Synthesis of Possible Cancer Chemotherapeutic Agents Based on Enzyme Rationale VI

Allylamine Derivatives

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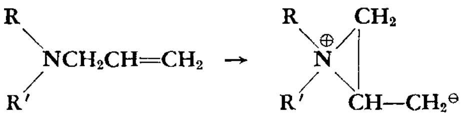
The participation of electron pair on the nitrogen atom with the olefinic π electron in allylamines makes possible the formation of an ethylenimonium-carbanion pair (I), a reactive intermediate analogous to the ethylenimonium ions that are formed



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in nitrogen mustards. The opportunity of such participation decreases as the electron density of the nitrogen is suppressed by the formation of an amide. This interesting participation reaction suggested the evaluation of a number of allylamine amides so that they could be mediated by the difference in amidase activity between normal and neoplastic tissues. Physical data support this novel participation, and preliminary antitumor screening data of several compounds are reported. *N,N'*-Diallyl β-aziridino propionamide was found to be an effective agent against Dunning leukemia in both solid and ascites forms.

In the design and preparation of new drugs, one of the interesting approaches is to take advantage of the difference in enzyme activity between normal and neoplastic cells (1–3).

A scheme is proposed to develop cancer chemotherapeutic agents which are acted upon *in vivo* by enzymes to liberate compounds toxic to the tumor, if the enzyme concentration is higher in the tumor. (Scheme I, where *A–B* represents a drug that can be acted upon at the site indicated by the dotted line, and where the asterisk designates the toxic moiety.)

$$A \text{-}\vdots\text{-} B \xrightarrow[\text{enzyme}]{} A^* + B$$

less or nontoxic than *A* — toxic agent

Scheme I

If the enzyme level is lower in tumor, then a more toxic compound that can be detoxified by enzyme in the normal cell but not in tumor cell should be used. (Scheme II.)

$$A \text{-}\vdots\text{-} B \xrightarrow[\text{enzyme}]{} A + B$$

more toxic than *A* or *B* to tumor — nontoxic

Scheme II

Thus, in such synthetic work, one is confronted with both what enzyme difference should be used and with what toxic agents should be incorporated. The choice of cytotoxic moiety, hemisulfur mustard, $ClCH_2CH_2SCH_2CH_2OH$, was used in early work (2). This was based on the low hematopoietic effect of this compound as reported by Seligman, Rutenberg, Persky, and Friedman (4). Subsequent preclinical work indicated that hemisulfur mustard compounds often exhibit neurotoxicity (5) which could complicate the clinical management of such types of drugs. Thus, the effort has continued along the line of synthesis of compounds with reduced neurotoxicity and/or drugs that incorporate other one-arm alkylating agents, such as hemi-nitrogen mustard[1] and aziridines (3). In addition, the authors also considered hexahydroazepine derivatives (6) as nonclassical alkylating agents. While much remains to be done in these compounds, the delay in hematopoietic effect in a pilot clinical trial (7) suggested that it would be worthwhile to consider also other nonclassical alkylating agents. The present paper reports a study of allylamine derivatives as potential alkylating agents, and the synthesis of allylamine amides as possible cancer chemotherapeutic agents based on the previously proposed rationale.

DISCUSSION

It is generally accepted that ethylenimonium ion is the active intermediate of nitrogen mustard

Received October 24, 1966, from the Harrison Department of Surgical Research, School of Medicine and Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104

Accepted for publication January 4, 1967.

Presented to the American Chemical Society, Atlantic City meeting, September 1965.

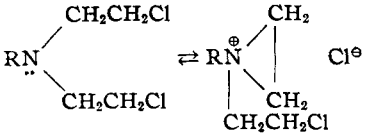
This investigation was supported by research grant CA-07339 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

The authors acknowledge the technical assistance of Mr. George Benson, Mr. H. Hoyt, and Mr. Jerry Ford, Dr. Gerald Peskin for advice in the dog experiments, and Dr. J. Venditti and Dr. H. Wood, Cancer Chemotherapy National Service Center, for the screening data.

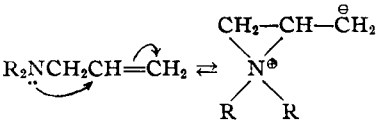
Previous paper: Tsou, K. C., and Su, H. C. F., *J. Med. Chem.*, 6, 693(1963).

