Preparation, Identification, and Quantitative NMR Determination of Silyl Derivatives of 6-Aminopenicillanic Acid, 7-Amino-3-methyl-Δ^3-cephem-4-carboxylic Acid, and 7-Amino-3-acetoxymethyl-Δ^3-cephem-4-carboxylic Acid

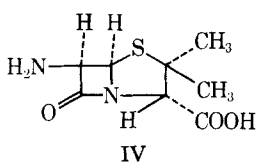
FULVIO BORTESI, SILVANO CAVALLI, and ALBERTO MANGIA ˣ

Abstract □ A rapid and accurate method for the quantitative determination of the extent and ratio of amino and carboxyl group trimethylsilylation of 6-aminopenicillanic acid, 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid, and 7-amino-3-acetoxymethyl-Δ^3-cephem-4-carboxylic acid is presented. The method utilizes NMR spectroscopy and is based on the difference in chemical shifts between *N*-trimethylsilyl and *O*-trimethylsilyl groups or, in cephalosporin derivatives, between the methyl group in the 3-position and free amino resonances. The spectra of the *N,O*-bis(trimethylsilyl) derivatives are discussed.

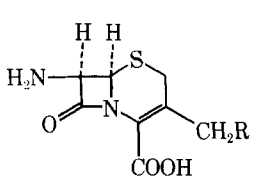
Keyphrases □ 6-Aminopenicillanic acid—silyl derivative, preparation and NMR analysis □ Cephemcarboxylic acids, substituted—silyl derivatives, preparation and NMR analyses □ NMR—analyses, silyl derivatives of 6-aminopenicillanic and substituted cephemcarboxylic acids □ Silyl derivatives—6-aminopenicillanic and substituted cephemcarboxylic acids, prepared, NMR analyses □ Antibacterial intermediates—6-aminopenicillanic and substituted cephemcarboxylic acids, silyl derivatives prepared, NMR analyses

Silylation (1) has a wide range of applications in analytical chemistry (2) and organic synthesis (1, 3) as a useful means of altering the solubility of materials, increasing their stability, and blocking reactive sites, allowing a successive, easy removal of the silyl group. All these advantages are present in the silylation of amino acids (4) using different silylating agents such as hexamethyldisilazane (I) (5), trimethylchlorosilane (II) (6), *N*-trimethylsilyldialkylamines (7), and *N,O*-bis(trimethylsilyl)acetamide (III) (8). Elemental analyses, refractive indexes, or IR spectra were the physical means used to determine the structure of *O*-mono- or *N,O*-disilylated products.

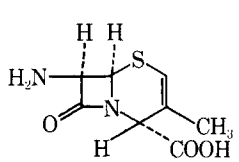
The silylation of 6-aminopenicillanic acid (IV), 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid (V), or 7-amino-3-acetoxymethyl-Δ^3-cephem-4-carboxylic acid (VI), prepared (9, 10) from naturally occurring penicillins and cephalosporins, is important in the commercial synthesis of semisynthetic β-lactam antibacterials (11, 12). Although there are several patents in this field, no analytical or physical data have been reported on these silylated β-lactam amino acids except monosilylated IV (13).



IV



V: R = H
VI: R = $OCOCH_3$



VII

Table I—NMR Quantitative Data

Experimental Condition[a]	Substrate	Silylation, %[b] $100 \times \frac{I_N}{I_O}$	Silylation, %[b] $100 \times \left(1 - \frac{I_A}{2} \times \frac{3}{I_M}\right)$	Solvent[c]
A	IV	15	—	VII
A	V	22	25	VIII
A	VI	16	18	VIII
2A	V	34	30	VIII
B	IV	42	—	VII
B	V	44	41	VIII
B	VI	40	42	VIII
2B	IV	92	—	VII
2B	V	95	—[d]	VIII
2B	VI	88	—[d]	VIII

[a] See *Experimental*. [b] Experiments were carried out at least three times, and the results are reproducible within ±5%. The values represent the percentage of silylation of the amino against the carboxyl group. In the first column, I_N = integration value of $(CH_3)_3SiN$ peak, and I_O = integration value of $(CH_3)_3SiO$ peak. In the second column, I_A = integration value of free amino group, and I_M = integration value of methyl resonance in position 3 for cephalosporin derivatives. [c] VII = deuterochloroform, and VIII = tetrachloroethylene. [d] The amino signal is broadened and the integration is difficult to evaluate due to its small value.

The described NMR method is a rapid and accurate tool to determine quantitatively the extent and ratio of amino and carboxyl group silylation directly in solution, avoiding handling of products sensitive to hydrolysis.

