Study on the $\triangle^3 \rightarrow \triangle^2$ Isomerization During Preparation of Cefuroxime Double Ester Prodrugs

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ABSTRACT

$\triangle^3 \rightarrow \triangle^2$ Isomerization during preparation of cephalosporin ester prodrugs has been frequently reported. Methods to eliminate the isomerization must be established. In the course of preparing cefuroxime double ester prodrugs, quarternary ammonium salts with different counter ions were usedas catalysts for alkylations and their effects on the isomerization were compared. The structures of $\triangle^3$ and $\triangle^2$ isomeric esters 1a and 1b were identified with^{1}H-^{1}H COSY 2D NMR and DEPT 135 NMR. The degree of isomerization during alkylation was monitored with HPLC. It was demonstrated that tetrabutyl ammonium hydrogen sulfate $(TBA^+HSO_4^-)$was a better catalyst than TBA^+I^- in regard to preventing any isomerization. The desired cefuroxime double ester 1a was obtained as a sole product in this reaction when the molar ratio of $TBA^+HSO_4^-$ to cefuroxime sodium was beyond 0. 35 We have successfully eliminated the $\triangle^3 \rightarrow \triangle^2$ isomerization commonly reported for preparation of cephalosporin esters.

Key word: $\triangle^3 \rightarrow \triangle^2$ isomerization, Cefuroxime prodrug, Quarternary ammonium salts.

INTRODUCTION

Structural modification by forming ester or double ester prodrugs has been a common approach for improving the oral bioavailability ofparenteral cephalosporin antibiotics[1-3]. We have been involved in perparing a series of cefuroxime prodrugs by attaching D-phenylglycine or other aminoacids onto the cepham nucleus at its 4-carboxyl group via a methylene linkage. The D-phenylglycine and analogous amino acid moieties were considered as drug delivery tools for intestinal absorption of cefuroxime[4]. Our double esterpredrugs were perpared by reaction of cefuroxime parent drug with the chloromethyl ester of the delivery moieties, as illustrated in Scheme 1 using D-phenylglycine as the example. The reactions, however routinely gave a mixture of the $\triangle^3$ and $\triangle^2$ ester products, i. e. compounds 1a and 1b in Scheme 1. The undesired $\triangle^2$ isomers were major products in most of the alkylation reactions. This phenomenon is common and has frequently beenreported[5-7]. Therefore, methods of regiospecific preparation of ceph-3-em esters without $\triangle^3 \rightarrow \triangle^2$ isomerization must be established. This report describes our efforts in pursuing methods of preparing the ceph-3-em esters (Scheme 1) and the identification of $\triangle^3$ and $\triangle^2$isomers by spectral analysis.

