hr. On cooling, 0.1 g. (75%) of faintly tan needles separated. The product was dissolved in 50 ml. of 0.01*N* hydrochloric acid. Addition of 2 ml. of concd. ammonium hydroxide to the hot solution resulted in the separation of white needles.

Anal. Calcd. for $C_{14}H_{16}N_{10}$: C, 51.84; H, 4.97; N, 43.19. Found: C, 51.76; H, 5.08; N, 43.16.

Ultraviolet spectrum. 0.1 *N* hydrochloric acid: λ_{max} 267 mμ, ϵ_{max} 29,500.

1,4-Bis-8-(6-furfurylaminopurinyl)butane. A mixture of 0.1 g. (0.000269 mole) of bischloropurine (IV), 1 ml. of furfurylamine, and 20 ml. of ethylene glycol monomethyl ether was heated to reflux for 4 hr. After standing overnight at room temperature, the solution was reduced in volume to 4 ml. under aspirator suction at 60°. Addition of 4 ml. of absolute ethanol resulted in the separation of 0.08 g. (60%) of tan crystals. Recrystallization from ethanol-Methyl Cellosolve (1:1) yielded pale yellow needles.

Anal. Calcd. for $C_{24}H_{24}N_{10}O_2$: C, 59.49; H, 4.99; N, 28.91. Found: C, 59.15; H, 5.32; N, 28.61.

Ultraviolet spectrum. 0.1 *N* hydrochloric acid: λ_{max} 279 mμ, ϵ_{max} 38,700.

1,4-Bis(8-purinyl)butane (VIII). A suspension of 0.2 g. (0.000532 mole) of bismercaptopurine (V) in 30 ml. of 10% ammonium hydroxide was heated to boiling and treated with 0.8 g. of ethanol-wet Raney nickel. The mixture was permitted to reflux for 2 hr. and filtered immediately. On standing, 0.03 g. (19.5%) of yellowish white crystals separated from the colorless solution. The products was recrystallized from boiling water.

Anal. Calcd. for $C_{14}H_{14}N_8$: C, 57.13; H, 4.79; N, 38.08. Found: C, 57.20; H, 5.10; N, 38.40.

Ultraviolet spectrum. *p*H 10: λ_{max} 272 mμ, ϵ_{max} 19,200.

Acknowledgment. In the synthetic work described, Miss Elaine Perry gave valuable and patient assistance.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MICHIGAN]

Synthesis of Potential Anticancer Agents. VII. *N,N*-Ethyleneureido Analogs of Some Amino Acids[1,2]

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The present status in cancer chemotherapy of amino acid analogs containing alkylating groups has been reviewed. Synthesis of isocyanato esters of five selected analogs and incorporation of ethylenimine functions are described.

Many of the early attempts to design anticancer agents led to compounds which could be broadly classified as either alkylating agents or antimetabolites. More recently there has been an increasing tendency to combine these functional features in the same molecule and, as a result, a number of biologically familiar substances, such as pyrimidines, sugars, amino acids, and even steroids, have been modified by incorporation of β,β'-bischloroethylamino (nitrogen mustard), ethylenimino, methanesulfonate, and other alkylating functions. An alternative conception of the carcinolytic activity observed with these newer compounds is based on the "carrier" hypothesis, which pictures the alkylating function as being transported by the familiar part of the molecule to the body site where the latter is normally metabolized. Baker[3] has pointed out that, for naturally occurring substances, *e.g.*, L-phenylalanine, the carrier and antimetabolite concepts are one and the same, since the *carrier* moiety in L-phenylalanine mustard fits the enzymatic site for the *metabolite*.

The field of amino acids and peptides with antimetabolic activity has been discussed in a recent symposium[4] which laid strong emphasis on the cytotoxic properties of some of these compounds.

Of the amino acid-alkylating function combinations prepared to date, the majority are nitrogen mustards[5] in which the mustard function occurs at a "remote" part of the molecule[3,6] or replaces the α-amino group.[7] Its incorporation as an amide[8] and as a urethan is also under investigation.[9] A few other alkylating functions when incorporated into an amino acid have known antitumor activity or are being evaluated, e.g., the diazoacetyl,[10-12] fluoroacetyl[3] and diazonium[3] substituents. On the

(1) The work presented was done under Research Grant CY-2961 from the National Cancer Institute to the University of Michigan.

(2) For Paper VI in this series, see W. R. Vaughan, R. S. Klonowski, *J. Org. Chem.*, **26**, 145 (1961).

(3) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(4) *Amino Acids and Peptides with Antimetabolic Activity*, G. E. W. Wolstenholme and C. M. O'Conner, eds., Churchill, London, 1958.

(5) F. H. Bergel, *Ann. N.Y. Acad. Sci.*, **68**, 1238 (1958).

(6)(a) H. E. Smith and J. M. Luck, *J. Org. Chem.*, **23**, 837 (1958); (b) G. E. McCasland, R. Horvat, J. Korntvedt, and A. Furst, *J. Org. Chem.*, **23**, 1568 (1958); (c) T. S. Osdene, D. N. Ward, W. H. Chapman, and H. Rakoff, *J. Am. Chem. Soc.*, **81**, 3100 (1959); (d) F. H. Bergel, J. M. Johnson, and J. A. Stock, *Chem. & Ind. (London)*, 1487 (1959).

