HETEROCYCLES, Vol. 60, No. 7, 2003, pp. 1681 - 1688
Received, 4th April, 2003, Accepted, 2nd June, 2003, Published online, 9th June, 2003

ISOLATION AND IDENTIFICATION OF AN UNUSUAL IMPURITY IN AN INTERMEDIATE OF RIMADYL® (CARPROFEN)

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Abstract – The isolation and identification of an unknown process related impurity in a key intermediate of Rimadyl® (carprofen) is described. The structure of the unknown was evaluated by NMR spectral analysis and eventually confirmed by single crystal X-Ray. A re-slurry in warm acetic acid was found to reduce the level of the unknown to an acceptable level.

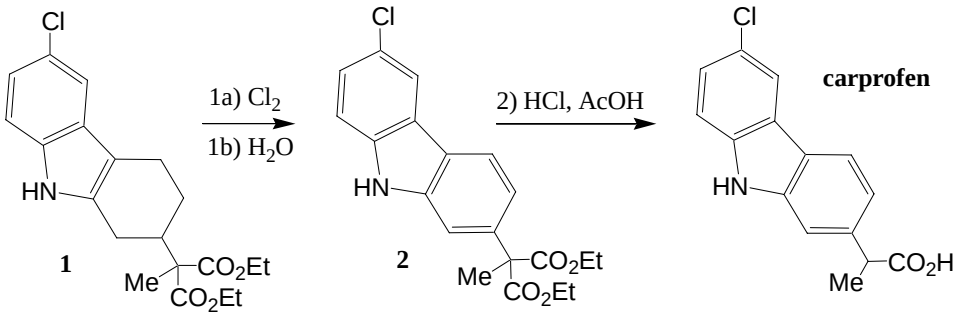
INTRODUCTION

Rimadyl® (carprofen)[1] is a nonsteroidal anti-inflammatory drug (NSAID) that was introduced in 1997 for the treatment of arthritis in dogs and cats. Due to the profound positive improvements observed with this medication,[2] the demand for Rimadyl® was in serious danger of exceeding available supply. An eleven metric ton lot of the malonate (**2**, the regulatory starting material for Rimadyl®) was available for processing, but was unable to provide finished drug substance of acceptable quality. The problem was traced to a process related side product of unknown structure that was present in **2** in the range of 0.3 to 0.9%. It was critical to determine the identity of the unknown impurity and a means by which it could be purged from **2** to ensure an uninterrupted supply of Rimadyl®.

RESULTS AND DISCUSSION

The last two steps of the Rimadyl® synthesis are shown in Scheme 1. Tetrahydrocarbazole **1** is first aromatized with molecular chlorine. HPLC and TLC analysis have determined that the offending impurity is formed during this transformation. The final step is a hydrolysis/decarboxylation of **2** that provides the active pharmaceutical ingredient Rimadyl®.[3] It was found necessary to purge the level of the unknown impurity in the carbazole ester (**2**) to < 0.08% in order to produce drug substance of acceptable quality.

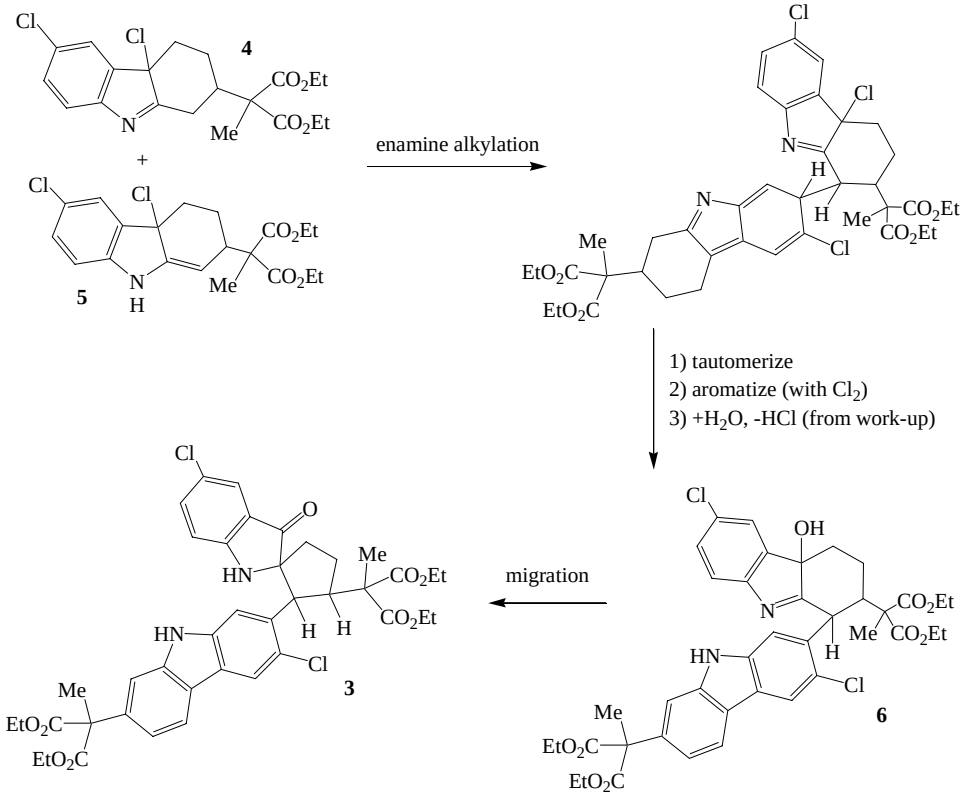
Scheme 1. The final two steps in the synthesis of Rimadyl® (carprofen).



