Evaluation of Nuclear-Substituted Styryl Ketones and Related Compounds for Antitumor and Cytotoxic Properties

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Abstract □ A number of nuclear-substituted styryl ketones, the related Mannich bases, and allyl alcohols were synthesized and evaluated for antitumor activity, principally in the L-1210 lymphoid leukemia and P-388 lymphocytic leukemia screening tests. The cytotoxicity of some of these compounds assessed in Eagle's 9KB carcinoma cell culture system was also recorded. Two of the Mannich bases showed promising levels of activity in the P-388 screening; of the results obtained to date, over one-third of the derivatives showed cytotoxicity at dose levels of 1–3 ppm. Other pharmacological results of these compounds are briefly reported.

Keyphrases □ Styryl ketones, nuclear substituted—synthesis, spectroscopy, and antitumor evaluation □ Mannich bases (related to styryl ketones)—antitumor evaluation □ Allyl alcohols from styryl ketones and related Mannich bases—antitumor evaluation □ Antitumor activity—nuclear-substituted styryl ketones and related Mannich bases and allyl alcohols

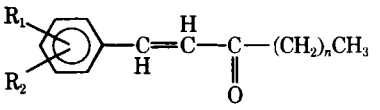
Approximately 70 years ago, it was shown that conjugated olefinic ketones undergo nucleophilic attack with thiols (1, 2) which, along with amines and carboxylate and phosphate anions, constituted the primary target sites for biological alkylating agents *in vivo* (3). Recent studies have demonstrated the reactivity of α,β-unsaturated ketones with thiols (4, 5) and amines (6), and enzyme-catalyzed alkylation of the thiol group of cysteine with α,β-unsaturated carbonyl compounds has been observed (7, 8).

It is known that Mannich bases have antitumor properties (9, 10) and the formation of the water-soluble Mannich bases derived from α,β-unsaturated ketones seems of interest. The higher chemical and biological reactivity of allyl alcohol compared to the saturated analog (11, 12) suggested the synthesis of substituted allyl alcohols as potential alkylating agents. Furthermore, it has been shown that the pyrrolizidine alkaloids with tumor-inhibiting properties possess an allylic ester function (13), which has been shown to react with thiols to give the corresponding S-alkylated product (14). The carbonyl reduction of both α,β-unsaturated ketones and the corresponding Mannich bases followed by esterification would lead to substituted allyl alcohols and esters that may have antitumor properties. The aim of the present investigation, therefore, was to synthesize a number of substituted styryl ketones (I), the related Mannich bases (II), and finally the related allyl alcohols and esters (III) for evaluation in various tumor systems, principally the L-1210 and P-388 leukemias *in vivo* and the KB cell culture system *in vitro*.

RESULTS AND DISCUSSION

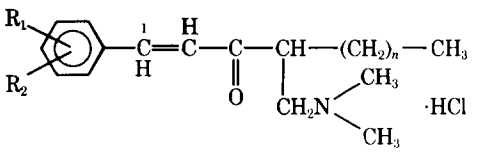
With the exception of I*i*, the styryl ketones were synthesized by the Claisen–Schmidt condensation between the appropriate benzaldehyde and either 2-octanone or 2-butanone. The possibility exists of condensation of the aromatic aldehyde with either the methyl or methylene group of the 2-alkanone. Base-catalyzed condensation between benzaldehyde and 2-octanone gave I*a*, whereas acid-catalyzed condensation gave an oil which was shown to consist mainly of 3-benzylidene-2-octanone (I*m*).

GLC analysis of the crude reaction products indicated that the base-catalyzed reaction did not give the methylene condensation product (I*m*) and that the acid-catalyzed reaction did not furnish the methyl condensation product (I*a*). However, the reaction product between benzaldehyde and 2-butanone was shown to consist of 84% I*j* and 16% I*n*, which was unexpected since earlier work had



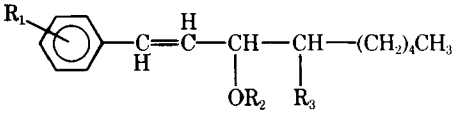
I

I*a*: $R_1 = R_2 = H$, $n = 5$
I*b*: $R_1 = 2\text{-Cl}$, $R_2 = H$, $n = 5$
I*c*: $R_1 = 3\text{-Cl}$, $R_2 = H$, $n = 5$
I*d*: $R_1 = 4\text{-Cl}$, $R_2 = H$, $n = 5$
I*e*: $R_1 = 2\text{-Cl}$, $R_2 = 4\text{-Cl}$, $n = 5$
I*f*: $R_1 = 2\text{-Cl}$, $R_2 = 6\text{-Cl}$, $n = 5$
I*g*: $R_1 = 3\text{-Cl}$, $R_2 = 4\text{-Cl}$, $n = 5$
I*h*: $R_1 = 4\text{-N(CH}_3)_2$, $R_2 = H$, $n = 5$
I*i*: $R_1 = 4\text{-}\overset{+}{\text{N}}(\text{CH}_3)_3\overset{-}{\text{I}}$, $R_2 = H$, $n = 5$
I*j*: $R_1 = R_2 = H$, $n = 1$
I*k*: $R_1 = 4\text{-Cl}$, $R_2 = H$, $n = 1$
I*l*: $R_1 = 4\text{-NO}_2$, $R_2 = H$, $n = 1$



II

II*a*: $R_1 = R_2 = H$, $n = 4$
II*b*: $R_1 = 2\text{-Cl}$, $R_2 = H$, $n = 4$
II*c*: $R_1 = 4\text{-Cl}$, $R_2 = H$, $n = 4$
II*d*: $R_1 = 2\text{-Cl}$, $R_2 = 4\text{-Cl}$, $n = 4$
II*e*: $R_1 = 2\text{-Cl}$, $R_2 = 6\text{-Cl}$, $n = 4$
II*f*: $R_1 = 3\text{-Cl}$, $R_2 = 4\text{-Cl}$, $n = 4$
II*g*: $R_1 = R_2 = H$, $n = 0$



III

III*a*: $R_1 = 4\text{-Cl}$, $R_2 = R_3 = H$
III*b*: $R_1 = R_2 = H$, $R_3 = CH_2N(CH_3)_2 \cdot HCl$
III*c*: $R_1 = R_2 = H$, $R_3 = CH_2\overset{+}{\text{N}}(CH_3)_3\overset{-}{\text{I}}$
III*d*: $R_1 = 2\text{-Cl}$, $R_2 = H$, $R_3 = CH_2N(CH_3)_2 \cdot HCl$
III*e*: $R_1 = 2\text{-Cl}$, $R_2 = CO$–C$_6$H$_4$–NO_2, $R_3 = CH_2N(CH_3)_2 \cdot HCl$
III*f*: $R_1 = H$, $R_2 = CO$–C$_6$H$_4$–NO_2, $R_3 = CH_2N(CH_3)_2 \cdot HCl$

Table I—Evaluation of Some (*E*)-1-(Substituted phenyl)-1-alken-3-ones (Series I) in the L-1210 and KB Test System

