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The Synthesis and Antimicrobial Activity of γ-Butyrolactone Derivatives

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This paper examines the synthesis of γ-butyrolactone compounds **2**, **3**, **4**, **5**, and **9**, and their potential antimicrobial nature and ability. The structures of all the compounds mentioned above were determined and confirmed with elemental analyses, and IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. The in vitro antimicrobial activity of compounds **2**, **3**, **4**, **5**, and **9** was examined and evaluated against the following pathogens: *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae, Aspergillus niger*, and *Candida albicans*. Compound **5** (20 mg/mL) demonstrated high antimicrobial activity against *S. epidermidis*, which was closely comparable to the antimicrobial activity of streptomycin.

Key Words: γ-Btyrolactone derivatives, antimicrobial activities

Introduction

γ-Butyrolactone is a very common structural feature of a number of organic compounds and is present in about 10% of all natural products.[1,2] A wide range and quantity of mono-, bi-, and tri-substituted monocyclic γ-butyrolactones are known, which are found to occur both naturally in our environment and as part of a more complex framework, particularly in bi-cyclic and tri-cyclic ring systems. These compounds display a broad range of biological ability, including strong antibiotic, anthelmintic, antifungal, antitumor, antiviral, anti-inflammatory, and cytostatic properties,[3−16] thus enabling them to serve as appealing leading structures for creating new drugs.[9,17−20] This paper examines the transformation process of the lactone **2** compounds into the multifunctional γ-butyrolactone derivative compounds **2**, **3**, **4**, **5**, and **9**, as well as their structural identification and biological activity.

Experimental

Melting points were determined with a Kofler apparatus and were uncorrected. Elementary analysis was performed on a Carlo Erba 1106 micro-analyzer, and satisfactory carbon and hydrogen values were obtained.

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IR spectra were recorded on Perkin-Elmer grating spectrophotometer models 137 and 337, either as film or KBr disks. NMR spectra were obtained with a Varian Gemini-200 (200 MHz for ^{1}H and 50 MHz for ^{13}C) in $CDCl_3$. TMS was used as an internal standard. All chemical shifts were reported as δ (ppm) values and coupling constants J (Hz). Every proton in the spectrum could be assigned by analyzing the DQF-COSY spectrum. Additionally, all of the protons were assigned on the basis of decoupled ^{1}H-NMR spectra (irradiation at specific frequencies during acquisition removed couplings to other nuclei). Signal multiplicities in the ^{13}C-NMR spectra were determined in the DEPT[21] experiment. Thin-layer chromatography was performed using a Merck Kieselgel 60 $PF_{254+366}$ and a Merck Kieselgel G. For column chromatography Silica gel 60 (230-400 mesh) was used.

***(4R)*-4-(Dichloro-methyl)-4-methyl-3a,4-hexahydro-2,7(3*H*,7aH)-benzofuran-dione 2**

At room temperature and over the course of 5 h a compound **1** benzene solution (5.50 g, 1.2 mmol) was added to a suspension of lead tetra acetate (LTA) (2.7 g 1.2 mmol) in cold benzene (20 mL) and boron trifluoride etherate (5 mL). The reaction mixture was then poured into cold water (150 mL) and the obtained solution was filtered with Celite 577 (Fluka). The organic layer was separated from the water layer, extracted with a saturated solution of sodium hydrogen carbonate and sodium chloride, and then dried with anhydrous sodium sulfate. Evaporation of the solvent generated a crude product that crystallized out from ether, affording 1.8 g. Yield: 71% of **2**, mp: 118 °C. Anal. for $C_{10}H_{10}Cl_2O_3$ (249.09). Calc. C: 48.39, H: 4.06. Found: C: 48.56, H: 4.07. IR (KBr): 1785, 1695, 1180, 810, 790 cm^{-1}.^{1}H-NMR ($CDCl_3$): δg.41 (s, 3H, CH_3), 2.51 (ABq, 2H, ^2J = 14.7 Hz, ^3J = 8.0 Hz, ^3J = 12.5 Hz H-3), 3.51 (m, 1H, ^3J = 8.0 Hz, ^3J = 12.5,Hz, ^3J = 9.7 Hz, ^4J = 1.7 Hz, H-3a), 5.14 (s, 1H, ^3J = 9.7 Hz, H-7a), 5.79 (s, 1H, $CHCl_2$), 6.31 (d, 1H, ^3J = 10.3 Hz, H-6), 6.75 (dd, 1H, ^3J = 10.3 Hz, ^4J = 1.7 Hz, H-5). ^{13}C-NMR ($CDCl_3$):gδg73.54 (C-2),43.27 (C-3), 29.67 (C-3a), 44.38 (C-4), 150.29 (C-5), 129.8 (C-6), 195.64 (C-7), 80.33 (C-7a), 18.98 (CH_3), 79.76 ($CHCl_2$).

