Chemistry of Heterocyclic Compounds, Vol. 34, No. 11, 1998

SYNTHESIS AND HUMAN LEUKOCYTE ELASTASE INHIBITORY ACTIVITY OF NOVEL 2-SPIRO(2′,2′-DIPHENYLCYCLOPROPANE) CEPHALOSPORIN SULFONES

Andhe V. Narender Reddy, Charles Y. Fiakpui, David P. Czajkowski, Paul Spevak, Jadwiga Kaleta, Ronald G. Micetich, and Samarendra N. Maiti

A new series of 2-spiro(2′,2′-diphenylcyclopropane) cephalosporin sulfones was synthesized as potent human leukocyte elastase inhibitors.

Proteolytic tissue damage by human neutrophil elastase (HNE) released from human polymorphonuclear leukocytes (PMN) by inflammatory stimuli, plays a major role in the destructive process associated with chronic inflammatory diseases such as rheumatoid arthritis [1], emphysema [2], adult respiratory distress syndrome [3], and cystic fibrosis [4]. It has been postulated that development of these degenerative diseases will result from genetic or chemically induced imbalance of the proteinase–antiproteinase system. Indeed, in the development of these pathologies, the natural plasma inhibitors of HNE, α_1-AT (also called α_1-PI), and (α_2-macroglobulin) (α_2-M) are thought to have diminished capacity to protect host connective tissues from degradation by the enzyme. Use of synthetic low molecular weight and selective HNE inhibitors that can be delivered to the site of unregulated PMN elastase activity could be an attractive approach in the treatment of the above-mentioned diseases. These possibilities have led to an intensive search for human neutrophil elastase inhibitors. Various modified cephalosporins have been found to be potential anti-elastase agents [5-14] in pathological conditions in which HNE is implicated. We have investigated the classes of 7a-methoxy-2-(1,3-dithiolan-2-ylidene)cephem sulfones [15] and found that some representatives are very potent inhibitors of HNE.

In the present study we will describe the synthesis and reactivity of a newly developed series of 2-spiro(2′,2′-diphenylcyclopropane) cephem sulfones as potent and selective HNE inhibitors.

