Articles

Endogenous Natriuretic Factors. 5. Synthesis and Biological Activity of a Natriuretic Metabolite of Diltiazem and Its Derivatives†

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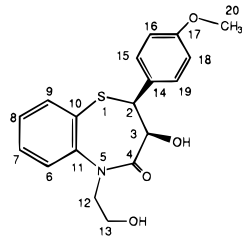
In our search for endogenous natriuretic factors from human uremic urine, we have previously identified a new metabolite of the drug diltiazem (Murray et al. *Life Sci.* **1995**, *57*, 2145−2161). The structure of this metabolite, (+)-(2*S*,3*S*)-3-hydroxy-5-(2-hydroxyethyl)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (LLU-β_1; **2**), was proved by unequivocal synthesis from a diltiazem synthon. The synthetic material also proved to be natriuretic as had the urinary isolate. Given the acetylation at C-3 in diltiazem, the 3-monoacetate (**8**) and diacetate (**3**) derivatives of **2** were prepared. The 4-nor-keto (**6**) derivative of **2** was also synthesized. Only the parent **2** induced natriuresis over a range of doses without accompanying kaliuretic activity at some doses.

Our laboratory has been engaged for several years in the isolation of endogenous natriuretic factors from human urine.[1−3] These studies have been directed toward the ultimate characterization of the putative natriuretic hormone described by deWardener.[4] We have employed various isolation procedures and have used as a search instrument an *in vivo* bioassay that measures sodium, potassium, and water excretion and permits the continuous monitoring of mean arterial pressure in the conscious rat. Of the several natriuretic isolates found in the course of these investigations,[1−3] we have concentrated our recent work to the characterization of what we have designated α, γ, and a series of compounds, β. From the complex mixture which composed the β isolate we have purified and identified one component to date, LLU-β_1 (**2**), a metabolite of the drug diltiazem.

The purification, spectroscopic characterization of the structure, and partial biological characterization of the isolate **2** were previously reported.[3] The analysis of the spectroscopic data is summarized here, and the confirmation of the structure of **2** by synthesis is described. Synthesis of derivatives of **2** and their biological activity as well as that of synthetic **2** are also reported.

Results and Discussion

The isolation of the natriuretic compound **2** was reported previously.[3] The structure of the natriuretic isolate was assigned by NMR and MS studies. The ^{1}H, ^{13}C, COSY, and HMQC NMR spectra revealed two aromatic rings, one that is *ortho* substituted and the other that is *para* substituted. A methoxy group was present as were separate CHCH and CH_2CH_2 groups, of which the latter was attached to heteroatoms at both ends. The remaining carbons detected in the ^{13}C NMR were observed in the region of 50−70 ppm. The lack of sample quantity prevented in-depth NMR studies. Mass spectroscopy revealed the loss of CHO from the molecular ion ($M^+ - 29$), suggesting an aliphatic hydroxy group. The molecular formula based on an exact mass of 345.1001 considering only CHNO did not match the NMR spectra by atom count. This suggested that another heteroatom may be present in the molecule. Intensive FAB MS study confirmed the presence of sulfur, thus establishing the molecular formula $C_{18}H_{19}NO_4S$, FW 345.41 Da. Silylation of **2** with trimethylsilyl bromide established the presence of two hydroxyl groups by mass spectra. The UV spectrum exhibits strong absorptions at 208 and 241 nm and a weak absorption at 272 nm. This suggested the presence of aromatic ring(s) conjugated with heteroatoms and a carbonyl. The analysis of spectroscopic data suggested the structure of **2** as 3-hydroxy-5-(2-hydroxyethyl)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one.



LLU-β_1 (**2**)

The compound **2** appeared to reasonably be the product of an oxidative deamination of the $CH_2CH_2N(CH_3)_2$ moiety of diltiazem to CH_2CH_2OH with concomitant deacetylation of the 3-*O*-acetate.[5] The presence of a diltiazem metabolite was not unexpected since the donors of the uremic urine were often treated with calcium channel blockers.

