10 mg of benzoyl peroxide, and 10 ml of CCl_4 was refluxed with stirring for 19 hr. The cooled solution was filtered through a Celite pad which was then washed with CCl_4. The combined filtrate and washings were spin evaporated *in vacuo* to leave an oil which moved as one major spot on tlc with EtOAc–petroleum ether (bp 60–110°) (1:18) and gave a positive test for active halide.[14] The oil was used without further purification.

***m*-Phenylpropoxybenzyl Chloride (Method C).**—To a stirred solution of 0.541 g (10.0 mmol) of NaOMe dissolved in 10 ml of absolute EtOH was added 1.24 g (10.0 mmol) of 3-hydroxybenzyl alcohol. After 5 min the solvent was spin evaporated *in vacuo*. The residue was dispersed in 10 ml of DMF, 1.86 g (9.3 mmol) of 3-bromopropylbenzene was added, and the mixture was heated on a steam bath for 1 hr. The cooled mixture was diluted with 25 ml of H_2O and was extracted with three 25-ml portions of $CHCl_3$. The combined organic extracts were washed with two 25-ml portions of 0.5 *N* NaOH and 25 ml of brine, and then dried ($MgSO_4$). The solvent was spin evaporated *in vacuo* (finally at ~1 mm) leaving an oil in nearly quantitative yield which moved as a single new spot on tlc in C_6H_6–EtOH (3:1).

The oil was dissolved in 20 ml of dry $CHCl_3$, warmed slightly on a steam bath and then treated with 2 ml of $SOCl_2$. The solution was stirred at ambient temperature for 1 hr during which time the evolution of gases ceased. The solvent was spin evaporated *in vacuo*, and the residue was redissolved in ~20 ml of benzene, then spin evaporated again. This was repeated several times to give a semisolid, greenish yellow oil in quantitative yield which moved as a single spot on tlc in EtOAc–petroleum ether (1:2) and gave a positive test for active halide.[14] The chloride was used without further purification.

1-(*m*-Hydroxybenzyl)uracil (29).—A solution of 1.37 g (4.4 mmol) of **34** and 16 ml of 30% anhydrous HBr–AcOH was stirred at ambient temperature for 19 hr. The solution was diluted with 100 ml of H_2O, then extracted with three 25-ml portions of $CHCl_3$, and spin evaporated *in vacuo*. The residue was dissolved in 50 ml of THF; the solution was filtered and spin evaporated. The residue was dissolved in a few milliliters of EtOAc and diluted with petroleum ether. The resultant solid was collected and recrystallized from EtOAc–petroleum ether; yield, 0.499 g (51%), mp 186–189°. Recrystallization of a portion from EtOAc–EtOH gave white rosettes, mp 188–191°. *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N.

5-Bromo-1-(*n*-butyl)uracil (41).—This compound was prepared from **4**[9] as previously described[2] for 6-benzylaminouracil; yield, 0.188 g (77%), mp 179–181°. The analytical sample was recrystallized from $CHCl_3$–petroleum ether to give transparent plates, mp 181–182°. *Anal.* ($C_8H_{11}BrN_2O_2$) C, H, N.

1-(*n*-Butyl)-5-nitrouracil (42).—To a stirred solution of 0.260 g (1.5 mmol) of **4** and 1 ml of concentrated H_2SO_4 was added 0.75 ml of red fuming HNO_3. After 0.5 hr at ambient temperature the reaction was quenched with ~10 g of crushed ice. The product was collected, washed with H_2O, and then recrystallized from H_2O; yield, 0.140 g (43%), mp 157–159°. The analytical sample had mp 158–159°. *Anal.* ($C_8H_{11}N_3O_4$) C, H, N.

1-(*n*-Butyl)-5-chlorosulfonyluracil (43).—A solution of 0.254 g (1.5 mmol) of **4** in 2 ml of $HOSO_2Cl$ was refluxed with stirring for 7 hr, cooled and then carefully added to about 40 g of crushed ice. The product was collected, washed with H_2O, then dried over P_2O_5. Recrystallization from $CHCl_3$ gave 0.198 g (49%) of white flakes, mp 176–184°, which was uniform on tlc with C_6H_6–EtOH (3:1). The analytical sample had mp 186–188° (if block preheated to 180°). *Anal.* ($C_8H_{11}ClN_2O_4S$) C, H, N.

1-(Butyl)-5-[*N*-(*n*-butyl)sulfamoyl]uracil (44).—To a stirred mixture of 81 mg (1.1 mmol) of *n*-$BuNH_2$, 110 mg (1.1 mmol) of Et_3N and 0.5 ml of DMF, cooled in an ice bath was added a solution of 0.267 g (1.0 mmol) of **43** in 0.5 ml of DMF. After 3 hr the resultant mixture was diluted with 5 ml of ice water and acidified to pH 1 with 1 *N* HCl. The product was collected, washed (H_2O), and then recrystallized from EtOH–H_2O; yield, 73 mg (24%) of soft white threads, mp 175–176°. *Anal.* ($C_{12}H_{21}N_3O_4S$) C, H, N.

1-(*n*-Butyl)uracil-5-sulfonanilide (45).—This compound was prepared by the same method as **44** using aniline; yield, 97 mg (32%) of yellow rosettes from *i*-PrOH, mp 192–196°. *Anal.* ($C_{14}H_{17}N_3O_4S$) C, H, N.

(14) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966).

Irreversible Enzyme Inhibitors. CLXXI.[1,2] Inhibition of FUDR Phosphorylase from Walker 256 Rat Tumor by 5-Substituted Uracils

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The inhibition of uridine phosphorylase from Walker 256 rat tumor, which can also cleave 5-fluoro-2′-deoxyuridine (FUDR) to FU, by 36 5-substituted uracils has been investigated. Strong hydrocarbon interaction with 5-benzyl (**9**) and 5-phenylbutyl (**12**) substituents was observed. Inhibition could be further enhanced by substitution of alkoxy groups on the *meta*-position of the benzyl moiety; the strongest inhibitors had *m*-CH_3O (**33**), *m*-C_2H_5O (**35**), or *m*-$C_6H_5CH_2O$ (**37**) groups and complexed to the enzyme 200–800 times more effectively than the substrate, FUDR.

