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.

REFERENCES

(1) L. Birkofer and A. Ritter, *Angew. Chem. Int. Ed. Engl.*, **4**, 417 (1965).
(2) A. E. Pierce, "Silylation of Organic Compounds," Pierce Chemical Co., Rockford, Ill., 1968.
(3) C. A. Roth, *Ind. Eng. Chem. Prod. Res. Dev.*, **11**, 134 (1972).
(4) A. E. Pierce, "Silylation of Organic Compounds," Pierce Chemical Co., Rockford, Ill., 1968, pp. 218–221.
(5) L. Birkofer and A. Ritter, *Chem. Ber.*, **93**, 424 (1960).
(6) K. Rühlmann, *J. Prakt. Chem.*, **9**, 86 (1959).
(7) K. Rühlmann, *Chem. Ber.*, **94**, 1876 (1961).
(8) J. F. Klebe, H. Finkbeiner, and D. M. White, *J. Am. Chem. Soc.*, **88**, 3390 (1966).
(9) H. W. O. Weissenburger and M. G. Van Der Hoeven, *Rec. Trav. Chim. Pays-Bas*, **89**, 1081 (1970).
(10) H. W. O. Weissenburger and M. G. Van Der Hoeven, U.S. pat. 3,499,909 (1970).
(11) American Home Products Corp., British pat. 1,201,542 (1970).
(12) Eli Lilly and Co., U.S. pat. 3,694,437 (1972).
(13) K. W. Glombitza, *Ann. Chem.*, **673**, 166 (1964).
(14) S. H. Langer, S. Connell, and I. Wender, *J. Org. Chem.*, **23**, 50 (1958).
(15) E. Van Heyningen and L. K. Ahern, *J. Med. Chem.*, **11**, 933 (1968).
(16) G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, **1965**, 1595.
(17) R. A. Archer and B. S. Kitchell, *J. Org. Chem.*, **31**, 3409 (1966).
(18) R. W. Ratcliffe and B. G. Christensen, *Tetrahedron Lett.*, **1973**, 4649.
(19) T. Jen, J. Frazee, and J. R. E. Hoover, *J. Org. Chem.*, **38**, 2857 (1973).
(20) J. P. Hardy and W. D. Cumming, *J. Am. Chem. Soc.*, **93**, 928 (1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 22, 1976, from *Pierrel S.p.A., Research and Development Laboratories, Via Degli Artigianelli 10, 20159 Milan, Italy.*
Accepted for publication January 18, 1977.
[x] To whom inquiries should be directed.

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

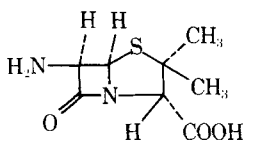
SILVESTRO SILINGARDI, MAURO Di BITETTO, and ALBERTO MANGIA [x]

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.

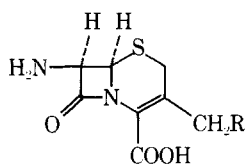
Keyphrases □ 6-Aminopenicillanic acid—GLC analysis, prepared samples □ Cephemcarboxylic acid, substituted—GLC analysis, prepared samples □ GLC—analyses, 6-aminopenicillanic acid and 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid, prepared samples □ Antibacterial intermediates—6-aminopenicillanic acid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

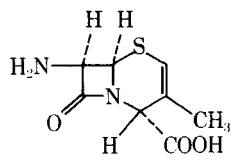
GLC determinations of some penicillins were reported (10, 11), but no procedure on cephalosporin antibiotics has been published. This paper reports the separation and quantitative determination of I and II[1] by GLC after silylation.



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.

Table I—GLC Data

Compound	Relative Retention Time[a] 2-m Column	2-m Column[b]	0.5-m Column	Relative Response Factor	*RSD* of Response Factor, %[c]
Phenanthrene	1.0 (4.2 min)	1.0 (2.3 min)	1.0 (1.3 min)	1.0	—
I	1.29	—	—	1.51	1.78
IV	—	1.92	2.42	1.62[d]	—
II	—	3.24	3.52	1.62	2.07

[a] For column conditions, see *Experimental.* [b] Oven temperature was 200°. [c] Relative standard deviations of response factors were obtained from eight separate preparations of reference standard for I and from six for II. [d] Estimated in a similar manner as II.

