Table I. Chromatographic Mobility of Some Iodinated Thyronines

Compd	Solvent A	Solvent B	Solvent C
1	0.42	0.24	0.32
2		0.22	0.22
3		0.14	0.20
4	0.19	0.21	0.29
5	0.73	0.34	0.375

5 which was removed during the preparative TLC after iodination. If the product was not to undergo a chromatographic separation, the small amount of 5 could be removed by increasing the volume of HBr to 15.5 ml and the time of hydrolysis to 5.5 h. A second recrystallization of the hydrochloride from 2 N HCl before conversion to the free amino acid was required to eliminate the larger amount of 4 formed: overall yield, 55%. Purity was checked by paper chromatography (see Table I) (solvent A) (ascending, 18°): mp 253°; $[\alpha]^{21}D$ +24.8° [c 1, 1 N HCl–EtOH (1:1)] (lit.[7] +24.9°). Anal. ($C_{15}H_{14}INO_4 \cdot H_2O$) I.

3,3′-Diiodo-L-thyronine (2). 1 (0.834 mg) in 40 ml of concentrated NH_4OH was treated dropwise with 1 N iodine solution (4.8 ml, 20% excess). After standing 1 h the solvent was removed under vacuum, adding 1-propanol if necessary to control foaming. The residue was redissolved in water by a few drops of ammonia and separated from an insoluble brown material. On addition of acetic acid to the filtrate, the amino acids precipitated as an off-white solid: 930–990 mg (80–85%). Batches (90–100 mg) were dissolved in ethanol–2 N ammonia (3:1) about 1 ml, and the solutions were streaked onto 20 × 20 cm preparative layer plates and developed in solvent B. They were dried and redeveloped several times until sufficient separation between the two heavy stripes was achieved, as determined by minimum exposure to uv light. A faint stripe of highest R_f, identified as 5, was discarded. The middle stripe held 2; the stripe of lowest R_f contained the triiodothyronine (3). The silica was eluted with ethanolic ammonia and filtered and the filtrate evaporated under vacuum. The dried products were recovered either by adding water and filtering or by dissolving in water by addition of a few drops of ammonia, transferring to a centrifuge tube, precipitating with acetic acid, washing the precipitate repeatedly with water, and lyophilizing. About 50% of the theoretical amount of 2 was obtained (mp 196°), whereas the less soluble 3 recovery averaged 75% (mp 204°). Using analytical plates with solvent B gave a single spot, but since 1 was not separated from the other compounds, solvent C was used. This also produced a single spot. 2 also gave a single peak by gas chromatography after silylation. The technique was that of Cahnmann:[21] column, 2 ft by 2.4 mm i.d. OV-1 (1%) on Supelcoport; oven programmed for 7.5°/min rise; nitrogen carrier (44 ml/min); starting temperature 178°. Under these conditions, compounds 4, 1, 2, and 3 emerged at 194, 210, 233, and 248°, respectively: $[\alpha]^{20}D$ +19.6° [c 1, 1 N HCl–EtOH (1:1)] (lit.[7] +18.8°). Anal. ($C_{15}H_{13}I_2NO_4 \cdot 2H_2O$) I. Anal. ($C_{15}H_{12}I_3NO_4 \cdot H_2O$) I.

Acknowledgment. The author is grateful to Dr. H. J. Cahnmann of the NIH, Bethesda, Md., for determinations of purity by silylation and gas chromatography.