Compound	Vehicle[a]	Route of Injection	Interval[b] of Injection, days	Number of Injections	Dose per Injection, mg/kg	Survivors[c] (out of 6)	Animal[d] Weight Difference, g	T/C, %[e]	KB[f]
I*a*	7	Subcutaneous	1	1	400	6	−1.8	100	>100
					200	6	−0.5	91	
					100	6	+0.5	94	
	4	Subcutaneous	4	3	400	0	—	—	
					200	0	—	—	
					100	0	—	—	
I*b*	7	Intraperitoneal	1	9	400	6	−1.6	93	23
					200	6	−1.7	92	
					100	6	+0.5	98	
I*c*	M	Intraperitoneal	4	3	400[g]	6	−0.5	91	>10
					200[g]	6	+0.2	102	
					100[g]	6	−1.0	104	
	T	Intraperitoneal	1	9	400	6	−3.3	95	
					200	6	−0.7	88	
					100	6	−1.0	88	
I*d*	T	Intraperitoneal	4	3	400	6	−1.4	93	30
					200	6	+0.4	98	
					100	6	−0.3	96	
	C	Intraperitoneal	1	9	400	6	−2.5	92	
					200	5	−2.1	90	
					100	6	−0.3	93	
I*e*	C	Intraperitoneal	4	3	400	6	−2.3	93	28
					200	6	−2.1	91	
					100	6	−1.4	86	
	C	Intraperitoneal	1	9	400	6	−3.1	95	
					200	6	−1.0	90	
					100	6	−0.7	95	
I*f*	7	Subcutaneous	1	1	350	6	−0.4	108	N.A.
I*g*	C	Intraperitoneal	4	3	400	6	−1.1	98	27
					200	6	−0.3	93	
					100	6	−1.0	101	
	C	Intraperitoneal	1	9	400	2	−3.6	—	
					200	5	−3.1	92	
					100	6	−1.6	92	
I*h*	4	Subcutaneous	1	1	400	6	−0.7	97	>100
					200	6	+0.1	105	
					100	6	+0.1	97	
	4	Subcutaneous	4	3	400	6	−1.6	102	
					200	6	−0.6	103	
					100	6	−1.6	105	
I*i*	4	Subcutaneous	1	1	400	0	—	—	24
					200	2	+1.3	—	
					100	6	−1.4	86	
	4	Subcutaneous	4	3	100	6	−1.0	106	
					50	6	−0.5	92	
					25	6	+0.9	94	
	4	Subcutaneous	1	9	100	0	—	—	
					50	6	−0.9	97	
					25	6	−1.3	93	
I*j*	M	Intraperitoneal	4	3	400	6	−0.4	91	N.A.
					200	6	−0.3	107	
					100	6	+0.2	96	
I*k*	M	Intraperitoneal	4	3	400	6	+0.5	102	25
					200	6	−0.2	104	
					100	6	−0.1	98	
	M	Intraperitoneal	1	9	400	6	−1.1	97	
					200	6	+1.2	97	
					100	6	+0.8	98	
I*l*	7	Intraperitoneal	4	3	400	6	−0.4	93	N.A.
					200	6	−0.8	93	
					100	6	−1.0	101	

[a] C = acetone, D = alcohol, M = hydroxypropylcellulose, S = saline sonified, T = saline with Tween 80, 2 = saline, 4 = steroid suspending solution, and 7 = unspecified vehicle. [b] 1 = injections given daily, and 4 = injections made every 4 days. [c] Number of survivors on 5th day after the first injection. [d] Average weight of test group minus average weight of control animals. [e] Ratio of survival time of treated animals to control animals expressed as a percentage. [f] The figures (in micrograms per milliliter) in the KB cell culture screen indicate the dose inhibiting 50% growth of human epidermoid carcinoma of the nasopharynx in Eagle's medium. N.A. = result not available. [g] The CDF_1 strain of mouse used.

shown that in dilute alkali the reaction product consisted of I*j* (15). The compounds in Series I submitted for screening were shown by spectroscopic means to be formed by condensation between the methyl portion of the appropriate 2-alkanone and the aromatic aldehyde.

The quaternary ammonium compound (I*i*) was prepared by quaternization of I*h* with methyl iodide. Attempts to condense *p*-nitrobenzaldehyde with 2-octanone to give 1-(*p*-nitrophenyl)-1-nonen-3-one using aqueous sodium hydroxide solution were unsuccessful. Other workers have also noted the failure of nitrobenzaldehydes to react with both γ-butyrolactone and 2-hexanone in the presence of aqueous alkali (16, 17). Piperidine acetate has

Table II—Evaluation of Some (*E*)-4-Dimethylaminomethyl-1-(substituted phenyl)-1-nonen-3-one Hydrochlorides (Series II) in the L-1210 and KB Test Systems

Compound	Vehicle[a]	Route of Injection	Interval[b] of Injection, days	Number of Injections	Dose per Injection, mg/kg	Survivors[c] (out of 6)	Animal[d] Weight Difference, g	T/C, %[e]	KB[f]
II*a*	4	Subcutaneous	1	1	400	5	−4.3	100	1.5
					200	5	+0.2	93	
					100	5	−2.9	93	
	7	Subcutaneous	4	3	400	1	−1.1	—	
					200	6	−1.1	90	
					100	6	−0.9	101	
	7	Subcutaneous	1	9	200	0	—	—	
					100	6	−1.4	102	
					50	6	−0.8	95	
II*b*	7	Intraperitoneal	1	9	400	0	—	—	2.4
					200	0	—	—	
					100	5	−3.8	72	
II*c*	4	Subcutaneous	1	1	400	0	—	—	1.7
					200	5	−2.9	116	
					100	6	−0.3	91	
	4	Subcutaneous	4	3	200	4	−1.3	79	
					100	6	−0.4	78	
					50	6	+0.3	95	
	7	Subcutaneous	1	9	50	6	−1.9	93	
					25	6	+0.2	100	
					12.5	6	−0.4	100	
	7	Subcutaneous	1	9	170	0	—	—	
					115	4	+0.2	100	
					75	6	+0.3	98	
II*d*	D	Intraperitoneal	4	3	400	0	—	—	1.0
					200	0	—	—	
					100	2	—	—	
	D	Intraperitoneal	4	3	50	6	−0.9	97	
					25	6	+0.5	97	
					12.5	6	−0.4	87	
	D	Intraperitoneal	1	9	50[g]	5	−3.6	82	
					25[g]	5	−2.2	102	
					12.5[g]	6	−0.7	102	
II*e*	M	Intraperitoneal	1	1	400	0	—	—	2.8
					200	1	—	—	
					100	5	−2.8	94	
	M	Intraperitoneal	1	9	100	2	—	—	
					50	6	−4.7	75	
					25	6	−3.6	106	
	7	Intraperitoneal	1	9	25	6	−1.4	104	
					12.5	6	−0.3	101	
					6.25	6	−0.1	101	
II*f*	C	Intraperitoneal	4	3	400	0	—	—	1.2
					200	0	—	—	
					100	0	—	—	
	C	Intraperitoneal	4	3	50	3	—	—	
					25	6	−1.4	92	
					12.5	6	+1.1	95	
	C	Intraperitoneal	1	9	25[g]	6	−3.9	97	
					12.5[g]	6	−1.0	94	
					6.25[g]	6	−0.5	92	
	2	Intraperitoneal	1	9	80[g]	0	—	—	
					55[g]	0	—	—	
					35[g]	1	—	—	
II*g*	S	Intraperitoneal	4	3	400	0	—	—	1.2
					200	0	—	—	
					100	6	−0.7	86	
	S	Intraperitoneal	4	3	50[g]	6	−0.6	102	
					25[g]	6	+0.4	106	
					12.5[g]	6	−0.5	102	
	S	Intraperitoneal	1	9	50	6	−2.1	107	
					25	6	−0.6	104	
					12.5	6	−0.8	103	
	S	Intraperitoneal	1	9	170	0	—	—	
					115	3	—	—	
					75	6	0	84	

