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# BIOTRANSFORMATION OF NITROSO NAPHTOLS BIOASSISTED BY BAKER'S YEAST

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***ABSTRACT.*** 1- Nitroso-2-naphtol, 2-nitroso-1-naphtol and 4-nitroso-1-naphtol were used as substrates for the reductive action of Baker's yeast. The expected amino-naphtols were not isolated, and some new products of their oxidation were identified, Sodium borohydride afforded unexpected products with 2-nitroso-1-naphtol. Ab initio were employed in elucidating the nature of the processes described.

## *INTRODUCTION*

Baker's yeast has been shown to exhibit selectivity as far as reduction of such groups as carbonyl, nitro or C=C is concerned. We have recently found aromatic nitroso compounds to be readily reduced to the corresponding amines [1], and have succeeded in isolating the products. Of the functional groups thus far considered as yeast substrates, nitroso is the most easily transformed. The mechanism of this reaction should involve firstly hidroxilamine formation (by the same mechanism as for carbonyl reduction), and then a hidride attack on the nitrogen atom. The simple experimental procedure we have developed [1] provides a convenient route for nitroso group reduction, as no demanding experimental conditions are required, and the amine is readily isolated as the hydrochloride.

In view of the data available [1] the nitroso-naphtols should be readily converted by means of Baker's yeast to the corresponding amino-naphtols. However, the latter are only stable under inert conditions, unless isolated as hydrochlorides or acetyl-derivatives. The usual experimental procedures employed with Saccharomices cerevisiae are not compatible with isolation of such labile compounds as amino-naphtols. This inconvenient has been dealt with by us in different ways, in a quest for developing experimental procedures of general use as far as labile compounds and Baker's yeast are involved. Such procedures should involve work under inert atmosphere, use of organic (hopefully dry) solvents; immobilized cells may also prove useful.

## *RESULTS AND DISCUSSION*

1-Nitroso-2-naphtol ***(I)***, 2-nitroso-1-naphtol ***(II)*** and 4-nitroso-1-naphtol ***(III)*** were used as substrates for the reductive action of Baker's yeast:

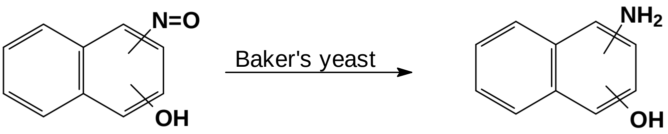


***Fig. 1.*** *General equation for nitroso naphtol reductions*

A number of aminmo phenols have already been obtained by this procedure [1]. The expected amino-naphtols should be difficult to issolate, as they are known to be unstable in the presence of oxygen and water (both of which unavoidable in experimental procedures involving Baker's yeast). Thus, a mixture of water, yeast and nitroso-naphtol should provide, besides little amino naphtol (if any), at least one of its oxidation products. Indeed, the reaction mixtures showed an initial partial decolorization, but they then acquired different (intense) colours.

While the final product of amino naphtol oxidation is the corresponding quinone (which should therefore be present in our reaction mixtures), a number of other compounds resulted from partial oxidation have been described (formulae in Fig.2). 2-Amino-1,4-naphtoquinon-$N^4$-(1-hydroxy, 2-naphtil-imine) (1) was obtained by bubbling air in ammonia solution [2]; its colour is violet, though initially a green compound (not isolated) is formed. (1) is solid, soluble in alcohols, and has also been obtained by means of cytochrome c oxidase [3]. The amino naphtoquinone and a amino either bu enzymic or chemical means [4]. Coupling of a naphtoquinone and a amino naphtol has led to (4) [5]. 2-Amino-1-naphtol afforded the corresponding 1,2-napthoquinone by peroxidase action, although highly unstable intermediates of oxidation were shown to be formed and to bind to proteins [6]. Fungal tyrosinase also afforded quinones [7].