The proof of structure by unequivocal synthesis of **2** was realized by coupling of the (+)-(2*S*,3*S*)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (**1**) with 2-bromoethanol in the presence of potas-

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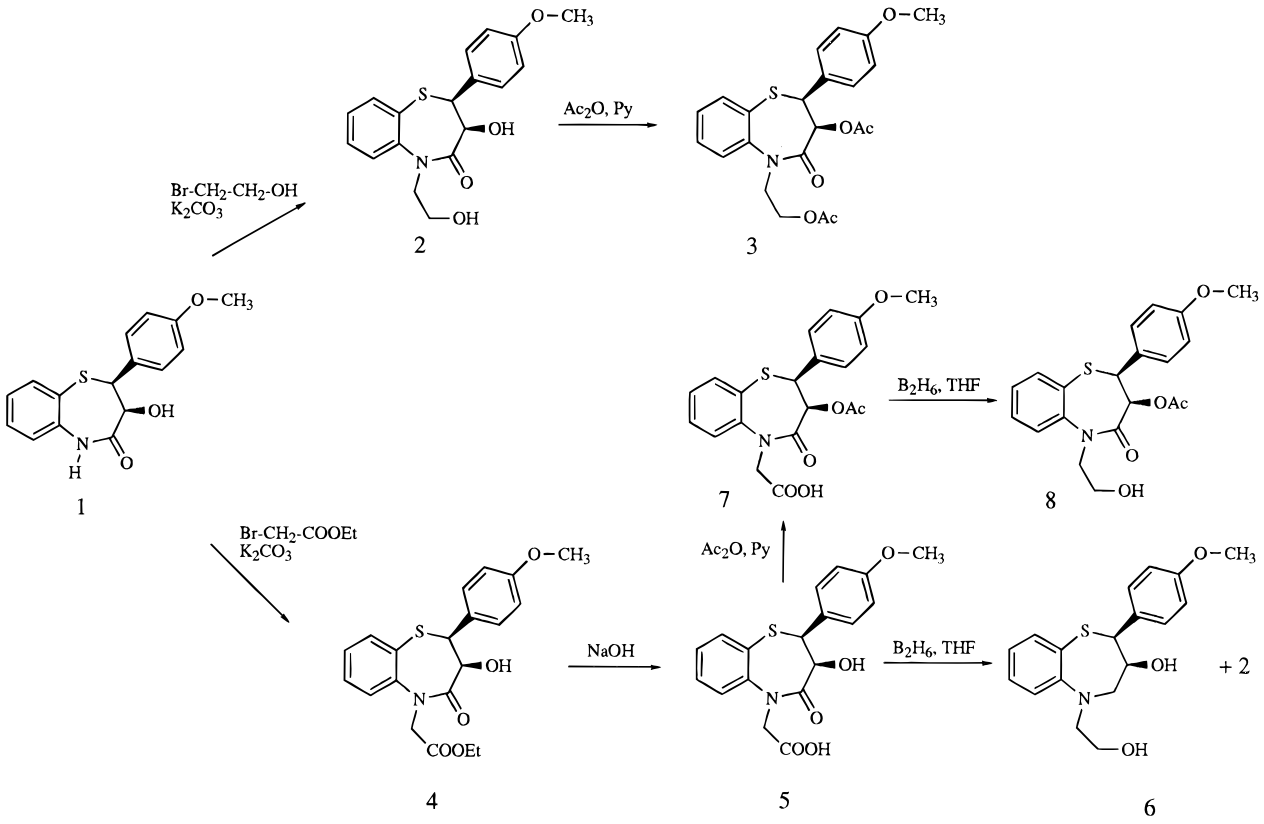

Figure 1. Synthetic scheme to target molecules **2**, **3**, **6**, and **8**. Compound **3** is a previously reported metabolite of diltiazem.[7]

sium carbonate in acetone according to the method of Sugawara et al.[6] (Figure 1). This also confirmed the stereochemistry at both C-2 and C-3 as *S*. The 3-*O*-acetyl derivative (**8**) was prepared by borane reduction of the carboxyl group of **7** under mild conditions (0 °C) to avoid the borane reduction of the carbonyl group. When **5** was treated with diborane at room temperature, the 4-carbonyl was reduced to methylene with concomitant reduction of the carboxyl group. This reaction may be accelerated by the presence of hydroxyl at position 3 forming a complex with diborane. Compound **7** (3-*O*-acetyl derivative of **5**) was less prone to the reductive deoxygenation by diborane of the 4-carbonyl, and only trace amounts of decarbonylated compound were detected by TLC.

