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Concise Synthesis of the Plant Growth Regulator Theobroxide

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A plant growth regulator, theobroxide, which produces potato tubers under noninduced long-day conditions, was synthesized in four steps from dihydrotoluene.

KEYWORDS: Plant growth regulator; theobroxide; practical synthesis

INTRODUCTION

The potato (*Solanum tuberosum* L.) belongs to the popular diet in many regions worldwide, especially in midlatitude countries. It is high in nutrients and also exhibits great fertility. Because *S. tuberosum* L. is a short-day plant, its cultivation has traditionally been limited to midlatitude countries. If potato production were to become possible in high-latitude countries (i.e., under long-day conditions), it could contribute to global efforts to solve nutrition problems in undernourished populations.

Theobroxide is an epoxy cyclohexene compound isolated from the culture filtrate of the fungus *Lasiodiplodia theobromae* (*1*). When sprayed on potato leaves, theobroxide induces tubers even under noninducing conditions (i.e., long days) (*2*). It has also been shown to produce flower buds in morning glory (*Pharbitis nil*) plants under long-day conditions (*2*).

The supply of theobroxide from this fungus is low, and thus efficient production by chemical synthesis is in great demand. From a practical point of view, decreasing the number of synthetic steps is one of the most important factors. We describe here a concise four-step synthesis of theobroxide (*3*−*8*).

MATERIALS AND METHODS

Preparation of 1-Methyl-1,2-dibromocyclohexa-4-ene (4). To a solution of diene **3** (13.8 g, 146 mmol) in CH_2Cl_2 (100 mL) was added pyridinium tribromide (50.1 g) in small amounts at −78 °C. After the solution had been stirred for 1 h, the reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (80 mL). The mixture was then extracted with CH_2Cl_2 (6 × 150 mL). The combined organic layers were washed with brine (500 mL) and then dried (Na_2SO_4). Removal of the solvent under conditions of reduced pressure gave a pale yellow oil, which was distilled (10 mmHg, bp 92.8−94.0 °C) to afford **4** (30.1 g, 81%) as a colorless oil: TLC (hexane/EtOAc, 15:1) $R_f = 0.6$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.68 (br s, 2H), 4.53 (br s, 1H), 3.29 (m, 1H), 2.84 (m, 1H), 2.73−2.67 (complex, 2H), 1.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 123.3, 122.0, 64.9, 56.5, 38.7, 35.2, 33.3; HRMS (EI), *m*/*z* calcd for $C_7H_{10}Br_2$ ($[M]^+$) 251.9149, found 253.9113.

Preparation of *rac*-Theobroxide (1) (**Figure 1; Scheme 1**). Urea hydrogen peroxide (30.1 g, 319 mmol) and sodium carbonate (50.2 g, 473 mmol) were mixed and dried under vacuum conditions for 1 h. Then, CH_2Cl_2 (100 mL) was added to the mixture under a nitrogen atmosphere at 0 °C, followed by the addition of alkene **4** (10.4 g, 40.9 mmol). Trifluoroacetic anhydride (25.2 g 120 mmol) was added in a dropwise manner, and the mixture was stirred for 1 h at 0 °C. The reaction was warmed to room temperature for 1 h and then was cooled to 0 °C. Ten percent aqueous Na_2SO_3 (200 mL) was added, and stirring was continued for 1 h at an ice-cold temperature. The mixture was extracted with CH_2Cl_2 (3 × 200 mL), and the combined organic layers were washed with brine (700 mL) and then dried (Na_2SO_4). Removal of the solvent under conditions of reduced pressure, followed by silica gel column chromotography (hexane/EtOAc, 10:1) gave 1-methyl-1,2-dibromo-4,5-epoxycyclohexanes **5** (10.7 g, 96%) as a diasteromeric mixture (*18*).

