tor/recorder. Using this system we also performed coinjection analysis on paired sets of samples containing either analogue or analogue + NPY 1:1 by weight. The purities quoted in Table I are a consensus of values determined in the three analytical systems described. The retention times quoted in Table I were determined from isocratic analysis of each peptide with the Hitachi system described above. The mobile-phase system used was 0.1% TFA/MeCN (percentages as specified).

Amino acid analysis of the peptides was performed following hydrolysis in 4 N methanesulfonic acid at 110 °C for 24 h. A Perkin-Elmer LC system comprising two Series 10 LC pumps, a ISS-100 sample injector, RTC 1 column oven, Kratos Spectroflow 980 fluorescence detector, and LCI-100 integrator was used. A Pierce AA511 ion-exchange column was maintained at 60 °C and post column derivatization with o-phthalaldehyde was performed at 52 °C. Samples containing the internal standard γ-aminobutyric acid were injected and a gradient 0–100% B in 25 min and then 100% B for 15 min was commenced 5 min after injection. The flow rate was 0.5 mL/min, and A and B buffers were Pierce Pico buffer (pH 2.20) and Beckman Microcolumn sodium citrate buffer (pH 4.95), respectively.

Optical rotations of peptides were measured in 1.0 M acetic acid ($c \sim 0.5$, i.e. 5 mg of lyophilized peptide/mL uncorrected for TFA counterions or water present after lyophilization). Values were calculated from the means of 10 successive 5-s integrations determined at 25 °C on a Perkin-Elmer 241 polarimeter (using the D line of Na emission) divided by the concentration of the sample in g/dL and are quoted as uncorrected specific rotations.

Conscious Rat Blood Pressure Assay. Male Sprague–Dawley rats (240–280 g, Bantin-Kingman Laboratories Fremont, CA) were used. All procedures performed on these animals were in accordance with the guidelines of the University of California, San Diego Committee on Investigations Involving Animal Subjects. All animals, when used in experiments were conscious and freely moving. Femoral catheters were constructed by heat bonding of 1-cm lengths of PE 10 tubing to pieces of PE 50 tubing. The PE 10 segment was inserted into the femoral artery. The remaining PE 50 was routed subcutaneously and exteriorized between the scapulae. Catheter placement was performed on the day of the experiment under pentobarbital anesthesia (40 mg/kg). There was at least a 2-h recovery period between the catheter placement and the start of the experiment. Saline solutions of the peptides were administered intraarterially in a 100-μL bolus. MAP and HR were measured with Gould-Statham P23Db pressure transducers and monitored with a Beckman R-611 dynograph or a Gould physiograph. A Cyborg A-D converter linked to an IBM-XT compatible computer was used for data collection. MAP was calculated as: [(systolic − diastolic/3) + diastolic]. Zero points were determined prior to administration of peptides by injection of saline vehicle alone.

Data Analysis. In all cases data points were calculated as ΔMAP or ΔHR by subtraction of mean pretreatment zero values ($n = 4$) from posttreatment values for each animal at each dose and time. These data were then grouped for each dose and time, and means ± SEM were calculated. Dose–response curves were fitted to the 1-min data by computer iteration (Bolt, Beranek and Newman Research Systems RS/1 Biocomputing Software) and ED_{50}'s (the effective dose required to elicit 50% of the maximal response) were calculated. Relative potencies were then calculated as ED_{50} (compound)/ED_{50} (NPY) for those compounds for which the experimentally determined maximum (max) was within 20% of that determined for NPY.

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Registry No. 1, 83589-17-7; 2, 118537-11-4; 3, 118537-10-3; 4, 118537-14-7; 5, 118536-94-0; 6, 118537-12-5; 7, 118536-95-1; 8, 118536-96-2; 9, 118575-01-2; 10, 118575-02-3; 11, 118536-97-3; 12, 118536-98-4; 13, 118536-99-5; 14, 118537-00-1; 15, 118537-01-2; 16, 118537-02-3; 17, 118537-03-4; 18, 118537-04-5; 19, 90880-35-6; 20, 118537-05-6; 21, 118537-06-7; 22, 118537-07-8; 23, 118537-08-9; 24, 118537-09-0; 25, 102961-52-4; 26, 118460-07-4; 27, 116208-53-8; 28, 118474-29-6; 29, 113676-81-6; 30, 118460-08-5; 31, 118460-09-6; 32, 101994-11-0; 33, 118536-93-9.

