and ultraviolet maximum at 235 mμ); (3) probable presence of a C=N– moiety in a five-membered ring (infrared band at 1570 cm^{-1} and ultraviolet maximum at 271 mμ); (4) probably structure 5,6-dihydro-6-(2-thienyl)imidazo[2,1-*b*]thiazole (VII).

The oily residue was crystallized with oxalic acid from 2-propanol to furnish 190 mg of an oxalate salt, after drying, mp 191–192°. Elemental analysis was in agreement with the proposed structure VII oxalate.

Anal. Calcd for $C_9H_8N_2S_2 \cdot C_2H_2O_4$: C, 44.28; H, 3,38; N, 9.39; S, 21.50. Found: C, 44.05; H, 3.36; N, 9.55; S, 21.51.

Compound VII (thiazothielite) was then synthesized by ring closure of thiazothienol (IV) with $SOCl_2$ in the presence of acetic anhydride. Synthetic VII and the metabolite isolated from fractions 23–64 were found to be the same compound (mmp 192–193°, ultraviolet and infrared spectra).

Acknowledgments.—The authors are indebted to the "Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw (IWONL)" for financial support of this project. Thanks are also due to W. A. Seth Paul and J. Loomans for infrared and ultraviolet measurements and interpretations, A. Sels and W. Verkest for microanalyses, and L. Stoffels and L. Roevens of the pilot plant for preparing generous supplies of starting materials.

Semisynthetic Penicillins. III. Heterocyclic Penicillins

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This report describes a group of penicillins related to 2-biphenylylpenicillin,[1] in which one of the two phenyl rings is replaced by a heterocyclic ring. The structure–activity relationship of these penicillins is discussed. The preparation of some new side-chain acids used in the synthesis of these penicillins is reported.

In part I of this series[2] the preparation of a group of 2-biphenylylpenicillins which are highly active against a resistant[3] strain of staphylococcus was reported. In a subsequent paper[4] a variety of further structural modifications of the 2-biphenylyl side chain was reported. These studies indicated that replacing either benzene ring with α- and β-naphthyl resulted in compounds having activity similar to that of the parent compound, while replacing either benzene ring with a cyclohexyl moiety, gave compounds having only modest activity against resistant staphylococci. These studies also demonstrated that in the modification of the biphenylyl group the proximal[5] ring must be sufficiently aromatic and that the distal ring must conform to very exacting structural requirements for the compound to be highly active against resistant staphylococci.

This paper describes the preparation of a group of heterocyclic analogs of 2-biphenylylpenicillin and a study of the effect on biological activity of replacing either ring with certain heterocyclic systems. These penicillins are tabulated in Table I.

Earlier conjecture as to the requirements of the size and substitution of the proximal ring in determining the degree of penicillinase resistance of the penicillin is supported by the activities of compounds **1–6**. Our previous studies, and the work of Doyle, *et al.*,[6] suggest that while the six-membered ring with only one *o*-phenyl substituent can confer penicillinase resistance on the penicillin, the phenyl-substituted unfused five-membered ring must be reinforced by *o,o'* disubstitution with respect to the carboxamido grouping to achieve the same effect. In this case **1–5**, without the *o,o'* disubstitution, are essentially inactive against the resistant strain, while oxacillin, 5-methyl-3-phenyl-4-isoxazolylpenicillin, having an *o,o'*-disubstituted five-membered heterocyclic ring, and **6**, having a phenyl-substituted, unfused six-membered ring, have activity against the resistant strain similar to their activity against the susceptible strain.

For the fused heterocyclic compounds **7–20** the same relationships hold as were found for the phenylpenicillins described in the preceding studies. Apparently the proximal phenyl ring can be replaced by a heterocyclic ring of sufficient size and aromaticity (*e.g.*, six-membered ring or fused-heterocyclic but not unfused five-membered ring) with the retention of activity against the resistant staphylococcus, provided that there is adjacent to the carboxamido group a phenyl or an equivalent substituent. As reflected in the preceding studies, when the adjacent carbon carries hydrogen or alkyl (**10**, **13**, **15**, and **16**) the activity against the resistant strain is lost, although the cyclohexyl ring can be a fairly efficient substitute for the distal phenyl substituent (see **14**, Table I, in ref 4: MIC of *o*-cyclohexylphenylpenicillin, susceptible 0.09 μg/ml, resistant 7.5 μg/ml). The *o*-phenyl substituent is less efficient in confering penicillinase resistance on the molecule when the carboxamido group is attached adjacent to the hetero atom rather than adjacent only to carbons (compare **7** and **11**, **14** and **17**).

The minimum inhibitory concentration values for **21** and **22** indicate that the distal phenyl group can be replaced by an aromatic heterocyclic system with the retention of activity against the resistant staphylococcus.

