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DIPHACINONE: ^{13}C NMR AS A PREDICTIVE TOOL IN TRICARBONYL CHEMISTRY

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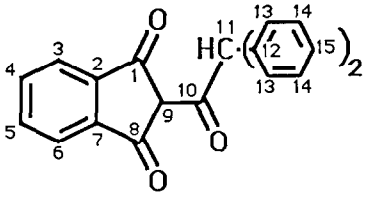
Summary: The reactivity of the tricarbonyl moiety of diphacinone towards carboxymethoxylamine has been related to the ^{13}C NMR spectral data of this potent rodenticide.

The development of a reliable and sensitive immunoassay for the detection of diphacinone (1) is an important environmental objective. This toxin, a representative of the indandione anticoagulants,[1] is a commonly used rodenticide and has also been used medicinally as an antithrombotic drug.[2] As a pestidice agent, diphacinone has been shown to be highly toxic to coyotes (LD_{50} = 0.6 mg/kg), rats (LD_{50} = 3.5 mg/kg), and rabbits (LD_{50} = 35 mg/kg), preventing vitamin K recycling from the inactive epoxide metabolite back to the functional quinone. This effectively blocks the zymogen cascade which is vital in proper hemostasis.[3] Herein we describe the unique ^{13}C NMR spectral characteristics of diphacinone and then demonstrate the synthetic implications of these data[4] in an elaboration of hapten 5.

Since the molecular weight of diphacinone is too low to elicit immune response,[5] it was necessary to covalently bind diphacinone to an immunogenic protein and we targeted the keto groups in 1 as the site for conjugation. However, a potentially problematic consequence of this strategy is that diphacinone offers two distinct carbonyl groups: cyclic carbonyls C(1) and C(8) and acyclic carbonyl C(10). Covalent attachment via the tricarbonyl moiety of 1 might therefore be complicated by the formation of two regioisomeric haptens [i.e. conjugation at C(1) or C(10)].



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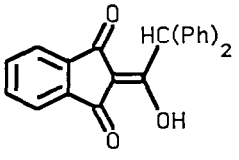
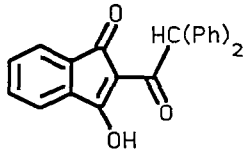


1a



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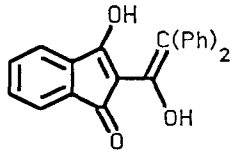


1c

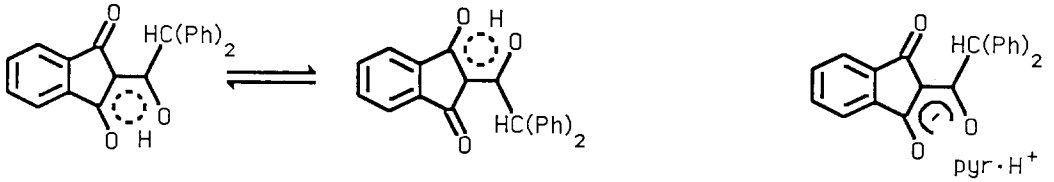
While a number of unique keto-enol tautomeric structures can be envisioned for diphacinone, NMR analysis indicates that C(11) is sp^3 hybridized in chloroform-*d*. Thus, the benzhydril proton appeared as a one proton singlet at 6.54 ppm in the ^1H NMR spectrum and C(11) appeared as a doublet ($^1J_{HC}$ = 132 Hz) in the proton coupled ^{13}C NMR spectrum. In contrast, C(9) appeared as a singlet and is therefore sp^2 hybridized, ruling out a significant contribution by tricarbonyl structure 1.[6] This data requires that diphacinone be represented by tautomeric structures 1a and/or 1b. Indeed, the ^{13}C spectral data listed in Table I are entirely consistent with a slow equilibration of these tautomers as 15 carbon resonances were detected in the noise decoupled ^{13}C spectrum. The chemical shift nonequivalence of C(1)/C(8), C(2)/C(7), C(3)/C(6), and C(4)/C(5) provides particularly compelling evidence that the C(1) and C(10) carbonyls of diphacinone are highly enolic while the C(8) carbonyl is not. Moreover, the chemical shifts of these carbonyl carbons corroborate this conclusion.[7]

