Synthesis and Antimicrobial Properties of 2*H*-Pyran-3(6*H*)-one Derivatives and Related Compounds

MINAS P. GEORGIADIS*[x], ELIAS A. COULADOUROS*, AND ANDREAS K. DELITHEOS[‡]

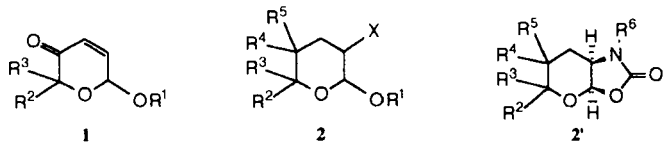
Received July 7, 1991, from the *Chemistry Laboratories, Agricultural University of Athens, Iera Odos 75, Athens 118 55, and the ‡Department of Experimental Pharmacology, Athens University School of Medicine, Athens 115 27, Greece. Accepted for publication March 23, 1992.

Abstract □ The synthesis of several derivatives of 2*H*-pyran-3(6*H*)-ones and their Michael adducts is described. Phenylthio, benzenesulfonyl, *p*-acetylaminobenzenesulfonyl, and *p*-bromophenyl substituents are beneficial for activity against gram-positive bacteria. 2-[4-(Phenylthio)phenyl]-2-methyl-6-methoxy-2*H*-pyran-3(6*H*)-one (**8a**) showed a minimum inhibitory concentration of 1.56 μg/mL against *Staphylococcus aureus* ATCC 2593, and 2-[4-(phenylthio)phenyl]-2-methyl-6-[(*p*-nitrobenzoyl)oxy]-2*H*-pyran-3(6*H*)-one (**9**) showed a minimum inhibitory concentration of 0.75 μg/mL against *Streptococcus* sp. C203M. In general, derivatives of 6-hydroxy-2*H*-pyran-3(6*H*)-ones with substituents at C-2 and C-6 showed significant activity against gram-positive bacteria. More specifically, the bulkier the C-2 substituent, the greater the antibacterial activity. Michael adducts of thiols (**13**) showed activity, which may be due to a retro-Michael reaction. In conclusion, the α,β-enone system is essential for the activity of 6-hydroxy-2*H*-pyran-3(6*H*)-ones, and the size and nature of substituents at C-2 are associated with antimicrobial activity.

2*H*-pyran-3(6*H*)-one derivatives (**1**), prepared by oxidation of furfuryl alcohols[1-4] or by multistep manipulations of sugars,[5-7] have been reported either as versatile synthetic intermediates[6-9] or as biologically important molecules with significant anticoccidial,[10] antibacterial,[10,11] pesticidal,[12] or herbicidal activity.[13] Furthermore, derivatives of **1** are components of naturally occurring anthracycline antibiotics.[14] There is ongoing interest in the synthesis and pharmacology of 2*H*-pyran-3(6*H*)-ones, and many new derivatives have been synthesized with additional properties such as acaricidal[15] and fungicidal[16] activities. However, little work has been done on the structure–activity relationships and the mode of action of these compounds.

We believe[17] that the double bond of compounds **1** is essential for their antimicrobial activity. Some experiments[3] performed with coenzyme A and cysteine showed that 2*H*-pyran-3(6*H*)-ones block –SH-containing molecules. Also, substituents at C-2 and C-6 play an important role.[10] More precisely, derivatives of **1** with a bulky aromatic group (biphenylyl, dibenzofuranyl, etc.) at R^2 and an ester or an ether functionality at R^1 exhibit better antimicrobial activity.[10] Finally, the Michael adducts with amines (**2**) have shown equal or better activity than the corresponding enones. This behavior may be due to an in vivo retro-Michael reaction[17] of compounds **2**.

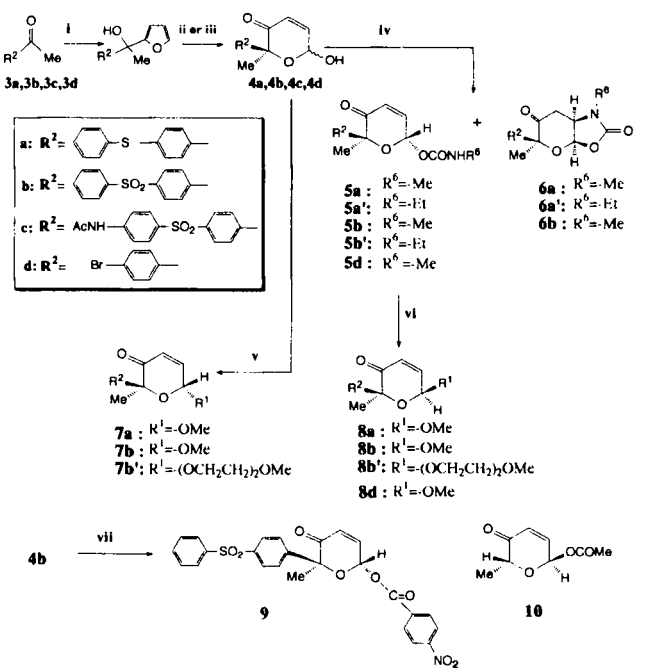
We have synthesized derivatives of 2*H*-pyran-3(6*H*)-ones in an attempt to increase their antimicrobial and anticoccidial properties. Our results concerning the antimicrobial properties of derivatives **1**, **2**, and **2'** may contribute to the understanding of the structure–activity relationships of this class of compounds.



1 **2** **2'**

Results and Discussion

Chemistry—Ketones of the general formula **3** were converted into the pyran analogues (**4**; Scheme I) by coupling with furyllithium and subsequent oxidative rearrangement of the furfuryl alcohol product with *m*-chloroperbenzoic acid or *N*-bromosuccinimide in water. This mixture of anomers was treated with methyl isocyanate or ethyl isocyanate in the presence of triethylamine to yield the desired carbamate (**5**) and a fused bicyclic byproduct (**6**). The assignment of the conformation of 2,2-disubstituted 2*H*-pyran-3(6*H*)-ones is based on our previous findings[18]; that is, a quotient of coupling constants $J_{5,6}/J_{4,6}$ of ~1 indicates a *trans*-2,6 substitution, and a quotient of ~2 indicates a *cis*-2,6 substitution. An X-ray analysis of the structures of **5b** and **8b** confirmed our results, as well as the axial orientation of the aromatic substituent.[18] For the carbamate derivatives, we proved that the β-anomer of **4** was converted to **5** (Scheme I), whereas the α-anomer was converted preferentially to the *endo*-Michael adduct, **6** (5:6 ratio, 80:20). The conformation and the ring-numbering system of a representative of each class of compounds presented in this work are shown in Scheme II.



