Equation 3 differs very little from eq 1. It indicates that the toxicity of these 7- and 10-substituted colchicines is strongly influenced by the presence of an amide nitrogen at position 7. There is a parabolic dependence on the partition coefficient with a log P close to that of eq 1 (1.19 vs. 1.17). A comparison of eq 1 and 3 leads to the conclusion that for 7- and 10-substituted colchicines toxicity and potency are inseparable. There is no clear way to manipulate substituents to decrease toxicity without decreasing potency.

In the previous study, there was some indication that ring A modified colchicines might behave differently from 7- and 10-modified analogues. This was based on the substantial overprediction of the potency of compound **2**, the 3-demethyl derivative. In the course of this investigation, three ring A substituted analogues were synthesized by literature methods and tested. These were the 4-formyl (**27**), 4-cyano (**28**), and 4-(hydroxymethyl) (**29**) derivatives.[9] The partition coefficients of these compounds were calculated as follows:

$$(1)\ \log P_{27} = \log P_1 + \pi_{CHO} = 1.03 - 0.65 = 0.38$$

$$(2)\ \log P_{28} = \log P_1 + \pi_{CN} = 1.03 - 0.57 = 0.46$$

$$(3)\ \log P_{29} = \log P_1 + \pi_{CH_2OH} = 1.03 - 1.03 = 0.0$$

The potency and toxicity of each of these three analogues was calculated by eq 1 and 3 and compared with observed values. The results are given in Table IV.

It is of considerable interest that all of the 4-substituted colchicine analogues (**27–29**) are overpredicted in terms of potency. Compounds **28** and **29** are overpredicted by almost 2 log units. All three derivatives were predicted to be considerably more toxic than they were observed to be. Therefore, eq 1 and 3 do not predict the potency or toxicity of 4-substituted colchicines. Ring A modification, especially at the 4 position, appears to result in colchicine derivatives that differ fundamentally from those which are modified at the 7 or 10 positions.

Table V presents the biological data of 4-formylcolchicine (**27**). In terms of maximum ILS produced, this compound appears to surpass the parent. It is considerably less toxic and has a much broader therapeutic range.

Table V. Comparison of 4-Formylcolchicine (27) and Colchicine (1) against in Vivo P388 Murine Leukemia[a]

	% ILS			
	4-formylcolchicine		colchicine	
dose, mg/kg	trial 1	trial 2	trial 1	trial 2
12.5		2		
6.25	105	105		
3.12	94	80		
1.56	60	75		
1.0			toxic	toxic
0.78	54	49		
0.5			78	89
0.39	51	49		
0.25			46	58
0.12			33	40
0.06			27	24

[a] The standard protocol[6] used by the National Cancer Institute for the P388 test system was followed. Hydroxypropylcellulose was used to suspend 4-formylcolchicine in saline; colchicine was dissolved in water. Drugs were administered ip on days 1-9 (nine injections) after tumor implantation. Median survival times of the control groups in trials 1 and 2 were 11.2 and 10.7 days, respectively.

It seems clear, as Hansch[10] has observed, that anticancer agents will yield satisfactory QSAR and that guidelines can be provided for the design of new agents. In this and the previous study, our analysis has shown that further modifications of positions 7 and 10 of colchicine are not likely to be profitable. Having established some important limitations on the design of colchicine analogues, a promising synthetic lead has been uncovered, i.e., modification of the 4 position of ring A. In addition, a strategy for obtaining toxicity from screening data has been described. While LD_{50} values thus obtained are not intended for translation into higher animals or man, they should provide a readily accessible and valuable parameter to the medicinal chemist in the search for improved anticancer agents. Work is in progress which is designed to further explore ring A modified colchicines.

(8) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
(9) Muller, G.; Bladé-Font, A.; Bardoneschi, R. *Justus Liebigs Ann. Chem.* **1963**, *662*, 105.
(10) Hansch, C.; Leo, A.; Schmidt, C.; Jow, P. Y. C.; Montgomery, J. *J. Med. Chem.* **1980**, *23*, 1095.

Preparation and Antibacterial Activity of Δ^1-Thienamycin

David H. Shih and Ronald W. Ratcliffe*

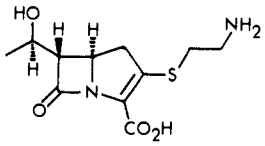
Department of Synthetic Chemical Research, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065.
Received October 20, 1980

Δ^1-Thienamycin (**2**), a double-bond isomer of thienamycin, was prepared by isomerizing *N*-[[(*p*-nitrobenzyl)oxy]carbonyl]thienamycin *p*-nitrobenzyl ester (**5b**) with DBU in Me_2SO followed by hydrogenolysis of the protecting groups. When evaluated in a disc-diffusion antibacterial assay, Δ^1-thienamycin was found to be essentially devoid of activity. The lack of antibacterial activity was ascribed to a chemically less reactive β-lactam amide bond than that found in thienamycin.

