hydrazine·HCl, 250 mg of NaOAc, and 1.0 ml of AcOH and refluxed for 40 min. The mixt was poured over a large amount of ice water and the ppt formed was collected by filtration. Recrystn from MeOH yielded 900 mg of **6**, mp 154–157°.

Receptor Binding Studies.—Uteri from 10 immature rabbits weighing less than 2 kg were removed, minced, and washed several times with buffer to rid the tissue of blood. Uteri were then homogenized in 0.4 vol of buffer (0.01 *M* Tris-HCl buffer, pH 8.0, contg 0.001 *M* EDTA and 0.25 *M* sucrose) at 4°. The homogenates were first centrifuged at 12,000*g* for 30 min, followed by 273,000*g* for 1.0 hr. Reaction mixts consisting of (a) 0.2 ml of buffer (0.01 *M* Tris-HCl, pH 8.0, contg 0.001 *M* EDTA, 0.25 *M* sucrose, and 25,000 cpm of progesterone-*t*/ml), (b) nonradioactive compds reported in this paper at a concn of 100 ng/ml, and (c) 50 μl of uterine cytosol were incubated at 4° for 16 hr. After incubation bound *vs.* unbound steroids were sepd as earlier reported, and the amt of bound progesterone-*t* was determined.

Acknowledgment.—We wish to thank Dr. I. Scheer and Dr. J. DaVanzo for their encouragement and interest. Thanks are also due to Mrs. E. Kaffitz, Miss C. DeDella, and Mrs. B. Fuller for their technical assistance.

β-Lactam Antimicrobial Agents Which Possess Antifungal Activity

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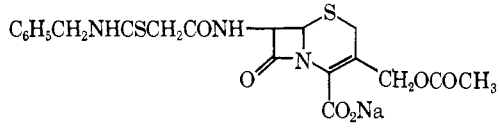
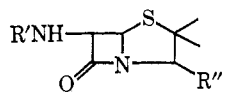
Although the cell walls of bacteria and fungi differ widely in their mucopeptide and mucopolysaccharide arrangement, we thought that it would be interesting to prepare some β-lactam-containing semisynthetic compounds and test them against certain fungi. Unexpectedly, we have found that a few of these antibiotics inhibit the growth of some pathogenic fungi *in vitro*.



I (**14**)



II (**15**), R′ = $C_6H_5OCH(CH_3)CO$; R″ = COSK
III (**16**), R′ = $C_6H_5OCH_2CO$; R″ = CHO

Chemistry.—The compounds listed in Tables I and II were prepared by two procedures unless otherwise noted. All of the thiocarbamoylmercaptomethylcephalosporanic acids were prepared by direct acylation of 7-aminocephalosporanic acid (7-ACA) with the *S*-carboxymethyl dithiocarbamate–carboxylic mixed anhydride (method A). The carbamoylmercaptomethylcephalosporins were prepared from the *S*-carboxymethyl *N*,*N*-disubstituted dithiocarbamates and 7-ACA in the presence of I_2 and NaI (method B).[1,2] *N*-Benzylthiocarbamoylmercaptoacetamidocephalosporanic acid (I) (**14**) was prepared from sodium 7-(2-bromoacetamido)cephalosporanate[3] and potassium *N*-benzyldithiocarbamate. Potassium 6-[(−)-α-phenoxypropionamido]thiopenicillanate (II) (**15**)[4] and 6-phenoxyacetamidopenicillanal (III) (**16**)[5] were prepared by published procedures.

Antifungal Activity.—Results of the tests are summarized in Table I. As can be seen, the most active compound is sodium (*N*-benzyldithiocarbamoylacetamido)cephalosporanate (I) (Table I, **14**). It is noteworthy that this cephalosporin showed two- to fourfold greater antifungal activity than the antifungal acid **17**[6] from which it was derived. It is also of interest that the penicillin aldehyde III (**16**) which is virtually without activity *in vitro* aganist bacteria, showed antifungal effects. Compound **14** was tested in an experimental systemic *Cryptococcus neoformans* infection of mice but was found to case no prolongation of survival time. Thus, the probability exists that, although these compounds are active *in vitro*, they are not present in an active form in animal tissues at high enough concentrations to give protection against systemic fungal infections.