There are two enzymes that start the detoxification of 5-fluoro-2′-deoxyuridine (FUDR) by cleavage to 5-fluorouracil (FU), namely, thymidine phosphorylase (EC 2.4.2.4)[3–5] and uridine phosphorylase (EC 2.4.-2.3).[4–6] Walker 256 rat tumor contains an FUDR cleaving enzyme that apparently is only uridine phosphorylase.[7] The latter can be inhibited by 6-aralkylaminouracils which could complex as much as 4-fold better than the substrate FUDR;[8] the aralkyl group apparently interacted with this uridine phosphorylase by hydrophobic bonding.[8] A much stronger hydrophobic interaction was observed with 1-aralkyl derivatives of uracil; the latter could complex as much as 180-fold better than the substrate, FUDR.[2] In the previous paper[2] we also observed that a hydrophobic

(1) This work was supported by Grant No. CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **13**, 458 (1970).

(3) B. R. Baker, *ibid.*, **10**, 297 (1967), paper LXXV of this series.

(4) B. Preussel, G. Etzold, D. Bärwolff, and P. Langen, *Biochem. Pharmacol.*, **18**, 2035 (1969) and references therein.

(5) P. Langen, G. Etzold, D. Bärwolff, and B. Preussel, *ibid.*, **16**, 1833 (1967).

(6) P. Langen and G. Etzold, *Biochem. Z.*, **339**, 190 (1963).

(7) (a) M. Zimmerman, *Biochem. Biophys. Res. Commun.*, **16**, 600 (1964); (b) see T. A. Krenitsky, J. W. Mellors, and R. K. Barclay, *J. Biol. Chem.*, **240**, 1281 (1965), for a more proper interpretation of Zimmerman's results.

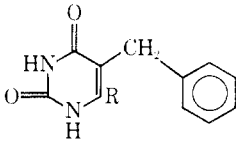
(8) B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **13**, 456 (1970), paper CLXIX of this series.

interaction could occur with two 5-substituted uracils. Therefore an extensive study has now been made with 5-substituted uracils and the results are the subject of this paper; the best inhibitor was complexed to this uridine phosphorylase from Walker 256 about 800 times better than FUDR.

Enzyme Results.—The effect of small 5 substituents was investigated first (Table I). 5-F (**2**) and 5-Br (**3**) enhanced the binding of uracil (**1**) by 4- and 12-fold, respectively; these results could be due to an increase of the acidity of uracil[9] and possible hydrophobic bonding by the Br atom.[9] Further enhancement of acidity[9] by introduction of a 5-NO_2 (**4**) increased binding 200-fold over uracil; that the nitro group of **4** did not enhance binding solely by its electron-withdrawing power was indicated by the poor binding (compared with **4**) by uracils substituted with the electronegative COOEt (**6**) and SO_2Cl (**7**) groups.

The inhibition of the enzyme by uracils substituted with 5-$C_6H_5(CH_2)_n$ groups, which were available from another study,[10] were then investigated. The in-plane 5-phenyl group of **8** gave no enhancement of binding. In contrast, the 5-benzyl (**9**) and 5-phenylbutyl (**12**) substituents gave an excellent 600- to 700-fold increment in binding; the intermediate sized phenethyl (**10**) and phenylpropyl (**11**) substituents were considerably less effective.

Insertion of 6-CF_3 (**13**) on uracil gave no change in binding; in contrast introduction of CF_3 group on **9** or **12** to give **14** and **15** resulted in a >100-fold loss in binding. These results cast light on the best conformation for binding of the 5 substituent of **9** and **12** to the enzyme. Only conformation **9a** with the



9a, R = H
14a, R = CF_3

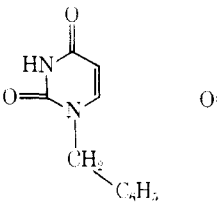
benzyl group being nearly coplanar with the uracil ring and pointing towards the 6 position (**9a**) would be hindered by the 6-CF_3 in conformation **14a**. Similar steric reasons can explain the poor binding of **16**.

A series of 5-$C_6H_5(CH_2)_nNH$ substituents were then investigated. The 5-C_6H_5NH group of **17** was 160-fold less effective than the 5-$C_6H_5CH_2$ group of **9**; similarly, the 5-$C_6H_5(CH_2)_3NH$ group of **20** was 50-fold less effective than the 5-$C_6H_5(CH_2)_4$ group of **12**. Little change in binding occurred with the other members of this series (**18**–**21**).

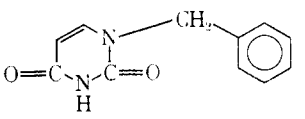
A similar loss in binding compared with **9** occurred with the 5-C_6H_5O group of **23**, although **23** was a 4-fold better inhibitor than **17**. However, the nonpolar C_6H_5S group of **24** was just as effective as the $C_6H_5CH_2$ group of **9**. The differences in binding among **9**, **17**, **23**, and **24** are best correlated by their relative hydrophobicity;[11] that is, the Hansch π constants[11] for CH_2, S, O, and NH are +0.5, +0.1, −0.5, and −1.2, respectively, which correlates with the order of their binding to the enzyme. The bond angles are not sufficiently different between C, S, O, and amine NH to account for these large differences.

It was previously shown[2] that a 1-CH_3 substituent (**25**) on uracil (**1**) did not change binding, but the 1-benzyl substituent of **26** gave a 20-fold increment in binding due to a hydrophobic interaction; therefore these two substituents were placed on 5-benzyluracil (**9**). As in the case of uracil, the 1-CH_3 substituent of **27** did not change the binding; in contrast, to **1** *vs.* **26** where a 20-fold increment was observed, **28** was only 2-fold more effective than **9**. Similarly, the 1-$C_6H_5O(CH_2)_3$ group (**29**) on uracil gave a 10-fold increment in binding, but no increment when attached to 5-benzyluracil (**9**) to give **30**. When the 1-$C_6H_5O(CH_2)_3$ group was inserted on **12**, an actual 14-fold loss in binding occurred with **31**. Nevertheless, further substitution on either benzyl group of **28** would be feasible for conversion into irreversible inhibitors.

