Chemistry of the Dihydrothiazine Ring Moiety of Cephalosporins. Synthesis of New C-4 Substituted Cephalosporins

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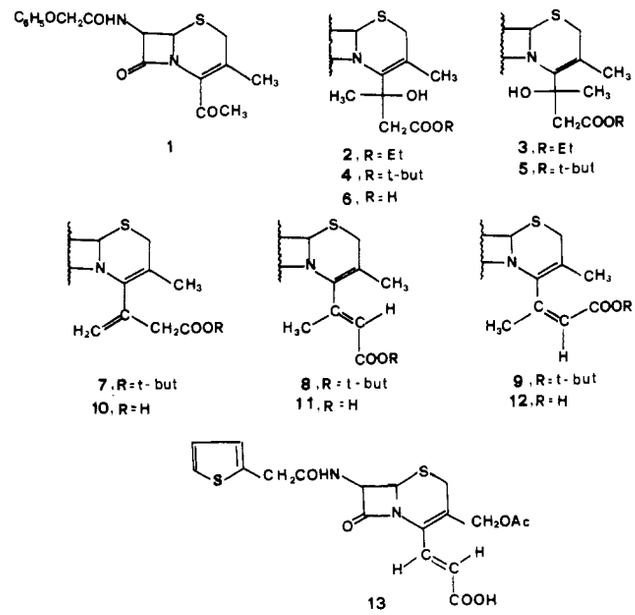
Received January 3, 1978

The Reformatsky reaction of a 4-acetyl-3-cephem derivative (1) with ethyl α-bromoacetate gave the expected mixture of β-hydroxy esters **2** and **3**; the same reaction of **1** with the *tert*-butyl α-bromo ester yielded only the diastereoisomer **4**. Dehydration of **4** with thionyl chloride and triethylamine in CH_2Cl_2 yielded a mixture of unsaturated esters **7**, **8**, and **9**, which were separated and transformed into the corresponding free acids **10**, **11**, and **12**. The structures of hydroxy esters **2** and **3** and the configurational correspondence between **2** and **4** were shown by their ^1H NMR spectra. The configurations of **2**, **3**, and **4** have been assumed on the basis of mechanistic considerations. Also, the structures of unsaturated esters **7–9** and of the corresponding acids **10–12** were demonstrated by ^1H NMR spectroscopy. The configurations of the couples **8,9** and **11,12** were established on the basis of the UV spectra of the acids **11** and **12**.

In recent years much effort has been devoted to the synthesis of new cephalosporin derivatives, but structural modifications at C-4 have not been extensively investigated.[1,2] Recently, some compounds having substituents on C-4 other than CO_2H[3,4] have shown biological activity.

In order to get better insight into the steric and electronic requirements at C-4 of Δ^3-cephems necessary for biological activity, we synthesized the new Δ^3-cephem derivatives **10–12**. In these compounds a carboxylic group is still present. Furthermore, compounds **10–12** have, with respect to cephalosporins, an additional double bond conjugated with the Δ^3-cephem double bond. The further resonance effect of this double bond could modify the resonances of the enamine and amide groups, whose properties are considered related to the biological activity of cephalosporins.[1,5] The recent report of the acid **13**,[6] structurally related to compounds **11** and **12**, prompted us to report the results so far obtained. The synthesis of our compounds has been achieved through a route completely different from that used for **13**, which allows us to obtain both geometrical isomers.