(7)(a) M. Izumi, *Pharm. Bull. (Japan)*, **2**, 275, 279 (1954); *J. Am. Pharm. Assoc.*, **44**, 132 (1955); W. L. Nyhan and H. Busch, *Chem. Eng. News*, May 5, 1958, p. 46.

(8) O. M. Friedman and R. Chatterji, *J. Am. Chem. Soc.*, **81**, 3750 (1959).

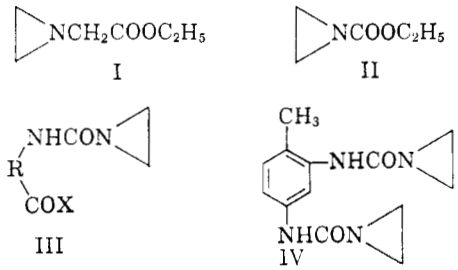
(9) R. B. Ross, *J. Chem. Ed.*, **36**, 370 (1959).

(10) H. A. de Wald and A. M. Moore, *J. Am. Chem. Soc.*, **80**, 3941 (1958); F. Weygand, H. J. Bestmann, and E. Klieger, *Chem. Ber.*, **91**, 1037 (1958).

(11) Y. Liwschitz, R. D. Irsay, and A. I. Vincze, *J. Chem. Soc.*, 1308 (1959).

(12) B. R. Baker, *Biochem. Pharmacol.*, **2**, 1037 (1959).

other hand, as far as we are aware, no ethylenimino derivatives of amino acids have so far been prepared with the exception of *N,N*-ethyleneglycine in the form of its ethyl ester (I),[13,14] *N,N*-ethyleneaspartic acid,[14] *N,N*-ethylenephenylalanine[14] and *N*-aziridinyl acetylserine.[14] III has not to our knowledge been investigated for antitumor properties and the other compounds failed to give a clear effect against animal tumors.[14]



N,N-Ethyleneurethan (II) has been reported[18] and found to be inactive against the Walker rat carcinoma 256.[15] This obviously is a special case.

Accordingly, it seemed to be of interest to examine some additional ethylenimino derivatives of amino acids for anticancer activity. The class of compounds represented by III attracted us particularly in view of the reported high activity of toluylene-2,4-bis-*N,N*-ethyleneurea (IV).[16]

At the outset we have prepared representative derivatives of III, namely the readily accessible esters (III. X = OCH_3, OC_2H_5). Other derivatives of interest are the amides (III. X = NH_2) and hydrazides (III. X = $NHNH_2$). All of these substances would be expected to undergo hydrolysis *in vivo* to the acids in view of the occurrence of many esterases and amidases of nonspecific nature. Hence, it is hoped that evaluation of the esters against selected animal tumors will give a good indication of the usefulness of compounds of the type of III as a class.

Also of significance in the present work are the recent findings of Berenblum and co-workers[17] that certain other *N*-carbonyl derivatives of amino acids, *e.g.*, the urethans, $C_2H_5OCONHR$, where RNH may be glycyl, alanyl, aspartyl, or glutamyl residues, were completely noncarcinogenic to the lungs and skin of mice. This contrasts strongly with the carcinogenic action of urethan itself and many of its analogs.

Finally, although monofunctional alkylating agents in general were formerly considered to have little or no carcinolytic activity, more recently the success reported with the monoaziridino derivative, Tetramin,[18] provides a strong incentive for the present approach. Moreover, the current theories of Baker[3,12,19,20] also furnish a plausible rationale for the present work.

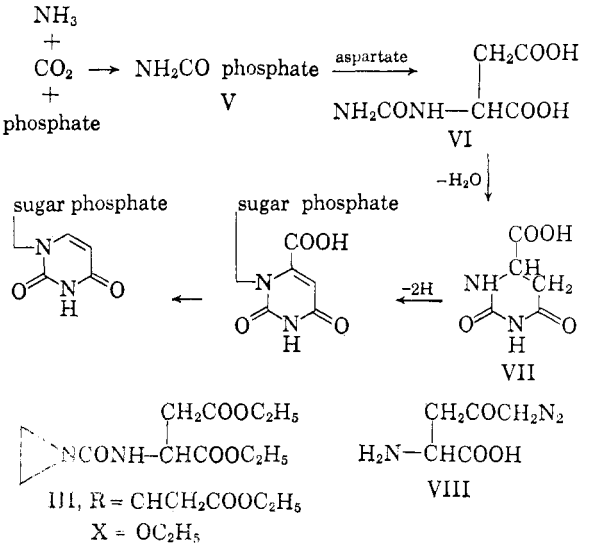
For exploratory purposes the compounds selected for synthesis were analogs of the simplest amino acids, glycine, DL-alanine and DL-phenylalanine (III. R = CH_2, CH_3CH, $C_6H_5CH_2CH$). However, each of these has an interest in its own right. Glycine plays a specific role as a precursor of serine and threonine, of sarcosine, creatinine, and glycocyamine, and of larger molecules such as porphyrins and purines.[21] It is conceivable that the enzymatic reactions leading to some of these may be more susceptible to blocking in tumors by the glycine analog (III. R = CH_2) than in normal tissue. It has been suggested[22] that inhibition of lactic dehydrogenase might be selective for tumors and have chemotherapeutic implications. Since the binding site of lactic acid on the enzyme is presumably the carboxyl group,[20] the alanine analog (III. R = CH_3CH) would be expected to function as a selective irreversible inhibitor through so-called "endoalkylation" by reaction of the ethylenimine residue with some active group in the co-enzyme or apo-enzyme which would prevent the antimetabolite from being desorbed. Phenylalanine is present in lower concentrations in tissues at a given time than the nonessential amino acids. Little is known as yet of the significance of this for tumors, but the success attending use of nitrogen mustard analogs of phenylalanine[2,6a-d,23] against various tumors prompts us to include this analog in our study.

