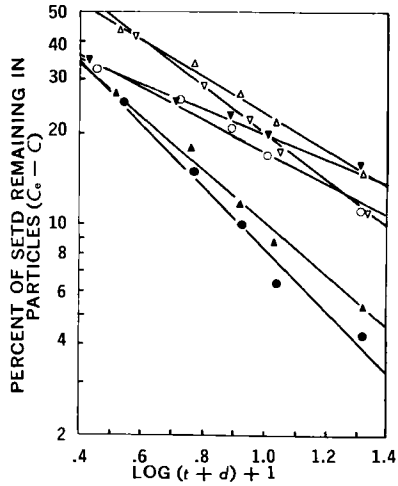

Fig. 7—Experimental data for percent SETD released from SETD-cetyl alcohol particles in alkaline pancreatin medium, plotted on lines predicted from dissolution model. Key: ●, $N_8S_0W_5$; Δ, $N_8S_1W_5$; ○, $N_8S_5W_5$; ▼, $N_{10}S_0W_5$; ▲, $N_{10}S_1W_5$; ▽, $N_{10}S_5W_5$.

particles. This would be the case particularly for those products that were somewhat tacky. Another factor is that many of the spray-congealed SETD-wax products were not easily wetted by the dissolution medium, at least initially. Some of the particles tended to agglomerate upon coming in contact with the dissolution medium, particularly in acid pepsin medium. As the dissolution process continued, these agglomerates were subsequently emulsified, disintegrated, or solubilized as were the individual particles.

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Keyphrases

Prolonged release dosage forms—spray congealed
Sulfaethylthiadiazole–wax formulations
Spray-congealed products—*in vitro* dissolution model
Particle size—wax, nozzle size effect
Dissolution rates—parameters affecting

Tumor-Inhibitory Activity of Pyrrolizidine Alkaloids

By C. C. J. CULVENOR

Eighteen pyrrolizidine alkaloids and several derivatives have been examined for tumor-inhibitory properties. Of these, 10 compounds show significant activity against one or more test tumors. Heliotrine, lasiocarpine, monocrotaline, spectabiline, and senecionine are highly active against the Walker 256 (intramuscular) system. The activity pattern is consistent with the tumor-inhibitory action being associated with the allylic ester function which imparts alkylating ability and is also responsible for hepatotoxicity.

PYRROLIZIDINE ALKALOIDS are found typically in species of *Senecio* (family *Compositae*, tribe *Senecioneae*), *Crotalaria* (family *Leguminosae*, tribe *Genistae*) and the subfamilies *Heliotropioideae* and *Boraginoideae* of the family *Boraginaceae* (1). They occur also in other genera of the tribes *Senecioneae* [*e.g.*, *Cacalia*, *Emilia* (2), *Erechtites*] and *Genistae* [*e.g.*, *Adenocarpus* (3), *Cytisus* (4)] as well as in the unrelated genera *Eupatorium* (*Compositae*) (5), *Thesium* (*Santalaceae*) (6), *Planchonella* (*Sapotaceae*) (7), *Lolium* (8), and *Festuca* (9) (*Gramineae*). The ability of some alkaloids of the group to cause an unusual chronic liver disease in animals (10) has promoted extensive study of their chemical and toxicological properties (*e.g.*, *References 1, 11, 12*). Platyphylline (I) (13) has no hepatotoxic action and has found use in the U.S.S.R. as an antispasmodic.

