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CHEMOTAXONOMIC AND MICROCHARACTER COMPARISONS OF SELECTED SPECIES OF *LIGULARIA* AND *SENECIO* SECTION *AMPLECTENTES*

by

ROBIN REED BARR

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Approved by:

J. M. Bradley
Major Professor

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INTRODUCTION

In the highlands of central Asia (e.g., the Altai and Himalayan Mountain regions), the genus Ligularia is a highly diversified group. A marked morphological resemblance has been noted between certain Asian species of Ligularia and certain species of Senecio section Amplectentes which are found in the Rocky Mountains of the United States (Weber, 1973). This morphological resemblance is of great phytogeographical interest because it is possible the two groups are more closely related than their geographical distance would suggest; they both may have descended from common ancestors which lived in the Arcto-Tertiary forest of Miocene times.

The taxonomy of the genus Ligularia was studied in detail by Good (1929), who treated the species in two genera, Ligularia and Cremanthodium, and by Handel-Mazzetti (1939), who recognized only one genus (Ligularia), and who produced the latest word on the subject. Neither of these treatments examined any North American specimens for possible inclusion in the genus. Ligularia was considered by Handel-Mazzetti (1939) to be connected to Senecio through the genus Cacalia.

Within the Senecioneae, generic delimitation of Senecio and Cacalia has always been a problem. Traditional works on the Senecioneae have treated Senecio as a very large genus composed of several semi-distinct groups. Bentham (1873a,b) did not separate Cacalia

from Senecio, but Hoffman (1889), in Die naturlichen Pflanzenfamilien, did separate them. Subsequently, Cacalia has been variously interpreted (Rydberg, 1924; Cuatrecasas, 1955, 1960; Pippen, 1968) and regrouped (Vuilleumier, 1969; Vuilleumier and Wood, 1969).

More recently, the Senecioneae have been divided into "Cacalioid" Senecioneae and "non-Cacalioid" Senecioneae, primarily on the basis of floral micromorphological characters (Robinson and Brettell, 1973f, g,i,j; 1974a,c; Wetter, 1977). Robinson and Brettell define "Cacalioid" Senecioneae as those with the complete inside surface of the style branch covered by stigmatic area and without enlarged anther collars. They define "non-Cacalioid" Senecioneae as those with the stigmatic area longitudinally divided by a line of non-stigmatic cells and usually with enlarged anther collars. These authors also use the form of endothecial cells to segregate genera within the "Cacalioid" Senecioneae.

Ligularia, which is considered to be derived from Senecio through Cacalia, would be expected to belong with the "Cacalioid" Senecioneae.

Weber (1973) noted the resemblances between Ligularia and Senecio sect. Amplectentes and transferred several species from Senecio to Ligularia. He cited similarities in root structure, leaf size

and bases, and phyllary structure. However, the latest word on North American Senecio (Barkley, 1978) places the questioned species in Senecio. Thus, there are three ways in which the morphologically similar Ligularia and Senecio species may be viewed. They may all be considered properly in Ligularia; they may all be considered properly in Senecio; or they may be considered distinct and properly separated as Ligularia and Senecio, as has been done traditionally.

The purpose of this study is to examine some of the questioned species of Senecio sect. Amplectentes and of Ligularia using characters other than gross morphology to make clearer their relative taxonomic positions. The characters examined are flavonoid content, sesquiterpene content, external pollen morphology, and briefly, style branches, anther collars, and anther endothecial cells.

This study was made possible by T. Elias, A.S. Tomb, and W.A. Weber, who visited Central Asia in the summer of 1978 and saw and collected the Ligularia specimens used here. W.A. Weber is responsible for many speculations on the similarities in the Southern Rocky Mountain--Central Asian mountain floras (Weber, 1965, 1973).

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POLLEN STUDY

Introduction. The literature is replete with examples of the usefulness of palynological data in systematics. The utility of pollen morphology ranges from the characterization of species (Stern, 1962) to the elucidation of subtribal relationships (Tomb et al., 1974), to the characterization of an order (Nowicke, 1975), depending on the group being studied.

Within the Compositae four pollen types have been found, based on transmission electron microscopy studies of the internal wall structure (Skvarla and Turner, 1966a,b; Skvarla et al., 1977). In general, each type is associated with a given tribe or set of tribes, though the types are not entirely restricted to those tribes. Stix (1960) and Skvarla (1965) established that externally, pollen grains of most of the tribes look very similar under light microscopy; exceptions to this are the pollen of the Vernonieae, Mutisieae, and Lactuceae. Among the 10 tribes with externally similar pollen, the basic morphology is a trizonocolporate pattern with an echinate ektexine. However, there are numerous variations on this basic pollen type which are apparent under scanning electron microscopy, including differences in spine length, spine width,

acuteness of spine tips, roundedness of spine bases, pore shape, and the pattern of surface perforations (Skvarla et al., 1977); these variations have not been studied previously in a systematic way in the Senecioneae.

This study used light microscopy and scanning electron microscopy to examine the pollen structure and sculpturing of several species of Senecio section Amplectentes and two species of Ligularia to see if pollen morphology supports the relationship suggested by the gross morphology.

Materials and Methods. The species examined from sect. Amplectentes were Senecio amplectens var. amplectens, S. amplectens var. holmii, S. bigelovii, and S. crassulus (sensu Barkley, 1978). In addition, three species in Senecio sect. Triangulares were examined to see if the Amplectentes had any pollen characteristics distinct from other sections of Senecio. Senecio integerrimus was also examined for comparison with S. crassulus, which is sometimes placed in sect. Integerrimi. The Siberian species examined were Ligularia altaica and Ligularia sibirica (sensu Handel-Mazzetti, 1938) (Table 1).

Pollen was obtained from florets of herbarium specimens in the case of Ligularia sibirica and L. altaica (KSC), and from the author's field collections in all other cases. Florets were removed and placed

for at least 18 hr in 10% potassium hydroxide. The tissue was then mashed through ca 200 mesh copper screen to remove larger pieces, and the pollen and smaller pieces of other material were rinsed through the screen with ca 15 ml 10% KOH. The resulting suspension of pollen was then processed through the acetolysis method of Erdtman (1960), as modified by Faegri and Iverson (1964); after this treatment only the resistant sporopollinin exine of the outer pollen wall remains. This is what was subsequently studied by light microscopy and scanning electron microscopy. The pollen residues were stored in 70% ethanol until used.

Prior to light microscopy, the pollen residues were mixed with warm glycerin jelly on a microscope slide, and a coverslip was placed on top. The slide was allowed to cool and then made permanent by ringing the coverslip with clear nail polish. Each slide represented pollen from one population. The parameters measured were polar diameter, equatorial diameter, spine base width, and spine length; thirty measurements of each parameter were made on each slide at 800x. Mean values and their standard deviations were calculated for each parameter on each slide; these values are tabulated in Table 2.

Prior to scanning electron microscopy, the pollen residues in 70% ethanol were placed on round coverslips

cemented to aluminum stubs with silver paste; the ethanol and water were allowed to air dry, leaving the pollen residue on the coverslip. Stubs were then coated with gold-paladium alloy in a Kenney vacuum evaporator. Electron micrographs were taken at 1500x of a polar view, an equatorial view, a colpus and pore, and the mesocolpal region for each pollen sample. Also a close-up micrograph of the spines of the mesocolpal region was taken at 5000x. This microscopy was done on the Kansas Agricultural Experiment Station's ETEC Autoscan U-2.

Results and Discussion. All pollen samples studied showed the most typical Compositae pollen sculpturing; all were trizonocolporate with an echinate surface (Figs. 1-11). Three minor kinds of variations on this basic theme in pollen morphology were found. There were variations in the size of the grain, in the length of the spines, and in the pattern of perforations around the spine bases.

Mean values for the measurements of the equatorial and polar diameters are shown in Table 2. These values demonstrate that the grains are all spherical and that most of the Senecio species are statistically the same size (28-30 um in diameter), with only two exceptions. The Ligularia species were significantly larger (33-36 um in diameter) than the average Senecio. The two

exceptions among the Senecio species were S. crassulus, which was smaller (26 um in diameter), and one population of S. amplectens var. holmii, which was larger (33 um in diameter) like the Ligularia species. The range in size variation is apparent from the SEM photomicrographs (Figs. 12-14).

Mean values for the measurements of spine length are also found in Table 2. These values show Ligularia species tend to have longer spines (4.1-5.2 um). Three samples of Senecio species had particularly short spines (S. triangularis, 2.6 um; S. crassulus, 2.7 um; and S. amplectens var. holmii, Barr 33a, 2.8 um). Figs. 15-16 demonstrate this variation.

Table 2 also shows mean values for the measurements of spine base width. This parameter is difficult to measure in a consistent manner in the light microscope. The resulting values showed some variation in base width, but most values are not statistically different.

Another character which did show variation was the pattern of perforations around the spine bases. In all the Senecio sect. Amplectentes and Ligularia species, the perforations appeared as rather large, irregular holes in the lower portion of the spines (Figs. 17-18). In two of the Senecio sect. Triangulares species, these perforations were interconnected to give a very rough, erose appearance to the spine bases (Figs. 19-20).

Senecio integerrimus (sect. Integerrimi) showed no features to distinguish it from the members of sect. Amplectentes. Thus, the position of S. crassulus in relation to these two sections was not clarified.

Fortuitously, a few broken pollen grains occurred in three of the pollen samples; this allowed examination of some of the internal wall structure under SEM. Broken grains were found of S. amplectens var. holmii, Ligularia sibirica, and L. altaica. The two Ligularia species appear to have the same internal wall structure, which is either the Senecioid or the Helianthoid type (Figs. 21-23); these two types are distinguished primarily by the absence or presence, respectively, of internal foramina in the tectum, and this feature cannot be seen consistantly under SEM. However, both types occur in the genus Senecio. The internal structure of Senecio amplectens var. holmii looked quite similar to that of the Ligularia species but showed more extensive disruption of the inner endexine, a characteristic of the Senecioid type (Fig. 24).

In conclusion, all Senecio and Ligularia species examined were found to be quite alike in external pollen morphology. Variation was found in the grain size, the spine length, and the pattern of perforations around the spine bases. The Ligularia pollen was found to be at the upper end of the size and spine

length ranges exhibited by the Senecio sect. Amplectentes species. In the pattern of perforations around spine bases, Ligularia pollen of the two species examined was more like species of sect. Amplectentes than were the species of sect. Triangulares. Thus, the external pollen morphology neither conclusively supports nor denies the suggested relationship between Senecio sect. Amplectentes and Ligularia.

