serum. Unfortunately, quantitative results were given for the resistant series only in the presence of serum. From his data (Table I) we have derived the following

TABLE I

OBSERVED AND CALCULATED ACTIVITY OF 2,6-DIALKOXYPHENYLPENICILLINS IN THE PRESENCE OF SERUM

No.	X_1[a]	X_2[a]	$\Sigma\pi$[b]	σ	*S. aureus* (resistant) log (1/*C*) Obsd.[c]	Calcd.[d]
1	CH_3	H	1.00	0	1.945	1.531
2	C_2H_5	H	2.00	0	1.395	1.287
3	C_6H_5	H	4.26	0	0.609	0.734
4	$C_6H_5CH_2$	H	5.80	0	0.638	0.357
5	$C_6H_5(CH_2)_2$	H	6.32	0	0.063	0.230
6	CH_3	3-Cl	1.76	0.34	1.709	1.930
7	CH_3	3-OCH_3	1.12	0.12	1.702	1.708
8	CH_3	4-OCH_3	0.96	−0.27	0.795	1.077

[a] See structure I. [b] See text for calculation of $\Sigma\pi$. [c] Taken from data of Sheehan. His values reported as γ/ml. were converted to moles/l. [d] Calculated from eq. 4.

equations for the tests on resistant bacteria in the presence of serum. Since substituent constants are not available for all of the functions in Table I, we have had to make certain assumptions. It is assumed that all of the OX_1 groups (Ib) have the same electronic effect on the ring and carbonyl group. Since all molecules have two such groups, σ was given a reference value of zero for these groups and only σ for X_2 was used in the calculations. The only group likely to be slightly out of line with this assumption is OC_6H_5.

$$\log \frac{1}{C} = -0.249\pi + 1.828 \qquad n = 8,\ r = 0.823,\ s = 0.412 \qquad (3)$$

$$\log \frac{1}{C} = -0.245\pi - 1.720\sigma + 1.776 \qquad n = 8,\ r = 0.929,\ s = 0.295 \qquad (4)$$

$$\log \frac{1}{C} = +0.010\pi^2 - 0.316\pi + 1.76\sigma + 1.853 \qquad n = 8,\ r = 0.930,\ s = 0.328 \qquad (5)$$

For π-values we have used those taken from phenoxyacetic acids[4] except for benzyl and phenylethyl which were developed from data in a later report.[5] To calculate the value of π for the benzyl and phenethyl groups we have subtracted the value of −1.80 for π for aliphatic OH from log P for benzyl alcohol (1.10) and log P for phenethyl alcohol[5] (1.36). Thus, $\Sigma\pi$ for derivative 5 in Table I was calculated as follows.

$$\Sigma\pi = 2(1.36 + 1.80) = 6.32 \qquad (6)$$

From the data in Table I we have derived eq. 3–5 by the method of least squares. Of these equations, 4 provides the simplest rationalization of the variance in biological activity. Most interesting is the fact that biological activity shows a similar dependence on π in eq. 1–4.

The fact that the π-term has a negative coefficient in each series of penicillins shows that the parent side chains [$C_6H_5OCH(CH_3)CO$ in Ia and 2,6-$(CH_3O)_2$-C_6H_3CO in Ib] are already too lipophilic for maximum activity. Unfortunately, both Gourevitch, *et al.*, and Sheehan were concerned only with functions having positive values for π. The above equations clearly indicate the disadvantage of such groups in the presence of serum.

In eq. 1 and 2, the addition of the σ-term did not result in improved correlation; however, σ does seem to play a role in the benzoic acid derivatives where the substituents are not insulated from the carbonyl group. A positive value for the σ-term indicates that groups which decrease the electron density on or in the region of the carbonyl group promote activity. The importance of σ must be accepted with some reservation since only three derivatives were used to evaluate this effect.

Our previous analysis[2] leads to the conclusion that bulky substituents in the side chain serve to prevent opening of the lactam ring. Keeping in mind that large groups will help shield both the amide linkage and the lactam ring, and that these groups should be much more hydrophilic than phenyl, a number of new approaches for improving the activity of these two classes of penicillins are evident. Of course, functions such as $-SO_2CH_3$ ($\pi = -0.50$, $\sigma = +0.60$) could be used to advantage. However, the phenyl group has such a large π-value (~2) that stable heterocyclic functions would make much better starting points. Pyridine (log $P = 0.65$), imidazole, or other nitrogen or oxygen heterocycles would be much more hydrophilic, and should give derivatives of much greater activity in the presence of serum.

