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Preparation, Hydrolysis, and Oral Absorption of α-Carboxy Esters of Carbenicillin

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Twelve α-carboxy esters of carbenicillin, a parenteral broad spectrum semisynthetic penicillin, were synthesized and examined as potential oral carbenicillin derivatives. The rates at which the esters were hydrolyzed *in vitro* to carbenicillin by animal and human tissues were compared and the carbenicillin serum levels arising after oral administration of the esters were measured in squirrel monkeys and human volunteer subjects. The α-carboxyphenyl ester of carbenicillin [carfecillin (British Pharmacopoeia approved name), BRL 3475] was selected for further study and is presently undergoing clinical trial.

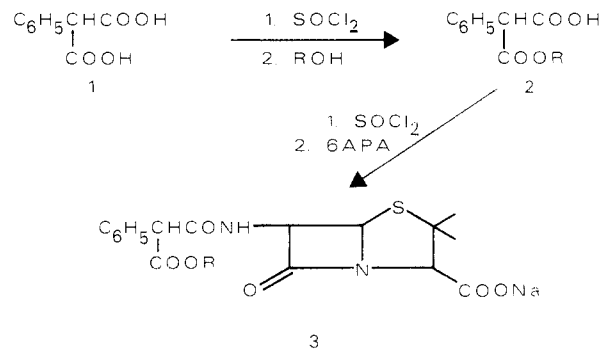
Carbenicillin (α-carboxybenzylpenicillin) has a broad spectrum of antibacterial activity and has been shown to be effective in the treatment of serious infections caused by *Pseudomonas aeruginosa* and other gram-negative bacteria. However, the compound is poorly absorbed from the gastrointestinal tract after administration by the oral route and its use is limited to parenteral administration. It has been found that certain α-carboxy esters of carbenicillin are absorbed by the oral route and undergo hydrolysis in the body to liberate carbenicillin.[1] One such ester (carbenicillin indanyl sodium) has been described in detail.[2]

Carbenicillin α-carboxy esters (that is, the ester group is in the side chain) possess a free thiazolidine carboxyl group and, hence, can be expected to demonstrate antibacterial activity *per se*. In general, we and others have found that the unhydrolyzed ester is more active than carbenicillin against gram-positive bacteria but shows a lesser degree of activity against most gram-negative bacilli.[3] However, the antibacterial spectrum shown by this class of compound will depend upon the extent of hydrolysis to carbenicillin that occurs in the course of the antibacterial test. Esters that are hydrolyzed rapidly to carbenicillin show an antibacterial spectrum similar to that of carbenicillin while esters with a slow rate of hydrolysis demonstrate activity primarily against gram-positive bacteria. In practice carbenicillin esters are of clinical interest only as a means of providing carbenicillin activity in the body after oral administration and in this context their intrinsic activity may be disregarded.

This report describes the preparation, *in vitro* hydrolysis rates, and oral absorption characteristics of a group of 12 α-carboxy esters of carbenicillin.

Chemistry. Scheme I illustrates the general route used to prepare α-carboxy esters of carbenicillin.[4] Phenylmalonic acid (1) was converted to its monoacid chloride by treatment with 1 equiv of thionyl chloride. The crude product was allowed to react with 1 equiv of alcohol or phenol (ROH) to afford the half-esters 2 described in Table I. The crystalline half-esters 2 were converted to their acid chlorides with excess thionyl chloride at 70° (higher temperatures caused degradation) and coupled directly to 6-aminopenicillanic acid (6-APA) in aqueous acetone. The penicillins 3 were isolated as their sodium salts and are described in Table II. A variation of this procedure has also been used to prepare penicillins of this type.[5]

Scheme I



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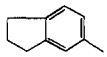
The α-carboxy esters, listed in Table II, as in the case of carbenicillin, contain an asymmetric center in their side chains and in solution their nmr spectra (Table III) suggested an approximately 1:1 mixture of epimers. However, in a number of instances (Table II) it was possible to obtain the penicillin sodium salts crystalline and these compounds were considered on nmr evidence to be single epimers in the solid state. Thus the nmr spectrum for the crystalline phenyl ester showed initially a singlet for the thiazolidine C-3 proton which rapidly changed to two singlets of equal intensity, consistent with racemization at the side-chain chiral center.†

Biological Properties. The 12 α-carboxy esters of carbenicillin, described in Table II, were examined for rates of hydrolysis to carbenicillin in aqueous solution and in the presence of isolated tissue homogenates. The 12 esters were also orally administered to both squirrel monkeys and human volunteers and levels of carbenicillin in blood and urine were measured. Details of these procedures are described in the Experimental Section. The results are discussed below.

