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 data[11] (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.

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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 occour 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 CO_2. (2) 1.0 ml of 2 N $K_2Cr_2O_7$ in 10 N H_2SO_4 for the absorbent of C_2H_5OH. (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.* 10^6—10^{10} 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.

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For the urea hydrolysis by urease: (1) 1 ml of 0.02 M urea, 2 ml of 1/15 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 H_2SO_4 respectively in two larger cups in inner chamber for the absorbent of CO_2 or NH_3.

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 OD_{585} 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 6×10^8 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%.

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