To a THF solution (15 mL) of 1-methyl-1,2-dibromo-4,5-epoxycyclohexane (4.45 g, 16.4 mmol) was added potassium *tert*-butoxide (3.76 g, 33.5 mmol) in small amounts at 0 °C. After the mixture had been stirred at the same temperature for 15 min, the insoluble materials were removed by centrifugation (3000 rpm, 15 min). The supernatant containing toluene oxide **2** was employed for the following steps without the removal of the solvent. **2**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.95 (d, 1H, $J = 7.8$ Hz), 5.85 (dd, 1H, $J = 4.3$, 7.8 Hz), 5.68 (dd, 1H, $J = 0.6$, 4.2 Hz), 4.69 (m, 2H), 1.79 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.2, 129.0, 122.1, 120.9, 50.4, 50.2, 22.1.

Oxygen was bubbled through a solution of rose bengal (157 mg, 0.149 mmol) in THF (400 mL) for 30 min at room temperature. The solution was cooled to −40 °C, and the solution of toluene oxide **2** (15 mL) was added under a high-pressure Hg lamp. Irradiation was continued for 3 h with stirring, and the temperature of the solution was maintained under −35 °C during the period. Thiourea (1.86 g, 24.4 mmol) in MeOH (30 mL) was added, and the mixture was warmed to room temperature. After the mixture had been stirred for 13.5 h, the solvent was removed under reduced pressure, and a dark red residue was purified by silica gel column chromatography (Et_2O/MeOH, 100:1), thus giving racemic theobroxide **1** (1.11 g, 47%) as a colorless solid: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.50 (dt, 1H, $J = 2.7$, 8.4 Hz), 4.45 (d, 1H, $J = 7.2$ Hz), 4.24 (s, 1H), 3.47 (br s, 1H), 3.35 (m, 1H), 3.28 (m, 1H), 1.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz)

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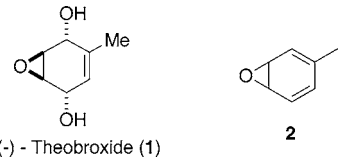

Figure 1. Structure of theobroxide **1** and a possible intermediate **2**.

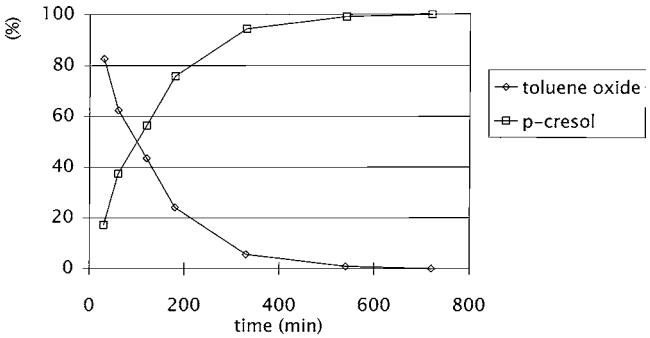

Figure 2. Stability of toluene oxide **2** in methanol-d_4 (400 MHz ^{1}H NMR) at 25 °C.

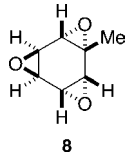

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Figure 3. Structure of triepoxide **7**.

δ 135.1, 121.5, 66.2, 63.0, 52.9, 51.8, 21.2; IR (KBr, cm^{-1}) 3250; HRMS (EI), *m*/*z* calcd for $C_7H_{10}O_3$ (M^+) 142.0630, found 142.0645.

Preparation of Silyl Ether (9). To a mixture of racemic theobroxide (**1**, 700 mg, 4.92 mmol) in DMF (20 mL) were added *tert*-butyldimethylsilyl chloride (713 mg, 6.13 mmol) and imidazole (1.00 g, 14.6 mmol) at 0 °C under a nitrogen atmosphere. After the mixture had been stirred for 5.5 h, the reaction was quenched with water, and the mixture was concentrated under reduced pressure. The resulting oily residue was purified by silica gel column chromatography (hexane/EtOAc, 50:1 to 0:1), thus giving **9** (459 mg, 36%, 51% based on recovered starting material) as a colorless oil. Disilyl ether (107 mg, 6%) was also obtained.

