Table IV. Effect of Polyelectrolyte on the Alkaline Fading Reaction of Phenolphthalein at 25° [a]

Catalyst	Concn of catalyst, equiv l.$^{-1}$	k_2, M^{-1} sec^{-1}	Initial absorbance at 553 nm
None	0	0.015	1.175
DECS	5.26×10^{-5}	0.018	1.116
	2.63×10^{-4}	0.024	1.023
	1.05×10^{-3}	0.041	0.857
C_2PVP	5.26×10^{-5}	0.020	1.076
	2.63×10^{-4}	0.037	0.836
	1.05×10^{-3}	0.081	0.466
C_3PVP	5.26×10^{-5}	0.017	1.097
	2.63×10^{-4}	0.021	0.857
C_4PVP	5.26×10^{-5}	0.017	1.053
	2.63×10^{-4}	0.017	0.720
BzPVP	5.26×10^{-5}	0.015	1.066
	2.63×10^{-4}	0.0064	0.707
	1.05×10^{-3}	0	0.315
NaPSS	1.05×10^{-3}	0.015	1.172
	7.90×10^{-3}	0.015	1.197

[a] [PP] = 4.21×10^{-5} M, [NaOH] = 1.05×10^{-1} M.

10^{-3} equiv l.$^{-1}$ (Table IV). Here, presumably, the attractive forces between the indicator and the catalyst are so strong as a result of both electrostatic and hydrophobic interactions that the attack of the hydroxyl ions on the indicator is sterically hindered. Similar effects of a hydrophobic polymer catalyst have been reported on a reaction between two ions with charges of the same sign.[35]

The last columns of Tables III and IV give the change of the initial absorbance at 553 nm, the maximum peak of PP. The red or blue shift of the peak could not be observed by the addition of catalyst. Kinetic data were practically independent of such changes in the initial absorbance at 553 nm. As is seen in the tables, the larger the catalytic action of the macrocation, the larger the decrease of the absorbance. The reverse does not, however, necessarily hold, since by the addition of strongly hydrophobic macrocation (*e.g.*, BzPVP), the absorbance decreased drastically, but the reaction was largely repressed. We may regard the decrease in the absorbance as a measure of the binding strength of the indicator with the catalyst.

Acknowledgment. The Hitachi stopped-flow spectrophotometer Model RSP-2 was purchased by a grant administered by the Ministry of Education.

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Differentiation of 1,4- and 1,5-Disubstituted Imidazoles[1]

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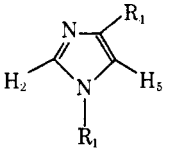
Abstract: A method is presented for distinguishing 1,4- and 1,5-disubstituted imidazoles by their proton cross-ring coupling constants. Other spectral methods also have been evaluated, as well as several methods which have been reported for differentiating such isomers. Comparison of these methods leads to the conclusion that the measurement of cross-ring coupling constants is the most generally satisfactory and reliable procedure. On this basis, structures are assigned to the carboxymethylhistidines, and the histamine metabolite is established as 1-β-D-ribofuranosyl-4-imidazoleacetic acid.

The considerable biological importance of the group of compounds incorporating the imidazole nucleus has stimulated much work on this heterocycle.[2] A remaining and important problem in this field has been the inability easily and reliably to differentiate 1,4- (I) and 1,5- (II) disubstituted imidazoles, either obtained from natural sources or prepared by ambiguous N-alkylation of the corresponding 4(5)-imidazoles. Isomer assignments have been made by analogy to similar reactions,[3] spectroscopic means,[4] conversion to known compounds,[5] or steric arguments.



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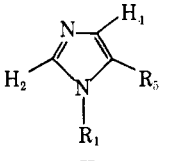

II

(1) Supported in part by the U. S. Army Research Office, Durham, N. C., and by the U. S. Atomic Energy Commission.

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(4) (a) M. Hoftner, V. Toomi, and A. Brossi, *J. Heterocycl. Chem.*, **3**, 454 (1966); (b) P. Rems, F. Kajfez, and V. Sunjic, *Bull. Sci., Cons. Acad. RSF Yougoslavie, Sect. A.*, **12**, 308 (1967); *Chem. Abstr.*, **69**, 55912*d* (1968); (c) J. S. G. Cox, C. Fitzmaurice, A. R. Katritzky, and G. J. T. Tiddy, *J. Chem. Soc. B*, 1251 (1967); (d) F. Kajfez, V. Sunjic, D. Kolhab, T. Fajdiga, and M. Oklobdzija, *J. Med. Chem.*, 11, 167 (1968); (e) H. Jaffe, *J. Biol. Chem.*, **238**, 2419 (1963); (f) J. B. Jones and D. W. Hysert, *Can. J. Chem.*, **49**, 3012 (1971).

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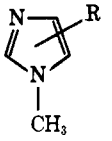
Table I. Ultraviolet Absorption of 1,4- and 1,5-Disubstituted Imidazoles



R	1,4-Disubstituted imidazoles λ_{max}, nm			1,5-Disubstituted imidazoles λ_{max}, nm		
	CH_3OH	0.1 *N* HCl	Δ, nm	CH_3OH	0.1 *N* HCl	Δ, nm
CHO	258	238	−20	261	237	−24
$CH_2CH(NH_2)COOH$	212	207	−5	206	205	−1
CH_2OH	204	207	+3	208	208	0
$CONHNH_2$	227	219	−8	240	219	−21
$COOCH_3$	233	219	−14	238	218	−20
$CONHNHSO_2C_6H_5$	235	217	−18	240	217	−23

