metrically at 340 nm for activity. The assay was performed at 30 °C in a mixture containing 64 μM NADPH, 45 μM dihydrofolate, 12 mM 2-mercaptoethanol, and 0.1 M potassium phosphate, pH 7.4, in a final volume of 1 mL. The reaction was initiated by addition of the dihydrofolate in a volume of 20 μL. Apparent K_is were obtained from Lineweaver–Burk double-reciprocal plots of the data for reversible inhibition (i.e., at time zero).

Acknowledgment. The work reported here was supported in part by research grants CA25394 (A.R.) and CA41461 (J.H.F.) from the National Cancer Institute, DHHS. We thank Robert Tansik for suggesting the use of rapid gel filtration to separate free inhibitor from enzyme in the *Candida* DHFR binary test. The capable technical assistance of Mary Radike-Smith and Suzanne Joyner in obtaining the enzymological results in this paper is gratefully acknowledged.

Synthesis and β-Lactamase Inhibitory Properties of 2β-[(1,2,3-Triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylic Acid 1,1-Dioxide and Related Triazolyl Derivatives[1]

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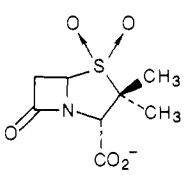
Benzhydryl 2β-[(1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide was prepared by heating benzhydryl 2β-(azidomethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide with (trimethylsilyl)acetylene. The ester group was removed by hydrogenolysis to give sodium 2β-[(1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide (**3i**, YTR-830), which was found to be a potent inhibitor of various bacterial β-lactamases. A series of related compounds was prepared in a similar way, and all of these compounds show excellent β-lactamase inhibitory properties.

The production of β-lactamase is a common defence mechanism of bacteria to β-lactam antibiotics. The enzyme (β-lactamase) catalyzes the hydrolysis of β-lactam antibiotics to the inactive penicilloic acids, and bacteria possessing this enzyme are therefore resistant to the killing effects of clinically important β-lactam antibiotics. Recently, a new approach effective against this defence mechanism of bacteria has been developed by utilizing "suicide" or "mechanism-based" irreversible inactivation of the enzyme. The significant breakthrough in this area came only in 1976 with the discovery of clavulanic acid,[2] which proved to be a very effective synergist. Over the past 8 years a number of naturally occurring and semisynthetic β-lactam compounds that inhibit or inactivate β-lactamase have been reported in the literature.[3] Penicillanic acid sulfone[4] 1 (sulbactam), first reported by the Pfizer group, is a potent synergist when used together with β-lactam antibiotics against resistant bacterial strains, thereby protecting the antibiotic from hydrolytic destruction by the enzyme.



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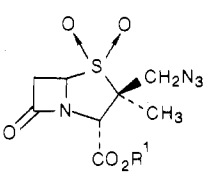

2

From investigations of the interaction of the enzyme with a number of β-lactamase inactivators including clavulanic acid, penam sulfones, and 6-halopenams, a possible mechanism of suicide inactivation has been proposed[3a] and is outlined in Scheme I.

The catalytic activity of the enzyme can be disrupted if the initially formed acyl–enzyme complex [B] can react by path a or path b rather than the normal deacylation pathway as illustrated in Scheme I. In a number of instances a branched reaction pathway (a or b) has been favored, and there is much evidence supporting the notion that the formation of a stable β-aminoacrylate system [F] is often responsible for enzyme inactivation.

In recent years since the β-lactamase has become an important target in β-lactam antibiotic research, the search for more effective inactivators of this enzyme has escalated. A recent report[5] from our laboratory has described the synthesis of 2β-(azidomethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide (**2**), which also possesses β-lactamase inhibitory property;[6] $IC_{50} = 4.0 \times 10^{-6}$ M. Since alkyl azides are remarkably reactive toward various reagents,[7,8] we have explored the feasibility of transforming the azido group of **2** into novel triazole systems **3** (Scheme II). These compounds, **3**, have proven to be even more effective β-lactamase inhibitors.