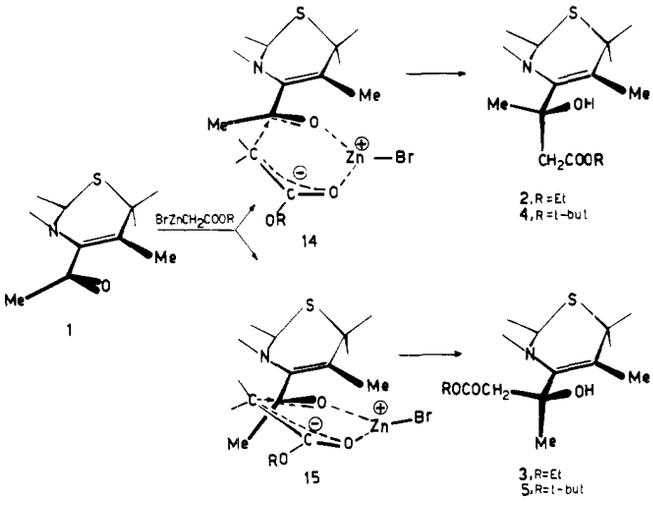


Results and Discussion

The Reformatsky reaction of the methyl ketone **1**[7] with ethyl α-bromoacetate in methylal–benzene gives a mixture of the diastereoisomeric β-hydroxy esters **2** and **3** in a ratio of about 2:1. These have been separated by column chromatography. When the Reformatsky reaction of **1** was carried out with *tert*-butyl α-bromoacetate in tetrahydrofuran, the hydroxy ester **4**, structurally corresponding to **2**, was obtained as the only product; the diastereoisomeric compound **5** was not detected. Treatment of the mixture of **2** and **3** with thionyl chloride and triethylamine in CH_2Cl_2 yielded a mixture of unsaturated ethyl esters, which were not characterized because of difficulty in their separation and saponification. However, similar treatment of the *tert*-butyl hydroxy ester **4** afforded mixtures of the unsaturated esters **7**, **8**, and **9**, which were separated by repeated thin-layer chromatography on silica gel. Esters **7**, **8**, and **9** were easily transformed into the corresponding free acids **10**, **11**, and **12** by hydrolysis with 99% formic acid. The hydroxy ester **4** could also be hydrolyzed under the same conditions to the corresponding hydroxy acid **6**.

The structures of hydroxy esters **2** and **3** and the configurational correspondence between **2** and **4** can be shown by their ^1H NMR spectra. Whereas in **2** and **4** the methylenic protons α to the COOR group appear as an apparent singlet, the corresponding ones of **3** exhibit a more complex signal; this reflects a different spatial relationship. The ^1H NMR spectra do not allow us to assign their configurations. The configurations of hydroxy esters **2**, **3**, and **4** have been tentatively[8] assumed on the basis of the preferential conformation of the ketonic carbonyl group of **1** with respect to the C(3)C(4)N(5) plane[7] (a perspective view of the molecule is reported in Figure 1) and the preferential approach of a reagent from the α side of the molecule.[7,9] According to the mechanism of the Reformatsky reaction,[10] the attack of the anion of the Reformatsky reagent on the carbonyl CO group in its preferential conformation should lead, through transition states **14** and **15** (see Scheme I), to the hydroxy esters of *R* configuration (**2** and **4**) or the hydroxy esters of *S* configuration (**3** and **5**), depending

Scheme I



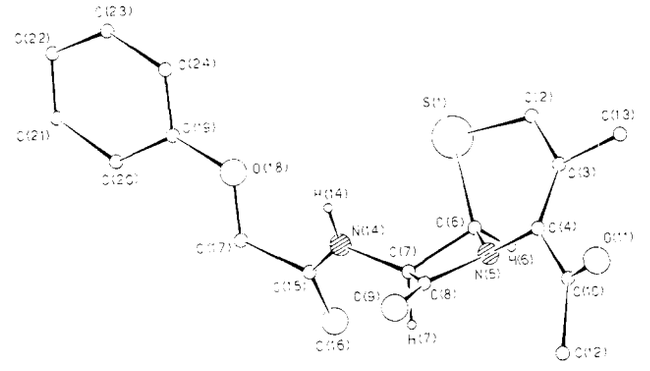


Figure 1. Perspective view of compound **1**.

on whether the attack of the reagent occurs from the α or β side of the dihydrothiazine ring. Due to the preferential approach of the reagent from the α side,[7,9] the products formed in higher amount should be **2** and **4**. The higher stereoselectivity of the reaction with the α-bromo *tert*-butyl ester (exclusive formation of **4**) may be attributed to the higher steric hindrance of this ester.

