Synthesis of Multi-substituted 4,5-Dihydrofuran Derivatives from (S)-Limonene and 1,3-Dicarbonyl Compounds and their Biological Activities

E. FINDIK*, A. DİNGİL, I. KARAMAN§, Y. BUDAK and M. CEYLAN

Department of Chemistry, Faculty of Arts and Sciences, Gaziosmanpasa University-60250, Tokat, Turkey.
§Department of Biology, Faculty of Arts and Sciences, Gaziosmanpasa University-60250, Tokat, Turkey.
esrafindk@gmail.com

Received 23 December 2008; Accepted 20 February 2009

Abstract: Multi-substituted 4,5-dihydrofuran derivatives were regioselectively synthesized by the reaction of α-carbo radical produced from 1,3-dicarbonyl compounds by oxidation with Mn(OAc)₃ in Acetic acid and S-(−)-limonene. All the compounds were tested for their antibacterial and antifungal activities by the disc-diffusion technique.

Keywords: Synthesis, Limonene, Biological activities.

Introduction

Monoterpenes are widely used in the pharmaceutical, cosmetic and food industries as active components of drugs and ingredients of artificial flavours and fragrances. Monoterpenes and limonene in particular, are an important class of naturally occurring chiral compounds widely used in organic synthesis as starting materials for optically pure molecules, as a core of chiral auxiliaries and as asymmetric ligands employed in enantioselective transformations. In nature, limonene is abundant in both enantiomeric forms, (R)-limonene and (S)-limonene. From a chemical point of view, these terpenes are very versatile since they present two double bonds that can be selectively converted to several functional groups. The biological activities of (R)-limonene and (S)-limonene have been studied against Staphylococcus aureus ATCC 12600, Bacillus cereus ATCC 11778, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 11775, Pseudomonas aeruginosa ATCC 9027, Klebsiella pneumoniae ATCC 13883 and Cryptococcus neoformans ATCC 90112 bacteria. They have shown considerable activity.

In addition, furans and their derivatives are available in nature and one of the most important heterocyclic compounds for the total synthesis of the complicated naturally occurring metabolites in organic chemistry. Furans and their derivatives have a broad spectrum of biological activity such as antiprotozoal, antiviral, antimicrobial, anticancer, HIV-1 activity and antimalarial.
Therefore, this study focuses on the synthesis of 4,5-dihydrofuran derivatives 3 and 4 with a limonene and dihydrofuran ring and the determination of their biological activities toward 12 different human pathogen microorganisms.

**Experimental**

Mn(OAc)$_3$, (S)-limonene, Acetic acid and 1,3-dicarbonyls were commercial products with highest reagent grade. IR spectra (neat or in CHCl$_3$) were recorded on a Jasco FT/IR-430 spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance III 400 instrument. As internal standards served TMS ($\delta$ 0.00) for $^1$H NMR and CDCl$_3$ ($\delta$ 77.0) for $^{13}$C NMR spectroscopy; $J$ values are given in Hz. The multiplicities of the signals in the $^1$H NMR spectra are abbreviated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad) and combinations thereof. Elemental analyses were obtained by a LECO CHNS 932 Elemental Analyzer.

**General procedure for the synthesis of benzofuran and 4,5-dihydrofuran derivatives**

A solution of Mn(OAc)$_3$ (2.97 g, 11.1 mmol) in 15 mL of glacial Acetic acid was heated under N$_2$ atmosphere at 80 °C until it dissolved. After Mn(OAc)$_3$ was dissolved completely, the solution was cooled to 60 °C and a solution of 1,3-dicarbonyl compounds variable (1.04 g, 7.4 mmol) and (S)-(-)-limonene (0.5 g, 3.7 mmol) in 5 mL acetic acid was added to this mixture. The reaction was finished when the dark brown color of the solution disappeared. Acetic acid was evaporated under reduced pressure; H$_2$O was added to the residue and extracted with ethyl acetate (3 x 20 mL). The combined organic phases were neutralized with saturated NaHCO$_3$ solution, dried (Na$_2$SO$_4$), and evaporated. Crude products were purified by column chromatography on silica gel using n-hexane/ethyl acetate (9:1) as eluent.

