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Synthesis and Biological Activity of 5-Fluoro- and 5-Methyl-1,3-oxazine-2,6(3*H*)-dione

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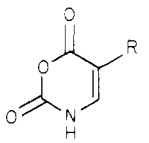
5-Fluoro-1,3-oxazine-2,6(3*H*)-dione (3-oxa-FU) was synthesized by reacting 3-oxauracil with fluoroxytrifluoromethane and decomposing the adduct in the presence of a catalytic amount of Et_3N. 5-Methyl-1,3-oxazine-2,6(3*H*)-dione (3-oxathymine) was prepared by polyphosphoric acid catalyzed ring closure of β-(*N*-ethoxycarbonylamino)-2-methacrylic acid and by treatment of citraconimide with sodium hypochlorite. As determined in vitro, 3-oxa-FU was markedly inhibitory to *S. faecium* ($ID_{50} = 9 \times 10^{-8}$ M) and *E. coli* ($ID_{50} = 1 \times 10^{-7}$ M) but was less active against leukemia L-1210 cells ($ID_{50} = 1 \times 10^{-5}$ M). At 1×10^{-4} M, 3-oxathymine was inactive in these cell systems. Inhibition of the growth of *S. faecium* by 3-oxa-FU was reversed competitively by the natural pyrimidines. The relatively rapid hydrolysis of the compounds in the growth media is a major factor in determining their biological effectiveness.

The isosteric replacement of the nitrogen atoms of the pyrimidine ring has been one of the approaches employed for obtaining potentially useful antitumor agents. One such modification, the replacement of nitrogen with oxygen, has been actively explored in recent years. Although 1,3-oxazine-2,6(3*H*)-dione (3-oxauracil) was first prepared by Rinkes, in 1927, by sodium hypochlorite oxidation of maleimide,[1] information on its biological activity became available only in 1972, when we reported the synthesis of 3-oxathymine and the in vitro inhibitory activity of these compounds against various microbial and tumor cell lines.[2] In the same year, Washburne et al. published a new synthesis of the agent, involving the reaction of maleic anhydride with trimethylsilyl azide.[3] In 1973, Skoda et al. demonstrated the inhibition of *E. coli* growth by 3-oxauracil,[4] and we reported the preparation of 3-oxa-2′-deoxyuridine, which is a much more potent inhibitor of cell growth than is the aglycon.[5] The synthesis of the ribonucleoside derivative of 3-oxauracil was published in 1974 by Chwang and Heidelberger,[6] and in a preliminary communication we presented the synthesis of the 5-fluoro derivative of 3-oxauracil.[7] Subsequently, the synthesis of 3-oxauracil variously substituted with halo, alkyl, and aryl groups was reported by Warren et al.,[8] McMillan and Washburne,[9] Farkas et al.,[10] and by Washburne and Park.[11]

Chemistry. The synthesis of 5-fluoro-1,3-oxazine-2,6(3*H*)-dione (I) was carried out by reacting 1,3-oxazine-2,6(3*H*)-dione dissolved in anhydrous acetone with fluoroxytrifluoromethane at −55 to −60 °C. The rapid disappearance of UV absorption at 260 nm indicated the formation of an adduct, as was previously reported to occur upon the interaction of this reagent with pyrimidine bases and nucleosides.[12]



I, R = F
II, R = CH_3

The attempted decomposition of the intermediate by procedures used in the synthesis of 5-fluoropyrimidines[13,14] led to the formation of decomposition products. This difficulty was overcome by adding catalytic amounts of Et_3N to the reaction mixture. When markedly greater amounts of catalyst were used, even under exclusion of protic solvents, the formation of I could not be detected by TLC.

The thymine analogue, 5-methyl-1,3-oxazine-2,6(3*H*)-dione (II) was prepared by polyphosphoric acid catalyzed ring closure of β-(*N*-ethoxycarbonylamino)-α-methacrylic acid at 75–80 °C or by treatment of the citraconimide with sodium hypochlorite at 0–5 °C.

