Octylsparsomycin (19) was prepared by a mixed anhydride procedure as follows. To a stirred, cooled (0 °C) solution of the acid **9**[2] (112 mg, 0.66 mmol) and triethylamine (86 mg, 0.86 mmol) in 5 mL of THF/DMF (1:1, v/v) was added ethyl chloroformate (103 mg, 0.86 mmol). Stirring was continued at 0 °C for 4 h. Subsequently, a solution of the amino alcohol **18** (140 mg, 0.5 mmol) in 5 mL of THF/DMF (1:1, v/v) was added dropwise. The reaction was stirred at room temperature for 48 h under the exclosure of light.

The solvents were removed in vacuo at room temperature. The crude product was chromatographed over silica gel 60 H by HPLC (eluent $MeOH/CH_2Cl_2$, 1:4, v/v), followed by gel filtration over Sephadex LH 20 (eluent $H_2O/MeOH$, 15:85, v/v). Compound **19** was obtained in 17% yield; no attempts were made yet to improve this yield: R_f 0.43 (eluent $MeOH/CHCl_2$, 1:4, v/v), 0.60 and 0.25 (eluents $MeOH/H_2O$, 85:15, v/v, and 75:25, v/v, respectively; RPTLC Merck precoated RP8-F254 plates); $[\alpha]^{25}_D$ +77.9° (*c* 0.086, $MeOH/H_2O$, 1:1, v/v); NMR (CD_3OD) δ 0.94–1.76 [m, 15 H, $(CH_2)_6CH_3$], 2.35 [s, 3 H, C(6) CH_3], 2.55–2.84 [m, 2 H, $SCH_2(CH_2)_6CH_3$], 2.84–3.49 [m, 2 H, $CHCH_2S(O)$], 3.60–3.85 (m, 2 H, $CHCH_2OH$), 3.90–4.16 [br s, 2 H, $S(O)CH_2S$], 4.40–4.67 (m, 1 H, $CHCH_2OH$), 6.95 and 7.61 (AB spectrum, 2 H, J_{AB} = 15.5 Hz, HC=CH). Anal. ($C_{20}H_{33}N_3O_5S_2$) C, H, N.

Acknowledgment. We thank Prof. R. J. F. Nivard for reading and criticizing the manuscript. Parts of the investigations were supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO) and by the Netherlands Cancer Foundation, Koningin Wilhelmina Fonds.

Registry No. 1, 1404-64-4; 2, 77880-75-2; 3, 77880-77-4; 4, 77880-76-3; 5, 88001-47-2; 6, 77826-40-5; 7, 88080-29-9; 8, 88001-48-3; 9, 28277-67-0; 10, 61787-30-2; 11, 60484-34-6; 12, 88001-49-4; 13, 88001-51-8; 14, 88001-53-0; 15, 88001-55-2; 16, 58462-97-8; 17, 88001-57-4; 18, 88001-58-5; 18 (*N*-Boc derivative), 88001-60-9; 19, 88001-59-6; sodium octyl mercaptide, 29524-77-4.

Structure–Activity Relationships among DNA Gyrase Inhibitors. Synthesis and Biological Evaluation of 1,2-Dihydro-4,4-dimethyl-1-oxo-2-naphthalenecarboxylic Acids as 1-Carba Bioisosteres of Oxolinic Acid[1]

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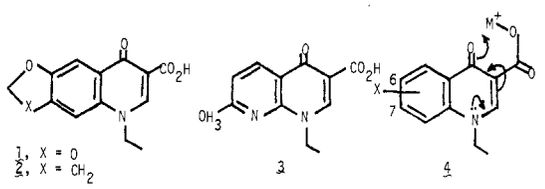
A series of oxolinic acid analogues was synthesized in an attempt to evaluate the role, if any, played by the N-1 atom in putative modes of action of antimicrobial DNA gyrase inhibitors. Carba analogues were prepared because these have no possibility of an internal resonance contribution of the nitrogen atom and yet could otherwise satisfy electronic requirements of putative modes of action. Successful routes were developed involving Friedel–Craft's cycloaddition of suitable aromatic compounds with 4,4-dimethylbutyrolactone, followed by ethoxycarbonylation, oxidation with dichlorodicyanobenzoquinone, and careful saponification. The *gem*-dimethyl group of these analogues prevents aromatization at the cost of nonplanarity. Only the unsubstituted parent compound, 1,2-dihydro-4,4-dimethyl-1-oxo-2-naphthalenecarboxylic acid (**9e**), possessed any appreciable antimicrobial activity in vitro. This may be due to a different mode of action, however, since **9e** gave no measurable inhibition of DNA gyrase in vitro. Thus, the N-1 atom plays a significant role in enzymic and bacteriological inhibition that cannot be compensated for by the presence of C-6 oxygen atoms.

