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Synthesis of 1-nitro-2-(*p*-hydroxyphenyl)[2-$^3H_1$]ethane.

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SUMMARY

A racemic mixture of (*R*)- and (*S*)-1-nitro-2-(*p*-hydroxyphenyl)[2-$^3H_1$]ethane was synthesized from 1-nitro-2-(*p*-hydroxyphenyl)ethene by selective reduction of the olefin with sodium boro[^{3}H]hydride. The nitroethene was obtained in one step by condensation of *p*-hydroxybenzaldehyde with nitromethane. The produced racemic mixture is to be used in studies regarding the involvement of 1-nitro-2-(*p*-hydroxyphenyl)ethane as intermediate in the biosynthesis of tyrosine-derived cyanogenic glucosides and glucosinolates.

Key Words: 1-nitro-2-(*p*-hydroxyphenyl)ethane, cyanogenic glucosides, glucosinolates

INTRODUCTION

Seedlings of *Sorghum bicolor* (L.) Moench synthesize the cyanogenic glucoside dhurrin (ß-D-glucopyranosyloxy-(*S*)-*p*-hydroxymandelonitrile) derived from tyrosine (1). The biosynthetic pathway involves *N*-hydroxytyrosine, (*E*)- and (*Z*)-*p*-hydroxyphenylacetaldehyde oxime, *p*-hydroxyphenylacetonitrile and (*S*)-*p*-hydroxymandelonitrile as intermediates (Fig.1) (2). A microsomal enzyme system catalyzes the conversion of tyrosine to (*S*)-*p*-hydroxymandelonitrile, which *in vivo* is glucosylated to dhurrin and which *in vitro* dissociates into *p*-hydroxybenzaldehyde and HCN (3). The involvement of an additional hydroxylation step in the pathway has been suggested (4,5), indicating the presence of an additional intermediate. 1-Nitro-2-(*p*-hydroxyphenyl)ethane has been suggested as candidate for this hitherto unknown intermediate (4). A nitro compound has also been suggested as intermediate in the biosynthesis of glucosinolates (6). Møller and Conn have reported that administration of 1-nitro-2-(*p*-hydroxy-

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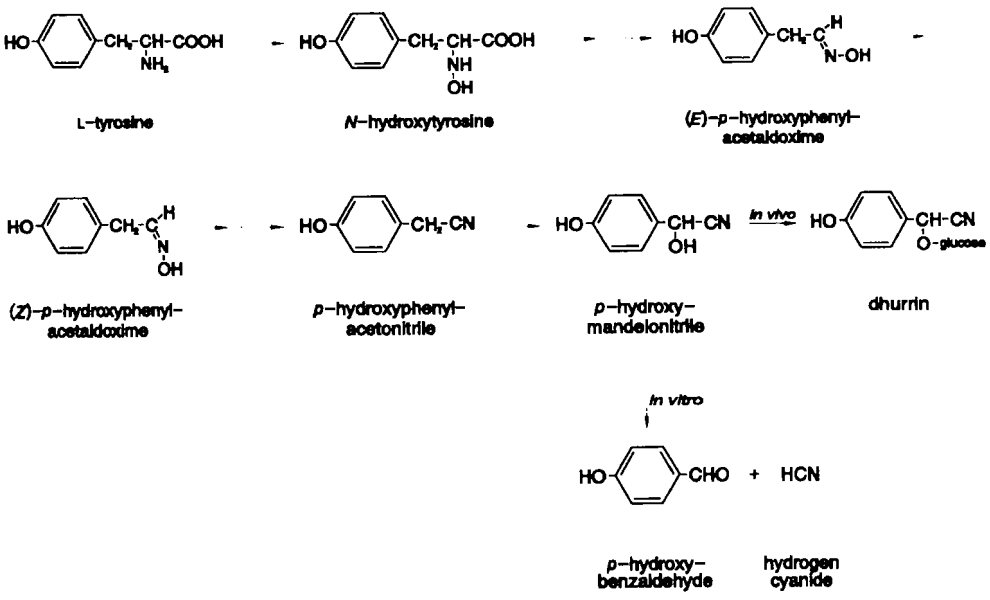

Fig. 1. The proposed biosynthetic pathway for the tyrosine-derived cyanogenic glucoside dhurrin from *Sorghum bicolor*. The 1-nitro-2-(*p*-hydroxyphenyl)ethane may be an intermediate between *N*-hydroxytyrosine and (*E*)-*p*-hydroxyphenylacetaldehyde oxime or between (*Z*)-*p*-hydroxyphenylacetaldehyde oxime and *p*-hydroxyphenyl-acetonitrile.