**Mixture of (R)-2,6,6-trimethyl-2-((S)-4-methylcyclohex-3-enyl)-2,3,6,7-tetrahydrobenzofuran-4(5H)-one (3a) and (S)-2,6,6-trimethyl-2-((S)-4-methylcyclohex-3-enyl)-2,3,6,7-tetrahydrobenzofuran-4(5H)-one (4a):** Viscous oil; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 5.24 (bs, 1H), 2.64 (d, $J$ = 14.3 Hz, 1H), 2.31 (t, $J$ = 13.7 Hz, 1H), 2.17-1.08 (m, 4H), 1.92-1.86 (m, 3H), 1.53 (s, 3H), 1.24 (d, $J$ = 4.7 Hz, 3H), 1.18-1.10 (m, 1H), 1.05 (s, 3H), 1.03 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = (175.20-175.03), (133.84-133.70), (119.79-119.73), 110.98, (95.39-95.17), 50.72, (43.03-42.84), 37.82, 35.01, (34.27-33.95), (30.31-30.21), (28.87-28.84), (28.33-28.28), (26.19-25.88), (24.78-24.45), 23.33, (23.27-23.17). IR (Liquid): 3046, 3010, 2960, 2928, 2890, 1633, 1402, 1240, 1028, 759 cm$^{-1}$. Anal. Calcd for C$_{18}$H$_{26}$O$_2$: C, 78.79; H, 9.55; Found: C, 78.47; H, 9.58.

**Mixture of (R)-2-methyl-2-((S)-4-methylcyclohex-3-enyl)-2,3,6,7-tetrahydrobenzofuran-4(5H)-one (3b) and (S)-2-methyl-2-((S)-4-methylcyclohex-3-enyl)-2,3,6,7-tetrahydrobenzofuran-4(5H)-one (4b):** Viscous oil; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 5.29 (bs, 1H), 2.69 (d, $J$ = 14.3 Hz, 1H), 2.39-2.34 (m, 3H), 2.27-2.26 (m, 2H), 1.98-1.87 (m, 5H), 1.74-1.66 (m, 2H), 1.59-1.57 (m, 4H), 1.29-1.26 (m, 4H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = (176.43-176.29), (133.98-133.85), (119.79-119.75), 112.63, (95.28-95.08), (43.10-42.94), 36.31, 35.27, 34.63, (30.34-30.28), (26.25-25.97), 24.51, 24.18, 23.97, (23.40-23.31), 21.70. IR (Liquid): 3009, 2927, 2889, 2839, 1632, 1402, 1265, 999, 856 cm$^{-1}$. Anal. Calcd for C$_{16}$H$_{22}$O$_2$: C, 78.79; H, 9.55; Found: C, 78.47; H, 9.58.

**Mixture of 1-((R)-5-methyl-5-((S)-4-methylcyclohex-3-enyl)-2-phenyl-4,5-dihydrofuran-3-yl)ethanone (3c) and 1-((S)-5-methyl-5-((S)-4-methylcyclohex-3-enyl)-2-phenyl-4,5-dihydrofuran-3-yl)ethanone (4c):** Viscous oil; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.51-7.49 (m, 2H), 7.46-7.41 (m, 3H), 5.37 (bs, 1H), 3.06 (d, $J$ = 14.8 Hz, 1H), 2.72 (dd, $J$ = 14.8,
10 Hz, 1H), 2.15-1.93 (m, 5H), 1.90 (s, 3H), 1.89-1.78 (m, 5H), 1.64 (s, 3H), 1.40 (s, 3H). 13C NMR (100 MHz, CDCl3): δ = 194.80, (165.73-165.65), (134.06-133.93), 131.49, 130.36, (129.02-128.98), (128.33-128.29), (119.96-119.93), 114.57, (91.04-90.81), (43.24-42.96), 40.49, 39.75, (30.47-30.37), 28.76, 26.39, 26.07, 24.14, 23.54, 23.37. IR (Liquid): 3059, 3008, 2965, 2926, 2836, 1623, 1591, 1376, 1267, 1243, 1072, 1062, 786,761 cm\(^{-1}\). Anal. Calcd for C_{20}H_{24}O_{2}: C, 81.04; H, 8.16; Found: C, 79.86; H, 7.96.