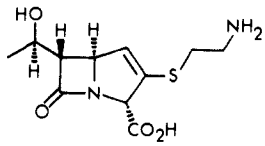
The recently discovered carbapen-2-em family of antibiotics, which includes thienamycin,[1] the epithienamycins,[2] the olivanic acids,[3] and PS-5,[4] exhibits unusual and highly desirable antibacterial properties. Of these, thienamycin

0022-2623/81/1824-0639$01.25/0

(1) appears most interesting because of its high in vitro



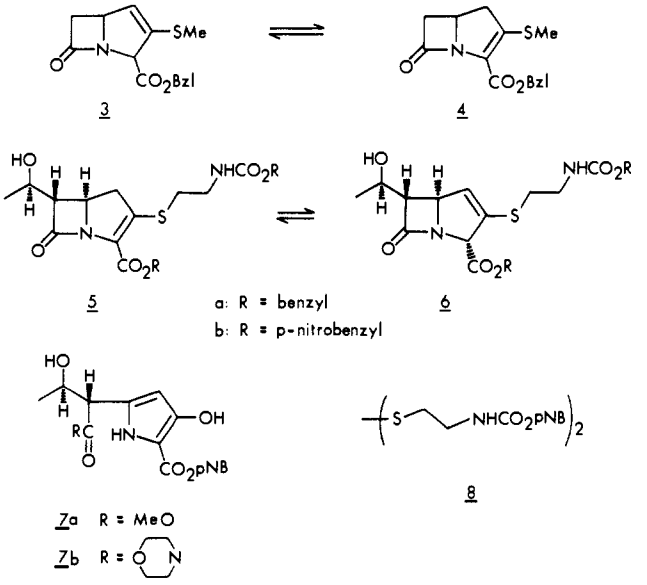
1



2

and in vivo activity[1c-e] against a broad range of Gram-positive and Gram-negative bacteria, including β-lactamase producers, anaerobes, and *Pseudomonas* species. This activity is thought to result from the unique combination of hydroxyethyl and (aminoethyl)thio side chains and from incorporation of the highly strained carbapen-2-em ring system.[5] Comparison of the in vitro antibacterial activity[6] of thienamycin with that of *N*-acetylthienamycin, the epithienamycins, and descysteaminylthienamycin[7] reveals that both the orientation and configuration of the hydroxyethyl group as found in thienamycin are necessary for high resistance to bacterial β-lactamases and optimum antibacterial potency and that the basic (aminoethyl)thio side chain also increases overall potency while imparting high antipseudomonal activity. Support for the carbapen-2-em nucleus requirement is found in the observation of modest antibacterial activity associated with the synthetic analogue[8] lacking both hydroxyethyl and (aminoethyl)thio side chains and of the greatly diminished antibacterial activity found for the ring-expanded analogue homothienamycin.[9] In order to better understand the structural parameters necessary for good antibacterial activity in the carbapenem series, we have prepared, as described herein, a double-bond isomer of 1, Δ^1-thienamycin (2), and have determined its antibacterial properties.

Chemistry. The first suggestion that thienamycin might be isomerized with base came from early synthetic work[10] in which carbapen-1-em **3** provided a mixture of **3**



and carbapen-2-em **4** on treatment with DBU in Me_2SO. When these conditions were applied to *N*-[(benzyloxy)carbonyl]thienamycin benzyl ester (**5a**), a mixture containing nearly equal proportions of **5a** and **6a** was similarly produced. The dynamics of the interconversion were demonstrated by equilibrating **6a** to the same mixture as obtained from **5a**.

The isomerization of the double bond from the Δ^2 to the Δ^1 position was confirmed by expected differences in the IR, UV, and ^1H NMR spectra of **5a** and **6a**. The infrared lactam carbonyl stretching frequency shifts from 1777 to 1770 cm^{-1} and the ester carbonyl frequency shifts from 1710 to 1745 cm^{-1} on isomerization of **5a** to **6a**, and the ultraviolet absorption near 320 nm, which is associated with the α-amido-β-thio-α,β-unsaturated carboxylate chromophore of **5a**, is completely absent in **6a**. The lower frequency lactam carbonyl absorption for Δ^1-isomer **6a** is

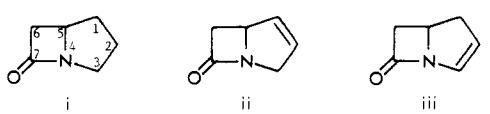
(1) (a) Kahan, J. S.; Kahan, F. M.; Goegelman, R.; Currie, S. A.; Jackson, M.; Stapley, E. O.; Miller, T. W.; Miller, A. K.; Hendlin, D.; Mochales, S.; Hernandez, S.; Woodruff, H. B.; Birnbaum, J. *J. Antibiot.* **1979**, *32*, 1. (b) Albers-Schonberg, G.; Arison, B. H.; Hensens, O. D.; Hirshfield, J.; Hoogsteen, K.; Kaczka, E. A.; Rhodes, R. E.; Kahan, J. S.; Kahan, F. M.; Ratcliffe, R. W.; Walton, E.; Ruswinkle, L. J.; Morin, R. B.; Christensen, B. G. *J. Am. Chem. Soc.* **1978**, *100*, 6491. (c) Kropp, H.; Kahan, J. S.; Kahan, F. M.; Sundelof, J.; Darland, G.; Birnbaum, J. Interscience Conference for Antimicrobial Agents and Chemotherapy, 16th, Chicago, IL, 1976; American Society for Microbiology: Washington, DC, 1976; Abstr 228. (d) Tally, F. P.; Jacobus, N. V.; Gorlach, S. L. *Antimicrob. Agents Chemother.* **1978**, *14*, 436. (e) Weaver, S. S.; Bodey, G. P.; LeBlanc, B. M. *Ibid.* **1979**, *15*, 518.

