Carcinogenicity of lactones. IV.[1] Alkylation of analogues of DNA guanine groups such as imidazole, *N*-methylimidazole, and guanosine by α,β-unsaturated acids[2]

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Studies on the reactions of carcinogenic γ-lactones and related compounds with analogues of guanine DNA residues indicate that the lactones themselves will not effect permanent alkylation of the guanine N-7 position since the Michael addition reactions involved would be readily reversible. In contrast, the α,β-unsaturated acids resulting from hydrolysis of such lactones are effective guanine N-7 alkylating agents owing to zwitterionic stabilization of the corresponding Michael addition products.

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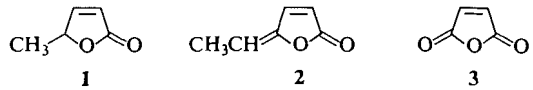
Previous studies (1) have shown that, of a number of γ-lactones which have been tested for carcinogenicity (2), a distinction of possible in vivo significance between active and inactive lactones can be made in that carcinogenic lactones such as 4-hydroxypent-2-enoic acid lactone (**1**) and 4-hydroxyhexa-2,4-dienoic acid lactone (**2**) behave as *alkylating* agents whereas noncarcinogenic lactones are *acylating* agents (1).



1 **2** **3**

4 **5**

It is now generally accepted (3, 4)[4] that one of the properties of carcinogenic alkylating agents responsible for their cancer-inducing effects is their capacity to effect genetic mutation by alkylating selectively at the N-7 positions of guanine residues of deoxyribonucleic acid (DNA). Accordingly it became of interest to determine whether or not the reactions of the alkylating carcinogenic lactones and related compounds with DNA would follow the same pattern.

Guanosine (**6**) has been widely used as a model compound for the guanine residues of DNA (5–7) but, to our surprise, our attempts to effect reaction of the lactones **1** and **2** and of structurally related carcinogens such as maleic anhydride (**3**) and sorbic acid (**4**) with guanosine under a wide variety of conditions[5] were all unsuccessful. That the above failures to effect alkylation were not the result of a defective experimental technique was demonstrated by the successful repetition of the reaction, carried out earlier by Roberts and Warwick (5) of β-propiolactone (**5**) with guanosine; this gave, after hydrolytic work-up, the reported *N*-7-(2-carboxyethyl)guanine (**8**) in 71% yield (see Chart 1). Attempts to isolate the intermediate nucleoside **7** in a pure form were unsuccessful since it appeared from paper chromatographic analysis that some β-propiolactone modification of the ribose moiety had also occurred.

From a detailed study of the above reaction and of the limits of detectability of compounds such as **7** and **8** by paper chromatographic analysis, the possibility that the reactions of **1** and **2** with guanosine were producing significant amounts of the N-7 alkylated products was excluded.

In view of the facility with which the carcinogenic lactones **1** and **2** had previously effected alkylation of various nucleophiles (1) their inability to modify guanosine was at first somewhat disturbing. However, a consideration of the pathways and intermediates which would be involved in such a reaction provided a rationale for the ineffectiveness of the lactones as alkylating agents of the guanine tertiary N-7 atom. As

[1]For Paper III see ref. 1.

[2]Abstracted from ref. 20. Presented in part at the 52nd Canadian Chemical Conference, Montreal, June, 1969.

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[4]A more complete discussion of the evidence is contained in ref. 20.

[5]These included heating in aqueous solution at several hydrogen ion concentrations in the p*H* range 5–9 and in dimethylformamide at 150° for several hours. In all cases guanosine, or guanine in the case of a reaction carried out at acid p*H*, was recovered almost quantitatively.

