Extensions of these chemical and biological studies will be detailed later.

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EVIDENCE FOR THE INVOLVEMENT OF GLUTATHIONE IN THE MECHANISM OF PENICILLIN ACTION

Sir:

Several authors have suggested the involvement of —SH groups in the antibacterial action of penicillin (see review[1]). The similarity in the molecular structure of glutathione and of penicillin[2,3] suggests the possible involvement of glutathione in the antibiotic action of penicillin. The following experiments (supported partly by the Cutter Laboratories, Berkeley, California) bear on this question.

When standard penicillin assay plates are flooded with a 1% solution of 2,6-dichlorophenolindophenol in a saturated aqueous solution of sodium bicarbonate the inhibition zones promptly stain intensely blue, and are sharply delineated from the faintly bluish uninhibited background by a narrow colorless rim that locates the ring of enhanced growth that circumscribes each zone. Similar patterns obtain on plates pretreated for five minutes with acetone, which blocks —SH groups from cysteine but not those from glutathione.[4] However, if —SH groups of glutathione are blocked by flooding the plates for ten minutes with a 10% solution of formaldehyde in saturated sodium bicarbonate the 2,6-dichlorophenolindophenol is no longer reduced to the colorless form in the ring of enhanced growth, which now stains deep blue.

The reducing activity in the regions of enhanced growth may be strikingly revealed also by flooding plates with a 0.5% aqueous solution of 2,3,5-triphenyltetrazolium chloride, whereupon these regions become intensely red, while the zones of inhibition remain uncolored. Pretreatment of the plates with 10% formaldehyde blocks this reaction. When such plates are subsequently flooded with the tetrazolium reagent, the red color fails to develop, except at the extreme outer margin of the ring of enhanced growth where it is very faint.

Such simple experiments do not themselves afford unequivocal proof of the participation of glutathione in the mechanism of penicillin action. However, it is generally assumed that —SH groups are involved. Our results indicate that some of these —SH groups are less reactive than those of cysteine, and in view of the work on the role of glutamine revealed by Gale and Taylor[5,6,7] it seems reasonable to deduce the involvement of glutathione.

(1) R. Pratt and J. Dufrenoy, *Bact. Rev.*, **12**, 79 (1948).
(2) E. Fischer, *Science*, **106**, 146 (1947).
(3) R. Pratt and J. Dufrenoy, *J. Bact.*, in press (1948).
(4) L. Genevois and P. Cayrol, *Enzymol.*, **6**, 352 (1939).
(5) E. F. Gale and E. S. Taylor, *Nature*, **158**, 676 (1946).
(6) E. F. Gale and E. S. Taylor, *J. Gen. Microbiol.*, **1**, 314 (1947).
(7) E. F. Gale, *Nature*, **160**, 407 (1947).

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IMPROVED ION EXCHANGE METHOD FOR SEPARATING RARE EARTHS IN MACRO QUANTITIES[1]

Sir:

Previous communications from this laboratory[2] described ion exchange methods by which rare earths were separated from one another in kilogram quantities. The process consisted essentially of absorbing the mixed rare earths on the top of long columns of commercial IR-100 Amberlite resin, in the acid cycle, and then eluting by means of citric acid solutions whose *p*H had been adjusted to the required value by the addition of ammonium hydroxide. While these processes represented an enormous saving, in man-hours required per gram of pure rare earth produced, over the old processes of fractional crystallization, etc., they were not ideal in the sense that when a mixture of rare earths was present, shapes of the elutions bands were such that there was a slight trailing of the preceding rare earth across the main band of the following one. This cut down the amount of pure rare earth obtained from any one pass of the column and frequently resulted in the necessity of recycling considerable quantities of the material.

Considerable work has been done in this Laboratory concerning the nature of the separation process. Good spectroscopic evidence has been obtained that at least four complexes of the rare earths with citrate solution exist and that each of these in turn becomes important as the *p*H range and citric acid concentrations are changed. Recently, it has been found that separation of the rare earths in large amounts can be markedly increased by eluting with a 0.1% citric acid solution in the *p*H range between 5.0 and 5.5. Under these conditions both the front and rear edges of the elution band (amount of rare earth eluted per liter plotted against liters of the eluant passed through the column) are steep and the tops of the eluting bands are flat. Furthermore, the bands separate from each other until the front edge of the one rare earth band is riding on the rear edge of the preceding band. Increasing the length of the column beyond the limit necessary to do this does not separate the bands any further, so there is good evidence that the one rare earth is replacing the

(1) This document is based in part on work performed under Contract No. W-7405 eng-82 for the Atomic Energy Project.
(2) Spedding, *et al.*, THIS JOURNAL, **69**, 2777, 2786, 2812 (1947).

other rare earth on the column as the material is eluted. It was also noted that in the cases tested the *p*H of the solution which comes from the column varies with the rare earth being eluted and differs by about 0.05 of a *p*H unit for adjacent rare earths. With binary mixtures of 50–50% neodymium–praseodymium and neodymium–samarium, it has been found possible to recover from 60 to 90% of each of the rare earths in such purity that the other rare earths could not be detected spectrophotometrically in these fractions.

