The residue was cooled, extracted with four 100-ml. portions of chloroform, the organic layer dried with drierite and the solvent removed by distillation on the steam-bath. The solvent-free residue was distilled *in vacuo*, b. p. 144° (0.06 mm.) in an all-glass-interjoint apparatus to give a light yellow oil, which could not be induced to crystallize; yield, 60%.

The base was identified as the dipicrate, which was prepared in anhydrous diethyl ether and recrystallized several times from a large volume of methanol, m. p. 168–169° (cor.).

Anal. Calcd. for $C_{30}H_{33}N_9O_{15}$: C, 47.44; H, 4.38. Found: C, 47.54; H, 4.50.

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RECEIVED AUGUST 4, 1944

COMMUNICATIONS TO THE EDITOR

CINCHONA ALKALOIDS PREPARED BY ION EXCHANGE

Sir:

An economical process for the isolation of alkaloids from low-grade cinchona barks has been a prime necessity since the loss of the Far Eastern sources of quinine and its raw materials. During a study of the acid extraction of South American bark, it was decided to investigate the use of cation-exchange adsorbents as a means of increasing the efficiency of extraction.

Three possibilities were seen for the application of ion exchange to cinchona extraction, namely: to recover alkaloids from the mother liquors of acid extracts of the bark after the major portion had been removed by alkaline precipitation; to purify the crude totaquine obtained from alkaline precipitation; to use ion exchange directly in the acid extraction of the bark in a cyclic system so that the bark is constantly percolated or extracted by an acid medium free from alkaloids. These experiments provide specific information for the first two of the above-mentioned applications. Cyclic extraction has been studied and will be reported upon at a later date.

Basic facts as to the adsorption capacity of the cation-exchanger for cinchona alkaloids were obtained using quinine as a representative alkaloid. Capacity determinations were run on a two-hundred mm. "Zeo-Karb" column[1,2] using quinine concentrations of 0.033 and 0.0033M and flow rates of approximately 5 and 50 ml./min., respectively.

The capacity of a 200-ml. bed of "Zeo-Karb" for quinine from acid solution (1% H_2SO_4) was found to be between 7 and 8 g. before breakthrough (Mayers reagent). To liberate the alkaloids from the column, ammoniacal alcohol was used as a combined regenerant and elution solvent. After the exchanger was used once or twice, recoveries were almost quantitative.

The purification of totaquine prepared by alkaline precipitation of an acid extract of the bark was now attempted by ion exchange. From 20 g. of the crude totaquine precipitate, 2.5 g. of a white crystalline material was obtained. A comparison of the properties of alkaline precipitated totaquine and the alkaloid prepared from it by ion-exchange is given in the table.

	Totaquine	
	Alkaline precipitated	Ion exchange
Color	Dark reddish brown	White
Form	Amorphous powder	Crystalline
Total alkaloids, %	23.4	94
Sol. in acid, %	Approx. 45	100
Sol. in $CHCl_3$, %	Approx. 20	100

The results suggest that ion exchange could prove a valuable aid in the extraction of cinchona by enabling the recovery of alkaloids which would otherwise be lost in the mother liquors following alkaline precipitation. Ion exchange also represents an excellent technique for purifying crude totaquine preparations, improving solubility, appearance, and removing non-alkaloidal, non-ionic contaminants.

The writer also has used this technique successfully in the isolation of atropine, scopolamine and morphine and will report more fully upon these experiments.

This work was performed at the Rutgers University College of Pharmacy in conjunction with the research program of the Foreign Economic Administration supervised by Professor Martin S. Ulan. The helpful advice of Dr. F. C. Nachod of the Permutit Company is gratefully acknowledged.

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(1) The Permutit Co., N. Y.

(2) F. C. Nachod and S. Sussman, *J. Chem. Ed.*, **21**, 56 (1944).

AN UNIDENTIFIED GROWTH FACTOR FOR A GAS GANGRENE CLOSTRIDIUM[1]

Sir:

In an investigation of the nutritional requirements of *Clostridium perfringens* it was found that a complex synthetic medium, such as that supporting the growth of *Clostridium tetani*, was inadequate for the growth of *Clostridium perfringens*.

(1) This work was supported by a grant from the Josiah Macy, Jr., Foundation.

