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Studies on UV-labelling Agents. I.[1] Stability of *N,N*-Dimethyl-4-diazomethyl Benzenesulfonamide

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The stability of the diazoalkane *N,N*-dimethyl-4-diazomethyl benzenesulfonamide (DDBS), used as a UV-labelling agent, was investigated.

The decomposition of DDBS in solvent depended mainly on the temperature and slightly on light and moisture. Thus, the decomposition in a benzene solution kept in a refrigerator and shielded from light and moisture was very slight. DDBS in solid form stored in container shielded from light was hardly decomposed in 30 weeks. DDBS has excellent stability compared with other UV-labelling agents for acidic substances.

Keywords——*N,N*-dimethyl-4-diazomethyl benzenesulfonamide; stable diazoalkane; UV-labelling agent; organic acid; stability of diazomethyl derivatives

Many attempts have been made to find UV-labelling agents suitable for acidic substances, especially carboxylic acids, and arylacyl halides,[2-4] methoxyaniline,[5] folytriazine agents,[6] and substituted isoureas[7] have been proposed. However, these agents are geneıally inadequate in terms of reactivity and ease of handling. Naphthyldiazoalkanes[8] are the newest reagents, but they cannot be used in solid form, and their solutions in hexane or ether are stable for only a few days.

Recently, Sekiya *et al.*[9,10] investigated stable diazoalkanes and found that *N,N*-dimethyl-4-diazomethyl benzenesulfonamide (DDBS) was available as a solid diazoalkane.

As DDBS reacts with acidic substances (Chart 1), the possible use of DDBS as a new UV-labelling agent was pointed out by Sekiya *et al.*[9,10] We have therefore investigated the stability of DDBS.

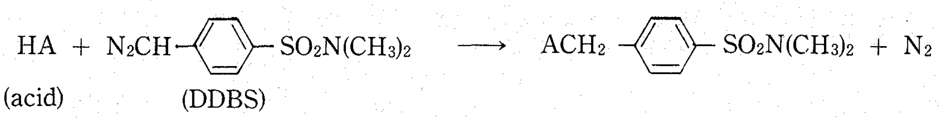

Chart 1

Experimental

Apparatus——Absorbance was measured in a cell of 10 mm optical path length with a Hitachi 124 double beam spectrophotometer equipped with a Hitachi QPD-54 recorder. Constant temperatures were obtained by using a Taiyo Thermo Unit Junior isothermo-bath or a Hitachi R462S refrigerator.

Reagents——DDBS[10] was recrystallized twice from diisopropyl ether to yield reddish-orange needles, mp 87—88°C (dec.). Benzene, diisopropyl ether, methanol and ethanol were of reagent grade, redistilled in glass apparatus.

Method——i) For Stability in Solution: DDBS in benzene or methanol solution (5 ml) was placed in a glass test tube (16 mm in inner diameter and 180 mm in length). The DDBS solution was then incubated for a definite time at a definite temperature in an isothermal bath. After that, the DDBS solution was diluted with the same solvent to a suitable concentration.

The absorbance of that solution was determined at a wavelength of 465 nm. The decomposition ratio of DDBS was calculated by determining the amount of unchanged DDBS from a predetermined analytical curve. In an experiment on the effect of moisture, a calcium chloride tube was fitted to a test tube to eliminate moisture. In an experiment on the effect of light, aluminium sheet was used to cover the test tube.

ii) For Stability in the Solid State: About 300 mg of DDBS was kept in a light-resistant glass test tube (brown-colored, 5 mm in inner diameter and 50 mm in length) with a Teflon stopper for 30 weeks.

One test tube was kept in a silica gel desiccator at 0—5°C (Ist condition) or at −10—−5°C (IInd one) or at room temperature (IIIrd one). In the fourth case, the test tube was allowed to stand in the laboratory (no air conditioning). Ten mg portions of the sample in a test tube were taken periodically for measurement of the decomposition ratio of DDBS. The sample was dissolved in benzene and the absorbance and decomposition ratio were determined as described above.

Results and Discussion

Stability in Solution

The effects of moisture and light on the stability of 5% DDBS in benzene solution at 40°C were observed. The results are shown in Table I.

At any time, the decomposition percentage of DDBS under dark-dry conditions was the lowest. However, the decomposition percentages of DDBS after incubation for 24 h under all three conditions were above 50%.

TABLE I. Effect of Light and Moisture on the Stability of 5% DDBS in Benzene Solution

Time (h)	Decomposition ratio (%)		
	Condition I	Condition II	Condition III
0.5	6.1	2.4	3.2
1.5	21.8	4.9	5.6
3.0	25.6	10.1	12.3
24.0	57.2	50.3	53.4

Each value represents the mean of 3 determinations.
Condition I: light — dry,
Condition II: dark — dry,
Condition III: dark — wet.
Details of the conditions used are given in the experimental section (method-i).

Next, the effect of temperature on the stability of 5% DDBS in benzene solution under dark-dry conditions was investigated.