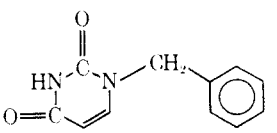
That hydrophobic bonding could be obtained with either 5- or 1-aralkyl groups, but not both, seemed incongruous at first. However a similar situation was encountered with hydrophobic bonding to dihydrofolate reductase with hydrophobically substituted pyrimidines;[12] in this case the data could be rationalized by rotation of the pyrimidine ring to give maximum hydrophobic interaction in only one area of the enzyme. Such an hypothesis could also rationalize the binding data obtained with **9**, **12**, and **25**–**31**. If 5-benzyluracil (**9**) binds in conformation **9a** relative to the enzyme,



26a



26b



26c

then **26** could be rotated from conformation **26a** to **26b** or **26c** in order to maximize binding; of these two latter conformations, **26c** would be preferred since the 2-oxo substituent of **26b** would interfere with the proper conformation necessary for the binding of the benzyl group to the enzyme (see **9a** *vs.* **14a** conformations).

The first study to support the concept that the 1-benzyl of **26** was complexed in conformation **26b** or **26c** to the same area occupied by the 5-benzyl of **9** was the effect of substitution on the benzyl moiety; the effects were essentially parallel. The order of increased binding of *meta* substituents[2] on **26** was H = 2,3-benzo (**38**) < *m*-CH_3O (**32**) < *m*-C_2H_5O(**34**) ≃ *m*-$C_6H_5CH_2O$ (**36**). The same order was followed for these *meta* substituents on 5-benzyluracil with the exception of one minor difference, that is H (**9**) = 2,3-benzo (**39**) < *m*-CH_3O (**33**) < *m*-C_2H_5O (**35**) < *m*-$C_6H_5CH_2O$ (**37**); **35** was not quite as potent as would be predicted from **34**.

(9) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 313 (1967), paper LXXIX of this series.

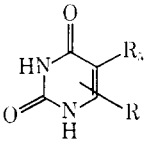
(10) B. R. Baker and J. L. Kelley, *ibid.*, **11**, 686 (1968), paper CXXXI of this series.

(11) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

(12) B. R. Baker and H. S. Shapiro, *J. Pharm. Sci.*, **55**, 308 (1966).

TABLE I

INHIBITION[a] OF WALKER 256 FUDR PHOSPHORYLASE[b] BY



No.	R_5	R	I_{50}, μM
1	H	H	2900[c,d]
2	F	H	770[d]
3	Br	H	250[d]
4	NO_2	H	15[d]
5	NH_2	H	>6000[d,e]
6	COOEt	H	1500[f]
7[g]	SO_2Cl	H	4800[h]
8[i]	C_6H_5	H	>2000[e]
9[i]	$C_6H_5CH_2$	H	5.3
10[i]	$C_6H_5(CH_2)_2$	H	140
11[i]	$C_6H_5(CH_2)_3$	H	49
12[i]	$C_6H_5(CH_2)_4$	H	4.0
13[j]	H	6-CF_3	3300
14[j]	$C_6H_5CH_2$	6-CF_3	660
15[j]	$C_6H_5(CH_2)_4$	6-CF_3	>400[e]
16	$C_6H_5CH_2$	6-C_6H_5	>800[e]
17[k]	C_6H_5NH	H	800
18	$C_6H_5CH_2NH$	H	270
19	$C_6H_5(CH_2)_2NH$	H	540
20	$C_6H_5(CH_2)_3NH$	H	~200[f]
21	$C_6H_5(CH_2)_4NH$	H	~400[f]
22	$C_6H_5CH_2OCH_2$	H	150
23[l]	C_6H_5O	H	190
24[m]	C_6H_5S	H	5.2
25	H	1-CH_3	3600[h]
26	H	1-$C_6H_5CH_2$	150[h]
27	$C_6H_5CH_2$	1-CH_3	6.2
28	$C_6H_5CH_2$	1-$C_6H_5CH_2$	2.6
29	H	1-$C_6H_5O(CH_2)_3$	300[h]
30[j]	$C_6H_5CH_2$	1-$C_6H_5O(CH_2)_3$	5.7
31[i]	$C_6H_5(CH_2)_4$	1-$C_6H_5O(CH_2)_3$	55
32	H	1-(*m*-$CH_3OC_6H_4CH_2$)	18[h]
33	*m*-$CH_3OC_6H_4CH_2$	H	1.9
34	H	1-(*m*-$C_2H_5OC_6H_4CH_2$)	7.7[h]
35	*m*-$C_2H_5OC_6H_4CH_2$	H	1.4
36	H	1-(*m*-$C_6H_5CH_2OC_6H_4CH_2$)	11[h]
37	*m*-$C_6H_5CH_2OC_6H_4CH_2$	H	0.50
38	H	1-(α-$C_{10}H_7CH_2$)	120[h]
39	α-$C_{10}H_7CH_2$	H	7.0
40[i]	$C_6H_5CH_2$	2-thio	200
41	$C_6H_5CH_2$	4-thio	9.0
42	H	1-$C_6H_5CH_2$-4-thio	>800[f]
43	H	1-(*m*-$C_2H_5OC_6H_4CH_2$)-4-thio	97
44[n]	H	3-CH_3	>9000[f]
45	*m*-$C_2H_5OC_6H_4CH_2$	3-CH_3	290
46	$C_6H_5CH_2$	1,3-$(CH_3)_2$	>1000[e]
47[o]	NO_2	1-CH_3	>4000[e]
48[o]	NO_2	3-CH_3	4000[f]

[a] The technical assistance of Maureen Baker, Julie Leseman, and Janet Wood is acknowledged. [b] I_{50} = concentration for 50% inhibition when assayed with 400 μM FUDR in pH 5.9 arsenate–succinate buffer containing 10% DMSO as previously described.[3,8] [c] Data from ref 8. [d] Dissolved in H_2O containing 1 equiv of KOH for assay. [e] No inhibition at the maximum solubility which is one-fourth of the concentration indicated. [f] Estimated from the inhibition observed at the maximum solubility or maximum concentration allowing light transmission, whichever is lower. [g] Synthesis: R. R. Herr, T. Enkoji, and T. J. Bardos, *J. Amer. Chem. Soc.*, **78,** 401 (1956). [h] Data from ref 2. [i] Synthesis: B. R. Baker and J. L. Kelley, *J. Med. Chem.*, 11, 686 (1968), paper CXXXI of this series. [j] Synthesis: B. R. Baker, M. Kawazu, and J. D. McClure, *J. Pharm. Sci.*, **56,** 1081 (1967). [k] Synthesis: F. R. Gerns, A. Perrotta, and G. H. Hitchings, *J. Med. Chem.*, **9,** 108 (1966). [l] Synthesis: C. N. Chang, S. A. Yang, T. T. Wang, and Y. Hu, *Yao Hsueh Hsueh Poa*, **10,** 600 (1963); *Chem. Abstr.*, **60,** 14504 (1964). [m] Synthesis: B. Roth and J. F. Bunnett, *J. Amer. Chem. Soc.*, **87,** 340 (1965). [n] Synthesis: C. W. Whitehead, *ibid.*, **74,** 4267 (1952). [o] Synthesis: D. J. Brown, E. Hoerger and S. F. Mason, *J. Chem. Soc.*, 211 (1955).

