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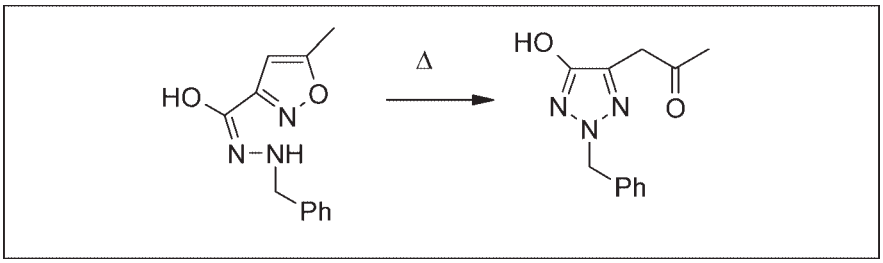

Isocarboxazid rearranges on heating to 5-acetonyl-2-benzyl-4-hydroxy-1,2,3-triazole in DMF at 150°C, in the ionic liquid, [bmin]HSO_4^- at 100°C or as a melt at 105°C. This is the first reported example of the Boulton–Katritzky rearrangement of an acyl hydrazide.

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INTRODUCTION

Control of process impurity formation is a critical aspect of pharmaceutical process research and development. Current guidelines by the ICH (International Committee on Harmonization) state that known impurities in the drug substance are acceptable at levels of 0.2%. Satisfactory levels of unknown impurities are 0.1% [1]. The melding of a mechanistic knowledge of impurity pathways coupled with solid process development has led to a generalized approach for statistical process control and control of impurity formation [2]. The necessity to characterize impurities and to synthesize quantities for analytical method development has afforded new synthetic methodology [3]. When an understanding of reactivity is required to elucidate potential degradation pathways in the drug product, experimental and computational methods have proven to be useful to accomplish this goal [4].

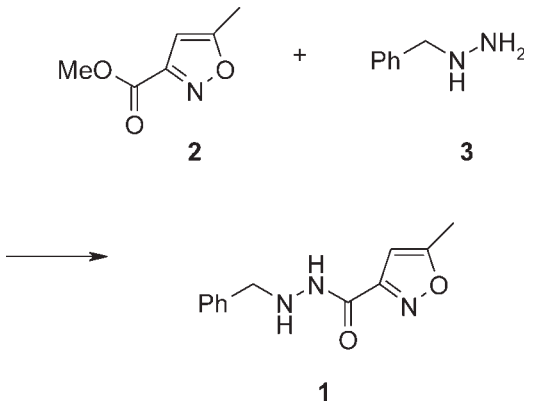
Isocarboxazid (**1**) is a monoamine oxidase inhibitor [5] and is used in the treatment of clinical depression [6]. The use of this compound in treatment is limited by side effects and its incompatibility with certain foods and the manifestation of the so-called ''cheese effect'' [7]. In the USP (United States Pharmacopoeia), the isocarboxazid (**1**) monograph requires a TLC test at a limit of 0.5% of two impurities: methyl 5-methylisoxazole-3-carboxylate (**2**) and 5-amino-1-benzyl-3-methylpyrazole (**6**). The respective visualization of these substances is done by short-wavelength ultraviolet light and by a spray of ferric chloride and potassium ferricyanide [8]. Isocarboxazid (**1**) is prepared by the reaction of the isoxazole ester **2** with benzylhydrazine (**3**) (Scheme 1) [9]. As a result of our efforts to characterize the pathway by which pyrazole **6** was formed, some interesting results on the thermolysis of isocarboxazid (**1**) were obtained. We wish to report on these findings.

