A New Class of Semisynthetic Penicillins and Cephalosporins Derived from D-2-(1,4-Cyclohexadienyl)glycine

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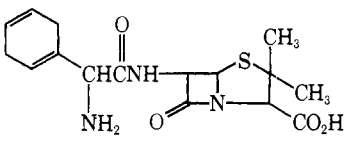
Derivatives of 1,4-cyclohexadienyl-substituted α-amino acids have been made from 6-aminopenicillanic acid, 7-aminocephalosporanic acid, and 7-aminodesacetoxycephalosporanic acid. The compounds exhibit considerable activity against a variety of Gram-positive and Gram-negative organisms *in vitro*.

As part of our study of new semisynthetic penicillins and cephalosporins, we became interested in structures derived from 1,4-cyclohexadienyl-α-amino acids. We were curious to see whether 1,4-cyclohexadienyl structures might exhibit activities different from those of their aromatic analogs. It was expected that the 1,4-cyclohexadienyl ring would be planar, similar, in that respect, to the benzene ring, but yet, chemically much different. Therefore, differences between the aromatic and 1,4-cyclohexadienyl analogs might be expected in their affinities for active sites on enzymes, dependent on the electrophilic and lipophilic properties involved. Contrasting enzyme site affinities may result not only in modified potency but also in differences in patterns of metabolism, absorption, excretion, and tissue penetration.

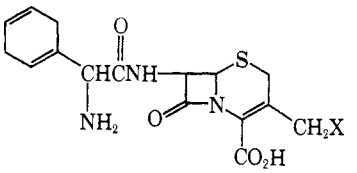
During the studies, several publications appeared describing a similar interest in the comparative properties of systems containing aromatic *vs.* 1,4-cyclohexadienyl structures. For instance, one[1] describes the antimicrobial action of 3-(1,4-cyclohexadienyl)alanine against *Leuconostoc dextranicum* 8086, an effect that can be competitively reversed by phenylalanine. The authors also suggest that the inhibitory action of the cyclohexadienyl compound is due to its structural similarity to the aromatic relative, and propose that the C atoms of the 1,4-cyclohexadiene ring are coplanar, a suggestion substantiated by nmr studies[2] on 1,4-cyclohexadiene and X-ray crystallographic studies[3] on 2-(1,4-cyclohexadienyl)glycine. We synthesized the cephalosporins and penicillins derived from D-2-(1,4-cyclohexadienyl)glycine (I, II, III) for comparison with their aromatic analogs.

D-α-Phenylglycine is commercially available. It was reduced by the well-known Birch reduction technique, without extensive racemization, to provide its D-1,4-cyclohexadienyl analog IV.

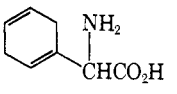
The synthesis of 6-[D-2-amino-2-(1,4-cyclohexadienyl)acetamido]penicillanic acid (I) was achieved by the method of Dane and Dockner,[4] as described in the Experimental Section. The method utilizes an acetoacetic ester enamine derivative of an amino acid to protect it during the coupling process with 6-aminopenicillanic acid (V).



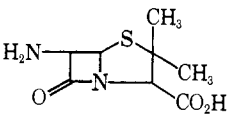
I



II, X = OAc
III, X = H

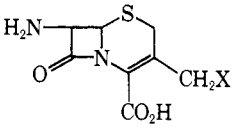


IV



V

7-[D-2-amino-2-(1,4-cyclohexadienyl)acetamido]-cephalosporanic acid (II) was prepared in several steps from 7-aminocephalosporanic acid (VI), and its desacetoxy (III) was prepared from 7-aminodesacetoxycephalosporanic acid (VII). Variations of published procedures[5] which employ the *N-tert*-butoxycarbonyl group to protect an amino acid during the coupling process were used for preparing amphoteric cephalosporins.