TABLE 1

LIST OF TAXA, POPULATIONS, AND VOUCHERS
POLLEN STUDY

Ligularia altaica DC, Elias, Weber, and Tomb 4748 (KSC)
L. sibirica (L.) Cass., Elias, Weber, and Tomb 4866 (KSC)
Elias, Weber, and Tomb 4849 (KSC)
Senecio amplectens A. Gray var. amplectens, Barr 33b (KSC)
S. amplectens var. holmii (Greene) Harrington,
Barr 23 (KSC)
Barr 33a (KSC)
S. bigelovii var. hallii A. Gray, Barr 27 (KSC)
S. crassulus A. Gray, Barr 14 (KSC)
S. integerrimus var. exaltatus (Nuttall) Cronquist,
Barr 15 (KSC)
S. fremontii var. blitoides (Greene) Cronquist,
Barr 34 (KSC)
S. triangularis Hooker, Barr 32 (KSC)
S. serra var. admirabilis (Greene) A. Nelson, Barr 31 (KSC)

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TABLE 2

POLLEN GRAIN MEASUREMENTS
(Micrometers)

Taxon	Mean Eq.* Diam.	S.D.** Eq. Diam.	Mean Polar Diam.	S.D. Polar Diam.	Spine Base Width	S.D. Base Width	Spine Length	S.D. Spine Length
L. altaica	34	1.9	34	1.9	4.6	0.96	4.1	0.53
L. sibirica								
Elias 4866	33	1.4	32	1.5	4.6	0.63	4.1	0.38
Elias 4849	36	1.6	35	1.7	5.8	0.77	5.2	0.54
S. amplectens								
var. ampl.	29	0.95	30	1.4	3.3	0.67	3.1	0.64
S. amplectens								
var. holmii								
Barr 23	33	1.8	32	1.3	4.5	0.75	3.8	0.49
Barr 33a	29	1.2	29	1.5	4.1	0.79	2.8	0.49
S. bigelovii	29	1.5	29	1.6	3.6	0.57	3.6	0.67
S. crassulus	26	1.2	25	1.3	4.0	0.50	2.7	0.34
S. integerrimus	30	2.5	29	2.0	4.4	0.61	3.2	0.58
S. fremontii	28	1.6	28	1.3	4.5	0.59	3.5	0.46
S. triangularis	29	1.6	29	1.6	4.0	0.37	2.6	0.30
S. serra	29	1.3	28	1.1	3.9	0.52	3.4	0.53

* Equatorial Diameter
** Standard Deviation

SEM Micrographs
of Pollen Grains of *Ligularia* spp. and *Senecio* spp.,
Colpus View

Figure 1. *Ligularia altaica*, Elias, Weber, and Tomb 4748 (KSC).

Figure 2. *L. sibirica*, Elias, Weber, and Tomb 4849 (KSC).

Figure 3. *Senecio amplectens* var. *amplectens*, Barr 33b (KSC).

Figure 4. *S. amplectens* var. *holmii*, Barr 23 (KSC).

Figure 5. *S. amplectens* var. *holmii*, Barr 33a (KSC).

Figure 6. *S. bigelovii*, Barr 27 (KSC).

Figure 7. *S. crassulus*, Barr 14 (KSC).

Figure 8. *S. integerrimus*, Barr 15 (KSC).

Figure 9. *S. fremontii*, Barr 34 (KSC).

Figure 10. *S. triangularis*, Barr 32 (KSC).

Figure 11. *S. serra*, Barr 31 (KSC).

Line = 10 micrometers.

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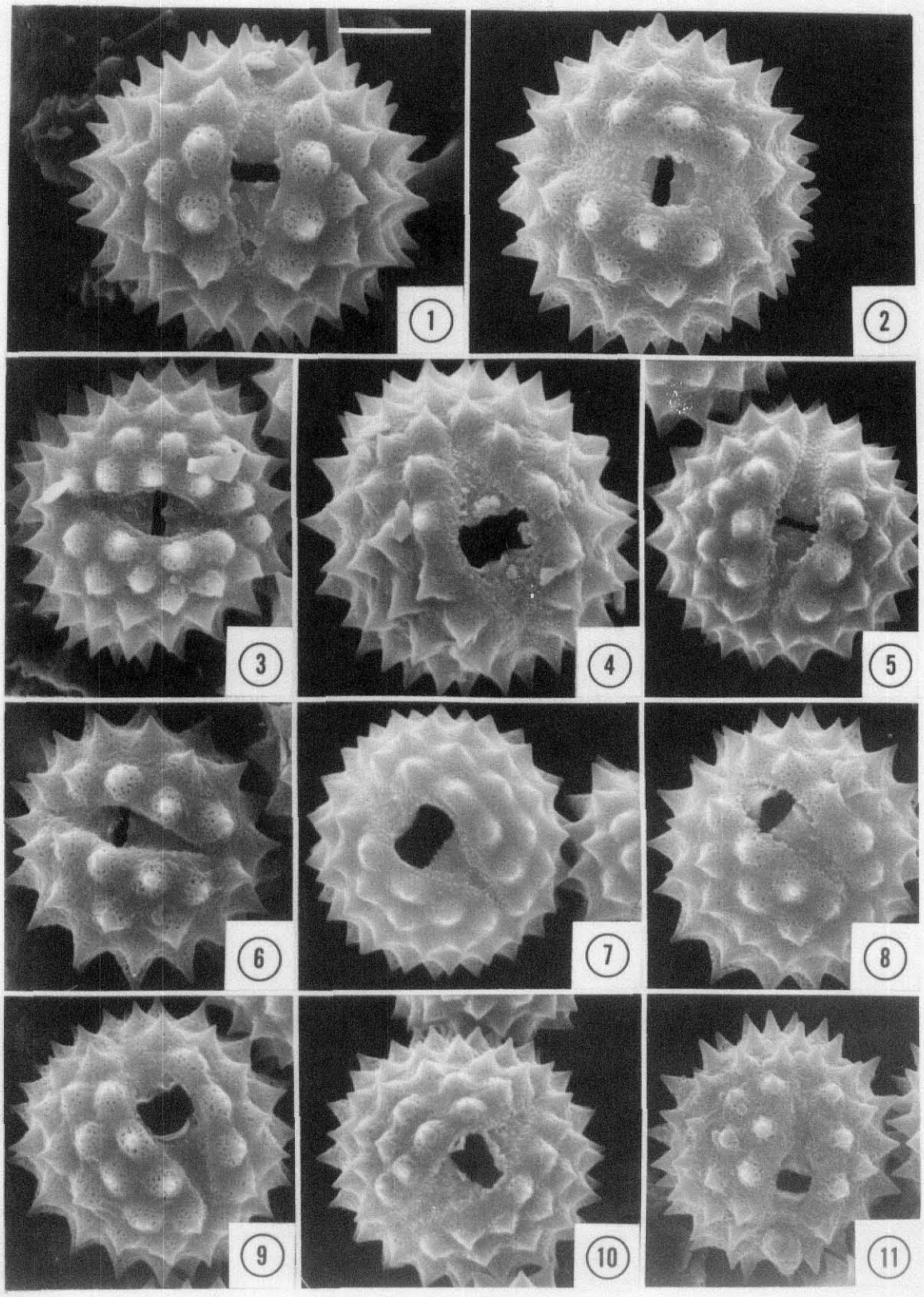
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Pollen of *Ligularia* spp. and *Senecio* spp.

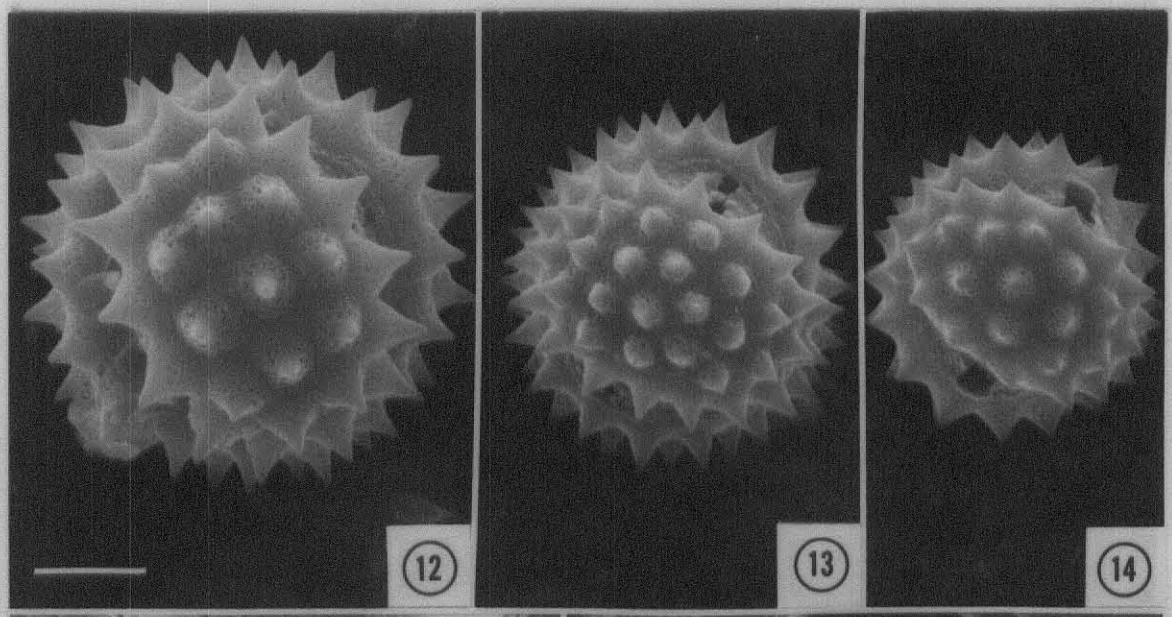
Figure 12. SEM micrograph of a pollen grain of *L. altaica*, equatorial view, line = 10 micrometers. *Elias*, *Weber*, and *Tomb* 4748 (KSC).

Figure 13. SEM micrograph of a pollen grain of *S. amplectens* var. *amplectens*, equatorial view, line = 10 micrometers. *Barr* 33b (KSC).

Figure 14. SEM micrograph of a pollen grain of *S. crassulus*, equatorial view, line = 10 micrometers. *Barr* 14 (KSC).

Figure 15. SEM micrograph of pollen surface spines of *L. sibirica*, mesocolpal region, line = 1 micrometer. *Elias*, *Weber*, and *Tomb* 4849 (KSC).