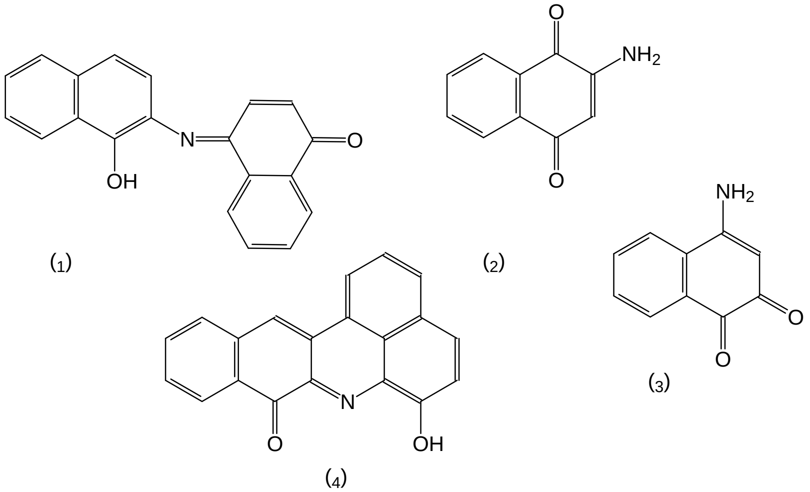


***Fig. 2.*** *Partial reduction products of the amino naphtols*

Amino naphtols have been used as electron (and proton) carriers in peroxidasic systems [8], on the basis of their quinonimine-amino naphtol equilibria. Free radicals, expected and proven to be formed on aminonapthol oxidation, have been shown to bind to proteins and nucleic acids (accounting for the biological activity) [9].

A different explanation for the intense colors of the reaction mixtures may arise when we consider the features of the nitroso group, either of the following reaction pathways being possible:

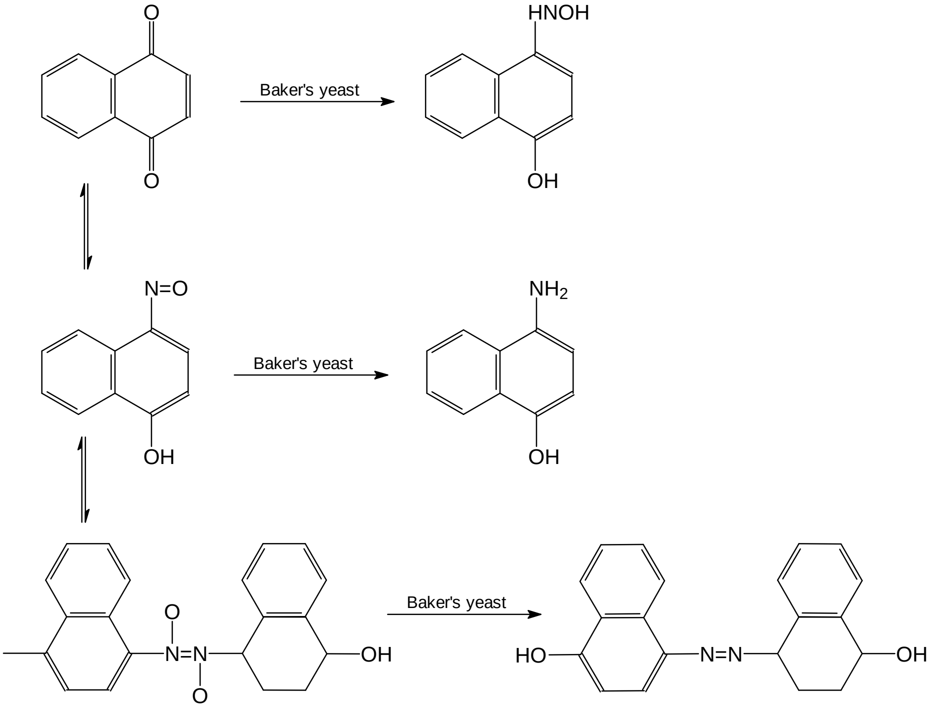
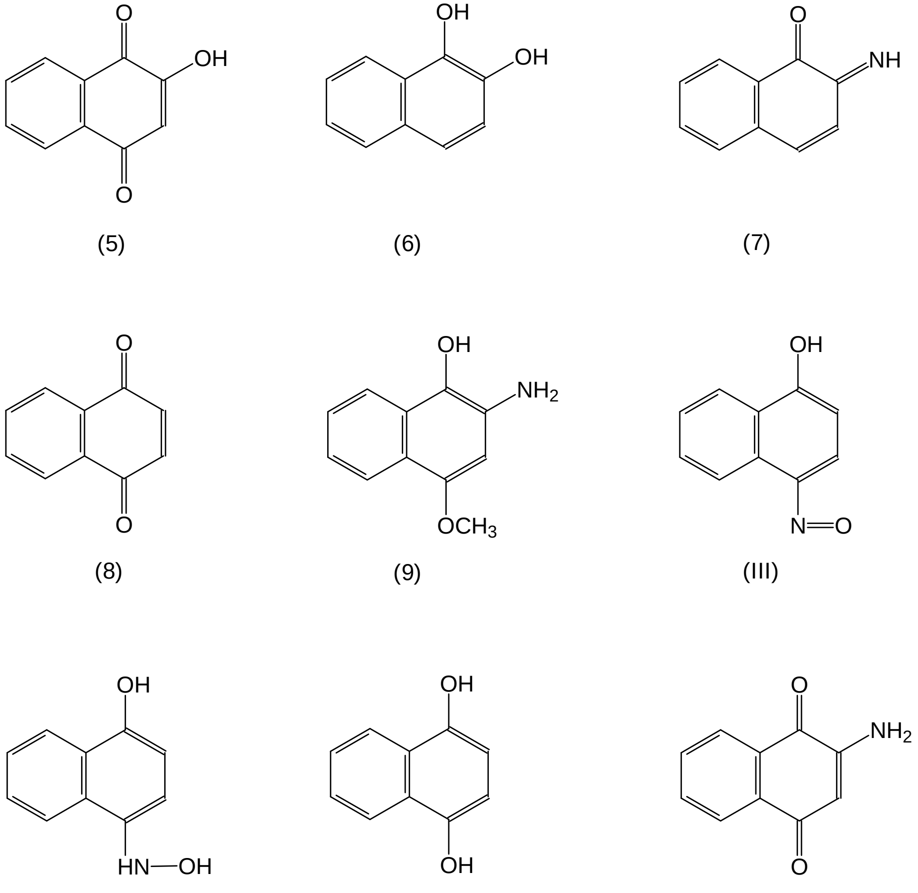