W[5]. The column packing was prepared by the standard slurry–filtration method. The columns were conditioned overnight at 300° in nitrogen flow and treated with repeated 10-μl injections of a column conditioner[6].

Reference Standards—Compounds I–III were obtained commercially[7]. 7-Amino-3-methyl-Δ^2-cephem-4-carboxylic acid (IV) was prepared by a known procedure (12).

Silylating Reagents and Silylation Procedures—*6-Aminopenicillanic Acid*—A 50% (v/v) solution of *N,O*-bis(trimethylsilyl)acetamide (V) in pyridine containing 0.82 mg of phenanthrene[8]/ml was prepared. The sample of I was carefully weighed (about 10 mg), and 2 ml of the silylating solution was added to each vial. After sealing, the vials were heated at 60° for 1 hr and allowed to stand at room temperature for 1 additional hr.

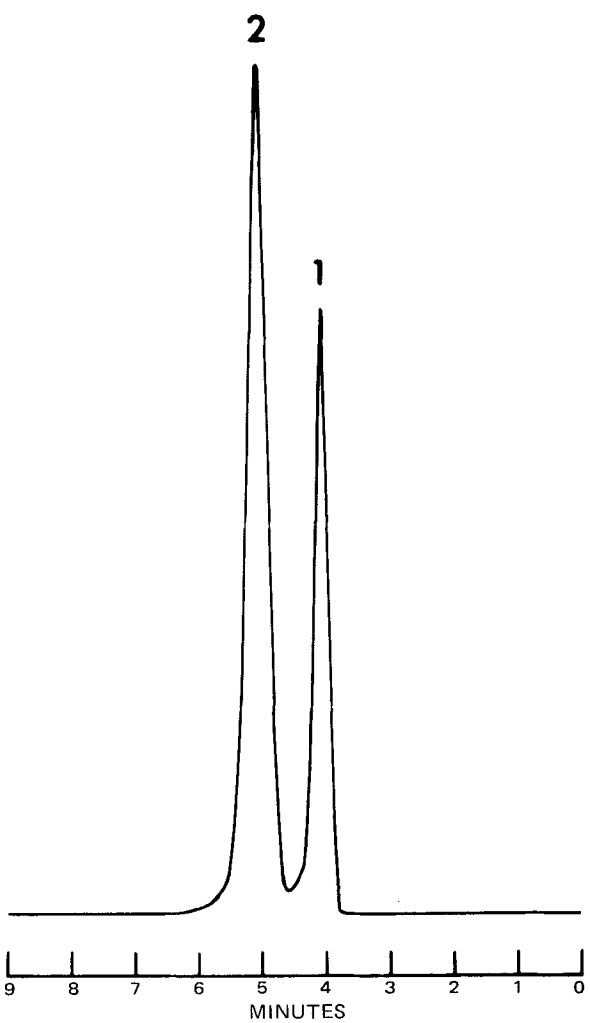


Figure 1—*Chromatogram of I. Key: 1, phenanthrene; and 2, I.*

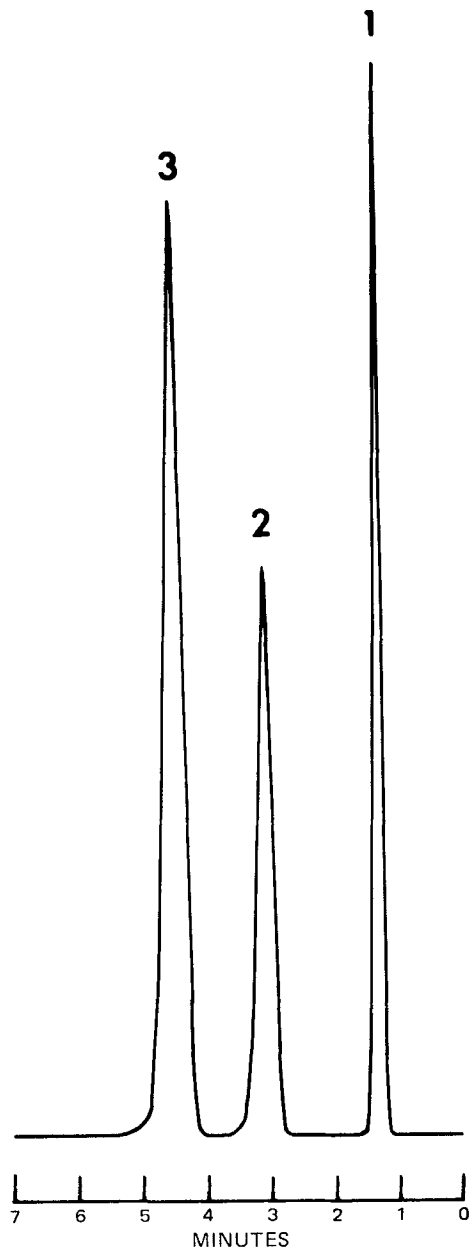


Figure 2—*Chromatogram of II (Sample 8; see Table II). Key: 1, phenanthrene; 2, IV; and 3, II.*

7-Amino-3-methyl-Δ^3-cephem-4-carboxylic Acid—The sample of II was accurately weighed (about 10 mg), and *sym*-dichloroethane (2 ml) containing phenanthrene (1.0 mg/ml), trimethylchlorosilane (328 mg = 0.38 ml), and hexamethyldisilazane (161 mg = 0.21 ml) were added to a 10-ml flask equipped with a reflux condenser. The mixture was stirred with refluxing for 1 hr (complete solution was attained within 20 min) and then cooled to room temperature; V was then added (203 mg = 0.245 ml) with additional stirring for 1 hr. Aliquots of 2 μl of the respective solutions were injected.

RESULTS AND DISCUSSION

Compound V is the best reagent to disilylate amino acids (13) using mild conditions. Derivatization for I was complete at room temperature in about 20 hr in chloroform or pyridine; shorter silylation times were obtained at 60°.

The silylation of II for quantitative purposes was much more difficult because of the ready formation of the Δ^2-compound (IV) (about 8–10%) with the reported conditions and at room temperature or with other

[5] Johns-Manville, New York, N.Y.