A second line of evidence to support the rotomer concept that **26** did not bind in conformation **26a**, but in conformation **26b** or **26c** was investigated. If only one of the two oxo groups on 5-benzyluracil (**9**) were necessary for binding, then it should be possible to eliminate binding conformation **26a** for **26**. That only the 2-oxo

group was necessary for binding is shown by comparison of **9** with its 2-thio (**40**) and 4-thio (**41**) derivatives. Less than a 2-fold loss in binding occurred with **41** compared with **9**, showing that the 4-oxo group was not complexed as an electron donor;[13] in contrast, a 38-fold loss in binding occurred when the 2-oxo group of **9** was replaced by 2-thio (**40**) indicating that the 2-oxo group was complexed as an electron donor to the enzyme.

If 1-benzyluracil (**26**) were complexed as rotomer **26a**, then no loss in binding should occur if its 4-oxo group were replaced by 4-thio; in contrast if **26** were complexed as rotomer **26b** or **26c**, then the 4-thio group would have to complex to the same region where the 2-thio and 2-oxo group of 5-benzyluracil are complexed with resultant loss in binding. Unfortunately, 1-benzyl-4-thiouracil (**42**) had considerable light absorption at the 300-mμ wavelength used for the assay; even so, it appeared that **42** was at least 5-fold less effective than its oxo counterpart, **26**. In order to avoid this light transmission problem, the more potent 1-(*m*-ethoxybenzyl)uracil (**34**) was converted into its 4-thio derivative (**43**); a 13-fold loss in binding occurred, indicating that the 4-oxo group of **34** was complexed to the enzyme and supporting the concept that **26** complexed as rotomer **26b** or **26c**.

As stated earlier, rotomer **26c** is preferred over **26b** since the 2-oxo substituent of **26b** would interfere with the benzyl group assuming its preferred conformation for binding (see **9a** *vs.* **14a** conformations).

That the 1-H of uracil (**1**) and 5-benzyluracil (**9**) was not complexed to the enzyme was shown by no loss of binding when converted into their 1-CH_3 derivatives, **25** and **27**. The question then arose as to whether or not the 3-H was complexed to the enzyme; although 3-methyluracil (**44**) was at least 3-fold less effective than uracil (**1**), the lack of sufficient light transmission thwarted the determination of an absolute number. Therefore, the 3-Me derivative **45** of 5-(*m*-ethoxybenzyl)uracil (**37**) was synthesized and evaluated; a 200-fold loss in binding occurred with **45**. This result indicates that the 3-H of a 5-substituted benzyluracil is complexed to the enzyme; however, this interpretation is equivocal, since the known binding of the 2-oxo group may be sterically hindered by an adjacent 3-Me. That the potent 5-nitrouracil (**4**) binds differently than 5-benzyluracil is indicated by the large loss in binding if either a 1-Me (**47**) or 3-Me (**48**) group is introduced.

Binding to this uridine phosphorylase from Walker 256 by substituted 5-benzyluracils occurs *via* (a) the 2-oxo group as an electron donor, (b) probably the 3-H as an electron acceptor, (c) the substituted 5-benzyl group by hydrophobic bonding; no binding occurs with the 1-H or 4-oxo groups. It is possible that C-5 of the uracil is also complexed by hydrophobic bonding; this would explain why 5-benzyluracils with substituents on the benzyl group complex 5- to 20-fold better in conformation **9a** than the corresponding 1-benzyluracils complex in conformation **26c** where hydrophobic bonding by C-5 of uracil is lost and the polar N-1-group of **26c** is repulsed from this hydrophobic region.

Rat liver has been shown unequivocally to contain both uridine phosphorylase and thymidine phosphorylase, since the two enzymes can be separated by chromatography on DEAE-Sephadex.[14] Langen, *et al.*[6] also showed that rat liver contained both thymidine phosphorylase and uridine phosphorylase, but that only the latter could be inhibited by 1-(2′-deoxy-β-D-glucopyranosyl)thymine (TDG); only a maximum of 50% inhibition of a rat liver extract could be achieved with deoxyuridine as a substrate. No inhibition of thymidine phosphorylase from horse liver, which is free of uridine phosphorylase, was observed with TDG. We have obtained results with a 0–45% ammonium sulfate fraction of rat liver with 5-benzyluracil (**9**) that are similar to the results Langen[6] obtained with TDG. When our enzyme preparation was stored at −15° in small aliquots in Tris buffer (pH 7.4), both the velocity and the inhibition by **9** decreased with time. After 2 months at −15°, the velocity was about half and no inhibition by **9** was seen at 500 μ*M*; these results indicate that the uridine phosphorylase is less stable than the thymidine phosphorylase of rat liver. This instability of uridine phosphorylase may account for why Zimmerman reported that only thymidine phosphorylase was present in rat liver.[7] We have found that the uridine phosphorylase (according to Zimmerman[7]) in Walker 256 rat tumor is stable at −15° as a 0–45% $(NH_4)_2SO_4$ fraction for many months and can be completely inhibited by high concentrations of 5-benzyluracil (**9**).

Rat tumors such as Walker 256,[7a] Dunning leukemia,[7a] Novikoff hepatoma,[7a] a butter-yellow induced hepatoma,[7b] and a methylcholanthrene-induced sarcoma[7a] have only uridine phosphorylase and no thymidine phosphorylase.[7] Since rat liver contains both enzymes, increased efficacy of FUDR treatment of these rat tumors should be seen in the presence of uridine phosphorylase inhibitors such as **9**, **33**, **35**, or **37**; the tumor will then not be able to detoxify FUDR by initial cleavage to FU, whereas the thymidine phosphorylase of rat liver could continue to cleave FUDR. However, even if such an experiment were successful, it is highly questionable whether or not it could be carried over to human tumors. Of 14 human tumors examined,[7,15] all contained thymidine phosphorylase and possibly unknown amounts of uridine phosphorylase. Any tumor containing thymidine phosphorylase could still cleave FUDR since this enzyme is probably not inhibited by the more potent compounds in Table I; however, any human tumor containing only uridine phosphorylase should respond like Walker 256. Since *E. coli* B thymidine phosphorylase is highly susceptible to inhibition by substituted uracils with hydrophobic substituents at the 6 position,[16] it is highly probable that mammalian thymidine phosphorylase also has a hydrophobic bonding region near the active-site; such studies on a mammalian thymidine phosphorylase are being pursued.