When steric interaction is invoked as the directive force,[4f] it has been assumed that alkylation to form the 1,5 isomer is more sterically hindered and therefore the 1,4 isomer should predominate. However, this argument is not generally applicable, especially in cases where the alkylating group is small. With the exception of the nitroimidazoles, no reliable rules for orientation in N-alkylation of 4(5)-imidazoles exist, and even this one exception obtains only if the alkylation is carried out under highly specific conditions.[3a] The structures of compounds such as pilocarpine,[5a] anserine,[5b,c] and the methylhistidines[5c] were originally assigned by degradation to the corresponding 1,4- or 1,5-dimethylimidazole whose structural assignments were in turn determined by conversion to acyclic compounds[6a] and syntheses.[6b]

Such chemical methods of differentiation impose severe limitations for many biologically important compounds of limited availability and complex structure. An investigation therefore was begun seeking a means of isomer differentiation which would require small amounts of material and little chemical manipulation, and would be applicable to imidazoles with a wide variety of substituents. Spectroscopic techniques should fulfill these requirements. To this end, using a series of 1-methyl-4- and -5-alkylimidazoles as models, nmr, uv, ir, and mass spectrometry have been tested for their ability to distinguish the 1,4 and 1,5 isomers. The unambiguous syntheses of these model compounds have been reported.[7]

Results

Mass Spectrometry. The use of mass spectrometry in determining the substitution pattern of some imidazoles has been reported.[8] In our studies, the mass spectrum of each member of four isomeric pairs of 1,4- and 1,5-disubstituted imidazoles was obtained, and a rationalization for the fragmentation of each compound was devised based upon these spectra and those previously reported.[8] Although differences were observed between the members of each pair, these differences were not of diagnostic value. The spectra of the 1,4 and 1,5 esters and aldehydes, taken as examples, are very similar within each pair. Primary ionization occurs in the side chain, leading to an intermediate common to both isomers, and therefore to very similar spectra. A different course of ionization occurs in the imidazoles with saturated substituents, ionization occurring first in the ring. But here again no diagnostic difference was observed for the two isomer types.

Ultraviolet Spectroscopy. Ultraviolet spectroscopy has been used[4a] to distinguish between 1-alkyl-4- and -5-nitroimidazoles. The assignment of structure is based upon the observation that for any given pair of isomers, the 1,5-substituted one exhibits a 5–20-nm bathochromic shift compared to the 1,4-substituted one. In Table I are tabulated the ultraviolet absorption maxima of a series of 1,4- and 1,5-disubstituted imidazoles both as the free bases and after protonation. Three kinds of distinguishing differences between isomers were looked for: (1) the relative difference in the wavelength of the absorption maxima of the free bases and conjugate acids, (2) differences in curve shapes, and (3) relative differences in shifts in the maxima upon protonation.

No definitive difference was noted in the shape of the absorption curves of the two isomer types. Unlike the 1,4-nitroimidazoles, which are reported[4a] to have an inflection between 220 and 260 nm but no maximum, the conjugated 1,4-imidazoles reported here have distinct maxima. From the data in Table I, it can also be seen that there is little difference in the relative protonation shifts. Indeed, protonation had a considerable leveling effect on the differences between the isomers, the values found for the isomeric conjugate acids being almost identical. A small but real difference was found, however, between the free bases of imidazoles with conjugating substituents, the 1,5 isomer absorbing at 3–13 nm longer wavelength. This is similar to the difference range reported[4a] for the nitroimidazoles. However, this characteristic does not extend to the imidazoles with nonconjugating substitutents. For these compounds, no consistent difference was observed.

Therefore, while ultraviolet spectroscopic differentiation of 1,4- and 1,5-disubstituted imidazoles may be applicable to imidazoles with conjugating substituents, there is no absolute difference between such isomers (*i.e.*, line shape) which may allow identification of a single compound. Furthermore, in the case

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of imidazoles bearing nonconjugating groups, not even consistent relative differences are found.

Infrared Spectroscopy. It has been reported[4b,d,e] that 1,4- and 1,5-disubstituted imidazoles in some cases can be differentiated by their infrared spectra. In one report,[4e] the differentiation of N^{Im}-methyl- and N^{Im}-carboxymethylhistidines was based upon the appearance of absorption maxima at 12 μ in the ir spectra of the 1,5-disubstituted isomers. The spectra of the 1,4-disubstituted isomers had minima at that point.

We have obtained spectra on samples of N^{Im}-methyl- and N^{Im}-carboxymethylhistidines in KBr pellets as reported.[4e] Spectra were also obtained for the two isomeric N-α-acetyl-1- and -3-methoxycarbonylmethylhistidine methyl esters. Each of the isomer pairs showed the described 12-μ maxima for the 1,5 isomer and minima for the 1,4 isomer. However, when several other model imidazoles were tested, no such difference consistently occurred between isomers. The 1,4 isomers of both the chloromethyl- and hydroxymethylimidazole have distinct absorption *maxima* at 12 μ. In other cases the spectra had the general appearance reported, but the distinction was equivocal.

Since infrared spectra recorded on crystalline materials in KBr pellets may reflect to a large extent interactions modified or brought about by the crystal structure, it is possible that the similarities in the KBr spectra of the methyl- and carboxymethylhistidines may be due to similarities in the crystal structures. The differences in the remainder of the disubstituted imidazoles could in part be explained, not by differences in the infrared spectra of the free molecules but by differences in crystal structure.

A study of the solution infrared spectra of the same compounds was therefore made. The approach used was derived from that reported,[4e] in that the isomers were compared in the 12-μ region. Bromoform was chosen as solvent for general solubility of imidazoles and because it is transparent from 9 to 14 μ. Where the compounds were not soluble in bromoform, acetonitrile, which is transparent from 11 to 13.2 μ, was used. Although there are differences in the solution ir spectra between the members of each pair, no diagnostic difference was found between the isomer types. It therefore appears that ir analysis is useful for distinguishing these isomer pairs only with N^{Im}-substituted histidines in KBr.