After evaluating a number of solvent systems, it was found that purification of **2** by re-slurry in warm acetic acid effectively purges the unknown impurity to below 0.08%.[4] In all other solvent systems evaluated, the impurity was found to co-crystallize with **2**.[5] Chromatography on the mother liquors provided a sample of the impurity in >95% purity. MS spectral data were consistent with a dimeric derivative of **2**. Further structure elucidation was attempted using NMR spectral techniques since a crystal suitable for X-Ray analysis was not available.

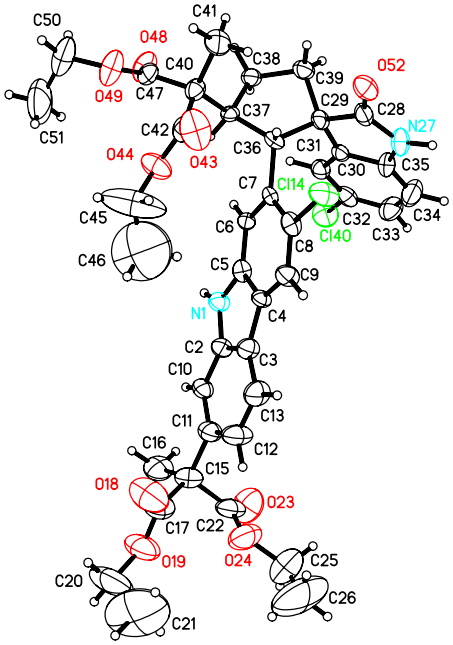
Based primarily on long-range carbon couplings, a structure (**3**) was proposed (Scheme 2, stereochemistry not defined).[6] The bond connecting the two carbazole halves is likely formed by reaction of the initially oxidized tetrahydrocarbazole (**4**) with its enamine tautomer (**5**). The spiro indolin-3-one (**3**) could be the result of a Pinacol-type rearrangement.[7]

Scheme 2. Formation of **3**.



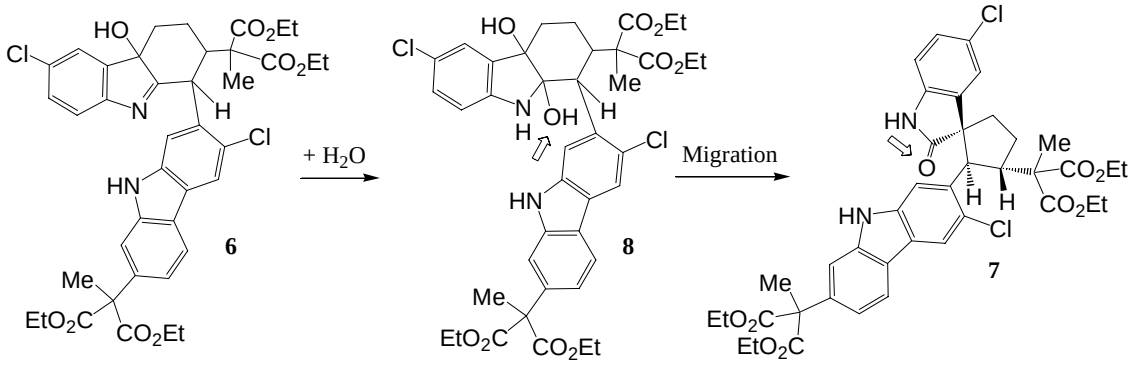
Eventually, a crystal suitable for X-Ray analysis was obtained by slow growth in dimethyl sulfoxide. The structure is shown in Scheme 3.