In summary, substituted 5-benzyluracils such as **33**, **35**, and **37** are potent reversible inhibitors of Walker 256 uridine phosphorylase (EC 2.4.2.3) that complex to the enzyme 200–800 times more effectively than the sub-

(13) See B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley & Sons, Inc., New York, N. Y., 1967, pp 75, 92 and 105.

(14) E. W. Yamada, *J. Biol. Chem.*, **243**, 1649 (1968).

(15) M. Zimmerman and J. Seidenberg, *ibid.*, **239**, 2618 (1964).

(16) (a) B. R. Baker and W. Rzeszotarski, *J. Med. Chem.*, **11**, 636 (1968), paper CXXI of this series; (b) B. R. Baker and S. E. Hopkins, *ibid.*, **13**, 87 (1970), paper CLXVII of this series.

strate, FUDR. Similar studies are needed on mammalian thymidine phosphorylase (EC 2.4.2.4).

Chemistry.—Nucleophilic substitution on 5-halouracils has been shown to be a useful method for introduction of alkylamino,[17] anilino,[18] or phenylthio[19] moieties into the uracil nucleus. We have similarly found that treatment of 5-bromouracil with ~10 equiv of the appropriate aralkylamine at 100° readily affords the desired **18–21** in high yield. Aniline does not react under these conditions;[17] however, **17** was prepared in this way at 200° in refluxing ethylene glycol according to Gerns, *et al.*[18]

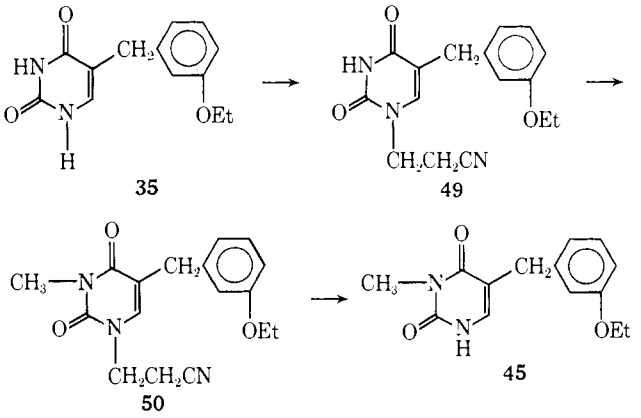
Although **24** has been synthesized from 5-chlorouracil and thiophenol by Roth and Bunnett,[19c] efforts to effect a similar displacement on 5-bromouracil with phenol under a variety of experimental conditions were unsuccessful. The desired **23** was obtained *via* the principle pyrimidine synthesis from ethyl phenoxyacetate.[20]

The 5-benzyl-2-thiouracils (**33a**, **35a**, **37a**, **39a**) were synthesized using a modification of the general literature procedure.[21] The starting hydrocinnamic esters were in turn prepared according to literature procedures or modifications thereof. Hydrolysis of the 2-thiouracils with aqueous ClAcOH[10] afforded the desired uracils (**33**, **35**, **37**, **39**) in excellent yield.

Although alkylation of uracil[2,22] and of 5-substituted uracils[10,22b] readily affords 1-alkylated products, a 3-fold excess of the uracil is required to avoid 1,3-disubstitution; moreover the reaction is best suited to large preparations. Consequently, the recently developed trimethylsilylation method of nucleoside synthesis[23] has been extended to the preparation of 5-substituted 1-methyl[24] and 1-alkyluracils.[25] This procedure allows use of an excess of the halogen yet gives only 1-alkylation. The procedure of Sakai, *et al.*[24a] was applied to 5-benzyluracil (**9**) and readily afforded **27** in excellent yield. This reaction has also been used with substituted benzyl bromides with similar results.[26]

Early efforts in this laboratory[22b] towards selective blockage of the N-3 position of uracil, possibly allowing selective alkylation at N-1, failed in development of a useful blocking group. More recently, Novacek and Lissmerova[27] have shown that acrylonitrile can be utilized to block selectively the N-1,[27a] N-3,[27b] or both[27a] positions of uracil. The cyanoethyl moiety can be easily removed with alcoholic NaOEt after the desired modification of the molecule.

Scheme I



The synthesis of 3-methyl-5-(*m*-ethoxybenzyl)uracil (**45**) (Scheme I) was accomplished by this method. When **35** was treated with an excess of acrylonitrile in Et_3N and H_2O in the presence of 1 equiv of NaOH, the 1-substituted uracil (**49**) (Scheme I) was formed in good yield. Alkylation of the anion of **49** with MeI gave **50** which was then treated with an equivalent of NaOEt in refluxing EtOH to afford **45** in 78% yield.

The 4-thiouracils (**41–43**) were available from **9**, **26**, and **34** using the previously described[28] P_2S_5 method for selective thiation of substituted uracils. The reaction gave quite variable results; in one instance retreatment with P_2S_5 was required before the reaction could be driven to completion.

Experimental Section[29]

5-Benzylaminouracil (18) (Method A).—A mixture of 0.953 g (5.0 mmol) of 5-bromouracil and 6.0 ml (55 mmol) of benzylamine was heated on a steam bath for 2 hr. The cooled mixture was diluted with 10 ml of H_2O, and the pH was adjusted to 5–6 with 12 *N* HCl. The product was collected, washed with H_2O, and recrystallized from MeOEtOH; yield, 0.628 g (58%), mp 279–284° dec. An additional recrystallization gave the analytical sample as white flakes of unchanged melting point. See Table II for additional data and other compounds prepared by this method.

5-(α-Naphthyl)-2-thiouracil (39a) (Method D).—To a dispersion of 0.802 g (20 mmol) of NaH (59.8% in mineral oil) in 25 ml of THF was added a solution of 4.57 g (20 mmol) of ethyl 3-(α-naphthyl) propionate[30] and 1.63 g (22 mmol) of ethyl formate in 25 ml of THF. The mixture was stirred at ambient temperature for 22 hr and then spin evaporated *in vacuo*.