The structures of the unsaturated esters **7–9** and the corresponding acids **10–12** have been shown by their ^{1}H NMR spectra: the presence of a signal due to the methylenic protons α to the ester group, two signals due to the two geminal vinyl protons for compounds **7** and **10**, and a methyl and an olefinic proton coupled by an allylic coupling constant for compounds **8, 9, 11,** and **12**. However, because of the presence of numerous functional groups, the ^{1}H NMR spectra do not permit the assignment of the relative configurations of the pairs **8** and **9** and **11** and **12**. The configurations of the acids **11** and **12**, and consequently those of the corresponding esters **8** and **9**, can be inferred from the UV spectra. Examination of the molecular models (Dreiding) shows that, due to strong steric interaction, only the *E* acid **11** is likely to exist in a conformation such as to allow an efficient conjugation between the crotonic system and the double bond of the Δ^3-cephem nucleus. Accordingly, only compound **11** shows a strong K band at particularly long wavelength [λ_{max} 292 nm (ϵ 11 000)],[11] whereas the spectra of **10** and **12** show broad maxima at 250–260 nm and only end absorption at 300 nm.

The new cephalosporin derivatives **10–12** were tested in vitro against several strains of gram-positive and gram-negative bacteria. These compounds exhibited minimum inhibitory concentrations (MIC's) of >100 μg/mL against any bacteria tested.[12]

Experimental Section

Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were taken on paraffin oil mulls on a Perkin-Elmer Infracord Model 137. The UV spectra were measured in methanol solution. ^{1}H NMR spectra of all compounds were detected with a Jeol C-60 HL spectrometer in $CDCl_3$ solution using Me_4Si as an internal standard. Those of compounds **8, 9, 11,** and **12** have been also measured on a Jeol PS-100 spectrometer in order to determine the allylic coupling constants (H_3C–C=CH). The proton magnetic resonance assignments have been firmly established on the basis of the area, the expected chemical shift, the multiplicity of the signal, and double resonance experiments.[13] The relative percentages of compounds **2** and **3** and **7, 8,** and **9** have been calculated for **2** and **3** on the basis of the integrals of the CH_3COH singlets and for **7, 8,** and **9** on the basis of the 3-CH_3 singlets in the ^{1}H NMR spectra of the crude reaction mixtures. Mass spectra were registered with a Varian-Mat CH-7 spectrometer. Preparative TLC was performed on 2-mm layer silica gel plates (Merck F_{254}) containing a fluorescent indicator; spots were detected under UV light (254 nm). Evaporations were made in vacuo (rotating evaporator). Magnesium sulfate was always used as drying agent.

4-Acetyl-3-methyl-7-phenoxyacetamido-3-cephem (1)[7] and *tert*-butyl α-bromoacetate[14] [bp 52–54 °C (8 mm); n^{25}_D 1.4431 (lit.[14] bp 69–70 °C (18 mm), n^{25}_D 1.4431)] were prepared as previously described.

Ethyl (3*R*)-(2) and (3*S*)-3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)-3-hydroxybutanoate (3). A mixture of zinc powder (3.0 g, 0.046 g-atom), a small crystal of iodine, and ethyl α-bromoacetate (4.9 g, 0.029 mol) in anhydrous dimethoxymethane (12 mL) was stirred and refluxed for 30 min. The cooled mixture was treated with a solution of **1** (1.2 g, 3.5 mmol) in anhydrous benzene (15 mL). The resulting mixture was stirred for 10 min at room temperature and then refluxed for 2 h. After cooling, the reaction mixture was hydrolyzed with crushed ice and 0.1 N hydrochloric acid and then layered with ethyl acetate and acidified with 10% hydrochloric acid to pH 2.5. The resulting mixture was filtered through asbestos, and the organic layer was separated, washed with 5% aqueous $NaHCO_3$ and water, and evaporated to dryness. The oily residue obtained (1.6 g) consisted of **2** and **3** in a ratio of 2:1 (^{1}H NMR). The crude residue was chromatographed through a 2.5 × 32 cm column of 60–100 mesh Florisil eluting with 1:1 ethyl acetate–hexane and collecting 5-mL fractions. Fractions 15–21 gave practically pure **2** (0.600 g) as an oil: ^{1}H NMR δ 1.30 (t, 3, J = 7.3 Hz, CH_2CH_3), 1.55 (s, 3, CH_3COH), 2.17 (s, 3, CH_3), 2.50 and 3.10 (d, 2, J = 17.2 Hz, SCH_2), 3.03 (s, 2, $CH_2COOC_2H_5$), 4.23 (q, 2, J = 7.3 Hz, CH_2CH_3), 4.85 (d, 1, J = 4.5 Hz, CHS), 5.5 (q, 1, J = 4.5 and 8.6 Hz, NHCH); MS *m/e* 434, 416, 244, 226, 198, 180. Anal. Calcd for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 57.74; H, 6.05; N, 6.17.