(2) (a) Cassidy, P. J.; Stapley, E. O.; Goegelman, R.; Miller, T. W.; Arison, B.; Albers-Schonberg, G.; Zimmerman, S. B.; Birnbaum, J. Interscience Conference on Antimicrobial Agents and Chemotherapy, 17th, New York, NY, 1977; American Society for Microbiology: Washington, DC, 1977; Abstr 81. (b) Stapley, E. O.; Cassidy, P.; Currie, S. A.; Daoust, D.; Goegelman, R.; Hernandez, S.; Jackson, M.; Mata, J. M.; Miller, A. K.; Monaghan, R. L.; Tunac, J. B.; Zimmerman, S. B.; Hendlin, D. Interscience Conference on Antimicrobial Agents and Chemotherapy, 17th, New York, NY, 1977; American Society for Microbiology: Washington, DC, 1977; Abstr 80.

(3) (a) Brown, A. G.; Corbett, D. F.; Eglington, A. J.; Howarth, T. T. *J. Chem. Soc., Chem. Commun.* **1977**, 523. (b) Corbett, D. F.; Eglington, A. J.; Howarth, T. T. *Ibid.* **1977**, 953. (c) Hood, J. D.; Box, S. J.; Verrall, M. S. *J. Antibiot.* **1979**, *32*, 295. (d) Brown, A. G.; Corbett, D. F.; Eglington, A. J.; Howarth, T. T. *Ibid.* **1979**, *32*, 961. (e) Box, S. J.; Hood, J. D.; Spear, S. R. *Ibid.* **1979**, *32*, 1239.

(4) (a) Okamura, K.; Hirata, S.; Okumura, Y.; Fakagawa, Y.; Shimauchi, Y.; Kouno, K.; Ishikura, T.; Lein, J. *J. Antibiot.* **1978** *31*, 480. (b) Okamura, K.; Hirata, S.; Koki, A.; Hori, K.; Shibamoto, N.; Okumura, Y.; Okabe, M.; Okamoto, R.; Kouno, K.; Fukagawa, Y.; Shimauchi, Y.; Ishikura, T. *Ibid.* **1979**, *32*, 262. (c) Sakamoto, M.; Iguchi, H.; Okamura, K.; Hori, S.; Fukagawa, Y.; Ishikura, T. *Ibid.* **1979**, *32*, 272.

(5) The nomenclature adopted throughout this paper is based on assignment of the terms carbapenam, carbapen-1-em, and carbapen-2-em to structures i, ii, and iii, respectively. This



i ii iii

nomenclature is based on the penam and cephem nomenclature generally employed in β-lactam chemistry.

(6) Leanza, W. J.; Wildonger, K. J.; Hannah, J.; Shih, D. H.; Ratcliffe, R. W.; Barash, L.; Walton, E.; Firestone, R. A.; Patel, G. F.; Kahan, F. M.; Kahan, J. S.; Christensen, B. G. "Structure–Activity Relationships in the Thienamycin Series", Proceedings of the Symposium on Recent Advances in the Chemistry of Beta-Lactam Antibiotics", Cambridge, England, 1980.

(7) Shih, D. H.; Hannah, J.; Christensen, B. G. *J. Am. Chem. Soc.* **1978**, *100*, 8004.

(8) Cama, L. D.; Christensen, B. G. *J. Am. Chem. Soc.* **1978**, *100*, 8006.

(9) Salzmann, T. N.; Ratcliffe, R. W.; Christensen, B. G. *Tetrahedron Lett.* **1980**, *21*, 1193.

(10) Schmitt, S. M.; Johnston, D. B. R.; Christensen, B. G. *J. Org. Chem.* **1980**, *45*, 1135.

suggestive of a chemically less reactive carbapenem ring system. Corroboration of the thermodynamically preferred exo orientation for the carboxylate group as shown in **6a** was obtained by NMR comparison with related structures;[11] the 3.4-Hz coupling constant between H3 and H5 is supportive of a trans relationship between those protons. The configuration of the carboxylate group in **6a** is the same as that found in the penicillins and structurally related Δ^2-cephalosporins.

Although **6a** could be hydrogenolytically deblocked to **2** in very low yield, it was found more convenient to prepare the title compound by isomerization and deprotection of the bis(*p*-nitrobenzyl) derivative **5b**. Thienamycin was converted to **5b** by successive N-acylation with *p*-nitrobenzyl chloroformate and esterification with *p*-nitrobenzyl bromide. Exposure of **5b** to DBU in Me_2SO as previously described[12] gave a mixture of starting material and Δ^1-isomer **6b**, which were readily separated by preparative TLC. The structure of **6b** was established by spectral comparison with the analogous *N*-[(benzyloxy)carbonyl]-benzyl ester **6a**. Removal of the more labile *p*-nitrobenzyl protecting groups by hydrogenolysis of **6b** in a buffered medium, followed by chromatography of the crude product on a neutral polystyrene resin, afforded Δ^1-thienamycin (**2**).