***Fig. 3.*** *Nitroso group isomerism and its possible influence on the nature of yeast mediated reductions*

As quinones and nitroso phenols [1] yield respectively the correspondig hydroquinones and amino phenols, the oxime in Fig. 3 should eventually provide the amine; azo derivatives are not substrates for Baker's yeast, and may be responsible for the intense colours of the reaction mixtures.

Six different experimental procedures (***A-E***) were applied for the attempted nitroso naphtol reduction, in order to study the nature of the products and to minimize the degree of amine oxidation. Thus HCl, AcOH, and DMF media were employed, as well as inert atmosphere. Structures of the products identified are presented in Fig. 4.



(5) (6) (7)

(8) (9) (III)

(10) (11) (2)

***Fig. 4.*** *Compounds obtained upon nitroso naphtol reduction, as described below*

***A.*** The classical proccedure involves mixing water and yeast and then adding a saturated solution of the substrate; the products are eventually extracted with organic solvents. In the case of the nitroso naphtols, extraction afforded intensely coloured solutions, which could not be extracted with HCl (no amino naphtol was present). TLC analysis proved them to be complex mixtures (at least 6 spots), which could not be totally resolved, as equillibria between the components occurred. GC-MS analysis of the sample resulted from 2-nitroso-1-naphtol proved the presence of 2-amino-1,4-naphtoquinone (2) and a methylated derivative (the samples were dissolved in methanol). The properties of the mixture suggested it to contain as the main component (1) [2,3]; however, (1) was not detected by GC-MS (and should not have been, in view of its molecular weight and polarity). A mixture, identical to the one above described, was obtained from ***(II)*** with $NaBH_4$ in basic aqueous methanol, followed by ether extraction. Two main components were shown to be present by TLC (one violet, one green); these two appear to be involved in an equilibrium, as the violet component prevails in etheric solution and the green one prevails aqueous NaOH medium. The solution turns red in acidic medium. Sodium borohydride may react with the amino naphtol as well as its tautomer oxime; its mechanism of action is not radicalic, as opposed to the oxidation processes (peroxidasic or not) expected to take place in the yeast suspension. The mixture obtained from ***(I)*** after solvent evaporation and recristallization from concd. HCl affroded a red precipitate, which could not be analized (did not melt up tp $360^0$C and provided no mass spectrum, was not soluble in the available deuterated solvents, had a poor IR spectrum and was obtained in very low yields).

