recorded by physiograph. The test material was administered by ip in 5% acacia suspension at 2 dose levels, 50 and 100 mg/kg. The volume of injection was 1.5 ml at both dose levels. A control injection of acacia suspension was also done at this volume. Pressure levels were determined just prior to injection and at 15-min intervals following for a period of 105 min. Table I presents data from the control rate and the 3 rats receiving the test compound.

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Antibacterial Activity of 6-(5-Membered heteroarylacetamido)penicillanic Acids

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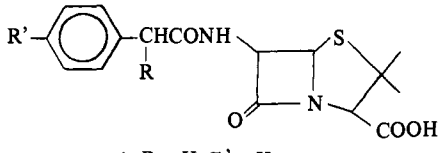
Analog synthesis[1] has been fruitful in obtaining semisynthetic penicillins with widely differing antibacterial spectra (for recent reviews on penicillins see ref 2). Thus, the insertion of a polar substituent into the phenylacetic acid moiety of penicillin G, **1**, has a profound effect on the activity of the penicillin.



1, R = H; R' = H
2, R = NH_2; R' = H
3, R = CO_2H; R' = H
4, R = H; R' = NH_2

The introduction of a free amino group into the "side chain" of penicillin G gives **2**† or **4**, penicillins with enhanced Gram-negative antibacterial activity, which also retain the Gram-positive activity of **1**.[2b,2d,3] The presence of CO_2H produces a broad-spectrum penicillin **3**,‡ clinically effective in treating infections caused by *Pseudomonas* and *Proteus* strains resistant to ampicillin.[4]

Another way in which the properties of the phenylacetic acid moiety in **1** can be altered is by replacing the Ph ring by an heteroaromatic ring. The 2- and 4-pyridylmethylpenicillins were reported to have better Gram-negative activities than **1**.[5] The 5-membered heteroaryl compds comprise a number of ring systems contg N, O, and S (occasionally Se) as part of the ring, and as a result provide a wide range in size and polarity. Since each ring and the CH_2 group of the acetic acid chain can be further substituted, a large variety of acetic acids are possible. This note describes the results obtained from penicillins derived from the heteroarylacetic acids, without additional substitution of the CH_2 group. The methods of preparation of the acetic acids and the derived penicillins are described in the Exptl Section.

Results and Discussion

In Table I the MIC values of **2** and the heteroarylmethyl penicillins against various bacteria are compared with those of **1**, measured under the same conditions.§,[6] The number of dilutions by which the MIC differs from that of **1** is given, + indicating that it is more active, – that it is less active, and = that it is as active. All of these penicillins show good Gram-positive antibacterial activity, but the activity against Gram-negative organisms show a greater spread of values, with **9**, **10**, **17**, and **33** being similar to **2**. Isomerism within a particular class of penicillin causes considerable variation in activity levels. This effect is quite evident with the isothiazoles **8**, **9**, and **10**; the 1,2,3-thiadiazoles **16**, **17**, **18**, and **19**; and the tetrazoles **25–45**. In the case of the tetrazoles the 1,5 isomers (**31**, **33**, **36**, **40**, and **42**) are more active than the 2,5 isomers (**32**, **34**, **37**, **41**, and **43**). This effect is probably a reflection of the differences in polarity between the isomers. The more polar the side-chain acid the better the Gram-negative antibacterial activity. Many of these penicillins are also more stable than **1** to acid.

Experimental Section#

Preparation of Heteroarylacetic Acids. The following general methods were used for the prepn of the side-chain acids.

A. From the Formic Acids. The heteroaryl carboxylic acids were converted to the acetic acids using the Arndt–Eistert synthesis.[7,8]

1,2,3-Thiadiazole-4-acetic Acid. 1,2,3-Thiadiazole-4-carbonyl chloride[9] with excess CH_2N_2 gave the diazo ketone (87%), mp 145–148° dec, which was converted to ethyl 1,2,3-thiadiazole-4-acetate (30%) [bp 105–109° (0.6 mm)] by a 20-hr reflux with EtOH–Ag_2O. Hydrolysis gave the acid (67%), mp 146–148°. *Anal.* ($C_4H_4N_2O_2S$) C, H, N, S.

