BBA 93371

Synthesis of α- and β-9-D-arabinofuranosyladenine by phenyl polyphosphate

β-9-D-Arabinofuranosyladenine has interesting biological properties. It is lethal to purine-requiring strains of *Escherichia coli* and prevents DNA synthesis in mouse L cells and in mouse ascites tumor cells. It produces breaks in the chromosomes of human leucocytes and inhibits the production of vaccinia and herpes virus[1]. On the other hand the α anomer is quite inactive in the biological test with *E. coli*.

Therefore simple methods are desirable for the synthesis of arabinofuranosyladenine and other arabinosyl purines. The classical method of FISCHER AND HELFERICH[2], involving the condensation of a peracylated arabinofuranosyl halide with a substituted adenine, leads only to the α anomer according to Baker's rule[3,4]. The β anomer was first prepared by rearrangement of derivatives of β-9-D-xylofuranosyladenine[5]. Later it was found that a halide of 2,3,5-tri-*O*-benzyl-D-arabinose could be condensed with *N*-benzoyladenine, predominantly in the β configuration[6,7]. Nevertheless, these methods are laborious and not very efficient. Therefore, COHEN[1] tried to condense D-arabinose and adenine using ethyl polyphosphate ester. However, the yields were low because the preliminary description[8] of this method was misleading. An improved procedure for the synthesis of adenosine and deoxyadenosine has been published recently[9] which can be successfully applied to the synthesis of α- and β-9-D-arabinofuranosyladenine.

When adenine and arabinose are heated for a few minutes in the presence of phenyl polyphosphate, prepared according to SCHRAMM AND BERGER[10], 25 % of the arabinose is transformed into a mixture of α- and β-9-D-arabinofuranosyladenine. As shown in Fig. 1, the anomers could easily be separated from each other and from small amounts of by-products by the technique of DEKKER[11].

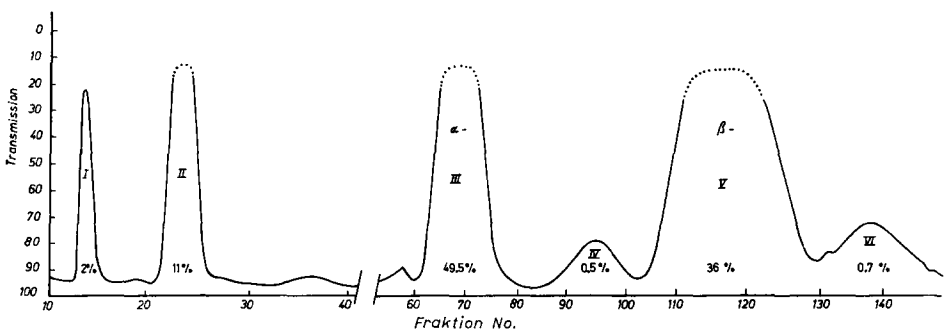

Fig. 1. Separation of α- and β-9-D-arabinosyladenine on Dowex 1 (OH^-) in 90 % methanol.

Preparation of α- and β-9-D-arabinofuranosyladenine. 5 g (37 mM) adenine were dissolved in 500 ml redistilled dimethylformamide and 3 ml conc. HCl. Phenyl polyphosphate (13 g), prepared as described previously[9,10], were added and the mixture was heated at 50° for 5 min. After addition of 1 g arabinose (7.4 mmoles) in dimethyl formamide (250 ml) the apparent pH of the solution, measured by means of a glass electrode, was 1.4. The solvent was removed by distillation under reduced pressure at 80° in a rotatory evaporator. The residue (yellow gum) was heated to 80° for 10

min and dissolved in 25 ml water. The separation of the nucleosides from adenine and acidic by-products could be accomplished by two different procedures. The final step, however, was a column chromatography according to DEKKER[11] in both cases.

(a) The aqueous solution was adjusted to pH 7 with 2 M NaOH. Phenyl polyphosphate and adenine partially precipitated and were removed by filtration. The filtrate was concentrated and adjusted to pH 10.6 with ammonia. The honey-like concentrate (20 ml) was applied immediately to a column of Dowex 1-X10 (formate) (50 cm × 3.0 cm, 200–400 mesh), equilibrated with 0.01 M ammonium formate, pH 10.6. The same solvent at pH 10.2 was used for elution. In the eluate, the ultraviolet absorption was recorded continuously on a Uvicord photometer (LBK produkter, Stockholm, type 4701 A) and fractions of 10 ml were collected. The first fractions containing polyphosphate and by-products with low R_F values were discarded. Fractions 91–240 contained a mixture of α- and β-9-D-arabinofuranosyladenine, which could be detected by paper chromatography in *n*-butanol–water. This fraction was concentrated to 20 ml for the final chromatography.