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The hepatotoxic alkaloids have an irreversible, antimitotic effect on liver cells which leads to grossly enlarged cells and cell nuclei (megalocytosis). They cause chromosome breakage (14) and are mutagenic (15). These properties suggested possible antitumor activity, and in 1958, several alkaloids from this laboratory were screened by the Cancer Chemotherapy National Service Center against sarcoma 180, lymphoid leukemia L1210, and Ehrlich ascites cell culture. Weak activity against sarcoma 180 was observed with heliotrine, but heliotrine *N*-oxide, seneciphylline, sarracine *N*-oxide, jaconine hydrochloride, lasiocarpine, and retronecine hydrochloride were inactive. About the same time, Pukhal'skaya *et al.* (16) also investigated the action of heliotrine, viridiflorine, platyphylline, seneciphylline, lasiocarpine, and heliosupine on a transplanted hepatoma in mice, Ehrlich's adenocarcinoma, and sarcoma 45. The hepatoma was inhibited to the extent of 21% by lasiocarpine, 10% by seneciphylline, and 8% by heliotrine. The Ehrlich's tumor was inhibited 54% by heliosupine and 19% by seneciphylline. Sarcoma 45 was inhibited 28% by heliosupine and 17% by lasiocarpine.

More extensive testing has since been undertaken in response to two developments. First, it was recognized that the hepatotoxic alkaloids, which conform to the general formula (II), behave chemically as alkylating agents and that several of their biological properties are those of alkylating agents (17). This provided further rationale for possible antitumor action, and in particular, suggested testing against the Walker 256 tumor systems which are sensitive to alkylating agents (18). Second, a high level of activity was observed in monocrotaline (III), which was isolated as the tumor-inhibitory constituent of *Crotalaria spectabilis* (19). The main activity was against adenocarcinoma 755, the highest degree of inhibition achieved being 95% at a dose of 90 mg./Kg. Further testing by CCNSC disclosed activity against plasmacytoma, but in many other systems monocrotaline was inactive (20). This paper reports results of testing of 18 alkaloids, three alkaloid *N*-oxides, and two derivatives from this laboratory and discusses the structure-activity pattern within the series in relation to tumor-inhibitory activity.

EXPERIMENTAL

Alkaloids—The alkaloids examined were either available from other studies or were reisolated by the techniques previously employed. They are crispatine (21), echinatine (22), europine (23), fulvine (21), heleurine (23), heliotrine (24), jacobine (25), jaconine hydrochloride (25), lasiocarpine (24), 1-methylenepyrrolizidine (26), 1-methoxymethyl-1,2-epoxypyrrolizidine (27, 28), 1-methoxymethyl-7β-hydroxy-1,2-dehydropyrrolizidine (28), monocrotaline (29), senecionine (30), seneciphylline (25), spectabiline (31), and supinine (23).

Alkaloid N-Oxides—Sarracine *N*-oxide was isolated directly from *Senecio mikanioides* Otto (32). Heliotrine *N*-oxide and monocrotaline *N*-oxide were prepared by oxidation of the parent alkaloids as described previously (*References 24* and *29*, respectively).

Derivatives—Retronecine hydrochloride was obtained by hydrolysis of monocrotaline using the procedure described by Culvenor and Smith (31). 1 - Chloromethyl - 7α - hydroxy - 1,2 - dehydropyrrolizidine was prepared by reaction of heliotridine with thionyl chloride as described previously (33).

Testing Procedure—Testing was carried out in accordance with the Cancer Chemotherapy National Service Center protocols (34). The dosing regimens are as follows: adenocarcinoma 755, in mice, one dose daily for 11 days; lymphoid leukemia L1210, in mice, one dose daily until death; sarcoma 180, in mice, one dose daily for 7 days; Walker 256 (intramuscular), in rats, one dose daily for 4 days; Walker 256 (subcutaneous), in rats, one dose daily for 5 days, by dose response at 4 doses; and plasmacytoma 1, in hamsters, one dose daily for 15 days. The criterion for significant activity accepted by CCNSC is at least 58% decrease in tumor size compared with controls for solid tumors, and 25% increase in survival time for leukemia 1210 and 50% inhibition of KB cell culture at a concentration of 1 mcg./ml.

Results—The results obtained are summarized in Table I, in which plus and minus signs indicate whether the level of inhibition in the particular tumor system meets the criteria given in the preceding paragraph. Detailed results are given for the active compounds in Table II, replicates being omitted except where it seemed advisable to indicate a variability or reproducibility in the level of action.