References and Notes

(1) I. J. Chopra and B. F. Crandall, *N. Engl. J. Med.*, **293**, 740 (1975).
(2) I. J. Chopra, J. Sack, and D. A. Fisher, *J. Clin. Invest.*, **55**, 1137 (1975).
(3) D. A. Fisher, *N. Engl. J. Med.*, **293**, 770 (1975).
(4) A. Burger and S. H. Ingbar, *Endocrinology*, **94**, 1189 (1974).
(5) I. J. Chopra, J. Sack, and D. A. Fisher, Excerpta Medica, Abstract No. 87, Seventh International Thyroid Conference, Boston, Mass., June 9–13, 1975.
(6) J. Roche, R. Michel, and W. Wolf, *C. R. Hebd. Seances Acad. Sci.*, **239**, 597 (1954).
(7) J. S. Varcoe and W. K. Warburton, *J. Chem. Soc.*, 2711 (1960).
(8) (a) C. L. Gemmill, J. J. Anderson, and A. Burger, *J. Am. Chem. Soc.*, **78**, 2434 (1956); (b) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).
(9) J. Roche, R. Michel, J. Nunez, and C. Jacquemin, *C. R. Hebd. Seances Acad. Sci.*, **244**, 1507 (1956); **245**, 77 (1957).
(10) R. I. Meltzer, U.S. Patent 2 954 399 (1960).
(11) P. Block, Jr., *J. Med. Chem.*, **10**, 950 (1967).
(12) G. Hillmann, *Z. Naturforsch.*, **11b**, 419 (1956); U.S. Patent 2 886 592 (1957). See also P. F. Bevilacqua, J. T. Plati, and W. Wenner, U.S. Patent 2 895 927 (1959).
(13) T. Shiba and H. J. Cahnmann, *J. Org. Chem.*, **29**, 1652 (1964).
(14) R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.*, **26**, 1977 (1961).
(15) A. Dibbo, L. Stephenson, T. Walker, and W. K. Warburton, *J. Chem. Soc.*, 2645 (1961).
(16) Ivor Smith, Ed., "Chromatographic and Electrophoretic Techniques", Vol. I, 2nd ed, Interscience, New York, N.Y., 1960, p 171.
(17) J. G. Heider and J. R. Bronk, *Biochim. Biophys. Acta*, **95**, 353 (1965).
(18) E. Stahl and J. Pfeifle, *Z. Anal. Chem.*, **200**, 377 (1964).
(19) F. M. Beringer, R. A. Falk, M. Karniol, I. Lillien, G. Masullo, M. Mausner, and E. Sommer, *J. Am. Chem. Soc.*, **81**, 350 (1959). See also B. Blank, F. R. Pfeiffer, C. M. Greenberg, and J. F. Kerwin, *J. Med. Chem.*, **6**, 554 (1963); E. C. Jorgensen and J. A. W. Reid, *ibid.*, **8**, 533 (1965).
(20) J. T. Plati, U.S. Patent 2 839 583.
(21) K. Funakoshi and H. J. Cahnmann, *Anal. Biochem.*, **27**, 150 (1969).

Antifungal Activity of 4-Substituted Crotonic Acid Esters

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Twenty-three 4-substituted crotonic acid esters were tested for antifungal activity against *Candida albicans*, *Aspergillus niger*, *Mucor mucedo*, and *Trichophyton mentagrophytes*. For the analogues of the methyl ester containing substituents in the 4 position, the following order of fungitoxicity was observed: I > Br > Cl > CH_3S > CH_3O > F = H. Of the homologues of the esters of the 4-iodo and 4-bromo compounds which included methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-hexyl, ethyl 4-iodocrotonate was most toxic to the four fungi at pH 7.0 in the presence of 10% beef serum (*C. albicans*, 18 μg/ml, *A. niger*, 40 μg/ml, *M. mucedo*, 5 μg/ml, *T. mentagrophytes*, 4 μg/ml). It is believed that the mechanism of fungitoxicity is due, in part, to a nucleophilic reaction involving SH-containing compounds. This is based on the correlation of fungitoxicity with the order of leaving groups in the nucleophilic reaction and the protection against the toxicity of the test compounds to the fungi by cysteine and glutathione.

As medicine becomes more complex, iatrogenic diseases follow.[1] In recent years, this has been manifested by an increase in the incidence of fungal infections.[2] Frequently, the attacking fungi are opportunistic invaders. They cause

infections that are secondary to debilitating diseases and to many types of medication which weaken the immune defenses of the host.[3-7]

Among the organisms implicated in deep fungal infections are *Candida* sp. (*C. albicans*, *C. tropicalis*, *C. parapsilosis* inter alia), *Cryptococcus neoformans*, *Aspergillus* sp. (*A. fumigatus*, *A. flavus* inter alia), species of the order Mucorales (*Rhizopus*, *Absidia*, and *Mucor* sp.), and *Histoplasma capsulatum*.[8] Very few antifungal agents are available for the treatment of deep fungal infections, and the drug of choice is amphotericin B, in spite of its mammalian toxicity and side effects.[7,8] Other agents that are of interest against *Candida* are 5-fluorocytosine and clotrimazole.[7]