[1] Tsou, K. C., unpublished data. Compounds cited in *Cancer Chemotherapy Rept.*, 1964.

(HN_2) (8) and that very likely biological alkylation is related to its formation (9). The formation of this transition state depends on the nucleophilic displacement of the nitrogen atom onto the carbon adjacent to the chlorine atom (Scheme III). Thus, it is not unreasonable to ask whether in allylamine derivatives a similar type of nucleophilic attack of electrons from the nitrogen atom could take place as shown in Scheme IV.



Scheme III



Scheme IV

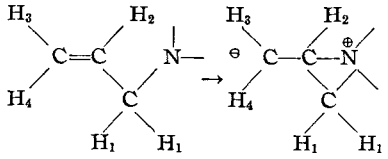
Such transition state could very well be favored if the carbanion could be stabilized by solvation as in the case of the chloride ion in HN_2. Thus, it is tempting to suggest that in the biological system allylamine might be stabilized through an internal ion pair and then act as potential alkylating agents. A careful investigation of the physical and chemical properties of some allylamine derivatives indeed suggested such possibilities could exist.

The pKa of allylamine (I), diallylamine (II), triallylamine (III), methyldiallylamine (IV), and dimethylallylamine (V) are listed in Table I (10). As the number of allyl group increases, the basicity decreases slightly in II, and significantly in III. This is in contrast to what is expected from the pKa values of ethylamine (10.63), diethylamine (10.93), and triethylamine (10.72), since increasing substitution generally results in slightly increased basicity. The substitution of methyl groups in IV and V also seems to result in a decrease in basicity, rather than the anticipated increase that one may expect from the hyperconjugation effect of the methyl group. It seems that these data could obviously be explained by an overlap of the electron pair on the nitrogen with the olefinic electrons. This is also suggested by their ultraviolet spectroscopy as shown in Table I, where additional suggestive evidence is apparent. Allylamine has a max. at 210 mμ which is clearly due to the participation of nitrogen electron with the π olefin, or a p–σ–π interaction in the triad =N—CH_2—CH= of diallylamine caused both bathochromic and hyperchromic effect as expected. The high intensity value (olefins are usually at 10–190 mμ) shows clearly that this is not due to the amine $n \rightarrow \sigma$ itself (usually ~1000) (11).

Examination of the infrared spectra of allylamine, diallylamine, and *N*-methyldiallylamine and other allylamines provides additional useful evidence of the formation of such "incipient ethylenimmonium ion." Thus, the strong 3075 cm.$^{-1}$ and 3005 cm.$^{-1}$ bands that are present in all aziridine derivatives are also in the substituted allylamines (12). In both diallylamine and methyldiallylamine, there was found the usual 2865 cm.$^{-1}$ methylene band and a very strong band at 2805 cm.$^{-1}$, but it is not so evident in monoallylamine. This 2805 cm.$^{-1}$ band can best be assigned as an unsymmetrical C—H stretching of the methylene group. The displacement of carbon 2–3 double bond to a single bond carbanion could contribute to an additional resonance vibration resulting in an increase in the intensity of this band.

A comparison of the nuclear magnetic resonance spectra of the allylamines reveals even more interesting data (Fig. 1).

As illustrated in Scheme V, if such intramolecular participation contributes to these compounds, the amine nitrogen should become less negative and the H_1 proton could be expected to shift to a higher field (or "curtain effect") (13). The chemical shifts



Scheme V

of H_1 in Table II indeed support this mechanism, especially when compared with propylamine. Thus, the theoretical H_1 value of dimethylallylamine (V) can be calculated from the Shoolery additive constant (14) to be 3.11, yet the found value is only 2.83. Correspondingly, an increase in carbanion character of the terminal allylic carbon is expected to cause chemical shifts of the terminal protons towards low field.[2] The slight increase in δ of H_3 in Table II obviously is in line with this expectation. There was no change in $J_{4,2}$ and $J_{3,2}$ in these compounds when compared with I in the liquid state, and there was also no significant change in J_{trans}/J_{cis} ratio. When one examines a molecular model, the approaching nitrogen to C_2 results actually in little change of the dihedral angle between $H_{4,2}$ and $H_{3,2}$; this is, very likely, why one may not expect any significant change in coupling constants.

