suitable for determining pyridine N-oxide reductase activity in whole cell suspensions of a given microorganism. 4-Hydroxymethylpyridine N-oxide has been employed as substrate; the enzymatically produced 4-hydroxymethylpyridine may then be determined spectrophotometrically. We are presently engaged in the isolation and study of the pyridine N-oxide reductase activity from *E. coli* 9723.

Carcinogenicity of Lactones. III.[1] The Reactions of Unsaturated γ-Lactones with L-Cysteine

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The reactions of a series of unsaturated γ-lactones and related compounds, ranging in carcinogenicity from inactive to potent, with cysteine have been studied, and it has been shown that such reactions will usually not provide meaningful indications of carcinogenicity or of other biological activities. In fact such a criterion can often prove misleading. However, a chemical basis has emerged which may have predictive value: carcinogenic lactones (for example, 4-hydroxypent-2-enoic acid lactone and 4-hydroxy-2,4-hexadienoic acid lactone) undergo Michael addition of the nucleophilic thiol group to the conjugated unsaturation(s) giving rise to S-alkylated adducts. No normal *in vivo* processes are known which can reverse such alkylations and any cellular modification of this type would probably be permanent. The inactive lactones (in which the double bonds are not conjugated with the carbonyl group) are also subject to rapid attack by the cysteine thiol group; however, addition occurs at the lactone carbonyl giving rise to S-acylated intermediates which then rearrange rapidly in neutral solution to yield N-acylated cysteines. In contrast to the effectively irreversible *in vivo* alkylation reactions cellular damage resulting from such acylation processes can readily be repaired by various proteolytic enzymes.

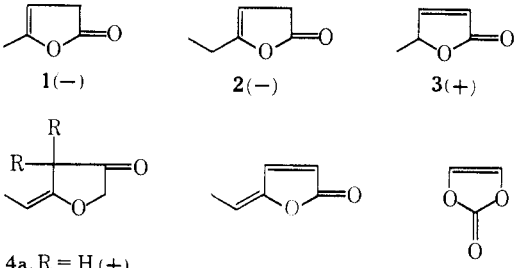
It is well known that compounds containing the lactone function exhibit a broad range of physiological properties[3] including carcinogenic[4] and antitumor[5] activities. Certain properties, for example, antibacterial activity, of many lactones (and of related compounds) are inhibited by the addition of cysteine and other sulfhydryl-containing compounds, and several attempts have been made to correlate physiological potency of lactones with their cysteine reactivity.[4,6,7]

One of the most recent studies of this kind was made by Dickens and Cooke[7] who measured the rates of thiol disappearance and hydrolysis for a number of cysteine–carcinogenic lactone reactions. However, the very approximate correlation between the rate of thiol disappearance and carcinogenicity which emerged was rather unsatisfactory. In retrospect, this is not surprising since cysteine is a trifunctional compound and attack by the thiol group need not be the initial nor the rate-determining step. Accordingly, a systematic examination of the products formed, and of the reaction pathways involved, was begun in the hope that some of the anomalies[7] might be clarified.[8]

Of the compounds examined by Dickens and Cooke those selected for detailed study are shown in Chart I with their relative carcinogenicities.[4,7,9]

Chart I

Relative Carcinogenicity (in parentheses) of Selected γ-Lactones and Related Compounds (from the Data of Dickens, *et al.*[4,7,9])



1(−) **2**(−) **3**(+)

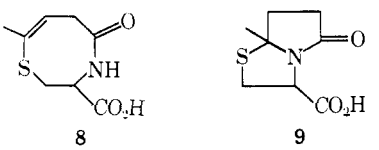
4a, R = H (+)
4b, R = Me (nd)

5(++) **6**(+++)

$CH_3CH{=}CHCH{=}CHCO_2H$

7(+++)

The reaction of 4-hydroxypent-3-enoic acid lactone (**1**) with cysteine was first investigated by Cavallito and Haskell.[6] However, since the assignment of structure 8



8 **9**

to the product isolated was made prior to the advent of modern spectroscopic analytical methods it was considered desirable to reexamine the reaction. A product identical with that described by Cavallito and Haskell

(1) Part II: J. B. Jones, C. H. Koo, I. P. Mellor, S. C. Nyburg, and J. M. Young, *Can. J. Chem.*, **46**, 813 (1968).

(2) Research Fellow of the National Cancer Institute of Canada, 1966–1968.

(3) Leading references to the extensive literature on this subject are given by (a) L. J. Haynes, *Quart. Rev.* (London), **2**, 46 (1948); (b) H. W. Buston and S. K. Roy, *Arch. Biochem.*, **22**, 1 (1949); (c) D. G. Wenzel and C. M. Smith, *J. Am. Pharm. Assoc.*, **47**, 792 (1958); (d) R. Rondanelli, *Arch. Intern. Pharmacodyn.*, **135**, 289 (1962).

(4) F. Dickens and H. E. H. Jones, *Brit. J. Cancer*, **15**, 85 (1961); **17**, 100, 691 (1963); **19**, 392 (1965).

(5) S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, *Tetrahedron Letters*, 149 (1968), and earlier papers; S. M. Kupchan, R. J. Hemingway, and R. W. Doskotch, *J. Med. Chem.*, **7**, 803 (1964), and later papers.

(6) C. J. Cavallito and T. H. Haskell, *J. Am. Chem. Soc.*, **67**, 1991 (1945), and references therein.

(7) F. Dickens and J. Cooke, *Brit. J. Cancer*, **19**, 404 (1965).

(8) In the final paragraph of their paper, Dickens and Cooke[7] also conclude that much chemical work is required in this area since the chemical literature contains surprisingly few relevant data.

(9) F. Dickens, H. E. H. Jones, and H. B. Waynforth, *Brit. J. Cancer*, **20**, 134 (1966).

was obtained and, following the detailed investigation described below, it was assigned structure **9**.[10]

When the lactone **1** was treated with cysteine in aqueous solution at pH 7 and worked up under strongly acid conditions,[6] the compound mp 194° described by Cavallito and Haskell was obtained in 85% yield. The ir spectrum contained bands indicative of a five-membered ring lactam (1665 cm^{-1}) and of a free carboxyl group (1740[12] and 3300–2800 cm^{-1}). The broad peak, 3300–2800 cm^{-1}, has previously been cited[11] as evidence for the N–H group of structure **8**, but the ir spectrum does not allow an unequivocal decision to be made in this regard. However, the pmr spectra in DMSO-d_6 and in pyridine-d_5 exclude the possibility of the latter interpretation being correct since no peaks due to an amide proton, or to a vinylic proton as required by **8**, were detectable in either solvent. Furthermore, the methyl protons appear as a sharp singlet, whereas those in structures such as **8** should show long-range coupling ($J \sim 1$–2 Hz) with the vinylic proton.[14] Of particular significance is the presence in the spectra in both solvents of the AA′BB′ system multiplet (δ 2–3) of the four C-6 and C-7 hydrogens. Only structure **9** would appear to be consistent with all of these spectroscopic data.[15] It is also of interest to note that, in contrast to the deceptively simple ABX splitting patterns observed for the C-3 and C-2 protons of **9** in DMSO-d_6, the corresponding protons of its carboxylate anion (in pyridine-d_5) behave as a remarkably clean ABX system. This situation might also obtain for structure **8** if the amide proton was undergoing sufficiently rapid exchange. However, that this is not the case is demonstrated by the fact that only one exchangeable proton (COOH) can be detected in either solvent in the range δ 0–16.