Scheme I—Synthesis of **4–10** from **3**. Key to reaction conditions: (i) furan: 15% butyllithium, tetrahydrofuran, −5 °C, room temperature (RT), 24 h; (ii) $CHCl_3$, *m*-chloroperbenzoic acid, 15 °C, 3–6 h; (iii) *N*-bromosuccinimide, tetrahydrofuran:water (4:1), 0 °C, 5–10 min; (iv) MeNCO or EtNCO–Et_3N, CH_2Cl_2, RT, 3 h; (v) MeI–Ag_2O, acetone, RT, 18 h; (vi) R^1H, catalytic 70% $HClO_4$; (vii) Et_3N, CH_2Cl_2, *p*-nitrobenzoyl chloride, RT, 3 h.

0022-3549/92/1100-1126$02.50/0

The anomeric ethers 7 and 8 (Scheme I) were prepared from 4 and 5, respectively by glycosidic coupling of 4[10] with methyl iodide–silver oxide or by acidic solvolysis of 5 (with perchloric acid in methanol or $MeOCH_2CH_2OCH_2CH_2OH$) with inversion of stereochemistry at C-6. Finally, ester 9 was synthesized from 4b (Scheme I) by coupling with *p*-nitrobenzoyl chloride, whereas ester 10 was prepared according to a previously described method.[1]

Derivatives of 2 were prepared by Michael addition of a nucleophile on C-5 (Scheme III). When ammonia was bubbled through a solution of 7b or 7b′ (methylene chloride:methanol, 1:2) for 6 h, an unstable amino adduct was produced (one ninhydrin-positive spot on thin-layer chromatogram). In situ reduction at 0 °C with sodium borohydride gave one main product, which was identified as the *threo* isomer 11 or 12, respectively. Under the reaction conditions, the thermodynamically favored 1,4 adduct was formed selectively. The vicinal coupling constants of the anomeric hydrogen were 7.8 and 6.9 Hz, respectively, for 11 and 12, indicating the equatorial orientation of the $-NH_2$ substituent.

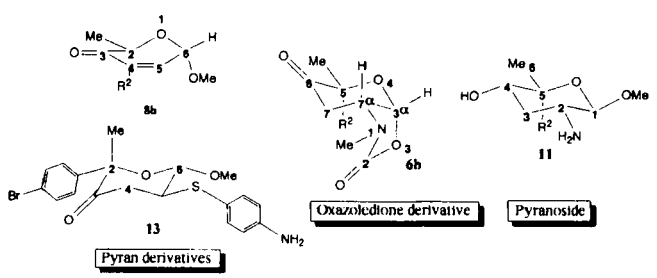
When the nucleophile was a thiolate (e.g., *p*-aminothiophenol), the Michael adduct was stable, and the addition occurred from the kinetically favored site (axial attack). The product (13) was flipped to the conformation having both the aryl substituents at equatorial positions, resulting in a vicinal coupling constant between H-5 and H-6 of ~8 Hz (diaxial orientation of hydrogens; Scheme II).

Finally, to investigate further the activity of the fused system 6, the hydroxyl or acylamino derivative 14 or 16, respectively, was prepared by simple chemical transformations (Scheme IV). Both the hydride reduction of the carbonyl group and the catalytic hydrogenation of the oxime gave only one isomer, 14 or 16, with an equatorial OH or $NHCOCH_3$ group (coupling constants between H-6 and axial H-7 were 12.7 and 14 Hz, respectively, for 14 and 15).

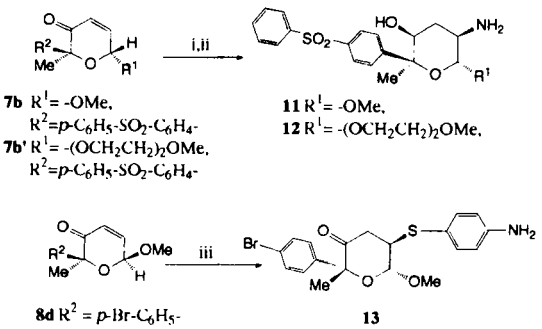
Antibacterial Activity—All the examined compounds were practically inactive against gram-negative bacteria (Table I). The 2*H*-pyran-3(6*H*)-one derivatives (1) showed significant activity against *Staphylococcus aureus* (gram positive). 6-Hydroxy or 6-acyloxy derivatives (Table I, 4 and 5) were less active than the corresponding ethers (7 and 8) or esters (9), in accordance with previous findings.[10]

Of the compounds listed in Table I, 10 were tested further against three gram-positive species, two gram-negative species, and a yeast (Table II). Two compounds (5a′ and 9) showed activity against gram-positive bacteria, the more promising being 9, which exerted particularly good activity against *Streptococcus* sp. Antifungal activity was poor for all compounds except 5a.

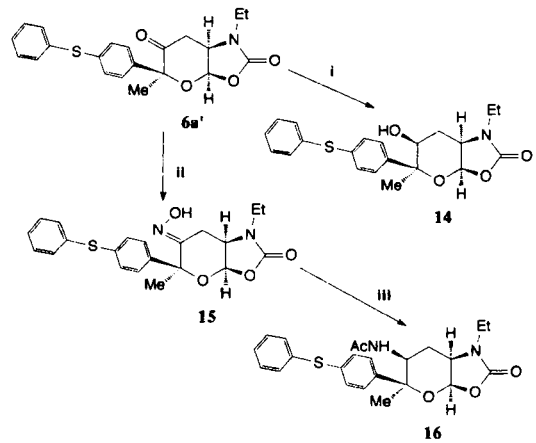
To combine the antimicrobial properties of dapsone and 2*H*-pyran-3(6*H*)-one, we synthesized some phenylthio, benzenesulfonyl, and *p*-aminobenzenesulfonyl derivatives of 1. Among the synthesized derivatives, those bearing a phenylthio substituent (namely, *p*-phenylthiophenyl; Table I, a compounds) gave better results. Methyl ether 8a was the best. The *p*-bromophenyl



Scheme II—Conformations and numbering of some characteristic compounds (R^2 = *p*-C_6H_5–SO_2–C_6H_4–).