(*4R, E and Z*)-4-(Dichloro-methyl)-7-hydroxy-imino-4-methyl-,3a,4,7,7a-hexahydro-benzofuran-2,(3*H*) one 3 and 4

The lactone **2** (249 mg, 1 mmol), hydroxylamine hydrochloride (100 mg, 1.3 mmol), and sodium acetate trihydrate (160 mg, 2 mmol) were all dissolved in a water/methanol mixture (10 mL methanol and 2 mL of water). The solution was heated under reflux for 1 h, then evaporated, extracted with dichloromethane, dried over anhydrous sodium sulfate, and subjected to column chromatography (fluent: benzene-ethyl acetate = 4:1) to give 216 mg. Yield: 82%, oil, a mixture of **3** and **4**. Anal. for $C_{10}H_{11}Cl_2NO_3$(264.11). Calc. C: 45.46 H: 4.16, N: 5.32. Found C: 46.01, H: 4.50, N: 6.03. IR (film):g570-2780, 1775, 1685, 1650, 1165, 820, 795 cm^{-1}.

Compound **3**.^{1}H-NMR ($CDCl_3$): δg.34 (s, 3H, CH_3), 2.40-2.60 (m, 2H, H-3), 3.32-3.42 (m, 1H, H-7), 5.42 (d, H, ^3J = 7.3 Hz, H-7a), 5.62 (s, 1H, $CHCl_2$), 5.98 (d, 1H, ^3J = 10.2 Hz H-5), 7.08 (d, 1H, ^3J = 10.25 Hz, H-6). ^{13}C-NMR ($CDCl_3$): δg74.37 (C-2), 46.65 (C-3), 31.46 (C-3a), 40.17 (C-4), 137.56 (C-5), 126.52 (C-6), 147.13 (C-7), 74.7 (C-7a).

Compound **4**. ^{1}H-NMR ($CDCl_3$): δg.36 (s, 3H, CH_3), 2.40-2.60 (m, 2H, H-3), 3.32-3.42 (m, 1H, H-3a), 5.68 (d, 1H, ^3J = 7.4 Hz H-7a), 5.84 (s, 1H, $CHCl_2$), 6.49 (d, 1H, ^3J = 9.9 Hz, H-5), 5.42 (d, 1H, ^3J = 9.9 Hz, H-6). ^{13}C-NMR ($CDCl_3$):gδ 174.31 (C-2), 46.41 (C-3), 31.30 (C-3a), 39.86 (C-4), 135.41 (C-5), 118.67 (C-6), 148.79 (C-7), 70.65 (C-7a).

(4*R,Z*)-4(Dichloro-methyl)-4-methyl-3a,4,8,8a–hexahydro-3*H*-furo[2,3-b]azepine-2,7-dione 5