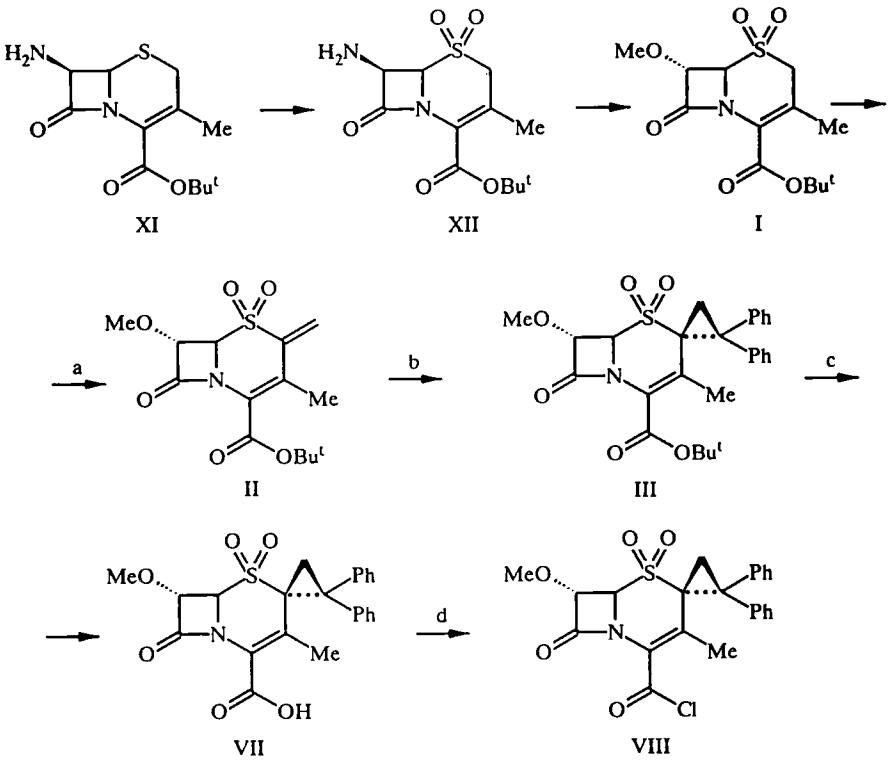
CHEMISTRY

In a general procedure, *tert*-butyl 7α-methoxy-3-methyl-3-cephem-4-carboxylate-1,1-dioxide (I), which was prepared from 7-aminodeacetoxycephalosporanic acid (7-ADCA) in three steps based on the procedure described by Blacklock et al. [16], was converted to the 2-exomethylene derivative II under Mannich conditions. Compound II was reacted at room temperature with freshly prepared diphenyldiazomethane to give *tert*-butyl 7α-methoxy-2-spiro(2′,2′-diphenyl)cyclopropane)-3-methyl-3-cephem-4-carboxylate 1,1-dioxide (III) as the major product in 85% yield along with the minor products IV-VI. The isolation of compound III as major product suggests that the addition of diphenyldiazomethane to the double bond was facially selective.

Removal of the *tert*-butyl protecting group from compound III with anhydrous formic acid at 35-40°C over 1 to 3 h or with TFA-anisole in methylene chloride at 0°C gave the corresponding acid (VII) in excellent yield (89-98%). The acid VII was then converted into the acid chloride VIII by reaction with oxalyl chloride in methylene chloride in the presence of a catalytic amount of DMF. The acid chloride VIII was smoothly transformed into various amides (IX) by reaction with appropriate amines in the presence of triethylamine. Similarly, the acid chloride VIII was

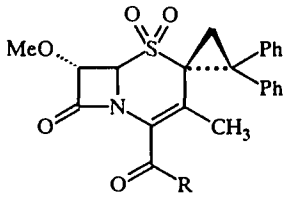
SynPhar Laboratories Inc., #2 Taiho Alberta Centre, 4290-91A Street, Edmonton, Canada T6E 5V2. Published in Khimiya Geterotsiklicheskikh Soedinenii, No. 11, pp. 1517-1523, November, 1998. Original article submitted July 15, 1998.

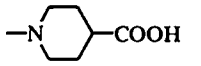
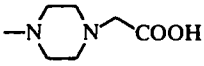
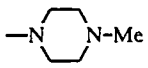
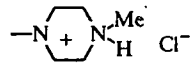
0009-3122/98/3411-1289$20.00



(a) aq. CH_2O—$Me_2NH \cdot HCl$/1,4-dioxane/*tert*-BuOH; (b) Ph_2CN_2/DCM; (c) HCOOH, 35...40 °C, 3 hrs; (d) $(COCl)_2$/DCM; (e) amines, DCM; (f) CuI/THF/R^2MgX

TABLE 1. Activity of 7α-Methoxy-2-spiro(2',2'-diphenyl)cyclopropane Cephrem Sulfones against Human Leukocyte Elastase

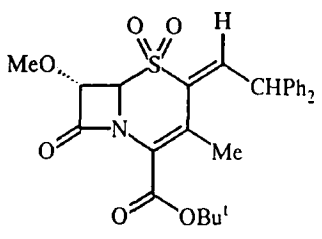


Inhibitor	R	IC_{50}, nM	Inhibitor	R	IC_{50}, nM
IXa		7.8	IXf		920
IXb		10.85	IXg		1600
IXc		27.4	Xa	—Et	10.3
IXd		125	Xb	—*n*-Bu	7.4
IXe		350	Xc	Bu^t	12.0

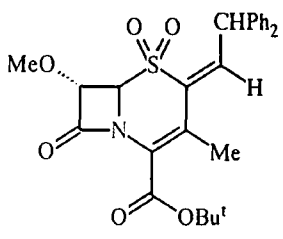
activated with a catalytic amount of CuI and converted into the corresponding 4-acyl cephems X upon reaction with suitable Grignard reagents. All the newly prepared compounds were tested for their elastase inhibitory activity against human leukocyte elastase and their values are presented in Table 1.