VI, X = OAc
VII, X = H

While the physical properties of the cyclohexadienyl compounds cited here are generally similar to those of the aromatic analogs, a distinct trend toward greater basicity should be noted. The pK_2 values for I and III are 7.62 and 7.57, respectively; for both ampicillin and cephalexin the comparable value is 7.24.

The results of a number of *in vitro* comparisons with other penicillins and cephalosporins are listed in Tables I and II. These data were determined in a twofold,

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(2) E. W. Garbisch, Jr. and M. J. Griffith, *J. Amer. Chem. Soc.*, **90**, 3590 (1968).

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(4) E. Dane and T. Dockner, *Angew. Chem.*, **76**, 342 (1964).

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TABLE I

In Vitro MICROBIOLOGICAL COMPARISON OF 6-[D-2-AMINO-2-(1,4-CYCLOHEXADIENYL)ACETAMIDO]PENICILLANIC ACID (I) WITH OTHER PENICILLINS

Organism	Squibb culture No.	Penicillin I	Penicillin G	Ampicillin	Oxacillin
Staphylococcus aureus	1276	0.02	0.01	0.03	0.05
Staph. aureus	2399	0.16	0.03	0.08	0.16
Staph. aureus[a]	2400	>50.0	>50.0	>50.0	0.63
Streptococcus pyogenes	3862	0.004	0.001	0.006	0.012
Salmonella schottmuelleri	3850	0.14	0.6	0.10	>50.0
Pseudomonas aeruginosa	3840	50.0	>50.0	>50.0	>50.0
Ps. aeruginosa	8329	12.5	>50.0	37.5	>50.0
Escherichia coli	2975	0.54	18.7	1.6	>50.0
Candida albicans	5314	>50.0	>50.0	>50.0	>50.0

[a] *Staph. aureus* SC 2400, penicillinase producer.

TABLE II

In Vitro MICROBIOLOGICAL COMPARISON OF 7-[D-2-AMINO-2-(1,4-CYCLOHEXADIENYL)ACETAMIDO]CEPHALOSPORANIC ACID (II) AND ITS DESACETOXY ANALOG (III) WITH OTHER CEPHALOSPORINS

Organism	Squibb culture no.	II	III	Cephaloglycin	Cephalexin
Staph. aureus	1276	0.27	0.27	0.27	0.37
Staph. aureus	2399	3.1	3.1	2.4	3.1
Staph. aureus[a]	2400	15.6	31.2	12.5	37.5
Strept. pyogenes	3862	0.03	0.01	0.01	0.01
S. schottmuelleri	3850	1.6	4.7	0.6	2.4
Ps. aeruginosa	3840	>50.0	>50.0	>50.0	>50.0
E. coli	2975	9.4	18.7	3.1	5.5
C. albicans	5314	>50.0	>50.0	>50.0	>50.0

[a] *Staph. aureus* SC 2400, penicillinase producer.

tube-dilution assay in antibiotic assay broth[6] or brain-heart infusion broth[6] as previously described.[7] It may be seen that these are highly potent, medium-spectrum antibiotics with activities comparable to those of the aromatic analogs. Additional microbiological properties, as well as the chemotherapeutic values of these compounds,[8] will be reported separately.

Experimental Section

Melting points (corrected) were taken on a Kofler hot stage. Proton nmr spectra were obtained on a Varian A-60 instrument, TMS standard. Combustion analyses were in accord with the calcd percentages within ± 0.4% unless otherwise indicated.