phenyl)ethane to the sorghum microsomes did not result in any detectable HCN production (3). This might, however, reflect the sensitivity of the spectrophotometric cyanide assay used. Due to the higher sensitivity of tracer methods, efforts were directed towards chemical synthesis of radioactively labelled 1-nitro-2-(*p*-hydroxyphenyl)ethane. In order to study whether this compound is an intermediate in tyrosine-derived cyanogenic glucosides and glucosinolates, the labelling must be positioned within that part of the molecule, which is retained in succeeding metabolites. Schiefer and Kindl have described a method for the synthesis 1-nitro-2-(*p*-hydroxyphenyl)[1-^{14}C]ethane (7). Labelling at position C-1 is not suitable for the study of the involvement of 1-nitro-2-(*p*-hydroxyphenyl)ethane as intermediate in the biosynthesis of cyanogenic glucosides, due to a 100% loss of the labelling when 1-nitro-2-(*p*-hydroxyphenyl)ethane is converted to (*S*)-*p*-hydroxymandelonitrile by the sorghum microsomal enzyme system. However, conversion of a racemic mixture of (*R*)- and (*S*)-1-nitro-2-(*p*-hydroxyphenyl)[2-$^{3}H_{1}$]ethane to (*S*)-*p*-hydroxymandelonitrile may result in retention of 50% of the radioactivity depending on the mechanism involved and on isotope effects. Similarly, enzymatic conversion of the labelled nitroethane into *desulfo*-*p*-hydroxybenzylglucosinolate may proceed with up to 100% retention of

the labelling. Thus, for studying the involvement of 1-nitro-2-(*p*-hydroxyphenyl)ethane as an intermediate in the biosynthesis of tyrosine-derived cyanogenic glucosides and glucosinolates a racemic mixture of (*R*)- and (*S*)-1-nitro-2-(*p*-hydroxyphenyl)[2-$^3H_1$]ethane seems suitable. A protocol for its synthesis is presented.

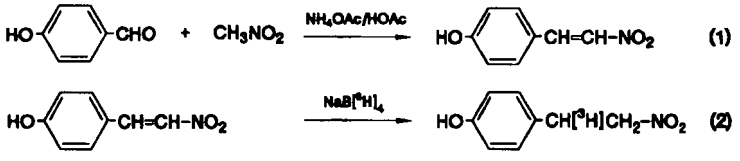

Fig.2. Reaction scheme for the synthesis of 1-nitro-2-(*p*-hydroxyphenyl)[2-$^3H_1$]ethane. Deuterium substituted and unlabelled nitro compounds were synthesized following the same protocol.

EXPERIMENTAL

Materials and methods. Sodium boro[^{3}H]hydride (spec.act. 51.8 Ci/mmol, radiochemical purity 95.9%) was from Amersham, (Buckinghamshire, England). Sodium boro[^{2}H]hydride (98% D) and nitromethane were purchased from Aldrich Chem.Co., (Milwaukee, WI). Silicagel (40 μm) was obtained from J.T.Baker Chemicals B.V. (Deventer, Holland). *p*-hydroxybenzaldehyde was purchased from Sigma (St.Louis, MO). Ethylacetate was redistilled prior to use.

Mass spectra were recorded on an AEI MS 3074 instrument using direct inlet and electron impact ionization at an electron energy of 70 eV and an ion source temperature of 200°C. ^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ on a Bruker AC250P NMR instrument using tetramethylsilane as internal standard. Melting points were determined using a Mettler FP80.

1-Nitro-2-(*p*-hydroxyphenyl)ethene.

A 0.5 l three-necked airtight flask containing *p*-hydroxybenzaldehyde (10 g, 82 mmol) and ammonium acetate (4 g, 52 mmol) was flushed with argon, and 100% acetic acid (37 ml) and nitromethane (6 ml, 110 mmol) were added. The flask was closed and the reaction mixture refluxed at 95°C (7). After 3 h, the reaction mixture was cooled in an ice bath. Addition of a trace amount of ether resulted in the formation of yellow needles composed of 1-nitro-2-(*p*-

hydroxyphenyl)ethene. The product was recrystallized from H_2O after removal of impurities with active charcoal. Mp. 152°C (rate: 0.2°C/min). Mass spectrum: *m/z* (relative intensity) 165 (35, M^+), 118 (100), 107 (25), 91 (41), 77 (17), 65 (68). ^{1}H-NMR spectrum: δ 6.90 ("d", 2H), 7.47 ("d", 2H), 7.52 (d, 1H), 7.97 (d, 2H, J=14Hz).