5-Methyl-1,2,3-thiadiazole-4-acetic Acid. The acid chloride (71%), bp 75–77° (0.4 mm), of 5-methyl-1,2,3-thiadiazole-4-carboxylic acid[10] with excess CH_2N_2 gave the diazo ketone (90%), mp 85–89°, which was converted to ethyl 5-methyl-1,2,3-thiadiazole-4-acetate (60%) [bp 100–105° (0.2 mm)] by a 2.5-hr reflux with EtOH–Ag_2O. Hydrolysis gave the acid (75), mp 91–93°. *Anal.* ($C_5H_6N_2O_2S$) C, H, N.

4-Methyl-1,2,3-thiadiazole-5-acetic Acid. The acid chloride (88%), bp 35° (0.4 mm), of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid[11] with excess CH_2N_2 gave the diazo ketone (75%), mp 55–57°, which was converted to methyl 4-methyl-1,2,3-thiadiazole-5-acetate (44%) [bp 115–117° (1.5 mm)] by a 4-hr reflux with MeOH–Ag_2O. Hydrolysis gave the acid (93%), mp 163–165° dec. *Anal.* ($C_5H_6N_2O_2S$) C, H, N, S.

B. From the Aldehydes. Cleavage of the aldehyde–rhodanine condensation product gave the acetic acid.[12]

C. From Methyl Heteroaryl Compds. The lateral lithiation of certain Me compds offers a convenient route to the acetic acids.[13]

D. From the Halomethyl Compds. These compds were obtd by the chloromethylation of the heteroaryl compd[14] or by bromination of the Me compds using NBS.[15,16] The halomethyl compds were converted to the acetic acids *via* the cyanomethyl derivs.[14,17-19]

E. From the Heteroaryl Methyl Ketones. These ketones were converted to the 4-heteroaryl-1,2,3-thiadiazoles[9,11] which underwent base-catalyzed ring opening to the 2-heteroarylalkynyl 1-thioether. These alkynyl thioethers were hydrolyzed to the acetic acids.[11]

F. From the NH Azoles. Haloacetic esters N-alkylate azoles contg an NH group in the ring, to form the *N*-acetic esters which can be hydrolyzed to the acids. In many of these alkylations a mixt of isomers may result. The tetrazoles, for example, produced both the 1- and the 2-acetic esters, which were sepd by distn, and the isomer structure was established from its nmr spectrum.[20]

G. Direct Synthesis. The heterocyclic compd was synthesized with a potential acetic acid group present. The starting material in most of these synthesis was cyanoacetic ester[20-22] or acetoacetic ester.[23]

2-Methyloxazole-4-acetic Acid. A mixt of ethyl γ-bromoacetoacetate (1 mole), NaAcO (2 mole), and HAcO (300 ml) was heated with stirring on a steam bath for 3 hr. NH_4AcO (3 moles) was added, and the mixt was heated for 1 hr more. The HAcO was re-

†D-(–) form, ampicillin from Bristol Labs, Syracuse, N. Y.

‡Carbenicillin from Beecham Labs.

§The penicillins were tested for their antibacterial activity by the late Dr. A. Gourevitch and his associates in the Microbiology Dept. of Bristol Labs, Syracuse, N. Y.

#For the instruments used see ref 7. All new acids were analyzed as indicated and the results were within ±0.4% of the calcd values.

Table I. *In Vitro* Antibiotic Activity of 6-(5-Membered Heteroarylacetamide)penicillins Compared to K-Pen G