Experimental Section[7]

Sodium 7-(*N*,*N*-Dimethyldithiocarbamoylacetamido)cephalosporanate (Table I, 1) (Method A).—To a soln of 1.8 g (0.01 mole) of *S*-carboxymethyl *N*,*N*-dimethyldithiocarbamate and 1 g (0.01 mole) of Et_3N in 50 ml of THF at 0° was added 1.2 g (0.01 mole) of isovaleryl chloride. The mixt was stirred for 10 min and a soln of 2.8 g (0.01 mole) of 7-ACA and 1.1 g (0.11 mole) of Et_3N in 25 ml of H_2O was added all at once. The soln was stirred for 0.5 hr and the THF was evapd under reduced pressure at 30° (15 mm). The aq residue was acidified with 1:1 H_3PO_4 and extd with EtOAc. The exts were washed with H_2O and dried (Na_2SO_4). The soln was treated with sodium 2-ethylhexanoate and the white cryst salt was collected and recrystd three times from aq *n*-BuOH to give 2.5 g of product (see Table II).

Sodium 7-(*N*-Benzyldithiocarbamoylacetamido)cephalosporanate (14).—To a soln of 11.7 g (0.03 mole) of sodium 7-(2-bromoacetamido)cephalosporanate[3] and 2.5 g of $NaHCO_3$ in 200 ml of H_2O at 10° was added, with vigorous stirring, 6.6 g (0.03 mole) of potassium *N*-benzyldithiocarbamate.[6] The soln was stirred in the cold for 1 hr as the pH was kept around 7 with 2 *N* $NaHCO_3$ soln. The soln was washed with Et_2O, layered with 100 ml of EtOAc, and acidified to pH 4 with 42% H_3PO_4. The org layer was sepd, and the aq phase was again extd with EtOAc. The exts were washed with H_2O, dried (Na_2SO_4), and evapd to an oil under 15 mm pressure at 35°. The residue, after trituration with dry Et_2O was collected by filtration and was dried over

(1) W. J. Gottstein and A. H. Eachus, U. S. Patent 3,391,141 (1968); *Chem. Abstr.*, **69**, 86992 (1968).

(2) W. J. Gottstein, A. H. Eachus, and L. C. Cheney, *J. Org. Chem.*, **35**, 1693 (1970).

(3) L. B. Crast, Jr., South African Patent 67/07,783 (1968); *Chem. Abstr.*, **70**, 68389 (1969).

(4) W. J. Gottstein, R. B. Babel, L. B. Crast, Jr., J. M. Essery, R. R. Fraser, J. C. Godfrey, C. T. Holdrege, W. F. Minor, M. E. Neubert, C. A. Panetta, and L. C. Cheney, *J. Med. Chem.*, **8**, 794 (1965).

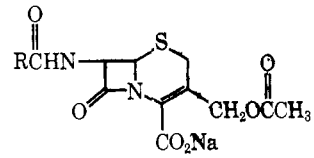
(5) W. J. Gottstein, G. E. Bocian, L. B. Crast, Jr., K. Dadabo, J. M. Essery, J. C. Godfrey, and L. C. Cheney, *J. Org. Chem.*, **31**, 1922 (1966).

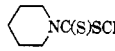
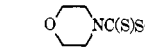
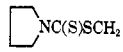
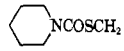
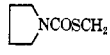
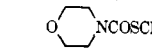
(6) A. Rieche, J. Hilgetag, D. Martin, and I. Kreyzi, *Arch. Pharm. (Weinheim)*, **296**, 310 (1963).

(7) Melting points were determined on a Fisher-Johns apparatus, and are uncorrected. Ir spectra were recorded on a Beckman IR 9 spectrometer, nmr spectra on a Varian A-60 spectrometer at a sweep width of 500 cps using D_2O as a solvent. All spectra were consistent with structure. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The authors wish to thank Mr. R. M. Downing and Mr. D. F. Whitehead for the microanalytical and spectral data, respectively.

