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Mechanism of imidazole and oxazole formation in [^{13}C-2]-labelled glycine and alanine model systems

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Abstract

Studies with ^{13}C-2-labelled glycine and alanine in model systems containing 2,3-butanedione, glyceraldehyde or glyoxal have indicated that imidazoles and oxazoles can be formed from α-dicarbonyl compounds through Strecker reaction and subsequent formation of α-amino carbonyl reactive intermediates. These intermediates can react with any aldehyde in the reaction mixture to form an imine which in turn can either cyclize to form oxazoles or react with an amino compound and then cyclize to form imidazole after an oxidation step. On the other hand, Amadori products, formed in α-hydroxycarbonyl containing systems, can undergo decarboxylation followed by reaction with ammonia to form an amino imine intermediate which, after reaction with any aldehyde followed by cyclization, can form imidazoles after a dehydration step. This latter mechanism fixes the C-2 atom of glycine as an N-alkyl substituent in imidazoles. In addition, model studies with α-dicarbonyl compounds, using ammonium carbonate as a source of ammonia and paraformaldehyde as a source of formaldehyde, also produced imidazoles and oxazoles.

Keywords: Amadori product; Maillard reaction; ^{13}C-labelled glycine and alanine; Imidazole; Oxazole formation mechanism; Py-GC/MS

1. Introduction

Different imidazole moieties have been identified in sugar-amine, dicarbonyl-amino acid model systems and in food and biological systems (Scheme 1). Pyrido[3,4-d]imidazoles are specific to histidine (Gi & Baltes, 1993), imidazolone (Thornalley, 1996) and pentosidine are specific to arginine (Scheme 1). Another arginine-specific imidazole moiety recently synthesized as a possible advanced glycation end-product (AGE), is ALI (arginine-lysine imidazole) (Al-Abed & Bucala, 2000). On the other hand, simple imidazoles and 1,3-disubstituted imidazolium salts are not amino acid-specific and could be formed in different model systems (Davidek, Velisek, Davidek, & Pech, 1991; Shibamoto & Bernhard, 1978). Pyrido[3,4-d]imidazoles and simple imidazoles were detected only in model systems and in some food products, arginine-specific imidazoles, on the other hand, were identified only in biological systems as protein modifications or AGEs. The 1,3-disubstituted imidazolium salt is the only moiety that is common to both model and biological systems (Chellan & Nagaraj, 1999). These salts were first identified (Velisek et al., 1989) in a model system containing glycine, glyoxal and formaldehyde and later in human lens and plasma proteins (Chellan & Nagarage, 1999) as glyoxal-lysine dimer (GOLD) and methylglyoxal-lysine dimer (MOLD). Simple imidazoles and oxazoles are major reaction products, along with pyrazines, in carbohydrate/ammonia mixtures (Kort, 1970). Imidazoles, with alkyl and polyhydroxyalkyl-substituents, have been identified in different model systems and in ammonia caramel (Kort, 1970) and molasses (Jezo, 1966). The detailed mechanism of formation of simple imidazoles and oxazoles has not been verified by labelling studies, although some mechanisms have been proposed based on model systems (Hwang, Hartman, & Ho, 1995; Kort, 1970; Shibamoto & Bernhard, 1978). In this study we provide evidence for the origin of imidazoles and oxazoles using [^{13}C-2]-labelled amino acids.

