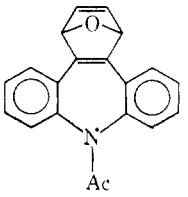
The greatest increase in survival time observed was 2.5 days at a dose of 640 mg/kg when the mice were treated with **10**. Furthermore, no activity was observed when **3**, **4**, **6**, **7**, and **9** were tested against *P. gallinaceum* in chicks.



12

Experimental Section

All melting points were obtained on a Thomas-Hoover Uni-Melt and are uncorrected. Satisfactory ir and nmr spectra were recorded for all new compounds. The ir spectra were obtained using a Perkin-Elmer Model 337 spectrophotometer, nmr spectra in $CDCl_3$ solns of the compounds using a Varian Model A-60A spectrophotometer (TMS internal standard). Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn., and Atlantic Microlab, Inc., Atlanta, Ga.

Preparation of 5*H*-Acetyl-10-bromodibenz[*b*,*f*]azepines (II).—To a soln of 5*H*-acetyl-10-bromo-10,11-dihydrodibenz[*b*,*f*]azepine[2a] (45 g) in 200 ml of EtOH was added 75 ml of a 50% aq KOH and the reaction mixture was maintained at 50–60° for 30 min. The soln was diluted with H_2O, extracted with Et_2O, washed (H_2O), and dried ($CaSO_4$) and the Et_2O removed to yield 29 g of crude 5*H*-acetyldibenz[*b*,*f*]azepine. Recrystallization from hexane–Et_2O gave 24 g; mp 121–122°.[3]

To a cooled soln of 24 g of 5*H*-acetyldibenz[*b*,*f*]azepine in 100 ml of $CHCl_3$, cooled in an ice bath, 16 g of Br_2 in 25 ml of $CHCl_3$ was added dropwise. After addn was complete, the soln was stirred for 0.5 hr, treated with charcoal, and filtered. The filtrate was cooled at −10° and the resulting precipitate was filtered, washed with hexane (mp 136–138°, yield 35 g), and used directly as follows.

A mixture of 35 g of 5*H*-acetyl-10,11-dibromo-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine and 35 g of *n*-Bu_2NH was warmed cautiously on a steam bath until an exothermic reaction occurred after which the soln was stirred and heated on the steam bath for 20 min. The reaction mixture was extracted with Et_2O, washed (H_2O), and dried ($CaSO_4$) and the Et_2O was removed under reduced pressure. The resulting residue crystallized on standing overnight. The crystals were filtered, washed with cold Et_2O, and recrystd from EtOH; mp 108–109°; lit. mp 109–110°;[3] yield 18 g.

5*H*-Acetyl-10-cycloalkylaminodibenz[*b*,*f*]azepines (IV).—In a typical example, to a soln of 2.05 g of 5*H*-acetyl-10-bromodibenz[*b*,*f*]azepine in 50 ml of *t*-BuOH, which had been dried over 4A molecular sieves, was added 0.8 g of KO-*t*-Bu and 8 g of *N*-methylpiperazine and the soln was refluxed for 15 hr. The reaction mixture was poured into H_2O, extracted with Et_2O, washed (H_2O), and dried ($CaSO_4$) and the Et_2O was removed under reduced pressure to yield a gummy residue which crystd from hexane–Et_2O on standing overnight. Recrystn from hexane–Et_2O gave a solid; mp 163–164°; yield 1.4 g.

5*H*-10-Cycloalkylaminodibenz[*b*,*f*]azepines (V).—A soln of 0.9 g of *N*-(5*H*-acetyldibenz[*b*,*f*]azepine-10-yl)-*N'*-methylpiperazine in 25 ml of 50% alcoholic KOH was refluxed for 2 hr, poured into H_2O, and extracted with Et_2O. The ether layer was washed (H_2O), dried ($CaSO_4$), and evaporated under reduced pressure. The resulting yellow solid was crystd from Et_2O–hexane; mp 170–171°; yield 0.6 g.