Selection of the other two agents described in this paper, the analogs of DL-aspartic acid and of β-alanine (III. R = $CHCH_2COOC_2H_5$, CH_2CH_2) is based on more concrete reasoning. The unsubstituted carbamyl derivative of aspartic acid, ureidosuccinic acid (VI), represents one stage of the now well established principal route of pyrimidine biosynthesis.[24] In addition to normal tissues, this scheme has also been identified in mouse tumors.[25]

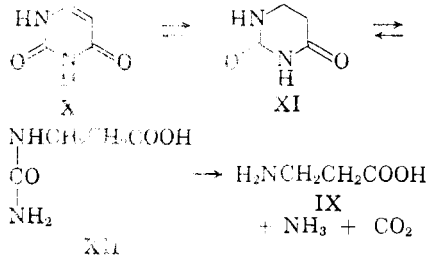
(13) H. Bestian, *Ann.*, **566**, 210 (1950).
(14) G. Sunagawa, K. Murayama, and N. Yoshida, *Yakugaku Zasshi*, **77**, 1173 (1957); *Chem. Abstr.*, **52**, 6304 (1958).
(15) J. A. Hendry and F. L. Rose, *Brit. J. Pharmacol.*, **6**, 357 (1951).
(16) P. A. Herbut and W. H. Kraemer, *Cancer Research*, **15**, 614 (1955).
(17) I. Berenblum, *Biochem. Pharmacol.*, **2**, 168 (1959).
(18) F. R. White, *Cancer Chemotherapy Reports*, No. **4**, 52 (1959).
(19) B. R. Baker, *Abstracts, 136th Meeting, American Chemical Society*, Atlantic City, N. J., September 1959, p. 25-O.
(20) B. R. Baker, *Cancer Chemotherapy Reports*, No. **4**, 1 (1959).
(21) Houben-Weyl, *Methoden der Org. Chem.*, 4th ed., Stuttgart, 11/2, 395 (1958).
(22) W. L. Nyhan and H. Busch, *Cancer Research*, **18**, 385, 1203 (1958).
(23) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).
(24) L. A. Heppel and J. C. Rabinowitz, *Ann. Rev. Biochem.*, **27**, 632 (1958).
(25) E. P. Anderson, C. Y. Yen, H. G. Mandel, and P. K. Smith, *J. Biol. Chem.*, **213**, 625 (1955).

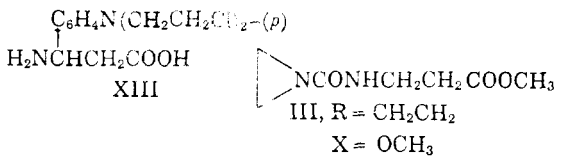
In fact, uracil, one of the products in this scheme, is known to be incorporated selectively into tumor tissue.[26] Hence, analogs of uracil or its precursors are of prime interest in the field of chemotherapy. The analog III (R = $CHCH_2COOC_2H_5$) would be expected to act as an inhibitor of the enzyme which catalyzes the conversion of VI to dihydroorotic acid (VII). Again, following Baker's simple rule,[20] the binding site of VI on this enzyme cannot be the reacting β-carboxyl or amino functions, and hence the α-carboxyl group must be involved. III (R = $CHCH_2COOC_2H_5$) can therefore be visualized as an endoalkylating inhibitor which is bound to the enzyme by the α-carboxyl group as in the case of the metabolite itself. However, the binding then becomes irreversible by reaction of the alkylating group with an active site on the enzyme (or co-factor) and thus the synthesis of pyrimidine nucleotides beyond ureidosuccinic acid (VI) is prevented. VI would then be expected to accumulate very quickly in the tumor since Cohen's group[27] has demonstrated the very high activity of the transcarbamylase catalyzing the reaction V → VI in several animal tumors. In fact, they suggested that the activity of this enzyme is specifically related to the rate of cell division and nucleic acid synthesis and acts as a regulatory mechanism for the rate of growth. It is of interest to note that Liwschitz and co-workers[11] have found that the aspartic acid analog (VIII) has only moderate activity against sarcoma 37 in mice. Possibly both of the carboxyl groups bind to the transcarbamylase in the synthesis of VII; hence to be fully effective an antimetabolite would require that the amino group rather than a carboxyl group be modified by incorporation of an alkylating function.

