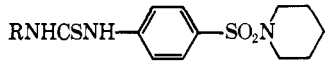
TABLE III
SUBSTITUTED THIOUREAS



No.	R	Yield, %	Mp, °C	Formula[d]
19	Me	89[a]	175	$C_{13}H_{19}N_3O_2S_2$
20	Et	91[a]	173	$C_{14}H_{21}N_3O_2S_2$
21	Bu	85[a]	183	$C_{16}H_{25}N_3O_2S_2$
22	*i*-Pr	89[a]	187	$C_{15}H_{23}N_3O_2S_2$
23	Pr	95[b]	158	$C_{15}H_{23}N_3O_2S_2$
24	Octyl	89[c]	99	$C_{20}H_{33}N_3O_2S_2$
25	Dodecyl	91[c]	93	$C_{24}H_{41}N_3O_2S_2$
26	Octadecyl	84[a]	84	$C_{30}H_{53}N_3O_2S_2$
27	Ph	65[a]	177	$C_{18}H_{21}N_3O_2S_2$
28	β-Naphthyl	79[a]	188	$C_{22}H_{23}N_3O_2S_2$

[a] Recrystallized from Me_2CO–H_2O. [b] Recrystallized from C_6H_6. [c] Recrystallized from C_6H_6–C_6H_{12}. [d] Analyzed for C, H, N, S.[5]

pounds **2** and **7** were effective at 10 ppm, and (b) **4** and **16** were effective at 100 ppm. None was effective against *Aspergillus niger* at concentrations below 1000 ppm. Since the influence of molecular size and chemical structure on antifungal activity is not well understood, no specific explanation can be given to account for these data. It may be pointed out, however, that the antifungal effectiveness of **2** and **7** against *Chaetomium globosum* is equivalent to that of a number of dithiosemicarbazones and the copper complexes of some of them[3] as well as of a commercial formulation of copper 8-hydroxyquinolinate and of dihydroxydichlorodiphenylmethane.[2] These latter compounds have also been shown to be ineffective against *Aspergillus niger* at concentrations below 1000 ppm.

Experimental Section

The reagents and solvents used in the syntheses described in this paper were the purest grade obtainable from commercial sources. Melting points were measured with a Fisher-Johns apparatus and are corrected. Elemental analyses were performed at the microanalytical laboratory of Drs. Weiler and Strauss in Oxford, England. Ir spectra of the new compounds described in this work were obtained from KBr pellets with a Model 21 Perkin-Elmer double-beam spectrophotometer (NaCl prism) over the frequency range 3500–700 cm^{-1}.

4-Isothiocyanatobenzenesulfonpiperidide (I) was obtained from a commercial source and was recrystallized repeatedly from benzene–hexane. The thiosemicarbazide of it (II) was prepared by adding dropwise hydrazine hydrate, 99–100% purity (0.1 mole), in EtOH (50 ml) to a solution of I (0.1 mole) in EtOH (250 ml). The mixture was heated on a steam bath for 30 min. The light brown precipitate which formed was separated by filtration, washed (H_2O, EtOH), dried, and purified by repeated crystallization from DMF–H_2O; yield 85%, mp 191°. *Anal.* ($C_{12}H_{18}N_4O_2S_2$) C, H, N, S. An ir absorption band observed at 1635 cm^{-1}, for II but none of the other compounds, is attributable to δ (NH_2).[6]

General Preparation for 1-Substituted 4-(4-Benzenesulfonpiperidide)thiosemicarbazides (III) (Table I).—Substituted hydrazine (0.01 mole), dissolved in warm H_2O or EtOH (50 ml), was added dropwise to a solution of I (0.01 mole) in EtOH (50 ml). The mixture was heated on a steam bath for 45 min, then cooled. The precipitate which formed was collected by filtration, washed (EtOH), dried, and recrystallized to constant melting point; ir absorptions: **2**, 3440 (OH); **5**, 3440 (OH), 1650 (C=O).

General Preparation for 4-(4-Benzenesulfonpiperidide)thiosemicarbazones (V) (Table II).—A solution of 4-(4-benzenesulfonpiperidide)thiosemicarbazide (II) (0.01 mole) in DMF (25 ml) was prepared. To this was slowly added a solution of aldehyde or ketone (0.01 mole) in EtOH (50 ml) containing 1 ml of glacial AcOH. The mixture was heated on a steam bath for 20 min; H_2O was then added until incipient precipitation. The precipitate which formed on cooling was collected by filtration, washed with cold 50% H_2O–EtOH, dried, and recrystallized to constant melting point; ir absorptions: **9**, **12**, **14**, 3440 (OH); **10**, 1630 (C=C); **11**, 1255 (COC).