RESULTS AND DISCUSSION

Although β-ketonitriles are known to have a propensity to dimerize and trimerize [10] and we are speculating about possible ways to form pyrazole **6**, we envisioned that a likely path for the formation of pyrazole **6** would be by loss of *N*-(benzylamino)isocyanate from the acidic α nitrogen of isocarboxazid (**1**) followed by isoxazole ring opening and formation of 3-oxobutanenitrile (**4**). Reaction of nitrile **4** with benzylhydrazine (**3**) would afford pyrazole **6** via the intermediacy of hydrazone **5** (Scheme 2). Many years ago, Gardener has commented on the formation of pyrazoles from isoxazole hydrazides and has indicated that their formation can be mitigated if the hydrazide-forming reaction is done at room temperature or under conditions by which the hydrazides will crystallize [11]. Alternatively, the route for the preparation of isocarboxazid (**1**) might be fundamentally flawed and the route could involve elimination of 3-oxobutanenitrile (**4**) in competition with loss of methanol.

Facile isoxazole ring opening of 3-acylisoxazoles is evident in the fact that when 3-acetyl-5-methylisoxazole (**7**) is reacted with sodium methoxide, nitrile **4** is formed. Nitrogen–oxygen bond cleavage of the isoxazole ring is initiated by the elimination of a neutral molecule, methyl acetate [12]. This chemistry parallels established behavior of isoxazole-3-yl lithium intermediates [13]. The enolate of nitrile **4** can be trapped with

Scheme 1. Synthesis of isocarboxazid (**1**).

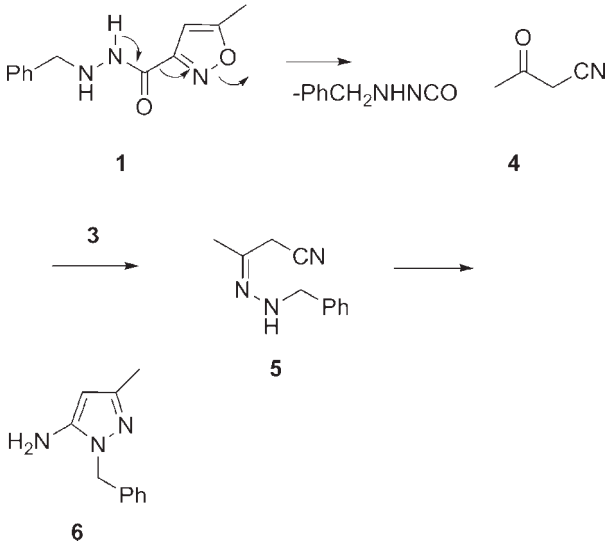


acetyl chloride and this route was used to prepare the (*E*) and (*Z*) isomers of 3-acetoxybut-2-ene nitrile (**8**) (Scheme 3) [14].

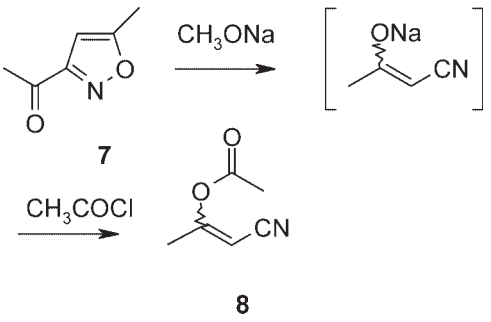
Unimolecular reactions of isocarboxazid (**1**) are known. As reported in the patent literature, isocarboxazid (**1**) rearranges to 5-acetonyl-2-benzyl-4-hydroxy-1,2-3-triazole (**9**) in refluxing toluene after an overnight hold in a 64% yield. No spectral data or purity assessment of the triazole **9** was reported [15]. We have repeated this experiment and found that the reaction follows first-order kinetics with $t_{1/2}$ of ~29 h (Scheme 4).

A significantly better conversion is observed when the rearrangement is conducted in DMF or in an ionic liquid at 150°C [16]. At 100°C in the ionic liquid, [bmin]HSO_4^-, close to the temperature of refluxing toluene, the reaction is essentially complete in 3 h as determined by HPLC analysis. Isocarboxazid (**1**) melts between 103 and 107°C and a 24% conversion to triazole **9** was observed when isocarboxazid (**1**) was heated

Scheme 2. Thermal degradation of isocarboxazid (**1**) to nitrile **4**.



Scheme 3. Base-catalyzed ring opening of isoxazole **7**.