No.	Penicillin[l]	Method of prepn of acid[a]	Mp, °C[b]	Ref[c]	*d*	*e*	*f*	*g*	*h*	*i*	*j*	Acid stability, min[k]
1	Penicillin G				0.01	0.012	0.011	42	1.3	8.0	22	4
2	Ampicillin				−3	−1	+1	+2	+3	+2	+3	300
3	2-A	B	68–69	12	−4	−2	−1	−2	−1	=	−1	24
4	3-A	A	61–62	24	−1	−1	+1	+1	+1	+1	+1	–
5	2-B	D	62–64	18	−2	−1	=	=	=	=	+1	12
6	3-B	D	79–81	17	−2	−2	−1	=	=	=	=	7
7	5-C-3-Me	C	104–105	13	−2	−1	−1	=	+1	–	+2	–
8	3-D	D	130–132	7	=	=	+1	+1	+1	+2	+4	240
9	4-D	A	117–119	7	−1	+1	+1	+2	+3	+2	+4	–
10	5-D	A	153–155 dec	7	−1	+1	+1	+2	+3	+2	+4	–
11	1-E	F	164–166	25	−4	−4	−2	=	−2	=	+2	129
12	4-F-2-Me	G	143–145		−4	−3	−3	−2	−2	−3	=	–
13	2-G	G	106 dec	22	−4	−3	−2	=	−2	−2	=	60
14	4-G	G	137–140	23	−4	−3	−2	=	−1	−1	=	21
15	4-G-2-Me	G	123–126	23	−4	−3	−2	−2	−1	−2	−1	33
16	4-H	A	145–147		−2	−2	−1	=	+1	+1	+3	–
17	5-H	G	179–180 dec	21	−3	−1	−1	+2	+1	+2	+4	–
18	4-H-5-Me	A	92–94		−2	−2	−1	+1	+1	=	+2	–
19	5-H-4-Me	A	163–164 dec		−2	−1	−1	=	=	−1	+1	–
20	2-I	G	78–83 dec	21	−4	−3	−2	=	−1	+1	+2	–
21	2-I-5-Me	G	84–85 dec	21	−4	−3	−2	=	−1	=	+2	–
22	3-J-4-Me	C	82–83	13	−3	−1	−1	=	+1	–	+2	300
23	1-K	G	208–210 dec	26	−5	−3	−2	+2	−2	=	+1	247
24	1-L	F	196–197	27	−7	−5	−5	−2	−4	−2	=	180
25	1-M	F	127–129	20	−4	−2	−2	+2	−1	=	+2	280
26	2-M	F	175–176	20	−3	−3	−1	+1	−1	=	+2	420
27	5-M-1-Me	G + F	83–84	20	−3	−3	−1	=	−1	=	+2	–
28	1-M-5-Me	F	184–186 dec	20	−4	−2	−2	+2	−1	=	+2	225
29	2-M-5-Me	F	155–156	20	−2	−1	=	=	−1	=	+1	–
30	5-M-2-Me	G + F	119–121	20	−4	−3	−2	+2	−1	=	+2	–
31	5-M-1-Et	G + F	96–98	20	−3	−3	−2	=	=	=	+2	–
32	5-M-2-Et	G + F	99–101	20	−2	−2	=	−1	−2	−1	−1	–
33	1-M-5-Et	F	135–136	20	−2	−1	−1	+2	=	+2	+3	–
34	2-M-5-Et	F	95–97	20	−2	−2	=	−1	−2	−1	=	–
35	1-M-5-*n*-Pr	F	106–108	20	−3	−1	−1	=	=	=	+2	–
36	5-M-1-*i*-Pr	G + F	100–101	20	−2	−2	−2	+1	=	+1	+2	–
37	5-M-2-*i*-Pr	G + F	109–112	21	−2	−2	=	−2	−2	−3	−1	–
38	5-M-1-NMe_2	G	115–117 dec	21	−4	−5	−4	−1	−2	−2	−1	–
39	2-M-5-NH_2	F	185–186 dec	20	−4	−3	−2	=	−2	=	+2	360
40	1-M-5-Cl	F		20	−2	−2	−1	+2	=	+1	+3	–
41	2-M-5-Cl	F	100–101	20	−3	−2	−1	=	−2	=	+1	480
42	1-M-5-CF_3	F	Oil	20	−3	−2	−2	=	−1	=	+1	–
43	2-M-5-CF_3	F	Oil	20	−2	−1	−1	−1	−2	−2	−1	–
44	1-M-5-SMe	F	173–174 dec	20	−2	−1	−1	−1	=	=	+2	–
45	2-M-5-SMe	F	127–129	20	−1	−1	=	=	−1	−1	=	–

