Tetrahedron Letters No. 25, pp 2179 - 2182, 1976. Pergamon Press. Printed in Great Britain.

A NEW REAGENT FOR THE SPIN LABELLING OF ALDEHYDES AND KETONES

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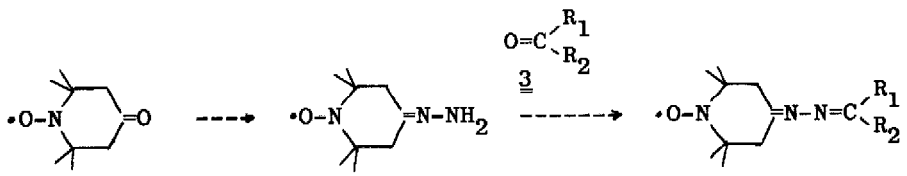
(Received in UK 21 April 1976; accepted for publication 10 May 1976)

To avoid the acidic conditions or redox steps in the existing methods of spin labelling[1-8] of carbonyl compounds, the hydrazone nitroxyl 2 was synthesised and found useful in the labelling of a number of aldehydes and ketones 3 by formation of the azines 4 (see Table).



1 2 4

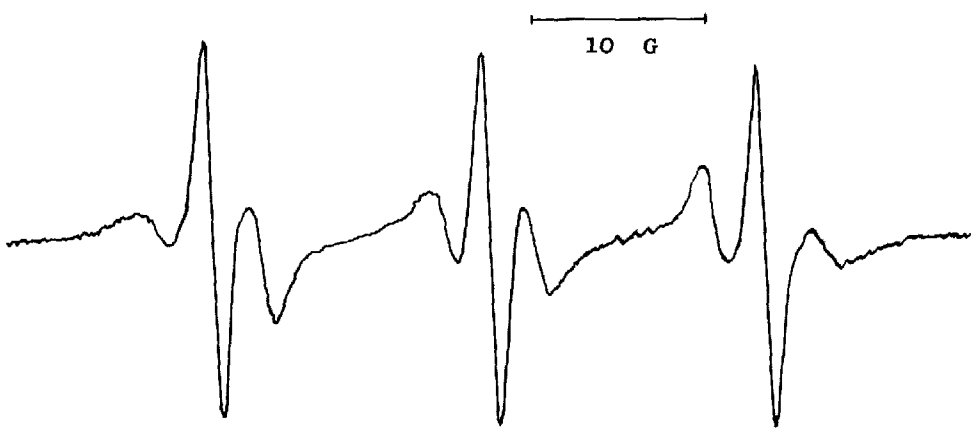
Ketone 1 (51g, 0.3mol) in methanol (1.5L) was stirred with hydrazine hydrate (22.5ml, 0.45mol) for 4.5h at room temperature. The solvent was removed in vacuo at 30°, the residue dissolved in a saturated 4° solution of Na_2HPO_4 in water (3L); 10N NaOH aq (90ml) was added. $K_3Fe(CN)_6$[9] (300g, 0.9mol) or Fremys salt[10] (300g, 1.1mol) was added. After nitrogen evolution had ceased, the solution was shaken for 5 min, then extracted with $CHCl_3$ (10 x 100ml). The combined extracts were dried with $MgSO_4$ and fil-

tered, the solvent being removed in vacuo at 30°. The residue was dissolved in $CHCl_3$ (about 30ml) and addition of hexane (600ml) yielded 2 (38g, 68%) as yellow crystals, m.p. 80°. Found: C, 58.81; H, 9.63; N, 22.60. Calc. for $C_9H_{18}N_3O$ (184.3): C, 58.66; H, 9.85; N, 22.81%.

Spin labelling with carbonyl compounds was carried out for 1-2 days in pH 7.5 aqueous phosphate buffer or in a pH 9 solution of borax in methanol. Aldehydes reacted at room temperature, condensations with ketones in methanol occurred at reflux temperature.

The new azines 4a - 4i and 4l were red oils or yellow to red crystals and were stable at room temperature in the absence of light, moisture and acid.

The dinitroxyl 4c showed a new type of spin-spin interaction[8] in the ESR spectrum (0.0001 M in oxygen-free methanol at room temperature):



The characteristic m/e = M-14 signal intensity[11] in the mass spectra was remarkably low compared to nitroxyls without azine or hydrazone groups.

Elemental analyses were carried out by Alfred Bernhardt, Elbach, Germany and Ilse Beetz, Kronach, Germany. All melting points are uncorrected.

Acknowledgment - The author wishes to acknowledge support of this work from Prof.G.Ruhenstroth-Bauer and thanks Dr.R.W.Barker for checking the English manuscript.

Table. Nitroxyl azines 4

aldehyde or ketone 3		azine 4	m.p.°/n_D^{20}	formula	m.w.	calc.: found:	C	H	N(%)	% yield at pH 7.5	pH 9
3a	acetaldehyde	4a	48-50	$C_{11}H_{20}N_3O$	210.3	calc.:	62.82	9.59	19.98	62	80
						found:	62.64	9.55	19.82		
3b	propionaldehyde	4b	1.5009	$C_{12}H_{22}N_3O$	224.3	calc.:	64.25	9.89	18.73	76	76
						found:	64.32	9.62	18.79		
3c	TEMP[a]-4-aldehyde	4c	106	$C_{19}H_{34}N_4O_2$	350.5	calc.:	65.11	9.78	15.99		54
						found:	65.04	9.70	15.81		
3d	vitamin B 6 aldehyde	4d	130dec	$C_{17}H_{25}N_4O_3$	333.4	calc.:	61.24	7.56	16.81	90	
						found:	61.18	7.58	16.84		
3e	acetone	4e	60	$C_{12}H_{22}N_3O$	224.3	calc.:	64.25	9.89	18.73	74	85
						found:	64.21	9.75	18.48		
3f	methyl ethyl ketone	4f	47-49	$C_{13}H_{24}N_3O$	238.3	calc.:	65.51	10.15	17.63	68	71
						found:	65.46	10.09	17.56		
3g	methyl propyl ketone	4g	40	$C_{14}H_{26}N_3O$	252.4	calc.:	66.62	10.38	16.65	73	59
						found:	65.67	10.29	16.76		
3h	methyl isopropyl ketone	4h	1.4957	$C_{14}H_{26}N_3O$	252.4	calc.:	66.62	10.38	16.65	55	
						found:	66.36	10.38	16.82		
3i	cyclohexanone	4i	51-52	$C_{15}H_{26}N_3O$	264.4	calc.:	68.14	9.91	15.90	33	72
						found:	68.24	9.73	15.81		
3k	TEMP[a]-4-ketone (= 1)	4k	163dec (184^{12})	$C_{18}H_{32}N_4O_2$	336.5	calc.:	64.25	9.59	16.65	92	95
						found:	64.52	9.42	16.98		
3l	cholestan-3-one	4l	141-3	$C_{36}H_{62}N_3O$	552.9	calc.:	78.20	11.30	7.60		75
						found:	78.10	11.09	7.43		

[a] TEMP = 2,2,6,6-tetramethyl-piperidine-1-oxyl

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