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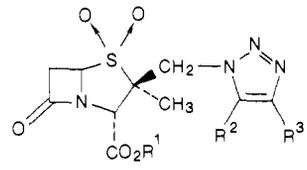
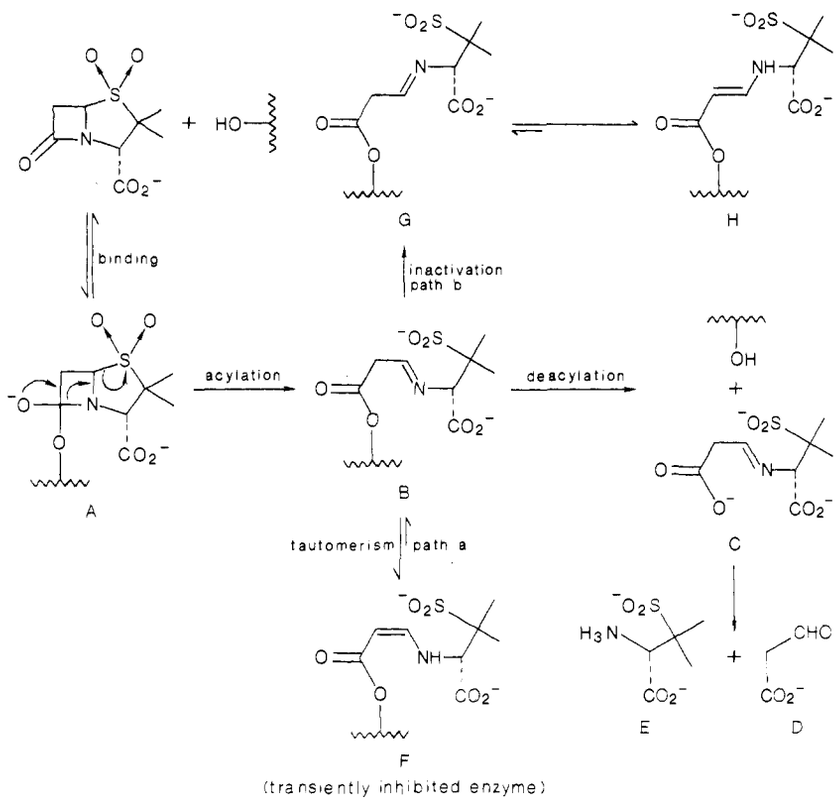
Table I



3

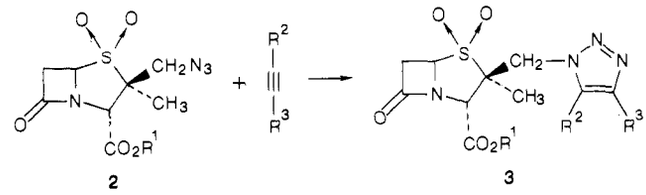
no.	R^1	R^2	R^3	mp, °C	ν max, cm^{-1}	^1H NMR (D_2O)
3a	Na	$COOCH_3$	$COOCH_3$	165 dec	1785, 1735, 1630	1.41 (s, 3 H), 3.40 (dd, 1 H), 3.80 (dd, 1 H), 3.98 (s, 3 H), 4.05 (s, 3 H), 4.51 (s, 1 H), 5.03 (dd, 1 H), 5.48 (d, 2 H)
3b	Na	H	$COOC_2H_5$	180 dec	1782, 1720	1.39 (t, 3 H), 1.46 (s, 3 H), 3.45 (dd, 1 H), 3.72 (dd, 1 H), 4.44 (q, 2 H), 4.50 (s, 1 H), 4.96–5.10 (m, 1 H), 5.18 (d, 1 H), 5.42 (d, 1 H), 8.72 (s, 1 H)
3c	Na	$COOC_2H_5$	H	180 dec	1788, 1736	1.39 (t, 3 H), 1.43 (s, 3 H), 3.40 (dd, 1 H), 3.71 (dd, 1 H), 4.46 (q, 2 H), 4.57 (s, 1 H), 4.95–5.05 (m, 1 H), 5.40 (d, 1 H), 5.82 (d, 1 H), 8.34 (s, 1 H)
3d	Na	H	$COOCH_3$	184 dec	1782, 1730	1.46 (s, 3 H), 3.45 (dd, 1 H), 3.73 (dd, 1 H), 3.97 (s, 3 H), 4.50 (s, 1 H), 4.81 (s, 2 H), 4.98–5.10 (m, 1 H), 5.18 (d, 1 H), 5.42 (d, 1 H), 8.72 (s, 1 H)
3e	Na	$COOCH_3$	H	180 dec	1778, 1730	1.41 (s, 3 H), 3.41 (dd, 1 H), 3.71 (dd, 1 H), 3.98 (s, 3 H), 4.56 (s, 1 H), 4.95–5.08 (m, 1 H), 5.40 (d, 1 H), 5.83 (d, 1 H), 8.34 (s, 1 H)
3f	K	H	COOK	178 dec	1780, 1610	1.47 (s, 3 H), 3.49 (dd, 1 H), 3.77 (dd, 1 H), 4.53 (s, 1 H), 5.0–5.1 (m, 1 H), 5.16 (d, 1 H), 5.41 (d, 1 H), 8.47 (s, 1 H)
3g	K	COOK	H	175 dec	1780, 1610	1.40 (s, 3 H), 3.43 (dd, 1 H), 3.71 (dd, 1 H), 4.58 (s, 1 H), 4.9–5.1 (m, 1 H), 5.36 (d, 1 H), 5.93 (d, 1 H), 8.04 (s, 1 H)
3h	Na	H	$Si(CH_3)_3$	170 dec	1780, 1630	0.32 (s, 9 H), 1.38 (s, 3 H), 3.1–3.7 (m, 2 H), 4.46 (s, 1 H), 4.9–5.0 (m, 1 H), 5.23 (AB q, 2 H), 8.16 (s, 1 H)
3i	Na	H	H	170 dec	1780, 1630	1.41 (s, 3 H), 3.45 (dd, 1 H), 3.72 (dd, 1 H), 4.48 (s, 1 H), 4.96–5.10 (m, 1 H), 5.25 (AB q, 2 H), 7.85 (d, 1 H), 8.13 (d, 1 H)
3j	H	H	NH_2	180 dec	1795, 1735	1.47 (s, 3 H), 3.39–3.84 (m, 2 H), 4.75 (s, 1 H), 5.06–5.10 (m, 1 H), 5.28 (m, 2 H), 8.69 (s, 1 H)