General Preparation for Substituted Thioureas (IV) (Table III).—A solution of a primary amine (0.01 mole) in H_2O or EtOH (50 ml) was slowly added to a solution of I (0.01 mole) in EtOH (50 ml). The mixture was heated on a steam bath for 45 min, then cooled. The precipitate which formed was collected by filtration, washed (cold 50% H_2O–EtOH), dried, and recrystallized to constant melting point.

It is reported[7] that alcohols react with isothiocyanates to yield thiourethans. Nevertheless, the syntheses described above produce higher yields of the desired products when the reactions are carried out in EtOH than when either $CHCl_3$ or Et_2O is used as solvent.

The antimicrobial activity of all the compounds prepared in this work toward two microorganisms was determined by screening procedures involving the tube dilution method described previously.[2] The test organisms used in the screening experiments were *Chaetomium globosum* strain USDA 1042.4, and *Aspergillus niger* strain USDA 215–5373.16. Concentrations of the compounds being tested of 10, 100 and 1000 ppm were employed; the criterion of effectiveness was simply the absence of fungal growth after a 2-week incubation period (*C. globosum*) or after 48 hr (*A. niger*).

Acknowledgments.—The authors are grateful to Miss G. Colin for screening the new compounds for antifungal activity and to R. Ironside and V. Boyko for recording the infrared spectra.

(6) D. M. Wiles and T. Suprunchuk, *Can. J. Chem.*, **47**, 1087 (1969).

(7) S. J. Assony in "Organic Sulphur Compounds," Vol. 1, N. Kharasch, Ed., Pergamon Press, New York, N. Y., 1961, p 333.

Synthetic Penicillins Derived from Cycloheptatrienecarboxylic Acids

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A common feature in the majority of the medicinally important synthetic penicillins[2] is the aromatic nature of the acyl group attached to the 6-aminopenicillanic acid. With the commercial availability of this compound,[3] a great number of semisynthetic penicillins[4,5] have been prepared in an effort to obtain clinically effective products. From the vast number of examples which are available in the literature, it is evident that minor changes in the nature and position of substituents on the aromatic ring or a side chain in the vicinity of the acyl carbonyl group causes profound changes in the biological activity. Thus, the quest for newer synthetic penicillins having a broader spectrum of biological activity continues.

To the best of our knowledge, the preparation of penicillins containing the cycloheptatrienecarbonyl moiety has not been reported. We now wish to describe

(1) To whom all correspondence should be addressed at the Department of Chemistry, University of Montreal, Montreal, Quebec, Canada.

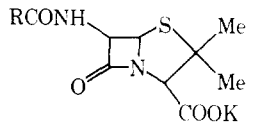
(2) J. O. Klein and M. Finland, *New Engl. J. Med.*, **269**, 1019, 1074, 1129 (1963).

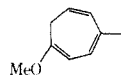
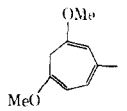
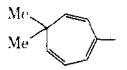
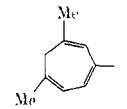
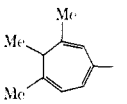
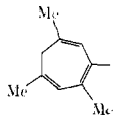
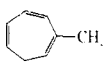
(3) F. R. Batchelor, F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson, *Nature*, **183**, 257 (1959).

(4) For leading references see Y. G. Perron, W. F. Minor, L. B. Crast, A. Gourevitch, J. Lein, and L. C. Cheney, *J. Med. Pharm. Chem.*, **5**, 1016 (1962).

(5) F. P. Doyle, J. H. C. Nayler, H. R. J. Waddington, J. C. Hanson, and G. R. Thomas, *J. Chem. Soc.*, 497 (1963).

TABLE I



No.	R	Purity,[b] %	Min inhib concn, μg/ml[c] *S. aureus* UC-76[d]	*S. aureus* TU-12404[d]	*Strep. pyogenes*	Ref[e]
1		82	0.8	50	0.05	6
2		90	0.4	100	0.05	7
3		88	6.3	100	3.1	9
4		96	6.3	100	3.1	10
5		95	3.1	50	1.6	11
6		73	3.1	50	0.8	*a*
7		95	0.4	6.3	0.2	12[f]
8		90	0.4	25	0.0063	13
9		90		25	0.0063	13
Penicillin G			0.0125–0.025	50–100	0.002	
Methicillin			0.8–1.6	1.6–3.1	0.1–0.2	

[a] Acid **6**, RCOOH, mp 116–117°, was prepared from the thermal decomposition of ethyl diazoacetate in 1,2,3-trimethylbenzene, followed by acid hydrolysis of the resulting ester. [b] Estimated by comparing the extinction coefficients of the pure cycloheptatrienecarboxylic acid and the penicillin derived from it. The purity of the penicillin as determined by the NH_2OH procedure[14] was in good agreement with the uv assay. [c] Measured in broth by serial twofold dilutions. The tubes were examined macroscopically for end-point determinations after incubation for 18 hr at 37°. For details see M. W. Fisher, *et al.*, *Antibiot. Ann.*, 293 (1959–1960). [d] *S. aureus* UC-76 and TU-12404 refer to penicillin-sensitive and -resistant strains, respectively. [e] The reference indicates the preparation of the cycloheptatrienecarboxylic acid derivative. [f] An alternative structure for the R group is the 1,4,6-triene isomer.