The good yield obtained in the deblocking of **6b** to **2**,[13] the IR lactam carbonyl stretching frequencies for isomers **5a** and **6a**, and our experience in isolating and handling the bisprotected thienamycin isomers **5** and **6** suggested a chemically less reactive Δ^1 isomer. In order to more clearly ascertain the relative reactivities of the carbapenem isomers toward nucleophilic reagents, equimolar mixtures of **5b** and **6b** in tetrahydrofuran solution were treated with either excess methanol in the presence of catalytic acid or with excess morpholine. The reactions were monitored by TLC and UV spectroscopy, both of which revealed gradual loss of only the Δ^2-isomer **5b**. Workup after complete disappearance of **5b** provided recovered Δ^1-isomer **6b**, hydroxypyrroles **7**, and disulfide **8**,[14] along with several minor, unidentified components. Control experiments with the separate **5b** and **6b** isomers confirmed that the products **7** and **8** originated from **5b**. Even after extended reaction times, little if any of the Δ^1-isomer **6b** was consumed. Analogous results were obtained using benzyl mercaptan and *N,N*-diisopropylethylamine in DMF as the nucleophilic system, although in this case product identification was not made. Schmitt and co-workers[10] have observed a similar reactivity order for simpler carbapenem isomers obtained by total synthesis. The results are in complete accord with a chemically reactive Δ^2 isomer and a relatively stable Δ^1 isomer, and the same reactivity order is expected to prevail for the deblocked products thienamycin (**1**) and Δ^1-thienamycin (**2**).

Biological Results and Conclusions

The antibacterial activity of Δ^1-thienamycin (**2**) was determined by a disc-diffusion assay employing the Kirby–Bauer method,[15] modified only by the use of an agar thickness of 0.2 cm. A panel of 40 laboratory strains representing two Gram-positive and ten Gram-negative species was used. For each species at least one isolate of typical antibiotic sensitivity was represented, while the remaining isolates were selected for their resistance to β-lactam antibiotics currently in use. When compared with thienamycin, the Δ^1 isomer was found to be essentially devoid of antibacterial activity. Minor zones of inhibition amounting to approximately 0.05% of the thienamycin activity were observed for the *Staphylococcus aureus* strains, while most other bacteria were found to be totally resistant to **2**.

The negligible antibacterial activity of Δ^1-thienamycin indicates that substituted carbapenems such as thienamycin must incorporate the Δ^2 double bond for good activity. Since synthetic carbapen-2-em-3-carboxylic acid, the simplest of all carbapenem antibiotics, exhibits modest activity[8] while carbapen-1-em-3-carboxylic acid is devoid of activity,[16] the requirement may be extended to other 2- and 6-substituted carbapenems. On the basis of the X-ray crystal structures of the acetonyl esters of carbapen-2-em-3-carboxylic acid and carbapen-1-em-3-carboxylic acid, Woodward[16] concluded that the Δ^1 isomer was extraordinarily reactive and that the lack of antibacterial activity was due to rapid destruction by indiscriminate reaction with any available nucleophiles. Our experience with the carbapenems **1**, **2**, **5**, and **6**, and particularly the relative reactivities of **5b** annd **6b** toward nucleophilic reagents, oppose that conclusion. For highly substituted carbapenems such as **5** and **6**, as well as for the less substituted derivatives **3** and **4**,[10] the Δ^2 isomers exhibit higher chemical reactivity than their Δ^1 counterparts.

The carbapenem antibiotics, like the classical penicillins and cephalosporins, presumably inhibit bacterial growth by acylating enzymes involved in the cross-linking step of cell-wall biosynthesis.[17] In the carbapenem series, acylating ability and potent antibacterial activity are associated mainly with a highly reactive β-lactam bond which is *double activated* by ring strain and the electronic effects of the adjacent double bond. The peripheral substituents, such as the hydroxyethyl and (aminoethyl)thio groups found in thienamycin, potentiate activity by providing additional binding sites to the target enzymes, by imparting β-lactamase stability, and by altering lipophilicity. Attempted synthesis of a carbapen-2-em derivative incoporating a 6-amido group, a substituent which would further enhance the chemical reactivity of the already sensitive β-lactam unit, resulted[18] in self-destruction of the ring system. By contrast, replacement of the 6(7)-amido group by hydroxyethyl in the less reactive penam and cephem ring systems[19] results in stable compounds nearly devoid of antibacterial activity.

The biological dualism observed in the carbapenem series has analogy in the related Δ^2- and Δ^3-cephem systems.[20] However, in the cephem case, ring strain is expected to play a less important role, and the ceph-3-em system is biologically more sensitive to the nature of the

(11) See ref 10 and papers cited therein.
(12) Schmitt, S. M.; Johnston, D. B. R.; Christensen, B. G. *J. Org. Chem.* **1980**, *45*, 1142.
(13) Similar deblocking of **5b** followed by XAD-2 chromatography provided thienamycin in only 20–25% yield.
(14) The isolation of hydroxypyrrole **7a** and disulfide **8** from the methanolysis reaction parallels a degradation previously observed for *N*-acetylthienamycin methyl ester; see ref 1b.
(15) Bauer, A. W.; Kirby, W. M.; Sherris, J. C.; Turk, M. *Am. J. Clin. Pathol.* **1966**, *45*, 493.
(16) Woodward, R. B. *Philos. Trans. R. Soc. London, Ser. B* **1980**, *289*, 239.
(17) Yocum, R. R.; Waxman, D. J.; Strominger, J. L. *Trends Biochem. Sci.* **1980**, *5*, 97.
(18) Narisada, M.; Uyeo, S.; Nagata, W. "Abstracts of Papers", 176th National Meeting of the American Chemical Society, Miami Beach, FL, Sept 1978; American Chemical Society: Washington, DC, 1978; Abstr MEDI 13.
(19) DiNinno, F.; Beattie, T. R.; Christensen, B. G. *J. Org. Chem.* **1977**, *42*, 2960.
(20) Sassiver, M. L.; Lewis, A. "Structure–Activity Relationships Among the Semisynthetic Antibiotics", Perlman, D., Ed.; Academic Press: New York, 1977; p 129.