EXPERIMENTAL

Materials—Compounds IV–VI were laboratory reference grade. Deuterochloroform[1] and tetrachloroethylene[2] were dried over phosphorus pentoxide (water content <0.01%); I[3] and II[3] were used without purification.

NMR Measurements—A 60-MHz spectrometer[4] was used at 33°, with benzene as the internal reference (δ 7.37 ppm from tetramethylsilane) to avoid overlapping between tetramethylsilane and trimethylsilyl groups. The NMR spectrum was run at a 500-Hz sweep width and a 250-sec scan time for identification purposes.

For quantitative measurements, the spectrum was run at 50-Hz sweep width in the 0–50-Hz region or at 100-Hz sweep width in the 80–180-Hz region (for methods of calculation, see Table I). These regions were integrated carefully five times. The spectra of disilylated compounds were also run at 100-Hz sweep width in the β-lactam region both at 60 and 100 MHz[5].

Sample Preparations—In a flask protected from moisture by a calcium chloride tube, I (1 mmole, 0.217 ml) (Condition A), a mixture (14) of I (0.66 mmole, 0.145 ml) and II (0.66 mmole, 0.084 ml) (Condition B),

[1] Ciba.
[2] C. Erba.
[3] Dow Corning.
[4] Varian model T 60 A.
[5] Varian model HA-100.

Table II—60-MHz Signals[a] of Monosilyl[b] Derivative Protons (δ)

Substrate	Solvent[c]	NH_2	C-7	C-6	C-5	C-3	C-2		$OSi(CH_3)_3$	$OCOCH_3$
IV	VII	1.83 bs	—	4.38 d[d]	5.48 d[d]	4.47	1.68	1.77	0.5	—
V	VIII	1.68 bs	4.6 d[e]	4.87 d[e]	—	2.16	3.1 d[f]	3.53 d[f]	0.5	—
VI	VIII	1.76 bs	4.66 d[e]	4.88 d[e]	—	4.9 d[g] 5.33 d[g]	3.3 d[f]	3.68 d[f]	0.55	2.15

[a] Singlet if not stated; bs = broad singlet; d = doublet. [b] Condition A; $NSi(CH_3)_3$, as partial resonance at 0.3 δ. [c] VII = deuterochloroform, and VIII = tetrachloroethylene. [d] J = 4.3 Hz. [e] J = 4.5 Hz. [f] J = 18 Hz. [g] J = 13.4 Hz.

or twice these quantities (Condition 2A or 2B) was added to a suspension of IV, V, or VI (1 mmole) in the chosen solvent (2 ml) at room temperature under magnetic stirring.

The mixture was heated at about 64° for IV (with deuterochloroform as the solvent) and at 120° (tetrachloroethylene) for V and VI until a complete solution was attained and then for an additional 0.5 hr (60–90 min total). After cooling to room temperature, a 1-ml sample was put in the NMR tube in a dry box. Alternatively, the solvent was removed *in vacuo* in a rotary evaporator, carefully protected from moisture, to eliminate the last traces of the silylating agents and the solution was remade with fresh dry solvent.

Isomerization of V—Triethylamine (2 mmoles, 0.28 ml) and trimethylchlorosilane (2 mmoles, 0.254 ml) were added to a suspension of V (1 mmole, 0.214 g) in tetrachloroethylene (4 ml) at room temperature under magnetic stirring. The suspension was refluxed for 2 hr. After cooling to room temperature, a 1-ml sample was analyzed by NMR spectroscopy. Besides other peaks, the spectrum showed a doublet (J = 1 Hz) at δ 2.08 ppm and a singlet at δ 2.26 ppm for methyl resonances on a Δ^2- and Δ^3-cephem moiety, respectively (15). By integration of these two peaks, 32.5% of the Δ^2-derivative (VII) was obtained. The same ratio was obtained in a larger scale preparation in methylene chloride. After hydrolysis and filtration, the recovery was practically quantitative[6]; NMR (D_2O + DCl): δ 1.93 (dd, CH_3), 5.0 (m, H_4), 6.08 (m, H_2), 5.0 (d, H_7, J = 4.5 Hz), and 5.3 (d, H_6, J = 4.5 Hz) ppm for VII *versus* 2.25 (s, CH_3), 3.56 (s, CH_2), 5.08 (d, H_7, J = 4.5 hz), and 5.23 (d, H_6, J = 4.5 Hz) ppm for V; [α] + 230° (c = 1, 0.5 *M* HCl).