D-2-(1,4-Cyclohexadienyl)glycine (IV).—A soln of 11.0 g (72.7 mmoles) of D-phenylglycine in 900 ml of distd NH_3 (which had been treated with 45 mg of Li after distn to remove traces of moisture) was slowly diluted with 370 ml of dry *t*-BuOH. Over a period of 2 hr, 1.65 g of Li was added in small portions until a permanent blue color appeared. The blue reaction mixture was then treated with 38 g of $Et_3N \cdot HCl$. The NH_3 was allowed to evap at room temp overnight, and the residual solvent was evapd at reduced pressure. The white residue was taken up in a small amt of MeOH–H_2O and added to 4 l. of cold 1:1 $CHCl_3$–Me_2CO to precipitate the crude product. After 20 min of stirring, the suspension was filtered, and the white filter cake was dried *in vacuo;* it was pulverized, dissolved in MeOH–H_2O, and submitted once more to the pptn process from 1:1 $CHCl_3$–Me_2CO. A nearly quant yield of white, cryst product, 11.8 g, was obtained: dec 297°; $[\alpha]^{25}D$ − 89.7° (2 *N* NaOH); nmr (NaOD, D_2O) δ 5.83 (b, 3, vinyl), 3.79 (s, 1, HC), 7.30 (b, 4, allylic). Further purification was conveniently performed by *in vacuo* concn of an aq soln of the amino acid in 28% NH_3, resulting in optically pure, white, cryst material: $[\alpha]^{25}D$ − 117.0° (2 *N* NaOH). *Anal.* ($C_9H_{11}NO_2$) C, H, N.

Methyl Acetoacetic Ester Enamine of D-2-(1,4-Cyclohexadienyl)glycine, Na Salt (VI).—Compd IV (306 mg, 2.00 mmoles), was dissolved by warming in a soln of 108 mg of NaOMe (2.00 mmoles) in 4.3 ml of reagent grade MeOH. Methyl acetoacetate (255 mg, 0.24 ml, 2.20 mmoles) was added, and the mixt was refluxed for 45 min. The MeOH was almost totally stripped off *in vacuo*. PhH (5 ml) was added and distd off to a small residual vol. The addition and distn of PhH was repeated to ensure complete removal of the MeOH and H_2O. The product crystd overnight from a small residual vol of PhH. It was filtered off, washed with PhH, and dried *in vacuo*, yielding 463 mg, mp 66–68°. This material was used without further purification.

6-[D-2-Amino-2-(1,4-cyclohexadienyl)acetamido]penicillanic Acid (I).—A soln of 358 mg (1.66 mmoles) of V and 0.23 ml of Et_3N in 2.5 ml of H_2O was prepared, with the final pH being 7.4; 0.85 ml of Me_2CO was added, and the soln was kept at −10°. A soln of 469 mg of methyl acetoacetate enamine of D-2-amino-2-(1,4-cyclohexadienyl)acetic acid, Na salt (1.72 mmoles) in 4.25 ml of Me_2CO was chilled to −20°. A microdrop of *N*-methylmorpholine was added, followed by the slow addition of 198 mg of ice-cold ethyl chloroformate. H_2O (0.43 ml) was added, and a turbid soln resulted. The mixt was stirred for 10 min at −20°. The turbid soln of mixed anhydride was then added to the 6-APA soln. The clear soln observed was stirred for 30 min at −10°, then raised to room temp, and acidified to pH 2.0 with dil HCl, and, with good stirring, the pH was kept at that level for 10 min. The soln was then extd with 5 ml of xylene. The aq phase was layered with 5 ml of MeCO–*t*-Bu, and the pH was adjusted to 5.0 with 1 *N* NaOH and chilled overnight. The resulting crystals were filtered off, washed with H_2O, and air-dried to yield 272 mg (44%), dec 202°, iodometric penicillin titration,[9] 97.4%. *Anal.* ($C_{16}H_{21}N_3O_4S \cdot 0.5H_2O$) C, H, N, S.

