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Preparative-Scale Synthesis of Two Metabolites Isolated from Soil Treated with Zoxium Fungicide and Kerb Herbicide

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The preparation in multigram scale of two metabolites 3-(3,5-dichloro-4-methyl-benzoylamino)-2-hydroxy-3-methyl-pentanoic acid and 3-(3,5-dichloro-benzoylamino)-3-methyl-2-oxo-butyric acid isolated from soil treated with either Zoxium fungicide or Kerb herbicide was efficiently accomplished using a common 5-step synthetic process starting from easily available raw materials.

KEYWORDS: Zoxium fungicide; Kerb herbicide; metabolites

INTRODUCTION

3,5-Dichloro-*N*-(3-chloro-1-ethyl-1-methyl)-4-methylbenzamide (Zoxium, RH-7281, compound **1**, **Figure 1**) is a new, high-performance fungicide currently under development by Rohm and Haas Co. for foliar use on potatoes, vines, and vegetables (*1*). RH-7281 exhibits excellent preventive efficacy against *Plasmopara viticola*, *Phytophthora infestans*, and *Pseudoperonospora cubensis.* It also has exceptionally low mammalian toxicity and is very safe to the environment (*1*). RH-7281 and Pronamide (Kerb herbicide, **2**) have similar structural features (**Figure 1**). Metabolism studies with ^{14}C-aromatic ring labeled **1** have shown that 3-(3,5-dichloro-4-methyl-benzoylamino)-2-hydroxy-3-methyl-pentanoic acid (**3**) can be isolated from treated soil (*2*, *7*, *8*). The structurally related 3-(3,5-dichloro-benzoylamino)-3-methyl-2-oxo-butyric acid (**4**) was reported by Yih and Swithenbank (*3*) in 1972 as a minor metabolite of **2** in both soil and alfalfa. Multigram quantities of metabolites **3** and **4** were required to complete the safety assessment of both Zoxium fungicide and Kerb herbicide. This paper describes the preparation on a multigram scale of these two metabolites (compounds **3** and **4**).

RESULTS AND DISCUSSION

Compounds **3** and **4** show a common 3-amino-2-oxo-3,3-dialkyl moiety. A general synthesis for the preparation of multigram amounts of these two compounds can be envisioned as starting from epoxides **5** or **10**, **Schemes 1** and **2**, readily prepared by the Darzens condensation of ketones and chloroacetate esters (*4*). Ring-opening of **5** or **10** with aqueous ammonia could yield the key intermediate β-hydroxyamine **6** or **11** (*5*). Benzoylation of the amino group present in **6** or **11** would afford **7** or **13**, followed by hydrolysis to the carboxylic acid **9** or **14**, and finally, oxidation of the hydroxy group should lead to the desired compounds **3** and **4** (**Schemes 1** and **2**).

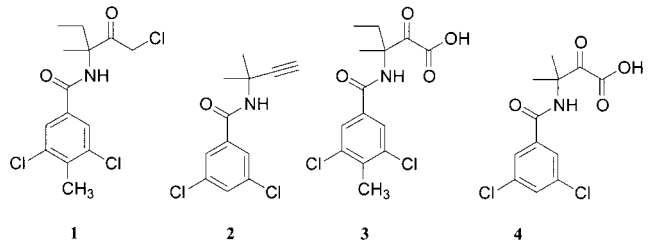

Figure 1.

Synthesis of compound **3** was accomplished in moderate yield by this pathway (**Scheme 1**) starting from methyl 2,3-epoxy-3-methyl pentanoate (**5**) (*4*), which was prepared in 59% yield from butanone and methyl chloroacetate following the method of J. R. Sjolande et al. (*3*) Ring-opening of **5** by treatment with aqueous ammonia in a sealed tube using a modified literature (*4*) procedure yielded the key α-hydroxy-β-aminoamide derivative **6** in 61% yield. Benzoylation of **6** with an equimolecular amount of 3,5-dichloro-4-methylbenzoyl chloride afforded benzamide **7** in 60% yield. On the contrary, the use of an excess of 3,5-dichloro-4-methylbenzoyl chloride in aqueous sodium hydroxide led to a 1:1 mixture of diacyl derivative **8** and benzamide **7**. Treatment of this mixture with ethanolic sodium hydroxide yielded the desired benzamide **7** in only 41% yield.

