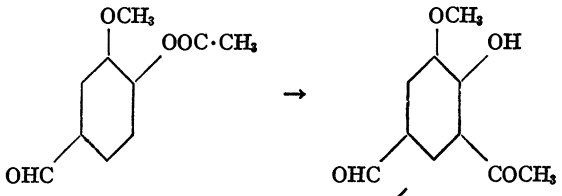
been made of a new type of polymerization reaction—the polymerization of a monomeric bifunctional ketoaldehyde. In these cases the reaction amounts to a condensation polymerization, but it could, in other cases, be an addition process.

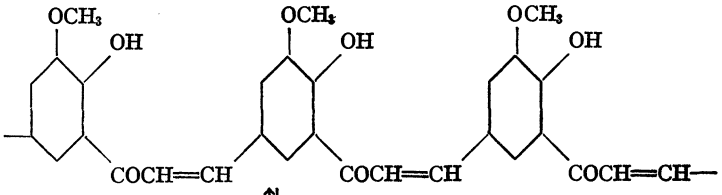
If water solubility were achieved by having enough phenolic hydroxyl groups present, materials built on the polydihydrobenzopyrone model would likely have tanning properties. It is conceivable that natural phlobatannins have just such a structure.

A detailed report of this work will appear elsewhere.

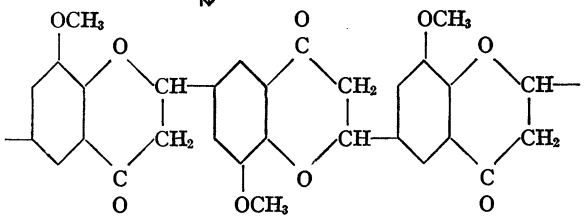
(II) → (III)



(IV)



(I)



IN THE LABORATORY

Ortho-Hydroxyphenylacetic Acid From an Amorphous Penicillin

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Recently Welch, Randall, and Price (*6*) have directed the interest of antibiotic investigators toward the significance of some impurities in certain batches of amorphous commercial penicillin. By means of a biological assay technique (*7*) which they developed these investigators were able to determine the presence of a nonpenicillin component which enhanced the activity of crystalline penicillin. Hobby, Burkhardt, Hyman, and Levert (*4*) have also demonstrated the presence of an enhancement factor in certain lots of impure penicillin.

This laboratory undertook the task of isolating and identifying the constituents in a batch of the amorphous commercial penicillin in which Welch, *et al.* had found the enhancement factor.

Since the material described by Welch, *et al.* as containing the enhancement factor was shown to be acidic by these authors, and in view of our previous results in the application of partition chromatography to the resolution of the penicillins (*2, 3*), this technique was applied to the present problem. An investigation of numerous buffer and solvent systems finally resulted in the use of the subsequent conditions as the method of choice.

The crude penicillin was extracted four times at room temperature from an aqueous pH 2 buffer solution into ether, and the combined ethereal phases were evaporated to dryness, thus destroying any penicillin present. The residue was taken up in chloroform and added to a prepared chromatographic column in which silicic acid was the adsorbent and a 20 per cent potassium phosphate buffer of pH 3.6 the immobile solvent. The precautions mentioned in an earlier report (*2*) were followed in the preparation of the column. The chromatographic fractions subsequently referred to include the colorless as well as the colored zones on the column. With chloroform as the initial mobile solvent, 10 zones were eluted from

the column. Ether was then substituted as the mobile solvent, and two additional bands were collected. Finally, an ether-butanol mixture (9:1) resulted in the development of two more zones. Each of the above fractions was analyzed by Welch, Randall, and Price (7) by their method for ascertaining the presence of the enhancement factor. A small amount of enhancement was exhibited in the third fraction, but the major portion was found in fractions 9 through 12.

TABLE 1

	Unknown	p-Hydroxyphenylacetic acid	o-Hydroxyphenylacetic acid
$FeCl_3$, test for phenols.....	Violet	Light green	Violet
M. P.....	149–150° C.	150° C.	149–150° C.
Neut. equiv	150.2–151.6	152.06	152.06
M. P. of methyl ether.....	124	85–86	124
Methyl ester.....	71–71.5	Oil	71.0

Mixed M. P. of unknown and p-hydroxyphenylacetic acid, 122–135° C.; of o-hydroxyphenylacetic acid and p-hydroxyphenylacetic acid, 122–135° C.; of unknown and o-hydroxyphenylacetic acid, 148° C.

Anal. Calculated for $C_8H_8O_3$: C, 63.13; H, 5.30. *Found:* C, 62.95; H, 5.78.

