2,2-dimethoxy-3,4,4-trimethyloxetane, 62841-92-3; 2,2-dimethoxy-3,3-dimethyl-4-isopropyloxetane, 62841-93-4, 2,2-dimethoxy-3,3-dimethyl-4-phenyloxetane, 62841-94-5; 2,2-dimethoxy-3,3-dimethyl-4-cyano-4-phenyloxetane, 62841-95-6; 2,2,3,3-tetramethoxy-4-isopropyloxetane, 62841-96-7; 2,2,3,3-tetramethoxy-4-phenyloxetane, 62841-97-8; 2,2,3,3-tetramethoxy-4-cyano-4-phenyloxetane, 60299-87-8; 2,3,3-trimethyl-2-cyano-1,5,8-trioxaspiro[3.4]octane, 62841-98-9; 3,3-dimethyl-2-trichloromethyl-1,5,8-trioxaspiro[3,4]octane, 62841-99-0; 2-cyano-2-phenyl-3,3-dimethyl-1,5,8-trioxaspiro[3.4]octane, 62842-00-6; 2-*p*-nitrophenyl-3,3-dimethyl-1,5,8-trioxaspiro[3,4]octane, 62842-01-7; 1,1-diethoxypropene, 21504-43-8; 3,3-diethoxypropene, 3054-95-3.

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A Novel Acylative Degradation of Uric Acid. Carbon-13 Nuclear Magnetic Resonance Studies of Uric Acid and Its Degradation Products

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Treatment of uric acid (1) with boiling isobutyric anhydride causes cleavage and rearrangement of the pyrimidine and imidazole rings to give a new heterocyclic derivative, 2-(1-methylethyl)-4-(1-hydroxy-2-methylpropylidene)aminoimidazole-5-carboxylic acid 5,1′-lactone (2). The structures of the lactone and related derivatives have been elucidated by infrared spectroscopy, ^1H and ^{13}C NMR, and mass spectrometry. Experiments with uric acids labeled with carbon-14 at either C-2, C-6, or C-8 confirmed that C-2 and C-8 were eliminated during the cleavage process. Uric acid, its 1,3-$^{15}N_2$ labeled derivative, and a series of degradation products and related model compounds have been studied by ^{13}C NMR spectroscopy, and the carbon-13 chemical shifts and coupling constants correlated with molecular structure.

In the course of a study of uric acid (1) to find volatile derivatives for chemical analysis of 1 in serum by isotope dilution mass spectrometry, we have examined its reactions with a series of aliphatic acylating agents, including acetic, propionic, *n*-butyric, and isobutyric acid anhydrides. Considerable work on the acetylation of uric acid has been described previously, notably conversion of 1 into 8-methylxanthine (3) by prolonged treatment (80 h) of 1 with a boiling mixture of acetic anhydride and pyridine.[1-4] However, there has been little or no work on the reaction of higher boiling, aliphatic acid anhydrides with 1.

Whereas reactions of 1 with boiling propionic or *n*-butyric anhydrides in the presence of pyridine, as observed in this laboratory, generally follow the path described earlier for acetic anhydride (e.g., conversion of 1 to 3),[1-4] treatment of 1 with boiling isobutyric anhydride either alone or in the presence of a tertiary amine (e.g., pyridine) gave surprising results. Reactions of 1 with a variety of oxidants[5] lead to either cleavage of the pyrimidine ring (e.g., with alkaline permanganate) to give allantoin, or degradation of the imidazole ring (e.g., with nitric acid) to produce alloxan, or cleavage of both rings (e.g., with permanganate in acetic acid) to give acyclic oxaluric acid. The structures and carbon-13 nuclear magnetic resonance (^{13}C NMR) data of some of the degradation products of 1 are shown in Table I.

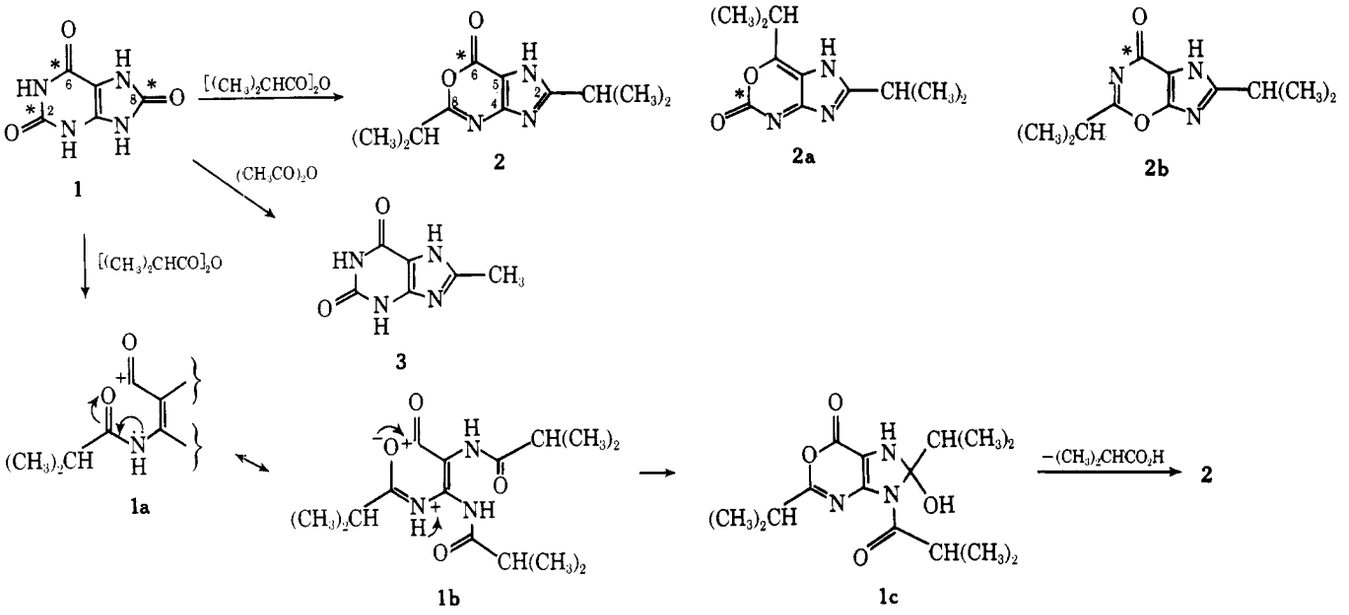
We report here a novel acylative degradation that involves simultaneous cleavage and rearrangement of the pyrimidine and imidazole rings in 1, with the formation of a new heterocyclic ring system. When mixtures of uric acid with isobutyric anhydride or isobutyric anhydride and pyridine were boiled under reflux for 8–24 h and 3–4 h, respectively, concentration followed by trituration of the resulting residues with ethyl acetate yielded 32–36% of a colorless crystalline material, mp 211–212 °C, that has proved to be 2-(1-methylethyl)-4-(1-hydroxy-2-methylpropylidene)aminoimidazole-5-carboxylic acid 5,1′-lactone (2).

