CHROM. 4212

Thin-layer chromatography of nucleic acid bases, nucleosides, nucleotides and related compounds

VIII. Qualitative *in situ* reflectance spectroscopy*

The qualitative analysis of nucleo derivatives has been reported in previous communications. Methods such as PC[3], TLC[1–6] in connection with fluorometric *in situ* techniques[1–3,5] and preliminary reflectance spectroscopy[1] have been mentioned. (For a review see ref. 7.) In the present work a detailed qualitative *in situ* investigation of nucleo derivatives is carried out with the new Zeiss Chromatogram Spectrophotometer. The reproducibility of absorption maxima and the shape of the spectra of numerous important compounds were studied for the purposes of identification. Other factors such as shifts of maxima with varying concentrations and time stability of spectra were also investigated in order to evaluate this method as a potential quantitative tool for nucleo derivative analysis in biological materials.

Experimental

Reagents. Chromatographically pure nucleic acid bases, nucleosides and nucleotides obtainable from regular suppliers for biochemical reagents were used without further treatment for the preparation of stock solutions. Chromatographic and other solvents as mentioned under *Procedure* are of reagent grade quality. MN-300 Cellulose (Macherey, Nagel, Düren, G.F.R.) was used as coating material.

Apparatus. For reflectance measurements, the Chromatogram Spectrophotometer (Zeiss, Oberkochen, G.F.R.) was used.

Procedure. Plates of 500 μ thickness were prepared with the cellulose purified according to PATAKI[4]. Two-dimensional chromatography was carried out as described previously[4]. An UV lamp (Camag Muttenz, Switzerland) was used at 254 nm for the detection of the spots. Dark spots on a light background are observed. After location of the spots, the spectra were measured on the Zeiss Instrument (set up M–Pr[1]) against the built-in instrument standard.

Results and discussion

Spectra. The reflectance spectra of some selected compounds and the absorption maxima of all the compounds studied are presented in Figs. 1 and 2 and in Table I. It can easily be seen that the spectra can be used in most cases for identification; particularly when observed in conjunction with the chromatographic mobility[4]. For example the three AMP-compounds show the same $\lambda_{max.}$ value (Table I), but on the two-dimensional chromatogram they can be separated very distinctly[4]. On the other hand, xanthine and guanine, which are separated in biological material with difficulty only, have $\lambda_{max.}$ values differing by about 22 nm (Table I). The spectra were generally obtained with 5 μg per spot. The sensitivity limit is approximately 1 μg per spot for most substances. Bathochromic shifts were generally observed for the reflectance spectra of nucleo derivatives in the adsorbed state, in comparison with transmission spectra of

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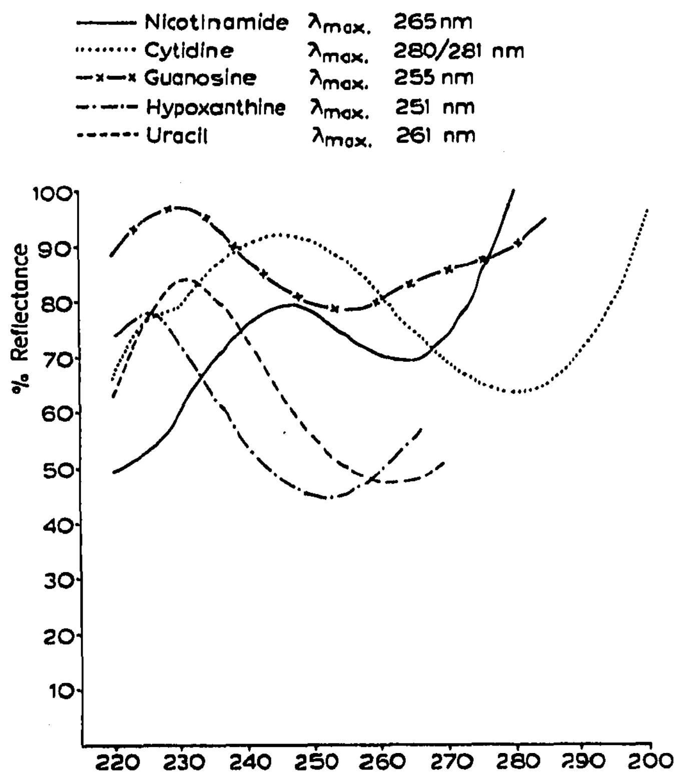
* For part VII *cf.* ref. 1.