The one piece of data which, at first, could not be accounted for in terms of structure **9** for the cysteine–**1** product was the evidence of Cavallito and Haskell[6] for one double bond provided by the iodine number determination.[6] However, it has been shown that when iodine numbers of compounds containing sulfide linkages are determined, the addition of iodine to the sulfur atom must be taken into account. For simple sulfides such addition is quantitative.[18]

The reaction of cysteine with levulinic acid is also reported to give **9**.[19] This is of interest since levulinic acid is the hydrolysis product of lactone **1**. The possibility that hydrolysis of **1** was the first step in the formation of **9** was eliminated by the observation that under the reaction conditions, the rate of reaction of cysteine with the lactone was much more rapid than that of the competing hydrolysis.

The formation of **9** as the final product of the reaction of cysteine with the Δ^3-lactone **1** obviously involves a multiple-step pathway requiring the participation of several intermediates. In view of our interest in identifying the biological site of alkylation by compounds exemplified by structures **1**–**7**, and in developing a reliable chemical method for predicting their carcinogenicity,[20] it became of interest to elucidate the mechanistic details of the reaction, and, in particular, to identify the nucleophilic group effecting the initial attack on the lactone.

The mechanism proposed originally[6] suggested the first step to be addition of the thiol group across the Δ^3 double bond, and this was reiterated recently by Black[11] in his speculation on the mechanism. However, in the current investigation it was observed that the reaction mixtures gave positive nitroprusside tests indefinitely[21] (provided that oxygen was excluded from the reaction), and only the crystalline product isolated from solution after acidification gave a negative test for free thiol.

Our previous work on the reaction of lactone **1** with amines had shown nucleophilic attack at the carbonyl group to be a very facile process.[20] This information together with a consideration of the pK_a's of the SH and $^+NH_3$ groups of cysteine,[22] suggested that, at pH 7, the most probable initial step was attack on the lactone carbonyl group by the cysteine thiol anion. That such a reaction could occur was demonstrated by the reaction, in benzene solution and in the presence of potassium carbonate, of α-toluenethiol with **1** to give the thiolester **10** in 60% yield.[23] Unfortunately, for

$$1 + C_6H_5CH_2SH \xrightarrow[\Delta]{K_2CO_3} \underset{\mathbf{10}}{CH_3COCH_2CH_2COSCH_2C_6H_5}$$

reasons of solubility, this reaction could not be carried out in aqueous solution and when the analogous reaction of **1** with N-acetylcysteine in water was attempted, no thiol ester formation could be detected.[24] This indication of the importance of the free cysteine amino group in the reactions leading to the formation of **9** was substantiated by the facile reaction at pH 7 of isopropenyl acetate (the acyclic analog of **1**) with cysteine to give N-acetylcysteine (60%) as the only isolable cysteine derivative.

$$CH_3C(=CH_2)OCOCH_3 + \text{cysteine} \longrightarrow CH_3CONHCH(CH_2SH)COOH$$

These results prompted us to search for similar

(10) While this paper was in preparation, Black[11] reported the results of his reinvestigation of this reaction, in which he concurred with the original assignment of structure **8** to the product isolated by Cavallito and Haskell.[6] For the product of the reaction of cysteine with lactone **3** his structural assignment, **15a**, is in agreement with the conclusions reported in this paper.

(11) D. K. Black, *J. Chem. Soc., C*, 1123 (1966).

(12) This frequency for the CO stretch of the carboxyl group is somewhat outside the normally accepted range[13] (1725–1700 cm^{-1}) of aliphatic acids; however, the related compound, cysteine hydrochloride monohydrate, also shows CO stretch at 1740 cm^{-1} (Nujol).

(13) C. N. R. Rao, "Chemical Applications of Infrared Spectroscopy," Academic Press Inc., New York, N. Y., 1963, p 193.

(14) In lactone **1**, the analogous CH_3 shows long-range coupling of this kind.

(15) That structure **9** was isomeric with **8** and was a possible structure for the Cavallito and Haskell compound was first pointed out in a review article by Syhora.[16] Following the submission of our manuscript, the report of the independent investigations of Hellstrom and his coworkers[17] appeared in which they also concluded that structure **9** was the correct one.

(16) K. Syhora, *Chem. Listy*, **53**, 311 (1959).

(17) N. Hellstrom, S. O. Almqvist, M. Aamisepp, and S. Rodmar, *J. Chem. Soc., C*, 392 (1968).

(18) E. E. Reid, "Organic Chemistry of Bivalent Sulfur," Vol. II, Chemical Publishing Co., New York, N. Y., 1960, pp 47–79.

(19) E. D. Bergmann and A. Kaluszyner, *Rec. Trav. Chim.*, **78**, 289 (1959).

(20) J. B. Jones and J. M. Young, *Can. J. Chem.*, **44**, 1059 (1966).

(21) That this positive test was due to the presence of an SH intermediate and not to traces of cysteine was confirmed by the subsequent isolation of the intermediate **11a**.

(22) J. T. Edsall and J. Wyman, Ed., "Biophysical Chemistry," Vol. I, Academic Press Inc., New York, N. Y., 1958, pp 496–504.

(23) Under the reaction conditions, *viz.*, reflux for 1 hr, the isomerization of **1** to **3** becomes a strongly competing reaction.