Synthetic **2** and its simple derivatives (**3**−**8**) were evaluated for their natriuretic, kaliuretic, and diuretic effects in the conscious female rat utilizing an assay involving metabolic caging.[8] Compounds **2** and **4**−**7** demonstrated natriuresis (Figure 2A). Compound **2** was natriuretic at 10 and 20 mg/kg ($p \leq 0.05$, Scheffe) while the derivatives (**3** and **8**) were not active at doses up to 50 mg/kg. Compounds **4**−**7** were also natriuretic at 10 mg/kg with only **5** and **7** exhibiting natriuresis at other doses (**5**, 30 and 50 mg/kg; **7**, 30 mg/kg, $p \leq 0.05$, Scheffe; Figure 2A). Compounds **5** and **7** were both kaliuretic at various doses (**5**, 20 and 50 mg/kg; **7**, 20, 30, and 50 mg/kg, $p \leq 0.05$, Scheffe; Figure 2B). Compound **2** also exhibited kaliuresis at 10 mg/kg ($p \leq 0.05$, Scheffe; Figure 2B). Compounds **2**, **5**, and **7** demonstrated diuresis ($p \leq 0.05$, Scheffe; Figure 2C). Diltiazem and furosemide were assayed as controls. Diltiazem exhibited diuresis at 20 mg/kg ($p \leq 0.05$, Scheffe; Figure 2C) and a reduction in kaliuresis at the same dose ($p \leq 0.05$, Scheffe; Figure 2B). Furosemide was natriuretic, kaliuretic, and diuretic at 30 mg/kg ($p \leq 0.05$, Scheffe; Figure 2). At 20 mg/kg it was also kaliuretic ($p \leq 0.05$, Scheffe; Figure 2B) but gave rise to a reduced natriuresis at the same dose ($p \leq 0.05$, Scheffe; Figure 2A). On the basis of these data, compound **2** is the least kaliuretic in the presence of natriuresis/diuresis induced in the rat, a highly desirable trait for a diuretic agent. As a consequence it is being evaluated further. Compound **2** exhibits a very narrow dose−response unlike many natriuretic agents. The reason for this is unclear and requires further studies. It could be speculated that **2** needs to reach a threshold concentration to achieve its effect which has a parabolic dose−reponse.

Compound **2** was investigated for its ability to antagonize various receptors. In binding assays toward the α1-adrenergic, α2-adrenergic, D1-, D2-, D3-, and D4-dopamine, 5-HT1a, 5-HT1dα, 5-HT1dβ, and 5-HT2 receptors it gave much less than 50% inhibition when tested at 1 μM. When tested at 30 μM as a K_{ATP} channel blocker in the rabbit mesenteric artery, it was inactive.[9]

Diltiazem has been shown in some studies to produce natriuresis.[10−14] Whether it is due to diltiazem directly[13,14] remains unclear. With the discovery of a natriuretic metabolite of diltiazem, it is possible that the natriuresis seen with diltiazem may be due at least in part to this (**2**) or other metabolites. When **2** is assayed by venous infusion and blood pressure monitored in the conscious female rat,[3] it has little or no effect on blood pressure when compared to diltiazem (data not shown).