MATERIALS AND METHODS

I. *Chemicals and Reagents*
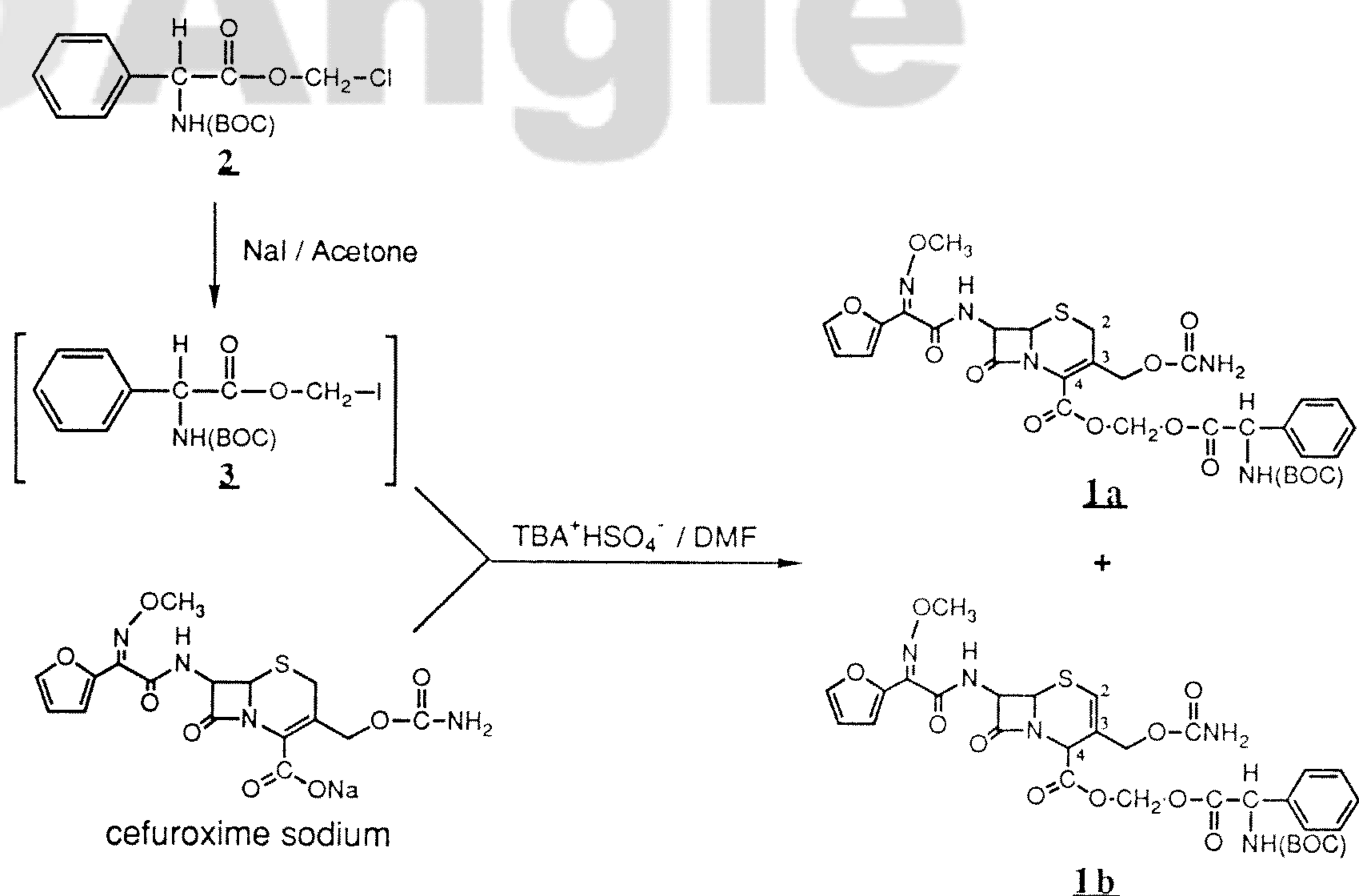
Scheme 1. Alkylation of Cefuroxime Sodium.

Cefuroxime sodium was kindly donated by U-Liang Pharmaceutical Company. Chloromethy 2-(BOC) amino-2-phenylacetate (compound 2) wassynthesized previously[4]. Solvents and reagents are commercial products from E. Merck, Aldrich, Wako or Kasai Companies. HPLC grade ethyl acetate, n-hexane and chloroform were purchased from Alpus Chemical Company. The internal standard acetophenetidin was pu chased from Sigma Chemical Company.

II. Alkylation Reactions of Cefuroxime Sodium-General Procedure

One and a half grams (5 mmole) of compound 2 and 3.75 g (25mmole) of sodium iodide were dissolved in 50 ml of acetone. The solution was stirred under nitrogen for 1 hr. Acetone was evaporated. The iodide intermediate 3 was obtained by extracting the residue with 50 ml of methylene chloride, which was then removed. The solid residue and 2.24 g (5 mmole) of cefuroxime sodium were then dissolved in 50 ml of N, N-dimethyl formamide (DMF). The resulting mixture was

stirred under nitrogen for 12 hr. DMF was removed *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with saturated sodium chloride solution, then dried with sodium sulfate. The light brown gummy liquid obtained after concentrating the solution was chromatographed in chromatotron with ethyl acetate: n-hexane: choroform = 35:25:40 as the mobile phase to give a pure compound. This product is identical with compound 1b.

Later, the same procedure was followed except that 2.04 g(6 mmole)of tetrabutylammonium hydrogen sulfate(TBA⁺HSO₄⁻)was added to the reaction mixture. The crude product was puified in an open silica gel column (300 g, 70-230 mesh, EtOAc: n-hexane = 3:2). The reaction gave 2.10 g (61%) of the product. This product is identical with compound 1a.