Table I. ^{13}C NMR Data for Diphacinone in Chloroform-*d* (carbon/ppm/relative intensity).*

C(1)/C(8)	C(2)/C(7)	C(3)/C(6)	C(4)/C(5)	C(9)	C(10)	C(11)	C(12)	C(13)	C(14)	C(15)
197.5/187.9	140.7/138.0	122.5/122.9	134.2/135.2	108.6	184.6	51.7	138.3	129.2	128.6	127.5
4.4/3.2	3.2/4.4	29.4/27.9	27.7/25.9	3.7	8.2	26.2	11.1	100.0	98.3	62.2

*The assignments for C(1) through C(8) are interchangeable in the same column.

When the ^{13}C spectrum is recorded at room temperature in the more polar solvent DMSO, C(1) and C(8) are chemical shift equivalent and resonate at 191.3 ppm (singlet), nearly the average chloroform-*d* chemical shift of keto-C(1) and enol-C(8). The chemical shift of C(10) is essentially unchanged at 182.6 ppm (doublet, $^2J_{HC}$ = 5.5 Hz). Interestingly, at -50°C in the mixed solvent 80% $CDCl_3$:20% DMSO, diphacinone gives a doublet at 182.0 ppm and singlets at 187.0 and 195.8 ppm and is thus nearly identical to the spectrum obtained in chloroform-*d* at room temperature. Upon warming the mixed solvent to room temperature, the C(1) and C(8) carbonyls coalesce and only the C(10) doublet at 183.0 ppm is observed in the carbonyl region of the spectrum.

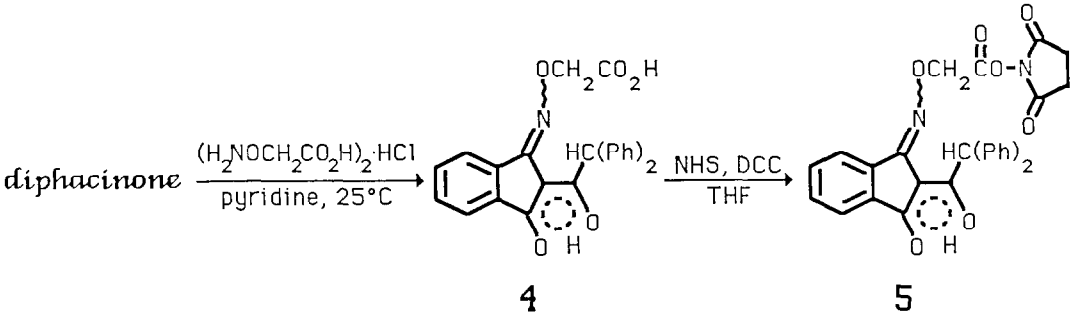


2 2' 3

Therefore the most accurate representation for diphacinone in chloroform-*d* is as depicted by tautomeric hybrids 2 or 2' and the rate of equilibration is slow on the NMR time scale. In contrast, DMSO promotes rapid equilibration but, importantly, C(10) remains equally enolized in either tautomeric hybrid. These data indicated that temporary protection of the C(10) carbonyl group in a nucleophilic addition to the "tricarbonyl" moiety might be realized by enolate formation: C(10) "always" participating in an enolate hybrid and thus unreactive

towards a nucleophilic reagent. The pyridine ^{13}C NMR of the diphacinone monoanion (3) corroborated this proposition. At room temperature, a C(10) doublet ($^{2}J_{HC}$ = 6.1 Hz) was observed at 191.5 ppm and a C(1)/C(8) singlet was observed at 191.9 ppm.[8] While no change was observed upon warming to 70°C, the C(1)/C(8) coalescence spectrum was obtained at 0°C. Upon cooling to -35°C, baseline resolved singlets for C(1) and C(8) were observed (190.9 or 193.0 ppm). Across this 105°C temperature range, the C(10) doublet remained unchanged at 191.5±0.05 ppm.