[a–g] See Table I.

been employed successfully as the catalyst in the synthesis of 3-(*p*-nitrobenzylidene)-2,4-pentanedione from 2,4-pentanedione and *p*-nitrobenzaldehyde (18), but use of this catalyst in the present investigation led only to the isolation of unreacted starting materials and a low yield of a multicomponent oil. The literature describes the preparation of an analog of 1-(*p*-nitrophenyl)-1-nonen-

Table III—Evaluation of Some Substituted (*E*)-1-Phenyl-1-nonen-3-ols and Related Compounds (Series III) against L-1210 and KB Test Systems

Compound	Vehicle[a]	Route of Injection	Interval[b] of Injection, days	Number of Injections	Dose per Injection, mg/kg	Survivors[c] (out of 6)	Animal[d] Weight Difference, g	T/C, %[e]	KB[f]
III*a*	T	Intraperitoneal	4	3	400[g]	6	−0.2	111	N.A.
					200[g]	6	−0.5	102	
					100[g]	6	−0.1	110	
	T	Intraperitoneal	1	9	400	6	−0.6	—	
					200	6	−3.8	91	
					100	6	−2.1	94	
III*b*	4	Subcutaneous	4	3	400	4	+0.8	61	6.4
					200	6	−0.6	75	
					100	6	−0.6	75	
	7	Intraperitoneal	4	3	50	6	+0.8	103	
					25	6	+0.1	106	
					12.5	6	−0.5	119	
	4	Subcutaneous	1	9	50	6	−0.9	97	
					25	6	−0.7	98	
					12.5	6	−0.9	97	
III*c*	T	Intraperitoneal	4	3	400	0	—	—	42
					200	0	—	—	
					100	0	—	—	
	2	Intraperitoneal	4	3	20	0	—	—	
					10	6	+0.6	98	
					5.0	6	+0.3	103	
	2	Intraperitoneal	1	9	10	6	−0.6	99	
					5.0	6	−0.7	97	
					2.5	6	+0.1	97	
III*d*	2	Intraperitoneal	4	3	400	1	—	—	3.2
					200	0	—	—	
					100	5	−0.8	88	
	2	Intraperitoneal	4	3	100	5	−1.6	95	
					50	6	−0.2	97	
					25	6	−0.6	96	
	2	Intraperitoneal	1	9	100	3	—	—	
					50	6	−0.5	94	
					25	6	+0.3	102	
III*e*	T	Intraperitoneal	4	3	400	6	+0.1	92	N.A.
					200	6	+0.1	92	
					100	6	+0.1	95	
III*f*	T	Intraperitoneal	4	3	400	5	−0.2	106	>10
					200	6	−0.6	105	
					100	6	−0.4	90	
	T	Intraperitoneal	1	9	400	3	—	—	
					200	6	−4.9	100	
					100	5	−3.4	97	

[a–g] See Table I.

3-one, namely I*l*, which was synthesized by an aldol condensation between 2-butanone and *p*-nitrobenzaldehyde followed by dehydration of one of the intermediate hydroxyketones (19). Two short-chain analogs of I*l*, namely I*j* and I*k*, were prepared by Claisen–Schmidt condensation of the appropriate aldehyde and 2-butanone.

The styryl ketones I were shown by GLC to be homogeneous. Examination of the PMR spectra of the styryl ketones in Series I showed that the olefinic protons at C-1 and C-2 resonated in the range of δ 7.32–7.80 and 6.37–6.85, respectively. An olefinic coupling constant of 16 Hz was observed, indicating the *(E)*-configuration of the double bond (17, 20). A strong IR band at 980 cm^{-1}, due to the carbon–hydrogen out-of-plane vibration characteristic of ethylenic compounds possessing the *(E)*-configuration (21), was noted in most compounds. Two carbonyl absorptions at 1695–1675 and 1690–1640 cm^{-1} due to the presence of s-*cis*- and s-*trans*-rotational isomers (17, 22, 23) were also observed.

In an attempt to prepare I*h*, using the conditions of the normal Claisen–Schmidt condensation, only unreacted starting materials were obtained, even when the time of heating under reflux was extended from 24 to 58 hr. However, Neilsen and Dubin (24) prepared I*h* by employing sodium hydroxide in a mixture of ethanol (92%) and water (8%), a solvent known to enhance dimer formation of styryl ketones (25, 26). In the present study, I*h* (48% yield) was obtained along with a colorless solid (IV) (~2% yield), which was shown to be a dimer of I*h*.

The preparation and properties of the Mannich bases II*a*–II*f* and the related compounds V*a* and V*b* were reported recently (27). The syntheses and spectral characteristics of the substituted allyl alcohols and esters (III) and the related compound (VI) are described in the literature (28) or under *Experimental*. In an attempt to prepare *(E)*-1-(*p*-dimethylaminophenyl)-1-nonen-3-ol by sodium borohydride reduction of I*h*, a mixture of the desired alcohol (70%) and unreacted I*h* (30%) was obtained, which was not separated by fractional crystallization or by preparative GLC.

The antineoplastic activity of a variety of epoxides (29, 30) suggested the preparation of 1,2-epoxy-1-(*p*-chlorophenyl)-3-nonanone by reaction of I*d* with hydrogen peroxide. The derivative obtained gave the predicted spectral characteristics for the epoxide and was homogeneous by TLC, but its rapid decomposition precluded its submission for screening.

The evaluation of the styryl ketones (I) against L-1210 lymphoid leukemia in mice is shown in Table I. None of the compounds showed activity. In the P-388 lymphocytic leukemia screen, I*c* showed no activity (Table V). In the KB test system *in vitro*, which is a measure of cytotoxicity and may indicate tumor-inhibiting properties (31), the chlorophenyl derivatives (I*b*, I*d*, I*e*, I*g*, and I*k*) and the quaternary ammonium iodide (I*i*) had a low level of activity, namely 23–30 ppm (Table I). At 400 mg/kg, no toxicity appeared in the mice, with the exception of Compounds I*g* and I*i*.