Results and Discussion

Spectroscopic Evidence for the Structure of 2. The infrared spectrum of 2 displayed a strong absorption at 1772 cm^{-1} that suggested the possibility of an ester or lactone group derived from an unsaturated alcohol. The ^{13}C NMR spectra of 2 (see Figure 1) and its proton NMR spectra revealed that two, chemically nonequivalent isopropyl groups had been introduced.[6] The presence of a nonacylated NH group in the structure of 2 was indicated by its proton NMR spectra in pyridine-d_5 and dry methyl-d_6 sulfoxide solutions, each of which displayed a broad, one-proton signal at low field that was displaced to higher field on addition of deuterium oxide to the solution. The chemical shift of this signal was found to be highly variable, in agreement with its assignment as an NH proton. The proton-decoupled ^{13}C NMR spectra (Figure 1a and 1b) of 2 display only five carbon resonances other than those of the isopropyl groups, which indicates that two carbon atoms have been eliminated from the reactants.

The apparent molecular ion in the electron impact (EI)

Scheme I



mass spectrum of **2** was found at *m/e* 221. Verification that this ion is indeed the molecular ion was obtained from the chemical ionization (CI) mass spectrum, which showed an M + 1 at *m/e* 222. The EI spectrum of **2** displayed ions at *m/e* 206 ($M^+ - CH_3$), 193 ($M^+ - CO$), 178 [$M^+ - CH(CH_3)_2$], and 43 [$(CH_3)_2CH^+$]. The molecular ion at *m/e* 221 corresponds to the molecular formula $C_{11}H_{15}N_3O_2$ for **2**.

The ultraviolet spectrum of **2** showed absorptions at 231, 239, 247, and 269 nm that are consistent with a conjugated lactone–imidazole ring system.

Degradation of ^{14}C-Labeled Uric Acids. On the basis of the foregoing evidence, the isomeric structures **2, 2a,** and **2b** were considered. Structure **2b** was thought to be less likely, since it is not a lactone. Structures **2** and **2a** could be formed by loss of either C-2 and C-8, or C-6 and C-8, respectively, from uric acid.

When uric-*2*-^{14}C acid and uric-*8*-^{14}C acid were treated separately with boiling mixtures of isobutyric anhydride and pyridine, products (**2**) were obtained that contained negligible radioactivity, thus confirming that C-2 and C-8 of uric acid are lost during this reaction. However, the application of these reaction conditions to uric-*6*-^{14}C acid led to a product that retained 97% of its original specific activity, thereby providing strong evidence in favor of structure **2** (see Scheme I). The loss of C-8 in this reaction (presumably as carbon dioxide) is consistent with the earlier observations[1-4] of acetylative cleavage of the imidazole ring of **1**, and with the scission of both rings of **1** during its alkaline oxidation, which has been confirmed by radiotracer techniques.[7,8]

That one nitrogen atom (N-1) is also eliminated from **1** during its reaction with isobutyric anhydride was confirmed by the gas chromatographic detection of isobutyramide and diisobutyramide in the crude product of the reaction, and by the isolation from it, of crystalline diisobutyramide. These simple amides and also urea diisobutyrate were prepared separately as reference compounds for the chemical and spectroscopic studies. The proton NMR spectrum (Figure 2a) of isobutyramide at 60 MHz displays two overlapping broad singlets at low field that were assigned as chemically nonequivalent ^{14}NH proton signals by comparison with the spectrum (Figure 2b) of the ^{15}N-labeled amide. The latter spectrum shows sharp, overlapping quartets, from which the coupling constants $^2J_{HNH}$ = 2.1 Hz and $^1J_{^{15}NH}$ = 88.9 and 87.4 Hz were readily measured. Thus isobutyramide is a further example of restricted rotation about the amide bond.[9,10]

Chemical Evidence for the Structure of 2. Ammonolysis of the lactone **2** yielded a crystalline amide (**4**) that also displayed the signals of two chemically nonequivalent isopropyl groups in its proton and ^{13}C NMR spectra (Scheme II). The

Scheme II

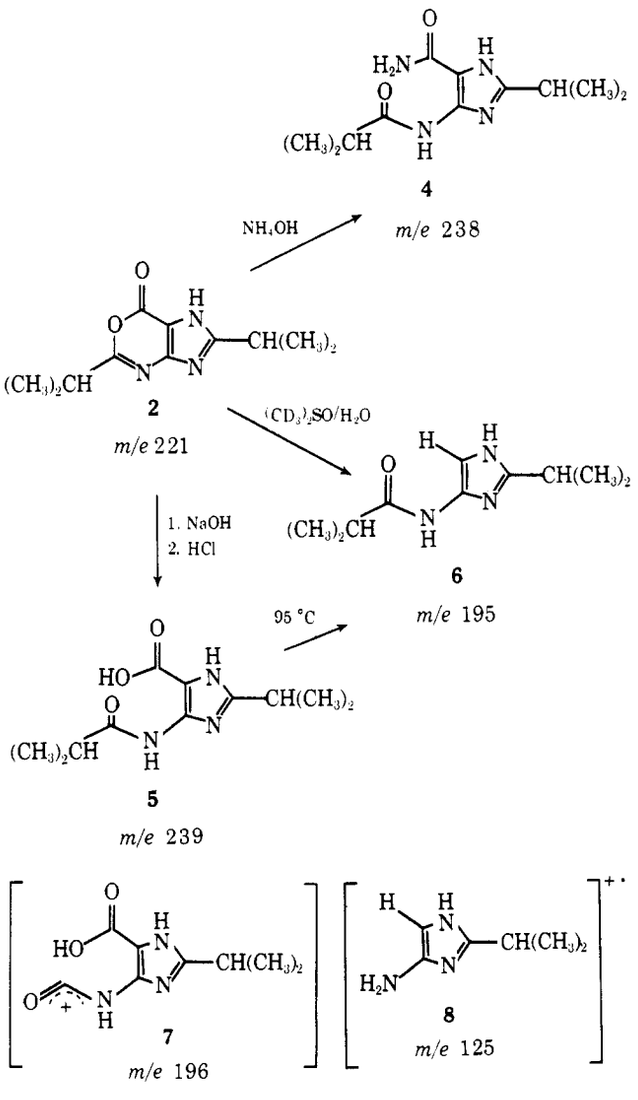
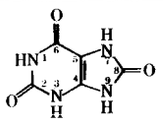
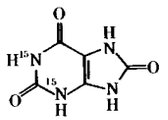
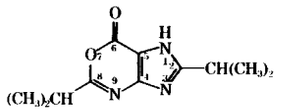
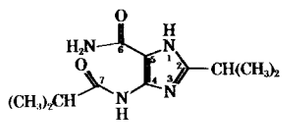
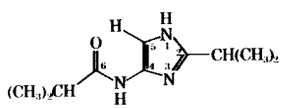
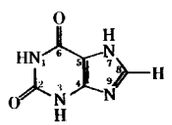
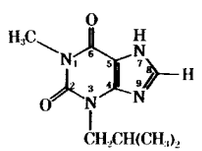
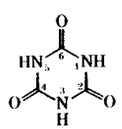
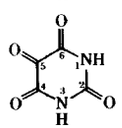
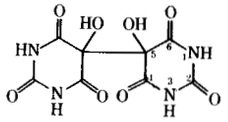
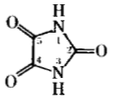
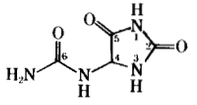
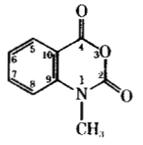
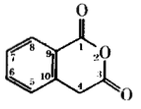


Table I. ^{13}C Chemical Shifts[a] of Uric Acid, Related Derivatives, and Other Model Compounds

