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The Yeast-Mediated Reduction of Nitrostyrenes in Organic Solvent Systems

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A range of nitrostyrenes have been reduced with dried baker's yeast in an organic solvent system. It was found that the reduction proceeded smoothly to give the corresponding nitroalkanes in good yield and with higher efficiency than the corresponding aqueous reaction system. No evidence for reduction of the nitro group was observed. In the case of β-methyl nitrostyrenes, racemic mixtures were formed, and it was shown that this is not due to racemization of the product.

Introduction

Baker's yeast has been widely used for the stereoselective reduction of carbonyl groups in aqueous reaction systems.[1] Recently, a number of reports have appeared describing a simpler system involving the use of yeast in organic solvent systems.[2–6] The yeast-mediated stereoselective reduction of carbon–carbon double bonds in aqueous systems has also been widely reported, and we were interested to see whether yeast-mediated reductions of this type could be transferred to organic solvent systems. Of particular interest was whether differences in the reduction reaction between aqueous and organic solvent systems could be observed. Nitrostyrenes were chosen for this study since they have been previously studied in aqueous reaction systems, and β-substituted nitrostyrenes have been shown to give rise to racemic products which is rare for yeast-mediated reactions.[7,8] It has been proposed that the formation of racemic products is due to racemization of the product under the mildly basic reaction conditions.[9] It was thought that this racemization would be less likely to occur in essentially neutral organic solvents.

Results and Discussion

Previous reports concerning the yeast-mediated reduction of ethyl acetoacetate in organic solvent systems have shown that a small amount of water needs to be added to the reaction system for reduction to occur.[5] β-Nitrostyrene (1a) was reduced by using dried baker's yeast (Mauri Foods Ltd, Australia) in light petroleum with varying amounts of water (0·2–1·2 ml water/g yeast) for 24 h at ambient temperature, and the results are summarized in Fig. 1. As has been previously reported,[5] no reduction occurred in the absence of added water, and optimum reduction occurred when 0·8 ml water/g yeast was added to the reaction mixture.

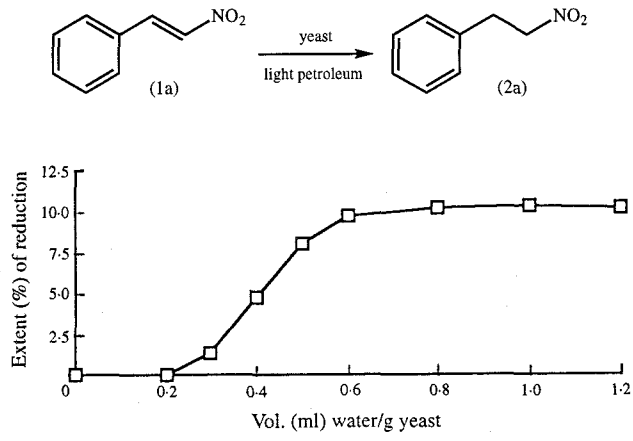

Fig. 1. Effect of added water on the yeast-mediated reduction of β-nitrostyrene (1a) (1 mmol nitrostyrene, 1 g yeast, 50 ml light petroleum, room temperature, 24 h). The extent of reduction was calculated from the gas chromatographic ratio of starting material to product.

As can be seen from these results 1 g of yeast was insufficient to completely reduce 1 mmol of β-nitrostyrene, so a series of reductions were performed utilizing increasing amounts of yeast while keeping the water/yeast ratio constant (0·8 ml/g). Reduction with 7 g of yeast was found to give complete reaction of β-nitrostyrene (1a) to the corresponding nitroalkane (2a). These reaction conditions (1 mmol substrate, 7 g yeast, 0·8 ml water/g yeast, 50 ml light petroleum) were considered to be the optimum conditions and were used for reduction of all of the nitrostyrenes in this

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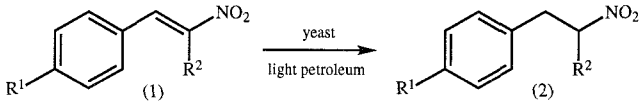
study. When the reduction reaction was carried out in toluene, carbon tetrachloride, chloroform or diethyl ether lower conversions were observed. Reactions involving these solvents were not examined further.