1-Nitro-2-(*p*-hydroxyphenyl)ethane.

1-Nitro-2-(*p*-hydroxyphenyl)ethene (2.6 g, 15.7 mmol) was dissolved in dried EtOH (100 ml) under argon. Sodium borohydride (417 mg, 11 mmol) was added and the reaction mixture stirred at room temperature. After 2 h, no 1-nitro-2-(*p*-hydroxyphenyl)ethene could be detected by TLC. pH was adjusted to 5 with 3.5 M urea in 20% HOAc (8), and ethanol removed *in vacuo*. 1-Nitro-2-(*p*-hydroxyphenyl)ethane was extracted with ethylacetate and purified by flash chromatography using ethylacetate/*n*-pentane (1:4) as the mobile phase. When taken to dryness, 1-nitro-2-(*p*-hydroxyphenyl)ethane formed a colourless oil. Yield: 36%. The colourless oil turned yellowish when left standing. Mass spectrum: *m/z* (relative intensity) 167(8, M^+), 120 (100), 107 (7), 103 (11), 91 (14), 77 (20). ^{1}H-NMR spectrum: δ 3.24 (t, 2H, J=7.3 Hz, Ph-CH_2), 4.56 (t, 3H, J=7.3 Hz, CH_2-NO_2), 6.78 ("d", 2H), 7.07 ("d", 2H).

1-Nitro-2-(*p*-hydroxyphenyl)(2-$^2H_1$)ethane.

1-Nitro-2-(*p*-hydroxyphenyl)ethene (11.5 mg, 70 μmol) was dissolved in 6 ml dried EtOH under argon. Sodium boro[^{2}H]hydride (2.9 mg, 70 μmol) was added and the reaction mixture stirred at 40°C. After 2 h, pH was adjusted to 5, and 1-nitro-2-(*p*-hydroxyphenyl)(2-$^2H_1$)ethane was purified as described above. Mass spectrum: *m/z* (relative intensity) 168 (11, M^+), 122(47), 121 (100), 120 (79), 108 (18), 104 (20), 92 (23), 78 (27), 77 (27), 65 (14). ^{1}H-NMR spectrum: δ 3.25 (m, 1H, Ph-CHD), 4.57 (d, 2H, CH_2-NO_2), 6.79 (2H), 7.08 (2H). ^{13}C-NMR spectrum (^{1}H-decoupled): δ 32.4 (t, J=20Hz, CHD-CH_2-NO_2), 77.2 (CH_2-NO_2), 115.8, 129.9, 154.8 (HO-C); C-1 in the benzene ring was too weak to be detected.

