Structure Confirmation of a Bioactive Lactone Isolated from *Otoba parvifolia* through the Synthesis of a Model Compound

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Received July 9, 2004

Synthesis of a model compound **4** structurally related to a bioactive lactone **3** isolated from *Otoba parvifolia* has been accomplished. The good match between the NMR data of both compounds suggests they have identical bicyclic [3.3.1] carbon skeletons.

Gottlieb and co-workers, when studying the fruit kernels of *Otoba parvifolia* (Mkgf), A. Gentry, isolated and identified a number of novel compounds.[1] One of these compounds was assigned the novel bicyclic lactone structure **1**. This lactone has promise for new antibiotic development since it shows activity in vitro as pronounced as tetracycline against important microorganisms such as *Staphylococus aureus*, *Staphylococus epidermis*, and *Peptococus* sp.[2]

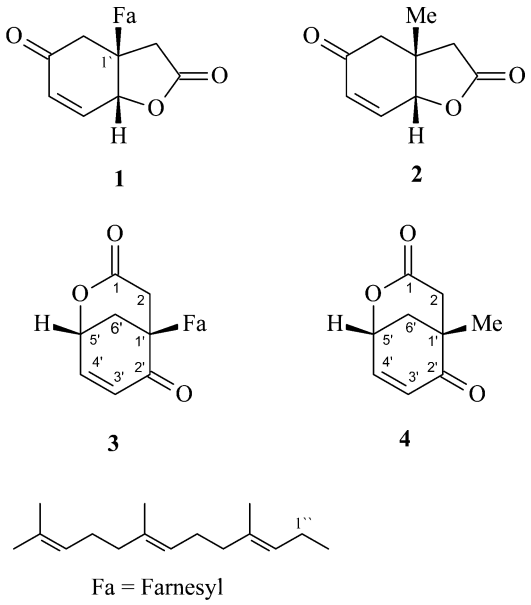
Attracted by the novel structure **1** and its promising biological activity, we synthesized the model compound **2** for structural confirmation.[3] However, a clear mismatching of NMR data was observed between the synthetic model and natural compounds, which led Gottlieb and co-workers to revise the structure of the natural lactone[4] by proposing the alternative bicyclic lactone structure **3**.

To verify the correctness of the revised structure **3**, comparison with another model compound has now been performed. Herein we present the synthesis and structural characterization of the model bicyclic lactone **4**, which contains the same bicyclic ring system as the revised structure **3**.

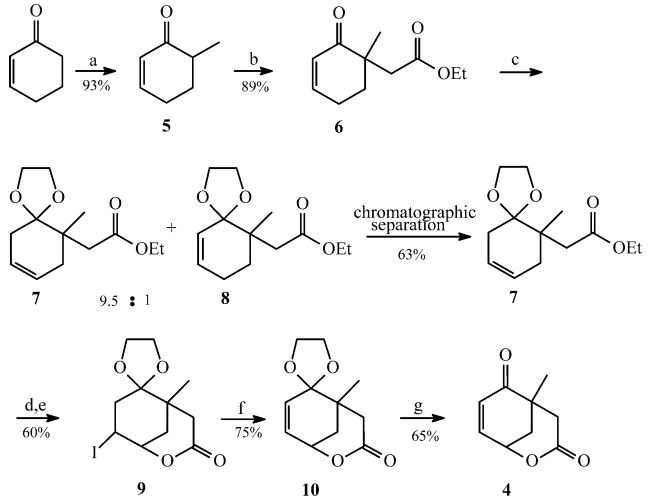
The strategy chosen for synthesis of compound **4** employed an iodolactonization as the key step (Scheme 1). The double alkylation of cyclohexenone was carried out under kinetic conditions and in the presence of HMPA,[5] affording compound **6** in 82% yield for the two steps. When ketone **6** was treated with ethylene glycol in the presence of PTSA in benzene,[6] a mixture of ketals **7** and **8** was obtained in a 9.5:1 ratio, as determined by GC. The regioisomers were separated by flash chromatography and the migration of the double bond was confirmed by the ^{1}H NMR spectrum of the major compound, which showed a multiplet for the vinylic hydrogens. Ester **7** was then hydrolyzed in the presence of KOH and the acid derivative submitted to iodolactonization conditions,[7,8] furnishing the iodo lactone **9** in 60% yield.

Dehydrohalogenation of the iodolactone **9** with DBU in benzene[9] afforded ketal lactone **10** in 75% yield. Removal of the ketal protecting group of **10** by transketalization with PTSA in acetone[6] generated the model compound **4** in 65% yield.