7-substituent.

Experimental Section

Organic solutions were dried with anhydrous magnesium sulfate and filtered, and the solvents were removed under vacuum on a rotary evaporator. IR spectra were determined with a Perkin-Elmer 267 spectrophotometer and UV spectra with Perkin-Elmer 202 and Cary 15 spectrophotometers. ^{1}H NMR spectra were determined with Varian HA-100 and SC-300 instruments using Me_4Si as internal standard [δ (ppm) 0]. Low-resolution mass spectra were determined with an LKB-9000 spectrometer at an ionizing voltage of 70 eV.

Benzyl (3*S*,5*S*,6*S*)-2-[[2-[[(Benzyloxy)carbonyl]amino]ethyl]thio]-6-[(*R*)-1-hydroxyethyl]carbapen-1-em-3-carboxylate (6a). A solution of *N*-[(benzyloxy)carbonyl]thienamycin benzyl ester (**5a**;[1b] 256 mg, 0.52 mmol) in anhydrous Me_2SO (1.65 mL) was treated with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU, 80 μL, 0.54 mmol) and stirred at room temperature under a N_2 atm for 10 min. The mixture was diluted with EtOAc (20 mL) and Et_2O (30 mL), washed with H_2O (3 × 10 mL), 1 M pH 3 phosphate buffer (10 mL), 5% $NaHCO_3$ (10 mL), and brine (10 mL), dried, and evaporated to a yellow oil (232 mg). The crude product was chromatographed on two 1 mm × 20 × 20 cm silica gel GF plates using 3:1 EtOAc–$CHCl_3$ as developing solvent. The UV-visible band at R_f 0.4 was eluted with EtOAc to provide **5a** (83 mg, 32%) as off-white crystals. The band at R_f 0.6 afforded Δ^1-isomer **6a** (73 mg, 29%) as a clear oil: IR ($CHCl_3$) 1770 (lactam C=O), 1745 (ester C=O), 1720 (urethane C=O) cm^{-1}; UV (dioxane) λ_{max} 223 nm, 248 (sh); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.32 (d, 3, J = 6.5 Hz, $CHCH_3$), 1.7 (s, 1, OH), 2.91 (m, 2, SCH_2), 3.02 (dd, 1, J = 2.6 and 7.2 Hz, H6), 3.36 (m, 2, CH_2N), 4.19 (m, 1, $CHCH_3$), 4.54 (ddd, 1, J = 1.5, 2.6, and 3.4 Hz, H5), 5.06 (dd, 1, J = 1.7 and 3.4 Hz, H3), 5.04 (br s, 1, NH), 5.08 (s, 2, NCO_2CH_2), 5.15 (s, 2, CO_2CH_2), 5.90 (dd, 1, J = 1.5 and 1.7 Hz, H1), 7.31 (s, 10, phenyl); MS, m/e 496 (M^+), 478, 410, 259.

Equilibration of Δ^1-Isomer 6a. A solution of **6a** (3.1 mg) in anhydrous Me_2SO (20 μL) was treated with DBU (1 μL) and kept at room temperature for 10 min. The reaction mixture was diluted with CH_2Cl_2 (3 mL), washed with H_2O (3 × 1 mL), dried, and evaporated to an oil (2.1 mg). This material was shown by IR and UV spectroscopy and by TLC to be a mixture of **5a** and **6a**.

***p*-Nitrobenzyl (5*R*,6*S*)-2-[[2-[[[(*p*-Nitrobenzyl)oxy]carbonyl]amino]ethyl]thio]-6-[(*R*)-1-hydroxyethyl]carbapen-2-em-3-carboxylate (5b).** A solution of thienamycin (565 mg, 94% pure by UV, 1.95 mmol) in ice-cold H_2O (55 mL) was treated with $NaHCO_3$ (1.68 g, 20 mmol) and diluted with dioxane (45 mL). The resulting solution was cooled in an ice bath and stirred while *p*-nitrobenzyl chloroformate (647 mg, 3 mmol) in dioxane (10 mL) was added dropwise over 11 min. After stirring an additional 10 min in the cold, the mixture was acidified to pH 7 with cold 1 M H_2SO_4 and extracted with cold Et_2O (4 × 50 mL). The aqueous phase was layered with cold EtOAc (50 mL) and stirred vigorously in an ice bath while 1 M H_2SO_4 was added to pH 2.3. The layers were separated and the aqueous portion was extracted with more EtOAc (2 × 10 mL). The combined EtOAc solution was washed with cold brine (30 mL) and then extracted thoroughly with cold 0.05 N LiOH (40 mL). The aqueous phase was separated, washed with EtOAc (50 mL), partially concentrated under vacuum to remove organic solvents, and lyophilized to provide crude *N*-[[(*p*-nitrobenzyl)oxy]carbonyl]thienamycin lithium salt (599 mg, 65%) as a yellow powder.

