Syntheses and Antifungal Activity of dl-Griseofulvin and Its Congeners. II

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Griseofulvin derivatives, dl-6'-demethyl-6'-ethylgriseofulvin (dl-5) and dl-6'-demethyl-6'-phenylgriseofulvin (dl-6) were prepared by application of a synthetic method developed by us. Antifungal activity of these derivatives decreased in the order of dl-griseofulvin (dl-1) > dl-5 > dl-6 (inactive). The reaction of these derivatives with ethanethiol gave two types of compounds, 2'-ethylthiogriseofulvin (15) and 4'-ethylthiogriseofulvin (16). The relationship between the ratios of isolated yield of 15 and 16 and antifungal the activity of griseofulvin derivatives is discussed.

Key words griseofulvin derivative; thiogriseofulvin; epigriseofulvin; isogriseofulvin; antifungal activity; thiol

dl-Epigriseofulvin (dl-2), the diastereomeric isomer of the antifungal agent dl-griseofulvin (dl-1), lacks the activity, and the racemate, dl-griseofulvin (dl-1), has half the antifungal activity of dl-1.2) These facts show that the steric environment around positions 1' and 6' of griseofulvin is important for the activity. We have continued to study the structure-activity relationship of 6'-substituted griseofulvin derivatives and found that the dl-6'-demethyl derivative (dl-3) and dl-6'-methyl derivative (dl-4) have decreased antifungal activities in comparison with dl-1.1) The focus of our study was derivatives in which the methyl group at position 6' of dl-1 is replaced. For this purpose, our new synthetic method was expected to be of use.3) Also, it is known that dl-griseofulvin (dl-1) reacts with thiol to give various thiogriseofulvin derivatives.4,5) In order to investigate this in detail, we examined the reaction of dl-1 or its derivatives with SH compounds. In this paper, we describe the synthesis and antifungal activity of dl-6'-demethyl-6'-ethylgriseofulvin (dl-5) and dl-6'-demethyl-6'-phenylgriseofulvin (dl-6), and the reaction of dl-1, dl-2, dl-5, and dl-6 with ethanethiol.

Synthesis dl-Ethyl (dl-5) and dl-phenyl (dl-6) derivatives could be synthesized by the application of our method, as shown in Chart 1. β-Alkoxyketones, (9a–c)

Fig. 1. Griseofulvin Derivatives

were prepared from trimethylsilylenol ether (7) and acetics (8a–c) by use of the Mukaiyama–Michael reaction.5) Condensation of benzo(furanone (10)6) and mono-sulfanyl ketones, which were prepared from 9a–c by oxidation with m-chloroperbenzoic acid (m-CPBA), gave the exo olefin compounds (11a–c) with migration of the double bond. We could not determine the configuration of the

 Chart 1

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Table 1. Antifungal Activities of Griseofulvin Derivatives (in Vitro)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>T.m. T-14</td>
</tr>
<tr>
<td>d-1 (d-griseofulvin)</td>
<td>3.13</td>
</tr>
<tr>
<td>dl-1 (dl-griseofulvin)</td>
<td>6.25</td>
</tr>
<tr>
<td>dl-2 (dl-epigriseofulvin)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>dl-5</td>
<td>12.5</td>
</tr>
<tr>
<td>dl-6</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

* a) T.m. T-14 = Trichophyton mentagrophytes T-14; b) T.m. T-16 = Trichophyton mentagrophytes T-16.

The ring-closure reaction of 11 could be achieved with high diastereoselectivity by alumina treatment to give 12 as the major product. In particular, the reaction of 11c with alumina gave only 12c, dl-Griseofulvin derivatives (dl-1, dl-5, and dl-6) were obtained successfully by oxidation of the 2-thiogriseofulvin derivatives (12) with m-CPBA, followed by addition-elimination reaction with sodium methoxide.