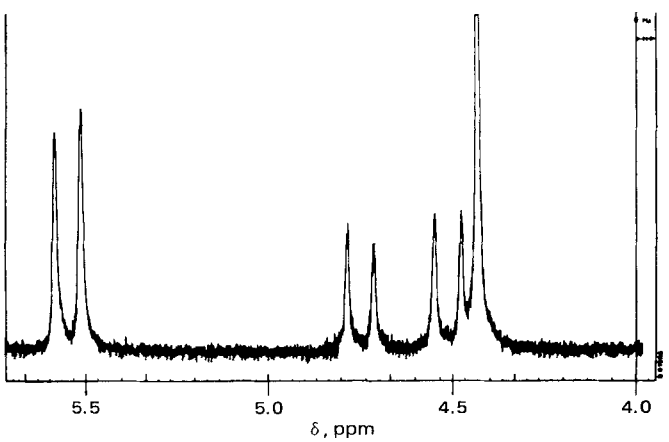


Figure 1—*The 60-MHz NMR spectrum of the β-lactam region of disilylated IV; singlet is the resonance for the 3-proton.*

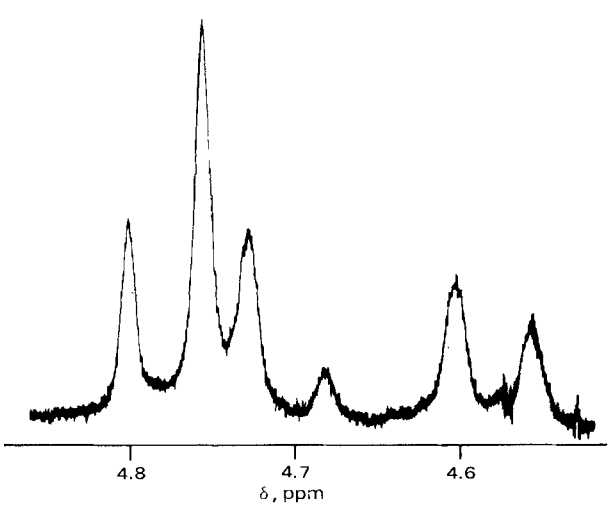


Figure 2—*The 100-MHz spectrum of the β-lactam region of disilylated V.*

RESULTS AND DISCUSSION

Compound I alone or I and II in an equimolar mixture were chosen as the silylating reagents because their low cost and availability in bulk amounts would allow the process to be extended to the industrial stage. In comparison with the β-lactam radicals, their quantity was varied from stoichiometric (Condition A or B) to twice as much (Condition 2A or 2B) to ascertain if further silylation of the amino group was possible. In preliminary experiments on V, the trimethylchlorosilane–triethylamine mixture (11) was discarded because of the ready formation of the Δ^2-cephem derivative (see *Experimental*).

The silylation of the amino against the carboxylic group may be determined quantitatively by the ratio of integrations over *N*- and *O*-trimethylsilyl peaks (δ 0.28–0.30 *versus* δ 0.5–0.55 ppm)[7] after the preliminary removal of I, II, and hexamethyldisiloxane whose signals (δ 0.17, 0.46, and 0.2 ppm, respectively, in deuterochloroform) interfere with the preceding peaks.

With cephalosporin derivatives, however, because there is complete agreement between integrations of the *O*-trimethylsilyl group and the methyl groups in the 3-position (indicating a quantitative silylation of the carboxyl group), the silylation of the amino group can also be calculated through the integrations over resonances of methyl groups and the free amino group (δ 2.16 *versus* about δ 1.66 ppm) without manipulation of the solution. Both methods of calculation gave similar results (Table I). The latter method is not applicable for IV because of the overlapping of free amino and methyl signals.

The three substrates behaved similarly on silylation. While a complete reaction on the carboxyl group was attained in all conditions, the amino group was silylated 15–25% with Condition A[8] and 90% with the more drastic operative conditions (2B), giving rise almost completely to disilylated compounds.

While the NMR signals of the protons of the monosilylated products (Table II) were similar to those of the corresponding amino acids (16, 17) and of their esters (18, 19), the pattern of the peaks for the two β-lactam protons of disilyl[9] derivatives appeared to be anomalous. For IV (Fig. 1), the 5-proton gave a normal doublet at δ 5.54 ppm (J = 5.5 Hz) whereas the 6-proton gave two doublets of equal intensity centered at δ 4.74 and 4.51 ppm (J = 5.5 Hz for both); irradiation of the resonance at δ 5.54 ppm led to the collapse of the doublets to two singlets.