As shown in Fig. 1, the decomposition of DDBS increased as the temperature became higher and the incubation time became longer. However, the decomposition percentage

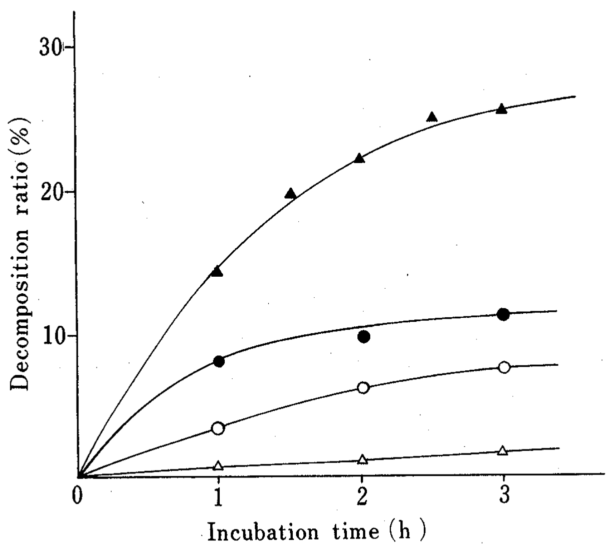

Fig. 1. Effect of Temperature on the Stability of 5% DDBS in Benzene Solution

Each point represents the mean of 3 determinations.
△—△ : 0—5°C, ○—○ : 20°C, ●—● : 40°C, ▲—▲ : 60°C.

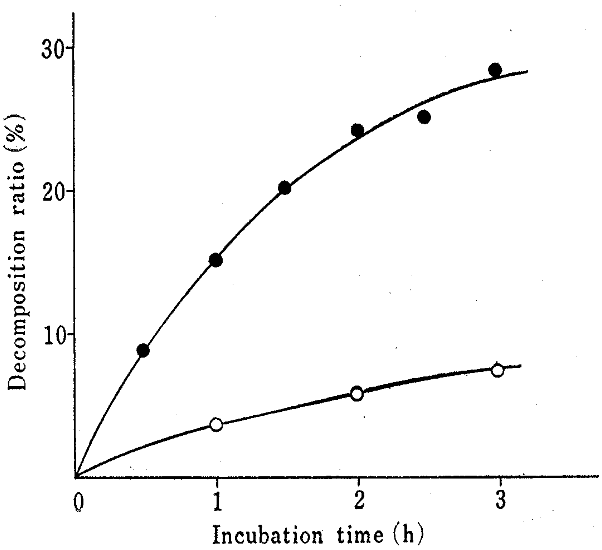

Fig. 2. Comparison of the Stability of DDBS Solutions in Benzene and Methanol

Each point represents the mean of 3 determinations.
○—○ : in benzene, ●—● : in methanol.

of DDBS in benzene solution kept in a refrigerator (0—5°C) was as low as 5.9% on the first day and 9.7% on the fourth day. The decomposition percentages of DDBS in benzene solution at each incubation time were obviously proportional to the temperature. It is clear that the decomposition of DDBS in benzene solution depends mainly on the temperature and slightly on light and moisture.

The stability of DDBS in benzene was compared with that in methanol. The experiments were carried out with 2.5% DDBS solutions at 40°C under dark-dry conditions. As shown in Fig. 2, the decomposition percentage of DDBS was lower in benzene than in methanol at any time. Therefore, it seems that the preferred solvent for the dissolution of DDBS should possess low polarity (*e.g.* benzene).

Stability in the Solid State

The results are shown in Table II. The decomposition percentage of DDBS in the case of condition IV (left in the laboratory without air conditioning) was as low as 3.9% even after 30 weeks. There was no significant difference ($p<0.05$) among the results obtained under the four conditions. This indicated that the decomposition of DDBS in the solid state was extremely low; it may be sufficient to store DDBS in a container shielded from light. In contrast, naphthyldiazoalkanes,[8] the newest derivatizing agent for the high-performance liquid chromatographic detection of fatty acids, must be synthesized just before experiments, and solutions of naphthyldiazoalkanes in hexane or ether are usable only for a few days. Therefore, it is apparent that DDBS is an extremely stable diazoalkane.

TABLE II. Stability of DDBS in the Solid State

Condition	Decomposition ratio (%)			
	5 weeks	10 weeks	15 weeks	30 weeks
I	2.4±1.44	3.4±0.75	3.4±1.56	3.4±1.62
II	2.2±1.43	2.5±0.55	2.6±2.19	1.7±0.45
III	3.4±2.76	3.6±0.99	3.5±1.60	3.9±2.32
IV	2.3±1.60	2.6±0.70	2.8±1.42	3.4±1.14

Each value represents the mean±S.D. of 5 experiments.
Conditions used are described in detail in the experimental section (method-ii)).

From the results described above, the new UV-labelling agent DDBS should be stored in solid form in a container shielded from light or kept in solution protected from light and moisture in a refrigerator.

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References and Notes

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