III. Identification of Compound 1a and Compound 1b
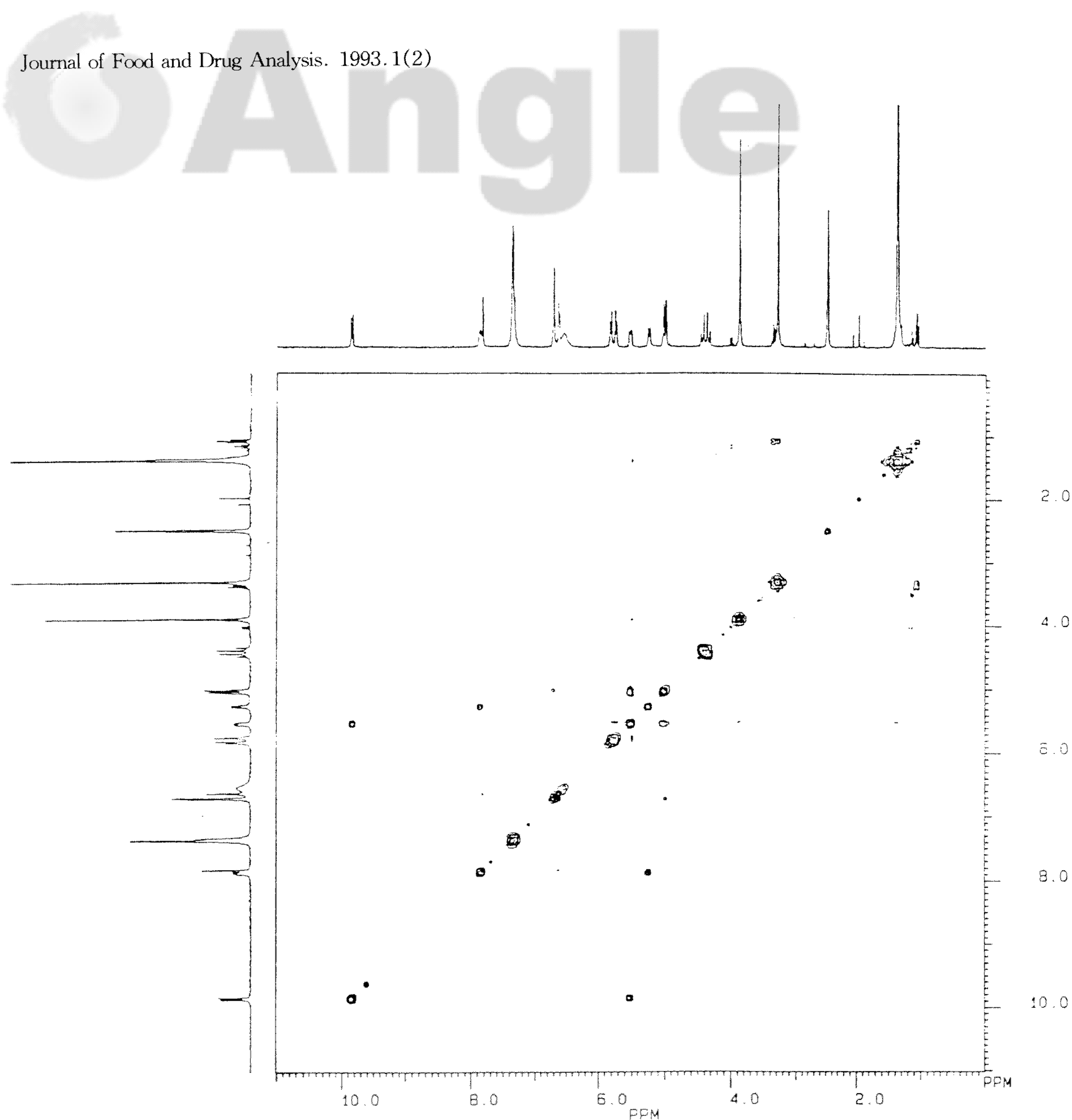
Compounds were identified by their physical

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Figure 1. ^{1}H-^{1}H COSY 2D NMR Spectrum of Compound 1b.

properties, such as melting point and elemental analysis, or with spectral analysis. Melting points were determined on a Buchi 510 capillary melting point apparatus and were uncorrected. Elemental analyses were performed in a Perkin-Elmer 240C elemental analyzer. Spectral data was obtained from Perkin-Elmer 1760 FT-IR spectrophotometer, Finnigan MAT 4510 EI mass spectrometer (70eV), Brucker AC 80 80 MHz or Brucker 300 MHz NMR spectrometer. Chemical shifts were recorded in parts per million(ppm) downfield from internal tetramethylsilane.