The NMR data of compound **4** showed good agreement with that of the natural lactone. A good match between the ^{13}C NMR data of both compounds (Table 1) was observed, except for the peaks attributed to C-2, C-1′, and



Scheme 1. Synthesis of Model Lactone **4**[a]



[a] Reagents: (a) LDA, THF, HMPA, −78 °C, MeI. (b) LDA, THF, HMPA, −78 °C, $BrCH_2COOEt$. (c) PTSA, $HOCH_2CH_2OH$, benzene, reflux 20 h. (d) KOH, THF/H_2O (3:1); HCl. (e) I_2, KI, $NaHCO_3$, THF, Et_2O. (f) DBU, benzene. (g) acetone, PTSA.

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10.1021/np049771x CCC: $27.50
Published on Web 11/03/2004

Table 1. ^{13}C NMR (δ) of Natural Lactone **3** and Model Compound **4**

C	natural lactone **3**	model compound **4**
1	169.0	168.5
2	39.7	41.6
1′	46.0	42.0
2′	200.3	200.5
3′	130.7	130.3
4′	144.5	144.4
5′	69.8	69.7
6′	33.6	36.9

Table 2. ^{1}H NMR Data of the Natural Lactone **3** and Model Compound **4**

H	natural lactone **3** (300 MHz)	model compound **4** (400 MHz)
2α	2.57 dd	2.72 dd
2β	2.67 d	2.58 d
3′	6.13 d	6.15 d
4′	7.13 ddd	7.15 ddd
5′	5.00−5.10 m	5.00−5.10 m
6′α	2.26 ddd	2.31 dt
6′β	2.20 ddd	2.25 ddd

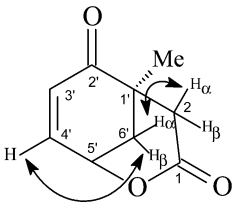

Figure 1. Long-range coupling (*W*-coupling) between H-4′ and H-6′β (J = 1.57 Hz) and H-6′α and H-2α (J = 2.02 Hz).

C-6′, which for **3** are under the influence of the farnesyl substituent and of the methyl substituent for the model compound **4**. The chemical shifts and multiplicity observed for the vinylic and allylic hydrogens of the model compound **4** are very closely related to those of the natural lactone **3** (Table 2). The double double doublet observed for the signal at δ 7.15 results from two vicinal couplings of H-4′ with H-3′ (J = 9.79 Hz) and with H-5′ (J = 5.96 Hz), along with a *W*-coupling with H-6′β (J = 1.57 Hz). Another *W*-coupling between H-2α and H-6′α (J = 2.02 Hz) is observed in the ^{1}H NMR spectrum of **4**, as shown in Figure 1. The small difference in the chemical shifts of H-2 and H-6′ observed between the model and natural substance can be attributed to the different substituents present on C-1′.

The mass spectrum showed that the protonated bicyclic lactone **4** dissociated upon collision activation (20 eV CID) by sequential loss of H_2O and CO molecules, and this dissociation chemistry is typical for protonated lactones.[10]

Thus, synthesis of model compound **4**, structurally related to bioactive lactone **3** isolated from *Otoba parvifolia*, has been performed. Comparison of spectral data suggests that **3** and **4** have identical bicyclic carbon skeletons. An enantioselective total synthesis of the natural lactone is being carried out in our laboratory, which it is hoped will permit the determination of the absolute configuration of the natural compound **3**.

Experimental Section

General Experimental Procedures. Reagents and solvent were purified and dried using standard methods. Reactions involving organometallic reagents were carried out under argon in oven-dried glassware. Reagents employed: HMPA (hexamethylphosphoramide), DBU (1,8-diazabicyclo[5.4.0]-undec-7-ene, LDA (lithium diisopropylamide), PTSA (*p*-toluenesulfonic acid). Reactions were monitorated by thin-layer chromatography (TLC: E. Merck, Type 5554 plates) and gas chromatography (GC) with a Varian 3800 GC (flame ionization detector, FID) equipped with a Varian CP-SIL 8 capillary column (30 m × 0.25 mm × 0.25 μm). Flash column chromatography was carried out with 230−400 mesh silica gel (E. Merck). IR spectra were recorded on a Bomer model MB 102 spectrometer with internal calibration. Low-resolution mass spectra were obtained on a GC/MS Varian Saturn 2000 spectrometer. High-resolution and high-accuracy MS and 20 eV MS/MS collision-induced dissociation (CID) spectra were acquired using electrospray ionization (ESI) on positive ion mode on a Q-Tof (Micromass, UK) mass spectrometer of hybrid quadrupole orthogonal time-of-flight configuration. Typical ESI conditions were as follows: capillary voltage of 1 kV, cone voltage of 40 V, and dessolvation gas temperature of 100 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions using a Bruker Advance-400 spectrometer. Deuterated solvents were used as lock and TMS as reference signal. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. The ^{13}C chemical shifts (δ) are reported in ppm relative to the center peak of $CDCl_3$ (δ 77.00).

