# Isolation of a Potential Antitumor Fraction from *Rumex hymenosepalus* (*Polygonaceae*) II. Identification of the Active Fraction

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**Continued investigation into the character of the antitumor fraction of *Rumex hymenosepalus* has resulted in the identification of leucodelphinidin and leucopelargonidin as components of this fraction. The activity was shown to reside in the polymeric fraction. The monomeric fraction was shown to be inactive.**

In a previous communication (1) the authors have reported that the roots and tubers of *Rumex hymenosepalus* yielded a tannin extract which demonstrated antitumor activity. The active extract was subsequently fractionated into two components, A and B. The presence of leucoanthocyanidin units in fraction B was confirmed by the conversion of leucocyanidin to cyanidin.

Further investigation of fraction B has led to the characterization of this fraction as consisting of polymeric leucoanthocyanidin units consisting of leucopelargonidin, leucodelphinidin, as well as the leucocyanidin previously reported. In subsequent tests for antitumor activity, it was found that the activity resided in the polymeric fraction, and attempts at purification of the polymeric material did not enhance the antitumor activity.

The identification of the polymeric nature of fraction B was carried out by paper chromatographic analysis, visible spectra analysis, and degradation by potassium fusion of the anthocyanidins produced from the polymeric leucoanthocyanidins.

Subsequent investigation of fraction A has shown that this fraction consisted of monomeric leucoanthocyanidin units of the same type as was found in the polymeric condensed unit. The leucoanthocyanidins—leucocyanidin, leucodelphinidin, and leucopelargonidin—were identified by conversion to their corresponding anthocyanidins and identification was again made by means of paper chromatography, visible spectra analysis, and degradation by potassium fusion. The results of the antitumor testing of this fraction indicated that the monomeric leucoanthocyanidins were inactive.

## EXPERIMENTAL

**Conversion of Fractions A and B from Leucoanthocyanidins to Anthocyanidins**—Both fractions A (water-soluble ethyl acetate extractable fraction of the tannin extract) and B (water-soluble ethyl acetate nonextractable fraction of the tannin extract), when boiled for 15 to 30 min. in ethanolic 10% hydrochloric acid, gave red solutions, indicating that both fractions consisted of leucoanthocyanidins which were convertible to anthocyanidins.

**Paper Chromatography of Anthocyanidins**—The anthocyanidins were applied to Whatman No. 1 filter paper for chromatographic analysis. Descending chromatography was used. The chromatogram was developed for 6 hr. and travelled 18 in. The following three solvent systems were utilized as they have proved effective for identifying anthocyanidins on paper (2, 3): (*a*) formic acid–3 *N* HCl (1:1), (*b*) water–acetic acid–concentrated HCl (10:30:3), (*c*) acetic acid–concentrated HCl–water (5:1:5).

All three solvent systems indicated the presence of three anthocyanidin pigments. The $R_f$ values in solvent system (*a*) matched those described in references for delphinidin, cyanidin, and pelargonidin. The $R_f$ values obtained in solvent systems (*b*) and (*c*) verified the presence of these three anthocyanidins. The same anthocyanidins were produced from both fractions A and B. (See Table I.)

**Visible Spectra Analysis of Anthocyanidins**—Although anthocyanidins characteristically exhibit intense absorption in the 500–550 mμ, addition of a few drops of aluminum chloride solution produces a bathochromic shift (15–50 mμ) of the principal $\lambda_{max.}$ of those anthocyanidin derivatives which contain adjacent hydroxyl groups.

Papers containing the three pigments corresponding to the anthocyanidins were extracted with methanol–0.1% HCl and subjected to visible light analysis in a Beckman DB spectrophotometer. The results of this visible analysis were compared with the values obtained by Harborne (4). Each material was also analyzed after the addition of 3 drops of aluminum chloride solution to the methanolic 0.1% HCl in which they were originally extracted.

The results of this analysis (Table II) verified the pigments that had been previously identified on paper. Delphinidin showed a maximum absorption at 546 mμ and a bathochromic shift of 23 mμ when aluminum chloride solution was added. Pelargonidin showed a maximum absorption in the visible region at 520 mμ and no bathochromic shift occurred on the addition of the aluminum chloride. Cyanidin showed a maximum absorption of 535 mμ, and a bathochromic shift of 20 mμ when aluminum chloride was added.