Scheme III—Synthesis of 11–13. Key to reaction conditions: (i) $MeOH:CH_2Cl_2$ (2:1), gaseous NH_3, 6 h; (ii) $NaBH_4$, 0 °C; (iii) *p*-aminothiophenol, CH_2Cl_2, room temperature, 6 h.



Scheme IV—Synthesis of 14–16 from 6a′. Key to reaction conditions: (i) $LiAlH_4$, room temperature, 1 h; (ii) MeOH–water, $H_2NOH \cdot HCl$–CH_3COONa, pH 4.5, 40 °C, 3 h; (iii) CH_3COOH–$(CH_3CO)_2O$, 5% Pd/C, H_2, 2 h.

derivatives (d compounds) showed marginal activity.

Reduction of the carbonyl group on the Michael adducts (11 and 12) decreased the antimicrobial activity. However, 13 had enhanced activity in comparison with the starting allylic ketone, 8d, which was most probably due to a retro-Michael reaction.

The oxazolone-type derivatives 14, 15, and 16 were almost inactive. A slight improvement of activity was found after replacing the ketone functionality with an acetamido group.

Experimental Section

Chemistry—Melting points were determined in open capillary tubes (Buchi melting-point apparatus) and are uncorrected. Thin-layer chromatography (TLC) was performed on 0.2-mm silica gel-precoated plastic sheets with fluorescent indicator (UV_{254}; Merck). IR spectra (neat for oils and potassium bromide pellets for solids) were obtained on a Perkin-Elmer model 283B spectrophotometer. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian 360 EM (60-MHz) spectrometer. Tetramethylsilane was used as an internal reference (chemical shift, 0.00 ppm). Elemental analysis was performed and mass spectra were recorded at the University of Thesalloniki.

The starting ketones 3a and 3b were prepared according to previously reported methods.[19] Ketone 3b was purchased from Fluka, and ketone 3c was prepared by coupling[20] *p*-aminothiophenol and *p*-bromoacetophenone with tetrabutylammonium bromide as a phase-transfer agent and subsequent acetylation of the amine with acetic acid, acetic anhydride, and Zn; mp, 154–156 °C (literature value,[21] 156–157 °C).

Spectroscopic data of new compounds are presented in Tables III and IV.

2-[*p*-[*p*-(Acetylamino)benzenesulfonyl]phenyl]-6-hydroxy-2-

Table I—Sensitivity of Control Strains of Bacteria to the New Compounds

Compound	MIC, μg/mL[a]	MBC, μg/mL[b]	Inhibitory Zone (mm) with Bacteria at Indicated Concentration (μg/disk)				
			S. aureus			*E. coli*[c]	*P. aeruginosa*[c]
			1	10	100		
4b	12.50	>200	—[d]	8	16	9	—
4c	n[e]	n	—	—	8	—	—
5a	3.12	50	—	11	14	—	—
5a'	6.25	200	7	12	17	—	—
5b	n	n	—	—	10	—	—
5b'	25	>200	—	7	15	—	—
5d	12.50	>200	—	9	13	—	—
7b	6.25	200	7	16	19	—	—
7b'	6.25	200	—	12	19	—	—
8a	1.56	50	15	18	20	—	—
8b	6.25	200	7	19	21	—	—
8b'	6.25	200	7	11	20	7	—
8d	6.25	200	—	13	17	8	7
9	3.12	100	9	10	11	7	8
10	25	>200	—	7	13	10	11
6a	n	n	—	—	—	—	—
6a'	n	n	—	—	—	—	—
6b	n	n	—	—	—	—	—
11	n	n	—	—	7	—	—
12	n	n	—	—	7	—	—
13	3.12	100	7	19	22	n	n
14	n	n	—	—	9	—	—
15	>25	>200	—	7	10	—	—
16	25	>200	—	8	12	—	—
Ampicillin	0.19	n	n	22	n	n	n

[a] MIC is the minimum inhibitory concentration. [b] MBC is the minimum bacterial concentration. [c] Compound concentration, 100 μg/disk. [d] No sensitivity. [e] n, Not tested.

Table II—Sensitivity of Bacteria and a Yeast to Some of the New Compounds[a]

Compound	MIC/MBC, μg/mL			
	B. subtilis[b] ATCC 6633	*S. aureus*[b] ATCC 9144	*Streptococcus*[b] C203M	*S. cerevisiae*[c] ATCC 2601
4b	50/100	50/50	12.5/12.5	—[d]
5a	50/100	100/100	6.25/6.25	6.25/12.5
5a'	6.25/25	12.5/12.5	3.12/3.12	—
5b	—	—	25/25	—
6a'	—	—	100/>100	—
9	3.12/6.25	3.12/6.25	0.78/0.78	—
11	—	—	—	—
12	—	—	—	—
14	—	—	50/100	—
16	50/>100	—	12.5/25	—

[a] Sensitivity against *E. coli* ATCC 11303, MIC > 100 and MBC > 100 for all tested compounds; sensitivity against *P. aeruginosa* NCTC 10701, MIC = 100 and MBC > 100 for all tested compounds, where MIC is the minimum inhibitory concentration and MBC is the minimum bacterial concentration. [b] Reported as MIC/M, μg/ml. [c] Reported as MIC/MFC, μg/ml, where MFC is the minimum fungicidal concentration. [d] —, No sensitivity (>100/>100).

methyl-2*H*-pyran-3(6*H*)-one (4c)—To a solution of freshly distilled furan (18 mL, 0.22 mol) in anhydrous tetrahydrofuran (50 mL) was added in a dropwise manner *n*-butyllithium (*n*-BuLi) in hexane (15%, 37 mL, 0.085 mol) under nitrogen at 0 to −5 °C. After being stirred for 1.5 h at 20 °C, the mixture was cooled to 0 °C, the starting ketone **3c** (10 g, 0.031 mol) diluted with tetrahydrofuran (30 mL) was added to the mixture in a dropwise manner, and the reaction mixture was stirred overnight at room temperature. After hydrolysis and usual workup of the mixture, evaporation under reduced pressure gave a brown-yellow oil of crude furfuryl alcohol, which was oxidized without further purification.