6-Methyl-2-cyclohexenone (5). To a solution of diisopropylamine (1.58 g, 15.6 mmol) in THF (8 mL) at 0 °C was added n-BuLi in hexanes (5.5 mL, 14.3 mmol). After stirring for 20 min the solution was cooled to −78 °C and 2-cyclohexenone (1.00 g, 10.4 mmol) in THF (10 mL) was added dropwise. The solution was stirred for 30 min followed by dropwise addition of iodomethane (2.96 g, 20.8 mmol). After stirring for 30 min at −78 °C, HMPA (6 mL) was added and the mixture was kept for another 2 h at −78 °C. Ether (20 mL) was added to the reaction mixture at 0 °C, and the organic layer was washed with saturated NH_4Cl (5 × 5 mL) and saturated NaCl (3 × 5 mL) and dried over Na_2SO_4. After filtration the solvent was removed by rotatory evaporation and the crude product was purified by flash chromatography with hexane/ethyl acetate (10:1 v/v) to give compound **5** (1.07 g, 93%): IR (KBr) ν_{max} 3033, 2964, 2872, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (3H, d, J = 6.8 Hz), 1.65−1.85 (1H, m), 1.95−2.10 (1H, m), 2.30−2.45 (3H, m), 5.98 (1H, dt, J = 10.0, 2.0 Hz), 6.94 (1H, dt, J = 10.0, 4.96 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.0, 25.5, 30.8, 41.6, 129.4, 149.6, 202.3; EIMS *m/z* 110 [M^+] (42), 81 (3), 68 (100), 53 (4), 39 (27); CIMS (CH_3CN) *m/z* 111 [M + 1]$^+$ (100).

Ethyl (1-Methyl-2-oxo-3-cyclohexenyl)acetate (6). To a solution of diisopropylamine (0.30 g, 2.95 mmol) in THF (3 mL) at 0 °C was added n-BuLi in hexanes (1.20 mL, 2.85 mmol). After stirring for 20 min the solution was cooled to −78 °C, followed by dropwise addition of 3-methyl-2-cyclohexenone (0.21 g, 1.9 mmol) in THF (3 mL). After 2 h at −78 °C ethyl bromoacetate (0.65 g, 3.82 mmol) was added dropwise. The resulting solution was stirred for 30 min at −78 °C, and HMPA (3 mL) was added. After stirring for 3 h at −78 °C ether (20 mL) was added followed by the addition of saturated NH_4Cl (5 mL). The organic solution was washed with saturated NH_4Cl (5 × 5 mL) and saturated NaCl (3 × 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a mixture of hexane/ethyl acetate (5:1, v/v), affording compound **6** (0.33 g, 89%): IR (KBr) ν_{max} 3033, 2977, 2934, 2872, 1735, 1677, 1215 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (3H, s), 1.20 (3H, t, J = 7 Hz,), 1.70−1.90 (2H, m), 2.18−2.42 (2H, m), 2.70 (1H, s), 2.78 (1H, s), 4.07 (2H, q, J = 7.0 Hz), 5.94 (1H, dt, J = 10.0, 1.7 Hz), 6.88 (1H, dt, J = 10.0, 3.9 Hz,); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 21.8, 22.9, 32.6, 41.6, 43.3, 60.1, 128.1, 148.7, 171.2, 201.9; EIMS *m/z* 197 [M + 1]$^+$ (100), 151 (86), 122 (11), 107 (11), 68 (42), 39 (22); CIMS (CH_3CN) *m/z* 197 [M + 1]$^+$ (100).