A solution of 243 mg of PCl_5 in 5 mL of dichloromethane was added dropwise to a solution of oximes **3** and **4** (264 mg, 1 mmol) in anhydrous dichloromethane (10 mL). The solution was stirred for 1 h at room temperature. After adding water the solution was extracted with saturated sodium chloride, dried over anhydrous sodium sulfate, and then evaporated. The crude product was subjected to column chromatography (eluent benzene:ethyl acetate = 4:1) to give **5**, 79 mg. Yield: 35%, oil, lactam. Anal. for $C_{10}H_{11}Cl_2NO_3$ (264.10). Calc. C: 45.46, H: 4.16, N: 5.32. Found: C: 45.98, H: 4.82, N: 5.90. IR (film): 3210, 3115, 1785, 1750, 1685, 1655, 1330, 1145, 820, 795 cm^{-1}.^{1}H-NMR ($CDCl_3$): δg.63 (s, 3H, CH_3), 2.55 (dd, 1H, ^2J =16.3 Hz, ^3J = 2.8 Hz, H-3$_{ax}$), 3.07 (d, 1H, ^2J =16.3 Hz, ^3J =9.2 Hz, H-3$_{eq}$), 3.38 (bd, 1H, ^3J = 7.3 Hz, ^3J = 2.8 Hz, ^3J = 9.2 Hz, H-3a,), 5.78 (s, 1H, $CHCl_2$), 5.84 (d, 1H, ^3J =12.7 Hz, H-6), 6.18 (d, 1H, ^3J = 12.7 Hz, H-5),6.38 (d, H, ^3J = 7.3 Hz, H-8a). ^{13}C-NMR ($CDCl_3$):gδg9.72 (C-3a), 148.49 (C-7), 104.70 (C-5), 115.11 (C-6), 51.87 (C-4), 27.65 (C-8a), 52.70 (C-3), 172.41 (C-2), 18.15 (CH_3), 79.76 ($CHCl_2$).

***(4R)*- 4-(Dichloro-methyl)-4-methyl-tetrahydro-benzofuran-2,7*(3H,7aH)*-dione 6**

A mixture of lactone **2** (1 g, 5 mmol) and 100 mg of 10% palladium/charcoal in 60 mL of ethyl acetate was hydrogenated at room temperature under the atmospheric pressure of 99-105 kPa. The mixture was filtered and the filtrate evaporated. Yield: 933 mg, 93%, mp 137 °C (crystallization from ether). Anal. for $C_{10}H_{12}O_3Cl_2$(250.02). Calc. C: 48.39, H: 4.06. Found C: 48.56, H: 4.07. IR (KBr): 1785 (C = O, γ-lactone), 1720 (C=O, ketone), 1180 (C-O, γ-lactone), 800 (C-Cl) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δg.18 (s, 3H, CH_3), 1.98 (m, 2H, H-5), 2.41-2.73 (ABq-d, 2H, $J_{(3,3a)}$ = 8.4 Hz, $J_{(3',3a)}$ = 6.8 Hz,$J_{(3,3')}$ = 12.43 Hz, H-3), 2.21-2.58 (m, 2H, H-6), 5.06 (d, 1H, $J_{(7a,3a)}$ = 9.6 Hz, H-7a). ^{13}C-NMR ($CDCl_3$): δg04.6 (C-7), 173.8 (C-2), 82.42 (C-7a), 29.87 (C-3a), 43.72 (C-3), 44.38 (C-4), 30.59 (C-5), 34.38 (C-6), 17.95 (CH_3), 79.76 ($CHCl_2$).

(4*R*,*E*)-4-(Dichloro-methyl)-7-(hydroxy-imino)-4-methyl-hexahydro-benzofuran 2,(3*H*)-one 7

Lactone **6** (249 mg, 1 mmol), hydroxylamine hydrochloride (100 mg, 1.3 mmol), and sodium acetate (160 mg, 2 mmol) were all dissolved in methanol (10 mL) containing 2 mL of water. The solution was heated under reflux for 1 h, then evaporated, extracted with dichloromethane, dried over anhydrous sodium sulfate, and subjected to column chromatography (eluent benzene:ethyl acetate = 4:1) to give the product **7**, 230 mg. Yield: 87%, mp 148 °C. Anal. for $C_{10}H_{13}Cl_2NO_3$ (266.12). Calc. C: 45.30, H: 4.90, N: 5.28. Found: C: 46.17, H: 4.53, N: 5.98. IR (KBr): 3320-3220 (OH), 1765 (C=O, γ-lactone), 1650, 1520 (C=N), 1310 (N-O), 1080 (C-O, γ-lactone), 800 (C-Cl) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δg.16 (s, 3H, CH_3), 1.80-1.88 (m, 2H, H-5), 2.46-2.83 (m, 4H, H-3, H-6), 3.10-3.30 (ddd, H, $J_{(3a,3)}$ = 7.1Hz, $J_{(3a,3')}$ = 6.8 Hz, $J_{(3a,7a)}$ = 7.2 Hz, H-3a), 5.10 (d, 1H, $J_{(7a,3a)}$ = 7.2 Hz, H-7a), 6.12 (s, 1H, ($CHCl_2$). ^{13}C-NMR ($CDCl_3$): δg78.2 (C-2), 42.61 (C-3a), 29.49 (C-3), 44.87 (C-4), 32.22 (C-5), 17.83 (C-6), 153.5 (C-7), 80.33 (C-7a), 18.93 (CH_3), 79.38, ($CHCl_2$).