Takeshita *et al.* reported the reduction of a series of nitrostyrenes using yeast in aqueous media and noted that the electronic nature of the substituent affected the ease of reduction.[7] To determine whether a similar trend was present in an organic solvent system, a series of nitrostyrenes (1a–k) with different substituents attached to the aromatic ring were reduced with yeast in light petroleum, and the results are presented in Table 1.

Table 1. Yeast-mediated reduction of nitrostyrenes in light petroleum



(1)/(2)	R^1	R^2	Yield[A] (%)
(a)	H	H	74
(b)	Me	H	76
(c)	OMe	H	82
(d)	CN	H	12
(e)	NO_2	H	8
(f)	H	Me	80
(g)	Me	Me	80
(h)	OMe	Me	43
(j)	CN	Me	52
(k)	NO_2	Me	30

[A] Isolated yield of spectroscopically pure material.

Generally, high isolated yields of nitroalkanes were obtained from the yeast-mediated reduction. Nitrostyrenes with electron-donating groups attached to the aromatic ring gave higher yields than those containing electron-withdrawing groups, a result which is consistent with the results reported for reduction in aqueous solvents. The low yields obtained for the reduction of (1d,e) are probably due to the formation of polymeric material. Reduction of nitrostyrenes with sodium borohydride can result in dimeric and polymeric by-products formed by a Michael addition of the partially reduced nitrostyrene to another molecule of nitrostyrene.[10,11] The presence of electron-withdrawing groups on the aromatic ring and the absence of a substituent on the double bond would greatly facilitate a Michael addition of this type.

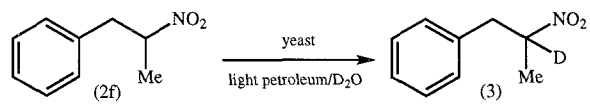
It was not possible to identify any dimeric or polymeric material in the reaction mixtures as a reasonable amount of lipid material is extracted from the yeast along with the reduction product. These products are readily separated from this yeast extract by vacuum distillation; however, separation or identification of other possible reaction products from the extract proved extremely difficult. Whilst hydrodimerization is the more likely cause of product loss, other yeast-mediated reactions obviously cannot be ruled out.

The organic solvent system behaves in a similar fashion to an aqueous system although it is clearly more efficient; comparable or higher yields of reduced products were obtained in the organic solvent system by using 7 g of yeast to reduce 1 mmol of nitrostyrene in 24 h compared with over 100 g yeast being required to reduce 1 mmol in 72 h in the aqueous system.[7]

It has been reported that the yeast-mediated reduction of the *p*,*β*-dinitrostyrenes (1e,k) in an aqueous reaction system resulted in the concomitant reduction of the nitro group attached to the aromatic ring to the corresponding amine.[7] No evidence of nitro reduction was observed in the organic solvent system.

Stereoselectivity

The reduction of *β*-methyl nitrostyrenes results in the formation of a chiral centre. In all cases the yeast-mediated reduction of *β*-methyl nitrostyrenes (1f–k) in an organic solvent system resulted in the formation of racemic products. The formation of racemic products also occurs when the reduction is performed in an aqueous reaction system.[7] The lack of stereoselectivity obtained from reduction in aqueous systems has been attributed to racemization of the product under the slightly basic reduction conditions.[9] It was considered doubtful that a similar racemization mechanism was operating in the organic reaction system. To determine whether racemization was occurring 2-nitro-1-phenylpropane (2f) was subjected to the yeast reduction conditions in the presence of deuterium oxide (Scheme 1). It was expected that if the nitroalkane was racemized under the reaction conditions deuterium incorporation should be observed in the *β*-position [see (3) in Scheme 1]. No evidence of deuterium incorporation into the isolated product could be detected by ^1H or ^2H n.m.r. This result strongly suggests that in an organic solvent system racemization of the resultant nitroalkane is not the cause of the formation of racemic products and that an alternative explanation is required to account for the formation of racemic products.



Scheme 1

Conclusion

The yeast-mediated reduction of a series of *β*-nitrostyrenes in an organic solvent system has been shown to proceed with higher efficiency than in an aqueous system, but without discernible stereoselectivity. This is the first reported example of a yeast-mediated reduction of a carbon–carbon double bond in an organic solvent system.