Oxolinic acid (**1**) and nalidixic acid (**3**) are employed in

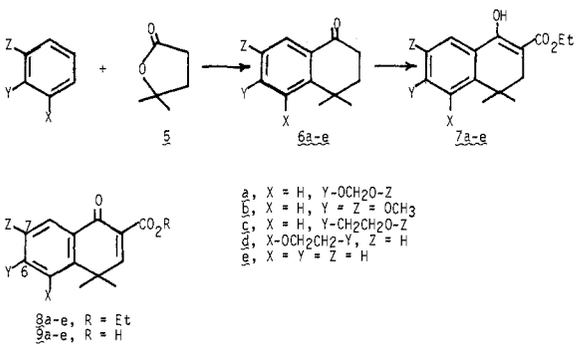


1, X = O
2, X = CH_2

3 **4**

the treatment of urinary tract infections. Their inhibitory effect on DNA gyrase[2] has stimulated extensive structure–activity studies from this[3] and other laboratories.[4] The 1-ethyl-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid moiety is a common feature of antimicrobial agents in this class. The majority of the synthetic work has been devoted to alterations in the annelated aromatic ring.[4] Modifications of the N-1 and C-2 substituents, the C-4 oxo group, and the C-3 carboxyl group have all been made. Successful variations in this area are limited predominantly to attachment of different groups to N-1.[4] So far, little attention has been paid to the role of the N-1 atom itself as a possible contributor to the molecular mode of action of these agents. Present published speculation highlights the chelating capacity of this ring system.[5] In such a coor-

Scheme I



5 **6a-e** **7a-e**

a, X = H, Y-OCH_2O-Z
b, X = H, Y = Z = OCH_3
c, X = H, Y-CH_2CH_2O-Z
d, X-OCH_2CH_2-Y, Z = H
e, X = Y = Z = H

8a-e, R = Et
9a-e, R = H

(1) A preliminary account of this work has been presented. See: "Abstracts of Papers", 185th National Meeting of the American Chemical Society, Seattle, WA, Mar 21, 1983; American Chemical Society: Washington, DC, 1983; Abstr MEDI 21.
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0022-2623/84/1827-0306$01.50/0

Table I. Ultraviolet and Proton Magnetic Resonance Spectra of Tetralones 6a-e

	UV λ_{max}, nm		^{1}H NMR, δ			
			C-8 H			
no.	found	estimated[a]	found	estimated[a]	C-5 H/C-7 H	*gem*-dimethyl
6e	245		8.00			1.36
6a	272	277	7.45	7.44	6.84	1.34
6b	274	277	7.42	7.44	6.75	1.35
6c	259	262	7.37	7.45	7.25	1.35
6d	265	262	7.59	7.50	7.11	1.44

[a] Based on substituent effects in 6e.[9]

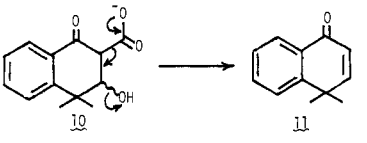
dination, the N-1 atom and to a lesser extent possibly also heteroatoms attached to C-7 could be important factors as electron donors (4).

This investigation describes the synthesis and antimicrobial evaluation of some carbocyclic analogues of oxolinic acid and related molecules in order to provide further insight into the importance of the oxoquinoline system of **1** and **3**.

The 1,2-dihydro-4,4-dimethyl-1-oxo-2-naphthalenecarboxylic acid skeleton (**9**) was chosen for study, since a quaternary carbon in the C-4 position is necessary in order to prevent aromatization. Further, two methyl groups possess a reasonable size judged from the known SAR of oxolinic acid analogues.[4] The desired compounds were thought to be easily preparable from the corresponding tetralone derivatives **6a–e** as shown in Scheme I. The 6,7-dimethoxy compound **9b** was included in the study since the ketone (**6b**) needed for its synthesis is readily available[6] and it would serve as a convenient starting material for the 6,7-methylenedioxy compound **9a**. The 6,7-ethyleneoxy derivative **9c** is of interest since **1** and **2** are approximately equipotent against *Escherichia coli* (minimum inhibitory concentrations: **1**, 0.1 mcg/mL; **2** 0.05 mcg/mL),[7] and a comparison could perhaps reveal the importance of resonance between the C-1 carbonyl group and the para ether oxygen atom in molecules lacking N-1. On the other hand, the 6,7-dimethoxy analogue of **1** possesses only modest in vitro activity against *E. coli* (minimum inhibitory concentration = 50 mcg/mL).[7] Ketone **6d** was obtained as a side product in the synthesis of **6c** and was transformed to **9d**, since 7,8-annelation is consistent with reasonably good bioactivity in the case of the quinolones.[3b,4] (Note that the numbering system is different for **4** and **9**.)

Chemistry. The key intermediates, ketones **6a–d**, were made by Friedel–Crafts reaction in polyphosphoric acid of lactone **5** and the appropriate aromatic derivative primarily following the method described for **6b** (Scheme I).[6] However, we were not able to reproduce the reported high-yield synthesis of **6b**. The pure ketone was obtained in 20% yield after flash chromatography. The reaction of **5** with 2,3-dihydrobenzofuran gave a mixture of ketones **6c** and **6d**, which were isolated in yields of 7 and 1.4%, respectively. That is, the positions ortho and para to the ether oxygen were predominantly alkylated rather than acylated. Direct reaction of **5** and 1,3-benzodioxole gave **6a** but in the extremely low yield of 0.1%. Therefore, the dimethoxy ketone (**6b**) was dimethylated with boron tribromide, and the resulting crude catechol was reacted with dibromomethane in dimethylformamide with cupric oxide as catalyst to give **6a** in an overall yield of 48%. Ketone **6e** was prepared in 67% yield by the method of Arnold et al.[8] using benzene, **5**, and aluminum chloride.