ENZYME ASSAY

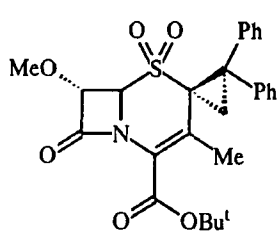
Human leukocyte elastase was obtained from Elastin Products, Missouri, USA. Substrate: MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide. Reaction mixture: 10 mM phosphate buffer (pH 7.6), 500 mM NaCl, 10% DMSO, 0.35 mM substrate. The enzyme activity was determined by monitoring the increase in absorbance at 410 nm caused by the hydrolysis of chromogenic substrates. Inhibition of enzyme by the compounds described was determined after a 10 min preincubation with the enzyme in reaction mixture minus substrate. Reaction was initiated by the addition of substrate. The concentration of human leukocyte elastase used for assay was 10 nM.



IV



V



VI

CONCLUSION

As can be seen from Table 1, the compounds IXa-c and Xa-c are potent elastase inhibitors.

When a carboxyl group is introduced into the piperidine ring or piperazine ring in order to obtain a water soluble elastase inhibitor, the inhibitory activity is diminished (compare IXc and IXf). When compound IXd (IC_{50} = 125 nM) was converted into its hydrochloride, the resulting salt compound IXe had an IC_{50} value of 350 nM. Similarly, when a carboxyl group was introduced, the resulting compound IXg was less active than compound IXd, suggesting the presence of a hydrophobic group at position 4 is probably important for strong elastase inhibitory activity.

EXPERIMENTAL

All column chromatographic purifications were accomplished over silica gel 60 (E.Merck, 230~400 mesh) with the appropriate solvent gradients. ^{1}H-NMR spectra were determined with a Bruker AC-200-F (200 MHz) spectrometer in appropriate deuterated solvents and are expressed in ppm downfield from TMS (internal standard).

7α-Methoxy-2-spiro(2′,2′-diphenyl)cyclopropyl-3-methyl-3-cephem-4-pyrrolidinecarboxamide-1,1-dioxide (IXb). Step A: *t*-Butyl 7β-amino-3-methyl-3-cephem-4-carboxylate (XI) was obtained from 7-ADCA according to the procedure [16] in 81% yield. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 4.95 (1H, d, J = 4.0 Hz); 4.70 (1H, d, J = 4.0 Hz); 3.59 (1H, d, J = 18.0 Hz); 3.12 (1H, d, J = 18.0 Hz); 2.25 (2H, br, s); 2.07 (3H, s); 1.55 (9H, s).

Step B: *t*-Butyl 7β-amino-3-methyl-3-cephem-4-carboxylate 1,1-dioxide (XII) was obtained from the ester XI (step A) in 90% yield as described in [16]. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 4.86 (1H, br); 4.70 (1H, br. d); 3.85 (1H, d, J = 18.0 Hz); 3.50 (1H, d, J = 18.0 Hz); 2.24 (2H, br); 2.05 (3H, s); 1.53 (9H, s).

Step C: *t*-Butyl 7α-methoxy-3-methyl-3-cephem-4-carboxylate 1,1-dioxide (I) was prepared from sulfone XII (step B) in 29% yield. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 5.15 (1H, br, s); 4.63 (1H, br, s); 3.75 (2H, ABq, J = 18.0 Hz); 3.57 (3H, s); 2.04 (3H, s); 1.54 (9H, s).