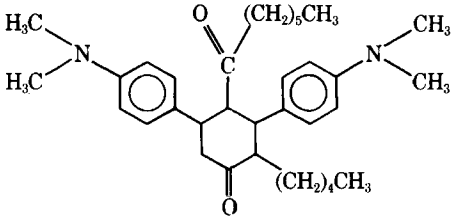
Table IV—Evaluation of IV, V*a*, V*b*, and VI in the L-1210 and KB Test Systems

Compound	Vehicle[a]	Route of Injection	Interval[b] of Injection, days	Number of Injections	Dose per Injection, mg/kg	Survivors[c] (out of 6)	Animal[d] Weight Difference, g	T/C, %[e]	KB[f]
IV	T	Intraperitoneal	4	3	400	6	−1.0	92	N.A.
					200	6	+1.1	90	
					100	6	−0.4	89	
	T	Intraperitoneal	1	9	400	6	−1.8	95	
					200	6	−0.5	95	
					100	6	+1.2	86	
V*a*	M	Intraperitoneal	4	3	400	2	—	—	2.1
					200	4	−1.3	106	
					100	6	−1.8	96	
	T	Intraperitoneal	1	9	400	0	—	—	
					200	0	—	—	
					100	4	−2.1	—	
V*b*	M	Intraperitoneal	4	3	400	6	−2.4	103	2.3
					200	5	−1.6	100	
					100	6	−1.0	102	
	M	Intraperitoneal	1	9	200	6	−0.1	95	
					100	4	+5.2	97	
					50	5	+0.2	103	
VI	M	Intraperitoneal	1	9	400	6	−2.6	113	N.A.
					200	5	−1.2	102	
					100	6	−1.3	103	

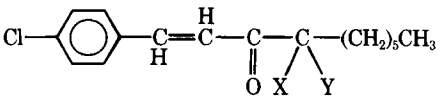
[a-f] See Table I.

The Mannich bases (II) showed no appreciable activity against the L-1210 lymphoid leukemia (Table II), although a high level of cytotoxicity (KB test) may be seen in this series for the results obtained to date. In the P-388 leukemia screen (Table V), II*d* and II*f* showed promising levels of activity, with increases in mean survival time of 30 and 42% at doses of 18 and 6.25 mg/kg, respectively. If the rate of reactivity of styryl ketones with biologically important nucleophiles is considered to be dependent on the fractional positive charge on C-1, then maximum activity would be expected to occur when the nuclear substituents are two chlorine atoms as in II*d*, II*e*, and II*f*. In the case of compounds containing one or two *ortho*-chloro atoms, II*d* and II*e*, steric impedence of an approaching nucleophile would occur, reducing reactivity; the effect would be less in the case of II*d*, which contains only one chlorine atom. In the case of II*f*, neither of the two chlorine atoms occupies the *ortho*-position on the aromatic ring so reaction with nucleophiles should be increased.

Activity against P-388 leukemia decreases in the order II*f*, II*d*, and II*e*, following a pattern of probable decreased chemical reactivity with nucleophiles at the cellular level. The toxicity in mice

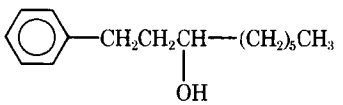


IV



V

V*a*: X = $CH_2N(CH_3)_3I$, Y = H
V*b*: X, Y = CH_2



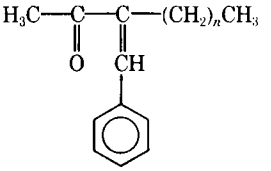
VI

for the chlorinated compounds II*b*–II*f* is higher than for the unsubstituted compound, II*a*. In addition, II*g*, possessing a shorter alkyl chain than II*a*, had greater toxicity. The mammalian toxicity increased in the dichloro derivatives II*d*–II*f* in the same sequence as the increases in activity in the P-388 screen. Compounds II*a*–II*c* showed no appreciable activity against the Ehrlich ascites tumor in mice (Table VI).

The allyl alcohol (III*a*) formed by reduction of I*d* showed only minor improvements in the L-1210 screen when the injections were given every 4 days. Both compounds were nontoxic at 400 mg/kg. Reduction of the Mannich bases II*a* and II*b* gave III*b* and III*d*, respectively. Both of these compounds had similar levels of toxicity and inactivity in the L-1210 screen as their precursors, although III*b* had a marginal level of activity (T/C 119%) when the injections were made every 4 days. While esterification of III*b* to give III*f* did not alter mammalian toxicity, quaternization of III*b* as the free base gave III*c*, with sharply increased mammalian toxicity. Esterification of III*d* gave III*e*, which showed reduced mammalian toxicity, but neither of the esters III*e* and III*f* had activity in the L-1210 screen. In the Ehrlich ascites screen (Table VI), III*b* and III*c* showed no activity but marginal activity (T/C 118%) was found for III*d*.

The possible metabolite of II*c*, namely V*b*, showed decreased toxicity in mice but no improvement in activity in the L-1210 screen (Table IV). The saturated alcohol (VI) was inactive and nontoxic at the dose levels examined.

Compounds I*a*, I*b*, I*d*, I*f*, I*h*, and I*i* were screened against *Streptococcus faecalis*, *Escherichia coli*, and *Candida albicans* at concentrations of 200 and 10 μg; the Mannich bases II*a*–II*c*, II*e*, and III*e* were screened against *S. faecalis* and *E. coli* at the same concentrations. Compound I*a* inhibited the growth of *S. faecalis* at 10 μg, and there was only partial growth of this organism when 10 μg of I*b*, I*d*, or I*f* was added to the medium. The remaining compounds were inactive against the organisms at a concentration of 200 μg.



I*m*: *n* = 4
I*n*: *n* = 0

In view of the fact that the potent diuretic ethacrynic acid possesses an α,β-unsaturated keto group attached to an aromatic ring containing two chlorine atoms, it was decided to examine II*d* and II*f* for possible diuretic activity. At 15 mg/kg, II*d* and II*f* showed marked antidiuretic activity with approximately 37 and 39%, respectively, of the urine output of the control rats. At 2 mg/kg, II*d* had 72% of the urine output of the control rats while II*f* had neither antidiuretic nor diuretic activity.

EXPERIMENTAL[1]

Substituted Styryl Ketones (I*a*–I*l*)—The preparation of I*a*, I*b*, I*d*–I*h*, I*j*, and I*k* was described previously (32). Utilization of the general method used earlier (32) gave *(E)*-1-(*m*-chlorophenyl)-1-nonen-3-one (I*c*) as colorless crystals (33%), mp 34°; IR (KBr): 1695 (s) (C=O), 1665 (s) (C=O), 1615 (s) (C=C), and 980 (s) (*trans*-CH=CH) cm^{-1}; NMR (CCl_4, 0.5 *M*): δ 7.33 (m, 5, C_1H and aromatic H), 6.55 (d, 1, $J_{2,1}$ = 16 Hz, C_2H), 2.53 (t, 2, $J_{4,5}$ = 6.5 Hz, C_4H_2), 1.87–1.10 (m, 8, CH_2), and 0.90 (m, 3, C_9H_3) ppm; mass spectrum: 250 (M^+, relative intensity 15%).

Anal.—Calc. for $C_{15}H_{19}ClO$: C, 71.84; H, 7.64. Found: C, 71.99; H, 7.51.