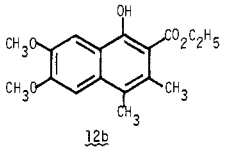
Ketones **6a–e** were reacted with excess diethyl carbonate in refluxing tetrahydrofuran with sodium hydride as base to give **7a–e** in excellent yields (Scheme I). Oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone in THF at room temperature with acetic acid as catalyst gave the unsaturated esters **8a–e** in yields of 73–96%. Hydrolysis at room temperature in aqueous ethanol with sodium hydroxide gave the desired acids **9a–e** in quantitative yields. However, raising the temperature to reflux resulted in extensive decarboxylation. For example, reflux of **8e** in 2 M sodium hydroxide for 2 h gave 40% **9e** and 40% unsaturated ketone **11**. The latter is probably formed by Michael attack of hydroxide and saponification (**10**), followed by decarboxylative β-elimination (eq 1). This conversion indicates



(1)

that the double bond of **8** or **9** is vulnerable to attack by nucleophiles.

We also attempted direct conversion of **8b** to **8a** via the catechol, but treatment of **8b** with boron trichloride gave an instantaneous Wagner–Meerwein rearrangement to naphthalene derivative **12b**.



The regioorientation of **6c** and **6d** was established by means of substituent effects[9] in their ^{1}H NMR and UV spectra (Table I). The orientation of the 6,7-ethyleneoxy group in **6c** is clear from the NMR and UV spectra. The NMR of **6d** is consistent with a C-5,6 or C-7,8 annelated derivative from which attachment of oxygen in the C-6 or C-7 position can be rulled out on the basis of substituent effects in the NMR. Furthermore, the UV data strongly indicate attachment of oxygen to the C-5 position. The *gem*-dimethyl protons of **6d** also show an anisotropic effect attributed to the ether oxygen (δ 1.44), and the C-7 hydrogen, being a double triplet (J = 7.8 and 1.2 Hz), indicates long-range coupling to the methylene group. This type of triplet (J = 1.1 Hz) was also observed for the C-5 hydrogen of **6c**. Finally, irradiation of the C-7 hydrogen in **6d** gave a 45% nuclear Overhauser enhancement (NOE) of the benzylic methylene signal, and, similarly, irradiation of this group gave a 33% NOE of the C-7 hydrogen. Also,

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Table II. Antibiotic and Anti DNA Gyrase Activity of Compounds 9a-e

	microorganism[a]						
no.	1	2	3	4	5	6	DNA gyrase
9a	500[b]	>500[b]	500[b]	>500[b]	500[b]	>500[b]	300[c]
9b	>100	>100	>100	>100	>100	>100	>1000
9c	500	>500	500	>500	500	>500	300
9d	>100	>100	>100	>100	>100	>100	300
9e	6.25	>250	50	>250	50	>250	2000
1	3.13	0.78	0.78	<0.10	50	>100	3
3	100	12.5	12.5	3.13	>100	>100	40

[a] Microorganism: 1, *Staphylococcus aureus* (ATCC 13709); 2, *Escherichia coli* (ATCC 9637); 3, *Salmonella gallinarum* (ATCC 9184); 4, *Klebsiella pneumoniae* AD (ATCC 10031); 5, *Mycobacterium smegmatis* ATCC 607); 6, *Candida albicans* (ATCC 10231). [b] Antibiotic potencies are expressed as minimum inhibitory concentrations in micrograms per milliliter. [c] Enzyme inhibition is expressed as the concentration in micrograms per milliliter that causes 50% inhibition of supercoil band formation.

irradiation of the *gem*-dimethyl protons gave no observable NOE. All these observations are consistent with the assigned structures of **6c** and **6d**.

Biological Results and Discussion

The in vitro antimicrobial assays were carried out by the standard agar-dilution/streak technique.[10] Derivatives **9a** and **9c** were only active at the highest test levels (500 mcg/mL). On the other hand, **9e** (1,2-dihydro-4,4-dimethyl-1-oxo-2-naphthalenecarboxylic acid) inhibited *Staphylococcus aureus*, ATCC 13709, at 6.25 mcg/mL and *Salmonella gallinarum*, ATCC 9184, and *Mycobacterium smegmatis*, ATCC 607, at 50 mcg/mL each. The other analogues were inactive.

For the enzyme assays, the DNA gyrase holoenzyme was prepared by the procedure described by Gellert et al.,[11] except that a heparin–Sepharose affinity column[12] was used instead of the DEAE-cellulose column step. DNA gyrase supercoiling activity was assayed by a gel electrophoresis technique in a manner similar to that described by Otter and Cozzarelli.[12] A 1% agarose horizontal gel slab was used. The amounts of relaxed plasmid (Col E_1) band and the supercoil band formed were determined by tracing the photographic negatives of the gel on a LKB Model 2202 Ultroscan densitometer. Due to the noncompetitive nature of the inhibition of this class of inhibitor, K_i may be determined as the concentration that caused 50% inhibition of supercoil band formation. Under these conditions, oxolinic acid (**1**) was 100–1000-fold more active than the carba bioisosteres **9a–e**. This level of potency is unimpressive.