Ethyl (6-Methyl-1,4-dioxaspiro[4.5]dec-8-en-6-yl)acetate (7). A solution of ketone **6** (0.631 g, 3.21 mmol), ethylene glycol (1.20 g, 19.3 mmol), and *p*-toluenesulfonic acid monohydrate (0.077 g, 0.4 mmol) in benzene (15 mL) was refluxed in a flask connected to a Dean Stark trap for 20 h. The mixture was diluted with ether (50 mL), washed with saturated $NaHCO_3$ (2 × 20 mL) and water (1 × 20 mL), and dried over

$MgSO_4$. The filtrate was concentrated in vacuo and purified by flash chromatography using a mixture of hexane/ether (1:1 v/v) to give ketal **7** (0.487 g, 63%): IR (KBr) ν_{max} 2979, 2360, 2340, 1727, 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.11 (3H, s), 1.26 (3H, t, J = 7.14 Hz), 2.14 (1H, ddd, J = 17.8, 3.94, 1.91 Hz), 2.21−2.23 (2H, m), 2.34−2.46 (3H, m), 3.94−4.00 (4H, m), 4.11 (2H, q, J = 7.14 Hz,), 5.55−5.59 (1H, m), 5.64−5.67 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3, 19.9, 33.3, 36.6, 39.7, 40.2, 60.0, 65.1, 65.2, 111.0, 123.7, 125.9, 172.6; EIMS m/z 241 $[M+1]^+$ (21), 240 $[M^+]$ (25), 195 (31), 186 (33), 152 (14), 125 (100), 113 (58); HRESIMS m/z 241.1411 $[M + H]^+$ (calcd for $C_{13}H_{21}O_4$, 241.1439).

4′-Iodo-1′-methyl-7′*H*-spiro[1,3-dioxolane-2,2′-[6]oxabicyclo[3.3.1]nonan]-7′-one (9). To a solution of **7** (0.143 g, 0.6 mmol) in ethanol (15 mL) was added 20% (w/v) aqueous KOH solution (0.25 mL, 0.9 mmol). The resulting mixture was refluxed for 6 h, and water was added (5 mL) followed by elimination of two-thirds of solvent under reduced pressure. The solution was acidified by the dropwise addition of 0.1 $mol \cdot L^{-1}$ HCl until pH 2 and extracted with ether (3 × 15 mL). After eliminating the solvent under reduced pressure, the crude product was dissolved in ether (3.5 mL) and a solution of saturated $NaHCO_3$ (3.5 mL) was added. After stirring for 30 min, a solution of I_2 (0.26 g, 1 mmol) and KI (0.52 g, 3.1 mmol) in water (5 mL) was added. The resulting mixture was vigorously stirred for 24 h and diluted with water (20 mL), the phases were separated, and the aqueous phase was extracted with ether (3 × 20 mL). The organic extracts were combined and washed with 10% (w/w) $Na_2S_2O_3$ (2 × 10 mL) and saturated brine (2 × 10 mL) and dried over $MgSO_4$. After filtration the solvent was removed under reduced pressure and the product was purified by flash chromatography using hexane/ethyl acetate (3:1 v/v) to give iodolactone **9** (0.121 g, 60%): IR (KBr) ν_{max} 2959, 1734, 1368, 1238, 1202, 1106, 1017, 945, 840, 807, 688, 589 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.01 (3H, s), 1.79 (1H, ddd, J = 14.6, 4.07, 2.30 Hz), 2.03 (1H, dd, J = 16.7, 5.33 Hz), 2.09 (1H, dt, J = 16.7, 1.48 Hz), 2.31 (1H, d, J = 19.3 Hz), 2.75 (1H, dt, J = 14.6, 2.00 Hz), 2.92 (1H, dd, J = 19.3, 2.30 Hz), 3.98−4.12 (4H, m), 4.54−4.56 (1H, m), 4.78−4.82 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.4, 21.8, 30.7, 34.0, 37.7, 40.2, 65.0, 65.2, 78.9, 109.0, 170.0; EIMS m/z 339 $[M + 1]^+$ (6), 321 (7), 277 (3), 211 (100), 167 (14), 157 (5), 149 (8), 113 (10), 99 (9), 69 (9); HRESIMS m/z 339.0240 $[M + H]^+$ (calcd for $C_{11}H_{16}IO_4$, 339.0094).

