THE SYNTHESIS OF RIBOSYL-CIS-ZEATIN AND THIN LAYER CHROMATOGRAPHIC SEPARATION OF THE CIS AND TRANS ISOMERS OF RIBOSYLZEATIN

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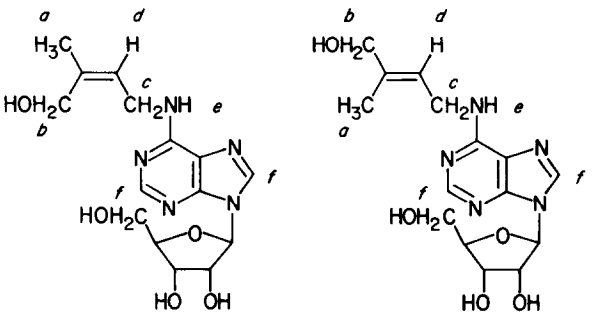
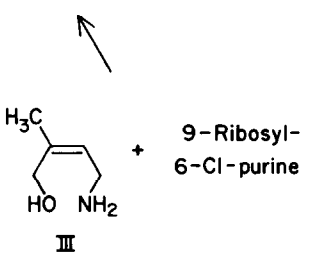
SUMMARY

Ribosyl-cis-zeatin has been synthesized. With this authentic ribonucleoside on hand for comparison, it has been shown that the cis and trans isomers of ribosylzeatin can be readily distinguished by thin layer chromatography on silica gel using chloroform-methanol (9:1) as eluant. Using this method, the ribosylzeatin from the tRNA of tobacco callus and wheat germ has been characterized as the cis isomer and that from pea epicotyls has been shown to be a mixture of both ribosyl-cis-zeatin and ribosyl-trans-zeatin.

The structure of the cytokinin isolated from the tRNA of peas, spinach, and corn (Hall et al., 1967; Hall and Srivastava, 1968; Babcock and Morris,1970)





is 6-(4-hydroxy-3-methyl-cis-2-butenylamino)-9-β-D-ribofuranosylpurine (ribosyl-cis-zeatin) (I). The assignment is based upon mass spectrometry and the isomeric relation to 6-(4-hydroxy-3-methyl-trans-2-butenylamino)-9-β-D-ribofuranosylpurine (ribosyl-trans-zeatin) (II). The latter has been isolated from immature corn kernels (Letham, 1966; Miller, 1965) and from chicory root (Bui-Dang-Ha and Nitsch, 1970), where it occurs unbound according to the evidence available.

We have now confirmed the structure of ribosyl-cis-zeatin by a stereoselective synthesis, developed a simple thin layer chromatography system for the separation of the cis and trans isomers, and utilized the tlc method to identify the stereochemistry of the ribosylzeatins obtained from the tRNA of several plant sources.

MATERIALS AND METHOD

6-(4-Hydroxy-3-methyl-cis-2-butenylamino)-9-β-D-ribofuranosylpurine (I). A solution of 500 mg of 6-chloro-9-β-D-ribofuranosylpurine, 2g of crude 4-hydroxy-3-methyl-cis-2-butenylamine (III) (Leonard et al., 1971), 10 ml of triethylamine, and 20 ml of ethanol was stirred under nitrogen for 2 hr. The precipitate which formed on standing was collected by filtration and washed successively with ethanol, ethyl acetate, and ether. Recrystallization from ethanol followed by vacuum drying afforded 220 mg (36%) of I, mp 202-205° (reported by Hall et al., 1967, 206°); $\lambda_{max}^{H_2O}$ 267 nm (ε 20,280), λ_{min} 230; $\lambda_{max}^{0.1N\ HCl}$ 264 (20,630), λ_{min} 232; $\lambda_{max}^{0.1N\ NaOH}$ 268 (20,560); λ_{min} 233. Anal. Calcd for $C_{15}H_{21}N_5O_5$: C, 51.27; H, 6.02; N, 19.93. Found: C, 51.44; H, 6.00; N, 19.88.

Thin layer chromatography was run on plates cut from Eastman Chromagram sheets (no.6060), silica gel with fluorescent indicator. Freshly prepared chloroform-methanol (9:1) served as the elution solvent. Samples of ribosylzeatin were obtained from the hydrolysates of tRNA isolated from tobacco callus (Burrows et al., 1971), wheat germ (Burrows et al., 1970), and pea epicotyls (Vreman and Skoog, work in progress). Fully characterized

synthetic samples of ribosyl-cis-zeatin (I) (see above) and ribosyl-trans-zeatin (II) (Shaw et al., 1966, 1967) were used as standards. The standards and the naturally occurring ribosylzeatin of undetermined side-chain stereochemistry were spotted together and separately on the same plate. The results are shown in Figure 1.