These results indicate that N-1 plays an important role in the antimicrobial and anti DNA gyrase activity of antimicrobial agents of the oxolinic acid class. Furthermore, the lack of a nitrogen atom in resonance with the carbonyl group cannot be compensated for by the presence of a C-6 oxygen atom, since compounds **9a** and **9c** are equipotent. Further investigation of the role of N-1 is under way, and a paper dealing with the oxa analogues will appear shortly.[14]

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded on a Beckman 33 spectrophotometer. Ultraviolet spectra were obtained on a Cary 219 spectrophotometer. Mass spectra were conducted on a Hitachi Perkin-Elmer RMS-4 mass spectrometer (EI, 70 eV). ^{1}H NMR spectra were recorded on a Varian Ft 80-A spectrometer with tetramethylsilane as internal standard. Microanalyses were performed on a Hewlett-Packard 185B at the university of Kansas, and the results are within ±0.4% of the theoretical values unless otherwise noted.

4,4-Dimethylbutyrolactone (5) was synthesized according to Arnold et al.[8] and according to Crombie et al.[13]

3,4-Dihydro-4,4-dimethyl-1(2*H*)-naphthalenone (6e) was prepared in 67% yield by the method of Arnold et al.[8]

3,4-Dihydro-6,7-dimethoxy-4,4-dimethyl-1(2*H*)-naphthalenone (6b) was prepared in 20% yield following the procedure of Sawa et al.[6]

Compounds **6a,c,d** were prepared and isolated in an analogous way. Compound **6a** was made from 1,3-benzodioxole (0.16 mol), lactone **5** (0.15 mol), and PPA (76 g) at 90 °C for 2.5 h in a yield of 35 mg (0.1%). See below for an alternative procedure. Compounds **6c,d** were obtained from 2,3-dihydrobenzofuran (0.067 mol), lactone **5** (0.05 mol), and PPA (76 g) at 95 °C for 3 h. The ketones were isolated by two consecutive separations by flash chromatography on SiO_2 with EtOAc/hexane (5%–25% EtOAc) to give 0.80 g (7%) of **6c** and 0.15 g (1.4%) of **6d**.

Compound **6c**: recrystallized from EtOAc/hexane; mp 143–144 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 [s, 6, $C(CH_3)_2$], 1.96 and 2.69 (A_2B_2, 4, CH_2CH_2), 3.22 (t, 2, Ar CH_2), 4.56 (t, 2, OCH_2), 7.25 (br t, J = 1.1 Hz, 1, 5-H), 7.37 (s, 1, 8-H); IR ($CHCl_3$) 1675 (CO) cm^{-1}; UV (MeCN) λ_{max} 259 nm (ϵ 9200); MS m/e (relative intensity) 216 (M, 38), 201 (100), 173 (36), 115 (13). Anal. ($C_{14}H_{16}O_2$) C, H.

Compound **6d**: recrystallized from hexane; mp 70–72 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 [s, 6, $C(CH_3)_2$], 1.95 and 2.64 (A_2B_2, 4, CH_2CH_2), 3.19 (t, 2, Ar CH_2), 4.60 (t, 2, OCH_2), 7.11 (dt, J = 7.8 and 1.2 Hz, 1, 7-H), 7.59 (d, J = 7.8 Hz, 1, 8-H); IR ($CHCl_3$) 1680 (CO) cm^{-1}; UV (MeCN) λ_{max} 265 nm (ϵ 8100); MS, m/e (relative intensity) 216 (M, 48), 201 (100), 173 (47), 115 (15). Anal. ($C_{14}H_{16}O_2$) C, H.

3,4-Dihydro-4,4-dimethyl-6,7-(methylenedioxy)-1(2*H*)-naphthalenone (6a). Boron tribromide (250 μL, 2.5 mmol) was injected over 15 min into a solution of **6b** (207 mg, 0.88 mmol) in 5 mL of CH_2Cl_2 at −70 °C under N_2. The temperature was allowed to rise to 0 °C during 1 h. The solution was stirred for another 3 h at 0 to 10 °C and then poured into ice and extracted twice with $CHCl_3$. The organic phase was washed twice with water, dried ($MgSO_4$), and concentrated in vacuo to give 148 mg of a dark brown residue, which was dissolved in 7 mL of dimethyl formamide. After the addition of anhydrous K_2CO_3 (218 mg, 1.58 mmol), CuO (6 mg, 0.07 mmol), and CH_2Br_2 (66 μL, 0.93 mmol), the mixture was stirred at 125 °C under N_2 for 2 h. The mixture was poured into ice–water and extracted three times with hexane/EtOAc (1:1). Washing with water, drying (Na_2SO_4), and evaporation gave 130 mg of crude material, which was purified by flash chromatography on SiO_2 with hexane/EtOAc (1:1) to give 92 mg (48%) pure **6a**: mp 136–138 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.34 [s, 6, $C(CH_3)_2$], 1.96 and 2.67 (A_2B_2, 4, CH_2CH_2), 5.98 (s, 2, OCH_2O), 6.84 (s, 1, 5-H), 7.45 (s, 1, 8-H); IR ($CHCl_3$) 1670 (CO) cm^{-1}; UV (MeCN) λ_{max} 272 nm (ϵ 8000); MS, m/e (relative intensity) 218 (M, 62), 203 (100), 175 (28), 145 (44). Anal. ($C_{13}H_{14}O_3$) C, H.