(1) 2-Biphenylylpenicillin has also been referred to as Ancillin in various publications.

(2) J. R. E. Hoover, A. W. Chow, R. J. Stedman, N. M. Hall, H. S. Greenberg, M. M. Dolan, and R. J. Ferlauto, *J. Med. Chem.*, **7**, 245 (1964).

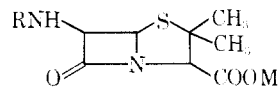
(3) The term "susceptible" is used for those staphylococci that are sensitive to penicillins G and V while the term "resistant" is used for those penicillinase-producing staphylococci unaffected by high levels (*e.g.*, 500–1000 μg/ml) of these antibiotics.

(4) R. J. Stedman, J. R. E. Hoover, A. W. Chow, M. M. Dolan, N. M. Hall, and R. J. Ferlauto, *J. Med. Chem.*, **7**, 251 (1964).

(5) See ref 4, p 252, for a definition of the proximal and distal rings.

(6) F. P. Doyle, J. C. Hanson, A. A. W. Long, J. H. C. Nayler, and E. R. Stove, *J. Chem. Soc.*, 5838 (1963).

TABLE I



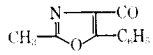
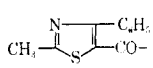
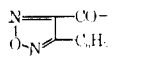
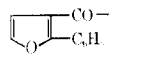
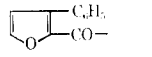
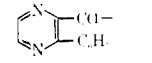
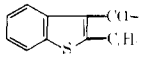
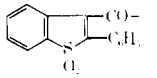
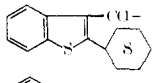
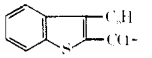
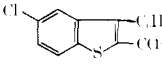
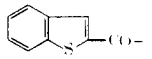
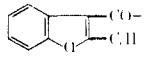
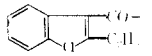
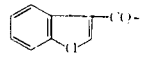
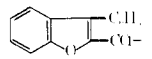
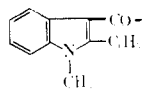
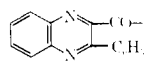
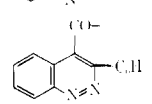
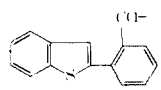
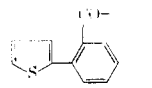
No.	R	Source of side-chain acid	Cryst. solvent[a]	Yield,[b] %	Dec. pt., °C[c]	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Infrared assay,[d] %	MIC for staphylococci,[e] μg/ml Suscept.	MIC for staphylococci,[e] μg/ml Resist.
1		*f*	E	56	...	$C_{19}H_{18}N_2O_6S$Na·0.5H$_2$O	52.77	52.84	4.43	4.63	93	0.62	80
2		*g*	I	53	179–187	$C_{19}H_{18}N_3O_4S_2K$·H$_2$O	48.18	48.19	4.26	4.57	91	1.5	125
3		*h*	C	16	175–176	$C_{17}H_{18}N_4O_6S$Na·1.5H$_2$O	46.68	46.60	4.15	4.20	80	0.18	500
4		*e*	I	38	153–159	$C_{19}H_{17}N_2O_6S$K·0.5H$_2$O	52.64	52.61	4.19	4.19	96	0.09	500
5		*j*	II	77	178–189	$C_{19}H_{17}N_2O_6S$K·0.5H$_2$O	52.64	52.75	4.19	4.27	99	0.09	1000
6		*k*	B	55	192–199	$C_{19}H_{17}N_3O_4S$Na·H$_2$O	52.05	52.10	4.37	4.62	92	3.7	1.8
7		*k*	G	11	195–205	$C_{23}H_{19}N_2O_4S_2K$·H$_2$O	54.31	54.05	4.16	4.30	89	0.37	0.45
8		*k*	E	10	221–225	$C_{25}H_{19}N_2O_6S_2K$·1.5H$_2$O	50.26	50.04	4.03	4.37	89	11.75	15
9		*k*	C	16	205–210	$C_{24}H_{26}N_2O_4S_2$Na·2H$_2$O	53.47	53.89	5.66	5.57	88	0.31	0.45
10		*l*	D	35	183–189	$C_{17}H_{18}N_2O_4S_2$Na·0.5H$_2$O·0.5EtOAc[m]	50.51	50.50	4.47	4.70	97	0.07	>1000
11		*a*	E	34	...	$C_{23}H_{18}N_2O_4S_2K$·0.5H$_2$O	55.29	55.30	4.03	4.11	81	11.22	15
12		*a*	F	62	194–198	$C_{23}H_{18}ClN_2O_4S_2K$·1.5H$_2$O	50.03	50.34	3.83	4.25	100	0.75	15
13		*p*	K	77	183–185	$C_{17}H_{15}N_2O_4S_2K$·3H$_2$O	43.57	43.58	4.52	4.25	102	0.09	500
14		*k*	J	63	188–195	$C_{24}H_{19}N_2O_6S$Na·0.5H$_2$O	59.09	59.09	4.34	4.48	90	0.18	0.45
15		*q*	E	18	...	$C_{18}H_{18}N_2O_6S$Na·0.5H$_2$O	54.41	54.06	4.81	4.93	86	0.05	250
16		*c*	L	17	216–218	$C_{17}H_{16}N_2O_6S$K·2H$_2$O	46.99	47.09	4.41	4.38	105	0.05	1000
17		*s*	I	70	188–195	$C_{23}H_{19}N_2O_6S$K·0.5H$_2$O	57.12	56.87	4.17	4.48	89	0.37	31
18		*t*	E	10	...	$C_{21}H_{22}N_3O_4S$Na·1.5H$_2$O	57.82	57.89	5.05	5.16	79	1.5	1.8
19		*a*	F	46	217–229	$C_{23}H_{19}N_3O_4S$K·H$_2$O	54.74	54.88	4.19	4.57	93	1.5	1.8
20		*e*	D	15	225–233	$C_{24}H_{18}N_3O_4S$Na·3H$_2$O	51.78	51.31	4.91	4.26	*w*	3.1	15
21		*k*	A	57	187–194	$C_{23}H_{19}N_2O_4S_2K$·1.5H$_2$O	53.36	53.15	4.28	4.19	98	0.18	0.90
22		*k*	C	57	185–190	$C_{15}H_{17}N_2O_4S_2K$·H$_2$O	49.76	49.59	4.18	4.19	100	0.37	0.45