Compd (registry no.)		C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	Others		
Uric acid (1) (69-93-2)			150.1		136.6	97.1	153.4		152.2					
Uric-1,3-$^{15}N_2$ acid[b] (62948-75-8)			150.1 t[c]		136.7 d	97.1[d]	153.3 d		152.3					
Lactone (2) (62948-76-9)			159.4[d]		152.7[d]	112.8[d]	153.8		169.8			$(CH_3)_2$	19.7	21.0
												CH	28.5	33.4
			159.5[e]		152.8	112.7	153.7		169.9			$(CH_3)_2$	19.6	20.9
												CH	28.6	33.5
			161.4[f]		154.1	113.3	155.1		171.8			$(CH_3)_2$	20.1	21.4
												CH	30.0	35.1
Amide (4) (62948-77-0)			161.6[d]		147.6[d]	112.2[d]	152.5	167.3				$(CH_3)_2$	20.1	21.3
												CH	27.8	33.3
Imidazole derivative[e,g] (6) (62948-78-1)			149.1		136.5	102.8	173.9					$(CH_3)_2$	19.5	21.6
												CH	27.8	34.1
Xanthine[h] (69-89-6)			151.3		148.8	106.7	155.5		140.4					
3-(2-Methylpropyl)-1-methylxanthine[i] (28822-58-4)			151.3		148.1	106.5	154.6		140.4			$(CH_3)_2$	19.9	
												CH_3N	27.0	
												CH_2	50.1	
												CH	27.8	
Cyanuric acid (504-19-8)			149.9		149.9		149.9							
Alloxan[j] (50-71-5)			149.8		156.3	168.8	156.3							

Alloxantin (76-24-4)			150.3		167.3	78.6	167.3						
Parabanic acid (120-89-8)			154.6		159.7	159.7							
Allantoin (97-59-6)			156.7		62.3	173.4	157.4						
1-Methylisatoic anhydride (10328-92-4)			147.6		158.9	129.3	123.6	137.2	114.7	142.1	111.3	CH_3	31.6
Homophthalic anhydride (703-59-3)		161.6		165.6	34.1	127.7[k]	135.0	127.6[k]	129.4	121.4	136.4		

[a] In ppm from internal tetramethylsilane, for solutions in methyl-d_6 sulfoxide. [b] ^{13}C–^{15}N coupling constants $J_{1,2} = J_{2,3} = J_{3,4} = 18.3$, $J_{1,5} = 8.5$, and $J_{1,6} = 11.0$ Hz. [c] Multiplicities of ^{13}C signals are indicated by d (doublet) or t (triplet). [d] Broad signal. [e] At 80 °C. [f] In methanol-d_4. [g] ^{13}C–H coupling constants $J_{4,H\text{-}5} = 7.3$ (tentative), $J_{5,H\text{-}5} = 190.4$, $J_{5,NCCH} = 3.7$ Hz. The assignment of the spacing in the C-4 doublet observed in the absence of proton decoupling as a coupling of C-4 with H-5 is tentative because of possible alternative assignments of this spacing as a coupling of C-4 with a methine proton of one of the 1-methylethyl groups. [h] ^{13}C–H coupling constants $J_{4,H\text{-}8} = 9.8$, $J_{5,H\text{-}8} = 4.9$, and $J_{8,H\text{-}8} = 211.2$ Hz. [i] ^{13}C–H coupling constants $J_{4,H\text{-}8} = 13.4$, $J_{5,H\text{-}8} = 4.9$, and $J_{8,H\text{-}8} = 211.2$ Hz, measured from the N-deuterated derivative 3-(2,2-dimethylethyl)-1-methylxanthine-7(9)-d. [j] Data taken from a spectrum of a mixture of alloxan and its hydrate. [k] Assignments interchangeable.

Table II. ^{13}C Chemical Shifts[a] of Carbonyl Derivatives

Compd		Registry no.	C=O	$(CH_3)_2$	CH
Urea	$(NH_2)_2CO$	57-13-6	160.5		
Urea diisobutyrate	$[(CH_3)_2CHCONH]_2CO$	62948-79-2	177.4, 149.9 (CHCONH)(NHCONH)	18.8	34.5
Isobutyramide	$(CH_3)_2CHCONH_2$	563-83-7	179.0	19.5	33.9
Diisobutyramide	$[(CH_3)_2CHCO]_2NH$	3668-74-4	177.4	18.8	34.5
Isobutyric anhydride	$[(CH_3)_2CHCO]_2O$	97-72-3	172.8	18.3	35.1

[a] In ppm from internal tetramethylsilane, for solutions in methyl-d_6 sulfoxide.

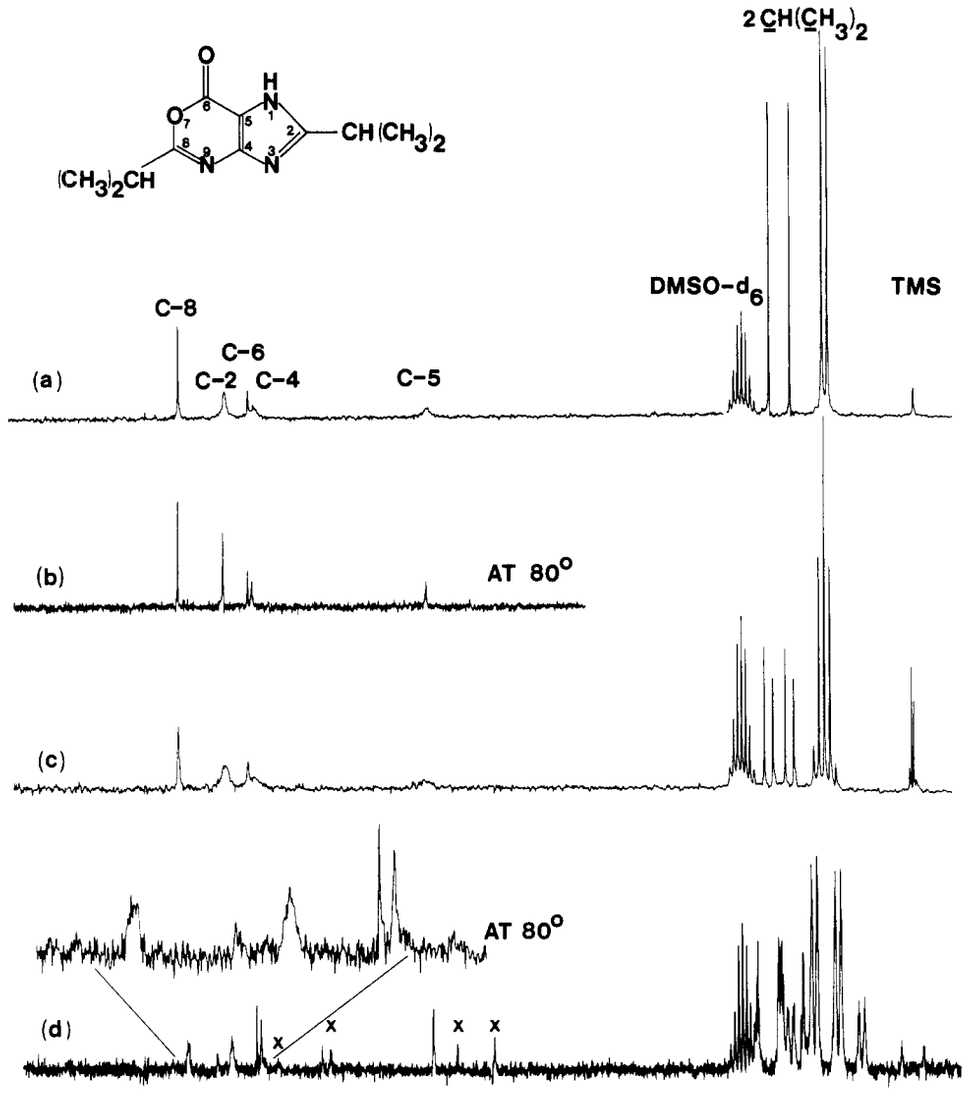


Figure 1. ^{13}C NMR spectra of 2-(1-methylethyl)-4-(1-hydroxy-2-methylpropylidene)aminoimidazole-5-carboxylic acid 5,1′-lactone (**2**) in methyl-d_6 sulfoxide at 22.6 MHz; (a) proton decoupled at 30 °C; (b) proton decoupled at 80 °C; (c) off-resonance, proton decoupled at 30 °C; and (d) proton coupled at 80 °C. The latter spectrum was obtained from a solution that contained 10% v/v of water, and it displays the signals (×) of a minor proportion of the imidazole derivative **6**.