The brown viscous residue was dispersed in 50 ml of absolute EtOH, treated with 1.52 g (20 mmol) of thiourea, and then refluxed with stirring for 6 hr. The cooled solution was spin evaporated *in vacuo*, and the residue was dissolved in 150 ml of H_2O. This solution was washed with six 50-ml portions of $CHCl_3$. The aqueous solution was heated to about 90° on a steam bath, cooled, and then clarified by filtration. Acidification to pH 5–6 with AcOH gave a solid which was recrystallized from a minimum of EtOH; yield, 0.701 g (13%), mp 266–268° dec. An additional recrystallization afforded the analytical sample as white threads of unchanged melting point. See Table II for additional data and other compounds prepared by this method.

(17) A. P. Phillips, *J. Amer. Chem. Soc.*, **73**, 1061 (1951).

(18) F. R. Gerns, A. Perrotta, and G. H. Hitchings, *J. Med. Chem.*, **9**, 108 (1966).

(19) (a) B. Roth and G. H. Hitchings, *J. Org. Chem.*, **26**, 2770 (1961); (b) B. Roth and L. A. Schloemer, *ibid.*, **28**, 2659 (1963); (c) B. Roth and J. F. Bunnett, *J. Amer. Chem. Soc.*, **87**, 340 (1965).

(20) C. N. Chang, S. A. Yang, T. T. Wang, and Y. Hu, *Yao Hsueh Hsueh Poa*, **10**, 600 (1963); *Chem. Abstr.*, **60**, 14504 (1964).

(21) T. B. Johnson and J. C. Ambelang, *J. Amer. Chem. Soc.*, **60**, 2941 (1938).

(22) (a) B. R. Baker and G. B. Chheda, *J. Pharm. Sci.*, **54**, 25 (1965); (b) B. R. Baker, M. Kawazu, D. V. Santi, and T. J. Schwan, *J. Med. Chem.*, **10**, 304 (1967).

(23) T. Nishimura and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 357 (1964).

(24) (a) T. T. Sakai, A. L. Pogolotti, Jr., and D. V. Santi, *J. Heterocycl. Chem.*, **5**, 849 (1968); (b) E. Wittenburg, *Chem. Ber.*, **99**, 2380 (1966).

(25) D. T. Browne, J. Eisinger, and N. J. Leonard, *J. Amer. Chem. Soc.*, **90**, 7302 (1969).

(26) Unpublished experiments.

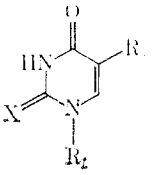
(27) (a) A. Novacek and M. Lissnerova, *Collect. Czech. Chem. Commun.*, **33**, 604 (1968); (b) *ibid.*, **33**, 1003 (1968).

(28) (a) B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **12**, 1039 (1969); (b) J. J. Fox, *et al.*, *J. Amer. Chem. Soc.*, **81**, 178 (1959).

(29) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had ir and uv spectra compatible with their assigned structures and each moved as a single spot on tlc on Brinkman silica gel GF. The analytical samples gave combustion values for C, H, and N within 0.3% of theory.

(30) (a) For the starting hydrocinnamate see S. I. Sergievskaya and A. S. Elina, *J. Gen. Chem. U. S. S. R.*, **13**, 864 (1943); *Chem. Abstr.*, **39**, 926 (1945); (b) the necessary cinnamate was prepared according to S. I. Sergievskaya, K. V. Levshina, and E. N. Petrova, *Zh. Obshch. Khim.*, **20**, 1478 (1950); *Chem. Abstr.*, **45**, 2458 (1951).

TABLE II
PHYSICAL PROPERTIES OF



No.	X	R_1	R_2	Method[a]	% yield	Mp, °C	Formula[b]
18	O	H	$C_6H_5CH_2NH$	A	58[c]	279–284 dec	$C_{11}H_{11}N_3O_2$
19	O	H	$C_6H_5(CH_2)_2NH$	A	56[c]	270–277 dec	$C_{12}H_{13}N_3O_2$
20	O	H	$C_6H_5(CH_2)_3NH$	A	54[c]	255–258 dec	$C_{13}H_{15}N_3O_2$
21	O	H	$C_6H_5(CH_2)_4NH$	A	63[c]	250–252 dec	$C_{14}H_{17}N_3O_2$
28	O	$C_6H_5CH_2$	$C_6H_5CH_2$	B[d]	49[e]	176–177	$C_{18}H_{16}N_2O_2$
33	O	H	*m*-$CH_3OC_6H_4CH_2$	C	96	243–245[e]	$C_{12}H_{12}N_2O_3$
33a	S	H	*m*-$CH_3OC_6H_4CH_2$	D[f]	24	192–194[g]	$C_{12}H_{12}N_2O_2S$
35	O	H	*m*-$C_2H_5OC_6H_4CH_2$	C	97	245–246[e]	$C_{13}H_{14}N_2O_3$
35a	S	H	*m*-$C_2H_5OC_6H_4CH_2$	D[h]	32[g]	206–208	$C_{13}H_{14}N_2O_2S$
37	O	H	*m*-$C_6H_5CH_2OC_6H_4CH_2$	C	83	266–268[e]	$C_{18}H_{16}N_2O_3$
37a	S	H	*m*-$C_6H_5CH_2OC_6H_4CH_2$	D[e]	15	169–170[g]	$C_{18}H_{16}N_2O_2S$
39	O	H	β-$C_{10}H_7CH_2$	C	86[f]	316–318	$C_{15}H_{12}N_2O_2$
39a	S	H	β-$C_{10}H_7CH_2$	D[e]	13[f]	266–268	$C_{15}H_{12}N_2OS$
41	O	H	$C_6H_5CH_2$(4-thiol)	E[m]	45[n]	265–279[e,o] dec	$C_{11}H_{10}N_2OS$
42	O	$C_6H_5CH_2$	(4-thiol)	E	92	112–114[g]	$C_{11}H_{10}N_2OS$
43	O	*m*-$C_2H_5OC_6H_4CH_2$	(4-thiol)	E[m]	41	118–119[e]	$C_{13}H_{14}N_2O_2S$