As a result of our interest in this area,[9] a study of the esters of 4-substituted crotonic acids was initiated. While this work was in progress, we became aware that others had reported the antifungal activity of ethyl 4-bromo-2-methylcrotonate and phenyl 4-bromocrotonate against plant pathogenic fungi.[10]

A systematic examination of the effect of structural alteration to antifungal activity of the 4-substituted crotonic acid esters was carried out. The alterations included replacement of the methyl group in the 4 position of methyl crotonate by hydrogen and substitution of a fluorine, chlorine, bromine, iodine, methoxyl, or thiomethyl substituent for hydrogen on carbon-4. The two most active derivatives were further modified by preparing the ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-hexyl esters.

The unsaturated esters were obtained by esterification of crotonic acid with the appropriate alcohol by azeotropic distillation with benzene in the presence of sulfuric acid as the catalyst. The *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-hexyl esters were prepared in this manner.[11,12] The methyl esters of 4-methoxy-,[13] 4-methylthio-,[14] 4-fluoro-,[15] and 4-chlorocrotonic acids[16] were prepared by methods found in the literature. Bromination of the 4 position of the crotonic esters was achieved by means of *N*-bromosuccinimide and benzoyl peroxide in carbon tetrachloride.[17] Of the six bromo esters prepared, the methyl,[17] ethyl,[18] and propyl[19] esters were known.

The esters of 4-iodocrotonic acid were prepared from the corresponding bromo esters by treatment with sodium iodide in acetone at room temperature. Of the iodo compounds prepared, only ethyl 4-iodocrotonate was previously reported, and it was prepared from ethyl 4-chlorocrotonate.[20] It should be mentioned that the distillates were darker than the products prepared for distillation. This was also observed previously.[20] The data characterizing the crotonic acid esters are given in Table I.

All of the compounds were tested against *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 1004), *Mucor mucedo* (ATCC 7941), and *Trichophyton mentagrophytes* (ATCC 9129) in Sabouraud dextrose agar (Difco) at pH 5.6 and 7.0 in the presence and absence of 10% beef serum (Miles Labs.) according to published methods,[9,21] and the results are listed in Table II. Since it was our desire to locate compounds which would be of potential medicinal interest, the highest level tested was 100 μg/ml, and, for the purpose of comparison, the most valuable results were those obtained at pH 7.0 in the presence of beef serum.

A comparison of the results obtained with the methyl 4-halocrotonates (Table II) shows the following order of antifungal activity: I > Br > Cl > F, with the iodo-compound being about twice as active as the bromo analogue on a molecular basis. To examine the effect on

Table I. Crotonic Acid Esters ($XCH_2CH{=}CHCOOR$)