Scheme I



We now report the synthesis and β-lactamase inhibitory properties of YTR-830 (**3i**, R^1 = Na, R^2 = R^3 = H), a potent β-lactamase inhibitor[9] and related 2β-[(1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylate 1,1-

Scheme II



2 3

R^1 = $CHPh_2$, $CH_2C_6H_4$-p-NO_2

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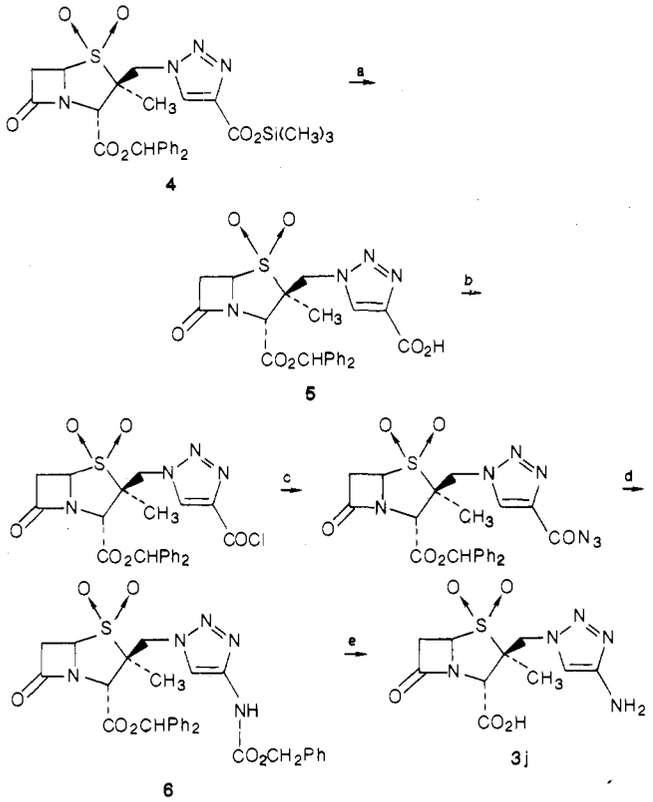
Scheme III[a]



[a] (a) THF–H_2O–AcOH; (b) $(COCl)_2$, Et_3N, CH_2Cl_2; (c) $Bu_4N^+N_3$, C_6H_6; (d) $C_6H_5CH_2OH$, Δ; (e) 10% Pd/C, EtOH, H_2.

dioxides (Table I) obtained via the cycloaddition reaction of substituted acetylenes to 2β-(azidomethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide **2** (R^1 = $CHPh_2$ or *p*-$CH_2C_6H_4NO_2$).[5]

Chemistry

All the triazoles except one, **3j**, were derived directly from either unsymmetrical or symmetrical acetylenes via a cycloaddition reaction. With unsymmetrical acetylenes a mixture of two positional isomers is expected. The direction of these dipolar additions is determined by both electronic and steric factors.[8,10] Regiospecific addition of unsymmetrical acetylenes to azides usually tends to give mainly the isomers with electron-withdrawing groups at the 4-position and electron-releasing groups at the 5-position.[11] A very bulky group, such as trimethylsilyl, tends to occupy the 4-position.[12] In our reactions, examination of the crude reaction mixtures by proton NMR indicated the formation of predominantly one isomer; in most of these cases the two isomers were separated by silica column chromatography. The structural assignment of the triazole isomers obtained via the cycloaddition is based on the data reported in the literature.[11,12]

The compounds were made by heating 2β-(azidomethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide (**2**; R^1 = $CHPh_2$ or *p*-$CH_2C_6H_4NO_2$) with an appropriate acetylene in a pressure vessel in a suitable solvent such as methylene chloride or benzene. In the case of relatively inactive acetylenes or with low-boiling acetylenes, excess of the acetylene was used as the solvent. The synthetic route for the preparation of **3j** is shown in Scheme III. Though benzhydryl 2β-[(4-carboxy-1,2,3-triazol-1-yl)-methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide (**5**) could be directly obtained by heating **2** (R^1 = $CHPh_2$) with propiolic acid, trimethylsilyl propiolate was used in place of propiolic acid to affect the cycloaddition, followed by cleavage of the silyl ester **4** with THF–H_2O–AcOH to obtain **5** in better yield.