Experimental Section

Materials. Chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI). All solvents were of analytical grade. Solvents were removed under reduced pressure at 40 °C. Gravity and medium-pressure chromatography was done on

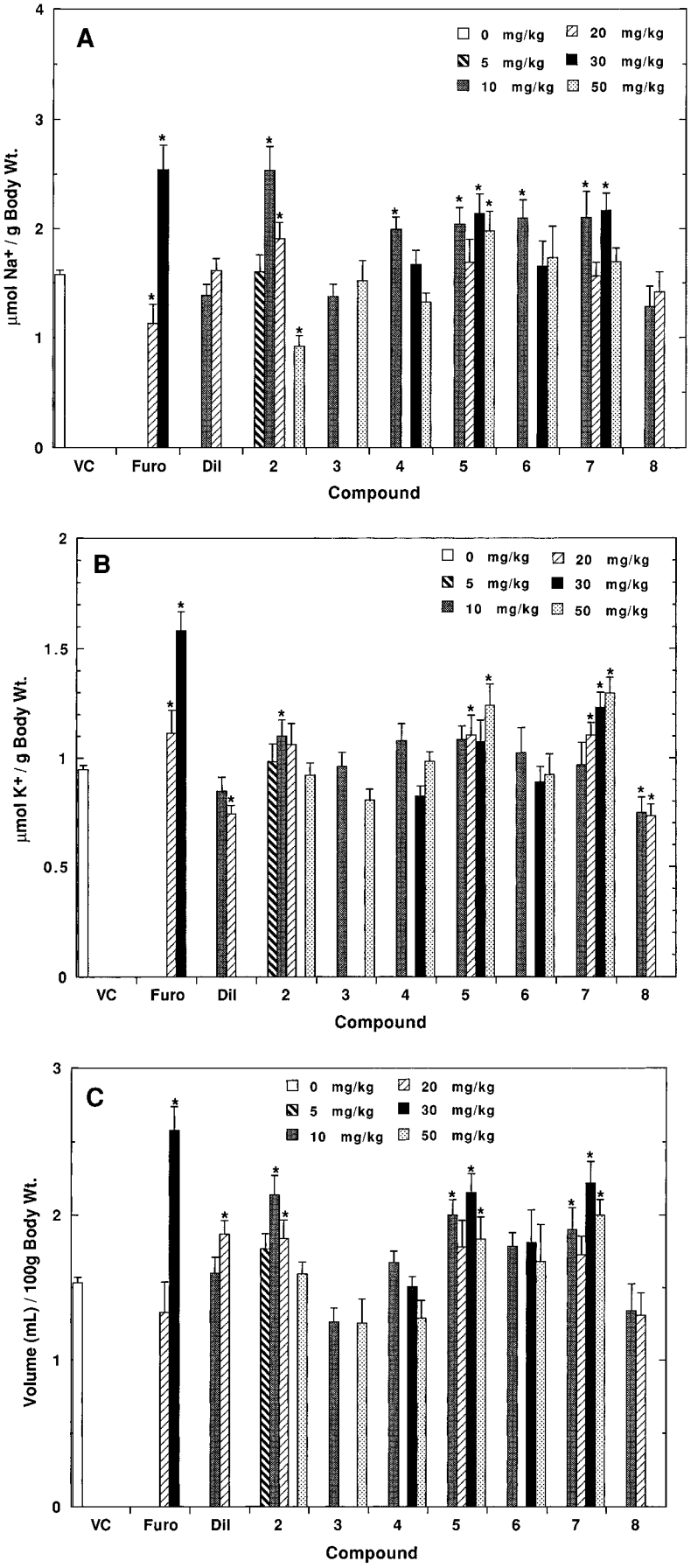

Figure 2. Natriuretic (A), kaliuretic (B), and diuretic (C) activity of intermediate and target compounds. The assay was performed as described in the Experimental section. All test substances were administered in 1% CMC. Significance was defined as $p \leq 0.05$ for Scheffe F test compared to the vehicle control and is represented by an asterisk (*). VC is vehicle control, 1% CMC, and Dil is the abbreviation for diltiazem. Furo is the abbreviation for furosemide. Error bars represent standard error. For each dose of vehicle or test compound $n \geq 9$ after removal of outliers (>2 SD).