† We thank Mr. N. Ward and Mr. A. E. Bird for these observations.

Table I. Monoesters of Phenylmalonic Acid

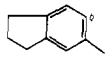
$C_6H_5CHCOOH$
|
COOR

R	Yield, %	Recrystn solvents	Mp, °C	Formula	Analyses
CH_3CH_2-	57	C_6H_6–pet. ether (60–80°)	78–79	$C_{11}H_{12}O_4$	
$C_6H_5CH_2-$	70	C_6H_6–pet. ether (60–80°)	62–64	$C_{16}H_{14}O_4$	C, H
$(CH_3)_2CH-$	62	C_6H_6–pet. ether (60–80°)	64–66	$C_{12}H_{14}O_4$	C, H
$CH_3(CH_2)_3-$	64	EtOAc–pet. ether (40–60°)	47–48	$C_{13}H_{16}O_4$	C, H
$(CH_3)_2CHCH_2-$	52	EtOAc–pet. ether (40–60°)	41–49	$C_{13}H_{16}O_4$	C, H
$CH_3(CH_2)_4-$	70	EtOAc–pet. ether (40–60°)	47–48	$C_{14}H_{18}O_4$	C, H
C_6H_5-	79	C_6H_6	115–117 dec	$C_{15}H_{12}O_4 \cdot 0.5C_6H_6$	H; C[a]
	71	C_6H_6–pet. ether (60–80°)	80–82[d]	$C_{18}H_{16}O_4$	C, H
$3\text{-}CH_3C_6H_4-$	68	C_6H_6–pet. ether (60–80°)	95–96	$C_{16}H_{14}O_4$	C, H
$4\text{-}CH_3C_6H_4-$	81	C_6H_6–pet. ether (60–80°)	122–124 dec	$C_{16}H_{14}O_4 \cdot 0.5C_6H_6$	H; C[b]
$2,5\text{-}(CH_3)_2C_6H_3-$	60	C_6H_6–pet. ether (60–80°)	100–102	$C_{17}H_{16}O_4$	C, H
$3,4\text{-}(CH_3)_2C_6H_3-$	70	C_6H_6	88–90	$C_{17}H_{16}O_4$	H; C[c]

[a] C: calcd, 73.25; found, 73.79. [b] C: calcd, 73.77; found, 72.64. [c] C: calcd, 71.82; found, 72.99. [d] D. C. Hobbs, *Antimicrob. Ag. Chemother.*, **2**, 272 (1972), quotes mp 97–99°.

Table II. α-Carboxy Esters of Carbenicillin

$C_6H_5CHCONHCH-CH(S)C(CH_3)_2$
|
COOR $CO-N-CHCOONa$

R	Yield, %	R_f[a]	$[\alpha]^{20}D$, deg (c 1, H_2O)	Formula	Analyses
CH_3CH_2-[b]	78	0.52	229.8	$C_{19}H_{21}N_2O_6SNa$	C, H, N, S
$C_6H_5CH_2-$[b]	60	0.57	195.5	$C_{24}H_{23}N_2O_6SNa \cdot H_2O$	C, H, N, S
$(CH_3)_2CH-$[b]	92	0.60		$C_{20}H_{23}N_2O_6SNa \cdot H_2O$	C, H, N, S
$CH_3(CH_2)_3-$	79	0.63		$C_{21}H_{25}N_2O_6SNa$	H, N, S; C[c]
$(CH_3)_2CHCH_2-$	77	0.61			
$CH_3(CH_2)_4-$	86	0.70			
C_6H_5-[b]	70	0.59	216.2		
	53	0.70		$C_{26}H_{25}N_2O_6SNa$	C, H, N, S
$3\text{-}CH_3C_6H_4-$	72	0.65	201.7		
$4\text{-}CH_3C_6H_4-$	62	0.60	207.4		
$2,5\text{-}(CH_3)_2C_6H_3-$	49	0.75			
$3,4\text{-}(CH_3)_2C_6H_3-$	32	0.70	176.8		

[a] R_f values determined by paper chromatography in butanol-ethanol-water (4:1:5, v/v top phase), followed by development on an agar plate seeded with *B. subtilis*. [b] Sodium salts obtained crystalline from ethanol. [c] C: calcd, 55.25; found, 54.48.