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(14) Högberg, T.; Vora, M.; Drake, S. D.; Mitscher, L. A. *Acta Chem. Scand., Ser. B.* in press.

3,4-Dihydro-4,4-dimethyl-2-(ethoxycarbonyl)-6,7-(methylenedioxy)-1(2*H*)-naphthalenone (7a). A mixture of sodium hydride (5.2 mmol), ketone **6a** (305 mg, 1.4 mmol), and diethyl carbonate (360 μL, 4.2 mmol) in 10 mL of dry tetrahydrofuran (THF) was refluxed under Ar overnight for 16 h. After cooling, the reaction was quenched with 0.5 mL of HOAc. Benzene was added, and the solvent was evaporated. After the addition of Et_2O, the mixture was washed with brine, dried ($MgSO_4$), and evaporated to give 463 mg. This residue was subjected to a simple flash chromatography on SiO_2 with EtOAc/hexane (1:9) to give 390 mg (96%) pure **7a**, which solidified during the evaporation: mp 84–87 °C; ^1H NMR shows ca. 90% of the enol form; ^1H NMR (enol, $CDCl_3$) δ 1.23 [s, 6, $C(CH_3)_2$], 1.34 (t, 3, OCH_2CH_3), 2.43 (s, 2, allyl CH_2 in enol form), 4.27 (q, 2, OCH_2CH_3), 5.97 (s, 2, OCH_2O), 6.82 (s, 1, 5-H), 7.31 (s, 1, 8-H), 12.49 (s, 1, enolic OH); IR ($CHCl_3$) 1735 (small, CO_2Et keto form), 1645 (CO_2Et enol form), 1615 (C=C enol) cm^{-1}; MS, *m/e* (relative intensity) 290 (m, 51), 275 (M – CH_3, 15), 245 (11), 244 (M – C_2H_5OH, 18), 229 (M – C_2H_5OH – CH_3, 100), 203 (17), 202 (30), 201 (25). Anal. ($C_{16}H_{18}O_5$) C, H.

The following four compounds were analogously prepared.

Compound **7b**: yield 72%; mp 85–91 °C (ca. 85% enol); ^1H NMR (enol, $CDCl_3$) δ 1.27 (s), 1.35 (t), 2.46 (s), 3.91 and 3.93 (2 s), 4.28 (q), 6.82 (s), 7.36 (s), 10.65 (s); IR (neat) 1740 (CO_2Et keto form), 1680 (CO keto form), 1645 (CO_2Et, enol form), 1615 (C=C, enol) cm^{-1}; MS, *m/e* (relative intensity) 306 (M, 58), 291 (14), 260 (23), 245 (100), 218 (25), 217 (22). Anal. ($C_{17}H_{22}O_5$) C, H.

Compound **7c**: yield 84%; oil; ^1H NMR (enol, $CDCl_3$) δ 1.24 (s), 1.33 (t), 2.43 (s), 3.19 (t), 4.27 (q), 4.55 (t), 7.16 (s, 1, 5-H), 7.24 (s, 1, 8-H), 10.73 (s); IR ($CHCl_3$) 1730 (small), 1645, 1615 cm^{-1}; MS, *m/e* (relative intensity) 288 (M, 40), 273 (34), 243 (6), 242 (4), 227 (100), 201 (15), 200 (30), 199 (19). Anal. ($C_{17}H_{20}O_4$) C, H.

Compound **7d**: yield 100%; oil; ^1H NMR (enol $CDCl_3$) δ 1.35 (s), 1.34 (t) 2.43 (s), 3.14 (t), 4.27 (q), 4.52 (t), 7.07 (dt, J = 7.7 and 1.2 Hz, 7-H), 7.41 (d, J = 7.7 Hz, 8-H), 12.44 (s); IR ($CHCl_3$) 1735, 1685, 1650, 1615 cm^{-1}; MS, *m/e* (relative intensity) 288 (M, 40), 273 (17), 243 (8), 242 (8), 277 (100), 201 (15), 200 (27), 199 (29). Anal. ($C_{17}H_{20}O_4$) C, H.

Compound **7e**: yield 81%; oil; ^1H NMR (enol, $CDCl_3$) δ 1,26 (s), 1.32 (t), 2.46 (s), 4.27 (q); MS, *m/e* (relative intensity) (M, 17), 232 (13), 231 (6), 200 (19), 185 (80), 105 (100). Anal. ($C_{15}H_{18}O_3$) C, H.