Experimental

General

Gas chromatography was performed on a Shimadzu GC-17A instrument with flame ionization detection. The column was an HP-1 (12 m by 0·22 mm) with a phase thickness of 0·33 μm. Bulb-to-bulb distillations were performed on a Buchi GKR-50 apparatus. ^{1}H n.m.r. spectra were recorded at 300 MHz on a Bruker DPX300 instrument and refer to solutions with tetramethylsilane as the internal reference (δ 0·0).

'Mauripan-Instant Dry Yeast' (*Saccharomyces cerevisiae*) was obtained from Mauri Foods Ltd, Australia, and stored at room temperature.

2-Nitro-1-phenylethene (1a) was purchased from Aldrich Chemical Company.

Light petroleum refers to the fraction boiling at 40–60°C.

Synthesis of Nitrostyrenes

All of the nitrostyrenes were prepared by the following general procedure, with nitromethane used to form the nitroethene derivatives (1b–e) and nitroethane for the nitropropene derivatives (1f–k).

(*E*)-2-Nitro-1-phenylprop-1-ene (1f; R^1 = H, R^2 = Me). Freshly distilled benzaldehyde (5 g, 50 mmol), nitroethane (5 g, 70 mmol), ammonium acetate (2 g, 30 mmol) and glacial acetic acid (20 ml) were refluxed for 2 h. The resultant liquid was poured into an ice water mixture (200 ml) and the solid collected and washed with cold water. Sublimation at 140°C gave the expected product (5·3 g, 65%), m.p. 62°C (lit.[12] 64–65°C). ^{1}H n.m.r. (300 MHz) δ 2·48, s, 3H, Me; 7·47, s, 5H, Ph; 8·11, s, 1H, H1.

(*E*)-1-(4′-Methylphenyl)-2-nitroethene (1b; R^1 = Me, R^2 = H) (3·3 g, 49%) had m.p. 81°C (lit.[13] 81°C). ^{1}H n.m.r. (300 MHz) δ 2·40, s, 3H, Me; 7·25, d, *J* 8·13 Hz, 2H, H3′,5′; 7·45, d, *J* 8·13 Hz, 2H, H2′,6′; 7·50, d, *J* 13·8 Hz, 1H, H2; 7·95, d, *J* 13·8 Hz, 1H, H1.

(*E*)-1-(4′-Methoxyphenyl)-2-nitroethene (1c; R^1 = OMe, R^2 = H) (3·7 g, 63%) had m.p. 84°C (lit.[12] 86–87°C). ^{1}H n.m.r. (300 MHz) δ 3·89, s, 3H, OMe; 6·97, d, *J* 8·9 Hz, 2H, H3′,5′; 7·52, d, *J* 8·9 Hz, 2H, H2′,6′; 7·53, d, *J* 13·6 Hz, 1H, H2; 7·98, d, *J* 13·6 Hz, 1H, H1.

(*E*)-1-(4′-Cyanophenyl)-2-nitroethene (1d; R^1 = CN, R^2 = H) was obtained after purification by sublimation in vacuum (120°C/0·1 mmHg) (2·39 g, 45%), m.p. 186°C (lit.[14] 186–188°C). ^{1}H n.m.r. (300 MHz) δ 7·62, d, *J* 13·8 Hz, 1H, H2; 7·66, d, *J* 6·3 Hz, 2H, H3′,5′; 7·76, d, *J* 6·6 Hz, 2H, H2′,6′; 8·01, d, *J* 13·8 Hz, 1H, H1.

(*E*)-2-Nitro-1-(4′-nitrophenyl)ethene (1e; R^1 = NO_2, R^2 = H) was obtained after purification by sublimation in vacuum (120°C/0·1 mmHg) (1·74 g, 36%), m.p. 200°C (lit.[15] 196–199°C). ^{1}H n.m.r. (300 MHz) δ 7·65, d, *J* 12 Hz, 1H, H2; 7·74, d, *J* 8·5 Hz, 2H, H2′,6′; 8·06, d, *J* 12 Hz, 1H, H1; 8·33, d, *J* 8·8 Hz, 2H, H3′,5′.