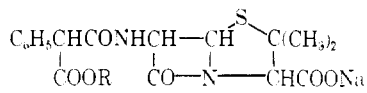
Hydrolysis by Squirrel Monkey Tissue Homogenate. The six alkyl esters (described in Table II) and the phenyl ester were examined for hydrolysis to carbenicillin by homogenates of squirrel monkey liver and small intestine. The results are presented in Table IV and represent the sum of both enzymatic and nonenzymatic hydrolysis. It will be seen that nonenzymatic hydrolysis was very small for the alkyl esters but made a significant contribution to the hydrolysis of the chemically labile phenyl ester. For all esters, the extent of hydrolysis in squirrel monkey liver homogenate was similar to that in the small intestine homogenate. The benzyl and phenyl esters were the most susceptible to hydrolysis and the isopropyl ester the least affected. The relationship of these results to carbenicillin blood levels in the squirrel monkey is discussed below.

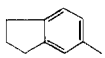
Hydrolysis by Human Liver Homogenate. Data for the conversion of the 12 carbenicillin esters to carbenicillin by aqueous human liver homogenate are presented in Table V The most striking difference between man and the squirrel monkey was for the benzyl ester, which hydrolyzed slowly in human liver homogenate but was completely hydrolyzed by squirrel monkey liver homogenate. The alkyl esters were slowly hydrolyzed by human liver homogenate as was the case with squirrel monkey liver homogenate. The aromatic esters were more susceptible to hydrolysis by human liver homogenate than the alkyl esters, even allowing for the increased contribution from aqueous hydrolysis.

In general, therefore, the aryl esters were more rapidly and more completely hydrolyzed to carbenicillin than were the alkyl esters in both aqueous solution and in the presence of tissue homogenates.

Further Studies on the Hydrolysis of the Phenyl Ester of Carbenicillin. The phenyl ester of carbenicillin was examined more extensively at ester concentrations of 5 and 100 μg/ml. Rates of hydrolysis were measured in human liver homogenate, human small intestine homogenate, and diluted human blood. The results are reported

Table III. Nmr Data (δ Values) for Penicillins



R	Solvent		$C(CH_3)_2$ (6 H)		C_3 proton (1 H)		PhCH-CO-(1 H)	β-Lactam protons (2 H)		C_6H_5- (5 H)	-CONH- (1 H)
H-	D_2O		1.51, 1.60		4.28, 4.30		4.58, 4.59	5.58		7.42	
CH_3CH_2-	DMSO	1.17 (3 H)	1.52		3.90, 3.93	4.10 (2 H)	4.98	5.38		7.35	8.83
$C_6H_5CH_2$-	D_2O		1.50		4.25, 4.29	4.93 (2 H)		5.53		7.13 (10 H)	
$(CH_3)_2CH$-	DMSO	1.18 (6 H)	1.50		3.92, 3.95	4.95 (1 H)	4.95	5.39		7.35	8.75
$CH_3(CH_2)_3$-	D_2O	0.70 (3 H) 1.33 (4 H)	1.52	4.10 (2 H)	4.27, 4.31		4.83	5.58		7.34	
$(CH_3)_2CHCH_2$-	DMSO	0.85 (6 H)	1.53	1.90 (1 H)	3.93, 3.97	3.90 (2 H)	5.06	5.11		7.36	8.40
$CH_3(CH_2)_4$-	D_2O	0.68 (3 H) 1.10 (6 H)	1.50	4.10 (2 H)	4.28, 4.32			5.61		7.35	
C_6H_5-	D_2O		1.50		4.29, 4.32			5.60		7.10 (10 H)	
	DMSO-D_2O		1.55	2.03 (2 H) 2.85 (4 H)	4.02, 4.05			5.48	6.92 (3 H)	7.45	
3-$CH_3C_6H_4$-	DMSO-D_2O		1.53	2.30 (3 H)	4.02, 4.05		5.40	5.40	7.08 (4 H)	7.41	9.10
4-$CH_3C_6H_4$-	D_2O		1.50	1.82 (3 H)	4.29, 4.31			5.63	6.70 (4 H)	7.30	
2,5-$(CH_3)_2C_6H_3$-	DMSO-D_2O		1.54	2.06, 2.28 (6 H)	4.03, 4.06			5.47	6.99 (3 H)	7.47	
3,4-$(CH_3)_2C_6H_3$-	D_2O		1.48	2.12 (6 H)	3.99, 4.02			5.17	6.50 (3 H)	7.47	

in Table VI. The phenyl ester showed marked hydrolysis in all three tissue preparations.