5,5-Dichloro-4-methyl-4-(5-oxo-tetrahydrofuran-3-yl)pentanenitrile 8

A solution of 243 mg of PCl_5in 5 mL of dichloromethane was added dropwise to a solution of oxime **7** (266 mg, 1 mmol) in 10 mL of anhydrous dichloromethane. The solution was stirred for 1 h at room temperature. After adding water the solution was extracted with dichloromethane. The extract was successively washed out with an aqueous 5% sodium carbonate and saturated sodium chloride solution. Then, it was dried over anhydrous sodium sulfate and the solvent was evaporated. The crude product was subjected to column chromatography (eluent benzene:ethyl acetate = 4:1) to generate compound **8** as oil,

191 mg. Yield: 72%. Anal. for $C_{10}H_{13}Cl_2NO_3$(266.12). Calc. C: 45.30, H: 4.90, N: 28. Found: C: 45.82, H: 5.10, N: 5.68. IR (film): 3380-3200 (OH), 2240 (CN), 1760 (C=O, γ-lactone), 1160 (C-O, γ-lactone), 800 (C-Cl) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δg.16, 1.42 (s, 3H, CH_3), 2.05-2.29 (m, 2H, H-3*), 2.48-2.65 (m, 2H, H-2*), 2.68-3.10 (m, 2H, H-4), 3.28-3.36 (m, 2H, H-3), 5.70-5.76 (s, 1H, $CHCl_2$), 6.42 (bs, 1H, H-2). ^{13}C-NMR ($CDCl_3$): δg72.7 (C-5), 46.5 (C-4), 30.3 (C-3), 128.2 (C-2), 12.2 (C-2'), 45.5 (C-4*), 27.9 (C-3'), 118.7 (CN), 18.8 (CH_3), 79.1 ($CHCl_2$).

5,5-Dichloro-4-methyl-4-(5-oxo-2,5-dihydrofuran-3-yl)pentanamide 9

Compound **8** (270 mg, 1.1 mmol) and hydrochloric acid (1:1, 10 mL) were refluxed for 1 h. The reaction mixture was then cooled to room temperature, extracted with chloroform, washed successively with saturated sodium hydrogen carbonate and sodium chloride, and dried with anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue was subjected to column chromatography (eluent benzene:ethyl acetate = 4:1) to produce compound **9** as oil, 142 mg. Yield: 52%. Anal. for $C_{10}H_{13}Cl_2NO_3$ (266.12). Calc. C: 45.30, H: 4.90, N: 5.28. Found C: 45.82, H: 5.10, N: 5.68. IR (film): 3350, 3180 (N-H, amide), 1780, 1750 (C=O, γ-lactone), 1690, 1650, (C=O, amide), 1605, 1405, (NH amide), 1160 (C-O, γ-lactone), 800 (C-Cl) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.42 (s, 3H, CH_3), 2.15-2.25 (m, 2H, H-3*), 2.34-2.44 (m, 2H, H-2*), 4.96 (ABq, 2H, $J_{(2,2')} = 14.53$, $J_{(2,4)} = 1.87$, H-2), 5.82 (s, 1H, $CHCl_2$), 6.12 (d, 1H, $J_{(4,2)} = 1.87$ H-4). ^{13}C-NMR ($CDCl_3$): δ 72.4 (C-1'), 167.7 (C-5), 118.3 (C-4), 120.3 (C-3), 71.4 (C-2), 33.8 (C-2*), 48.5 (C-4'), 18.45 (C-3'), 12.8 (CH_3), 76.1 ($CHCl_2$).