TABLE II (*Continued*)

[a] Recrystallizations were carried out without heating. Solvents: A, methanol; B, methanol–ether; C, methanol–isopropyl ether; D, methanol–ethyl acetate; E, ethanol–ether; F, ethanol–isopropyl ether; G, ethanol–*n*-pentane; H, 1-butanol–isopropyl ether; I, chloroform–ether; J, acetone; K, acetone–ether; L, water–isopropyl alcohol. [b] Yields of purified products. [c] At the decomposition point, which was dependent on the rate of heating, the penicillin changed from a solid to a bubbling liquid; darkening and shrinking had usually already occurred. Where no decomposition point is reported, the same changes took place, but over a range of temperature, neither the beginning nor the end of which was well defined. [d] See ref 2 for the method. Assays are calculated for the appropriate hydrates. [e] Measured in broth by serial dilutions. End points were determined by macroscopic readings after incubation for 18 hr at 37°. Inoculum, 10^6 organisms/ml. The susceptible staphylococcus: coagulase positive, not phage typable, MIC 0.04 μg/ml of penicillin G, 1.8 μg/ml of methicillin, and 0.22 μg/ml of 2-biphenylylpenicillin. The resistant staphylococcus: Finland 400, phage type 54, MIC >1000 μg/ml of penicillin G, 3.7 μg/ml of methicillin, and 0.45 μg/ml of 2-biphenylylpenicillin. [f] The corresponding ethyl ester was prepared according to the method of C. Tanaka and N. Saito, *Yakugaku Zasshi*, **82,** 136 (1962), and hydrolyzed to the acid according to the directions of F. Korte and K. Störiko, *Ber.*, **93,** 1033 (1960). [g] Prepared from ethyl α-chlorobenzoylacetate by the action of phosphorous pentasulfide and acetamide, mp 212°. *Anal.* Calcd for $C_{11}H_9NO_2S$: C, 60.26; H, 4.14. Found: C, 60.47; H, 4.34. E. B. Knott, *J. Chem. Soc.*, 1656 (1947), using a similar reaction reported mp 216°. [h] F. Korte and K. Störiko, *Ber.*, **94,** 1956 (1961). [i] Prepared according to the general procedure of A. W. Johnson, *J. Chem. Soc.*, 895 (1946). [j] The corresponding ethyl ester was prepared according to the procedure of D. M. Burness, *J. Org. Chem.*, **21**, 102 (1956), and hydrolyzed to the acid with 10% NaOH in 64% yield; mp 151–153°. *Anal.* Calcd for $C_{11}H_8O_3$: C, 70.21; H, 4.29. Found: C, 70.40; H, 4.35. [k] This paper. [l] M. Martynoff, *Compt. Rend.*, **236,** 385 (1953). [m] Infrared spectrum of analytical sample, dried at 100° *in vacuo*, indicated the presence of ethyl acetate. [n] Prepared in 80% yield from 2-(methylthio)benzophenone and chloroacetic acid by the procedure of R. D. Schuetz and L. Ciporin, *J. Org. Chem.*, **23,** 206 (1958), mp 199–200°; S. Middleton, *Australian J. Chem.*, **12,** 218 (1959), reported mp 199–200°. [o] R. D. Schuetz and L. Ciporin, *J. Org. Chem.*, **23,** 206 (1958). [p] R. A. Shirley and M. D. Cameron, *J. Am. Chem. Soc.*, **72,** 2788 (1950). [q] M. Bisagni, Ng. Ph. Buu-Hoï, and R. Royer, *J. Chem. Soc.*, 3688 (1955). [r] E. H. Huntress and W. M. Hearon, *J. Am. Chem. Soc.*, **63,** 2762 (1941). [s] J. N. Chatterjea, *J. Indian Chem. Soc.*, **33,** 339 (1956). [t] A. F. Ames, D. E. Ames, C. R. Coyne, T. F. Grey, I. M. Lockhart, and R. S. Ralph, *J. Chem. Soc.*, 3388 (1959). [u] Prepared by the procedure of A. Wahl, *Bull. Soc. Chim. France*, **1,** 461 (1907), except that nitrogen oxides were generated more conveniently *in situ* by adding an aqueous $NaNO_2$ solution to glacial acetic acid containing ethyl isonitrosobenzoylacetate, mp 162.5–163° dec; Wahl reported mp 166–167°. *Anal.* Calcd for $C_{15}H_{10}N_2O_2$: C, 71.99; H, 4.03. Found: C, 72.32; H, 4.06. [v] Prepared from N-benzylideneaminoisatin according to the procedure of H. E. Baumgarten and J. L. Furnas, *J. Org. Chem.*, **26,** 1536 (1961), except that a more dilute (0.5 *N*) NaOH solution was used, mp 238° dec; R. Stollé and W. Becker, *Ber.*, **57,** 1123 (1924), reported mp 244°; Baumgarten and Furnas reported mp 224–224.5°. *Anal.* Calcd for $C_{15}H_{10}N_2O_2$: C, 71.99; H, 4.03; N, 11.19. Found: C, 71.81; H, 3.93; N, 11.27. [w] Infrared assay not done.