Step D: *t*-Butyl 7α-methoxy-3-methyl-2-methylene-3-cephem-4-carboxylate 1,1-Dioxide (II). A mixture of *t*-butyl 7α-methoxy-3-methyl-3-cephem-4-carboxylate 1,1-dioxide (I, from Step C, 40 g), dimethylamine hydrochloride (11.5 g), aq. formaldehyde (37%, 16 ml), DMF (235 ml), and dioxane (66 ml) was heated to reflux at 90°C for 1 h. TLC (hexane—ethyl acetate, 4:1) indicated that the reaction was complete. Solvent was removed under reduced pressure and methylene chloride (750 ml) was added. The organic layer was washed with water (4×200 ml), brine, and dried over anhydrous Na_2SO_4. After concentration, the residue was treated with ethyl acetate (25 ml). The solid was filtered off and dried under vacuum to give 26 g of pure product II. The mother liquor was concentrated and purified over a silica gel column using hexane—ethyl acetate (3:1) as eluent to give 2.7 g of additional amount of product II. Total mass, 28.7 g (68.5% yield). 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.55 (1H, d, J = 2.0 Hz); 6.05 (1H, d, J = 2.0 Hz); 5.25 (1H, d, J = 1.5 Hz); 4.80 (1H, d, J = 1.5 Hz); 3.58 (3H, s); 2.1 (3H, s); 1.55 (9H, s). Found, %: C 50.98; H 5.64; N 4.26. $C_{14}H_{19}NO_6S$. Calculated, %: C 51.05; H 5.81; N 4.25.

Step E: *t*-Butyl 2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-3-cephem-4-carboxylate 1,1-Dioxide (III). To a solution of *t*-butyl 7α-methoxy-3-methyl-2-methylene-3-cephem-4-carboxylate 1,1-dioxide II from step D (133 mg) in 8 ml of methylene chloride was added diphenyldiazomethane (86 mg). The mixture was stirred at room temperature for 3 h. Solvent was removed under reduced pressure and the residue was digested with ether. The precipitated solid was collected by filtration and air-dried, 170 mg (85% yield). 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.20-7.48 (10H, m); 5.05 (1H, d, J = 2.0 Hz); 4.95 (1H, d, J = 2.0 Hz); 3.48 (3H, s); 2.95 (1H, d, J = 7.0 Hz); 2.33 (1H, d, J = 7.0 Hz); 1.55 (9H, s); 1.05 (3H, s). Found, %: C 65.22; H 5.78; N 2.80. $C_{27}H_{29}NO_6S$. Calculated, %: C 65.43; H 5.89; N 2.83.

Step F: 4-Carboxy-2-spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-3-cephem-1,1-dioxide (VII). A solution of ester III from Step E (10 g) in dry distilled formic acid (180 ml) was heated at 50°C for 2 h. TLC of the mixture (hexane—ethyl acetate, 1:2) indicated completion of the reaction. Formic acid was removed under reduced pressure and the residue was treated with methylene chloride and evaporated again to give a foam which was digested with hexane. Hexane was decanted off and the residue was dried under vacuum to give the pure acid VII in 85% yield as a foam. 200 MHz ^{1}H NMR ($CDCl_3$) δ 7.24-7.49 (10H, m); 5.05 (1H, br. s); 5.03 (1H, br. s); 3.47 (3H, s); 2.94 (1H, d, J = 6.8 Hz); 2.38 (1H, d, J = 6.8 Hz); 1.23 (3H, s). Found, %: C 61.48; H 6.60; N 2.98. $C_{23}H_{21}NO_6S$. Calculated, %: C 1.72; H 6.53; N 3.13.