The β-lactam peaks for disilylated V and VI were partially overlapped, being much closer together (also with the signals of the exocyclic methylene protons in VI). Two doublets centered at δ 4.56 and 4.83 ppm (J = 4.8 Hz) and a sharp singlet at δ 4.81 ppm were present in both their spectra at 60 MHz. This anomaly was resolved for V by the 100-MHz spectrum (Fig. 2), showing a doublet for the 6-proton (4.78 ppm) and two doublets of equal intensity (4.71 and 4.58 ppm) for the 7-proton, with a pattern similar to that of disilylated IV (J = 4.7 Hz for all doublets).

The splitting of the proton alpha to the trimethylsilylamino group in two doublets of like intensity might be explained by a HCNH proton–proton coupling (12.6–14 Hz) that is, however, roughly 50% as large as

[6] Analogously, the recovery of mono- and disilylated derivatives was quantitative, giving NMR spectra superimposable with the starting ones.

[7] The assignments of these resonances were made by comparison with the respective ones of 2,2,2-trichloroethyl-7-trimethylsilylamino-3-methyl-Δ^3-cephem-4-carboxylate and trimethylsilyl-7-phenylacetamido-3-methyl-Δ^3-cephem-4-carboxylate silylated with III or I, respectively.

[8] The present results agree with Glombitza's data (13) for IV.

[9] The peaks of the other protons had the same chemical shifts as monosilyl derivatives.

other values reported (16). Its increase is similar to that observed in *N*-trimethylsilylcyclohexylamine (20) between the proton on nitrogen and the cyclohexyl α-proton, indicative perhaps, in the same way, of a probable *trans*-coplanar arrangement of $H_\alpha C$ and NH bonds in these β-lactam derivatives.

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GLC Determination of 6-Aminopenicillanic Acid and 7-Amino-3-methyl-Δ^3-cephem-4-carboxylic Acid

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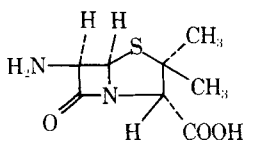
Abstract □ A quantitative GLC determination of 6-aminopenicillanic acid and 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid is presented. The results obtained are in good agreement with those of known chemical procedures. The method is free from interference by related substances.

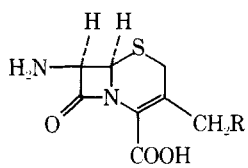
Keyphrases □ 6-Aminopenicillanic acid—GLC analysis, prepared samples □ Cephemcarboxylic acid, substituted—GLC analysis, prepared samples □ GLC—analyses, 6-aminopenicillanic acid and 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid, prepared samples □ Antibacterial intermediates—6-aminopenicillanic acid and 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid, GLC analyses, prepared samples

6-Aminopenicillanic acid (I), 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid (II), and 7-amino-3-acetoxymethyl-Δ^3-cephem-4-carboxylic acid (III) are the key intermediates in the preparation of several semisynthetic β-lactam antibacterials (1). Their characterization can be made from IR (2) and UV (3, 4) spectra or by TLC procedures (5–7), and chemical methods are known for their quantitative analysis (8, 9).

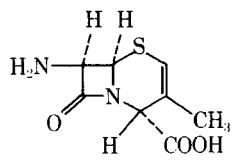
GLC determinations of some penicillins were reported (10, 11), but no procedure on cephalosporin antibiotics has been published. This paper reports the separation and quantitative determination of I and II[1] by GLC after silylation.



I



II: R = H
III: R = $OCOCH_3$



IV

EXPERIMENTAL

Apparatus—A gas chromatograph[2] equipped with a flame-ionization detector, maintained at 200°, was used with gas flow rates of 40 ml/min for hydrogen and 300 ml/min for air. The areas of the peaks were calculated by an electronic integrator[3]. A column oven temperature of 180° with the injector at 220° and nitrogen flow rates of 40 ml/min was used for the analysis of I. For the analysis of II, the column oven temperature was programmed from 160 to 210° with an increase of 6°/min, the injector was at 220°, and nitrogen flow rates were 57 ml/min.

Glass columns, 2 mm i.d. × 2.0 m for the analysis of I or 2 mm i.d. × 0.5 m for II, were packed with 4% OV-17[4] on 80–100-mesh HP Chromosorb

[1] In spite of several trials, III was partially decomposed in the operative conditions.

[2] Perkin-Elmer model 900.
[3] Perkin-Elmer model SIP 1.
[4] Applied Science Laboratories, State College, Pa.