The reaction of **2** (R^1 = $CHPh_2$) with vinyl acetate or (trimethylsilyl)acetylene resulted in the formation of the parent triazole **3i**.

The IR and NMR data of all these compounds were consistent with the structures. An observation worth noting is that the methylene protons attached to the azido group undergo a downfield shift in their NMR absorption on conversion to the triazole. In the starting azides, absorption at 3.75–4.0 ppm was observed; after the cycloaddition to acetylenes the methylene protons absorbed at 5.1–5.9 ppm. This shift might be attributed to the presence of the new adjacent aromatic system. The ester protective groups ($CHPh_2$ and *p*-$CH_2C_6H_4NO_2$) were removed by catalytic hydrogenation over palladium charcoal to provide the free acids, which were then converted to the corresponding salts (Na or K) and tested for their β-lactamase inhibitory and antibacterial activities.

Table II. β-Lactamase Inhibitory Activities of the Prepared Triazoles

compd	50% inhibitory concn,[a] M	compd	50% inhibitory concn,[a] M
3a	3.0×10^{-7} [b]	**3g**	1.7×10^{-6}
3b	5.4×10^{-8}	**3h**	5.1×10^{-7}
3c	3.4×10^{-7}	**3i**	6.9×10^{-7}
3d	4.9×10^{-8}	**3j**	7.0×10^{-7}
3e	3.0×10^{-7}	sulbactam	1.0×10^{-5}
3f	6.0×10^{-7}		

[a] Microiodometric assay method; see ref 13. [b] β-Lactamase inhibitory activity was determined by pH-stat alkalimetric titration method; see ref 15.

Biology

The β-lactamase inhibitory activity of the prepared triazoles (**3a–j**) was determined by a microiodometric assay[13] using the penicillinase (β-lactamase) from *Bacillus sp.* and penicillin G as substrate. The results are summarized in Table II. Among the prepared triazoles, compound **3i** (YTR-830) was selected for detailed studies because of its relative ease of preparation and purification. In the case of this compound the additional problem of isomer separation is absent. Also the activity of this compound is the best in this series. A comparison of the β-lactamase inhibitory activity of compound **3i**, clavulanic acid, and sulbactam on isolated β-lactamases using the microiodometric assay[13] method is summarized in Table III. These data clearly indicate that compound **3i** is significantly better than sulbactam and clavulanic acid.[14] The minimum inhibitory concentrations (MIC) of ampi-

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Table III. Comparison of Inhibitory Activities (IC_{50}) of Sulbactam, Clavulanic Acid, and **3i** on Isolated β-Lactamases

strains	β-lactamase type	substrate[d]	IC_{50},[a] μM		
			sulbactam	clavulanic acid	**3i**
E. coli OXA1	OXA1[b]	ABPC	22	3.2	5.4
E. coli TEM2	TEM2[b]	PCG	320	21	7.0
Staphylococcus aureus TH-14	penicillinase	PIPC	6.5	0.08	0.26
P. morganii TH-64	cephalosporinase	CET	1.0	NI[c]	0.15
Proteus vulgaris TH-147	cephalosporinase	CEX	0.8	0.16	0.06

[a] Microiodometric assay method; see ref 13. [b] Penicillinase. [c] NI, no inhibition. [d] Substrate concentration, 40 μM. ABPC, ampicillin; PCG, penicillin G; PIPC, piperacillin; CET, cephalothin; CEX, cephalexin.

Table IV. Synergistic Combinations of Ampicillin with the Prepared Triazoles

compound	minimum inhibitory concentration (MIC),[a] μg/mL						
	S. aureus S-54K	*S. aureus* ATCC90124	*E. coli* TH-13[b]	*E. coli* TH-397[b]	*Proteus mirabilis* 121K	*P. vulgaris* IID OX-19	*Serratia marcescens* TH-5[b]
ampicillin	25	25	200	400	>400	200	400
ampicillin + **3a**	0.39	≤0.2	25	25	1.56	0.78	100
ampicillin + **3b**	0.2	0.1	3.13	6.25	0.78	1.56	6.25
ampicillin + **3c**	0.2	0.2	25	12.5	0.78	0.78	25
ampicillin + **3d**	0.2	0.2	3.13	3.13	0.78	0.39	12.5
ampicillin + **3e**	0.2	0.2	6.25	6.25	0.78	0.39	25
ampicillin + **3f**	0.2	0.2	6.25	3.13	0.78	1.56	6.25
ampicillin + **3g**	0.78	0.78	0.05	6.25	0.39	1.56	1.56
ampicillin + **3h**	0.78	0.39	100	50	25	1.56	100
ampicillin + **3i**	0.2	0.1	3.13	6.25	0.78	1.56	3.13
ampicillin + sulbactam	0.2	0.2	6.25	0.78	6.25	1.56	3.13

[a] MIC values were determined by a micro broth dilution method; see ref 16. [b] The organisms were clinical isolates. Inoculum size, 10^7 cfu/mL. Concentration of triazole, 10 μg/mL.