N-Bromosuccinimide (5.5 g, 0.03 mol) was added in portions to a tetrahydrofuran:water (4:1) solution of the crude furfuryl alcohol at 0 °C. After the reaction was complete (as monitored by TLC), the mixture was diluted with ether and successively washed with potassium iodide (15%) and sodium thiosulfate (15%). The organic layer was washed with water to neutrality, dried over magnesium sulfate, and evaporated under reduced pressure to yield a yellow oil. The oil was chromatographed on a silica gel column with ether:ethyl acetate:hexane (3:3:4) as eluant. After evaporation of the solvents and crystallization of the residue from methanol, 5.3 g of analytically pure **4c** as a white solid was isolated (mp, 137–138 °C). The yield for these two steps was 67%.

6-Hydroxy-2-[*p*-(phenylthio)phenyl]-2-methyl-2*H*-pyran-3(6*H*)-one (4a)—This compound was synthesized as described previously.[4]

6-Hydroxy-2-[*p*-(benzenesulfonyl)phenyl]-2-methyl-2*H*-pyran-3(6*H*)-one (4b)—This compound was synthesized as described previously.[18]

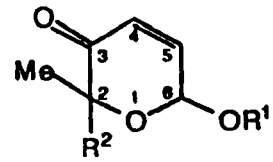
6-Hydroxy-2-(*p*-bromophenyl)-2-methyl-2*H*-pyran-3(6*H*)-one (4d)—Ketone **3d** (12.5 g, 0.06 mol) was treated with *n*-BuLi as before to yield the corresponding furfuryl alcohol, which was subsequently oxidized with *N*-bromosuccinimide to yield 10.3 g of **4d** as a colorless oil (yield for two steps, 71%).

2-[*p*-(Phenylthio)phenyl]-2-methyl-6-[[(methylamino)carbonyl]oxy]-2*H*-pyran-3(6*H*)-one (5a)—To a solution of **4a** (11 g, 0.035 mol) and methyl isocyanate (5 mL, 0.1 mol) in dry ether (150 mL) was added triethylamine (12 mL, 1.2 mol) in a portionwise manner. After the end of the reaction was confirmed by TLC, the mixture was washed with water to neutrality, and the organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure to yield an oil, which was chromatographed on a silica gel column with ether:hexane (6:4) as eluant to give 11.8 g (yield, 90%) of a rapidly eluting compound that proved to be **5a** (white crystals from ether–hexane; mp, 134–135 °C) and 0.2 g (yield, 3%) of a slowly eluting compound that proved to be 1,5-dimethyl-5-[*p*-(phenylthio)-phenyl]dihydro-[1*H*,7*H*]-5*H*-pyrano[3,2-*d*]oxazole-2,6-dione (**6a**; white crystals from acetone:hexane; mp, 111–112 °C).

2-[*p*-(Benzenesulfonyl)phenyl]-2-methyl-6-[[(methylamino)carbonyl]oxy]-2*H*-pyran-3(6*H*)-one (5b) and 1,5-Dimethyl-5-[*p*-(benzenesulfonyl)phenyl]dihydro-[1*H*,7*H*]-5*H*-pyrano[3,2-*d*]oxazole-2,6-dione (6b)—These compounds were synthesized as described previously.[18]

2-[*p*-(Phenylthio)phenyl]-2-methyl-6-[[(ethylamino)carbonyl]oxy]-2*H*-pyran-3(6*H*)-one (5a') and **5-[*p*-(Phenylthio)phenyl]-1-ethyl-5-**

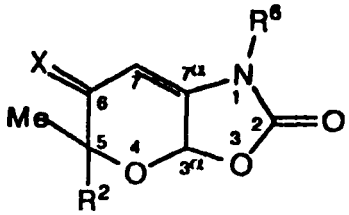
Table III—Characteristics of New Compounds of the General Formula