DISCUSSION

It is apparent from Table I that tumor-inhibitory activity is widely exhibited among pyrrolizidine alkaloids, 10 of 23 compounds tested being active at a significant level against one or more tumor systems. The tumors most frequently inhibited were the Walker 256 (intramuscular) and adenocarcinoma 755, the level of activity against the former being in some cases very high. Thus complete or nearly complete destruction of this tumor was observed with heliotrine (IV) at a dose level of 125 mg./Kg., and T/C values[1] below 30 were attained at fairly low dose levels with fulvine (V), heliotrine *N*-oxide (VI), lasiocarpine (VII), monocrotaline (III), spectabiline (VIII), and senecionine (IX).

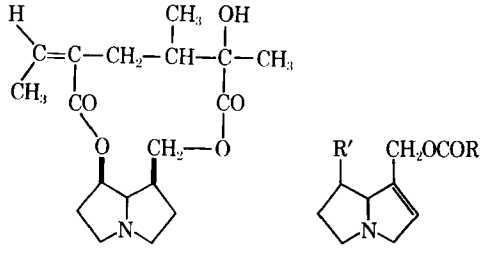
Against adenocarcinoma 755, the strongest activity is found in the bases of monocrotaline type, monocrotaline, spectabiline, and fulvine, and in the chloro-derivative (X); crispatine (XI), heliotrine, and supinine (XII) exhibit weak activity. Heliotrine, lasiocarpine, monocrotaline, and the chloro-

[1] T/C is the ratio of tumor weight or survival time of test animals to control animals, expressed as percent.

TABLE I—ANTITUMOR SCREENING RESULTS[a]

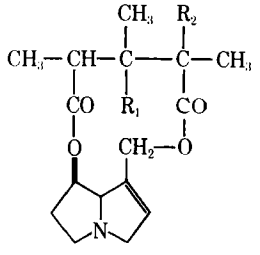
Alkaloid	NSC No.	CA	LE	SA	WM	WA	KB
Crispatine (XI)	89933	+	−	−	−	−	−
Echinatine (XVIII)	89937	−	−		−	−	−
Europine (XIII)	89939					−	−
Fulvine (V)	89932	+	−	−	+	+	−
Heleurine (XIV)	89940	−	−	−	−	−	−
Heliotrine (IV)	30620	+		+	+	−	−
Heliotrine *N*-oxide (VI)	30621	−	−	−	+		−
Jacobine (XVI)	89936		−		−		−
Jaconine (XVII) hydrochloride	30624		−	−	−		−
Lasiocarpine (VII)	30625		−	+	+	+	−
1-Methylenepyrrolizidine (XIX)	89941		−		−	−	
1-Methoxymethyl-1,2-epoxypyrrolizidine (XX)	89944						−
1-Methoxymethyl-7β-hydroxy-1,2-dehydropyrrolizidine (XXI)	89942						−
Monocrotaline (III)	28693	+	−	+	+	−	−
Monocrotaline *N*-oxide (XXV)	108378	−	−				
Sarracine *N*-oxide (XXIII)	30623	−	−	−	−		
Senecionine (IX)	89935	−	−		+	−	−
Seneciphylline (XV)	30622	−	−	−	−	−	−
Spectabiline (VIII)	89934	+	−		+	+	−
Supinine (XII)	89938	+	−	−	−	−	−
Retronecine (XXII) hydrochloride	30618		−	−			
1-Chloromethyl-7α-hydroxy-1,2-dehydropyrrolizidine (X)		+		+			−

[a] The tumor systems are CA, adenocarcinoma 755; LE, lymphoid leukemia L1210; SA, sarcoma 180; WM, Walker 256 intramuscular; WA, Walker 256 subcutaneous; KB, cell culture.