Synthesis and in Vitro Activity of 1β-Methyl C-2 Quaternary Heterocyclic Alkylthio Carbapenems

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New 1β-methylcarbapenems having various (substituted) quaternary heterocyclic alkythio groups at the C-2 position were synthesized and tested for antibacterial activity and renal dipeptidase susceptibility. Compounds having the 1β-methyl substituent were found to possess an increased stability to the enzyme. In addition, combination of the 1β-methyl substituent and the C-2 quaternary heterocyclic alkylthio side chain generated compounds with excellent antipseudomonal activity and improved stability toward hydrolysis by renal dipeptidase.

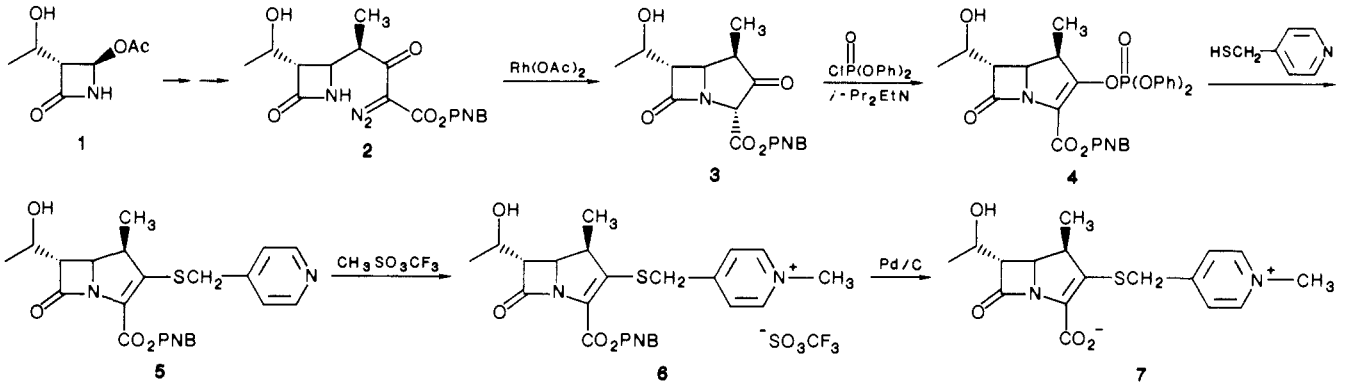
Carbapenems, as exemplified by the natural product thienamycin, have the widest spectrum of antibacterial activity of all β-lactams.[1] However, the discovery that they are susceptible to hydrolysis by a mammalian enzyme, renal dipeptidase, has hampered development in this class of compounds.[2] Since the disclosure of the first practical carbapenem synthesis by the Merck group, many research institutes have prepared various substituted carbapenems to address this deficiency.[3] The vast majority of these

(1) Ratcliffe, R. W.; Albers-Schonberg, G. The Chemistry of Thienamycin and Other Carbapenem Antibiotics. In *Chemistry and Biology of β-Lactam Antibiotics*. Morin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; Vol. 2, p 227.

(2) Graham, D. W.; Ashton, W. T.; Barash, L.; Brown, J. E.; Brown, R. D.; Canning, L. F.; Chen, A.; Springer, J. P.; Rogers, E. F. *J. Med. Chem.* **1987**, *30*, 1074.

0022-2623/89/1832-0601$01.50/0

Scheme I[a]



[a] PNB = $CH_2C_6H_4$-4-NO_2.

have been substituted amino alkylthio derivatives at C-2, analogous to the naturally occurring thienamycin, which appears to be a necessary structural feature for retaining activity against *Pseudomonas aeruginosa*.[4]

In a previous paper,[5] we have shown that carbapenem analogues having a C-2 quaternary heterocyclic alkylthio group are excellent broad antibacterial agents including activity against *Pseudomonas aeruginosa*. These derivatives were also shown to be less susceptible toward hydrolysis by renal dipeptidase. The susceptibility of imipenem toward this enzyme results in extensive renal metabolism in humans, which leads to low urinary recovery.

In continuation of our studies in the C-2 quaternary (alkylthio)carbapenem series, we became interested in preparation of the 1β-methyl analogues hoping to obtain further improved renal dipeptidase stable carbapenems while retaining the broad antibacterial spectrum including excellent antipseudomonal activity.

The preparation of 1β-methylcarbapenem analogues was particularly attractive in view of the observations by Shih et al.[6] that some 1β-methylcarbapenem analogues showed not only increased stability to renal dipeptidase but also improved chemical stability. The new class of carbapenems described in this paper retained the broad antibacterial activity including the excellent antipseudomonas activity similar to imipenem, and also showed a remarkable stability toward hydrolysis by renal dipeptidase.