Proton magnetic resonance is particularly suited to a structural study of 1,4- and 1,5-disubstituted imidazoles. To this end, several parameters were considered which might reflect the substitution pattern: absolute and relative proton chemical shifts of the free bases, absolute and relative proton chemical shifts of the conjugate acids, relative deshielding upon protonation, coupling constants, and solvent–solute interactions.

a. Coupling Constants. The aromatic protons of 1,4- (H-2 and H-5) and 1,5- (H-2 and H-4) disubstituted imidazoles are coupled to each other across the ring. These coupling constants are designated $J_{2,5}$ and $J_{2,4}$, respectively; $J_{2,5}$ is larger than $J_{2,4}$ and measures in the range of 1.1–1.5 Hz, while $J_{2,4}$ measures in the range 0.9–1.0 Hz. The coupling constants for a variety of imidazoles measured in $CDCl_3$ and DMSO are recorded in Table II.

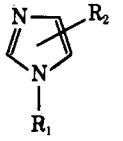
On the basis of these data, we propose that under specified conditions the cross-ring coupling constants can be used to distinguish 1,4- and 1,5-disubstituted imidazoles, and, furthermore, that the cross-ring coupling constants can provide an absolute determination of the substitution pattern. Coupling constants were first established for three isomeric pairs of compounds of known orientation.[7] Other pairs were then synthesized, and the separate isomers were identified by their respective cross-ring coupling constants. Within the pairs thus synthesized, one isomer clearly fits the pattern of the 1,5 compounds and the other of the 1,4 compounds. In no case did a measured coupling constant vary from this pattern.

1-Methoxycarbonylmethyl-5-imidazoleacetonitrile, methyl 1-methoxycarbonylmethyl-5-imidazolecarboxylate, N-α-acetyl-1-methoxycarbonylmethyl-5-histidine methyl ester, and 1-triacetylribofuranosyl-5-imidazoleacetonitrile apparently display steric interactions between the 1 and 5 substituents which obscure the coupling constants. Space-filling models indicate that, especially in the case of the ribosyl derivative, this interaction is sufficient to hinder free rotation in the substituents.

To test this hypothesis, the variations in the cross-ring coupling constant, $J_{2,4}$, in methyl 1-methoxycarbonylmethyl-5-imidazolecarboxylate with temperature were determined. At ambient magnet temperature (30°), H-2 and H-4 appeared as broadened singlets. When the probe temperature was raised to 85°, a doublet structure began to appear, and at 120°, $J_{2,4}$ of 0.91 Hz was easily measurable. The carboxymethylhistidine derivative assigned the 1,4 structure had $J_{2,5}$ = 1.1 Hz, and its isomer, probably owing to 1,5-steric interaction, exhibited no resolvable cross-ring coupling constant. An attempt to measure $J_{2,4}$ for the supposed 1-carboxymethyl-5-histidine derivative by raising the temperature caused loss of resolution and broadening of the peaks in this case. Decoupling from the β-methylene protons also did not prove fruitful. Since this supposed $J_{2,5}$ value is at the low end of the range, and in the absence of a measurable $J_{2,4}$, a second approach was taken to demonstrate that the lack of a measurable $J_{2,4}$ was indeed due to 1,5 steric interaction.

The relatively bulky methoxycarbonyl was replaced by the much smaller cyano group by alkylating N-acetylhistidine methyl ester with iodoacetonitrile. Two isomeric 1-cyanomethyl-4(5)-histidine derivatives were isolated. Models indicate that 1,5-steric interaction should be greatly reduced. One, assigned the 1,4 structure, had $J_{2,5}$ = 1.10 Hz; and the other, assigned the 1,5 structure, had $J_{2,4}$ = 0.99 Hz. Even though $J_{2,5}$ in this case is not much larger than $J_{2,4}$, the measurable $J_{2,4}$ makes the assignment certain. Both the 1,4-cyanomethyl- and 1,4-carboxymethylhistidine derivatives have been hydrolyzed to the same 1,4-carboxymethylhistidine. This hydrolysis product is identical with that assigned the 1,4 structure by Jaffe.[4e] A second confirmation of this approach was the measurement of $J_{2,4}$ = 0.94 Hz for ethyl 1-cyanomethyl-5-imidazolecarboxylate, at ambient magnet temperature, and $J_{2,5}$ = 1.20 Hz for the corresponding 1,4 isomer. Thus, whereas $J_{2,4}$ for methyl 1-methoxycarbonylmethyl-5-imidazolecarboxylate was measurable only at high temperature, the $J_{2,4}$ for the cyanomethyl compound did not show evidence of 1,5-steric interaction.

Table II. Nmr Chemical Shifts and Coupling Constants for Ring Protons of 1,4- and 1,5-Disubstituted Imidazoles