We determined the structures of 12b and 13b from the 1H-NMR data in comparison with those of the thiogriseofulvin derivatives, 12a and 13a, whose structures were elucidated by conversion to known compounds, dl-griseofulvin (dl-1) and dl-epigriseofulvin (dl-2), respectively. In the 1H-NMR spectra of 12a and 13a, differences were observed in the chemical shifts of the 6' proton and 6' methyl or ethyl protons, as shown in Fig. 2. The 6' methine proton of 12a was observed at lower field than that of 13a. On the other hand, the methyl protons at the 6' position of 12a were observed at higher field than those of 13a. Similar results were obtained for 12a and 13a. We could not determine the structure of 12c because 13c could not be isolated for comparison. However, we think that the relative configuration of 12c is the same as that of 12a or 12b, because 12c was obtained under the same reaction conditions as 12a and 12b.

Antifungal Activity The antifungal activity of griseofulvin derivatives was examined and the minimum inhibitory concentration (MIC) values against Trichophyton mentagrophytes are listed in Table 1. The bulkiness of the 6'-substituent of griseofulvin derivatives strongly affected the activity. The antifungal activity of the dl-6-ethyl derivative (dl-5) exhibited 1/4 value of dl-1. On the other hand, dl-6'-phenyl derivative (dl-6) lacked the activity. The activities of the 6'-substituted derivatives decreased in order of Me (dl-1) > Et (dl-5) > Ph (dl-6). We thought that the reaction products might change depending on the kind of griseofulvin derivative.

Treatment of d-griseofulvin (d-1) with ethanethiol in the presence of p-toluenesulfonic acid in methylene chloride gave d-ethylthiogriseofulvin (d-14a) in 52% yield and d-ethylthioisogriseofulvin (d-15a) in 27% yield. Under the same conditions, 14b (30% yield) and 15b (24% yield) were obtained from the dl-6'-ethyl derivative (dl-5), and 14c (12% yield) and 15c (48% yield) from the dl-6'-phenyl derivative (dl-6). The structures of the 2'-ethylthio derivatives (d-14a and 14b) and 4'-ethylthio derivatives (15a-c) were confirmed by transformation to the corresponding starting materials (d-1 and d-5) and d-isogriseofulvin (d-16a), respectively (Chart 2).

The position of the ethylthio group of the major product obtained from the reaction of d-epigriseofulvin (d-2) with ethanethiol was different from that of d-1 with ethanethiol. Treatment of d-2 with ethanethiol in the presence of p-toluenesulfonic acid in methylene chloride gave mainly d-4'-ethylthioisogriseofulvin (d-15d) in 43% yield, with 1'-2'-ethylthioisogriseofulvin (1-14d) in 14% yield, as shown in Chart 3. The structure of each product confirmed by transformation to the known product d-2 or d-16d, respectively.

Results and Discussion Our new synthetic method for dl-griseofulvin was useful for the synthesis of 6'-substituted griseofulvin derivatives.

The activities of 6'-substituted derivatives decreased in order of Me (d-1) > Me (dl-1) > Et (dl-5) > Ph (dl-6) = epi Me (d-2). Thus, the steric factor of the 6'-substituent is important role for the activity and the methyl group is the best substituent in this respect. The reaction of the 6'-derivatives with ethanethiol gave two products, the 2'- and 4'-ethylthio derivatives. The ratios of isolated yield of 2'-ethylthio derivatives against 4'-ethylthio derivatives
in the reaction decreased in order of Me (d-1) > Et (d-5) > epi Me (d-2) = Ph (d-6).

We could not explain the similarity of the order of antifungal activity to that of the product ratio in the reaction of these derivatives with ethanethiol. However, considering the fact that griseofulvin binds in vivo with microtubulin,¹ we speculate that binding between griseofulvin and an SH group may play an important role in manifestation of the antifungal activity.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-102 spectrometer. Mass spectra (MS) were recorded on a VG-70SE spectrometer.¹ H-NMR spectra were run on a Hitachi R-1500 (60 MHz) or a Varian VXR-500 (500 MHz) spectrometer. Optical rotations were measured on a JASCO DIP-4 spectrometer. Analytical HPLC was performed on Chemosorb 5Si-U (Chromco). Mecke Silica gel 60 (230–400 mesh) was employed for column chromatography. Extracts were dried over anhydrous MgSO₄.