Absorption Studies. Results in Table VII show the mean peak carbenicillin concentrations measured in the blood of squirrel monkeys after oral administration of a single dose of 25 mg/kg of the esters. In contrast to carbenicillin, all 12 esters were absorbed by the oral route producing maximum carbenicillin concentrations ranging from 5 to 20 μg/ml in blood. The highest levels were obtained with the 3-methylphenyl ester followed by the 5-indanyl, 4-methylphenyl, phenyl, and *n*-butyl esters.

There did not appear to be any direct relationship between *in vitro* hydrolysis rates in the squirrel monkey tissue preparations (small intestine and liver) shown in Table IV and peak carbenicillin concentrations in blood. For example, although both the phenyl ester and the benzyl ester were rapidly hydrolyzed *in vitro,* the peak carbenicillin blood concentrations produced by the phenyl ester were about twice those of the benzyl ester. Similarly, although the *n*-butyl, isobutyl, and *n*-pentyl esters were all hydrolyzed relatively slowly and at about the same rates by the tissue preparations, the two former compounds produced peak carbenicillin blood levels almost twice those of the *n*-pentyl ester. The isopropyl ester was hydrolyzed at a very low rate *in vitro* and produced relatively low blood carbenicillin concentrations.

For comparison, the mean peak serum carbenicillin concentrations obtained in human volunteers are also shown in Table VII and it can be seen that there was no good correlation between the results in squirrel monkey and man. Thus, the phenyl, 3-methylphenyl, and 5-indanyl esters gave relatively high carbenicillin levels in both groups but the 4-methylphenyl ester, which produced relatively high levels in the squirrel monkey, gave low serum concentrations of carbenicillin in man. On the other hand, the isopropyl ester which produced low levels in the monkey gave relatively high levels in man. The lack of correlation between results in the squirrel monkey and in man is not entirely unexpected, since this would require not only similar absorption rates in the two species but also the rates at which the esters were hydrolyzed to carbenicillin by the body tissues would have to be the same.

The carbenicillin concentrations measured in serum and urine of human volunteers dosed with the esters are shown

Table IV. Ester Hydrolysis by Squirrel Monkey Liver and Intestine Homogenates[a]

Ester	% hydrolysis to carbenicillin after 2.5 hr in Buffer, pH 7	Intestine	Liver
Ethyl	<1	5	5
Benzyl	<5	100	100
Isopropyl	<1	0.3	1.2
n-Butyl	<1	19	24
Isobutyl	<1	17	31
n-Pentyl	<1	28	33
Phenyl	23	100	100

[a]Substrate concentration 1 mg/ml, tissue concentration 2% w/v, temperature 37°.

Table V. Ester Hydrolysis by Human Liver Homogenate[a]

Ester	% hydrolysis to carbenicillin in Liver at 30 min	Liver at 60 min	Liver at 180 min	Buffer, pH 7, at 180 min
Ethyl	1	2	23	<1
Benzyl	<1	1	7	<5
Isopropyl	<1	<1	<1	<1
n-Butyl	1	2	17	<1
Isobutyl	<1	1	5	<1
n-Pentyl	2	4	11	<1
Phenyl	16	46	60	23
5-Indanyl	41	71	83	18
3-Methylphenyl	58	64	85	16
4-Methylphenyl				23
2,5-Dimethylphenyl	9	16	35	7
3,4-Dimethylphenyl	57	73	84	25

[a]Substrate concentration 2 mg/ml, tissue concentration 4% w/v, temperature 37°.

in Table VIII.‡ The compounds were administered in gelatin capsules without excipients as a single dose equivalent to 500 mg of carbenicillin free acid. Table VIII also shows the amount of unhydrolyzed ester recovered from the urine of these subjects. Carbenicillin itself is poorly absorbed in human subjects after oral administration and under the conditions described here no carbenicillin was detected in the blood (<1 μg/ml) and less than 1% of the dose was found in the urine. In contrast, after administration of the esters, carbenicillin was measured in serum with all compounds, except the *n*-pentyl ester (<1.0 μg/ml), and the amount of carbenicillin excreted in the urine varied from 15 to 36% of the dose administered (*n*-pentyl ester, 6%). The highest peak carbenicillin serum concentrations (approximately 7.0 μg/ml) were obtained with the phenyl, 5-indanyl, and isobutyl esters and with these compounds approximately one-third of the dose was excreted in the urine as carbenicillin.

It was noteworthy that after administration of the alkyl esters significant amounts of unhydrolyzed ester were found in the urine along with carbenicillin, whereas unhydrolyzed ester was not detected in urine with the aryl esters. The difference between the alkyl and aryl esters is presumably a reflection of the slower rates of hydrolysis of the former as reported above.