Figure 16. SEM micrograph of pollen surface spines of *S. crassulus*, mesocolpal region, line = 1 micrometer. *Barr* 14 (KSC).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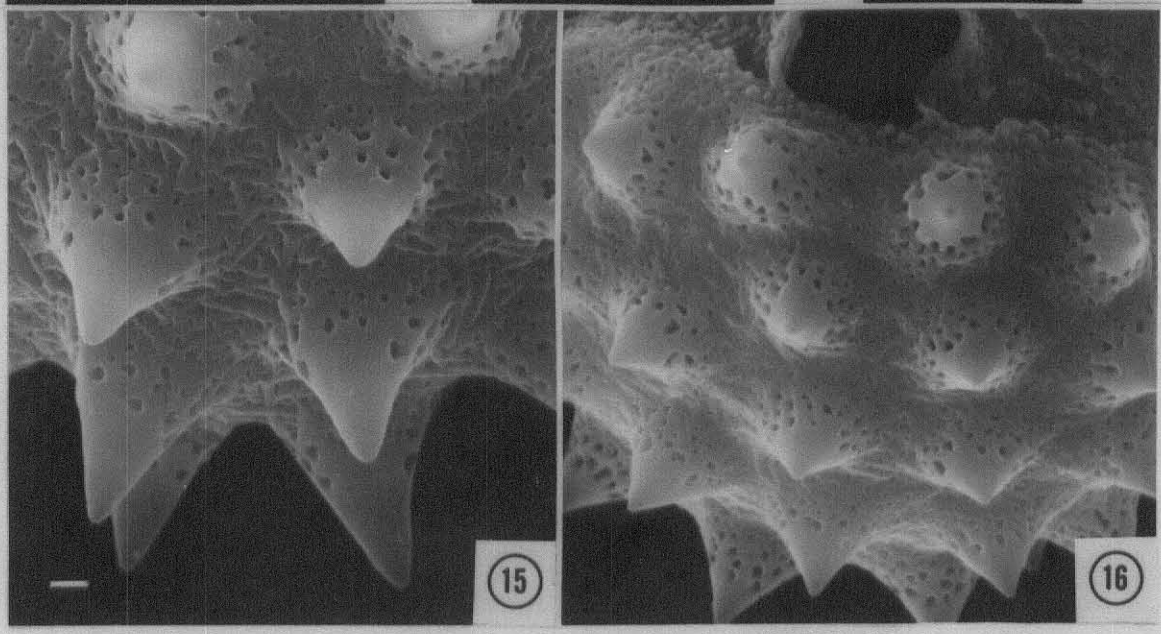
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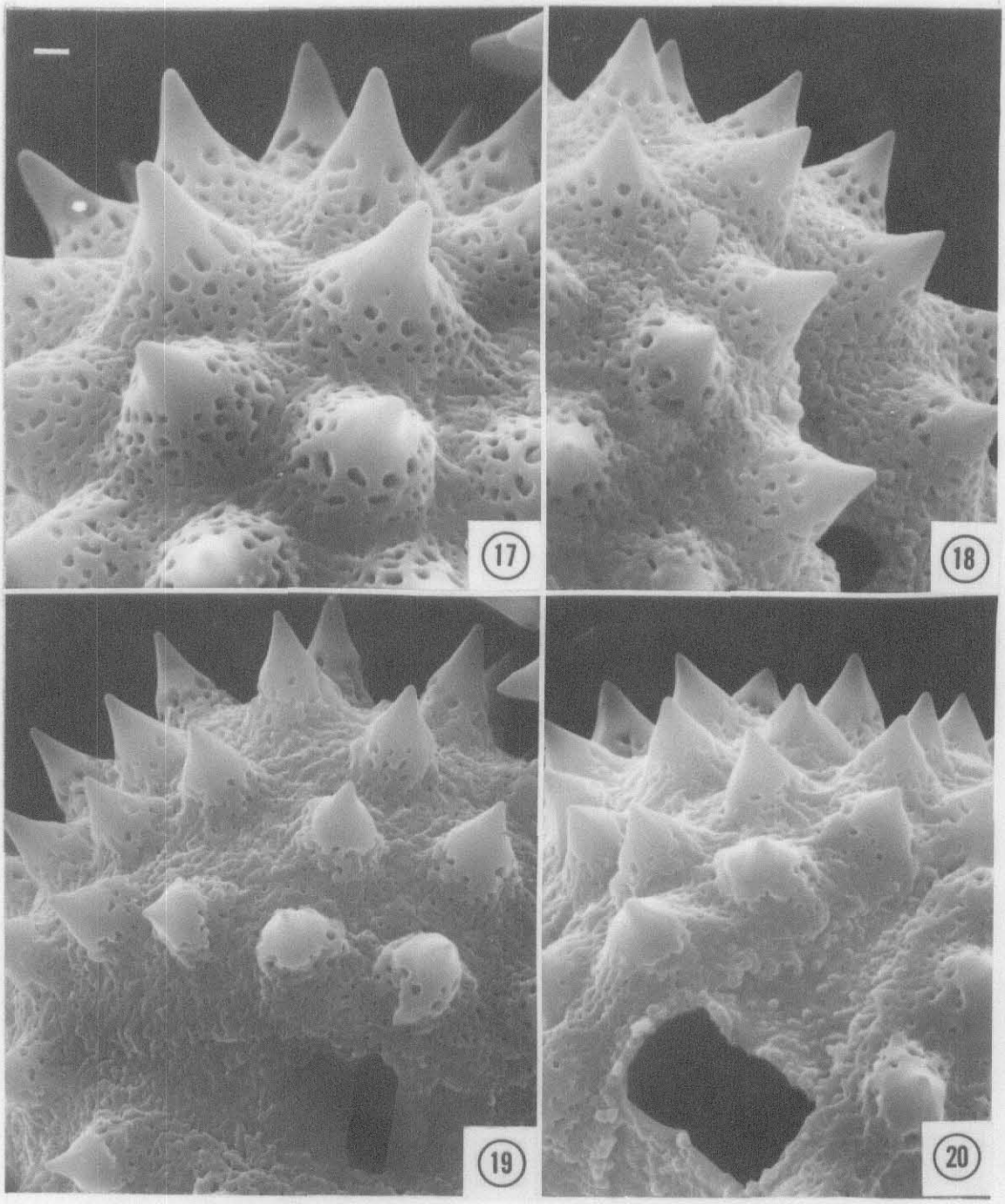
Pollen of *Senecio* spp. and *Ligularia altaica*

Figure 17. SEM micrograph of pollen surface of *Ligularia altaica*, mesocolpal region, line = 1 micrometer. *Elias*, *Weber*, and *Tomb* 4748 (KSC).

Figure 18. SEM micrograph of pollen surface of *S. bigelovii*, mesocolpal region, line = 1 micrometer. *Barr* 27 (KSC).

Figure 19. SEM micrograph of pollen surface of *S. serra*, mesocolpal region, line = 1 micrometer. *Barr* 31 (KSC).

Figure 20. SEM micrograph of pollen surface of *S. triangularis*, mesocolpal region, line = 1 micrometer. *Barr* 32 (KSC).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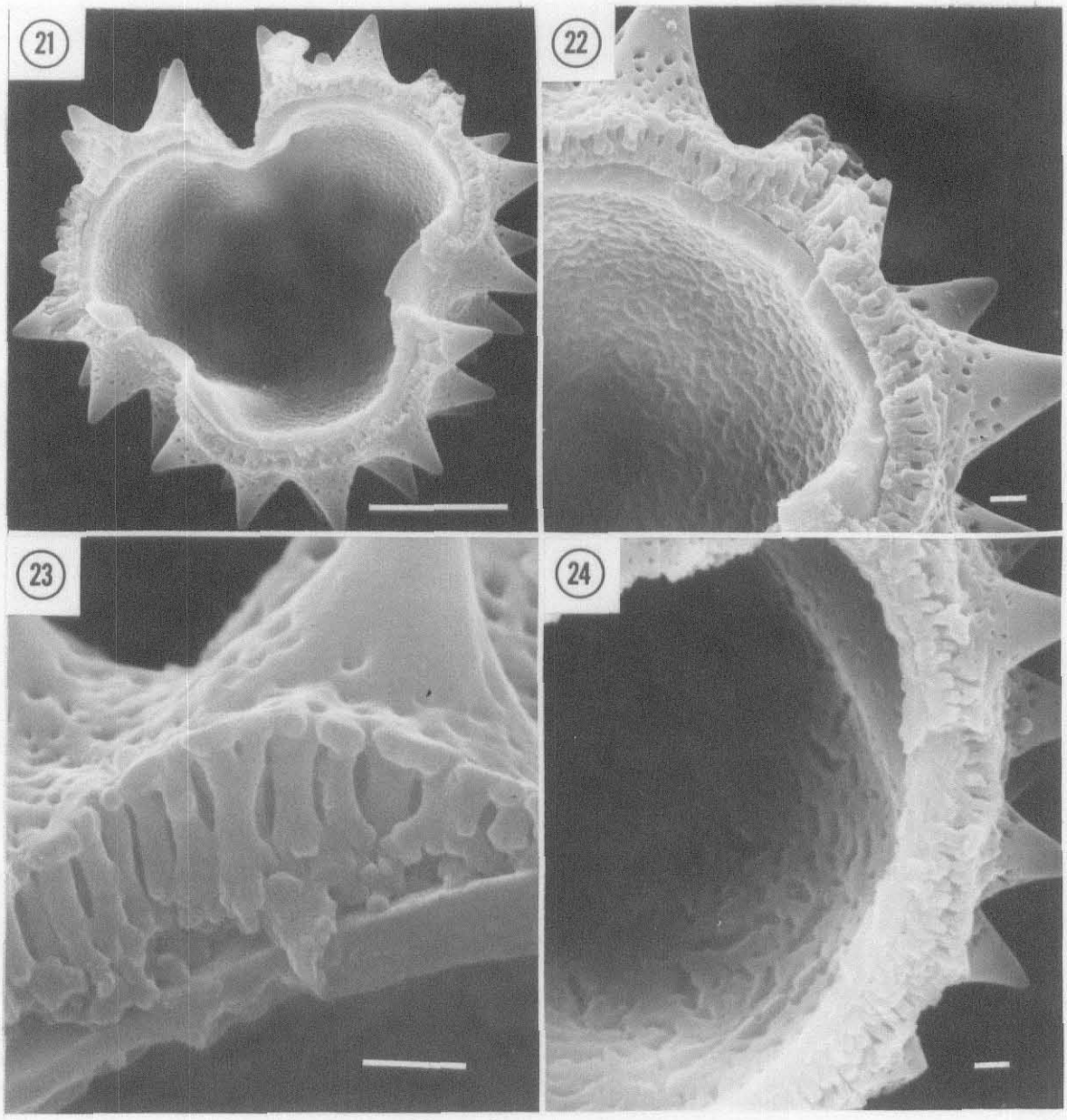
Pollen of *Ligularia* spp.
and *Senecio amplectens* var. *holmii*

Figure 21. SEM micrograph of a broken pollen grain of *L. sibirica*, polar view of equatorial wall, line = 10 micrometers. *Elias*, *Weber*, and *Tomb* 4849 (KSC).