retention of these signals and of five other carbon signals at low field in the ^{13}C NMR spectrum of **4** indicated that the reaction of **2** with ammonia achieved merely opening of the lactone ring, without further degradation. The proton NMR spectrum of **4** also showed two broad NH resonances of differing intensity. The EI mass spectrum of **4** showed a molecular ion at *m/e* 238 (confirmed by chemical ionization). Pertinent fragment ions were observed at *m/e* 223 ($M^+ - CH_3$), 205 ($M^+ - CH_3 - H_2O$), 177 ($M^+ - CH_3 - H_2O - CO$), 167, 151, 125, and 110. The presence of an amide group in **4** was confirmed by the observation of a band at 1650 cm^{-1} in its infrared spectrum. The facile opening of the lactone ring of **2** by ammonium hydroxide resembles the hydrolyses of structurally related 1,3-benzoxazinone derivatives in the presence of nucleophilic reagents.[11]

Hydrolysis of **2** with sodium hydroxide yielded a syrupy acid **5**, which displayed a molecular ion at *m/e* 239 in its EI mass spectrum, and also a characteristic fragment ion at *m/e* 196 that was assigned to the carbonium ion **7**. A fragment ion at *m/e* 125 was assigned to the imidazole moiety **8**. On heating at 95 °C, the acid **5** was readily decarboxylated to an imidazole derivative **6** that was characterized by its molecular ion at *m/e* 195.

A slow conversion of the lactone **2** into the imidazole derivative **6** was monitored by ^{13}C NMR spectroscopy of **2** under gentle hydrolytic conditions (see Figure 3). Compound **6** was distinguished by a wide quartet in its proton-coupled ^{13}C NMR spectrum (Figure 3b) at mid-field. The larger spacing (190.4 Hz) in this quartet was assigned as the coupling between C-5 of the imidazole ring and its directly attached proton (H-5). A comparably large value (211.2 Hz) was measured for the coupling of H-8 with C-8 in the imidazole rings of xanthine and its 3-(2-methylpropyl)-1-methyl derivative (Table I). The values $J_{2,H-2} = 208$ Hz and $J_{4,H-4}$ ($J_{5,H-5}$) = 199 Hz have been measured previously for imidazole.[12] Presumably, the slow conversion of **2** into **6** in hot, aqueous methyl sulfoxide proceeds by an initial, aqueous hydrolysis of lactone **2** to the acid **5**, which then decarboxylates to give the imidazole derivative **6**. Lactone **2** was also readily hydrolyzed (with strong effervescence) by concentrated hydrochloric acid either on brief warming or at room temperature. However, careful processing of this reaction mixture yielded a product that was somewhat sensitive to air and light, but which showed a predominant ion in its mass spectrum at *m/e* 125 (**8**).

On the basis of the foregoing chemical and spectroscopic evidence, the product isolated from the reaction of uric acid with boiling isobutyric anhydride is assigned structure **2**. The mechanism of formation of this structure may be rationalized

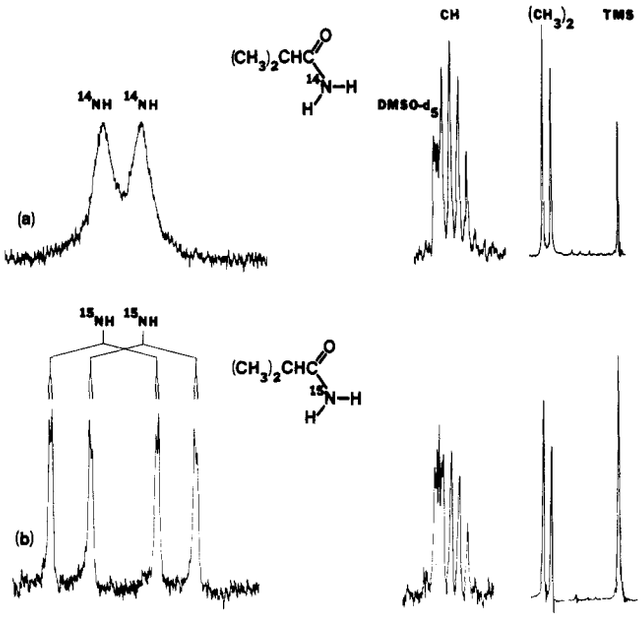


Figure 2. Proton NMR spectra of solutions in methyl-d_6 sulfoxide at 60 MHz; (a) isobutyramide; (b) isobutyramide-^{15}N.

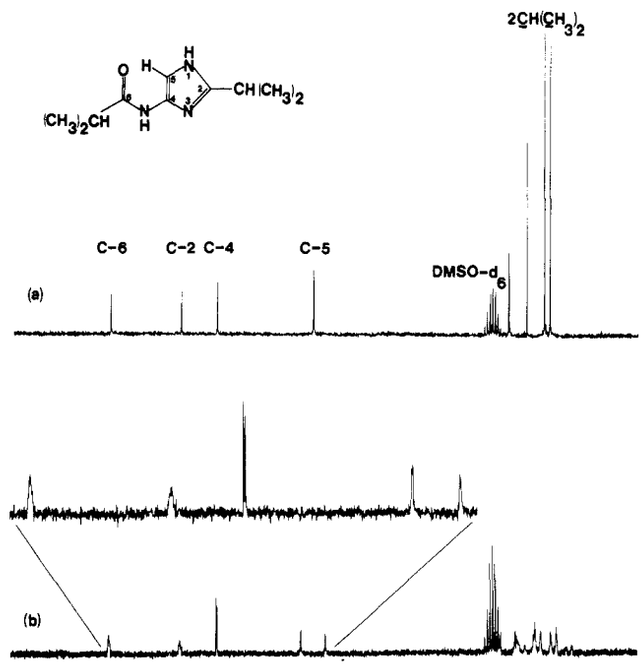


Figure 3. ^{13}C NMR spectra of 2-(1-methylethyl)-4-(2-methyl-1-oxo-propyl)aminoimidazole (**6**) in 5:1 v/v methyl-d_6 sulfoxide–water at 22.6 MHz; (a) proton decoupled at 80 °C; (b) proton coupled at 30 °C.

in terms of cleavage of the five- and six-membered rings of **2** by acyl exchange to give a resonance stabilized, *N,N',N''*-triisobutyryl intermediate (**1a** ↔ **1b**), which undergoes ring closure to the intermediate **1c**, which then forms **2** by elimination of isobutyric acid.