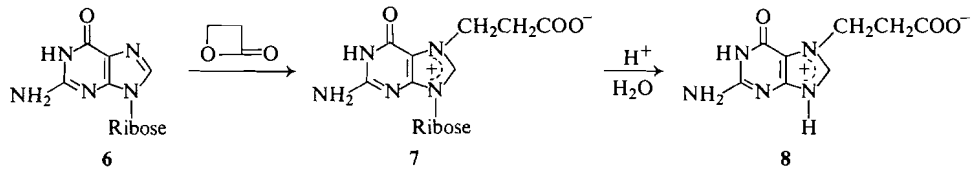


CHART 1

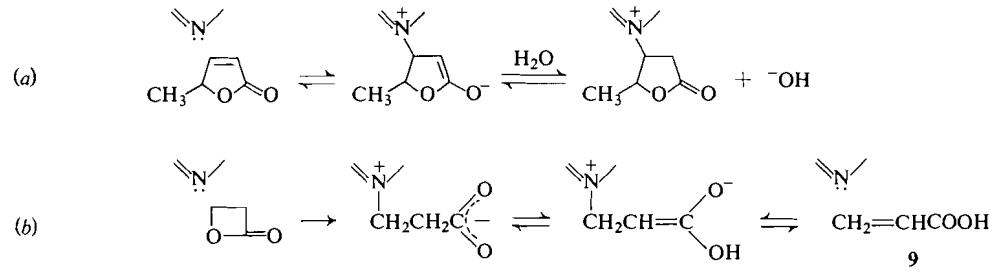


CHART 2

Chart 2*a* indicates, the Michael reaction of lactones such as **1** (or **2**) with a tertiary base would be expected to be unfavorable to product formation when the equilibrium is considered in terms of the relative acidities and basicities of the starting compounds and products involved. On the other hand, with other alkylating agents (5, 6), including β-propiolactone as illustrated in Chart 2*b*, formation of the product would be favored by its stabilization as a normal or zwitterionic salt. Furthermore it was noted that the zwitterionic product stabilization factors operating in the S_N2 reaction of β-propiolactone with tertiary amines would also obtain for the addition of such nucleophiles to α,β-unsaturated acids such as acrylic acid (**9**) (see Chart 2*b*) and that such stabilization might well be sufficient to ensure the accumulation of significant amounts of the Michael addition product.

In view of the difficulties experienced with the guanosine reactions with respect to product purification, spectral interpretations etc., it was decided to test the validity of the above stabilization hypotheses with simpler model compounds containing the guanine N-7 type of function. Purine was considered first but was discarded since its reaction with β-propiolactone led to a multiplicity of products. Fortunately however, the even simpler guanine model compounds, imidazole (**10***a*) and *N*-methylimidazole (**10***b*), proved satisfactory and initially attention was concentrated on the reactions of these two compounds. Unfortunately, *N*-methylimidazole, which is the better model of the latter two since it has one nitrogen atom substituted in a manner analogous to N-9 of guanine nucleotides, effected polymerization of both β-propiolactone and acrylic acid, and it was necessary to use the less satisfactory model imidazole in order to obtain any characterizable products.

That the tertiary nitrogen atom of imidazole was in fact a suitable model for N-7 in guanosine was demonstrated by its facile reaction with one equivalent of β-propiolactone (**5**) in diethylether to give the products of mono- and bis-alkylation, **11** (54%) and **12** (16%) respectively (Chart 3). When acetonitrile, in which **11** is soluble, was used as the solvent the yield of **12** was increased to 50% and on addition of a second equivalent of β-propiolactone the yield of **12** became quantitative.[6]

The charged nature of the imidazolium ring of **11** and **12** was confirmed by the downfield shift in the proton magnetic resonance (p.m.r.) spectra of both compounds of the vinylic protons of the imidazole rings (δ 7.51, C-4 and C-5 H and 8.75 p.p.m., C-2 H) relative to those of imidazole (δ 7.15 and 7.71 p.p.m.) and *N*-methylimidazole (δ 7.23 and 7.77 p.p.m.) respectively.

Extension of the model work to acrylic acid (**9**) (Chart 3) confirmed that zwitterionic stabilization

[6]Similar reactions of this lactone have been observed with pyridine (8), 2-aminopyridine (9), and trialkylamines (10).

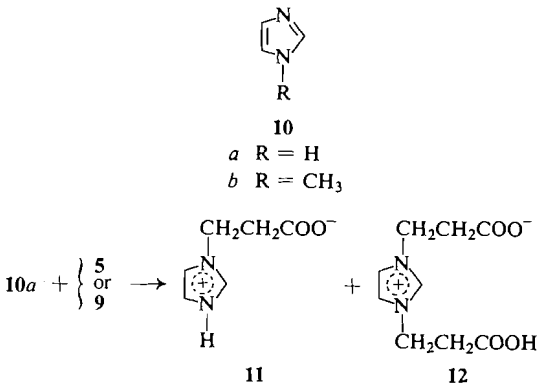


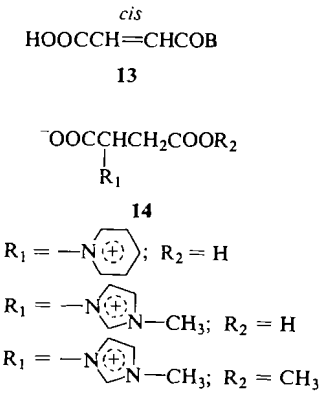
CHART 3

of the Michael addition products of reactions of α,β-unsaturated acids as illustrated in Chart 2*b* was important since the bis-adduct **12** was indeed formed when imidazole was treated with two equivalents of acrylic acid in methanolic or aqueous solution. Although the reaction proceeded somewhat more slowly than with β-propiolactone, a good (54%) yield of **12** was obtained after several hours reaction at steam bath temperature.

This demonstration of the importance of zwitterionic stabilization in the Michael addition of the guanine N-7 type of nucleophile to α,β-unsaturated compounds raised the possibility that, since the carcinogenic lactones and related compounds did not react with DNA, their carcinogenic properties might obtain from their initial in vivo hydrolysis to α,β-unsaturated acids which could then effect alkylation of DNA at the guanine N-7 position.