‡ We thank Dr. K. H. Jones for supervising the studies in human volunteers.

Table VI. Hydrolysis of Carbenicillin Phenyl Ester by Human Tissue Homogenates

Hydrolyzing system	Ester concn (μg/ml) remaining after 0 min	30 min	60 min	180 min
Buffered saline, pH 7.4	5.0 (0)[a]	4.4 (12)	4.4 (12)	2.7 (46)
1% human liver homogenate	5.0 (0)	3.8 (24)	3.3 (34)	1.3 (74)
10% human gut homogenate	5.0 (0)	2.3 (54)	1.3 (74)	<0.5 (>90)
10% human blood	5.0 (0)	3.3 (34)	2.3 (54)	0.6 (88)
90% human blood	5.0 (0)	1.2[b] (76)		
Buffered saline, pH 7.4	100 (0)			67 (33)
1% human liver homogenate	100 (0)	83 (17)	66 (34)	10 (90)
10% human liver homogenate	100 (0)	13[b] (87)		
10% human gut homogenate	100 (0)	54 (46)	34 (66)	11 (89)

[a]Figures in parentheses are per cent hydrolysis. [b]Ester concentration remaining after 20 min.

Table VII. Carbenicillin Blood Levels in the Squirrel Monkey for Esters of Carbenicillin[a]

Ester	Mean carbenicillin blood levels (μg/ml) at 0.5 hr	1.0 hr	2.0 hr	4.0 hr	6.0 hr	Mean peak level (μg/ml) in humans[b]
Ethyl	**8.5**	7.2	2.4			3.8
Benzyl	**7.8**	6.8	5.5			2.5
Isopropyl	**5.3**	**5.3**	3.1	<2.5	<2.5	5.5
n-Butyl	**13.9**	10.9	4.1	<1.5	<1.5	3.2
Isobutyl	**12.5**	9.6	3.6	<1.5	<1.5	7.2
n-Pentyl	**7.3**	6.8	2.0			<1.0
Phenyl	11.9	**14.0**	10.2			7.0
5-Indanyl	**17.7**	13.6	5.1			6.7
3-Methylphenyl	**19.7**	14.2	8.8	1.7	<1.5	5.8
4-Methylphenyl	**15.0**	11.4	3.2			3.6
2,5-Dimethylphenyl	**4.6**	3.8	2.4	<1.5	<1.5	4.4
3,4-Dimethylphenyl	**10.0**	8.0	3.4	<1.5	<1.5	3.9

[a]Single oral dose of 25 mg of ester/kg. [b]Taken from Table VIII.

The preferred compound of the 12 esters tested appeared to be the phenyl ester, on the basis of peak carbenicillin serum concentrations, area under the blood level curve, urinary excretion of carbenicillin, and absence of unhydrolyzed ester. This compound (carfecillin, BRL 3475) was therefore selected for detailed examination. In further bioavailability studies (to be reported elsewhere) the favorable pharmacokinetic properties of the compound were confirmed and studies with selected formulations (tablets and syrups) resulted in carbenicillin serum and urine levels significantly greater than those indicated in Table VIII.

As a result of these observations the phenyl ester of carbenicillin is currently undergoing clinical trial in the treatment of urinary tract infections.

Table VIII. Carbenicillin Blood Levels in Human Volunteers for Esters of Carbenicillin[a]

Ester	Mean carbenicillin serum levels (μg/ml) at 30 min	60 min	120 min	240 min	Urinary 0–6-hr excretion, % of dose: Carbenicillin	Unhydrolyzed ester
Ethyl	1.1	2.8	3.8	1.1	21	15
Benzyl	<1.0	1.9	2.5	1.3	15	8
Isopropyl	1.2	3.6	5.5	1.8	30	25
n-Butyl	1.3	3.2	3.0	1.1	19	8
Isobutyl	<1.0	6.6	7.2	1.9	36	10
n-Pentyl	<1.0	<1.0	<1.0	<1.0	6	11
Phenyl	5.2[b]	7.0	4.6	<1.0	33[c]	<2
5-Indanyl	1.5	3.7	6.7	1.9	34	<2
3-Methylphenyl	5.5[b]	5.8	4.1	<1.5	33	<2
4-Methylphenyl	2.6	3.0	3.6	<1.0	20	<2
2,5-Dimethylphenyl	1.7	4.4	4.3	1.0	26	<2
3,4-Dimethylphenyl	<1.5[b]	2.5	3.9	<1.5	29	<2

[a] Single oral dose of ester equivalent to 500 mg of carbenicillin. [b] 40-min assay figure. [c] A further 3.8% of the dose was recovered as the penicilloic acid of carbenicillin.