[6] Silyl 8, Pierce Chemical Co., Rockford, Ill.
[7] Pierrel Analytical Laboratories.
[8] Schuchardt, München, Germany.

Table II—Comparison of Analytical Data[a] (Percent Purity)

Sample	GLC Procedure	Colorimetric Assay[b]	Iodometric Assay[c]
Compound I			
1	98.7	100.3	98.5
2	99.3	99.5	98.6
3	95.2	96.7	97.5
Compound II			
4	95.2	94.6	90.0
5	88.2	87.7	88.7
6	96.6	93.6	93.3
7	99.7	97.4	94.6
8	57.8[d]	81.5	86.5
9	93.3	93.4	89.3

[a] Standard deviations of known procedures are calculated on our results (average of at least two determinations). [b] For I samples, $\sigma = \pm 1.30\%$. For II samples, $\sigma = \pm 0.96\%$. [c] For I samples, $\sigma = \pm 1.25\%$. For II samples, $\sigma = \pm 3.04\%$. [d] The sample has a content of 29.3% of IV. The ratio of II to IV was 2.08 as determined by NMR spectroscopy *versus* 1.97 as calculated by GLC.

strong silylating agents such as *N,O*-bis(trimethylsilyl)formamide (about 30% of IV), *N*-trimethylsilylimidazole (60% of IV), and *N*-trimethylsilylacetamide (about 10% of IV). In the last two trials, incomplete silylation was obtained.

Hexamethyldisilazane alone or with trimethylchlorosilane[9] does not form IV during derivatization of II (12), which is, however, incomplete. Quantitative silylation on the amino group, which is much more difficult (14) to react than the carboxylic group, was attained in a second step by adding V at room temperature. This procedure leaves the double bond of the dihydrothiazinyl ring unaltered. Furthermore, a shorter column was finally chosen to minimize previously observed decomposition phenomena. The analytical data are reported in Table I.

The analytical results of samples of I and II obtained by GLC (Figs. 1 and 2), compared with the colorimetric determination of Marrelli (9) and with the iodometric assay of Alicino (8), are reported in Table II. The GLC determinations of these substrates are independent of related substances such as IV, which interfere in the chemical methods (see Sample 8, Table II). Furthermore, IV as an impurity can be detected accurately up to 0.5% in samples of II by this GLC method.