The discovery of the literature report that maleic acid (**13**, B = OH), the hydrolysis product of the carcinogenic maleic anhydride, reacted with pyridine in aqueous ethanol (11) to give the betaine **14***a* provided a further indication that such a rationale for the in vivo carcinogenic action was reasonable and the subsequent investigations were designed to evaluate the generality of such reactions and of the likelihood of their occurring in vivo.

The reaction of *N*-methylimidazole with maleic acid proceeded smoothly in methanol solution to give 2-(*N*-methylimidazolium)succinic acid betaine **14***b* in 65% yield. In the p.m.r. spectrum of the compound the succinic acid protons appeared as an ABX system and the imidazolium vinylic protons again showed the

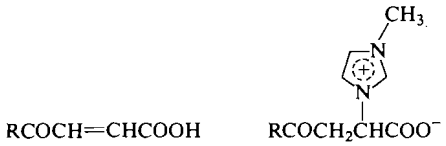


downfield shifts to be expected if the ring was positively charged.

3-Carbomethoxyacrylic acid (maleic acid monomethyl ester; **13**, B = OCH_3) was evaluated next since this could be regarded as a model for the products of in vivo attack of maleic anhydride by any in vivo nucleophiles other than water. Again a ready reaction with *N*-methylimidazole was observed and the product **14***c* was isolated in 57% yield. The p.m.r. spectrum included the characteristic patterns noted above for **14***b*. The assignment of structure **14***c* to the compound, rather than that of the isomeric product which would have resulted from Michael addition in the opposite sense, was made on the assumption that the carbomethoxy group would have a greater directing influence than carboxyl particularly since under the reaction conditions the latter would exist predominantly as the conjugate base. However, the isomeric structure cannot be rejected entirely.

In order to evaluate the influence of the stereochemistry of the double bond on such Michael additions the reaction of fumaric acid with *N*-methylimidazole in aqueous solution was examined. In contrast to the facile room temperature addition observed with the *cis* isomer maleic acid, with fumaric acid the product **14***b* was formed much less readily and even after heating the reactants for several hours at temperatures in excess of 100°, a yield of 18% only was obtained. Nevertheless, although the *trans* acid is clearly much less reactive, significant addition does occur.[7]

[7]This estimate of the effect on this particular type of Michael reaction of the geometry of the ethylenic linkage was valuable since some subsequent reactions were carried out on the more conveniently prepared *trans* analogues of in vivo compounds in which the corresponding unsaturation would be *cis*.



RCOCH=CHCOOH

15

a R = CH_2CH_3
b R = CH_3
c R = C_6H_5

$RCOCH_2\overset{+}{C}HCOO^-$

16

a R = CH_2CH_3
b R = CH_3
c R = C_6H_5

The compounds considered next were those related to the hydrolysis product, *cis*-4-oxohex-2-enoic acid (**15***a*), of carcinogenic lactone **2**.[8] *trans*-Acetylacrylic acid (**15***b*), being readily prepared from levulinic acid, was selected as a suitable analogue of **15***a* but unfortunately its reaction with *N*-methylimidazole produced only intractable gums from which none of the desired compound **16***b* could be isolated. Accordingly *trans*-benzoylacrylic acid (**15***c*) was substituted[9] and from the reaction with *N*-methylimidazole in methanol solution the expected zwitterionic product was obtained in 66% yield. The structure was assigned as **16***c*, although again the isomeric formula could not be wholly excluded on the basis of the available data. The p.m.r. spectrum was as expected for **16***c* but unlike those of **14***b* and **14***c* the C-2 and C-3 protons appeared as an A_2X pattern.

Compounds **14***b*, **14***c*, and **16***c* were extremely hygroscopic and satisfactory elemental analyses could only be obtained for the succinic acid derivatives **14***b* and **14***c*.[10] As a result of these difficulties the structural assignment of **16***c* rests primarily on the similarities of the infrared (i.r.) and p.m.r. spectra of the three compounds. Recourse was had to mass spectroscopy in an attempt to establish molecular weights for the three compounds but in no case was a molecular ion observed. However, the spectra (70 eV) of **14***b*, **14***c*, and **16***c* were strikingly similar showing base peaks at *m/e* 82 for **14***b* and **14***c* and a 40% of the base peak for **16***c*. This corresponds to ionized *N*-methylimidazole and its subsequent fragmentation pattern was common to all three spectra. In addition the total mass spectrum of each was compatible with thermal decomposition, involving a reverse Michael reaction, into the respective reactants having occurred prior to ionization.[11]

That Michael reactions with unsaturated acids are not facile for all conjugated unsaturated acids was demonstrated by the total failure of the carcinogen sorbic acid (**4**), or of *trans*-but-2-enoic acid (crotonic acid), to react with *N*-methylimidazole even at temperatures in excess of 150°. In these cases, however, the lack of reaction may be ascribed to the *trans*-stereochemistry of the ethylenic linkages and also to the fact that the adverse electronic effects of the methyl vinyl and methyl groups respectively serve to increase the electron density at the β-carbon atom thereby making nucleophilic attack less facile than on acrylic acid itself. For acetyl- and benzoylacrylic acids the electron-withdrawing β-substituents facilitate Michael addition in the opposite direction.