D-2-[*N*-(*tert*-Butoxycarbonyl)amino]-2-(1,4-cyclohexadienyl)acetic Acid (VIII).—A soln of 30 g (0.201 mole) of IV in 201.5 ml of 1 *N* NaOH was diluted with 100 ml of H_2O and 300 ml of 95% EtOH. To this was added 28.8 g (20.28 ml, 0.201 mole) of *N*-*tert*-butoxycarbonyl azide, with no significant rise in temp. The soln was mixed for 16–18 hr. A total of 13.9 g of unreacted amino acid pptd during this period. After filtration, the white cryst material was washed with H_2O, and the wash was combined with the first filtrate. The aq soln was diluted with 100 ml of H_2O, and the pH was lowered from 8.5 to 2, using about 46 ml of 6 *N* HCl. The ppt was washed thoroughly with H_2O yielding 24.6 g (49%) of white crystals, mp 64–67°. *Anal.* ($C_{13}H_{19}NO_4$) C, H, N.

7-[D-2-[*N*-*tert*-Butoxycarbonyl)amino]-2-(1,4-cyclohexadienyl)acetamido]desacetoxycephalosporanic Acid (IX).—A soln of 4.77 g (19.08 mmoles) of VIII in 100 ml of THF containing Et_3N (1.96 g, 2.7 ml) was chilled to −10°. With good agitation, isobutyl chloroformate (2.57 g, 2.6 ml) was added over a period of 2 min; $Et_3N \cdot HCl$ pptd from the soln. The slurry was mixed at −5° for 15 min. To this was added a cold soln of 4 g (18.68 mmoles) of VII in 70 ml 50% THF–H_2O contg 1.9 g (2.65 ml) of Et_2N. The temp was kept between 0 and −10° for 1 hr. A clear soln resulted when the temp was allowed to rise to 25°. Agitation was continued for an additional 2 hr, then 100 ml of H_2O was added, and the soln was extd 3 times with 75 ml of EtOAc. The aq fraction was layered with 100 ml of EtOAc, and the pH was lowered to 3, using 6 ml of 3 *N* HCl. During this extn, some solids formed, and separation of the layers became difficult. It was necessary to filter the mixture to continue the extn with two 50-ml aliquots of EtOAc. The combined EtOAc fractions were dried (Na_2SO_4) before being concd on a rotary evaporator. The result was a dry, yellow foam weighing 5.37 g (64%), which was used without further purification.

7-[D-2-Amino-2-(1,4-cyclohexadienyl)acetamido]desacetoxycephalosporanic Acid, Hydrate (III).—At 0°, 10 ml of cold F_3CCO_2H was added to 5.37 g of IX. Swirling the flask for several min resulted in a clear soln, which was kept at 25° for 15 min before stripping off the excess acid. The clear residue was triturated with Et_2O and mixed very well to form small colorless crystals. This product was slightly hygroscopic and was filtered under dry N_2. The crystals were washed with Et_2O. The yield was 4.15 g, 79.1%, mp 140–142° dec. A satisfactory anal. was

(6) Supplied by the Baltimore Biological Laboratories.

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(9) J. F. Alicino, *Chem. Eng. News*, 648 (1961).

not obtained. A soln of 7.88 g of the CF_3COOH salt in 350 ml of H_2O required filtration to remove a small amt of insol material. The pH of this clear soln was raised from 2 to 5 by gradually adding about 50 g of Amberlite IR4B resin that had been washed several times with H_2O. Good agitation was necessary, and the time required for conversion was about 10 min. Darco (1 g) was added to the soln after filtration and mixed in for 5 min. The light yellow soln resulting from this treatment was concd on a rotary evaporator using a vacuum pump. The concn was stopped when crystn of the product began, at a vol of approx 25 ml. Storage in the cold for several hours, followed by the addition of 150 ml of EtOH with strong agitation, led to the formation of small particles that were easily filtered. The product was washed with EtOH and dried *in vacuo* at 45–50°. The anhyd product absorbed 1 mole of H_2O from the atm. The yield was 3.56 g (58%) of colorless crystals; iodometric assay, 97.9%, as monohydrate. A second crop (0.77 g) was obtained by concn of the mother liquor. *Anal.* ($C_{16}H_{19}N_3O_4S \cdot H_2O$) C, H, N.