Mention should also be made of the observations of Mickelson[28] which provide further evidence for the high rate of formation of VI and for significant differences in the metabolism of aspartic acid in normal and tumorous tissue. It was found that whereas most of the amino acids were present to approximately the same extent in normal mouse organs and in mouse sarcoma 180, the aspartic acid content of the latter was only about half that of normal liver. From this it was concluded that aspartic acid must be utilized more rapidly by the tumor than the other amino acids and also more rapidly than by normal tissue. Aspartic acid and its analogs thus become very interesting substances in the search for an exploitable biochemical difference between tumor and normal cells.



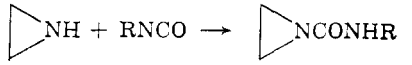
Finally, β-alanine (IX) too is implicated in pyrimidine metabolism, Fritzson[29] has shown that the principal degradation pathway of uracil (X) in the rat is as shown by X–IX, *i.e.*, by what amounts to a "dihydroorotic acid" pathway. Grisolia and co-workers[30] have demonstrated that the reverse process also takes place and that β-ureidopropionic acid (XII) and 5,6-dihydrouracil (XI)





are pyrimidine precursors and utilized for RNA biosynthesis, although to a less specific and extensive degree than orotate or its precursors. In the light of these observations investigation of the antitumor action of the analog of IX (III, R = CH_2CH_2) would seem to present almost as much interest as that of III (R = $CHCH_2COOCH_3$). Bergel[6d] has reported that the β-alanine analog (XIII) shows strong tumor-inhibiting action.

The simplest route to substituted ureas is by the interaction of amines and isocyanates, *e.g.*,



(26) J. Smrt, J. Beranek, and F. Sorm, *Coll. Czech. Chem. Communs.*, **25**, 130 (1960).

(27) E. Calva, J. M. Lowenstein, and P. P. Cohen, *Cancer Research*, **19**, 101 (1959).

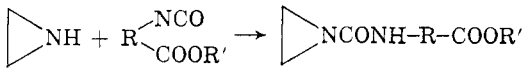
(28) M. N. Mickelson and L. Barvick, *J. Natl. Cancer Inst.*, **17**, 65 (1956); M. N. Mickelson and R. S. Flippin, *Arch. Biochem. Biophys.*, **64**, 246 (1956).

(29) P. Fritzson and A. Pihl, *J. Biol. Chem.*, **226**, 229 (1957).

(30) L. C. Mokrasch and S. Grisolia, *Biochem. et Biophys. Acta*, **33**, 444 (1959) and references cited therein.

Since carboxylic acids and isocyanates interact to give a multiplicity of products, although the primary reaction is always the formation of a mixed carbamic–carboxylic acid anhydride,[31–36] the esters were used.

The isocyanato esters are stable, well-characterized compounds[32,37–39] so that the reaction



XIV III (X = OR′)

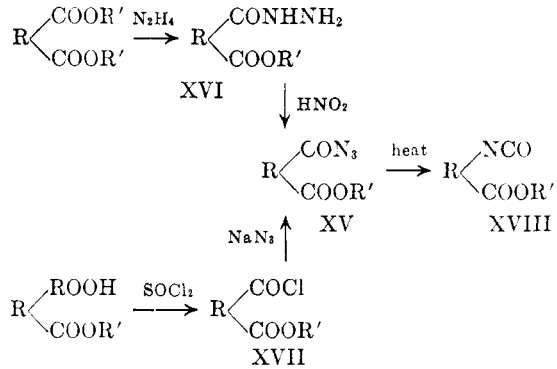
becomes the obvious route to III (X = OR′). In fact, even isocyanato acid chlorides are known,[38] so that the ethyleniminoamides

(III. X = -N) appear to be readily accessible.

Three approaches to the isocyanato esters are available: (1) reaction of halogeno esters with inorganic isocyanates (2) Curtius rearrangement of ester azides (N_3CO—R—COOR′) and (3) action of phosgene on amino acid ester hydrochlorides. For optically active α-isocyanates only (3) is applicable[32] since there is no possibility of racemization or inversion, whereas (1) and (2) involve the asymmetric carbon atom. However, since in the first approach we have been concerned with DI-compounds, all three routes have been explored.

Under heterogeneous conditions, the halogen in ethyl chloroacetate is insufficiently reactive toward freshly prepared silver isocyanate; after two days in boiling ether almost all the starting material was recovered unchanged. This experience should be contrasted with that of Donleavy and English.[40] Ethyl bromoacetate was practically unchanged on boiling in benzene with silver isocyanate for two days, although a trace of higher-boiling material with infrared absorption at 2250 cm.$^{-1}$ (isocyanate) was obtained.

The azide route is practical only for the β-amino acid analog. By this method the requisite azides (XV) can theoretically be obtained from the action of nitrous acid on the half hydrazides (XVI) by the "wet" method,[41] isolated in solution, and dried, all at low temperatures. However, the stepwise hydrazinolysis of diesters is very tedious,[42] the yield of XVI is poor and there are many side reactions.



In contrast, the "dry" method involving the interaction of active sodium azide with ester chlorides (XVII) in a hot inert solvent[37] avoids the isolation of the azides (XV) and gives good yields of XVIII. The limiting factor is the accessibility of the half ester chlorides of dibasic acids.