Although the above results with imidazole and *N*-methylimidazole provide a strong indication that the α,β-unsaturated acid hydrolysis products of the carcinogenic lactones are capable of alkylating guanine N-7 positions, the data are obviously not extrapolatable to nuclear DNA without many reservations. Accordingly the experiments were repeated using guanosine in aqueous solution as a closer model for the in vivo situation and the results obtained are summarized in Chart 4.

The first reaction attempted was that with acrylic acid since it was expected that the products would be identical with those obtained with β-propiolactone and for which isolation and purification procedures had been established. Guanosine and acrylic acid were heated[12] in aqueous solution at 80° for 3 h and after hydrolytic work-up of the reaction mixture a 59% yield[13] of *N*-7-(2-carboxyethyl)guanine (**8**) was obtained which was identical with the β-propiolactone product obtained previously. As in the previous imidazole studies the reaction of acrylic acid with

[8]The acid derived from lactone **1** itself was not studied at this stage since it was felt that the reactions of acrylic acid were sufficiently representative of this type of function. Furthermore analogues of the hydrolysis product of the more potent carcinogen **2** were of more immediate interest.

[9]Ease of preparation was again a factor in the choice of *trans*-benzoylacrylic acid as an appropriate model. In addition, its Michael reactions with, for example, cysteine (12) had been studied previously. Although it possesses considerable biological activity in its own right (13) it has not yet been evaluated as a carcinogen.

[10]This compound formed a hydrate which was relatively stable.

[11]A detailed analysis of the mass spectral data and fragmentation pathways is given in ref. 20, p. 119–124.

[12]Heating was necessary in order to bring the guanosine into solution.

[13]Based on reacted guanosine.

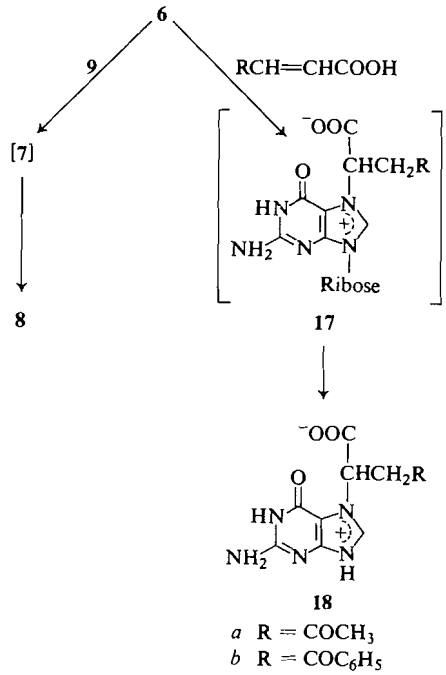


CHART 4

guanosine was much slower than for β-propiolactone but nevertheless proceeded quite readily.

Equally encouraging results were obtained when guanosine was reacted under similar aqueous conditions with the model of the hydrolysis acid of lactone **2**, acetylacrylic acid (**15***b*), and a 29% yield[13] of the N-7 alkylated guanine **18***a* was obtained following the hydrolytic work-up procedure. The material proved somewhat difficult to handle and no satisfactory elemental analyses were obtained. The structural assignment was made on the basis of the i.r. and p.m.r. spectra and of the relative ultraviolet (u.v.) absorptions in 1 *N* aqueous hydrochloric acid, 1 *N* aqueous sodium hydroxide, and p*H* 7 phosphate buffer solutions which were as required for an N-7 alkylated guanine (5, 6).

The reaction of benzoylacrylic acid with guanosine was also investigated but a trace only of the desired product **18***b* was detectable.[14] Although the compounds **18** are undoubtedly produced *via* the intermediacy of compounds **17**, no attempt was made to isolate the latter since such zwitterionic guanosine derivatives are known to be very susceptible to hydrolysis of the glycosidic linkage (6).

Disappointingly, maleic acid appeared to be such an excellent catalyst for the hydrolysis of the guanosine glycoside link that no guanine N-7 alkylation could be achieved prior to the formation of the insoluble hydrolysis product guanine.[15]

Not unexpectedly, in view of the negative *N*-methylimidazole results, sorbic acid and crotonic acid failed to react with guanosine even under the forcing conditions of heating under reflux in *N*,*N*-dimethylformamide solution.