Figure 22. SEM micrograph of pollen internal wall structure of *L. sibirica*, line = 1 micrometer. *Elias*, *Weber*, and *Tomb* 4849 (KSC).

Figure 23. SEM micrograph of pollen internal wall structure of *L. altaica*, line = 1 micrometer. *Elias*, *Weber*, and *Tomb* 4748 (KSC).

Figure 24. SEM micrograph of pollen internal wall structure of *S. amplectens* var. *holmii*, line = 1 micrometer. *Barr* 23 (KSC).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FLORAL MICROSTRUCTURE STUDY

The genus Ligularia is considered by Handel-Mazzetti (1939) to have been derived from Senecio through Cacalia. However, the generic delimitation of Cacalia from Senecio has long been a problem. Recently some progress has been made at distinguishing the two genera using floral micromorphological characters. In fact, two larger groups of "Cacalioid" and "non-Cacalioid" species have been distinguished within the Senecioneae (Robinson and Brettell, 1973f,g,i,j, 1974a,c; Wetter 1977). The characters considered useful for such a distinction are the style branch stigmatic areas, the anther collars, and the anther endothecial cells (Nordenstam, 1978). In "Cacalioid" species, the stigmatic area is continuous, the anther collars are not enlarged, and the endothecial cells have polar thickenings. In "non-Cacalioid" species, the stigmatic area is divided longitudinally by a line of non-stigmatic cells, the anther collars are usually enlarged, and the endothecial cells have radial thickenings. It was hoped that the examination of these characters would make clearer the relative taxonomic positions of the questioned species of Senecio sect. Amplectentes.

Materials and Methods. Mature disc florets were removed from the herbarium specimens listed in Table 3.

Materials were examined using light microscope (LM) techniques. The florets were softened and dissected in Pohl's solution, and material was then mounted on slides in Hoyer's solution. The slides were then allowed to sit for several days, during which the Hoyer's solution hardened and cleared the specimens. Material was then examined and photographed using a Zeiss photomicroscope II.

Style branches were sectioned to make visible the morphological continuity or discontinuity of the stigmatic area. Materials were dissected in Pohl's solution, rinsed in water, and sectioned according to the method of Lersten (1974), except specimens were placed directly in distilled H_2O, and paraffin was substituted for Tissuemat. Unstained 10 um sections were then mounted, examined, and photographed.

Photographs of stigmatic areas, anther collars (the morphologically distinct, anther end of the filament), and endothecial cells (cells lining the inside wall of the pollen sacs) were taken for each specimen.

Results and Discussion. The two varieties of Senecio amplectens were identical to each other with respect to these microcharacters. Both showed a stigmatic area divided into two regions by a longitudinal cleft (Figs. 25-26), radial thickenings in the endothecial cells (Figs. 27-28), and basally dilated anther

collars (Figs. 29-30). These are the features that would be expected of "non-Cacalioid" species. The two Ligularia species were also identical to each other with respect to these microcharacters. Both showed a stigmatic area divided into two regions by a longitudinal cleft (Figs. 31-32), polar thickenings in the endothecial cells (Figs. 33-34), and anther collars of relatively uniform diameters (not basally dilated) (Figs. 35-36). These traits of the endothecial cells and the anther collars are typical of "Cacalioid" species. The cleft stigmatic area was more of a surprise, since most "Cacalioid" species have stigmatic areas that are entire. However, other "Cacalioid" species with cleft stigmatic areas have been noted (Wetter, 1977).

In conclusion, Senecio amplectens appears to be a good "non-Cacalioid" species and the two Ligularia species are good "Cacalioid" species. Thus, on the basis of these microcharacters, S. amplectens var. amplectens and S. amplectens var. holmii are distinct from Ligularia but fit perfectly well with Senecio.

TABLE 3

LIST OF TAXA, POPULATIONS, AND VOUCHERS
MICROSTRUCTURE STUDY

Ligularia altaica DC, Elias, Weber, Tomb 4748 (KSC)
L. sibirica (L.) Cass., Elias, Weber, Tomb 4326 (KSC)
Senecio amplectens var. holmii (Greene) Harrington, Barr 23 (KSC)
S. amplectens A. Gray var. amplectens, Barkley and Robinson 229 (KSC)
Russell 61-115 (KSC)
Hartman 2273 (KSC)

Floral Microstructures of *Senecio* spp.

Figure 25. LM of the style branch of *Senecio amplectens* var. *amplectens*.

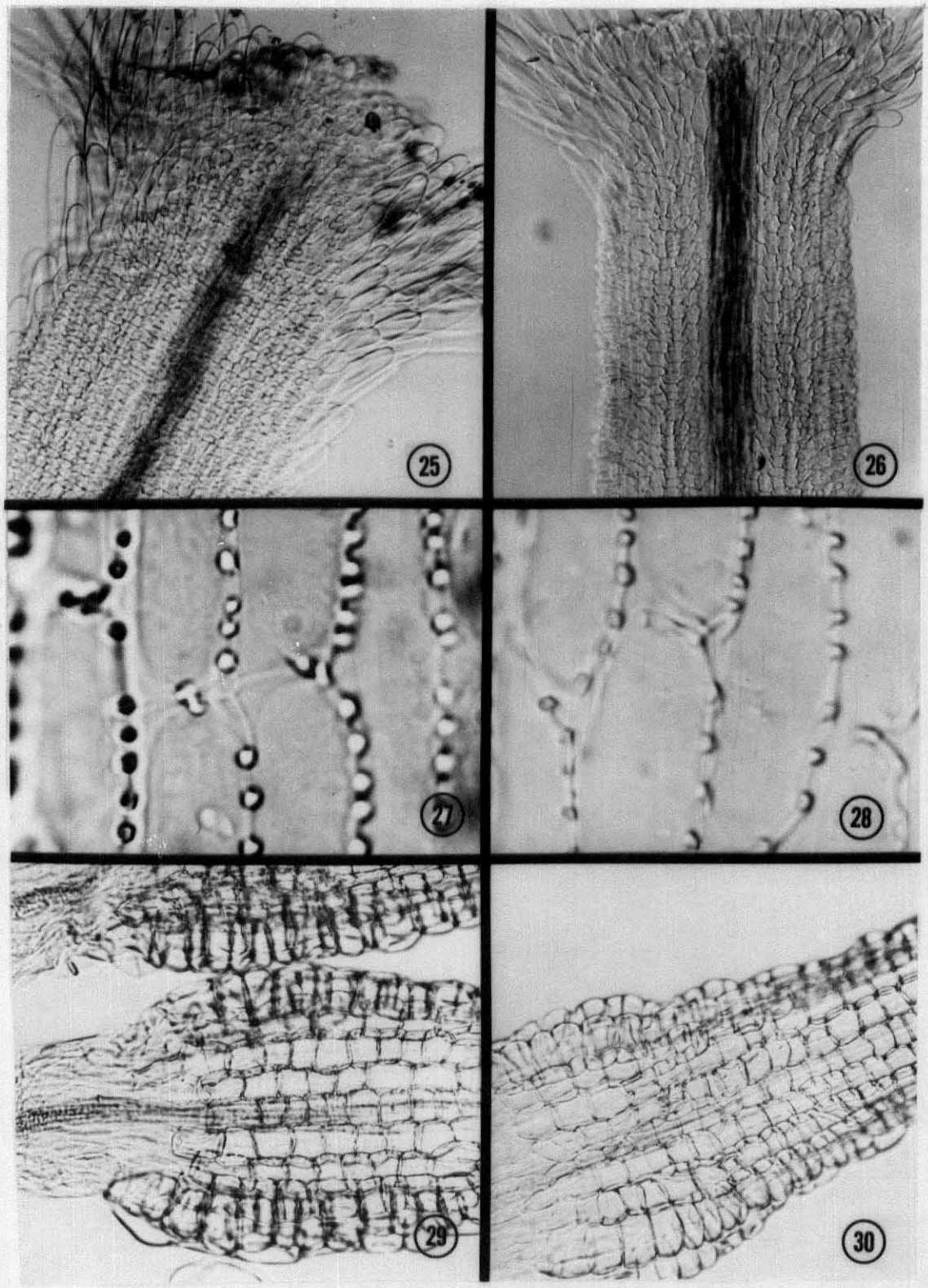
Figure 26. LM of the style branch of *S. amplectens* var. *holmii*.

Figure 27. LM of anther endothecial cells of *S. amplectens* var. *amplectens*.

Figure 28. LM of anther endothecial cells of *S. amplectens* var. *holmii*.

Figure 29. LM of anther collar of *S. amplectens* var. *amplectens*.

Figure 30. LM of anther collar of *S. amplectens* var. *holmii*.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Floral Microstructures of *Ligularia* spp.

Figure 31. LM of style branch of *Ligularia sibirica*.

