and then started to flash (brilliant yellow green flame) at five-minute intervals. After six of these flashes the chlorination was stopped. There was a heavy deposit of carbon on the inside of the flask and condenser. Distillation of the mahogany-red reaction product gave a 32% yield of dichloro-dioxane (b. p., 58–60° (5 mm.)) instead of the expected 96.6% yield.[7] The forerun (25% of the reaction product) was dioxane and the distillation residue was a black tar, non-volatile at 250° (5 mm.). The 58–60° boiling fraction contained about 24% of a colorless solid which melted at 20–28°. Triple crystallization from ethanol raised the melting point to 30°. Wilson, et al.,[5] isolated a solid isomer of 2,3-dichlorodioxane (m. p., 30°) from a liquid product which had stood several weeks. Both our liquid and solid products were 2,3-dichlorodioxane, as proved by hydrolysis to glyoxal which was identified by means of its p-nitrophenylhydrazone and dioxime, and by conversion to the known naphthodioxanes.[1]

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A CHEMICAL ASSAY METHOD FOR PENICILLIN G

Sir:

It is now recognized that commercial penicillin is a mixture of at least four different penicillins, G, X, F and K, apparently differing markedly in efficacy, and that the less satisfactory results since May, 1944, of penicillin treatment of early syphilis may be due to the variation in the relative proportion of these penicillins, particularly to an increase in the content of less effective K in commercial penicillin.

We have developed a rapid, convenient and accurate chemical method for determining the minimum penicillin G content of clinical and "crystalline" penicillin which depends on the sparing solubility of the N-ethylpiperidine salt of penicillin G in amyl acetate–acetone mixtures.

K, F, and the degradation products of G apparently do not here interfere (penicillin X has not been tested). Although the method is most useful in definitely establishing a minimum penicillin G content, the recovery appears to be essentially quantitative when the G content is over 50% and the potency is over 800 U./mg. With highly purified crystalline sodium penicillin G, the recovery as N-ethylpiperidine salt is 98.6% (average of 11 assays).

Procedure.—By means of a 2-ml. syringe inserted through the rubber cap, the contents of a weighed penicillin vial (100,000 or 200,000 units) is transferred quantitatively to a chilled centrifuge tube, using a total of 3 ml. of ice-cold distilled water. The vial may then be opened, dried and tared. To the aqueous solution is added exactly 2 ml. of ice-cold amyl acetate saturated with

the N-ethylpiperidine salt of penicillin G (the solubility is approximately 0.6 mg./ml.). With shaking and cooling in an ice-bath, 0.5 ml. of a 20% phosphoric acid solution is added and the mixture is centrifuged. About 1.8 ml. of the amyl acetate layer containing the penicillins is removed and dried over sodium sulfate (0.1 g.) using a sintered glass micro filter crucible for the filtration of the drying agent. The pH of the spent aqueous layer should be about 2.

Exactly 1 ml. of the dried amyl acetate solution is transferred to a 10-ml. micro beaker in an ice-bath. After dilution with 1 ml. of acetone saturated with the N-ethylpiperidine salt of penicillin G (the solubility is about 2 mg./ml.) 0.5 ml. of a 10% solution of N-ethylpiperidine in amyl acetate saturated with the amine salt (about 2 mg./ml.) is added. After two hours at 0–5°, the mixture is filtered through a tared micro filter stick, washed with 1 ml. of cold acetone (saturated with amine salt), and dried in vacuo at room temperature for one hour.

The practically colorless N-ethylpiperidine salt of penicillin G melts (capillary) with decomposition at 152–154° when placed in a bath at 140° and heated 3° per minute. Anal. Calcd. for $C_{23}H_{33}O_4N_3S$: C, 61.71; H, 7.43; N, 9.39. Found: C, 61.55; H, 7.50; N, 9.51.

The physical and biological constants of the N-ethylpiperidine salt of penicillin G correspond very well with values for sodium penicillin G on a molar basis. Against S. aureus, the activity is 1328 U./mg. The ultraviolet absorption in water is $E_M = 271$ at 2575 Å. (the benzyl maximum),[1] and the optical rotation is $|\alpha|^{23}D +240°$ (1% in water).

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(1) Determined by Dr. N. R. Trenner.

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ORIENTED FILAMENTS OF AMYLOSE AND ALKALI AMYLOSE

Sir:

By deësterifying oriented potato amylose acetate we have obtained excellent fiber diagrams corresponding to A, B, V and branched chain alcohol-precipitated amylose powder patterns, and previously unreported alkali amylose. Heretofore only a B fiber pattern has been obtained.[1]

Alkali amylose is produced directly on deacetylation of clamped filaments at 25° in 2% potassium hydroxide solution in 75% methanol or ethanol or in saturated butanol. Contained alcohol is not an integral part of the fiber structure, since identical patterns are given by amylose

(1) Rundle, Daasch and French, THIS JOURNAL, 66, 130 (1944).

