Carcinogenicity of lactones. V.[1] The reactions of 4-hydroxypent-2-enoic acid lactone with α-toluenethiol, benzylamine, methylamine, imidazole, and guanidine[2]

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The Michael additions of α-toluenethiol, methylamine, and benzylamine to 4-hydroxypent-2-enoic acid lactone have been confirmed to be facile. The analogous additions of imidazole and glycine amide are much less readily accomplished and no stable Michael addition product could be detected with guanidine as the base. In the p*H* range 6–9, subsequent lactone ring opening reactions of the Michael addition products were observed only when methylamine (pK_a 9.3) and benzylamine (pK_a 10.6) were used as nucleophiles.

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Introduction

During previous investigations related to the carcinogenicity of lactones (1) it was noted that all the carcinogenic γ-lactones studied chemically (1, 2), of which **1** may be regarded as representative, had proven to be alkylating agents and it was concluded (1) that the most probable initial in vivo sites of alkylation would be the nucleophilic functional groups of proteins. It was also noted that the products of such Michael addition reactions, for example **2***a–f*, still allowed the possibility of subsequent in vivo nucleophilic attack at the lactone carbonyl group to give products of the type **3***a–d* (eq. [1]).

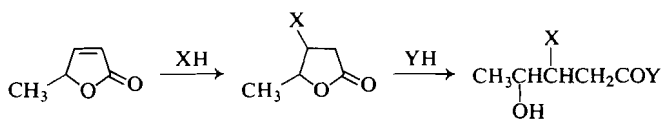
The amino acid residues which were considered to be the most readily alkylatable by the lactones were those containing the thiol (cysteine), imidazolium (histidine), and primary amino (*N*-terminal and lysine etc.) functions. As a preliminary step towards delineating the types and selectivities of the permutations and combinations possible for such carcinogen-nucleophile reactions we have examined the reactions of lactone **1** with some simple model compounds containing the above functional groups. Since a purely chemical survey only was intended at this stage little effort was made to duplicate physiological conditions except that whenever feasible reactions were carried out in aqueous solution within the p*H* range 6–9.

[1]For Part IV see ref. 1.
[2]Abstracted from the M.Sc. thesis of J.N.B., University of Toronto, Toronto, Ontario, 1968.

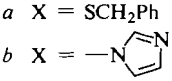
Results and Discussion

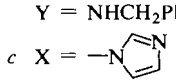
(a) Michael Addition Reactions

Attention was first directed towards the reaction of lactone **1** with α-toluenethiol. Selection of the latter as a simple model for a cysteine thiol residue was dictated largely by its easy recognition in the proton magnetic resonance (p.m.r.) spectra of the expected products (3). However, its low solubility in the preferred solvent, water, required the reactions to be carried out in ethereal or alcoholic media. As expected, the Michael addition of α-toluenethiol to lactone **1**

[1] 