Fractions 35–45 yielded a semisolid residue which when crystallized from acetone–hexane afforded pure **3** (0.030 g): mp 97.5–98 °C; ^{1}H NMR δ 1.30 (t, 3, J = 7.3 Hz, CH_2CH_3), 1.50 (s, 3, CH_3COH), 2.17 (s, 3, CH_3), 2.60–3.25 (two AB systems, SCH_2 and $CH_2COOC_2H_5$), 4.27 (q, 2, J = 7.3 Hz, CH_2CH_3), 5.07 (d, 1, J = 4.5 Hz, CHS), 5.63 (q, 1, J = 4.5 and 8.3 Hz, NHCH); MS *m/e* 434, 416, 244, 225, 198, 180. Anal. Calcd for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 58.22; H, 6.29; N, 6.46.

***tert*-Butyl (3*R*)-3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)-3-hydroxybutanoate (4).** A mixture of zinc powder (10.1 g, 0.155 g-atom), a small crystal of iodine, and anhydrous tetrahydrofuran (30 mL) was stirred and heated to reflux under a nitrogen atmosphere. Then a solution of *tert*-butyl α-bromoacetate (24.7 g, 0.126 mol) and **1** (7.6 g, 0.022 mol) in anhydrous tetrahydrofuran (130 mL) was added over 5 min, heating to reflux during the addition. Refluxing was continued for an additional hour. After cooling, the resulting mixture was worked up as described for the synthesis of **2** and **3**, yielding an oily residue (9.9 g) consisting of **4** (^{1}H NMR). Crude **4** was chromatographed through a 3 × 63 cm column of 60–100 mesh Florisil eluting with a 4:6 mixture of ethyl acetate–hexane and collecting 5-mL fractions. Fractions 17–41 gave practically pure **4** (2.85 g) as an oil. Rechromatography of fractions 13–16 (0.94 g) yielded a further amount of pure **4** (0.35 g): ^{1}H NMR δ 1.50 (s, 12, CH_3COH and C_4H_9), 2.18 (s, 3, CH_3), 2.42 and 3.05 (d, 2, J = 17.2 Hz, SCH_2), 3.05 (s, 2, $CH_2COOC_4H_9$), 4.90 (d, 1, J = 4.5 Hz, CHS), 5.55 (q, 1, J = 4.5 and 8.8 Hz, NHCH); MS *m/e* 462, 444, 272, 253, 198, 180. Anal. Calcd for $C_{23}H_{30}N_2O_6S$: C, 59.72; H, 6.54; N, 6.06. Found: C, 59.45; H, 6.38; N, 6.18.

(3*R*)-3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)-3-hydroxybutanoic Acid (6). A solution of **4** (0.090 g, 0.2 mmol) in 99% HCOOH (10 mL) was stirred under a nitrogen atmosphere at room temperature for 2 h, and then the solvent was evaporated under reduced pressure. The residue was taken up with ethyl acetate and extracted with 5% aqueous $NaHCO_3$. The aqueous phase was washed with ethyl acetate, cooled to 0 °C, layered with ethyl acetate, and then acidified under stirring with 10% hydrochloric acid to pH 2.5. The organic phase was separated, washed (H_2O), filtered, and evaporated to give a semisolid residue (0.058 g) which by crystallization from 2-propanol yielded **6** (0.035 g), mp 147–149 °C. Anal. Calcd for $C_{19}H_{22}N_2O_6S$: C, 56.14; H, 5.46; N, 6.89. Found: C, 56.00; H, 5.46; N, 6.81.

