the two tetracyclines, AM and TM, react similarly, while CM, which is structurally dissimilar, acts differently. It appears, therefore, that a study such as that described is able to distinguish between compounds with like and unlike modes of action.

Following the analytical precedent set by JOHNSON *et al.*[3], these antibiotics appear qualitatively to fall into the Type II class of inhibitors[4,5], *i.e.* their activity declines with decrease in temperature. All observed Type II inhibitors are also known to be biochemically "noncompetitive".

Furthermore, WOOLLEY[6] has demonstrated, that in *Escherichia coli*, CM acts as a noncompetitive inhibitor. The data shown in this report, then, uphold WOOLLEY's contention and in addition indicate that TM and AM function in the same manner. Therefore, it is tentatively concluded that the general mechanism of action of the three antibiotics is the same in that they are Type II inhibitors. However, their specific mechanisms or sites of action seem to be different, and appear to depend upon their chemical structure.

A more critical analysis of these results is untenable at this time. Such analysis must await further studies at intermediate temperatures and varied antibiotic concentrations, as well as growth carried out in constant temperature chambers or water baths of greater sensitivity than those utilized in this investigation.

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A sensitive reaction on paper of ninhydrin with hydroxyproline and proline

The sensitivity of the ninhydrin (triketo-hydrindene hydrate) reaction with amino acids separated on two dimensional paper chromatograms has been described by PRATT AND AUCLAIR[1]. The reagent has a low sensitivity for both hydroxy-proline and proline due to the formation of a yellow pigment which is difficult to differentiate from the white paper background.

A more satisfactory qualitative test was evolved using the following procedure. The filter paper chromatograms, after development with the solvent system of REDFIELD[2] and steaming for 10 minutes to remove adsorbed diethylamine, were dipped in a 0.1% (w/v) solution of ninhydrin in benzene containing 0.1% (v/v) collidine. The papers were then placed in dry steam for 1 minute to produce magenta spots from hydroxy-proline and proline, which exhibited an intense red fluorescence in u.v. light. The limit of detection by u.v. fluorescence was 0.008 μg and 0.025 μg amino-nitrogen for hydroxy-proline and proline respectively.

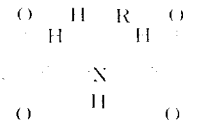
A pigment of similar colour is known to occur as an intermediate in the hydroxy-proline ninhydrin reaction[3]. This substance, subsequently referred to as pigment A, can be isolated from the aqueous reaction media by extraction with benzene[4], and is non-fluorescent in u.v. light. It has an absorption spectrum in the 350 to 750 mμ range identical with that of the fluorescent pigment (pigment B) as measured whilst adsorbed on the paper. This was achieved by placing paper strips carrying the pigment in a glass cuvette containing benzene. Pigments A and B both exhibit an absorption maximum at 570 mμ and similar strong absorption 260–280 mμ. Pigment B was found to be inextractable from the paper by both polar and non-polar solvents except to a small extent by acetone. The latter gave a magenta-coloured solution, non-fluorescent in u.v. light with absorption spectrum identical with that of pigment A in acetone—each maximal at 550 mμ. This suggests that B is formed by adsorption of A on the paper and this is supported by the observation that addition of A to the paper produced a magenta spot which became fluorescent on steaming for one minute. Moreover, both A and B on prolonged heating yielded a yellow pigment of identical absorption spectra with that published by MOORE AND STEIN[5].

The conversion of A to B is specific to cellulose, being formed only on paper, cotton wool or cellulose powder. No reaction was obtained with starch, dextran, cellobiose or the monosaccharides. Moreover, the reaction requires an aqueous media (supplied by steam in the case of filter paper chromatograms). Thus no reaction will occur on acetylated paper and heating benzene solutions of pigment A with cellulose powder does not produce pigment B.

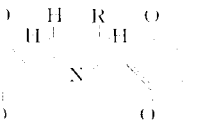
Spectroscopic analysis of the fluorescent light from pigment B indicated that it consisted of a broad red band including part of the yellow. The absorption peak at 570 mμ cannot be responsible for the fluorescence which only occurred in the presence of filtered u.v. light.

The chemical structure of pigment A was elucidated by GRASSMANN AND VON ARNIM[1] who proposed structures (1) and (2) but it is probable that in polar solvents structure (3) may make an important contribution.

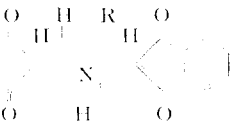
(1)



(2)



(3)



The above experimental data are consistent with the following interpretation, that pigment B is due to stabilization of structure (3) on the cellulose chains. This would account for the formation of B occurring only in aqueous media, its instability to alkali and stability to dilute acid and the immediate intensification of colour when A is converted to B on filter paper. Moreover, structure 3 when adsorbed on the linearly orientated cellulose chains could be fixed in a planar form which would be compatible with its fluorescent properties[6-8].

Due to the virtual non-extractibility of the pigment from the paper the possibility of its use for quantitative estimation of hydroxy-proline is limited to densitometric measurement on the paper. However the reaction will serve as a rapid and extremely sensitive test for hydroxy-proline (and to a less extent proline) on filter paper chromatograms.

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Evidence for a new precursor of methionine in *Neurospora crassa*

A "leaky" methionine-requiring mutant of *Neurospora crassa*, UT 27[1], accumulates a compound which promotes the growth of a series of *Neurospora* mutants blocked in different stages of methionine biosynthesis[2]. The chemical and biological properties of this compound indicate that it is a new intermediate in the conversion of homocysteine to methionine.

Mutant mycelia were obtained after growth for a period of two weeks in aerated carboys containing 2.5 liters of minimal medium. The harvested mycelia were extracted with water in a blendor and concentrated *in vacuo*. Occasional cultures exhibiting near wild type growth did not yield active extracts.