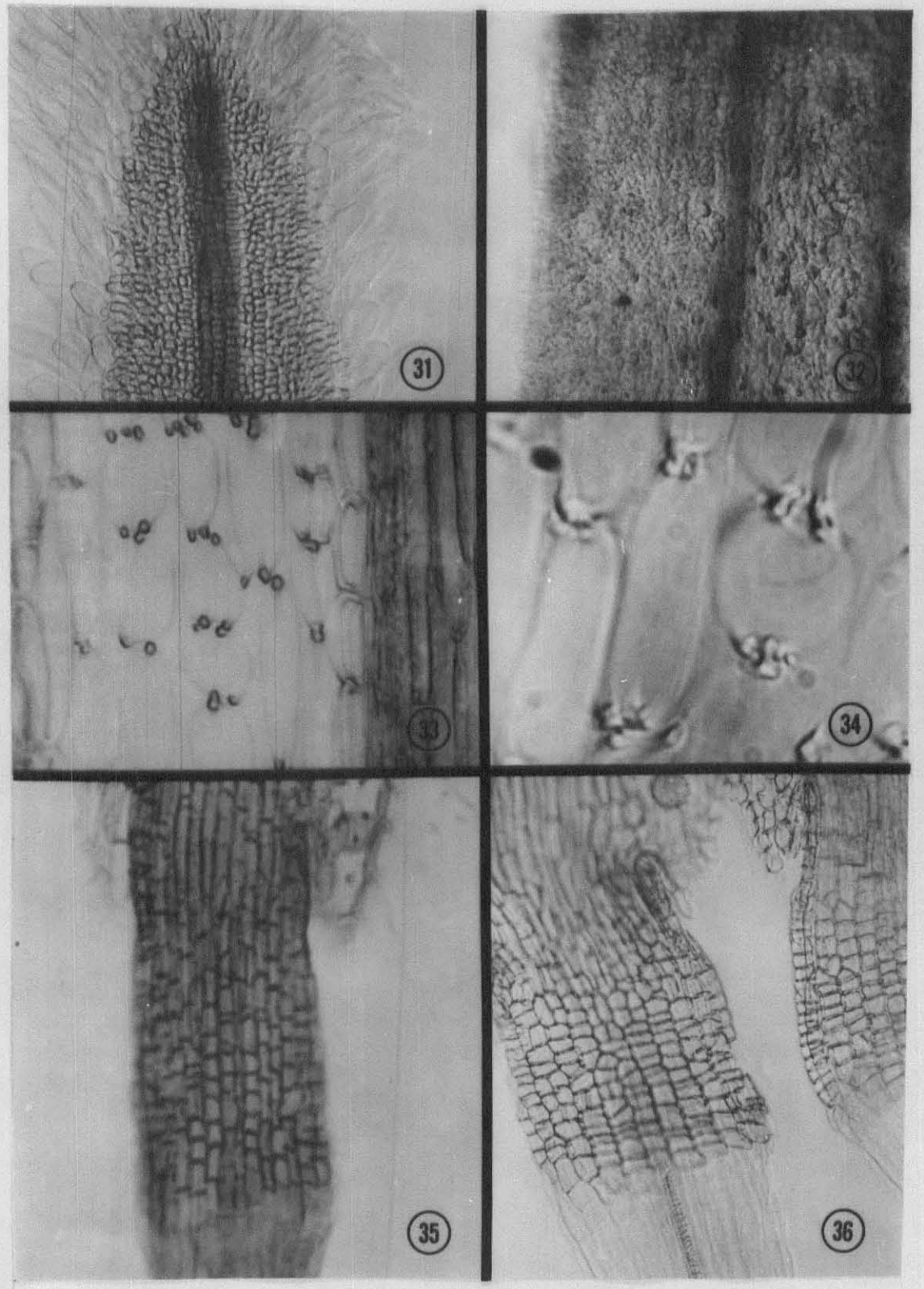
Figure 32. LM of style branch of *L. altaica*.

Figure 33. LM of anther endothecial cells of *L. sibirica*.

Figure 34. LM of anther endothecial cells of *L. altaica*.

Figure 35. LM of anther collar of *L. sibirica*.

Figure 36. LM of anther collar of *L. altaica*.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FLAVONOID STUDY

Introduction. Chromatographic spot patterns of flavonoids were shown to be taxonomically useful by Alston and Turner (1959, 1963) and Markham and Mabry (1968) for the legume Baptisia. Since then, flavonoid spot pattern analysis has been used frequently as an important taxonomic character. Partial or complete flavonoid structural analysis has extended the usefulness of flavonoid data and has been applied to numerous taxonomic groups (Moore et al., 1970, Giannasi and Chuang, 1976) including Senecio (Glennie et al., 1971).

Flavonoids are phenolic compounds found in most tissues of all higher plants and many lower groups. Typical flavonoid structures are shown in Fig. 37. Their widespread occurrence indicates they do have one or several functions in plants, though the functions are not entirely known. Some flavonoids serve as flower pigments, some as intermediates for biosynthesis of lignins and tannins, and some may be involved in growth and development (Smith, 1976). The sugars frequently found attached to flavonoids increase solubility and may aid in transport or storage of flavonoids.

Flavonoids are synthesized by condensation of three acetate units and a phenyl propanoid intermediate

from the shikimic acid pathway. They may be subsequently modified by dehydroxylation, methylation, O-glycosylation, and sometimes hydroxylation.

Flavones and flavonols are two major categories of flavonoids whose derivatives are found in almost bewildering array in the Compositae (Harborne, 1977). Within the Senecioneae, derivatives of the common flavonoids apigenin, luteolin, kaempferol, and quercetin, along with other more unusual forms have been reported (Robins, 1977).

In this study, it was hoped a flavonoid analysis would support or contradict the postulated relationship between certain species of Senecio sect. Amplectentes of the Rocky Mountains and species of Ligularia found in the mountain regions of Middle Asia.

Materials and Methods. Plant material was collected in bulk and air dried in gunny sacks. Leaf and stem material (ca 3 g) were ground dry for about one minute in a Waring blender. Flavonoids were extracted with 25 ml methanol:water (3:1) for three to five hours. The plant material was removed by filtration and re-extracted two more times. Plant material was then discarded. Extracts were combined and the solvent was removed under water pump vacuum. A sticky, green residue remained. One ml of methanol

was added to the residue, some of which was insoluble. The suspension was allowed to settle and the methanol and soluble components were removed. This flavonoid solution was spotted on pre-washed 46 X 57 cm Whatman 3MM chromatography paper. Pre-washing of the paper involved running an excess of 15% glacial acetic acid in water through the paper by normal descending chromatographic techniques; the solvent was allowed to drip off the end of the paper. The process was usually carried out over night. The paper was dried thoroughly after washing and after development in each solvent system. The flavonoids were separated by 2-dimensional descending paper chromatography according to the method of Mabry et al. (1970). Development in the 57 cm direction used t-butanol:glacial acetic acid:water (3:1:1) and required 18 hr; the second direction was developed in 15% glacial acetic acid in water and required 4 hr.

Chromatograms were viewed under short wavelength ultraviolet radiation (UV); under these conditions flavonoids typically appear deep purple, fluorescent light blue, orange, or green, and spots of such appearance were outlined. Chromatograms were also exposed for several seconds to NH_3 vapors and viewed under UV. Any change in color was noted. Spots which showed typical flavonoid color and which were present in a

quantity large enough to be definitely detectable to the eye were eluted in the following way. A spot was cut out of the chromatogram and cut into strips about 5 mm wide. For each spot cut out, a blank of equal area was cut from another part of the chromatogram which showed no compounds. The strips for the spot were clipped together and suspended from a horizontal bar. A Pasteur pipette was modified so that the tip opening was very small, and then it was positioned above the strips with the tip touching one end of the strips, as shown in Fig. 38. Two ml of spectroscopic grade methanol were placed in the pipette and allowed to run slowly through the strips. The methanol dripped into a 5 ml beaker below. The methanol, containing the eluted flavonoid, was transferred to a 1 cm cuvette. The blank was eluted and transferred to a 1 cm cuvette in the same manner. The flavonoid was then analyzed against its blank in a Cary-14 spectrophotometer. Five spectra were obtained for each spot. A spectrum was obtained 1) in methanol; 2) immediately after the addition of sodium methoxide to the methanol; 3) after waiting 5 minutes following the addition of sodium methoxide to the methanol; 4) in methanol plus $AlCl_3$; 5) in methanol plus $AlCl_3$ plus HCl. Figs. 39-40 show these spectra for 3-O-galacto-quercetin (compound 6). All reagents were prepared, stored, and used as

described by Mabry et al. (1970). Structures were then postulated for each flavonoid based on color, mobility in the solvent systems, and UV spectra.

The sugars attached to the flavonoids were identified in the following manner. A spot was eluted as described for UV analysis. A length of glass tubing was cut into 10 cm sections and one end was sealed over a flame to form a hydrolysis tube. The eluted flavonoid in methanol was placed in a tube, and the methanol was evaporated under a stream of nitrogen. To hydrolyze the sugars off the flavonoid, 0.5 ml of 2N HCl was placed in the tube. The tube was then evacuated and sealed over a flame and placed in a heat block at 100°C for 2 hr. After hydrolysis the tube was opened, the hydrolysis mixture was frozen and placed in a vacuum dessicator over night to remove the water and HCl. The residue, containing the sugars, was then dissolved in 50 ul of 0.5M borate buffer, pH 8.6. Half of this volume was applied to the anion exchange high performance liquid chromatography system of Barr and Nordin (in preparation). This method involves separation of the sugars on a Dionex DA-X8-11 column, detection using a color reaction with bicinchoninate reagent (Mopper and Gindler, 1973), and a Dionex P-2 absorption monitor. Absorbances were recorded on a strip chart. A solution of standard

sugars was run prior to the flavonoid sugars to allow identification. This system can detect sugars in amounts as low as 1 nmole.

Flavonoid data were analyzed by the paired affinity (PA) method of Ellison et al. (1962), in which

$$PA = \frac{\#\text{ spots in common}}{\#\text{ spots in species A} + \#\text{ spots in species B}},$$

and by the minimum biosynthetic-step indices (J_{bs}) of Levy (1977), in which

J_{bs} = BSI / (BSI + MBSD) ,

BSI = biosynthetic-step identity = # biosynthetic steps in common to a pair of species.

MBSD = minimum biosynthetic-step distance
= # biosynthetic steps exclusive to one or the other member of a pair of species.

The former method does not require that structures be known, while the latter requires postulated structures (Table 5) and biosynthetic pathways (Fig. 41).

Table 4 lists taxa used, along with collection and voucher information.

Results and Discussion. Table 5 lists the flavonoids with their colors, R_f values, and postulated structures. Table 6 shows the distribution of the 18 flavonoids among the taxa studied. No clear patterns are apparent. Four of the flavonoids appear to be distributed throughout the groups studied (compounds 3,5,6, and 10). Two compounds are restricted to species of sect. Amplectentes, among the species studied, but are not

found in all members of the section (compounds 11 and 12). Two flavonoids appear in only two species (compounds 1 and 16). The remaining ten flavonoids are found in only one species each. These results seem to indicate the Ligularia species are no more related to species of sect. Amplectentes than to members of the other Senecio sections examined.

After paired affinity values were calculated and plotted a few patterns did appear. The varieties of S. amplectens showed substantial affinity for each other (Figs. 44-46), though the two populations of S. amplectens var. holmii did not show as much mutual affinity as might have been expected. All the S. amplectens showed some affinity for the other two sect. Amplectentes species, with one exception (Figs. 47-48); one population of S. amplectens var. holmii did not show any affinity for S. crassulus. All the S. amplectens also showed as much affinity for the two Ligularia species as for each other (Figs. 44-46). In addition, all the S. amplectens showed some affinity for S. serra and two samples showed affinity for S. fremontii (Figs. 44-46). Senecio bigelovii showed the most straight-forward results; it showed affinities for all the other species in sect. Amplectentes and no affinities for anything else (Fig. 47). S. crassulus likewise showed affinities for the other species of

sect. Amplectentes, with the one exception mentioned above, but it also showed affinities for Ligularia sibirica and S. triangularis (Fig. 48). It is interesting to note that none of the species of sect. Amplectentes, including S. crassulus, showed any affinity for S. integerrimus (Fig. 49).