Biology. To eliminate biologically active degradation products, e.g., halogenoacetaldehyde,[15] that may have formed upon storage of the relatively labile 3-oxa-FU, the compound was recrystallized from ethyl acetate–methylene chloride each time before its use. Under these conditions, 3-oxa-FU was found to be an effective inhibitor of bacterial cell growth, but it was only moderately active against leukemia L-1210 cells (Table I). In contrast, 3-oxauracil

Table I. Comparative Biological Effects of Some 1,3-Oxazines

compd	molar concn for 50% inhibn of the growth of: leuk L-1210	*S. faecium*	*E. coli*
5-fluoro-3-oxauracil	1×10^{-5}	9×10^{-8}	1×10^{-7}
3-oxauracil	4×10^{-5}	2×10^{-5}	4×10^{-7}
3-oxathymine	$>10^{-4}$	$>10^{-4}$	3×10^{-4}

Table II. Relative Effectiveness of Natural Pyrimidines in Preventing the Inhibition of *S. faecium* Growth Caused by 3-Oxa-5-fluorouracil

metabolite	inhibn index $([I]/[S]_{50})^a$
thymine	120
thymidine	110
2′-deoxyuridine	100
uracil	90
uridine	90
2′-deoxycytidine	10
cytidine	8
cytosine	7

[a] [I]/[S] for 50% growth inhibition at substrate concentrations ranging from 10^{-3} to 10^{-6} M.

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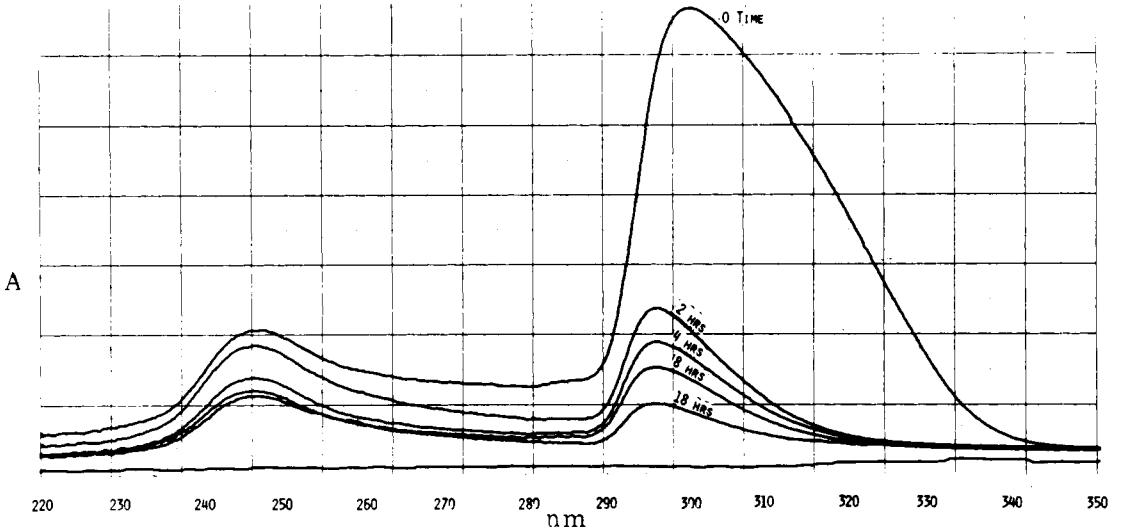

Figure 1. Degradation of 3-oxa-5-fluorouracil in the bacterial growth medium (pH 7.2).

was markedly inhibitory to *E. coli* but only moderately active against *S. faecium* and against L-1210 cells. 3-Oxathymine was essentially inactive in these cell systems.

To gain information concerning the nature of the drug effect, an inhibition analysis was carried out, using the *S. faecium* cells, which demonstrated that the inhibition of cell growth by 3-oxa-FU is competitively reversed by the natural pyrimidines (Table II). The relative efficiency with which the pyrimidines prevented the inhibition suggests that the DNA biosynthetic pathway may be the primary target of the compound. Skoda and his associates[4] had previously observed that the inhibition of *E. coli* growth by 3-oxauracil is similarly reversed by the natural pyrimidines, whereas the inhibitory effects of various 5-halo (bromo, chloro, and iodo) derivatives of 3-oxauracil were not reversed by preformed pyrimidines but were prevented by glutathione.[15]