Substituents	Chemical shifts $\delta_{1,5}$ H-2, H-4[b]	Chemical shifts $\delta_{1,4}$ H-2, H-5[b]	Cross-ring coupling constants $J_{2,4}$ CDCl$_3$	$J_{2,4}$ DMSO	Position[a] 4/5	$J_{2,5}$ CDCl$_3$	$J_{2,5}$ DMSO
$R_1 = CH_3$							
$R_2 =$							
$COOCH_3$	7.68, 7.52	7.44, 7.55	0.94	1.05	U/D	1.37	1.22
CH_2OH	7.36, 6.83	6.78, 7.30	Insol	0.99	U/D	1.35	1.20
CHO	7.72, 7.59	7.58, 7.68	0.92	1.00		1.17	1.20
CH_3	7.26, 6.67	6.47, 7.18					
$CONHNH_2$			0.95	1.00	U/D	1.15	1.21
$CH_2CHNH_2CO_2H$			Insol	1.00		Insol	1.20
$CONHNHSO_2C_6H_5$				1.00			1.20
CH_2CN				1.02	U/—		
NO_2			1.07		D/D	1.28	
$R_1 = CH_2COOCH_3$							
$R_2 =$							
$COOC_2H_5$	7.61, 7.50	7.48, 7.60		0.90 (120°)	U/D		1.20
CH_2CN	7.46, 6.92	6.91, 7.39			—/D		1.20
$CH_2CHCOOCH_3$ | $NHCOCH_3$					—/D		1.10
$R_1 = CH_2CN$							
$R_2 =$							
$CH_2CHCOOCH_3$ | $NHCOCH_3$	7.54, 6.83	6.85, 7.49		0.99	U/D		1.10
$COOC_2H_5$	7.78, 7.72	7.70, 7.80		0.94	—/D		1.20
$R_1 = COCH_3$[c]							
$R_2 =$							
$COOC_2H_5$					—/U	1.3	
R_1 = triacetyl-β-D-ribofuranosyl							
$R_2 =$							
CH_2CN		7.16, 7.66			—/D	1.20	

[a] Position: U = H-4 (or -5) was upfield relative to H-2; D = H-4 (or -5) was downfield relative to H-2. [b] The specific assignment of a resonance line to either proton of the pair H-2, H-4, or H-2, H-5 cannot be made. [c] G. S. Reddy, L. Mandell, and J. H. Goldstein, *J. Chem. Soc.*, 1414 (1963).

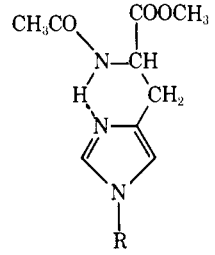
The difference in $J_{2,4}$ and $J_{2,5}$ in 1,4- and 1,5-disubstituted imidazoles can be rationalized by the geometry of the imidazole system. The cross-ring coupling constants for several heterocycles have been reported.[9] For pyridine, $J_{2,4}$ = 1.9 Hz, $J_{2,5}$ = 0.9 Hz, and $J_{2,6}$ and $J_{3,5}$ = 1.6 Hz; for furan $J_{2,4}$ = 1.4 Hz; and for pyrrole, $J_{2,4}$ = 2.1 Hz. These are all similar to the values we find for the imidazoles. At least two geometrical factors may produce differences in the J values: (1) the angles the carbon–hydrogen bonds make to each other and (2) the relative separations of the carbons. Both of these factors will affect the interactions of the two protons involved. In the case of pyridine as solvent, the cross-ring coupling constants decrease with separation of the carbons. For some imidazoles for which the X-ray crystal structures[10] have been determined, the C-2–N-1 and N-1–C-5 distances are less than the N-3–C-4 distance. Therefore, one may assume that the C-2–C-5 distance is less than the C-2–C-4 distance in the 1,4- and 1,5-disubstituted imidazoles, and on this basis $J_{2,4}$ should be less than $J_{2,5}$.

(9) J. A. Pople, W. J. Schneider, and H. J. Bernstein, "High Resolution NMR," McGraw-Hill, New York, N. Y., 1959, p 266.
(10) C. Sandmark and C. Branden, *Acta Chem. Scand.*, 21, 993 (1967).

b. Solvent–Solute Interaction. In all protic solvents studied, the cross-ring coupling constants were generally not evident and the aromatic protons appeared as broadened singlets. In aprotic solvents, however, the aromatic absorptions sharpened, and at least one in each case appeared as a doublet. In some cases, the appearance of the doublet structure was extremely sensitive to the presence of a hydrogen bonding source. Thus, when the cross-ring coupling constants of 1-methoxycarbonyl-4- and -5-methylimidazoles were measured in chloroform as a function of the addition of small amounts of methanol, it was found that a 1% solution of methanol was sufficient to completely obscure the coupling. When DMSO was employed as the solvent, however, the doublet structure, while less well resolved, was still measurable even at a concentration of 40% H_2O. DMSO has thus been generally used as the solvent for these studies because of the lower sensitivity of the coupling constants in this solvent. DMSO as an excellent hydrogen acceptor apparently competes effectively with the imidazoles for protons available for hydrogen bonding.

Since the imidazoles are in relatively low concentration compared to the DMSO solvent, the imidazoles are essentially non-hydrogen-bonded if only traces

of a proton source are present. As the protic source increases, the coupling constants become smaller and less well defined. That $J_{2,5}$ for the 1,4-*N*-α-acetylhistidine derivatives is smaller than for other 1,4-imidazoles can thus be explained as resulting from a hydrogen bond source for which the imidazole effectively competes with DMSO. That source is the *N*-acetylamino proton as shown in structure III. The



IIIa, R = CH_2COOH
b, R = CH_2CN

normal $J_{2,4}$ of the 1-cyanomethyl-5-histidine derivative is due to little or no 1,5-steric interaction and inability to form an intramolecular hydrogen bond.

c. Chemical Shifts of Free Base. A series of 1-methyl-4- and -5-alkyl-substituted imidazoles was compared on the basis of chemical shift, and the results are tabulated in Table II. The absolute chemical shifts have no relationship to the isomeric feature but reflect more the nature of the substituent. The relative chemical shifts, however, do reflect differences in the isomers in that H-2 and H-5 resonances in the 1,4 isomers are at slightly higher field than H-2 and H-4 resonances in the 1,5 isomers.[4f] There is not, however, enough consistency in the differences to make this a reliable characteristic in differentiating isomers.