***tert*-Butyl 3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)but-3-enoate (7) and (*E*)- (8) and (*Z*)-3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)but-2-enoate (9).** A solution of $SOCl_2$ (0.67 g, 5.6 mmol) in anhydrous methylene dichloride (5 mL) was added under stirring and a nitrogen atmosphere to a cooled (0 °C) mixture of **4** (1.92 g, 4.14 mmol) and triethylamine (1.5 g, 15 mmol) in anhydrous methylene dichloride (30 mL). When the addition was complete, the resulting mixture was stirred and refluxed for 75 min, left for 2 h at room temperature, and then washed in succession with 3% hydrochloric acid, 5% aqueous $NaHCO_3$, and water. Evaporation of the filtered organic phase yielded an oily residue (1.7 g) consisting of **7**, **8**, and **9** in a ratio of about 4:1:2.5 (^1H NMR). The crude oil was subjected to preparative TLC eluting with a 4:6 mixture of ethyl acetate and hexane, and the three bands observed were extracted with $CHCl_3$. Extraction of the top band of the chromatogram gave practically pure **8** (0.090 g): ^1H NMR δ 1.44 (s, 9, C_4H_9), 2.08 (s, 3, CH_3), 2.15 (d, 3, J = 1.2 Hz, C**H**$_3$C=CH), 2.95 and 3.36 (d, 2, J = 16.5 Hz, SCH_2), 5.09 (d, 1, J = 4.5 Hz, CHS), 5.67 (q, 1, J = 4.5 and 9.0 Hz, NHC**H**), 5.81 (m, 1, CH_3C=C**H**); MS *m/e* 444, 388, 343, 315, 265, 253, 236, 224, 198, 192, 182, 180. Anal. Calcd for $C_{23}H_{28}N_2O_5S$: C, 62.14; H, 6.35; N, 6.30. Found: C, 61.82; H, 6.65; N, 6.58.

Extraction of the middle band of the chromatogram gave a solid residue (0.080 g) which crystallized from acetone–hexane to yield pure **7** (0.040 g): mp 179–180 °C; ^1H NMR δ 1.48 (s, 9, C_4H_9), 1.98 (s, 3, CH_3), 3.00 and 3.31 (d, 2, J = 16.5 Hz, SCH_2), 3.37 (s, 2, C**H**$_2$COOC$_4$H$_9$), 5.15 (d, 1, J = 4.5 Hz, CHS), 5.21 and 5.49 (s, 2, CH_2=C), 5.61 (q, 1, J = 4.5 and 9.6 Hz, NHC**H**); MS *m/e* 444, 388, 343, 315, 254, 198, 180. Anal. Calcd for $C_{23}H_{28}N_2O_5S$: C, 62.14; H, 6.35; N, 6.30. Found: C, 61.85; H, 6.31; N, 6.22.

Extraction of the lowest band of the chromatogram afforded impure **9** (0.230 g), which was chromatographed again under the same conditions used for the crude reaction mixture. Extraction of the middle band of the latter chromatogram yielded 90% pure **9** (0.060 g) as an oil: ^1H NMR δ 1.48 (s, 9, C_4H_9), 1.82 (s, 3, CH_3), 2.26 (d, 3, J = 1.4 Hz, C**H**$_3$C=CH), 3.08 and 3.48 (d, 2, J = 18.5 Hz, SCH_2), 4.98 (d, 1, J = 4.5 Hz, CHS), 5.70–5.84 (m, 2, NHC**H** and CH_3C=C**H**); MS *m/e* 444, 388, 343, 315, 254, 198, 192, 180. Anal. Calcd for $C_{23}H_{28}N_2O_5S$: C, 62.14, H, 6.35; N, 6.30. Found: C, 61.94; H, 6.02; N, 6.58.