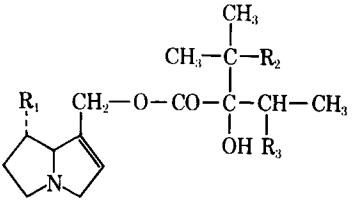


I

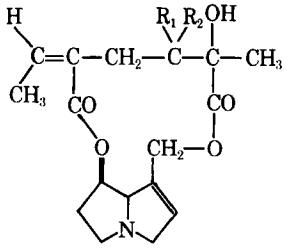
II



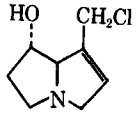
III, $R_1 = OH$, $R_2 = OH$
V, XI, $R_1 = OH$, $R_2 = H$
VIII, $R_1 = OCOCH_3$, $R_2 = OH$
XXV, III with N→O in place of N



IV, $R_1 = OH$, $R_2 = H$, $R_3 = OCH_3$
VI, IV with N→O in place of N
VII, $R_1 = CH_3-CH=C(CH_3)-CO-O$, $R_2 = OH$, $R_3 = OCH_3$
XII, $R_1 = H$, $R_2 = H$, $R_3 = OH$
XIII, $R_1 = OH$, $R_2 = OH$, $R_3 = OCH_3$
XIV, $R_1 = H$, $R_2 = H$, $R_3 = OCH_3$
XVIII, $R_1 = OH$, $R_2 = H$, $R_3 = OH$



IX, $R_1 = H$, $R_2 = CH_3$
XV, $R_1R_2 = CH_2$



X

derivative were active against sarcoma 180, and lasiocarpine, spectabiline, and fulvine were active (the last two only weakly) against the Walker 256 (subcutaneous) system. Activity was essentially confined to the solid tumors, all compounds being inactive against leukemia 1210 and KB cell culture, except that heliotrine, lasiocarpine, europine (XIII) and heleurine (XIV), evinced weak activity against the latter (ED_{50} 15–23 mcg./ml.) at a level lower than the CCNSC minimum standard (ED_{50} 1 mcg./ml.).

The active alkaloids are all of the allylic ester type which have a potential for alkylation (17). Of the bases of monocrotaline type (11-membered ring diesters), monocrotaline and spectabiline are highly active against WM and CA, fulvine of moderate activity, and crispatine of weak activity overshadowed by a high toxicity. The alkaloids of senecionine type (12-membered ring diesters) also appear to be generally of too high toxicity to the host to permit expression of activity against the

TABLE II—TUMOR-INHIBITORY ACTIVITY OF PYRROLIZIDINE ALKALOIDS[a]