X	R	Yield, %	Bp (mm), °C		*n*D		ν_{neat}, cm^{-1}		Formula	Analyses
			Obsd	Lit.	25°	°C	C=O	C=C		
H	CH_3			Purchased			1722	1661		
CH_3O[a]	CH_3	74	64 (7.5)	54–57.5 (8.0)[c]	1.4454	1.4333 (25)[a]	1722	1664	$C_6H_{10}O_3$	C, H
CH_3S	CH_3	54	111–112 (19)	95–97 (12)[e]	1.4542		1724	1652		
F	CH_3	15	127–131	46 (16)[f]	1.4019		1730	1672		
Cl[b]	CH_3	77	68–71 (8.5)	68.5 (11)[c]	1.4609	1.4650 (24)[c]	1728	1663		
Br	CH_3	70	86–88 (7.0)	78–82 (8.0)[d]	1.4986	1.5021 (20)[d]	1725	1658		
Br	C_2H_5	48	86–89 (5.5)	66–67 (0.3)[g]	1.4907	1.492 (16)[g]	1723	1658		
Br	n-C_3H_7	37	102–105 (5.5)	108–109 (15)[h]	1.4870	1.487 (18)[h]	1725	1659		
Br	n-C_4H_9	45	116–117 (5.5)		1.4827		1725	1655	$C_8H_{13}BrO_2$	C, H, Br
Br	n-C_5H_{11}	33	127–130 (5.0)		1.4814		1720	1656	$C_9H_{15}BrO_2$	C, H, Br
Br	n-C_6H_{13}	60	125–129 (2.7)		1.4807		1719	1652	$C_{10}H_{17}BrO_2$	C, H, Br
I	CH_3	83	55–57 (0.2)		1.5574		1720	1642	$C_5H_7IO_2$	C, H, I
I	C_2H_5	70	55–58 (0.1)	90–92 (2.0)[i]	1.5423		1728	1645		
I	n-C_3H_7	70	68–71 (0.1)		1.5331		1710	1648	$C_7H_{11}IO_2$	C, H, I
I	n-C_4H_9	75	77–81 (0.2)		1.5426		1725	1648	$C_8H_{13}IO_2$	C, H, I
I	n-C_5H_{11}	65	91–94 (0.2)		1.5190		1721	1642	$C_9H_{15}IO_2$	C, H, I
I	n-C_6H_{13}	53	100–102 (0.05)		1.5126		1720	1645	$C_{10}H_{17}IO_2$	C, H, I

[a] Prepared by method in ref 13; product not fully characterized. [b] Prepared by method in ref 16. [c] H. O. House, V. K. Jones, and G. A. Frank, *J. Org. Chem.*, 29, 3327 (1964). [d] L. N. Owen and M. U. S. Sultanbawa, *J. Chem. Soc.*, 3098 (1949). [e] See ref 14. [f] See ref 15. [g] See ref 18. [h] See ref 19. [i] See ref 20.

Table II. Antifungal Activity of Crotonic Acid Esters and Derivatives at pH 5.6 and 7.0 in Sabouraud Dextrose Agar in the Absence and Presence of Beef Serum[a]

XCH=CHCOOR		Levels of inhibition[b]															
		C. albicans				*A. niger*				*M. mucedo*				*T. mentagrophytes*			
		pH 5.6		pH 7.0		pH 5.6		pH 7.0		pH 5.6		pH 7.0		pH 5.6		pH 7.0	
X	R	−[c]	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
FCH_2	CH_3	NA[d,e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
$ClCH_2$	CH_3	NA	NA	NA	NA	NA	NA	NA	NA	60	80	80	90	4	40	60	60
$BrCH_2$	CH_3	30	30	30	30	50	50	50	60	6	8	10	14	3	5	5	6
$BrCH_2$	C_2H_5	20	30	30	40	80	90	70	80	5	7	6	7	<1	2	2	4
$BrCH_2$	n-C_3H_7	18	30	30	40	80	90	60	80	4	5	4	7	<1	3	3	7
$BrCH_2$	n-C_5H_9	16	30	30	40	60	100	50	80	4	4	4	7	<1	4	3	8
$BrCH_2$	n-C_5H_{11}	20	50	30	50	70	NA	70	NA	3	14	14	30	<1	4	2	10
$BrCH_2$	n-C_6H_{13}	20	70	20	90	NA	NA	NA	NA	14	40	16	50	<1	14	4	30
ICH_2	CH_3	16	30	20	18	30	40	30	40	9	10	9	8	<1	3	2	4
ICH_2	C_2H_5	8	18	10	18	40	50	40	40	<1	4	3	5	<1	2	2	4
ICH_2	n-C_3H_7	10	16	12	20	30	60	30	60	2	5	3	6	<1	3	2	7
ICH_2	n-C_5H_9	10	20	12	30	30	70	20	70	2	5	4	7	<1	3	<1	7
ICH_2	n-C_5H_{11}	5	40	12	50	40	NA	30	NA	4	9	3	12	<1	4	2	10
ICH_2	n-C_6H_{13}	16	80	30	90	NA	NA	NA	NA	14	40	14	50	<1	8	3	20
Candicidin		<1	<1	<1	2	2	5	5	7	<1	<1	<1	<1	<1	<1	3	9
Amphotericin B		<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