Experimental Section[7]

3-Phenylpyrazine-2-carboxylic Acid.—A stirred mixture of 7.3 g (0.04 mole) of 3-phenylpyrazine-2-carbonitrile,[8] 7.3 g of NaOH, and 75 ml of ethylene glycol containing 15 drops of water was heated at reflux overnight (19 hr). The reaction mixture was poured into 500 ml of ice–water and filtered, and the filtrate made strongly acidic with concentrated HCl. The light tan solid which crystallized was filtered, washed with water, and dried. Recrystallization from benzene–petroleum ether using Norit A afforded 5.85 g (72%) of colorless solid, mp 143–144° dec.

Anal. Calcd for $C_{11}H_8N_2O_2$: C, 65.99; H, 4.03; N, 13.99. Found: C, 66.11; H 4.07; N, 14.10.

2-Phenyl-3-thianaphthenecarboxylic Acid.—To a stirred solution of 700 ml (115 g, 1.8 moles) of *n*-butyllithium in hexane and 700 ml of anhydrous ether, cooled in an ice bath and in an atmosphere of nitrogen, was added during a period of 0.5 hr a solution of 201 g (1.5 moles) of redistilled thianaphthene in 200 ml of anhydrous ether. The mixture was stirred a further 2.5 hr and then refrigerated overnight. To the crude 2-thianaphthenyllithium thus prepared were added 84.5 g (0.75 mole) of redistilled chlorobenzene and 12.8 g of redistilled piperidine, and the resulting mixture was refluxed for 7 hr during which period two additional portions of 12.8 g of piperidine were added after 2 and 4 hr. The reaction mixture was cooled, and stirred at room temperature overnight. After cautious addition of 2 l. of water, the phases were separated and the aqueous phase was extracted with four portions of $CHCl_3$ totaling 3 l. The $CHCl_3$ layer was washed three times with 1 l. of 3 *N* HCl and dried. The $CHCl_3$ extract was evaporated to a crystalline mass which was filtered, washed with cold alcohol and dried to give 100.2 g (64% based on chlorobenzene) of **2-phenylthianaphthene**, mp 171–173° (lit.[9] mp 175–176°, 174–175°, 175.5–176°).

Anal. Calcd for $C_{14}H_{10}S$: C, 79.96; H, 4.79; S, 15.25. Found: C, 79.94; H, 4.78; S, 15.19.

To a suspended mixture of 96 g (0.457 mole) of 2-phenylthianaphthene and 74 g (0.902 mole) of NaOAc in 880 ml of $CHCl_3$ was added dropwise with stirring a solution of 26.2 ml (0.511 mole) of Br_2 in 180 ml of $CHCl_3$. The resulting mixture was refluxed 1.5 hr and then stirred a further 3 hr without heat. To this mixture was added 220 ml of water, and the phases were separated. The chloroform extract was washed once with 220 ml of 5% NaOH and twice with 450 ml of saturated NaCl solution. It was then dried and filtered, and the $CHCl_3$ filtrate was evaporated to an oil which crystallized. The crystalline mass was macerated with a small amount of petroleum ether and filtered to give 130 g (98%) of **3-bromo-2-phenylthianaphthene**, mp 61–64°.