The Ligularia species showed affinities with each other and with S. amplectens; neither showed any affinity with S. bigelovii (Figs. 42-43). Both also showed substantial affinity with S. serra.

The biosynthetic-step index of Levy (1977) showed striking similarities between the following pairs:
Ligularia sibirica -- Senecio amplectens var. amplectens
L. sibirica -- S. serra
S. amplectens var. amplectens -- S. amplectens var. holmii #23

Of the three samples of Senecio amplectens (Figs. 52-54), two show striking similarities to one another (above pair), as would be expected. The third (S. amplectens var. holmii #33a) shows very little similarity with the other two; this is in agreement with the results of the Ellison (1962) paired affinity analysis (Figs. 44-46). This is a very interesting result since S. amplectens var. amplectens and S. amplectens var. holmii #33a were growing intermixed at the same site, while S. amplectens var. holmii #23 was collected ca 200 miles from the other two. This suggests some

interesting differentiation between the two varieties growing together and may be chemical character displacement.

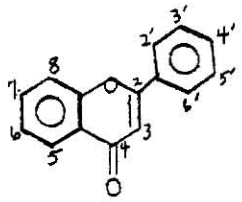
Senecio crassulus (Fig. 56) shows moderate similarities to S. amplectens var. holmii #33a and S. bigelovii. However, it shows low similarities to S. amplectens var. amplectens and S. amplectens var. holmii #23. It also shows little similarity to S. integerrimus (Fig. 57).

Senecio bigelovii (Fig. 55), on the other hand, shows moderate similarities to all the other sect. Amplectentes and low similarities to everything else.

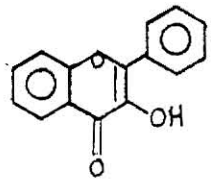
Ligularia sibirica (Fig. 51) shows a striking similarity to Senecio amplectens var. amplectens, with the other sect. Amplectentes ranging to very low on the scale. L. sibirica also shows a striking similarity to S. serra. Thus, it might be said that L. sibirica seems related to both S. amplectens and S. serra, or it might be said that L. sibirica is no more related to any of the sect. Amplectentes than to members of the other Senecio sections examined. Ligularia altaica (Fig. 50) shows only moderate to low similarities to all the other taxa and doesn't show any more affinity for sect. Amplectentes species than for anything else. Conversely, sect. Amplectentes does not clearly side more with either the Ligularia species or the other Senecio sections.

It might have been expected that the members of sect. Amplectentes would show consistantly high similarities to each other. However, this was not the result; some taxa are quite similar while others are very dissimilar. The relatedness of the taxa within sect. Amplectentes was not being doubted. Therefore, it seems that flavonoid composition may not vary with taxonomic relatedness but randomly or with other unknown factors. Thus, no taxonomic re-alignments are recommended here on the basis of flavonoid composition.

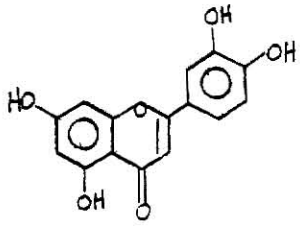
Figure 37. Representative flavonoid structures.



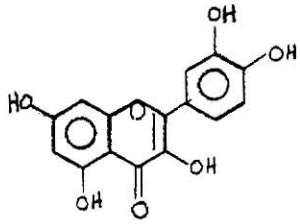
a flavone



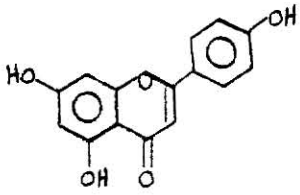
a flavonol



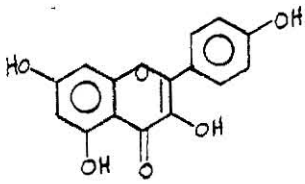
Luteolin



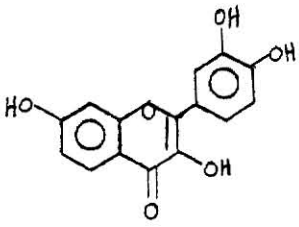
Quercetin



Apigenin



Kaempferol



Fisetin

TABLE 4

LIST OF TAXA, POPULATIONS, AND VOUCHERS
FLAVONOID STUDY

Ligularia altaica DC, Elias, Weber, and Tomb 4748 (KSC)
L. sibirica (L.) Cass., Elias, Weber, and Tomb 4849 (KSC)
Senecio amplectens A. Gray var. amplectens, Barr 33b (KSC)
S. amplectens var. holmii (Greene) Harrington,
Barr 23 (KSC)
Barr 33a (KSC)
S. bigelovii var. hallii A. Gray, Barr 27 (KSC)
S. crassulus A. Gray, Barr 14 (KSC)
S. integerrimus var. exaltatus (Nuttall) Cronquist,
Barr 15 (KSC)
S. fremontii var. blitoides (Greene) Cronquist,
Barr 34 (KSC)
S. triangularis Hooker, Barr 6 (KSC)
S. serra var. admirabilis (Greene) A. Nelson, Barr 31 (KSC)

Figure 38. Apparatus used to elute a flavonoid from the chromatographic paper.

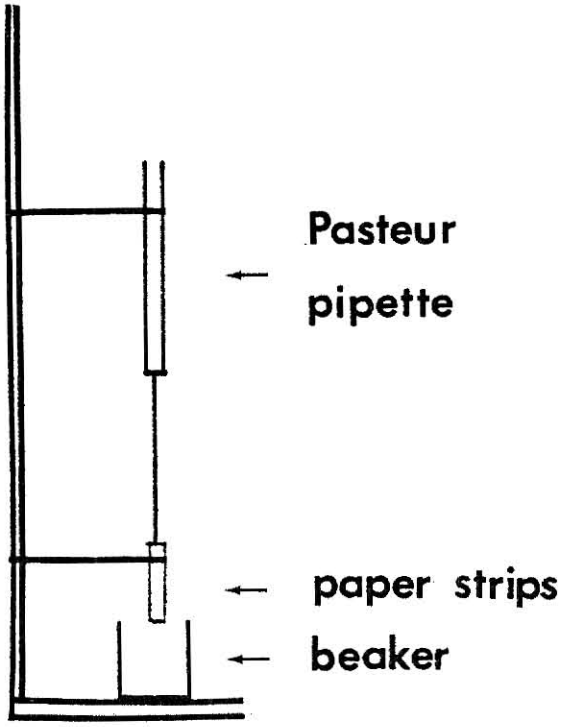

Pasteur
pipette
paper strips
beaker

Figure 39. UV spectra of 3-O-galacto-quercetin (compound 6) in methanol and immediately after the addition of sodium methoxide. The spectrum after 5 minutes in the presence of sodium methoxide was identical to the spectrum taken immediately.

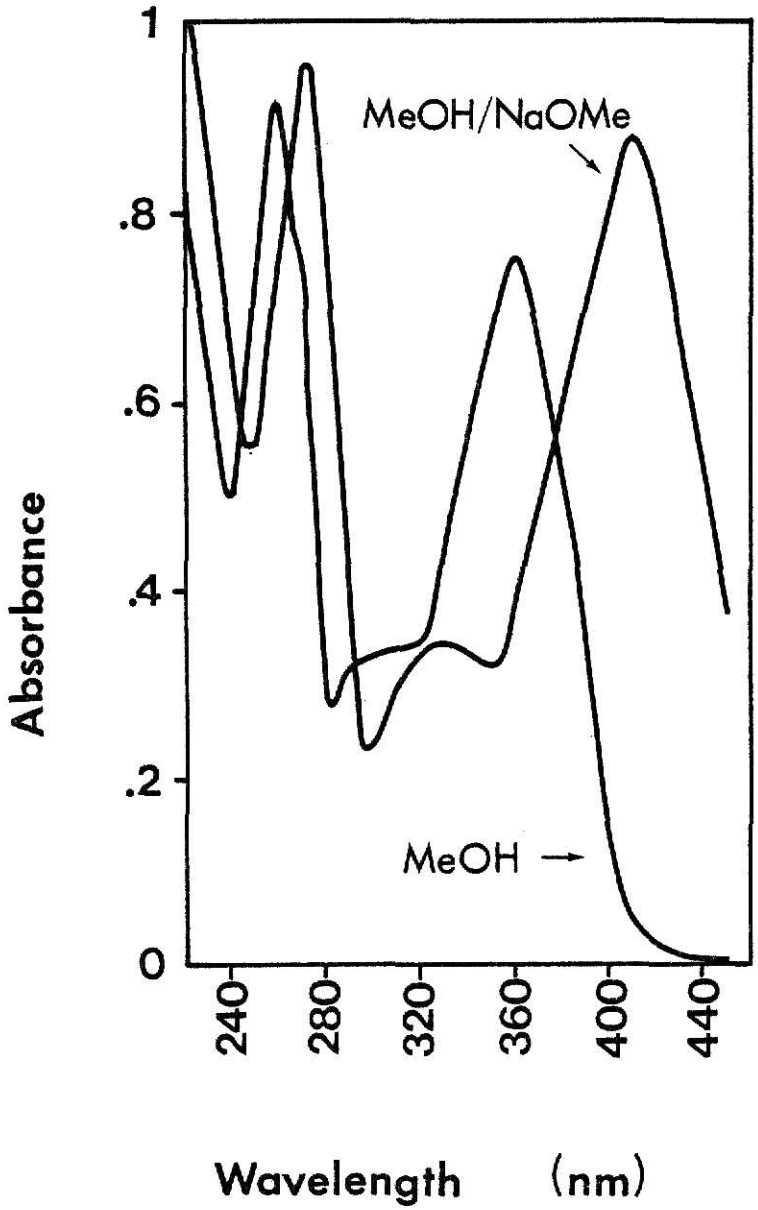

1
.8
.6
.4
.2
0
Absorbance
MeOH/NaOMe
MeOH
240
280
320
360
400
440
Wavelength (nm)

Figure 40. UV spectra of 3-O-galacto-quercetin (compound 6) in methanol plus $AlCl_3$ and in methanol plus $AlCl_3$ plus HCl.

