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

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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 $\text{Mn}(\text{OAc})_3$ 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^{1,2}. Monoterpenes and limonene in particular, are an important class of naturally occurring chiral compounds³ widely used in organic synthesis⁴ as starting materials for optically pure molecules⁵, as a core of chiral auxiliaries⁶ and as asymmetric ligands employed in enantioselective transformations⁷. In nature, limonene is abundant in both enantiomeric forms, (*R*)-limonene and (*S*)-limonene⁸. 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 groups⁹⁻¹¹. The biological activities of (*R*)-limonene and (*S*)-limonene have been studied¹² 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 nature³ and one of the most important heterocyclic compounds¹⁴ for the total synthesis of the complicated naturally occurring metabolites in organic chemistry¹⁵. Furans and their derivatives have a broad spectrum of biological activity such as antiprotozoal¹⁶, antiviral¹⁷, antimicrobial¹⁷, anticancer¹⁷, HIV-1 activity¹⁸ and antimalarial¹⁹.

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 CHCl₃) were recorded on a Jasco FT/IR-430 spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance III 400 instrument. As internal standards served TMS (δ 0.00) for ¹H NMR and CDCl₃ (δ 77.0) for ¹³C NMR spectroscopy; *J* values are given in Hz. The multiplicities of the signals in the ¹H 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 N₂ 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 colour of the solution disappeared. Acetic acid was evaporated under reduced pressure; H₂O was added to the residue and extracted with ethyl acetate (3 x 20 mL). The combined organic phases were neutralized with saturated NaHCO₃ solution, dried (Na₂SO₄), 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; ¹H NMR (400 MHz, CDCl₃): δ = 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). ¹³C NMR (100 MHz, CDCl₃): δ = 194.57, (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 C₁₈H₂₆O₂: 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, ¹H NMR (400 MHz, CDCl₃): δ = 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). ¹³C NMR (100 MHz, CDCl₃): δ = (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 C₁₆H₂₂O₂: C, 78.01; H, 9.00; Found: C, 77.81; H, 8.77.

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; ¹H NMR (400 MHz, CDCl₃): δ = 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). ^{13}C NMR (100 MHz, CDCl_3): 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 $\text{C}_{20}\text{H}_{24}\text{O}_2$: 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, CDCl_3): 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). ^{13}C NMR (100 MHz, CDCl_3): 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 (CHCl_3): 3010, 2947, 2925, 2840, 1702, 1644, 1436, 1381, 1241, 1097, 762 cm^{-1} . Anal. Calcd for $\text{C}_{15}\text{H}_{22}\text{O}_3$: 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-dihydrofuran-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, CDCl_3): 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). ^{13}C NMR (100 MHz, CDCl_3): 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 (CHCl_3): 2968, 2927, 2727, 1698, 1646, 1445, 1380, 1240, 1220, 974, 785, 762 cm^{-1} . Anal. Calcd for $\text{C}_{16}\text{H}_{24}\text{O}_3$: 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-dihydrofuran-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, CDCl_3): 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). ^{13}C NMR (100 MHz, CDCl_3): δ = 168.21, (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 $\text{C}_{18}\text{H}_{29}\text{NO}_2$: 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, CDCl_3): 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). ^{13}C NMR (100 MHz, CDCl_3): 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 $\text{C}_{15}\text{H}_{22}\text{O}_2$: 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 method²¹⁻²² using 100 µL of suspension containing 10⁸ CFU/mL of bacteria and 10⁶ 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 Mattos²⁰ from the Mn(OAc)₃ mediated radical cyclizations of cyclohexane-1,3-dione (**2a**), acetylacetone (**2b**), ethyl acetoacetate (**2c**), and benzoylacetone (**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)₃ mediated radical cyclizations of dimedon (**2e**), methyl acetoacetate (**2f**), ethyl *N,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)₃, respectively, under N₂ 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 ¹³C-NMR). All efforts to separate the diastereomeric mixture consisting of **3a-g** and **4a-g** by column chromatography, crystallization, and distillation failed.

Scheme 1

The structures of the compounds were determined on the basis of spectroscopic data (¹H NMR, ¹³C NMR, IR and Elemental analysis) and comparison with literature data²⁰. All spectroscopic data were in accord with the proposed structures.

Table 1. Synthesized compounds.

Entry	Olefin	1,3-Dicarbonyl	Products	Total yield, %	
1	1				85
2	1				81
3	1				87
4	1				63
5	1				74
6	1				84
7	1				57

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 Pyogenes* ATCC 176 and five gram negative bacteria viz., *Proteus Vulgaris* KUEN 1329, *Escherichia coli* 111, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteridis* ATCC 13076, *Enterococcus faecalis* ATCC 29122 by using disc-diffusion method. A reference standard drug SCF (Sulbactam (30 µg) + Cefoperazone (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. faecalis* ATCC 29122. Compound (**3c + 4c**) showed significant activity against *C. utilis* KUEN 1031 and *S. homilis*. Compound (**3f + 4f**) showed remarkable activity against *S. homilis* and *S. enteridis* ATCC 13076. Compound (**3g + 4g**) has shown high potency especially against *C. utilis* KUEN 1031 and *E. faecalis* 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 (Gaziosmapa University).

Table 2. Antimicrobial activity inhibition of diastereomers of **3 + 4**.

Compounds	Microorganisms and inhibition zone diameter around test disc, mm											
	A	B	C	D	E	F	G	H	I	J	K	L
3a + 4a	-	-	8	11	14	-	13	14	-	14	16	-
3b + 4b	-	-	9	-	14	-	11	14	8	8	10	-
3c + 4c	15	17	17	11	13	14	13	13	13	9	10	14
3d + 4d	15	14	15	12	13	8	12	13	12	12	9	-
3e + 4e	13	14	14	14	15	-	12	15	9	10	11	8
3f + 4f	16	16	16	11	12	16	12	13	15	14	11	16
3g + 4g	12	19	15	14	13	16	14	10	14	11	13	16
SCF	22	24	19	22	23	25	26	18	17	17	16	26
CH₃OH	-	7	7	9	-	7	8	19	7	78	8	7

SCF: Sulbactam (30 µg)+cefoperazona (75 µg) = positive control CH₃OH: Methanol = negative control - : No effect A: *Proteus Vulgaris* KUEN 1329, B: *Candida utilis* KUEN 1031, C: *Streptococcus homilis*, D: *E. coli* 111, E: *Bacillus cereus* DSM 4312, F: *Pseudomonas aeruginosa* ATCC 9027, G: *Bacillus subtilis* ATCC 6633, H: *S. Aureus* ATCC 29213, I: *Salmonella enteridis* ATCC 13076, J: *St. Pyogenez* ATCC 176, K: *Enterococcus faecalis* ATCC 29122, L: *Candida albicans* ATCC 1213.

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