Test System	Dose, mg./Kg.	Survivors	Animal Wt. Diff., T-C(g.)	T/C,[b] %	Tester[c]
Crispatine					
CA	90	0/6		Toxic	A
	45	0/6		Toxic	
	22.5	6/6	−1.3	50	
LE	20	6/6	−1.2	96	C
WM	20	5/6	−10	62	
	75	1/6	−5		
	50	0/6			
	25	2/6	−20		
	12.5	5/6	−10	104	
WA	3.5	6/6	−18	61	C
KB				(>100)[d]	C
Fulvine					
CA	90	0/6		Toxic	A
	45	5/6	−0.8	36	
	22.5	6/6	−1.6	56	
LE	400	4/4	−1.5	104	C
	200	4/4	0.8	98	
	100	4/4	0.7	98	
	50	4/4	0.4	98	
	50	6/6	0.0	112	
WM	200	4/6	−12	44	
	150	4/6	−9	49	
	100	5/6	−1	29	
	50	1/6	9		
	50	6/6	7	65	
	50	6/6	−2	26	
WA	3.8	6/6	−11	49	C
KB				(>100)[d]	
Heliotrine					
CA	90	4/5	−2.6	45	A
	45	5/5	−1.2	60	
	22.5	5/5	−0.7	104	
SA	125	5/6	−1.4	47	C
	125	6/6	−1.8	87	
WM	188	1/6	−5		
	125	6/6	−9	4	C
	125	5/6	−6	10	
	125	5/6	2	4	
	125	6/6	7	0	
	125	6/6	−7	5	
	125	5/6	−8	0	
	83	6/6	4	30	
	55	6/6	0	44	
WA	10	6/6	0	86	C
KB				(15)[d]	C
Heliotrine N-Oxide					
LE	88	6/6	−1.0	92	
SA	125	4/6	−0.1	65	
WM	100	6/6	6	45	C
	100	6/6	4	23	
	100	6/6	1	40	
EA	125	4/6	2.6	120	
Lasiocarpine					
CA	90	0/5			A
	45	1/5			
	22.5	2/5			
LE	27.9	6/6	−1.6	89	C
SA	66	0/6			C
	44	3/6	1.1		
	40	4/6	−2.4	40	
	26.6	6/6	−2.6	48	
	18	6/6	−2.1	73	
WM	40	5/6	−9	52	C
	40	4/6	−13	26	
	20	6/6	−11	18	
	20	6/6	−16	36	
	20	6/6	−17	34	
	20	6/6	−25	58	
	10	6/6	−12	64	
	5	6/6	−7	66	
WA	3.0	6/6	−10	32	C
KB				(25)[d]	C
Monocrotaline					
CA	240	5/5	−2.8	23	A
	180	5/5	−0.9	32	
	200	6/10	−4.8		C
	100	9/10	−5.4	9	
	200	10/10	−5.1	7	
	100	8/10	−5.1	7	
	50	9/10	−4.4	44	
	25	10/10	−2.6	65	
	180	7/10	−4.6	7	
	90	9/10	−3.1	15	
	45	10/10	−1.9	34	
	22.5	10/10	−1.3	45	
LE	135	7/7	−0.5	117	C
	90	7/7	−0.5	109	
	60	7/7	0.0	113	
	40	7/7	0.0	110	
	90	6/6	0.4	136	
SA	100	6/6	−1.7	33	C
	100	6/6	−1.0	47	
	100	2/6	−4.3		
	100	7/7	−2.5	43	
	100	6/6	−1.4	34	
	50	6/6	0.7	48	
WM	100	6/6	−1	0	C
	50	6/6	−2	4	
	25	6/6	−1	14	
	12.5	6/6	−5	17	
	6.25	6/6	−2	17	
	50	5/6	−16	22	
	25	5/6	−11	25	
	12.5	6/6	−4	43	
	6.25	6/6	−6	58	
WA	10	6/6	−17	74	C
Pl	84	10/10	−8	4	
	63	10/10	−7	3	
	42	9/10	−5	25	
	30	8/10	−3	25	C
	15	9/10	−9	38	
	7.5	8/10	−6	59	
KB				(>100)[d]	C
Spectabiline					
CA	60	5/6	...	18	A
	45	5/6	...	23	
LE	50	6/6	−1.2	112	C
WM	75	6/6	−3	7	C
	50	6/6	4	5	
	33	6/6	3	14	
	22	6/6	5	25	
	100	3/6	−23		C
	75	6/6	−26	4	
	50	6/6	−22	8	
	25	6/6	−11	14	
	12.5	6/6	−8	25	
	20	6/6	−7	14	
	10	6/6	−9	19	
	5	6/6	−7	46	
WA	4.5	6/6	−13	47	C
KB				(>100)[d]	
Supinine					
CA	90	6/6	−1.3	51	A
	45	6/6	0.3	88	
	22.5	6/6	1.2	122	
LE	200	6/6	0.6	95	C
WM	200	6/6	−0.7	99	C
WA	50	6/6	2	132	C
KB				(>100)[d]	C
1-Chloromethyl-7α-hydroxy-1,2-dehydropyrrolizidine					
CA	90	0/6		Toxic	A
	45	6/6	−3.4	82	
	22.5	6/6	−0.7	26	
Senecionine					
LE	50	6/6	−0.5	98	C
	30	4/4	−2.0	98	
	20	4/4	0.1	96	
WM	35	3/4	1	5	C
	30	2/4	2		
	25	4/4	−6	16	
	20	4/4	−10	21	
	15	4/4	3	57	
	40	0/4			
	30	2/4	−10		
	20	3/4	−2	22	
	40	6/6	−9	85	
	27	6/6	−6	45	
	18	6/6	−7	66	
	12	6/6	−5	93	
WA	5.5	6/6	−13	110	
KB				(>100)[d]	C