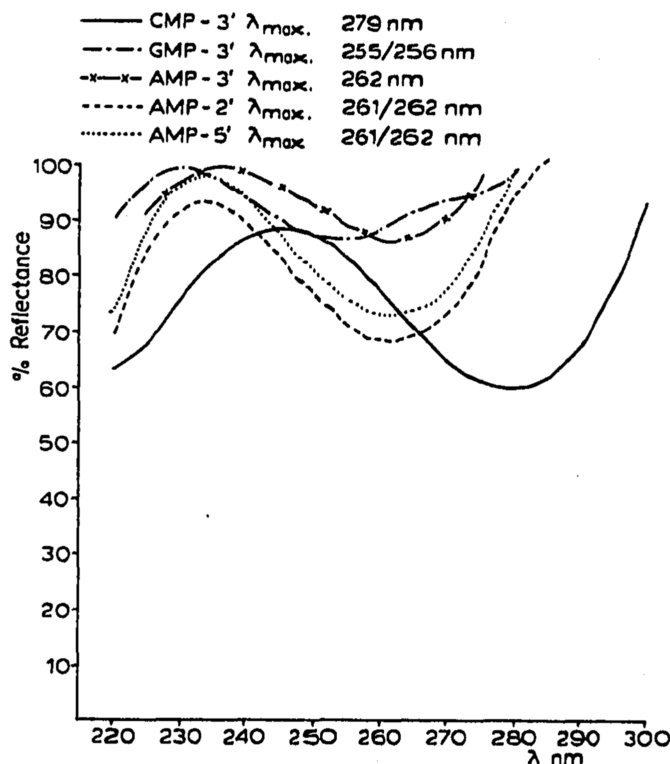


Fig. 1. Reflectance spectra of some nucleo derivatives and related compounds adsorbed on cellulose. Concn. ~ 5 µg per spot.

Fig. 2. Reflectance spectra of some nucleotides adsorbed on cellulose Concn. ~ 5 µg per spot.

TABLE I

REFLECTANCE MAXIMA OF NUCLEO DERIVATIVES AND RELATED COMPOUNDS ADSORBED ON CELLULOSE

Compound	$\lambda_{max.}$ (*nm*)	*Compound*	$\lambda_{max.}$ (*nm*)
Xanthine	270–271	Nicotinamide	265
Guanine	248/277–278	Cytidine	280–281
Uridine	265	Guanosine[a]	255
Inosine	250	AMP-3′	262
Uric acid	288	AMP-2′	261–262
Nicotinic acid	263	AMP-5′	261–262
Hypoxanthine	251	CMP-3′	279
Uracil	261	GMP-3′	255–256

[a] A wrong value was reported in the preliminary communication[1].

the same compounds in solution. This is in agreement with previous findings[1,8–10]. Reflectance spectra of some nucleotides have also been reported by LIEU *et al*[11]. The spectra were recorded on a Beckman DK-2 instrument and are in good agreement with our $\lambda_{max.}$ values.

Time study. Hypoxanthine has been studied with regard to time influence on the spectrum. After the chromatographic procedure, the spectrum was measured at various intervals over a period of 24 h. No significant change was observed, either with regard to peak shift or intensity of maxima. Fluctuations of intensities at $\lambda_{max.}$ values

over 24 h were determined from nine values and found to have a standard deviation of ± 0.82%.

Concentration study. Dilution series of hypoxanthine, thymine and uracil, ranging from approximately 0.5 to 5.0 μg per spot, were chromatographed and the reflectance spectra taken. Maximum shifts observed with changing concentrations were ± 2 nm, which makes this method suitable for quantitative work as well as for characterization. Further investigations on the quantitative analysis of this group of compounds are in progress. Sensitivity limits for the measurement of spectra were found to be between 0.5 to 1.0 μg per spot.

Reproducibility. Six individual spectra of hypoxanthine were recorded independently from separate chromatograms over a period of six days. $\lambda_{max.}$ values were found to fluctuate only by a maximum of 2 nm units, thus this technique is well suited for identification purposes.

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