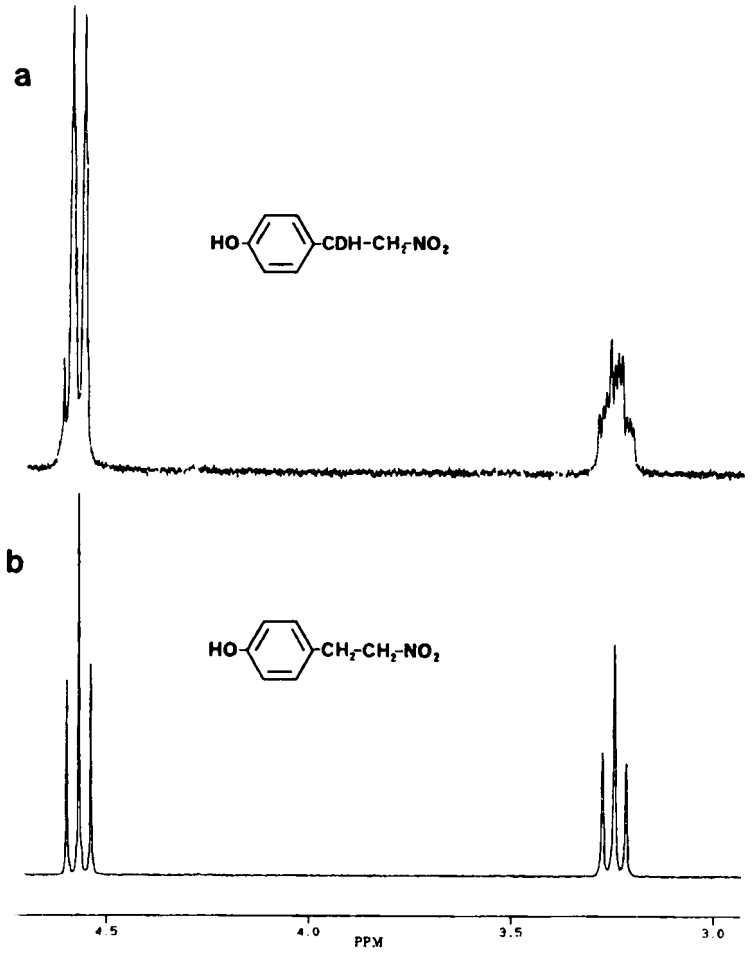

Fig.3. 250 MHz ^{1}H-NMR spectra in $CDCl_3$ of a) 1-nitro-2-(*p*-hydroxyphenyl)(2-$^2H_1$)ethane, b) 1-nitro-2-(*p*-hydroxyphenyl)ethane.

1-Nitro-2-(*p*-hydroxyphenyl)[2-$^3H_1$]ethane.

1-Nitro-2-(*p*-hydroxyphenyl)ethene (2.7 mg, 16.2 μmol) was dissolved in dried ethanol (1 ml) under argon. Then sodium boro[^{3}H]hydride (1 Ci, spec.act. 51.8 Ci/mmol) was added and the reaction mixture was stirred at 40°C under argon. After 3.5 h, pH was adjusted to 5 as described above and the reaction mixture was concentrated under a stream of nitrogen. The nitroalkane was purified by preparative TLC developed in ethylacetate/*n*-pentane (1:4). The nitroalkane was located on the TLC-plate by comparison with an authentic standard. The area was scraped off the TLC-plate and the nitroalkane eluted with ethylacetate. Radioactivity yield: 2.7%. Spec.act. 13.0 Ci/mmol, as deduced from the specific activity of the sodium boro[^{3}H]hydride.

RESULTS AND DISCUSSION

Nitroalkanes may be prepared by condensation of an aldehyde or ketone with a lower nitroalkane under basic conditions to produce the nitroalcohol, followed by an acylation and reductive elimination to produce the nitroalkane (9,10,11). A different procedure has been used for the synthesis of 1-nitro-2-(*p*-hydroxyphenyl)[1-^{14}C]ethane as described by Schiefer and Kindl (7). This procedure involves an initial condensation of *p*-hydroxybenzaldehyde with nitro[^{14}C]methane under acidic conditions to produce 1-nitro-2-(*p*-hydroxyphenyl)[1-^{14}C]ethene in one step (Fig.2, reaction 1). We adapted the procedure of Schiefer and Kindl using unlabelled nitromethane to produce a bulk of 1-nitro-2-(*p*-hydroxyphenyl)ethene.

The reduction of the 1-nitro-2-(*p*-hydroxyphenyl)ethene to 1-nitro-2-(*p*-hydroxyphenyl)ethane as described by Schiefer and Kindl involves acetylation of the phenol group in pyridine prior to reduction of the nitroethene with $LiAlH_4$ in tetrahydrofuran. In the present study, the procedure has been simplified to involve direct reduction of the nitroethene by sodium borohydride in ethanol without the initial acetylation step (Fig.2, reaction 2). Attempts to increase the ratio of ethene to borohydride were not successful. When the ratio was increased to more than 1.5 hardly any reaction occurred. The nitroalkane was liberated from its salt by addition of an urea-acetic acid solution as described by Kornblum and Graham (8).

To determine the position of the radioisotope after reduction of the nitro-ethene with sodium boro[^{3}H]hydride, an initial experiment was carried out using sodium boro[^{2}H]hydride. When sodium boro[^{2}H]hydride was used, the reaction mixture had to be heated to 40°C for the reaction to occur. ^{1}H- and ^{13}C-NMR spectra and mass spectra showed that the deuterium atom, as expected (12), was incorporated at the C-2 position (Fig.3). The presence of a symmetry plane in 1-nitro-2-(*p*-hydroxyphenyl)ethane in relation to the *cis* stereochemical course of additions of hydrogen atoms to alkenes during borohydride reduction results in the production of a racemic mixture of (*R*)- and (*S*)-1-nitro-2-(*p*-hydroxyphenyl)[2-$^{3}H_1$]ethane. The low ethene:borohydride ratio might at least partially explain the low radioactivity yield.

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