[a] See Experimental Section. [b] Melting point of acetic acid. [c] For acetic acids. [d] *Staphylococcus aureus* Smith. [e] *Diplococcus pneumoniae.* [f] *Streptococcus pyogenes.* [g] *Escherichia coli.* [h] *Staph. enteritidis.* [i] *Salmonella typhosa.* [j] *Klebsiella pneumoniae.* [k] Half life at pH 2. [l] The first number of the code refers to the position of attachment of the acetic acid, the letter refers to the ring system, the second number and group indicates the position and type of substitution, A, furan; B, thiophene; C, isoxazole; D, isothiazole; E, pyrazole; F, oxazole; G, thiazole; H, 1,2,3-thiadiazole; I, 1,3,4-oxadiazole; J, 1,2,5-oxadiazole; K, 1*H*-1,2,3-triazole; L, 1*H*-1,2,4-triazole; M, 1*H*-tetrazole.

moved *in vacuo*, ice H_2O added, and the mixt extd with Et_2O. The combined exts were dried ($MgSO_4$), concd, and distd to give ethyl 2-methyloxazole-4-acetate (20%) [bp 60° (0.3 mm)]. Hydrolysis gave the acid (55%), mp 139–140° dec. *Anal.* ($C_6H_7NO_3$) C, H, N.

Preparation of Penicillins. The penicillins were made from the acetic acids either by the active ester method[7] or *via* the imidazolides as described below. In every instance the purity of the penicillin was estd as better than 80% from tlc, ir, and nmr spectral analysis.

Preparation of Penicillins *via* the Imidazolides. The well-powd acid (1–5 mmoles) was mixed with an inert dry solvent such as $CHCl_3$, THF, or DMF (2–8 ml) in a dry flask under N_2. (DMF is the solvent of choice since both the acids and the imidazolides were found to be sol in it.) After complete soln, the flask was cooled to −10°, and an equiv of carbonyldiimidazole was added. Rapid gas evoln usually occurred, although in certain cases a higher reactn temp (not exceeding 40°) was reqd. When the gas evoln ceased (about 5–90 mins) the mixt was evacuated for about 5 min and cooled to −10°. An equiv of the Et_3NH salt of 6-APA (as a 5% CH_2Cl_2 soln) was added. Excess Et_3N could be used without adverse effect. After stirring 2 hr the solvent was removed *in vacuo*. The oily residue was dissolved in *n*-BuOH (2–3 ml) and a soln of potassium 2-ethylhexanoate in *n*-BuOH (2 *M*, about 30% excess) added. After diln with Et_2O and cooling, the K salt of the penicillin was filtered and washed with Et_2O. The salt was dissolved in the min amt of MeOH and repptd with Et_2O, filtered, washed with Et_2O, and dried.

Acid Stability. The stability of the penicillin to low pH was estd by determining the half-life at 37° of the compd in a soln buffered to pH 2.0 with citric acid–HCl.

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Antibacterial 2,3-Dihydro-2-(5-nitro-2-thienyl)-quinazolin-4(1*H*)-ones

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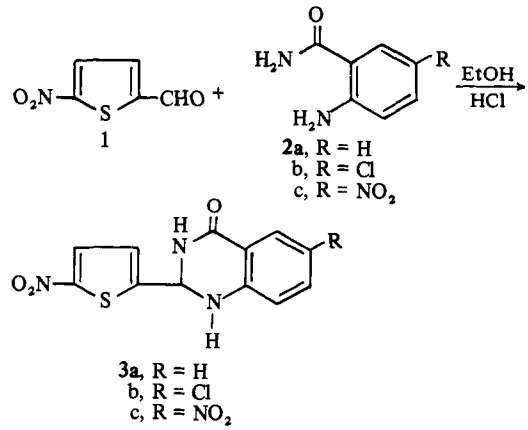
The anthelmintic activity of a series of 2-(5-nitro-2-thienyl)quinazolines has recently been reported.[1] During the course of this research, three 2,3-dihydro-2-(5-nitro-2-thienyl)quinazolin-4(1*H*)-one derivatives were prepared as intermediates. These 2,3-dihydroquinazolin-4(1*H*)-ones have shown significant activity against organisms implicated in bacterial vaginitis.