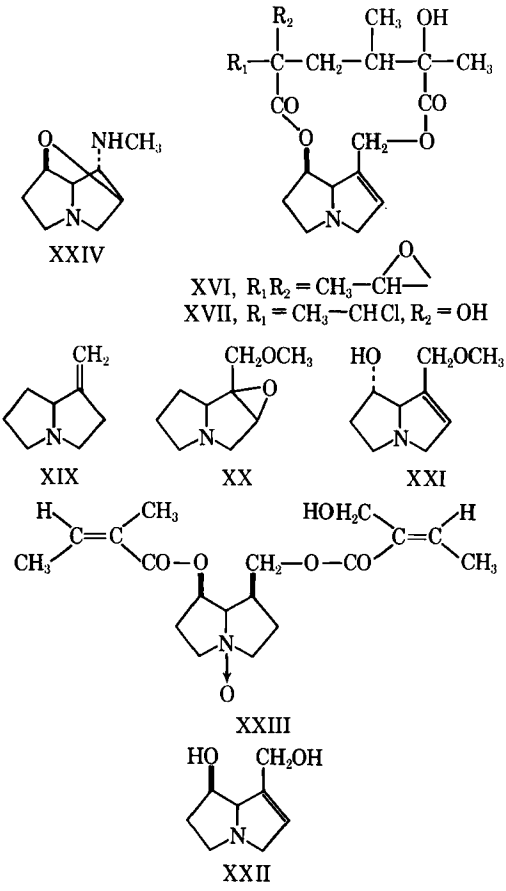
[a] The tumor systems are CA, adenocarcinoma 755; LE, lymphoid leukemia L1210; SA, sarcoma 180; WM, Walker 256 intramuscular; WA, Walker 256 subcutaneous; KB, cell culture; EA, Ehrlich ascites; Pl, plasmacytoma 1. [b] T/C is the ratio of tumor weight or survival time of test animals to control animals, expressed as percent. [c] A denotes testing by Dr. R. J. Stein, Abbott Laboratories, North Chicago. C denotes testing by Cancer Chemotherapy National Service Center, Bethesda, Md. [d] ED_{50} in mcg./ml.

tumors, although senecionine itself has shown high activity against WM at low dose levels. Senecionine (XV) almost meets the criterion for activity against WM at a dose of 50 mg./Kg., while jacobine (XVI) and jaconine (XVII) are inactive at nontoxic levels. Of the bases of heliotrine type (esters of monocarboxylic acids) heliotrine is strongly active against WM, lasiocarpine moderately active, and echinatine (XVIII), supinine, and heleurine essentially inactive.

On the whole, these results suggest that there is for tumor-inhibitory activity, an optimum level or set of levels for one or more of the interrelated properties, water solubility, lipid-water partition coefficient, and base strength. High hepatotoxicity is correlated with low water solubility and low base strength (2) as found for example in lasiocarpine and the macrocyclic diester alkaloids, excepting monocrotaline which has a moderate water solubility occasioned by two hydroxyl groups. Echinatine is very highly soluble in water and weakly hepatotoxic; it might be expected to have only poor access to the target cells. Supinine and heleurine are more highly basic than the other alkaloids [pK_a 9.6, compared with heliotrine, 8.5, and lasiocarpine, 7.8 (35)] and are so highly ionized at physiological pH that they partition only very slightly into a lipid phase (2). Thus monocrotaline and heliotrine appear to come closest to the optimum in properties although spectabiline and lasiocarpine, both more lipid-soluble, are almost as active. The restriction of even weak KB activity to the esters of monocarboxylic acids and the fact that europine (highly soluble in water) and heleurine (high base strength) are among the weakly inhibitory bases is probably a reflection of the different environment of the cell culture. High water solubility is apparently less damaging to activity in this system than high lipid solubility. Alternatively, there is the intriguing possibility that the macrocyclic alkaloids are too bulky to pass the cell membranes.

