Alkaline Degradation Product of Cephradine

ALLEN I. COHEN[▲], PHILLIP T. FUNKE, and MOHINDAR S. PUAR

Abstract □ Cephradine yields an unique alkaline degradation product, which has been identified by proton magnetic resonance, ^{13}C-NMR, and mass spectrometry as 2-[6-(1,4-cyclohexadine-1-yl)-2,5-dioxo-3-piperazinyl]-5,6-dihydro-5-methyl-2*H*-1,3-thiazine-4-carboxylic acid, sodium salt.

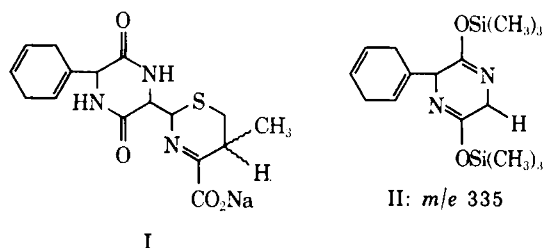
Keyphrases □ Cephradine—structure determination of alkaline degradation product as 2-[6-(1,4-cyclohexadine-1-yl)-2,5-dioxo-3-piperazinyl]-5,6-dihydro-5-methyl-2*H*-1,3-thiazine-4-carboxylic acid, sodium salt □ 2-[6-(1,4-Cyclohexadine-1-yl)-2,5-dioxo-3-piperazinyl]-5,6-dihydro-5-methyl-2*H*-1,3-thiazine-4-carboxylic acid, sodium salt—cephradine alkaline degradation product, structure determination □ Cephem derivatives—structure determination of cephradine alkaline degradation product

The alkaline hydrolysis of penicillins and, more recently, of ampicillin (α-aminobenzylpenicillin) was investigated extensively (1). Similar studies into the aminolysis products of cephalosporins were also reported (2). The structure of the alkaline degradation product of cephradine, a new semisynthetic cephem derived from D-2-(1,4-cyclohexadienyl)glycine and desacetoxy-7-aminocephalosporanic acid (3), is reported here.

EXPERIMENTAL[1]

Materials—Cephradine[2], 7-[D-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hydrate, was prepared by the procedure of Dolfini *et al.* (3). The sample was characterized by NMR and by mass spectrometry as its trimethylsilyl derivative (Fig. 1). Anhydrous sodium carbonate (reagent grade), sodium hydroxide, or 20% sodium deuteroxide in deuterium oxide was used in the degradation studies. Either *N*,*O*-bis(trimethylsilyl)acetamide[3] or trimethylchlorosilane[3] was employed as the silylating agent.

Preparation of Degradation Product—Cephradine (3.0 g.) and sodium carbonate (9.0 g.) were mixed thoroughly. Then 1.6 g. of the mixture was transferred to a vial, 5 ml. of water was added, and the vial was capped and agitated. The vial was stored at 5° for 1 week. The white precipitate that formed was collected on a sintered-glass funnel. The filter cake was washed with five 2-ml. portions of cold water and then with three 1-ml. portions of 95% ethanol and was dried by suction. The crystals were further dried in a vacuum oven at 40° for 3 hr. The mass spectrum of the trimethylsilyl derivative of the isolated products shows a molecular ion of *m/e* 565.2297, corresponding to the addition of three trimethylsilyl groups, $C_{25}H_{43}N_3O_4SSi_3$ (calc. 565.2280). PMR (dimethyl sulfoxide-d_6 and tetramethylsilane): δ 1.06 (3H, d, J = 7 Hz.), 2.95 (2H, m), 5.05 (1H, m, J = 3 Hz.), 2.6 (5H, m), 4.19 (1H, t, J = 3 Hz.) [which, on deuterium exchange, becomes a doublet (J = 3 Hz.)], 4.93 (1H, $w_{1/2}$ = 3 Hz.) (which has a $w_{1/2}$ = 2 Hz. after deuterium exchange), 5.72 (1H), 5.67 (2H), 8.18 (1H, exchangeable), and 8.10 (1H, d, J = 3 Hz., exchangeable); ^{13}C-NMR (dimethyl sulfoxide-d_6; parts per million from tetramethylsilane, δ = 0): δ 16.8, 23.7, 25.7 (2C), 28.7, 39.6 (dimethyl sulfoxide), 58.8, 59.4, 63.8, 122.6, 123.3, 124.2, 130.2, 166.2, 166.7, and 167.7. There are at least 15 carbon atoms; the formula requires 16.