Product 1a has mp. 111-113℃；IR(KBr)ν_{max} 3500-2900(broad), 2978, 2938, 1785($\nu_{c=o}$), 1720-1698(m, $\nu_{c=o}$), 1521, 1259, 1159, 1044, 1022 cm^{-1}；^{1}H NMR (300 MHz；DMSO-d$_6$)：δ1. 38(s, 9H, t-Butyl), 3.51& 3.61 (ABq, J = 18.2Hz, 2H, H-2), 3.89(s, 3H, -N-O-CH$_3$), 4.57&4.74(ABq, J = 13.3Hz, 2H, CH$_2$-OCONH$_2$), 5.21(d, J = 4.7Hz, 1H, H-6), 5.28(d, J = 7.6Hz, 1H, Φ-CH), 5.79-5.89(m, 3H, H-7 + -O-CH$_2$-O-), 6.63-6.69(m, 3H, -CONH$_2$ + furan H-4), 7.32-7.37(m, 6H, Φ-H + furan H-3), 7.83-7.87(m, 2H, -NHCOO + furan H-5), 9.78(d, J = 7.9Hz, 1H, -NHCO-)ppm；

elemental analysis for $C_{30}H_{33}N_5O_{12}S$: Calc C: 52. 40, H: 4. 84, N: 10. 18, Found C: 51. 96, H: 5. 00, N: 9. 78.

Product 1b has mp. 101-102℃；IR（KBr）ν_{max} 3425（broad）, 2970, 2935, 1771（$\nu_{c=o}$）, 1715-1692 （m, $\nu_{c=o}$）, 1520, 1330, 1260, 1160, 1060, 1045, 981 cm^{-1}；^{1}H NMR （300 MHz；DMSO-d$_6$）：δ1. 38（s, 9H, t-Butyl）, 3. 90（s, 3H, O-CH$_3$）, 4. 36&4. 45 （ABq, J = 12. 6Hz, 2H, -CH$_2$-O-CONH$_2$）, 5. 00-5. 04（m, 2H, H-6 + CH-COO）, 5. 26（d, J = 7. 5Hz, 1H, Φ-CH）, 5. 51-5. 55（m, 1H, H-7）, 5. 75&5. 83 （ABq, J = 5. 9Hz, 2H, -O-CH$_2$-O-）, 6. 54（broad, 2H, CONH$_2$）, 6.62-6. 64（m, 1H, furan H-4）, 6. 71 （d, J = 2.5Hz, 1H, -S-CH = C-）, 7. 33-7. 36（m + s,

6H, Φ-CH + furan H-3）, 7. 83-7. 89（m, 2H, NH-COO + furan H-5）, 9. 85（d, J = 7. 6Hz, 1H, -NH-CO-）ppm.

IV. *Chromatographic Conditions*

HPLC was used to monitor the isomerization reaction during the alkyation of cefuroxime sodium with compound 3. The HPLC system contains a Waters 600E pump, a Water 484 Tunable Absorbance Detector and a Waters 745 Data Module. A Vercopax-5μm silica column was used as the stationary phase. A degassed solvent system containing ethyl acetate, *n*-hexane and chloroform at a ratio of 35:25:40 was used as the mobile phase at a
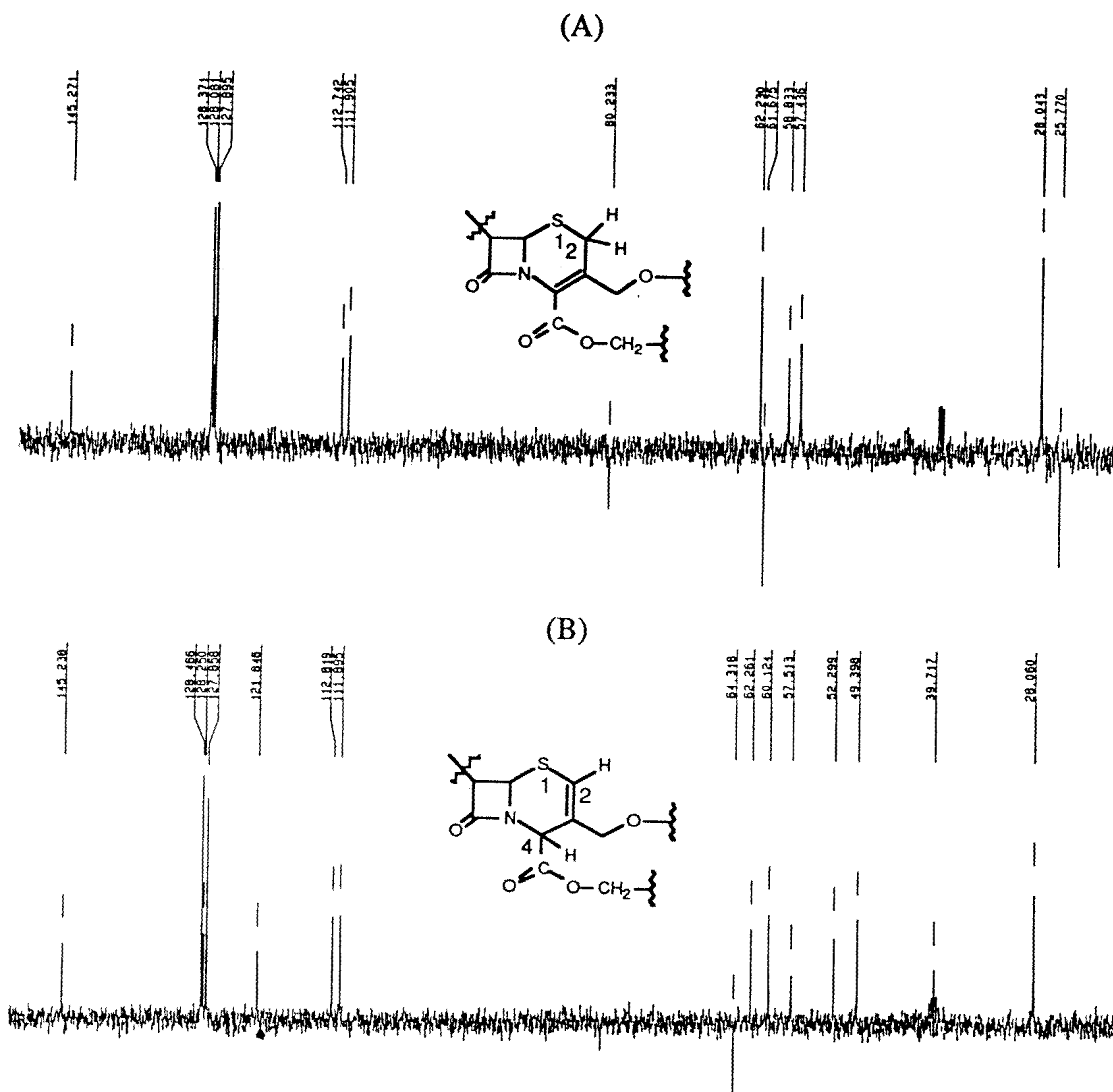
(A)