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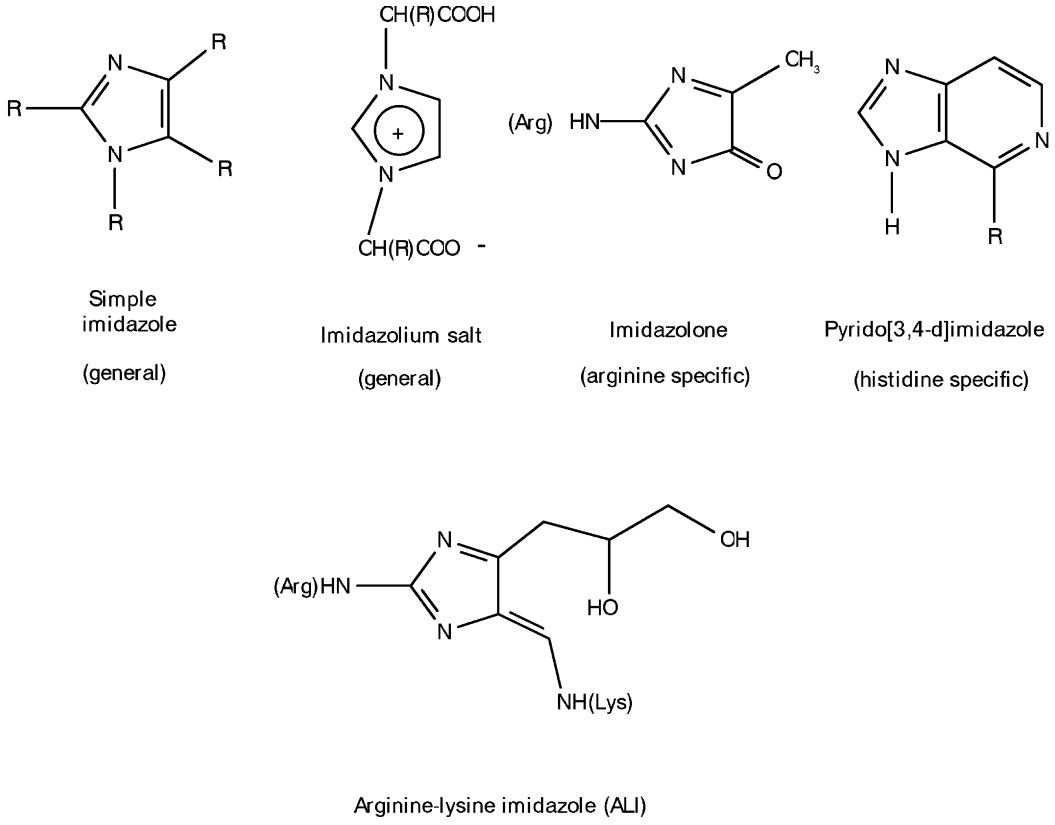


Scheme 1. Selected examples of imidazole moieties identified in different Maillard model systems.

2. Materials and methods

2.1. Materials

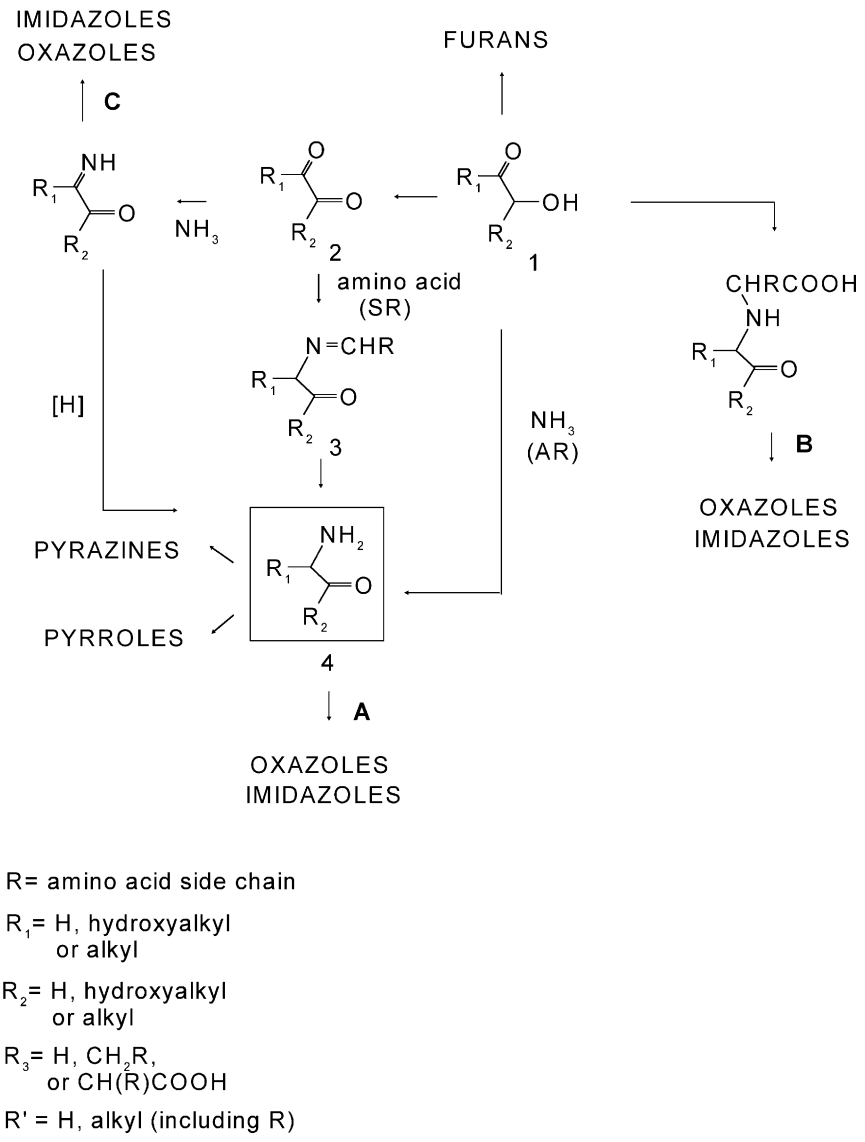
All reagents and chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI). [2-^{13}C]glycine (90% enriched) and DL[2-^{13}C]alanine (99% enriched) were purchased from Cambridge Isotope Laboratories (Andover, MA).