7-[D-2-Amino-2-(1,4-cyclohexadienyl)acetamido]cephalosporanic acid (II) was prepd in the same manner as III using VIII and isolated as light tan crystals: mp 265–270° dec. *Anal.* ($C_{18}H_{21}N_3O_6S \cdot H_2O$) C, H, N.

Acknowledgment.—We are indebted to Joseph Alicino and Joseph Hydro and to Mrs. Mary Young for analytical data, to Dr. Allen Cohen and Miss Barbara Keeler for spectroscopic studies, and to Dr. Harold Jacobson for p*K* determinations.

Irreversible Enzyme Inhibitors. 180.[1,2] Irreversible Inhibitors of the C'1a Component of Complement[3] Derived from *m*-(Phenoxypropoxy)benzamidine and Phenoxyacetamide

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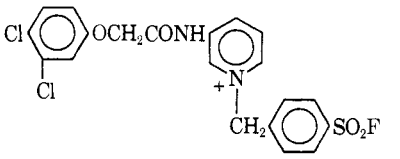
A new assay for irreversible inhibition of the C'1a component of complement has been established. A series of substituted pyridines quaternized with fluorosulfonylbenzyl bromide related to **1** in structure were previously shown to be good inhibitors of whole guinea pig complement; many of these compounds are excellent irreversible inhibitors of the C'1a component of complement. The good correlation in irreversible inhibition of C'1a and inhibition of whole complement by analogs of **1** strongly suggests that the main site of action by compounds of type **1** is inhibition of C'1. In contrast, the lack of correlation of irreversible inhibition of C'1a and inhibition of whole complement by benzamidines of type **5** strongly suggests that the main site of action of the benzamidines is one of the other 8 components of complement. *m*-[*m*-(*p*-Fluorosulfonylphenylureido)phenoxypropoxy]benzamidine (**5**) is the most potent inhibitor of guinea pig complement yet observed; **5** is about 1000 times as potent as benzamidine and 3000 times as potent as *N*-tosyl-L-arginine Me ester (TAME).

Inhibitors of the serum complement system could have medicinal utility for organ transplantation and in treatment of some arthritic states.[4,5] The serum complement system is a mixture of 11 distinct proteins.[5–7] One of the functions of complement is to kill foreign cells such as bacteria and protozoa; however, it can also lyse foreign mammalian cells and causes rejection of organ transplants.[5–7] Some of the proteins of the complement system are proteases with "tryptic" or "chymotrypic" properties;[5–7] therefore it is not surprising that complement is inhibited by certain inhibitors of trypsin[4] or chymotrypsin[8,9] when measured by the lysis of sheep red blood cells (RBC) by guinea pig complement and antibody.[4,10]

Two types of chymotryptic inhibitors of complement have emerged from this laboratory as exemplified by **1**[8]



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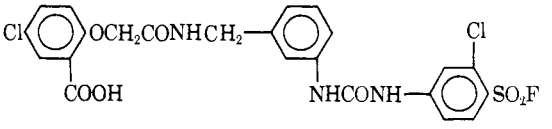

2

and **2**;[9] in both cases, removal of the SO_2F moiety resulted in loss of their activity, indicating that the SO_2F group was necessary for activity, presumably by irreversible inhibition[11] of one of the complement enzymes. That **1** was an irreversible inhibitor of the C'1a component of complement was shown by Becker.[8]

Benzamidine, a strong trypsin inhibitor,[12,13] is a weak inhibitor of complement;[4] inhibition is enhanced 6-fold by introduction of a *m*-phenoxypropoxy substituent (**3**)[4] which is further enhanced to 400-fold by substitution of *m*-(*p*-nitrophenylurea) on the phenoxy moiety (**4**)[3] (Table III). Notable is the fact that **3** and **4**, which are most probably reversible inhibitors in con-

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series see B. R. Baker and W. T. Ashton, *J. Med. Chem.*, **13**, 1165 (1970).

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