tagonizes strychnine lethality.[7-9] In the present series myorelaxant activity was present in those compounds with depressant activity. At 100 mg/kg (oral and ip) **1** and **2** completely protected[10] against strychnine-induced convulsions while **3**, **4**, and **5** protected only one of four experimental animals. At 500 mg/kg (ip) **1** and **2** partially protected all and completely protected three of four test animals. Although **1** and **2** were shown by comparison experiments to have the same order of potency as meprobamate, the sedative properties of the former compounds preclude any pointed consideration of their muscle relaxant properties.

None of the compounds in this series antagonized the parkinson-like tremors induced by tremorine-[11,12] or pentylenetetrazole-induced convulsions.

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Isolation and Identification of Cephalexin from Human Urine

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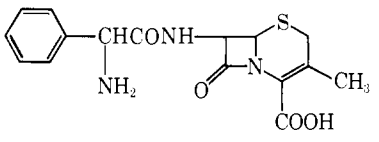
Cephalexin, 7-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid is a broad-spectrum cephalosporin antibiotic. The oral administration of



doses of cephalexin in mice[1] as well as in human volunteers[2] demonstrated nearly complete absorption, high serum and urine concentration, and antibacterial activity. Experiments conducted on cephalexin-^{14}C in rats and mice[3] indicated that unchanged cephalexin-^{14}C was the only radioactive substance appearing in urine or bile. Absorption and excretion at the administered dose level was also essentially quantitative. Human urine after single cephalexin doses was shown *via* paper chromatography–bioautography techniques[4] to contain only one substance active against *Sarcina lutea* with an R_f value identical with that of cephalexin. Since the criteria of the chromatography–bioautography are not necessarily specific, it was desirable to secure definitive evidence that the antibiotic was excreted unchanged. This paper reports the direct isolation and complete identification of cephalexin from urine after single cephalexin doses.

The urine samples were obtained from a group of ten normal volunteers, who after fasting overnight were given an oral dose of 500 mg of cephalexin. Urine specimens were then collected during 0–6 hr after drug administration, the total volumes were measured, and all aliquots were frozen for microbiological assay *via* the cup–plate method using *Sarcina lutea* to establish the cephalexin concentration and the total cephalexin contained in urine as shown in Table I.

TABLE I[a]

	Urine		
Volunteer no.	Volume, ml	Cephalexin, μg/ml	Total cephalexin, mg[b]
1	190	2700	533
2	370	1620	598
3	450	1225	551
4	195	1260	209
5	420	1340	563
6	295	150	44
7	600	917	550
8	290	1995	578
9	285	1890	539
10	360	1480	533
Average		1458	470

[a] Many groups of normal volunteers' urine samples had been tested; this table was used as a typical example. The oral dose was 500 mg. [b] Actual dose of cephalexin in the 250-mg capsule was 265 ±11 mg.

To isolate the cephalexin excreted in the urine and establish its identity, ion-exchange chromatography was employed. The cephalexin in urine can be isolated *via* its anion using AG2-X8 resin in acetate form or *via* its cation using Dowex 50 W-X2 resin in sodium form. However, the anionic resin was better than the cationic resin since no complication by salt formation was encountered. The eluted fractions from the column were followed by means of the ninhydrin test, uv absorption maximum at 262 mμ,[5] paper chromatography–bioautography, and microbiological assays. Only those fractions with a positive ninhydrin test and highest absorbance (A) were combined and used for crystallization. Two crystalline forms of cephalexin, namely, cephalexin hydrochloride and cephalexin anhydrate (acetonitrile solvated) were obtained, and their identity was established by ir, uv, and nmr spectra and by direct comparison of X-ray diffraction patterns with respective authentic samples. In addition, tlc also revealed that it had the same R_f value as cephalexin, and bioautography also indicated that their activities against *Sarcina lutea* were comparable.[6] The fractions with lower absorbance at 262 mμ were not used for crystallization of cephalexin. Instead they were assayed to establish the total content of cephalexin in all the fractions from the column as shown in Table II. The total content of

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(6) The microbiological assay of the isolated cephalexin anhydrate using the cup–plate method had an average value of 1045 μg/mg, compared with standard sample of cephalexin at 1000 μg/mg.

TABLE II

Fractions	Vol, ml	pH of effluent	Max, mμ	Optical ratio	Absorbance at λ max	Calcd[a] concn, mg/ml	Calcd[b] total content, mg	Assayed concn, μg/ml	Total content, mg
I	43	8.5							
II	8	8.0	232 and 262 (sh)	20	0.52	0.60	1.8	620	5.0
III	1	5.0	262	100	0.67	3.85	15.4	2580	11.3
IV	8	3.0	262	50	0.57	1.64	13.1	1760	12.1
V	8	3.0	262	10	0.63	0.36	2.9	436	3.5
VI	8	2.0	262	2	0.60	0.08	0.6	119	1.0

[a] Column 5 × column 6/slope (17.4). [b] Column 7 × column 2. [c] Column 9 × column 2.

the cephalexin in these fractions was also calculated from the slope[7] of a standard curve of concentration of cephalexin *vs.* absorbance at the 262-mμ peak. From columns 8 and 10 in Table II, it is apparent that the assay value and the calculated value do adequately represent the level of cephalexin in urine and cephalexin was truly excreted unchanged.