(24) Further support for the postulated initial attack by SH can be drawn from the recent data of Hellstrom, *et al.*,[17] who noted that mercaptoacetic acid reacted with **1** in H_2O to give the corresponding thiol ester, which subsequently underwent rapid hydrolysis.

intermediates in the cysteine–**1** reaction mixture. When the latter was worked up by chromatography on an ion-exchange column of pH 5 in place of the usual acidification procedure an oil was obtained containing the ketoamide **11a** (75% by pmr). Compound **11a** could not be purified since ketoamides of this type are unstable and cyclize readily to the corresponding hydroxypyrrolidinones;[20] thus, when the **11a**-containing oil was kept for several days in chloroform solution, cyclization followed by dehydration occurred to give an 80% over-all yield of **9**.

$$RCOCH_2CH_2CONHCHCO_2R_1$$
$$\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad CH_2SH$$

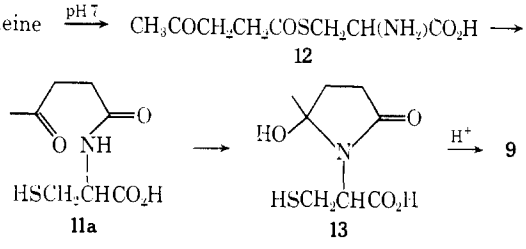
11a, R = CH_3; R_1 = H
b, R = C_2H_5; R_1 = H
c, R = C_2H_5; R_1 = CH_3

The other Δ^3-lactone studied, 4-hydroxyhex-3-enoic acid lactone (**2**),[25] behaved analogously and in aqueous solution at pH 7 underwent rapid, quantitative reaction with cysteine to give N-(4-oxohexanoyl)cysteine (**11b**). Although this product showed no tendency toward pyrrolidinone formation it could not be crystallized. In constrast, the reaction of **2** with methyl cysteinate afforded (in 90% yield) the methyl ester **11c** as a crystalline solid which was completely characterized.

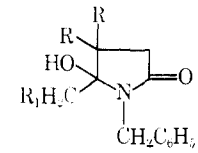
Although the data indicated the initial nucleophilic attack to be by the cysteine amino group, this conclusion lost much of its appeal with the discovery that amino acids lacking the thiol group, such as glycine, reacted not at all with **1** at pH 7 and only slowly at pH 9. After **1** hr under the latter conditions the reaction mixture contained some of the expected amide and its pyrrolidinone derivative. In addition, levulinic acid, from hydrolysis of the starting lactone, was produced.

These apparently conflicting data are best accommodated by the mechanism shown in Chart II in which

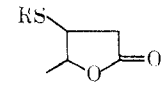
CHART II



initial attack by the thiol group to form the thiolester **12** is followed by a rapid S to N acyl migration leading to **11a**. That such migrations occur rapidly is adequately documented in the literature[26] and, in general, amines and amino acids react with thiolesters to give the corresponding N-acyl compounds almost quantitatively at neutral pH.[27] Further confirmation of the validity of this step was provided by the facile reaction of benzylamine with benzyl 4-oxopentanethiolate (**10**). The initial product, N-(4-oxopentanoyl)-benzylamine, cyclized spontaneously to the hydroxypyrrolidinone **14a** during the reaction. Our previous



14a, R, R = H; R_1 = H
b, R, R = Me; R_1 = Me



15a, R = $CH_2CH(NH_2)CO_2H$
b, R = $CH_2C_6H_5$

studies have shown that compounds such as **11a** and **13** will be in equilibrium in aqueous solution[20] and both of these compounds would give the positive thiol test observed prior to acidification.

As expected from previous studies with amines,[20] the carcinogenic lactone **3** underwent a facile Michael addition reaction when treated with cysteine at pH 7 in aqueous solution.

The product formed gave a negative thiol test and a positive ninhydrin reaction indicating that addition of the thiol group to the double bond had occurred. The ir and pmr spectral data were consistent with this view and structure **15a** was assigned to the product.[10]

Although primary amines are well known to be excellent Michael reaction nucleophiles, the preferential attack in this case by the thiol group was expected from a consideration of the rate data of Friedman[28] on Michael additions to acrylonitrile and α,β-unsaturated carbonyl compounds which show thiol anions of the cysteine type to be two to three orders of magnitude more effective than primary amines as Michael addition nucleophiles.

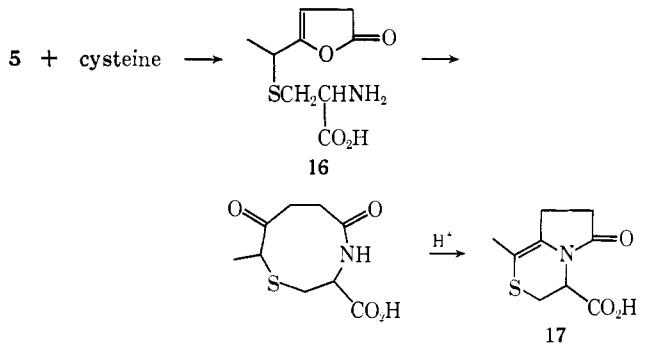
Under anhydrous conditions, and in the presence of potassium carbonate, 4-hydroxypent-2-enoic acid lactone (**3**) also reacted readily with α-toluenethiol to give the Michael addition product **15b**. No products resulting from the addition of a second mole of toluene thiol was observed.[29] Upon distillation **15b** underwent a reverse Michael reaction; that such a process could occur fairly readily for **15a** also was indicated by the faintly positive thiol test obtained when **15a** was treated with a nitroprusside–KCN reagent. By analogy with the related amino compounds,[1] the methyl group and **15b** are probably *trans* to one another.

The carcinogenic 2,4-dienoic lactone **5** also underwent a rapid reaction with cysteine at pH 7. The product obtained, following work-up with HCl, analyzed for $C_9H_{11}NO_3S$, indicating that addition of cysteine to the lactone, followed by loss of the elements of water, had occurred. Its ir spectrum contained bands at 3500–2700 (broad) and 1725 cm^{-1}, characteristic of a carboxyl group, one at 1680 cm^{-1} ascribed to an amide carbonyl, and in addition a relatively strong absorption at 1655 cm^{-1} which was indicative of a hetereo atom tetrasubstituted double bond. The only structure consistent with these data and with the pmr spectra (see Experimental Section) was that of the bicyclic compound **17**. The uv absorption maximum at 256 mμ (ϵ 8000) is consistent with such a heterocyclic system.

A likely pathway for the formation of **17** from the reaction of the dienoic lactone **5** with cysteine is shown in Chart III. Evidence for the initial step of the sequence was provided by a model study in which a mixture of 4-hydroxy-2,4-dienoic acid lactone (**5**) and α-toluenethiol was treated with a catalytic quantity

(25) This lactone was prepared as outlined in Chart IV.
(26) *E.g.*, L. A. Cohen and B. Witkop, *Angew. Chem.*, **73**, 263 (1961).
(27) S. Yamagishi, *Yakugaku Zasshi*, **78**, 1133 (1958).
(28) M. Friedman, J. F. Cavins, and J. S. Wall, *J. Am. Chem. Soc.*, **87**, 3672 (1965).
(29) *Cf.* W. Reppe, *et al.*, *Ann. Chem.*, **596**, 194 (1955).