Antimicrobial activity

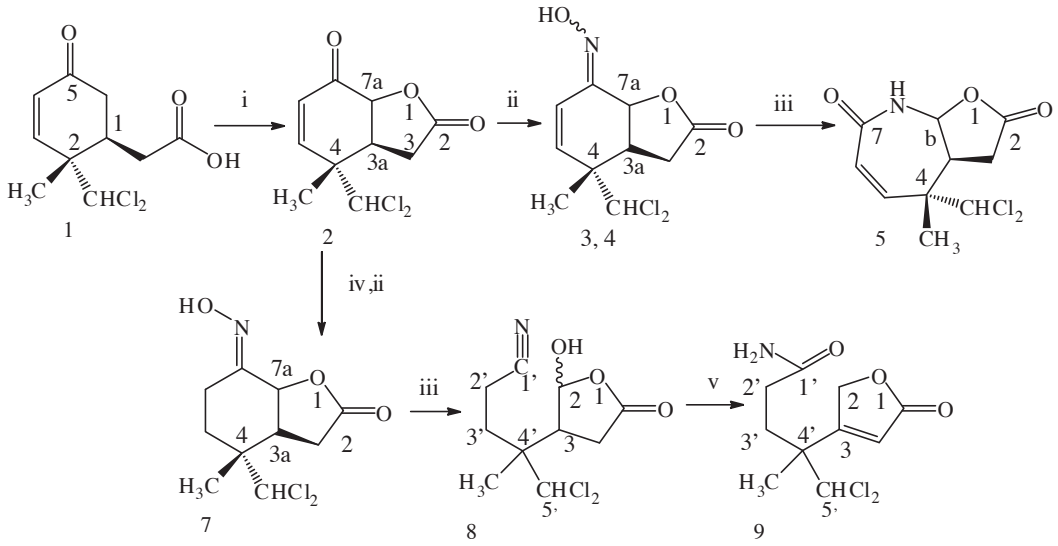
Microorganism Tests

Bacteria used in this experiment were *Enterobacter aerogenes* (IPH), *Enterobacter cloacae* (IPH), *Escherichia coli* (DBFS), *Pseudomonas aeruginosa* (FAB 096), *Staphylococcus aureus* (IPH), *Staphylococcus epidermidis* (IPH), and *Streptococcus agalactiae* (IPH). Fungi used in this experiment were *Aspergillus niger* (IPH) and *Candida albicans* (FSBKg-31).

The antibacterial activity of the compounds' ethanol solutions was investigated by the nutrient agar broth[22,23] disc-diffusion method. The investigation was performed using the bacteria reseeded in nutrient broth for 24 h at 37 °C, and the fungi were reseeded in Sabouraud dextrose broth for 48 h at 25 °C. The cultures were adjusted with sterile water (10^6 CFU/mL for bacteria and 10^4 CFU/mL for fungi) to match and correspond to the density of inoculants. A suspension (1 mL) containing nutrient agar broth was added to the reseeding plates to achieve uniform microbial growth on both control and test plates. The compounds were dissolved in 96% ethanol solution (20 mg/mL and 5 mg/mL for compound **5**) and then sterilized. Under aseptic conditions the empty sterilized disks (Whatman no. 5, 10 mm in diameter) were permeated with the compounds' ethanol solutions and then placed on the agar surface. The plates were left out for 30 min at room temperature to permit compound solution diffusion and then were incubated at 37 °C. After 18-h incubation the zones of inhibition were measured and recorded, (mm). Ethanol was used as a control. Standard commercial drugs that were currently on the market were used for antimicrobial activity control and measurement. Penicillin G (from Galenika a.d., Belgrade) was used as a control drug for antibacterial activity and nystatin (from Panfarma d.o.o. Belgrade) was used as a control drug for antifungal activity. Each test was conducted in triplicate and repeated 3 times.

Results and Discussion

Compound **1,**[24,25] 2-((1S,2S)-2-dichloro-methyl-2-methyl-5-oxo-cyclohex-3-enyl) acetic acid, was synthesized according to a previously described procedure[26] until its structure was confirmed on the basis of spectral data, further concurring with the literature. Acetoxylation[27,28]of 2-(2-(dichloro-methyl)-2-methyl-5-oxocyclohex-3-enyl) acetic acid **1** with lead(IV) acetate in the presence of BF_3 etherate produced 4-(dichloro-methyl)-4-methyl-3a,4,–hexahydro-2,7(3*H*,7a*H*)-benzofuran-dione **2**. Compound **2** via oximes[29] **3** and **4** transformed into lactamic lactone 4-(dichloro-methyl)-4-methyl-3a,4,8,8a–tetrahydro-3*H*-furo[2,3-b]azepine-2,7-dione **5** by Beckmann rearrangement.[30–32] The key step in synthesizing 5,5-dichloro-4-methyl-4-(5-oxo-tetrahydrofuran-3-yl) pentanamide **9** involves the Beckmann fragmentation of 4-(dichloro-methyl)-7-(hydroxy-imino)-4-methyl-hexahydro benzofuran-2,(3*H*)one **7** with phosphorus pentachloride (PCl_5)in a dichloromethane solution (Scheme).