Mixture of (R)-methyl 2,5-dimethyl-5-(S)-4-methylcyclohex-3-enyl)-4,5-dihydrofuran-3-carboxylate (3d) and (S)-methyl 2,5-dimethyl-5-(S)-4-methylcyclohex-3-enyl)-4,5-dihydrofuran-3-carboxylate (4d): Viscous oil; 1H NMR (400 MHz, CDCl3): δ = 5.32 (bs, 1H), 3.64 (s, 3H), 2.76 (d, J = 14.4 Hz, 1H), 2.44-2.38 (dd, J = 12.4, 10.8 Hz, 1H), 2.12 (s, 3H), 1.95-1.87 (m, 3H), 1.78-1.62 (m, 3H), 1.59 (s, 3H), 1.26-1.22 (m, 4H).

13C NMR (100 MHz, CDCl3): δ = (167.26-167.11), (13.95-133.77), (120.01-119.92), 100.68, (90.62-90.40), 50.58, (43.10-42.92), 38.93, 38.16, (30.42-30.32), (26.22-25.95), 24.60, 24.15, (23.29-23.26), 14.13. IR (CHCl3): 3010, 2947, 2925, 2840, 1702, 1644, 1436, 1381, 1241, 1097, 762 cm\(^{-1}\). Anal. Calcd for C_{13}H_{22}O_{2}: C, 71.97; H, 8.86; Found: C, 71.97; H, 8.86.

Mixture of (R)-ethyl 2,5-dimethyl-5-((S)-4-methylcyclohex-3-enyl)-4,5-di-hydrofuran-3-carboxylate (3e) and (S)-ethyl 2,5-dimethyl-5-((S)-4-methylcyclohex-3-enyl)-4,5-dihydrofuran-3-carboxylate (4e): Viscous oil; 1H NMR (400 MHz, CDCl3): δ = 5.25 (bs, 1H), 4.04 (q, J = 8 Hz, 2H), 2.71 (d, J = 14 Hz, 1H), 2.39-2.33 (dd, J = 13.6, 9 Hz, 1H), 2.06 (s, 3H), 1.90-1.82 (m, 3H), 1.72-1.59 (m, 3H), 1.53 (s, 3H), 1.19-1.15 (m, 7H). 13C NMR (100 MHz, CDCl3): δ = (169.79-166.65), (133.77-133.60), (120.01-119.91), 100.86, (90.34-90.12), 59.10, (43.06-42.88), 38.94, 38.20, (30.37-30.28), 26.18, 25.91, 24.04, 23.34, 23.22, (14.36-14.06). IR (CHCl3): 2968, 2927, 2727, 1698, 1646, 1445, 1380, 1240, 1220, 974, 785, 762 cm\(^{-1}\). Anal. Calcd for C_{16}H_{23}O_{2}: C, 72.69; H, 9.15; Found: C, 72.96; H, 8.84.

Mixture of (R)-N,N-diethyl-2,5-dimethyl-5-((S)-4-methylcyclohex-3-enyl)-4,5-di-hydrofuran-3-carboxamide (3f) and (S)-N,N-diethyl-2,5-dimethyl-5-((S)-4-methylcyclohex-3-enyl)-4,5-dihydrofuran-3-carboxamide (4f): Viscous oil; 1H NMR (400 MHz, CDCl3): δ = 5.32 (bs, 1H), 3.33 (q, J = 7.2 Hz, 4H), 2.81 (ddd, J = 14.2, 5.6, 1.6 Hz, 1H), 2.41 (d, J = 14.4 Hz, 1H), 2.02-1.88 (m, 3H), 1.79 (d, J = 1.6 Hz, 3H), 1.74-1.64 (m, 2H), 1.59 (s, 3H), 1.39-1.37 (m, 1H), 1.25 (s, 3H), 1.25-1.18 (m, 1H), 1.09 (t, J = 7.2 Hz, 6H). 13C NMR (100 MHz, CDCl3): δ = 166.21, (155.26-155.26), (134.02-133.82), (120.14-120.07), (103.36-103.32), (88.85-88.69), (43.11-42.97), 41.80, 40.83, (30.48-30.44), 26.40, 26.17, 24.67, 24.04, 23.57, 23.34, 13.78, (13.45-13.42). IR (Liquid): 3425, 2968, 2929, 2835, 1636, 1433, 1358, 1134, 785 cm\(^{-1}\). Anal. Calcd for C_{18}H_{29}NO_{2}: C, 74.18; H, 10.03; N, 4.81; Found: C, 67.18; H, 9.92; N, 4.42.