The marked difference in the potency of 3-oxa-FU in the bacterial as compared to the mammalian cell systems may be due to the relative instability of the compound in the growth media. As shown in Figure 1, the relatively rapid degradation of 3-oxa-FU in the bacterial growth medium (pH 7.2) is reflected by the time-dependent decrease in UV absorbence at 306 nm. The compound is strongly acidic and at the pH of the medium is completely dissociated (Figure 2). A similar pattern of degradation is seen in the medium used for the growth of the leukemic cells. The greater potency of the analogue against the bacteria is probably due to the fact that the doubling time of these cells is approximately 60 min, during which time extensive degradation of the compound has not yet occurred. In contrast, the doubling time of the leukemic cells is 10–12 h, during which period the compound is almost completely broken down. Indeed, the observed[16] inability of pyrimidines to prevent the moderate inhibition of the growth of L-5178 cells by 3-oxauracil and its ribofuranosyl derivative may be due to degradation products which accumulate during the 3-day incubation period. Skoda and his co-workers have demonstrated[15,17] that in a synthetic growth medium only 15% of 3-oxauracil remains after 16 h of incubation at 37 °C and that the 5-halogeno derivatives possess half-lives of 4–5 h, the respective acetaldehydes being the major end products formed.

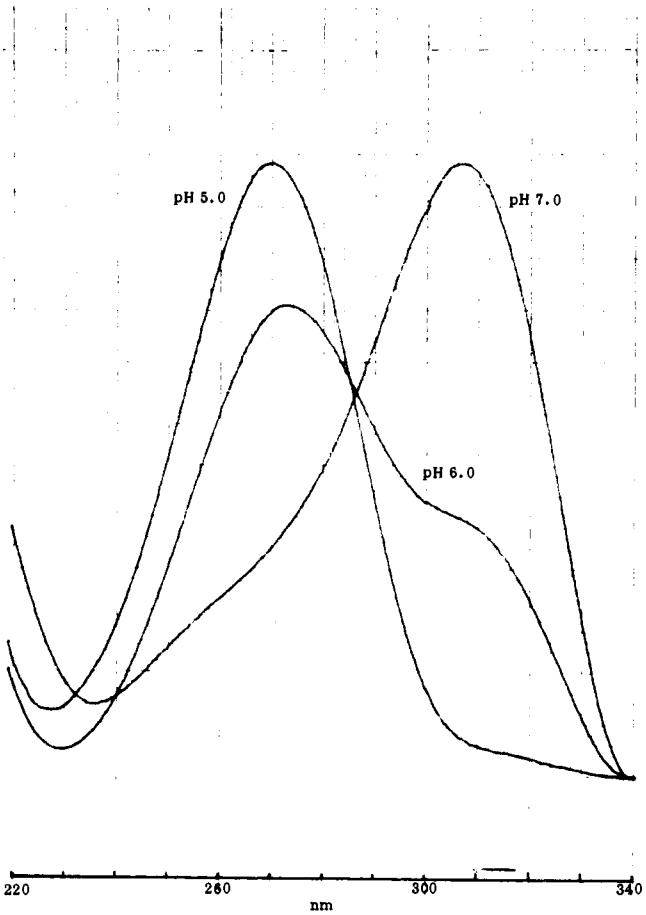

Figure 2. UV spectra of 3-oxa-5-fluorouracil at various pH values.

Experimental Section

Melting points were determined on a Fisher-Jones melting point apparatus and are uncorrected. UV and NMR spectra were, respectively, recorded on a Beckman Model 25 and a Varian XL-100 spectrometer. A Dupont CC 21-491 double-focusing spectrometer was used for obtaining mass spectra. Satisfactory analyses (C, H, N, and F) within ±0.4% of the theoretical values were obtained from Robertson Laboratory, Florham Park, N.J. Evaporations were carried out under reduced pressure in a rotary evaporator. Thin-layer chromatography was performed on precoated plastic sheets (silica gel N-HR/UV_{254}, Brinkman Instruments, Inc.). Column chromatography was carried out on silica gel 60–200 mesh, J. T. Baker no. 3405.

5-Fluoro-1,3-oxazine-2,6(3*H*)-dione. 3-Oxauracil (5.65 g) was dissolved in 400 mL of dry acetone and cooled to −55 to −60 °C. CF_3OF was added slowly (one bubble/s) with stirring. The mixture was allowed to reach room temperature and its volume was reduced to approximately 200 mL. An additional 100 mL

of acetone was added, and, after cooling the solution to −5 °C, 15 drops of Et_3N were added with stirring and the mixture was kept at 4 °C for 24 h. It was then evaporated to a syrup and 100 mL of benzene was added; after reducing the volume to 10 mL, the solution was applied to a dry silica gel column (3 × 100 cm), and the product was eluted with a mixture of benzene–acetone (9:1): yield 855 mg (13%); mp 117–121 °C dec; MS *m/e* 131 (M^+); UV λ_{max} (pH 7.0) 307 nm (ϵ 7729); λ_{max} (pH 6.0) 272 nm (ϵ 5947), 308 (ϵ 3144) sh; λ_{max} (pH 5.0) 270 nm (ϵ 7729).