Experimental Section

Chemistry. All melting points were taken on a Büchi melting point apparatus and were not corrected. Nmr spectra were obtained on a Varian A-60 spectrometer using tetramethylsilane as an internal reference when dimethyl sulfoxide was the solvent and 3-trimethylsilylpropane sulfonic acid sodium salt when water was the solvent. Where analyses are indicated in the tables only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of theoretical values.

Monoesters of Phenylmalonic Acid (2). General Method. Phenylmalonic acid (27.0 g, 0.14 mol) in dry ether (80 ml) was treated with thionyl chloride (17.85 g, 10.8 ml, 0.15 mol) and dimethylformamide (2 drops). The mixture was heated in a warm water bath at reflux for 2 hr or until a clear solution was obtained. The solution was evaporated under reduced pressure to remove any residual thionyl chloride and hydrogen chloride and the oily residue redissolved in dry ether (80 ml). The solution was treated with the hydroxy compound (0.15 mol) and the mixture refluxed for 2 hr. The reaction mixture was cooled to room temperature and washed with water (20 ml). The organic layer was extracted with saturated sodium bicarbonate solution until the extracts were alkaline. The combined extracts were washed with ether (30 ml) and the wash was discarded. The aqueous layer was acidified with 5 *N* hydrochloric acid to pH 1. The precipitated oil was extracted with methylene chloride (3 × 100 ml). The combined organic extracts were washed with water (4 × 100 ml) to remove any unreacted phenylmalonic acid, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The oily residue crystallized on standing and was recrystallized from appropriate solvents.

α-Carboxy Esters of Carbenicillin (3). General Method. The monoester of phenylmalonic acid (0.01 mol) mixed with thionyl chloride (5 ml) was heated in a warm water bath at 70° for 1 hr. The solution was evaporated under reduced pressure, diluted with dry benzene (5 ml), and reevaporated to remove residual thionyl chloride. The crude acid chloride, dissolved in dry acetone (50 ml), was added to a solution of 6-aminopenicillanic acid (2.16 g, 0.01 mol) in water (50 ml), 1 *N* sodium hydroxide (10 ml), 1 *N* sodium bicarbonate (15 ml), and acetone (25 ml) cooled to 12°. The mixture was stirred at room temperature for 2 hr and washed with ether (3 × 50 ml) and the wash discarded. The aqueous solution was layered with fresh ether (50 ml) and acidified, with stirring, with 1 *N* hydrochloric acid to pH 2.0. The ether layer was separated and the aqueous layer reextracted with ether (2 × 50 ml). The combined ether extracts were washed with water (20 ml) and extracted with 1 *N* sodium bicarbonate until the combined extracts were at pH 7.0. The neutral aqueous extract was freeze-dried to give the penicillin sodium salt as a noncrystalline solid. Solution of the amorphous product in ethanol and standing at room temperature gave in some instances the penicillin sodium salt as a crystalline solid.

***In Vitro* Hydrolysis Studies. Hydrolysis by Squirrel Monkey Tissue Preparations.** The liver and small intestine (pyloric to caecal junction) were obtained from a freshly killed squirrel monkey (death from CO_2 asphyxiation). The gut was washed through immediately with 0.05 *M* potassium phosphate buffer, pH 7.0, containing 0.15 *M* sodium chloride to remove any contents before the mucosa degenerated. The tissues were homogenized at 20% w/v in ice-cold buffered saline and further diluted with phosphate buffer, pH 7.0, before use. Reaction mixtures consisted of ester at a final concentration of 1 mg/ml in 2% w/v homogenate. Controls to determine aqueous hydrolysis rates consisted of ester at 1 mg/ml in 0.05 *M* potassium phosphate buffer, pH 7.0. Reaction mixtures were incubated at 37° and spotted (5 μl) onto 1-cm wide chromatography tape (Whatman Grade 1) at 0 and 150 min. Standard solutions of carbenicillin were prepared in homogenate or buffer and also spotted (5 μl) onto chromatography tapes. The chromatograms were developed overnight (16 hr) by descending chromatography in a *n*-butanol–ethanol–water (4:1:5 v/v top phase) system at 4°. The tapes were then dried and laid on agar plates (Oxoid blood agar base CM 55 3.5% w/v) seeded with spores of *Bacillus subtilis* ATCC 6633. The plates were incubated at 40° for 7 hr, after which time the zones of inhibition appearing at R_f 0.05 (carbenicillin) were measured.