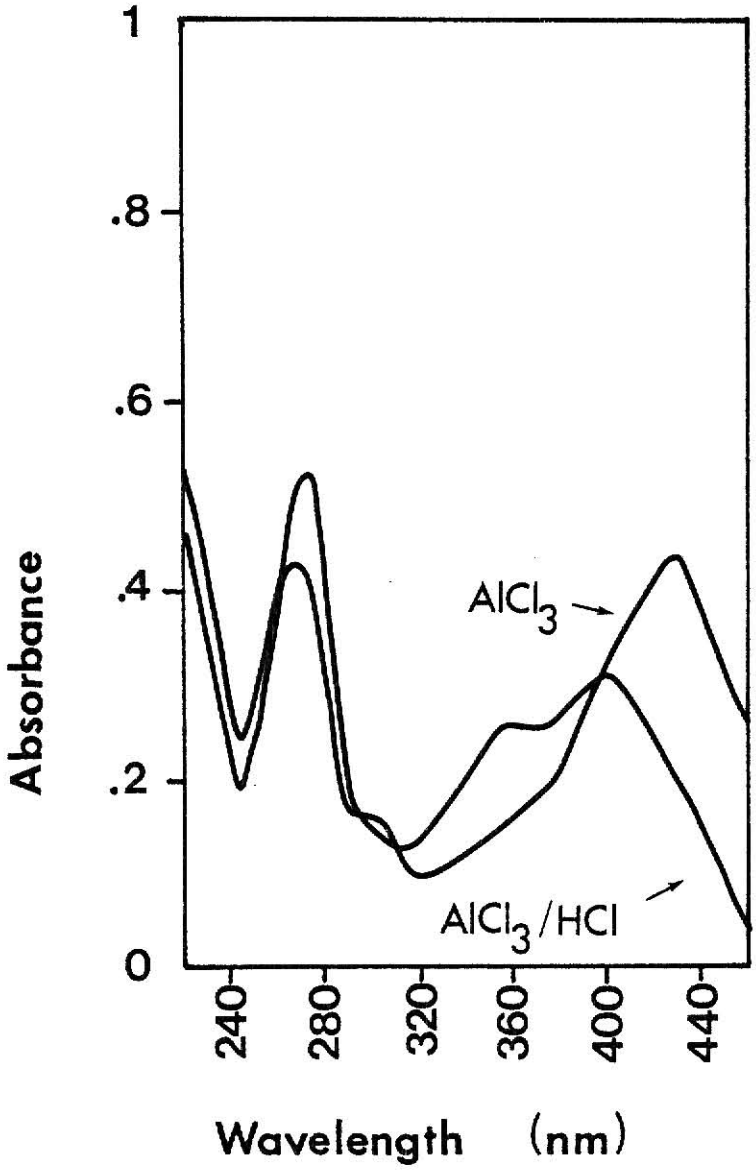

1
.8
.6
.4
.2
0
Absorbance
AlCl3
AlCl3/HCl
240
280
320
360
400
440
Wavelength (nm)

TABLE 5

FLAVONOID CHARACTERISTICS

Compound Number	UV color	UV/NH_3 color	R_f TBA	R_f HOAc	Postulated Structure *
1	light	light green	0.65	0.39	FL(3,4'-di-OH) 3-O-glc
2	purple	yellow	0.38	0.11	L 7-O-glc 3'-O-Me
3	purple	yellow	0.64	0.51	K 3-O-di-glc
4	purple	yellow	0.65	0.03	K 3-O-Me
5	purple	yellow	0.46	0.47	Q 3-O-glc
6	purple	yellow	0.54	0.43	Q 3-O-gal
7	purple	no change	0.37	0.58	Q 3-O-glc 7-O-gal 3'-O-Me 4'-O-Me
8	fluorescent light blue	light green	0.81	0.65	FL(3,4'-di-OH 8-gly) 3-O-Me
9	light	light green	0.74	0.54	FL(3,4;6-tri-OH) 3-O-glc 4'-O-Me

*See key to symbols, Figure 41.

TABLE 5 continued.

FLAVONOID CHARACTERISTICS

Compound Number	UV color	UV/NH_3 color	R_f TBA	R_f HOAc	Postulated Structure*
10	fluorescent light blue	no change	0.62	0.77	FL(3,4'-di-OH) 3-O-di-glc
11	fluorescent light blue	brighter	0.61	0.73	FL(3,4',6-tri-OH) 3-O-di-glc
12	light	no change	0.64	0.84	FL(3,4',7-tri-OH) 3-O-di-glc 7-O-glc
13	light blue	light green	0.57	0.83	FL(3,3'-di-OH) 3-O-di-glc
14	purple	yellow	0.56	0.82	Q 3-O-rha 7-O-rha 3'-O-Me
15	fluorescent light blue	little change	0.55	0.76	F 3-O-rha 7-O-rha 3'-O-Me
16	purple	yellow	0.34	0.68	Q 3-O-glc 7-O-rha 3'-O-Me
17	light	no change	0.62	0.85	FL(3,3',4'-tri-OH)H_2 3-O-di-glc
18	purple	yellow	0.55	0.25	A 7-O-glc

*See key to symbols, Figure 41.

Figure 41. Proposed biosynthetic pathways of the flavonoids. Based on the principles of flavonoid biosynthesis (Hahl and Grisebach, 1975; Harborne, 1967) and modeled after Levy (1977).

Key to symbols:

A = apigenin*
L = luteolin*
Q = quercetin*
K = kaempferol*
F = fisetin*
FL_n = flavonol nucleus$_n$
FL( ) = structure of flavonol nucleus
FL( )H_2 = structure of dihydroflavonol
Me = methyl group
glc = glucose
gal = galactose
rha = rhamnose
gly = unknown sugar

Numbers followed by a dash indicate point of attachment of the following group to the flavonoid nucleus.

Arrows indicate flow along a biosynthetic pathway.

Brackets indicate the preceding structure is repeated.

White numbers indicate the compound number as shown in TABLE 5.

*See Figure 37 for structures.

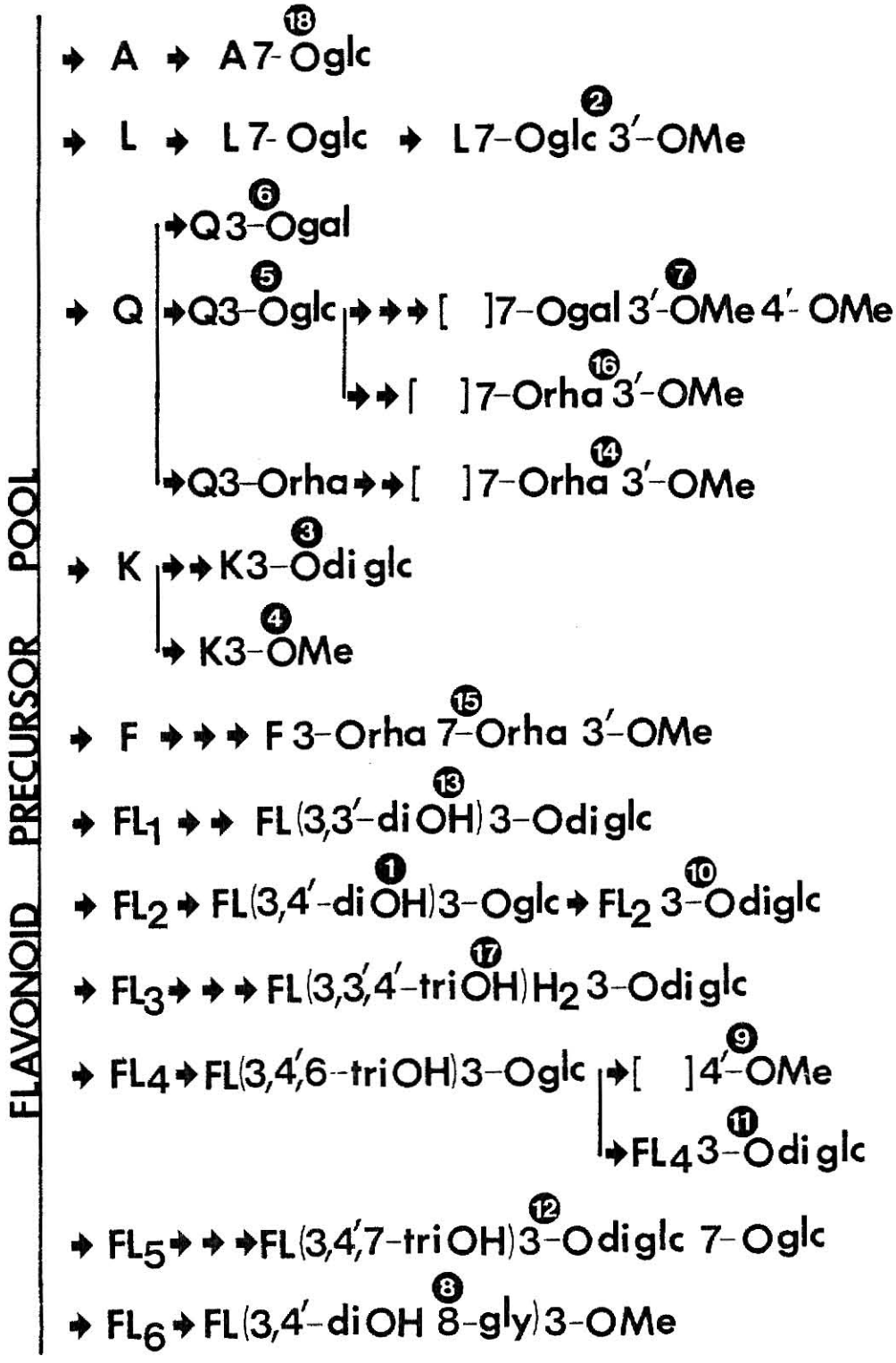

FLAVONOID PRECURSOR POOL
→ A → A 7-Oglc (18)
→ L → L 7-Oglc → L 7-Oglc 3′-OMe (2)
→ Q → Q 3-Ogal (6)
→ Q → Q3-Oglc (5) → → → [ ]7-Ogal 3′-OMe 4′-OMe (7)
→ → [ ]7-Orha 3′-OMe (16)
→ Q3-Orha → → [ ]7-Orha 3′-OMe (14)
→ K → → K3-Odiglc (3)
→ K3-OMe (4)
→ F → → → F 3-Orha 7-Orha 3′-OMe (15)
→ FL1 → → FL(3,3′-diOH) 3-Odiglc (13)
→ FL2 → FL(3,4′-diOH)3-Oglc → FL2 3-Odiglc (1) (10)
→ FL3 → → → FL(3,3′,4′-triOH)H2 3-Odiglc (17)
→ FL4 → FL(3,4′,6--triOH)3-Oglc → [ ]4′-OMe (9)
→ FL4 3-Odiglc (11)
→ FL5 → → → FL(3,4′,7-triOH)3-Odiglc 7-Oglc (12)
→ FL6 → FL(3,4′-diOH 8-gly)3-OMe (8)