Scheme 3. Single crystal X-Ray of the impurity (**7**).



The initial predictions based on the NMR spectral data were correct with the exception of a reversal of the carbonyl and the spirocyclic center.[8] Our original mechanistic construct would be consistent with this structure, provided that **6** reacted with a *second* molecule of water. The diol (**8**) could then undergo a Pinacol-type rearrangement to provide the impurity (**7**).[9, 10]

Scheme 4. Formation of **7**.



In summary, a purification method was developed which purged impurity (**7**) to an acceptable level from a large lot of malonate (**2**), the precursor to Rimadyl®. It was necessary to purge this impurity to < 0.08% in order to provide finished goods of acceptable quality. A combination of mass spectroscopy, NMR spectroscopy, and single-crystal X-Ray analysis was used to solve the structure. The unknown impurity was found to be an oxidative dimer of **2**, which had undergone a Pinacol-type carbon migration to form an indolin-2-one impurity (**7**).

EXPERIMENTAL

The NMR spectral data were collected on a Bruker DMX500 equipped with a 5mm z-gradient inverse broadband probe. All experiments were run using standard pulse sequences. The two-dimensional experiments were acquired using inverse mode with z-gradients. The following experiments were acquired: proton, carbon, DEPT (Distortionless Enhancement by Polarization Transfer), gradient COSY (COrrelation SpectroscopY), gradient HMQC (Heteronuclear Multiple Quantum Correlation spectroscopy), long range gradient HMQC(CNST2=6), 200ms NOESY (Nuclear Overhauser Effect SpectroscopY), and long range gr-COSY. MS spectra were obtained on a Micromass Platform II mass spectrometer using APcI (APcI+) with a 50:50 water/acetonitrile mobile phase. The single crystal X-Ray structure was obtained at room temperature on a Siemens R3RA/v diffractometer with a resolution of 1Å. Space group = C2/c, R-index = 7.09%. Crystallographic calculations were facilitated by the SHELXTL system. X-Ray information will be submitted to the Cambridge Crystallographic Database.

Preparation of (6-chloro-2-carbazolyl)methylmalonic acid diethyl ester (2).

2.5 Kg of (6-chloro-1,2,3,4-tetrahydro-2-carbazolyl)methylmalonic acid diethyl ester are introduced into a 100 L reaction vessel and 75 L of toluene are added. The mixture is heated to 75^{o}C with stirring and the vessel is evacuated to –0.6 bar. 940 G. of chlorine gas are passed in slowly within 4 h. The solution is cooled to 20^{o}C. 10 L of deionized water are added. The pH of the aqueous phase is adjusted to 8-9 with 1.25 kg of sodium bicarbonate and the aqueous phase is separated. 10 L of deionized water are added to the toluene phase, the mixture is stirred and the aqueous phase is separated. The combined aqueous phases are extracted with 15 L of methylene chloride. The methylene chloride phase is evaporated in vacuo, the toluene phase is added and the mixture is concentrated to a volume of 5 L in vacuo. It is then cooled to 0^{o}C, and stirred at this temperature overnight. The product is removed by filtration under suction and washed with 1 L of toluene. After drying overnight in vacuo at 60^{o}C, there are obtained 2.1 kg (85% of theory) of (6-chloro-2-carbazolyl)methylmalonic acid diethyl ester having a melting point of 134^{o}-136^{o}C.

ACKNOWLEDGEMENTS

We would like to thank Dr. Jon Bordner and Debra L. DeCosta for the determination of the single-crystal X-Ray of structure (**7**). We also thank Juhani Varhiala and Anthony Neyerapalli for their contributions, and Robert W. Dugger for insight with regard to the dimerization mechanism.

REFERENCES AND NOTES

1. L. Berger and J.W. Scott, US 4,234,487, 1980; *Chem. Abstr*., 1979, **91**, 20319.
2. Freedom of Information Summary, NADA 141-053.
3. Rimadyl® is marketed as a racemic mixture.
4. P.D. Hammen, P.R. Rose, J. L. Tucker, K. M. DeVries, and D. M. Rescek, US 6,013,808 2000; *Chem. Abstr*., 2002, **137**, 140436.