With this data in hand, the (O-carboxymethyl)oxime moiety appeared to be an ideally suited substrate/protein tether and was implemented by the nucleophilic attack of carboxymethoxylamine on the monoanion of diphacinone as follows. Diphacinone (340 mg, 1 mmol) was dissolved in pyridine (10 mL) and stirred at 25°C. Carboxymethoxylamine hemihydrochloride (110 mg, 0.5 mmol) was added and the mixture allowed to stir 24 h at 25°C at which time the pyridine was removed at reduced pressure. The oily residue was dissolved in chloroform (20 mL) and the oxime was separated from unreacted diphacinone by extraction with 4% aq. $NaHCO_3$. The combined aqueous layers were acidified with 10% aq. HCl and extracted with dichloromethane (2 x 20 mL). The combined extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give an inseparable 57:43 mixture of two (O-carboxymetheyl)oximes (4). That this was a stereo-



isomeric and not a regioisomeric mixture of oximes was unambiguously established by ^{13}C NMR (Table II). Selective irradiation of the benzhydril proton in the two isomers of 4 [δ 6.24

Table II. ^{13}C NMR Data for Oximes 4 in $CDCl_3$ (ppm/relative intensity/multiplicity).*

193.4	188.1	181.6	175.5	175.2	174.2	156.9	150.2	71.3	71.2	54.2	50.6
28.5	25.2	48.7	65.9	62.2	76.5	31.8	26.2	58.3	55.9	84.9	100.0
br d†	br d†	d**	d**	br t**	br t**	s	s	t‡	t‡	d‡	d‡

*Only the nonaromatic carbon resonances are reported. †Due to ^{3}J coupling. **Due to ^{2}J coupling. ‡Due to ^{1}J coupling.

and 6.83 (Rf 99, 538, 840 Hz)] resulted in a collapse of the two carbonyl doublets at 181.0 and 174.8 ppm while the oxime signals at 156.4 and 149.7 ppm were not effected: proof that in each isomer C(10) is a carbonyl carbon and not an oxime carbon. Subsequent elaboration of 4 to succinimide ester 5, the hapten employed in our successful ELISA,[9] was accomplished in the usual manner (N-hydroxysuccinimide and dicyclohexylcarbodiimide in THF).[10]

These results illustrate the utility of ^{13}C NMR in developing synthetic strategy. Additional aspects of the ^{13}C spectrum for the cross conjugated "tricarbonyl" moiety will be reported in due course.

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4 ^{1}H NMR were obtained at room temperature on a Varian EM 390 spectrometer (90 MHz) and ^{13}C NMR were recorded on a JEOL P.G. 100 F.T. 1A spectrometer (25.04 MHz). Proton broadband decoupling was achieved by 2.5 kHz noise modulation at Rf 99, 539, 195 Hz (10 ppm downfield of TMS with internal lock on chloroform-*d*) and 20 watts power. Selective irradiations were effected with 2 watts of monochromatic decoupler power.

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6 (a) Interestingly, 2-phenyl-1,3-indandione exists unenolized in chloroform-*d*: the ^{1}H NMR displaying a singlet at 4.25δ (benzylic proton)[5b] and the ^{13}C NMR displaying a doublet at 59.7δ (methine α to the two carbonyls). (b) Spectra #3618M, Sadtler Standard Proton NMR Spectra, Sadtler Research Laboratories; Philadelphia, PA. (c) Spectra #10268C, Sadtler Standard 13-Carbon NMR Spectra, ibid.

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