Selective hydrolysis of pentanamide **7** to the corresponding carboxylic acid in the presence of the benzamide was accomplished by a treatment with sodium nitrite in aqueous acid yielding **9** in a 71% yield. The latter can be easily oxidized to the desired **3** using DMSO/oxalyl chloride (*6*) with 51% yield. The overall yield from **5** to **3** was 21%.

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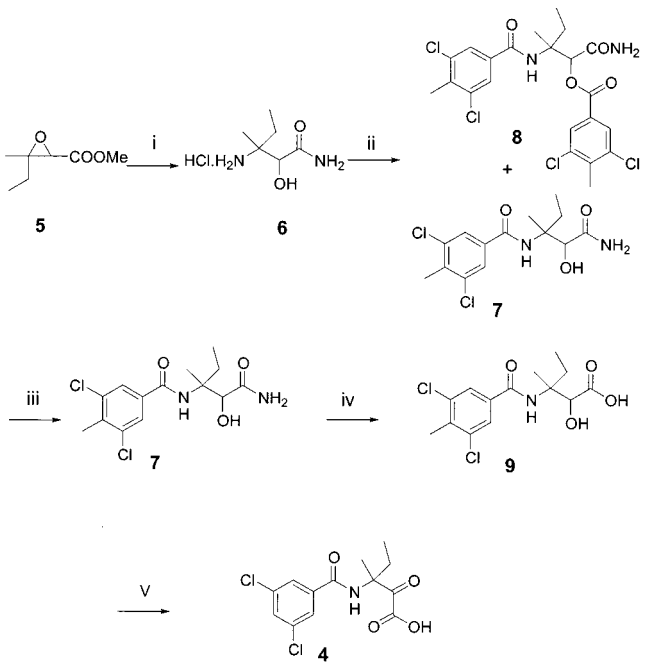
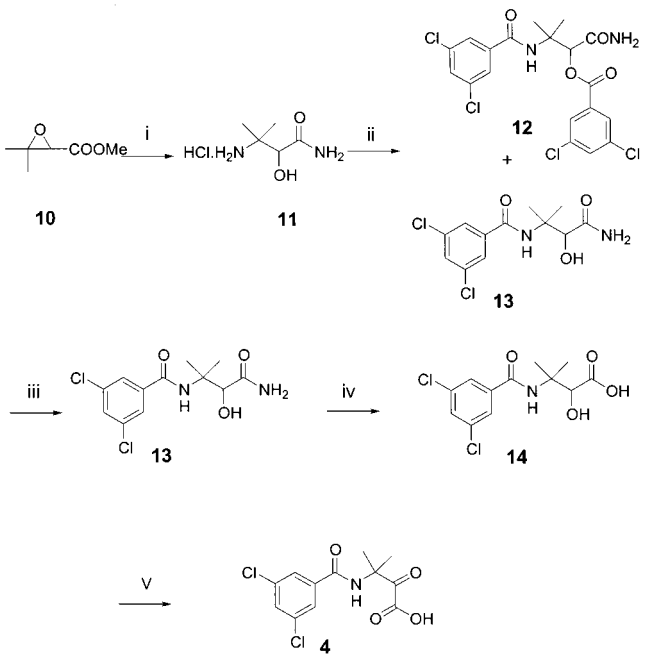
Scheme 1[a]



[a] (i) 30% NH_3(aq), 100 °C, 8 h. (ii) 3,5-Dichloro-4-methylbenzoyl chloride, NaOH. (iii) NaOH, ethanol, reflux 3 h. (iv) HNO_2, H_2O. (v) DMSO/oxalyl chloride, TEA.

Scheme 2[a]



[a] (i) 30% NH_3(aq), 100 °C, 8 h. (ii) 3,5-Dichlorobenzoyl chloride, NaOH. (iii) NaOH, ethanol, reflux 3 h. (iv) HNO_2, H_2O. (v) DMSO/oxalyl chloride, TEA.