A mixture of the crude salt (1.3 mmol) and *p*-nitrobenzyl bromide (702 mg, 3.25 mmol) in anhydrous HMPA (6.3 mL) was stirred under a N_2 atmosphere at room temperature for 2.5 h. The mixture was diluted with EtOAc (100 mL), washed with H_2O (5 × 50 mL), 5% $NaHCO_3$ (50 mL), and brine, dried, and evaporated to a yellow semisolid. This material was triturated with EtOAc and Et_2O and dried under vacuum to provide **5b** (438 mg, 38% overall yield) as a pale yellow solid: mp 170–172 °C; IR (Nujol mull) 1776 (lactam C=O), 1695 (ester and urethane C=O); UV (dioxane) λ_{max} 268 nm (ϵ 21900), 318 (14300); ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 1.05 (d, 1, J = 6 Hz, OH), 1.27 (d, 3, J = 6.5 Hz, $CHCH_3$), 3.09 (m, 2, SCH_2), 3.33 (dd, 1, J = 2.5 and 6.8 Hz, H6), 3.33 (dd, 1, J = 8 and 18 Hz, H1a), 3.45 (m, 2, CH_2N), 3.52 (dd, 1, J = 10 and 18 Hz, H1b), 4.13 (ddd, 1, $CHCH_3$), 4.28 (ddd, 1, J = 2.5, 8, and 10 Hz, H5), 5.28 (s, 2, NCO_2CH_2), 5.31 and 5.55 (2 d, 2, J = 14 and 5, CO_2CH_2), 6.92 (m, 1, NH), 7.67 (d, 2, J = 9 Hz, aryl), 7.83 (d, 2, J = 9 Hz, aryl), 8.27 (d, 4, J = 9 Hz, aryl); MS, m/e 542 (M − 44), 540, 500, 304. Anal. ($C_{26}H_{26}N_4O_{10}S$) C, H, N, S.

***p*-Nitrobenzyl (3*S*,5*S*,6*S*)-2-[[2-[[[(*p*-Nitrobenzyl)oxy]carbonyl]amino]ethyl]thio]-6-[(*R*)-1-hydroxyethyl]carbapen-1-em-3-carboxylate (6b).** A solution of **5b** (176 mg, 0.3 mmol) and DBU (50 μL, 0.33 mmol) in anhydrous Me_2SO (1.0 mL) was stirred under a N_2 atmosphere at room temperature for 10 min. The solution was diluted with EtOAc (50 mL), washed with H_2O (3 × 20 mL), pH 3 phosphate buffer (10 mL), 5% $NaHCO_3$ (20 mL) and brine, dried, and concentrated to approximately 5 mL volume, at which point crystallization began. After storing overnight in a refrigerator, the mixture was filtered to remove the precipitate, which was washed with cold EtOAc and Et_2O and dried under vacuum to provide a pale yellow powder (55 mg) shown to be **5b** by IR and TLC. The filtrate and washings were evaporated under vacuum to a yellow semisolid (110 mg). This material was dissolved in CH_2Cl_2 and the solution charged onto a column of EM silica gel 60 (8 g, packed under 3:1 EtOAc–$CHCl_3$). The products were eluted with 3:1 EtOAc–$CHCl_3$, 3.5-mL fractions being collected every 3 min. Fractions 11–21 gave the Δ^1-isomer **6b** (42 mg, 24%) as a clear oil, which was lyophilized from PhH to give a white powder: IR ($CHCl_3$) 1774 (lactam C=O), 1755 (ester C=O), 1728 (urethane C=O) cm^{-1}; UV (dioxane) λ_{max} 262 nm (ϵ 22400); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.34 (d, 3, J = 6.1 Hz, $CHCH_3$), 1.68 (d, 1, J = 4.4 Hz, OH), 2.99 (m, 2, SCH_2), 3.08 (dd, 1, J = 2.5 and 7.3 Hz, H6), 3.43 (m, 2, CH_2N), 4.25 (m, 1, $CHCH_3$), 4.59 (ddd, 1, J = 1.5, 2.5, and 3.5 Hz, H5), 5.15 (dd, 1, J = 1.8 and 3.5 Hz, H3), 5.20 (s, 2, NCO_2CH_2), 5.28 (s, 2, CO_2CH_2), 5.99 (dd, 1, J = 1.5 and 1.8 Hz, H1), 7.48 (d, 2, J = 8.7 Hz, aryl), 7.50 (d, 2, J = 8.7 Hz, aryl), 8.20 (d, 4, J = 8.7 Hz, aryl); MS, m/e 586 (M), 568 (M − 18), 540, 500, 304. Anal. ($C_{26}H_{26}N_4O_{10}S$) C, H, N, S.

Fractions 34–55 gave additional Δ^2-isomer **5b** (15 mg) as a white solid. The total yield of recovered **5b** was 34%.