1′-Methyl-7′*H*-spiro[1,3-dioxolane-2,2′-[6]oxabicyclo-[3.3.1]non[3]en]-7′-one (10). To a solution of iodo lactone **9** (115 mg, 0.34 mmol) in benzene (10 mL) was added DBU (0.10 g, 0.65 mmol). After stirring for 3 h at room temperature, the mixture was filtered and the solid rinsed with ether (3 × 10 mL). The combined organic extracts were washed with saturated NH_4Cl (2 × 10 mL) and saturated NaCl (2 × 10 mL) and dried over $MgSO_4$. After removing the solvent under reduced pressure, the crude product was purified by flash chromatography (hexane/ethyl acetate, 3:1 v/v) to give compound **10** (54 mg, 75%): IR (KBr) ν_{max} 2981, 2962, 2926, 2895, 1719, 1692, 1448, 1397, 1376, 1204, 1107, 1006, 954, 924, 799 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.09 (3H, s), 1.88 (1H, ddd, J = 14.1, 2.88, 1.64 Hz), 2.27 (1H, dt, J = 14.1, 1.43 Hz), 2.41 (1H, d, J = 19.8 Hz), 3.10 (1H, dd, J = 19.8, 1.43 Hz), 3.84−4.12 (4H, m), 4.76−4.79 (1H, m), 5.79 (1H, d, J = 9.79 Hz), 6.07 (1H, ddd, J = 9.79, 5.40, 1.64 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.4, 35.1, 37.5, 39.3, 65.5, 65.8, 70.1, 107.9, 127.4, 131.9, 171.3; EIMS m/z 210 $[M^+]$ (3), 151 (2), 139 (5), 128 (100), 112 (8), 99 (10), 83 (12), 55 (21); HRESIMS m/z 211.0937 $[M + H]^+$ (calcd for $C_{11}H_{15}O_4$, 211.0970).

5-Methyl-2-oxabicylo[3.3.1]non-7-ene-3,6-dione (4). To a solution of compound **10** (51 mg, 0.24 mmol) in acetone (15 mL) was added PTSA (5 mg, 0.030 mmol). After stirring for 5 h at room temperature, the solvent was removed under reduced pressure and ether (20 mL) was added to the residue. The organic phase was washed with saturated $NaHCO_3$ (2 × 10 mL) and dried over $MgSO_4$. The filtrate was concentrated in vacuo and purified by flash chromatography (hexane/ethyl acetate, 1:1 v/v), giving model compound **4** (26 mg, 65%) as a white noncrystalline solid: IR (KBr) ν_{max} 2933, 1736, 1690, 1230, 1013 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.27 (3H, s), 2.25 (1H, ddd, J = 14.1, 3.24, 1.57 Hz), 2.31 (1H, dt, J = 14.1, 2.02 Hz), 2.58 (1H, d, J = 19.1 Hz), 2.72 (1H, dd, J = 19.1, 2.02 Hz), 5.00−5.10 (1H, m), 6.15 (1H, d, J = 9.79 Hz), 7.15 (1H, ddd, J = 9.79, 5.96, 1.57 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.3, 36.9, 41.6, 42.0, 69.7, 130.3, 144.4, 168.5, 200.5; EIMS m/z 167 $[M + 1]^+$ (3), 149 (4), 138 (35), 120 (33), 121 (10), 109 (23), 108 (14), 95 (47), 93 (31), 83 (54), 81 (50), 79 (35), 77 (34), 67 (10), 55 (100), 39 (43); HRESIMS m/z 167.0781 $[M + H]^+$ (calcd for $C_9H_{11}O_3$, 167.0708).

Acknowledgment. F.A.M. thanks the Brazilian Council for the Development of Science and Technology (CNPq) for financial support. M.N.E. thanks CNPq and the São Paulo State Research Foundation, FAPESP.

References and Notes

(1) Ferreira, A. G.; Motidome, M.; Gottlieb, O. R.; Fernandes, J. B.; Vieira, P. C.; Cojocaru, M.; Gottlieb, H. E. *Phytochemistry* **1989**, *28*, 579−583.
(2) Ferreira, A. G. Personal communication. Biological tests were performed at The Centro de Pesquisas da Rhodia, Paulínia, SP, Brazil.
(3) Ferreira, J. B.; Boscaini, R. C.; Marques, F. A. *Nat. Prod. Lett.* **1993**, *2*, 313−316.
(4) Ferreira, A. G.; Fernandes, J. B.; Vieira, P. C.; Gottlieb, O. R.; Gottlieb, H. E. *Phytochemistry* **1995**, *40*, 1723−1728.
(5) Paquette, L. A.; Okazaki, M. E.; Caille, J.-C. *J. Org. Chem.* **1988**, *53*, 477−481.
(6) Marques, F. A.; Ferreira, J. T. B.; Piers, E. *J. Braz. Chem. Soc.* **2000**, *11*, 502−511.
(7) Pearson, A. J.; Hsu, S.-Y. *J. Org. Chem.* **1986**, *51*, 2505−2511.
(8) Cuzzupe, A. N.; Di Florio, R.; Rizzacasa, M. A. *J. Org. Chem* **2002**, *67*, 4392−4398.
(9) Wakamatsu, T.; Hara, H.; Ban, Y. *J. Org. Chem.* **1985**, *50*, 108−112.
(10) Donovan, T.; Brodbelt, J. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 47−59.

NP049771X