Step G: 2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-pyrrolidinocarbonyl-3-cephem-1,1-dioxide (IXb). Carboxylic acid VII from Step F (1.84 g, 4.187 mmol) was dissolved in 5 ml of methylene chloride and oxalyl chloride (797 mg, 6.280 mmol) was added followed by a drop of DMF. The mixture was stirred at room temperature for 1 h. Solvent was removed under reduced pressure and the light brown foam was dried under vacuum to give acid chloride VIII. The brown foam was redissolved in dry methylene chloride (15 ml), cooled in an ice bath, and a solution of pyrrolidine (596 mg, 8.374 mmol) in dry methylene chloride (8 ml) was added dropwise. The mixture was stirred at ice temperature for 1 h, washed with cold water, dil. HCl, water, brine, dried, and concentrated to give 1.57 g of the crude product, which was purified over a silica gel column using hexane—ethyl acetate (1:1) as eluent to give pure compound IXb in 50.5% yield (1.04 g). Crystallization from ether gave a pale yellow solid, m. p. 222°C (decomposed). 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.17-7.50 (10H, m); 5.05 (1H, d, J = 1.5 Hz); 4.94 (1H, d, J = 1.5 Hz); 3.46 (3H, s); 3.09-3.68 (4H, m); 2.91 (1H, d, J = 6.7 Hz); 2.23 (1H, d, J = 6.7 Hz); 1.71-2.04 (4H, m); 0.92 (3H, s). Found, %: C 65.66; H 5.48; N 5.62. $C_{27}H_{22}N_2O_5S$. Calculated, %: C 65.83; H 5.72; N 5.68.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-piperidinocarbonyl-3-cephem-1,1-dioxide (IXc). Carboxylic acid VII (1.0 g, 2.275 mmol, from Step F) was dissolved in 15 ml of methylene chloride and oxalyl chloride (0.3 ml, 3.413 mmol) was added followed by a drop of DMF. The mixture was stirred at room temperature for 1 h. Solvent was removed under reduced pressure. The light brown foam was redissolved in dry methylene chloride (5 ml), cooled in an ice bath, piperidine (388 mg, 4.55 mmol) dissolved in 5 ml of dry methylene chloride was added dropwise, and the reaction mixture was stirred at ice temperature for 2 h, washed with water, brine, dried, and concentrated to give 980 mg of crude product which was purified on a silica gel column using hexane—ethyl acetate (2:1) as eluent to give 491 mg of pure compound IXc.

200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.20-7.50 (10H, m); 5.04 (1H, d, J = 1.5 Hz); 4.95 (1H, d, J = 1.5 Hz); 3.46 (3H, s); 3.34-3.64 (4H, m); 2.91 (1H, d, J = 6.8 Hz); 2.23 (1H, d, J = 6.8 Hz); 1.46-1.68 (6H, m); 0.90 (3H, s). Found, %: C 66.11; H 5.83; N 5.45. $C_{28}H_{30}N_2O_5S$. Calculated, %: C 66.38; H 5.97; N 5.53.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-[(pyrazol-1-yl)carbonyl]-3-cephem-1,1-dioxide (IXa). This compound was prepared by the same procedure as described for compounds and the title compound was obtained as a pale yellow amorphus powder. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.45 (1H, s), 8.15 (1H, s); 7.1-7.6 (10H, m); 6.75 (1H, m); 5.4 (1H, d, J = 2.0 Hz); 5.25 (1H, d, J = 2.0 Hz); 3.4 (3H, s); 2.8 (1H, d, J = 7.0 Hz); 2.55 (1H, d J = 7.0 Hz); 0.78 (3H, s). Found, %: C 63.69; H 4.62; N 8.44. $C_{26}H_{23}N_3O_5S$. Calculated, %: C 63.78; H 4.73; N 8.58.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-(N-methylpiperazinocarbonyl)-3-cephem-1,1-dioxide (IXd). This compound was prepared by the same procedure as described for compounds IXb and IXc. The title compound IXd was obtained as a pale yellow solid after crystallization from ether, m. p. 202-204°C. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.17-7.50 (10H, m); 5.05 (1H, d, J = 2.1 Hz); 4.95 (1H, d, J = 2.1 Hz); 3.77-3.85 (1H, m); 3.49-3.62 (3H, m); 3.46 (3H, s); 2.91 (1H, d, J = 7.1 Hz); 2.36-2.54 (4H, m); 2.28 (3H, s); 2.24 (1H, d, J = 7.1 Hz), 0.89 (3H, s). Found, %: C 64.35; H 5.68; N 8.12. $C_{28}H_{31}N_3O_5S$. Calculated, %: C 64.47; H 5.99; N 8.05.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-[(N-methylpiperazio)carbonylimide]-3-cephem-1,1-dioxide Hydrochloride Salt (IXe). Dry hydrogen chloride gas was bubbled into a solution of 7α-methoxy-2-spiro(2′,2′-diphenyl)cyclopropyl-3-methyl-3-cephem-4-[(N-methylpiperazino)carbonyl] 1,1-dioxide (0.0531 g, 0.102 mmol) in methylene chloride (1.0 ml) at 0°C for 2 min. Dry ether (15 ml) was added to the mixture and the precipated solid was collected by filtration, washed thoroughly with dry ether, and dried to give 0.042 g of the title compound, m.p. 218°C, decomp. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 13.4 (1H, br); 7.20-.7.50 (10H, m); 4.99 (2H, br. s); 4.70 (1H, br, d); 3.15-4.10 (5H, m); 3.47 (3H, s); 2.95 (2H, d J = 7.0 Hz); 2.90 (2H, m); 2.78 (3H, s); 2.29 (2H, d, J = 7.0 Hz); 0.88 (3H, s). Found, %: C 60.19; H 5.49; N 7.39. $C_{28}H_{32}ClN_3O_5S$. Calculated, %: C 60.26; H 5.78; N 7.53.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-(4-carboxypiperidinocarbonyl)-3-cephem-1,1-dioxide (IXf). Carboxylic acid VII (2.87 g, 6.53 mmol) was taken in dry methylene chloride (40 ml) and oxalyl chloride (1.10 g) was added followed by two drops of DMF. The mixture was stirred at ice temperature for 15 min and at room temperature for 1 h. Solvent was removed under reduced pressure. The residue was redissolved in methylene chloride (50 ml), cooled to 0°C, a solution of *t*-butyl isonipecotinate (1.21 g, 6.53 mmol) in dry methylene chloride (50 ml) was added followed by TEA (0.667 g, 6.53 mmol). The reaction mixture was stirred at 0°C for 30 min and at room temperature for 2.5 h. After the reaction was complete, the mixture was diluted with methylene chloride (100 ml) and washed successively with water, 1 N HCl, water, aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a foam which was purified over a silica gel column