Scheme. Reagents and conditions. i) LTA, BF_3Et_2O, C_6H_6, 25 °C, 5 h. ii) $NH_2OH.HCl$, AcONa, H_2O, CH_3OH, 60 °C, 1 h. iii) PCl_5, CH_2Cl_2, 25 °C, 1 h. iv) Pd/H_2, AcOEt, 100 kPa, 4 h. v) HCl, H_2O, reflux, 1 h.

Test results of the compounds' antimicrobial activity are presented in the Table. The antimicrobial activity of the synthesized compounds, compared to the control drugs, indicated that compounds **2**, **3**, **4**, **5**, and **9** inhibited the growth of all the tested microorganisms.

Bioassays demonstrated that for all the tested microorganisms, compounds **2**, **9**, and **5** exhibited greater biological activity than the mixture of **3** and **4** oximes. The 20 mg/mL derivate **5** concentration was more active than the others, with an inhibition zone ranging from 27 to 30 mm against *E. coli*, *E. cloacae*, *E. aerogenes*, and *S. aureus* (29, 30, 27, and 30 mm, respectively); therefore its antibacterial activity was equivalent to that of penicillin G's. A low compound **5** concentration (5 mg/mL) and the compound **2** concentration of 20 mg/mL were not active against *C. albicans*. Moreover, a mixture of compounds **3** and **4** at the same concentration did not generate any antimicrobial activity against *S. epidermidis, S. agalactiae*, or *E. aerogenes*, or against either of the tested fungi.

Table. Antimicrobial activity of lactone derivatives.

Microorganisms:	Type of compounds[a,b,c]					Antibiotics (control)	
	2	**3** and **4**	**5**		**9**	penicillin G	Nystatin
	20 mg/mL	20 mg/mL	20 mg/mL	5 mg/mL	20 mg/mL	30 mg/mL	10 mg/mL
Staphylococcus aureus (G+)	21	19	17	14	15	26	-
Staphylococcus epidermidis (G+)	25	0	26	16	24	26	-
Streptococcus agalactiae (G+)	21	0	24	15	21	27	-
Escherichia coli (G-)	21	19	29	18	22	23	-
Pseudomonas aeruginosa (G-)	13	13	17	14	15	19	-
Enterobacter cloacae (G-)	20	19	30	19	24	21	-
Enterobacter aerogenes (G-)	20	0	27	18	20	21	-
Aspergillus Niger	0	0	18	0	14	-	20
Candida albicans	16	0	16	0	15	-	21

[a] Mean value ± SD, n = 3, the zone of inhibition in mm

[b] "0" absence of antimicrobial activity, "-" did not exhibit a zone of inhibition.

[c] Solvent control ethanol was negative.

References

1. Hoffmann, H. M. R.; Rabe, J. *Angew Chem.* **1985**, *24*, 94-110.
2. Devon; T. K.; Scott, A. I. *Handbook of Naturally Occurring Compounds*, Academic Press, New York, Vol. 1, pp 249-250, 1975.
3. Yang, X.; Shimizu, Y.; Steiner, J. R.; Clardy, J. *Tetrahedron Lett.* **1993**, *34*, 761.
4. Davidson, B. S.; Ireland, C. M. *J. Nat. Prod.* **1990**, *53*, 1036.
5. Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, *18*, 37.
6. Pettus, J. A. Jr.; Wing, R. M.; Sims, J. J. *Tetrahedron Lett.* **1977**, *18*, 41.
7. Hung, T. V.; Mooney, B. A.; Prager, R. H.; Tippett, J. M. *Aus. J. Chem.* **1981**, *34*, 383-395.
8. Gammill, R. B.; Wilson, C. A.; Bryson, T. A. *Synth. Commun.* **1975**, *5*, 245-251.
9. Grieco, P. A. *Synthesis* **1975**, 67-82.
10. Grieco, P. A.; Noguesz, J.A; Masaki, Y.; Hiroi, K.; Nishizawa, I. *J. Med. Chem.* **1977**, *20*, 71-76.
11. Huang, S.; Piantadosi, C.; Pagano, J. S.; Geissman, T. A.; Lee, K. H. *Cancer. Res.* **1971**, *31*, 1649-1656.