(E)-1-(*p*-Dimethylaminophenyl)-1-nonen-3-one methiodide (I*i*) was prepared by heating under reflux a mixture of I*h* (20.00 g, 0.077 mole), methyl iodide (21.86 g, 0.154 mole), and ethanol (20 ml) as brown platelets (19.69 g, 64%) from methanol, mp 142° dec; IR (KBr): 1695 (s) (C=O), 1665 (s) (C=O), and 1620 (C=C) cm^{-1}; NMR ($CDCl_3$): δ 7.80 (m, 5, C_1H and aromatic H), 6.75 (d, 1, $J_{2,1}$ = 16 Hz, C_2H), 4.03 [s, 9, $^+N(CH_3)_3$], 2.67 (t, 2, $J_{4,5}$ = 6.5 Hz, C_4H_2), 2.00–1.10 [m, 8, $(CH_2)_4$], and 0.90 (m, 3, C_9H_3) ppm.

Anal.—Calc. for $C_{18}H_{28}INO$: C, 53.87; H, 7.03. Found: C, 54.05; H, 7.22.

(E)-1-(*p*-Nitrophenyl)-1-penten-3-one (I*l*) was prepared in 7% yield from 2-butanone and *p*-nitrobenzaldehyde, according to a literature method (19), as orange crystals from ethanol, mp 108.5° [lit. (19) mp 108–110°]; IR (KBr): 1695 (s) (C=O), 1670 (s) (C=O), and 1620 (s) (C=C) cm^{-1}; NMR ($CDCl_3$, 0.5 *M*): δ 8.23 (m, 2, aromatic H), 7.63 (m, 3, C_1H and aromatic H), 6.85 (d, 1, $J_{2,1}$ = 16 Hz, C_2H), 2.75 (q, 2, $J_{4,5}$ = 7.5 Hz, C_4H_2), and 1.18 (t, 3, $J_{5,4}$ = 7.5 Hz, C_5H_3) ppm.

Anal.—Calc. for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.55; H, 5.45; N, 6.87.

In an attempt to prepare *(E)*-1-(*p*-nitrophenyl)-1-nonen-3-one, 2-octanone (16.03 g, 0.125 mole) and *p*-nitrobenzaldehyde (3.78 g, 0.025 mole) were added to piperidine acetate (from 2.1 g from piperidine and 2.0 g acetic acid). The mixture was stirred at room temperature for 2 hr and then poured onto ice; the precipitate was collected and recrystallized from ethanol to give unreacted *p*-nitrobenzaldehyde (0.445 g) (melting point and NMR evidence). Extraction of the aqueous phase with ether (3 × 40 ml) gave a brown oil (0.63 g), shown by GLC analysis to consist of at least seven components.

Preparation of 3-Benzylidene-2-alkanones (I*m* and I*n*)—A stirred mixture of 2-octanone (12.82 g, 0.10 mole) and benzaldehyde (10.61 g, 0.10 mole) was cooled (−5°), and hydrogen chloride gas (1.82 g, 0.05 mole) was bubbled through the reactants. The mixture was stirred at room temperature for 24 hr and heated under reflux for 2.5 hr to give a dark-brown oil, which was distilled to give a yellow oil, 6.60 g, bp 130°/9.0 mm [lit. (33) bp 161–162°/14 mm]. GLC analysis showed that two products were present, the major one (I*m*) to the extent of 95%; IR (neat): 1660 (s) (C=O) and 1620 (m) (C=C) cm^{-1}; NMR (CCl_4): δ 7.29 (m, 6, phenyl H and benzylidene H), 2.31 (broad s, 5, C_1H_3 and C_4H_2), 1.60–1.10 [m, 6, $(CH_2)_3$], and 0.90 (m, 3, C_8H_3) ppm.

GLC analysis of the crude reaction product between benzaldehyde and 2-butanone indicated a mixture of I*j* (84%) with a longer retention time than I*n* (16%). The mixture was separated using preparative GLC (10% Carbowax 20M, 215°). The characteristics of I*n* are as follows: IR (neat): 1665 (s) (C=O) and 1625 (s) (C=C) cm^{-1}; NMR (CCl_4): δ 7.30 (m, 6), 2.40 (s, 3), and 2.00 (d, 3, J = 2Hz) ppm; mass spectrum: 160 (M^+, relative intensity 100%).

3,5-Bis(*p*-dimethylaminophenyl)-4-heptanoyl-2-pentylcyclohexanone (IV)—This compound was obtained from the reaction between *p*-dimethylaminobenzaldehyde (74.58 g, 0.50 mole) and 2-octanone (64.10 g, 0.50 mole) in ethanol (340 ml) and 25% aqueous sodium hydroxide solution (30 ml). After the reaction mixture had been stirred at room temperature for 1 hr, it was set aside for 22 hr at room temperature; then I*h* (61.98 g, 48%) was removed by filtration. The mother liquor was concentrated *in vacuo* to give a semisolid. TLC on silica gel, using benzene–acetic acid–95% ethanol (10:1:1 v/v), showed that I*h* and a less mobile component were present. The semisolid was washed with the minimum quantity of ethanol to give a colorless powder (3.97 g), which was removed by filtration. Recrystallization from ethanol–ethyl acetate (1:1 v/v) gave IV (2.293 g, 1.8%), with an R_f value corresponding to the less mobile compound on TLC, mp 176–177° [lit. (24) mp 184–186°]; IR (KBr): 1720 (s) (C=O) and 1695 (s) (C=O) cm^{-1}; mass spectrum: 518 (M^+, relative intensity 100%).

Anal.—Calc. for $C_{35}H_{50}N_2O_2$: C, 78.71; H, 9.72; N, 5.40. Found: C, 78.90; H, 9.74; N, 5.18.

Substituted 4-Dimethylaminomethyl-1-phenyl-1-nonen-3-one Hydrochlorides (II*a*–II*g*) and V*a* and V*b*—The preparation of II*a*–II*f* and V*a* and V*b* was described previously (27). *(E)*-5-Dimethylamino-4-methyl-1-phenyl-1-penten-3-one hydrochloride (II*g*) was prepared by the general method (27), crystallizing as a colorless hygroscopic powder (43%) from acetone, mp 114–115° [lit. (34) mp 115–117° for 1:1 maleate salt]; IR (KBr): 1685 (s) (C=O), 1660 (w) (C=O), 1615 (s) (C=C), and 975 (s) (*trans*-CH=CH) cm^{-1}; NMR ($CDCl_3$, 0.5 *M*): δ 12.5–11.7 (broad s, 1, ^+NH, exchanged with D_2O), 7.75 (d, 1, $J_{1,2}$ = 16 Hz, C_1H), 7.47 (m, 5, phenyl H), 6.82 (d, 1, $J_{2,1}$ = 16 Hz, C_2H), 3.73 (m, 2, C_5H_2), 3.33–2.50 [m, 7, C_4H and $^+N(CH_3)_2$], and 1.33 (d, 3, J = 6.5 Hz, C_4CH_3) ppm.

Anal.—Calc. for $C_{14}H_{20}ClNO$: C, 66.26; H, 7.94; N, 5.52. Found: C, 65.75; H, 7.90; N, 5.48.