cillin in combination with the triazoles (10 μg/mL) were determined against a series of β-lactamase-producing bacteria (Table IV). The bacteria cultivated in Muller Hinton Broth (Difco) and diluted to 10^7 cfu/mL were inoculated into the same medium containing ampicillin and the prepared triazoles in a specific concentration and incubated at 37 °C for 20 h. The growth of the microorganisms was observed to determine the minimal inhibitory concentration (MIC) for rendering the inoculated medium free from turbidity. The MIC values of the triazoles, when used alone, were all more than 25 μg/mL. Some of the prepared triazoles were also tested in combination with ampicillin, mecillinam, piperacillin, and cephalexin against 30 strains of *Escherichia coli* collected from patients. Table V represents the results in which MIC_{50} and MIC_{70} indicate the minimal inhibitory concentration of each antibiotic combined with 10 μg/mL of the test compound, required to inhibit the growth of 50% and 70%, respectively, of the strains. These data thus show that the triazoles exhibit excellent β-lactamase inhibitory properties.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. The ^1H NMR spectra were recorded on either a Varian EM-360 or a Bruker AM-300 spectrometer and are reported in parts per million downfield from Me_4Si. Infrared spectra were recorded with a Nicolet DX FT-IR. Only significant maxima are listed. Microanalyses were performed by the Department of Chemistry, University of Alberta, and are indicated by symbols of elements only; the results were within ±0.4% of the theoretical values. All microbiological results are from the average of two experiments.

Sodium 2β-[[4,5-Bis(methoxycarbonyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (3a). A mixture of 0.870 g (0.002 mol) of benzhydryl 2β-(azidomethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide[5] (**2**, R^1 = $CHPh_2$) and 0.618 g (0.004 mol) of dimethyl acetylenedicarboxylate in dry benzene (15 mL) was heated under nitrogen at reflux for 18 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate–chloroform (1:3) as the eluent to give 0.495 g of **3** (R^1 = $CHPh_2$, R^2 = R^3 = $COOCH_3$): mp 75–77 °C; IR (KBr) 1800, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (s, 3 H), 3.48 (t, 2 H), 3.97 (s, 3 H), 3.98 (s, 3 H), 4.59 (m, 1 H), 4.95 (s, 1 H), 5.26 (s, 2 H), 6.97 (s, 1 H), 7.36 (s, 10 H). Anal. ($C_{27}H_{26}N_4O_9S$) C, H, N.

A solution of 116 mg of the ester **3** (R^1 = $CHPh_2$, R^2 = R^3 = $COOCH_3$) in 100 mL of THF was added to a suspension of 58 mg of 10% Pd/C and 17 mg of $NaHCO_3$ in 100 mL of H_2O. The mixture was hydrogenated at normal pressure and temperature until absorption of hydrogen ceased. The catalyst was removed

Table V. MIC_{50} and MIC_{70} of Triazoles with β-Lactam Antibiotics against *E. coli*[a]

compound	MIC (μg/mL) against 30 strains of *E. coli*	
	MIC_{50}	MIC_{70}
ABPC	400	>400
ABPC + **3b**	6.25	50
ABPC + **3c**	50	100
ABPC + **3f**	6.25	6.25
ABPC + **3g**	25	100
ABPC + **3i**	3.13	6.25
ABPC + SBT	12.5	100
MPC	3.13	12.5
MPC + **3b**	0.2	0.39
MPC + **3c**	0.2	0.39
MPC + **3f**	0.1	0.1
MPC + **3g**	0.05	0.39
MPC + **3i**	0.1	0.2
MPC + SBT	0.1	0.39
PIPC	50	200
PIPC + **3b**	1.56	6.25
PIPC + **3c**	6.25	25
PIPC + **3f**	1.56	3.13
PIPC + **3g**	6.25	50
PIPC + **3i**	1.56	1.56
PIPC + SBT	12.5	25
CEX	25	100
CEX + **3b**	12.5	100
CEX + **3c**	12.5	100
CEX + **3f**	6.25	25
CEX + **3g**	3.13	12.5
CEX + **3i**	12.5	50
CEX + SBT	6.25	12.5

[a] ABPC, ampicillin; MPC, mecillinam; PIPC, piperacillin; CEX, cephalexin; SBT, sulbactam. Concentration of triazole, 10 μg/mL.

by filtration. Tetrahydrofuran was distilled out of the filtrate and the residue was washed twice with chloroform. The resultant aqueous solution was concentrated under reduced pressure and chromatographed with MCI gel (CHP-20P, Mitsubishi Kasei Co.) and eluted with water–acetone (9:1). The eluate was lyophilized to afford a white powder, which weighed 53 mg.