[a] *C. albicans* and *M. mucedo* were incubated at 37° for 20 h and *T. mentagrophytes* and *A. niger* at 28° for 5 days. [b] Minimal concentrations of compound in μg/ml causing 100% inhibition of test organisms. Activity to 10 μg/ml was obtained in increments of 1 μg/ml, from 10 to 20 μg/ml in increments of 2 μg/ml, and from 20 to 100 μg/ml, the highest level tested, increments of 10 μg/ml were used. All tests were carried out in "I" plate Petri dishes. [c] − and + = absence or presence of 10% beef serum. [d] NA = not inhibitory below 100 μg/ml. [e] Methyl acrylate and the methyl, ethyl, propyl, butyl, pentyl, and hexyl esters of crotonic acid were inactive against all four fungi. Methyl 4-methoxycrotonate was also inactive, but methyl 4-methylthiocrotonate was inhibitory to *M. mucedo* (70 μg/ml) and *T. mentagrophytes* (90 μg/ml) at pH 5.6 in the absence of beef serum.

fungitoxicity of the chain length of the alkyl group in the ester function, the 4-bromo and 4-iodocrotonic esters from methyl to *n*-hexyl were tested. The order of antifungal activity against the four fungi of the esters of the bromocrotonate series was methyl > butyl > propyl > ethyl > pentyl > hexyl and of the iodocrotonate series was ethyl > methyl > propyl > butyl > pentyl > hexyl.

All of the iodocrotonates were more fungitoxic than the corresponding bromocrotonates, and the most active of all the compounds tested in this study was ethyl 4-iodocrotonate. The minimum inhibiting concentration for the four test organisms was as follows: *C. albicans*, 18 μg/ml; *A. niger*, 40 μg/ml; *M. mucedo*, 5 μg/ml; *T. mentagrophytes*, 4 μg/ml.

Since allylic compounds are known to undergo nucleophilic substitution,[22] it was desired to determine whether this played any part in the mode of fungitoxic action of the 4-substituted crotonic esters. Methyl 4-bromocrotonate was added to the growth medium at pH 7.0 at a concentration of one increment above the minimal inhibitory level for each organism (Table II). Cystine, cysteine, and glutathione, respectively, were added to the medium in molar ratios of 1:1 to 10:1 in increments of 1 equiv to one with respect to the toxicant, and the Petri dishes were inoculated and incubated as described above. Beef serum was omitted in order to avoid the complications which might be caused by the presence of a multitude of additional metabolites being added to the medium. Cystine had no effect on the toxicity of methyl 4-bromocrotonate. Cysteine and glutathione allowed all four fungi to grow in the presence of the toxicant. The ratio of SH-containing compound to toxicant for each fungus which prevented the inhibition of growth is shown in Table III. Methyl 4-methylthiocrotonate was studied in the same manner as methyl 4-bromocrotonate, against *M. mucedo* and *T. mentagrophytes*. The results were similar for both compounds (Table III).

It appears that the mechanism of fungitoxicity of the 4-substituted crotonic acid esters may possibly be based, at least in part, on nucleophilic substitution. The reduction in toxicity of the compounds by beef serum, protection of the fungi by SH-containing metabolites, and the order of fungitoxicity due to the different substituents in the 4 position are consistent with the order of leaving groups in the nucleophilic reaction.[22] That the size of the ester affects fungitoxicity in the homologous series indicates that chain length of the molecule affects its activity, as is the case with the fatty acids and their methyl esters.[21]

Table III. Protection by SH-Containing Metabolites against the Fungitoxicity of 4-Substituted Methyl Crotonates

Fungus	Inhibitory level of toxicant, μg/ml	Molar ratio of protectant to inhibitor		
		Cystine	Cysteine	Glutathione
$BrCH_2CH=CHCOOCH_3$				
C. albicans	40	>10[a]	2	3
A. niger	60	>10	<1	<1
M. mucedo	12	>10	4	8
T. mentagrophytes	6	>10	10	9
$CH_3SCH_2CH=CHCOOCH_3$				
M. mucedo	80	>10	<1	<1
T. mentagrophytes	70	>10	<1	<1

[a] A molar ratio of cystine to toxicant of 10:1, the highest ratio tested, allowed all cultures to remain inhibited.