Compound **4** was synthesized in a similar way starting from acetone and 3,5-dichlorobenzoyl chloride (**Scheme 2**). Thus, epoxide **10** was prepared in 44% yield by the condensation of acetone and methyl chloroacetate. Ring-opening of **10** using aqueous ammonia yielded β-hydroxyamine **11** in 32% yield, which by treatment with 3,5-dichlorobenzoyl chloride yielded the diacyl derivative **12** in 69% yield. Selective hydrolysis of **12** in ethanolic sodium hydroxide afforded compound **13** in quantitative yield, which in turn can be hydrolyzed to the corresponding carboxylic acid (**14**) by treatment with nitrous acid in dioxane in 68% yield. Finally, oxidation of **14** yielded **4** in 77% yield.

CONCLUSIONS

The preparation in multigram scale of two metabolites (**3** and **4**) isolated from soil treated with either Zoxium fungicide or Kerb herbicide was efficiently accomplished using a common 5-step synthetic process starting from either acetone or butanone.

EXPERIMENTAL PROCEDURES

Infrared spectra were recorded in a Nicolet Magna 560 Fourier transform infrared spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined in a Varian Gemini-300 operating at field strengths of 300 and 75.5 MHz, respectively, or a Varian-200 operating at a field strength of 200 MHz. Chemical shifts are reported in parts per million (δ), and coupling constants (J) are reported in Hertz using, in the case of ^{1}H NMR, tetramethylsilane or sodium 2,2,3,3-tetradeuteriotrimethylsilylpropionate as an internal standard and setting, in the case of ^{13}C NMR, the references at the signal of the solvent. Standard and peak multiplicities are designated as follows: s, singlet; t, triplet; m, multiplet. Tetrahydrofuran (THF) was distilled from $LiAlH_4$ and kept over 4 Å molecular sieves. Triethylamine was freshly distilled before use. Dimethyl sulfoxide was distilled in vacuo and kept over 4 Å molecular sieves.

Methyl 2,3-Epoxy-3-methylpentanoate (5). A mixture of distilled butanone (61.2 g, 0.85 mol) and of methyl chloroacetate (92.2 g, 0.85 mol) was added dropwise to a stirred cooled (ice bath) suspension of sodium methoxide (51.0 g, 0.94 mol) in diethyl ether (165 mL) over a period of 3 h. The reaction mixture was allowed to warm gradually to room temperature and stirred overnight. The resulting orange mixture was heated on a water bath for 3 h and poured into ice water:H_2SO_4 (98%) (380 mL:7.5 mL); diethyl ether (300 mL) was added, and the layers were separated. The aqueous phase was extracted once with diethyl ether (300 mL), and the combined ether extracts were washed with water (400 mL), 10% aqueous $NaHCO_3$ (4 × 400 mL), and water (400 mL) and dried over anhydrous magnesium sulfate. The ether was removed under vacuum, and the resulting oily residue was distilled in vacuo through a short Vigreux column to afford 80.2 g (65%) of **5** (diastereomeric mixture) as a colorless liquid collected at 54 °C (4 mm Hg). IR (film, cm^{-1}): 2973, 2942, 2883 (C−H), 1757, 1735 (C═O). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 3H, −OCH_3), 3.36 (s, 1H, H2), 1.77−1.56 (m, 2H, H4), 1.40, 1.35 (s, 3H, CH_3), 0.99 (t, 3H, H5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.0, 168.8 (C1), 63.6, 63.4 (C3), 59.3, 58.0 (C2), 52.0 (OCH_3), 30.4, 25.2 (C4), 20.8, 15.7 (CH_3), 9.4, 8.8 (C5).