using hexane—ethyl acetate (1:1) as eluent to give amide IXf as a white foam (3.29 g, 83.09%). The compound thus obtained was dissolved in anhydrous formic acid (70 ml) and was stirred at room temperature for 2 h. After the completion of the reaction, the mixture was freeze-dried to give a white solid which was washed thoroughly with a mixture of hexane—ether (4:1), the solid was collected by filtration, and air dried (3.78 g, 94.74%). 200 MHz ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 11.90 (1H, br. s); 7.21-7.48 (10H, m); 5.04 (1H, br. s); 5.00 (1H, br. s); 3.63-3.82 (1H, m); 3.46 (3H, s); 3.03-3.14 (2H, m); 2.90 (1H, d, J = 6.7 Hz); 2.49-2.51 (1H, m); 2.26 (1H, d, J = 6.7 Hz); 1.58-2.05 (4H, m); 0.87 and 0.90 (3H, 2 singlets). Found, %: C 62.89; H 5.43; N 4.98; $C_{29}H_{30}N_2O_7S$. Calculated, %: C 63.26; H 5.49; N 5.09.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-(4-N-carboxymethyl)piperazinocarbonyl-3-cephem-1, 1-dioxide (IXg). This compound was prepared by the same procedure as described for compound IXf. The starting material was 2-spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-(4-*tert*-butoxycarbonylmethyl)piperazinocarbonyl-3-cephem 1,1-dioxide. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.22-7.49 (10H, m); 5.03 (1H, d, J = 1.9 Hz); 4.96 (1H, d, J = 1.9 Hz); 3.43-4.03 (5H, m); 3.46 (3H, s); 3.26 (2H, s); 2.72-2.80 (4H, m); 2.92 (1H, d, J = 7.0 Hz); 2.25 (1H, d, J = 7.0 Hz); 0.89 (3H, s). Found, %: C 61.33; H 5.49; N 7.40. $C_{29}H_{31}N_3O_7S$. C 61.58; H 5.53; N 7.43.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-propionyl-3-cephem-1,1-dioxide (Xa). To a suspension of carboxylic acid VII (360 mg, 0.8191 mmol) in dry methylene chloride (15 ml) cooled in an ice bath was added oxalyl chloride (135 mg, 1.065 mmol) followed by one drop of DMF. The mixture slowly went into solution. After 5 min, another drop of DMF was added, ice bath was removed, and the mixture was stirred at room temperature for 40 min. Solvent was removed under reduced pressure. The residue was dissolved in dry THF (10 ml), cooled to –70°C, cuprous iodide (164 mg, 0.8601 mmol) was added followed by ethyl magnesium bromide (0.532 ml, 2 M solution in THF), and the reaction mixture was stirred at –70°C for 15 min, poured into ice-cold water, extracted with methylene chloride, washed with aq. sodium bicarbonate solution, brine, dried, and concentrated to give a light yellow solid which was purified over a silica gel column using hexane—ethyl acetate (7:3) as eluent, 190 mg. Crystallization from methylene chloride—ether gave pure white solid, m.p. 206-208°C, decomp. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.18-7.49 (10H, m); 5.05 (1H, d, J = 1.7 Hz); 4.97 (1H, d, J = 1.7 Hz); 3.48 (3H, s); 2.97 (1H, dq); 2.94 (1H, d, J = 7.1 Hz); 2.67 (1H, dq); 2.34 (1H, d, J = 7.1 Hz); 1.19 (3H, t); 0.975 (3H, s). Found, %: C 66.32; H 5.47; N 2.98. $C_{25}H_{25}NO_5S$. Calculated, %: C 66.49; H 5.58; N 3.10.