Substituted 1-Phenyl-1-nonen-3-ols (III*a*–III*d*) and Related Compounds (III*e*, III*f*, and VI)—The preparation of III*a*, VI, and diastereoisomeric modifications of III*b*–III*d* and III*f* was reported previously (28). The samples evaluated in the L-1210 screens (Table III) were the major diastereoisomers in the cases of III*d* and III*f* and a preponderance of the major isomers from the mixture in the cases of III*b*, mp 122–122.5° [lit. (28) mp 126°] and III*c*, mp 110–112° [lit. (28) mp 112.5–113.5°]. The alcohols III*b* (mp 120–121.5°) and III*c* (mp 110–112°) were screened against the Ehrlich ascites tumor (Table VI). The III*d* alcohol was a pure sample of the major isomer (NMR evidence).

The synthesis of III*e* was accomplished by the esterification of a diastereoisomeric mixture of *(E)*-1-(*o*-chlorophenyl)-4-dimethylaminomethyl-1-nonen-3-ols (5.10 g, 0.0164 mole) (28) with *p*-nitrobenzoyl chloride (3.70 g, 0.020 mole) in ether, using the method previously described (28), except that the time of stirring at room temperature was 24 hr. The colorless crystals deposited (6.73 g), mp 140–144°, were recrystallized from ethanol to give III*e* (1.33 g, 16%), mp 177° dec. The compound was homogeneous on silica gel thin-layer plates [R_f 0.67; *n*-butanol–acetic acid–water (12:3:5 v/v)]; IR (KBr): 1715 (s) (C=O), 1530 (s) (NO_2), and 965 (s) (*trans*-CH=CH) cm^{-1}; NMR ($CDCl_3$): δ 12.93–12.27 (broad s, 1, ^+NH, exchanged with D_2O), 8.28 (s, 4, *p*-$NO_2C_6H_4$), 7.37 (m, 5, C_1H and

[1] Melting points were determined on a Gallenkamp MF-370 apparatus. Boiling points and melting points are uncorrected. The NMR spectra were determined using a Varian T-60 spectrometer, with tetramethylsilane as the internal standard. IR absorption spectra were recorded on a Unicam SP-200G spectrophotometer previously calibrated with polystyrene. Band intensities are denoted as s (strong), m (medium), and w (weak). Mass spectra were determined at 70 ev on an AE1 MS-12 single-focusing mass spectrometer, operated by Mr. D. Bain of the Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. The instrument used a heated inlet system operating near the melting point of the compound, and samples were introduced by direct probe technique. Elemental analyses were performed by Dr. F. B. Strauss, Microanalytical Laboratories, Oxford, England, and by Mr. R. M. Smith of the College of Pharmacy, University of Saskatchewan, Saskatoon, who used a Coleman model 33 carbon–hydrogen analyzer.

TLC plates, 0.5 mm thick, were prepared using silica gel G (E. Merck and Co.) and subsequently heated at 120° for 2 hr prior to use. The chromatograms were developed (20–120 min), and the compounds were detected with a spray composed of a 1% (w/v) aqueous solution of potassium permanganate containing 1% sulfuric acid. GLC was undertaken on a Pye 104 model 64 instrument using the conditions previously described (27). For preparative GLC, a Hewlett-Packard 5750B research instrument was employed. The aluminum columns [1.8 m × 0.952 cm (6 ft × 0.375 in.) o.d.] were packed with 10% Carbowax on Chromosorb G, 60–80 mesh, acid washed and silanized. Organic extracts were washed with water and dried over anhydrous magnesium sulfate.

Table V—Activity of Some Styryl Derivatives against P-388 Lymphocytic Leukemia

Compound	Vehicle[a]	Route of Injection	Interval[b] of Injection, days	Number of Injections	Dose per Injection, mg/kg	Survivors[c] (out of 6)	Animal[d] Weight Difference, g	T/C, %[e]
Ic	2	Intraperitoneal	4	3	400	6	+1.2	100
					200	6	+0.5	0
					100	6	+0.7	88
	T	Intraperitoneal	1	9	400[g]	6	−0.7	104
					200[g]	6	−0.8	104
					100[g]	6	−0.5	108
IId	2	Intraperitoneal	1	9	25[g]	6	−2.1	115
					12.5[g]	6	−1.9	125
					6.25[g]	6	−0.6	90
	2	Intraperitoneal	1	9	18[g]	6	−3.3	130
					12.5[g]	6	−2.2	120
					8.3[g]	6	−0.2	110
	D	Intraperitoneal	1	9	35	6	−3.5	110
	2	Intraperitoneal	1	9	18	5	−2.8	122
					12	6	−1.9	122
					8.0	6	−2.3	122
IIe	M	Intraperitoneal	1	9	25	4	−5.4	60
					6.25	6	−5.8	110
					12.5	6	−0.7	100
	M	Intraperitoneal	4	3	12.5	6	−0.6	104
					6.25	6	+0.2	109
					3.12	6	+0.5	104
					40	6	−4.0	90
					28	6	−2.5	115
					18	6	−4.9	105
	2	Intraperitoneal	1	1	28	6	−1.7	100
					14	6	−1.0	110
					7.0	6	+0.3	110
					95	3	—	—
					65	6	−1.8	104
					40	6	−1.7	109
IIf	2	Intraperitoneal	1	9	80	0	—	—
					55	0	—	—
					35	0	—	—
					25	5	−4.1	136
					12.5	6	−3.7	126
					6.25	6	−2.2	142
					4.12	6	−1.7	105
					2.75	5	+1.1	110
	2	Intraperitoneal	1	1	400	0	—	—
					200	0	—	—
					100	2	—	—
					50	6	−1.6	100
					25	6	0	106
					12.5	6	+0.3	106
					6.25	6	+0.1	106
IIg	2	Intraperitoneal	1	9	110	2	—	—
					75	6	−0.8	109
					37.5	6	0	109
					18.7	6	−0.2	100
	2	Intraperitoneal	4	3	225	0	—	—

[a–e,g] See Table I.

o-ClC_6H_4), 7.27 (m, 2, C_2H and C_3H), 3.53–2.37 [m, 9, C_4H and $CH_2N(CH_3)_2$], 2.07–1.10 [m, 8, $(CH_2)_4$], and 0.90 (m, 3, C_9H_3) ppm.

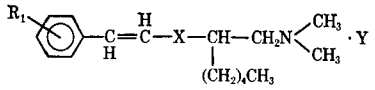
Anal.—Calc. for $C_{25}H_{32}Cl_2N_2O_4$: C, 60.60; H, 6.51; N, 5.67. Found: C, 60.64; H, 6.55; N, 5.75.

Attempted Preparation of *(E)*-1-(*p*-Dimethylaminophenyl)-1-nonen-3-ol—A solution of sodium borohydride (1.135 g, 0.03 mole) in water (18 ml, adjusted to pH 8.5 with aqueous sodium hydroxide solution) was added dropwise to a solution of Ih (7.780 g, 0.03 mole) in methanol (45 ml) at 0° under nitrogen. The reaction mixture was stirred at 0° for 1 hr and then at room temperature for 19 hr, after which the solvents were removed *in vacuo* to give a yellow residue; this residue was added to water (150 ml) at pH 4. The pH of the reaction mixture was adjusted to pH 7.5 and extracted with ether to give a yellow solid (7.30 g). TLC of the solid on silica gel, using *n*-butanol–acetic acid–water (12:3:5 v/v), showed the presence of at least two poorly resolved components. NMR spectroscopy indicated a mixture of Ih (~30%) and the desired alcohol (~70%); NMR ($CDCl_3$): δ 6.62 (dd, $J_{2,1}$ = 16 Hz, $J_{2,3}$ = 6.5 Hz, C_2H), 4.20 (q, J = 6 Hz, C_3H), and 1.90 (s, C_3OH, exchanged with D_2O) ppm. Fractional crystallization from petroleum ether, hexane, methylene chloride, and ethanol was unsuccessful. The crude solid changed to a red oil upon storage in a vacuum desiccator.