TABLE I

In Vitro ANTIFUNGAL ACTIVITY[a]



Compd	R or compd	Lowest level of inhibition, T. mentagrophytes	M. canis	C. neoformans	H. capsulatum
1	$(CH_3)_2NC(S)SCH_2$	250	>250	63	63
2	$(C_2H_5)_2NC(S)SCH_2$	250	>250	250	31
3		250	250	>250	31
4		>250	>250	63	16
5	$C_6H_5NCH_3C(S)SCH_2$	>250	>250	>250	250
6		250	250	250	31
7	$C_6H_5CH_2N(CH_3)C(S)SCH_2$	>250	>250	>250	16
8	$(CH_3)_2NCOSCH_2$	16	63	63	>250
9	$(C_2H_5)_2NCOSCH_2$	31	31	63	125
10		>250	>250	>250	125
11		250	>250	>250	125
12	$C_6H_5N(CH_3)COCH_2$	125	>250	>250	250
13		125	>250	>250	250
14	I	4	8	8	1.6
15	II	13	25	250	16
16	III	62	13	50	63
17[b]	$C_6H_5CH_2NHC(S)SCH_2COH$	8	32	2	4

[a] Agar dilution tests; minimum inhibitory concn, μg/ml. [b] A. Rieche, J. Hilgetag, D. Martin, and I. Kreyzi, *Arch. Pharm.* (*Weinheim*), **296**, 310 (1963).

TABLE II

Compd	Method of prepn	Solvent	Decompn point, °C	Yield, %	Formula	Analyses[a]
1	A	Aq *n*-BuOH	210	60	$C_{15}H_{18}NaN_3O_8S_3$	C, H, N
2	A	EtOAc	195–199	24	$C_{17}H_{22}NaN_3O_8S_3 \cdot H_2O$	C, H, N
3	A	Aq *n*-BuOH	182–185	32	$C_{18}H_{22}NaN_3O_8S_3$	C, H, N
4	A	Aq *n*-BuOH	250	51	$C_{17}H_{20}NaN_3O_7S_3$	C, H, N
5	A	Aq *n*-BuOH	192	58	$C_{20}H_{20}NaN_3O_6S_3$	C, H, N
6	A	Aq Me_2CO	160	42	$C_{17}H_{20}NaN_3O_6S_3 \cdot 2H_2O$	C, H,
7	A	Amorphous from EtOAc	>210	10	$C_{21}H_{22}NaN_3O_6S_3$	C, H, N
8	B	EtOAc–Et_2O	95–96	40	$C_{15}H_{19}N_3O_7S_2 \cdot H_2O$ (free acid)	C, H, N
9	B	Aq *n*-BuOH	162–165	20	$C_{17}H_{22}NaN_3O_7S_2 \cdot H_2O$	C, H, N
10	B	DMF–Me_2CO	158–159	45	$C_{18}H_{22}NaN_3O_7S_2$	C, H, N
11	B	Aq *n*-BuOH	210–212	37	$C_{17}H_{20}NaN_3O_7S_2$	C, H, N
12	B	Aq *n*-BuOH	185–187	25	$C_{20}H_{20}NaN_3O_7S_2$	C, H, N
13	B	Aq *n*-BuOH	169–170	40	$C_{17}H_{20}N_3NaO_8S_2$	C, H, N

[a] Anal. results were within 0.4% of the theoretical values.

P_2O_5 at 15 mm to give 8 g of product. The acid was dissolved in EtOAc and treated with solid sodium 2-ethylhexanoate. Crystals were collected by filtration, washed with hot EtOAc, and dried overnight over P_2O_5 at 15 mm, yield, 4 g (26%) of a light yellow cryst product. Recrystn from Me_2CO–EtOH gave an anal. sample, mp 185° dec. *Anal.* ($C_{20}H_{20}N_8NaO_6S_3$) C, H, N.