Compound	Formula	M_r	Analysis[a]			Mass Spectral data[b]	IR (KBr) Absorptions, cm^{-1}				^1H NMR ($CDCl_3$) Chemical Shifts, ppm (J, Hz)					
			C, %	H, %	N, %		R^2	R^1	C = O	C–O–C	R^2	R^1	H-4 $J_{4,5}$	H-5 $J_{5,6}$	H-6 $J_{4,6}$	Me
4c	$C_{20}H_{19}NO_6S$	401.4	—[c]	—[c]	—[c]	401 (1), 134 (35), 85 (100)	3050 3400 1320	3350	1690	1010 1105	3.1 s 7.8 m	5.4 m	6.2 dd J = 10	6.9 dd J = 1.2	5.9 dd J = 1.2	1.7 s
4d	$C_{12}H_{11}BrO_3$	283.1	—[c]	—[c]	—[c]	—[c]	1480 3060 820	3420	1690	1220 1010	7.0 m	3.7 s	5.9 dd J = 10	6.6 dd J = 1.4	5.2 t J = 1.4	1.3 s
5a	$C_{20}H_{19}NO_4S$	369.5	65.02 64.74	5.18 5.08	3.79 3.46	295 (10), 228 (47), 369 (10), 77 (5), 83 (100), 295 (10)	3080 1580 744	3480 1720 1530	1690	1215 1010	7.2 s	2.8 d 4.8 q J = 5	6.0 dd J = 10	6.6 dd J = 1.6	6.2 t J = 1.5	1.6 s
5a'	$C_{21}H_{21}NO_4S$	383.5	65.78 65.49	5.52 5.27	3.65 3.42	—[c]	3060 1590 750	3380 1725 1530	1685	1215 1010	7.2 s	—[d]	6.1 dd J = 10	6.6 dd J = 2.0	6.3 t J = 1.5	1.7 s
5b'	$C_{21}H_{21}NO_6S$	415.5	60.71 60.88	5.09 5.19	3.37 3.41	245 (100), 77 (90), 415 (1), 327 (30), 260 (70)	3060 1300 1160	3440 1765 1695	1690	1210 1010	7.5 m	—[e]	6.1 dd J = 10	6.6 dd J = 2.0	6.3 t J = 2.0	1.6 s
5d	$C_{14}H_{14}BrNO_4$	340.2	49.43 51.03	4.15 4.10	4.12 3.93	—[c]	3090 1490 525	3400 1715 1535	1690	1215 1000	7.2 m	2.8 d 5.0 q J = 5.2	5.9 dd J = 10	6.5 dd J = 1.8	6.1 t J = 1.8	1.6 s
8b'	$C_{23}H_{26}O_7S$	446.5	61.87 62.07	5.87 5.90	—[c] —[c]	245 (22), 186 (30), 77 (6), 327 (100), 285 (28), 260 (10)	3040 1320 1160	2800 2850	1680	1205 1000	7.4 m	3.4 m 3.1 s	5.9 dd J = 10	6.6 dd J = 2.1	5.1 t J = 1.6	1.7 s
8d	$C_{13}H_{13}BrO_3$	297.2	52.54 52.22	4.41 4.64	—[c] —[c]	297 (7), 266 (40), 83 (20), 98 (100), 223 (40), 184 (15)	3090 1490 555	2840	1685	1110	7.1 m	3.3 s	5.9 dd J = 10	6.6 dd J = 2.8	5.1 t J = 1.1	1.6 s
9	$C_{25}H_{19}NO_8S$	493.5	60.85 60.66	3.88 3.55	2.84 2.73	493 (1), 245 (95), 260 (28), 77 (12), 327 (2), 167 (100)	3050 1315 1160	1740	1695	1100	7.4 m	8.0 m	6.1 dd J = 10	6.7 dd J = 2	6.5 t J = 1.4	1.7 s
7b'	$C_{23}H_{26}O_7S$	446.5	61.87 61.95	5.87 5.71	—[c] —[c]	260 (1), 245 (13), 186 (100), 77 (23), 327 (87), 285 (13)	3050 1310 1160	2850 2800	1680	1200 1000	7.4 m	3.6 m 3.3 s	5.9 dd J = 10	6.6 dd J = 2.7	5.1 dd J = 1.4	1.6 s

[a] Top row, calcd; bottom row, found. [b] *m/z* (relative intensity). [c] Not determined. [d] –NH–, 4.2 m; Et–, 3.2 m and 1.2 t; J = 6.8 Hz. [e] –NH–, 4.9 m; Et–, 3.2 m and 1.2 t; J = 3.3 Hz.

Table IV—Characteristics of New Compounds of the General Formula



Compound	Formula	M_r	Analysis[a] C, %	H, %	N, %	Mass Spectral Data[b]	IR (KBr) Absorptions, cm^{-1} R^2	R^6	X	OCON	^1H NMR ($CDCl_3$) Chemical Shifts, ppm (J, Hz) R^2	R^6	H-3α $J_{3\alpha,7\alpha}$	H-7α $J_{7\alpha,7}$	H-7a,7e $J_{7a,7e}$	Me
6a	$C_{20}H_{19}NO_4S$	369.5	65.02	5.18	3.79	369 (50), 228 (90),	3080	2800	1715	1738	7.2 m	2.9 s	6.1 d	4.3 q	2.8 dq	1.6 s
			64.75	4.92	3.92	213 (100), 99 (24),	1480			1520			J = 7.8	J = 3.4	J = 16.8	
						184 (38), 341 (20)	760			1250						
6a'	$C_{21}H_{21}NO_4S$	383.5	65.78	5.52	3.65	383 (95), 213 (62),	3070	2820	1715	1735	7.2 m	3.1 m	6.0 d	4.4 q	2.7 dq	1.6 s
			66.01	5.55	3.46	228 (100), 42 (11),	1580			1528		2.3 s	J = 7.1	J = 3.5	J = 14.5	
						113 (45), 355 (33)	745			1280		J = 7.0				
14	$C_{21}H_{23}NO_4S$	385.5	65.43	6.01	3.63	385 (20), 213 (52),	3080	2850	3490	1765	7.2 m	3.4 m	5.6 d	3.6 m	2.1 m	1.5 s
			65.38	6.05	3.70	228 (100), 42 (8),	1580			1517		1.9 t	J = 5.4		—[c]	
						113 (27), 56 (11)	745			1280		J = 7.6				
15	$C_{21}H_{22}N_2O_4S$	398.5	63.30	5.56	7.03	—[d]	3050	2825	3350	1735	7.1 s	3.2 m	5.9 d	3.9 q	3.4 m	1.7 s[e]
			63.07	5.37	6.90		1580		1650	1010		2.1 t	J = 7.6	J = 3.5		
							750			1270		J = 7.2				
16	$C_{23}H_{25}N_2O_4S$	425.5	64.92	5.92	6.58	—[d]	3060	2860	3300	1770	7.1 s	3.1 m	5.9 d	4.1 m	2.0 m	1.8 s
			64.73	5.85	6.39		1490		1645	1250		2.1 t	J = 7.6		—[f]	
							750					J = 7.2				

[a] Top row, calcd; bottom row, found. [b] *m/z* (relative intensity). [c] –OH, 2.4 d, J = 8.5; H-6 (after addition of D_2O), 4.1 dd, $J_{6,7a}$ = 12.7 Hz, $J_{6,7e}$ = 3.2 Hz. [d] Not determined. [e] = N–OH, 8.1 s. [f] –COMe, 2.2 s; H-6, 3.3 dd, $J_{6,7a}$ = 14 Hz, $J_{6,7e}$ = 3 Hz.

methyldihydro-[1*H*,7*H*]-5*H*-pyrano[3,2-*d*]oxazole-2,6-dione (6a')—Alcohol **4a** (10 g, 0.032 mol) was treated with ethyl isocyanate (9 mL, 0.13 mol) and triethylamine (4 mL, 0.4 mol) as before. Column chromatographic separation with ether:hexane (1:1) as eluant afforded 2.5 g of **5a'** (white crystals from ether; mp, 123 °C; yield, 77%) and 0.4 g of **6a'** (white crystals from ether–hexane; mp, 153–154 °C; yield, 14%).