(3*S*,5*S*,6*S*)-2-[(2-Aminoethyl)thio]-6-[(*R*)-1-hydroxyethyl]carbapen-1-em-3-carboxylic Acid (2, Δ^1-Thienamycin). A solution of **6b** (24.8 mg, 0.042 mmol) in THF (4 mL), EtOH (4 mL), and 0.05 M pH 7 phosphate buffer (8 mL) was added to 10% Pd/C (72 mg), and the mixture was hydrogenated at atmospheric pressure and room temperature for 30 min. The mixture was filtered and the filtrate extracted wiih Et_2O (5 × 30 mL). The clear, aqueous phase was concentrated under vacuum to 5 mL and charged onto a column of XAD-2 resin (2.2 × 13 cm), which was eluted with H_2O. The progress of the chromatography was monitored by a 254-nm UV detector. The appropriate fractions were combined, concentrated under vacuum to 1 mL, and lyophilized to afford Δ^1-thienamycin (**2**; 7.2 mg, 63%) as a white powder; IR (Nujol) 1757 cm^{-1}; UV (H_2O) λ_{max} 240 nm (ϵ 3400); ^{1}H NMR (300 MHz, D_2O) δ 1.30 (d, 3, J = 6.3 Hz, $CHCH_3$), 3.2 (m, 2, SCH_2), 3.26 (dd, 1, J = 2.7 and 6.2 Hz, H6), 3.3 (m, 2, CH_2N), 4.27 (apparent pentet, 1, J = 6.3 Hz, $CHCH_3$), 4.65 (m, 1, H5), 4.95 (dd, 1, J = 1.5 and 3.5 Hz, H3), 6.09 (dd, 1, J = 1.5 and 1.8 Hz, H1).

Methanolysis of 5b and 6b. A solution of **5b** (5.9 mg, 0.01 mmol) and **6b** (5.9 mg, 0.01 mmol) in THF (1.0 mL) was diluted with MeOH (1.0 mL) and treated with 1 M HCl (2 μL, 0.002 mmol). The resulting solution was stored in a capped flask at room temperature. Aliquots were removed periodically and examined by TLC (silica gel GF, 3:1 EtOAc–$CHCl_3$) and by UV spectroscopy (10 μL in 2 mL of dioxane), reduction in the 318-nm absorption being associated with loss of **5b**. After 2 h, both TLC and UV revealed complete consumption of **5b**. The reaction mixture was diluted to 10 mL with EtOAc, washed with H_2O (3 × 2 mL), 5% $NaHCO_3$ (2 mL), and brine, dried, and evaporated to yield a pale yellow oil (10.3 mg). This material was chromatographed on a 0.25 × 85 × 200 mm silica gel GF plate using 3:1 EtOAc–$CHCl_3$ as developing solvent. Three UV-visible bands at R_f 0.36 (band A), 0.50 (band B), and 0.60 (band C) were removed and eluted with EtOAc.

Band A provided Δ^1-isomer **6b** (4.9 mg, 83% recovery) as a clear oil.

Band B provided the hydroxypyrrole **7a** (2.9 mg) as a clear oil: IR (CH_2Cl_2) 3410, 1726, 1670, 1580, 1525, 1350 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (d, 3, J = 6.5 Hz, CH_3CH), 2.86 (br s,

1, exchanges with D_2O, OH), 3.60 (d, 1, J = 4 Hz, $CHCO_2Me$), 4.42 (m, 1, CH_3CH), 5.42 (AB q, J = 15 Hz, CO_2CH_2), 5.82 (d, 1, J = 2.5 Hz, collapses to singlet with D_2O, pyrrolyl), 7.60 (d, 2, J = 9 Hz, aryl), 8.27 (d, 2, J = 9 Hz, aryl), 8.97 (br s, 1, exchanges with D_2O, NH); MS, m/e 378 (M), 334 (M - CH_3CHO), 302, 198, 181. The NMR spectrum also revealed that **7a** was contaminated with a small amount of another pyrrole thought to be the product retaining the cysteamine side chain.

Band C provided the disulfide **8** (1.6 mg) as a clear oil: IR (CH_2Cl_2) 3450, 1727, 1520, 1350 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.82 (t, 2, J = 6 Hz, SCH_2), 3.55 (q, 2, J = 6 Hz, CH_2N), 5.20 (s, 2, CO_2CH_2), 5.34 (br s, 1, NH), 7.52 (d, 2, J = 8.5 Hz, aryl), 8.23 (d, 2, J = 8.5 Hz, aryl); MS, m/e 510 (M), 357, 314, 256, 255, 254, 233, 136.

Replication of the experimental conditions on the separate isomers revealed that **7a** and **8** were derived from **5b** and that **6b** was stable even after prolonged exposure.

Aminolysis of 5b and 6b. A solution of **5b** (5.9 mg, 0.01 mmol) and **6b** (5.9 mg, 0.01 mmol) in THF (1.0 mL) was treated with morpholine (17.4 μL, 0.2 mmol) and left at room temperature in a capped flask. Aliquots were removed periodically and examined by TLC and UV spectroscopy (5 μL in 2 mL of dioxane), both of which showed complete disappearance of **5b** after 4 days. The reaction mixture was diluted to 10 mL with EtOAc, washed with H_2O (2 × 2 mL), pH 3 buffer (2 mL), 5% $NaHCO_3$ (2 mL), and brine, dried, and evaporated to a pale yellow oil (13 mg). The crude product was chromatographed on a 0.25 × 85 × 200 mm silica gel GF plate using 3:1 EtOAc–$CHCl_3$ as developing solvent. UV visualization showed minor bands at R_f 0.04 and 0.45 and three major bands at R_f 0.13 (band A), 0.30 (band B), and 0.52 (band C). The major bands were removed and eluted with EtOAc.