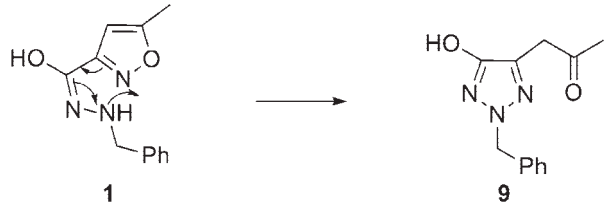


at 105°C in a reaction vial for 2 h. For this drug substance, a validated HPLC method showed limits of detection (LOD) of 15 μg for the triazole **9** and 7.5 μg for the pyrazole **6** and no triazole **9** or pyrazole **6** was detected in the crude isocarboxazid (**1**).

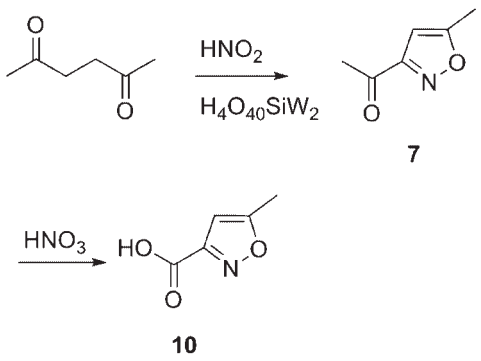
By analogy with the Boulton–Katritzky rearrangement of monoazoles, the driving force for this reaction would be the formation of the more aromatic triazole ring as opposed to the isoxazole ring [17]. In general, 1,2,4-oxadiazoles are the heterocycle involved in this rearrangement, although it has been observed in the case of an isoxazole [18]. Because of the increased acidity of the α-hydrogens of the hydrazide as opposed to an amide [19], we favor that the rearrangement occurs from the enol or imidic acid tautomer of the hydrazide. The rate acceleration that is observed in the ionic liquid as opposed to the nonpolar solvent toluene would support such an assertation. Nucleophilic attack of nitrogen on nitrogen followed by isoxazole nitrogen–oxygen bond cleavage would afford triazole **9**.

The expired Roche patent for the preparation of isocarboxazid (**1**) involves a purification of isocarboxazid (**1**) by the formation of a hydrochloride salt. Free-basing of the salt yields isocarboxazid (**1**) [9]. In a control experiment, we have found that isocarboxazid (**1**) is unstable in base and we would offer that pyrazole **6** originates, when isocarboxazid (**1**) is regenerated by treatment of the hydrochloride with base. For our current process, which is the result of additional development studies, the process has been refined and does not involve the formation of a hydrochloride salt.

Scheme 4. Thermolysis of isocarboxazid (**1**).



Scheme 5. Synthesis of isoxazole acid (**10**).



3-Oxobutanenitrile (**4**) or any precursors are not present in the starting ester **2** as both the isoxazole ester **2** and 5-methylisoxazole-3-carboxylic acid (**10**) are of exceptional quality [9,20]. Isoxazole acid (**10**) was prepared by the nitric acid oxidation of 2,5-hexanedione [20]. We have found that the violent exotherm of this oxidation [12] can be tempered when 3-acetyl-5-methylisoxazole (**7**) is prepared *in situ* by a heteropoly acid-catalyzed nitrosation with nitrous acid. When nitric acid is added to this reaction mixture, no uncontrolled exotherm is observed (Scheme 5). No yield improvement has yet been realized, however, as the heteropolyacid may also catalyze an aldol reaction or cyclization to 2,5-dimethylfuran.