Anal. Calcd for $C_{14}H_9BrS$: C, 58.14; H, 3.14. Found: C, 57.81; H, 2.99.

The bromo compound (115 g, 0.398 mole) was refluxed for 25 hr with a mixture of 345 g (0.388 mole) of CuCN and 288 ml of redistilled 1-methyl-2-pyrrolidone.[10] The resulting complex was decomposed with $FeCl_3$,[11] and the crystalline solid thus obtained was recrystallized from methanol to give 67.0 g of **3-cyano-2-phenylthianaphthene**, mp 93–96° (second crop 6.5 g, mp 88–93°); total yield, 73.5 g (79%). This material was sufficiently pure for conversion to the acid.

Anal. Calcd for $C_{15}H_9NS$: C, 76.56; H, 3.86; N, 5.95. Found: C, 76.52; H, 3.84; N, 5.76.

The nitrile (55 g, 0.234 mole) was hydrolyzed by refluxing for 19 hr with 55 g (1.38 moles) of NaOH, 400 ml of ethylene glycol, and 1 ml of water. The cooled mixture was diluted with 2 l. of water and filtered, and the filtrate was made strongly acidic with concentrated HCl to precipitate a white solid. This was filtered, washed with water, and dried to give 68.7 g of the crude **2-phenyl-3-thianaphthenecarboxylic acid**, mp 159–180°. Crystallization from dilute aqueous alcohol gave 51.6 g (87%) of a white solid, mp 188–189°.

Anal. Calcd for $C_{15}H_{10}O_2S$: C, 70.84; H, 3.96. Found: C, 70.78; H, 4.05.

2-Phenyl-3-thianaphthenecarboxylic Acid 1,1-Dioxide.—The procedure described here is similar to that which has been used

(7) Corrected capillary melting points and decomposition points (Thomas–Hoover melting point apparatus) are reported. Infrared spectra were recorded with a Perkin-Elmer Infracord. Ultraviolet data were obtained with a Cary Model 14 spectrophotometer. Ethanol was anhydrous S.D.A. 2B grade. Petroleum ether was the fraction of bp 30–60°. $MgSO_4$ was used as drying agent unless otherwise stated. Evaporations were carried out under aspirator vacuum.

(8) G. Karmas and P. E. Spoerri, *J. Am. Chem. Soc.*, **78,** 2141 (1956).

(9) J. E. Banfield, W. Davies, N. W. Gamble, and S. Middleton, *J. Chem. Soc.*, 4791 (1956); O. Dann and M. Kokorudz, *Ber.*, **91,** 172 (1958); A. W. Horton, *J. Org. Chem.*, **14,** 761 (1949). Several attempts to prepare this compound by the methods of Banfield, *et al.*, gave very low yields.

(10) M. S. Newman and H. Boden, *ibid.*, **26,** 2525 (1961).

(11) L. Friedman and H. Shechter, *ibid.*, **26,** 2522 (1961).

for preparing other thianaphthene 1,1-dioxides.[12] A stirred mixture of 12.7 g (0.05 mole) of 2-phenyl-3-thianaphthenecarboxylic acid, 31 ml of 30% H_2O_2, and 75 ml of glacial acetic acid was heated to reflux for 0.5 hr. The reaction mixture was poured into 250 ml of water and cooled, and the precipitated yellow solid was filtered, washed with water, and dried. The crude solid was redissolved in 5% $NaHCO_3$ solution and filtered, and the filtrate was acidified with 3 *N* HCl to give a yellow precipitate. Crystallization from aqueous ethanol gave 9.6 g (67%) of **2-phenyl-3-thianaphthenecarboxylic acid 1,1-dioxide** as a yellow solid, mp 191–193°.

Anal. Calcd for $C_{15}H_{10}O_4S$: C, 62.93; H, 3.52. Found: C, 62.99; H, 3.37.

