
0	......	0.003
15.0	......	1.0
1.5	......	1.0
0.25	......	0.60
0.10	......	.029
15.0	NaOH[c]	.36
15.0	CH_3COOH[d]	.51
15.0	HCl[e]	.003

[a] 100 γ of pyridoxamine was present in all tubes. Total volume in each case was 2.0 cc. Tubes were autoclaved at 15 lb. steam pressure for thirty minutes. The *p*H was 6.8 except where otherwise indicated. [b] Unheated pyridoxal was used as standard, with an activity of 1.0. If pyridoxamine is being converted to pyridoxal, the activity should approach 1.0 as a limit. Unheated pyridoxamine in the same test was 0.003 times as active as pyridoxal. [c] 0.1 cc. of 20% sodium hydroxide added. [d] 0.1 cc. of glacial acetic acid added. [e] 1.0 cc. of 6 *N* hydrochloric acid added.

discarded. Sulfate was exactly removed from the aqueous residue with barium hydroxide. An aliquot assayed for pyridoxal indicated formation of 67.5 mg. of pyridoxal (0.40 millimole); an aliquot assayed microbiologically for glutamic acid[17] indicated formation of 53.1 mg. of *dl*-glutamic acid (0.36 millimole).

The solution was concentrated *in vacuo* to 10 cc., then filtered. Two grams of sodium acetate was dissolved in the filtrate, then 2 cc. of a solution containing 1 g. of hydroxylamine hydrochloride was added. The mixture was heated on the steam-bath for five to ten minutes, then cooled for several hours. The slightly yellow crystalline precipitate weighed 36 mg. It was dissolved in a small volume of hot ethanol, treated with Darco G-60, filtered, and recrystallized twice from a hot ethanol-water mixture. The pure white crystals were microscopically identical with those of an authentic sample of pyridoxal oxime. The two samples melted at 225-226° (dec.). There was no depression of the mixed melting point. Harris, *et al.*,[15] give 225-226° as the decomposition point of this oxime. On assay with *L. casei*, both products showed identical activity, about 0.2 that of pyridoxal itself, and far greater than that of pyridoxamine.

The crude pyridoxal oxime isolated from the reaction mixture accounts for 49% of the pyridoxal indicated by microbiological assay to be present in the original reaction mixture.

Attempts to isolate the small amount of glutamic acid present in this reaction mixture failed. The reaction was therefore carried out a second time. Nine-tenths gram of α-ketoglutaric acid and 0.9 g. of pyridoxamine were dissolved in 60 cc. of water. After adjusting to *p*H 6.8 with barium hydroxide, the solution was autoclaved at 15 lb. pressure for thirty minutes, cooled, and diluted to 150 cc. Microbiological assays of an aliquot indicated formation of 290 mg. of glutamic acid (1.97 millimoles) and 330 mg. of pyridoxal (1.99 millimoles). The solution was adjusted to *p*H 7.5, then concentrated to dryness. The dried material was triturated repeatedly with 10-cc. portions of boiling ethanol, and the extracts discarded. The insoluble material was dissolved in 75 cc. of water, and the barium ion exactly removed with sulfuric acid. The filtrate was concentrated to dryness and repeatedly extracted with hot ethanol. The insoluble material weighed 580 mg. and contained by microbiological assay about 50% of *dl*-glutamic acid. This material was further purified by preparing the calcium salts and twice precipitating from water with ethanol, as described by Block and Bolling.[18] The purified calcium salts thus obtained were freed from calcium with oxalic acid. After filtering, excess concentrated hydrochloric acid was added, and the mixture concentrated to a thin sirup. After standing overnight, the crystals were centrifuged out, washed with a little concentrated hydrochloric acid, then recrystallized twice by the same procedure. The pure white crystals weighed 171 mg., and melted at 193-194° (dec.). An authentic sample of *dl*-glutamic acid hydrochloride melted at the same temperature; there was no depression of the mixed melting point. Wolff[19] gives 193° for the melting point of this compound. Calculated for $C_5H_{10}O_4NCl$: N, 7.63; Cl, 19.3; glutamic acid, 80.1. Found: N, 7.69; Cl, 19.1; glutamic acid, 81.4 (microbiological assay).