the preparation of a series of such semisynthetic penicillins and to comment briefly on their biological activity. The cycloheptatrienecarboxylic acids employed in this investigation were prepared by published procedures[6–13] with minor modifications, using the well-known thermal decomposition of ethyl diazoacetate in appropriate aromatic hydrocarbons. The penicillins were prepared by treating the appropriate acid chloride with 6-aminopenicillanic acid and isolated in the form of amorphous potassium salts. The homogeneity of the products was verified in each case by tlc and their purity was determined qualitatively by ir spectra. Quantitative estimation of the purity was done by uv spectral measurements and the colorimetric hydroxylamine procedure.[14]

Table I lists the penicillins prepared, together with their purity and antibacterial activity. Consideration of the data reveals that the *in vitro* activity of the analogs against penicillinase-sensitive and -resistant strains of *Staphylococcus* is in general inferior to that of penicillin G and methicillin. The only promising *in vitro* activity was exhibited by the 2,4,6-trimethyl-1,3,6-cycloheptatrienecarbonyl penicillin (or the 1,4,6-triene isomer), which approached that shown by methicillin in the same test. It is interesting to note that the 1,3,6-cycloheptatrienecarbonyl penicillin can be considered to be a nonbenzenoid isomer of penicillin G. Thus, in the cycloheptatrienecarbonyl series, the nature and positions of substituents in the ring cause considerable changes in the levels of antibacterial activity as in the aromatic penicillin analogs. In mice, the penicillin analog mentioned above was well tolerated at the

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(7) J. R. Bartels-Keith, A. W. Johnson, and A. Langemann, *J. Chem. Soc.*, 4461 (1952).
(8) R. B. Johns, A. W. Johnson, and M. Tisler, *ibid.*, 4605 (1954).
(9) R. B. Johns, A. W. Johnson, and J. Murray, *ibid.*, 198 (1954).
(10) A product of the Crown-Zellerbach Co.
(11) E. Buchner and K. Dolbruck, *Ann.*, **358**, 1 (1908).
(12) E. Buchner and K. Schottenhammer, *Ber.*, **53**, 865 (1920).
(13) F. Korte, K.-H. Buchel, and F. F. Wiese, *Ann.*, **664**, 114 (1963).
(14) J. H. Ford, *Anal. Chem.*, **19**, 1004 (1947).

highest dose tested of 100 mg/kg. Additional *in vivo* data can be found in Table II.

TABLE II

Penicillin	Approx single sc dose[a] ED_{50}, mg/kg *S. aureus* UC-76	*S. aureus* H-228	Resistance index in mice[b]
Penicillin G	1	80	80
Oxacillin	16	25	1.6
2,4,6-Trimethylcycloheptatrienecarbonyl penicillin	70	450	6.4

[a] Standard mouse protection tests [M. W. Fisher, M. C. Manning, L. A. Gagliardi, M. R. Gaetz, and A. L. Erlandson, *Antibiot. Ann.*, 293 (1959–1960)] involving single subcutaneous dose therapy (0.5 ml) concurrent with lethal intraperitoneal mucinized challenges with either *S. aureus* UC-76 (sensitive) or H-228 (resistant); groups of ten mice were used. [b] *In vivo* resistance index obtained by comparing data from the two *S. aureus* strains.

Experimental Section

Tlc was carried out on silica gel HF plates in the solvent system BuOH–H_2O–AcOH (80:80:2) with 6-aminopenicillanic acid (Aldrich Chemical Co.) as standard. The spots were detected under uv light and with the alkaline NH_2OH–$FeCl_3$ spray.

General Procedure for the Preparation of Penicillins.—The particular cycloheptatrienecarboxylic acid was refluxed in Et_2O in the presence of excess $SOCl_2$ for 2–3 hr. Evaporation of the solution and removal of excess reagent gave the corresponding acid chloride which was examined by ir spectroscopy for the absence of C=O peaks. The preparation of the penicillins followed the procedure described below.