(B)

Figure 2. DEPT 135 NMR of Compound 1a (A) and Compound 1b (B).
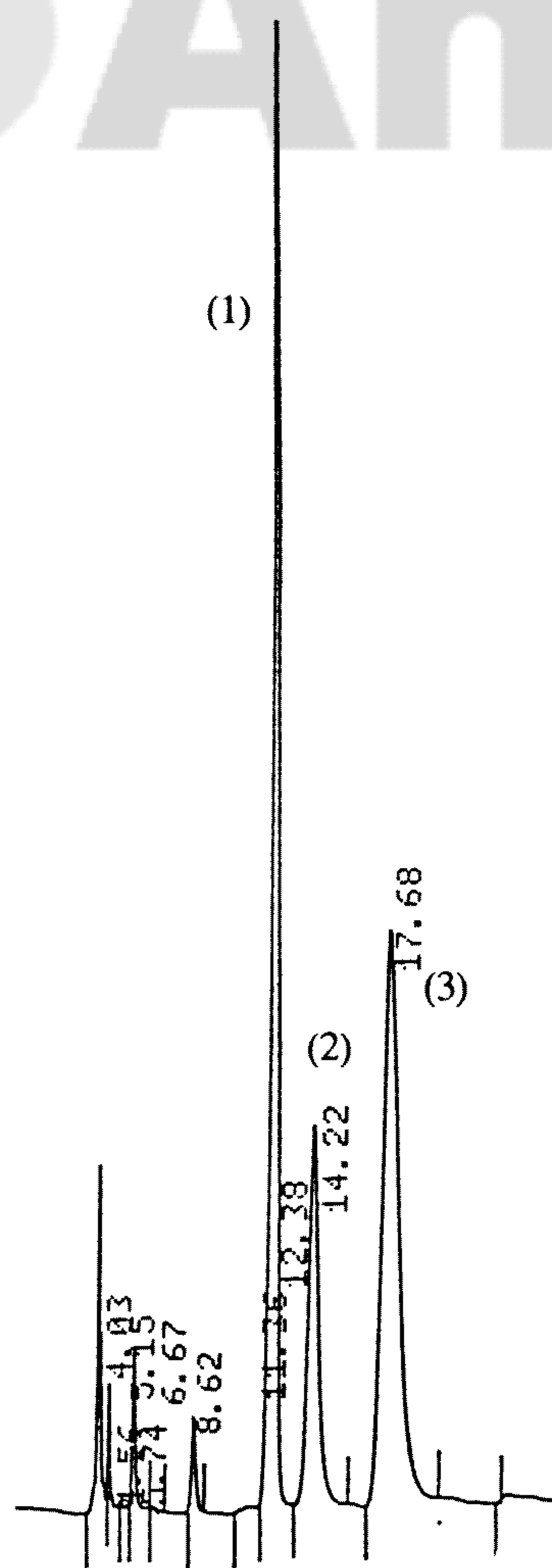
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Figure 3. Chromatogram of Reaction Mixture from Alkylation of Cefuroxime Sodium, spiked with (1) Acetophenetidin (Internal Standard), (2) Compound 1a and (3) Compound 1b.

