Studies with β-phenethylbiguanide

1. Microdetection colorimetric tests

As a part of studies on the human metabolism of β-phenethylbiguanide (β-PEBG), an oral hypoglycemic agent, several location reagents have been found to be useful for detection of the unaltered compound on paper chromatograms in the microgram and submicrogram range. The observations support the view that β-PEBG behaves as a monosubstituted guanidine in these color tests.

Experimental

Three reagents giving positive color tests with β-PEBG are the pentacyanoaquoferriate (PCF), Sakaguchi, and α-naphthol–diacetyl reagents. These reagents were prepared as previously described[1], except the bromine liquid, 0.3 ml, was dissolved in 100 ml of 2 % NaOH in water for preparation of the Sakaguchi reagent. The α-naphthol and 2,3-butanedione (diacetyl) were Eastman Grade (Eastman Kodak Co.), and other chemicals were A.R. grade obtained from J. T. Baker Chemical Co. or The Matheson Company, Inc. Tests were performed with β-PEBG free base and with various salts of β-PEBG including the monohydrochloride, the dihydrochloride, and the sulfate. The sensitivity limitations were obtained by spotting the appropriate amount of β-PEBG on unwashed Whatman No. 2 chromatography paper and performing the color test using a dipping technique without prior development of the chromatogram.

Pertinent data concerning these color tests together with structural representations of β-PEBG are illustrated in Fig. 1. The color produced with the α-naphthol–diacetyl reagent appears to be the most sensitive, with a detection limitation of 0.05 μg β-PEBG compared to 0.25 μg obtained with either the PCF or Sakaguchi

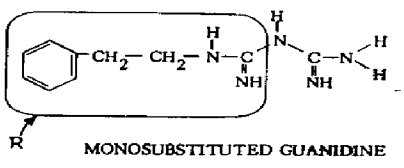
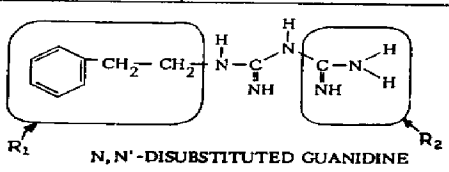
	PENTACYANOAQUOFERRIATE REAGENT	SAKAGUCHI REAGENT	α-NAPHTHOL-DIACETYL REAGENT
COLOR OF SPOT	PINK	ORANGE	PURPLE
DETECTION LIMITS	0.25 μg	0.25 μg	0.05 μg
TIME OF DEVELOPMENT	IMMEDIATE ROOM TEMPERATURE	IMMEDIATE ROOM TEMPERATURE	3 MINUTES 100° C
TYPE OF GUANIDINE AND TEST GIVEN (+) POSITIVE (-) NEGATIVE	MONOSUBSTITUTED (+) N,N-DISUBSTITUTED (+) N,N'-DISUBSTITUTED (-)	MONOSUBSTITUTED (+) N,N-DISUBSTITUTED (-) N,N'-DISUBSTITUTED (-)	MONOSUBSTITUTED (+) N,N-DISUBSTITUTED (+) N,N'-DISUBSTITUTED (-)
STRUCTURAL REPRESENTATIONS OF β-PEBG	 MONOSUBSTITUTED GUANIDINE	 N,N'-DISUBSTITUTED GUANIDINE	

Fig. 1. Summary of colorimetric microdetection tests.

reagents. Following paper chromatography as little as 1 μg β-PEBG has been detected with these reagents. The detection limitations of smaller amounts of β-PEBG following chromatography have not been investigated.

No differences have been noted between the color tests given by β-PEBG free base and the salts mentioned. The alkali in these color tests would be expected to convert these salts to the free base[2] by proton removal. Therefore, it appears likely the free base is produced as an intermediary in reactions involving the salts investigated.

These location reagents have been reported to be useful for distinguishing structurally dissimilar guanidines, and to give positive color reactions with certain monosubstituted guanidinium compounds, but not with N,N'-disubstituted guanidines[1]. β-PEBG can be represented structurally as a monosubstituted or an N,N'-disubstituted guanidine. Since β-PEBG gives a positive test with all three reagents, the data have been interpreted as indicating β-PEBG behaves as a monosubstituted guanidine in these color tests. None of the colorimetric reactions mentioned is specific for β-PEBG, and detection requires prior purification of β-PEBG from other guanidinium compounds.

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A new technique for preparative paper chromatography

Preparative partition-chromatography techniques have been reviewed in several recent treatises on chromatography[1–3]. Many techniques that depend upon increasing the mass of the stationary phase are cumbersome and tedious or have low capacity[4–10]. The continuous methods[11,12], which require elaborate, synchronous mechanical equipment, are not used extensively although commercial equipment has been available.

The technique outlined in this report requires only readily available laboratory supplies and equipment. It has high, adjustable capacity and resolving power, simplicity, convenience, and versatility that facilitates multi-directional fractionations and the use of glass fiber, cellulose acetate, ion-exchange or any partition media available in tape form.