9: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.38 (dt, 1H, J = 5.0, 1.7 Hz), 4.45 (br d, 1H, J = 5.0 Hz), 4.16 (s, 1H), 3.39 (m, 1H), 1.88 (m, 1H), 1.82 (br s, 3H), 0.91 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 134.9, 122.2, 66.5, 63.9, 53.0, 52.2, 25.8 (3C), 21.1, 18.2, −4.5, −4.7; IR (KBr, cm^{-1}) 3217; HRMS (FAB), *m*/*z* calcd for $C_{13}H_{25}O_2Si$ ($[M + 1]^+$) 257.1573, found 257.1570.

Preparation of Acetate (10). Alcohol (**9**, 409 mg, 1.59 mmol) and lipase TL (405 mg, Meito Sangyo Co., Ltd., Nagoya, Japan) in vinyl acetate (16 mL, 171 mmol) were stirred at room temperature for 1 week. The mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc, 50:1 to 2:1), giving **10** (152 mg, 32%) as a colorless oil. Unreacted **9** (236 mg) was also recovered. The ^{1}H NMR of **9** was measured in $CDCl_3$ with 50 mol % of tris[3-(heptafluoropropylhydroxymethylene)−(+)-camphorato] europium, Eu(hfc)$_3$. The enantiomeric excess of **10** thus obtained was >99% ee.

10: $[\alpha]_D^{26}$ +10° (*c* 0.26, EtOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.47 (complex, 2H), 4.46 (m, 1H), 3.26 (m, 1H), 3.19 (m, 1H), 2.14 (s, 3H), 1.69 (s, 3H), 0.92 (s, 9H), 0.13 (s, 3H), 0.13 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.9, 129.1, 124.6, 66.7, 63.5, 52.9, 51.5, 45.2, 25.8(3C), 20.9, 20.6, −4.4, −4.5; IR (neat, cm^{-1}) 1747; HRMS (FAB), *m*/*z* calcd for $C_{15}H_{27}O_3Si$ ($[M + H]^+$) 299.1679, found 299.1655.

Preparation of (−)-Theobroxide (1). (+)-**10** (8.3 mg, 27.8 μmol) was dissolved in THF (1.0 mL). A TBAF (1 M in THF)/acetic acid (5:1, 0.1 mL) mixture was added at room temperature. After the mixture had been stirred for 12 h, the TBAF/acetic acid mixture was added, and the resulting mixture was further reacted for 18 h. The reaction was poured into saturated aqueous $NaHCO_3$, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Removal of the solvent in vacuo gave a pale yellow oil, which was subjected to preparative thin-layer chromatography (silica gel, hexane/EtOAc, 1:1) to afford (−)-2-*O*-acetyl theobroxide (5.1 mg, quant.).

To a solution of (−)-2-*O*-acetyl theobroxide (35.2 mg, 186 μmol) in MeOH (2 mL) was added dropwise 28% aqueous NH_3 (1.0 mL) at room temperature. The reaction was stirred for 17 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (hexane/EtOAc, 1:1 to 0:1), thus giving (−)-**1** (24.7 mg, 93%) as colorless needles: $[\alpha]_D^{26}$ −5° (*c* 0.4, EtOH).

RESULTS AND DISCUSSION

We envisaged that the singlet oxygen oxidation of 4-toluene oxide **2** would afford theobroxide in a straightforward manner. Although the preparation of **2** has been described in the literature (*9*), neither the detailed procedure nor the yields of each synthetic step have been described to date. Thus, we optimized the yields by improving reaction conditions.