flow rate of 1.0ml/min. Isomeric double esters 1a, 1b, and the internal standard acetophenetidin were monitored at 260 nm.

V. *Preparation of Stock Solutions for $\triangle^3 \rightarrow \triangle^2$ Isomerization Study*

Three hundred milligrams (1 mmole) of compound 2 and 750 mg (5mmole) of sodium iodide were dissolved in 50 ml of acetone and the solution was heated under reflux for 5 hr. The acetone was removed in vacuo. The iodide intermediate 3 was obtained by extracting the residue with 50ml of methylene chloride. Methylene chloride was evaporated, then the residue was accurately dissolved in 10.00 ml of DMF to form stock Solution

A. Solution B was prepared by dissolving 448 mg (1 mmole) of cefuroxime sodium and 100 mg of acetophenetidin in 10.00 ml of DMF. $TBA^+ HSO_4^-$ (340 mg, 1 mmole), was dissolved in 10.00 ml of accurately measured DMF to form Solution C. The stock Solution D was prepared by dissolving 370 mg (1 mmole) of $TBA^+ I^-$ in 10.00 ml of DMF.

VI. $\triangle^3 \rightarrow \triangle^2$ *Isomerization Study*

Alkylation reactions of cefuroxime sodium were carried out with the general procedure described above. In each reaction, $TBA^+ HSO_4^-$ was added to the reaction mixture with the molar ratio of $TBA^+ HSO_4^-$ to cefuroxime sodium as 0.00, 0.05, 0.10, 0.15, 0.25, 0.35, 0.55, 0.75, 1.00 and 1.20. In another series of reactions, $TBA^+ I^-$ was used to replace $TBA^+ HSO_4^-$ with the molar ratio of 0.00, 0.05, 0.15, 0.50, 0.70, 0.80, 0.90 and 1.00. Acetophenitidin was used as internal standard in each reaction. The reactions were monitored with HPLC for the isomerization. One hundred microliters of the reaction mixture was pretreated with a silica gel cartridge and $10\mu l$ of the sample was injected into HPLC.