Chemistry

The synthesis of C-2 quaternary (alkylthio)carbapenems was described by us in a previous paper.[5] For the synthesis of 1β-methylcarbapenem analogues, the 1β-methyl bicyclo keto intermediate **3** was prepared by the $Rh(OAc)_2$-catalyzed cyclization of the diazo intermediate **2** as described by the Merck group.[6] The stereoselective synthesis of compound **2** was accomplished by us[7] and others[8] from the readily available azetidinone **1**.

As shown in Scheme I, treatment of **3** with diphenylphosphoryl chloride in the presence of diisopropylethylamine generated in situ the phosphonate **4**, which was further converted into the intermediate **5** in 40% overall yield by addition of 3-(mercaptomethyl)pyridine in the presence of diisopropylethylamine.

Quaternization of the pyridine ring was accomplished by action of methyl trifluoromethanesulfonate at room temperature. There was no indication of reaction between the alkylating agent and the sulfur atom of the C-2 side chain. This decreased reactivity of the sulfur atom in **5** appears to be due to the conjugation of the sulfur atom with the α,β-unsaturated ester functionality. Catalytic hydrogenation of **6** over Pd/C and purification of the crude product by a medium-pressure C_{18} Bondapak column (Waters Associates) gave the zwitterionic product **7** as an amorphous solid. Similarly, other 1β-methylcarbapenem analogues **11**, **13**, and **14** were prepared by the reaction sequence described above.

Biological Studies

Compounds were assayed for their antimicrobial activity and their rate of hydrolysis by renal dipeptidase. The results of these biological studies are shown in Table I. It is clear from these few examples that the 1β-methyl group has a minimal effect on the antibacterial properties relative to their C-1 unsubstituted counterparts. These molecules still retain excellent MICs against Gram-positive and a spectrum of Gram-negative bacteria. There is also little or no effect of this substitution on activity against *Pseudomonas* (compare **7** vs **9**, **10** vs **11**, **12** vs **14**). As had been found in the nonsubstituted series[5] (**8** vs **9**), the positive charge from quaternization of pyridine also causes a marked improvement in antipseudomonal activity with the 1β-methyl series (**13** vs **14**).

By far the most striking effect of the 1β-methyl substituent on activity is in the stability to enzymatic hydrolysis by renal dipeptidase (for example, compare **9** vs **7**, **10** vs **11**, **12** vs **14**). Improvements of 40–60-fold are realized, and rates at or below 1% of the rate with imipenem have been achieved.

(3) For a review in the synthesis of carbapenem β-lactam antibiotics, see: Kametani, T.; Fukumoto, K.; Ihara, M. *Heterocycles* **1982**, *17*, 463.

(4) For example, see: (a) Sato, M.; Takemura, M.; Atarashi, S.; Higashi, K.; Nagahara, T.; Furukawa, M. *J. Antibiot.* **1987**, *40*, 1292. (b) Miyadera, T.; Sugimura, Y.; Hashimoto, T.; Tanaka, T.; Iino, K.; Shibata, T.; Sugawara, S. *J. Antibiot.* **1983**, *36*, 1034.

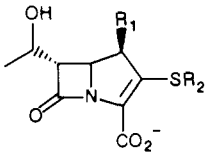
(5) Kim, C. U.; Misco, P. F.; Luh, B. Y.; Hitchcock, M. J. M. *J. Antibiot.* **1987**, *40*, 1707.

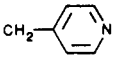
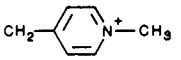
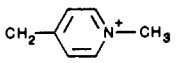
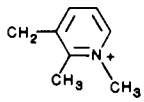
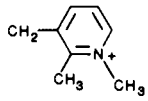
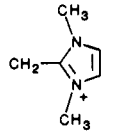
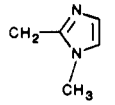
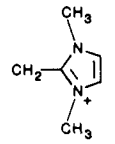
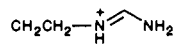
(6) (a) Shih, D. H.; Baker, F.; Cama, L.; Christensen, B. G. *Heterocycles* **1984**, *21*, 29. (b) Shih, D. H.; Fayter, J. A.; Cama, L. D.; Christensen, B. G.; Hirschfield, J. *Tetrahedron Lett.* **1985**, *26*, 583. (c) Shih, D. H.; Cama, L.; Christensen, B. G. *Tetrahedron Lett.* **1985**, *26*, 587.