A solution containing 0.39 g (0.002 mole) of 2,4,6-trimethylcycloheptatrienecarbonyl chloride[6] in 20 ml of CH_2Cl_2 was added dropwise to a stirred solution of 6-aminopenicillanic acid (0.42 g, 0.002 mole) and Et_3N (0.7 ml) in 7 ml of CH_2Cl_2 at 0°. The solution was stirred for 2 hr after the addition was completed and then allowed to warm to room temperature. Evaporation to dryness followed by addition of Me_2CO (20 ml) and filtration afforded a pale yellow solution which was concentrated to dryness. The residue was dissolved in 15 ml of H_2O, the solution was covered with 30 ml of EtOAc, and the pH was adjusted to 2.2 with 10% aqueous H_2SO_4 at 0°. The organic phase was separated, washed rapidly (H_2O), dried (Na_2SO_4), and filtered. A solution of 50% potassium 2-ethylhexanoate in BuOH was added to the filtrate and the whole was concentrated to a small volume (*ca.* 5 ml). Et_2O was then added to the neutral solution until precipitation was complete. After standing overnight at 0°, the precipitated K salt of the penicillin was filtered, washed (Et_2O), and dried (P_2O_5); yield, 0.3 g of an almost colorless solid. Tlc revealed the presence of only one spot. *Anal.* ($C_{19}H_{22}KN_2O_4 \cdot 2H_2O$) C, 50.08; H, 4.90; N, 6.23; S, 7.12. Found: C, 50.62; H, 4.60; N, 5.93; S, 6.72.

Acknowledgment.—The authors thank Dr. C. L. Heifetz of these laboratories for carrying out the biological tests.

Synthetic Penicillins Derived from Benznorcaradienecarboxylic Acids

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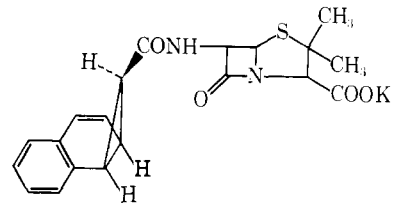
The enhanced antibacterial activity of certain commercially available penicillins against penicillinase-producing strains of *Staphylococcus aureus* has been attributed[2] to a "steric effect" exerted by the bulky group(s) attached to the acyl portion. Thus, in some penicillins[3] containing *ortho*-substituted aromatic acyl groups, such as methicillin, diphenicillin, nafcillin, etc., the cleavage of the lactam ring by penicillinase-producing bacteria is considerably retarded or even prevented, presumably due to the inability of the active sites of the enzyme to achieve the prerequisite fit with the specific groups on the penicillin.

We wish to describe the preparation of a series of novel semisynthetic penicillins **1**, derived from benznorcaradienecarboxylic acids (1a,7b-dihydro-1H-cyclopropa[*a*]naphthalene-1-carboxylic acids). From con-



1

siderations of molecular models of these derivatives, it was anticipated that the unique spacial arrangement[4] of the acyl group in the vicinity of the lactam ring would confer some interesting antibacterial properties, particularly against penicillinase-producing strains of *S. aureus*.

The majority of the benznorcaradienecarboxylic acids required for this study were prepared by published procedures. The penicillins were prepared by the acid chloride method and were isolated in the form of amorphous potassium salts. Their homogeneity was established qualitatively by tlc and ir spectral data, and quantitatively by a uv spectral assay and colorimetric procedures.

Results of the antibacterial tests[5] are listed in Tables I and II. It can be seen that, in general, the derivatives exhibit *in vitro* antibacterial activities against penicillinase-producing strains, which are somewhat superior to penicillin G, but none approach the levels shown by oxacillin. The most promising compound appeared to be the unsubstituted penicillin **1**, which has a favorable ratio of activities *vs.* the two *S. aureus* strains. It is of interest to note that minor variations in the nature and positions of substituents in the substituted compound caused appreciable variations in *in vitro* activity, particularly against *Streptococcus pyogenes* (**15** and **16**). Compound **1** was well tolerated in mice at the highest dose tested of 100 mg/kg. Additional data on *in vivo* tests using **1** are provided in Table II, together with comparative data on penicillin G and oxacillin.

Experimental Section

Melting points are uncorrected. Tlc were developed in the solvent system BuOH–H_2O–AcOH (80:80:2) using silica gel HF plates and spots were detected by uv visualization and the alkaline NH_2OH–$FeCl_3$ spray.

1a,2,3,7b-Tetrahydro-2,3-dichloro-1H-cyclopropa[*a*]naphthalene-1-carboxylic Acid (13).—A solution containing 1.86 g (0.01 mole) of 1a,7b-dihydro-1H-cyclopropa[*a*]naphthalene-1-

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(2) R. Knox, *Nature*, **192**, 492 (1961).

(3) For a review see, J. O. Klein and M. Finland, *New Engl. J. Med.*, **269**, 1019, 1074, 1129 (1963).

(4) R. Huisgen and G. Juppe, *Chem. Ber.*, **94**, 2332 (1961).

(5) M. W. Fisher, M. C. Manning, L. A. Gagliardi, M. R. Gaetz, and A. L. Erlandson, *Antibiot. Ann.*, 293 (1959–1960).