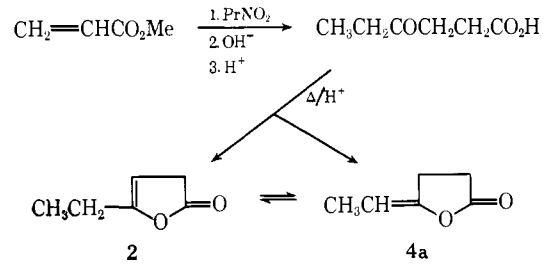
CHART III



of triethylamine in a pmr tube. A peak appeared at δ 5.25 which is ascribable only to the vinylic proton of Δ^3-lactone such as **16**.[30] After this peak had reached its maximum intensity it began to decline and was gradually replaced by the peaks of the two vinylic protons of the corresponding Δ^2-lactone.

The next lactone selected for study was 4-hydroxyhex-4-enoic acid lactone (**4a**). This compound was reported to possess moderate carcinogenic activity by Dickens and Jones[4] but a search of the literature revealed no other report of this compound. Accordingly it was synthesized as shown in Chart IV. As this chart

CHART IV



indicates cyclization of 4-oxohexanoic acid afforded a mixture of **2** and **4a** which at its room temperature equilibrium contained 40% of the latter isomer.

All attempts to obtain a pure sample of **4a**, including careful distillation and chromatography, were unsuccessful owing to the facility with which **4a** isomerizes to the corresponding Δ^3 compound. On one occasion an extremely rapid distillation of the cyclization product afforded a mixture containing 60% (by pmr) of **4a**; however, on keeping overnight this too reverted to the equilibrium composition.[31]

By careful and slow distillation of the lactone equilibrium mixture, a 90% yield of the more volatile component, 4-hydroxyhex-3-enoic acid lactone (**2**), could be obtained. The reactions (with cysteine, etc.) described earlier for this compound were carried out immediately following the distillations; on keeping, pure samples of **2** gradually equilibrated to the 60:40 mixture of Δ^3- and Δ^4-lactones. The equilibration process was markedly accelerated by the addition of *p*-toluenesulfonic acid.

In order to enable the reactions of lactones such as **4** to be studied, the synthesis of 3,3-dimethyl-4-hydroxyhex-4-enoic acid lactone (**4b**) was effected. This compound, for which isomerization to the Δ^3-lactone is precluded, was prepared by the free-radical condensation of 3,3-dimethylacrylic acid with propionaldehyde followed by distillation in the presence of *p*-toluenesulfonic acid of the 3,3-dimethyl-4-oxohexanoic acid so produced.

The results of attempted reactions of **4b** with cysteine in aqueous solution at pH 7, with cysteine methyl ester in aqueous ethanol, and with α-toluenethiol and triethylamine in benzene were disappointing since the lactone was recovered unchanged in all cases. However, that its chemical behavior was analogous to Δ^3-lactones was established by its reaction with benzylamine to give 1-benzyl-4,4-dimethyl-5-ethyl-5-hydroxypyrrolidin-2-one (**14b**) in 91% yield. In contrast to the other pyrrolidinones encountered during our investigations, **14b** was obtained as a crystalline solid although all attempts to recrystallize this material from a variety of solvents led to its contamination with the dehydration product, 1-benzyl-4,4-dimethyl-5-ethylidenepyrrolidin-2-one.

During our attempts to rationalize the ready reaction of Δ^3-lactones **1** and **2**, and isopropenyl acetate, with cysteine with the lack of reaction of the structurally similar compound **4b**, it was observed that in addition to the normal carbonyl absorption, the ir spectrum of **4b** showed a very strong band at 1710 cm^{-1}. In contrast, for **1**, **2**, and isopropenyl acetate the corresponding band at 1680–1690 cm^{-1} was a much weaker one. Unfortunately any possible empirical predictive value this observation might have had was invalidated when the series of C=COCO-containing compounds studied was extended to vinyl acetate. The ir spectrum of this ester in the 1650–1800-cm^{-1} region is very similar to that of **4b** but on treatment with cysteine in aqueous solution at pH 7, it gives the expected product, N-acetylcysteine.

In view of its potency as a tumor-producing agent,[4] its slow rate of reaction with cysteine,[7] and its structural resemblance to Δ^3-lactones, it was decided to extend the investigation to vinylene carbonate (**6**).

As far as could be determined, no reaction with cysteine occurred even though the pH of the solution dropped rapidly and carbon dioxide was evolved; furthermore, the cysteine could be quantitatively recovered from the reaction mixture. Treatment of vinylene carbonate alone with a sodium bicarbonate buffer at pH 7 also resulted in carbon dioxide evolution and the slow formation of a fluffy precipitate. Perusal of the literature showed that vinylene carbonate is readily hydrolyzed to glycolaldehyde which may then polymerize to tetroses and eventually to caramels.[32] Although it is possible that nucleophilic attack on vinylene carbonate by the cysteine thiol or amino group occurs,[33] it seems more likely that the reaction involved is simply hydrolysis to glycolaldehyde and that the

(30) The analogous proton in 4-hydroxypent-3-enoic acid lactone (**1**) occurs at δ 5.15.[20]

(31) In view of the facility with which this isomerization occurs it appears that caution should be exercised when considering the carcinogenicity ascribed[4] to this structure. In fact, it seems probable that the tumor induction observed[4] is due to a compound other than **4a**. This conclusion is supported by the observation that, at present, **4a** is the one anomaly in the structure–activity relationships that have emerged from the studies of Dickens and his coworkers.[4]

(32) A. H. Saadi and W. H. Lee, *J. Chem. Soc., B*, 4 (1966).

(33) The rapid pH drop and CO_2 evolution would seem to indicate some cysteine participation.

carcinogenicity of vinylene carbonate is in reality due to this compound[34] as a result of *in vivo* hydrolysis.

Sorbic acid (**7**), which was also found to be a potent carcinogen[4] and which may be regarded as structurally analogous to **5**, did not react with cysteine in the pH range 5–7. Under these conditions the sorbic acid would exist almost completely as the conjugate base and nucleophilic attack would not be expected to be facile; nevertheless an *in vivo* enzyme mediated process cannot be ruled out.

Conclusions

The data presented argue strongly against the advisability of using reactions with cysteine, or rate determinations based on such reactions, as criteria for predicting the carcinogenicity of lactones and related compounds.[36] Furthermore, it is apparent that owing to the multiple reactions which can occur with cysteine, such a test cannot be satisfactorily used to implicate *in vivo* reactions with thiol residues. If such model studies are to have validity it would seem advisable to use a cysteine derivative in which all the reactive functions other than the thiol group are protected.

However, from the above cysteine reactions, and from previous data,[4,20] a significant distinction between carcinogenic and noncarcinogenic lactones has emerged.

All carcinogenic lactones undergo attack resulting in alkylation of the nucleophile, whereas the inactive lactones all react to give acylated products.

This classification is in accord with the structural groupings derived by Dickens and Jones[4,41] and it appears to provide a basis for predicting whether or not lactones or related compounds will be carcinogenic;[42] if generally applicable, such a chemically based test will obviate much time-consuming biological evaluation.