Work is being continued and the details of this process will be presented in a paper soon to be submitted for publication.

CONTRIBUTION NO. 29
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LEAF XANTHOPHYLLS

Sir:

Recently, a violaxanthin-like xanthophyll called xanthophyll-epoxide has been reported as a new leaf pigment.[1] However, earlier observations indicate that this leaf xanthophyll is spectroscopically identical with violaxanthin, obtained originally from pansies (*Viola*).[2] Moreover, leaf violaxanthin and pansy violaxanthin are chromatographically identical in Tswett columns of magnesia or of sugar.[3]

Karrer and co-workers also claim that, in spite of other similarities, violaxanthin and leaf violaxanthin (their xanthophyll-epoxide) yield different pigments when treated with acids[1]

violaxanthin ⟶ auroxanthin
xanthophyll-epoxide ⟶ flavoxanthin.

By contrast, I have found violaxanthin from the two sources to react with acids in the following way

pansy violaxanthin ⟶ flavoxanthins ⟶ auroxanthin
leaf violaxantin ⟶ flavoxanthins ⟶ auroxanthin

Obviously, pansy violaxanthin and leaf violaxanthin are identical with respect to their reaction with acids. This xanthophyll, whether obtained from pansies or from leaves, should, therefore, be called violaxanthin, not xanthophyll-epoxide.

In spite of Karrer's assertions to the contrary,[1] numerous experiments confirm the complexity of the leaf pigment mixture. The leaves of some fifty plants, ranging from ferns to angiosperms, have yielded the following pigments: chlorophylls a and b (with traces of chlorophylls a′ and b′), neoxanthin, zeaxanthin, violaxanthin, lutein, cryptoxanthin-like pigments and β-carotene ± α-carotene. In leaves of eleven species of cycads representing six genera, taraxanthin, identical with taraxanthin from dandelions, accompanies the pigments just enumerated. In most of these plants, lutein is the principal xanthophyll, violaxanthin is slightly less abundant, neoxanthin occurs in small amounts, and zeaxanthin and the cryptoxanthin-like pigments are present in very small proportions. Traces of flavoxanthins are sometimes found in the leaf extracts.

When the pigments of fresh leaves are extracted with methanol or acetone, transferred to petroleum ether, adsorbed in columns of powdered sugar, and washed with petroleum ether containing 0.5% propanol, the following sequence of adsorbed pigments is obtained: neoxanthin, violaxanthin, (flavoxanthins), chlorophyll b, (taraxanthin), lutein plus zeaxanthin[4] plus chlorophyll b′, chlorophyll a, chlorophyll a′, crytpoxanthin-like pigments and the non-adsorbed carotenes.

1,2-Dichloroethane, formerly employed for the resolution of leaf xanthophylls by adsorption,[5] decomposes easily, especially in the presence of moisture, yielding hydrochloric acid. Unless special precautions are observed, the action of this acid on the leaf xanthophylls dissolved in dichloroethane may decrease the amount of violaxanthin and increase the amounts of flavoxanthins and isolutein.[5]

All these facts confirm the identity of violaxanthin from leaves and from pansies. They indicate that flavoxanthins can be converted into auroxanthin. They illustrate the complexity and the lability of the leaf xanthophylls. They point to precautions to be observed in the handling of leaf xanthophylls, and they illustrate problems in nomenclature arising from the use of different names for a single substance.

(1) Karrer, Krause-Voith and Steinlin, *Helv. Chim. Acta*, **31**, 113 (1948).

(2) Kuhn, Winterstein and Lederer, *Z. physiol. Chem.*, **197**, 141 (1931).

(3) Strain, Manning and Hardin, *Biol. Bull.*, **86**, 169 (1944).

(4) Strain, THIS JOURNAL, **70**, 588 (1948).

(5) Strain, "Leaf Xanthophylls," Carnegie Inst. Wash., Publ. 490, Washington 1938.

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A SYNTHESIS OF STREPTIDINE

Sir:

There has been reported[1] the synthesis of hexaacetylstreptamine from D-glucosamine by a method which establishes its configuration, and that of streptidine, as all-*trans*. We wish to record herein the conversion of hexaacetylstreptamine to streptidine sulfate monohydrate, thus completing the synthesis of the latter from D-glucosamine. Hexaacetylstreptamine was saponified with aqueous sodium hydroxide under reflux and the product was crystallized as the sulfate. The

(1) M. L. Wolfrom and S. M. Olin, Abstracts of Papers, 113th Meeting, Am. Chem. Soc., Chicago, Illinois, April 19–23, p. 5Q (1948).