5-Methyl-1,3-oxazine-2,6(3*H*)-dione. A. 3-Ethoxycarbonylamino-2-methylacrylic acid[18] (17.3 g) was added to hot (75–80 °C) polyphosphoric acid (170 g, 82–84% P_2O_5) with stirring. The mixture was stirred at 75–80 °C for a total of 45 min, poured into 100 mL of ice–water, and stirred to a smooth slurry. The precipitate was filtered, washed with cold water, and dried over P_2O_5: yield 8.5 g; mp 129–131 °C. The product was recrystallized from ethyl acetate: yield 6.2 g (56%); mp 134–135.5 °C; UV λ_{max} (EtOH) 271 nm (ϵ 6500); NMR (Me_2SO-d_6) δ 1.83 (d, 3, J_{CH_3-4} = 1.5 Hz), 7.56 (q, 1, J_{4-CH_3} = 1.5 Hz, H-4).

B. A cold (0 °C) 5.25% solution (30 mL) of NaOCl containing 1 g of NaOH was added to a cold (0 °C) suspension of citraconimide (2.22 g) in 8 mL of H_2O. The mixture was stirred at 0 °C for 5 h, and cold dilute H_2SO_4 was used to adjust the pH to ~3. The precipitated product was filtered, washed with water, and dried, yield 1.78 g (72%).

Biological. The procedures for determining the growth-inhibitory capacity of the compounds and for carrying out the inhibition analyses have been published previously.[19,20]

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A Structural Modification Study of Procarbazine

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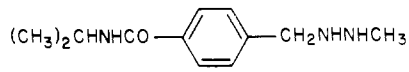
Eight analogues of the antineoplastic compound procarbazine were prepared by varying one portion of the molecule, keeping either the methylhydrazinomethyl or the *N*-(1-methylethyl)benzamido portion of procarbazine intact. Preliminary screening results indicated that none of the analogues tested in leukemias L1210 and P388 were as active as the original compound.

N-(1-Methylethyl)-α-(2-methylhydrazino)-*p*-toluamide (procarbazine, **1**), a methylhydrazine derivative synthesized



1

in 1963,[2a] has demonstrated pronounced tumor-inhibitory effects,[2b] has a marked influence on the growth of several transplantable tumors,[3] causes chromosome breakage in mouse cancer cells,[4] inhibits rat prostatic 5α-reductase and araginase activity,[5] and has been studied clinically, either singly or in combination.[6-16] Procarbazine is particularly useful in patients with Hodgkin's disease and non-Hodgkin's lymphoma. Metabolism and mechanism of action studies of this unique agent have also been reported.[17-23]

Procarbazine was found to be carcinogenic[24] and to produce cardiovascular[25] and severe CNS toxicity in patients with hepatic metastasis.[11] The latter toxicity could be related to the procarbazine acting either as an inhibitor of monoamine oxidase[6,26,27] or through depletion of the cofactor pyridoxal phosphate.[28] A structural modification study of this agent has therefore been conducted in this laboratory in order to uncover more desirable compounds. Compounds **2**–**7** represent our preliminary selection of structural variations wherein the methylhydrazinomethyl moiety of **1** is kept intact with the modification of the amide portion or varying the hydrazine unit but leaving the rest of the molecule unaltered.

Chemistry. The pyrimidine derivative **2** was prepared by the condensation of 5-(chloromethyl)uracil[29] and *N*-acetyl-*N*-methylhydrazine,[30] followed by removal of the protecting group. The thiosemicarbazone **3** was obtained from 4-[(2-methylhydrazino)methyl]benzaldehyde.[31]

The benzyloxy isostere of procarbazine (**4**) was prepared as follows. Condensation of 4-(bromomethyl)-*N*-(1-methylethyl)benzamide (**8**) with *N*-[(benzyloxy)carbon-

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