Rates of reaction do not appear to provide a satisfactory basis of distinction since the noncarcinogenic lactones (*e.g.*, **1**) often undergo reaction with nucleophiles as rapidly as those which are carcinogenic.

A consideration of the possible *in vivo* processes which can occur provides a reasonable explanation for the alkylation–acylation distinction. The reaction of a noncarcinogenic lactone with a biological nucleophile[43] will produce an acyl derivative, such as a thiolester or an amide, and there are several enzyme-catalyzed processes capable of reversing such reactions. Thus any cellular damage so caused can easily be repaired. On the other hand, if the tumor-producing lactones alkylate the appropriate biological nucleophiles, although the reverse reactions may occur on thermodynamic grounds, no enzymes are known which catalyze such processes. Furthermore, the products of such initial alkylating reactions, such as **15** and **16**, are capable of undergoing further nucleophilic attack at the carbonyl group; this introduces the possibility for cross-linking[44] nucleic acids and/or proteins.[45]

Experimental Section[46]

Materials.—L-Cysteine was obtained from Mann Research Laboratories and was used without further purification. 4-Hydroxypent-3-enoic acid lactone (**1**) and vinylene carbonate (**6**) were obtained from Aldrich Chemical Co. Compounds **1**–**7** were purified by distillation or by recrystallization immediately prior to each use. 4-Hydroxypent-2-enoic acid lactone (**3**) was prepared from **1** as previously described.[20,47]

4-Hydroxyhex-3-enoic Acid Lactone (2).—Base (trimethylbenzylammonium hydroxide)-catalyzed condensation[48] of 1-nitropropane (180 g, 2 mol) and methyl acrylate (86 g, 1 mol) gave methyl 4-nitrohexanoate (148 g, 85%), which was then transformed into the *aci* salt by treatment with NaOH (70 g, 1.6 mol) in H_2O (600 ml). Slow addition of the *aci* salt solution to a cooled (<5°) mixture of concentrated H_2SO_4 (178 ml) in H_2O (800 ml) followed by thorough extraction of the aqueous acid solution (Et_2O) and evaporation of the ethereal extracts yielded 4-oxohexanoic acid (85 g, 65%). *p*-Toluenesulfonic acid (3 g) was added to the crude acid and the mixture was distilled slowly at 10 mm. Fractionation of the distillate (70 g) gave pure **2** (45 g, 40%): bp 68–70° (9 mm); ir ($CHCl_3$), 1795, 1675, and 1110 cm^{-1}. Higher boiling fractions contained varying amounts of the isomeric 2-, 3-, and 4-enoic lactones.

Attempted Preparation of 4-Hydroxyhex-4-enoic Acid Lactone (4a).—After keeping at room temperature for 2 weeks, or in the presence of *p*-toluenesulfonic acid for 1 day, **2** isomerized to a mixture containing 60% of **2** and 40% of **4a** at equilibrium. Careful fractionation of this mixture (40 g) yielded only the more volatile Δ^3-lactone **2** (36 g, 90%). On one occasion, a rapid distillation afforded a fraction containing 60% of the Δ^4 isomer **4a** but this reverted overnight to the composition of the equilibrium mixture. No preparative separation of the isomers could be achieved by chromatography on Florisil or on alumina or by glpc on a variety of columns.

4-Hydroxy-2,4-hexadienoic Acid Lactone (5).—To 4-hydroxyhex-3-enoic acid lactone (**2**) (22 g, 0.20 mol) in CS_2 (25 ml) was added Br_2 (30 g, 0.19 mol) in CS_2 (25 ml), the temperature being maintained below 12° during the addition. The dibromolactone solution so obtained was not isolated but was diluted with CS_2 (200 ml). Hydroquinone (5 mg) was added to the solution and Et_3N (40 g, 0.4 mol) was then added dropwise with stirring. The resulting black mixture was kept overnight, and was then filtered and evaporated. The residual oil (20 g) was distilled to give **5** (11 g, 50%): bp 88–90° (10 mm); ir (CCl_4), 1785, 1770 (sh), and 1740 cm^{-1}.

3,3-Dimethyl-4-hydroxyhex-4-enoic Acid Lactone (4b).—3,3-Dimethylacrylic acid (15 g, 0.15 mol) and propionaldehyde (70 g, 1.2 mol) were refluxed with daily additions of benzoyl peroxide

(34) Glycolaldehyde is also suspected of being responsible for the antiviral properties of vinylene carbonate; such antiviral activities are common to a large group of glyoxals, α-hydroxyaldehydes, and related compounds.[35]

(35) B. D. Tiffany, J. B. Wright, R. B. Moffett, R. V. Heinzelman, R. E. Strube, B. D. Aspergren, E. H. Lincoln, and J. L. White, *J. Am. Chem. Soc.*, **79**, 1682 (1957).

(36) This conclusion is probably applicable to most other attempts which have been made to correlate cysteine reactivity with various biological effects,[37,38] particularly since the prior addition of cysteine to a compound does not always preclude activity.[39,40]

(37) G. B. Marcus, A. M. Municio, and S. Vega, *Chem. Ind.* (London), 2053 (1964).

(38) R. Schoental and D. J. Rive, *Biochem. J.*, **87**, 22P (1963).

(39) F. H. Kirchner, J. H. Bailey, and C. J. Cavallito, *J. Am. Chem. Soc.*, **71**, 1210 (1949).

(40) P. H. Daniel, E. Lasfargues, and E. Delaunay, *Compt. Rend. Soc. Biol.*, **149**, 1621 (1955).

(41) The alkylation–acylation selection rule predicts that **4a** and **4b** should not be carcinogenic. In view of the uncertainty regarding the stability of **4a**,[31] it is intended to submit **4b** for biological evaluation.

(42) This distinction might well apply to other lactone activities, such as their properties as antibacterials, mitotic inhibitors, etc.[3]

(43) The biological effects of lactones almost certainly result from their ability to react with the functional groups of proteins and/or nucleic acids. Although there is evidence that many alkylating agents, including β-propiolactone, exert their effect by modification of the nucleic acid bases, particularly of guanine,[14] it is as yet uncertain which of the possible *in vivo* reactions are responsible for the carcinogenicity of lactones such as **3** and **5**.[45]

(44) P. Brookes and P. D. Lawley, *Brit. Med. Bull.*, **20**, 91 (1964).

(45) Experiments investigating these possibilities are in progress.

(46) All melting points were determined on a Fisher-Johns melting point block and are corrected. Pmr spectra were recorded on a Varian A-60 or HA-100 spectrometer in $CDCl_3$, DMSO-d_6, and pyridine-d_5 with Me_4Si, or in D_2O with 3-(trimethylsilyl)propanesulfonic acid sodium salt as internal standards; ir spectra were recorded on a Perkin-Elmer 237-B or a Beckman IR-8 spectrophotometer. Where not quoted, the ir and pmr spectra were as expected. Unless otherwise stated solvents were removed by rotary evaporation at room temperature.