1 **2** **3**

a X = SCH_2Ph
b X = 
c X = $NHCH_2Ph$
d X = $NHCH_3$
e X = $NHCH_2CONH_2$
f X = $NHC(=NH)NH_2$

a X = SCH_2Ph, Y = $NHCH_3$
b X = SCH_2Ph, Y = $NHCH_2Ph$
c X =  , Y = $NHCH_2Ph$
d X = $NHCH_2Ph$, Y = $NHCH_3$

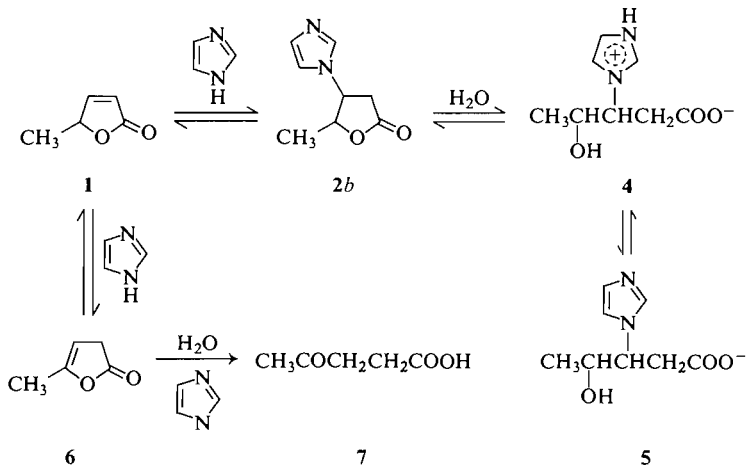


CHART 1

proceeded extremely smoothly in ether solution (containing triethylamine as catalyst) at room temperature to give 3-benzylthio-4-hydroxypentanoic acid lactone (**2***a*) in 89% yield. This result confirmed the earlier data (2) that reactions of carcinogenic δ-lactones with cysteine-like thiol groups are facile.

The reaction considered next, of the histidyl analogue imidazole with **1**, proved more complex than in the above and related studies (3). On mixing lactone **1** with one equivalent of imidazole in aqueous solution at 20°, Michael addition to give **2***b* did occur but owing to the relatively lengthy reaction times required at room temperature to ensure significant conversion of the starting compounds to products, several further and competitive reactions took place. The total situation, which was deduced mainly from periodic p.m.r. analyses, is summarized in Chart 1.

The desired product **2***b* and the zwitterionic compound **4** were both isolable from the reaction mixture. The carboxylate **5** and levulinic acid (**7**) were not isolated but were identified in the mixture by comparison of the relevant p.m.r. peaks with those of authentic spectra.

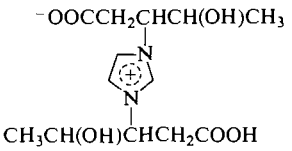
The complications encountered with imidazole can be largely attributed to its well documented assistance of ester and lactone hydrolysis and to its general base catalytic properties (4). Thus the rate of hydrolysis of **2***b* to **4** would be expected to be enhanced by the presence of imidazole as also would the equilibration of **1** and its isomeric lactone **6**. Although the latter is much less favored thermodynamically, its greater susceptibility to nucleophilic attack (3) by water, either alone or assisted by imidazole, would ensure a steady accumulation of levulinic acid (**7**). Some representative p.m.r. data indicating the composition of the mixture as the reactions proceeded are recorded in Table 1. A further p.m.r. monitored study showed that treatment of either the Michael product **2***b* or the zwitterion **4** with dilute hydrochloric acid afforded a 1:1 equilibrium mixture of both compounds together with a trace of levulinic acid. The use of more concentrated hydrochloric acid as a catalyst resulted only in the formation of increased amounts of levulinic acid. Dilute sodium hydroxide solution effected the conversion of both **2***b* and **4** into the imadazolium carboxylate **5**.

TABLE 1

Yields of products of the reaction of imidazole with 4-hydroxypent-2-enoic acid lactone (**1**) as a function of time*

Product	δ (p.p.m.)† of C-5 CH_3	Percentage present in reaction mixture‡ 24 h	45 h	250 h	500 h
1	1.45	33	29	25	6
2*b*	1.55	30	27	<1	—
4	1.17	10	20	60	67
5	1.10	16	13	—	—
7	2.10	8	11	15	27

*Reactions carried out at 20° in aqueous solution 1.5 *M* in each reagent.
†With sodium 3-(trimethylsilyl)propane sulfonate as the internal standard.
‡Calculated from the area of the C-5 CH_3 peak in the 60 MHz p.m.r. spectrum.



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Owing to the difficulties encountered during the work-up procedures for compounds **2***b* and **4**, the isolated yields were not as high as might have been expected from the concentrations known to be present in the reaction mixture (cf. Table 1). Furthermore, during the work-up process secondary reactions, including reverse Michael processes, occurred and in addition a certain amount of artefactual material, for which the chemical shifts of the imidazolium vinylic protons in the p.m.r. spectrum at δ 7.6 and 9.14 p.p.m. were considered indicative of the hydrolyzed bis-addition product **8**, was always formed.[3]

The complexity of the reaction of **1** with imidazole under the conditions surveyed make it impossible to draw any conclusions pertinent to the in vivo situation. However, it is apparent that alkylation of histidine residues by carcinogenic γ-lactones is a distinct possibility.