The semisynthetic compds were tested for activity against *Trichophyton mentagrophytes*, *Microsporum canis*, *Cryptococcus neoformans*, and *Histoplasma capsulatum* by a twofold serial diln method. The compds were dissolved in DMSO, brought to vol with sterile H_2O (1:9) at 1 mg/ml, and serially dild twofold in appropriate liq media. These medicated dilns were dispensed in 2-ml amts into sterile, capped, 1.56-cm diameter test tubes prior to inoculation. The tests for activity against *T. mentagrophytes* and *M. canis* were carried out in Sabouraud's dextrose broth (Difco), *C. neoformans* in antibiotic medium No. 3 (Difco), and *H. capsulatum* in Salvin's Y-P medium.[8]

Stock suspensions of the test cultures were prepd as follows and then used at the rate of 0.05 ml of inoculum per 2 ml of

(8) S. B. Salvin, *J. Bacteriol.*, **54**, 655 (1947).

medicated broth: *T. mentagrophytes* and *M. canis* were grown on Sabouraud's dextrose agar slants at 28° for 7–10 days. Surface growth from an agar slant culture was taken up into 5 ml of sterile H_2O, homogenized, and dild 500-fold with sterile H_2O. *C. neoformans* was grown on Sabouraud's dextrose agar slants at 22° for 2 days. The surface growth from an agar slant culture was taken up into 5 ml of sterile H_2O, homogenized, and dild 25-fold with sterile H_2O. *H. capsulatum* was grown in Salvin's Y-P semifluid medium at 22° for 7 days; 0.5 ml of medium containing surface growth was uniformly suspended in 25 ml of sterile H_2O.

Minimum inhibitory concns (MICs), which are considered to be the min concns of the test compds in micrograms per milliliter which prevent grossly detectable growth of the test organisms, were detd after inoculated tubes were incubated for a suitable period of time at the desired temp. In the case of *T. mentagrophytes* and *M. canis*, this was 5 days at 28°, while *C. neoformans* and *H. capsulatum* were incubated for 2 and 4 days, respectively, at 37°.

Insect Chemosterilants. 10. Substituted Dithiobiurets[1a,b]

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Sexual sterilization of female insects can be achieved with many compounds of different structural types.[2] Apparently, the complex biochemical processes in oogenesis can be interrupted or modified at several points with the same ultimate effects, *i.e.*, the female does not produce eggs or the eggs are infertile. Fertility of male insects, on the other hand, is not as easily affected by chemicals and only 3 well-defined classes of male chemosterilants are known: alkylating agents,[3] phosphoramides,[4] and *s*-triazines.[5]

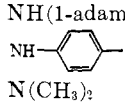
In this communication we wish to describe a new class of male insect chemosterilants, derivatives of dithiobiuret (Table I). Compounds **2** through **20** were synthesized according to Scheme I. Dialkylthiocarbamoyl chlorides were converted to the corresponding thiocarbamoyl isothiocyanates[6a] with KSCN in Me_2CO; the rather unstable[6b] thiocarbamoyl isothiocyanates were then treated, without isolation, with amines to provide the dithiobiurets.

SCHEME I

$$R_2R_1NCSCl \xrightarrow{KSCN} R_2R_1NCSNCS \xrightarrow[R_3R_4NH]{} R_2R_1NCSNHCSNR_3R_4$$

Several of the 1,1,5,5-tetrasubstituted dithiobiurets, particularly the unsymmetrically substituted ones, were unstable. Although all but **8**, **12**, and **14** were crystalline, **8**–**12** and **14** tended to decompose on attempted purification or on prolonged standing at room temperature, and satisfactory elemental analyses were not obtained; the compounds were characterized by their ir and nmr spectra. An extreme example was 1,1,5,5-tetraethyldithiobiuret (too unstable for biological test-