(47) J. Thiele, *Ann.*, **319**, 144 (1902).

(48) R. B. Moffett in "Organic Syntheses," Coll. Vol. IV, N. Rabjohn, Ed., John Wiley and Sons, Inc., New York, N. Y., 1963, p 652.

(0.5 g).[49] The progress of reaction was monitored by pmr and when a 50% conversion to 3,3-dimethyl-4-oxohexanoic acid had occurred (7 days), the solution was evaporated and vacuum-distilled in the presence of *p*-toluenesulfonic acid. Chromatography of the distillate (7 g) on Florisil (200 g, C_6H_6 elution) followed by redistillation yielded pure **4b** (4.1 g, 25% based on 3,3-dimethylacrylic acid): bp 82–83° (9 mm); ir ($CHCl_3$), 1795 (vs), 1710 (vs), 1105, and 993 cm^{-1}. *Anal.* ($C_8H_{12}O_2$) C, H.

Reaction of 4-Hydroxypent-3-enoic Acid Lactone (1) with L-Cysteine. (a) With Acid Work-up.—The lactone 1 (2 g, 20 mmol) was added with stirring to a solution of L-cysteine (2.4 g, 20 mmol) in H_2O (15 ml) which had previously been adjusted to pH 7.[50] Since a two-phase system was formed, stirring was continued throughout and the mixture was maintained in the range pH 6–7 by periodic titration with base.[50] Within 15 min 20 mmol of base had been added and the reaction mixture had become homogeneous; it was then kept at room temperature overnight. Acidification (to pH 1.5) of the solution with 37% HCl, followed by evaporation under reduced pressure and trituration of the residue with 1 *N* HCl (5 ml), yielded 1-aza-2-carboxy-4-thia-5-methylbicyclo[3.3.0]octan-8-one (**9**) (3.4 g, 85%). On recrystallization from absolute EtOH, thick needles, mp 194°, were obtained which gave a negative SH (nitroprusside) test but which evolved CO_2 on treatment with aqueous $NaHCO_3$; ir (Nujol), 3300–2800 (acid OH), 1740 (sharp, COOH), and 1665 cm^{-1} (broad, amide CO); pmr (DMSO-d_6, 60 MHz), δ 1.62 (s, 3, CH_3), 2.1–2.9 (m, 4, CH_2CH_2CO), 3.58 (d, 2, J = 7 Hz, SCH_2), 4.86 (t, 1, J = 7 Hz, SCH_2CHN), and 10.5 ppm (s, 1, COOH). The spectrum obtained in D_2O was similar and confirmed the presence of only one exchangeable proton; pmr (pyridine, 60 MHz), δ 1.80 (s, 3, CH_3), 2.00–2.90 (AA′BB′, m, 4, CH_2CH_2), 3.46–4.08 (ABX, m, 2, SCH_2), 5.41 (ABX, d of d, 1, J_{AX} = 8.6 Hz, J_{BX} = 6 Hz, CH_2CHN), and 14.8 ppm (s, 1, COOH). The 100-MHz spectra provided no additional data. Mass spectrum (70 eV) showed *m/e* 201 (parent ion). *Anal.* ($C_8H_{11}NO_3S$) C, H, N, S.

When the reaction was carried out in 1 *M* potassium–sodium phosphate buffer (pH 7), the product **9** was obtained in 75% yield after acidification and recrystallization.

No significant reaction was observed at pH 7 with N-acetyl-L-cysteine or with glycine.

(b) Without pH Control.—The lactone 1 (2.0 g, 20 mmol) was added with stirring to a solution of L-cysteine (2.4 g, 20 mmol) in H_2O (25 ml) at pH 7[50] as described above. Within 25 min the pH had fallen to a terminal value of 3.6 and work-up of the reaction mixture afforded **9** in only 10% yield.

(c) With pH Control and with Ion-Exchange Resin Work-Up.—This experiment was carried out as in part a except that after standing overnight the neutral solution was filtered through a column of Rexyn AG-50 (100 g, Fisher) and the fractions giving a positive free SH test were collected and combined. Evaporation (bath temperature <20°) gave a clear colorless oil (3.7 g) which was dissolved in $CHCl_3$ and allowed to crystallize. The crystalline material so produced (0.75 g, mp 191–194°) was filtered off and was shown to be identical with **9** obtained in part a. Pmr spectroscopic examination of the $CHCl_3$ filtrate indicated the presence of the acyclic intermediate N-(4-oxopentanoyl)-L-cysteine (**11a**): pmr ($CHCl_3$), δ 1.60 (broad s, 1, SH), 2.1 (s, 3, CH_3CO), 2.3–2.8 (A_2B_2, m, 4, $COCH_2CH_2CO$), 3.55 (m, 2, SCH_2C), 4.9 (m, 1, CHNH), and 7.2 ppm (broad d, 1, NHCO). This material could not be isolated in a pure state, and on keeping in $CHCl_3$ for 3 days at room temperature it became entirely converted to **9** which precipitated from solution.

Reaction of 4-Hydroxypent-3-enoic Acid Lactone (1) with α-Toluenethiol.—The lactone 1 (5 g, 50 mmol) and α-toluenethiol (6.2 g, 50 mmol) in C_6H_6 (10 ml) were refluxed for 1 hr in the presence of K_2CO_3 (0.5 g). The solution was then filtered and concentrated. Pmr examination of the resulting solution showed the presence of the thiolester **10** (60%) and 3-(benzylthio)-4-hydroxypentanoic acid lactone (**15b**) (40%). (Compound **15b** results from isomerization of **1** to the Δ^2-lactone **3** followed by Michael addition.) Chromatography of the fully evaporated reaction mixture (11 g) on Florisil (300 g), with petroleum ether (bp 40–60°)–C_6H_6 elution, yielded pure benzyl 4-oxopentanethiolate (**10**) (6.0 g, 54%): bp 122–124° (0.06 mm); ir ($CHCl_3$), 1725 (ketone C=O) and 1692 cm^{-1} (thiolester C=O). (Attempted distillation at 10 mm caused the thiolester to dissociate to a mixture of starting lactone and α-toluenethiol.) *Anal.* ($C_{12}H_{14}O_2S$) C, H, S.

Reaction of Benzyl 4-Oxopentanethiolate (10) with Benzylamine.—Benzylamine (0.7 g, 6.5 mmol) was added dropwise to a stirred solution of **10** (1.39 g, 6.25 mmol) in C_6H_6 (5 ml). After further stirring for 1 hr at 20° the solution was evaporated to an oil (2.1 g) containing 1-benzyl-5-hydroxy-5-methylpyrrolidin-2-one[20] (**14a**).