The synthesis started with 1-methylcyclohexa-1,4-diene **3**, which is commercially available. The trisubstituted alkene of **3** was chemoselectively brominated with pyridinium tribromide. The attempted epoxidation of the resulting alkene with *m*-chloroperbenzoic acid at room temperature failed. When trifluoroperacetic acid was generated in the presence of **4** from urea hydrogen peroxide and trifluoroacetic anhydride, the desired epoxide **5** was obtained in 96% yield as a 1.2:1 mixture of diastereomers. The treatment of epoxides with potassium *tert*-butoxide gave a solution of 4-toluene oxide **2**. After the removal of insoluble KBr by centrifugation, the supernatant could be employed for the next synthetic step.

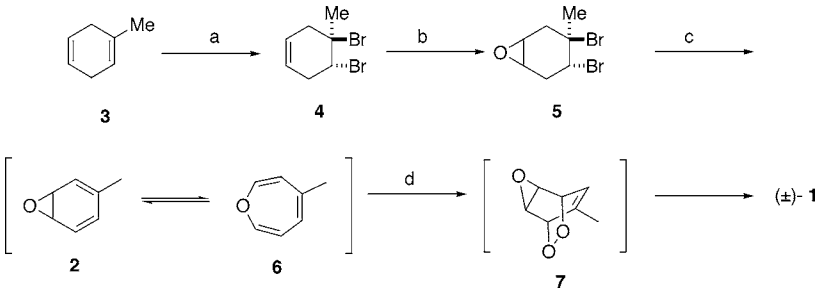
It was previously noted that the benzene oxide and its derivatives were especially sensitive to traces of acid. In early studies (*9*, *10*), glassware washed with 10% aqueous NaOH was employed to handle arene oxides. Witkop reported that toluene oxide is more unstable than benzene oxide (*9*). Thus, we first conducted reactions in plastic tubes (polypropylene); however, this precaution turned out to be unnecessary. The stability of toluene oxide **2** in various solvents was investigated by NMR experiments. The oxide **2** was unexpectedly stable at room temperature either in $CDCl_3$ or in THF-d_8 (data not shown). There was no indication of any degradation products after 12 h. Although arene oxides similar to **2** could be in equilibrium with the corresponding oxepin, the NMR spectra showed only one set of signals.

In contrast, when protic methanol-d_4 was employed as the solvent, **2** was quantitatively transformed to *p*-cresol via the NIH shift (*19*). The half-life of **2** was found to be 100 min in methanol at room temperature (**Figure 2**).

The addition of singlet oxygen to the toluene oxide was then explored. Although the corresponding transformation with oxepin−benzene oxide was achieved by Foote (H_2O_2, aqueous NaOCl, MeOH) (*11*) in 37% yield, the application of these conditions to **2** did not afford the desired adduct; instead, *p*-cresol was obtained. Presumably, **2** cannot survive in protic reaction media, as indicated by the above-mentioned NMR experiments.

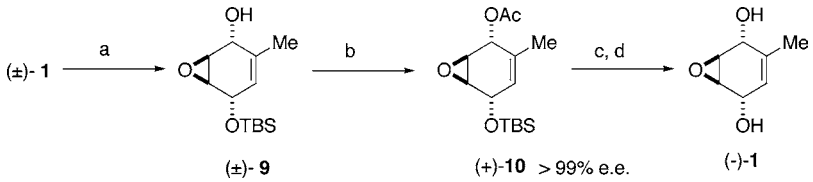
Photosensitized oxidation was then investigated. Oxygen was passed through a THF solution of rose bengal and toluene oxide

Scheme 1. Preparation of *rac*-Theobroxide via 4-Toluene Oxide[a]



[a] Reagents and conditions: (a) Pyr-HBr_3, CH_2Cl_2, −78 °C (81%); (b) urea hydrogen peroxide, TFAA, Na_2CO_3, CH_2Cl_2, 0 °C to room temperature (96%); (c) t-BuOK, THF, 0 °C; (d) O_2, rose bengal, THF, *hν*, −35 °C; then thiourea, rt (47% in two steps).