Attempted Reduction of *N*-(5*H*-acetyldibenz[*b*,*f*]azepine-10-yl)-*N'*-methylpiperazine with LAH.—To a stirred suspension of 0.5 g of LAH in 25 ml of THF maintained at 0° was added a soln of 1 g of **2** in 25 ml of THF under N_2. The mixture was stirred for 30 min and then at room temp for 30 min, decomposed with H_2O in the usual manner, extracted with Et_2O, washed (H_2O), and dried ($CaSO_4$), and Et_2O was removed under reduced pressure to yield 0.7 g of yellow solid which on crystn from Et_2O–hexane gave a mp of 170–171°. The compd was identified by its ir and nmr spectra and by mmp with a sample material obtained from the above experiment. It was converted back into **2** by refluxing with AcCl in C_6H_6 in a manner similar to that described previously.[2c]

5*H*-Methyl-10-cycloalkylaminodibenz[*b*,*f*]azepines (VI).—A soln of *N*-(5*H*-dibenz[*b*,*f*]azepin-10-yl)piperidine (0.5 g), 0.3 g of NaH in 50 ml of PhMe was refluxed under N_2 for 2 hr. The soln was cooled and 1.5 g of Me_2SO_4 in 10 ml of PhMe was added dropwise and refluxing was contd for an additional 20 hr. The reaction mixture was cooled, excess NaH was decompd with H_2O, extracted with C_6H_6, washed (H_2O), and dried ($CaSO_4$) and the solvent was removed under reduced pressure. The residue was dissolved in hexane; after storage at −10° 0.3 g, mp 135–136° was obtained. Recrystallization from hexane raised the melting point to 138–139°.

Trapping of the Hetaryne with Furan.—A soln of 2.1 g of 5*H*-acetyl-10-bromodibenz[*b*,*f*]azepine and 1.0 g of KO-*t*-Bu in 15 ml of *t*-BuOH, and 30 ml of furan was refluxed for 20 hr. The reaction mixture was poured into H_2O, extracted with Et_2O, washed (H_2O), and dried ($CaSO_4$). Evapn of the Et_2O gave a residue which was triturated with hexane and crystallized from EtOH. The yield of **12** was 0.5 g which melted at 236–237°; nmr τ 8.15 (3 H singlet), 4.12 (2 H singlet), and 2.7 (10 H multiplet). *Anal.* ($C_{20}H_{15}NO_2$) C, H, N.

Acknowledgments.—We are indebted to Drs. D. P. Jacobus, T. R. Sweeney, and E. A. Steck for the test results. We wish to thank Dr. Steck for helpful discussions.

(5) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

Synthesis and Chemotherapeutic Activity of Two Metabolites of Trimethoprim

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2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (trimethoprim[1]) (**1**) shows antibacterial[1] and antimalarial[2] activity and potentiates[3] sulfonamides such as 5-methyl-3-sulfanilamidoisoxazole (sulfamethazole[4]) to provide a clinically useful broad spectrum antibacterial agent.[5] Of the metabolites of **1**, isolated from the urine of man and animals and identified[6] as M_1 (**2**), M_2 (**4**), M_3 (**5**), and M_4 (**6**), the synthesis of **5** and **6** has recently been accomplished.[7] We now report a facile synthesis of the two major metabolites **2** and **4** and their chemotherapeutic activity.

Treatment of **1** with 48% HBr cleaved preferentially[8] the middle of the three MeO groups to provide the monophenol **2**, previously obtained by a multistep synthesis.[9] Oxidation of **1** with MnO_2 gave the ketone **3** which was reduced with $NaBH_4$ to afford the alcohol **4**.

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(1) B. Roth, E. A. Falco, G. H. Hitchings, and S. R. M. Bushby, *J. Med. Pharm. Chem.*, **5**, 1103 (1962).

(2) D. C. Martin and J. D. Arnold, *J. Clin. Pharmacol.*, **7**, 336 (1967).

(3) E. Grunberg and W. F. DeLorenzo, *Antimicrob. Ag. Chemother.*, **1966**, 430 (1967), and ref cited therein.

(4) GANTANOL.

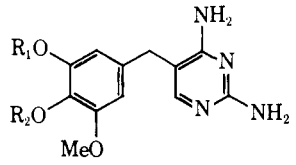
(5) BACTRIM; E. Böhni, *Chemotherapy Suppl.*, **14**, 1 (1969).

(6) D. E. Schwartz, G. Englert, and W. Vetter, *Arzneim.-Forsch.*, **20**, in press (1970).