**Degradation by Potassium Fusion of Anthocyanidins**—In order to substantiate further chemically the structure of the anthocyanidins, the anthocyanidins were degradated by fusion with potassium hydroxide (5). This procedure resulted in phenolic and acidic portions which were then subjected to paper chromatography.

**Paper Chromatography Identification of Fragments**—Commercial samples of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, and phloroglucinol were obtained, and chromatography of the degradation products of potassium fusion and commercial samples was carried out in the follow-

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TABLE I—$R_f$ VALUES OF ANTHOCYANIDINS DEVELOPED FROM FRACTIONS A AND B[a]

| Solvent System | $R_f$ Value | Color | Ref. | $R_f$ Value (2, 3) |
|---|---|---|---|---|
| (*a*) | 0.11 | Violet brown | Delphinidin | 0.11 |
| | 0.22 | Pink red | Cyanidin | 0.22 |
| | 0.50 | Light orange | Pelargonidin | 0.55 |
| (*b*) | 0.29 | Brown | Delphinidin | 0.29 |
| | 0.50 | Pink red | Cyanidin | 0.50 |
| | 0.71 | Orange | Pelargonidin | 0.71 |
| (*c*) | 0.20 | Choc. brown | Delphinidin | 0.22 |
| | 0.32 | Red | Cyanidin | 0.34 |
| | 0.55 | Light orange | Pelargonidin | 0.55 |

[a] All spots on paper responded to ammonia fumes by becoming blue or blue-gray, and upon exposure to concentrated hydrochloric acid fumes reverted to their original colors. A commercial sample of cyanidin obtained from K & K Laboratories, Inc., was used to verify $R_f$ values with those of the literature.

TABLE II—VISIBLE SPECTRA ANALYSIS OF ANTHOCYANIDINS FROM FRACTIONS A AND B[a]

| Pigment | $\lambda_{max.}$ Methanolic 0.1% HCl, mμ | $\lambda_{max.}$ with Aluminum Chloride, mμ | $\lambda_{max.}$ (4) Methanolic 0.1% HCl, mμ | $\lambda_{max.}$ (4) Aluminum Chloride, mμ |
|---|---|---|---|---|
| Delphinidin | 546 | 569 | 546 | 569 |
| Cyanidin | 535 | 555 | 535 | 553 |
| Pelargonidin | 520 | 520 | 520 | 520 |

[a] Measurement made on a Beckman DB spectrophotometer.

TABLE III—$R_f$ VALUES OF COMMERCIAL SAMPLES AND FUSION FRAGMENTS OF ANTHOCYANIDINS FROM FRACTIONS A AND B

| Sample | Solvent System (*1*) $R_f$ Spot Color | Solvent System (*2*) $R_f$ Spot Color | Solvent System (*3*) $R_f$ Spot Color |
|---|---|---|---|
| Gallic acid | 0.73 Blue gray | 0.13 Blue gray | 0.48 Blue gray |
| Fusion fragment | 0.73 Blue gray | 0.13 Blue gray | 0.48 Blue gray |
| Protocatechuic acid | 0.89 Green | 0.455 Green | 0.58 Green |
| Fusion fragment | 0.89 Green | 0.455 Green | 0.58 Green |
| *p*-Hydroxybenzoic acid | 0.773 Yellow | 0.773 Yellow | 0.685 Yellow |
| Fusion fragment | 0.95 Yellow | 0.773 Yellow | 0.685 Yellow |
| Phloroglucinol | 0.77 Light gray | 0.28 Light gray | 0.65 Light gray |
| Fusion fragment | 0.77 Light gray | 0.28 Light gray | 0.65 Light gray |

ing three solvent systems: (*1*) butanol–acetic acid–water (4:1:5) (upper phase only), (*2*) phenol–water (9:1), (*3*) 5% glacial acetic acid.

Five per cent ferric chloride solution was used as the chromogenic spray to identify the fragments produced on Whatman No. 1 filter paper. The results of the paper chromatography of the products of potassium fusion are shown in Table III. These results further verified the identity of the anthocyanidins produced from fractions A and B.

## SUMMARY AND CONCLUSIONS

It has been determined that the active antitumor fraction of ground roots and tubers of *R. hymenosepalus* consists of polymeric leucoanthocyanidin units. These units are leucocyanidin, leucodelphinidin, and leucopelargonidin. The exact method of polymerization of these units in the active fraction has not yet been determined.

Monomeric flavanoidal units of the same type contained in the polymer were investigated and found to be devoid of antitumor activity.

## REFERENCES

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