(7) Kim, C. U.; Luh, B.; Partyka, R. A. *Tetrahedron Lett.* **1987**, *28*, 507.

(8) Alternate methods to prepare 1β-methylcarbapenems. (a) Iimori, T.; Shibasaki, M. *Tetrahedron Lett.* **1986**, *27*, 2149. (b) Nagao, Y.; Kumagai, T.; Tamai, S.; Abe, T.; Kuramoto, Y.; Taga, T.; Aoyagi, S.; Nagase, Y.; Ochiai, M.; Inoue, Y.; Fujita, E. *J. Am. Chem. Soc.* **1986**, *108*, 4673. (c) Fuentes, L. M.; Shinkai, I.; Saltzman, T. N. *J. Am. Chem. Soc.* **1986**, *108*, 4675. (d) Fuentes, L. M.; Shinkai, I.; King, A.; Purick, R.; Reamer, R. A.; Schmitt, S. M.; Cama, L.; Christensen, B. G. *J. Org. Chem.* **1987**, *52*, 2563.

Table I. In Vitro Activity



compd	R_2	R_1	antibacterial activity,[a] μg/mL S.a.[c]	E.c.	E.cl.	S.m.	P.v.	P.a.	renal dipeptidase hydrolysis (rel rate)[b]
8		H	0.03	0.03	0.5	0.13	0.03	32	3.84
9		H	0.016	0.03	0.13	0.13	0.03	0.5	0.84
7		CH_3	0.016	0.016	0.06	0.03	0.03	0.5	0.02
10		H	0.016	0.008	0.016	0.03	0.03	0.5	0.40
11		CH_3	0.016	0.004	0.016	0.16	0.03	2	0.01
12		H	0.004	0.008	0.03	0.016	0.008	2	0.20
13		CH_3	0.016	0.008	0.016	0.016	0.016	16	0.94
14		CH_3	0.008	0.004	0.06	0.03	0.016	1	0.003
imipenem		H	0.008	0.016	0.06	0.03	0.03	0.25	1.00

[a] Determined by serial 2-fold dilution of compound in Mueller-Hinton agar and inoculation of the agar surface or broth with an appropriately diluted 18–24-h broth culture. Agar plates and tubes of broth were incubated at 37 °C for 17 h, and the lowest concentration causing inhibition of visible growth was considered to be the MIC. [b] Pure hog renal dipeptidase was prepared as described.[10] Solutions of carbapenem (0.10 mM) in buffer (50 mM) MOPS, pH 7.1, were freshly prepared. The UV/vis spectrum of a 2.5-mL aliquot was measured and then 0.025 mL of 1 M NH_2OH was added to degrade the β-lactam bond. The spectrum was again measured after 30 min at 25 °C and again at 5–10-min intervals until no further decrease in absorbance was observed. The different spectrum between intact and degraded β-lactam was used to calculate a λ max and ε. A similar aliquot of carbapenem solution was incubated at 25 °C and the rate of change in absorbance at λ max was determined. Enzyme was then added to give a rate of at least 10^{-4} absorbance units per second. The rate of enzymatic hydrolysis was corrected for spontaneous hydrolysis and then converted with ε to nmol min^{-1} (mL of enzyme)$^{-1}$. All rates are reported relative to that observed with imipenem. [c] S.a., *Staphylococcus aureus*, pen-res A-9606; E.c., *Escherichia coli* A-15119; E.cl., *Enterobacter cloacae* A-9695; S.m., *Serratia marcescens* A-20019; P.v., *Proteus vulgaris* A-21559; P.a., *Pseudomonas aeruginosa*.

Discussion

The systematic synthetic modification of carbapenems to achieve high-potency antimicrobial activity coupled with enzymatic and chemical stability has been the goal of many researchers. Shih et al.[6] have shown the beneficial effect of a 1β-methyl substituent in terms of improvements in enzymatic and chemical stability with no loss of antibacterial potency. In spite of this, there has been little published on the biological properties of carbapenems incorporating this feature. One exception is 3-[[2-(dimethylamino)pyrrolidin-4-yl]thio]-6-(1-hydroxyethyl)-4-methyl-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.[9] This has good activity but is only 4 times more stable than imipenem to human renal dipeptidase. Urinary recovery in animals was low and varied with species (12–50%), correlating well with the differences in hydrolysis rates measured with the isolated enzymes.