Summary and Conclusions

The total data suggest that for lactones analogous to **1** and **2**, and for related compounds such as maleic anhydride, it may well be the unsaturated acids, or their derivatives, resulting from in vivo hydrolysis that are the proximate carcinogens. This suggestion is still in accord with the *alkylating–acylating* distinction (1) drawn between carcinogenic and non-carcinogenic lactones and on this basis a plausible, albeit at the present time speculative, rationale of their possible in vivo effects can be presented.

Acylation damage by inactive lactones to protein and other cellular nucleophiles should be, in general, non-specific[16] and thus no one site should be selectively or extensively attacked. Furthermore such acyl derivatives, and also the lactones themselves, are susceptible to hydrolysis[17] to give readily metabolizable ketoacids. In the cases of the alkylating carcinogenic lactones it is envisaged that they also will react initially with protein nucleophiles since our experience (1) is that such Michael additions should be more rapid than any other in vivo process.[16] However, the reversible nature of these reactions will allow at least some of the active lactone to proceed, under thermodynamic control, from one nucleo-

[14]The much poorer yields obtained with acetyl- and benzoylacrylic acids compared with acrylic acid itself are probably largely due to the presence of the less favorable *trans* double bond geometry in **15***b* and *c*.

[15]However, this evidence does not exclude the possibility of alkylation of DNA by maleic acid under in vivo conditions. Furthermore, attack by a cellular nucleophile other than water would give rise to compounds such as **13** which are analogous to the oxoacrylic acids for which the reactions with guanosine *were* observed.

[16]Studies with other carcinogenic alkylating agents have given similar indications (5, 14).

[17]A broad spectrum of proteolytic enzymes could assist in this hydrolytic repair process.

philic site to another and in this way it could eventually reach the nucleus of the cell. (Further nucleophilic attack at the carbonyl group of the Michael addition products is also a possibility). Hydrolysis at any stage during such migration would produce the corresponding α,β-unsaturated acid which if formed in the vicinity of the nuclear DNA would be capable of effecting its essentially irreversible alkylation.

The somewhat circuitous route to DNA that the above hypothesis implies may account in part for the relatively low carcinogenic potencies and non-cumulative effects of many of the lactone group of compounds evaluated by Dickens *et al.* (2) since it provides ample opportunity for the compounds or their derivatives to enter the normal metabolic or detoxification pathways. Thus conjugated unsaturated acids, or their derivatives or precursors, are likely to be potent carcinogens only if their structures are such that metabolic degradation etc. does not provide for their adequate elimination from the cell prior to reaching the nucleus. Many further studies involving biological testing of selected compounds of this type are required and these are to be carried out.

Sorbic Acid

An important exception to the above rationale is sorbic acid which has been recorded (2) as a fairly potent carcinogen. Our current and previous (1) inability to demonstrate any alkylating capacity for sorbic acid[18] indicates strongly that it is not itself a carcinogen. Its acceptance as a food additive following thorough testing on dogs (16) is also interesting in this regard. Without further testing (which would now appear to be very desirable) it will not be possible to rationalize these conflicting data (2, 16). However, it appears very likely that upon oral administration sorbic acid is metabolized via the normal fatty acid pathway. On the other hand, when subcutaneously injected, sorbic acid probably does not enter the normal metabolic pathways but remains in the region of application where it exerts its carcinogenic effect either because of its unsaturated acid nature or possibly as a result of its conversion to other carcinogenic compounds. This latter possibility deserves further investigation since it has been shown (17) that sorbic acid can undergo autooxidation at the C-4,5 double bond, giving rise to 4-acylacrylic acids of a type expected to be carcinogenic on the basis of the data presented in this communication.

[18]Under very forcing conditions (150° under pressure) sorbic acid does react with amines (15).

Experimental

The instruments and conditions used for spectral, m.p., etc. determinations are as listed previously (1). Paper chromatography was carried out on Whatman #1 filter paper with 1-propanol–water (5:2) as the ascending developing solvent. A Mineralight UVS-11 lamp was used for the detection of fluorescent compounds on paper chromatograms.

Compounds

4-Hydroxypent-2-enoic acid lactone (**1**) and 4-hydroxyhexa-2,4-dienoic acid lactone (**2**) were prepared as described previously (1). Maleic anhydride, maleic acid, fumaric acid, sorbic acid, β-propiolactone, acrylic acid, imidazole, and *N*-methylimidazole were obtained from Fisher or Aldrich and guanosine was purchased from Sigma. Where necessary the commercially supplied compounds were recrystallized or redistilled immediately prior to use. 3-Acetylacrylic acid, m.p. 124–126° (lit. (18) m.p. 125–126°), and 3-benzoylacrylic acid, m.p. 95–96° (lit. (19) m.p. 96–97°), were prepared according to the literature methods.