More recently, Ferretti and Tesi (16) reported an observation of Prévost reaction in *N*-allylmorpholine in which they obtained a 2-morpholino-1,3-propandiol instead of the expected 1,2-diol. Independent of their work, a similar reaction of iodine chloride with *N*-allylpiperidine, followed by hydrolysis, has also been found by us to yield 2-piperididino-1,3-propanediol. These reactions thus provide indirect support for the formation of an ethylenimonium ion among allylamines.

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[2] The study of the chemical shift of proton attached to a carbanion has not hitherto been reported. It should especially be noted that the present case is not that of a delocalized allyl anion, CH_2=CH—$CH_2^{\ominus}$, where one might expect a shift to high field due to shielding. Where electron density can be localized on the terminal carbon, as in the present case, an anisotropic effect would predict a downfield shift. (See *Reference 13*, p. 176.) The nearest example the authors know of is the investigation of *N*-ethylallenimine by Roberts, who stated that the "vinyl proton does not change significantly with temperature" (15). While Roberts did not say whether it also shifted up or down slightly, it is important to note that the vinyl proton remained discrete, did not exchange with the ring proton, and therefore did not become involved in the following scheme:

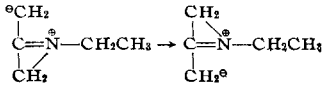

TABLE I—PHYSICAL PROPERTIES OF ALLYLAMINES

Compd.	B.p., ° C.	pKa[a]	U.V. Spectra[b] $\lambda_{max.}$ (mμ)	U.V. Spectra[b] $\epsilon_{max.}$	Retention Time,[c] sec.
Allylamine (I)	54–55	9.49	210.0	13,870	89
Diallylamine (II)	60–61.5	9.29	211.0	32,630	137
Triallylamine (III)	148–50.5	8.31	215.0	44,660	942
Methyldiallylamine (IV)	110.5–111.5	8.79	215.0	39,090	315
Dimethylallylamine (V)	60–61.5	8.72	213.0	26,550	120

[a] See *Reference 10.* [b] In 95% ethanol. [c] On amine-220 column (6 ft. × 1/4 in., 5% on Chromosorb P, 60–80 mesh); column temperature, 78°C.; collector temperature, 56°C., injector, 153°C.; detector, 227°C.; helium flow, 60 ml./min.; 1 μl. injection.