For the synthesis of analogs of α-amino acids, this acid is malonic or a substituted malonic acid, in which the β-dicarbonyl system renders the half ester chloride particularly reactive. Staudinger[43] has shown that the malonic acid derivative itself (XVII. R = CH_2, R′ = C_2H_5) tends to split out hydrogen chloride to give carboethoxyketene which then polymerizes in various ways. Staudinger as well as Snyder[44] prepared XVII (R = CH_2) from the half ester salt with thionyl chloride in ether in 40–45% yield. In contrast, the first recorded preparation[45] of XVII (R = CH_2) employed the half acid ester itself and thionyl chloride without a solvent to give a 30% yield. We have used thionyl chloride in ether at 40° on the half acid ester (XVII. R = CH_2, R′ = C_2H_5) in three identical runs to obtain yields of 77%, 28%, and 21% respectively. The quality of the thionyl chloride seems to be the controlling factor. Purification of commercial thionyl chloride using quinoline and linseed oil was unsatisfactory, and eventually the commercial material was subjected to simple distillation immediately before use.

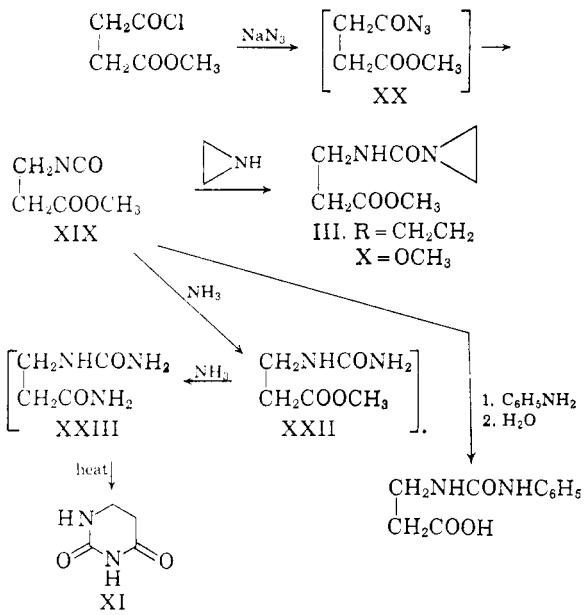
However, we have found that the best method for the preparation of XVII (R = CH_2; R′ = C_2H_5) is that of Hauser and co-workers[46] involving interaction of phthaloyl chloride and the half acid ester of malonic acid at 110° without a solvent, which gives yields of 75%. If this could be

(31) T. Wieland, *Angew. Chem.*, **63**, 12 (1951).
(32) S. Goldschmidt and M. Wick, *Ann.*, **575**, 217 (1952).
(33) W. R. Sorenson, *J. Org. Chem.*, **24**, 978 (1959).
(34) C. Neuberg and A. Manasse, *Ber.*, **38**, 2365 (1905).
(35) A. L. Levy, *Nature*, **165**, 152 (1950).
(36) H. Giesemann and E. Oertel, *J. prakt. Chem.*, [4] **8**, 292 (1959).
(37) B. Flaschenträger and F. Halle, *Zeit. physiol. Chem.*, **192**, 253 (1930).
(38) W. Siefken, *Ann.*, **562**, 442 (1957).
(39) A. C. Smith, Jr., and C. C. Unruh, *J. Org. Chem.*, **22**, 442 (1957).
(40) J. J. Donleavy and J. English, Jr., *J. Am. Chem. Soc.*, **62**, 218 (1940).
(41) P. A. S. Smith, *Org. Reactions*, **3**, 337 (1946).
(42) T. Curtius and K. Hochswender, *J. prakt. Chem.*, [2], **125**, 218 (1930).
(43) H. Staudinger, *Ber.*, **50**, 1016 (1917); S. J. Davis, *Chem. & Ind. (London)*, 323 (1953).
(44) H. R. Snyder and M. M. Robison, *J. Am. Chem. Soc.*, **74**, 5945 (1952).
(45) F. Marguery, *Bull. soc. chim. France*, [3], **33**, 541 (1905).
(46) D. S. Breslow, E. Baumgarten, and C. R. Hauser, *J. Am. Chem. Soc.*, **66**, 1287 (1944).

applied to substituted malonic acid esters, route (2) above would be practical for general use. At present we have prepared only XVII (R = CH_2, R′ = C_2H_5) by this method.

The azide (XV. R = CH_2, R′ = C_2H_5) is readily obtained when the acid chloride is treated with freshly prepared finely divided sodium azide. When the reaction is carried out in boiling benzene the isocyanate (XVIII. R = CH_2, R′ = C_2H_5) is formed directly in good yield.

In contrast to the rather difficultly accessible malonic acid derivatives, ester chlorides of α, ω-dibasic acids are easily obtained. For the preparation of β-alanine derivative (III. R = CH_2CH_2-$COOCH_3$, X = OCH_3) methyl β-isocyanatopropionate (XIX) was readily prepared from the ester azide (XX) which again was not isolated.



