Journal of Chromatography, (1979) 459–461

CHROM. 11,461

Note

Simple procedure for the separation of lumiflavine and lumichrome from photolysates of riboflavine

A. T. SUZUKI, N. OHISHI and K. YAGI
Institute of Biochemistry, Faculty of Medicine, University of Nagoya, Nagoya 466 (Japan)
(Received September 13th, 1978)

Lumiflavine and lumichrome have been used as model compounds for studies on the spectral behaviour of the isoalloxazine nucleus of flavine compounds. For the large scale preparation of lumiflavine and lumichrome the procedures of chemical synthesis[1] are essential. However, on a small scale, the photolysis of riboflavine is suitable. In the latter case, each of these compounds must be separated from other photolysates. For this purpose, crystallization[2], paper chromatography[3], column chromatography either with cellulose powder[4] or with silica gel[5], and thin-layer chromatography with silica gel[6] have been adopted. These purification methods are practical for the preparation of minute amounts of the sample. Our recent studies on the physicochemical nature of flavines, such as ^{15}N nuclear magnetic resonance (NMR) spectroscopy of ^{15}N-labelled lumiflavine and lumichrome[7], required more than 10–20 mg of these samples. Thus, a separation method suitable for a larger scale is needed. This paper deals with a simple chromatographic technique which fulfils the above requirement.

EXPERIMENTAL

Diethylaminoethyl-cellulose, DE-23, was obtained from Whatman, Maidstone, Great Britain. Silica gel 60 thin-layer plates were purchased from E. Merck, Darmstadt, G.F.R.

For the preparation of lumiflavine, 10 mg of riboflavine dissolved in 300 ml of 0.5 *N* NaOH were irradiated under a fluorescent lamp for 3 h in a cold room. The solution was neutralized by adding glacial acetic acid and the photolysates were extracted with chloroform. The chloroform was evaporated *in vacuo* and the residue was dissolved in 2 l of water. This mixture of photolysates was applied to the DE-23 column (OH^-, 40 × 4.6 cm). Elution was performed with 0.5 *M* sodium acetate.

For the preparation of lumichrome, riboflavine dissolved in distilled water (10 mg per 300 ml) was irradiated under the same conditions as above, except that it was photolyzed for 100 h. The solution was directly applied to the column, and eluted with 0.9% NaCl.

Thin-layer chromatography (TLC) was performed using the upper layer of *n*-butanol–acetic acid–water (4:1:5) or acetic acid–2-butanone–methanol–benzene (5:5:20:70) as developing solvent.

Fluorescent photolysates were detected under a UV lamp ($\lambda_{max.} = 365$ nm). The concentrations of riboflavine, lumiflavine and lumichrome were determined from their absorbancies[8].

Since flavines are extremely sensitive to light, all procedures were carried out in a dark room.

RESULTS AND DISCUSSION

When an aqueous solution of the photolysates (2 l) was adsorbed on the anion exchanger, the photolysates were separated into two bands: a narrow, bright yellowish fluorescent band near the top of the column, followed by a broad, dark yellowish band. A typical chromatographic separation is shown in Fig. 1. The first fraction (I), which has strong absorption in the ultraviolet region and a weak fluorescence, was found to be eluted from the ion exchanger, DE-23, itself. The second (II) and the third (III) fractions were identified as lumiflavine and lumichrome, respectively, by their R_F values and absorption spectra. They migrated on a silica gel plate as a single spot when developed with either of the solvents mentioned above.

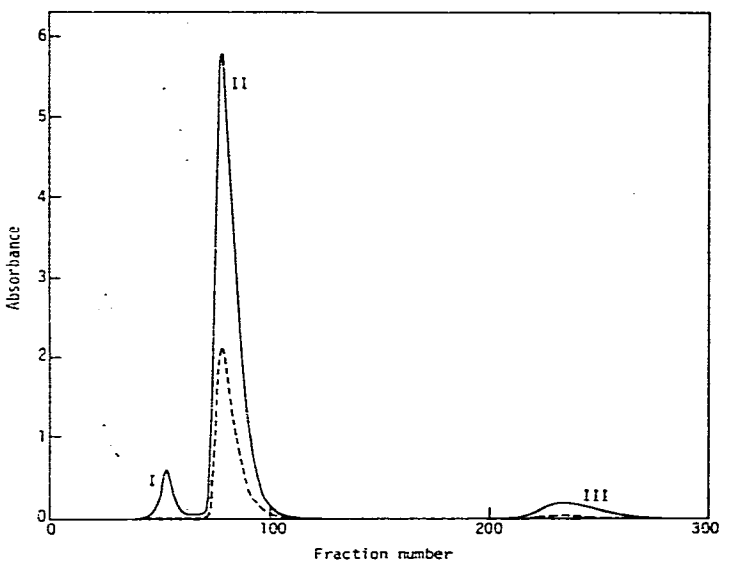

Fig. 1. Elution pattern of lumiflavine and lumichrome. An aqueous solution (2 l) of the photolysates was obtained as specified in the text and applied to the column (40 × 4.6 cm) of DE-23. Elution was performed with 0.5 *M* sodium acetate. Fractions of 10 ml were collected. The flow-rate was 7.5 ml/min. Eluates were checked by their absorbance at 265.5 nm (solid line) and at 444 nm (dotted line). I = Eluate from DE-23 column itself; II = lumiflavine; III = lumichrome.

The eluates were acidified with acetic acid, extracted with chloroform, washed twice with water and the chloroform phases were evaporated *in vacuo*. The yields of lumiflavine and lumichrome were 75% and 6%, respectively, of the initial amount of riboflavine.

It was also possible to apply the neutralized mixture of the photolysates to the DE-23 column without extraction with chloroform. In this case, the adsorption band on the column is fairly broad. By elution with 0.5 *M* sodium acetate, riboflavine and another yellow substance (probably, 10-carboxymethyl flavine[6,9]), besides the fractions shown in Fig. 1, were found to be eluted just before the peak (II) of lumiflavine. The separation of lumiflavine from these substances could be accomplished by means of the extraction with chloroform.

Another anion exchanger, DEAE-cellulose (Brown, Berlin, N.H., U.S.A.), could also be used, but the adsorption bands became wider.

For the preparation of lumichrome, the mixture of photolysates obtained by photolysis in water was directly applied to the DE-23 column. In this case, lumichrome was separated as a main peak. The yield was *ca.* 72%.

The present chromatographic technique using the DE-23 column is convenient and provides a simple separation of lumiflavine and lumichrome from photolysates of riboflavine.

REFERENCES

1 P. Hemmerich, S. Fallab and H. Erlenmeyer, *Helv. Chim. Acta*, 39 (1956) 1242.
2 J. Koziol and E. Knobloch, *Biochim. Biophys. Acta*, 102 (1965) 289.
3 J. Koziol, *Photochem. Photobiol.*, 5 (1966) 41.
4 A. Koziolowa and J. Koziol, *J. Chromatogr.*, 34 (1968) 216.
5 E. C. Smith and D. E. Metzler, *J. Amer. Chem. Soc.*, 85 (1963) 3285.
6 G. E. Treadwell, W. L. Cairns and D. E. Metzler, *J. Chromatogr.*, 35 (1968) 376.
7 K. Yagi, N. Ohishi, A. Takai, K. Kawano and Y. Kyogoku, *Biochemistry*, 15 (1976) 2877.
8 J. Koziol, *Methods Enzymol.*, 18 (1971) 253.
9 C. Fukamachi and Y. Sakurai, *Vitamins*, 7 (1954) 939.