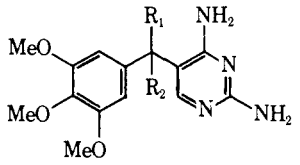
(7) G. Rey-Bellet and R. Reiner, *Helv. Chim. Acta*, **53**, 945 (1970).

(8) For related preferential O-demethylations, see S. Teitel and A. Brossi, *J. Med. Chem.* **13**, 333 (1970), and ref cited therein.

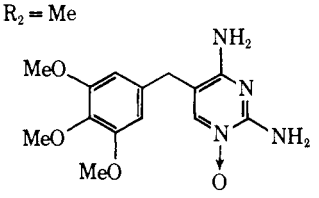
(9) B. Roth and J. Z. Strelitz, Netherland Patent 6702397 (1967).



1, $R_1 = R_2 = Me$
2, $R_1 = Me$; $R_2 = H$
6, $R_1 = H$; $R_2 = Me$



3, $R_1 + R_2 = O$
4, $R_1 = H$; $R_2 = OH$



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Biological Results.[10]—Compound **4** was tested *in vitro* against various Gram-positive and Gram-negative bacteria and against three pathogenic fungi. Minimum inhibitory concentrations observed were 250 μg/ml against *Staphylococcus aureus* 209 and *Salmonella typhosa* F and 1000 μg/ml against *Escherichia coli* J and *Mycobacterium tuberculosis* H37Rv. The compound was inactive when tested against other representative Gram-positive and Gram-negative bacteria and against *Candida albicans*, *Trichophyton mentagrophytes*, and *Microsporum audouini*.

When tested *in vivo* against mice infected with *S. typhosa* P58a, **2** protected 50% of the animals at a dose of 206 mg/kg orally but was inactive at 1000 mg/kg against other representative bacterial infections. Compound **4** was without antibacterial activity when tested *in vivo* at 1000 mg/kg orally. No *in vivo* antifungal or antiviral activity was observed with either **2** or **4** nor was any antiprotozoal, anthelmintic, or antitumor activity observed with **4**.

When **2** and **4** were tested *in vivo* at a fixed concentration of 50 mg/kg orally in combination with graded doses of sulfisoxazole, **2** potentiated the activity of sulfisoxazole against infections with *E. coli* 257, *Staph. aureus* Smith, and *Proteus vulgaris* 190. No potentiation was observed when **2** was tested in combination with sulfisoxazole against other representative Gram-positive and Gram-negative bacteria. Compound **4** failed to exhibit a potentiative effect on the activity of sulfisoxazole against the organisms tested.

Experimental Section

2,4-Diamino-5-(3,5-dimethoxy-4-hydroxybenzyl)pyrimidine (2).—A mixture of 120 g (0.41 mole) of trimethoprim[1] (**1**) and 1 l. of 48% HBr was stirred at 95–100° for 100 min, the soln cooled, and 240 ml of 50% NaOH added. The acidic mixture was stored at room temp for 2 hr, the crystals filtered, washed with ice-water, dissolved in 500 ml of boiling H_2O, and neutralized with NH_4OH. The resulting crystals were filtered, washed (H_2O), and air-dried to give 84.5 g (75%) of **2**, mp 264–266°, identical (mmp, spectroscopic, and chromatographic properties) with an authentic sample.[9] *Anal.* ($C_{14}H_{16}N_4O_3$), C, H, N.

2,4-Diamino-5-(3,4,5-trimethoxybenzoyl)pyrimidine (3).—A mixture of 50 g (0.17 mole) of **1** and 56 g of MnO_2 in 1 l. of 99% HOAc was stirred and refluxed for 4 hr and then stored at room temp overnight and the crystalline $Mn(OAc)_2$ filtered and washed with 150 ml of HOAc. The combined filtrates were rendered strongly acidic with 35–40 ml of concd HCl and evapd, the residua hydrochloride was slurried with H_2O, filtered, dissolved in hot H_2O, and neutralized with NH_4OH. The resulting ppt was collected and crystd from 65% EtOH to give 29 g (56%) of **3**, mp 198–199°. *Anal.* ($C_{14}H_{16}N_4O_4$), C, H, N.

