mental evidences exhibit the presence of α-glycol at 8β and 14β. C-17 side chain of marsdenin (IV) may be β-H oriented from its ORD data11) (negative Cotton effect, a = −88.1). Finally, we propose the structure of (IV) for marsdenin and of (V) for ester-C as shown in Chart 1.

Acknowledgement We are grateful to Mrs. Tohma and Miss Maeda for elemental analysis. We also wish to thank Miss Kishio for NMR measurement and Hitachi Seisakusho for Mass spectrum measurement.

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo

KOJI HAYASHI
HIROSHI MITSUHASHI

Received September 6, 1968


Multi-Order Microdiffusion Analysis

The microdiffusion analysis was established by Conway and his co-workers and up to this time, the method has estimated only one component released from a sample. However, this is anyhow a special case and in general there occur two or more volatile components. If the previous method is named one order microdiffusion analysis, the following method should be called a multi-order microdiffusion analysis. Namely, the components may be estimated in a unit operation whereby to release more than two volatile components simultaneously and to absorb them in two or more absorbents respectively. The technique is generally named a "multi-order microdiffusion analysis."

In this paper, two order microdiffusion analysis was applied to two experiments; to a simultaneous determination of ethanol and carbon dioxide in alcohol fermentation, and to that of ammonia and carbon dioxide in hydrolysis of urea by urease. Theoretical speculation will be discussed in the future but from the experimental data the followings were revealed:

Apparatus: Conway-Ishizaka's semi-micro unit (the outer chamber: inner diameter 90 mm, inner height 17 mm; the inner chamber: outer diameter 65 mm, inner diameter 60 mm, inner height 8.5 mm) contains two larger cups (inner diameter 22.1 mm, inner height 12 mm) for the use of absorbents and if necessary, such as in the case of alcohol fermentation, one additional smaller cup (inner diameter 12.5 mm, inner height 12 mm) was used instead of outer chamber for the use of fermentation medium, because the culture solution decreased considerably by the strong dehydration ability of acidic oxidative absorbent.

Procedure: It was carried out according to the general operation of unit, but taking the sample solution in a smaller cup in the case of alcohol fermentation. Incubation and diffusion temperature: 30—37°.

Reagents: For the alcohol fermentation: (1) 1.0 ml of 1 N KOH for the absorbent of CO₂. (2) 1.0 ml of 2 N K₂Cr₂O₇ in 10 N H₂SO₄ for the absorbent of C₂H₅OH. (3) 0.5 ml of Henneberg's culture medium (4% saccharose) for the analytic test and 0.5 ml of Saccharomyces cerevisiae var. Sake suspension (ca. 10⁸—10¹⁰ cells/ml). For the blind test, it was treated without saccharose. (4) As the inhibitor of fermentation, 0.5 ml of 15% T.C.A was used.
For the urea hydrolysis by urease: 1 ml of 0.02 M urea, 2 ml of 0.15 M phosphate buffer and 1 ml of urease (1 mg/ml) solution for the analytic test in outer chamber. (2) For the blind test, it was used three controls. (3) 1 ml of 0.1 N KOH or 0.1 N H₂SO₄ respectively in two larger cups in inner chamber for the absorbent of CO₂ or NH₃.

Estimation: Determination of carbon dioxide and ethanol were carried out respectively by an ameliorated titration method with EDTA-Ba-Mg chelate and by a reformed spectrophotometric method of OD₉₅₀ in the estimation of alcohol fermentation, and in the case of urea hydrolysis all over by neutralization methods.

Results: In the case of alcohol fermentation: 1. The range of estimation was less than 40 mg of saccharose. 2. Fermentation required 6 hr and diffusion at least 24 hr. 3. Desirable number of yeast for fermentation of 20 mg saccharose at the 4% was more than 6×10⁸ cells and the rate of fermentation 97.5%. 4. The method was much easier and more convenient at the treatment with a number of samples than Warburg's manometry. The range of estimation was 40 times as much as that of manometry. 5. From the fundamental examination it was revealed that pH of test solution was applicable at 4—5 while the range of determination and the value of standard deviation (σ) were as follows. Ethanol: less than 22 mg and σ=1.6%. Carbon dioxide: less than 22 mg and σ=4.9%.

In the case of urea hydrolysis: 1. Incubation temperature was 37° and the incubation-diffusion time less than 20 hr. 2. The range of applicable pH was about 8.8. 3. The rate of recovery was about 96% and the value of standard deviation about 1.8%.

Faculty of Pharmaceutical Sciences,
Nagoya City University,
Tanabe-dori 3-1, Mizuho-ku, Nagoya

Received September 19, 1968

Hideko Ishihara
Otoharu Ishizaka