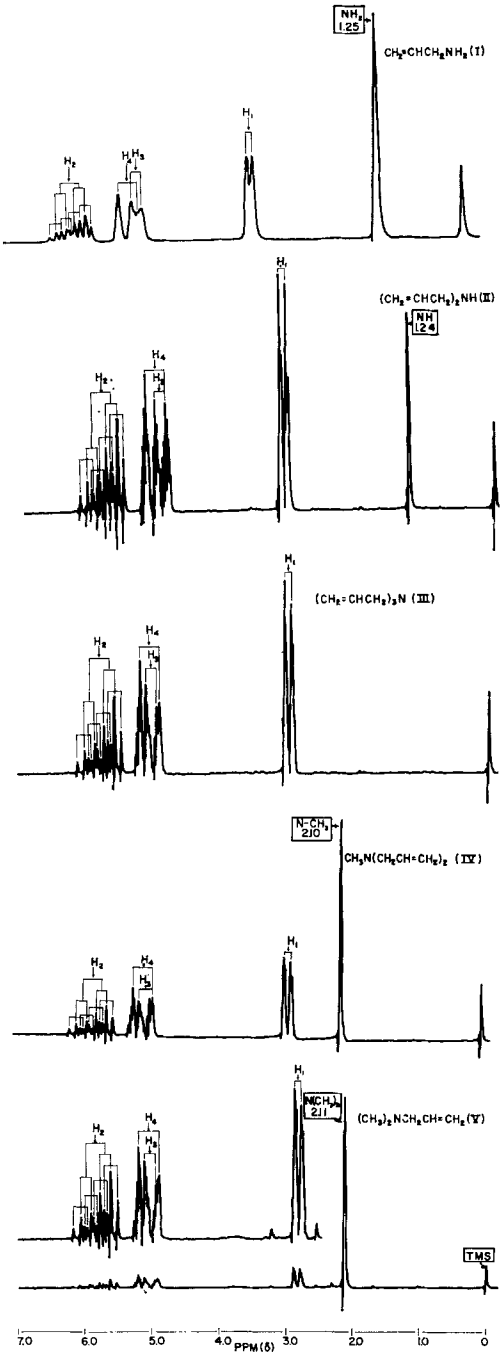

Fig. 1—Nuclear magnetic resonance spectra of allylamines.

While much of the assessment of the chemical data was in progress, exploratory screening of allylamine derivatives was simultaneously begun in different tumor systems through the generous cooperation of Cancer Chemotherapy National Service Center. Even though most of the data have appeared in their report (17), a discussion of these data in a summarized manner is certainly appropriate and not repetitious.

Allylamine, diallylamine, and triallylamine were compared in the sarcoma 180 system. The protocol of the screening has been described (17), and the results are summarized in Table III. It is of interest to note that diallylamine was found to have some tumor inhibitory activity in the sarcoma 180 system, even though the reproducibility in the confirmatory test was poor. No additional screening has been run in other systems.[8]

In another study, the authors were evaluating amidase hydrolysis of normal and neoplastic tissue on substrates of the *N*-acylaziridines (3), and occasionally high hydrolysis rate was noted in neoplastic tissue. First, if allylamine were to be incorporated in an amide, the electron density of the nitrogen atom would be suppressed and thereby reduce the opportunity for the allylamine to cyclize to an ethylenimonium ion. And yet, upon enzymatic hydrolysis, the allylamines could then undergo the incipient ethylenimonium ion formation as postulated.

In reviewing the literature on amidase activity, one noted that the hydrolysis of amides has been reported high in several tumors. Greenstein (18) reported fivefold increase in the hydrolysis activity of leucyl glycine in hepatoma over mouse liver. Goldbarg and Rutenberg (19) reported also the higher rate of hydrolysis of leucyl β-naphthylamide in cancer of the pancreas, using a method originally developed by Green, Tsou, Bressler, and Seligman (20). Burstone (21) and Hosoda, Takase, and Yoshida (22) also reported independently high activity in gastric cancer with the use of the same substrate. It thus seems of interest that a number of α- and β-amino diallyl amides might simulate closely the environmental requirement of the enzyme.

To survey the first requirement, *N*-allylacrylamide, *N,N'*-diallylacrylamide, and *N,N'*-diallylmethacrylamide were chosen for exploratory purposes. The use of acrylic acid derivatives was preferred because interesting results had been found when acrylamide (NSC No. 7785) was screened in its activity against adenocarcinoma 755. These allylamides were also compared in the CA-755 system. As can be seen readily (Table IV), *N,N*-diallylmethacrylamide did pass the primary screening

[8] One must add that the analogous methallylamines are not active in the CCNSC screening systems.

TABLE II—NUCLEAR MAGNETIC RESONANCE DATA OF ALLYLAMINES

Compd.	Chemical Shift[a] H_1	H_2	H_3	H_4	Coupling Constants[b] $J_{4,2}$	$J_{3,2}$	$J_{1,2}$	$J_{3,4}$
I	3.20	5.88	4.89	5.07	17.0	10.0	5.0	2.0
II	3.15	5.87	5.00	5.06	17.5	9.5	6.0	1.5
III	3.00	5.83	5.05	5.06	17.5	9.5	6.0	1.5
IV	2.90	5.80	5.00	5.02	17.5	9.5	6.0	1.5
V	2.83	5.87	5.03	5.06	17.5	9.5	6.0	2.0

[a] In p.p.m. from tetramethylsilane (TMS)$_1$ ± .02. [b] In c.p.s. ± 0.5.

