Use of Dyes for Better Reliability in a Microbiological Assay

Keyphrases ☐ Microbiological assay—seed layer dye ☐ Dye incorporation—agar plate seed layer ☐ Seed layer detection—dye method

Sir:

Microbiological assays by agar plate methods use a seed layer containing the test organism. This seed layer is usually poured onto a base layer, which has been solidified. Under normal conditions it is difficult to observe whether a particular plate has received the seed layer or not. To solve this problem, food dyes were evaluated for coloring the seed layer to permit visual assurance that a seed layer has been added to each plate.

FD & C Blue No. 1[1] was used for coloring the seed layer in penicillin assays. A 0.1% stock solution was prepared in sterile distilled water and used 1 ml./100 ml. of seed layer. The assays were done by the standard FDA procedure[2] except that all six cylinders in each of the three plates was filled with 0.05 unit/ml. of potassium penicillin G. Three plates without dye in the seed layer served as control. The results are given in Table I. Assays were performed by using *Sarcina lutea* ATCC 9341 as the test organism.

Table I—Effect of Blue Dye in Seed Layer on Zone Size in Penicillin Assay.

Plate No.	Zone Size, mm.[a] Normal Seed Layer	Zone Size, mm.[a] Seed Layer with Blue Dye
1	19.00	18.66
2	18.33	18.83
3	19.00	19.33
Av.	18.77	18.94

[a] Average of six zones per plate.

The zones obtained with dye in the seed layer are quite comparable with those obtained without dye. Moreover, the plates containing dye are a distinct blue, thus ensuring the presence of seed layer on top of the base layer. Finally, we found that the zones in the colored and uncolored plates were equally sharp. Colored seed layers should be useful in other microbiological assays; these are under evaluation.

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[1] H. Kohnsramm & Co., Inc.
[2] Procedures for detecting and measuring penicillin contamination in drugs, Department of Health, Education and Welfare, Food and Drug Administration, Bureau of Scientific Standards and Evaluation, Division of Antibiotics and Insulin Certification, Washington, D. C., October 1965.

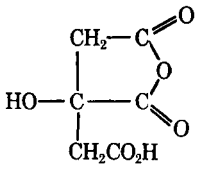
Synthesis and Isolation of Citric Acid Anhydride

Keyphrases ☐ Citric acid anhydride—synthesis, isolation ☐ NMR spectroscopy—structure

Sir:

We wish to report the synthesis, isolation, and characterization of a new crystalline derivative of citric acid. The compound which apparently has a melting point of 121–123° has been identified as the monomolecular unsymmetrical anhydride of citric acid. Several compounds are mentioned in *Chemical Abstracts* as citric anhydride, but upon further inquiry, it is found that these compounds are actually dehydrated citric acid, which is aconitic acid, or the anhydrous crystalline form of citric acid. Transient formation of a true anhydride of citric acid in aqueous solution was suggested (1–3) earlier but never isolated.

The method of preparation which has been found feasible in converting citric acid to its anhydride is based on interacting the solid acid with an excess of acetic anhydride. The critical aspect of the procedure is in controlling the reaction conditions so that neither acylation nor dehydration involving the alcohol group occurs. We have obtained relatively good yields by suspending finely powdered anhydrous citric acid in an excess of acetic anhydride in acetic acid and heating at 36–38° with good stirring. Based on its apparent equivalent weight as an acid in water, its NMR spectrum, elemental analysis, and other physical evidence, the crystalline anhydride recovered from the reaction mixture has the structure shown below (I).



I

The anhydride reacted readily with aniline to yield the expected monoanilides and hydrolyzed in water to yield citric acid. Further details on its chemistry will be presented later.

Since citric acid is so widely used in the food, drug and chemical industries, we feel that the anhydride may find similar utility. Some of the more apparent

uses would appear to be as: (*a*) a latentiated acidifier in spontaneous carbonation of aqueous systems; (*b*) a desiccant in food and drug products; (*c*) a reagent in synthesis of various citric acid derivatives.

(1) T. Higuchi, S. Eriksson, H. Uno, and J. Windheuser, *J. Pharm. Sci.*, **53**, 280(1964).
(2) J. Robinson, A. Repta, and T. Higuchi, *ibid.*, **55**, 1196(1966).
(3) A. Repta, J. Robinson, and T. Higuchi, *ibid.*, **55**, 1200(1966).