5-Methoxy-1,1-bis(methylthio)-1-heptan-3-one (9b) Under an Ar atmosphere, 8b (11.0 ml, 89.6 mmol) was added dropwise at 0°C to a solution of 7 (10.0 g, 42.6 mmol) in dry CH₂Cl₂ (21.6 ml). Ph₃CClO₄ (0.72 g, 2.14 mmol) was added portionwise at 0°C, and the reaction mixture was stirred at the same temperature for 50 min, then poured into ice water (400 ml), and 10% aqueous NaHCO₃ (100 ml) was added. The whole was extracted with CH₂Cl₂. The CH₂Cl₂ layer was washed with saturated NaCl solution, dried, and evaporated in vacuo. The residue was purified by distillation to give 9b (6.11 g, 61%) as a slightly green oil, bp 120–130°C/0.2 mm Hg. IR (Nujol) v: 1640 cm⁻¹.¹ H-NMR (60 MHz, CDCl₃) δ: 0.92 (3H, t, J = 7.0 Hz, CH₃-CH₂), 1.30–1.80 (2H, m, CH₂-CH₃), 2.45, 2.47 (each 3H, each s, each CH₃), 2.39–2.82 (2H, m, COCH₃), 3.34 (3H, s, OCH₃), 3.67 (1H, sextet, J = 5.7 Hz, CH₂OCH), 6.08 (1H, s, C = CH). FAB-MS (positive ion mode) m/z: 235 [(M + 1)⁺].

5-Methoxy-1,1-bis(methylthio)-6-phenyl-1-hexan-3-one (9c) Under an Ar atmosphere, TrClO₄ (0.3 g, 0.9 mmol) was added portionwise at 0°C to a solution of 8c (3.0 g, 18 mmol), and 7 (2.0 g, 8.9 mmol) in dry CH₂Cl₂ (2 ml). The reaction mixture was stirred at the same temperature for 15 min, then aqueous NaHCO₃ (10%, 100 ml) was added, and the whole was extracted with AcOEt. The AcOEt layer was washed with saturated NaCl solution, dried, and evaporated in vacuo. The residue was purified by column chromatography (SiO₂, AcOEt:hexane = 1:9) to give 9c (1.2 g, 45%) as an oil. IR (Nujol) v: 1640 cm⁻¹.¹ H-NMR (60 MHz, CDCl₃) δ: 2.51, 2.34 (each 3H, each s, each CH₃), 2.29–2.96 (2H, m, COCH₃), 3.16 (3H, s, OCH₃), 4.67 (1H, t, J = 5.4 Hz, CH₂OCH), 5.94 (1H, s, C = CH), 7.26 (5H, s, C₆H₅). FAB-MS (positive ion mode) m/z: 283 [(M + 1)⁺].

7-Chloro-4,6-dimethoxy-2-[5-methoxy-1-(methylthio)-3-oxoheptyliden]-(3H)-benzofuranone (11b) m-Chloroperbenzoic acid (5.85 g, 33.9 mmol) was added portionwise at 0°C to a solution of 9b (5.3 g, 22.6 mmol) in dry CH₂Cl₂ (500 ml). The reaction mixture was stirred at the same temperature for 15 min and 10% aqueous Na₂SO₃ was added, then the whole was extracted with CH₂Cl₂. The CH₂Cl₂ layer was washed with 10% aqueous NaHCO₃ and saturated NaCl solution, dried, and evaporated in vacuo. Potassium tert-butoxide (2.77 g, 24.6 mmol) was added portionwise at 0°C to a mixture of the residue (about 5.1 g) and dry tetrahydrofuran (THF) (180 ml). The whole was stirred at the same temperature for 20 min. Benzoquinone 10b (5.61 g, 22.4 mmol) dissolved in dry THF (40 ml) was added at 0°C. The reaction mixture was stirred at the same temperature for 20 min, then poured into ice water (600 ml), and 10% aqueous HCl solution (150 ml) was added. The whole was extracted with CH₂Cl₂. The CH₂Cl₂ layer was washed with water,
dried, and evaporated in vacuo. The residue was purified by column chromatography (SiO₂, AcOEt: hexane = 1:1) and recrystallization (CH₂Cl₂ and petr. ether) to give 11b (6.13 g, 68%) as yellow prisms, mp 139—141 °C. IR (Nujol) ν: 1715, 1680 cm⁻¹. 1H-NMR (500 MHz, CDCl₃) δ: 0.89 (3H, t, J = 7.4 Hz, CH₃-CH₂), 1.50—1.60 (2H, m, CH₂-CH₂), 2.53 (4H, s, S, CH₂), 2.65 (6H, dd, J = 16.1, 4.5 Hz, COOCH₃). 2.80 (1H, dd, J = 16.1, 7.8 Hz, COOH), 3.22 (3H, s, CH₃), 3.64—3.71 (1H, m, CH(OCH₃)), 3.96, 3.99 (each 3H, each s, each OCH₃), 4.14, 4.34 (each 1H, each d, each J = 7.2 Hz, COCH₂), 6.15 (1H, s, CH=O). FAB-MS (positive ion mode) m/z: 417 [(M⁺ + 1)+2], 415 [(M⁺ + 1)]. Anal. Caled. For C₁₉H₂₃Cl₂O₄S; C, 55.0; H, 5.59. Found: C, 55.26; H, 5.32.

