Effect of Phosphoric Acid on Digitalis Compounds

It has been shown by Cox¹ that pregnane-3 α ,17 α ,20 α -triol (pregnanetriol) and related compounds react with 70 per cent ortho-phosphoric acid to produce a brilliant fluorescence. Herman and Bruton^{2,3} have shown that pregnanetriol and related compounds and digitalis compounds react intensely with antimony pentachloride, which thus can be used as a paper chromatographic detecting agent. Since pregnanetriol reacts with both phosphoric acid and antimony pentachloride, by analogy, those steroids which react strongly with antimony pentachloride could be expected to react with 70 per cent ortho-phosphoric acid. Heftmann *et al.*⁴ have noted briefly that aniline-phosphoric acid is useful as a detecting agent for cardiac glycosides.

We have applied ortho-phosphoric acid to digitalis compounds and other steroids and have found that the natural fluorescence of the digitalis compounds under ultra-violet light is markedly enhanced. Each steroid was dissolved in chloroform and applied to Whatman No. 1 chromatogram paper in 50- μ gm. amounts or less. The paper was dipped into 70 per cent orthophosphoric acid and placed on a sheet of tinfoil. The paper was examined in visible and ultra-violet light (366 and 254 m μ) before and after heating at 80° C. for 5 min.

Fig. 1. Fluorescent photograph of digitalis compounds treated with 70 per cent orthophosphoric acid. The compounds are: a, digitoxigenin; b, digoxin; c, digitoxin

Table 1 indicates the results obtained with digitalis compounds and other steroids and related compounds. Fig. 1 is a fluorescent photograph of the digitalis compounds treated with 70 per cent ortho-phosphoric acid. The Whatman No. 1 paper containing the treated digitalis compounds was placed on type IF contrast 5 Haloid photographic paper (Xerox Corp., Rochester, New York) and exposed for 5 sec. at approximately 18 in. using a 254 m μ ultra-violet light⁵. Both digitoxin and digitoxigenin fluoresce with a bright yellow colour and are reproduced well on the Haloid photographic paper. The digoxin fluoresces with a bright blue colour but appears much less intensely in the fluorescent photograph. Haloid photographic paper is insensitive to wave-lengths of

light beyond 450 m μ so that fluorescence at wave-lengths beyond 450 m μ can be recorded as an unexposed area.

We were able to detect a minimum of 10 μ gm. of each of the three digitalis compounds in visible light using 70 per cent phosphoric acid. With ultra-violet light (366 m μ) we could detect a minimum of 5- μ gm. with the fluorescence being more brilliant after heating.

This technique should be useful as a detecting method for digitalis compounds.

JOSEPH BRUTON
ROBERT H. HERMAN
LLOYD E. CLAYTON

Department of Metabolism,
Division of Medicine,
Walter Reed Army Institute of Research,
Washington 12, D.C.

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² Herman, R. H., and Bruton, J., *Nature*, **190**, 444 (1961).

³ Herman, R. H., *Nature*, **190**, 268 (1961).

⁴ Heftmann, E., Berner, P., Hayden, A. L., Miller, H. K., and Mosettig, E., *Arch. Biochem. Biophys.*, **51**, 329 (1954).

⁵ Herman, R. H., Clayton, L. E., and Bruton, J., *Nature*, **193**, 169 (1962).

O-Methylation in Fish

DURING an investigation of the fate of some foreign organic compounds in fish, the metabolism of 3,4-dihydroxyphenylacetic acid (homoprotocatechuic acid) was investigated with the purpose of obtaining some information on methylation reactions in these animals. DeEds *et al.*¹ have noted that this compound is methylated at the 3-hydroxyl group in rats and rabbits. A later report by Scheline *et al.*² showed that homoprotocatechuic acid labelled with carbon-14 fed to rabbits at a dose-level of 100 mgm./kgm. resulted in about 85 per cent of the radioactivity being excreted in the urine within 44 hr. While the main excretory product was the original acid, nearly six per cent of the dose was found as 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid).

In the experiments described here 12 cod, *Gadus callarias*, weighing 420–1,370 gm. (average 740 gm.) were given homoprotocatechuic acid dissolved in saline. The dose-levels ranged from 35 to 120 mgm./kgm. (average 72 mgm./kgm.) and the compound was injected intraperitoneally (nine animals) and intramuscularly (three animals). The method used for identifying metabolites was similar in part to that of Wood³ and consisted of placing the treated fish in a small plastic tank containing 5 l. of water. The water was aerated and maintained at 10° C. by keeping the tank in a constant flow of cold water. After 20 hr. the

Table 1. EFFECT OF PHOSPHORIC ACID ON DIGITALIS COMPOUNDS AND OTHER STEROIDS AND RELATED COMPOUNDS

Compound	Visible light	Before heating		Visible light	After heating	
		366 m μ	254 m μ		366 m μ	254 m μ
Digoxin	yellow colour	bright blue fluorescence	bright blue fluorescence	green-yellow colour	bright blue fluorescence	blue fluorescence
Digitoxin	faint yellow colour	bright yellow fluorescence	dark yellow fluorescence	green-yellow colour	bright yellow fluorescence	yellow fluorescence
Digitoxigenin	no colour	bright yellow fluorescence	dark yellow fluorescence	green-yellow colour	bright yellow fluorescence	yellow fluorescence
Ergosterol	yellow colour	yellow absorbance	purple absorbance	pale-yellow colour	yellow absorbance	orange absorbance
7-Dehydrocholesterol	no colour	no colour	purple absorbance	no colour	yellow absorbance	orange absorbance
Vitamin D ₂	no colour	no colour	purple absorbance	no colour	no colour	no colour
Vitamin D ₃	no colour	no colour	purple absorbance	no colour	no colour	no colour
Adrenosterone	no colour	no colour	purple absorbance	no colour	no colour	purple absorbance