1,2-Epoxy-1-(*p*-chlorophenyl)-3-nonanone—A solution of hydrogen peroxide (4.25 g, 0.125 mole, 14.2 ml of 30% solution) in aqueous sodium hydroxide solution (5% w/v, 15 ml) was added dropwise to a solution of Id (3.135 g, 0.0125 mole) in dioxane (30 ml) at 10°. The mixture was stirred at 10–12° for 0.5 hr and, after standing at room temperature for 16 hr, water (350 ml) was added and the resultant precipitate was removed by filtration and dried overnight in a vacuum desiccator protected from light. The colorless precipitate (2.505 g, 75%), mp 61–62°, was recrystallized from petroleum ether (bp 60–80°), which did not alter the melting point. The title epoxide migrated as a single spot on silica gel thin-layer plates, using benzene–acetic acid–water (10:1:1 v/v) as the developing solvent; IR (KBr): 1710 (s) (C=O) cm^{-1}; NMR (CCl_4, 0.5 *M*): δ 7.20 (m, 4, aromatic H), 3.87 (d, 1, $J_{1,2}$ = 2.0 Hz, C_1H), 3.25 (d, 1, $J_{2,1}$ = 2.0 Hz, C_2H), 2.40 (m, 2, C_4H_2), 1.73–1.07 [m, 8, $(CH_2)_4$], and 1.07–0.67 (m, 3, C_9H_3) ppm.

A sample of the epoxide became yellow, mp 48–49°, after stand-

Table VI—Activity of Some Styryl Derivatives against Ehrlich Ascites Tumor in Mice



Compound	R_1	X	Y	Dose, mg/kg[a]	T/C, %
II*a*	H	C=O	HCl	10	103
II*b*	2-Cl	C=O	HCl	25	111
				10	108
II*c*	4-Cl	C=O	HCl	25	110
				10	111
III*b*	H	CH(OH)	HCl	25	101
III*c*	H	CH(OH)	CH_3I	25	104
III*d*	2-Cl	CH(OH)	HCl	50	118
				25	117

[a] The same dose was given for 7 consecutive days. The control animals received injections of physiological saline.

ing at room temperature for 3 days; after 5 days, it became a yellow oil. Another sample of the title compound was stored at 0°; after 10 and 30 days, it melted at 59–60 and 51–54°, respectively, after which it became a yellow oil [IR (neat) showed absorptions at 3350 and 1630 cm^{-1}, which were absent in the pure epoxide].

Screening of Compounds—The screening results listed in Tables I–V were carried out by the Drug Research and Development Division of the National Cancer Institute, Bethesda, Md., using the N.C.I. protocols (35). Unless otherwise stated, the BDF_1 strain of mouse was used as the host. In the evaluation of the compounds against the Ehrlich ascites tumor[2] (Table VI), the compounds were dissolved in physiological saline and injected intraperitoneally 24 hr after inoculation with 10^6 tumor cells. Ten tumor-bearing female Swiss mice were used in each experiment, with the 10 control animals receiving physiological saline intraperitoneally.

The antimicrobial evaluations were carried out using several strains of microorganisms[3]. The compounds were dissolved in methanol and diluted with sterile broth. *S. faecalis* (stock number 40) and *E. coli* (stock number 7) were added to the culture and incubated at 37°; growth was checked at 18 and 24 hr. The culture media containing the compounds and *C. albicans* (stock number 96) were incubated at 25°.

In the screen to assess the potential of II*d* and II*f* as diuretic agents, adult male albino rats of the Wistar strain, weighing between 225 and 275 g, were fasted overnight (18 hr) with water *ad libitum*. The rats were divided into groups of eight, one control group and one group for each dose level of 2 and 15 mg/kg. Each rat received an oral load of 2.4 ml of 0.9% NaCl/100 g of body weight, administered by means of a graduated syringe fitted with a stomach tube. The saline–drug solution or suspension (1.0 ml) was administered to each rat intraperitoneally and, in addition, the control group received 1 ml/kg of 0.9% NaCl intraperitoneally.

The rats were placed in metabolism cages and urine was collected and recorded for a 6-hr period. It was found that 25 control groups of eight rats excreted 52.58% of the volume of the saline load with a standard deviation of 11.04%. At 15 mg/kg, II*d*-, II*f*-, and hydrochlorothiazide-treated rats excreted 19.37[4], 20.50, and 137.38% of the volume of the saline load, respectively. At 2 mg/kg, II*d*-, II*f*-, and hydrochlorothiazide-treated rats excreted 37.65, 52.79, and 113.52% of the volume of the saline load, respectively.

REFERENCES

(1) T. Posner, *Chem. Ber.*, **35**, 799(1902).

(2) *Ibid.*, **37**, 502(1904).

(3) W. J. C. Ross, "Biological Alkylating Agents," Butterworth and Co., London, England, 1962, chap. 3.

(4) C. E. Lough, D. J. Currie, and H. L. Holmes, *Can. J. Chem.*, **46**, 771(1968).

(5) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, **168**, 376(1970).

(6) F. W. Menger and J. H. Smith, *J. Amer. Chem. Soc.*, **91**, 4211(1969).

(7) E. Boyland and L. F. Chasseaud, *Biochem. J.*, **104**, 95(1967).

(8) *Ibid.*, **109**, 651(1968).

(9) W. von Werner, W. Jungstand, and W. Gutsche, *Arzneim.-Forsch.*, **20**, 246(1970).

(10) H. Schoenenberger, T. Bastug, L. Bindl, A. Adam, D. Adam, A. Petter, and W. Zwez, *Pharm. Acta Helv.*, **44**, 691(1969); through *Chem. Abstr.*, **72**, 65748f(1970).

(11) J. Packer and J. Vaughan, "A Modern Approach to Organic Chemistry," Oxford, London, England, 1958, p. 184.

(12) W. A. Uricchio, *Proc. Helminthol. Soc.*, **18**, 136(1951); through *Chem. Abstr.*, **48**, 6650c(1954).

(13) C. C. J. Culvenor, *J. Pharm. Sci.*, **57**, 1112(1968).

(14) C. C. J. Culvenor, A. T. Dann, and A. T. Dick, *Nature*, **195**, 570(1962).

(15) J. D. Gettler and L. P. Hammett, *J. Amer. Chem. Soc.*, **65**, 1824(1943).

(16) H. Zimmer and J. Rothe, *J. Org. Chem.*, **24**, 28(1959).

(17) J. R. Dimmock, P. L. Carter, and P. D. Ralph, *J. Chem. Soc. B*, **1968**, 698.

(18) I. P. Sword, *J. Chem. Soc. C*, **1970**, 1916.