7-Chloro-4,6-dimethoxy-2-[(methoxy-1)-methylthio]-3-oxo-5-phenylpent-2-en-1-yl[2,3-b]thiophene-2-carboxylic acid (12a) Chlortetracycline (2.5 mg, 0.23 g, 1.3 mmol) was added portionwise to 0 °C so that the solution of the residue (about 0.18 g) in dry THF (5 mL). Benzoquinone (0.24 g, 0.24 mmol) and 10% Pd/C (3 mg, 0.03 mmol) were added at 0 °C, and the reaction mixture was stirred at the same temperature for 15 min, then acidified with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with water, dried, and evaporated in vacuo. The residue was purified by column chromatography (SiO₂, AcOEt: hexane = 1:1) and recrystallization (AcOEt and hexane) to give 11a (0.23 g, 64%), mp 143—145 °C. IR (Nujol) ν: 1710, 1675 cm⁻¹. 1H-NMR (500 MHz, CDCl₃) δ: 2.46 (3H, s, S, CH₂), 2.54—3.13 (2H, m, CO₂CH₂), 3.20 (3H, s, OCH₃), 3.98 (6H, s, OCH₂), 4.07—4.54 (2H, m, CH₂CH₂), 4.55—4.88 (1H, m, CH-O-CO), 6.18 (1H, s, CH=O), 7.36 (5H, s, Ar-H). FAB-MS (positive ion mode) m/z: 465 [(M⁺ + 1)+2], 463 [(M⁺ + 1)]. Anal. Caled. For C₁₉H₁₉Cl₂O₄S; C, 59.67; H, 5.01. Found: C, 59.48; H, 4.86.