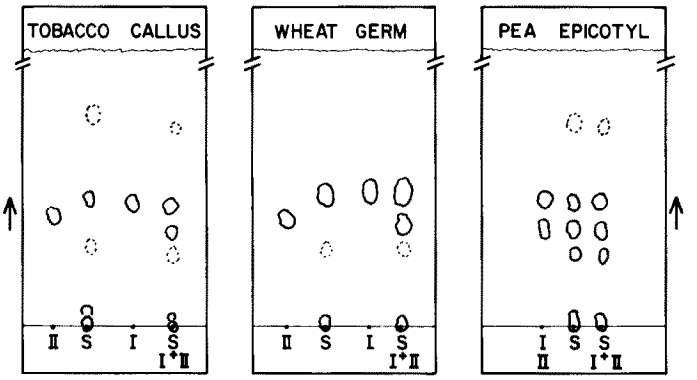


Figure 1. Thin layer chromatography on silica gel using $CHCl_3/CH_3OH$ (9/1) of ribosyl-cis-zeatin (I), ribosyl-trans-zeatin (II) and samples (S) of naturally occurring ribosylzeatins from different tRNA sources.

RESULTS AND DISCUSSION

The route which has been successful in our Laboratory for the stereoselective synthesis of cis-zeatin (Leonard et al., 1971) was adapted to the synthesis of ribosyl-cis-zeatin (I) by condensation of 6-chloro-9-β-D-ribofuranosylpurine with 4-hydroxy-3-methyl-cis-2-butenylamine (III). The product was characterized by microanalysis, melting point (202-205° vs. 206° for the modified ribonucleoside from tRNA), and mass spectrum (practically identical with that of its isomer II). The relationship between synthetic cis and synthetic trans (Shaw et al., 1966) ribonucleosides as geometrical isomers was established by their nmr spectra. The comparison in Table I, omitting the ribose moiety, has parallels in the spectra of isomer pairs of isoprene alcohols (Katzenellenbogen, 1969; Corey et al., 1970) and of cis- and trans-zeatin (Leonard et al., 1971).

Gas-liquid partition chromatography has been used for the separation of

Table I

Comparative Nmr Spectra

Protons	Cis (I)	Multiplicity		Trans (II)	Protons
a	δ1.76	s(3)		δ1.74	a
b	} 4.0-4.4	m(4)	s(2)	3.92	b
c			m(2)	4.25	c
d	5.3-5.6	m(1)		5.4-5.74	d
e	7.90	t(1)		7.98	e
f	8.25	s(1)		8.26	f
f	8.40	s(1)		8.40	f

trimethylsilylated derivatives of cytokinins, including ribosylzeatin (Upper et al., 1970; Most et al., 1968), and for the resolution of cis- and trans-zeatin ribonucleosides (Babcock and Morris, 1970). On paper chromatography, the compound assigned the cis structure was reported to migrate slightly faster than the ribosyl-trans-zeatin in several systems (Hall et al., 1967). We turned to thin layer chromatography as a convenient and widely accepted technique and found that the use of chloroform-methanol (9:1) as eluant provided a highly acceptable means of separation and identification (Figure 1).

The chromatography was run on silica gel with a fluorescent indicator and chloroform-methanol (9:1) as the elution solvent. Authentic samples of synthetic ribosyl-cis-zeatin and synthetic ribosyl-trans-zeatin were spotted separately and together. The R_f values obtained, 0.20 for cis and 0.14 for trans, are a good indication the relative mobilities of the two isomers in this system. In general, a mixture must contain about 5% of an isomer for it to be detectable.

Using both standards or a single standard we were able to identify the stereochemistry of the ribosylzeatins isolated from natural sources.

For example, our earlier identification of ribosylzeatin (Burrows et al., 1970) as a component of wheat germ tRNA did not include assignment of side-chain stereochemistry. Chromatography (Figure 1) established clearly that this sample was the cis isomer. The sample of ribosylzeatin which was isolated previously from tobacco callus tRNA (Burrows et al., 1971) was also identified as ribosyl-cis-zeatin (I). By contrast the tRNA isolated from pea epicotyls (Vreman and Skoog, work in progress) yielded a mixture of both cis and trans isomers in roughly equal amounts. This result is somewhat surprising since the trans isomer had not previously been observed as a component of tRNA. The significance of this particular finding will be considered in a sequel.

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