The good agreement between the data reported in Table II supports the use of the GLC procedure and indicates that it might supplement the chemical methods.

[9] The 1:3 molar ratio of these two silylating reagents was found to be the most suitable after preliminary experiments.

REFERENCES

(1) E. H. Flynn, "Cephalosporins and Penicillins, Chemistry and Biology," Academic, New York, N.Y., 1972.

(2) B. Casu and P. Ventura, *J. Pharm. Sci.*, **63**, 211 (1974).

(3) R. J. Stedman, K. Swered, and J. R. E. Hoover, *J. Med. Chem.*, **7**, 117 (1964).

(4) Eli Lilly and Co., British pat. 948858 (Feb. 5, 1964).

(5) E. J. Vandamme and J. P. Voets, *J. Chromatogr.*, **71**, 141 (1972).

(6) C. J. Budd, *ibid.*, **76**, 509 (1973).

(7) M. Pokorny, N. Vitezic, M. Japely, and U. Valcavi, *Farmaco, Ed. Prat.*, **31**, 23 (1976).

(8) J. F. Alicino, *Anal. Chem.*, **33**, 648 (1961).

(9) L. P. Marrelli, *J. Pharm. Sci.*, **57**, 2172 (1968).

(10) E. Evrard, M. Claesen, and H. Vanderhaeghe, *Nature*, **201**, 1124 (1964).

(11) C. Hishta, D. L. Mays, and M. Garofalo, *Anal. Chem.*, **43**, 1530 (1971).

(12) F. Bortesi, S. Cavalli, and A. Mangia, *J. Pharm. Sci.*, **66**, 1767 (1977).

(13) J. F. Klebe, H. Finkbeiner, and D. M. White, *J. Am. Chem. Soc.*, **88**, 3390 (1966).

(14) C. A. Roth, *Ind. Eng. Chem. Prod. Res. Dev.*, **11**, 134 (1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 22, 1976, from *Pierrel S.p.A., Research and Development Laboratories, Via Degli Artigianelli 10, 20159 Milan, Italy.*

Accepted for publication January 19, 1977.

The authors thank Professor M. Riva and Dr. C. Pistoja for their encouragement.

[x] To whom inquiries should be directed.

Fluorescence Properties of Glafenine

W. BAEYENS [x] and P. DE MOERLOOSE

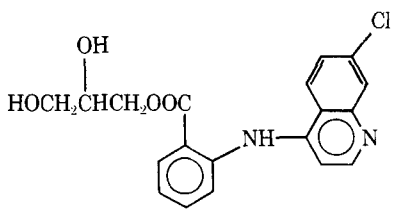
Abstract □ The fluorescence properties of glafenine are partly caused by the anthranilic acid nucleus of the molecule. Correlations are made between fluorescence capacities and UV absorbance. Analytical determinations can be carried out in different solvents, *e.g.*, ether, benzene, and ethanol. Linearity of the emission intensity with the concentration, limiting detectable sample concentrations, and Stokes shifts are reported.

Keyphrases □ Glafenine—fluorescence characteristics in various solvents, effect of pH □ Fluorescence characteristics—glafenine in various solvents, effect of pH □ Analgesics—glafenine, fluorescence characteristics in various solvents, effect of pH

Glafenine[1] (I), 2-[(7-chloro-4-quinolinyl)amino]benzoic acid 2,3-dihydroxypropyl ester, is used frequently for its analgesic properties. It is a pale-yellow powder, mp 165°, and is nearly insoluble in water, slightly soluble in organic solvents, and soluble in aqueous acid solutions. Since this substance contains the anthranilic acid nucleus, the following report is concerned with the question of whether this molecule possesses native fluorescence characteristics.

Anthranilic acid, *o*-aminobenzoic acid, exhibits a strong native fluorescence (λ_{exc} = 300 nm and λ_{em} = 405 nm at pH 7) (1), for which pH dependence (2), decay time (3), and assay in human urine (4) have been reported.

Solutions of glafenine in diluted mineral acids show an intense yellow color but do not fluoresce. In neutral and



I

[1] Glifanan, 200-mg tablets, Roussel-Labunis, France.