2-Cyclohexyl-3-thianaphthenecarboxylic Acid.—A solution of 2-thianaphthenyllithium[13] was prepared by adding 53.6 g (0.4 mole) of redistilled thianaphthene in 60 ml of dry ether to a solution of 370 ml (0.6 mole) of *n*-butyllithium (15% in hexane) in 370 ml of dry ether with ice-salt-bath cooling. To this ice-cold solution, stirred under a nitrogen atmosphere, was added 58.8 g of cyclohexanone in 100 ml of ether over a 15–20-min period. Stirring was continued for 2 hr as a white precipitate formed; then 700 ml of ice-cold, saturated NH_4Cl solution was slowly added. The nearly colorless organic layer was separated, and the aqueous layer was extracted with additional ether. The combined organic layers were dried (Na_2CO_3). The solvent was evaporated to give a light yellow oil which crystallized upon standing overnight. After recrystallization from hexane there was obtained 76.5 g (82% based on thianaphthene) of colorless **2-(1-hydroxycyclohexyl)thianaphthene**, mp 93–94°. The analytical sample was recrystallized a second time from hexane, mp 94.5–96°.

Anal. Calcd for $C_{14}H_{16}OS$: C, 72.37; H, 6.94. Found: C, 72.40; H, 7.15.

A mixture of 11.6 g (0.05 mole) of 2-(1-hydroxycyclohexyl)-thianaphthene, 1.5 g of anhydrous oxalic acid, and 100 ml of toluene was refluxed through a Dean–Stark trap until the theoretical quantity of water was collected (1 hr). The reaction mixture was washed twice with 25 ml of 5% $NaHCO_3$ solution, once with 25 ml of water, and then dried. Evaporation of the solvent left a pale yellow, crystalline residue which was recrystallized from methanol to give 8.85 g of white plates, mp 90–91.5°. The mother liquors yielded an additional 0.6 g of product having the same melting point. Total yield of **2-(1-cyclohexenyl)-thianaphthene** was 9.45 g (88%); λ_{max}^{EtOH} 290 mμ (ε 26,862), 234 mμ (ε 23,124).

Anal. Calcd for $C_{14}H_{14}S$: C, 78.45; H, 6.58. Found: C, 78.50; H, 6.54.

Hydrogenation of 2-(1-cyclohexenyl)thianaphthene (21.4 g, 0.1 mole) was carried out with 2.5 g of 10% Pd-C in 150 ml of ethyl acetate on a Parr apparatus under 3.5 kg/cm² of hydrogen for 30 min. The absorption of hydrogen appeared essentially complete. The spent catalyst was filtered, and the solvent was evaporated to give 21 g of oil which soon crystallized. Recrystallization from methanol gave 18.9 g of **2-cyclohexylthianaphthene** as a white solid, mp 55–57°. The addition of a small amount of water to the mother liquor yielded a second crop: 0.63 g; mp 54–55°; total yield 90%; λ_{max}^{EtOH} 230 mμ (ε 31,476), 262 mμ (ε 9432).

Anal. Calcd for $C_{14}H_{16}S$: C, 77.72; H, 7.45. Found: C, 77.78; H, 7.38.

With ice-bath cooling, dry HCl was passed into a stirred suspension of 2.6 g of paraformaldehyde in 30 ml of glacial acetic acid until all of the paraformaldehyde had dissolved. A total of 8.0 g of HCl was absorbed. The cooling bath was removed, and 13 g (0.06 mole) of 2-cyclohexylthianaphthene was added in one portion. The temperature slowly rose to a maximum of 33°, and was allowed to drop to room temperature over a 5-hr period. The yellow, oily suspension was refrigerated overnight and poured into 250 ml of water, and the aqueous mixture was extracted with two 125-ml portions of ether. The combined extract was washed with 5% $NaHCO_3$ solution until the washings were alkaline, and then with 50 ml of water. The washed ethereal solution was dried and filtered, and the filtrate was evaporated to give a viscous light yellow syrup which crystallized from ether. Recrystallization from petroleum ether gave 11.8 g (74%) of **3-chloromethyl-2-cyclohexylthianaphthene**, mp 83–85°. The analytical sample was recrystallized a second time from the same solvent: mp 84–85.5°. The product gave a positive halogen test with alcoholic $AgNO_3$ at room temperature.

Anal. Calcd for $C_{15}H_{17}ClS$: Cl, 13.39. Found: Cl, 13.11.

The chloromethyl compound was converted to the formyl compound by the method of Hass and Bender.[14] To 200 ml of absolute alcohol were added in the order given 1.84 g (0.08 g-atom) of Na, 7.4 g (0.083 mole) of redistilled 2-nitropropane, and 21.2 g (0.08 mole) of 3-chloromethyl-2-cyclohexylthianaphthene. The mixture was stirred for 17 hr at room temperature with protection from atmospheric moisture. Following a 5-min reflux period the NaCl which formed was removed by filtration, and the alcohol was distilled under reduced pressure. The residual yellow oil was taken up in 200 ml of ether. The ethereal solution was washed once with 100 ml of water, twice with 50 ml of 2.5 *N* NaOH, twice with 50 ml of water, and then dried. Evaporation of ether and distillation of the residue yielded 15.2 g (78%) of **2-cyclohexyl-3-formylthianaphthene** as a yellow oil, bp 170–177° (0.35–0.70 mm), λ_{max}^{film} 5.95 μ (formyl band).