TABLE I
DITHIOBIURET CHEMOSTERILANTS
$R_1CSNR_2CSR_3$

Compd	R_1	R_2	R_3	Mp, °C	Formula[a]	% hatch[b] at injected dose 5 μg/♂[f]	10 μg/♂[f]
1	NH_2	H	NH_2	189–191[c]	$C_2H_5N_3S_2$	94 (0)	91 (0)
2	$N(CH_3)_2$	H	NH_2	126–127[d]	$C_4H_9N_3S_2$	92 (60)	(100)
3	$N(CH_3)_2$	H	$NHCH_3$	123–124	$C_5H_{11}N_3S_2$	70 (90)	(100)
4	$N(CH_3)_2$	H	NHC_2H_5	81–82	$C_6H_{13}N_3S_2$	79 (50)	(100)
5	$N(CH_3)_2$	H	NH(1-adamantyl)	144–145	$C_{14}H_{23}N_3S_2$	93 (0)	78 (0)
6	$N(CH_3)_2$	H		103–105	$C_{10}H_{12}ClN_3S_2$	90 (4)	82 (90)
7	$N(CH_3)_2$	H	$N(CH_3)_2$	112–115	$C_6H_{13}N_3S_2$	15 (10)	1 (80)
8	$N(CH_3)_2$	H	$N(C_2H_5)_2$	Oil[e]	$C_8H_{17}N_3S_2$	15 (70)	(100)
9	$N(CH_3)_2$	H	Pyrrolidyl	95–105[d]	$C_8H_{15}N_3S_2$	59 (10)	44 (40)
10	$N(CH_3)_2$	H	Piperidyl	103–128[d]	$C_9H_{17}N_3S_2$	28 (25)	(100)
11	$N(CH_3)_2$	H	Morpholinyl	163–165[d]	$C_8H_{15}N_3OS_2$	14 (50)	(100)
12	$N(CH_3)_2$	H	$N(CH_3)CH_2CH_2OH$	Oil[e]	$C_7H_{15}N_3OS_2$	41 (0)	17 (8)
13	$N(CH_3)_2$	H	$N(CH_2CH_2OH)_2$	96–100 dec	$C_8H_{17}N_3O_2S_2$	92 (0)	81 (0)
14	$N(CH_3)_2$	H	$N(CH_3)C_6H_5$	Oil[a]	$C_{11}H_{15}N_3S_2$	(100)[g]	(100)
15	Pyrrolidyl	H	Pyrrolidyl	147–153 dec	$C_{10}H_{17}N_3S_2$	24 (0)	20 (20)
16	Piperidyl	H	Piperidyl	133–138	$C_{12}H_{21}N_3S_2$	93 (0)	87 (10)
17	Morpholinyl	H	Morpholinyl	161–163 dec	$C_{10}H_{17}N_3O_2S_2$	29 (40)	13 (90)
18	Pyrrolidyl	H	Piperidyl	125–130 dec	$C_{11}H_{19}N_3S_2$	45 (0)	26 (20)
19	Pyrrolidyl	H	Morpholinyl	137–141	$C_{10}H_{17}N_3OS_2$	54 (0)	36 (20)
20	Piperidyl	H	Morpholinyl	138–144 dec	$C_{11}H_{19}N_3OS_2$	18 (10)	47 (80)
21	$N(CH_3)_2$	CH_3	$N(CH_3)_2$	Oil	$C_7H_{15}N_3S_2$	88 (60)	95 (70)
22	$N(CH_3)_2$	C_6H_5	$N(CH_3)_2$	130	$C_{12}H_{17}N_3S_2$	94 (0)	90 (10)

[a] New compds analyzed satisfactorily for C, H, N, S, except where indicated. [b] Average hatch among controls was 95 ± 5%. [c] Commercial sample (American Cyanamid). [d] J. S. Davidson, *J. Chem. Soc. C*, 2069 (1966). [e] Unstable, not obtd anal. pure. [f] Values in parentheses indicate per cent mortality in 48 hr. [g] At 1 and 2 μg, the hatch was 55 and 42%, respectively.

(1) (a) Paper 9: P. H. Terry and A. B. Bořkovec, *J. Med. Chem.*, **13**, 782 (1970); (b) mention of a pesticide does not constitute a recommendation by the U. S. Department of Agriculture.

(2) A. B. Bořkovec, "Insect Chemosterilants," Interscience, New York, N. Y., 1966.

(3) A. B. Bořkovec, *Ann. N. Y. Acad. Sci.*, **163**, 860 (1969).

(4) P. H. Terry and A. B. Bořkovec, *J. Med. Chem.*, **10**, 118 (1967).

(5) A. B. Bořkovec and A. B. DeMilo, *ibid.*, **10**, 457 (1967).

(6) (a) J. S. Davidson, *J. Chem. Soc. C*, 2069 (1966); (b) L. A. Spurlock and P. E. Newallis, *J. Org. Chem.*, **33**, 2073 (1968).