silica gel (0.040−0.063 mm) (Bodmann ICN). NMR spectra were recorded on either a General Electric GN-500 (^{1}H 500.135 MHz, ^{13}C 125.768 MHz) or QE-300 (^{1}H 300.150 MHz, ^{13}C 75.480 MHz) spectrometer. Infrared spectra were acquired on a Perkin-Elmer 1600 Series FTIR, and data were processed on PC with IRDM v. 2.5 and PGEM v. 3.02 software. Optical rotation was measured on a Perkin-Elmer 241 polarimeter at 22 °C. EIMS spectra were acquired on a Finnigan 4000 GC/MS instrument, and data were processed on PC with Galaxy 2000 v. 5.5 software (X-Spec Inc.). Elemental analyses were performed by Galbraith Laboratories Inc. (Knoxville, TN). Melting points were determined on a Fisher-Johns hot stage and are uncorrected. HPLC purification of synthetic compounds was done on a Beckman System Gold instrument (model 126 pump, model 168 diode array UV detector) controlled by System Gold software v. 5.1 on PC. The column used was a SPHEREX 10 ODS, 250 × 21.2 mm (Phenomenex). Thiazepn GL [(+)-(2*S*,3*S*)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one] was donated by Tanabe Seiyaku Co. Ltd., Osaka, Japan.

The synthesis of **4, 5,** and **7** was performed according to the method of Sugawara et al.[6]

(+)-(2*S*,3*S*)-3-Hydroxy-5-(2-hydroxyethyl)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (2). To the mixture of (+)-(2*S*,3*S*)-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (**1**, 10.0 g) and K_2CO_3 (10.0 g) in acetone (110 mL) at reflux was added 2-bromoethanol (10.0 g) in acetone (30 mL) over 1 h. The reaction mixture was stirred under reflux. The addition of K_2CO_3 (5 g) and 2-bromoethanol (5 g) was repeated three times, after 6, 26, and 32 h of stirring. The reaction mixture was stirred at reflux for another 20 h. Potassium carbonate was filtered and washed with acetone, and the combined acetone filtrates were concentrated under reduced pressure. The residue was dissolved in ethyl acetate (300 mL), and the organic layer was washed with sulfuric acid (20%, 50 mL), water (50 mL), saturated aqueous sodium bicarbonate (50 mL), and water (50 mL) and then dried (Na_2SO_4). The oily residue was purified on silica gel column, eluent EtOAc−hexane (2:1), yield 10.7 g (87.9%). Product **2** was crystallized from ethanol: mp 104−105 °C; R_f = 0.23 (EtOAc−hexane, 2:1); $[\alpha]^{22}_D = +141°$ (*c* 0.84, MeOH); ^{1}H NMR (CD_3OD) δ 7.73 (2d, 1H, *J* 8.1 Hz, H-9), 7.68 (2d, 1H, *J* 7.7 Hz, H-6), 7.57 (2dd, 1H, *J* 7.5, 8.0 Hz, H-7), 7.40 (d, 2H, *J* 8.7 Hz, 15, H-19), 7.35 (2dd, 1H, *J* 7.6 Hz, H-8), 6.89 (d, 2H, *J* 8.7 Hz, 16, H-18), 4.89 (d, 1H, *J* 7.3 Hz, H-2), 4.33 (d, 1H, *J* 7.3 Hz, H-3), 4.27 (m, 1H, H-12a), 3.92 (m, 1H, H-13b), 3.87 (m, 1H, H-12b), 3.79 (s, 3H, 20), 3.66 (m, 1H, H-13a) ppm; ^{13}C NMR (CD_3OD) δ 173.27 (C-4), 161.15 (C-17), 147.18 (C-11), 136.21 (C-9), 132.62 (C-15, C-19), 132.04 (C-7), 129.96 (C-14), 128.78 (C-8), 128.18 (C-10), 126.28 (C-6), 114.18 (C-16, C-18), 70.47 (C-3), 59.79 (C-13), 57.93 (C-2), 55.53 (C-20), 53.05 (C-12) ppm; UV λ (ϵ) (MeOH) 208 (33 573), 241 (24 432), 272 (4315) nm; IR (KBr) ν 1670, 1654 (amide) cm^{-1}; EIMS *m*/*z* 121 (100, C_8H_9O, $C_7H_5^+$ − OMe, tropilium − OMe), 136 ($C_7H_6NS^+$), 150 , 180, 208, 316 (M^+ − 29, M^+ − CHO), 345 (0.5, M^+).