In this solvent system the esters had R_f values of 0.5 and above. Zone diameters for the standards were plotted against log concentrations to give a linear standard line from which the concentrations of carbenicillin in the reaction mixtures were obtained. Percentage conversions were calculated using the formula $Mc/422 \times 10$, where M = molecular weight of sodium salt ester (molecular weight of disodium carbenicillin = 422) and c = concentration (μg/ml) of carbenicillin in the reaction mixture.

Hydrolysis by Human Liver Homogenate. Reaction mixtures consisted of ester (2.0 mg/ml) in 4% (w/v) human liver homogenate in 0.05 *M* potassium phosphate buffer, pH 7.0. The liver was obtained postmortem and stored at −20° until required when it was homogenized as described below. Control reactions consisted of ester at 2.0 mg/ml in 0.05 *M* potassium phosphate buffer, pH 7.0. Tests and controls were incubated at 37° and spotted onto chromatography tapes at 0, 30, 60, and 180 min. The chromatography and assay procedures were as above.

Hydrolysis of Carbenicillin Phenyl Ester by Human Tissue Preparations. Human liver and jejunum were obtained postmortem and immediately frozen and stored at −20° until needed. Before use they were thawed and homogenized for 60 sec at 5° in 0.05 *M* potassium phosphate buffer, pH 7.4, containing 0.15 *M* sodium chloride using an Ultra Turrax homogenizer (Janke and Kunkel) to give a 10% w/v homogenate. Blood was taken from healthy volunteers, heparinized (10 units of heparin/ml of blood), and diluted with the above buffered saline. Reaction mixtures consisted of the phenyl ester at 5.0 or 100 μg/ml in 10% v/v blood, 10% w/v gut homogenate, 1% w/v liver homogenate, or buffered saline (control). Reaction mixtures were incubated at 37° and assayed at 0, 30, 60, and 180 min for residual ester by the following procedure.

Ten milliliters of a 5.0 μg/ml reaction mixture or 1.0 ml of a 100 μg/ml reaction mixture was mixed with 0.1 *M* hydrochloric acid in a centrifuge tube, using a sufficient amount to bring the sample to pH 5.5. *n*-Butyl acetate (10 ml) at 0° was added, and the tube was sealed, shaken vigorously, and then spun at 38,000g for 5 min in a refrigerated centrifuge (*ca.* 5°) to separate the phases.

Five milliliters of the organic phase was removed and evaporated to dryness *in vacuo*. The residue was redissolved in 0.2 ml of *n*-butyl acetate and 50 μl spotted onto each of two antibiotic assay disks (Whatman 1.3 cm). Standard solutions of the phenyl ester were made up in blood, gut homogenate, or buffered saline at 0° and immediately extracted by the same procedure. Test and standard disks were placed on an agar plate (3.5% Oxoid blood agar base CM 55) seeded with *Sarcina lutea* NCTC 8340 and incubated overnight (16 hr) at 30° and the zones of inhibition were measured. Concentration of ester remaining in the reaction mixtures was obtained by reference to a standard line of zone diameter for ester against log concentration.

Absorption Studies. Absorption in Squirrel Monkeys. The esters were administered orally by intubation as solutions of the sodium salts in water (2 ml), at a dose of 25 mg/kg, to groups of five squirrel monkeys (Saimiri sciureus) weighing 500-1000 g. Venous blood samples were removed at 0.5, 1, 2, 4, and 6 hr after dosing. The blood samples were heparinized, stored at 4°, and assayed on the day of sampling.

Absorption in Human Volunteers. The compounds were administered in plain gelatin capsules without excipients to groups of ten healthy fasting volunteer subjects as a single oral dose corresponding to 500 mg of carbenicillin. Venous blood was taken 0.5, 1, 2, 3, and 4 hr after administration of the ester and urine was collected over a 6-hr period. In order to reduce hydrolysis *in vitro* sera were kept in an ice bath until microbiological assay which was usually commenced within 1 hr of collection of the specimens. The specimens of urine were examined for carbenicillin, unhydrolyzed ester, and penicilloic acid.

Microbiological Assay. The concentrations of carbenicillin found in body fluid specimens after administration of the esters were measured by standard large plate microbiological assay with *Pseudomonas aeruginosa* NCTC 10701 as assay organism.[6] This organism is sensitive to relatively low concentrations of carbenicillin but is insensitive to unhydrolyzed carbenicillin esters. Specimens of squirrel monkey blood or human sera were assayed against standard solutions of carbenicillin prepared in squirrel monkey blood or in pooled human serum. Urine specimens were assayed against carbenicillin standard solutions prepared in 0.05 *M* phosphate buffer, pH 7.0. The assay plates were incubated overnight at 30°, inhibition zone diameters measured, and the concentrations of carbenicillin derived from the standard lines.