Chemistry. The general synthetic method for the preparation of the compounds **3a**, **3b**, and **3c** involves the reaction of 5-nitro-2-thiophenecarboxaldehyde with a suitably substituted anthranilamide in acidic EtOH.

Table I

Bacterial strain	MIC, μg/ml[a] 3a	3b	3c	nitrofurazone
Staphylococcus aureus	>50	>50	>50	12
Streptococcus faecalis	>50	–	–	25
Corynebacterium liquefaciens	50	25	50	25
Escherichia coli Es-2	1.5	12.5	12.5	3
E. coli Es-L	50	>50	>50	12
Salmonella typhosa	12.5	50	>50	6
Hemophilus vaginalis	0.4	6	1.5	0.8

[a]Minimal inhibitory concentration is the lowest concentration of compound that prevents visible growth after 24 hr of incubation.

Table II

Compound	Concentration, μg/ml	Medium with serum–mucin	Medium without serum–mucin
3a	0.5	1.2×10^3	1.3×10^{3b}
3b	1	8.1×10^4	7.8×10^4
3c	1	7.5×10^4	3.0×10^3
Nitrofurazone[a]	0.5	6.1×10^5	7.9×10^5
Control		5.2×10^7	6.6×10^7

[a]For comparison. [b]Compounds were considered active when they produced a 3 log reduction in the viable *Hemophilus vaginalis* concentration both in the presence and the absence of serum and mucin after 6-hr incubation at 37°.

Biological Method. The compounds in Table I were screened for bacteriostatic activity by methods reported previously.[2] However, since *Hemophilus vaginalis* requires more fastidious growth conditions than the other microorganisms tested, the composition of the growth medium was altered. The compounds were also tested for bactericidal activity in a medium with serum and mucin added. Both tests are described in the Experimental Section and the results are shown in Table II.

Biological Results. *In vitro* testing results indicate that the most active compound is 2,3-dihydro-2-(5-nitro-2-thienyl)quinazolin-4(1*H*)-one (**3a**). It is more active than the reference standard nitrofurazone, in the serum–mucin bactericidal test. It also has MIC's comparable to nitrofurazone against *H. vaginalis* and *Escherichia coli*, organisms usually implicated in bacterial vaginitis.

Experimental Section†

Bacteriostatic Test. Brain–heart infusion broth (Difco) with 0.1% agar added was heated to expel residual O_2 and then cooled to 40°. Calf serum was added at a 10% concentration. A 0.3-ml aliquot of a 24-hr culture was added to 5 ml of complete medium and after incubation for 18–20 hr at 37°, the culture was adjusted in fresh complete medium to 40% transmittance in a Spectronic 20 colorimeter at a setting of 440 nm. Calf serum was also added to the medium after diluting the test compound. The inoculum, 0.2 ml of the diluted culture, was added to 2-ml vol of the previously diluted compound. Tubes were incubated at 37° for 24 hr and the lowest compound concentration with no visible growth was considered the minimal inhibitory concentration (MIC).

Bactericidal Test. Appropriate concentrations of the compounds were added to 2 test tubes. Serum and mucin at 25% and 1%, respectively, were added to 1 tube and to the other tube serum at 5% and H_2O equivalent to the addition made to the first tube. Equal amounts of double strength Casman's broth were then added to each tube. Finally, a 16-hr broth culture of *Hemophilus vaginalis* was centrifuged and resuspended in Casman's broth to an O.D. of 0.3 at 620 nm is a Spectronic 20 colorimeter. Approximately 1×10^8 cells (0.1 ml) were added to each tube. Tubes, containing a

†Melting points were determined in open capillary tubes using a Mel-Temp melting point apparatus and are uncorrected. Microanalytical results obtained for the elements indicated were within ±0.4% of the theoretical value.