XIX was further characterized by conversion to the known β-(*N*-phenylureido)propionic acid (XXI).[47]

The action of aqueous ammonia on XIX led eventually to 5,6-dihydrouracil (XI). Whereas Goldschmidt and Wick[32] obtained ureido ethyl esters from ammonia and the corresponding isocyanates in straightforward manner, the more reactive methyl ester (XIX) under the same conditions gave a mixture of XXII and XXIII as judged by analytical data. Further heating of this mixture with aqueous ammonia gave the dihydropyrimidine.

Finally, for more general application method (3) is the preferred one. Siefken[38] has prepared the simple glycine analog (XVIII. R = CH_2, R′ = C_2H_5) by merely heating the amino acid ester hydrochloride with phosgene in toluene, and Goldschmidt and Wick[32] have applied this method to the esters of a number of common amino acids. The isocyanato esters are stable distillable liquids. The lower numbers are extremely lachrymatory and react very rapidly with atmospheric moisture to give the corresponding ureas. The higher molecular weight compounds are almost odorless and much less reactive to water. They all show infrared absorption at 2260 cm.$^{-1}$ (isocyanate) and 1740 cm.$^{-1}$ (ester).

The reactions with phosgene invariably ran smoothly but the most tedious aspect of method (3) is the preparation of the amino acid esters. Goldschmidt and Wick[32] stressed the desirability of thorough esterification in order to obtain high yields of the isocyanates. The usual criterion of complete esterification by the classical Fischer method is crystallinity of the ester hydrochloride; several repetitions of the esterification process may be necessary to achieve this. In the case of the glycine, α-alanine, and phenylalanine derivatives, the crystalline hydrochlorides were used. However, such a small yield of diethyl aspartate hydrochloride was obtained that finally the free ester was liberated by cold aqueous potassium carbonate, distilled, and its solution in xylene treated with dry hydrogen chloride (which formed a soluble salt) before passing in phosgene. The yield of isocyanate was not inferior to that obtained from the crystalline salt.

The ester isocyanates all reacted readily with ethylenimine in cold ethereal solution. When moisture is excluded analytically pure *N,N*-ethyleneureido esters result after evaporation of the solvent. This purity of the products was fortunate because the esters crystallized only with extreme difficulty. Distillation is objectionable since highly substituted ureas are readily cleaved to the original isocyanates and amines by heat.[48] It was possible to distill the lower molecular weight compounds but extensive decomposition occurred during the process. At 10^{-3} mm. pressure the phenylalanine analog decomposed completely with some violence. All of these *N,N*-ethyleneureas show infrared absorption at 3300 cm.$^{-1}$ (NH) and 1740 cm.$^{-1}$ (ester or amide).

Results of screening of these compounds against animal tumors will be reported elsewhere.

EXPERIMENTAL[49,50]

The isocyanato esters (XVIII). The esters listed in Table I were prepared in high yields from the corresponding amino acid ester hydrochlorides[51,52] and phosgene (Method

(47) F. Lengfeld and J. Stieglitz, *Am. Chem. J.*, 15, 515 (1893).

(48) P. A. Herbut and W. H. Kraemer, *Cancer Research*, **15**, 130, 166 (1955).

(49) All melting and boiling points are uncorrected except as noted.

(50) Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Mich.

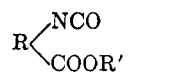
(51) E. Fischer *et al.*, *Ber.*, **34**, 452 (1901); **38**, 2376 (1905); **40**, 500 (1907). *Ann.*, **357**, 14 (1907).

(52) T. Curtius, *et al.*, *Ber.*, **18**, 1293 (1885); **37**, 1266 (1904); *J. prakt. chem.*, [2] **37**, 159 (1888); **38**, 472 (1888).

3) in boiling anhydrous toluene or xylene.[32,36] Reaction occurs smoothly and there are no isolation problems. Phosgene, dried by passage through a wash bottle containing sulfuric acid, was passed into the stirred refluxing solution or suspension and thence through a calcium chloride tube to a hood. At the conclusion of the reaction the solution was swept with dry nitrogen for 30 min. After removal of the solvent the esters were distilled under reduced pressure.

TABLE I

ISOCYANATO ESTERS



R	R′	Method	B.P./Mm.	n_D
CH_2	C_2H_5	2 and 3	71/13	
CH_2CH_2	CH_3	2	75/11	
CH_3CH	C_2H_5	3	69/11	
$C_6H_5CH_2CH$	C_2H_5	3	120–121/ 1.5	1.5034 (21°)
$C_2H_5OOCCH_2CH$	C_2H_5	3	130/10	1.4370 (25°)

Alternately, certain of the isocyanato esters were prepared from the appropriate ester acid chlorides and active sodium azide prepared from hydrazine hydrate and *n*-butyl nitrite in the presence of sodium methoxide according to Naegeli[53] (Method 2). The sodium azide need not be freshly prepared for this purpose. A month-old sample stored over potassium hydroxide was perfectly satisfactory. Instead of the 150% excess azide previously recommended[37] as little as 25% excess gives excellent results. The following procedures are typical.