RESULTS AND DISCUSSION

Alkylation of cefuroxime sodium with iodomethyl compound 3, prepared *in situ* from its chloromethyl ester precursor 2, was carried out under a variety of reaction conditions. Primary NMR spectra of the isolated product indicated it to be a mixture of the desired double ester 1a and its $\triangle^2$ isomeric compound 1b. Several reports suggested that $\triangle^3 \rightarrow \triangle^2$ isomerization of cepham esters were subjected to general and specific base catalysis either in reactions[8] or in biological systems[9,10]. In order to avoid basic conditions, Mobashery et al. reported a method of using cepham acids in preparing ceph-3-em esters unaccompanied by $\triangle^3 \rightarrow \triangle^2$ isomerization[11]. This method was applied in our study. However, alkylation with iodomethyl ester 3
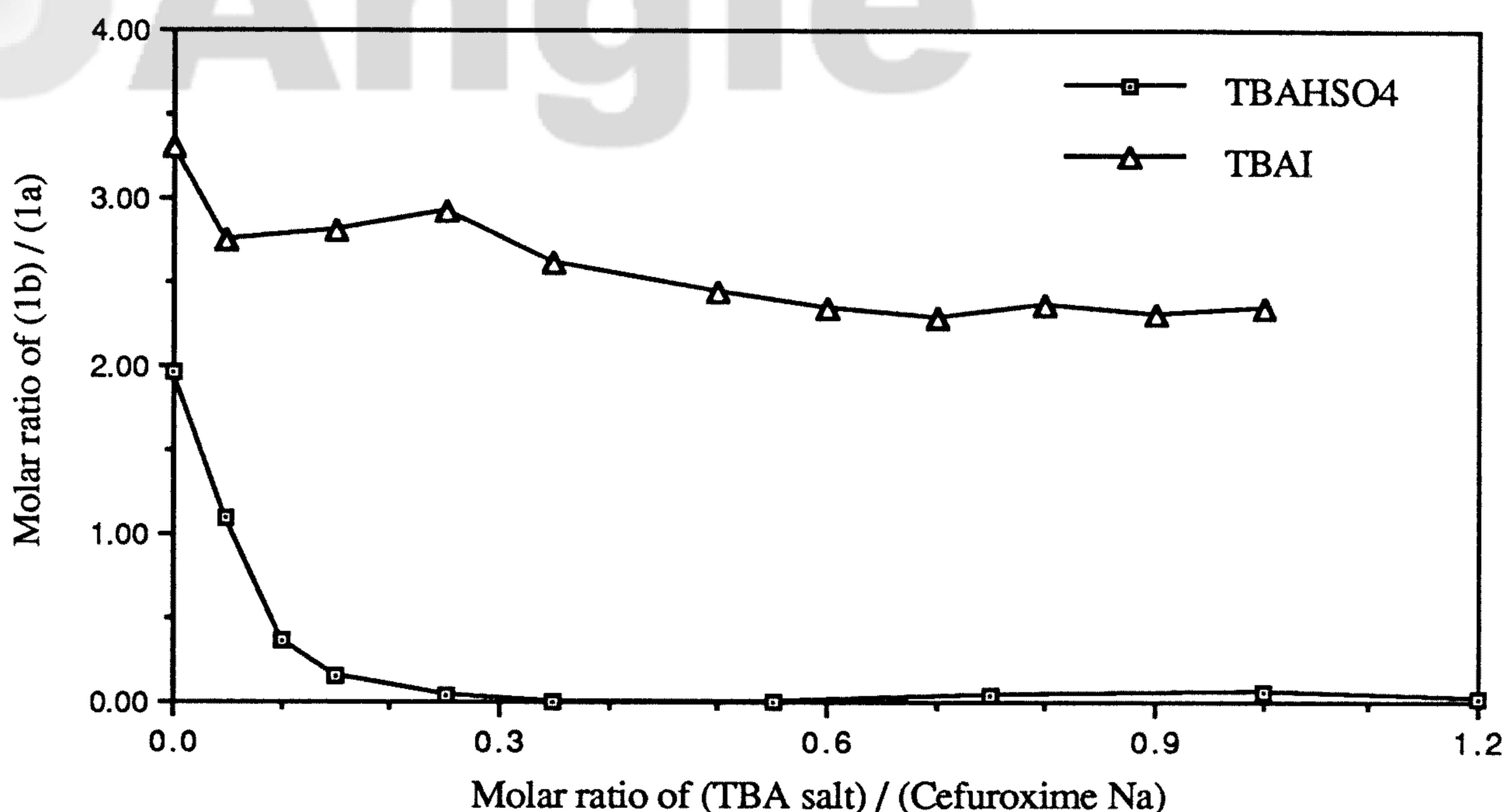
Figure 4. Effect of Molar Ratio of $TBA^+HSO_4^-$/Cefuroxime Sodium and TBA^+I^-/Cefuroxime Sodium on the $\triangle^3 \rightarrow \triangle^2$ Isomerization.

proceeded very slowly and the double ester product 1a was obtained with a very low yield. The poor nucleophilicity of the free carboxylic acid might account for the poor reaction. Metal salts of cefuroxime seemed to be a better choice of starting materials for alkylation and a method for eliminating the $\triangle^3 \rightarrow \triangle^2$ isomerization caused by the basicity of carboxylic acid metal salts *per se* must be estabilished.

Quaternary ammonium salts are commonly used as phase transfer catalysts in alkylations of carboxylic compounds. We felt that a catalyst with a strong acidic counter ion may not only act as a catalyst *per se* for alkylation but also the counter ion may act as a buffer in keeping the reaction media from being basic. Thus, $TBA^+ HSO_4^-$ and TBA^+I^- were used as catalysts in two series of reactions repesenting the acidic and the neutral conditions respectively.