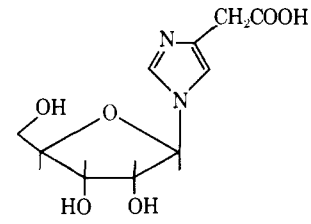
The common practice of assigning H-2 to the most downfield resonance of 1-substituted imidazoles becomes a very doubtful procedure as a result of this study. As was stated earlier, one of the aromatic protons invariably shows a more resolved doublet than does the other. In many cases only one appears as a doublet. The loss of resolution in the other resonance may be caused by ^{14}N quadrupole coupling. The question then becomes which resonance arises from which aromatic proton.

Whether the upfield or downfield proton shows the doublet structure is noted in Table II. With the exceptions of the *N*-acetyl-, chloro-, and nitroimidazoles, in the 1,5 isomers it is the upfield resonance while in the 1,4 isomers it is the downfield resonance. Where the 4(5) substituent has a proton α to the ring (*i.e.*, $-CH_3$, $-CHO$, $-CH_2OH$), the 4(5) proton is sometimes coupled to these α protons and can be identified on this basis. In those compounds where coupling to α protons occurs, it is this coupled resonance which shows the best resolved doublet structure when decoupled from the α protons. It is therefore the H-4(5) resonance which shows the best resolved doublet.

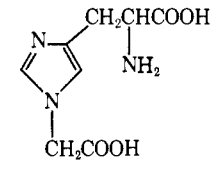
It follows from this that while H-2 is relatively more shielded than H-4, it is relatively less shielded than H-5 in some of these compounds. The shielding effect of a nitro group[4c] and the deshielding effect of a chloro group explain why these compounds do not follow this pattern. The effects are in the expected directions, both H-4 and H-5 appearing downfield to H-2 in the nitroimidazoles and upfield to H-2 in the chloroimidazoles. Therefore, while it is possible to distinguish some 1,4- and 1,5-disubstituted imidazoles on this basis, it is more important for future assignments to note that H-2 is not always the most deshielded proton in these imidazoles. Further evidence to support these contentions is obtained from 1-methyl-4-hydroxymethylimidazole. In methanol, the downfield resonance shows some residual coupling. After a small amount of trifluoroacetic acid is added, the doublet resonance appears relatively upfield even though both resonances have shifted downfield. The resonance which shifts from relatively upfield to downfield, and therefore shows the greatest shift upon protonation, is H-2. The one exhibiting the residual coupling is the H-5 and is downfield to H-2 in the free base.

Application

During the course of this study, it became possible to determine the substitution pattern of two biologically important 1,4(5)-disubstituted imidazoles as a demonstration of the usefulness of the method. The first was the ribofuranosyl derivative of imidazole-4(5)-acetic acid (IV) which is found in the urine of mice



IV



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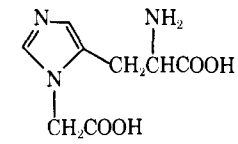

VI

and men as a metabolite of histamine. Previous studies had failed to determine the actual substitution pattern by any reliable means. The fusion[1c] of imidazoleacetonitrile with tetraacetyl-β-D-ribofuranose gave two major products (A and B). Separation of these two products by column chromatography provided a pure sample of the more abundant material (A) and a significantly purified sample of the other (B). The nmr, ir, and mass spectra of each product indicated that it was the expected triacetyl nucleoside. On the basis of a cross-ring coupling constant of $J = 1.25$ Hz, A was shown to be 1-triacetylribofuranosyl-4-imidazoleacetonitrile. A portion of A was hydrolyzed to the free imidazoleacetic acid riboside, which was identical with the natural product, which is therefore 1-β-D-ribofuranosyl-4-imidazoleacetic acid (IV).

The other disubstituted imidazoles of interest were the isomeric 1-carboxymethyl-4(5)-histidines (V and VI). Although their structures had been previously determined by ir spectroscopy, the results of our studies left some doubt as to the validity of that criterion. Thus, *N*-α-acetylhistidine methyl ester was alkylated with iodoacetonitrile to give *N*-α-acetyl-1-cyanomethyl-4-histidine methyl ester and *N*-α-acetyl-1-cyanomethyl-5-histidine methyl ester. Each compound could be

assigned a substitution pattern by its cross-ring coupling constant. The 1,4-disubstituted isomer was hydrolyzed to 1-carboxymethyl-4-histidine (V) which proved to be identical with that isomer previously assigned[4e] the same structure.

Conclusion

Of the several possible methods of distinguishing 1,4- and 1,5-disubstituted imidazoles reported here and previously reported, one is of general applicability. That is the use of cross-ring coupling constants which we have demonstrated can be used to differentiate these isomers. These coupling constants, except when obscured by 1,5-steric interaction, have proved highly reliable, so that it is necessary to have only one isomer in order to determine its structure. This makes the nmr method particularly useful in the identification of natural products, avoiding costlier degradation procedures.

Experimental Section

All nmr spectra are reported as δ values and were measured on a Varian HA-100 spectrometer equipped for variable temperature and homonuclear decoupling. The spectra were usually obtained in $CDCl_3$ (TMS-lock) or DMSO-h_6 (DMSO-lock) solution at a concentration of 3–30 mg/200–300 μl. Coupling constants were measured on a 50-Hz sweep width, 100-sec scan speed, 1-Hz filter, a power level of 70 decibels, and an observing field power low enough to prevent saturation as indicated by broadening of the lines. The coupling constants were measured as the distance between the half-widths at half-height of the two lines of the doublet. Generally six to ten measurements were made, and the standard deviation in any given J value is ± 0.03 Hz. Acid shifts were measured in CD_3OD solutions with trifluoroacetic acid to ensure complete solubility. To effect the shift, 5-μl aliquots of TFA were added directly to the sample in the nmr tube after the free base spectrum was recorded. Infrared specta were obtained in KBr wafers or on about 0.6% solutions in acetonitrile or bromoform on Perkin-Elmer 237 and 137 spectrophotometers. Ultraviolet spectra were recorded on a Cary 14 spectrophotometer in methanol solution. Acid shifts were measured by adding aqueous HCl to 0.1 N for each sample. Mass spectra were recorded on a Consolidated Electrodynamics Corp. mass spectrometer, Type 21-103C, at 70 eV using standard injection techniques.