Ethyl isocyanatoacetate. To a solution of the acid chloride of monoethyl malonate[44,46] (3.19 g., 0.021 mole) in 150 ml. of anhydrous toluene was added 3.45 g. (0.053 mole) of active sodium azide. The flask containing the vigorously stirred solution was immediately connected to an eudiometer *via* a reflux condenser and calcium chloride tube and slowly warmed. Reaction was substantially complete in about 25 min. when the temperature was 90–95° and the brisk evolution of nitrogen ceased. The volume of wet nitrogen collected at 742 mm. and 30° was 559 ml., which represents 110% of the theoretical amount. The suspension was cooled and filtered from separated salts. The yield of material, which does not require distillation, was 7 g. (substantially quantitative).

Methyl β-isocyanatopropionate (XIX). This was prepared as in the above instance from 30.1 g. (0.2 mole) of the acid chloride of monoethyl succinate[54] and 16.25 g. (0.25 mole) of active sodium azide in 800 ml. of benzene. At about 75° the reaction was complete in 1 hr. and 112% of the theoretical amount of wet nitrogen was evolved. The yield of redistilled ester was 19.6 g. (70%).

Methyl β-phenylureidopropionate (XXII). When 1.5 g. (0.016 mole) of freshly distilled aniline was added to 1.3 g. (0.009 mole) of the above isocyanato ester much heat was evolved and the mixture crystallized at once. After the mixture had stood for 30 min., excess aniline was washed out with ether. The remaining pure methyl β-phenylureidopropionate, which has not been previously described, melted at 111–112°. It was saponified with 5 ml. of 2*N* aqueous sodium hydroxide for 35 min. at 40°. Acidification to *p*H 4.5 with hydrochloric acid gave 1.05 g. (54%) of β-phenylureidopropionic acid, m.p. 173–174° dec., after recrystallization from water; reported m.p., 171–172° dec.[55] and 174°[56].

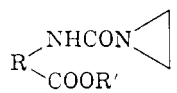
5,6-Dihydrouracil (XXIV). Addition of 5 ml. of concd. aqueous ammonia to 1.3 g. of XIX resulted in an even more violent reaction than that of XIX with aniline. After standing for 1 hr., the mixture was evaporated to dryness on the steam bath over 30 min. The residue was insoluble in benzene, readily soluble in water, and finally was recrystallized with difficulty from ethanol-chloroform. It melted at 142–200°. Analysis indicated that the material was β-ureidopropionamide (XXIII) contaminated with a small amount of methyl β-ureidopropionate.

Anal. Calcd. for $C_4H_9N_3O_2$: C, 36.63; H, 6.92; N, 32.05. Calcd. for $C_5H_{10}N_2O_3$: C, 41.09; H, 6.90; N, 19.18. Found: C, 38.20; H, 6.58; N, 29.96.

The above material was further treated with 5 ml. of concd ammonia, warmed to dissolve it, and left to evaporate at room temperature. This was repeated twice more and the product melted at 274° (corr.) after recrystallization from methanol; reported[47] m.p. for XXIV is 275.°

TABLE II

N,N-ETHYLENIMINOUREIDO ESTERS



R	R′	M.P.	B.P./mm.	n_D	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
CH_2	C_2H_5	51–52°	103–105/0.12	1.4633(29°)	48.83	7.03	16.27	48.92	7.00	16.04
CH_2CH_2	CH_3	26–27°	112–113/0.3	1.4742(26°)	48.83	7.03	16.27	49.17	6.95	15.89
CH_3CH	C_2H_5	—	92–93/10^{-2}	1.4653(22°)	51.60	7.58	15.04	51.48	7.46	15.11
$C_6H_5CH_2CH$	C_2H_5	50–52°	dec. 150/10^{-3}	1.5225(22°)	64.10	6.92	10.68	63.61	6.76	11.12
$C_2H_5OOCCH_2CH$	C_2H_5	—	—	1.4685(25°)	51.15	7.03	10.85	51.16	6.93	11.31

Anal. Calcd. for $C_4H_6N_2O_2$: C, 42.10; H, 5.30; N, 24.56. Found: C, 42.11; H, 5.23; N, 24.49.

The N,N-ethyleneureido esters. (III. X = OR) were prepared by reaction of the isocyanato esters with ethylenimine in anhydrous ether. As a rule the substances crystallized spontaneously on refrigeration in substantially pure state. When possible, they were distilled. A typical procedure will be given and data on the esters is given in Table II.

Methyl β-(N,N-ethyleneureido)propionate. To a solution of 15.3 g. of methyl β-isocyanatopropionate in 100 ml. of anydrous ether, a solution of 6.15 g. of ethylenimine in 30 ml. of dry ether was added dropwise with stirring while the temperature of the mixture was held at 5–8° by external cooling. The reaction was appreciably exothermic. After warming slowly to room temperature the mixture was

(53) C. Naegeli and G. Stefanovitsch, *Helv. Chim. Acta*, 11, 650 (1928); C. Naegeli and E. Vogt-Markus, *Helv. Chim. Acta*, 15, 64 (1932).

(54) J. A. Cason, *Org. Syntheses*, 25, 19 (1945).

(55) S. Hoogewerff and W. A. van Dorp, *Rec. trav. chem.*, 9, 49 (1890).