When a mixture of **2** and **3** was dehydrated exactly as described above for **4**, a mixture of dehydratation products (^1H NMR) was obtained which could not be separated.

3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)but-3-enoic Acid (10). Unsaturated ester **7** (0.042 g, 0.094 mmol) was hydrolyzed in 99% formic acid (4.2 mL) exactly as described above for the preparation of **6**, yielding a residue (0.036 g) which on crystallization from 2-propanol gave **10** (0.026 g): mp 185–187 °C; ^1H NMR δ 1.98 (s, 3, CH_3), 5.05 (d, 1, J = 4.5 Hz, CHS), 5.12 and 5.43 (s, 2, CH_2=C), 5.43 (q, 1, J = 4.5 and 10.5 Hz, NHC**H**); UV λ_{max} 251 nm (ϵ 8100). Anal. Calcd for $C_{19}H_{20}N_2O_5S$: C, 58.75; H, 5.19; N, 7.21. Found: C, 58.86; H, 5.34; N, 7.14.

(*E*)-3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)but-2-enoic Acid (11). A solution of **8** (0.025 g, 0.056 mmol) in 99% formic acid (2.5 mL) was hydrolyzed as described above for the preparation of **6**, affording a solid residue (0.017 g) which on crystallization from 2-propanol gave **11** (0.010 g): mp 171–173 °C; ^1H NMR δ 2.13 (s, 3, CH_3), 2.16 (d, 3, J = 1.2 Hz, C**H**$_3$C=CH), 3.08 and 3.43 (d, 2, J = 16.5 Hz, SCH_2), 5.10 (d, 1, J = 4.5 Hz, CHS), 5.69 (q, 1, J = 4.5 and 9.0 Hz, NHC**H**), 5.81 (m, 1, CH_3C=C**H**); UV λ_{max} 292 nm (ϵ 11 000). Anal. Calcd for $C_{19}H_{20}N_2O_5S$: C, 58.75; H, 5.19; N, 7.21. Found: C, 58.84; H, 5.17; N, 7.05.

(*Z*)-3-(3-Methyl-7-phenoxyacetamido-3-cephem-4-yl)but-2-enoic Acid (12). Ester **9** (0.045 g, 0.10 mmol) in 99% formic acid (5 mL) was hydrolyzed as described for the preparation of **6**. An oily residue (0.031 g) was obtained which on crystallization from 2-propanol gave **12** (0.010 g): mp 189–192 °C; ^1H NMR δ 1.83 (s, 3, CH_3), 2.29 (d, 3, J = 1.2 Hz, C**H**$_3$C=CH), 5.80 (m, 1, CH_3C=C**H**); UV λ_{max} 262 and 268 nm (ϵ 8000). Anal. Calcd for $C_{19}H_{20}N_2O_5S$: C, 58.75; H, 5.19; N, 7.21. Found: C, 58.44; H, 4.79; N, 6.90.

Acknowledgment. This work has been supported in part by a grant from the Consiglio Nazionale delle Ricerche.

Registry No.—**1**, 66323-25-9; **2**, 66323-26-0; **3**, 66323-27-1; **4**, 66323-28-2; **6**, 66323-29-3; **7**, 66323-30-6; **8**, 66323-31-7; **9**, 66323-32-8; **10**, 66323-33-9; **11**, 66323-34-0; **12**, 66323-35-1; ethyl α-bromoacetate, 105-36-2; *tert*-butyl α-bromoacetate, 5292-43-3.

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(8) An x-ray crystal analysis of the acid **6**, corresponding to the ester **4**, was performed in order to determine unequivocally the configurations of esters **2–4**. Compound **6** crystallizes in the space group $P2_1$ with a = 11.253 (1) Å, b = 20.137 (7) Å, c = 8.917 (3) Å, β = 102.62 (1)°, and Z = 4. Till now only a small molecular fragment has been detected, but we were unable to completely resolve the structure.
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