Reaction of Isopropenyl Acetate with L-Cysteine.—Isopropenyl acetate (1 g, 10 mmol) was added at 20° to a stirred solution of L-cysteine (1.24 g, 10 mmol) in H_2O (10 ml) adjusted to pH 7.[50] Base[50] was added periodically to maintain the pH at 6–7, 10 mmol being consumed during 1 hr. Acidification of the solution with concentrated HCl followed by evaporation gave a NaCl-contaminated oil which was extracted with a minimum of hot EtOH. The EtOH solution was filtered and cooled and the L-cysteine hydrochloride monohydrate (0.6 g, mp 175–183°) which separated was filtered off. Reevaporation of the filtrate followed by recrystallization (*i*-PrOH) gave N-acetyl-L-cysteine (1.0 g, 60%), mp 106–107°.

Reaction of 4-Hydroxyhex-3-enoic Acid Lactone (2). (a) With L-Cysteine.—The lactone **2** (2.2 g, 20 mmol) was added at room temperature, with stirring, to a solution of L-cysteine (2.4 g, 20 mmol) in H_2O (25 ml) at pH 7,[50] and the pH was maintained at this level by the periodic addition of base[50] (up to 20 mmol) during 1 hr. After 4 hr the solution was made strongly acidic with concentrated HCl and evaporated to an oil containing N-(4-oxohexanoyl)-L-cysteine; pmr ($CDCl_3$), δ 1.06 (t, 3, J = 7.2 Hz, CH_3CH_2CO), 1.67 (broad t, 1, SH), 2.50 (q, 2, J = 7.2 Hz, CH_3CH_2CO), 2.72 (A_2B_2, q, 4, $COCH_2CH_2CO$), 2.94 (m, 2, CH_2SH), 4.85 (m, 1, CH_2CHNH), and 7.34 ppm (d, 1, J = 7.5 Hz, NH). All attempts to further purify the compound, including recrystallization and distillation, were unsuccessful.

(b) With Methyl L-Cysteinate.—A solution of methyl L-cysteinate hydrochloride (1.71 g, 10 mmol) in H_2O (10 ml) was adjusted to pH 7[50] and 4-hydroxyhex-3-enoic acid lactone (1.1 g, 10 mmol) in MeOH (5 ml) was added, the pH being maintained at 7 in the usual way. After 1 hr the solution was acidified (concentrated HCl), concentrated, and extracted thoroughly (Et_2O). The ethereal extracts were dried ($MgSO_4$) and evaporated to give methyl N-(4-oxohexanoyl)-L-cysteinate (**11c**) (2.23 g). Recrystallization (Et_2O) gave needles (2.2 g, 90%): mp 59.5–60.5°; ir ($CHCl_3$), 3440, 1750, 1720, and 1682 cm^{-1}; pmr ($CDCl_3$), δ 1.03 (t, 3, J = 7.2 Hz, CH_3CH_2), 1.60 (s, 1, SH), 2.49 (q, 2, J = 7.2 Hz, CH_3CH_2CO), 2.68 (A_2B_2, q, 4, $COCH_2CH_2CO$), 2.95 (m, 2, SCH_2), 3.77 (s, 3, CH_3OCO), 4.82 (m, 1, SCH_2CH), and 7.03 ppm (d, 1, J = 7.5 Hz, NHCO). The product gave a positive nitroprusside test for free SH. *Anal.* ($C_{10}H_{17}NO_4S$) C, H, N, S.

Reaction of 4-Hydroxypent-2-enoic Acid Lactone (3) with L-Cysteine.—The lactone **3** (1 g, 10 mmol) was added to a stirred solution (pH 7) of L-cysteine (1.24 g, 10 mmol) in H_2O (15 ml). After 15 min at room temperature, the solution was evaporated and the residue was recrystallized from EtOH–H_2O to give 3-(S-L-cysteinyl)-4-hydroxypentanoic acid lactone (**15a**) as needles (2.0 g, 90%): mp 188–189° dec; ir (Nujol), 3200–2400, 2125–2000, 1785–1755, and 1640–1540 cm^{-1}. The product gave a positive ninhydrin test, but showed no coloration with nitroprusside unless warmed with aqueous KCN. *Anal.* ($C_8H_{13}NO_4S$) C, H, N, S.

Reaction of 4-Hydroxypent-2-enoic Acid Lactone (3) with α-Toluenethiol.—To the lactone **3** (1 g, 10 mmol) and α-toluenethiol (1.2 g, 10 mmol) in C_6H_6 (10 ml) was added 5 drops of Et_3N and the mixture was refluxed for 1 hr. Evaporation and distillation gave 3-benzylthio-4-hydroxypentanoic acid lactone (**15b**) as a colorless oil (2.0 g, 91%): bp 134–136° (0.05 mm); ir ($CHCl_3$), 1780 cm^{-1}; pmr ($CDCl_3$), δ 1.27 (d, 3, J = 6.5 Hz, CH_3CH), 2.2–3.2 (m, 3, $SCHCH_2CO$), 3.74 (s, 2, $C_6H_5CH_2S$), 4.1–4.5 (m, 1, 4-H), and 7.73 ppm (s, 5, C_6H_5). *Anal.* ($C_{12}H_{14}O_2S$) C, H, S. Attempted distillation of this material at 10 mm yielded only starting lactone and α-toluenethiol from the reverse Michael reaction.

Reaction of 4-Hydroxy-2,4-hexadienoic Acid Lactone (5). (a) With L-Cysteine.—The lactone **5** (2.2 g, 20 mmol) in MeOH (5 ml) was added at 20° with stirring to a pH 7[50] solution of L-cysteine (2.4 g, 20 mmol) in H_2O (20 ml). In order to maintain pH 7, base[50] (20 mmol) was added during 3 hr. The solution was allowed to stand overnight (negative nitroprusside test) and was then acidified with concentrated HCl. Partial evaporation followed by cooling and scratching of the concentrated solution

(49) R. L. Huang, *J. Chem. Soc.*, 1342 (1957).