[a] B: see method D in ref 10 except that the reaction mixture was stirred at ambient temperature overnight; C: see method B in ref 10 except reaction was refluxed about 18 hr. [b] All compounds were analyzed for C, H, and N. [c] Recrystallized from MeOEtOH. [d] Product leached from starting material with hot $CHCl_3$. [e] Recrystallized from EtOAc. [f] For the starting hydrocinnamate see R. Robinson and J. Walker, *J. Chem. Soc.*, 192 (1936) except that Raney Ni was used as the hydrogenation catalyst. [g] Recrystallized from *i*-PrOH. [h] For the starting hydrocinnamate see D. A. Peak, R. Robinson, and J. Walker, *J. Chem. Soc.*, 725 (1936), except that Raney Ni was used as the hydrogenation catalyst. [i] For the starting hydrocinnamate see the Experimental Section. [j] Recrystallized from EtOH–MeOEtOH. [k] For the starting hydrocinnamate and cinnamate esters see ref 30. [l] Recrystallized from EtOH. [m] The product precipitated directly from the aqueous mixture. [n] Recrystallized from EtOH–H_2O. [o] Decomposition range varied with rate of heating. [p] A second charge of P_2S_5 was required since reaction was incomplete after 4 hr.

1-Benzyl-4-thiouracil (42)(Method E).—A mixture of 2.00 g (9.1 mmol) of **26**, 5 g of P_2S_5, 50 ml of pyridine, and several drops of H_2O was refluxed with stirring for 5 hr. The reaction mixture was cooled, poured into 500 ml of H_2O, and stirred for several hours. The oily mixture was extracted with three 50-ml portions of $CHCl_3$. The combined extracts were washed with five 200-ml portions of H_2O, dried ($MgSO_4$), and spin evaporated *in vacuo*. The dark syrup was then repeatedly dissolved in EtOH and spin evaporated to remove the last traces of pyridine. The resultant oil was dissolved in 50 ml of hot *i*-PrOH (charcoal), filtered through Celite and spin evaporated *in vacuo* to give 1.99 g (92%) of yellow crystals, mp 110–112°. Several recrystallizations from *i*-PrOH gave the analytical sample as yellow needles, mp 112–114°. See Table II for additional data and other compounds prepared by this method.

Ethyl *m*-Benzyloxyhydrocinnamate.—Ethyl *m*-hydroxycinnamate[31] was hydrogenated over Raney Ni using the general procedure of ref 30a. The homogeneous syrup was in turn alkylated with benzyl chloride as in Method C of ref 2 to give a homogeneous oil which was used without further purification.

5-Benzyloxymethyluracil (22).—A mixture of 0.802 g (5.0 mmol) of 5-chloromethyluracil[32] and 8 ml of benzyl alcohol was refluxed with stirring for 0.5 hr during which time dissolution occurred. The solution was cooled, and the white precipitate was collected and washed with $CHCl_3$; yield, 0.772 g (67%), mp 212–213°. Recrystallization from H_2O gave white granules, mp 208–209° (lit. mp 189–190°,[33a] 211–213°,[33b] and 202–203°[33c] from an alternate method).

5-Benzyl-1-methyluracil (27).—A mixture of 1.00 g (5.0 mmol) of **9**, 10 ml of hexamethyldisilazane, and ~0.2 ml of chlorotrimethylsilane protected from moisture was refluxed with stirring for 6.5 hr during which time dissolution occurred. The cooled solution was treated with 14 ml of MeI and refluxed for an additional 16 hr. The reaction mixture was spin evaporated *in vacuo*, and the residue was triturated with 5 ml of 50% aqueous AcOH. The resultant mixture was diluted with 10 ml of H_2O, then the product collected and washed (H_2O); yield, 0.984 g (92%), mp 151–153°. Recrystallization from *i*-PrOH gave 0.780 g (72%) of white flakes, mp 185–186°. *Anal.* ($C_{12}H_{12}N_2O_2$) C, H, N.

5-Benzyl-1,3-dimethyluracil (46).—A cooled and filtered solution of 3.36 g (17 mmol) of **9** in 100 ml of 1 *M* NaOH was treated with 5 ml of Me_2SO_4 and then heated on a steam bath for 1 hr. The cooled mixture was extracted with two 25-ml portions of $CHCl_3$. The combined extracts were washed with two 25-ml portions of 0.5 *N* NaOH, dried ($MgSO_4$), and then spin evaporated *in vacuo*. The residual oil was dissolved in ~30 ml of Et_2O, diluted with petroleum ether (bp 60–110°) and spin evaporated *in vacuo*, finally at ~90° to give a semisolid; trituration with petroleum ether gave 1.81 g (47%) of white granules, mp 66–68°. Recrystallization from petroleum ether–THF gave the analytical sample as white rosettes, mp 68–69°. *Anal.* ($C_{13}H_{14}N_2O_2$) C, H, N.

1-(β-Cyanoethyl)-5-(*m*-ethoxybenzyl)uracil (49).—To a partial solution of 2.46 g (10 mmol) of **35**, 0.40 g (10 mmol) of NaOH, 12 ml of Et_3N, and 40 ml of H_2O at ~70° was added 1.40 g (26 mmol) of acrylonitrile. After 4.5 hr at ~70° the stirred solution was treated with charcoal and filtered hot. Acidification with AcOH with ice bath cooling gave a white solid which was recrystallized from EtOH; yield, 1.86 g (63%), mp 167–174°. The analytical sample was obtained by recrystallization from EtOH as white needles, mp 176–177°. *Anal.* ($C_{16}H_{17}N_3O_3$) C, H, N.

5-(*m*-Ethoxybenzyl)-3-methyluracil (45).—To 3.5 mmol of NaOEt [formed from 0.143 g of NaH (57% in mineral oil) and 10 ml of absolute EtOH] in EtOH was added 1.00 g (3.3 mmol) of **49**. To the stirred solution was added 2 ml of MeI which was left at ambient temperature for 29 hr, then spin evaporated *in vacuo*. The residue was dissolved in 50 ml of $CHCl_3$, washed

(31) F. H. Stodola, *J. Org. Chem.*, **29**, 2490 (1964).

(32) W. A. Skinner, M. G. M. Schelstraete, and B. R. Baker, *ibid.*, **25**, 149 (1960).