The formyl compound was converted to the nitrile by the general method of Blatter and co-workers,[15] and thence to the acid by alkaline hydrolysis.[16]

A stirred mixture of 7.35 g (0.03 mole) of 2-cyclohexyl-3-formylthianaphthene, 28.6 g of $(NH_4)_2HPO_4$, 125 ml of redistilled 1-nitropropane, and 41 ml of glacial acetic acid was refluxed for 20 hr. After removal of all volatile material under reduced pressure, the residual syrup was shaken with 100 ml of hot water and left overnight. The aqueous mixture was extracted with two 100-ml portions of ether. The combined extract was washed free of acid with 5% Na_2CO_3 and dried. Evaporation of the ether left 7.12 g of crude **3-cyano-2-cyclohexylthianaphthene**, λ_{max}^{Nujol} 4.44 μ (cyano band). This was hydrolyzed without purification by refluxing for 19 hr with 7.0 g of NaOH in 70 ml of ethylene glycol containing 5 drops of water. The cooled reaction mixture was diluted with 140 ml of water, extracted twice with 50 ml of ether to remove some nonacidic material, stirred with decolorizing carbon, and filtered, and the filtrate was made strongly acidic with 3 *N* HCl. The precipitated light tan solid (2.0 g) was collected and purified by recrystallization from glacial acetic acid using decolorizing carbon. The yield of slightly pinkish colored crystals of **2-cyclohexyl-3-thianaphthenecarboxylic acid** was 1.52 g (19% based on the aldehyde), mp 211–212.5°.

Anal. Calcd for $C_{15}H_{16}O_2S$: C, 69.20; H, 6.19. Found: C, 69.23; H, 6.19.

2-Phenylbenzofuran-3-carboxylic Acid.—A KOCl solution[17] was prepared from 9.7 g of $Ca(OCl)_2$ ("HTH") and to this solution was added in one portion 4.4 g (0.0186 mole) of 2-phenyl-3-acetylbenzofuran.[18] Heating the rapidly stirred mixture at 65° for 2 hr resulted in the evolution of $CHCl_3$ and complete consumption of the KOCl (negative test with acidified KI solution). A second portion of KOCl was prepared, using one-half the quantities, and added to the reaction mixture. After an additional hour of stirring at 65°, during which time more chloroform was evolved, there remained an excess of KOCl, which was destroyed by adding a little $NaHSO_3$. The reaction mixture was acidified while hot with 3 *N* HCl. After cooling, the pale yellow solid which formed was filtered, dried, and purified by recrystallization from aqueous ethanol, decolorizing with charcoal. The yield of purified **2-phenylbenzofuran-3-carboxylic acid** was 3.2 g (72%), mp 194–195° (lit.[19] mp 195°).

Anal. Calcd for $C_{15}H_{10}O_3$: C, 75.62; H, 4.23. Found: C, 75.79; H, 4.54.

***o*-(2-Thianaphthenyl)benzoic Acid.**—A mixture of 39 g (0.15 mole) of 2-iodothianaphthene[20] and 78 g (0.3 mole) of methyl *o*-iodobenzoate was stirred in a bath at 225°, and 90 g of copper bronze was added gradually over a period of 3 hr. The reaction

(12) (a) F. G. Bordwell, B. B. Lampert, and W. H. McKellin, *J. Am. Chem. Soc.*, **71**, 1702 (1949); (b) A. H. Schlesinger and D. T. Mowry, *ibid.*, **73**, 2614 (1951).

(13) D. A. Shirley and M. D. Cameron, *J. Am. Chem. Soc.*, **74**, 664 (1952).

(14) H. B. Hass and M. L. Bender, *ibid.*, **71**, 1767 (1949); "Organic Syntheses," Coll. Vol. IV, N. Rabjohn, Ed., John Wiley and Sons, Inc., New York, N. Y., 1963, p 932.

(15) R. M. Blatter, H. Lukaszewski, and G. deStevens, *J. Am. Chem. Soc.*, **83**, 2203 (1961).

(16) Direct oxidation of 2-cyclohexyl-3-formylthianaphthene to the acid with Ag_2O or $KMnO_4$ was unsuccessful.

(17) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 429.

(18) R. Royer, E. Bisagni, and C. Hudry, *Bull. Soc. Chim. France*, 933 (1961).

(19) J. N. Chatterjea, *Experientia*, **12**, 18 (1956).

