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Note

Thin-layer chromatography of some 5-nitroimidazoles of pharmaceutical interest

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Nitroimidazoles are effective against anaerobes[1,2] and their ability to increase the effect of radiation on hypoxic cells is well known[3,4]. Given the possible clinical usefulness of these compounds, we tried to develop separations for several 5-nitroimidazoles that have recently been tested for their potential radiosensitizing action[5]. We report here a systematic study of the thin-layer chromatographic (TLC) properties of 14 5-nitroimidazoles on thin layers of silica gel.

EXPERIMENTAL

Samples and reagents

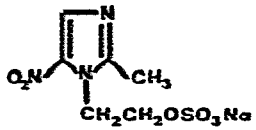
The DA nitroimidazoles shown in Fig. 1 were supplied by the Istituto De Angeli (Milan, Italy), MY 40/20 by MIDI (Milan, Italy), metronidazole by Farmitalia (Milan, Italy); and 2-methyl-4(5)-nitroimidazole by Aldrich Europe (Beerse, Belgium).

All of the chemicals used (Merck, Darmstadt, G.F.R.) were of analytical-reagent grade.

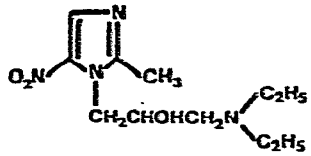
Thin-layer plates and chromatographic procedures

TLC was performed on 0.25 mm pre-coated layers of silica gel G-60 F_{254} (Merck) using the solvents listed in Table I. The plates were pre-washed with the eluent mixture to be used. Aliquots (5 μl) of ethanolic solutions containing from 10^{-4} to 10^{-3} M of nitroimidazoles were spotted. DA 3831 was chromatographed as the ammonium salt prepared according to Braun and Geenen[6]. After spotting, the plates were developed in a chromatographic tank lined with filter-paper at 30 °C using the ascending technique; the run of the developing eluent was 10 cm. The developed layers were air dried and the spots located by fluorescence quenching under 254-nm radiation. All of the chromatograms were then sprayed with 1.5% titanium(III) chloride in 10% acetic acid and heated at 80 °C for 20 min to reduce the nitro group. The plates were then sprayed with one of the following chromogenic reagents: (1) 0.5% *p*-dimethylaminobenzaldehyde in ethanol–concentrated hydrochloric acid (75:25); (2) diazotized sulphanilic acid (prepared according to Grimmett and Richards[7]; (3) 0.2% ninhydrin in acetone. These reagents were prepared immediately before use.

