derivatives, nasutin-A has the constitution $C_{16}H_8O_7$, with no alkoxy group present. The ultra-violet absorption spectrum of the nasutin in ethanol (Fig. 1) reveals the existence of an extended chromophore and the infra-red spectrum (potassium bromide disk) shows peaks at 3,300 and 3,290 cm^{-1} ($2 \times$ phenolic OH), 3,076 cm^{-1} (aromatic CH), 1,735 and 1,705 cm^{-1} ($2 \times \alpha\beta$ -unsaturated ester or lactone). Nasutin-A dissolves in mild alkali (pH 8.5) to form a deep yellow solution from which it is reprecipitated by acid. With stronger alkali, however, the colour is rapidly discharged and acidification then affords the colourless nasutic acid (λ_{max} 223 and 313 $m\mu$ in ethanolic sodium hydroxide), which slowly reforms the nasutin on standing in contact with mineral acid (rapidly on being heated). Nasutin-A would therefore appear to have a coumarin-like structure with, probably, two phenolic groups and two lactone rings. No information is yet available concerning the nature of the remaining oxygen atom.

B. P. MOORE

C.S.I.R.O., Division of Entomology,
P.O. Box 109, City,
Canberra.

Inhibition of Antibiotic Formation by Bromthymol Blue and Other Indicators in *Streptomyces rimosus*

ANTIBIOTIC production can be altered either by the induction of stable genotypic changes through mutation of a culture or by the addition of compounds which modify the biosynthesis of the antibiotic. Alikhanian *et al.*¹ described the antagonistic effects of high inorganic phosphate levels on oxytetracycline formation and the isolation of mutants which required the addition of increased phosphate for maximal antibiotic production. Ševčík, Musilek and Komersová² examined the effect of various inhibitors of terminal oxidases on production of oxytetracycline and oxidation of glucose by washed *S. rimosus* mycelia, and found that such compounds as potassium cyanide, sodium azide and quinacrine markedly inhibited synthesis of oxytetracycline. Arsenite has also been found to inhibit production of chlortetracycline³. Gádó, Horváth and Magyar⁴ reported on the reversal of the inhibitory effect of iron on formation of oxytetracycline by methylene blue in *S. rimosus*. There is only limited information available, however, with the previously described compounds as to the specificity with which they inhibit tetracycline formation as opposed to antagonizing growth processes in general.

In this communication are summarized several observations on the inhibition of antibiotic formation by a variety of indicators—compounds which have not previously been reported to inhibit biosynthesis of oxytetracycline. Recently it has also been shown that DL-ethionine⁵ is a potent inhibitor specifically of antibiotic synthesis in *S. rimosus* without affecting cell growth.

The procedures used for all shake flask fermentations, preparation of inocula, antibiotic assays and determinations of growth were similar to those previously described⁶. Composition of the basal medium follows (g. per l.): glucose 10.0 (autoclaved separately); β -alanine 10.0; NaCl 5.0; K_2HPO_4 2.0; $MgSO_4 \cdot 7H_2O$ 1.0; $CaCl_2 \cdot 2H_2O$ 0.4; $(NH_4)_2HPO_4$ 0.2; $FeSO_4 \cdot 7H_2O$ 0.02 and $ZnSO_4 \cdot 7H_2O$ 0.01.

When bromthymol blue was used as an acid-base indicator in a fermentation medium with *S. rimosus* strain N.R.R.L. 2234, it was found that antibiotic formation was markedly inhibited. This initial observation was confirmed and extended in further experiments. Several other sulphonphthalein indicators, bromcresol green, bromphenol blue and bromcresol purple, were also tested. Of these, bromcresol green was also a potent antagonist of antibiotic formation. Other types of indicators found to be potent inhibitors of antibiotic production were methylene blue and phenolphthalein. Phenylsulphonphthalein, triphenyltetrazolium chloride and litmus were only slightly active as inhibitors (Table 1).

Table 1. SUMMARY OF THE 'INDICATOR-DYE TYPE' CHEMICAL COMPOUNDS SCREENED FOR POSSIBLE INHIBITION OF ANTIBIOTIC FORMATION WITH *Streptomyces rimosus*

Test compound*	Percentage inhibition of antibiotic yield	Percentage of inhibition of cell mass
Bromthymol blue	95	15
Bromcresol green	80	0
Bromphenol blue	21	3
Bromcresol purple	5	8
Methylene blue	89	33
Phenolphthalein	95	26
Phenylsulphonphthalein	17	0
Triphenyltetrazolium chloride	31	12
Litmus	15	0

* All test compounds were added at a level of 20 $\mu g/m.l.$

It is difficult to explain the inhibitory activity of certain compounds in the sulphonphthalein series, namely, bromthymol blue and bromcresol green, and the inability of related analogues to decrease markedly antibiotic production. When pK values are compared, one finds values of 7.0 for bromthymol blue, 4.7 for bromcresol green, 4.0 for bromphenol blue and 6.3 for bromcresol purple. No apparent correlation appears to exist between the pK values and antagonistic activity.

The recent work of Miller *et al.*⁷, in which a new co-factor, cosynthetic factor I, has been discovered in *S. aureofaciens* which acts as a hydrogenating catalyst in reducing 7-chloro-5a(11a)-dehydro-tetracycline to chlorotetracycline, would suggest that hydrogen acceptors such as methylene blue could interfere with reactions of this type. Whereas methylene blue and triphenyltetrazolium chloride are both active hydrogen acceptors, one finds that the former compound is about three times as potent in inhibiting antibiotic formation as the latter compound on an approximately equimolar basis.

These results show that bromthymol blue and several other indicators can act as highly specific inhibitors of tetracycline formation by *S. rimosus* without adversely affecting growth.

W. A. ZYGMUNT

Department of Nutritional
and Biochemical Research,
Mead Johnson Research Center,
Mead Johnson and Co.,
Evansville, Indiana.

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