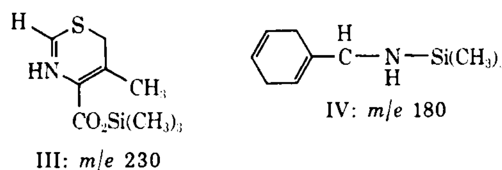
RESULTS AND DISCUSSION

The PMR assignments of the isolated degradation product, dissolved in dimethyl sulfoxide-d_6, are: C-3 methyl δ 1.06 (d, 7 Hz.), vinyl protons δ 5.72 (1H) and δ 5.67 (2H), methylene and C-3 cephem protons δ 2.62 (5H), α-proton of glycyl δ 4.93, and —NH— δ 8.10 and 8.18. The respective chemical shifts of the C-6 and C-7 cephem protons are δ 5.05 (d, 3 Hz.) and 4.19 (t, 3 Hz.); the latter peak becomes a doublet of 3 Hz. coupling on deuterium exchange. The C-2 protons near δ 3 appear as an *ABX* system coupled to the C-3 methine proton. The presence of a methyl doublet and the methine proton at C-3 coupled to the vicinal methylene protons indicates that the double bond has migrated to the C-4,5 position.

The ^{13}C-NMR spectrum was also used to establish the structure of the degradation product. There are 14 peaks (one has an intensity for two carbons), accounting for 15 of the 16 carbons. Because it is a tertiary carbon of an azomethine group, the 16th carbon occurs as a broad resonance due to a longer relaxation time which results from nuclear quadrupole coupling and π-electron polarization. The pulse interval used in the Fourier experiment did not permit sufficient time for complete relaxation. Additionally, the nitrogen quadrupole coupling can give rise to broadened resonances. The three ^{13}C resonances at δ 166.2, 166.7, and 167.7 clearly demonstrate the presence of three carbonyl groups in the alkaline degradation product (4). The resonances at δ 122–130 account for four of the other five carbon atoms bearing double bonds. The five carbon resonances between δ 16 and 29 are the alkyl carbons bearing two allylic double bonds or one electronegative group, whereas the three resonance lines between δ 59 and 64 account for three carbon atoms bearing two electronegative groups and/or two allylic double bonds.

Structure I for the alkaline degradation product is consistent with the NMR data and the formula derived from the mass spectral data. While the NMR data do not permit assignment of the configuration of the C-3 methyl group, the appearance of a single doublet suggests that one product is formed.

The mass spectrum provides additional support for the structural assignment. Trimethylsilylation with chlorotrimethylsilane resulted in the precipitation of sodium chloride. The molecular ion of *m/e* 565.2297 agrees with the formula $C_{25}H_{43}N_3O_4SSi_3$ (calc. 565.2280) (Fig. 2). The most significant fragment ion is the *m/e* 335 ion, II, which demonstrates the presence of a disilyl moiety resulting from the direct cleavage of the C_6—C_7 bond. Cephradine, on the other



[1] Proton magnetic resonance (PMR) spectra were obtained on a Varian Associates XL-100 spectrometer employing the deuterium field-frequency lock system. The ^{13}C-magnetic resonance (^{13}C-NMR) spectrum was taken on a JEOL PS-100 equipped with a Fourier transform pulser and a Nicolet computer. At 25.1 MHz., the effective sweep range was 5000 Hz. (200 p.p.m.). Low- and high-resolution mass spectra were taken on an AEI MS-902 mass spectrometer. Data were acquired *via* a frequency-modulated analog magnetic tape, which was subsequently processed on a Digital Equipment Corp. PDP-11 computer, employing Squibb programs.

[2] Batch NNO11ND, E.R. Squibb and Sons, New Brunswick, N. J.

[3] Pierce Chemical Co., Rockford, Ill.