Attention was next directed towards the reactions of the primary amino group. The facile Michael reactions of amines analogous to lysine side chains (pK_a 9.4–10.6 (5)) such as benzylamine (pK_a 9.3 (6)) and methylamine (pK_a 10.6 (6)), with lactone **1** to give **2***c* and **2***d* respectively had been well documented previously (3) and no further work in this area was carried out.

It is of interest to compare the ease with which the Michael addition product can be isolated from the primary amine reactions with the situation encountered with the secondary amine of markedly less basicity and nucleophilicity, imidazole. In the latter case the attainment of equilibrium in the Michael addition reaction appears to be relatively slow, thereby allowing significant isomerization of **1** to the β,γ-unsaturated lactone **6** to occur. In contrast to the apparently sluggish Michael addition capabilities of such secondary amines, their capacity for nucleophilic attack at the carbonyl group of **6**, once the latter is formed, is much superior and accordingly the products of the latter reactions build up under conditions of kinetic control (7).

It was noted that the above primary amines could not be regarded as good models for *N*-terminal amino groups (pK_a 7.6–8.4 (5)). A more suitable model for the latter appeared to be glycine amide and accordingly a study of its reaction with **1** in aqueous solution was carried out. The reactions which occurred turned out to be similar to, but much less facile than, those encountered with imidazole as a nucleophile and in view of the very slow rate of formation of the Michael addition product **2***e* it may be concluded that extensive in vivo alkylation of *N*-terminal amino groups by **1** would be improbable.

Of the remaining amino acid functions known to be readily susceptible to chemical modification (8) only the guanidinium (of arginine) group was considered further in this preliminary survey. It was included on the basis of the abundance of arginine residues in many histones although, in view of its quoted pK_a range of 11.6–12.6 (5), it was not regarded as a serious in vivo lactone alkylation possibility.

Guanidine (pK_a 13.65 (6)) was taken as the simplest analogue. The reagents, guanidine hydrochloride and **1**, were mixed in aqueous solution and in order to achieve reasonable rates of reaction it was found necessary to maintain the p*H* of the solution at values > 10. None of the hoped for Michael addition product **2***f* could be isolated from the reaction mixture and on the basis of a combination of p.m.r. and infrared (i.r.) analyses of the amide products it was concluded that the sequence of reactions indicated in Chart 2 was occurring with the initial step again being guanidine base-catalyzed isomerization of **1** to the β,γ-unsaturated lactone **6**. That the latter compound would in fact then react very rapidly with guanidine was established by mixing guanidine with **6** in either aqueous or methanolic solution. The product from the aqueous reaction showed an i.r. red carbonyl absorption at 1660 cm^{-1} compatible with the amide structure **9** but, presumably as a result of its susceptibility to subsequent nucleophilic attack, it could not be purified. However, the structure assignment was corroborated by the observation that on treatment with aqueous hydrochloric acid, the product hydrolyzed very rapidly to give levulinic acid and guanidinium hydrochloride. Furthermore, on attempted prep-

[3]The formation of such a product would not be unexpected in view of the ease with which β-propiolactone and acrylic acid are known to form such bis-adducts on reaction with imidazole (1).

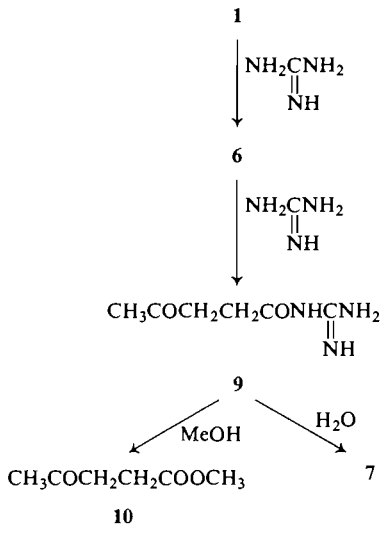

CHART 2

aration of the same compound **9** under anhydrous methanolic conditions the expected products, methyl levulinate (**10**) and guanidinium hydrochloride, were isolated.