Increasing the hydrophilic character of the penicillins will be even more important when they are being designed for use against gram-negative bacteria. Analysis of the mechanism of action of phenols[3] against gram-positive and gram-negative bacteria showed that optimal lipophilic character for the phenol coefficient for the latter group is very much lower than for the former.

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Dihydrazides, a New Class of Anthelmintics[1a]

RAYMOND CAVIER

Chaire de Parasitologie, Faculté de Pharmacie, Paris

AND RICHARD RIPS[1b]

Institut d'Anesthesiologie de la Faculté de Médecine, Paris, France

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More than half a century ago, Curtius[2] described the synthesis of hydrazides of dicarboxylic acids. Such derivatives were studied later for their sweet taste, in the search for a saccharin substitute.[3] More recently, it appeared that hydrazides of malonic and other dicarboxylic acids might have tuberculostatic

(1) (a) This work was supported by a grant given by the Institut National de la Santé et de la Recherche Médicale. (b) To whom inquiries should be addressed.

(2) (a) T. Curtius, *J. prakt. Chem.*, [2] **91**, 1 (1915); (b) T. Curtius and H. Clemm, *ibid.*, **62**, 189 (1900); (c) T. Curtius, G. Schöfer, and N. Schwann, *ibid.*, **51**, 180 (1895).

(3) (a) J. J. Blanksma and H. A. Bakels, *Rec. trav. chim.*, **58**, 497 (1939); (b) J. J. Blanksma and H. de Graaf, *ibid.*, **57**, 3 (1938).

TABLE I
$RNHCO(CH_2)_nCONHR$

No.	R	n	Yield, %	M.p., °C.	*S. obvelata*, 200 mg./kg. × 4 days, % clear	*S. obvelata*, 200 mg./kg. × 4 days, Deaths	*H. nana*, 200 mg./kg., once, % clear	*H. nana*, 200 mg./kg., once, Deaths
I	NH_2	0	96	243[a]	22	1/10	0	1/10
II	NH_2	1	89	154[a]	72.4	1/30	0	0/10
III	NH_2	2	88	168[a]	64.7	3/20	10	0/10
IV	NH_2	3	86	176[b]	77	1/10	0	1/10
V	NH_2	4	80	200[c]	66	1/10	10	0/10
VI	$(CH_3)_2C{=}N$	0	89	210[d]	0	9/10	30	0/10
VII	$(CH_3)_2C{=}N$	1	94	185[e]	47.3	1/20	10	0/10
VIII	$(CH_3)_2C{=}N$	2	93	202[f]	50	2/10	14	3/10
IX	$CH_3CH{=}N$	1	96	192[f]	61.5	7/20	22	1/10
X	$(CH_3)_2CHCH{=}N$	1	92	173[f]	0	8/10	40	0/10
XI	$(CH_3)_2CHCH_2C(CH_3){=}N$	1	89	154[g]	25	2/10	20	0/10
XII	$(CH_3)_2CH$	1	60	117[h]	0	7/10	20	0/10

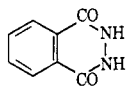
[a] Lit.[2c] m.p. 235° for I, 152° for II, 167° for III. [b] Lit.[2b] m.p. 176° for IV. [c] Lit.[2a] m.p. 171° for V. [d] H. De Graaf [Thesis, University of Leiden, 1930, p. 138; *Chem. Abstr.*, **24,** 5724 (1930)] gives 210°. [e] Lit.[3b] m.p. 180°. [f] Lit.[3a] m.p. 200° for VIII, 188° for IX, 173° for X. [g] *Anal.* Calcd. for $C_{15}H_{28}N_4O_2$: C, 60.78; H, 9.52; N, 18.91; O, 10.79. Found: C, 60.37; H, 9.46; N, 19.1; O, 11.2. [h] R. W. West [*J. Chem. Soc.*, **127,** 748 (1925)] gives 114°.

TABLE II
$RNHNHCOCH_2CONHNHR$

No.	R	Yield, %	M.p., °C.	*S. obvelata*, 200 mg./kg. × 4 days, % clear	*S. obvelata*, 200 mg./kg. × 4 days, Deaths	*S. obvelata*, 250 mg./kg., once, % clear	*S. obvelata*, 250 mg./kg., once, Deaths	*S. obvelata*, 500 mg./kg., once, % clear	*S. obvelata*, 500 mg./kg., once, Deaths
XIII	CH_3CO	83	244[a]	21	1/20	0	4/20	15	2/15
XIV	C_2H_5CO	82	255[b]	0	0/10	0	2/10	12.5	2/10

[a] C. Bülow and R. Weidlich [*Ber.*, **39,** 3372 (1906)] give 228°. [b] *Anal.* Calcd. for $C_{11}H_{20}N_4O_4$: C, 48.52; H, 7.41; N, 20.57; O, 23.50. Found: C, 48.17; H, 7.17; N, 21.07; O, 23.69.