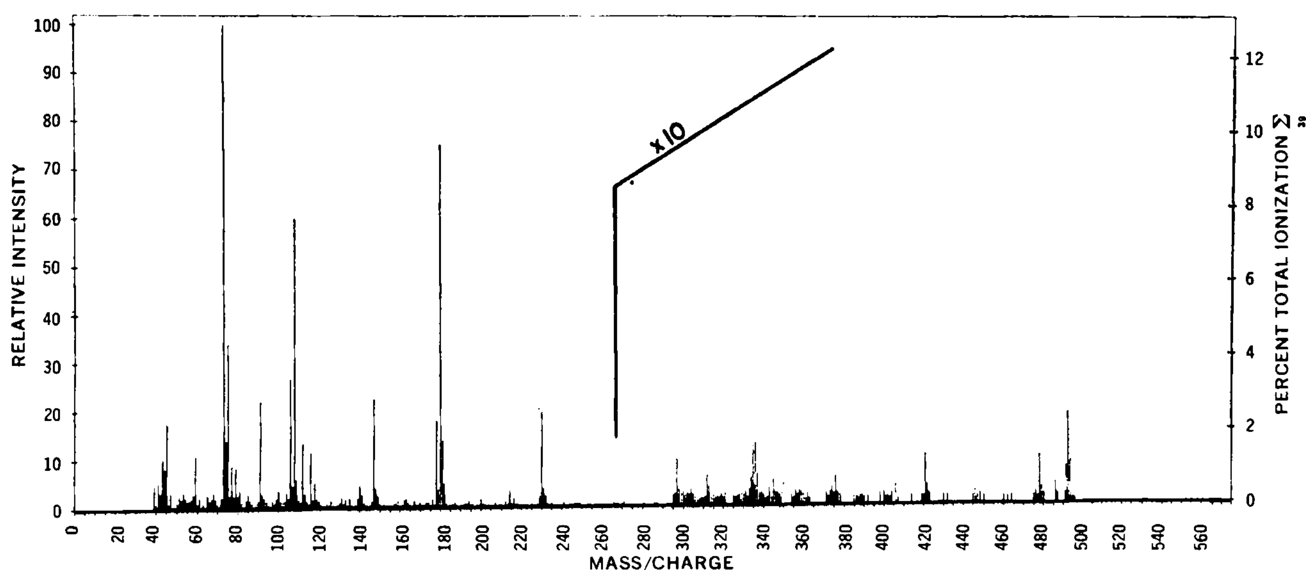


Figure 1—*Low-resolution mass spectrum of the trimethylsilyl derivative of cephradine.*

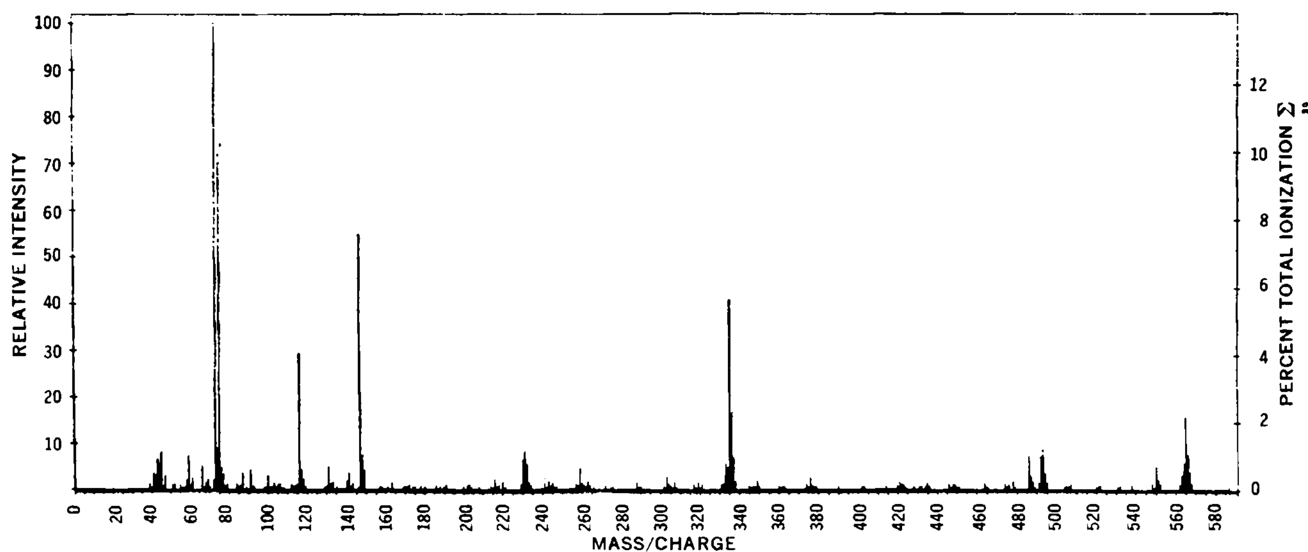


Figure 2—*Low-resolution mass spectrum of the trimethylsilyl derivative of the alkaline degradation product of cephradine.*

hand, was disilylated primarily by *N,O*-bis(trimethylsilyl)acetamide, as demonstrated by the *m/e* 493 ion (Fig. 1), and was also partially trisilylated due to addition to the amide group under the forcing conditions of the trimethylsilylation. Trimethylsilylated cephalosporins yield a *m/e* 230 ion, III, arising through the β-lactam cleavage, proton addition, and cleavage of the C_6—C_7 bond.