Attempted Reactions of 4-Hydroxypent-2-enoic Acid Lactone (1) and 4-Hydroxyhexa-2,4-dienoic Acid Lactone (2) with Guanosine (6)

Solutions of guanosine (1 mole equ.) and the lactones (1–5 mole equ.) in water or 50% aqueous *N,N*-dimethylformamide of different p*H*'s in the range 5–9 (adjusted with aqueous hydrochloric acid or sodium hydroxide respectively) were kept for varying periods (2–24 h) at temperatures ranging from 25 to 100°. In all cases unreacted guanosine or its hydrolysis product guanine were recovered to the extent of > 95%.

Reaction of β-Propiolactone with Guanosine

A mixture of guanosine (0.4 g, 1.4 mmole) and β-propiolactone (0.5 g, 7 mmole) in water (4.5 ml) was warmed to 80° at which temperature complete solution took place. The solution was then rotary evaporated at 25° to give a colorless viscous oil. (No attempt was made to purify this material further since paper chromatographic analysis indicated the presence of five compounds, including guanosine (R_f 0.43; dark blue fluorescence) and **8** (R_f 0.5 (light blue fluorescence)). Hydrolysis with 1 *N* hydrochloric acid (10 ml) at 100° for 1 h followed by neutralization with concentrated aqueous ammonium hydroxide, afforded a precipitate which was filtered, washed with water, and dried *in vacuo* over P_2O_5. Paper chromatographic analysis of the solid obtained showed only a single spot (light blue fluorescence) of R_f 0.5 and recrystallization from 40% aqueous *N,N*-dimethylformamide (70 ml) gave 7-(2-carboxyethyl) guanine, (0.23 g), m.p. > 290° (lit. (6) m.p. > 290°), i.r. (Nujol) 1741 (sh), 1725, 1695, and 1650 cm^{-1}; p.m.r. (2 *N* NaOD) δ 2.77 (2H, t, J = 7 Hz, CH_2COO^-), 4.50 (2H, t, J = 7 Hz,

$CH_2CH_2COO^-$), 5.00 (*H*OD) and 7.83 p.p.m. (1H, s, guanine C-8 *H*).

Reaction of β-Propiolactone with Imidazole

(a) In Diethyl Ether

β-Propiolactone (1.0 g, 13.9 mmole) was added dropwise with stirring to imidazole (1.0 g, 14.7 mmole) in diethyl ether (40 ml) at 20°. Within 10 min a crystallizing oil began to separate and after keeping overnight the mixture was filtered. The solid obtained (1.71 g) was fractionally recrystallized from methanol–chloroform solution to give *1-(2-carboxyethyl)imidazole* (**11**) as leafy plates (1.06 g) m.p. 152.5–153°; i.r. (Nujol) 3180, 2400, 1900 and 1715 cm^{-1}; p.m.r. (D_2O) δ 2.81 (2H, t, J = 6.5 Hz, $CH_2CH_2COO^-$), 4.50 (2H, t, J = 6.5 Hz, $CH_2CH_2COO^-$), 4.89 (*H*OD), 7.50 (2H, broad s, NC*H*=C*H*N) and 8.75 p.p.m. (1H, broad s, N=C*H*N).

Anal. Calcd. for $C_6H_8N_2O_2$: C, 51.25; H, 5.75; N, 19.99. Found: C, 51.23; H, 5.92; N, 19.89.

Also obtained was *1,3-bis(2'-carboxyethyl)imidazolium betaine* (**12**) as fine needles (0.24 g) m.p. 203–204°; i.r. (Nujol) 3180, 3100, 1710 and 1640 cm^{-1}; p.m.r. (D_2O) δ 2.90 (4H, t, J = 6.5 Hz, $CH_2CH_2COO^-$), 4.49 (4H, t, J = 6.5 Hz), $CH_2CH_2COO^-$), 4.82 (*H*OD), 7.56 (2H, d, J = 1.5 Hz, NC*H*=C*H*N) and 8.85 p.p.m. (1H, broad s, N=C*H*N).

Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H 5.70; N, 13.20. Found: C, 51.00; H, 5.80; N, 13.06.

(b) In Acetonitrile

When the above reaction was repeated in acetonitrile (50 ml), the bis-addition product **12** was obtained in 50% yield. When two equ of β-propiolactone were used, the recrystallized yield of **12** was increased to 92%.

The Reaction of Acrylic Acid with Imidazole

Acrylic acid (4 g, 55 mmole) and imidazole (2.0 g, 29 mmole) were dissolved in methanol (40 ml) and the solution was slowly concentrated to 10 ml during 3 h by heating on a steam bath. Rotary evaporation of the concentrate afforded a solid which on recrystallization from aqueous methanol gave 3.13 g of 1,3-bis(2-carboxyethyl)-imidazolium betaine (**12**) identical in all respects to that obtained previously from the β-propiolactone reaction.