TABLE III
MISCELLANEOUS HYDRAZIDES

No.	Formula	Yield, %	M.p., °C.	*S. obvelata*, 200 mg./kg. × 4 days, % clear	*S. obvelata*, 200 mg./kg. × 4 days, Deaths	*H. nana*, 200 mg./kg., once, % clear	*H. nana*, 200 mg./kg., once, Deaths
XV	$CH_3CONHNH_2$	80	135[a]	0	7/10	0	3/10
XVI	$(=CHCONHNH_2)_2$	70	220[b]	0	9/10	0	1/10
XVII		86	340[c]	12.5	2/10	22	1/10
XVIII	1,3-$(CONHNH_2)_2C_6H_4$	85	236[c]	83	4/10	20	0/10
XIX	1,4-$(CONHNH_2)_2C_6H_4$	77	320[c]	30[d]	0/10		

[a] H. McKennis, A. S. Yard, J. H. Weatherby, and J. A. Hagy [*J. Pharmacol. Exptl. Therap.*, **126,** 109 (1959)] report the product to be deliquescent contrary to previous authors who found 135°. [b] H. Radenhausen [*J. prakt. Chem.*, **52,** 451 (1895)] gives 220°. [c] Lit.[3a] m.p. >320° for XVII, 320° for XIX, and 227° for XVIII. [d] One dose of 250 mg./kg. produces no clearance and no deaths; with 500 mg./kg. clearance is 10% with no deaths.

activity,[4] but this hope failed. Interest in inhibitors of monoamine oxidase led to an assay of a great number of hydrazides, and in a recent French patent[5] the reduction product of oxalic acid diisopropylhydrazide is said to be 7.5 to 15 times more potent than iproniazid. However, to our knowledge, dihydrazides have never been tried as anthelmintics despite the fact that cyanoacethydrazide is very active against nematodes[6] and that numerous parasiticides are dibasic compounds.[7]

$$\underset{A}{(CH_3)_2C{=}}\underset{B}{NHCO}\underset{C}{CH_2}\underset{B}{CONH}\underset{A}{N{=}C(CH_3)_2}$$

I

We began this study with diisopropylidenemalonylhydrazide (I), thinking that it may present various "active centers"[8] (A, as in chrysanthemum monocarboxylic acid; B, as in cyanoacethydrazide; C, that it may have the necessary distance between two groups; this distance is for example 5 C for dithiazanin, 1 C for dichlorophen, etc.). Indeed this compound gave a clearance of 88% in mice infested with *Syphacia obvelata*. We then decided to modify systematically each portion.

Structure–Anthelmintic Activity Relationships.—Group "A" was studied with substituted malonylhydrazide: the smallest substitution was found the most successful on *S. obvelata*, decreasing from VII (47.3%) to X (0%); on *Hymenolepis nana* it progressed from VII to X but without significant differences. The influence of "C" was determined using molecules bearing the same isopropylidene substituent in position

(4) (a) D. Libermann, N. Rist, F. Grumbach, M. Moyeux, B. Gauthier, A. Rouaix, J. Maillard, J. Himbert, and S. Cals, *Bull. soc. chim. France*, 1430 (1954); (b) E. Jeney and T. Zsolnai, *Zentr. Bakteriol. Parasitenk. Abt. I. Orig.*, **180,** 398 (1960).

(5) G. Marinier, French Patent M 1609 (Jan. 7, 1963); *Chem. Abstr.*, **58,** 11174*b* (1963).

(6) J. K. Walley, *Vet. Rec.*, **69,** 815 (1957).

(7) (a) R. Cavier, *Biol. Med.* (Paris), **49,** 201 (1960); (b) B. S. Kaushiva, *J. Sci. Ind. Res.* (India), **15C,** 199 (1956).

(8) W. Perkow, *Arzneimittel-Forsch.*, **12,** 1185 (1962).

TABLE IV
EFFECT OF DOSES IN THE TREATMENT AGAINST *S. obvelata*.