2-Dehydroxy-2-[(methylthio)glycine in vacuo. The reaction mixture was stirred at 0 °C for 30 min, then 10% aqueous Na₂SO₄ (40 mL) was added, and the whole was extracted with CH₂Cl₂. The CH₂Cl₂ layer was washed with saturated aqueous NaHCO₃ and saturated NaCl solution, dried, and evaporated in vacuo. Under an Ar atmosphere, NaOMe (0.30 mol in MeOH, 16.5 mL, 4.05 mmol) was added dropwise to a solution of the residue in dry benzene (14 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and poured into ice water. The whole was acidified with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with saturated NaCl solution, dried, and evaporated in vacuo. The residue was purified by column chromatography (SiO₂, AcOEt: hexane = 2:1) to give 6B (0.06 g, 49%) as colorless needles, mp 209—210 °C (CH₂Cl₂ and Et₂O). IR (Nujol) ν: 1700, 1670 cm⁻¹. 1H-NMR (500 MHz, CDCl₃) δ: 0.87 (3H, d, J = 7.4 Hz, CD₃), 2.21 (3H, s, S, CH₂), 2.44 (1H, dd, J = 17.0, 4.9 Hz, C₂S-H₃), 2.91 (1H, d, J = 13.7, 6.6, 4.9 Hz, C₂S-H₂), 3.04 (1H, dd, J = 17.1, 13.7 Hz, C₂S-H₃), 3.97, 4.03 (each 3H, each s, each OCH₃), 5.91 (1H, s, C₂S-H₃), 6.14 (1H, s, C₂S-H), ELMS m/z: 370 [(M⁺ + 1)+2], 368 [(M⁺ + 1)]. Anal. Caled. For C₁₉H₂₁Cl₂O₄S; C, 59.24; H, 5.47; S, 3.05.
Under an Ar atmosphere, NaOMe (0.30 mmol in MeOH, 0.4 mL, 0.12 mmol) was added dropwise to a solution of the residue in dry benzene (0.4 mmol) at 0°C. The reaction mixture was stirred at 0°C for 30 min and poured into ice water. The mixture was acidified with 10% aqueous HCl solution and extracted with CH2Cl2. The CH2Cl2 layer was washed with saturated NaCl solution, dried, and evaporated in vacuo. The residue was purified by preparative TLC (TLC: hexane = 2: 1) to give 2 (17 mg, 43%) as colorless needles, mp 252–254°C (CHCl3 and petr. ether) (lit. 256–257°C, 256–257°C) [114].

**Reaction of d-Griseofulvin (d-14a) with EtSH** Under an Ar atmosphere, EtSH (1.05 mL, 14.2 mmol) was added dropwise to a solution of d-1 (1.00 g, 2.83 mmol) and t-PhSOH (0.54 g, 2.83 mmol) in dry CH2Cl2 (7 mL). The reaction mixture was stirred at room temperature for 40 min, then aqueous NaClO solution (50 mL) was added and the whole was acidified with 10% aqueous KOH solution and extracted with CH2Cl2. The CH2Cl2 layer was washed with saturated NaCl solution, dried, and evaporated in vacuo. The residue was purified by column chromatography to give d-14a (eluent: AcOEt: hexane = 1: 1) [214].

**Reaction of d'-Griseofulvin (d-14a) and d'-4'-demethoxy-4'-ethylthioisogriseofulvin (d-15b)** in the residue was determined by HPLC (column, Chromosorb 5Si-U; column temperature, room temperature; eluent: AcOEt: hexane = 1: 1; flow rate, 2.0 mL/min; wavelength, 254 nm). Separation by column chromatography gave d-14a (eluent: AcOEt: hexane = 1: 1 and d-15a (eluent: AcOEt: hexane = 1: 1); d-14a (558 mg, 52%), mp 184–185°C (lit. 180–181°C) [215].

**Reaction of d'-6'-Dimethyl-6'-ethylthiogriseofulvin (d-5) with EtSH** Under an Ar atmosphere, EtSH (1.20 mL, 16.2 mmol) was added dropwise to a solution of 5 (5.19 g, 32.4 mmol) and t-PhSOH (0.56 g, 32.4 mmol) in dry CH2Cl2 (7.6 mL). The reaction mixture was stirred at room temperature for 40 min, then aqueous NaClO solution (50 mL) was added. The whole was acidified with 10% aqueous KOH solution and extracted with CH2Cl2. The CH2Cl2 layer was washed with saturated NaCl solution, dried, and evaporated in vacuo. The ratio (2: 1) of d'-6'-demethoxy-6'-dimethyl-6'-ethylthio (14b) and d'-4'-demethoxy-6'-dimethyl-6'-ethylthioisogriseofulvin (15b) in the residue was determined by HPLC (column, Chromosorb 5Si-U; column temperature, room temperature; eluent: AcOEt: hexane = 1: 1; flow rate, 1.0 mL/min; wavelength, 254 nm). Separation and purification of the residue by column chromatography gave 15b (eluent: AcOEt: hexane = 1: 2) and 14b (eluent: AcOEt: hexane = 1: 1).