(50) The pH adjustments were made with 1 *N* NaOH.

yielded 1-aza-2-carboxy-4-thia-5-methylbicyclo[4.3.0]non-5-en-9-one (**17**) (3.2 g 75%) as fine needles, mp 140–160° dec. Five recrystallizations from AcOH gave needles, mp 145–165° dec; from Me_2CO, small cubes, mp 141–156° dec; and from $CHCl_3$, plates, mp 165–175° dec; ir ($CHCl_3$), 3500–2700, 1720, 1675, and 1650 cm^{-1}; pmr (DMSO-d_6), δ 1.75 (s, 3, $CH_3C{=}C$), 2.25–2.95 (A_2B_2, m, 4, CH_2CH_2CO), 2.95 (calcd) (d of d, 1, J_{AB} = 13 Hz, J_{AX} = 3.7 Hz, SCH_2CH), 3.24 (calcd) (d of d, 1, J_{BA} = 13 Hz, J_{BX} = 3.2 Hz, SCH_2CH), 5.04 ppm (broad t, 1, J = 3.2 Hz, SCH_2CH); COOH proton not detectable; pmr (pyridine), δ 1.19 (s, 3, CH_3), 2.06 (A_2B_2, broad s, 4, CH_2CH_2), 2.57 (calcd) (d of d, 1, J_{AB} = 13 Hz, J_{AX} = 3.5 Hz, SCH_2), 3.18 (calcd) (d of d, 1, J_{BA} = 13 Hz, J_{BX} = 3 Hz, SCH_2CH), 5.06 (t, 1, J = 3 Hz, SCH_2CH), and 15.88 ppm (s, 1, COOH); uv (MeOH), 256 mμ (ϵ 8000). *Anal.*[51] ($C_9H_{11}NO_3S$) C, H, N, S.

(b) **With α-Toluenethiol.**—A solution of **5** (0.56 g, 5 mmol) and α-toluenethiol (0.60 g, 5 mmol) in dioxane (3 ml) was kept at 20° for 24 hr during which time no reaction (by pmr) had occurred. Et_3N (1 mg) in dioxane (0.5 ml) was then added, and further pmr spectra were recorded at hourly intervals. A peak at δ 5.25, corresponding to the vinylic proton of a Δ^2-unsaturated γ-lactone, appeared and gradually increased in intensity during 5 hr; it then declined and was replaced by peaks, at δ 6.10 and 7.45 ppm, of the vinylic protons of the benzylthio analog of the Δ^2-unsaturated lactone **16**.

Reaction of 3,3-Dimethyl-4-hydroxyhex-4-enoic Acid Lactone (4b) with Benzylamine.—Benzylamine (0.53 g, 5 mmol) in petroleum ether (bp 40–60°, 2 ml) was added slowly to a stirred solution of the lactone **4b** (0.704 g, 5 mmol) in petroleum ether (bp 40–60°, 6 ml) and Et_2O (2 ml). After 18 hr the precipitated crystalline mass was collected and was thoroughly dried *in vacuo* to give 1-benzyl-4,4-dimethyl-5-ethyl-5-hydroxypyrrolidin-2-one (1.12 g, 91%): mp 102–103°; ir ($CHCl_3$), 3600, 3400, and 1688 cm^{-1}. Attempts to recrystallize this material from a variety of solvents led to its contamination with increasing amounts of the dehydrated material, 1-benzyl-4,4-dimethyl-5-ethylidenepyrrolidin-2-one. Elemental analysis was therefore performed on the material which had precipitated from the reaction solution. *Anal.* ($C_{15}H_{21}NO_2$) C, H, N.

Lactone **4b** did not react with cysteine in aqueous solution at pH 7 or with methyl cysteinate in EtOH.

Reaction of Vinyl Acetate with L-Cysteine.—Vinyl acetate (1.72 g, 20 mmol) was added to a stirred solution of L-cysteine (2.4 g, 20 mmol) in H_2O (15 ml) at pH 7.[50] Dilute NaOH (20 mmol) was added periodically to maintain pH 6–7,[50] and the solution was allowed to stand overnight at room temperature. Acidification with concentrated HCl and isolation in the usual manner gave N-acetyl-L-cysteine (1.0 g, 30%), mp 108°.

Reaction of Vinylene Carbonate with L-Cysteine.—Vinylene carbonate (1.72 g, 20 mmol) was added to a stirred solution of L-cysteine (2.4 g, 20 mmol) in H_2O (15 ml) at pH 7.[50] An exothermic reaction occurred and carbon dioxide was evolved. After 3 hr the solution was evaporated; on treatment with 1 *N* HCl and with ethanol, the residual gum gave L-cysteine hydrochloride monohydrate (3.1 g, 90%), mp 175–185°.

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(51) The analytical sample was obtained *via* AcOH recrystallization followed by thorough drying.

Potential Anticancer Agents. IV. 5-Substituted Pyrimidine-6-carboxaldehydes and Derivatives[1]

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Various 5-substituted pyrimidine-6-carboxaldehydes and derivatives were synthesized and tested for inhibition of growth of the Ehrlich ascites carcinoma and inhibition of incorporation of L-phenylalanine-1-^{14}C and glycine-1-^{14}C into proteins and orotic acid-5-^{3}H, thymidine-2-^{14}C, and formate-^{14}C into nucleic acids of tumor cells *in vitro*. The following compounds were found to be particularly active as inhibitors: 2-mercapto-4-hydroxy-5-(4-chlorobenzyl)pyrimidine-6-carboxaldehyde (VII-3), 2-mercapto-4-hydroxy-5-(4-bromobenzyl)pyrimidine-6-carboxaldehyde (VII-6), 2-ethylthio-4-hydroxy-5-(4-chlorobenzyl)pyrimidine-6-carboxaldehyde (VII-16), and 2-ethylthio-4-hydroxy-5-(4-bromobenzyl)pyrimidine-6-carboxaldehyde (VII-17). The best compounds of this series are equally as effective as 5-fluorouracil (FU) in inhibiting formate incorporation into DNA and growth of the tumor. They are more effective than FU in inhibiting incorporation of formate and orotic acid into RNA, thymidine into DNA, and phenylalanine and glycine into proteins. Although these compounds inhibit incorporation of formate into DNA and RNA, they have only negligible inhibitory activity against the folate reductases. Smaller *in vivo* and *in vitro* inhibitions are obtained with the 5-bromopyrimidine-6-carboxaldehydes. The diamino-5-phenylpyrimidinealdehyde exhibits the least inhibitory activity against the Ehrlich ascites carcinoma cells *in vivo*, but it shows greater inhibition of folate reductases although considerably less than antifols such as aminopterin.

2-Mercapto-4-hydroxypyrimidine-6-carboxaldehydes,[3] 2-phthalimidoaldehydes,[4] 5-fluoropyrimidine-6-carboxaldehydes,[5] and their derivatives have been synthesized and tested as inhibitors of growth and protein synthesis in Ehrlich ascites carcinoma in mice. The 5-fluoropyrimidine-6-carboxaldehydes have shown strong inhibitory activity against incorporation of amino acids and formate into proteins as well as thymidine, orotic acid, and formate into nucleic acids.

Several derivatives of different types of aldehydes have also been found to inhibit the growth of neoplasms, for example, pyridine-2-carboxaldehyde thiosemicarbazone,[6] octadecylthiosemicarbazones of aldehydes,[7] indole-3-carboxaldehyde *p*-bromophenylhydrazone,[8] and 3-ethoxy-2-ketobutanal bisthiosemicarbazone.[9]

Antagonism to the utilization of folates was recognized a number of years ago as a general property of

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