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-Hydroxy-phenylacetic acid	o-Hydroxy-phenylacetic acid
FeCl₃, 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."

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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

absorbed by rubber. In fact, rubber tubing will completely remove mustard from an air stream, only to give it off later when the tube is more or less saturated.

The easiest way to obtain a given partial pressure of mustard in an air stream is probably to make a saturator by taking a U-tube, inserting a folded filter paper in each arm in such
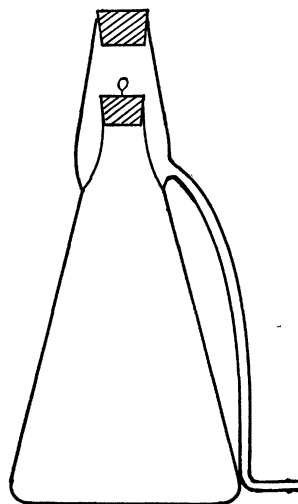

FIG. 1

a way as to give a large surface, and then pouring in enough mustard to dampen the filter paper by capillary rise. This U-tube can then be put in a beaker of water of any desired temperature below room temperature to give the desired partial pressure. We have recently submitted to the *Journal of the American Chemical Society* an article giving vapor pressure data on mustard which fit the equation $\log_{10}p = 9.4819$–$3117.2/(t + 273.1)$, in which p is the vapor pressure in millimeters and t is the centigrade temperature.

Oxidation of β-Carotene With Osmium Tetroxide

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The successful oxidation of carotene at the central double bond to form vitamin A aldehyde, accomplished by Hunter and Williams (3) using hydrogen peroxide, indicated that a catalyst was necessary to improve their low yield (0.5 per cent). Osmium tetroxide, used by Criegee (1) for the oxidation of anethole and other ethylenic compounds, seemed to have the characteristics of being such a substance, since, with hydrogen peroxide, both aldehydes and glycols are formed (4).

If one of the central double bonds can be formed into the epoxide simultaneously with formation of a diglycol at the other, then the resulting compound is identical with the postulated intermediate of the Cannizzaro reaction. This, according to Fredenhagen and Bonhoeffer (2), undergoes rearrangement prior to fission, which would account for β-carotene having half the biological value of vitamin A by weight, and corresponding lower values for other provitamin A compounds.

In applying the reaction of Criegee it has been found that a 1 per cent ethereal solution of osmium tetroxide cannot be prepared from the solid and kept, as it is all reduced in a few hours. An aqueous solution has therefore been employed. Similarly, 30 per cent hydrogen peroxide (aqueous) has been used, although in both cases the reaction proceeds faster with a slightly higher yield if the solutions used are anhydrous in the initial stages.
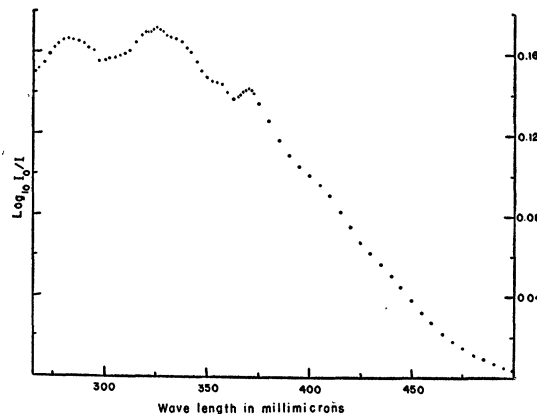

FIG. 1. Absorption curve of oxidation products of β-carotene in cyclohexane.

This work is still in progress, and the details will be published at a later date. However, a typical set of conditions for the reaction is as follows:

About 10 grams of anhydrous sodium sulfate is introduced into a 500-ml., three-necked flask fitted with a reflux condenser, a sealed stirrer, and a burette. For the initial stages the reflux condenser should be fitted with a loosely packed, calcium chloride drying tube. A 50-ml. aliquot of a solution of carotene (90 per cent β- and 10 per cent α-carotene) in anhydrous, alcohol-free ethyl ether is run in from the burette, followed by 1 ml. of a 2 per cent aqueous solution of osmium tetroxide, the solution is stirred for 5 minutes or until a color change is observed, and 2 ml. of 30 per cent hydrogen peroxide is then introduced dropwise. The solution is stirred for 10 minutes, 3 ml. of a 0.5M sodium bicarbonate solution is added slowly, and the stirring is continued until there is no further effervescence. A mixture of 50 ml. of 95 per cent ethyl alcohol and 5 ml. of 50 per cent potassium hydroxide solution is added. The solution is stirred for a further 10 minutes, then decanted into a 500-ml. separatory funnel, and 50 ml. of ethyl ether is added. The funnel is then shaken thoroughly, the lower layer run off, and the supernatant washed as in vitamin A analysis.

An aliquot of the resulting solution is dried over anhydrous sodium sulfate, evaporated under reduced pressure, and taken up in cyclohexane. The absorption spectrum of this solution shows maxima at 283, 325, and 370 mμ (Fig. 1). If the intensity of the blue color with antimony trichloride, measured at 620 mμ, is due to vitamin A, the yield is 30–40 per cent of the theoretical conversion of β-carotene to vitamin A.

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