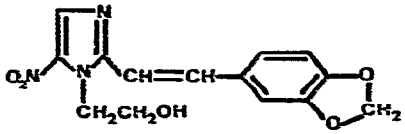
DA 3837



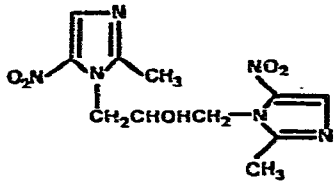
DA 3829



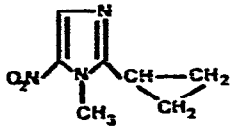
DA 3832



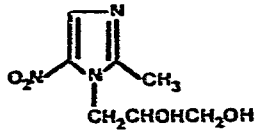
DA 3853



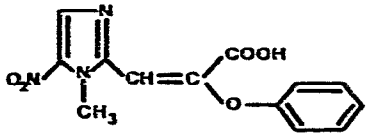
DA 3851



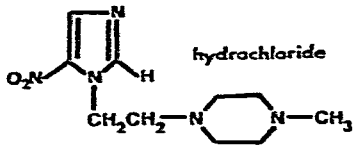
DA 3838



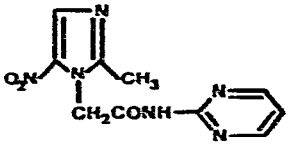
DA 3831



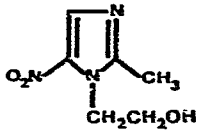
DA 3915



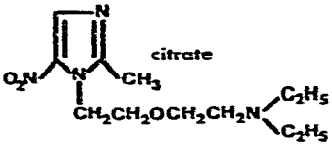
DA 3839



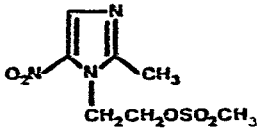
Metronidazole



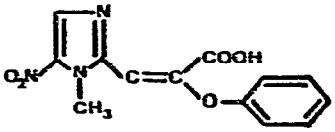
DA 3854



MY 40/20



DA 3804



2-methyl 4(5) nitroimidazole

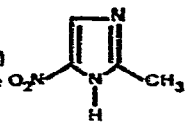


Fig. 1. Structures of nitroimidazoles.

RESULTS

The $R_F \times 100$ values given in Table I represent the average of a minimum of three separate chromatographic runs. The $R_F \times 100$ values differed by less than ± 3 from run to run. The solvent systems used allowed the effective chromatographic separation of all of the nitroimidazoles examined. For example, although DA

TABLE I

$R_F \times 100$ VALUES AND COLOUR REACTIONS OF 5-NITROIMIDAZOLES IN VARIOUS SOLVENT SYSTEMS

Compound	*Eluting solvent**					*Colour reactions***		
	A	*B*	*C*	*D*	*E*	*1*	*2*	*3*
2-Methyl-4(5)-nitroimidazole	43	66	80	71	52	bl	br	tn
Metronidazole	38	53	80	70	65	gr	tn	br
MY 40/20	41	58	78	80	77	gr	lt-yl	br
DA 3837	4	10	83	17 (tl)	10	gr	tn	gr-gy
DA 3832	65	87	82	92	88	yl-gr	cs	cs
DA 3851	63	77	85	87	85	yl-gr	bg	tn
DA 3831	0	6	87	3 (tl)	4	or	bg	gr-br
DA 3839	34	30	74	66	61	lt-gr	pl-yl	cs
DA 3854	1	0	77	19 (tl)	60	gr	tn	v
DA 3804	13 (tl)	4 (tl)	66	11 (tl)	33 (tl)	br	br	r-v
DA 3829	14 (tl)	6 (tl)	81	54 (tl)	72	gr	tn	pk
DA 3853	30 (tl)	37	82	66	64	lt-gr	tn	br
DA 3838	28 (tl)	45	73	60	47	lt-gr	tn	br
DA 3915	2	0	68	14 (tl)	31 (tl)	lt-gr	tn	r

* Solvent systems used: A = chloroform–diethyl ether–methanol–dichloromethane (6:2:2:1); B = ethyl acetate–methanol–*n*-hexane (7:2:1); C = 25% ammonia–ethanol (1:9); D = acetone–25% ammonia (99:1); E = acetone–ethyl acetate–25% ammonia (65:15:4). tl = tailing.

** For chromogenic reagents 1–3, see text. Colours: bg = beige; bl = blue; br = brown; cs = colourless; gr = green; gy = grey; lt = light; or = orange; pk = pink; pl = pale; r = red; tn = tan; v = violet; yl = yellow.

3804 and DA 3829 are tailed and poorly resolved in solvent systems A and B, they could be successfully resolved by using solvent system C.

Table I also lists the colour reactions of the nitroimidazoles after spraying with the chromogenic reagents. Optimal coloration of the spots occurred within 5 min when solution 2 was used and after heating (10 min at 80 °C) with reagents 1 and 3. The sensitivity of the colour reactions was found to be at least 0.1 μg. The coloured Ehrlich and Pauli derivatives were very stable on silica gel and the chromatograms could be preserved for 1 week or even longer, unlike the colours obtained with ninhydrin, which darkened within 24 h. According to Stambaugh and Manthei[8], a green colour obtained with ninhydrin can be considered tentative proof of a methyl group in the 2-position. This cannot be taken as a general rule for all 2-methylnitroimidazoles when silica gel is used as the support for TLC. In fact, 2-methyl-4(5)-nitroimidazole and DA 3838, both of which have a methyl group in the 2-position, gave a pale yellow and a deep blue colour, respectively, when sprayed with ninhydrin after reduction with titanium(III) chloride.

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