2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-4-pentanoyl-3-cephem-1,1-dioxide (Xb). To a suspension of acid VI (700 mg, 1.5928 mmol) in dry methylene chloride (25 ml) cooled in an ice bath was added oxalyl chloride (263 mg, 2.0706 mmol) followed by two drops of DMF. The reaction mixture was stirred at ice temperature for 15 min and at room temp. for 45 min. Solvent was removed and the residue was dissolved in dry THF (15 ml), cooled to –70°C, cuprous iodide (319 mg, 1.6724 mmol) was added followed by *n*-butyl magnesium chloride (1.035 ml, 2 M in THF) and the reaction mixture was stirred at –70°C for 15 min, poured into ice-cold water, and extracted with methylene chloride. The aqueous layer was saturated with NaCl and re-extracted with methylene chloride. The combined organic layers were washed with aq. $NaHCO_3$ solution, brine, dried, and concentrated to give a light yellow foam (600 mg), which was purified over a silica gel column using hexane—ethyl acetate as eluent. Crystallization from methylene chloride—ether gave pure compound Xb as white crystals, m.p. 170-175°C. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.22-7.48 (10H, m); 5.04 (1H, d, J = 2.0 Hz); 4.97 (1H, d, J = 2.0 Hz); 3.49 (3H, s); 2.93 (1H, d, J = 6.8 Hz); 2.73-2.95 (2H, m); 2.34 (1H, d, J = 6.8 Hz); 1.33-1.72 (4H, m); 0.98 (3H, t); 0.98 (3H, s). Found, %: C 67.39; H 5.98; N 2.88. $C_{27}H_{29}NO_5S$. Calculated, % C 67.62; H 6.09; N 2 92.

4-*t*-Butylcarbonyl 2-Spiro(2′,2′-diphenylcyclopropane)-7α-methoxy-3-methyl-3-cephem-1,1-dioxide (Xc). This compound was prepared in the same manner as described for compounds Xa and Xb. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.16-7.52 (10H, m); 5.06 (1H, d, J = 1.6 Hz); 4.96 (1H, d, J = 1.6 Hz); 3.48 (3H, s); 2.88 (1H, d, J = 6.8 Hz); 2.32 (1H, d, J = 6.8 Hz); 1.30 (9H, s); 0.87 (3H, s). Found, %: C 67.58; H 5.87; N 2.68. $C_{27}H_{29}NO_5S$. Calculated, %: C 67.62; H 6.09; N 2.92.

