TABLE II

Fractions	Vol, ml	pH of eluents	Max, mμ	Dilution ratio	Absorbance	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.

Acknowledgments.—I am grateful to Drs. Edwin R. Shepard and Richard T. Rapala for valuable suggestions and stimulating discussions, to K. S. Yang for his help in the crystallization of cephalexin, to H. W. Smith for carrying out the X-ray diffraction work, and to Patricia Hughes for technical assistance.

(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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Received April 22, 1969

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

(1) K. A. Zirvi and C. H. Jarboe, *J. Med. Chem.*, **11**, 183 (1968).
(2) R. T. Buckler and C. H. Jarboe, *ibid.*, **9**, 768 (1966).
(3) K. A. Zirvi and C. H. Jarboe, *ibid.*, **12**, 923 (1969).
(4) C. A. Winter, *J. Pharmacol. Exptl. Therap.*, **94**, 7 (1948).
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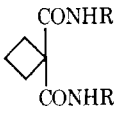
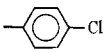
TABLE I: DIAMIDES OF CYCLOBUTANE-1,1-DICARBOXYLIC ACID



No.		Yield, %	Mp, °C[a]	Crystn solvent[b]	Formula	Analyses	Gross effect[c]	R'[d]
1	CH_3	50	204	A	$C_8H_{14}N_2O_2$	N	S	1.1
2	$(CH_2)_2CH_3$	56	145	A	$C_{12}H_{22}N_2O_2$	N	S	8.8
3	$(CH_2)_5CH_3$	50	79	P	$C_{18}H_{34}N_2O_2$	N	S	1.9
4	$CH(CH_3)_2$	40	195	A	$C_{12}H_{22}N_2O_2$	N	S	2.3
5	$C(CH_3)_3$	45	195	P	$C_{14}H_{26}N_2O_2$	N	S	2.3
6		70	212	A	$C_{18}H_{30}N_2O_2$	N	N	1.2
7		65	232	A	$C_{20}H_{34}N_2O_2$	N	N	0.9
8		75	258	A	$C_{18}H_{16}Cl_2N_2O_2$	N	N	1.2

[a] Corrected. [b] A, acetone; P, pentane. [c] Drug dose was 1000 mg/kg orally. N = no effect, S = slight reduction in spontaneous motor activity, subjectively graded and compared with control animals receiving medium only. [d] Drug dose was 500 mg/kg orally. R' = (drug + pentobarbital sleep time)/(pentobarbital sleep time). Pentobarbital dose, 50 mg/kg ip administered 30 min after test drug.

true potentiation and becomes significant when greater than 1.5. The fact that **1** is active as a depressant but inactive as a barbiturate potentiator lends some support to the suggestion that there is functional independence of sites for the two types of activity.[3]

Two of the compounds, **1** and **2**, were also tested for anticonvulsant[6] (pentylenetetrazole antagonism), antistrychnine lethality,[7] and antitremorine[8] effects.[3] Neither showed antagonism to pentylenetetrazole- or strychnine-induced convulsions. However, they completely protected 40–60% of the test animals from tremorine-induced tremors at an oral dose of 1000 mg/kg.

Experimental Section

Microanalyses were performed by Midwest Microlab Inc., Indianapolis, Ind. Where analyses are indicated only by elemental symbols, analytical results for those elements were within ±0.4% of theoretical values.

Preparation of Diamides.—The methods used in synthesizing diamides of cyclobutane-1,1-dicarboxylic acid were essentially the same as those reported for producing diimides of that acid.[1] Crystallization solvents and yields are reported in Table I.

Acknowledgments.—This work was made possible by research Grant NB-7548 of the National Institutes of Health, U. S. Public Health Service.

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Substituted Chromones as Coronary Vasodilators

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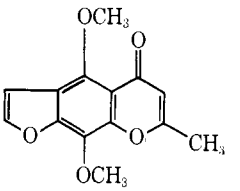
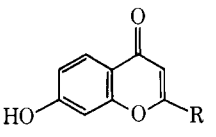
Received January 13, 1969

Khellin or 2-methyl-5,8-dimethoxyfuranochromone (I) and some of its analogs are known to be coronary vasodilators.[1-3] This paper describes the synthesis and pharmacological properties of a number of new substituted chromones (III–XII) which were prepared as potential coronary vasodilators (Table I). In addition to the fact that III–XII share the characteristic features of active chromones,[2,3] these compounds could also be considered khellin analogs in which the furane ring of the khellin molecule (I) is not a part of the rigid furanochromone structure, but instead is attached to the chromone molecule through an ether linkage as in VI, VII, and XI.



I

Chemistry.—2-Furyl-7-hydroxychromone (II, R = 2-furyl) was synthesized from 2,4-dihydroxyacetophenone and 2-furoyl chloride by a standard three-step procedure.[4] The synthesis of 2-aryl-7-hydroxychromones (II, R = C_6H_5 and $C_6H_4OCH_3$-4) is already described in the literature.[5]



II

The chromones II (R = C_6H_5, $C_6H_4OCH_3$-4, and 2-furyl) were treated with various chloromethyl intermediates and acid chlorides to give the desired compounds III–XII.

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