Sodium 2β-[[4-(Ethoxycarbonyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (3b) and Sodium 2β-[[5-(Ethoxycarbonyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (3c). A solution of 2.1 g (0.005 mol) of *p*-nitrobenzyl 2β-(azidomethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide[5] (**2**, R^1 = $CH_2C_6H_4NO_2$) in dry benzene (62 mL) was heated under nitrogen at reflux with 0.63 g (0.006 mol) of ethyl propiolate for 37 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate–hexane as eluent. The fast-running fraction was *p*-nitrobenzyl 2β-[[5-(ethoxycarbonyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide, 0.7 g (27%), as a foam: IR (KBr) 1795, 1755, 1727 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (s, 3 H), 1.39 (t, 3 H), 3.48–3.60 (m, 2 H), 4.39 (q, 2 H), 4.58–4.70 (m, 1 H), 5.11 (s, 1 H), 5.14 (d, 1 H), 5.25 (d, 1 H), 5.31 (d, 1 H), 5.56 (d, 1 H), 7.54 (d, 2 H), 8.09 (s, 1 H), 8.25 (d, 2 H).

The second eluted product was *p*-nitrobenzyl 2β-[[4-(ethoxycarbonyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide, 1.6 g (62%), as foam: IR (KBr) 1800, 1760, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (s, 3 H), 1.41 (t, 3 H), 3.50–3.65 (m, 2 H), 4.42 (q, 2 H), 4.60–4.75 (m, 2 H), 5.09 (s, 2 H), 5.36 (s, 2 H), 7.59 (d, 2 H), 8.28 (d, 2 H), 8.30 (s, 1 H). Anal. ($C_{20}H_{21}N_5O_9S$) C, H, N.

To a solution of 340 mg of *p*-nitrobenzyl 2β-[[4-(ethoxycarbonyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide in 15 mL of EtOAc was added a suspension of 60 mg of 10% Pd/C and 110 mg of $NaHCO_3$ in 15 mL of H_2O. The mixture was hydrogenated at a low pressure (1–5 kg/cm^2) and room temperature. The catalyst was removed by filtration and the aqueous layer was washed with benzene. The aqueous solution was concentrated under reduced pressure and subjected to column chromatography using MCI gel (CHP-20P, Mitsubishi Kasei Co.). The column was eluted with water–acetone (9:1) and the eluate was lyophilized to afford 200 mg of the desired product **3b** as a white amorphous solid in 76% yield.

The procedure used for the preparation of **3b** was used for the preparation of **3c** with 0.340 g of *p*-nitrobenzyl 2β-[[5-(ethoxycarbonyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide. A total of 0.222 g (83%) of the triazole **3c** was isolated as a white amorphous solid.

The compounds **3d** and **3e** were prepared in the same way with methyl propiolate in the cycloaddition step.

Dipotassium 2β-[(4-Carboxy-1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (3f) and Dipotassium 2β-[(5-Carboxy-1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (3g). A mixture of 4 g (0.009 mol) of **2** (R^1 = $CH_2C_6H_4NO_2$) and 8.2 g (0.04 mol) of *p*-nitrobenzyl acetylenecarboxylate in benzene (100 mL) was refluxed under nitrogen for 12 h. The solvent was removed under reduced pressure and the residue was purified over silica column. The major component (3.6 g) was *p*-nitrobenzyl 2β-[[4-[[(*p*-nitrobenzyl)oxy]carbonyl]-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide (**3**, R^1 = $CH_2C_6H_4NO_2$, R^2 = H, R^3 = $COOCH_2C_6H_4NO_2$): IR (KBr) 1800, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (s, 3 H), 3.3–3.8 (m, 2 H), 4.67 (s, 1 H), 4.60–4.76 (m, 1 H), 5.12 (s, 2 H), 5.37 (s, 2 H), 5.48 (s, 2 H), 7.5–7.7 (m, 4 H), 8.1–8.3 (m, 4 H), 8.37 (s, 1 H).

The other isomer **3** (R^1 = $CH_2C_6H_4NO_2$, R^2 = $COOCH_2C_6H_4NO_2$, R^3 = H) weighed 0.9 g: IR (KBr) 1800, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (s, 3 H), 3.3–3.7 (m, 2 H), 4.6–4.7 (m, 1 H), 5.07 (s, 1 H), 5.1–5.6 (m, 4 H), 5.46 (s, 2 H), 7.4–7.7 (m, 4 H), 8.15 (s, 1 H), 8.1–8.4 (m, 4 H).

Hydrogenation was carried out by using 3.6 g of **3** (R^1 = $CH_2C_6H_4NO_2$, R^2 = H, R^3 = $COOCH_2C_6H_4NO_2$), 2.0 g of $NaHCO_3$, 0.68 g of 10% Pd/C in 100 mL of EtOAc and 100 mL of H_2O at room temperature for 1 h. The catalyst was removed by filtration and the aqueous layer was separated and washed twice with EtOAc. The pH of the aqueous layer was adjusted to 1.5–1.7 with 6 N HCl. The aqueous solution was saturated with NaCl and extracted three times with EtOAc. The organic extracts were combined and dried over anhydrous $MgSO_4$. Evaporation of the solvent under reduced pressure provided 2β-[(4-carboxy-1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylic acid 1,1-dioxide. The free acid thus obtained was dissolved in 20 mL of butanol and was treated with stirring with potassium 2-ethylhexanoate in butanol. The white crystals were collected and weighed 2.0 g after air-drying. The other isomer **3g** was prepared in the same manner.