The structure-activity pattern and the high activity against the intramuscular Walker 256 system both support the idea that activity is connected with the alkylating ability of the allylic ester system (II). It is also significant that the chloro-derivative (X), which has the ester grouping replaced by a halogen atom and has strong *in vitro* alkylating reactivity, is active against two test tumors. Insofar as they have been tested, the other nonester alkaloids (XIX, XX, and XXI), as well as retronecine (XXII) and sarracine *N*-oxide (XXIII), the ester of a saturated pyrrolizidine aminoalcohol, have no inhibitory action. Thus antitumor activity seems quite definitely to be associated with the same functional region in the molecule as hepatotoxicity (17). A recent report by Aizenman *et al.* (36) that loline (XXIV) and *N*-methylloline have a weak inhibitory action against Ehrlich carcinoma suggests that it may become necessary to extend this concept of the structure-activity relationship, but this is scarcely warranted on the present evidence. It is possible that the shape of the pyrrolizidine ring, which is not unlike that of a purine, is another factor in promoting activity.

The *N*-oxides of monocrotaline and heliotrine were tested in the hope of achieving a more favorable ratio of desired activity to acute toxicity. Heliotrine *N*-oxide is active against the Walker 256



tumor but superiority over the tertiary base has not been demonstrated. Monocrotaline *N*-oxide is inactive against CA and LE but has not been tested against WM. From the same point of view, heleurine and supinine were also disappointing. Both are weakly hepatotoxic and thus presumably capable of biological alkylation, but do not appear to shorten the life span of rats (37). They were essentially inactive at the dose levels used.

The known acute and chronic effects of pyrrolizidine alkaloids militate against successful exploitation of the tumor-inhibitory activity which has been uncovered in this group of compounds. However, it may be possible to utilize the selective and apparently permanent and complete antimitotic action exerted by the alkaloids on parenchymal liver cells. If there is a similar effect on tumor cells derived from liver cells, it may be possible to achieve complete inhibition of hepatomas while leaving the liver capable of sustaining life for long periods.

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Keyphrases

Pyrrolizidine alkaloids
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Lipid solubility—antitumor activity
Alkylating ability—antitumor activity

Prediction of Stability in Pharmaceutical Preparations XV

Kinetics of Hydrolysis of 5-Trifluoromethyl-2′-deoxyuridine

By HANS J. NESTLER* and EDWARD R. GARRETT†

The antiviral 5-trifluoromethyl-2′-deoxyuridine, F_3TdR, is hydrolyzed by hydrogen ions and solvent to 5-trifluoromethyluracil, F_3T, and deoxyribose. The F_3T is readily hydrolyzed to 5-carboxyuracil by hydroxyl ion attack on the undissociated and anionic species. At elevated temperatures, 5-carboxyuracil is decarboxylated by hydroxyl ion catalysis to uracil. The F_3TdR is readily hydrolyzed to 5-carboxy-2′-deoxyuridine by hydroxyl ion attack on the undissociated and anion species through an observable kinetic intermediate at high alkalinity, probably 5-hydroxydifluoromethyl-2′-deoxyuridine. The optimum pH range for stabilization is 1–4 where the half-life at 30° is 280 days. The material is readily degraded at pH 7.4 where 1.5 days is the half-life at 30°.

THE KNOWN antitumor and antiviral activity of the 5-halogenated uracils and 5-halogenated pyrimidine nucleosides (1) initiated the synthesis of 5-trifluoromethyl-2′-deoxyuridine (trifluorothymidine, F_3TdR, I) (2). The structure I sterically resembles 5-iodo-2′-deoxyuridine, IDU, which is active against *Herpes simplex* in human keratitis (3). The van der Waals radius of iodine is 2.15 Å. and that of the trifluoromethyl group is 2.44 Å. (4). Since a degradation product of IDU, 5-iodouracil tends to antagonize its activity (5), complete studies of its kinetics of solvolysis were performed in order to determine optimum conditions for pharmaceutical stabilization (6–9).

Preliminary experiments with F_3TdR, I, have

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* Present address: Institute of Organic Chemistry, University of Wurzberg, Germany.
† To whom inquiries concerning this manuscript should be addressed.