Mixture of 1-((R)-2,5-dimethyl-5-((S)-4-methylcyclohex-3-enyl)-4,5-dihydrofuran-3-yl)ethanone (3g) and 1-(S)-2,5-dimethyl-5-((S)-4-methylcyclohex-3-enyl)-4,5-dihydrofuran-3-yl)ethanone (4g): Viscous oil; 1H NMR (400 MHz, CDCl3): δ = 5.30 (bs, 1H), 2.80 (d, J = 14 Hz, 1H), 2.45 (dd, J = 13.4, 8.4 Hz, 1H), 2.12 (bs, 3H), 2.11 (bs, 3H), 1.98-1.90 (m, 4H), 1.75-1.59 (m, 3H), 1.57 (s, 3H), 1.23 (bs, 3H). 13C NMR (100 MHz, CDCl3): δ = 194.68, (167.03-166.88), (133.95-133.80), (119.89-119.83), 111.66, (90.78-90.58), (43.06-42.89), (39.69-39.96), (30.37-30.28), 29.22, (26.20-25.95), 24.56, 24.13, (23.37-23.28), 15.17. IR (Liquid): 3010, 2926, 1698, 1653, 1600, 1436, 1378, 1244, 937, 784, 762, 626 cm\(^{-1}\). Anal. Calcd for C_{13}H_{22}O_{2}: C, 76.88; H, 9.46; Found: C, 76.67; H, 9.06.
Microbiology
Preparation of microorganisms

A total of 12 microbial cultures belonging to ten bacterial and two fungal species were used in this study (Table 1). The cultures were grown in Mueller-Hinton Broth (Merck) for all the bacterial strains by 24 h of incubation at 36 °C. *C. albicans* and *C. utilis* were grown in Sabouraud Dextrose Broth (Merck) by incubation for 24 h at 25 °C.

Disc-diffusion assay

Antimicrobial tests were carried out by disc-diffusion method\textsuperscript{21-22} using 100 µL of suspension containing 10\textsuperscript{6} CFU/mL of bacteria and 10\textsuperscript{6} CFU/mL of yeast spread on Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) medium, respectively. The blank discs (Oxoid = 6 mm in diameter) were impregnated with 20 µL of the each substances (105 µg/disc) and placed on the inoculated agar. Negative controls were prepared using the same solvents (Methanol) employed to dissolve each substances. Sulbactam (30 µg) + Cefoperazona (75 µg) (105 µg/disc) was used as positive reference standard to determine the sensitivity of a strain of each microbial species tested. The inoculated plates were incubated at 36 °C for 24 h for clinical bacterial strains, 48 h for yeast and 72 h for fungi strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

Results and Discussion

Previously, multi-substituted 4,5-dihydrofuran derivatives 3a-d and 4a-d have been synthesized by de Mattos\textsuperscript{20} from the Mn(OAc)\textsubscript{3} mediated radical cyclizations of cyclohexane-1,3-dion (2a), acetylacetone (2b), ethyl acetoacetate (2c), and benzoylaceton (2d) with limonene, respectively. We resynthesized this compounds 3a-d and 4a-d from the (S)-limonene (1) using the same method. In addition, we studied Mn(OAc)\textsubscript{3} mediated radical cyclizations of dimedon (2e), methyl acetoacetate (2f), ethyl \textit{N},\textit{N}-diethyl-3-oxobutanamide (2g) with (S)-limonene (1). Radical cyclization reactions were performed in 2:1:3 molar ratio 1,3-dicarbonyl:(S)-limonene: Mn(OAc)\textsubscript{3}, respectively, under N\textsubscript{2} atmosphere, at 80-60 °C, in acetic acid. The results are given in Scheme 1 and Table 1. The reactions of 1,3-dicarbonyl compounds (2a-g) with (S)-limonene resulted in the formation of the corresponding cyclization products (3a-g and 4a-g) in 57-87% yield as a 1:1 mixtures of diastereoisomers (determined by \textsuperscript{13}C-NMR). All efforts to separate the diastereomeric mixture consisting of 3a-g and 4a-g by column chromatography, crystallization, and distillation failed.