Band A provided the hydroxypyrrole **7b** (2.1 mg) as a clear oil: IR (CH_2Cl_2) 3425, 1725 (sh), 1710, 1665, 1625, 1580, 1525, 1350 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.11 (d, 3, J = 7 Hz, CH_3CH), 3.5–3.8 (m, 9, morpholino and CHCO), 4.33 (m, 1, CH_3CH), 4.67 (br s, 1, exchanges with D_2O, OH), 5.40 (AB q, 2, J = 14 Hz, CO_2CH_2), 5.75 (d, 1, J = 2.5 Hz, collapses to singlet with D_2O, pyrrolyl), 7.61 (d, 2, J = 9 Hz, aryl), 8.29 (d, 2, J = 9 Hz, aryl), 8.99 (br s, 1, exchanges with D_2O, NH); MS, m/e 433 (M), 415 (M - H_2O), 389 (M - CH_3CHO), 253, 236, 209, 136, 114.

Band B afforded Δ^1-isomer **6b** (4.6 mg, 78% recovery), and band C afforded the disulfide **8** (1.2 mg).

Replication of the reaction conditions on the separate isomers revealed that **7b** and **8** were derived form **5b**, whereas **6b** was unchanged even after prolonged reaction times.

Acknowledgment. The authors thank W. J. Leanza for experimental guidance in preparing the bisprotected thienamycin derivatives, Dr. R. A. Firestone for helpful discussions during this work, Ms. Jean S. Kahan for the antibacterial assays, and Dr. B. G. Christensen for his support and encouragement during this investigation.

Book Reviews

Medical and Biological Applications of Electrochemical Devices. Edited by J. Koryta. Wiley, New York. 1980. ix + 331 pp. 15.5 × 23.5 cm. $79.00.

Medical and biological applications of electrochemical techniques can be divided into two categories. The first concerns with utilizing electrochemical methods for the study of chemical actions of molecules, proteins, and enzymes of biological significance. In the second category, electrochemical techniques are treated as powerful analytical tools in the determination of the amount of the chemical substance of interest in biological samples. This book deals with the latter aspect with an emphasis on the direct measurements in biological media. The authors present, in a comprehensive way, those electrochemical devices which have been introduced to the laboratory and clinic during the last 2 decades.

The first chapter is a concise review of relevant concepts in electroanalytical chemistry. The principles and practices of ion selective electrodes are treated in detail in Chapters 2–5. These include both liquid-membrane and solid-state ion selective electrodes and their applications in the measurements of ion activities in whole blood, plasma, urine, single cells, and excitable tissue. The next three chapters cover voltammetric (polarographic) methods applied to analysis of oxygen in biological materials, to in vivo measurements in kidneys and brain tissues, and to determination of 1,4-benzodiazapin derivatives in body fluid. Finally, Chapter 9 described enzyme electrodes and their possible biomedical utilizations.

This monograph will be a valuable reference for those electrochemists who are involved in biomedical research and for those medicinal chemists, pharmacologists, and clinical chemists who are constantly seeking for improvements on their analytical procedures. It offers a comprehensive background knowledge, some practical considerations, and an exhaustive literature survey on the recent applications of ion selective electrodes, enzyme electrodes, and voltammetric electrodes in medicine and biology. The overall quality is excellent, and the editor (Koryta) and contributors ought to be congratulated. It is a must for a science library. The price, $79.00, would be the only thing which might prevent the addition of this book to the personal collections of those who are interested in the specific topics.

Department of Chemistry **Hung-Yuan Cheng**
University of Maryland
College Park, Maryland 20742

Forensic Toxicology. Edited by J. S. Oliver. University Park Press, Baltimore. 1980. 320 pp. 14 × 22 cm. $39.95.

This book consists of the papers presented at the European Meeting of the International Association of Forensic Toxicologists, held at the University of Glasgow in August, 1979. Thirty-six individual contributions are included here, dealing with a wide variety of analytically related problems in forensic toxicology. These analytical techniques include thin-layer chromatography, gas–liquid chromatography, mass spectrometry, high-performance liquid chromatography, and mass spectrometry. Most analytical methods and approaches deal with organic compounds, such as drugs, hydrocarbons, pesticides, etc., but there are also discussions of the trace analysis of metals and organometallic compounds. As expected, almost all of the matrices analyzed are of biological origin, such as whole blood, plasma, tissues, and related matter. Dealing with forensic related matters, the analyses emphasize drug overdose, arson fatalities, accidental and intentional poisonings, and similar situations. Much of the analytical approaches are somewhat standard by this time, but certain sample preparations, extractions, workups, etc. are often novel, practical, and extremely useful. Of special interest to this reader was a discussion of extractive dialysis by Brandenberger and Bucher.

There is little question that this must have been a very successful scientific meeting, as the vast majority of the papers presented are of interest to anyone involved in forensic related research areas. However, it would appear that the emphasis has been on analytical chemistry as applied to forensic related matters, with relatively little discussion or emphasis on the toxicological aspects. Perhaps a better title for the conference and book might have been analytical toxicology or analytical chemistry as applied to forensic toxicology. Nevertheless, the book is of general interest to anyone involved in trace organic/inorganic analyses, especially