TABLE III—COMPARISON OF ANTITUMOR ACTIVITIES OF ALLYLAMINE, DIALLYLAMINE, AND TRIALLYLAMINE IN SARCOMA 180

Dose, mg./Kg.	Survivors	Wt. Change, Gm. T/C[a]	Tumor Wt., mg. T/C[a]	% Inhibition
Allylamine, NSC No. 7600				
31.2	3/6	−3.0/−2.0	672/903	16
16.0	4/6	−1.0/−3.0	575/640	11
Diallylamine, NSC No. 20948				
415.0	2/6	+0.6/+3.2	430/1132	62
208.0	2/6	−2.6/+2.9	495/870	43
104.0	4/6	−1.9/+1.5	403/803	50
104.0	6/6	−2.4/−0.7	413/1339	69
104.0[b]	6/6	+2.3/+0.9	579/725	20
Triallylamine, NSC No. 32635				
500.0	4/6	−1.9/−2.1	415/711	42

[a] T/C, tumor/control. [b] In this group, one mouse has tumor weight 1090; one mouse did not respond (730).

TABLE IV—COMPARISON OF ACRYLAMIDE, *N*-ALLYLACRYLAMIDE, *N*-DIALLYLACRYLAMIDE, AND *N,N*-DIALLYLMETHACRYLAMIDE IN ADENOCARCINOMA 755

Dose, mg./Kg.	Survivors	Wt. Change, T/C[a]	Tumor Wt., mg., T/C[a]	% Inhibition
Acrylamide				
80	0/10	...	...	
40	8/10	1.7/3.2	146/627	77
40	9/10	0.1/0.9	238/645	64
40	10/10	2.3/3.1	533/757	30
20	10/10	2.1/2.4	2421/2795	14
N-Allylacrylamide				
55.8	10/10	−2.2/−0.3	1185/753	None
N,N-Diallyacrylamide				
110.0	9/10	−1.4/−0.5	784/948	19
N,N-Diallylmethacrylamide				
94	0/10	0.0/+4.4	0/1257	Toxic
47	7/10	−8.8/+2.2	39/711	94
47	9/10	−0.7/+2.2	1318/1817	27
47	10/10	+0.2/+2.3	1416/1830	23

[a] T/C, tumor/control.

system, even though it failed later in sequential tests. In the second group, one of the earliest compounds that we prepared is *N,N*-diallyl β-aziridinopropionamide (DAAP). It was prepared readily by the addition of ethylenimine to diallylacrylamide. While this series of compounds is still being extended, DAAP was found to be active in screening against the Dunning leukemia system (Table V), both in solid and ascites form. Curative doses

TABLE V—EVALUATION OF *N,N*-DIALLYL β-AZIRIDINOPROPIONAMIDE[a] IN DUNNING LEUKEMIA SYSTEM (SALINE, i.p.)

Dose, mg./Kg.	Survivors	Cures	Survival Time, Days	%
Solid (DA)				
40	6/6	6	30.0/10.0	300
20	6/6	6	30.0/10.0	300
10	6/6		21.0/10.0	210
5	6/6		16.0/10.0	160
Ascites (DL)				
40	6/6	5	30.0/13.5	222
20	6/6	6	30.0/13.5	222
10	6/6		25.0/13.5	185
5	6/6		17.5/13.5	129

[a] NSC No. 74431.

were effected in 20–40 mg./Kg. range. The remarkable low toxicity of DAAP was also found to be true in dog experiments where severe leucopenia were never noticed in 20–40 mg./Kg. dose in contrast to many known difunctional alkylating agents. Dimethyl β-aziridinopropionamide was also prepared in order to compare the alkylating effect of the β-aziridino group. Such a study will be reported elsewhere in a lymphoma system (23). The corresponding β-methylaziridino derivative was also made, but was found to be less effective.

It is obvious that much remains to be done to extend these observations and to verify our speculative rationale. Whether the low toxicity and effective antitumor activity of DAAP provides a worthwhile clue to our enzyme rationale remains to be shown. Such study is in progress.

EXPERIMENTAL

Allylamine, diallylamine, and triallylamine were obtained from the Shell Chemical Co. They were distilled free from inhibitor and sealed in ampuls under nitrogen for screening. For spectroscopic study, they were further purified by gas chromatography as shown in Table I.

N-Allylacrylamide and *N,N*-diallylacrylamide were obtained from the Monomer-Polymer Laboratory, Borden Chemical Co., and again purified just before sending to the screener.