\[ \text{O} \quad \text{O} \]
\[ R' \quad R \]
\[ \text{O} \quad \text{O} \]
\[ \text{R} \quad \text{R'} \]
\[ 3a-g \]
\[ \text{Mn(OAc)} \textsubscript{3} \text{H}_2\text{O} \text{AcOH, 80 °C to 60 °C} \]
\[ \text{H} \]
\[ \text{H} \]
\[ \text{R} \quad \text{R'} \]

Scheme 1

The structures of the compounds were determined on the basis of spectroscopic data (\textsuperscript{1}H NMR, \textsuperscript{13}C NMR, IR and Elemental analysis) and comparison with literature data\textsuperscript{20}. All spectroscopic data were in accord with the proposed structures.
Table 1. Synthesized compounds.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Olefin</th>
<th>1,3-Dicarbonyl</th>
<th>Products</th>
<th>Total yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibacterial and antifungal activity

The newly synthesized compounds (3a-g and 4a-g) were screened for their antibacterial activity against two yeasts bacteria viz., Candida albicans ATCC 1213, Candida utilis KUEN 1031), five gram positive bacteria viz., Streptococcus homilis, Bacillus cereus DSM 4312, Bacillus subtilis ATCC 6633, Staphylococcus Aureus ATCC 29213, Streptococcus Pyogenez ATCC 176 and five gram negative bacteria viz., Proteus Vulgaris KUEN 1329, Escherichia coli 111, Pseudomonas aeruginosa ATCC 9027, Salmonella enteridis ATCC 13076, Enterococcus fecaelis ATCC 29122 by using disc-diffusion method. A reference standard drug SCF (Sulbactam (30 µg) + Cefoperazona (75 µg)) and methanol were used as positive and negative control, respectively. The experiments were performed in triplicate in order to minimize the errors. Zone of inhibition produced by each compound was measured in mm. The results of antibacterial studies were given in Table 2.

The screening results revealed that the majority of compounds 3a-g and 4a-g showed remarkable antimicrobial activity. In particular compound (3a + 4a) showed the same activity with positive control (SCF) against the E. fecaelis ATCC 29122. Compound (3c + 4c) showed significant activity against C. utilis KUEN 1031 and S. homilis. Compound (3f + 4f) showed remarkable activity against and S homilis and S enteridis ATCC 13076. Compound (3g + 4g) has shown high potency especially against C. utilis KUEN 1031 and E fecaelis ATCC 29122. Compounds (3a+4a) and (3b+4b) have not shown any inhibition against P. Vulgaris KUEN 1329, C. utilis KUEN 1031, P. aeruginosa ATCC 9027 and C. albicans ATCC 1213. The authors are indebted to the Department of Chemistry and Biology (Gaziosmapaş University).
Table 2. Antimicrobial activity inhibition of diastereomers of 3 + 4.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Microorganisms</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a + 4a</td>
<td>-</td>
<td>8</td>
<td>11</td>
<td>14</td>
<td>-</td>
<td>13</td>
<td>14</td>
<td>-</td>
<td>14</td>
<td>16</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3b + 4b</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>11</td>
<td>14</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3c + 4c</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td>13</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>9</td>
<td>10</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3d + 4d</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3e + 4e</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>-</td>
<td>12</td>
<td>15</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3f + 4f</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>11</td>
<td>12</td>
<td>16</td>
<td>12</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>11</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>3g + 4g</td>
<td>12</td>
<td>19</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>16</td>
<td>14</td>
<td>10</td>
<td>14</td>
<td>11</td>
<td>13</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>SCF</td>
<td>22</td>
<td>24</td>
<td>19</td>
<td>22</td>
<td>23</td>
<td>25</td>
<td>26</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;OH</td>
<td>-</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>-</td>
<td>7</td>
<td>8</td>
<td>19</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


References