^{13}C NMR Studies of Uric Acid and Its Degradation Products. A suitable starting point for assignments of the ^{13}C NMR spectra of uric acid and its derivatives was the known assignment for purine, which has been confirmed by deuteration.[13] In many heterocyclic derivatives of the purine type, C-5 is characteristically shielded and resonates at considerably higher field than does C-4.[14,15] Thus, for uric acid, the ^{13}C resonance at highest field (97.1 ppm) was assigned to C-5. Additional evidence for assignments was obtained from the ^{13}C spectrum of uric-*1,3*-$^{15}N_2$ acid, which displayed only one resonance that was not split by coupling with ^{15}N. This resonance (152.3 ppm) was assigned to C-8, which is relatively remote from the ^{15}N nuclei at the 1 and 3 positions. The remaining four ^{13}C resonances of 1-*1,3*-$^{15}N_2$ were split by coupling with ^{15}N and the moderately large spacings (11.0–18.3 Hz) observed were assigned as couplings of ^{15}N nuclei with directly bonded, sp^2-hybridized carbons. The magnitudes of these coupling constants are similar to the values $J_{^{15}N^{13}C=O}$ = 13.4 and 14.6 Hz measured previously for[16] methyl 5-deoxy-2,3-*O*-isopropylidene-5-phthalimido-β-D-ribofuranoside-*5*-^{15}N and[16,17] 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose-*6*-^{15}N, respectively, and to the value $J_{^{15}N^{13}C=O}$ = 18.3 Hz found[18] for dixanthylurea-$^{15}N_2$. Because C-2 of 1-*1,3*-$^{15}N_2$ might be expected to be coupled equally to each of its directly bonded ^{15}N nuclei, the triplet (spacings $J_{1,2} = J_{2,3}$ = 18.3 Hz) at 150.1 ppm was assigned to C-2. Doublets at 153.3 ppm ($J_{1,6}$ = 11.0 Hz) and 136.7 ppm ($J_{3,4}$ 18.3 Hz) were assigned to C-6 and C-4, respectively, on the basis of the chemical shifts expected for these amide carbonyl carbon and olefinic carbon nuclei (see Tables I and II).

The assignment of C-8 of xanthine and its 3-(2-methylpropyl)-1-methyl derivative was indicated by the observation of wide doublets ($J_{8,H\text{-}8}$ = 211.2 Hz) in the proton-coupled ^{13}C spectra of these compounds. C-4 and C-5 were each characterized by narrow doublets due to coupling (4.9–13.4 Hz) of these nuclei with H-8. The C-2 resonance was assigned on the basis of the similarity of its ^{13}C chemical shift (151.3 ppm) to that (149.9 ppm) of cyanuric acid (Table I). By elimination, the ^{13}C resonance at lowest field was assigned to C-6, as was also the case for uric acid. Made in this way, the ^{13}C assignments for xanthine and its 3-(2-methylpropyl)-1-methyl derivative (see Table I) agree with those published previously for xanthosine.[14]

The ^{13}C assignments for lactone **2**, amide **4**, and imidazole derivative **6** were based partially on comparisons with the data shown in Tables I and II for uric acid, its degradation products, and various related model compounds, including 1-methylisatoic and homophthalic anhydrides, which each contain an oxycarbonyl function attached to an aromatic ring. Where possible, the assignments were confirmed by the use of proton-coupled spectra and off-resonance proton decoupling techniques. In the proton-decoupled ^{13}C NMR spectrum (Figure 1a) of lactone **2** obtained at ambient probe temperature (30 °C), three of the five carbon resonances at low field are broad. This observation and the fact that the three broad resonances are sharper at 80 °C (Figure 1b) and in the spectrum of **2** in methanol-d_4 measured at 30 °C suggest the presence of a proton exchange process or tautomeric equilibrium,[19-22] which would, indeed, be expected of structure **2**. For example, the NH proton could reside at either nitrogen atom in the imidazole ring of **2**, or at its carbonyl oxygen atom. The annular prototropy of the N-7 and N-9 atoms in purine[20] and N-1 and N-2 atoms in pyrazole[22] may be compared. The assignment of the aliphatic carbon resonances of **2** at high field (Figure 1a) was confirmed by the partially decoupled spectrum (Figure 1c) which contains two overlapping quartets due to nonequivalent pairs of methyl groups and two doublets that represent nonequivalent methine protons. The proton coupled spectrum (Figure 1d) of **2** obtained at 80 °C displays two complex multiplets at lowest field that were assigned to C-2 and C-8 on the basis that only the carbon nuclei bearing the isopropyl groups are near enough to the methyl protons to be significantly spin-coupled to them. The resonance at lowest field was assigned as that of C-8, since this nucleus is attached to an electronegative oxygen atom. In the group of five quaternary carbon resonances of **2**, the resonance at highest field was assigned to C-5 and that at next lowest field to C-4, in

agreement with the assignments for uric acid. The chemical shift of the remaining resonance at 153.8 ppm (C-6) is quite similar to that (158.9 ppm) of C-4 of 1-methylisatoic anhydride and to that (161.6 ppm) of C-1 of homophthalic anhydride (see Table I). The broadening of the C-2, C-4, and C-5 signals at ambient temperature is consistent with a tautomeric equilibrium involving the imidazole nitrogen atoms (compare purine[20]), since proton exchange between these sites would be expected to have the most influence on the chemical shifts of the carbon nuclei that are directly bonded to the imidazole nitrogen atoms.

By comparison with the ^{13}C chemical shifts (177.4 ppm) of the carbonyl carbon nuclei of the isobutyryl groups in diisobutyramide and urea diisobutyrate (see Table II), the sharp resonance at lowest field (167.3 ppm) in the ^{13}C NMR spectrum of the amide **4** was assigned to the carbonyl carbon nucleus (C-7) of the isobutyramido group. The quaternary carbon resonance at highest field (112.2 ppm) was assigned to C-5, and the resonance at next lowest field (147.6 ppm) to C-4, by analogy with **1** and **2**. Since the chemical shift of C-2 would be expected to be relatively unchanged by conversion of **2** into **4**, the resonance of **4** at 161.6 ppm was assigned to C-2. The chemical shift of the remaining quaternary carbon resonance at 152.5 ppm (C-6) is comparable with the shifts (153.4 and 155.5 ppm) of C-6 in uric acid and in xanthine, respectively. At 80 °C, narrowing of the C-2, C-4, and C-5 resonances of **4** (in methyl-d_6 sulfoxide) was observed, which again suggested the presence of an exchange process involving the NH proton of the imidazole moiety.[19-22]

For the imidazole derivative **6**, the assignment of the ^{13}C signal at 102.8 ppm to C-5 was confirmed by the observation of a large coupling (190.4 Hz) of this nucleus with H-5 in the proton coupled spectrum of **6** (Figure 3b). This spectrum showed a doublet at next lowest field (136.5 ppm) that was assigned to C-4 by analogy with compounds **1**, **2**, and **4**. The spacing (7.3 Hz) in this doublet was assigned tentatively as a coupling of C-4 with H-5.[12] The proton-coupled ^{13}C spectrum of **6** also displayed two narrow complex multiplets (at 149.1 and 173.9 ppm) that were assigned on the basis of their complexity to the nuclei (C-2 and C-6), which are bonded to isopropyl groups. The resonance of **6** at lowest field (173.9 ppm) was assigned to C-6 by comparison with the chemical shifts of carbonyl carbon nuclei in urea diisobutyrate, isobutyramide, and diisobutyramide (Table II).