N,N-Diallylmethacrylamide—To a solution of methacrylyl chloride (24) (20.92 Gm., 0.2 mole) in 50 ml. of dry benzene was added a mixture of diallylamine (19.5 Gm., 0.2 mole) and triethylamine (22.2 Gm., 0.2 mole) in 50 ml. of dry benzene. The addition was completed in 1 hr. The reaction mixture was stirred at room temperature overnight and filtered to remove triethylamine hydrochloride. The filtrate was evaporated under aspirator to

remove solvent and was distilled under high vacuum to yield 30.0 Gm. (89%) of the product, b.p. 90–92°/15 mm.

Anal.[4]—Calcd. for $C_{10}H_{15}NO$ (165.23): C, 72.69; H, 9.15; N, 8.48. Found: C, 72.57; H, 9.03; N, 8.19.

N,N-Diallyl β-Aziridinopropionamide—A 8.3-Gm. quantity of pure ethylenimine was added to 12 Gm. of *N,N*-diallylacrylamide (distilled, b.p. 108–110°/3.0 mm.) in 10 min. The solution was stirred for 2 hr. and allowed to stand overnight at room temperature. It was then distilled under reduced pressure to give 12.7 Gm. (80%) of the product, b.p. 110–114°/0.5 mm.; n_D^{20} = 1.4906; $d.^{25}$ = 0.9752. I.R. 3.17 μ (aziridine), 3.26, 3.32, 6.02, 6.78 μ.

Anal.—Calcd. for $C_{11}H_{18}N_2O$: C, 68.01; H, 9.34; N, 14.42. Found: C, 67.84; H, 9.41, N, 14.43.

N,N-Diallyl β-(2-Methyl)aziridinopropionamide—A 11.4 Gm. quantity of propyleneimine was added to 15.1 Gm. of *N,N*-diallylacrylamide in 10–15 min. with stirring and cooling. The solution was stirred continuously for 2 hr. and then allowed to stand overnight. The reaction mixture was then distilled under reduced pressure to yield 16.0 Gm. (77%) of *N,N*-diallyl β-(2-methyl)aziridinopropionamide, b.p. 101–102°/0.4 mm.; n_D^{20} = 1.4867; $d.^{26}$ = 0.9766.

Anal.—Calcd. for $C_{12}H_{20}N_2O$: C, 69.19; H, 9.68; N, 13.45. Found: C, 69.16; H, 9.90; N, 13.72.

N,N-Dimethyl β-Aziridinopropionamide—Ethylenimine (13.2 Gm., 0.3 mole) was added to a solution of dimethylacrylamide (14.9 Gm., 0.15 mole) in 30 min. with stirring. The mixture was allowed to stand overnight. The solvents were removed under aspirator and the product distilled under high vacuum to give 7.8 Gm. of the product, b.p. 71–72°/0.4 mm.; n_D^{20} = 1.4750; $d.^{24}$ = 0.9741.

Anal.—Calcd. for $C_7H_{14}N_2O$: C, 59.12; H, 9.92; N, 19.71. Found: C, 59.06; H, 9.89; N, 19.76.

Reaction of Allylpiperidine with Iodine Monochloride—To a benzene solution of ICl (1.6 Gm.) was added 1.3 Gm. of allylpiperidine slowly, and the reaction mixture was warmed for 10 min. and allowed to stand overnight. After removal of benzene, the residue turned reddish and failed to crystallize even though some yellow crystals appeared first. Purification of this oil was not successful. This mixture was then heated in an alkali suspension (4 *N* NaOH) and extracted with three 50-ml. portions of ether. The combined ether extract was dried with sodium sulfate and evaporated to dryness. An oily residue again resulted. This was distilled under reduced pressure to yield 0.6 Gm. of a lemon yellow oil, b.p. 149–152°/3 mm.; n_D^{27} = 1.4930, and analyzed to be 2-piperidino-1,3-propanediol (25).

Anal.—Calcd. for $C_8H_{17}NO_2$: C, 60.34; H, 10.77; N, 8.80. Found: C, 60.12; H, 10.52; N, 8.99.

[4] Microanalysis by Dr. S. M. Nagy, Belmont, Mass.