Experimental Section

Preparation of the Ion-Exchange Column.—The anionic resin, Bio-Rad AG2-X8 (100–200 mesh, 10 g) was transferred from the Cl^- form to the ^-OH form by treating with 200 ml of 1 *N* NaOH. It was then washed free of alkali with deionized H_2O to about pH 6.7 and poured into a 1.0 × 30 cm column. The resin was back washed to arrange the particles according to sizes and the flow rate was adjusted to 2 ml/min (0.14 ml/ml of wet resin per min). AcOH (1 *N*) was then run through the column until the effluent was pH 2.5 or less. It was then washed free of AcOH with H_2O and then with 0.1 *N* NaOAc until the effluent was pH 8.2. The column was ready for the experiments described below.

Separation and Identification of Cephalexin Excreted from Urine.—A human volunteer's urine sample (450 ml, microbiological assay[1] 1140 μg/ml, total content 513 mg) collected in the first 6 hr after oral administration of two 250-mg capsules of cephalexin was chilled and centrifuged. To an aliquot of 50 ml of the supernatant was added 20 mg of urease and the solution was stirred vigorously. The pH of the solution was raised after stirring for a few minutes and brought back continuously to the isoelectric point of cephalexin at pH 4.5 with the dropwise addition of concentrated HCl until the pH no longer was altered. The solution was centrifuged and the supernatant was poured into the ion-exchange column prepared above. It was then eluted with 1 *N* AcOH and the various fractions were collected as shown in Table II.

The fractions were evaluated by the following tests: uv maxima at 262 mμ, paper chromatography–bioautography,[8] and tlc.[9] The results of uv absorptions and microbiological assays are shown in Table II. The tlc (R_f 0.67) and bioautography (R_f 0.60) of fractions II–VI showed only one active spot against *S. lutea*.

Fractions III and IV were combined and evaporated to dryness under vacuum at room temperature. The solid residue was crystallized in dilute HCl–MeCN to give 13 mg of crystalline cephalexin anhydrate (MeCN solvated).

In a separate experiment, the *S. lutea* active eluents from the column were evaporated to dryness and crystallized in *n*-PrOH–concentrated HCl to give cephalexin hydrochloride in crystalline form. Both the cephalexin anhydrate and cephalexin hydrochloride were identified by ir, uv, and nmr spectra and by direct comparison of X-ray diffraction patterns with respective authentic samples.

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(7) The slope of the standard curve was 17.4 [A (ml)/mg].

(8) The sample (2–5 mg) was spotted on a strip of Whatman No. 1 paper (46.4 × 19 cm) and was eluted by a descending solvent system composed of BuOH–AcOH–H_2O (3:1:1). After the solvent front had reached about 2.54 cm from the other end of the paper it was air dried and laid on top of an agar plate seeded with suitable microorganism for 10 min before the plate was incubated overnight. The R_f values of the antibiotics were calculated from the clear zone shown on the plate. Both cephalexin standard and the urine sample before and after the column chromatography showed an identical spot with R_f 0.60.

(9) The tlc utilized the Brinkmann precoated silica gel plates (plastic sheets were used when a bioautograph was needed) with fluorescent indicator and the solvent system was MeCN–H_2O (3:1). The cephalexin standard and the urine fractions showed one spot with R_f 0.67 in tlc.

Diamides of Cyclobutane-1,1-dicarboxylic Acid

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We have reported several diimides of cyclobutane-1,1-dicarboxylic acid to be inactive either as general CNS depressants or potentiators of barbiturate sedation.[1] Of the compounds studied only cyclobutane-1,5-spiro-2,6-diketo-4-thiohexahydropyrimidine was active. Its effect was that of barbiturate potentiation. This compound has prompted consideration of various other cyclobutane-1,1-dicarboxylate derivatives and at this time we wish to report studies on some of its simple diamides.

With the exception of compounds **6**–**8** all members of the series are di-*N*-alkylamides.

Bioassay of **2** and **4** for general sedative effects was carried out using intraperitoneal injection of the drugs.[2] At 1000 mg/kg no decrease in spontaneous activity nor any signs of toxicity were seen. However, when given orally as 1% suspensions in gum tragacanth[3] both caused decreases in spontaneous activity and responsiveness to irritant stimuli. Consequently, all testing on remaining members was done using oral administration of the drugs. Compounds **1**, **3**, and **5** showed noticeable depressant activity (Table I). Thus, all of the simple alkyl amides were shown to have a sedative effect. However, the alicyclic and aromatic amides were inactive.

When analyzed for pentobarbital-potentiating activity[3-5] at a dose of 500 mg/kg, **2**–**5** were clearly active. A sleep prolongation factor, R' (Table I), was used as the criterion for effect. For compounds not causing loss of the righting reflex, R' is a measure of

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