Replacement of the 5-carboxamide substituent of amide **4** by a hydrogen atom evidently causes significant redistribution of the charge densities in the imidazole ring, so that for derivatives **4** and **6**, the chemical shifts of C-2 are substantially different (see Table I).

The ^{13}C NMR spectra of alloxan, alloxantin, parabanic acid, and allantoin (see Table I) were assigned by comparison with each other, and by correlation with the spectra of cyanuric acid (Table I) and the simple amides (Table II). For alloxantin and allantoin, the ^{13}C resonance at highest field is in each case assigned to the nucleus of an sp^3-hybridized carbon atom (C-5 and C-4, respectively). The chemical shifts of C-2 in parabanic acid and allantoin are similar to that of C-8 in uric acid, as would be expected.

The ^{13}C spectra of 1-methylisatoic and homophthalic anhydrides were assigned by use of proton-coupled spectra and from the substituent effects expected on the basis of previous studies of aromatic compounds.[23]

The ^{13}C chemical shifts and coupling constants shown in Table I provide basic data for further elucidation of the chemistry of uric acid and other purines.

Experimental Section[24]

Melting points are uncorrected and were determined in open capillaries in a silicone oil bath apparatus. Infrared spectra were recorded by use of Perkin-Elmer spectrophotometers, Models 137 and 257 (grating), and ultraviolet spectra with a Cary spectrophotometer, Model 14. Mass spectra were obtained with a Hewlett-Packard instrument, Model 5930A, by use of the direct insertion probe and Model 5933A data system. Methane was employed for chemical ionization spectra. Proton NMR spectra were measured either with a Varian A-60 spectrometer, or in the pulse Fourier transform mode at 90 MHz by means of a Bruker spectrometer, Model HFX-11, equipped with a Model BSV-2 pulse amplifier and Nicolet Model BNC-12 data system. ^{13}C NMR spectra were obtained at 22.6 MHz.

Qualitative and quantitative analyses of reaction products were performed by TLC on layers (0.25- and 2-mm thick) of silica gel that included a fluorescent indicator (Brinkman Silica Gel GF 254, or HF 254 and 366). The plates were developed with 1:1 v/v ethyl acetate–hexane for lactone **2**, 2:3 v/v ethyl acetate–methanol for amide **3**, and 1:4 v/v methanol–ethyl acetate for amine salts (e.g., products from hydrolysis of **2** with concentrated hydrochloric acid). GLC analyses were performed with a Hewlett-Packard gas chromatograph, Model 5750, equipped with a dual flame ionization detector, a glass column (10 ft, × 0.25 in.) of 2% OV-101 on Chromosorb WHP, and temperature programming (190–250 °C). Microanalyses were performed by Schwarzkopf Laboratories, Woodside, N.Y. 11377.

2-(1-Methylethyl)-4-(1-hydroxy-2-methylpropylidene)aminoimidazole-5-carboxylic Acid 5,1′-Lactone (2). Procedure A. A mixture of uric acid (2 g), isobutyric anhydride (180 mL), and pyridine (20 mL) was boiled under reflux for 4 h to give an orange or slightly brown solution. The mixture was filtered (if necessary) and concentrated under vacuum and then under nitrogen flush with some methanol present to a syrupy residue that was dissolved in chlorofrom (or methanol) and left overnight in the hood to induce slow crystallation. The crude product was triturated with cold ethyl acetate and thereby isolated in several crops: first crop (~450 mg) after 24 h, and second crop (300 mg) after 48 h; total yield 850–950 mg (32–36%). Careful recrystallization from 1:1 v/v ethyl acetate–cyclohexane gave microcrystalline **2**: mp 211–212 °C; UV λ_{max} (MeOH) 231 nm (sh, ϵ 8.66×10^3), 239 (9.71×10^3), 247 (8.48×10^3), 269 (6.88×10^3); IR ν_{max} (Nujol) 3120 w (NH), 1772 s (lactone C=O), 1609 m, 1590 m, and 1540 m cm^{-1} (C=C and C=N); proton NMR (C_5D_5N, 60 MHz) δ 11.30 or 12.5 (br, 1, NH), 3.29 (sp, 1, J = 7 Hz, CH), 2.91 (sp, 1, J = 7 Hz, CH), 1.46 [d, 6, J = 7 Hz, $C(CH_3)_2$], 1.27 [d, 6, J = 7 Hz, $C(CH_3)_2$].

Anal. Calcd for $C_{11}H_{15}N_3O_2$: C, 59.71; H, 6.83; N, 18.99. Found: C, 60.12; H, 6.77; N, 18.96.

Procedure B. A mixture of uric acid (2 g) and isobutyric anhydride (180 mL) was boiled under reflux for 8 h. The reaction mixture was filtered and concentrated (with some methanol present, nitrogen flush) to a syrup that crystallized on standing overnight. Compound **2** was recovered in several crops; yield 800 mg (30.5%); the product was identical with that obtained by procedure A.

Diisobutyramide, mp 174–175 °C, crystallized from the mother liquors of **2**, which showed TLC R_f values of 0.32 (lactone **2**), 0.42 (diisobutyramide), and 0.14, 0.50, 0.57, 0.65, and 0.71 (unknown), and GLC retention times (min, ±3%) of 5.63 (diisobutyramide), 14.88 (lactone **2**), and 3.78, 5.19, 11.65, 12.13, 12.60, 12.91, 14.64, 15.15, 16.25, 17.01, and 24.41 (unknown).

Experiments with ^{14}C-Labeled Uric Acids. Commercially available ^{14}C-labeled uric acids were used and their radiochemical purity was checked by paper chromatography in *tert*-butyl alcohol–2-butanone–water–ammonium hydroxide (4:3:2:1 v/v) and in 1-butanol–acetic acid–water (2:1:1 v/v); chromatograms were scanned with a radiochromatogram scanner. Purity was also checked by addition of a known activity of ^{14}C-labeled uric acid to unlabeled uric acid, followed by dissolution of the mixture in lithium carbonate solution, crystallization by acidification, and determination of specific activity. The uric-2-^{14}C acid and uric-6-^{14}C acid were determined by these criteria to be of suitable purity, but the uric-8-^{14}C acid was found to be impure, and, therefore, was recrystallized to constant specific activity. All samples were radioassayed by counting with a liquid scintillation counter.

Uric-2-^{14}C Acid. A mixture of uric-2-^{14}C acid (27.125 mg at 0.0386 μCi/mg, 1.047 μCi total), isobutyric anhydride (15 mL), and pyridine (1 mL) was boiled under reflux for 4 h. The mixture was then concentrated (N_2 flush), and the residue was subjected to TLC on silica gel GF 254 (8 × 8 × 0.025 cm) by development with 1:1 v/v ethyl acetate–hexane. The band corresponding to lactone **2** was scraped off, extracted with methanol, and its radioactivity determined. The main band contained only 0.003 μCi, indicating that C-2 is lost during formation of lactone **2**.

Uric-8-^{14}C Acid. Uric-8-^{14}C acid (25.343 mg at 0.0170 μCi/mg, 0.431 μCi total) was heated with isobutyric anhydride–pyridine for

4 h. Preparative TLC gave a lactone band that contained only 0.006 μCi, indicating that C-8 is lost when **2** is formed.

Uric-6-^{14}C Acid. Uric-6-^{14}C acid (937 mg at 0.0419 μCi/mg) was converted to lactone **2**, which was recrystallized to a constant activity of 0.0314 μCi/mg. The theoretical specific activity of the lactone if all radiolabel at position 6 is retained is 0.0318 μCi/mg.