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Mass Spectrometry of Lysergic Acid Diethylamide

Keyphrases ☐ Lysergic acid diethylamide—analysis ☐ Fragmentation pattern—lysergic acid diethylamide ☐ Mass spectroscopy—analysis

Sir:

The need for sensitive and specific tests for lysergic acid diethylamide (LSD), a dangerous and widely abused drug, has led to the development of analyses based on thin-layer and gas chromatographic techniques (1–3). IR spectroscopy offers an additional criterion of identity (3, 4). The authors have found that mass spectrometry provides an unequivocal method for establishing the presence of LSD in only trace amounts. The present communication suggests the fragmentation pattern for the compound and demonstrates the forensic value of the technique.

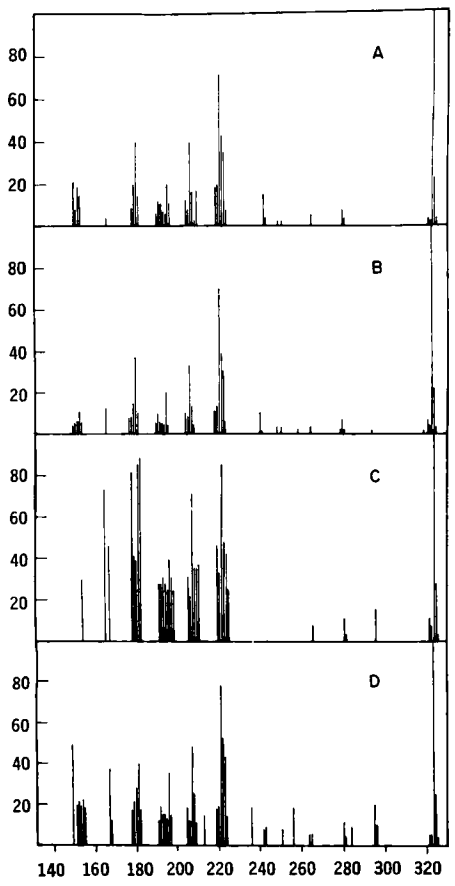

Figure 1—*Mass spectra (70 ev.) of LSD. Instrument, Perkin-Elmer RMU6D equipped with MG150A direct inlet; source temperature, 150°; inlet block temperature, 300°. Samples:* A, *LSD tartrate (5 mcg.);* B, *LSD base;* C, *sugar cube scraping (about 2 mg.);* D, *sugar cube extract.*

The mass spectrum (Fig. 1*A*) of LSD tartrate (LSD 25 of Sandoz) closely resembles that (Fig. 1*B*) of the base showing that the salt dissociates readily. Fragment ions from tartaric acid appear only at low *m/e* values (highest *m/e* value: 105, base peak 76) and therefore do not interfere with the analytically significant portion of the spectrum. In both spectra the base peak is the molecular ion M(*m/e* 323). The parent ion as well as the fragment ions tend to lose hydrogen atoms in order to acquire increased conjugation. Thus, small peaks are observed at M-1 and M-2, possibly due to cleavage of hydrogen atoms at C-4 and C-5. Spectra of aged samples (exposed to light and air) show small peaks at M-3 and M-4 also. Near the most prominent fragments (*e.g.*, at *m/e* 221, 207, 196, and 181) clusters of peaks differing in mass number by 1 are observed. Consequently all metastable peaks, except that at *m/e* 242.8, were broad with maxima at the approximate *m/e* values shown in Scheme I.

The fragmentation of LSD appears to be directed by the amido carbonyl group and the tertiary amino group, and proceeds through characteristic α- and β-cleavages of the former and α- and C,N-bond cleavages of the latter (5). Metastable peaks for some of these transitions were located and are shown in Scheme I.

The most prominent cluster of fragment ions is in the region *m/e* 221–223; these ions are formed *via* the loss of the side chain (see Scheme I). Fragment *m/e* 223 yields *m/e* 222 and *m/e* 221 by H-atom loss. A broad metastable peak at *m/e* 194 could arise from loss of CH_3 from *m/e* 222 yielding the highly conjugated moiety *m/e* 207. Other modes of fragmentation of *m/e* 223 probably involve fission of the piperidine ring. α-Cleavage of the 7,8-bond with hydrogen transfer and subsequent expulsion of CH_2—N=CH_2 produces *m/e* 181 which yields the fully conjugated species *m/e* 154. The identities of *m/e* 154 and *m/e* 167 have been established earlier (6) by high-resolution mass spectrometry. Alternative fission of the piperidine ring may proceed *via* rupture of 8,9- and 7,N-bonds, the resulting ion *m/e* 196 yielding *m/e* 167 through loss of CH_2=NH. Minor but characteristic ions appearing