(56) E. Fischer and H. Leuchs, *Chem. Zentr.*, 1902, I, 762.

allowed to stand for 3–4 hr. Removal of the ether and excess ethylenimine *in vacuo* gave 19.8 g. (97%) of a thick oil which solidified in the refrigerator.

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Synthesis of Potential Anticancer Agents. VIII. Bicyclic Nitriles and Related Compounds.[1,2] I

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Thirteen new compounds available from Diels-Alder reactions involving fumaronitrile, maleonitrile, acrylonitrile, and tetracyanoethylene as dienophiles and cyclopentadiene, cyclohexadiene, cycloheptadiene, and cyclooctatetraene as dienes are reported. Maleonitrile adds to cyclohexadiene in the *endo* sense.

In the course of a study directed toward examination of various aspects of rearrangements and other reactions of functionally substituted bicyclic hydrocarbons derivable *via* the Diels-Alder synthesis from 1,3-cycloalkadienes and mono- and difunctional dieneophiles, the adduct of furmaronitrile (I)[3-5] and cyclohexadiene[6] was prepared.

The ready accessibility of analogs of this substance prompted us to prepare a series of related compounds, inasmuch as substances of this type have not as yet been examined for possible anticancer activity.

Four nitrile-dieneophiles have been used in the present study: fumaronitrile (I), maleonitrile (II)[7] acrylonitrile (III), and ethylenetetracarbonitrile (tetracyanoethylene) (IV).[8]

Thus far four cyclic dienes have been used for the preparation of new compounds: cyclopentadiene (V), cyclohexadiene (VI), cycloheptadiene (VII), and cyclooctatetraene (VIII).

From the reaction series beginning with III and V the following compounds were prepared: bicyclo[2.2.1]hep-5-ene-2-carbonitrile (X),[4,9] bicyclo[2.2.1]heptane-2-carbonitrile (XI),[9] and the phenyl azide adduct of X (3a, 4,5,6,7,7a-hexahydro-1-phenyl-2,7-methano-benzotriazol-5(or 6)carbonitrile (XII)).[10,11] The new compound, *N*-phenyl-5,6-iminobicyclo[2.2.1]heptane-2-carbonitrile (XIII),[12] was prepared by heating XII to expel nitrogen.

In an attempt to reduce the nitrile group of XI catalytically there was obtained, instead of the expected product,[13] *N*,*N*-bis-2-norbornanemethylamine hydrochloride (XIV).

While the simple adducts of IV with V (bicyclo[2.2.1]hept-5-ene-2,2,3,3-tetracarbonitrile (XV[14])), and with VI (bicyclo[2.2.2]-oct-5-ene-2,2,3,3-tetracarbonitrile, XVI[14]), were both known, their hydrogenation products, bicyclo[2.2.1]heptane-2,2,-3,3-tetracarbonitrile (XVII) and bicyclo[2.2.2]octane-2,2,3,3-tetracarbonitrile (XVIII) are herein reported for the first time.

Reaction of I with VI has not been previously reported. Accordingly, the initial adduct, bicyclo-

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(1) Previous paper in this series, Robert C. Elderfield and R. Stanley McElhinney, *J. Org. Chem.*, 25, 1917 (1961).

(2) Work supported in part by Research Grant CY-2961 from the National Cancer Institute to The University of Michigan, and in part by an Institutional Grant to The University of Michigan (IN-40A) from the American Cancer Institute.

(3) A. T. Blomquist and E. C. Winslow, *J. Org. Chem.*, 10, 149 (1945).

(4) This compound has been previously examined for toxicity and activity against sarcoma, carcinoma, and myeloid leukemia in mice and was found to be inactive. *Cf.* E. M. Gal, F. Fung, and D. M. Greenberg, *Cancer Res.*, 12, 565 (1952).

(5) A gift of a small sample of fumaronitrile from the Monsanto Chemical Company is gratefully acknowledged. Further supplies were prepared by James Hudson of this laboratory.

(6) A. T. Blomquist and J. Kwiatek, *J. Am. Chem. Soc.*, 73, 2098 (1951).

(7) Prepared by James Hudson of this laboratory.

(8) Generously supplied by the E. I. du Pont de Nemours & Co. Laboratories.

(9) The *endo* and *exo* isomers were not separated. See K. Alder, K. Heimbach, and R. Reubke, *Chem. Ber.*, 91, 1516 (1958).

(10) K. Alder, H. Krieger, and H. Weiss, *Chem. Ber.*, 88, 144 (1955).

(11) Possible positional isomerism of the nitrile group in this compound renders the structure ambiguous. No attempt has been made to resolve this ambiguity. Likewise, the stereochemistry of the azide addition is ambiguous, although addition *cis* to the methylene bridge of XX is probable on steric grounds.

(12) The stereochemistry of the ethyleneimine system is equivocal. If one accepts the suggested *exo*-configuration for the nitrogen portion of the precursor, XII, the configuration in XIII is probably *exo* also.

(13) Reduction of bicyclo[2.2.2]octane-2-carbonitrile[9] afforded the primary 2-aminomethylbicyclo[2.2.2]octane hydrochloride as reported.[10]

(14) W. J. Middleton, R. E. Heckert, E. L. Little, and C. G. Grespan, *J. Am. Chem. Soc.*, 73, 2783 (1958).