prepared in the three alcohols, and also (with greater difficulty) in aqueous alkali. On extracting alkali from the filaments with absolute methanol, the fiber pattern disappears, and is not restored by humidification. Soaking in 75% alcohol containing 2% alkali restores the original pattern, whereas 75% alcohol alone produces the V structure. The fiber pattern (Fig. 1) can be indexed on the basis of an orthorhombic unit cell having $a_0 = 9.0$ Å., $b_0 = 22.7$ Å. and $c_0 = 12.7$ Å. and containing twelve $C_6H_{10}O_5 \cdot (KOH)_x$ groups. x has not been established and may be variable, since the alkali uptake of the fibers giving this pattern depends on the alkali concentration in the deacetylating medium. Lithium, sodium and cesium hydroxide amylose have similar structure and composition.
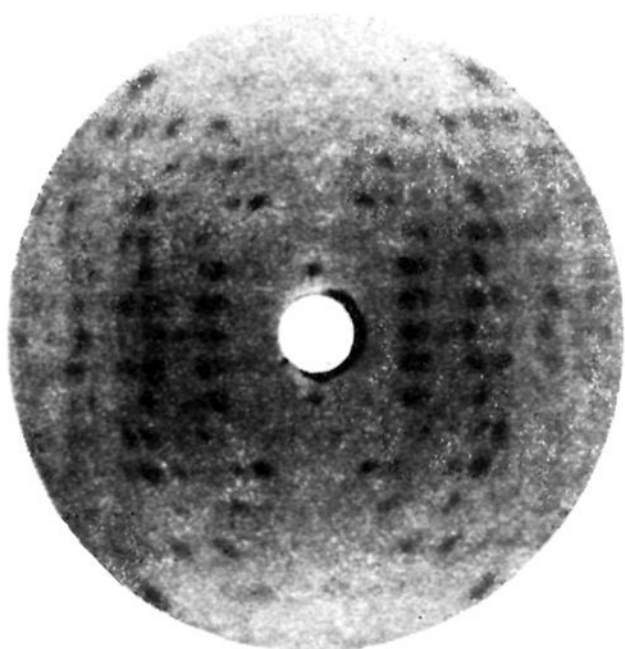

Fig. 1.—Fiber diagram of potassium hydroxide amylose. Fiber axis vertical, filtered Cu radiation, 5 cm. distance.

Transformation of the alkali amylose structure to the V structure is effected most easily in 75% methanol, allowing the clamped filaments to contract 10 to 15%. The diffraction pattern is independent of the primary alcohol and shows a fiber repeat period of 8 Å. Lateral spacings vary with moisture content. Extraction of alkali with 75% t-butanol results in a fiber pattern with similar fiber period, 7.9 Å., but lateral reflections are considerably shifted and indicate a monoclinic unit cell.

Filaments giving fiber patterns that correspond to the A (cereal starch) structure are obtained by exposing alkali amylose to high humidity (80%) for several days. The fiber identity period is 10.5 Å., at variance with the unit cell proposed[2] from powder patterns.

In saturated water vapor the A structure changes to the B (tuber starch) structure. Complete conversion and sharpest fiber patterns result from boiling vapor-treated filaments in water.

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(2) Bear and French, THIS JOURNAL, **63**, 2298 (1941).

THE STRUCTURE OF KETENE

Sir:

In a recent paper Hannay and Smyth[1] have found ketene to possess a dipole moment considerably less than those of aldehydes and ketones and have concluded that "the oxygen has much less negative charge than the ordinary carbonyl oxygen." This fact they explain in terms of four resonance structures.

In two papers[2,3] the printing of one of which has been greatly delayed owing to post-war difficulties in this country, we have been able—mainly from a study of the ionization potentials of the non-bonding electrons on the oxygen atoms—to arrange a long series of carbonyl molecules in the order of increasing C=O bond polarity. As a result it has become clear that as the polarity increases so the bond weakens, that is, the stretching force constant and bond energy decrease and the bond length increases. It is therefore not satisfactory to describe the polarity of a carbonyl bond in terms of covalent–ionic resonance, for such a description would imply an *increase* of bond strength with increasing bond polarity (up to 50% ionic character) and a greater strength in the polar than in the non-polar bond.

Our work showed that ketene had a C=O bond polarity considerably less than that of the aldehydes, with which fact the work of Hannay and Smyth is in accord. A simple and satisfactory explanation is possible without recourse to the postulation of resonance forms. It is essential to realize that the central carbon atom in ketene is really an "acetylenic" or "digonal" one, that is, it is forming two hybrid s–p bonds at 180° and two π bonds. The two π bonds have their central planes at right angles, so that conjugation does not occur between them. Now a carbon atom exerting a hybrid valency has a stronger electronegativity the greater the proportion of s character in that valency. In other words a carbon atom of a CH bond exerts a greater "pull" on those CH electrons if the carbon is part of a triple than of a single C–C bond. This statement may either be regarded as a strongly founded theoretical conclusion[4] or as a simple deduction from such experimental facts as the acidity of acetylene. It is therefore clear that the C=O bond in ketene will have considerably reduced C^+O^- polarity relative to formaldehyde. The other properties of ketene (for example, low carbonyl bond length, high carbonyl bond stretching force constant, high carbonyl bond energy, all relative to aldehydes and ketones) then follow from this reduced carbonyl polarity.[3]

Carbon dioxide is another example of a molecule containing a "digonal" carbon atom. In this case the competition of the two C=O bonds is a further factor reducing the bond polarity. Ac-

(1) Hannay and Smyth, THIS JOURNAL, **68**, 1357 (1946).
(2) Walsh, *Trans. Faraday Soc.*, **42**, 56 (1946).
(3) Walsh, *ibid.*, in press.
(4) Coulson, private communication.