The other significant ion in cephradine, at *m/e* 180, produced by the cleavage of the α-carbonyl bond of the trimethylsilylated glycyl portion of the molecule, IV, is absent in the spectrum of the alkaline degradation product. The degradation product exhibits an $M^+ - 79$ ion at *m/e* 486, arising from the cleavage of the dihydrophenyl moiety.

In one continuing program, NMR spectrometry is employed in the determination of stability of drugs in acidic, neutral, and basic aqueous media. The NMR spectrum of a 10-mg./ml. D_2O–NaOD solution of cephradine was determined for 12 hr. at 30° or for 5 hr. at 60°. A progressive decrease in the C-3 methyl proton resonance at δ 1.82 was observed, with the corresponding appearance of a new singlet proton at δ 1.24. When the hydrolysis was performed in water, the methyl proton resonance appeared as a doublet with a coupling constant, *J*, of 7 Hz. That the migration does not occur in the C-2,3 position was demonstrated by the retention of the C-2 methylene proton resonances and, when water was used as the solvent, by the additional vicinal coupling of these protons to the newly introduced methine proton. Thus, double-bond migration to the C-4,5 position occurs with the concurrent introduction, depending on the solvent used, of a proton or of deuterium at C-3 after the cleavage of the β-lactam bond. It appears that the major product[4] of this degradation is identical to the product isolated after treatment with sodium carbonate. A partial tautomerization to the C-3,4 position was demonstrated by redissolving the product isolated from water in D_2O–NaOD and observing the rapid decrease in the C-3 methine proton signal in the PMR spectrum.

The alkaline degradation product represents a unique structure in the penam and cephem series. For example, the glycyl penicillin analog, ampicillin, is reported to yield a penicilloic acid and the decarboxylated derivative (1). Indelicato *et al.* (5) prepared a similar compound, with the retention of the double bond at the C-3,4 position, by refluxing cephalexin in benzene overnight. In a basic aqueous medium, cephradine yields a product that has undergone migration of the double bond to the C-4,5 position and protonation at C-3. Under the same conditions, ampicillin does not yield a cyclic product (1); a cyclic product is not obtained in refluxing benzene either (5). The failure of the cyclization of ampicillin has been attributed to steric effects of the C-2 dimethyl and C-3 methine

[4] Additional studies are being conducted to separate the degradation products formed in acid, neutral, and alkaline media and to elucidate their structures.

groups (5). In cephalosporin studies (2), a double-bond migration was reported only when accompanied by dehydroacetoxylation; otherwise, the double bond remained at the C-3,4 position.

REFERENCES

(1) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, **58**, 447(1969).
(2) J. M. T. Hamilton-Miller, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **116**, 371(1970).
(3) J. E. Dolfini, H. E. Applegate, G. Bach, H. Basch, J. Bernstein, J. Schwartz, and F. L. Weisenborn, *J. Med. Chem.*, **14**, 117 (1971).
(4) E. Brettmaier, G. Jung, and W. Voelter, *Angew. Chem. Int. Ed. Engl.*, **10**, 673(1971).
(5) J. M. Indelicato, T. T. Norvilas, and W. J. Wheeler, *J. Chem. Soc. Chem. Commun.*, **1972**, 1162.

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▲ To whom inquiries should be directed.

Phytochemical Investigation of *Virola peruviana*, A New Hallucinogenic Plant

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Abstract ☐ Although *Virola peruviana* has been described in ethnobotanical studies as being used as a hallucinogen, no proof of the presence of chemical agents explaining this activity existed previously. The present study resulted in the isolation of 5-methoxy-*N,N*-dimethyltryptamine as well as the identification of *N,N*-dimethyltryptamine and 5-methoxytryptamine, thus confirming the hallucinogenic aspect of *V. peruviana*. In addition, myoinositol and the lignans lirioresinol-A dimethyl ether and lirioresinol-B dimethyl ether were isolated and identified. A mixture of *n*-alkanols was isolated and shown to consist of octacosanol, triacontanol, and dotriacontanol, whereas a mixture of sterols was isolated and shown to consist of β-sitosterol, campesterol, and stigmasterol.