2.2. Pyrolysis GC/MS analysis

A Hewlett-Packard GC/mass selective detector (5890 series II GC/5971B MSD) interfaced to a CDS Pyroprobe 2000 unit, through a valved interface (CDS 1500), was used for Py-GC/MS analysis. In all experiments, solid samples of a mixture of labelled or unlabelled glycine or alanine/carbonyl compound (1:1 ratio, 2 mg) were placed inside the quartz tube (0.3 mm thickness); this was plugged with quartz wool and placed inside the coil probe. The pyroprobe was set at 250 °C at a heating rate of 50 °C/ms with a total heating time of 20 s. The pyroprobe interface temperature was set at 250 °C. The samples were introduced under splitless mode. The 3 ml/min constant flow was maintained by an Electronic pressure controller (Hewlett-Packard). Capillary direct MS interface temperature was 180 °C; ion source temperature was 280 °C. The ionization voltage was 70 eV and the electron multiplier was 2047 volts. The mass range analyzed was 17–200 amu. The column was an HP-PLOT-Q (30 m×0.32 mm×0.20 μm (Hewlett-Packard, Mississagua, ON). The column initial temperature (30 °C) was held for 2 min, then increased to 100 °C at a rate of 30 °C/min, then further increased to 250 °C at a rate of 7 °C/min, and held for 35 min. The reported percent label incorporation values (corrected for natural abundance and for % enrichment) are the averages of duplicate analyses and are rounded off to the nearest multiple of 5%. The identity and purity of the peaks were determined using NIST AMDIS version 2.1 software.

3. Results and discussion

The sugar-derived reactive intermediates formed in Maillard model systems can be described, generally, as possessing either α-dicarbonyl or α-hydroxycarbonyl moieties having alkyl and/or hydroxyalkyl substituents (structures **1** and **2** in Scheme 2). These reactive intermediates eventually constitute the backbone of most heterocyclic compounds formed during the Maillard reaction (Yaylayan, 1997). The incorporation of nitrogen atoms, during Maillard reaction, to these moieties (**1** and **2**) can be achieved through either imine formation, Strecker reaction (SR) or Amadori rearrangement



Scheme 2. Different pathways of formation of imidazoles and oxazoles. AR = Amadori rearrangement; SR = Strecker reaction, [H] = reduction.

(AR) with ammonia, amines or amino acids. Interestingly, the most important nitrogen containing reactive intermediate (**4**, α-amino carbonyl moiety) can be formed by both intermediates (**1** and **2**), either through Amadori rearrangement of **1** with ammonia or through Strecker reaction of **2** with amino acids. The importance of **4** is well established in the formation of pyrazines and recently (Yaylayan & Keyhani, 2001) it has also been shown to be the precursor of pyrroles through aldol condensation with aldehydes (Knorr pyrrole synthesis). In addition, Hwang et al. (1995) have suggested that intermediate **4** can even play a role in the formation of oxazoles. Since oxazoles and imidazoles are structurally related compounds we attempted to investigate the common origin of these two important heterocyclic compounds through labelling studies using the convenient pyrolysis/gas chromatography/mass spectrometry (Py-GC/MS) as an integrated reaction, separation and identification system (Yaylayan, 1999). Although, under pyrolytic conditions, more products are formed than with aqueous reactions, most of the products identified in aqueous systems are also formed under pyrolytic conditions, albeit in different amounts. In addition, experimental evidence was provided that the position and label distribution in the common products observed in the same model systems, in aqueous and pyrolytic reactions, are identical (Yaylayan & Wnorowski, 2000). This indicates the similarity of mechanisms of formation of these common products under both conditions. Consequently, mechanistic conclusions derived from label incorporation in the products observed under pyrolytic conditions, that are common to both systems, have relevance to the aqueous reactions.