Verification of Specifically Labeled Uric Acids by Oxidation to Allantoin. Uric-6-^{14}C acid (100 mg at 0.009 15 μCi/mg) was oxidized to allantoin with potassium permanganate. All of the radioactivity of the uric-6-^{14}C acid would be lost if it were labeled only at position 6. The specific activity of the allantoin found was 7.8×10^{-6} μCi/mg; the theoretical specific activity if all radioactivity were retained would be 9.7×10^{-3} μCi/mg.

Uric-2-^{14}C acid (201.5 mg at 0.0411 μCi/mg) was oxidized to allantoin. The radioactivity should be completely retained if the label is at position 2. The theoretical specific activity of the allantoin was 0.0437 μCi/mg; the activity found was 0.0378 μCi/mg (86% of theoretical).

2-(1-Methylethyl)-4-(2-methyl-1-oxo-propyl)aminoimidazole-5-carboxamide (4). A suspension of lactone **2** (200 mg) in concentrated ammonium hydroxide (2 mL) was carefully stirred at 40 °C (not above!) until dissolution was complete (3–4 min). The pale yellow solution was concentrated in a vacuum desiccator over concentrated sulfuric acid, phosphorus pentoxide, and sodium hydroxide for 72 h. The greenish syrup was dissolved in methanol (3 mL), and the solution was filtered through a layer of carbon (the use of an excess of decolorizing carbon imparts a red color). The clear filtrate was stirred and diluted with ethyl acetate to incipient turbidity. Amide **4** was isolated in several crops: total yield, 100–105 mg (47–49%); mp 185–186 °C (crystallized from 1:2 v/v pyridine–ethyl acetate); UV λ_{max} (MeOH) 213 nm (ϵ 10.6×10^3), 233 (9.3×10^3), 277.5 (14.6×10^3); IR ν_{max} (Nujol) 3190 m (NH), 1650 m (amide C=O), 1590 s (amide CNH) cm^{-1}; proton NMR (C_5D_5N, 90 MHz) δ 9.83 (br, 1, NH), 6.28 (br, 7, NH, NH_2, and H_2O), 3.21 (sp, 1, J = 7 Hz, CH), 2.74 (sp, 1, J = 7 Hz, CH), 1.37 [d, 6, J = 7 Hz, $C(CH_3)_2$], 1.28 [d, 6, J = 7 Hz, $C(CH_3)_2$].

Anal. Calcd for $C_{11}H_{18}N_4O_2$: C, 55.44; H, 7.61; N, 23.51. Found: C, 55.72; H, 7.59; N, 23.79.

2-(1-Methylethyl)-4-(2-methyl-1-oxo-propyl)aminoimidazole-5-carboxylic Acid (5). A suspension of lactone **2** (150 mg) in water (15 mL) was stirred and titrated with 0.2 mol/L sodium hydroxide to phenolphthalein. Dissolution of **2** was complete after 8 min, and the alkaline solution was kept for 20 h at room temperature. After neutralization (hydrochloric acid), the solution was extracted with 9:1 v/v ethyl acetate–dichloromethane (four 30-mL portions), and the combined extracts were dried (Na_2SO_4) and concentrated to a syrupy residue (45 mg). TLC indicated the presence of one major component, and two minor components. The EI mass spectrum showed *m/e* 239 (M^+) and fragment ions at *m/e* 196 ($M^+ - 43$), 178 (*m/e* 196 $- H_2O$), and 125 (imidazole derivative). On heating at 95 °C for 30 min, this material was partially decarboxylated to **6**. The EI mass spectrum of the product displayed characteristic peaks at *m/e* 196 and 195, indicating the presence of unchanged **5** and the imidazole derivative **6**, respectively.

Hydrolysis and Decarboxylation of 2 to 6 Monitored by ^{13}C NMR Spectroscopy. A solution of **2** (0.14 g) in 10:1 v/v methyl-d_6 sulfoxide–water (0.33 mL) was maintained at 80 °C in the NMR probe and was analyzed periodically over a period of 30 days by ^{13}C NMR spectroscopy, under either proton-coupled, proton-decoupled, or off-resonance proton-decoupled conditions. ^{13}C spectra taken after 2–4 days displayed four resonances in the isopropyl region in addition to those of **2** and four resonances at lower field in addition to the five resonances of **2** at low field. After 5 days, more water (0.03 mL) was added to the solution. In a spectrum taken after 6 days, the corresponding carbon resonances of **2** and **6** were of approximately equal intensity, except for the C-5 signal of **6** which showed substantial enhancement of intensity due to the Overhauser effect of an attached proton. After 30 days at 80 °C, the nine ^{13}C resonances of **2** had disappeared from the spectrum, and only the eight ^{13}C resonances of **6** remained (see Figure 3): proton NMR [$(CD_3)_2SO$, 60 MHz] δ 10.05 (s, 2, NH), 7.12 (s, 1, C=CH), 2.78 (m, 2, J = 7 Hz, CH), 1.26 [d, 6, J = 7 Hz, $C(CH_3)_2$], 1.11 [d, 6, J = 7 Hz, $C(CH_3)_2$].

Acid Hydrolysis of Lactone 2. Treatment of **2** (0.1 g) with concentrated hydrochloric acid (1 mL) at 60 °C for 5 min, followed by neutralization ($NaHCO_3$), extraction (9:1 v/v EtOAc–CH_2Cl_2), and concentration gave a syrupy residue. This was chromatographed in subdued light on a layer of silica gel HF 254 + 366 in 3:7 v/v MeOH–EtOAc. The mass spectrum of the fraction with $R_f \sim 0.47$ resembled that of the imidazole derivative **8** (*m/e* 125). In a separate experiment, lactone **2** (0.15 g) was stirred with concentrated hydrochloric acid (3 mL) at room temperature for 30 min. The clear solution was then concentrated (vacuum desiccator, KOH, 72 h) and the solid residue was extracted with warm pyridine. The mass spectrum of the *pyridine insoluble* fraction [about 50%, mp > 320 (d) from MeOH–Et_2O] showed a fragment ion at *m/e* 126 which was assigned to the cation of amine **8**. Mass spectrometry of the *pyridine soluble* fraction indicated that it contained starting material (*m/e* 221) and the hydrochloride of amine **8** (*m/e* 126).

Isobutyramide-^{15}N. Ammonium-^{15}N hydroxide was generated by mixing an ice-cold solution of ammonium-^{15}N chloride (0.166 g) in water (5 mL) with 1.0 mol/L sodium hydroxide; isobutyric anhydride (1 mL) was then added and the mixture stirred in a closed vessel at room temperature for 1 h, and then at 85 °C for 30 min. The solution was cooled and extracted with ethyl acetate, and the extracts dried (Na_2SO_4) and concentrated to give isobutyramide-^{15}N (40 mg): mp 128–129 °C; IR ν_{max} (KBr) 1625 s cm^{-1} (C=O); proton NMR [$(CD_3)_2SO$, 60 MHz] δ 7.20 (q, 1, $^2J_{HNH}$ = 2.1 Hz, $^1J_{^{15}NH}$ = 88.9 Hz, NH), 6.65 (q, 1, $^2J_{HNH}$ = 2.1 Hz, $^1J_{^{15}NH}$ = 87.4 Hz, NH), 2.34 (sp, 1, J = 7 Hz, CH), 0.99 [(d, 6, J = 7 Hz, $C(CH_3)_2$]; MS (EI) *m/e* 88 (M^+). Nonlabeled isobutyramide was prepared by boiling a mixture of ammonium carbonate and isobutyric anhydride under reflux for 30 min, followed by concentration (N_2 flush) and recrystallization from ethyl acetate. The isobutyramide had: mp 128–129 °C; proton NMR [$(CD_3)_2SO$, 60 MHz] δ 7.20 (br, 1, NH), 6.67 (br, 1, NH), 2.34 (sp, 1, J = 7 Hz, CH), 0.99 [d, 6, J = 7 Hz, $C(CH_3)_2$]; MS (EI) *m/e* 87 (M^+).