Similar reactions were carried out with methylallylamine, cyclohexylallylamine, and triallylamine, but the products were not separated as cleanly as in the case of allylpiperidine.

Gas chromatography was determined with a Wilken's Autoprep 700 under the conditions described in Table II.

Infrared spectra were determined on a Perkin-Elmer 421 diffraction-grating spectrophotometer, using 0.025 mm. KBr cell.

Nuclear magnetic resonance spectra were determined on a Varian HR-60 NMR spectrophotometer in the Department of Chemistry through the generous cooperation of Dr. C. C. Price.

Dog Toxicity Study of N,N-Diallyl β-Aziridinopropionamide—Three dogs each were given 20 and 40 mg./Kg. in a single injection of this compound in isotonic saline (50%) intravenously, and blood samples were withdrawn periodically and blood counts carried out in the Hematology Laboratory, University Hospital of Pennsylvania. All dogs survived. One dog was given 75 mg./Kg. This dog, however, died of distemper.

REFERENCES

(1) Seligman, A. M., Nachlas, M. M., Manheimer, L. H., Friedman, O. M., and Wolf, G., *Ann. Surg.*, **130**, 333 (1949).

(2) Tsou, K. C., Su, H. C. F., Segarbath, C., and Mirachi, U., *J. Org. Chem.*, **26**, 4987(1961).

(3) Tsou, K. C., Su, H. C. F., and Hoegerle, K., *J. Med. Chem.*, **6**, 435(1963).

(4) Seligman, A. M., Rutenberg, S. M., Persky, L., and Friedman, O. M., *Cancer*, **5**, 354(1952).

(5) Green, T. H., *Obstet. Gynecol.*, **13**, 383(1959).

(6) Tsou, K. C., and Hoegerle, K., *J. Med. Chem.*, **6**, 47(1963).

(7) Goodman, L. E., Bakal, D., Tsou, K. C., Kramer, S. P., Ulfohn, A., Gaby, S. D., Dorfman, H. E., Aybar, O., Candana, E., Aschi, M. G., and Seligman, A. M., *Cancer*, **18**, 3, 307(1965).

(8) Bartlett, P. D., Ross, S. D., and Swain, C. G., *J. Am. Chem. Soc.*, **71**, 1415(1949).

(9) Price, C. C., *Ann. N. Y. Acad. Sci.*, **68**, 663(1958).

(10) King, J. F., "Elucidation of Structure by Physical Methods," Bentley, D. W., ed., Interscience Publishers, New York, N. Y., 1963, pp. 337–338.

(11) Mason, S. F., *Quart. Rev.*, **15**, 287(1961).

(12) Steward, J. E., *J. Chem. Phys.*, **30**, 1259(1959).

(13) Pople, J. A., Schneider, W. G., and Bernstein, H. F., "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 166.

(14) Jackman, L. M., "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 59.

(15) Roberts, J. D., "Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York. N. Y., 1959, p. 76.

(16) Ferretti, A., and Tesi, G., *Tetrahedron Letters*, **1964**, 2975.

(17) Leiter, J., Bourke, A. R., Fitzgerald, D. B., Shepartz, S. A., and Wodinsky, I., *Cancer Res. (Suppl.)*, **22**, 221(1962).

(18) Greenstein, J. P., "Biochemistry of Cancer," Academic Press Inc., New York, N. Y., 1954, p. 379.

(19) Goldbarg, J. A., and Rutenburg, A. M., *Cancer*, **11**, 283(1958).

(20) Green, M. N., Tsou, K. C., Bressler, R., and Seligman, A. M., *Arch. Biochem. Biophys.*, **57**, 458(1955).

(21) Burstone, M. S., *J. Natl. Cancer Inst.*, **16**, 1149 (1956).

(22) Hosoda, S., Takase, S., Yoshida, K., and Tohoku, J., *Expil. Med.*, **73**, 86(1960).

(23) Miller, E. M., and Tsou, K. C., *Proc. Am. Assoc. Cancer Res.*, 1966.

(24) Gotkis, D., and Cloke, J. B., *J. Am. Chem. Soc.*, **56**, 2710(1934).

(25) Cromwell, N. H., and Tsou, K. C., *J. Org. Chem.*, **15**, 1219(1950).