(20) R. Gaertner, *J. Am. Chem. Soc.*, **74**, 1950 (1952).

mixture was stirred for another hr with the bath at 250°, cooled, and extracted with 1 l. of acetone in four portions. The acetone solution was evaporated to give an oil which was refluxed with 300 ml of 2 *N* KOH in aqueous ethanol for 8 hr. The cooled hydrolysate was concentrated to remove alcohol, diluted with water to 1 l. and filtered to remove a little insoluble material. The filtrate was washed twice with benzene and then made acidic with 6 *N* H_2SO_4 to give 45 g of crude solid, which was extracted at room temperature with 500 ml of benzene in three portions. The residue consisted of 17.8 g of diphenic acid. The filtrate was concentrated to a small volume to give 13.7 g of crystalline solid, mp 154–156°. From the mother liquor a second crop of 3 g (mp 150–153°) was obtained; total yield 16.7 g (44%). Recrystallization from ethanol–water gave 13.7 g (36%) of the acid as colorless crystals, mp 155.5–156°.

Anal. Calcd for $C_{15}H_{10}O_2S$: C, 70.84; H, 3.96. Found: C, 71.08; H, 4.03.

***o*-(2-Thienyl)benzoic Acid.**—Copper bronze (60 g) was added in small portions during 20 min to a stirred mixture of 26 g (0.124 mole) of *o*-iodothiophene and 60 g (0.23 mole) of methyl *o*-iodobenzoate heated in a bath at 180°. The resulting sludge was stirred under reflux for a further 4 hr with the bath at 210–220°. The reaction mixture was processed in the same manner as described for the preparation of *o*-(2-thianaphthenyl)benzoic acid. The crude product weighed 5.5 g (22%), mp 84–91°. Recrystallization from aqueous acetic acid gave 3.65 g (14%) of the pure acid as colorless crystals, mp 93–94°.

Anal. Calcd for $C_{11}H_8O_2S$: C, 64.69; H, 3.95. Found: C, 64.83; H, 4.02.

Preparation of Penicillins.—The methods used for the preparation of penicillins are those reported in the first paper of this series.[2] In most cases the acid chlorides of the side-chain acids were used to react with 6-aminopenicillanic acid in aqueous acetone in the presence of $NaHCO_3$ and the penicillins were isolated as their sodium or potassium salts. In two cases, penicillins **18** and **20** of Table I, an anhydrous system consisting of acid chloride–triethylamine–chloroform was used. The resulting penicillins were isolated in the usual manner.

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Activity of a Series of Piperdines against *Haemonchus contortus* Larvae

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An *in vitro* test procedure using larvae of *Haemonchus contortus*, a gastrointestinal nematode of sheep, has been used to determine the antilarval activity of a series of 1,4-substituted piperidines and related compounds. Structure–activity relationships are discussed. The most effective compound found in this series of piperidines, many of which are new to the literature, was 1-phenethyl-4-phenylpiperidine hydrochloride which produced a 90% reduction in developing larvae at a concentration of 1:8000.

Larvae of horse strongyles have been used to examine the antilarval activity of compounds for many years.[2] Many series of organic and inorganic compounds have been tested by this method.[3–5] The methods and early literature have been well reviewed.[6]

The test was orginally designed to find a compound which would kill larvae on pasture, but has also proved useful in discovering compounds capable of killing the parasitic stages within the host animal. The organic phosphate, O,O-dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate[7] which was found to be efficient against larvae of horse strongyles[8] has subsequently been found to be a useful anthelmintic treatment for adult *Haemonchus* and other nematode species of sheep[9] and cattle[10] when given orally or subcutaneously.[11]

Using a similar approach, the aim of our investigation was to discover a compound having sufficient activity against larvae of sheep helminths, so that when incorporated in a heavy pill, similar to the cobalt bullet,[12] and lodged permanently in the reticulorumen, the small quantity of compound continuously released would maintain a concentration in the rumen liquor sufficient to kill the majority of infective larvae as they were ingested with forage. About 1600 random organic compounds were tested. One of the first test compounds to show moderate activity was 3-phenethyl-3-azaspiro[5.5]undecane hydrochloride, one of a group of 1,4-substituted piperidines that had been synthesized for general pharmacological studies,[13] and others were synthesized and tested in pursuit of activity at a useful level. While useful activity was not attained in this series of compounds, interesting structure–activity relationships were noted and these are reported.

Testing Procedure.—Feces containing eggs of *H. contortus* were collected in fecal bags from lambs with pure infestations. The lambs were held indoors in concrete pens to prevent infestation with other helminths.

The pellets of feces were broken and mixed thoroughly. If necessary, sufficient helminthologically sterile feces were added to ensure that the moisture content was not too high. Five-gram samples were weighed into small tubes, 5 × 2 cm, containing a small wad of nonabsorbable cotton wool and the end was covered with muslin held on by a rubber band. The compound to be tested in the form of its hydrochloride salt was introduced in aqueous solution or as a fine sus-

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(6) N. D. Levine, *Trans. Illinois State Acad. Sci.*, **43**, 233 (1950).
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