Two derivatives of intermediate **2** (2,3-butanedione and glyoxal) and one derivative of intermediate **1** (glyceraldehyde) were reacted with [^{13}C-2]-labelled glycine

and/or alanine in the quartz tube of the pyrolyzer and the resulting pyrograms were analyzed for the presence of oxazoles and imidazoles. Tables 1–3 summarize the oxazoles and imidazoles detected in different model systems and percent incorporation of labelled C-2 atom of glycine or alanine in these structures. Other oxazoles and imidazoles were also detected but not listed, due to the interference of impurities in their peaks with label incorporation calculations.

Analysis of the label incorporation patterns of different imidazoles and oxazoles (Tables 1–3) have indicated that they can be generated in model systems containing amino acids and both α-hydroxycarbonyl (**1**) and α-dicarbonyl (**2**) type precursors, either through the formation of α-aminocarbonyl derivatives, such as intermediate **4** (Pathway A in Scheme 2) or through the formation of Amadori product (Pathway B in Scheme 2). Intermediate **4** can react with any aldehyde in the reaction mixture to form the imine **3′** which is the immediate precursor of oxazolines, as shown in Scheme 3. Alternatively, **3′** can react with any amine in the reaction mixture and after a cyclization step followed by oxidation (Weenen, 1998), can generate imidazoles. If the starting α-dicarbonyl (**2**) precursors possess a γ-hydroxy moiety, then dehydration can also produce imidazoles in the absence of oxidative conditions. On the other hand, the Amadori product, formed by the interaction of amino acids with α-hydroxycarbonyl moieties, can undergo decarboxylation and form the intermediate **8** as shown in Scheme 4. For this intermediate to be converted into imidazole, it requires to react with ammonia to form the imine **9**. This imine can undergo condensation with any aldehyde in the reaction mixture to produce imidazole after cyclization and dehydration steps. Mechanistically, it is not possible for this pathway to produce oxazoles. The α-hydroxycarbonyl precursors can produce oxazoles through Amadori rearrangement with ammonia as shown in Scheme 2.

Evidence for pathway A (Schemes 2 and 3) comes from model studies with 2,3-butanedione, glyoxal and [^{13}C-2]-labelled amino acids. In the 2,3-butanedione model system containing glycine, unlabelled acetaldehyde and formaldehyde can be generated from the decomposition of the starting α-dicarbonyl compound and labelled formaldehyde (Strecker aldehyde of glycine) can be generated from the Strecker reaction. According to the proposed pathway shown in Scheme 3, the 2,4,5-trimethyloxazole, formed from the acetaldehyde reaction, should not incorporate any label but 4,5-dimethyloxazole, formed from the formaldehyde reaction, should incorporate a single label in the proportion of $^{13}CH_2O/CH_2O$ found in the mixture. Based on the values listed in Table 1, it appears that only 15% of the formaldehyde originated from the Strecker reaction and 85% from 2,3-butanedione degradation. In this model system, the 1,4,5-trimethylimidazole detected can be

Table 1
Percent ^{13}C-label distribution in different imidazole and oxazoles formed in [^{13}C-2]glycine/2,3-butanedione model system

Compound	M	M + 1	M + 2
1,4,5-Trimethyl-1H-imidazole	45	50	10
2,4,5-Trimethyl-oxazole	100	0	0
4,5-Dimethyl-oxazole	85	15	0