Diisobutyramide. Treatment of either urea, biuret, cyanuric acid, or isobutyramide with an excess of boiling, refluxing isobutyric anhydride, followed by concentration and crystallization from hot water yielded diisobutyramide: mp 174–175 °C; IR ν_{max} (Nujol) 1720 cm^{-1} (C=O); proton NMR ($CDCl_3$, 60 MHz) δ 8.94 (br, 1, NH), 3.06 (sp, 2, J = 7 Hz, CH), 1.18 [d, 12, J = 7 Hz, $C(CH_3)_2$]; MS (EI) *m/e* 157 (M^+).

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Registry No.—**5**, 62973-61-9; ammonium-^{15}N hydroxide, 62948-80-5; isobutyramide-^{15}N, 62962-45-2; ammonium carbonate, 10361-29-2.

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(6) For convenience in tabulation and description of the ^{13}C chemical shifts, compounds **2**, **4**, **5**, and **6** are all described herein as imidazole derivatives, as indicated in Table I. Compound **2** may also be named systematically as 2,5-bis(1-methylethyl)imidazo[4,5-*d*][1,3]oxazine-7(1*H*)-one. This name was kindly determined by Dr. R. S. Tipson (personal communication), by consultation with the Chemical Abstracts Service.
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Conformational Analysis of Prostaglandins F_1 Based on Proton Nuclear Magnetic Resonance Spectral Data

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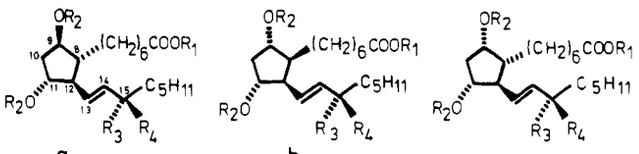
The ^1H NMR spectral parameters of prostaglandin $F_{1\alpha}$ (**5c**), prostaglandin $F_{1\beta}$ (**5a**), 8-*epi*-prostaglandin $F_{1\alpha}$ (**5b**), and derivatives are discussed. Evidence is presented for the occurrence of a restricted number of conformations in the three series. The solvent-dependent variation of the ^1H NMR spectral parameters of prostaglandin $F_{1\alpha}$ is interpreted on the basis of intramolecular hydrogen bonding between the 9- and 11-hydroxyl groups.

Studies of the conformational behavior of prostaglandins are important since it has been shown that there exist strict stereostructural requirements for certain characteristic actions[2a] and for substrate suitability with prostaglandin-metabolizing enzymes.[2b] The restraints applied to the prostaglandin molecule to fix a preexisting receptor site[2c,d] as a stable conformational isomer, or conformer, are so far little understood. Especially x-ray analysis[3] and theoretical calculations[4] have brought some knowledge about the conformation of prostaglandin $F_{1\beta}$ and of prostaglandins of the E series. The results of these studies are generally interpreted on the basis of a conformation (designated "hairpin" [5]) in which the two side chains are closely aligned. In these studies abstraction is made of the molecular environment, as no solvent effects are taken into account. In a series of refined experiments using different techniques Andersen[6] has investigated the occurrence of the "hairpin" conformation in solvated prostaglandins. While a lot of work is done in understanding the relation of the side chains, little attention has been paid to the conformational behavior of the five-membered ring in this molecule.[7] Accurate ^1H NMR spectral data of prostaglandins are scarcely found in the literature (see, however, ref 8). These data should be suited for the study of the conformation of the cyclopentane part of the prostaglandin molecule. We will discuss the ^1H NMR spectral data of the prostaglandins $F_{1\alpha}$, $F_{1\beta}$, and 8-epi-$F_{1\alpha}$ in chloroform-d_1 and methanol-d_4, and we will present evidence for the occurrence of a restricted number of conformations. Hydrogen bonding in aprotic medium, between the 9- and 11-hydroxyl groups of prostaglandin $F_{1\alpha}$ will be proven. The latter fact may be of crucial importance in understanding the ability of prostaglandins to pass through discrete conformational states as the environment changes.

Results and Discussion

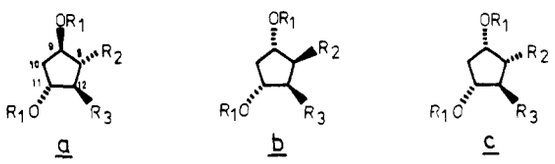
In Tables I–III the relevant ^1H NMR spectral parameters are found for products with three different configurations, **a** (as found in prostaglandin $F_{1\beta}$), **b** (as found in 8-epiprostaglandin $F_{1\alpha}$), and **c** (as found in prostaglandin $F_{1\alpha}$; Scheme I). Whereas products **1–6** are prostaglandins,[9] compounds **7–10** are used as references.[10] Comparison of the ^1H NMR spectral parameters of a large number of differently functionalized 1,4-dihydroxy- (and diacetoxy-) 2,3-dialkylcyclopentanes[11] indicates comparable pseudorotational itinerary energetics

Scheme I



a **b** **c**

1 ; $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$
2 ; $R_1 = CH_3$, $R_2 = COCH_3$, $R_3 = H$, $R_4 = OH$
3 ; $R_1 = CH_3$, $R_2 = COCH_3$, $R_3 = OH$, $R_4 = H$
4 ; $R_1 = R_2 = R_3 = H$, $R_4 = OH$
5 ; $R_1 = R_2 = R_4 = H$, $R_3 = OH$
6 ; $R_1 = CH_3$, $R_2 = R_4 = H$, $R_3 = OH$



a **b** **c**

7 ; $R_1 = COCH_3$, $R_2 = R_3 = CH_3$
8 ; $R_1 = H$, $R_2 = R_3 = CH_3$
9 ; $R_1 = COCH_3$, $R_2 = -(CH_2)_6CH{=}C(CH_3)_2$, $R_3 = -CH_2CH{=}C(CH_3)_2$
10 ; $R_1 = H$, $R_2 = -(CH_2)_6CH{=}C(CH_3)_2$, $R_3 = -CH_2CH{=}C(CH_3)_2$

for products with the same configuration. We will therefore assume throughout the discussion that the conformational behavior of prostaglandins is similar to that of the model compounds **7** and **8** as far as the cyclopentane is concerned. Although only sums of vicinal coupling constants are available to substantiate this assumption in the case of prostaglandins with **a** and **b** configurations, individual coupling constants will be used for prostaglandins with the "natural" **c** configuration.

One can expect that the conformational behavior of products with the **a** configuration will be dictated by the requirement of the two trans alkyl side chains to be diequatorial[12] on the base of torsional strain. Calculations[13] of the potential energy of the ten twist and the ten envelope conformations encountered during the itinerary of pseudorotation[14,15] of **7a** show that the C_2 conformation with the methyl groups in the most puckered part of the molecule ($^8_{12}T$; Scheme II) is the minimal-energy form. This conformation is, however, only