In this paper we show that the 1β-methyl substituent alone is insufficient to confer good stability to hydrolysis by hog renal dipeptidase (**13**). However, when coupled with a quaternary side chain at C-2, these carbapenems are extremely stable to enzymatic hydrolysis. This feature together with their excellent antibacterial spectrum makes them interesting candidates for further evaluation.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus

(9) SM-7338. Sunagawa, M.; Matsumura, H.; Inoue, T.; Fukasawa, M.; Kato, M. 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, October, 1987; Abstract No. 752.

(10) Hitchcock, M. J. M.; Farrell, C. A.; Huybensz, S.; Luh, B. Y.; Phelps, D. J. *Anal. Biochem.* 1987, *163*, 219.

and are uncorrected. The UV spectra were run in EtOH; IR spectra were recorded on a Beckman 5240 spectrophotometer using KBr pellets; NMR spectra were obtained on a Varian HA-100 spectrometer using $(CH_3)_4Si$ as an internal standard. All solid compounds were characterized by UV, IR, and NMR.

***p*-Nitrobenzyl 3-[(Diphenylphosphoryl)oxy]-6α-[1(*R*)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (4).** A solution of 1.75 g (4.85 mmol) of *p*-nitrobenzyl 6α-[1(*R*)-hydroxyethyl]-3,7-dioxo-4-methyl-1-azabicyclo[3.2.0]heptane-2-carboxylate[6,6] in 20 mL of acetonitrile is cooled to 0 °C under a nitrogen atmosphere. A solution of 726 mg (7.18 mmol) of diisopropylethylamine in 2 mL of acetonitrile is added followed by a dropwise addition of 1.51 g (5.60 mmol) diphenyl chlorophosphate in 12 mL of acetonitrile over a period of 3 min. The resulting solution is stirred at 0 °C for 20 min to provide the phosphonate 4 in situ, which was used for further chemical transformation without isolation.

***p*-Nitrobenzyl 3-[(Pyridin-4-ylmethyl)thio]-6α-[1(*R*)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (5).** To a cooled (−15 °C) solution of 1.2 g (2 mmol) of the phosphonate 4 in 10 mL of acetonitrile was added 390 mg (3 mmol) of diisopropylethylamine followed by 370 mg (3 mmol) of 2-(mercaptomethyl)pyridine under N_2. The reaction mixture was allowed to stir for 60 min at −15 °C and then an additional 60 min at 0 °C. The reaction was diluted with ethyl acetate, washed with ice water and brine, and dried ($MgSO_4$). Evaporation of solvents in vacuo gave a yellow oil, which was purified by SiO_2 column; elution of the column with 20% ethyl acetate in methylene chloride gave 375 mg (40% yield) of compound 5 as a white amorphous foam: IR (KBr) 3400, 1775, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (3 H, d, J = 6.7 Hz), 1.29 (3 H, d, J = 6.7 Hz), 3.14 (1 H, q, J = 6.2 and 2.0 Hz), 3.40 (1 H, m), 4.0 (1 H, d, J = 7.6 Hz), 4.12 (1 H, d, J = 7.6 Hz), 4.18 (1 H, q, J = 6.7 and 2.0 Hz), 4.25 (1 H, m), 5.25 (1 H, d, J = 11.3 Hz), 5.40 (1 H, d, J = 11.3 Hz), 7.15–8.2 (4 H, m).

3-[[(*N*-Methyl-4-pyridinio)methyl]thio]-6α-[1(*R*)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (7). To a solution of 1.0 g (3 mmol) of compound 5 in 10 mL of methylene chloride was added 450 mg (3.3 mmol) of methyl trifluoromethanesulfonate and stirred at 23 °C for 90 min. Evaporation of methylene chloride in vacuo gave the quaternized pyridine as a foam, which was hydrogenated immediately without any further purification. The crude pyridinium salt was dissolved into tetrahydrofuran–ether–pH 7 buffer (1:1:1, 100 mL each) followed by 600 mg of 10% palladium on charcoal. The mixture was hydrogenated at 35 psi on the per shaker for 45 min. The mixture was filtered through a Celite pad, and the catalyst was washed with water (2 × 10 mL). The combined filtrate and washings were extracted with ether (2 × 100 mL) and lyophilized to give a yellow powder, which was purified on a C_{18} Bondapak reverse-phase column (10 g), eluting with 5% acetonitrile in water under 8 psi pressure. Each 15-mL fraction was assayed by high-pressure liquid chromatography, and fractions having an ultraviolet absorption at 300 nm max were collected and lyophilized to give 58 mg (11% yield) of the title compound as a pale yellow powder: IR (KBr) 3410, 1750, 1650 cm^{-1}; UV max (H_2O) 293 nm (ε 7295); ^1H NMR (D_2O) δ 1.15 (3 H, d, J = 6.5 Hz), 1.20 (3 H, d, J = 6.5 Hz), 3.20 (1 H, m), 3.45 (1 H, q, J = 6.0 and 2.0 Hz), 4.11 (1 H, q, J = 8.0 and 2.0 Hz), 4.20 (1 H, m), 4.35 (3 H, s), 7.95 (2 H, d, J = 5.2 Hz), 8.72 (2 H, d, J = 5.2 Hz). Anal. Calcd for $C_{17}N_{20}N_2O_4S_2 \cdot {}^1/_2H_2O$: C, 51.90; H, 6.36; N, 7.12. Found: C, 51.92; H, 5.71; N, 6.88.