Sodium 2β-[[4-(Trimethylsilyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (3h) and Sodium 2β-[(1,2,3-Triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (3i). A mixture of benzhydryl 2β-(azidomethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide[5] (**2**, R^1 = $CHPh_2$; 0.5 g, 0.001 mol) and (trimethylsilyl)-acetylene (0.335 g, 0.003 mol) in methylene chloride (2 mL) was heated in a sealed vessel at 95 °C for 20 h. The product was purified by gradient elution chromatography over silica gel with ethyl acetate–hexane. The fast-moving fraction, benzhydryl 2β-[[4-(trimethylsilyl)-1,2,3-triazol-1-yl]methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide, was obtained as a white foam (60%): mp 172–175 °C; IR (KBr) 1805, 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.32 (s, 9 H), 1.05 (s, 3 H), 3.3–3.7 (m, 2 H), 4.5–4.7 (m, 1 H), 4.65 (s, 1 H), 5.08 (AB q, 2 H), 7.00 (s, 1 H), 7.3–7.5 (m, 10 H), 7.67 (s, 1 H). Anal. ($C_{26}H_{30}N_4O_5SSi$) C, H, N.

The slow-moving fraction, benzhydryl 2β-[(1,2,3-triazol-1-yl)-methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide, was obtained as a white amorphous solid in low yield (10%): mp 206–208 °C dec; IR (KBr) 1800, 1760 cm^{-1}; ^1H NMR (Me_2SO-d_6, 300 MHz) δ 1.13 (s, 3 H), 3.37 (d, 1 H), 3.80 (dd, 1 H), 4.98 (d, 1 H), 5.28 (s, 1 H), 5.29 (d, 1 H), 5.32 (d, 1 H), 7.02 (s, 1 H), 7.32–7.56 (m, 10 H), 7.80 (s, 1 H), 7.98 (s, 1 H). Anal. ($C_{23}H_{22}N_4O_5S$) C, H, N.

The corresponding sodium salts **3h** and **3i** were prepared by the procedure described before.

2β-[(4-Amino-1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylic Acid 1,1-Dioxide (3j). A. Benzhydryl 2β-[(4-carboxy-1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide (**5**) was prepared by heating a mixture of **2** (R^1 = $CHPh_2$; 0.5 g, 0.001 mol) and propiolic acid (0.083 g, 0.001 mol) in CH_2Cl_2 (2 mL) under nitrogen for 24 h. The solvent was removed. To the residue was added benzene and the insoluble material was filtered off. Hexane was added slowly with stirring to the filtrate and the precipitated solid was collected, which weighed 0.23 g after air-drying: mp 151–156 °C dec; IR (KBr) 1805, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.07 (s, 3 H), 3.2–3.8 (m, 2 H), 4.5–4.7 (m, 1 H), 4.69 (s, 1 H), 5.12 (br s, 2 H), 7.02 (s, 1 H), 7.1–7.6 (m, 10 H), 8.33 (s, 1 H).

B. Alternatively a mixture of **2** (R^1 = $CHPh_2$; 440 mg, 1 mmol) and trimethylsilyl propiolate (156 mg, 1.09 mmol) in dry benzene (2 mL) was heated under nitrogen at reflux for 24 h. The solvent was removed under reduced pressure and the residue was dissolved in THF (10 mL). To this solution was added 10% AcOH (8 mL) and the mixture was stirred at room temperature for 2 h. The major portion of THF was removed under reduced pressure. The residue was diluted with water and was extracted with CH_2Cl_2, washed with H_2O and then with brine, and finally dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the desired acid **5** (400 mg) as a light yellow foam, which was pure enough for further reaction.

To a stirred ice-cold solution of **5** (0.25 g, 0.0005 mol) in CH_2Cl_2 (15 mL) was added a solution of triethylamine (1.23 mL, 0.0707 g, 0.0007 mol) in CH_2Cl_2 (5 mL). A solution of oxalyl chloride (2.20 mL, 0.0761 g, 0.0006 mol) in CH_2Cl_2 (5 mL) was added slowly (10–15 min) and the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 45 min. A solution of freshly prepared tetrabutylammonium azide[17] (0.213 g, 0.00075 mol) in benzene (10 mL) was added over 30 min, and the mixture was stirred at room temperature for 3 h. Benzyl alcohol (0.163 g, 0.0015 mol) was added and the mixture was heated under nitrogen at

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reflux for 2 h and then allowed to stand overnight at room temperature. The mixture was poured into cold water (150 mL) and extracted with methylene chloride (3 × 50 mL). The methylene chloride extract was washed with 5% $NaHCO_3$ solution and finally with water and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. Purification on a silica column using hexane–ethyl acetate (3:1) as eluent gave 0.067 g of the desired product **6**. The compound **6** was further purified by crystallization from ether–hexane–ethyl acetate, affording 0.036 g of the pure compound: mp 78–80 °C; IR (KBr) 1805, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04 (s, 3 H), 3.52–3.57 (m, 2 H), 4.59–4.65 (m, 1 H), 4.64 (s, 1 H), 5.09 (s, 2 H), 5.39 (m, 2 H), 7.01 (s, 1 H), 7.26–7.50 (m, 16 H), 8.23 (s, 1 H). Anal. ($C_{31}H_{29}N_5O_7S$) C, H, N.