REFERENCES

1. L. Ekerot and K. Ohlsson, Adv. Exp. Med. Biol., **167**, 335 (1984).
2. A. Janoff, Am. Rev. Respir. Dis., **132**, 417 (1985).
3. T. A. Merritt, C. G. Cochrane, K. Holcomb, B. Bohl, M. Hallman, D. Strayer, D. Edwards, and L. Gluck, J. Clin. Invest., **72**, 656 (1983).

4. S. Suter, L. Schaad, L. Roux, V. E. Nydegger, and F. A. Waldvogel, J. Infect. Dis., **149**, 523 (1984).
5. J. B. Doherty, B. M. Ashe, P. L. Barker, T. J. Blacklock, J. N. Butcher, G. O. Chandler, M. E. Dahlgreen, P. Davies, C. P. Dorn, Jr., P. E. Fluke, R. A. Firestone, W. K. Hagmann, T. Halgren, W. B. Knight, A. L. Maycock, M. A. Navia, L. O'Grady, J. M. Pisano, S. K. Shah, K. R. Thompson, H. Weston, and M. Zimmerman, J. Med. Chem., **33**, 2513 (1990).
6. P. E. Finke, B. M. Ashe, W. B. Knight, A. L. Maycock, M. A. Navia, S. K. Shah, K. R. Thompson, D. J. Underwood, H. Weston, M. Zimmerman, and J. B. Doherty, J. Med. Chem., **33**, 2522 (1990).
7. S. K. Shah, K. A. Brause, G. O. Chandler, P. E. Finke, B. M. Ashe, H. Weston, W. B. Knight, A. L. Maycock, and J. B. Doherty, J. Med. Chem., **33**, 2529 (1990).
8. P. E. Finke, S. K. Shah, B. M. Ashe, R. G. Ball, T. J. Blacklock, R. J. Bonney, K. A. Brause, G. O. Chandler, M. Cotton, P. Davies, P. S. Dellea, C. P. Dorn, Jr., D. S. Fletcher, L. A. O'Grady, W. K. Hagmann, K. M. Hand, W. B. Knight, A. L. Maycock, R. A. Mumford, D. G. Osinga, P. Sohar, K. R. Thompson, H. Weston, J. B. Doherty, J. Med. Chem., **35**, 3731 (1992).
9. M. Alpegiani, P. Bissolino, E. Perrone, G. Cassinelli, and G. Franceschi, Tetrahedron Lett., **32**, 6207 (1991).
10. M. Alpegiani, P. Bissolino, D. Borghi, R. Corigli, S. D. Nero, E. Perrone, G. Razzano, and V. Rizzo, Bioorg. Med. Chem. Lett., **2**, 1127 (1992).
11. M. Alpegiani, A. Baici, P. Bissolino, P. Carminati, G. Cassinelli, S. D. Nero, G. Franceschi, P. Orezzi, E. Perrone, V. Rizzo, and N. Sacchi, Eur. J. Med. Chem., **27**, 875 (1992).
12. US Pat. 5,264,429, S. N. Maiti, D. Czajkowski, P. Spevak, K. Adachi, and R. G. Micetich; Chem. Abstr., **115**, 255894 (1991).
13. US Pat. 5,264,430, S. N. Maiti, D. Czajkowski, P. Spevak, K. Adachi, and R. G. Micetich; Chem. Abstr., **118**, 212756 (1993).
14. US Pat. 5,258,377, S. N. Maiti, A. V. N. Reddy, D. Czajkowski, P. Spevak, C. Fiakpui, and R. G. Micetich; Chem. Abstr., **118**, 124299 (1993).
15. S. N. Maiti, D. Czajkowski, A. V. M. Reddy, P. Spevak, J. Kaleta, and R. G. Micetich, Bioorg. Med. Chem. Lett., **6**, 823 (1996).
16. T. J. Blacklock, J. W. Butcher, P. Sohar, T. R. Lamanec, and E. J. J. Grabowski, J. Org. Chem., **54**, 3907 (1989).