2-[*p*-(Benzenesulfonyl)phenyl]-2-methyl-6-[[(ethylamino)carbonyl]oxy]-2*H*-pyran-3(6*H*)-one (5b)—Alcohol **4b** (6.9 g, 0.02 mol) was treated with ethyl isocyanate (7 mL, 0.1 mol) and triethylamine (3 mL, 0.3 mol) as before. Column chromatographic separation with ether:ethyl acetate (9:1) as eluant afforded 7.5 g of **5b'** (white crystals from ethyl acetate–hexane; mp, 155 °C; yield, 90%). The oxazoledione by-product was not isolated.

2-(*p*-Bromophenyl)-2-methyl-6-[[(methylamino)carbonyl]oxy]-2*H*-pyran-3(6*H*)-one (5d)—Alcohol **4d** (10 g, 0.035 mol) was treated with methyl isocyanate (5 mL, 0.11 mol) and triethylamine (4 mL, 0.4 mol) as before. Column chromatographic separation with ether:hexane (1:1) as eluant afforded 10 g of **5d** (white crystals from ether–hexane; mp, 134–135 °C; yield, 83%). The oxazoledione by-product was not isolated.

2-[*p*-(Phenylthio)phenyl]-2-methyl-6-methoxy-2*H*-pyran-3(6*H*)-one (*trans*-7a)—Alcohol **4a** (0.9 g, 0.003 mol), methyl iodide (4.5 g, 0.014 mol), and silver oxide (3.5 g, 0.015 mol) were vigorously stirred with acetone (30 mL) at room temperature for 24 h, with exclusion of moisture and light. The reaction mixture was filtered through infusorial earth (Celite), concentrated, and chromatographed on a silica column with ether:hexane (80:20) as eluant to yield the *trans* (**4a**; colorless oil; 0.75 g; yield, 80%) and *cis* **8a**; white solid from ether; mp, 86–87 °C; 0.09 g; yield, 10%) ethers in a ratio of 8:1.

2-[*p*-(Benzenesulfonyl)phenyl]-2-methyl-6-methoxy-2*H*-pyran-3(6*H*)-one (*trans*-7b)—This compound was synthesized as described previously.[18]

2-[*p*-(Phenylthio)phenyl]-2-methyl-6-methoxy-2*H*-pyran-3(6*H*)-one (*cis*-8a)—Carbamate **5a** (1 g, 2.6 mol) was diluted in absolute methanol (0.05 M), and 70% perchloric acid (0.4 mL) was added in a dropwise manner at room temperature. The mixture was stirred for 1 h, quenched with sodium bicarbonate solution, and diluted with ether. The organic layer was washed with water, dried over magnesium sulfate, and evaporated to a pale yellow solid, which was recrystallized from ether to yield white crystals of **8a** (0.7 g; yield, 80%; mp, 86–87 °C).

6-[2-[2-(Methoxy)ethoxy]ethoxy]-2-[*p*-(benzenesulfonyl)phenyl]-2-methyl-2*H*-pyran-3(6*H*)-one (*cis*-8b' and *trans*-7b')—Carbamate **5b** (5 g, 0.012 mol) was suspended in 50 mL of $CH_3(OCH_2CH_2)_2OH$, and 70% perchloric acid (3.4 mL) was added in a dropwise manner at room temperature. After 1 h of stirring, the reaction was over. The usual workup and column chromatographic separation (ether:hexane, 9:1) gave *cis*-**8b'** as a colorless oil (2.9 g; yield, 63%) and *trans*-**7b'** as a colorless oil (0.7 g; yield, 12.5%).

2-[*p*-(Benzenesulfonyl)phenyl]-2-methyl-6-methoxy-2*H*-pyran-3(6*H*)-one (*cis*-8b)—This compound was synthesized as described previously.[18]

2-(*p*-Bromophenyl)-2-methyl-6-methoxy-2*H*-pyran-3(6*H*)-one (*cis*-8d)—A suspension of carbamate **5d** (3 g, 0.009 mol) in absolute methanol (30 mL) was treated with 70% perchloric acid (2.3 mL) as before. The mixture gradually turned clear, and subsequently, white crystals of the product precipitated. The mixture was cooled to −5 °C to yield after filtration 1.9 g of white crystals of ether **8d** (yield, 72.5%; recrystallization from acetone gave analytically pure compound; mp, 113–114 °C).

2-[*p*-(Benzenesulfonyl)phenyl]-2-methyl-6-[(*p*-nitrobenzoyl)oxy]-2*H*-pyran-3(6*H*)-one (9)—Alcohol **4d** (0.9 g, 2.6 mmol) and *p*-nitrobenzoyl chloride (0.6 g, 3.2 mmol) were dissolved in 25 mL of methylene chloride at 0 °C with exclusion of moisture. Triethylamine (0.2 mL, 0.02 mol) was added in a dropwise manner while the reaction mixture was stirred; the reaction was over after 0.5 h. The reaction mixture was quenched with methanol, diluted with ethyl acetate, and the organic layer washed with a saturated solution of ammonium chloride and water to neutrality, dried over magnesium sulfate and concentrated under reduced pressure. The crude product was passed through a small column of silica with ether as the elution solvent, and after evaporation of the solvents, the product was crystallized from acetone–hexane to give 1.2 g of white crystals of **9** (mp, 150–152 °C; yield, 83%).