Excellent growth was obtained if such a defined medium[2] was supplemented with yeast extract, peptone, or a crude biotin concentrate. These crude products could not be replaced by riboflavin, nicotinic acid, *p*-aminobenzoic acid, inositol, pimelic acid, adenine, guanine, uracil, yeast nucleic acid, oleic acid, glucosamine, asparagine, serine, choline, or folic acid.[3]

A preparation of the growth factor was obtained from yeast extract by precipitation with lead hydroxide, after first removing much inactive material with lead acetate at *p*H 4.0. The lead hydroxide precipitate was fractionally decomposed by first removing color and other impurities with alcoholic 0.1 *N* hydrochloric acid. The lead was then removed by decomposing the residue with hydrogen sulfide. The resulting solution was deanionized with Amberlite IR-4, and was then treated with Norite. The colorless and active solution so obtained was free of thiamin, nicotinic acid, pyridoxine, folic acid, biotin, *p*-aminobenzoic acid, and riboflavin. The recovery was 2–10%. The actual degree of purification was over 200-fold, but this is in all probability a low value since Amberlite IR-4 contributes considerable solids to the final preparation.

The growth factor may be partially adsorbed on the cation-exchange resin Amberlite IR-100 and on Decalso (at *p*H 0.5, but not at *p*H 3.0). Little or no adsorption on Norite (*p*H 3.0 to 7.5), Amberlite IR-4 or Permutite (*p*H 7.0) was observed. The active principle was not precipitated by uranium, silver, barium (water or 70% ethanol), phosphotungstic acid or 95% ethanol. Both mercuric salts and picric acid precipitated the growth factor, while lead only did so from crude extracts. Extraction by ether from acid, alkaline or neutral solution was not obtained. The above properties indicate that the growth factor is not identical with either sporogenes vitamin[4] or with strepogenin.[5]

Inactivation experiments have provided some data on the chemical behavior of the growth factor. Treatment in absolute ethanol with excess anhydrous hydrochloric acid leads to inactivation, while the activity may be regenerated by mild alkaline hydrolysis. Nitrous acid completely inactivates the substance, whereas oxidation with potassium permanganate at *p*H 7.5 does not affect it. While stable to normal acid or alkali at room temperature, complete inactivation occurs in five minutes at 100°. No labile phosphate is present in the best preparations. More complete elucidation of the chemical properties must await the isolation of the growth factor in pure form. With this in view large scale preparations are now in progress.

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(2) Besides the described growth factor, salts, amino acids and glucose, the organism requires biotin, pantothenic acid, thiamin and pyridoxine for growth.

(3) Kindly supplied by Dr. Roger Williams.

(4) Pappenheimer, *Biochem. J.*, **29**, 2057 (1935).

(5) Woolley and Sprince, reported before the Biochemical Division of The American Chemical Society, New York, N. Y., September 14, 1944.

DEPENDENCE OF POLYMER PROPERTIES ON TEMPERATURE OF POLYMERIZATION

Sir:

In a recent paper on polystyrene, Alfrey, Bartovics and Mark[1] showed that the values of certain characteristic constants (μ, a, K and k') deduced from osmotic pressure and viscosity data, although practically independent of molecular weight, are quite dependent on the temperature at which the polymerization occurred. This unexpected result they interpreted, properly, as indicating "a different internal architecture of the macromolecules" in the different samples produced at different temperatures. They suggested that different amounts of branching of the polymer chains in the different samples might account for the observed results.

Against the branching hypothesis it may be pointed out that more branching would be expected in a polymer produced at a high temperature than in one produced at a low temperature, that on the average (for the same molecular weight) the high-temperature polymer molecules would therefore have a less extended form than the low-temperature polymer molecules, and that the former would therefore be characterized by a smaller value of the exponent a in the equation relating viscosity to molecular weight. Actually, the reverse is the case.

An alternative explanation seems to me more reasonable. At each CHR group in a polymer chain there are two different, non-equivalent dispositions of the H and the R relative to the plane containing the C–C bonds which join this group to the adjacent C atoms in the chain. Once a chain is formed, the disposition of H and R relative to this plane cannot be changed, without a reaction equivalent to a Walden inversion; mere rotation around single bonds will not do it. In a polymer produced at a low temperature one would expect a tendency toward some *regular* sequence of dispositions of H and R, such as one in which all of the R groups would be on the same side of the plane of the zigzag carbon chain if the molecule were stretched out, or one in which the R groups alternated from one side to the other. In a polymer produced at a high temperature, however, a more *random* sequence would be expected. Difference in randomness of these dispositions would produce differences in the average degree of coiling of the chains (hence in a and K), in the solute–solvent and solute–solute interac-

(1) T. Alfrey, A. Bartovics and H. Mark, THIS JOURNAL, **65**, 2319 (1943).