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.

The encouragement of Dr. A. J. Liebmann is gratefully acknowledged and similarly the competent assistance of Philip Schwed.

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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-

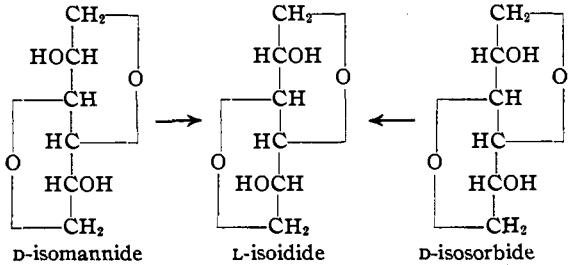
(1) Bell, Carr and Krantz, *J. Phys. Chem.*, **44**, 862 (1940).
(2) Hockett, Fletcher, Soltzberg and Goepp, Abstracts, Detroit Meeting, Am. Chem. Soc., April, 1943. Paper given before Division of Sugar Chemistry.
(3) (a) Soltzberg, Goepp and Freudenberg, Abstracts, Detroit Meeting, Am. Chem. Soc., April,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

TABLE II

DIANHYDROHEXITOLS AND THEIR DERIVATIVES

	1,4;3,6-Dianhydro-D-sorbitol (D-*iso*sorbide)	1,4;3,6-Dianhydro-D-mannitol (D-*iso*mannide)	1,4;3,6-Dianhydro-L-iditol (L-*iso*idide)
M. p., °C.	61.9–64.0	86.7–89.5	63.7–64.5
Specific rotation (H_2O)	44.8 (c, 2.22; 24.4°)	.....	20.8 (c, 2.02; 24.5°)
Specific rotation ($CHCl_3$)	32.8 (c, 2.47; 26.2°)	62.2 (c, 1.88; 26.2°)	.....[a]
Specific rotation (C_5H_5N)	64.9 (c, 2.32; 28.2°)	139.4 (c, 2.22; 28.2°)	33.27 (c, 2.24; 28.2°)
M. p. of dibenzoate	101.5–102.2	132.0–132.4	111.0–111.3
Specific rotation of dibenzoate ($CHCl_3$)	23.1 (c, 1.14; 25.0°)	225.7 (c, 1.34; 20°)	141.9 (c, 2.15; 25.2°)
Specific rotation of dibenzoate (C_5H_5N)	13.78 (c, 2.39; 28.2°)	235.9 (c, 2.12; 28.2°)	110.5 (c, 2.07; 28.2°)

[a] The compound proved too insoluble in chloroform to obtain an accurate rotation.



isomannide has the 1,4;3,6 configuration, the structures of D-isomannide, D-isosorbide, and the new L-iditol dianhydride (for which the trivial name L-isoidide is proposed) are now considered as established.

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NEW BOOKS

The Theory of Resonance and its Application to Organic Chemistry. By GEORGE WILLARD WHELAND, Assistant Professor of Chemistry, University of Chicago. John Wiley and Sons, Inc., 440 Fourth Avenue, New York, N. Y., 1944. vi + 316 pp. Illustrated. 14.5 × 21.5 cm. Price $4.50.

It has frequently been said in recent years that the concept of resonance and its concomitant functions has been the most important contribution to structural organic chemistry since the formulation of the electron pair bond by G. N. Lewis. This statement is again made in the preface to the present work by one who has had a considerable part in making it so. But, as with all basically new conceptions, attempts have been made to apply it to cases in which it is not applicable and to draw from it conclusions which it does not truly contain. The source of these misconceptions lies mainly in the mechanical analogies which have been used in the exposition of an idea that really has no complete counterpart in classical mechanics. These analogies must in the nature of things be limited and the lack of appreciation of the limitations of these analogies has led to a certain amount of futile effort to demonstrate them experimentally, and to much non-pertinent controversy both oral and written.

In this, the first book devoted entirely to an exposition of resonance and its applications in organic chemistry, the attempt is made to resolve some of the difficulties. So, in the author's own words: "A comprehensive discussion of resonance offers difficulties. On the one hand, this theory has been found to have its most interesting applications, and to be of greatest value, in the field of organic chemistry. For that reason, it should preferably be presented in terms with which the organic chemists are familiar. On the other hand, its basis lies in the mathematic depths of quantum mechanics. For that reason, it can be presented precisely and completely only in highly mathematical language. Some sort of working compromise must therefore be reached."—And he has achieved a good measure of his purpose. In the first two chapters a more or less general or abstract discussion of the principles of valence and resonance is given. These are applied in the next six chapters to a series of selected fields, namely, energy of molecules, steric effects, dipole moments, molecular spectra, chemical equilibrium, and rates of reaction.

The point of view throughout naturally follows closely that of Pauling. Most of the discussion represents a well-documented review of previously published work. There are, however, a number of points upon which entirely original opinions are expressed and in which there is still room for considerable argument. The chapters on resonance energy and steric effects are especially well done and a very useful feature of the book is the inclusion as an appendix of a table, complete to June, 1943, of interatomic distances in organic molecules. The book should certainly be in the hands of all organic chemists working in any field involving resonance interpretations.

M. CALVIN