Sufficient quantities of fractions 9 through 12 were prepared for the purpose of purifying and identifying the active principle. After evaporation of the solvent the residues were all glass-like and varied from an amber to dark reddish-brown color. Crystallization was effected by dissolving the free acid in a minimum amount of ether and adding enough benzene or petroleum ether to bring the system to a point just short of precipitation or, if precipitation occurred, the mixture was

TABLE 2

INFRARED DATA

(Absorption Bands in Microns)

Unknown	o-Hydroxyphenylacetic acid	p-Hydroxyphenylacetic acid
13.75	13.75	12.60
13.18	13.18	12.10
11.75	11.75	11.95
11.42	11.42	11.62
10.60	10.60	11.09
10.32	10.32	10.41
7.85	7.85	9.80

heated slightly to clarify and crystallization initiated at room temperature. Depending on the type of crystallization employed, either needles or rosettes of needle-like crystals were recovered. A series of recrystallizations were required in order to achieve clean, white crystals exhibiting a sharp melting point at 149–150°C. From the physical and chemical tests to which the unknown and derivatives of the unknown were subjected, it became evident that either p- or o-hydroxyphenylacetic acid was the compound involved. A sample of p-hydroxyphenylacetic acid was obtained through the courtesy of Chas. Pfizer & Company, and a sample of o-hydroxyphenylacetic acid was synthesized in this laboratory. These two compounds and their derivatives were compared with the unknown and its derivatives. The data in Tables 1 and 2 demonstrate conclusively that the crystalline component separated from the active fraction of the crude penicillin used is o-hydroxyphenylacetic acid.

For the sake of brevity only the significant differences between the absorption bands of o- and p-hydroxyphenylacetic acids in the infrared are included in Table 2. The compounds were mascerated in mineral oil and the mixture analyzed in a Perkin-Elmer infrared spectrophotometer.

The o-hydroxyphenylacetic acid was prepared by a modification (5) of the procedure of Czaplicki, von Kostanecki, and Lampe (1). Preliminary experiments with o-hydroxyphenylacetic acid have not conclusively demonstrated an enhancement effect on the penicillin blood levels. These studies by Welch, *et al.* are being continued.

Studies are now in progress toward the isolation and identification of the component in active "fraction 3."

References

1. CZAPLICKI, S., VON KOSTANECKI, S., and LAMPE, V. *Ber.*, 1909, **42**, 827.
2. FISCHBACH, H., EBLE, T. E., and MUNDELL, M. *J. Amer. pharm. Ass.*, (Sci. ed.), 1947, **36**, 220–223.
3. FISCHBACH, H., MUNDELL, M., and EBLE, T. E. *Science*, 1946, **104**, 84–85.
4. HOBBY, G. L., BURKHARDT, B., and HYMAN, B. *Proc. exp. Biol. Med.*, 1946, **63**, 296–301; HOBBY, G. L., LEVERT, T. F., and HYMAN, B. (To be published.)
5. LEVINE, J., EBLE, T. E., and FISCHBACH, H. (To be published.)
6. WELCH, H., RANDALL, W. A., and PRICE, C. W. *J. Amer. pharm. Ass.*, (Sci. ed.), in press.
7. WELCH, H., RANDALL, W. A., and PRICE, C. W. (To be published.)

The Laboratory Preparation of Mustard Gas

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The current interest in mustard gas in producing gene mutations suggests the desirability of describing a convenient procedure for making and handling this material. Because of shipping restrictions it is easier to make mustard in the laboratory than to buy it. The wide variation in susceptibility to mustard between individuals or in the same individual after repeated exposures makes the handling of the material an important consideration.

Mustard can conveniently be prepared by warming $\beta\beta'$-dihydroxy ethyl sulfide (Eastman Kodak No. T1224) with concentrated hydrochloric acid. The reaction can be followed by observing the separation of a heavy oil which settles to the bottom. In practice we have used a large excess of acid in order to drive the reaction to completion. Twenty-five ml. of the $\beta\beta'$-dihydroxy ethyl sulfide may be heated with 75 ml. of concentrated hydrochloric acid at 60° C. for 30 minutes. A longer time will do no harm. The aqueous layer is then poured off, the oil being washed rapidly with a little distilled water and transferred to a storage bottle. This preparation should be carried out in a hood with good ventilation, the aqueous layer poured into a cream of bleaching powder to destroy any mustard, and the hands washed promptly with bleaching powder to remove any mustard absorbed from the gas phase.

The flask shown in Fig. 1 is convenient for storing, since it permits one to remove a sample without contaminating the air of the laboratory from the storage flask. If an aspirator is turned on before the first stopper is taken out, the downcurrent of air will sweep away any mustard diffusing out of the flask when the second stopper is removed. We have used cork stoppers covered with metal foil, since mustard is readily