**Determination of the Structures of d-14a, 14b and 1-4d. General Procedure** Under an Ar atmosphere, m-CPPBA (65.2 mg, 0.378 mmol) was added to a solution of 14b (100 mg, 0.252 mmol) in CH2Cl2 (4.5 mL) at 0°C. After 20 min, the reaction mixture was stirred at 0°C for 20 min, then 10% aqueous Na2SO3 was added and the whole was extracted with CH2Cl2. The CH2Cl2 layer was washed with saturated aqueous NaHCO3, and a saturated NaCl solution, dried, and evaporated in vacuo. Under an Ar atmosphere, NaOMe (0.30 mmol in MeOH, 1.0 mL, 0.30 mmol) was added dropwise to a solution of the residue in dry benzene (0.9 mL) at 0°C. The reaction mixture was stirred at 0°C for 20 min, and poured into ice water. The whole was washed with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with water, dried, and evaporated in vacuo. Recrystallization of the residue from a mixture of CH2Cl2 and Et2O gave d-5.

By the same procedure, d-14a and 1-4d gave d-1 and d-2, respectively.

**Transformation of d-15a, 15b, 15c and d-15c to the Corresponding Bisulfide (d-6) and Bisphenyl (d-6'). General Procedure** Under an Ar atmosphere, m-CPPBA (52.2 mg, 0.302 mmol) was added to a solution of 15b (80 mg, 0.202 mmol) in CH2Cl2 (3.5 mL) at 0°C. The reaction mixture was stirred at 0°C for 20 min, then 10% aqueous Na2SO3 was added and the whole was extracted with CH2Cl2. The CH2Cl2 layer was washed with saturated aqueous NaHCO3 and a saturated NaCl solution, dried, and evaporated in vacuo. Under an Ar atmosphere, NaOMe (0.30 mmol in MeOH, 0.8 mL, 0.24 mmol) was added dropwise to a solution of the residue in dry benzene (0.8 mL) at 0°C. The reaction mixture was stirred at 0°C for 20 min and poured into ice water. The whole was washed with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with water, dried, and evaporated in vacuo. Recrystallization of the residue from a mixture of CH2Cl2 and petroleum ether gave 16b.

By the same procedure, d-15a, 15c and d-15d gave d-16a, 16c and
d-Isogriseofulvin (d-16a, 52%) as colorless needles, mp 182—184°C (lit.²⁵ 200—201°C).

d-L-Methyl-6-ethylisogriseofulvin (16b, 66%) as colorless needles, mp 219—223°C. IR (KBr) ν: 1700, 1655 cm⁻¹. ¹H-NMR (500 MHz, CDCl₃) δ: 0.90 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.18—1.28, 1.54—1.63 (each 1H, each m, CH₃CH₂), 2.56—2.65 (2H, m, C₅-H and C₆-H), 3.06 (1H, dd, J = 18.9, 13.3 Hz, C₅-H), 3.77 (3H, s, C₃-OCH₃), 3.91, 3.99 (each 3H, each s, each OCH₃), 5.44 (1H, s, C₃-H), 6.07 (1H, s, C₅-H). El-MS m/z: 368 [M⁺ + 2], 366 [M⁺]. Anal. Caled for C₁₄H₁₄O₄S: C, 58.94; H, 5.22. Found: C, 58.35; H, 5.15.

d-L-Methyl-6-phenylisogriseofulvin (16c, 68%) as colorless needles, mp 207—208°C. IR (Nujol) ν: 1705, 1660 cm⁻¹. ¹H-NMR (500 MHz, CDCl₃) δ: 3.24—4.01 (3H, m, C₅-H and C₆-H), 3.79 (3H, s, C₃-OCH₃), 3.85 (6H, s, OCH₃ x 2), 5.56 (1H, s, C₃-H), 5.94 (1H, s, C₅-H). FAB-MS (positive ion mode) m/z: 417 [(M+1)⁺ + 2], 415 [(M+1)⁺]. Anal. Caled for C₂₂H₂₅ClO₂: C, 58.81; H, 5.14. Found: C, 58.92; H, 4.48.

d-Episogriseofulvin (d-16d, 37%) as colorless needles, mp 200—202°C (lit.³ 200—202°C).

**Antifungal Activity** Assays and evaluation of antifungal activities were carried out according to the methods described previously.¹

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**References**