***B.*** A different approach implied addition of HCl (in various amounts) to the yeast suspension (before substrate addition). After filtering and boling with active C, the clear colourless solutions were mixed with DMF and the water removed, White precipitates were thus obtained; altough these were expected to be amino naphtol hydrochlorides, they did not redissolve in any solvent, did not release the amines in basic media, had poor IR spectra, did not melt up to $360^0$C, tested positive with nihnydrin; they dissolved in HCl medium and reprecipitated when NaOH was added. It was concluded that these materials were of proteic nature.

***C.*** HCl was added only before filtering the reaction mixture. Organic solvent extraction then afforded a mixture similar to that obtained in the ***A***. procedure. The aqueous phase was treated as in procedure ***B***., with similar results. The organic phases were analyzed by means of GC-MS. Identified in the mixture obtained from ***(I)*** was 2-hydroxy-1,4-naphtoquinone (5). The mixture obtained from ***(II)*** contained 1,2-naphtalenediol (6) and (in very low concentrations) 1,2 naphtoquinon-2-imine (7). The mixture obtained from ***(III)*** contained 1,4-naphtalenediol (11), 1,4-naphtoquinone (8), 4-nitroso, 1-naphtol ***(III)***, and 4-hydroxylamino 1-naphtol (10). As mentioned above, hydroquinone should ne expected to form: (10) may arise from either of the two tautometric states of ***(III)***; (8) should be present whenever the corresponding quinone is present, given that benzoquinone is known to be readily reduced in yeast medium to benzohydroquinone.

***D.*** $Ac_2O$ was added at times to the reaction mixture of ***(II)***, as amino naphtols have been acetylated under similar conditions [10]. Organic solvent extraction eventually afforded a mixture similar to that obtained by the procedures ***A*** and ***C***. A brown solid precipitated from the aqueous phase (m.p. 230-$6^0$ C) and GC-MS analysis proved it to contain 2-hydroxy 1,4-naphtoquinone (5) and 2-amino,4-mehtoxy,1-naphtol (9); the latter may have been methylated in situ while performing the analysis, as methanol was the solvent. Non-methylated (9) is expected to form upon (2) reduction by the yeast.

***E.*** The yeast was suspended in DMF and the mixture was placed under inert atmosphere. Should theoxidation processes have been due only to the oxygen/water system, they should have been greatly reduced. This was not the case; although the reaction mixtures did acquire intense colours, they were not identical to those obtained under aerobic conditions. Concd. HCl was eventually added, followed by the work-up of procedure ***C*** (the proteic material was also obtained).

No azo-derivatives were isolated; although the latter may well not be detectable by the Gc-MS system employed in analysis, we conclude that the processes taking place after nitroso group reduction are of oxidative or peroxidative nature, and at least part of them are not $O_2$-dependent (as proven by procedure ***E***). Some of the oxidation products were in turn reduced by the yeast.

Ab initio calculations were performed on the amino naphtols expected to form, and the results are presented in Table 1. These data provide insights as far as the oxidation processes of the amino naphtols in our experiments are concerned.

The stabilities of the three amino naphtols decrease in the order: 2-amino 1-naphtol>1-amino 2-naphtol>4-amino 1-naphtol; the energies are given in hartrees. The high energy values of their HOMO indicates the amino naphtols as very good electron donors, while the high values for LUMO suggests them to be poor electron acceptors. The HOMO-LUMO gaps are relatively large. Also given in Table 1 are the carbon atoms expected to be the most reactive on the basis of their relative charges.