Methyl 2-Amino-5-[*p*-(benzenesulfonyl)phenyl]-2,3,6-trideoxy-α-DL-*ribo*-pyranoside (11)—Gaseous ammonia was bubbled through a solution of ether **7b** (0.4 g, 1 mmol) in methanol:methylene chloride (30:15, v/v) for 6 h. The mixture was concentrated under reduced pressure, diluted with 30 mL of methanol, and cooled to 0 °C before an excess sodium borohydride was added. The pH of the reaction was buffered by the addition of acetic acid (1 mL), and the progress of the

reaction was followed by TLC. After the reaction was over, the mixture was diluted with ether, and the organic layer was washed to neutrality with water, dried over magnesium sulfate, and evaporated under reduced pressure. Column chromatographic separation (ethyl acetate:hexane:triethylamine, 8:2:0.1) gave, after evaporation of the solvents, **11** as a white solid (mp, 169–170 °C; 0.3 g; yield, 75%). The yield of the other isomer of the addition was <10%, and the isomer was not purified further and characterized. Mass spectra: *m/z* (relative intensity) 377 (M^+ 1), 260 (1), 274 (100), 116 (80), 98 (30), 72 (50), 53 (80), and 88 (35); IR (KBr): 3050 and 1440 (aromatic), 3330 and 1590 (–NH_2), and 3400 (–OH)cm^{-1}; ^1H NMR ($CDCl_3$): 7.3 (m, 9H, aromatic), 3.45 (s, 3H, OMe), 2.2 (s, 2H, –NH_2), 3.3 (bd, 1H, –OH), 1.5 (s, 3H, angular Me), 4.2 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 2.7 (m, 1H, H-2), 2.0 (m, 2H, H-3), and 3.5 (m, 1H, H-4) ppm.

Anal.—Calcd for $C_{19}H_{23}NO_5S$ (377.5): C, 55.97; H, 5.51; N, 2.84. Found: C, 55.69; H, 5.50; N, 2.57.

2-[2-(Methoxy)ethoxy]ethyl] 2-Amino-5-[*p*-(benzenesulfonyl)phenyl]-2,3,6-trideoxy-α-DL-*ribo*-pyranoside (12)—A solution of ether **7b'** (0.4 g, 1 mmol) in methanol:methylene chloride (30:15, v/v) was treated with gaseous ammonia and sodium borohydride as before. Column chromatographic purification (ethyl acetate:hexane: triethylamine, 6:4:0.1) gave 0.35 g of alcohol **12** as the main product (yield, 67%, crystals from methanol; mp, 186–187 °C). Mass spectra: *m/z* (relative intensity) 465 (M^+ 1), 260 (5), 274 (67), 346 (26), 77 (13), and 44 (100); IR (KBr): 3080, 3020, and 1595 (aromatic); 2920–2840 (several bands, polyether); and 3350 (bd, –NH_2 –OH)cm^{-1}; ^1H NMR ($CDCl_3$): 7.3 (m, 9H, aromatic), 3.3 (m, 8H, 2 –CH_2CH_2–), 2.9 (s, 3H, –OMe), 1.3 (s, 3H, angular Me), and 4.5 (d, 1H, H-1, $J_{1,2}$ = 6.9 Hz) ppm (peaks from H-2, H-3, H-4, –OH, and –NH_2 could not been resolved).

Anal.—Calcd for $C_{23}H_{31}NO_7S$ (465.6): C, 59.33; H, 6.72; N, 3.01. Found: C, 59.51; H, 6.85; N, 2.87.

5-(*p*-Aminobenzenethio)-2-(*p*-bromophenyl)-2-methyl-6-methoxydihydro[4*H*,5*H*]-2*H*-pyran-3(6*H*)-one (13)—*p*-Aminothiophenol (2.5 g, 0.02 mol) and **8d** (3 g, 0.01 mol) were dissolved in 50 mL of acetone saturated with potassium carbonate. The mixture was stirred for 2 h, quenched with a saturated solution of sodium carbonate, and diluted with ether, and the organic layer washed with water to neutrality. Column chromatographic purification (ether: hexane, 1:1) gave, after evaporation of the solvents, 3.5 g of thioether **13** as a white solid (crystals from hexane; mp, 111–112 °C; yield, 82%). Mass spectra: *m/z* (relative intensity) 422 (M^+ 15), 266 (1), 184 (5), 151 (100), 125 (42), and 98 (99); IR (KBr): 3080 and 1490 (aromatic); 3490, 3400, and 1600 (–NH_2); and 1720 (carbonyl) cm^{-1}; ^1H NMR ($CDCl_3$): 7.2 (m, 4H, *p*-bromophenyl), 6.4 (m, 4H, *p*-aminophenyl), 3.7 (s, 2H, –NH_2), 3.3 (s, 3H, –OMe), 1.6 (s, 3H, angular Me), 4.6 (d, 1H, H-6, $J_{5,6}$ = 5.2 Hz), 3.1 (m, 1H, H-5), and 2.6 (m, 2H, H-4) ppm.

Anal.—Calcd for $C_{19}H_{20}BrNO_3S$ (422.4): C, 54.03; H, 4.77; N, 3.32; S, 7.59; Br, 18.92. Found: C, 54.02; H, 4.64; N, 3.38; S, 7.53; Br, 18.88.

1-Ethyl-5-methyl-5-[*p*-(phenylthio)phenyl]-6-hydroxyperhydropyrano[3,2-*d*]oxazole-2-one (14)—To a solution of **6a'** (0.2 g, 0.5 mmol) in anhydrous tetrahydrofuran (25 mL) at 0 °C, lithium aluminum hydride (10 mg, 0.25 mmol) was added in portions. The cooling bath was removed, and the mixture was stirred for 1 h more. The reaction was quenched with a stoichiometric amount of saturated ammonium chloride, filtered, and concentrated under reduced pressure to afford, after crystallization from acetone–hexane, white crystals of **14** (0.16 g; yield, 82%; mp, 200–202 °C).

1-Ethyl-5-methyl-5-[*p*-(phenylthio)phenyl]perhydropyrano[3,2-*d*]oxazole-2,6-dione-6-oxime (15)—A mixture of **6a** (1.5 g, 4 mmol) in 40 mL of methanol was stirred for 12 h with a mixture of hydroxylamine hydrochloride (1.8 g) and sodium acetate (3 g) in water (15 mL) at 40 °C. After the reaction was over, methanol was removed under reduced pressure, the mixture was cooled, and the product was filtered off. Recrystallization from ether–hexane gave 1.36 g (yield, 88%) of oxime **15** as white needles (mp, 157–158 °C).