(+)-(2*S*,3*S*)-3-Acetyl-5-(2-acetylethyl)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (3). 2 (0.7 g) was dissolved in pyridine (10 mL), and acetic anhydride (5 mL) was added at room temperature. The reaction mixture was kept at room temperature for 1 h, and then the solvent was removed under vacuum. Ethanol (20 mL) was added to the residue and removed under vacuum, the addition and removal of ethanol was repeated four more times. The product was dried under vacuum overnight. The residual oily product was purified on a silica gel column, solvent EtOAc−hexane (1:1): yield 0.776 g (89.2%) as an oil; R_f = 0.41 (EtOAc−hexane, 2:1); $[\alpha]^{22}_D = +148°$ (*c* 0.50, MeOH); crystallized from acetone−hexane; mp 107−108 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 1H, *J* 7.5 Hz, Ph), 7.51 (2d, 1H, *J* 7.2 Hz, Ph), 7.45 (d, 1H, Ph), 7.41 (d, 2H, *J* 8.9 Hz, Ph), 7.28 (dd, 1H, *J* 7.1 Hz, Ph), 6.89 (d, 2H, *J* 8.9 Hz, Ph), 5.19 (d, 1H, *J* 7.5 Hz, H-2), 5.02 (d, 1H, *J* 7.5 Hz, H-3), 4.37−4.30 (m, 1H), 4.70−4.61 (m, 1H, CH_2), 3.82 (s, 3H, OCH_3), 3.89−3.79 (m, 1H), 1.91 (s, 3H, OAc), 1.88 (s, 3H, OAc) ppm; UV λ (ϵ) (MeOH) 210 (31 202), 240 (23 863), 273 (3519) nm; IR (KBr) ν 1748, 1734, 1678 (amide, ester), 1250, 1234, 1222 (ester) cm^{-1}; EIMS *m*/*z* 121, 136, 150 (100), 151, 152, 178, 240, 298, 341, 369, 429 (0.2, M^+).

(+)-(2*S*,3*S*)-3-Hydroxy-2,3-dihydro-5-(2-hydroxyethyl)-2-(4-methoxyphenyl)-1,5-benzothiazepin (6). (+)-(2*S*, 3*S*)-3-Hydroxy-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one-5-acetic acid (**5**) (2.0 g) was suspended in tetrahydrofuran (10 mL) under nitrogen. The mixture was cooled in an ice−water bath and borane−tetrahydrofuran complex (1 M, 27 mL) added over 30 min. The clear solution was stirred under nitrogen at 0−5 °C for 1 h and then for 2 h at room temperature. The reaction mixture was cooled to 0−5 °C, and ice was added slowly to quench the excess borane. Tetrahydrofuran was removed under reduced pressure and the semisolid dissolved in a mixture of ethyl acetate (150 mL) and water (50 mL). Water was removed, the organic phase was washed once more with water (50 mL), saturated aqueous sodium bicarbonate (50 mL), and water (50 mL) and dried (Na_2SO_4), and then the solvent was removed under reduced pressure: yield 1.40 g (76.1%); crystallized from benzene; mp 132−133 °C; R_f = 0.27 (EtOAc−hexane, 2:1); $[\alpha]^{22}_D = +341°$ (*c* 0.47, MeOH); ^{1}H NMR, ($CDCl_3$) δ 7.61 (d, 1H, *J* 7.7 Hz, Ph), 7.42 (d, 2H, *J* 8.7 Hz, Ph), 7.28 (dd, 1H, *J* 7.2 Hz, Ph), 7.14 (d, 1H, *J* 7.2 Hz, Ph), 6.95 (dd, 1H, *J* 7.4 Hz, Ph), 6.85 (d, 2H, *J* 8.7 Hz, Ph), 4.06 (d, 1H, *J* 4.3 Hz, H-2), 3.78 (s, 3H, OCH_3), 3.75 (m, 2H, CH_2), 3.6 (m, 1H, H-3), 3.3 (m, 2H, CH_2), 2.90 (d, 2H, *J* 14.5 Hz, CH_2-4) ppm; ^{13}C NMR ($CDCl_3$) δ 158.89, 155.70, 134.07, 131.70, 130.46, 129.27, 128.27, 122.93, 120.56, 113.85, 70.60, 60.98, 60.05, 57.44, 55.23, 54.93 ppm; UV λ (ϵ) (MeOH) 203 (30 080), 234 (19 973), 269 (8342) nm; IR (KBr) ν 1480, 1462, 1442, 1252 (tertiary amine) cm^{-1}; EIMS *m*/*z* 121, 150 (100), 181, 300, 331 (3, M^+).