The Reaction of Maleic Acid with N-Methylimidazole

Maleic acid (2.32 g, 20 mmole) was dissolved in a mixture of diethylether (50 ml) and methanol (10 ml) and *N*-methylimidazole (1.62 g, 20 mmole) was then added with stirring. An oil began to separate which became crystalline following removal of the solvent at 90°. Recrystallization of the solid from 50% aqueous ethanol (40 ml) gave *2-N-(N'-methylimidazolium)succinic acid betaine* (**14***b*) as needles, (2.53 g) m.p. 232–233° (decomposition); i.r. (Nujol) 3300–2400, 1700 (COOH) and 1650–1550 cm^{-1} (COO^-); p.m.r. (2 *N* NaOD) δ 2.94 (H_A, d of d, J_{AB} = 16.5 Hz, J_{AX} = 10 Hz, $CH_XCH_AH_B$-COOH), 3.18 (calcd.) (H_B, d of d, J_{BA} = 16.5 Hz, J_{BX} = 5 Hz, $CH_XCH_AH_BCOOH$), 3.92 (3H, s, CH_3N), 4.70 (HOD), 5.23 (H_X, d of d, J_{XA} = 10 Hz, J_{XB} = 5 Hz, $CH_XCH_AH_BCOOH$), 7.52 (2H, AB q, NC*H*=C*H*N), and 8.84 p.p.m. (1H, broad s, N=C*H*N).

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 48.49; H, 5.09; N, 14.14. Found: C, 48.33; H, 5.18; N, 14.04.

The Reaction of Maleic Acid Monomethyl Ester with N-Methylimidazole

A solution of maleic anhydride (2.0 g, 20 mmole) in methanol (50 ml) was refluxed for 10 min and *N*-methylimidazole (1.62 g, 20 mmole) was then added. The solution was evaporated slowly on the steam bath during 3 h and the resulting solid was recrystallized from chloroform–ethanol. *2-N-(N'-Methylimidazolium)-3-carbomethoxypropionic acid betaine* (**14***c*) separated as needles (2.42 g), m.p. 157–158° (decomposition); i.r. (Nujol) 3530, 3190, 3075, 1730 ($COOCH_3$), and 1645–1600 cm^{-1} (COO^-); p.m.r. (D_2O), δ 3.35 (H_AH_B, approaching d, J_{AX} = 8 Hz, J_{BX} = 6 Hz, $CH_XCH_AH_B$-$COOCH_3$), 3.74 (3H, s, OCH_3), 4.00 (3H, s, NCH_3) 4.70 (*H*OD), 5.37 (H_X, d of d, J_{XA} = 8 Hz, J_{XB} = 6Hz, NCH_X-CH_AH_B), 7.60 (2H, m, NC*H* = C*H*N), and 8.96 p.p.m. (1H, broad s, N=C*H*N).

Anal. Calcd. for 2 $C_9H_{12}N_2O_4 \cdot 1\ H_2O$: C, 48.86; H, 5.92; N, 12.66. Found: C, 48.73; H, 6.14; N, 12.84.

The Reaction of 3-Benzoylacrylic Acid with N-Methylimidazole

A solution of *N*-methylimidazole (1.62 g, 20 mmole) and 3-benzoylacrylic acid (3.52 g, 20 mmole) in methanol (50 ml) was kept at 50° for 1.5 h and was then rotary evaporated. The residue was recrystallized from chloroform–methanol (10:1, 40 ml) and *2-N-(N'-methylimidazolium)-4-oxo-4-phenylbutanoic acid betaine* (**16***c*) separated as a microcrystalline powder (3.40 g) m.p. 95–106°; i.r. (Nujol) 3450, 3375, 3260, 3130, 3050 (sharp bands), 1685 (CO), and 1640–1625 cm^{-1} (COO^-); p.m.r. (D_2O) δ 4.12 (3H, s, NCH_3), 4.20 (2H, d, J = 6Hz, $CHCH_2COPh$), 5.00 (*H*OD), 5.72 (1H, t, J = 6Hz, $CHCH_2COPh$), 7.5–8.2 (5H, 2H, complex m, C_6H_5-NC*H*=C*H*N), and 9.20 p.p.m. (N=C*H*N).

Reaction of Acrylic Acid with Guanosine

Guanosine (0.40 g, 1.4 mmole) and acrylic acid (0.53 g, 7 mmole) were dissolved in water (6 ml) at 80°. The temperature was maintained at this level for 1.5 h and the solution was then kept at room temperature overnight. The unreacted guanosine (0.17 g) was removed by filtration and the filtrate was diluted with an equal volume of 2 *N* aqueous hydrochloric acid and heated under reflux for 1 h. After cooling the solution and neutralization with concentrated aqueous ammonium hydroxide, 7-(2-carboxyethyl)guanine (0.11 g) separated which was identical in all respects with the material obtained from the guanosine-β-propiolactone reaction.