1-Methylimidazoles. Methyl 1-methyl-4-imidazolecarboxylate, methyl 1-methyl-5-imidazolecarboxylate, 1-methyl-4-hydroxymethylimidazole, 1-methyl-5-hydroxymethylimidazole, 1-methyl-4-imidazolecarboxaldehyde, and 1-methyl-5-imidazolecarboxaldehyde were prepared as previously described.[7]

1-Methyl-4-nitroimidazole and 1-methyl-5-nitroimidazole were prepared by alkylation of 4(5)-nitroimidazole with dimethyl sulfate.[3c] The product was chromatographed on a silica gel column in 10% methanol–chloroform. Two products were obtained. One was identified as 1-methyl-4-nitroimidazole by nmr, mp 134° (lit.[3c] mp 134°). The other was a glass and was characterized by nmr as 1-methyl-5-nitroimidazole.

1-Methoxycarbonylmethylimidazoles and 1-cyanomethylimidazoles were prepared by alkylation of the appropriate 4(5)-imidazoles with methyl iodoacetate[11] or iodoacetonitrile,[12] respectively, by the general procedure described in the literature.[13] Alkylations were carried out in refluxing acetone for 2 hr over solid, anhydrous potassium carbonate. The reaction mixture was then filtered, the precipitate was washed with acetone, and the combined filtrate and washings were evaporated. The residue was digested with 10% methanol in chloroform, filtered, and chromatographed on silica gel using 10% methanol in chloroform for elution. The various isomers, which were all oils, were identified by nmr. Their composition was established by high-resolution mass spectroscopy.

Ethyl 1-methoxycarbonylmethyl-4-imidazolecarboxylate: nmr ($CDCl_3$) 1.33 (3 H, CH_3 (Et), t), 3.72 (3 H, s, OCH_3), 4.28 (2 H, q, $-CH_2$ (Et)), 4.72 (2 H, s, $-NCH_2-$), 7.48 (1 H, d, H-2), 7.60 (1 H, d, H-5).

Ethyl 1-methoxycarbonylmethyl-5-imidazolecarboxylate: nmr ($CDCl_3$) 1.32 (3 H, t, CH_3(Et)), 3.70 (3 H, s, $-OCH_3$), 4.26 (2 H, q, $-CH_2-$ (Et)), 4.77 (2 H, s, N-CH_2), 7.50 (1 H, d, H-4), 7.61 (1 H, d, H-2).

1-Methoxycarbonylmethyl-4-imidazoleacetonitrile: nmr ($CDCl_3$) 3.58 (2 H, s, CH_2CN), 3.65 (3 H, s, OCH_3), 4.66 (2 H, s, N–CH_2), 6.91 (1 H, d, H-2), 7.39 (1 H, d, H-5).

1-Methoxycarbonylmethyl-5-imidazoleacetonitrile: nmr ($CDCl_3$) 3.66 (3 H, s, OCH_3), 3.71 (2 H, s, $-CH_2CN$), 4.73 (2 H, s, N–CH_2), 6.92 (1 H, s, H-4), 7.46 (1 H, s, H-2).

Ethyl 1-cyanomethyl-4-imidazolecarboxylate: nmr ($CDCl_3$) 1.3 (3 H, t, CH_3), 4.3 (2 H, q, CH_2), 5.3 (2 H, s, CH_2CN), 7.7 (1 H, d, H-2), 7.8 (1 H, d, H-5).

Ethyl 1-cyanomethyl-5-imidazolecarboxylate: nmr ($CDCl_3$) 1.36 (3 H, t, CH_3), 4.30 (2 H, q, CH_2), 5.28 (2 H, s, CH_2CN), 7.72 (1 H, d, H-4), 7.78 (1 H, d, H-2).

1-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-4- and -5-imidazoleacetonitrile. Imidazole-4(5)-acetonitrile[14] (3 g) and 9 g of 1,2,3,5-tetraacetyl-β-D-ribofuranose[15] were powdered and placed in an evacuated three-necked, 50-ml, round-bottom flask. The flask was lowered into a preheated salt bath at 205°, and, when the temperature of the melt reached 160°, the vacuum was released temporarily while 0.05 g of chloroacetic acid was added. Aspirator vacuum was reapplied until the internal temperature reached 182°, at which point boiling began, and a high vacuum (0.10 mm) was applied with continued heating for 10 min. The mixture was removed from the salt bath, triturated while hot with 50 ml of benzene, the benzene mixture filtered, and the residue washed with hot benzene. The benzene solution was extracted with cold, saturated sodium carbonate (4 × 25 ml) and water (2 × 25 ml) and dried over magnesium sulfate. After the drying agent was removed, the solvent was stripped leaving a syrup. This syrup was shown by tlc on Kieselgel, eluting with chloroform–ethanol–acetic acid, 90:6:4, to contain starting material and two major products: imidazole-4(5)-acetonitrile, R_f 0.36; product B, R_f 0.52; product A, R_f 0.74. Column chromatography of the oil on Kieselgel, eluting with 5% methanol in chloroform and collecting 10-ml fractions at 1 ml/min, gave pure A and B significantly purified.