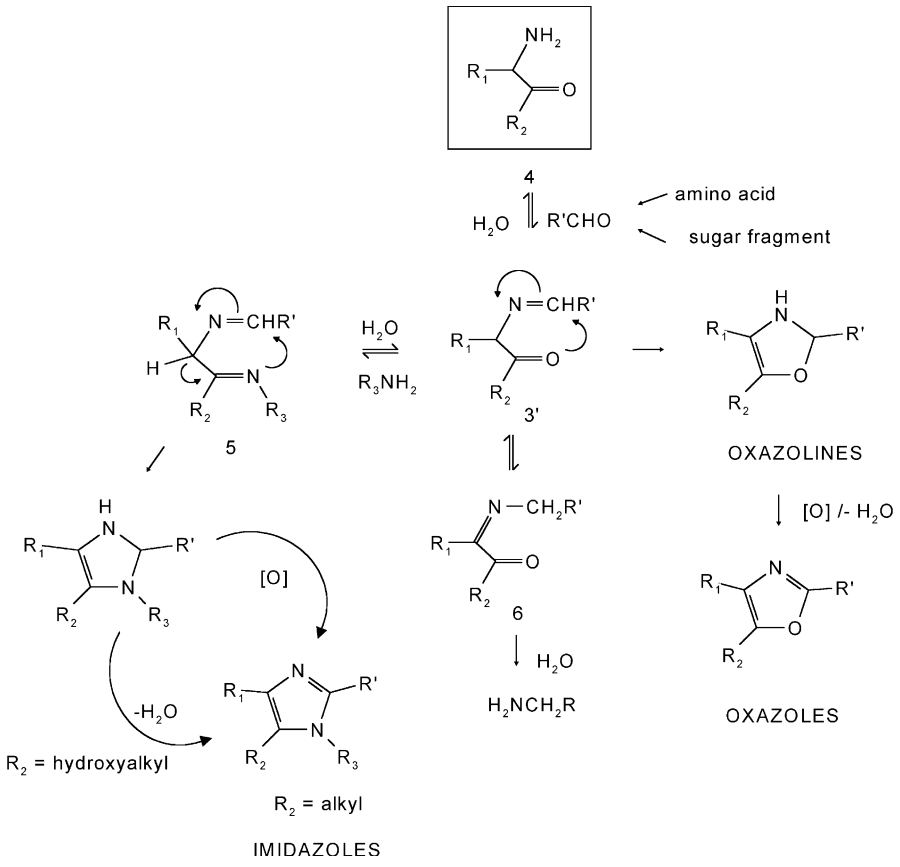
Table 2
Percent ^{13}C-label distribution in different imidazoles and oxazoles formed in glyoxal model systems containing [^{13}C-2]glycine or [^{13}C-2]alanine

Compound	M	M + 1	M + 2	M + 3	M + 4
[^{13}C-2]glycine/glyoxal					
1,4,5-Trimethyl-1H-imidazole[a]	0	0	0	30	70
1-Methyl-1H-imidazole	0	30	70		
1,3-Oxazole	30	70			
[^{13}C-2]alanine/glyoxal					
1-Ethyl-1H-imidazole	0	100			

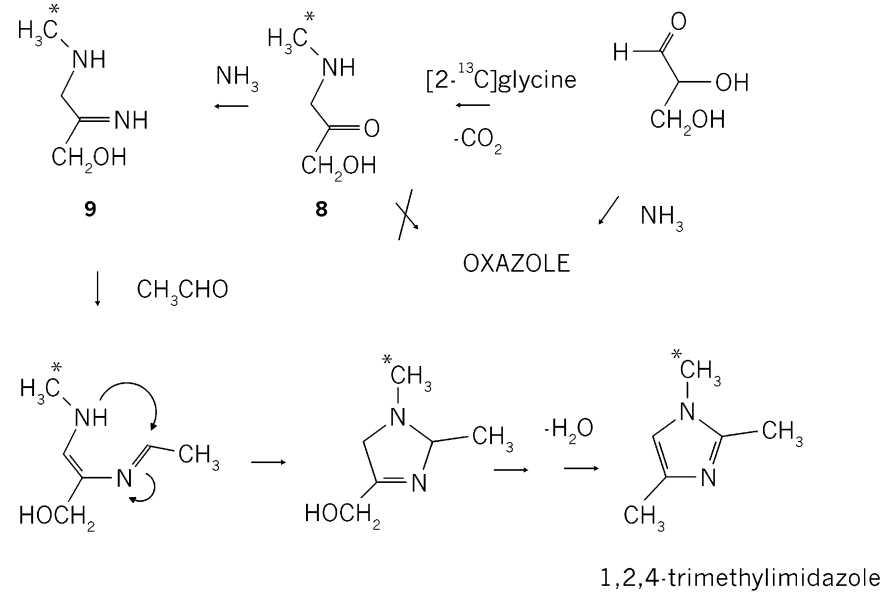
[a] From 2,3-butanedione formed in situ with the incorporation of two ^{13}C-2 atoms of glycine (see Keyhani and Yaylayan, 1996).