(+)-(2*S*,3*S*)-3-Acetyl-5-(2-hydroxyethyl)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (8). 7 (3.51 g) was dissolved in tetrahydrofuran (10 mL) then cooled in an ice-water bath to 0−5 °C under nitrogen. Borane−tetrahydrofuran complex (13 mL, 1 M solution in THF) was added in small portions over 15 min. The reaction mixture was stirred for 2 h at 0−5 °C, additional borane−tetrahydrofuran complex (3 mL, 1 M solution in THF) was added, and stirring was continued for 1 h at 0−5 °C. The solvent was removed on a rotary evaporator and the residue dissolved in a mixture of water and ethyl acetate (300 mL, 2:1). The water layer was removed, the organic phase was washed with saturated $NaHCO_3$ solution (100 mL) and then dried (Na_2SO_4), and solvent was removed. The product was purified on a silica gel (0.040−0.063 mm) column (2.5 × 40 cm), eluent EtOAc−hexane (2:1): yield 1.79 g (53%); crystallized from ethanol−hexane; mp 142−143 °C; R_f = 0.22 (EtOAc−hexane, 2:1); $[\alpha]^{22}_D = +115°$ (*c* 0.81, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.7−6.8 (8H, 2 × Ph), 5.17 (d, 1H, *J* 7.52 Hz, H-2), 5.04 (d, 1H, *J* 7.52 Hz, H-3), 4.43 (m, 2H, CH_2), 3.9 (m, 2H, CH_2), 3.82 (s, 3H, OMe), 1.92 (s, 3H, OAc) ppm; UV λ (ϵ) (MeOH) 210 (31 383), 240 (22 937), 269 (4766) nm; IR (KBr) ν 1722, 1672 (amide, ester) 1256 (ester) cm^{-1}; EIMS *m*/*z* 121 (100), 136, 150, 208, 240, 298, 316, 387 (0.11, M^+).

Natriuretic Assay. This assay is a modification of the assay by Lipschitz et al.[8] Rats are caged individually in metabolic cages for 1−2 days before the beginning of an experiment. Rats are also acclimated to oral dosing for at least one day by administering 3 mL/kg of tap water by oral gavage. Two hours before the experiment, food and water are removed from the cages. A total of 18 rats, in three groups of six, one group as a control and two experimental groups, are used in the experiment. For a series of experiments the control group and the experimental groups remain as such throughout the course of the study. The rats are volume expanded with 25 mL/kg of physiological saline administered orally. The test substances are administered at various doses by an additional gavage of 1 mL/kg of 1% carboxymethyl cellulose (CMC) suspension of the compound. One percent CMC then serves as the vehicle control. Urine is collected for 5 h. The volume is determined, and the sodium and potassium concentrations are measured using a Beckman E2A electrolyte analyzer. Before ANOVA analysis, outliers, defined as data points greater than 2 standard deviations from the mean, are removed. The experimental groups are compared to the

vehicle control group using ANOVA with the Scheffe *F* test and those with a $p \leq 0.05$ are considered significant.