A: nmr ($CDCl_3$) 7.66 (1 H, d, H-5, J = 1.25 Hz), 7.16 (1 H, d, H-2, J = 1.25 Hz), 5.77 (1 H, m, H-1′), 5.32 (2 H, m, H-2′,3′), 4.31 (3 H, m, H-4′,5′), 3.64 (2 H, s, H-α), 2.08 (3 H, s, Ac), 2.06 (3 H, s, Ac), 3.03 (3 H, s, Ac); *m/e* 365 (M^+), 366 (M + 1), 292, 259, 139, 97, 69, 43.

B: ir (mull) 2260 (CN), 1745 (OAc) cm^{-1}; mass spectrum, same as for isomer A.

1-β-D-Ribofuranosyl-4-imidazoleacetic Acid (IV). A sample of 1-(2′,3′,5′-triacetyl-β-D-ribofuranosyl)-4-imidazoleacetonitrile, product A, was hydrolyzed in $Ba(OH)_2$ as described[16] for the hydrolysis of 1-β-D-ribofuranosylimidazole-4(5)-acetonitrile. This removes the acetyl groups and hydrolyzes the cyano group in one step. The product, purified by ion-exchange chromatography as described,[16] crystallized on standing. Its nmr spectrum indicated that the derivative was a ribosylimidazoleacetic acid isomer: nmr (D_2O) 3.8 (4 H, broad s, CH_2COOD and H-5′), 4.3 (3 H, m, H-4′,2′,3′), 5.9 (1 H, s, H-1′), 7.5 (1 H, broad s, im H), 8.8 (1 H, broad s, im H); mp 180–182°; $[\alpha]^{20}D$ −51.0° (lit.[16] mp 185°; $[\alpha]^{20}D$ −51.4°).

Anal. Calcd for $C_{10}H_{15}O_6N_2Cl$: C, 40.7; H, 5.1; N, 9.5. Found: C, 41.2; H, 5.5; N, 9.5.

***N*-α-Acetylhistidine Methyl Ester.** Histidine methyl ester dihydrochloride[17] (21.5 g) was dissolved in methanol (250 ml). To this solution was added a solution of sodium (4.6 g) in 100 ml of methanol followed by diethyl ether (200 ml). The mixture was allowed to stand overnight and was then filtered; the filtrate was evaporated under reduced pressure. The methylhistidine residue was dissolved in $CHCl_3$ (400 ml) and maintained below −5° while CH_3COCl (3.1 g) in $CHCl_3$ (50 ml) was slowly added with *vigorous* stirring. When addition was complete, the reaction

(11) L. Aronstein and J. M. A. Kramps, *Ber.*, 14, 604 (1881).
(12) D. B. Luten, Jr., *J. Org. Chem.*, 3, 595 (1939).
(13) P. Neelakantan and G. Thyagarajan, *Indian J. Chem.*, 189 (1969).
(14) H. Bauer and H. Tabor, *Biochem. Prep.*, 5, 97 (1957).
(15) R. D. Guthrie and S. C. Smith, *Chem. Ind. (London)*, 547 (1968).
(16) H. Bauer, *J. Org. Chem.*, 27, 167 (1962).
(17) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Wiley, New York, N. Y., 1961, p 2671.

mixture was stirred a further 15 min in the cold and finally 15 min at room temperature. It was then filtered and the solvent evaporated at 35°. The residue, a viscous light-brown oil, was triturated with petroleum ether, the solid which formed was allowed to settle, the petroleum ether was removed, and the trituration was repeated to give the product as a fine white powder, pure by tlc (15% MeOH–$CHCl_3$): mp 123–124°; nmr ($CDCl_3$) 7.55 (1 H, s, H-2), 6.82 (1 H, s, H-4(5)), 3.09 (2 H, d, H_α, J = 6.0 Hz), 4.74 (1 H, t, H_β, J = 6.0 Hz), 7.82 (1 H, d, H_N, J = 8.0 Hz), 2.00 (3 H, s, NAc), 3.72 (3 H, s, OCH_3).

Anal. Calcd for $C_9H_{13}O_2N_3$: C, 51.2; H, 6.2; N, 19.9. Found: C, 51.3; H, 6.0; N, 20.0.

Alkylation of *N*-α-Acetylhistidine Methyl Ester with Methyl Iodoacetate. *N*-α-Acetylhistidine methyl ester (1 g) was dissolved in 50 ml of dry acetone, and powdered potassium carbonate (anhydrous, 0.981 g, 1.5 equiv) was added followed by a solution of methyl iodoacetate (0.948 g, 1 equiv) in 20 ml of dry acetone. The resulting mixture was heated at reflux and samples were taken for tlc at 2, 12, and 24 hr. After 24 hr, the product distribution had stabilized; the mixture was cooled and filtered and the filtrate evaporated. The residue was digested with 15% methanol in chloroform, the solid material was removed, and the filtrate was evaporated. The residue was dissolved in a small amount of 15% methanol–chloroform, applied to a column of Kieselgel packed in the same solvent (120 g, 60 cm × 2.4 cm) and eluted with the same solvent. Two products were eluted; the first (592 mg) was the expected 1,4-imidazole (C) and the second (152 mg) was the expected 1,5-imidazole (D).

Anal. of C: Calcd for $C_{12}H_{17}N_3O_6$: C, 50.9; H, 6.1; N, 14.8. Found: C, 50.7; H, 6.0; N, 14.9.