Table 3
Percent ^{13}C-label distribution in different imidazoles formed in glyceraldehyde model systems containing [^{13}C-2]glycine or [^{13}C-2]alanine

Compound	M	M + 1	M + 2
[^{13}C-2]glycine/glyceraldehyde			
1,2,4-Trimethyl-imidazole	0	100	0
[^{13}C-2]alanine/glyceraldehyde			
1-Ethyl-4-methyl-1H-imidazole	0	100	0



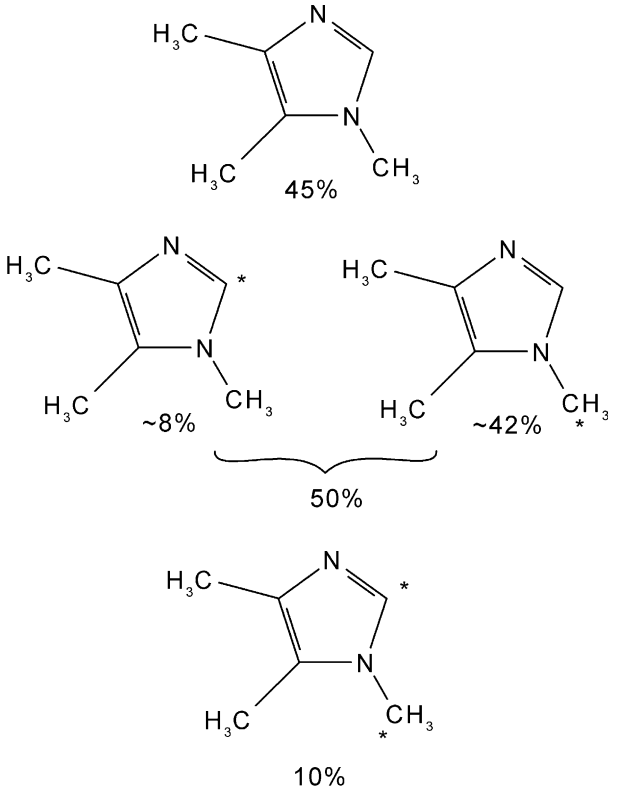
Scheme 3. Proposed mechanism of formation of imidazoles and oxazoles in Maillard model systems through pathway A. [O] = oxidation.



Scheme 4. Proposed mechanism of formation of 1,2,4-trimethylimidazole in glyceraldehyde/glycine model system through pathway B. (*) indicates C-2 atom of glycine.

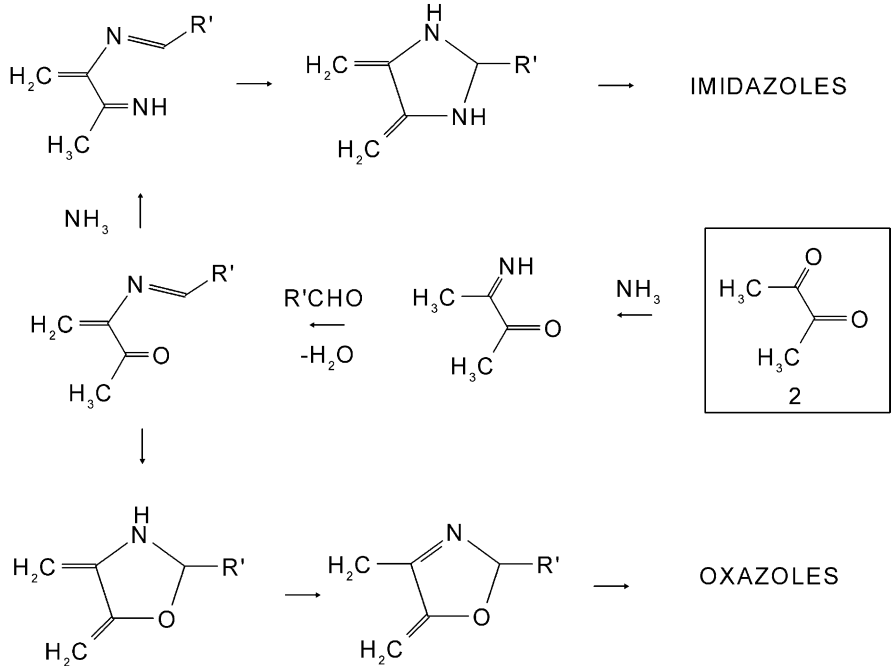
formed by the interaction of formaldehyde with intermediate **4**, followed by reaction with methylamine to produce imidazole as shown in Scheme 3. As demonstrated by Davidavek, Velisek, and Pokorny (1990), aldehydes can be converted into their corresponding amines through isomerization of imine intermediates (**3′** to **6** in Scheme 3) followed by hydrolysis as illustrated in Scheme 3. Therefore, the methylamine observed in this