Racemic α-(2,4-Diamino-5-pyrimidyl)-3,4,5-trimethoxybenzyl Alcohol (4).—To a stirred and refluxing soln of 12.5 g (0.04 mole) of **3** in 250 ml of MeOH was added 3 g of $NaBH_4$ over 1 hr. The mixture was stirred an additional hr and evapd and the residue crystd first from H_2O and then from EtOH to give 11.3 g (90%) of **4**, mp 199–200°, identical (mmp, spectroscopy, and chromatography) with metabolite M_2.[11]

(10) For *in vitro* and *in vivo* test methodologies, see E. Grunberg, J. Berger, G. Beskid, R. Cleeland, H. N. Prince, and E. Titsworth, *Chemotherapia*, **12**, 272 (1967), and ref 3.

(11) We are indebted to our colleagues Drs. R. Reiner and G. Rey-Bellet Chemical Research Department, F. Hoffmann-La Roche & Co., A. G., Base for this comparison.

Preparation and Antimicrobial Activity of *N*-Thiadiazolylcarbamic Acid Esters

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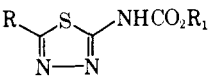
Recently we reported the adverse effect of *N*-thiadiazolylcarbamic acid *n*-butyl ester on measles virus in Vero cells.[1] In continuation of our search for potent antiviral and antimicrobial agents in 1,3,4-thiadiazolyl series,[1,2] compounds listed in Table I were prepared by interaction of alkyl chloroformates with appropriate 3-aminothiadiazoles (eq I).

TABLE I



No.	R	R_1	Yield, %	Mp, °C	Formula[a]
1	H	CH_3	68	230	$C_4H_5N_3O_2S$
2	H	C_2H_5	73	206	$C_5H_7N_3O_2S$
3	H	*i*-C_3H_7	71	191	$C_6H_9N_3O_2S$
4	H	*n*-C_4H_9	83	110	$C_7H_{11}N_3O_2S$
5	H	*i*-C_4H_9	89	147	$C_7H_{11}N_3O_2S$
6	H	$CH_2C_6H_5$	69	146	$C_{10}H_9N_3O_2S$
7	CH_3	CH_3	86	215	$C_5H_7N_3O_2S$
8	CH_3	C_2H_5	78	177	$C_6H_9N_3O_2S$
9	CH_3	*i*-C_3H_7	90	164	$C_7H_{11}N_3O_2S$
10	CH_3	*n*-C_4H_9	75	142	$C_8H_{13}N_3O_2S$
11	CH_3	*i*-C_4H_9	84	140	$C_8H_{13}N_3O_2S$
12	CH_3	$CH_2C_6H_5$	82	205	$C_{11}H_{11}N_3O_2S$
13	C_2H_5	CH_3	73	175	$C_6H_9N_3O_2S$
14	C_2H_5	C_2H_5	66	145	$C_7H_{11}N_3O_2S$
15	C_2H_5	*i*-C_3H_7	74	140	$C_8H_{13}N_3O_2S$
16	C_2H_5	*n*-C_4H_9	69	130	$C_9H_{15}N_3O_2S$
17	C_2H_5	*i*-C_4H_9	82	150	$C_9H_{15}N_3O_2S$
18	C_2H_5	$CH_2C_6H_5$	71	180	$C_{12}H_{13}N_3O_2S$
19	CF_3	CH_3	86	196	$C_5H_4F_3N_3O_2S$
20	CF_3	C_2H_5	91	183	$C_6H_6F_3N_3O_2S$
21	CF_3	*i*-C_3H_7	88	144	$C_7H_8F_3N_3O_2S$
22	CF_3	*n*-C_4H_9	90	158	$C_8H_{10}F_3N_3O_2S$
23	CF_3	*i*-C_4H_9	92	150	$C_8H_{10}F_3N_3O_2S$
24	CF_3	$CH_2C_6H_5$	89	180	$C_{11}H_8F_3N_3O_2S$

[a] All compounds were analyzed for C, H, and the analytical results were satisfactory. Ir and nmr spectra were as expected.

(1) H. Mirshamsi, I. Lalezari, M. Kamali, G. Niloufari, and N. Rezvani, *Arch. Virusforsch.*, **29**, 267 (1970).

(2) I. Lalezari and N. Sharghi, *J. Heterocycl. Chem.*, **3**, 336 (1966).