Methyl 2,3-Epoxy-3-methylbutyrate (10). To a cooled (5 °C) mixture of acetone (26.7 g, 0.46 mol), diethyl ether (160 mL), and methyl chloroacetate (50 g, 0.46 mol) was added sodium methoxide (39.8 g, 0.74 mol) portionwise over a period of 2 h. After the addition was completed, the reaction mixture was stirred at 5 °C for an additional 8 h. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was poured into a mixture of crushed ice:HCl (36%) (200:30 mL. The phases were separated, and the organic phase was washed with water (2 × 100 mL), saturated aqueous $NaHCO_3$ (1 × 100 mL), water (1 × 100 mL), and brine (1 × 100 mL) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo to give 26.4 g (44%) of the desired product as a pale yellow liquid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 3H), 1.5 (s, 3H), 3.4 (s, 1H), 3.8 (s, 3H).

3-Amino-2-hydroxy-3-methylpentanamide Hydrochloride (6). A mixture of **5** (76.9 g, 0.53 mol) and 30% aqueous ammonia (340 mL) was heated in an autoclave at 100 °C for 8 h. The resulting mixture was cooled to room temperature overnight, and the solvent was removed in vacuo. The residue was dissolved in methanol (500 mL) and was acidified to pH 1 with concentrated HCl. The solvent was removed under vacuum. The resulting orange oil was taken up in the minimum amount of methanol, diethyl ether was added slowly until cloudiness, and the solution was cooled yielding a solid that was collected by

vacuum filtration yielding 59.1 g (61%) of 3-amino-2-hydroxy-3-methylpentanamide hydrochloride **6**. IR (KBr, cm^{-1}): 3415, 3314, 3190 (N−H, O−H), 2980, 2921 (C−H), 1672 (C═O). ^{1}H NMR (300 MHz, D_2O): δ 4.19 (s, 1H, H2), 1.88−1.60 (m, 2H, H4), 1.36 (s, 3H, CH_3), 0.99 (t, 3H, H5). ^{13}C NMR (75.5 MHz, D_2O): δ 177.8 (C1), 74.0 (C2), 60.6 (C3), 29.1 (C4), 20.3 (CH_3), 8.2 (C5).

3-Amino-2-hydroxy-3-methylbutyramide Hydrochloride (11). Compound **11** was prepared in analogous fashion from **10** as a white solid in 32% yield. ^{1}H NMR (200 MHz, D_2O): δ 0.3 (s, 3H), 1.35 (s, 3H), 4.1 (s, 1H), 4.7 (s, 7H).

***N*-[1-(Carbamoyl-hydroxy-methyl)-1-methyl-propyl]-3,5-dichloro-4-methyl-benzamide (7). Procedure A.** To a solution of sodium hydroxide (6.6 g, 0.164 mol) and **6** (10.0 g, 0.055 mol) in water (51 mL) was added dropwise a solution of 3,5-dichloro-4-methylbenzoyl chloride (24.5 g, 0.109 mol) in toluene (66 mL) over a period of 1 h, and the resulting mixture was stirred for 2 h at room temperature. The solid formed was collected by vacuum filtration, washed with water, toluene, and methanol, and dried in vacuo at 50 °C overnight to afford 17.1 g of a yellow solid 1:1 mixture of 3,5-dichloro-4-methyl-benzoic acid 1-carbamoyl-2-(3,5-dichloro-4-methyl-benzoylamino)-2-methyl-butyl ester (**8**) and **7**. This mixture was suspended in a solution of sodium hydroxide (1.31 g, 33 mmol) in ethanol (125 mL) and refluxed for 3 h. The reaction mixture was cooled to room temperature, and the solvent was removed in vacuo. The residue was slurred in water, and the solid was collected by vacuum filtration, washed with toluene, and dried in vacuo at 50 °C overnight to yield 7.5 g of **7**. IR (KBr, cm^{-1}): 3445, 3334, 3283 (N−H, O−H), 2974, 2939, 2879 (C−H), 1678, 1650 (C═O). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.49 (s (D), 1H, NH), 7.82 (s, 2H, Ph), 7.58 (s, 2H, NH_2), 6.15 (s, 1H, OH), 4.15 (s, 1H, H2), 2.50 (s, 3H, Ph-CH_3), 2.14−1.93 (m, 2H, H4), 1.27 (s, 3H, CH_3), 0.81 (t, 3H, H5). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 176.3 (C1), 162.7 (Ph-CO), 136.4, 135.5, 134.5, 126.4 (Ph), 70.9 (C2), 58.9 (C3), 26.4 (C4), 19.2 (Ph-CH_3), 17.3 (CH_3), 7.7 (C5).