4,4-Dimethyl-2-(ethoxycarbonyl)-6,7-(methylenedioxy)-1-(2*H*)-naphthalenone (8a). A mixture of keto ester **7a** (375 mg, 1.29 mmol), 2,3-dichloro-5,6-dicyanobenzoquinone (425 mg, 1.87 mmol), and 50 μL of HOAc in 10 mL of dry THF was stirred under Ar at room temperature overnight for 16 h. Benzene was added, and the solvent was evaporated. The residue was subjected to flash chromatography on SiO_2 with EtOAc/hexane (1:3). Concentration in vacuo gave 347 mg (93%) of an oil, which solidified: mp 137–139 °C; ^1H NMR ($CDCl_3$) δ 1.37 (t, 3, OCH_2CH_3), 1.49 [s, 6, $C(CH_3)_2$], 4.36 (q, 2, OCH_2CH_3), 6.03 (s, 2, OCH_2O), 6.90 (s, 1, 5-H), 7.47 (s, 1, 8-H), 7.60 (s, 1, 3-H); IR ($CHCl_3$) 1730 (CO_2Et), 1710, 1665 (CO), 1620 (C=C) cm^{-1}; MS, *m/e* (relative intensity) 288 (M, 62), 273 (M – CH_3, 35), 243 (M – OC_2H_5, 27), 299 (24), 228 (M – CH_3 – OC_2H_5, 11), 217 (17), 216 (40), 215 (m – $CO_2C_2H_5$, 100), 201 (28). Anal. ($C_{16}H_{16}O_5$) C, H.

The following four compounds were analogously prepared.

Compound **8b**: yield 84%; mp 122–125 °C; ^1H NMR ($CDCl_3$) δ 1.30 (t), 1.44 (s), 3.86 and 3.89 (2 s), 4.28 (q), 6.79 (s), 7.42 (s), 7.59 (s); IR ($CHCl_3$) 1735, 1715 (shoulder), 1660, 1605 cm^{-1}; MS, *m/e* (relative intensity) 304 (M, 100), 289 (83), 259 (30), 258 (15), 245 (22), 232 (36), 231 (100), 217 (19). Anal. ($C_{17}H_{20}O_5$) C, H.

Compound **8c**: yield 96%; mp 93–94 °C; ^1H NMR ($CDCl_3$) δ 1.37 (t), 1.51 (s), 3.27 (t), 4.34 (q), 4.59 (t), 7.37 (s, 1, 5-H), 7.53 (2 overlapping s, 2, 8-H and 3-H); IR ($CHCl_3$) 1735, 1720 (shoulder), 1665, 1620 cm^{-1}; MS, *m/e* (relative intensity) 286 (M, 53), 271 (71), 241 (24), 240 (13), 227 (39), 214 (32), 213 (100), 199 (32). Anal. ($C_{17}H_{18}O_4$) C, H.

Compound **8d**: yield 73%; oil; ^1H NMR ($CDCl_3$) δ 1.38 (t), 1.58 (s), 3.25 (t), 4.36 (q), 4.67 (t), 7.24 (dt, J = 7.8 and 1.1 Hz, 1, 7-H), 7.46 (s, 1, 3-H), 7.79 (d, J = 7.8 Hz, 8-H); IR ($CHCl_3$) 1730, 1715 (shoulder), 1660, 1605 cm^{-1}; MS, *m/e* (relative intensity) 286 (M, 70), 271 (61), 241 (27), 240 (6), 227 (17), 226 (16), 225 (12), 214 (35), 213 (100), 199 (22). Anal. ($C_{17}H_{18}O_4$) C, H.

Compound **8e**: yield 81%; oil; ^1H NMR ($CDCl_3$) δ 1.38 (t), 1.54 (s), 4.37 (q), 7.57 (s), 7.2–7.6 (m), 8,21 (dt); IR ($CHCl_3$) 1730, 1710, 1665, 1605; MS, *m/e* (relative intensity) 244 (M, 18), 229 (8), 199 (33), 184 (9), 172 (66), 171 (100), 157 (30). Anal. ($C_{15}H_{16}O_3$) C, H.

1,2-Dihydro-4,4-dimethyl-6,7-(methylenedioxy)-2-naphthalenecarboxylic Acid (9a). Ester **8a** (245 mg, 0.85 mmol) was stirred for 4 h at room temperature with 15 mL of EtOH and 4 mL of 2 M aqueous NaOH. The EtOH was evaporated. The aqueous residue was diluted with $NaHCO_3$ solution and washed with Et_2O. The aqueous layer was acidified and extracted twice with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated to give 219 mg (99%) of crystalline acid. Recrystallization from CH_2Cl_2/hexane afforded 195 mg (88%) pure **9a**: mp 211–213 °C dec; ^1H NMR ($CDCl_3$) δ 1.54 [s, 6, $C(CH_3)_2$], 6.11 (s, 2, OCH_2O), 6.98 (s, 1, 5-H), 7.63 (s, 1, 8-H), 8.28 (s, 1, 3-H), 13.62 (s, <1, COOH); IR ($CHCl_3$) 2790 (OH, H bonded), 1750 (COOH), 1645 (CO, H bonded), 1590 (C=C) Cm^{-1}; M S, *m/e* (relative intensity) 260 (M, 67), 245, (M – CH_3, 35), 216 (M – CO_2, 100), 215 (28), 203 (13), 202 (27), 201 (M – CO_2 – CH_3, 81), 173 (201 – CO, 16). Anal. ($C_{14}H_{12}O_5$) C, H.