(b) To avoid the column chromatography, the aqueous solution can be neutralized with Dowex 1-X2 (OH^-). The resin was removed by filtration and washed several times with 90 % methanol. Usually 2 l 90 % methanol were needed until no nucleosides were chromatographically detectable in the extracts. The aqueous filtrate and the alcoholic extracts were concentrated to 20 ml.

The concentrates obtained by Procedure a or b were rechromatographed on a Dowex 1-X2 (OH^-) column (50 cm × 3 cm). 90 % methanol was used for elution and fractions of 10 ml were collected and the absorption recorded. A complete separation of the α and β anomers and some by-products was achieved (Fig. 1).

Peak III contained chromatographically pure α-9-D-arabinofuranosyladenine. The fractions were evaporated and the crystalline substance was recrystallized from hot water. m.p. 208°, $[\alpha]_D$: +69° (in water); literature[3] m.p.: 208°, $[\alpha]_D$: +69°.

β-9-D-Arabinofuranosyladenine appeared in Peak V. The fractions were evaporated and the residue was dissolved in a few ml of methanol. β-9-D-Arabinofuranosyladenine crystallized from the concentrated solution and could be recrystallyzed from methanol–water. m.p.: 253°, $[\alpha]_D$: −5° (in water); literature[5] m.p.: 257°, $[\alpha]_D$: −5°. Since β-9-D-arabinofuranosyladenine was only slightly soluble in water, the $[\alpha]_D$ value is somewhat uncertain.

The R_F values in *n*-butanol–water (Solvent A) were: α-9-D-arabinofuranosyladenine (Peak III) 0.31, β-9-D-arabinofuranosyladenine (Peak V) 0.28, adenine 0.50. Thin-layer chromatography in triethylamine–*n*-butanol–water–ether (1:7:2:2, by vol.) (Solvent B) gave the following R_F values: α-9-D-arabinofuranosyladenine 0.32, β-9-D-arabinofuranosyladenine 0.28, adenine 0.37. The yield (based on absorbance units) of both anomers together was 20–25 % calculated on the amount of arabinose taken, the yield of the α anomer being slightly higher (49.5 %) than that of the β anomer (36 %).

The ultraviolet spectra of the anomers in acidic and neutral solution were identical with those of the ribosyl derivatives. After hydrolysis in HCl, adenine and arabinose were obtained. The amount of by-products was small (14 % of the sum of both anomers). Table I gives the R_F values found in Solvent A and Solvent B. The ultraviolet spectra of Peaks II, IV and VI showed a maximum at 260 mμ in neutral and acidic solution. Therefore, they are probably 9-*N*- derivatives.

TABLE I

R_F VALUES IN SOLVENTS A AND B

Peak No.	*R_F value*	
	Solvent A	*Solvent B*
I	Inhomogeneous	Inhomogeneous
II	0.30	0.30
III	0.31	0.32
IV	0.15	0.17
V	0.28	0.29
VI	0.17	0.19
Adenine	0.50	0.37

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Received July 15th, 1968

Biochim. Biophys. Acta, 169 (1968) 263–265

BBA 93370

Isolation of chloroplasts free from nuclear DNA contamination

Investigations of chloroplast DNA in the past have relied heavily on the ability to separate mixtures of nuclear and chloroplast DNA's by CsCl density gradient ultracentrifugation after isolation and purification from the organelles[1,2]. The success of this technique depends not on the purification of chloroplasts free from nuclear DNA contamination but on the differences in buoyant density of the two DNA's and the ability to separate them on CsCl density gradients. In our investigations of *Vicia faba* chloroplast DNA, it was found that the chloroplast and nuclear DNA were of the same or similar densities (Fig. 1). WELLS AND BIRNSTIEL[5] report a density of 1.694–1.695 g/cm^3 for *Vicia faba* chloroplast DNA which is similar to the density found here for both chloroplast and nuclear DNA's. These results agree with the recent re-examination of buoyant density of tobacco and spinach chloroplast DNA by WHITFELD AND SPENCER[6]. Contrary to other published data, they found that the buoyant densities of tobacco and spinach chloroplast DNA's in CsCl were the same as or similar to the corresponding nuclear DNA's. The available evidence