Experimental Section[12]

Butyl 4-Bromocrotonate. A mixture of butyl crotonate[11] (50 g, 0.35 mol), *N*-bromosuccinimide (63 g, 0.35 mol), and benzoyl peroxide (0.5 g, 0.002 mol) in 500 ml of dry CCl_4 was heated under reflux with stirring and exclusion of moisture for 10 h. The reaction mixture was cooled to room temperature with continued stirring, and the succinimide was removed by filtration. The filtrate was subsequently washed with H_2O (3 × 100 ml) and dried (Na_2SO_4). After removing the solvent in the rotary evaporator,

the residue was distilled under vacuum. The yield of product was 35 g (45%), bp 129–135° (16 mm).

Butyl 4-Iodocrotonate. To a solution of NaI (19.3 g, 0.133 mol) in $(CH_3)_2CO$ (150 ml) at room temperature was added butyl 4-bromocrotonate (22.1 g, 0.1 mol). After stirring for 1 h, the NaBr formed was removed by filtration. The solids were washed with $(CH_3)_2CO$ and the combined filtrates were poured into 600 ml of Et_2O. The ether solution was washed with 5% $Na_2S_2O_3$ solution (100 ml), followed by H_2O (2 × 200 ml), and dried (Na_2SO_4). The solvent was removed in the rotary evaporator, and the residue was distilled under reduced pressure. The yield of product was 18.9 g (70%), bp 100–110° (0.1 mm).

Acknowledgment. We wish to thank Dr. B. Brown, S. B. Penick and Company, for a sample of candicidin. Thanks are also due to T. Engelmann for technical assistance.

References and Notes

(1) G. F. Parkhurst and G. D. Vlahides, *J. Am. Med. Assoc.*, **202**, 131 (1967).
(2) L. E. Zimmerman, *Am. J. Clin. Pathol.*, **25**, 46 (1955).
(3) Z. T. Manowski and B. J. Littleton, *Antibiot. Chemother.*, 4, 253 (1954).
(4) R. M. Torack, *Am. J. Med.*, **22**, 872 (1957).
(5) H. Sidransky and M. A. Pearl, *Dis. Chest*, **39**, 630 (1961).
(6) M. S. Seelig, *Am. J. Med.*, **40**, 887 (1966).
(7) D. Armstrong, L. S. Young, R. D. Meyer, and A. H. Blevins, *Med. Clin. North Am.*, **55**, 729 (1971).
(8) A. S. Levine, R. G. Graw, Jr., and R. C. Young, *Semin. Hematol.*, **9**, 141 (1972).
(9) H. Gershon and L. Shanks, *Can. J. Microbiol.*, **21**, 1317 (1975).
(10) J. Perronnet and P. Girault, Fr. Demande 2 221 437 (1974); *Chem. Abstr.*, **82**, 155 398c (1975).
(11) S. Hersek, *Istanbul Univ. Fen Fak. Mecm. Seri C*, **31**, 43 (1966).
(12) Methyl acrylate, crotonic acid, and methyl and ethyl crotonate were purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis., and amphotericin B was purchased from Calbiochem, La Jolla, Calif. The synthetic procedures are general. Infrared spectra were obtained with a Perkin-Elmer Model 221 spectrophotometer on neat samples. Refractive indices were taken with an Abbe-3L, B & L refractometer. Gas chromatography was performed on a Varian Aerograph Model 1200 gas chromatograph with a flame ionization detector to which is attached a Varian Aerograph Model 20 recorder. The purity of the compounds was established by gas chromatographing on a column of 3% Dexsil 400 on Anachrom A (90–100 mesh) purchased from Analabs, New Haven, Conn. All samples tested microbiologically were at least 95% pure.
(13) M. U. S. Sultanbawa, P. Veeravagu, and T. Padmanathan, *J. Chem. Soc.*, 1262 (1960).
(14) L. Birkofer and I. Hartwig, *Chem. Ber.*, **87**, 1189 (1954).
(15) F. L. M. Pattison and B. C. Saunders, *J. Chem. Soc.*, 2745 (1949).
(16) J. W. E. Glattfeld and E. Rietz, *J. Am. Chem. Soc.*, **62**, 974 (1940).
(17) K. Ziegler, A. Späth, E. Schaaf, W. Schumann, and E. Winkelmann, *Chem. Ber.*, **551**, 80 (1942).
(18) S. P. Massie, *Iowa State Coll. J. Sci.*, **21**, 41 (1946); *Chem. Abstr.*, 41, 3044*b* (1947).
(19) R. Rambaud, *Bull. Soc. Chim. Fr.*, 1, 1342 (1934).
(20) G. Braun, *J. Am. Chem. Soc.*, **52**, 3167 (1930).
(21) H. Gershon and R. Parmegiani, *J. Med. Chem.*, **10**, 186 (1967).
(22) A. Streitwieser, Jr., *Chem. Rev.*, **56**, 571 (1956).

