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Some critical remarks on the colours of secondary amines on paper with sodium nitroprusside reagent

In paper electrophoretic and chromatographic studies on the occurrence of biogenic amines and basic amino acids in the faeces¹⁻³, rumen fluid⁴ and urine⁵ of dairy cows, we used ninhydrin as a colour reagent, though in some cases diazotized sulfanilic acid (Pauly's reagent) was used for the final detection of aromatic compounds. Generally good results had been found with the ninhydrin reagent, but the colours with the secondary amines, dimethylamine and diethylamine, were often faint.

TABLE I

RELATIVE INTENSITY OF BIOGENIC AMINES AND BASIC AMINO ACIDS COLOURED WITH SODIUM NITROPRUSSIDE REAGENT

Compound	Concentration in μg	Paper: Schleicher & Schüll 2043 b			
		Untreated	Treated with*		
			A	B	C
Methylamine	2.8	+	-	-	-
Dimethylamine	1.7	++	-	-	(+)
Ethylamine	2.5	+	-	-	-
Diethylamine	3.6	+	-	-	+
Propylamine	3.1	+	-	-	-
Butylamine	3.8	+	-	-	-
Amylamine	3.8	+	-	-	-
Phenylethylamine	3.1	+	-	-	-
Tyramine	3.9	+	-	(+)	-
Diaminopropane	3.1	+	-	(+)	-
Putrescine	3.3	+	-	+	+
Cadaverine	3.5	+	-	+	+
Spermine	4.0	+	-	((+))	((+))
Spermidine	4.0	+	-	(+)	((+))
Ethanolamine	2.0	+	-	-	(+)
3-Aminopropane	3.0	+	-	-	((+))
4-Aminobutane	4.0	+	-	((+))	((+))
Agmatine	3.4	+	-	(+)	-
Lysine	3.4	+	-	(+)	(+)
Arginine	3.4	+	-	-	(+)
Histidine	2.9	+	-	-	-
Ornithine	2.3	+	-	(+)	-
β -Alanine	2.5	+	-	-	-
γ -Aminobutyric acid	2.5	+	-	-	-
ϵ -Aminocaproic acid	4.0	+	-	-	-
Taurine	3.0	+	-	-	-
Tryptamine	3.7	+	-	-	-
Histamine	3.9	+	-	-	-
Carnosine	4.0	+	-	-	-
Norephedrine	3.3	+	-	-	-
Kynurenine	3.4	+	-	-	-
Glutamine	4.0	+	-	-	-
Citrulline	2.5	+	-	-	-

* A = Phenol saturated with water; B = *n*-butanol-acetic acid-water (4:1:5); C = ethylene glycol monomethyl ether-propionic acid-water (70:15:15), saturated with sodium citrate.
+ = Strong; (+) = faint; ((+)) = very faint; - = negative.

In later studies we therefore decided to make use of the colours produced by secondary amines with the sodium nitroprusside reagent recommended by STEIN VON KAMIENSKI⁶. According to this author, this reagent should be highly sensitive and specific for secondary amines.

On spraying an electrochromatogram of a faeces extract, in which dimethylamine and diethylamine were present, however, we found with this reagent a blue spot in the position of spermidine. This unexpected result decided us on a closer study of this colour reagent.

A great number of biogenic amines and basic amino acids were treated on chromatographic paper (Schleicher & Schüll, 2043b) with the sodium nitroprusside reagent. All compounds tested in this way gave a blue-purple spot after 30-60 minutes. The experiment was repeated with chromatographic paper treated beforehand with the solvents used^{2, 3}. The results of these experiments are given in Table I.

Although STEIN VON KAMIENSKI⁶ stated that primary amines give pale red-blue spots with this reagent above 10 μg , we found, however, that this also happened below 10 μg , and that, moreover, the colour was the same as with secondary amines.

Furthermore it appears that the sensitivity depends strongly on the solvent used. Thus we were unable to confirm the great sensitivity and specificity of this reagent.

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