No.	Treatment, mg./kg.	No. of mice	Deaths	Total elimin.		Partial elimin.
				a	*b*	*c*
II	250, 4 days	10	2	8	100	0
	200, 4 days	30	1	21	72.4	3
	150, 4 days	20	1	10	52.6	3
	100, 4 days	20	1	7	36.8	2
	50, 4 days	10	0	2	20	2
III	250, 4 days	10	1	8	88	0
	200, 4 days	20	3	11	64.7	1
	150, 4 days	10	0	5	50	1
	100, 4 days	10	0	4	40	0
VII	250, 4 days	10	1	8	88	1
	200, 4 days	20	1	9	47.3	3
	150, 4 days	10	0	2	20	2
	120, 4 days	10	1	2	22	1
	100, 4 days	10	2	1	12.5	0
	500, once	10	0	2	20	1
	400, once	10	1	2	22	2
	200, once	10	1	2	22	0
IX	250, 4 days	10	8	1	...	0
	200, 4 days	20	7	8	61.5	1
	150, 4 days	10	0	5	50	1
	100, 4 days	10	2	4	12	1

[a] Number of mice totally free of parasite. [b] Percentage calculated on the number of living mice at the end of the test. [c] Mice with one or two pinworms in cecum.

"A." On *S. obvelata* compounds with C = 0 had no activity, while those with C = 2 and C = 1 are quite similar in potency (50 and 47%, respectively). The introduction of a double bond when C = 2 decreases this activity (XVI). Replacement of the hydrazide by an amide function (study of "B") also led to inactive compounds. The influence of anti *Hymenolepis nana* activity is not restricted to a given structure. The finding that the smallest "A" substitution was the best led us to test compounds with no "A" substitution, *i.e.*, simple hydrazides of dicarboxylic acids. Odd numbers of carbons as in II and IV seem to be the best (72.4 and 77%) and in the range of the commercial derivatives of piperazine. Introduction of an aromatic ring without changing the number of carbons between the hydrazide groups as in isophthalic hydrazide slightly increases the activity (from 72 to 83%) and considerably increases toxicity (deaths range from 1/30 for II to 4/10 for XVIII). We also tried to diminish the absorption of the active molecules by forming salts and blocking the hydrazide group with an acyl moiety which can undergo lysis in the organism. No appreciable changes were noted. In conclusion, the best structure seems to be the one of a diacid hydrazide, the most active of the compounds tested being glutaric acid dihydrazide.

Other Pharmacological Results.—A more general pharmacological study, including determination of acute toxicity in mice, measurement of variation of blood pressure and respiration in cats and dogs, and action on isolated organs: frog's heart, rat duodenum, guinea pig ileum, alone and as antagonists of acetylcholine, barium chloride, and histamine, has been performed on some of these compounds, without finding any action of interest. The toxicity is low and, in particular, they did not show any convulsive effects, contrary to what one might expect from previous studies on hydrazides.[9]

(9) H. L. Williams and J. A. Bain, *Intern. Rev. Neurobiol.*, **3**, 319 (1961).

Experimental

Synthesis.—Most of the hydrazines tested have already been described in the literature. They were easily prepared by addition of hydrazine to the corresponding ester, without solvent (sometimes followed by reflux) and careful recrystallization of the precipitate. In case of acylation the hydrazide was refluxed in the appropriate aldehyde, ketone, or anhydride.

Anthelmintic Tests. *Hymenolepis nana.*—Three weeks after infection by ingestion of 50 eggs of *H. nana*, fasted white mice received 25 mg./kg. of the test compound orally. They were then given a purgative (Na_2SO_4) 4 hr. later. The next day, the animals underwent autopsy and the percentage of parasite-free animals was determined[10] (see Tables I–IV).

Syphacia obvelata.—Mice infected with *S. obvelata* by contact with highly contaminated mice received the test compound, orally, 8–11 days after infection over a period of 4 days. On autopsy 48 hr. after completion of the treatment, the percentage of parasite-free animals was determined[11] (see Tables I–IV).

(10) R. Cavier, *Ann. pharm. franc.*, **14**, 545 (1956).

(11) R. Cavier, *Bull. Soc. Pathol. Exotique*, **54**, 850 (1961); **55**, 412 (1962).

Deaza Analogs of 6-Mercaptopurine[1]

JOHN A. MONTGOMERY AND KATHLEEN HEWSON

Southern Research Institute, Birmingham, Alabama 35205

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It is well known that 6-mercaptopurine is converted *in vivo* to 6-mercaptopurine ribonucleotide by cells whose growth is inhibited by 6-mercaptopurine. There is good evidence that the product of this "lethal synthesis" inhibits growth by negative pseudo-feedback, blocking the conversion of phosphoribosyl pyrophosphate to aminoribosyl phosphate, thus inhibiting *de novo* synthesis of purine nucleotides.[2]

(1) This investigation was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

(2) L. L. Bennett, Jr., L. Simpson, J. Golden, and T. L. Baker, *Cancer Res.*, **23**, 1574 (1963); R. W. Brockman, *ibid.*, **23**, 1191 (1963).