(19) M. Stiles, D. Wolf, and G. V. Hudson, *J. Amer. Chem. Soc.*, **81**, 628(1959).

(20) M. Regitz, F. Menz, and A. Liedhegener, *Justus Liebigs Ann. Chem.*, **739**, 174(1970).

(21) A. D. Cross, "Introduction to Practical Infra-Red Spectroscopy," Butterworth Scientific Publications Ltd., London, England, 1960, p. 58.

(22) M. E. Kronenberg and E. Havinga, *Rec. Trav. Chim.*, **84**, 17(1965).

(23) W. P. Hayes and C. J. Timmons, *Spectrochim. Acta*, **24A**, 323(1968).

(24) A. T. Nielsen and H. J. Dubin, *J. Org. Chem.*, **28**, 2120(1963).

(25) I. M. Heilbron and F. Irving, *J. Chem. Soc.*, **1928**, 2323.

(26) *Ibid.*, **1929**, 931.

(27) J. R. Dimmock and W. G. Taylor, *J. Pharm. Sci.*, **63**, 69(1974).

(28) W. G. Taylor and J. R. Dimmock, *Can. J. Chem.*, **52**, 2522(1974).

(29) J. A. Stock, in "Drug Design," vol. II, E. J. Ariens, Ed., Academic, New York, N.Y., 1971, p. 542.

(30) A. Goldin and H. B. Wood, Jr., in "Biological Effects of Alkylating Agents," *Ann. N.Y. Acad. Sci.*, **163**, 976(1969).

(31) N. R. Farnsworth, *J. Pharm. Sci.*, **62** (5), iv(1973).

(32) P. J. Smith, J. R. Dimmock, and W. G. Taylor, *Can. J. Chem.*, **50**, 871(1972).

(33) M. T. Bogert and D. Davidson, *J. Amer. Chem. Soc.*, **54**, 334(1932).

(34) C. R. Ganellin and R. G. W. Spickett, *J. Med. Chem.*, **8**, 619(1965).

(35) *Cancer Chemother. Rep.*, **25**, 1(1962).

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[4] Figure is the mean percentage excretion from five animals.

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Indolizines II: Search for Potential Oral Hypoglycemic Agents

A. U. DE [x] and B. P. SAHA

Abstract □ A few 1,2-bis(*N*-alkylaminomethyl)indolizines, simple indolizinecarboxylic acids, and several 6-alkoxyindolizine-2-carboxylic acids were synthesized and screened as possible oral hypoglycemic agents. The absence of any significant hypoglycemic activity excludes these compounds from the predicted structural lead provided by some hypoglycemic *Vinca* alkaloids, such as vincamine, vindoline, and vindolinine, having the indolizine ring as one structural component. But an extension of the rationale that indolizines are also the structural components of some carcinolytic *Vinca* alkaloids, such as vincristine and vinblastine, used in cancer chemotherapy provided encouraging results. One indolizine derivative showed significant antineoplastic activity in Ehrlich ascites carcinoma.

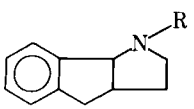
Keyphrases □ Indolizine derivatives—synthesized and screened as possible oral hypoglycemic agents □ Hypoglycemic agents, potential—synthesis and screening of indolizine derivatives □ Antineoplastic agents—screening of indolizine derivatives

The rationale for undertaking the synthesis of some *N*-alkyl 1,2,3,3a,4,8b-hexahydroindeno[1,2-*b*]pyrroles (I) and 2-(*N*-alkylaminomethyl)indolizines (II) as possible oral hypoglycemic agents was discussed previously (1, 2). Three additional compounds (I, R = *n*-pentyl, and II, R = *n*-pentyl or *n*-hexyl) were prepared according to the reported methods (1, 2) and biologically evaluated (Table I).

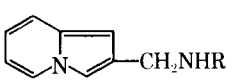
The failure of the compounds (II) to show any significant activity might be due to some undesirable biotransformation taking place through the very active 1- and 3-positions of II in any of the four intermediate steps of absorption, transport, barrier passage, and metabolism. Therefore, indolizine derivatives of type III, having only one active position free, were synthesized (Scheme I).

EXPERIMENTAL

Chemistry—Ethyl 2-pyridyl acetate (III*b*), prepared from 2-picolyllithium (III*a*) and ethyl carbonate according to the modification proposed by Goldberg *et al.* (3), was condensed with ethyl bromopyruvate to furnish indolizine 1,2-dicarboxylate (III*c*) (4). The diester was treated with various alkylamines to give 1,2-bis(*N*-alkylcarboxamido)indolizines (III*d*) and subsequently reduced with lithium aluminum hydride to 1,2-bis(*N*-alkylaminomethyl)-indolizines (III). The reaction between III*c* and an amine might also lead to an imide (III*e*) under the conditions followed, but the usual elemental analysis and IR and NMR spectra confirmed the diamide structure (III*d*).



I



II

Table I—Hypoglycemic Activity[a]

Compound	Compound Type	R	Maximum Blood Sugar Lowering, %
1	I	H	5
2	I	*n*-C_5H_{11}	7
3	II	*n*-C_5H_{11}	12
4	II	*n*-C_6H_{13}	10
5	III	CH_3	5
6	III	C_2H_5	Nil
7	III	*n*-C_3H_7	Nil
8	III	*n*-C_4H_9	Nil
9	III	*n*-C_5H_{11}	5
10	IV	H	5
11	IV	COOH	4
12	V	CH_3	3
13	V	C_2H_5	11
14	Tolbutamide		40

[a] Hypoglycemic tests were carried out by Central Drug Research Institute, Lucknow, India.

The synthesized compounds (III) were inactive. The introduction of two basic side chains with one active position free in III abolished the activity in comparison to II. It appeared that introducing an activating group and an acid function in the indolizine moiety while keeping positions 1 and 3 free might lead to better biological response. Moreover, various carboxylic acids and their derivatives, such as 5-methylpyrazole-3-carboxylic acid, 5-methylisoxazole-3-carboxylic acid (5), salicylic acid, and mesoxalic acid (6), have significant activity.

The simple indolizinecarboxylic acids (IV: R = H or COOH) were already known (4, 7) and prepared accordingly. 2-Methylpyridine-5-sulfonic acid (V*b*), obtained from 2-picoline (V*a*) by sulfonation with fuming sulfuric acid (8), was fused with potassium hydroxide to 5-hydroxy-2-methylpyridine (V*c*) and subsequently methylated with diazomethane to 5-methoxy-2-methylpyridine (V*d*: R = methyl) according to a modification of the method of Marion and Cockburn (9).

Attempts at methylation of V*c* with methyl iodide and dimethyl sulfate completely failed. However, ethylation of V*c* with diethyl sulfate led to 5-ethoxy-2-methylpyridine (V*d*: R = ethyl). The failure in alkylation with dimethyl sulfate and methyl iodide might be due to the weaker polarized character in C—O and C—I bonds of these molecules in comparison to diethyl sulfate, the ionic character being a necessary feature for this type of reaction.

Condensation of 5-alkoxy-2-methylpyridines (V*d*) with ethyl bromopyruvate and their subsequent cyclization with sodium bi-