An authentic sample of 5-amino-1-benzyl-3-methylpyrazole (**6**) was prepared by an adaptation of the Magnus β-ketonitrile process for the synthesis of 3-oxobutanenitrile (**4**) [21]. The procedure entails the reaction of β-ketonitrile **4** with benzylhydrazine (**3**), followed by ring closure of hydrazone **5** in a mixture of ethanol and hydrochloric acid [22]. The basis of this nitrile **4** process involves reaction of the enolate of acetonitrile with ethyl acetate and an inverse addition of the enolate suspension to an acetic acid solution of benzylhydrazine (**3**) [21]. Magnus' laboratory procedure involves the use of DMSO to solubilize the enolate. However, we found that stiff plastic tubing outfitted with standard adapters could accomplish this transfer in much the same way as the enolate suspension was transferred in the Magnus plant process. Although the yield was only 25%, byproducts could be readily removed from the relatively soluble pyrazole **6** and the pyrazole **6** was isolated as its hydrochloride salt **6a**. For comparison's sake, chromatography of the reaction mixture afforded the free base and the ^{1}H NMR spectrum agreed with the published values for this compound [23].

In conclusion, our requirement to control the formation of pyrazole **6** led to a more detailed investigation of the thermal rearrangement of isocarboxazid (**1**) to triazole **9**. By analogy with the Boulton–Katritzky rearrangement of monoazoles, the reaction would appear to proceed through the enol tautomer of the hydrazide. There is increased interest in monoamine oxidase inhibitors [24] and the crystal structures of monoamine oxidase A [25] and monoamine oxidase B [26] as well as the crystal structure of isocarboxazid (**1**) [27] have been solved. Combinatorial routes to 3-acylisoxazoles have been described [12,28]. The unmasking of a ketone functionality in this pericyclic rearrangement may have biochemical implications. The recent work of Edmonson and others have suggested that an aldehyde group may play a role in the inhibition of monoamine oxidase by arylalkylhydrazine-based therapeutics [26].

EXPERIMENTAL

Isocarboxazid (**1**) was a product of internal manufacture. An authentic sample of 5-amino-1-benzyl-3-methylpyrazole hydrochloride (**6a**) was obtained form the U.S. Pharmacopoeia, Rockville, MD [29]. The validated HPLC method consisted of gradient elution with a variable composition of 25 m*M* phosphate buffer and acetonitrile. The flow rate was 1.0 mL per minute. The initial conditions of the method consisted of 80% aqueous buffer and 20% acetonitrile. This condition was maintained for 10 min and over a 10 min period was changed to 60% buffer and 40% acetonitrile. After a further 10 min period, the conditions were changed to 50% aqueous buffer and 50% acetonitrile. The column temperature was maintained at 40°C and the wavelength was 232 nm. The buffer was prepared by dissolving 3.40 g of potassium hydrogen phosphate in 1 L of distilled water and by adjusting the pH to 2.5 with phosphoric acid. The column had dimensions of 250 mm × 4.6 mm and contained Luna C18(2), 5 μ, 100 Å packing. The column manufacturer was Phenomenex. The injection volume was 10 μL.

The respective retention times are 19.4 min for isocarboxazid (**1**), 15.9 min for the triazole **9**, 10.9 min for the isoxazole ester **2**, 6.9 min for the pyrazole **6**, 5.6 min for the isoxazole acid **10**, and 3.5 min for 5-methylisoxazole-3-carboxylic acid hydrazide (**11**). Similar retention time behavior is observed when 0.1% trifluoroacetic acid (v/v) is used as the aqueous phase.

For the validated method, 20 mg of isocarboxazid (**1**) was dissolved in 5.0 mL of acetonitrile in a 100 mL volumetric flask. The diluent was 0.1% trifluoracetic acid (v/v) and the solution was diluted to volume with diluent. Serial dilutions of this solution allowed for the determination of the LOD from six replicate injections. The LOD of the known compounds was determined in a similar manner. The LOD for isocarboxazid (**1**), triazole **9**, isoxazole ester **2**, pyrazole **6**, isoxazole acid **10**, and isoxazole hydrazide **11** was 30, 15, 15, 7.5, 15, and 15 μg, respectively.

