

The above technique provides a complete method for the separation, detection and estimation of the known urinary 17:20-dihydroxy-20-methyl-steroids. Use of the method in estimating these steroids and in detecting new compounds in the urine of normal and diseased people will be described in detail elsewhere.

I acknowledge a grant from the New South Wales State Cancer Council, and thank Prof. C. W. Emmens for his interest in this work. Drs. Gallagher and Hirschmann and Prof. G. F. Marrian kindly gave steroids.

R. I. Cox

Department of Veterinary Physiology,
University of Sydney.

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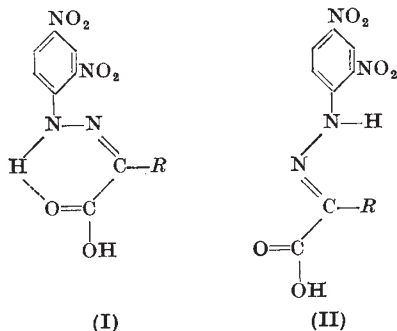
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A New Reaction for the Differentiation of *cis*- and *trans*-Isomers of Nitrophenylhydrazones of some α -Keto Acids

RECENTLY, it has been reported by several investigators that there exist *cis*- and *trans*-isomers in 2:4-dinitrophenylhydrazones of pyruvic acid^{1,2}, benzoylformic acid³ and α -keto- β,β -dimethyl- γ -butyrolactone⁴ as shown in (I) and (II) respectively. This was supported by infra-red spectra, since the wave-numbers of the stretching bands of CO and NH of the *cis*-form were smaller than those of the *trans*-form. Our interest in the structures of the isomers of these hydrazones has led us to the discovery of a new chelating reaction, capable of differentiating the two forms.



When 0.1 gm. of zinc powder was added to a mixture of 10 μ moles of nickel chloride and 30 μ moles of the *cis*-hydrazone in 5 ml. of 70 per cent aqueous ethanol (the reaction never occurred in absolute alcohol) and shaken for a few minutes, a red colour appeared. This did not happen with the *trans*-hydrazone. Maximum coloration was obtained at a 3:1 molar ratio of hydrazone to nickel. No coloration was produced when the nickel chloride solution was added to a hydrazone solution which had been shaken with zinc powder and then filtered to remove the powder. From these facts, the coloration is presumed to result from chelation of nickel with the carboxyl

group and with a metastable group produced by the reduction of the nitro group. Nickel was found to be replaceable by cobalt, iron and mercury. These colouring reactions were also observed with several other nitro-compounds (Table 1). The shifts in the value of λ_{\max} on chelation with nickel were as follows. Pyruvic acid 2:4-dinitrophenylhydrazone: *cis*, 370 $m\mu$ to 380 $m\mu$; *trans*, 356 $m\mu$ to 358 $m\mu$. Benzoylformic acid *o*-nitrophenylhydrazone: *cis*, 425 $m\mu$ to 447 $m\mu$; *trans*, 412 $m\mu$ to 365 $m\mu$. The chelated compounds of the *cis*-forms showed more intense absorption in the longer wave-length region than those of the corresponding *trans*-forms.

Table 1. COLOUR REACTIONS WITH SOME NITROPHENYLHYDRAZONES AND NITROPHENOLS

Compounds	Colouring reaction
2:4-Dinitrophenylhydrazone of pyruvic acid	<i>cis</i> +
	<i>trans</i> -
2:4-Dinitrophenylhydrazone of benzoylformic acid	<i>cis</i> +
	<i>trans</i> -
<i>o</i> -Nitrophenylhydrazone of benzoylformic acid	<i>cis</i> +
	<i>trans</i> -
<i>m</i> -Nitrophenylhydrazone of benzoylformic acid	<i>cis</i> -
	<i>trans</i> -
<i>p</i> -Nitrophenylhydrazone of benzoylformic acid	<i>cis</i> -
	<i>trans</i> -
<i>o</i> -Nitrophenol	-
<i>m</i> -Nitrophenol	-
<i>p</i> -Nitrophenol	-

Judging from the results shown in Table 1, the presence of a nitro-group at the *ortho* position in the benzene ring is indispensable for this colour reaction. Furthermore, this colour reaction is confined to α -keto acids, in which the nitro- and carboxyl-groups are situated in positions suitable for chelating around the nickel atom. Thus in the hydrazones of symmetrical keto acids only that of mesoxalic acid (α -keto acid) shows a positive reaction, whereas acetonedicarboxylic acid (β -keto acid) and γ -ketopimelic acid (γ -keto acid) give a negative reaction.

This reaction can be used to differentiate the geometrical isomers of 2:4-dinitrophenylhydrazones of α -keto acids, but it is less effective if the hydrazone isomerizes rapidly.

HIROHIKO KATSUKI
KŌICHIRO SUMIZU
TARUMI MORIWAKI
SHŌZŌ TANAKA

Department of Chemistry,
Kyoto University.

ICHIRO HAYASHI

Laboratory of Organic Chemistry,
Osaka Women's University,
Japan.

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The α - β Transformation in Keratin

BENDIT¹ has recently underlined the necessity for refinement of some of the earlier ideas² on the mechanism of the α - β transformation in wool keratin. The problem of a precise quantitative investigation of the transition is difficult, largely because of the intervention of relaxation and creep, the effects of which on the crystalline phases are unknown. Recently³, a technique for obtaining the desired diffraction data in a short time has been described, and its use, together with standard diffractometric technique, has enabled some interesting preliminary results about the transformation to be obtained.