12. Rao, Y. S. *Chem. Rev.* **1976**, *76*, 625-694.

13. Pelter, A.; Satyanarayana, P.; Ward, R. S. *Tetrahedron Lett.* **1981**, *22*, 1549-1550.

14. Geissman, T. A.; Irwin, M. A. *Pure and Appl. Chem.* **1970**, *21*, 167-180.

15. Howie, G. A.; Stamos I. K.; Cassady, J. M. J. *Med. Chem.* **1976**, *19*, 309-313.

16. Kupchan, S. M.; Earkin, M. A.; Thomas, A. M. J. Med. Chem. **1971**, *14*, 1147-1152.

17. Askin, D.; Wallace, M. A.; Vacca, J. P.; Reamer, R. A.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1992**, *57*, 2771-2773.

18. Chamberlain, R.; Koch, C. *J. Org. Chem.* **1993**, *58*, 2725-2737 and references therein.

19. Lee, K.; Choi, Y.; Gullen, S.; Wirtz, S. S.; Schinazi, R. F.; Cheng, Y. C.; Chu, C. K. *J. Med. Chem.* **1999**, *42*, 1320-1328.

20. Garcıa, C.; Martin T.; Martin, V. S. *J. Org. Chem.* **2001**, *66*, 1420-1428.

21. DEPT ^{13}C NMR: Quaternary carbons are not observed, while others are positive, negative, or null depending on the "angle" (45°, 90°, 135°) used in the experiment. This technique effectively identifies the most basic structural units: C, CH, CH_2, and CH_3.

22. Kavanagh, F. *Analytical microbiology*, Acad. Press, New York, 1972.

23. NCCLS, Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard NCCLS Publication M2/A4, Villanova, PA, USA, 1990.

24. Compound **1**. 2-((1*S*, 2*S*)-2-dichloro-methyl-2-methyl-5-oxo-cyclohex-3-enyl)acetic acid ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.36 (s, 3H, CH_3), 2.41 (part B, ABq, J = 15.81 Hz, J = 7.41 Hz, 1H, H-6), 2.53 (part A, ABq, 1H, J = 15.81 Hz, J = 2.60 Hz, J = 0.79 Hz, H-6), 2.69 (part A, ABq, J = 15.30 Hz, J = 10.1 Hz, 1H, H-9), 3.05 (part B, ABq, 1H, J = 15.31 Hz, J = 3.91 Hz, H-9), 3.15 (m, 1H, J = 7.41 Hz, J = 2.60 Hz, J = 10.1 Hz, J = 3.91 Hz,H-1), 5.98 (s, 1H, $CHCl_2$), 6.15 (d, 1H, J = 9.9 Hz, H-4), 7.05 (dd, 1H, J = 9.9 Hz, J = 0.90 Hz, H-3).

25. The chiral center is designated according to the well-known priority-sequence rules.

26. Stefanovic, M.; Jeremic, D.; Solujic, S.; Sukdolak, S. *J. Serb. Chem. Soc.* **1986**, *51*, 429-434.

27. Bhalerao, U. T.; Rapopart, H. *J. Am. Chem. Soc.* **1971**, *93*, 4835-4940.

28. Solujic, S.; Sukdolak S.; Ratkovic, Z. *Tetrahedron Lett.* **1991**, *32*, 4577-4578.

29. Shoppee, C. W.; Krueger, G. *J. Chem. Soc.* **1961**, 3641-3655.

30. Conley, R. T.; Longe, R. J. *J. Org. Chem.* **1963**, *28*, 210-214.

31. Oka, K.; Hore, S. *J. Org. Chem.* **1978**, *43*, 3790-3791.

32. Suginome, H.; Kaji M.; Yamada, S. *J. Chem. Soc., Perkin Trans.* **1988**, *1*, 321-326.