3-[[(1,2-Dimethyl-3-pyridinio)methyl]thio]-6α-[1(*R*)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (11). This compound was obtained as yellow powder in 14% yield from compound 4 in the same manner as that described for compound 7: IR (KBr) 3400, 1750, 1600 cm^{-1}; UV max (H_2O) 296 nm (ε 8500); ^1H NMR (D_2O) δ 1.25 (3 H, d, J = 6.5 Hz), 1.30 (3 H, d, J = 6.5 Hz), 2.95 (3 H, s), 3.40 (1 H, m), 3.50 (1 H, q, J = 6.2 and 1.8 Hz), 4.2–4.4 (4 H, m), 4.35 (3 H, s), 7.82 (1 H, t, J = 8.5 and 6.3 Hz), 8.40 (1 H, d, J = 8.5 Hz), 8.72 (1 H, d, J = 6.3 Hz).

3-[[(*N*-Methylimidazol-2-yl)methyl]thio]-6α-[1(*R*)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (13). This compound was obtained as a yellow amorphous powder in 41% yield from compound 4 in the same manner as that described for compound 7: IR (KBr) 3400, 1750, 1620 cm^{-1}; UV max (H_2O) 293 nm (ε 8700); ^1H NMR (D_2O) δ 1.15 (3 H, d, J = 6.5 Hz), 1.25 (3 H, d, J = 6.5 Hz), 3.30 (1 H, m), 3.45 (1 H, q, J = 6.0 and 2.2 Hz), 3.55 (3 H, s), 4.2–4.6 (4 H, m), 7.25 (2 H, s).

3-[[(1,3-Dimethyl-2-imidazolio)methyl]thio]-6α-[1(*R*)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (14). This compound was obtained as a yellow powder in 32% yield from compound 4 in the same manner as that described for compound 7: IR (KBr) 3400, 1758, 1600 cm^{-1}; UV max (H_2O) 294 nm (ε 7194); ^1H NMR (D_2O) δ 1.10 (3 H, d, J = 6.3 Hz), 1.25 (3 H, d, J = 6.3 Hz), 3.30 (1 H, m), 3.42 (1 H, q, J = 6.0 and 2.2 Hz), 3.85 (6 H, s), 4.2–4.6 (4 H, m), 7.40 (2 H, s).

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Antitumor Agents. 100.[1] Inhibition of Human DNA Topoisomerase II by Cytotoxic Ether and Ester Derivatives of Podophyllotoxin and α-Peltatin

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A principal mechanism of action of the clinical antitumor drugs etoposide (1) and teniposide (2) is the inhibition of catalytic activity of type II DNA topoisomerase and concurrent enzyme-mediated production of lethal DNA strand breaks. Substitution of the glycosidic moiety of 1 or 2 by ester and ethers, as well as the esterification and etherification of α-peltatin (4) including its glucosidic ethylidene and thenylidene cyclic acetals (25 and 26), has afforded compounds of much less activity than that of 1. The in vitro cytotoxicity (KB) appears to have no correlation with the inhibitory activity of the human DNA topoisomerase II.

The clinically useful nonintercalative antitumor drugs etoposide (1) and teniposide (2) most likely produce their therapeutic effect by inducing DNA strand breaks which lead to tumor cell death.[2] This cleavage reaction is brought about by the formation of a "cleavable complex"

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(2) Ross, W.; Rowe, T.; Glisson, B.; Yalowich, J.; Liu, L. *J. Antibiot.* in press.

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