5-Acetonyl-2-benzyl-4-hydroxy-1,2,3-triazole (9). Under a nitrogen purge, isocarboxazid (**1**) (50.00 g) and 150 mL of anhydrous DMF were combined and heated to 150°C. When first at temperature, HPLC analysis showed the reaction to be 85% complete. After 2 h at 150°C, the conversion was essentially 100% and the triazole **9** was the only component present by HPLC. The reaction mixture was cooled and was added to 2.5 L of distilled water. Gummy material, which was associated with a

white solid formed and the solution was decanted. The solid was washed with water. The solid was dissolved in 500 mL of isopropanol (IPA) and the IPA was evaporated to a minimum volume and was filtered. There was obtained 13.86 g of the triazole **9** as a pale yellow solid in a 27.4% yield and the solid was 100% pure by HPLC analysis. The crude solid was recrystallized from a 10:1 mixture of cyclohexane and IPA. Pure triazole **9** as a white solid was obtained in a 90.2% yield. The triazole **9** had melting point 95–96°C; IR (potassium bromide): 3033–2983 (br, OH), 1717 (C=O), 1226, 1180 cm^{-1}; ^1H NMR (deuteriochloroform) δ 2.29 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 7.27–7.36 (m, 5H aromatic), 9.24 (b, 1H, OH); ms: *m/z* 232 (M + 1), 175 (M +1-CH_3COCH_2), 132; Anal. Calcd for $C_{12}H_{13}N_3O_2$ (231.26): C, 62.33; H, 5.67; N, 18.17. Found: C, 62.44; H, 5.74; N, 18.32.

Thermolysis of isocarboxazid (1) in [bmin] HSO_4^-. Isocarboxazid (**1**) (21.55 g) and 67.55 g of 1-butyl-3-methylimidazolium hydrogen sulfate were combined and heated to 150°C. At temperature, the reaction was essentially complete as evident by HPLC analysis. The reaction was cooled and the product was extracted with 5 × 150 mL of MTBE. The MTBE layer was isolated by vacuum decantation using a suction flask and the ionic liquid remained in the flask. After evaporation, there was obtained 11.41 g of the triazole **9** as a white solid in a 52.9% yield. The ionic liquid phase was extracted with 2 × 150 mL of ethyl acetate and 6.96 g of the remaining 10.14 g of triazole **9** was obtained. 20% IPA in ethyl acetate (150 mL) was used to extract the remaining amount of triazole **9**. After evaporation, 4.30 g of solid was obtained. The total mass balance was 105% with the overage being the ionic liquid, which was extracted when ethyl acetate or a mixture of IPA and ethyl acetate was used. No triazole **9** was detected in the ionic liquid phase. The ionic liquid was heated at 100°C for an overnight hold to remove solvent.

The ionic liquid was used in a recycle with 20.00 g of isocarboxazid (**1**) at 100°C. The reaction was monitored by HPLC once the reaction mixture reached temperature and every hour afterward. The reaction was 100% complete in 3 h.

Thermolysis of isocarboxaxzid (1) in toluene. Isocarboxazid (**1**) (50.00 g) and 150 mL of anhydrous toluene were combined under a nitrogen purge and the batch was heated to reflux. The solution was monitored periodically by HPLC over a time period of 60 h and the disappearance of isocarboxazid (**1**) was ascertained by this method. At an 88% conversion to triazole **9**, there was 4.6% of a polar impurity present along with <2.0% of other components.

Thermolysis of neat isocarboxazid (1). 20 mg of isocarboxazid (**1**) was heated at 105°C in a reaction vial for 2 h. The extent of the rearrangement was determined by HPLC. Triazole **9** was present to the extent of 24.4% by HPLC and trace levels (<0.2%) were observed for five unknowns.

5-Amino-1-benyzl-3-methylpyrazole hydrochloride (6a). Under a nitrogen purge, potassium *tert*-butoxide (87.90 g, 0.783 mol) and 825 mL of anhydrous tetrahydrofuran were combined in a 2 L flask with a mechanical stirrer. A mixture of 69.0 mL (62.2 g, 0.706 mol) of ethyl acetate and 45.0 mL (35.4 g, 0.862 mol) of acetonitrile was added over a 30 min period and the temperature was maintained below 30°C. The light beige suspension was stirred for 1 h at room temperature.