***Table 1.*** *Results of ab initio calculations for amino naphtols*

| Compound | 1-amino 2-naphtol | 2-amino 1-naphtol | 4-amino 1-naphtol |
|---|---|---|---|
| Energy | -510,385098 | -510,386683 | -510,377495 |
| HOMO | -0,2861 | -0,2773 | -0,2504 |
| LUMO | 0,09484 | 0,1110 | 0,1192 |
| HOMO-LUMO gap | 0,3845 | 0,3883 | 0,3697 |
| highest negative charge on carbon | $C_3$, $C_6$, $C_8$ | $C_4$, $C_7$ | $C_2$ |

## *EXPERIMENTAL*

All reactives were Merck of Reactivul products. 2-nitroso-1-naphtol (II) and 4-nitroso-1-naphtol (III) were prepared according to the data available [11].

Procedure ***A***: 80 g yeast are suspended in water, and then 0,5 g nitroso naphtol is added (as satd. solution in methanol). The mixture is stirred 0,5-2 h, and then filtered; the filtrate is extracted with ethyl acetate/benzene, the solvent from the organic phase evaporated and the residue analyzed by GC-MS.

Procedure ***B***: 80 g yeast are suspended in water, 8 ml concd. HCl are added, followed by steps as in procedure ***A***. The aqueos phase is boiled with activated C, 10 ml DMF are added and the water evaporated; filtering and washing with benzene afforded 0,3-0,6 g of white precipitate. The amount of HCl may vary, without affecting the results.

Procedure ***C***: 80 g yeast are suspended in water, and then 0,5 g nitroso naphtol is added (as satd. solution in methanol). The mixture is stirred 0,5-2 h, and then 8 ml concd. HCl is added. Work-up follows for the organic phase as in procedure ***A*** and for the aqueous phase as in procedure ***B***. 0,1 g white precipitate were thus obtained.

Procedure ***D***: 80 g yeast are suspended in water, and then 0,5 nitroso naphtol is added (as satd solution in methanol). Every 20 minutes 5 ml $Ac_2O$ are added, until the initial colour has faded. The mixture is filtered; the filtrate is extracted with ethyl acetate/benzene, the solvent from the organic phase evaporated; the aqueos phase pH is made neutral, affording a brown precipitate, m.p. 230-236$^0$ C.

Procedure ***E***: 80 g yeast are suspended in dry DMF under nitrogen; nitrogen is bubbled through the suspension, and then 0,5 g nitroso naphtol is added (as satd. solution in methanol). The mixture is stirred 3,5 h, and then 15 ml concd. HCl are added, followed by steps as in procedure ***C***.

Sodium borohydride reduction: to a aqueos methanolic soln of nitroso-derivative (0,1 g in 15 ml, 10% NaOH) 0,1 g sodium borohydride are added. After 20 minutes the efervescence ceases and the reaction is complete. The mixture is extracted with ether and after solvent evaporation a violet powder is obtained.

IR spectra of the white precipitates: 3400, 1580, 1100 cm$^{-1}$, all broad and strong.

Organic phase extracts mass spectra:

***(III)***: M/e: 173, m/e: M-30, M-17, M-30-28, M-16-26, 76,77.

(2): M/e: 173, m/e: 76, 105, 27, M-27, M-27-26, M-28

(5): M/e: 174, m/e: 75,76,77,105, M-28, M-28-28.

(6): M/e: 160, m/e: 131, 104, 75, 50, M-29-29, M-29.

(7): M/e: 157, m/e: M-16, M-28, M-27, M-29, M-16-27, 76,77,105,29.

(8): M/e: 158, m/e: M-28, M-28-28, M-30, M-28-26, 50, 75, 76,77,105.

(9): M/e: 189, m/e: M-15, M-17, M-30, M-30-16, M-30-28, M-30-16-25, M-30-16-29, M-30-16-28, 105,89,76.

(10): M/e: 175, m/e: M-17, M-17-28, M-17-28-26, M-29, M-29-17, M-29-31, M-29-44, 74,75,76,77,105.

(11): M/e: 160, m/e: M-29, M-29-27, 76, 50.

Calculation details: ab initio calculations were made in the Spartan package [12], using the 3-21G(*) basis.

## *ACNOWLEDGEMENTS*

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