(b) Subsequent Reactions of the Initially Formed Michael Products

As outlined at the beginning of this communication, in principle each of the Michael addition products obtained above is susceptible to further attack at the carbonyl group by each of the nucleophiles evaluated. However, of the complete permutation of possible reactant combinations of the Michael products **2***a–d* with all of the above model protein nucleophiles, very few ring opening reactions were observed. In fact at 20° in aqueous or aqueous methanolic solutions in the p*H* range 6–9, the only compounds for which formation of compounds **3** could be satisfactorily[4] demonstrated were for the primary amine analogues of the ε-amino group of lysine; the products **3***a–d* of reaction of **2***a–d* with benzylamine and methylamine were produced in 50–80% yields.

Experimental

The instruments used for spectral etc. determinations, were as listed previously (9).

[4]Guanidine also reacted with compounds **3** and although spectral data indicated that attack at the carbonyl group was occurring, this could not be fully substantiated.

Compounds

4-Hydroxypent-2-enoic acid lactone (**1**) and 3-benzylamino-4-hydroxypentanoic acid lactone (**2***c*) were prepared by the literature methods (3); all other materials used were purchased from Fisher or Aldrich.

The Reaction of 4-Hydroxypent-2-enoic Acid Lactone with α-Toluene Thiol

A solution of 4-hydroxypent-2-enoic acid lactone (2.0 g, 20 mmole), α-toluene thiol (2.54 g, 20 mmole), and triethylamine (2.06 g, 20 mmole) in diethyl ether was stirred for 1 h at 20°. The solvent was then removed by rotary evaporation at 20° and the residual oil distilled to give *3-benzylthio-4-hydroxypentanoic acid lactone* (**2***a*) (4.0 g), b.p. 160–161° (0.7 mm); i.r. ($CHCl_3$) 1780 cm^{-1}; p.m.r. ($CDCl_3$) δ 1.26 (3H, d, J = 6.5 Hz, CH_3CH), 2.6 (2H, m, $CHCH_2CO$), 2.9 (1H, m, $CHCH$(S)CH), 3.73 (2H, s, $SCH_2C_6H_5$), 4.3 (1H, m, CH_3CH(O)CH), and 7.30 p.p.m. (5H, s, C_6H_5).

Anal. Calcd. for $C_{12}H_{14}O_2S$: C, 64.90; H, 6.30; S, 14.40. Found: C, 65.29; H, 6.08; s, 14.53.

The Reaction of 3-Benzylthio-4-hydroxypentanoic Acid Lactone with Methylamine and Benzylamine

(a) With Methylamine

A solution of 3-benzylthio-4-hydroxypentanoic acid lactone (2.04 g, 9 mmole), aqueous methylamine (1 ml, 9 mmole CH_3NH_2) in 85% aqueous methanol (14 ml) was stirred for 16 h at 20° and was then rotary evaporated. The p.m.r. analysis indicated the presence of 80% of *3-benzylthio-4-hydroxy-N-methylpentanoamide* (**3***a*) at this stage but all attempts, including distillation, to effect further purification resulted in a reverse Michael reaction. The spectral data obtained were: i.r. ($CHCl_3$) 1668 cm^{-1}; p.m.r. ($CDCl_3$) δ 1.21 (3H, d, J = 6.5 Hz, CH_3CH), 2.47 (2H, m, $CHCH_2CO$), 2.75 (3H, d, J = 4.5 Hz, $CONHCH_3$), 3.2 (1H, m, $CH.CH$(S)CH_2), 3.42 (1H, s, O*H*), 3.78 (1H, m, CH_3CH(OH)CH), 3.80 (2H, s, $SCH_2C_6H_5$), 6.2 (1H, m, $CONHCH_3$), and 7.3 p.p.m. (5H, s, C_6H_5).