Hydrolyses of *N*-α-Acetyl-1-methoxycarbonyl-4-histidine Methyl Ester (C). A solution of 200 mg of C in 6 *N* HCl (80 ml) was refluxed for 6 hr, cooled, and evaporated *in vacuo* at 40°. The residue was dissolved in CO_2-free distilled water and applied to an ion-exchange column (Dowex 2-X10, 15 × 1.5 cm).[18] The column was washed with 50 ml of CO_2-free distilled water and then 1 *N* acetic acid. The effluent was collected in 5-ml fractions and the amino acid located by ninhydrin on filter paper. After evaporation of the acetic acid, the N^{Im}-carboxymethylhistidine was recrystallized from ethanol–water. Its ir was identical with that reported.[4e]

Anal. Calcd for $C_8H_{11}N_3O_4$: C, 41.6; H, 5.7; N, 18.2. Found: C, 42.1; H, 5.6; N, 18.1.

Alkylation of *N*-α-Acetylhistidine Methyl Ester with Iodoacetonitrile. The alkylation was carried out in the same way as the alkylation with methyl iodoacetate described above. The crude product was chromatographed on Kieselgel in methanol–glacial acetic acid–chloroform, 2:1:7, and the elution of products was followed by tlc. The first product to elute is the 1,5 isomer, the second the 1,4 isomer, followed closely by starting material. Samples containing one product only were pooled, the solvent was removed by rotary evaporation at 30° (20 mm), and the remaining acetic acid was removed by lyophilization. The powdery residue was extracted with ethyl acetate and the solid residue was removed by centrifugation. This material is silica, leeched from the column in the presence of acetic acid. Evaporation of the ethyl acetate solution gave the particular cyanomethylhistidine as its acetic acid salt. These salts are quite stable and do not dissociate even at 1 μ (room temperature). Their stability at higher temperatures, however, was not tested. The cyanomethylhistidine salts were each dissolved in enough saturated Na_2CO_3 solution to maintain pH ~10. The water was removed by lyophilization and the residue extracted with ethyl acetate. The 1-cyanomethyl-4-histidine derivative was recovered from this solution and recrystallized from ethyl acetate–hexane. A small amount of the 1-cyanomethyl-5-histidine derivative was likewise recovered, but could not be crystallized. Its structure was determined by mass spectral, hydrolytic, and nmr analysis. The room temperature reaction gave a 1,4/1,5 ratio of about 10:1.

***N*-α-Acetyl-1-cyanomethyl-5-histidine methyl ester:** mass spectrum *m/e* 250 (M+), 207, 191, 160, 152, 149, 121, 120, 88, 82, 81, 43; nmr ($CDCl_3$) 1.98 (3 H, N-Ac, s), 2.99 (2 H, 5-CH_2–, d), 3.64 (3 H, -OCH_3, s), 4.76 (1 H, -C*H*NAc, m), 5.71 (2 H, CH_2CN, s), 6.83 (1 H, H-4, s), 7.1 (1 H, NHAc, m), 7.54 (1 H, H-2, d, J = 0.99 Hz).

***N*-α-Acetyl-1-cyanomethyl-4-histidine methyl ester:** mass spectrum *m/e* 250 (M+), 207, 191, 160, 149, 121, 120, 88, 81, 45, 43; nmr ($CDCl_3$) 1.95 (3 H, N-Ac, s), 3.00 (2 H, 4-CH_2, d), 3.63 (3 H, OCH_3, s), 4.70 (1 H, C*H*NAc, m), 4.92 (2 H, CH_2CN, s), 6.85 (1 H, H-2, s), 7.26 (1 H, NH, d, J = 8 Hz), 7.49 (1 H, H-5, d, J = 1.10 Hz).

Anal. Calcd for $C_{11}H_{14}N_4O_3$: C, 52.8; H, 5.6; N, 22.4. Found: C, 52.8; H, 6.1; N, 22.2.

(18) A. M. Crestfield, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 2413 (1963).

A Total Synthesis of (±)-α- and (±)-β-Copaenes and Ylangenes

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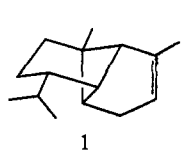
Abstract: Syntheses of racemic α-copaene (**1**), α-ylangene (**2**), β-copaene (**33**), and β-ylangene (**34**) are described. The *cis*-decalin "envelope" ring component of these substances was constructed by the orientationally selective reaction of 3-carbomethoxy-2-pyrone (**3**) with 4-methyl-3-cyclohexenone (**4**) which afforded the keto ester **5**. Epoxidation of the ethylene ketal of **5** using peracid afforded the α-oxide **14** which was further transformed into the dienone ketal **18**, a key intermediate for the synthesis of the four title compounds. Reduction of **18** using lithium in liquid ammonia (to **19**), tosylation, deketalization, and internal S_N2 cyclization then afforded the tricyclic ketone **23** which was further converted to (±)-**1** and (±)-**2** by a sequence which transformed the carbonyl group into >CH-*i*-C_3H_7. Reduction of the dienone ketal **18** using zinc–acetic acid led to the *exo*-methylene keto ketal **28** from which (±)-β-copaene (**33**) and (±)-β-ylangene (**34**) were obtained by a sequence paralleling that used for the synthesis of **1** and **2** from **19**.

The tricyclic sesquiterpenes, α-copaene (**1**) and α-ylangene (**2**), pose an interesting synthetic problem which centers about the unusual ring system.[1] For some time, we have been studying an approach which involves direct formation of a *cis*-decalin structure using the Diels–Alder reaction and subsequent generation of the tricyclic system by four-membered ring closure.



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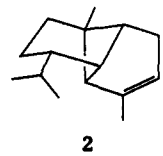

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(1) One solution has already been devised by Heathcock; see (a) C. H. Heathcock, *J. Amer. Chem. Soc.*, **88**, 4110 (1966); (b) C. H. Heathcock, R. A. Badger, and J. W. Patterson, Jr., *ibid.*, **89**, 4113 (1967).