Scheme 2. Optical Resolution of *rac*-Theobroxide **1** by Lipase[a]



[a] Reagents and conditions: (a) TBSCl, imidazole, DMF, 0 °C (51%); (b) lipase TL, vinyl acetate, rt (32%, >99 ee); (c) TBAF, acetic acid, THF, rt (99%); (d) aq NH_3, MeOH, rt (93%).

2, and the solution was irradiated with a high-pressure Hg lamp at −35 °C. Upon the reduction of endoperoxide **7** (**Figure 3**) (*12*−*14*) with thiourea, theobroxide was afforded in 37% overall yield in only four steps from starting material **3**.

Because THF was employed as the reaction medium, the formation of *p*-cresol was not observed in the above photosensitized oxidation reaction. The structures of the side products of the oxidation were not fully characterized; however, the formation of toluene trioxide **8** was revealed by ^{1}H NMR analysis. A related benzene−trioxide was previously reported by Berchtold (*15*). To prevent **7** from rearranging into the trioxide **8**, photooxidation was conducted in the presence of thiourea; this approach was taken to reduce the endoperoxide intermediate in situ before the rearrangement, but this resulted in no significant improvement in the yield (*16*).

At this stage, we were pleased to know that racemic theobroxide exhibited similar levels of tuber-inducing activity as those of a natural sample (*17*).

However, we did perform the enzymatic resolution of racemic theobroxide, as the natural theobroxide exists in the (−)-form.

Racemic theobroxide **1** was monosilylated at a less hindered hydroxyl group (regioselectivity 30:1). Treatment of **9** with lipase TL (**Scheme 2**; Meito Sangyo Co., Ltd., Nagoya, Japan) in vinyl acetate as the solvent afforded chiral acetate (+)-**10** (32%). Unreacted alcohol was recovered in a 57% yield. The optical purity of (+)-**10** was determined as being >99% ee by ^{1}H NMR employing Eu(hfc)$_3$ as the chiral shift agent. The removal of TBS ether and acetate gave (−)-theobroxide **1** in a quantitative yield.

In summary, we developed a concise and high-yield access to theobroxide with the singlet oxygen oxidation of arene oxide as the key step. The optical resolution of racemic theobroxide was also achieved with lipase. These results will promote field tests of theobroxide, which may enable the production of potato plants under long-day conditions.

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(18) These diastereomers (ratio 1.2:1) could be separated from each other by silica gel column chromatography (hexane/EtOAc, 15:1). The major isomer was further purified by recrystalization from hexane/EtOAc (15:1), and the minor isomer was recrystalized from hexane.

(19) The major isomer: TLC (hexane/EtOAc, 5:1) $R_f = 0.4$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.35 (dd, 1H, $J = 2.4$, 5.6 Hz), 3.28 (s, 2H), 3.01 (ddd, 1H, $J = 2.4$, 5.6, 16.8 Hz), 2.73 (dd, 1H, $J = 2.2$, 16.8 Hz), 2.59 (d, 2H, $J = 1.4$ Hz), 1.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 65.2, 52.0, 50.2, 49.5, 37.6, 33.2, 32.4; HRMS (EI) *m*/*z* calcd for $C_7H_{10}BrO$ ($[M - Br]^+$) 188.9915, found 188.9904. The minor isomer: TLC (hexane/EtOAc, 5:1) $R_f = 0.3$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.38 (dd, 1H, $J = 7.4$, 3.9 Hz), 3.20−3.16 (m, 2H), 2.91 (ddd, 1H, $J = 0.5$, 15.9, 16.4 Hz), 2.73 (dd, 1H, $J = 16.4$ Hz), 2.66 (dd, 2H, $J = 3.9$, 16.2 Hz), 2.46 (ddd, 1H, $J = 3.5$, 7.4, 15.9 Hz), 1.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 61.4, 56.1, 50.5, 50.4, 39.9, 33.9, 30.3; HRMS (EI) *m*/*z* calcd for $C_7H_{10}BrO$ ($[M - Br]^+$) 188.9915, found 188.9904.

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