(*E*)-1-(4′-Methylphenyl)-2-nitroprop-1-ene (1g; R^1 = Me, R^2 = Me) (4·3 g, 52%) had m.p. 52–54°C. ^{1}H n.m.r. (300 MHz) δ 2·42, s, 3H, Me; 2·48, s, 3H, Me; 7·28, d, *J* 8·1 Hz, 2H, H3′,5′; 7·37, d, *J* 8·1 Hz, 2H, H2′,6′; 8·09, s, 1H, H1. The n.m.r. data agree with those previously reported.[16]

(*E*)-1-(4′-Methoxyphenyl)-2-nitroprop-1-ene (1h; R^1 = OMe, R^2 = Me) (4·5 g, 71%) had m.p. 44°C (lit.[12] 44–45°C). ^{1}H n.m.r. (300 MHz) δ 2·47, s, 3H, Me; 3·88, s, 3H, OMe; 6·95, d, *J* 8·8 Hz, 2H, H3′,5′; 7·42, d, *J* 8·8 Hz, 2H, H2′,6′; 8·10, s, 1H, H1.

(*E*)-1-(4′-Cyanophenyl)-2-nitroprop-1-ene (1j; R^1 = CN, R^2 = Me) (3·25 g, 69%) had m.p. 103–108°C. ^{1}H n.m.r. (300 MHz) δ 2·47, s, 3H, Me; 7·51, d, *J* 8·1 Hz, 2H, H3′,5′; 7·77, d, *J* 8·2 Hz, 2H, H2′,6′; 8·06, s, 1H, H1. The n.m.r. data agree with those previously reported.[16]

(*E*)-2-Nitro-1-(4′-nitrophenyl)prop-1-ene (1k; R^1 = NO_2, R^2 = Me) (4·64 g, 74%) had m.p. 114°C (lit.[17] 114–115°C). ^{1}H n.m.r. (300 MHz) δ 2·47, s, 3H, Me; 7·59, d, *J* 9 Hz, 2H, H2′,6′; 8·10, s, 1H, H1; 8·33, d, *J* 9 Hz, 2H, H3′,5′.

Reduction of Nitrostyrenes

The general procedure is outlined for the preparation of (2a).

2-Nitro-1-phenylethane (2a; R^1 = H, R^2 = H). A mixture of (*E*)-2-phenyl-1-nitroethene (1·00 g, 6·7 mmol), light petroleum (250 ml), water (6 ml) and yeast (47 g) was placed in a 500-ml round-bottom flask and stirred at room temperature. After 24 h, gas chromatography indicated that only product was present in the reaction mixture. The reaction mixture was filtered and the yeast washed with ethyl acetate (50 ml), petroleum spirit (50 ml) and ethanol (50 ml), and the solvent removed under reduced pressure. Bulb-to-bulb distillation, (140°C/1 mmHg) (lit.[18] 120–130°C/15 mmHg) gave the required product as an oil (0·74 g, 74% yield). ^{1}H n.m.r. δ 3·33, t, *J* 7·35 Hz, 2H, H1; 4·62, t, *J* 7·35 Hz, 2H, H2; 7·33, m, 5H, Ph.

1-(4′-Methylphenyl)-2-nitroethane (2b; R^1 = Me, R^2 = H)[19] was isolated as an oil (76%) after bulb-to-bulb distillation, 140°C/1 mmHg (lit.[18] b.p. 63–64°C/1 mmHg). ^{1}H n.m.r. (300 MHz) δ 2·35, s, 3H, Me; 3·30, t, *J* 7·5 Hz, 2H, H1; 4·58, t, *J* 7·5 Hz, 2H, H2; 7·00, d, *J* 9·0 Hz, 2H, H3′,5′; 7·11, d, *J* 9·0 Hz, 2H, H2′6′.

1-(4′-Methoxyphenyl)-2-nitroethane (2c; R^1 = OMe, R^2 = H)[11] was isolated as an oil (82%) (lit.[20] b.p. 135°C/0·06 mmHg). ^{1}H n.m.r. (300 MHz) δ 3·27, t, *J* 7·0 Hz, 2H, H1; 3·80, s, 3H, OMe; 4·58, t, *J* 7·0 Hz, 2H, H2; 6·88, d, *J* 9·0 Hz, 2H, H3′5′; 7·13, d, *J* 9·0 Hz, 2H, H2′,6′.