Procedure B. A solution of sodium hydroxide (16.5 g, 0.413 mol) and **6** (25.0 g, 0.137 mol) in water (128 mL) was added dropwise with vigorous stirring over a 1 h period to a solution of 3,5-dichloro-4-methylbenzoyl chloride (15.4 g, 0.069 mol) in toluene (41 mL). After the addition was completed, the reaction mixture was stirred for 2 h at room temperature. The resulting solid was collected by vacuum filtration, washed with water, toluene, and methanol, and dried in vacuo at 50 °C overnight to give 11.6 g (51%) of a white solid 14:1 mixture of **7** and an unidentified impurity.

***N*-(2-Carbamoyl-2-hydroxy-1,1-dimethyl-ethyl)-3,5-dichloro-benzamide (13).** A 2:1 mixture of 3,5-dichloro-benzoic acid 1-carbamoyl-2-(3,5-dichloro-benzoylamino)-2-methyl-propyl ester (**12**) and *N*-(2-carbamoyl-2-hydroxy-1,1-dimethyl-ethyl)-3,5-dichloro-benzamide was prepared using the same procedure as above in 69% yield. ^{1}H NMR (200 MHz, DMSO-d_6): mix δ 1.35 (s), 0.4 (s), 1.5 (s), 1.6 (s), 3.4 (s, b), 4.2 (s), 5.7 (s), 6.1 (s, b), 7.45 (s, b), 7.5 (s, b), 7.7 (s, b), 7.75 (s), 7.95 (t), 8.0 (t), 8.4 (s, b), 8.45 (s, b). Hydrolysis of the ester was carried out in standard procedure to yield quantitatively butyramide **13**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.4 (d, 6H), 4.2 (s, b, 1H), 6.0 (s, b, 1H), 7.4 (d, b, 2H), 7.8 (s, 3H), 8.4 (s, b, 1H).

***N*-[1-(Carbamoyl-hydroxy-methyl)-1-methyl-propyl]-3,5-dichloro-4-methyl-benzamide (9).** To a suspension of **7** (17.0 g, 0.051 mol) in dioxane (283 mL) was added dropwise concentrated hydrochloric acid (43 mL, 0.516 mol), followed by portionwise addition of solid sodium nitrite (35.2 g, 0.510 mol). The resulting mixture was stirred overnight at room temperature. The reaction mixture was poured into iced water (680 mL) and extracted with diethyl ether (2 × 340 mL). The combined organic layers were dried over anhydrous magnesium sulfate, the ether was removed in vacuo, and water (250 mL) was added to the resulting residue. The resulting suspension was cooled at 0 °C and 50% sodium hydroxide solution was added until most of the solid was dissolved (approximately 180 mL). The suspension was filtered, and the filtrate was brought to pH 1 with concentrated hydrochloric acid. Trituration in hexane afforded 12.0 g of **9** (71%) as a light yellow solid. IR (KBr, cm^{-1}): 3400−3080 (O−H, N−H), 2972, 2939, 2881 (C−H), 1719, 1652 (C═O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.22 (s (D), 1H, NH), 7.82 (s, 2H, Ph), 4.42 (s, 1H, H2), 2.47 (s, 3H, Ph-CH_3), 2.20−2.11, 1.81−1.74 (m, 2H, H4), 1.21 (s, 3H, CH_3), 0.79 (t, 3H, H5). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 174.6 (C1), 164.2 (CONH), 136.8, 135.6, 134.8, 127.0 (Ph), 72.4 (C2), 59.0 (C3), 27.3 (C4), 19.0 (Ph-CH_3), 17.6 (CH_3), 8.0 (C5).