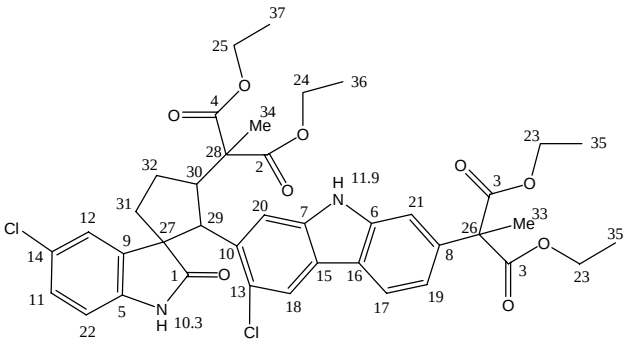
5. In the evaluation of other solvent systems, the impurity (**7**) was initially solubilized then re-precipitated with time to contaminate malonate (**2**). This type of kinetic crystallization is not uncommon, and product (**2**) could be easily and cleanly isolated on laboratory scale. However, upon scale-up the time required for filtration lead inevitably to re-precipitation of the impurity and contamination of **2**.

6. NMR Spectral Data Analysis:

The standard methods of NMR data analysis were used to determine a possible structure for the unknown impurity and are briefly described below.

The proton spectrum integrated for 8 aromatic hydrogens, 32 aliphatic hydrogens, and 2 broad exchangeable, for a total of 42 hydrogens. Detailed examination of the data led to the possibility of two equivalent ethyl groups, two other ethyl groups, and the presence of two singlet methyls. The carbon spectrum gave rise to 37 carbon peaks (Table 1). Upon close inspection, three of the peaks (171.92, 62.24, and 14.70 ppm) were determined to be two equivalent carbons, for a total of 40. The carbon peaks could be subdivided into the following groups: 1 keto (182 ppm), 4 carboxy (171-173 ppm), 18 vinyl (110-142 ppm), 4 alkoxy (61-63 ppm), and 13 alkyl carbons (13-60 ppm).

Table 1. NMR spectral data for the unknown (**7**).



7

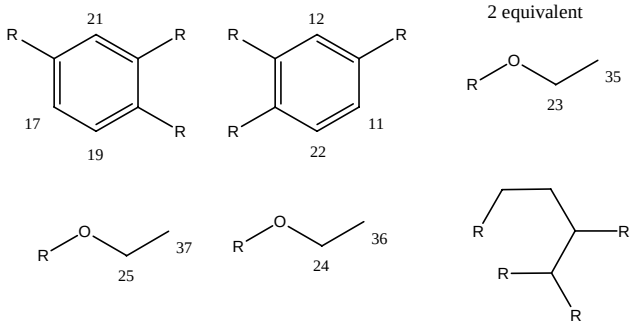
#	^{13}C – ppm	# attached protons	^{1}H – ppm	comments
1	182.23	0		
2	172.03	0		
3	171.92	0		2 equivalent
4	171.14	0		
5	141.62	0		
6	141.09	0		
7	138.94	0		
8	137.05	0		
9	134.43	0		
10	133.91	0		
11	128.00	1	6.94	
12	126.04	1	6.95	
13	125.93	0		
14	125.48	0		
15	122.17	0		
16	121.31	0		
17	121.01	1	7.99	
18	120.85	1	7.92	
19	119.25	1	7.10	
20	113.08	1	7.56	
21	110.83	1	7.44	
22	110.78	1	6.57	

23	62.24	2	4.20	2 equivalent
24	61.76	2	4.10/4.05	
25	61.21	2	3.18/2.91	
26	59.58	0		
27	59.42	0		
28	56.09	0		
29	50.38	1	4.29	
30	48.40	1	3.69	
31	37.86	2	2.10	
32	27.92	2	2.43/2.21	
33	23.54	3	1.86	
34	17.09	3	1.48	
35	14.70	3	1.21	2 equivalent
36	14.63	3	1.17	
37	13.98	3	0.79	
NH			11.9	
NH			10.3	

Tabulating the proton and carbon data above led to a molecular formula containing at least 40 carbons, 42 hydrogens, and 9 oxygens for a total of 666 mass units. The mass spectrum showed an extra 98 mass units that must now be accounted for. Since carprofen contains one chlorine and one nitrogen, and this impurity was suspected to be dimeric in nature, 2 chlorine and 2 nitrogen were added for a formula of $C_{40}H_{42}N_2O_9Cl_2$, which agreed with the MS spectrum data.

The number of hydrogens attached to carbon were determined by the DEPT experiment. The DEPT spectra showed the presence of 10 methines (8 in the vinyl region), 6 methylenes (4 attached to oxygen), and 6 methyls. The chemical shifts of the hydrogens attached to carbons were determined by the HMQC experiment optimized for a J_{CH} of 145 Hz. The two proton signals at 11.9 and 10.3 ppm are not attached to carbons and were therefore confirmed to be exchangeable protons attached to nitrogen.