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References

(1) Benaksas, E. J.; Murray, E. D., Jr.; Rodgers, C. L.; Pham, T.; Bigornia, A. E.; DeWind, S. A.; Giebel, R.; Brubacher, E. S., Wechter, W. J. Endogenous Natriuretic Factors 1: Sodium Pump Inhibition Does Not Correlate With Natriuretic Or Pressor Activities From Uremic Urine. *Life Sci.* **1993**, *52*, 1045−1054.

(2) Levine, B. H.; Murray, E. D., Jr.; Bigornia, A. E.; DeWind, S. A.; Wechter, W. J. Endogenous Natriuretic Factors 2: Characterization of Natriuretic and Vasopressive Substances from Human Uremic Urine. *J. Cardiovasc. Pharmacol.* **1993**, *22* (Suppl. 2), S63−S68.

(3) Murray, E. D., Jr.; Kantoci, D.; DeWind, S. A.; Bigornia, A. E.; D'Amico, D. C.; King, J. G., Jr.; Pham, T.; Levine, B. H.; Jung, M. E.; Wechter, W. J. Endogenous Natriuretic Factors 3: Isolation and Characterization of Human Natriuretic Factors LLU-α, LLU-β_1, and LLU-γ. *Life Sci.* **1995**, *57*, 2154−2161.

(4) deWardener, H. E.; Mills, I. H.; Clapham, W. F.; Hayter, C. J. Studies on the Efferent Mechanism of the Sodium Diuresis which Follows the Administration of Intravenous Saline in the Dog. *Clin. Sci.* **1961**, *21*, 249−258.

(5) Nakamura, S.; Ito, Y.; Fukushima, T.; Suigawara, Y.; Ohashgi, M. Metabolism of Diltiazem III. Oxidative Deamination of Diltiazem in Rat Liver Microsomes. *J. Pharmacobio-Dyn.* **1990**, *13*, 612−621.

(6) Sugawara, Y.; Ohashi, M.; Nakamura, S.; Usuki, S.; Suzuki, T.; Ito, Y.; Kume, T.; Harigaya, S.; Nakao, A.; Gaino, M.; Inoue, H. Metabolism of Diltiazem I. Structures of New Acidic and Basic Metabolites in Rat, Dog and Man. *J. Pharmacobio-Dyn.* **1988**, *11*, 211−223.

(7) Maurer, H. M. Identification of Antiarryhthmic drugs and their Metabolites in Urine. *Arch. Toxicol.* **1990**, *64*, 218−230.

(8) Lipschitz, W. L.; Hadidian, Z.; Kerpcsar, A. Bioassay of Diuretics. *J. Pharm. Exp. Ther.* **1943**, *79*, 97−110.

(9) Preliminary data kindly provided by Dr. James H. Ludens (The Upjohn Co.).

(10) Seino, M.; Abe, K.; Nishiro, N.; Omata, K.; Sato, K.; Tsunoda, K.; Yoshinaga, K. Role of the Renal Kinin-Prostaglandin System in Diltiazem-Induced Natriuresis. *Am. J. Physiol.* **1986**, *250*, F197−F202.

(11) Zimmerman, B. G.; Raich, P. C. Renal Hemodynamic Effects of a Selected Calcium Antagonist. *Am. J. Cardiol.* **1988**, *62*, 69G−73G.

(12) Krishna, G. G.; Riley, L. J., Jr.; Deuter, G.; Kapoor, S. C.; Narins, R. G. Natriuretic Effect of Calcium-Channel Blockers in Hypertensives. *Am. J. Kidney Dis.* **1991**, *18*, 566−572.

(13) Lu, S.; Roman, R. J.; Mattson, D. L.; Cowley, A. W., Jr. Renal Medullary Interstitial Infusion of Diltiazem Alters Sodium and Water Excretion in Rats. *Am. J. Physiol.* **1992**, *263*, R1064−R1070.

(14) Nagao, T.; Yamaguchi, I.; Narita, H.; Nakjima, H. Calcium Entry Blockers: Antihypertensive and Natriuretic Effects in Experimental Animals. *Am. J. Cardiol.* **1985**, *56*, 56H−61H.

JM950559N