In order to unambiguously identify the products, the crude mixture of alkylations was purified in chromatotron, then NMR spectra was performed for both components. Compound 1a was

clearly identified from the NMR spectrum. The structure of Compound 1b was further elucidated with ^1H-^1HCOSY 2D NMR and DEPT 135 NMR. The AB quartet signal at 3.51 and 3.61 ppm($J =$ 18.2 Hz) of C-2 proton on compound 1a disappeared in the spectrum of compound 1b. Instead, as indicated in Figure 1 for compound 1b, the signal at 6.71 ppm was designated to a vinylic proton. Correlation of the doublet with singnal at 5.00 ppm implied an allylic coupling between the vinylic proton and the proton at C-4. The DEPT 135 spectra of both compounds were compared (Figure 2). Signal at 25.77 ppmfor compound 1a designated to the secondary carbon of C-2 was no more present in the spectrum of compound 1b. In stead, a new signal appeared at 121.85 ppm in this spectrum and was designated to the vinylic C-2 carbon. Signals at 57.44, 58.83 and 62.23 ppm in the spectrum of compound 1a were from the primary methoxyl carbon, the tertiary C-6, C-7 and benzylic carbons, of which two were overlaped. In comparison with the spectrum of compound 1a, an additional peak appeared between 49.40 ppm and

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62.26 ppm in that of compound 1b. The peak was designated to the C-4 tertiary Distinct carbon. structures for compounds 1a and 1b were thus clearly confirmed.

$\triangle^3 \rightarrow \triangle^2$ isomerization during alkylation was monitored with HPLC. Samples from reaction mixtures were analyzed for the presence of the iso- meric compound 1a and 1b. Retention times for in- ternal standard acetophenetiden, 1a and 1b were 11.36, 14.22 and 17.68 minutes respectively (Fig- ure 3). Molar ratio of products 1b/1a were plotted against the ratio of TBA salt/cefuroxime sodium. As indicated in Figure 4, more of $\triangle^2$ isomer 1b was obtaine than the desired $\triangle^3$ isomer 1a, when the molar ratio of $TBA^+ HSO_4^-$/cefuroxime sodium was less than 0.05. Isomerization was significantly inhibited when the ratio was beyond 0.15. When the molar ratio of $TBA^+ HSO_4^-$ to cefuroxime sodi- um reached 0.35, compound 1a was obtained as the sole product. In reaction catalyzed by TBA^+ I^-, $\triangle^3 \rightarrow \triangle^2$ was obviously favored and the $\triangle^2$ i- somer was the major product in all reactions, no matter what the ratio of catalyst to cefuroxime sodium was. The results indicated that the counter ions of phase transfer catalysts have significant ef- fect on the $\triangle^3 \rightarrow \triangle^2$ isomerization, probably due to the difference of capability in acidifying the reac- tion media.

CONCLUSION

$\triangle^3 \rightarrow \triangle^2$ Isomerization during alkylations of cefuroxime sodium was monitored under various reaction conditions. The $\triangle^3$ and $\triangle^2$ isomeric com- pounds 1a and 1b were identified by[1]H-^{1}H COSY 2D NMR and DEPT 135 NMR spectra. The acidi- ty of counter ions of phase transfer catalysts had ef- fect on the $\triangle^3 \rightarrow \triangle^2$ isomerization. We have clearly demonstrated that $TBA^+ HSO_4^-$ was a better cata- lyst than $TBA^+ I^-$ in regard to preventing the isomerization. The desired cefuroxime double ester 1a was obtained as a sole product when the molar ratio of $TBA^+ HSO_4^-$ to cefuroxime sodium was

beyond 0.35. We have successfully eliminated the $\triangle^3 \rightarrow \triangle^2$ isomerization commonly reported for preparation of ester or double ester prodrugs of cephalosporin antibiotics.

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合成 Cefuroxime 雙酯先驅藥中四級銨鹽
對 $\triangle^3 \rightarrow \triangle^2$ 異構化反應之影響

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摘　要

在合成 cefuroxime 雙酯先驅藥的過程中，我們不斷觀察到產物有 $\triangle^3 \rightarrow \triangle^2$ 異構化之現象，此現象在文獻上亦多所記載。根據報導，此異構化反應多在鹼性條件下發生，因此如何避免反應溶液被鹼催化為合成該類化合物之必要條件。本研究考慮使用四級銨鹽作為酯化反應形成雙酯先驅藥之催化劑。為研究該類催化劑之抗衡離子(counter ion)的酸鹼性對異構化之影響，我們選取 $TBA^+HSO_4^-$ 及 TBA^+I^- 作為催化劑，觀察催化劑含量對 $\triangle^3$ 及 $\triangle^2$ 產物(化合物 1a 及 1b)比例之影響。結果顯示酸類催化劑 $TBA^+HSO_4^-$ 確實可影響異構化程度及 $\triangle^3/\triangle^2$ 產物之比例。當 $TBA^+HSO_4^-$/cefuroxime sodium 之莫耳數比例超過 0.35 時，所欲之 $\triangle^3$ 化合物 1a 幾為唯一之產物。而中性 TBA^+I^- 催化劑則使 $\triangle^2$ 產物 1b 成為反應之主產物。