A solution of 0.070 g of the ester **6** in 20 mL of ethyl acetate was added to a suspension of 0.014 g of 10% Pd/C in 20 mL of H_2O. The mixture was hydrogenated at a low pressure (1–5 kg/cm^2) at room temperature. After the absorption of hydrogen ceased, the catalyst was removed by filtration and the aqueous layer was separated, washed twice with ethyl acetate, and then lyophilized to afford a white powder, which weighed 0.030 g.

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Heteroarotinoids. Synthesis, Characterization, and Biological Activity in Terms of an Assessment of These Systems To Inhibit the Induction of Ornithine Decarboxylase Activity and To Induce Terminal Differentiation of HL-60 Cells

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The synthesis of certain heteroarotinoids has been achieved, namely the systems (2*E*,4*E*,6*E*)-3,7-dimethyl-7-(1,2,3,4-tetrahydro-4,4-dimethyl-6-thiochromanyl)-2,4,6-heptatrienoic acid (**1a**), ethyl (2*E*,4*E*,6*E*)-3,7-dimethyl-7-(1,2,3,4-teterahydro-4,4-dimethyl-6-thiochromanyl)-2,4,6-heptatrienoate (**1b**), (2*E*,4*E*,6*E*)-3,7-dimethyl-7-(1,2,3,4-tetrahydro-4,4-dimethyl-6-chromanyl)-2,4,6-heptatrienoic acid (**1c**), 2-phthalimidoethyl 3,7-dimethyl-7-(1,2,3,4-tetrahydro-4,4-dimethyl-6-thiochromanyl)-2,4,6-heptatrienoate (**1d**), methyl (*E*)-*p*-[2-(4,4-dimethyl-6-chromanyl)-1-propenyl]benzoate (**2a**), (*E*)-*p*-[2-(4,4-dimethyl-6-chromanyl)-1-propenyl]benzyl alcohol (**2b**), (*E*)-*p*-[2-(4,4-dimethyl-6-chromanyl)-1-propenyl]benzonitrile (**2c**), (*E*)-*p*-[2-(4,4-dimethyl-6-chromanyl)-1-propenyl]benzaldehyde (**2d**), methyl 4-[2-(2,3-dihydro-3,3-dimethyl-5-benzofuranyl)-1-propenyl]benzoate (**3a**), and (*E*)-*p*-[2-(2,3-dihydro-3,3-dimethyl-5-benzofuranyl)-1-propenyl]benzoic acid (**3b**). Characterization via elemental, IR, ^1H NMR, and ^{13}C NMR analyses was completed for these heterocycles. The biological activity of these heteroarotinoids was assayed by either the suppression of the 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induced synthesis of ornithine decarboxylase (ODC) in mouse skin or the induction of differentiation of human (HL-60) promyelocytic cells. In the ODC assay, systems **1a–c** exhibited strong activity (within 10% of or less than the control) whereas alcohols **2b** and **3a** showed good activity (within 50% of the control) as compared to either 13-*cis*-retinoic acid or *trans*-retinoic acid. Moderate activity was observed with **2a** and **2b** while **1d** and **2c** were essentially inactive. With the HL-60 assay, **1a** and **1c** were approximately 2- and 5-fold less active, respectively, than *trans*-retinoic acid. In contrast, **2a**, **3a**, and **3b** induced differentiation of only a very small percentage of the cells. Acids **1a** and **1c** were the most active heteroarotinoids in the two biological assays. Consequently, the presence of the heteroatom does not eradicate the activity of the heteroarotinoids and thus they may have potential as chemotherapeutic agents.

Retinoids have recently received considerable attention as agents that may have utility for both cancer prevention and treatment.[1] A particularly interesting series of synthetic retinoids contain aromatic rings within the system[2-5] and have been called "arotinoids" and/or "retinoidal benzoic acid derivatives". Since neither of these terms can be used without some ambiguity, in this paper we prefer to use "heteroarotinoids" for **1–3** since the systems possess both an aryl ring fused to a partially saturated ring and a heteroatom. [The use of the term "heteroarotinoids" was introduced previously[3] and its meaning in our work refers to the presence of an aryl ring and a heteroatom within the skeletal framework. The term "arotinoid" has previously[2] been reserved for systems containing an aryl ring within the side chain or fused to the cyclohexyl ring.] In system **3**, there is an aryl ring fused to a five-membered ring. Three groups[3-5] have studied a few examples of such rare heterocycles that one might expect would inhibit a

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