1-(4′-Cyanophenyl)-2-nitroethane (2d; R^1 = CN, R^2 = H)[7,21] was isolated as an oil (12%). ^{1}H n.m.r. (300 MHz) δ 3·36, t, *J* 7·5 Hz, 2H, H1; 4·65, t, *J* 7·5 Hz, 2H, H2; 7·34, d, *J* 8·3 Hz, 2H, H3′5′; 7·58, d, *J* 8·3 Hz, 2H, H2′,6′.

2-Nitro-1-(4′-nitrophenyl)ethane (2e; R^1 = NO_2, R^2 = H) (8%) had m.p. 97–99°C (lit.[22] 98–99°C). ^{1}H n.m.r. (300 MHz) δ 3·44, t, *J* 7·5 Hz, 2H, H1; 4·71, t, *J* 7·5 Hz, 2H, H2; 7·42, d, *J* 8·4 Hz, 2H, H2′,6′; 8·18, d, *J* 8·7 Hz, 2H, H3′,5′.

1-Phenyl-2-nitropropane (2f; R^1 = H, R^2 = Me) was isolated as an oil (80%) after bulb-to-bulb distillation, 140°C/1 mmHg (lit.[18] b.p. 87–89°C/1 mmHg). ^{1}H n.m.r. (300 MHz) δ 1·58, d, *J* 6·7 Hz, 3H, Me; 3·04, dd, *J* 6·7, 14·0 Hz, 1H, H1; 3·34, dd, *J* 6·7, 14·0 Hz, 1H, H1; 4·82, sextet, *J* 6·6 Hz, H2; 7·03, m, 5H, Ph.

1-(4′-Methylphenyl)-2-nitropropane (2g; R^1 = Me, R^2 = Me) was isolated as an oil (80%) (lit.[18] b.p. 65–67°C/0·5 mmHg). ^{1}H n.m.r. (300 MHz) δ 1·53, d, *J* 6·63 Hz, 3H, Me; 2·33, s, 3H, Me; 2·95, dd, *J* 6·8, 14·0 Hz, 1H, H1; 3·28, dd, *J* 6·8, 14·0 Hz, 1H, H1; 4·76, sextet, *J* 6·6 Hz, H2; 7·05, d, *J* 8·0 Hz, 2H, H3′,5′; 7·12, d, *J* 8·0 Hz, 2H, H2′,6′.

1-(4′-Methoxyphenyl)-2-nitropropane (2h; R^1 = OMe, R^2 = Me)[23] was isolated as an oil (43%). ^{1}H n.m.r. (300 MHz) δ 1·51, d, *J* 6·6 Hz, 3H, Me; 2·94, dd, *J* 7·47, 14·10 Hz, 1H, H1; 3·24, dd, *J* 7·47, 14·10 Hz, 1H, H1; 3·77, s, 3H, OMe; 4·71, sextet, *J* 6·7 Hz, 1H, H2; 6·88, d, *J* 8·3 Hz, 2H, H3′,5′; 7·09, d, *J* 8·5 Hz, 2H, H2′,6′.

1-(4′-Cyanophenyl)-2-nitropropane (2j; R^1 = CN, R^2 = Me) was isolated as an oil (52%) (lit.[20] b.p. 150°C/0·5 mmHg). ^{1}H n.m.r. (300 MHz) δ 1·57, d, *J* 6 Hz, 3H, Me; 3·08, dd, *J* 14·2, 5·9 Hz, 1H, H1; 3·34, dd, *J* 14·2, 8·3 Hz, 1H, H1; 4·81, m, 1H, H2; 7·30, d, *J* 8·1 Hz, 2H, H3′,5′; 7·60, d, *J* 8·2 Hz, 2H, H2′,6′.

2-Nitro-1-(4′-nitrophenyl)propane (2k; R^1 = NO_2, R^2 = Me)[7,21] (30%) had m.p. 50–53°C. ^{1}H n.m.r. (300 MHz) δ 1·60, d, *J* 6 Hz, 3H, Me; 3·16, dd, *J* 14·3, 5·8 Hz, 1H, H1; 3·41, dd, *J* 14·3, 8·4 Hz, 1H, H1; 4·85, m, 1H, H2; 7·38, d, *J* 8·7 Hz, 2H, H2′,6′; 8·14, d, *J* 8·9 Hz, 2H, H3′,5′.

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