LETTERS TO THE EDITOR

An Identity Test for Pheniodol

SIR,—During recent work in our laboratories an identification test suitable for routine analysis was required for pheniodol, α-phenyl-β-(4-hydroxy-3 : 5-diiodophenyl)-propionic acid, but no assistance in this matter was obtained by a survey of the literature. Accordingly, we developed the following test which has proved satisfactory in the hands of several independent workers.

Dissolve 0·5 g. in 15 ml. of 10 per cent. sodium hydroxide solution, warming if necessary. Add 1 g. of zinc dust and boil the mixture under a reflux condenser for 20 minutes. Cool, filter, add excess of dilute hydrochloric acid and collect the α-phenyl-β-(4-hydroxyphenyl)-propionic acid. Wash with water and recrystallise the product from alcohol-water mixture. The crystals, after drying at 100°C., melt at 180° to 181°C. The filtrate, from the acid after separation from the original reaction mixture, affords reactions characteristic of iodides.

It is felt that this test may be of interest to your readers.

Wellcome Chemical Works, Dartford. G. E. FOSTER.
October 21, 1948 W. D. WILLIAMS.

Silicotungstic Acid

SIR,—In the discussion at the British Pharmaceutical Conference on our paper entitled "The Chemical Determination of Aneurine in Tablets and Ampoule Solutions" (see *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 370, 423) a question was asked as to the composition of the silicotungstic acid used in our experiments. The composition may be of importance, as is reputed to be the case in the method of the Association of Official Agricultural Chemists for the determination of nicotine (*Methods of Analysis*, 6th Ed., p.74). We obtained the silicotungstic acid for our work from only one source and the suppliers have advised us that the composition varies slightly but approximates closely to $H_4SiW_{12}O_{40},24H_2O$. This corresponds to the formula quoted by the A.O.A.C. for the nicotine determination, $4H_2O,SiO_212WO_3,22H_2O$.

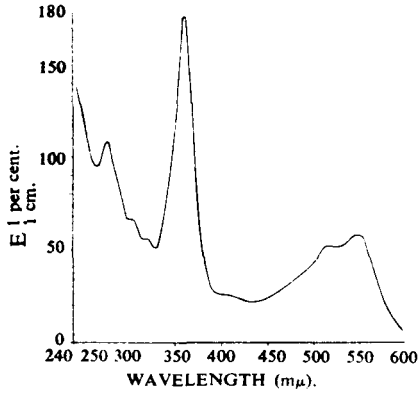
Roche Products, Ltd., Welwyn Garden City. D. C. M. ADAMSON.
November 3, 1948. F. P. HANDISYDE.

The Isolation of the Crystalline Anti-Pernicious Anæmia Factor from Liver

SIR,—Work on the anti-pernicious anæmia (A-P-A) factor present in liver, in progress in these Laboratories for some years, has led to the isolation of a red crystalline compound from anahæmin, probably identical with the vitamin B_{12} of Rickes *et al*[1] and with the crystalline A-P-A- factor of Smith and Parker[2]. The methods of purification employed by us, however, differ in certain respects from those hitherto revealed. Thus the observation that the A-P-A factor is extracted by *n*-butanol from its aqueous solutions in the presence of fairly high concentrations of ammonium sulphate[3] enabled us to effect enrichment of the fractions at various stages of the process. Chromatography was reserved only for the final purification. Columns of bentonite or aluminium silicate[3] were used, and, under carefully controlled conditions, proved eminently satisfactory by giving rise to the formation of sharply defined red bands. These, after dissection and elution, gave material which readily crystallised in small red needles from aqueous acetone.

Our crystalline product, after drying *in vacuo*, contained 4·0 per cent. of cobalt, a figure identical with that reported by Smith[4]. In aqueous solutions

it shows characteristic light absorption (see Fig.). A main band appears in the visible region of the spectrum with a maximum at 500 mμ and a "shoulder" at approximately 520 mμ, whilst two distinct maxima occur in the ultra-violet, one at 361 mμ and the other at 278 mμ, with inflections at 322 mμ and 304 mμ.



Several batches of the crystals have been hydrolysed with 20 per cent. hydrochloric acid in sealed tubes at 100°C., and the hydrolysates examined by uni-dimensional paper-strip partition chromatography, using the technique described by Consden *et al*[5]. The chromatograms obtained have consistently revealed the presence of only one substance reacting with ninhydrin. Using aqueous *iso*butyric acid as the solvent, a pronounced purple spot appears on the paper at a point approximately mid-way between the positions occupied by the α-amino-acids valine and *nor*valine. With aqueous phenol or *n*-butanol as solvents, however, the colour of the spot is greatly diminished in intensity although its position with respect to the two amino-acids remains substantially unchanged. Experiments to detect the presence of purines[6] in A-P-A hydrolysates have not, so far, been successful. Vigorous hydrolysis of the A-P-A factor with 20 per cent. hydrochloric acid under reflux for 8 hours failed to rupture the cobalt-containing complex present in the molecule. Removal of a hydrophilic fragment undoubtedly takes place as the product is readily and quantitatively extracted from the diluted hydrolysate with *n*-butanol (cf. Smith[7]). The product is an almost black, amorphous solid which is acidic in character as it is soluble in dilute alkalis and is reprecipitated unchanged on neutralisation. This "acid" is insoluble in ether, chloroform and acetone, but is rendered soluble in these solvents by the addition of a trace of hydrochloric acid. Its light absorption (measured in dioxan) shows a maximum at 557·5 mμ with a "shoulder" at 530 mμ, and is thus similar (in the visible part of the spectrum) to the absorption of the A-P-A factor itself. The retention of a cobalt co-ordination complex in the molecule after the somewhat drastic acid hydrolysis is surprising, but is paralleled by the behaviour of certain metal porphyrins. Supplies of the methyl ester of the "acid" are now being accumulated to enable us to undertake its more detailed study.

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Research Department,
The British Drug Houses, Ltd., London, N.1.
November 24, 1948.

B. ELLIS.
V. PETROW.
G. F. SNOOK.

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