(b) With Benzylamine

3-Benzylthio-4-hydroxypentanoic acid lactone (0.51 g, 2 mmole), benzylamine (0.25 g, 2 mmole), and 85% aqueous methanol (3 ml) were stirred together for 24 h at 20° and the solution was then rotary evaporated. The residual solid was chromatographed on Florisil (75 g, column prepared in chloroform) and chloroform–acetone (1:1) elution gave a fraction containing *3-benzylthio-4-hydroxy-N-benzylpentanoamide* (**3***b*). Recrystallization from benzene–cyclohexane afforded 0.38 g of material m.p. 76–77°; i.r. ($CHCl_3$) 1668 cm^{-1}; p.m.r. ($CDCl_3$) δ 1.17 (3H, d, J = 6.5 Hz, CH_3CH), 2.42 (2H, m, $CHCH_2$-CO), 3.05 (2H, m, O*H* and $CHCH$(S)CH_2), 3.75 (2H, s, $SCH_2C_6H_5$), 3.75 (1H, m, CH_3CH(OH)CH), 4.38 (2H, d, J = 6.0 Hz, $CONHCH_2C_6H_5$), 6.1 (1H, m, $CONHCH_2$), 7.22 (5H, s, $SCH_2C_6H_5$), and 7.25 p.p.m. (5H, s, $NHCH_2C_6H_5$).

Anal. Calcd. for $C_{19}H_{23}NO_2S$: C, 69.30, H, 7.02; N, 4.25; S, 9.72. Found: C, 69.38; H, 7.20; N, 4.29; S, 9.72.

The Reaction of 4-Hydroxypent-2-enoic Acid Lactone with Imidazole

(a) A solution of 4-hydroxypent-2-enoic acid lactone (1.44 g, 15 mmole) and imidazole (1.01 g, 15 mmole) in

water (10 ml) was kept for 3 weeks at 20°. The precipitate (0.55 g) of *3-imidazolyl-4-hydroxypentanoic acid betaine* (**4**) was collected and the aqueous filtrate concentrated by rotary evaporation at 20°. Trituration of the residue with chloroform–benzene afforded a further 0.47 g of the zwitterion **4**. Recrystallization of a portion from water–acetone afforded a sample, m.p. 185°,[5] i.r. (KBr) 1600 cm^{-1} (COO^-); p.m.r. (D_2O) δ 1.17 (3H, d, J = 6.5 Hz, CH_3CH), 2.9 (2H, m, $CHCH_2CO$), 4.15 (1H, m, $CHCH$-$(N)CH_2$), 4.65 (1H, m, $CH_3CH(OH)CH$), 7.54 (2H, m, $NCH{=}CHN$), and 8.88 p.p.m. (1H, m, $N{=}CHN$).

Anal. Calcd. for $C_8H_{12}N_2O_3$: C, 52.16; H, 6.57; N, 15.21. Found: C, 52.36; H, 6.68, N, 15.14.

No further compounds could be isolated in a pure form from this reaction (see Discussion).

(b) 4-Hydroxypent-2-enoic acid lactone (1.5 g, 15 mmole) and imidazole (3.3 g, 45 mmole) were dissolved in water (15 ml) and the solution was stirred for 21 h at 20°. Water (10 ml) was then added and the aqueous solution was extracted with chloroform (4 × 25 ml). The dried ($MgSO_4$) chloroform extracts were evaporated (20°) and 2 *N* aqueous hydrochloric acid (10 ml) was added to the glassy product. Rotary evaporation at 20° afforded *3-imidazolyl-4-hydroxypentanoic acid lactone hydrochloride* which on recrystallization from aqueous methanol gave needles (0.4 g), m.p. 162–164°; i.r. (KBr) 1784 cm^{-1}; p.m.r. (D_2O) δ 1.53 (3H, d, J = 6.5 Hz, CH_3CH), 3.35 (2H, m, $CHCH_2CO$), 5.12 (2H, m, $CH_3CHC(O)CH$ and $CHCH(N)CH_2$), 7.65 (2H, m, $NCH{=}CHN$), and 8.96 p.p.m. (1H, m, $N{=}CHN$).