Figure 1. The COSY data showed the presence of 7 spin systems consisting of:

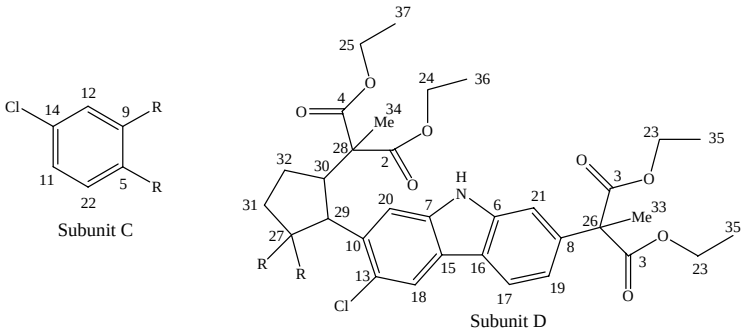


Note: The numbering scheme used is from the order of the carbon shifts in the NMR spectrum (Table 1).

The long range HMQC correlated the spin systems found in the COSY spectrum through non-protonated carbons. The experiment was optimized for three bond couplings (J=6 Hz) though at times 2, 4, and 5 bond couplings were also observed. Differentiation of these correlations was made by the intensity of the crosspeak and other long-range correlations seen. It was quickly determined that one carbazole unit had

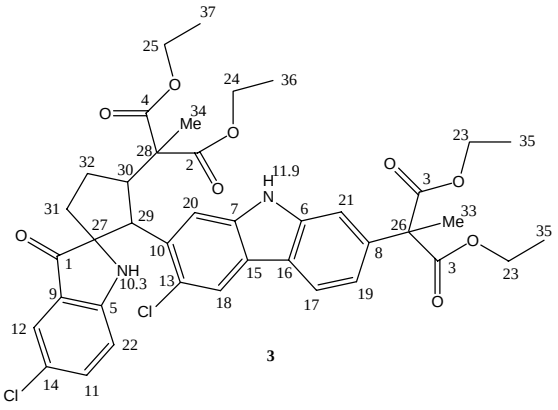
remained intact, but that the second one had rearranged. Long-range correlations from H29 to C20, C10 and C13 determined the site of attachment between the two sections of the dimer. Other key long range correlations observed were C27 to H29 and H31; H34 to C2 and C4; NH (11.20) to C15, C16, C6 and C7; and C2 to H30, leading to the partial structure shown in Figure 2.

Figure 2. Partial structure of unknown **7** based on NMR spectral correlations.



The final step was to join subunits C and D to form a five membered ring containing C5, C9 and C27, as well as the remaining carbonyl (C1) and NH moieties. Weak correlations were observed between the carbonyl C1 and H29 and H31, and between the NH (10.3 ppm) and C27, C9, and C5. The lack of correlations between the phenyl group (subunit C) and either NH or carbonyl C1 did not permit an unambiguous final assignment. A NOESY experiment was acquired at this time to determine the position of the NH relative to the phenyl group (subunit C). A correlation was seen from the NH to H22, providing some evidence for the position of the NH. The placement of the carbonyl C1 was determined by observing the strengths of the various cross peaks seen, leading to the proposed structure (**3**) in Figure 3. Once the X-Ray structure was determined, the NMR spectral data was reviewed and also found to be consistent with the structure (**7**). The two structures differ only in the reversal of C1 and C27.

Figure 3. Initial proposed structure of the unknown based upon NMR spectral data.



Note: The numbering scheme used is from the order of the carbon shifts in the NMR spectrum (Table 1).

7. For analogous literature precedent, see: R. M. Williams, J. F. Sanz-Cervera, F. Sancenon, J. A. Marco, and K. Halligan, *J. Am. Chem. Soc.*, 1998, **120**, 1090, and references cited therein.

8. Re-evaluation of the carbon coupling data at this point showed that the original assignment for the position of the carbonyl was ambiguous.

9. For analogous literature precedent, see X. Zhang and C. S. Foote, *J. Am. Chem. Soc.*, 1993, **115**, 8867, and references cited therein.

10. An alternate mechanism has been suggested which proceeds through an intermediate similar to **8** (Scheme 4) but with a chloride in place of the benzylic hydroxyl group. For an analogous transformation, see G.S.R. Subba Rao and S.N. Janaki, *Tetrahedron Lett.*, 1988, **25**, 3105.