(33) (a) J. Farkas and F. Sorm, *Collect. Czech. Chem. Commun.*, **28**, 1620 (1963); (b) M. Prystas and F. Sorm, *ibid.*, **31**, 1053 (1966); (c) R. Brossmer and E. Rohm, *Angew. Chem.*, **76**, 50 (1964).

with three 10-ml portions of 0.5 *N* NaOH, once with 10 ml of brine, and then dried ($MgSO_4$). Spin evaporation *in vacuo* left a syrup which showed no uv change from pH 1 to 13.

This syrup was treated with 3.5 mmol of NaOEt (formed as described above) in 25 ml of absolute EtOH and refluxed with stirring for 4 hr. The cooled solution was acidified to pH 5 with AcOH, then spin evaporated *in vacuo*. The residue was triturated with several milliliters of *i*-PrOH, cooled, the product collected and then washed with *i*-PrOH; yield, 0.680 g (78%), mp 146–149°. Recrystallization from *i*-PrOH-EtOH gave clones of white pins, mp 153–154°. *Anal.* ($C_{14}H_{16}N_2O_3$) C, H, N.

Irreversible Enzyme Inhibitors. CLXXII.[1,2] Proteolytic Enzymes. XVI.[3] Covalent Bonding of the Sulfonyl Fluoride Group to Serine Outside the Active Site of α-Chymotrypsin by *exo*-Type Active-Site Directed Irreversible Inhibitors

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Three active-site directed irreversible inhibitors with a terminal SO_2F group (1–**3**) of α-chymotrypsin have been shown to covalently link a serine OH group by two independent methods. The sulfonate group on α-chymotrypsin in each case was displaced by mercaptoethylamine and resultant *S*-aminoethyl-L-cysteine identified after hydrolysis; the second method converted each sulfonate of serine into pyruvic acid which was identified enzymatically and by conversion into its 2,4-dinitrophenylhydrazone. Reaction of **2** with α-chymotrypsin was unequivocally shown not to occur on the active-site serine-195, but a serine outside the site tentatively identified as serine-223; these results establish the hypothesis that properly designed active-site directed irreversible inhibitors can covalently link an amino acid residue outside the active site by the *exo* mechanism.

Active-site directed irreversible inhibitors of enzymes operate by two steps.[5] A reversible complex is first formed between the enzyme and inhibitor; if a proper leaving group on the inhibitor is juxtaposed to a nucleophilic group on the enzyme, then a rapid and selective neighboring group reaction occurs with formation of a covalent bond that inactivates the enzyme.[5] There are two classes of active-site directed irreversible inhibtors, the *endo* type that forms a covalent bond inside the active-site and the *exo* type that forms a covalent bond outside the active-site.[5]

The *endo* type of irreversible inhibitor is of interest in protein structure studies for "labeling" amino acids inside the active-site; a now classical example is 1-chloro-4-phenyl-3-tosylamido-2-butanone (TPCK)[6] which specifically forms a covalent bond with histidine-57 in the active site of α-chymotrypsin.[7]

The *exo* type of irreversible inhibitor has a considerably wider utility in drug design than the *endo* type.[8] The best leaving group yet found[9] for the *exo* type of covalent bond is the F of the SO_2F moiety; such a moiety has the electrophilic capacity to react rapidly with a serine OH of an enzyme,[10] but direct chemical proof of covalent bond formation with a serine outside the active site had yet to be achieved. The SO_2F moiety properly positioned on an appropriate reversible inhibitor could inactivate a variety of enzymes, presumably by the *exo* type of active-site directed irreversible inhibition. Examples are dihydrofolic reductase,[9] xanthine oxidase,[11] guanine deaminase,[12] trypsin,[13] α-chymotrypsin,[14–16] the C'1a component of complement,[17] and cytosine nucleoside deaminase.[18] With dihydrofolic reductase, SO_2F-type inhibitors have been found that can inactivate an L1210 mouse leukemia enzyme with no appreciable inactivation of the enzyme in normal liver, spleen, and intestine of the mouse.[19]

Three successive questions can be asked about an active-site directed irreversible enzyme inhibitor of the SO_2F type that presumably operates by the *exo* mechanism. (1) Does the SO_2F form a covalent bond with a serine? (2) If the enzyme has a serine in the active-site, as in the case of α-chymotrypsin, has the covalent bond formed with the active-site serine (*endo*) or has the covalent bond been formed with a serine outside the active site (*exo*)? (3) If the covalently

(1) This work was supported in part by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series see B. R. Baker, and J. L. Kelley, *J. Med. Chem.*, **13**, 461 (1970).

(3) For the previous paper on proteolytic enzymes see B. R. Baker and M. Cory, *ibid.*, **12**, 1053 (1969).

(4) Visiting research professor from the Department of Biology, Atomic Energy Commission, France, 1968–1969.

(5) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley & Sons, New York, N. Y., 1967, pp 17–21.

(6) G. Schoellmann and E. Shaw, *Biochemistry*, **2**, 252 (1963).

(7) E. B. Ong, E. Shaw, and G. Schoellmann, *J. Biol. Chem.*, **240**, 694 (1965).

(8) B. R. Baker, *Biochem. Pharmacol.*, **11**, 1155 (1962).

(9) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series.

(10) For discussion of the chemistry of the SO_2F group, see ref 9.

(11) (a) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **11**, 650 (1968), paper CXXIII of this series; (b) B. R. Baker and J. A. Kozma, *ibid.*, **11**, 652 (1968), paper CXXIV of this series; (c) B. R. Baker and J. A. Kozma, *ibid.*, **11**, 656 (1968), paper CXXV of this series; (d) B. R. Baker and W. F Wood, *ibid.*, **12**, 211 (1969), paper CXLVI of this series.

(12) B. R. Baker and W. F. Wood, *ibid.*, **12**, 214 (1969), paper CXLVII of this series.

(13) (a) B. R. Baker and E. H. Erickson, *ibid.*, **11**, 245 (1968), paper CXV of this series; (b) B. R. Baker and E. H. Erickson, *ibid.*, **12**, 112 (1969), paper CXLIV of this series.

(14) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 233 (1968), paper CXIII of this series.

(15) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 118 (1969), paper CXLV of this series.

(16) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 221 (1969), paper CL of this series.

(17) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 902 (1969), paper CLXI of this series.

(18) B. R. Baker and J. L. Kelley, *ibid.*, **12**, 1046 (1969), paper CLXIII of this series.

(19) (a) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII of this series; (b) for a recent review see B. R. Baker, *Accounts Chem. Res.*, **2**, 129 (1969).