The Reaction of 3-Acetylacrylic Acid with Guanosine

Guanosine (2.83 g, 10 mmole) in water (50 ml) was heated on a steam bath (90°) until complete solution had occurred. 3-Acetylacrylic acid (1.78 g, 15 mmole) was then added and the mixture was maintained at a temperature of 90°. The progress of the reaction was monitored by paper chromatographic analysis which indicated that several compounds were formed and that the optimum reaction time was 5 h. After this time the guanine (0.36 g) which had precipitated was filtered off from the hot solution and the filtrate was cooled and seeded with guanosine. After keeping overnight at 0°, the mixture was again filtered to give guanosine (0.86 g) and the colored filtrate was treated with charcoal and

TABLE 1

Ultraviolet data for 7-(2′-acetyl-1′-carboxyethyl) guanine

Solvent	λ_{max}	ε	λ_{min}	$\varepsilon_{280}/\varepsilon_{260}$
1 *N* HCl	251 270	7610 5760	230.5	0.68
1 *N* NaOH	244 279	5050 5820	257	1.55
0.1 *M* PO_4^{2-}, p*H* 7	245 283	5720 6120	237 264	1.50

was then evaporated. The solid residue obtained was digested with ethanol (75 ml) under reflux and the ethanolic solution obtained was kept at 0° until precipitation of the product was complete (12 h). Filtration afforded *7-(2′-acetyl-1′-carboxyethyl)guanine* (0.36 g), m.p. > 200° (decomposition); i.r. (Nujol) 3500–2500 and 1725–1550 cm^{-1} (broad, diffuse bands); p.m.r. (2 *N* D_2SO_4) δ 2.28 (3H, s, CH_3CO), 3.52–3.78 (3H, AA′X d of d, J_{AX} = 7.3 Hz, $J_{A'X}$ = 5 Hz, $J_{AA'}$ not observed, $CH_3COCH_AH_BCH_XN$-7), 5.16 (*H*OD), 5.97 (H_X, d of d, J_{XA} = 7.3 Hz, $J_{XA'}$ = 5 Hz, N-7$CH_XCH_AH_BCOCH_3$), and 8.60 p.p.m. (1H, s, guanine C-8H). The u.v. data are tabulated in Table 1.

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1. J. B. Jones and J. M. Young. J. Med. Chem. **11**, 1176 (1968) and references therein.
2. F. Dickens, H. E. H. Jones, and H. B. Waynforth. Brit. J. Cancer, **20**, 134 (1966) and references therein.
3. e.g. P. Brookes. Cancer Res. **26**, 1994 (1966) and references therein.
4. e.g. N. H. Colburn and R. K. Boutwell. Cancer Res. **28**, 653 (1968) and references therein.
5. J. J. Roberts and G. P. Warwick. Biochem. Pharmacol. **12**, 1441 (1963) and previous papers.
6. P. Brookes and P. D. Lawley. J. Chem. Soc. 3923 (1961); 1348 (1962).
7. N. H. Colburn, R. G. Richardson, and R. K. Boutwell. Biochem. Pharmacol, **14**, 1113 (1965).
8. T. L. Gresham, J. E. Jansen, F. W. Shaver, R. A. Bankert, and F. T. Fiedorek. J. Amer. Chem. Soc. **73**, 3168 (1951).
9. C. D. Hurd and S. Hayao. J. Amer. Chem. Soc. **77**, 117 (1955).
10. R. Tsuzuki, K. Ichikawa, and M. Kase. J. Org. Chem. **26**, 1808 (1961).
11. L. H. Flett and W. H. Gardner. Maleic anhydride derivatives. J. Wiley and Sons, New York, N.Y., 1952. p. 120.
12. F. H. Kirchner, J. H. Bailey, and C. J. Cavallito. J. Amer. Chem. Soc. **71**, 1210 (1949).
13. B. J. Cramer, W. Schroeder, W. J. Moran, C. H. Nield, M. Edwards, C. I. Jarowski, and B. Puetzer. J. Amer. Pharm. Ass. Sci. Ed. **37**, 439 (1948).
14. W. C. J. Ross. Biological alkylating agents. Butterworth and Co. (Publishers) Ltd., London, 1962.
15. A. J. Verbiscar and K. N. Campbell. J. Org. Chem. **29**, 2472 (1964).
16. K. Lang. Arzneimittel-Forsch. **10**, 997 (1960).
17. M. Hamada. J. Agr. Chem. Soc. (Japan), **16**, 181 (1940).
18. W. G. Overend, L. M. Turton, and L. G. Wiggins. J. Chem. Soc. 3500 (1950).
19. G. Baddeley, G. Holt, and S. M. Makar. J. Chem. Soc. 3289 (1952).
20. J. M. Young, Ph.D. Thesis. University of Toronto, Toronto, Ontario. 1969.