Penicilloic Acid in Urine. The carbenicillin penicilloic acid concentrations in urine collected in the 6-hr period after administration of the esters to human volunteers were measured by an iodometric method.[7]

Unhydrolyzed Ester in Urine. The amounts of unhydrolyzed ester found in the urine of fasting subjects were measured by chromatography and bioautography, as described above (see section on hydrolysis by squirrel monkey tissue preparations). The inhibition zones formed by the urine specimens were measured and compared with those produced by buffer standard solutions of the esters.

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5-Aryl-2,3-dihydro-5*H*-imidazo[2,1-*a*]isoindol-5-ols. A Novel Class of Anorectic Agents

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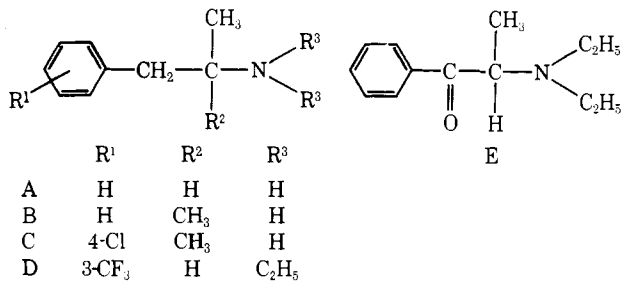
A series of 5-aryl-2,3-dihydro-5*H*-imidazo[2,1-*a*]isoindol-5-ols (IV), prepared by the $LiAlH_4$ reduction of the corresponding 9b-aryl-1,2,3,9b-tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-ones (II), was evaluated for suppression of food consumption in rats. One member of this series, 5-*p*-chlorophenyl-2,3-dihydro-5*H*-imidazo[2,1-*a*]isoindol-5-ol (**6**, mazindol), was evaluated in squirrel and capuchin monkeys and found to have anorexic activity approximately equal to *d*-amphetamine.

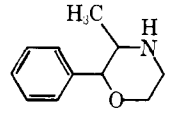
It has become increasingly apparent in recent years that obesity is a major health hazard in the United States and its control in individuals with diabetes and heart conditions is particularly desirable.[1] In the overall management of obesity the anorectic drugs[2] continue to occupy a useful role by diminishing the desire for intake of food.

At the time we began our work amphetamine (A), phentermine (B), chlorphentermine (C), fenfluramine (D), diethylpropion (E), and phenmetrazine (F) were the most widely used single drug entities in the United States and Europe for appetite control.[3] All of these drugs are characterized by the presence of a β-phenethylamine moiety G in their structure.

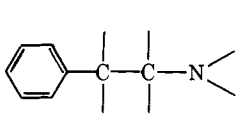


	R^1	R^2	R^3
A	H	H	H
B	H	CH_3	H
C	4-Cl	CH_3	H
D	3-CF_3	H	C_2H_5

E



F



G

It was the goal of our research effort to prepare an anorectic substance that did not contain a β-phenethylamine moiety as part of its structure. In the present work we present our findings on one such class, the 5-aryl-2,3-dihydro-5*H*-imidazo[2,1-*a*]isoindol-5-ols IV.†

Chemistry. Scheme I depicts the synthesis used to prepare the 5-aryl-2,3-dihydro-5*H*-imidazo[2,1-*a*]isoindol-5-ols IV reported in this paper. Condensation of a 2-benzoylbenzoic acid I with ethylenediamine gave the 9b-aryl-1,2,3,9b-tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-ones II.[5] Treatment of II with $LiAlH_4$ in tetrahydrofuran (THF) gave the labile 9b-aryl-2,3,5,9b-tetrahydro-1*H*-imidazo[2,1-*a*]isoindol-5-ols III.[6] The latter compounds were not isolated but directly converted to IV by air oxidation[4c,7] in THF-CH_3OH.

It has been demonstrated by ultraviolet spectral data that 5-phenyl-2,3-dihydro-5*H*-imidazo[2,1-*a*]isoindol-5-ol (IV, compound **1**) exists as the tautomeric 2-(2-imidazolin-2-yl)benzophenone form IVa in acid solution.[6] An X-ray single-crystal structure analysis of **1**·HBr has also confirmed the existence of the tautomeric form IVa in the

† Similar results have been published in the patent literature by groups from American Home Products and Hoffmann-La Roche.[4]