The following four acids were analogously prepared.

Compound **9b**: yield 97%; mp 188–190 °C dec (from EtOAc/hexane); ^1H NMR ($CDCl_3$) δ 1.56 (s), 3.98 and 4.02 (2 s), 6.93 (s), 7.67 (s), 8,30 (s), 13.69 (s); IR ($CHCl_3$) 2750, 1750, 1645, 1585 cm^{-1}; MS, *m/e* (relative intensity) 276 (M, 100), 261 (57), 232 (86), 231 (19), 218 (22), 217 (57), 189 (31). Anal. ($C_{15}H_{16}O_5$) C, H.

Compound **9c**: yield 98%; mp 197–198 °C dec (From EtOAc/hexane); ^1H NMR ($CDCl_3$) δ 1.56 (s), 3.33 (t), 4.66 (t), 7.43 (s, 1, 5-H), 7.57 (s, 1, 8-H), 8.32 (s, 1, 3-H), 12.8 (broad); IR ($CHCl_3$) 2770, 1750, 1645, 1595 cm^{-1}; MS, *m/e* (relative intensity) 258 (M, 70), 243 (100), 214 (75), 213 (31), 200 (53), 199 (95), 171 (32). Anal. ($C_{15}H_{14}O_4$) C, H.

Compound **9d**: yield 89%; mp 217–220 °C dec (from EtOAc/hexane); ^1H NMR ($CDCl_3$) δ 1.62 (s), 3.32 (t), 4.73 (t), 7.33 (d, J = 7.8 Hz, 1, 7-H), 7.87 (d, J = 7.8 Hz, 1, 8-H), 8.29 (s, 1, 3-H), 13.56 (s); IR ($CHCl_3$) 2780, 1750 1640, 1580 cm^{-1}; MS, *m/e* (relative intensity) 258 (M, 60), 243 (46), 214 (100), 213 (36), 200 (22), 199 (75), 171 (56). Anal. ($C_{15}H_{14}O_4$) C, H.

Compound **9e**: yield 97%; mp 117–118 °C (from CH_2Cl_2/hexane); ^1H NMR ($CDCl_3$) δ 1.59 (s), 7.4–7.8 (m), 8.30 (dm), 8.37 (s), 13.39 (s); IR ($CHCl_3$) 2760, 1745, 1640, 1590 cm^{-1}; MS, *m/e* (relative intensity) 216 (M, 1), 201 (4), 172 (100), 157 (55). Anal. ($C_{13}H_{12}O_3$) C, H.

Decarboxylative Hydrolysis of Compound 8e to Compounds 9e and 11. Ester **8e** (37.8 mg, 0.15 mmol) was heated to reflux in 5 mL of aqueous sodium hydroxide for 2 h. After cooling, the aqueous layer was extracted with Et_2O. From the aqueous phase was isolated 13.0 mg (40%) of acid **9e**. Drying ($MgSO_4$) and evaporation of the ethereal layer afforded 10.3 g (40%) of unsaturated ketone **11** as an oil: ^1H NMR ($CDCl_3$) δ 1.41 [s, 6, $C(CH_3)_2$], 6.30 and 6.85 (2 d, J = 10.2 Hz, 2, 2-H and 3-H), 7.2–7.6 (m, 3), 8.10 (dt, J = 7.4 and 1.2 Hz, 1, 8-H); MS, *m/e* (relative intensity) 172 (M, 97), 157 (M – CH_3, 82), 129 (M – CH_3 – CO, 100), 128 (75), 127 (35), 115 (17), 102 (15). Peak match: found, *m/z* 172.08837; calcd for $C_{12}H_{12}O$, 172.08875.

Attempted Demethylation of Compound 8b (12b). A solution of boron trichloride (1.2 mmol, 1 M) in CH_2Cl_2 was injected into unsaturated ester **8b** (92 mg, 0.30 mmol) in 8 mL of CH_2Cl_2 under Ar with ice cooling. The cooling was removed after 10 min, and, after stirring for 1 h at room temperature, the mixture was cooled and quenched with 5 mL of dry MeOH and evaporated. Another 5-mL portion of MeOH was added, and the mixture was heated to reflux for 5 min and evaporated. This was repeated twice. The crystalline residue was washed with MeOH and filtered to give 39 mg (42%) of **12b**: mp 127–129 °C; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3, OCH_2CH_3), 2.45 and 2.56 (2 s, 6, 2 CH_3), 4.01 (overlapping s, 6, 2 OCH_3), 4.46 (q, 2, OCH_2CH_3), 7.16 (s, 1, 8-H), 7.68 (s, 1, 5-H), 11.18 (s, 1, OH); MS, *m/e* (relative intensity) 304 (M, 18), 258 (M – C_2H_5OH, 100). Anal. ($C_{17}H_{20}O_5$) C, H.