Synthesis and Antimicrobial Evaluation of Substituted 5,6-Dihydro-5-nitrouracils

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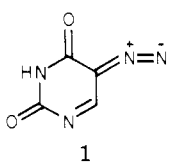
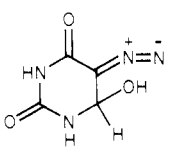
Reaction of 5-nitrouracil derivatives with sodium borohydride in methanol–water, followed by neutralization of the product with acid, has produced 5,6-dihydro-5-nitrouracil (**5**), 5,6-dihydro-6-methyl-5-nitrouracil (**7**), 5,6-dihydro-5-nitro-1-(4-nitrophenyl)uracil (**10**), and 5,6-dihydro-5-nitro-1-(β-D-ribofuranuronic acid ethyl ester)uracil (**12**). In assays for antimicrobial activity using strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Trichophyton mentagrophytes*, significant inhibition of growth was not found.

It has been demonstrated that 5-diazouracil (1) inhibits cell division,[1] causes an increase in the mean cell volume,[2] and has a broad-spectrum in vitro activity against pathogenic bacteria.[3] The antimicrobial activity of 1 is, however, accompanied by severe mammalian toxicity. Since assigned structures[4] for the 5-diazouracils and 5-diazouridines indicate the presence of a substituent at C-6, as in the hydrated form of 5-diazouracil (**2**), we sought to prepare other uracil derivatives containing a 5,6-dihydro structure with a strongly electronegative group at C-5. The synthesis of 5,6-dihydro-5-nitrouracil and related derivatives by reduction of the corresponding 5-nitrouracil with sodium borohydride plus the evaluation for antimicrobial activity is herein reported.



1



2

Chemistry. In an early investigation[5] of 5-nitropyrimidines, treatment of 5-nitro-1-(β-D-ribofuranuronic acid isopropyl ester)uracil with sodium borohydride and aluminum chloride or with potassium borohydride and lithium chloride yielded a new product which was identified as the 5,6-dihydro-5-nitro derivative. Reduction of the 5,6 bond in N^4-acetylcytidine[6] and 5-acetyluracil[7] has been reported using sodium borohydride. More recently it was found[8] that reduction of 6-nitroquinoxaline to give 6-nitro-1,2,3,4-tetrahydroquinoxaline occurred upon treatment with sodium borohydride in methanol. No reduction of quinoxaline itself under the same conditions was found, indicating the necessity of the nitro group for reaction with the hydride.

When a solution of 5-nitrouracil (**3**) in methanol–water was treated with sodium borohydride, a precipitate formed. This was identified as the sodium salt of 5,6-dihydro-5-nitrouracil (**4**). Treatment of a solution of **4** in water to pH 3 with 1 N hydrochloric acid gave 5,6-dihydro-5-nitrouracil (**5**). Similarly, 6-methyl-5-nitrouracil (**6**) yielded 5,6-dihydro-6-methyl-5-nitrouracil (**7**) (Scheme I). The ^1H NMR spectra of **7** in Me_2SO-d_6 revealed a mixture of two products, presumed to be the cis and trans isomers. The signal for the C_5H (δ 5.81 $J_{5,6}$ = 9.5 Hz) seems to indicate a predominance of the trans isomer,[9] which could be expected with the bulky methyl and nitro groups