Anal. Calcd. for $C_8H_{11}ClN_2O_2$: C, 47.41; H, 5.47; Cl, 17.49; N, 13.82. Found: C, 47.48; H, 5.62; Cl, 17.44; N, 13.82.

3-Imidazolyl-4-Hydroxypentanoic Acid Lactone Hydrochloride from the Zwitterion 4

3-Imidazolyl-4-hydroxypentanoic acid betaine (**4**, 0.38 g, 2 mmole) was treated with 2 *N* aqueous hydrochloric acid at 60° for 30 min and the solution was then rotary evaporated. Recrystallization of the residue from aqueous methanol gave the desired lactone hydrochloride m.p. 162–164° which was identical in all respects with the product obtained above.

The Reaction of 3-Imidazolo-4-hydroxypentanoic Acid Lactone with Benzylamine

A solution of 3-imidazolo-4-hydroxypentanoic acid lactone hydrochloride (0.17 g, 9 mmole) and benzylamine (0.25 g, 27 mmole) in water (5 ml) was stirred for 3 h at 20° and was then filtered. (The filtrate was evaporated and the solid obtained recrystallized from acetone – petroleum ether (b.p. 40–60°) to give benzylamine hydrochloride (0.11 g, 8 mmole) m.p. 245–248°). The residue was recrystallized from aqueous acetone to yield *3-imidazolyl-4-hydroxy-N-benzylpentanoamide* (**3***c*) (0.17 g), m.p. 156–158°; i.r. (KBr) 1635 cm^{-1}; p.m.r. (D_2O) δ 1.18 (3H, d, J = 6.5 Hz, CH_3CH), 2.88 (2H, m, $CHCH_2$-CO), 4.22 (2H, s, $NDCH_2C_6H_5$), 4.25 (1H, m, $CHCH$-CH_2), 5.15 (1H, m, CH_3CHCH), 7.50 (2H, m, $NCH{=}CHN$), 7.51 (5H, s, C_6H_5), and 8.67 p.p.m. (1H, m, $N{=}CHN$).

Anal. Calcd. for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 65.82; H, 7.20; N, 15.37.

Reaction of 4-Hydroxypent-3-enoic Acid Lactone (6) with Guanidine

(a) In Aqueous Solution

To a solution of 4-hydroxypent-3-enoic acid lactone (1.74 g, 18 mmole) and guanidine hydrochloride (1.65 g, 18 mmole) in water (3 ml) was added 1 *N* aqueous sodium hydroxide solution (18 ml, 18 mmole). Spectroscopic analysis after 15 min at 20° indicated that complete reaction had occurred to give mainly *N-guanidyl-4-oxopentanoamide* (**8**)[6] [i.r. 1660 cm^{-1}, p.m.r. (H_2O), δ 2.20 (3H, s, CH_3CO), 2.41 (2H, m, $CH_3COCH_2CH_2CO$), and 2.74 p.p.m. (2H, m, $CH_3COCH_2CH_2CO$)]. The mixture was concentrated at 20°, ethanol (10 ml) added, and the precipitated sodium chloride filtered off. Aqueous hydrochloric acid 2*N* (10 ml) was added to the filtrate and the solution was again rotary evaporated and the residue was extracted with acetone. The remaining solid (0.86 g) m.p. 184–186° was identified as guanidine hydrochloride and the acetone solution, after evaporation and distillation, gave levulinic acid (7) (0.89 g), b.p. 84–86° (0.12 mm) which was identical with an authentic sample.

(b) In Methanol Solution

When the above reaction was carried out in anhydrous methanol (5 ml) solution[6] containing sodium methoxide (19 mmole, from sodium 0.45 g), the products isolated were guanidine hydrochloride (0.51 g), m.p. 184–186° and methyl levulinate (**10**, 0.72 g), b.p. 44–45° (15 mm) identical with an authentic sample.

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[5]At this temperature a change of crystalline form occurred which was followed by sublimation at 200°.

[6]All attempts to isolate **8** from this solution were unsuccessful owing to its extreme susceptibility to further nucleophilic attack.