Rearrangement of Compound 8e to 12e. A solution of **8e** (37 mg, 0.15 mmol) in 8 mL of CH_2Cl_2 saturated with HCl was stirred at room temperature. There was some **8e** left after 4 h. After running overnight, the solvent was evaporated to leave pure salicylate **12e** as a solid: mp 109–111 °C (sublimation); NMR

($CDCl_3$) δ 1.42 (t), 2.47 and 2.55 (2 s), 4.45 (q), 7.1–8.5 (m), 11.96 (s); MS, m/e (relative intensity) 244 (M, 24%), 198 (M – C_2H_5OH, 100).

Acknowledgment. We greatly appreciate the technical assistance of Karen Black, Koni Stone, Mahboob Vora, and Wai-Cheong Wong. This research was supported in part by a grant (AI 13155) from the NIH.

Registry No. **5**, 3123-97-5; **6a**, 88296-04-2; **6b**, 57644-56-1; **6c**, 88296-05-3; **6d**, 88296-06-4; **6e**, 2979-69-3; **7a**, 88296-07-5; **7b**, 88296-08-6; **7c**, 88296-09-7; **7d**, 88296-10-0; **7e**, 88296-11-1; **8a**, 88296-12-2; **8b**, 88296-13-3; **8c**, 88296-14-4; **8d**, 88296-15-5; **8e**, 88296-16-6; **9a**, 88296-17-7; **9b**, 88296-18-8; **9c**, 88296-19-9; **9d**, 88296-20-2; **9e**, 88296-21-3; **11**, 16020-16-9; **12b**, 88296-22-4; **12e**, 88296-23-5; 1,3-benzodioxole, 274-09-9; 2,3-dihydrobenzofuran, 496-16-2.

Structure-Activity Studies on the N-Terminal Region of Glucagon

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Using solid-phase methodology and preparative medium- and high-performance reverse-phase liquid chromatography, we have synthesized glucagon and its Arg^{12} analogue in approximately 5% yields. The synthetic glucagon was fully active relative to natural material, and the Arg^{12} peptide exhibited 50% activity. Since perhaps the most critical part of the glucagon-family peptides is the N-terminal hexapeptide region, both batches of resin were split during synthesis in order to prepare two series of analogues based on glucagon and [Arg^{12}]glucagon with changes in the His-Ser-Gln-Gly-Thr-Phe sequence. The following new analogues were tested for their effects on blood glucose levels in normal male rats relative to glucagon and gave the following activities: [Ac-His^1,Arg^{12}]glucagon, 46%; [3-Me-His^1,Arg^{12}]glucagon, 30%; [Phe^1,Arg^{12}]glucagon, 31%; [Des-His^1,Arg^{12}]glucagon, 4%; [D-Ala^2,Arg^{12}]glucagon, 44%; [D-*p*-Cl-Phe^1,D-Ala^4,Arg^{12}]glucagon, 9%; [D-Phe^4]glucagon, 655%; [Ala^2]glucagon, 9%. These data indicate that the amino or imidazole nitrogens of the histidine residue are not essential for biological activity. However, an aromatic group in position 1 may be important, since the Phe^1 analogue is almost as active as glucagon in our bioassay. The superagonist activity with [D-Phe^4]glucagon, which was synthesized to test the hypothesis that a β-bend conformation occurs at this position in glucagon by analogy with luteinizing hormone-releasing hormone and other Gly-containing peptides, indicates that this is indeed the case and has important implications for the receptor-recognition requirements of the glucagon–secretin–vasoactive intestinal peptide family of peptides.

Glucagon, a 29 amino acid hormone (Figure 1), is a member of a closely related group of peptides with a high degree of sequence homology (secretin, VIP,[1] GIP, PHI, and hpGRF) and is well known for its role in the activation of adenylate cyclase,[2] its numerous and well-documented effects upon maintenance of fuel homeostasis, and its possible involvement in the pathogenesis of diabetes mellitus.[3]

A number of studies have been done to evaluate the role of specific amino acids in the interaction of glucagon with its receptor.[4-9] This research was focused on the development of a wide variety of modified derivatives, all of them, with few exceptions,[8] produced by the semisynthetic approach. These were used to investigate the molecular mechanisms involved in glucagon action, as well as to search for clinically useful antagonists of this hormone.

The general conclusion from these numerous studies is that virtually the entire molecule participates in the receptor-recognition process. The entire sequence is also believed to be necessary for the full expression of hormonal activity.[5] However, the lipophilic carboxyl-terminal region appears not to have an essential function in the mechanism of action but is important only for the high receptor affinity of the hormone.[9,10] In fact, the N-terminal hexapeptide of glucagon is all that is required for biological recognition and action.[11] Preservation of the charge of the single lysine at position 12 also appears essential for binding.

Although glucagon, secretin, and VIP have closely related sequences, glucagon is the most effective on hepatic adenylate cyclase and appears to act through a receptor distinct from those that bind secretin and VIP.[12-14] In-

(1) Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.* **1971**, *247*, 977. Other abbreviations used are VIP, vasoactive intestinal peptide; GIP, gastric inhibitory polypeptide; hpGRF, human pancreatic growth hormone-releasing factor; LH-RH, luteinizing hormone-releasing hormone; HPLC, high-performance liquid chromatography; RP-MPLC, reverse phase medium-pressure liquid chromatography; TFA, trifluoroacetic acid; DMF, dimethylformamide; EDTA, ethylenediaminetetracetic acid.

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