Thus interaction between pyridoxamine and α-ketoglutaric acid appears to proceed according to equation (1b), and the process is fully reversible.

Reaction of Pyridoxal with Other Amino Acids.—Results of autoclaving pyridoxal with hydrolyzed casein and with other amino acids on its growth-promoting properties for three organisms are shown in Table III.

TABLE III

EFFECT OF AUTOCLAVING PYRIDOXAL WITH INDIVIDUAL AMINO ACIDS ON ITS GROWTH-PROMOTING ACTIVITY FOR VARIOUS ORGANISMS[a]

Amino acid	*L. casei*	Activity[b] for *S. fecalis* R	Yeast
None	1.00	1.0	1.0
Hydrolyzed casein (10%)	0.006	2.1	0.91
l(+)Glutamic acid	.006	2.1	.97
l(+)Lysine	.013	2.0	.80
dl-Methionine	.33	1.9	.84
l-Tyrosine	.39	2.2	.84
dl-Phenylalanine	.43	1.8	.84
l-Aspartic acid	.43	1.6	.86
l(+)Arginine	.43	2.2	.98
l-Leucine	.51	1.6	1.0
dl-Isoleucine	.53	1.7	0.96
dl-Alanine	.67	1.4	.97
dl-Valine	.80	1.4	.86
dl-Threonine	.80	1.4	1.0
l-Cystine	.83	1.4	1.0
Glycine	.90	1.6	1.3
l-Hydroxyproline	.90	0.97	1.0
l-Proline	1.0	1.0	0.97
dl-Serine	0.95	1.1	.72
β-Alanine	.95	1.0	.90
l-Tryptophan	.003	0.00	.00
l-Histidine	.013	0.34	.12

[a] 100 γ of pyridoxal was added in each case to a 0.1 *M* solution of the amino acid at *p*H 6.8. Cystine and tyrosine were not completely soluble at this concentration. The mixtures were autoclaved at 15 lb. steam pressure for thirty minutes in a total volume of 2 cc., then diluted and assayed. [b] Pyridoxamine in the same test showed the following activities: *L. casei*, 0.006; *S. fecalis* R, 2.1; yeast, 0.95. Activity of the autoclaved mixtures should approach these values to the degree that pyridoxal is converted to pyridoxamine.

The amino acids appear to fall into four groups: (1) those like glutamic acid and lysine, which appear to effect almost complete conversion of pyridoxal to pyridoxamine

(17) This assay was made on the medium of McMahan and Snell (*J. Biol. Chem.*, **152**, 83 (1944)), from which glutamic acid was omitted, with *S. fecalis* R as test organism. So far as has been determined, the method is specific for glutamic acid.

(18) R. J. Block and D. Bolling, "Determination of the Amino Acids," revised ed., Burgess Pub. Co., Minneapolis, Minn., 1940.

(19) L. Wolff, *Ann.*, **260**, 120 (1890).

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.

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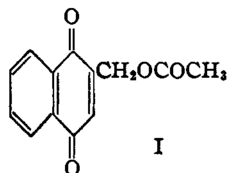
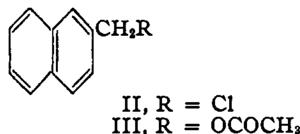
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLLEGE OF ARTS AND SCIENCES, AND THE DEPARTMENT OF BIOCHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF ROCHESTER]

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.

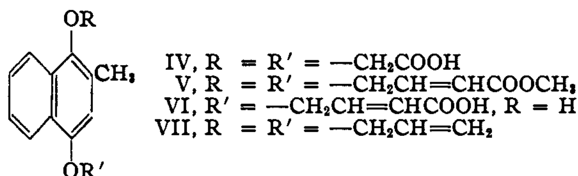




Experiment showed that this did not occur; a ten-fold excess of I did not inhibit the normal activity of 2-methyl-1,4-naphthoquinone when administered simultaneously.[2] This result agrees with the idea that vitamin K activity depends on conversion of the substance into 2-methyl-1,4-naphthoquinone (or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

(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.