3-(3,5-Dichloro-benzoylamino)-2-hydroxy-3-methyl-butyric Acid (14). Compound **14** was prepared in an analogous fashion from **13** as a white solid in 68% yield; mp = 162−164 °C (lit mp = 161−163 °C). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.4 (d, 6H), 4.5 (s, 1H), 5.6 (s, b, 1H), 7.8 (s, 3H), 8.0 (s, 1H), 12.6 (s, b, 1H).

3-(3,5-Dichloro-4-methyl-benzoylamino)-2-hydroxy-3-methyl-pentanoic Acid (3). A solution of dimethyl sulfoxide (5.0 g, 0.066 mol) in dichloromethane (46 mL) was added dropwise to a solution of oxalyl chloride (6.0 g, 0.048 mol) in dichloromethane (73.2 mL) keeping the temperature below −55 °C. The resulting mixture was stirred for 5 min, and then, a solution of **9** (10.0 g, 0.030 mol) in tetrahydrofuran (73 mL) was added dropwise over a 20 min period. Stirring was continued at −50 to −60 °C for 30 min. Triethylamine (15.0 g, 0.150 mol) in dichloromethane (23.2 mL) was added, and the mixture was stirred for another 10 min. The reaction mixture was allowed to warm to room temperature over a 30 min period and diluted with water: dichloromethane (1:1, 1000 mL), and the two layers were separated. The organic layer was evaporated in vacuo, and water was added to the residue, acidified to pH 1 with concentrated hydrochloric acid, and extracted with ethyl acetate. The organic extracts were combined, dried over anhydrous magnesium sulfate, filtered, evaporated in vacuo, and washed with hexane to give 5.0 g (50%) of **3** as a white solid. IR (KBr, cm^{-1}): 3358−3084 (O−H, N−H), 2988, 2974, 2884 (C−H), 1742, 1727 (C═O). ^{1}H NMR (300 MHz, acetone-d_6): δ 8.43 (s (D), 1H, NH), 7.85 (s, 2H, Ph), 2.49 (s, 3H, Ph-CH_3), 2.20−2.06 (m, 2H, H4), 1.57 (s, 3H, CH_3), 0.91 (t, 3H, H5). ^{13}C NMR (75.5 MHz, acetone-d_6): δ 195.8 (C2), 165.4, 163.0 (C1, CONH), 138.4, 135.9, 134.1, 127.6 (aromatic protons), 63.4 (C3), 21.2 (Ph-CH_3), 17.6 (CH_3), 7.9 (C5).

3-(3,5-Dichloro-benzoylamino)-3-methyl-2-oxo-butyric Acid (4). Compound **4** was prepared in an analogous fashion from **14** as a white solid in 77% yield; mp = 155−157 °C. ^{1}H NMR (200 MHz, acetone-d_6): δ 1.6 (s, 6H), 7.7 (m, 1H), 7.85 (m, 2H), 8.5 (s, b, 1H).

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(2) Compound **3**, or 3 -(3,5-dichloro-4-methylbenzamido)-2-oxo-3-methylpentanoic acid, was recently isolated and identified from soil metabolism, soil column leaching, and aquatic sediment studies conducted with ^{14}C-RH-7281. For the soil metabolism study, metabolically active soil samples were treated with ^{14}C-RH-7281 to approximate the field rate of 0.2 kg a.i./ha. For the aged column leaching study, treated soil samples from the soil metabolism study were loaded onto a column of fresh soil and leached with water to simulate a rainfall event. For the water sediment study, water/sediment systems in flasks were incubated with ^{14}C-RH-7281. For each of these studies, material balance analyses and identification of metabolites were performed. A complete report of this information will be reported in later publications. Compound **3** was isolated as a discrete ^{14}C entity in each of these studies. Proof of structure by mass spectrometry required isolation of sufficient quantities of material for HPLC/MS analyses. Material for mass spectrometer identification was isolated from the combined samples of three studies. Extracts from the water phase of the water sediment study, extracts from soil column segments from the column leaching study, and soil extracts from the soil metabolism study were combined to obtain sufficient material. Confirmation of structure was obtained by chemical synthesis of compound **3** and comparison of mass spectra and chromatographic behavior.

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