Keyphrases ☐ *Virola peruviana*—phytochemical investigation, hallucinogenic constituents isolated and identified ☐ Hallucinogens—isolation and identification of hallucinogenic constituents of *Virola peruviana* ☐ Phytochemistry—isolation and identification of hallucinogenic constituents of *Virola peruviana*

In the Rio Apaporis, Puinave Indians refer to *Virola peruviana* as yá-kee, suggesting its possible utilization in preparing the hallucinogenic snuff by the same name (1). Schultes and Holmstedt (1) suggested that this species may be used as a hallucinogen and determined that it contained alkaloids (1). Other virola species, *i.e.*, *V. theiodora*, *V. rufula*, *V. multinervia*, *V. venosa*, *V. calophylloidea*, *V. calophylla*, and *V. sebifera*, are well documented as hallucinogenic plants, with their active principles being determined as *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine, and/or *N*-methyltryptamine (2–5).

Table I—Chromatographic Separation of Fraction A

Fraction Number	Eluent	Isolate	Yield, mg.
1–34	Benzene	—	—
35–39	Benzene	*n*-Alkanols	26
40–64	Benzene	—	—
65–81	Benzene-acetone (9:1)	Phytosterols	205
82–154	Benzene-acetone (9:1)	—	—
155–169	Benzene-acetone (6:1)	—	—
170	Benzene-acetone (6:1)	Lirioresinol-A dimethyl ether	185
171–174	Benzene-acetone (6:1)	Lirioresinol-B dimethyl ether	216
175–200	Benzene-acetone (6:1)	—	—
201–234	Benzene-acetone (3:1)	—	—
235–245	Acetone	—	—
246–280	Methanol	—	—

The purpose of this investigation was to determine whether *V. peruviana* contained chemical constituents that would explain its use as a hallucinogen.

EXPERIMENTAL[1]

Plant Material—The plant material[2] used represented the bark of *Virola peruviana* (A.DC.) Warburg (Myristicaceae), collected near Leticia, Peru, during October 1968.

Extraction and Fractionation—Coarsely milled bark (4.7 kg.) of *V. peruviana* was defatted with 35 l. of petroleum ether (b.p. 30–60°) using a soxhlet apparatus for 24 hr. The combined extracts were filtered and evaporated to dryness to yield 4.0 g. of Fraction A. The defatted plant material was then exhaustively extracted in the soxh-

[1] All chemicals used were of reagent grade quality. Melting points were determined by means of a Thomas-Hoover apparatus or a Kofler hot plate and are uncorrected. Optical rotations were measured in a Carl Zeiss optical polarimeter. The UV spectra were recorded in methanol using a Beckman model DB-G spectrophotometer. IR spectra were taken using a Beckman model IR-18A instrument. Mass spectral analyses were made using the Hitachi Perkin-Elmer model RMD-6D single-focusing mass spectrometer. NMR spectra were taken using a Bruker model HFX-5 magnetic resonance spectrometer. GC analyses were carried out by means of a Perkin-Elmer model 881 linear programmed temperature gas chromatograph, equipped with a hydrogen flame-ionization detector and a Sargent model SR,S-72180-20 1-mv. recorder; a 1-sec. full-scale response was used. A borosilicate coiled glass column, 1.8 m. (6 ft.) × 2.0 mm. (i.d.), was packed with either 3% OV-1 or 5% OV-101 on 100–120-mesh Gas Chrom Q. Silica gel GF_{254} plates were used for monitoring the chromatographic separation of Fraction A; the solvent system used for development of plates was the same as the eluent used to elute the fractions. The same type plates were used for monitoring the chromatographic fractions from Fraction D, but development was with benzene-ethyl acetate-diethylamine (7:2:1). Spots were visualized after spraying the plates with sulfuric acid (70%) or Ehrlich reagent and heating at 110° for 5 min.

[2] A voucher specimen (2245) was authenticated by Dr. J. Wurdack, Smithsonian Institution, Washington, D. C. A specimen has been deposited at that address.