Benzylhydrazine dihydrochloride (**3a**) (55.65 g, 0.285 mol), 150 mL of ethanol, and 75 mL of concentrated ammonium hydroxide was added to a separate 3 L flask. Glacial acetic acid (225 mL) was added. The pH of the solution was 5 by paper and the suspension was cooled to 30°C. Under vacuum, the enolate suspension was transferred to the flask containing benzylhydrazine (**3**) with 1.0 cm (outer diameter) stiff plastic tubing, which had fitted into two standard 29/42 tubing adapters. The addition time was <5 min and the temperature was maintained at <30°C. The vessel was rinsed with 150 mL of THF. The suspension was stirred at room temperature for 22.5 h.

The solvent was removed under 10 mm of vacuum and a temperature of <45°C. Ethanol (500 mL) was added and this was followed by the addition of 120 mL of concentrated hydrochloric acid. The reaction mixture was refluxed for 24 h [22]. The reaction mixture was cooled and sodium carbonate was added. The pH of the solution was 5 to wet paper. The batch was filtered and washed with ethanol (2 × 300 mL). The filtrate was evaporated at 40–45°C under vacuum. After 500 mL of ethanol was removed, a white solid formed. The suspension was filtered and washed with ethanol. The concentration and filtration was repeated twice. To the residue was added 550 mL of isopropanol. The slurry was cooled, filtered, and washed with 2 × 50 mL of IPA. Concentrated hydrochloride acid (25 mL) was added. The slurry was cooled in an ice-bath, filtered, and dried. There was obtained in two crops, 16.16 g of 5-amino-1-benzy-3-methylpyrazole hydrochloride (**6a**) as a white solid in a 25.3% yield. Pyrazole hydrochloride **6a** had mp 221–223°C and the purity by HPLC was 100%.The melting point of the USP reference standard was 223–227°C [29].

5-Amino-1-benzyl-3-methylpyrazole (6). Using a similar protocol, the free base **6** was isolated in a 29% yield after chromatography on silica gel and with an eluent consisting of a mixture of 60% ethyl acetate and 40% heptane. Pyrazole **6** had mp 64–66°C (lit mp 67–70.5°C [23]) and ^1H NMR (deuteriochloroform) δ 2.14 (s, 3H), 3.46 (b, 2H), 4.92 (s, 2H), 5.40 (s, 1H), 7.15–7.36 (m, 5H, aromatic). The ^1H NMR agrees with the reported values [23]. Pyrazole **6** was freely soluble in IPA, the recrystallization solvent for isocarboxazid (**1**). The addition of hydrochloric acid causes the hydrochloride **6a** to precipitate from solution. Pyrazole **6a** had mp 223–227°C.

5-Methylisoxazole-3-carboxylic acid (10). 2,5-Hexanedione (42.8 g; 0.375 mol), 0.54 g (0.05 mol %) of silicotungstic acid, 17.9 mL (0.34 mol) of concentrated sulfuric acid, and 80 mL of water were combined and cooled to 5°C. Sodium nitrite (65.0; 0.94 mol) was added over a 100 min period and the temperature was maintained below 40°C. Copious gas evolution was observed with some nitrogen dioxide vapors. The mixture was held overnight. The reaction mixture was filtered and washed with ~5 mL of water. The filtrate contained two phases.

The reaction mixture was heated to between 75 and 80°C. 70% Nitric acid (85 mL, 1.3 mol) was added slowly over a 1 h period. The heat was shut off after the addition of 15 mL nitric acid. The batch temperature was maintained at 100–102°C for 19.5 h. The reaction was cooled to 15°C and the product was isolated by filtration. After drying to a constant weight, there was obtained 21.33 g of 5-methylisoxazole-3-carboxylic acid (**10**) as a white solid in a 45% yield. The purity by HPLC was 99% (Zorbax-SC-Cyano column, eluent: 95% 25 m*M* phosphate buffer and 5% methanol, flow rate: 1.0 mL

per min) and the melting point was 171–173°C (decomposition). A reference standard, which was prepared by the Cusmano method [20] and was recrystallized from acetone had a melting point of 172–173°C with decomposition.

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