model system can also have two origins, one from decarboxylation reaction of glycine and hence labelled, and the other from reaction of formaldehyde with intermediate **6** and therefore with mixed label distribution. Consequently, four different isotopomers can be formed, as shown in Scheme 5. The proportion of $^{13}CH_2O/CH_2O$ calculated above (15:85), from the values of label incorporation in 4,5-dimethyloxazole (Table 1), is consistent with the values calculated from% label incorporation in 4,5-dimethyloxazole. The data in Table 1 confirm the proposed mechanism shown in Scheme 3 and the origin of both methylamine and formaldehyde. Similar reasoning can justify the label incorporations observed in glyoxal/amino acid model systems and listed in Table 2.



Scheme 5. Different isotopomers of 2,4,5-trimethylimidazole formed in 2,3-butanedione/[^{13}C-2]glycine model system through pathway A. (*) indicates C-2 atom of glycine.

Evidence for Pathway B, which stipulates the intermediacy of a decarboxylated Amadori product (**8** in Scheme 5) in the formation of imidazoles, comes from model studies with glyceraldehyde containing [^{13}C-2]-labelled amino acids (see Table 3). In the glycine model system, the major peak in the pyrogram was 1,2,4-trimethylimidazole, whereas, in the alanine system, the major peak was a pyrazine (3-ethyl-2,5-dimethylpyrazine) indicating the stability of glycine Amadori product, relative to alanine, to undergo 1,2-enolization and β-elimination, to produce pyruvaldehyde, necessary for the formation of pyrazines. Both model systems generated imidazoles with 100% incorporation of one ^{13}C-2 atom of the amino acid (see Table 3). The decarboxylation of Amadori product (**8**) ensures 100% incorporation of the C-2 atom of amino acid as N-methyl group. In the case of glycine, 1,2,4-trimethylimidazole can be formed, as depicted in Scheme 4, by further interaction of **8** with ammonia, followed by acetaldehyde and a dehydration step. Since, in this model sys-



Scheme 6. Proposed mechanism of formation of imidazole and oxazole from 2,3-butanedione/NH_3/CH_2O in the absence of amino acid.

tem, only unlabelled acetaldehyde can be generated (from glyceraldehyde degradation) only singly-labelled product is expected to form, consistent with experimental observation. Similar reasoning can justify the formation of 1-ethyl-4-methylimidazole, through the same pathway, by the interaction of **8** with ammonia followed by reaction with formaldehyde.

Since simple aldehydes and amines play an important role in the generation of oxazoles and imidazoles, we decided to replace the amino acid in the model system with a source of ammonia and formaldehyde to investigate the possibility of oxazole and imidazole formation through pathway C (see Schemes 2 and 6). When 2,3-butanedione was reacted with ammonium carbonate (source of ammonia) in the presence of paraformaldehyde (source of formaldehdye), trimethyl- and dimethyloxazoles were formed as major products but dimethyl- and trimethylimidazoles as minor products. These data indicate the existence of another path (pathway C in Schemes 2 and 6) to oxazoles and imidazoles initiated through the reaction of α-dicarbonyls with ammonia, followed by reaction with an aldehyde, as detailed in Scheme 6.

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