6-Acetylamino-1-ethyl-5-methyl-5-[*p*-(phenylthio)phenyl]perhydropyrano[3,2-*d*]oxazole-2-one (16)—A mixture of oxime **15** (550 mg, 1.4 mmol), acetic acid (12 mL), acetic anhydride (40 mL), and 10% Pd/C (80 mg) was stirred under hydrogen (1 atmosphere) for 2 h. The mixture was filtered through Celite and distilled as an azeotrope with toluene to yield a solid, which after recrystallization from acetone, gave 490 mg of **16** as white crystals (mp, 160–161 °C; yield, 84%).

Pharmacology—*Measurement of Antibacterial Action*—Assessment of the antibacterial activity of the synthesized compounds was carried out with the following test organisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. These bacteria are commonly used as control organisms in interpreting disk sensitivity tests in routine diagnostic work. In addition, three gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 9144, and *Streptococcus* sp. C203M), two gram-negative microorganisms (*E. coli* ATCC 11303 and *P. aeruginosa* NCTC 10701), and a yeast (*Saccharomyces cerevisiae* ATCC 2601) were used. The compounds were appropriately diluted in a 20% dimethylsulfoxide solution in water, which extended no inhibitory effect on the growth of the bacteria tested.

For the disk assay, Petri dishes were filled with 15 mL of Muller–Hinton (MH) agar (Difco). Disks of Whatman filter paper no. 3 (5 mm in diameter) impregnated with 0.01 mL of 0.1-, 1-, and 10-mg/mL solutions of each compound were placed on the surface of the agar that was seeded with a bacterial suspension (5×10^6 cells/mL). After incubation of the plates at 37 °C for 24 h, the diameter of the inhibition zone was measured with a ruler to the nearest millimeter. Minimal inhibitory concentration (MIC) was determined by broth dilution, with a double concentration of each compound in MH broth at a final volume of 2 mL and an inoculum of $\sim 2 \times 10^5$ microorganisms. The MIC was defined as the lowest concentration showing no visible turbidity after overnight incubation at 37 °C.

To determine the minimal bactericidal concentration (MBC; the lowest concentration of the compound giving no growth) aliquots were taken with a 2-mm-diameter loop from each visually clear tube of broth, spread on the surface of whole 90-mm-diameter MH plates, and incubated for 48 h to allow the diffusion of any trace of the compound carried over with the inoculum. For *S. cerevisiae*, yeast nitrogen broth and yeast nitrogen agar instead of MH broth and MH agar were used, respectively.

References and Notes

1. Achmatowicz, O., Jr.; Bukowski, P.; Szechner, B.; Swierzchowska, Z.; Zamojski, A. *Tetrahedron* **1971**, *27*, 1973–1996.
2. Lefebvre, Y. *Tetrahedron Lett.* **1972**, *13*, 133–136.
3. Couladouros, E. A. Ph.D. Thesis; Agricultural University of Athens, Athens, Greece, 1984.
4. Georgiadis, M. P.; Couladouros, E. A. *J. Org. Chem.* **1986**, *51*, 2725.
5. Fraser-Reid, B.; McLean, A.; Usherwood, E.; Yunker, M. *Can. J. Chem.* **1970**, *48*, 2877.
6. Holder, N. *Chem. Rev.* **1982**, *82*, 287–332.
7. Grapsas, I.; Couladouros, E. A.; Georgiadis, M. P. *Polish J. Chem.* **1990**, *64*, 823–826.
8. Zamojski, A.; Banaszek, A.; Grynkiewicz, G. *Adv. Carbohydr. Biochem.* **1982**, *40*, 1–129.
9. Martin, S. F.; Gluchowski, C.; Campbell, C. L.; Chapman, R. C. *Tetrahedron* **1988**, *44*, 3171–3180 and references therein.
10. Laliberte, R.; Medawar, G.; Lefebvre, Y. *J. Med. Chem.* **1973**, *16*, 1084.
11. Otsuka Pharmaceutical Company Ltd., Jpn. Kokai Tokkyo Koho 80 55,176; 80 55,177; 80 55,178, 1980; *Chem. Abstr.* **1981**, *94*, 15551v, 15552w, 15553x.
12. Asani, M.; Shimada, H.; Yoshikawa, K.; Shimizu, Y.; Takao, H., Otsuka Pharmaceutical Company Ltd., Jpn. Kokai Tokkyo Koho JP 57,188,585, 1982; *Chem. Abstr.* **1980**, *93*, 63644d.
13. Sammes, P. G.; Street, L. J.; Kirby, P. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2729.
14. Oki, T.; Kitamura, I.; Matsuzawa, Y.; Shibamoto, N.; Ogasawara, T.; Yoshimoto, A.; Inui, T.; Naganawa, H.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1979**, *32*, 801.
15. Takao, H.; Osaki, N.; Yasutomi, N. PCT Int. Appl. WO 84 02,910, 1984; *Chem. Abstr.* **1984**, *101*, 210985e.
16. Otsuka Pharmaceutical Company Ltd., Jpn. Kokai Tokkyo Koho JP 57,188,585, 1982; *Chem. Abstr.* **1983**, *99*, 38363c.
17. Georgiadis, M. P. *J. Med. Chem.* **1976**, *19*, 346.
18. Georgiadis, M. P.; Couladouros, E. A.; Polissiou, M. G.; Filippakis, S.; Mentzafos, D.; Terzis, A. *J. Org. Chem.* **1982**, *47*, 3054.
19. Szmant, H. H.; Palopoli, F. P. *J. Am. Chem. Soc.* **1950**, *72*, 1757.
20. Landini, D.; Rolla, F. *Org. Synth.* **1978**, *58*, 143.
21. Szmant, H. H.; Irwin, D. A. *J. Am. Chem. Soc.* **1956**, *78*, 4386.

Acknowledgments

Part of this work was taken from the Ph.D. thesis of EAC (see ref 3). We thank Prof. N. Alexandrou of the University of Thessaloniki for the elemental analysis and mass spectra. We also thank Dr. S. M. Hammond of Astra Alab AB, Sweden, for testing some of our compounds against microorganisms (Table II). Finally, we thank Bristol-Myers Squibb Hellas and UNI-PHARMA Hellas for their financial support.