COMMUNICATIONS TO THE EDITOR

CHEMICAL METHOD FOR THE DETERMINATION OF PENICILLIN

Sir:

The early observation of Abraham and Chain[1] that when penicillin is inactivated by alkali an acid group is formed affords a basis for a chemical method for the analysis of penicillin. A procedure is followed similar to the usual ester determination.

We have developed a more precise and convenient method based on the finding of Foster[2] that when penicillin is inactivated by penicillinase an acid group is formed. This reaction makes it possible to determine the penicillin content of unbuffered aqueous solutions by a simple alkalimetric titration method.

The method consists of adjusting separately to *p*H 8.0 an aliquot of penicillin of 5,000 to 20,000 units, in a volume of approximately 10 ml., and 1–2 ml. enzyme solution prepared in these laboratories, containing 1000–2000 penicillinase units.[3] One set of electrodes, in conjunction with a *p*H meter, is used for the adjustment of the penicillin solution and a second set for the adjustment of the penicillinase solution. The enzyme solution is then added to the penicillin solution and the mixture is maintained around *p*H 6.8 by gradual addition of 0.02 *N* sodium hydroxide. For the measurement of the *p*H the second set of electrodes is used. After a few minutes the penicillin is inactivated and the *p*H becomes constant. The titration is then completed by a rapid adjustment to *p*H 8.0.

When the method was tested with pure crystalline penicillin (G) recoveries were over 98%. Apparently good accuracy is also obtained with less pure commercial preparations. Salts of four manufacturers, using different processes, have been tested and the results of this method found to correspond with those obtained by the turbidimetric microbiological assay method. The precision of this chemical method was also tested extensively with several batches of penicillin salt solutions. The replicate analyses showed a dispersion in results of the order of 1%.

The method presented was found to be applicable to all aqueous penicillin solutions of potencies over 200–500 units/ml., which do not contain an excessive amount of buffer. The method was developed for the analysis of penicillin G and apparently gives accurate results with commercial preparations. As a chemical method of analysis its precision is subject to error to the extent of similar chemical analyses but such error is significantly less than that found in present microbiological assay methods. A detailed description of the method will be presented in the near future.

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(1) Abraham and Chain, *Brit. J. Exp. Path.*, **23**, 103 (1942).
(2) Foster, *Science*, **101**, 205 (1945).
(3) McQuarrie, Liebmann, Kluener and Venosa, *Arch. Biochem.*, **5**, 307 (1944).

1,4;3,6-HEXITOL DIANHYDRIDE L-ISOIDIDE

Sir:

The attempted reduction of D-isomannide and of D-isosorbide[1] to the corresponding bidesoxy derivative by hydrogenating at 200° over Raney nickel at 250 atmospheres pressure, has given, in both cases, a mixture of hexitol dianhydrides from which by benzoylation and fractional crystallization a new dianhydride of L-iditol has been isolated, identical with the crystalline dianhydride obtained by the direct acid-catalyzed anhydrization of L-iditol.

TABLE I

CONSTANTS OF DIANHYDRO-L-IDITOL DIBENZOATE

Source	M. p.,[a] °C.	Spec. rotation[b]
A = D-isomannide	111.1–111.9	$[\alpha]^{25.2}D$ 141.9 ($CHCl_3$, *c*, 2.15)
B = D-isosorbide	111.0–111.3	$[\alpha]^{23.4}D$ 140.3 ($CHCl_3$, *c*, 2.03)
C = L-iditol	110.9–111.6	

[a] All melting points are corrected. Mixed melting points among the products were undepressed. [b] Rotations are for the D line of sodium.

Assuming ring stability during hydrogenation, one or two hydroxyls, at most can be involved in the transformation. L-Iditol can be obtained from D-mannitol solely by epimerization at hydroxyls 2 and 5. Hence these hydroxyls must be free, and the anhydro rings must involve carbons 1, 3, 4 and 6. Similarly, sorbitol can yield L-iditol solely by a 5-epimerization.

The reaction is thus interpreted as a racemization resulting from the dehydrogenation and hydrogenation of the secondary carbinol groups. The reaction is being studied further, and experimental details will be published.

Since Hockett and co-workers[2] have shown that isosorbide results in high yield from the further acid-catalyzed anhydrization of both 1,4-sorbitan[3] and 3,6-sorbitan and Wiggins[4] has shown that D-

(1) Bell, Carr and Krantz, *J. Phys. Chem.*, **44**, 862 (1940).
(2) Hockett, Fletcher, Soltzberg and Goepp, Abstracts, Detroit Meeting, Am. Chem. Soc., April, 1943. Paper given before Division of Sugar Chemistry.
(3) (a) Soltzberg, Goepp and Freudenberg, Abstracts, Detroit Meeting, Am. Chem. Soc., April, 1943. Papers given before Division of Sugar Chemistry. (b) Hockett, *ibid.*
(4) Wiggins, *J. Chem. Soc.*, 4 (1945).