TABLE 6

FLAVONOID DISTRIBUTIONS

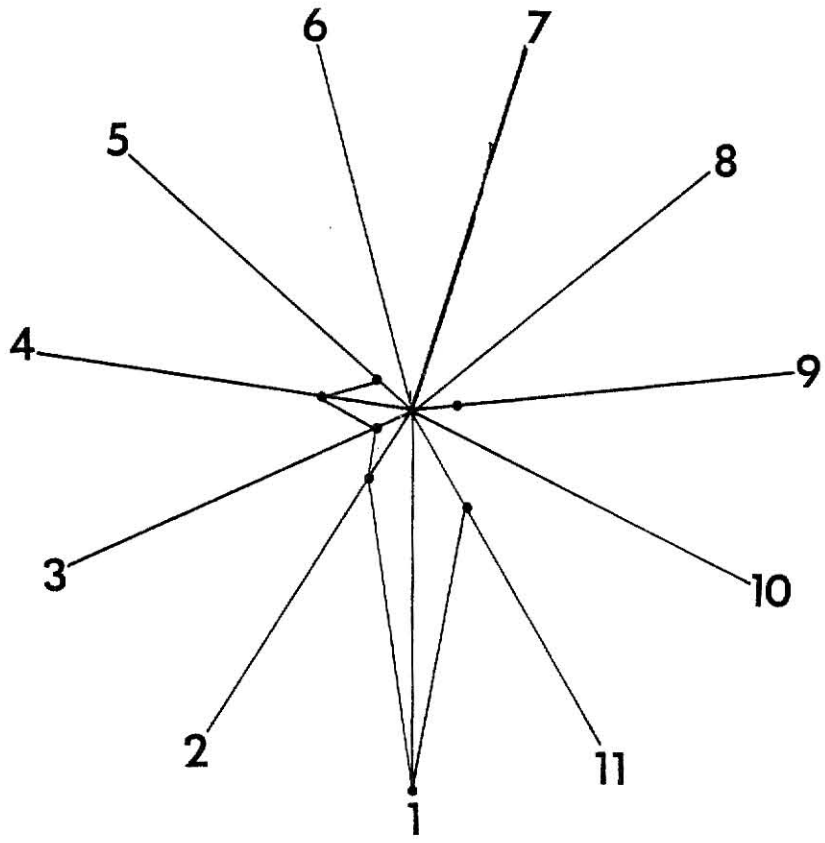
Taxon	10	6	5	3	1	16	11	12	2	4	7	8	9	13	14	15	17	18
L. altaica	+	+			+							+	+					
L. sibirica	+	+	+	+														
S. amplectens var. amplectens	+	+	+	+				+										
S. amplectens var. holmii 23	+	+						+										
S. amplectens var. holmii 33a		+					+							+				
S. bigelovii							+	+			+							
S. crassulus			+				+											
S. integerrimus						+									+	+		
S. fremontii	+					+												
S. triangularis			+						+	+								+
S. serra	+	+		+	+												+	

+ means compound is present.
Numbers refer to compounds as shown in TABLE 5.

Figure 42. Diagram of paired affinity indices based on flavonoid data of *Ligularia altaica* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *L. altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

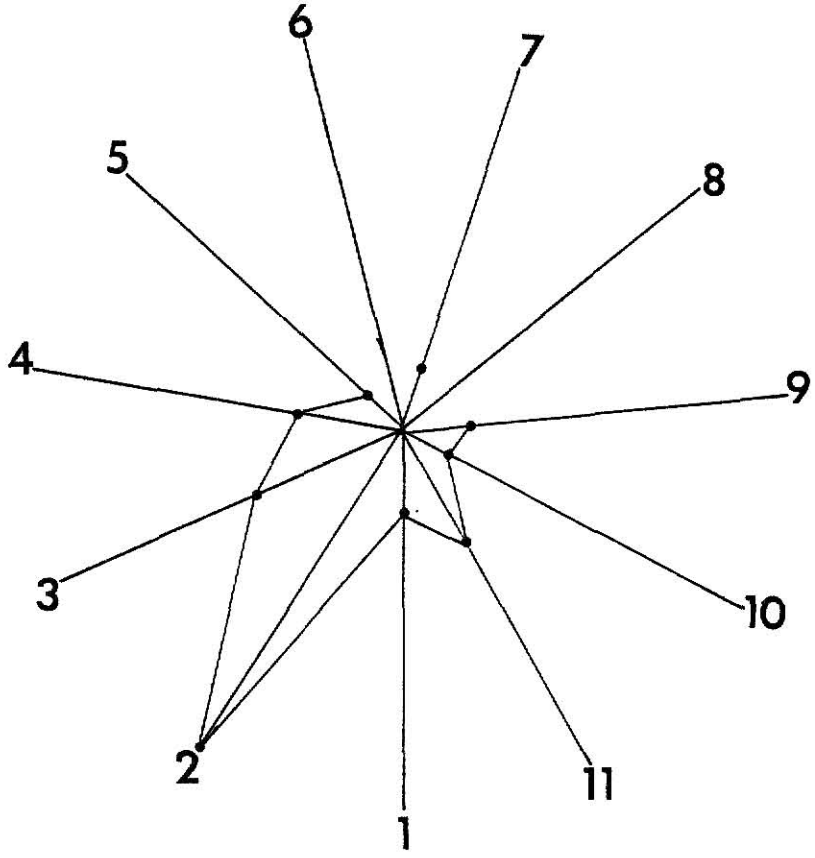


1 L. altaica

Figure 43. Diagram of paired affinity indices based on flavonoid data of *Ligularia sibirica* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *L. altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, *Barr* 23
5 = *S. amplectens* var. *holmii*, *Barr* 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

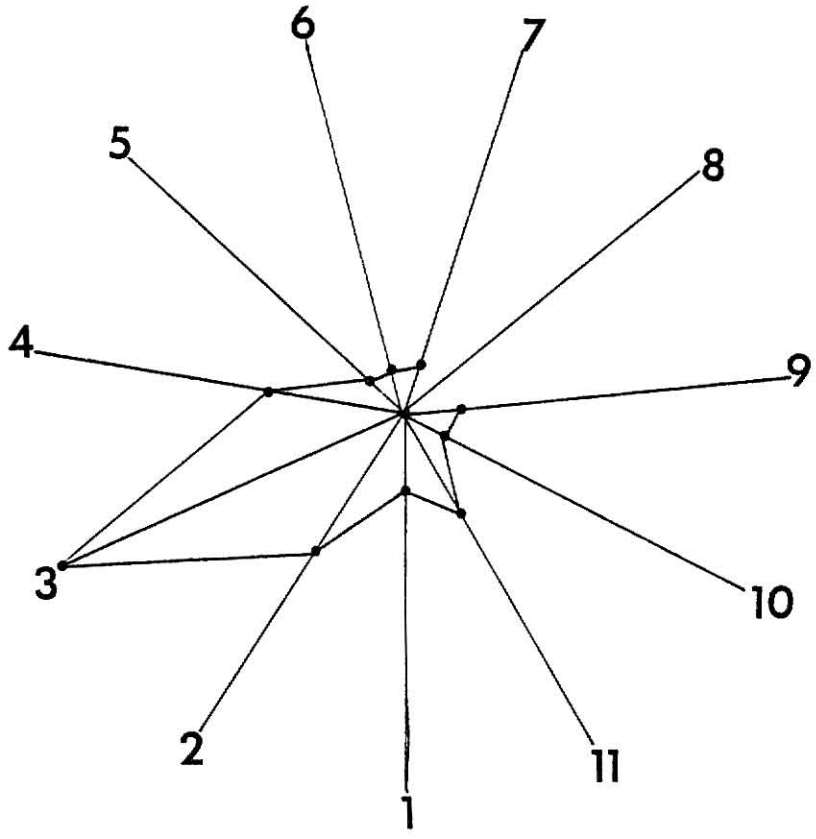


2 L. sibirica

Figure 44. Diagram of paired affinity indices based on flavonoid data of *Senecio amplectens* var. *amplectens* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *S. amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

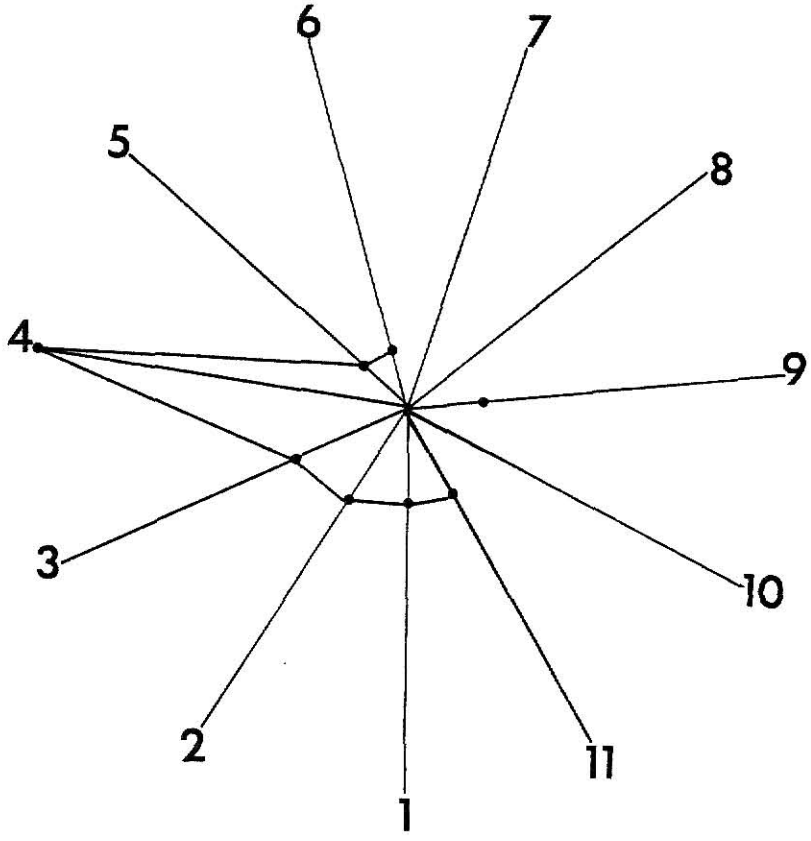


3 S. amplectens var. amplectens

Figure 45. Diagram of paired affinity indices based on flavonoid data of Senecio amplectens var. holmii, Barr 23, compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = Ligularia altaica
2 = L. sibirica
3 = S. amplectens var. amplectens
4 = S. amplectens var. holmii, Barr 23
5 = S. amplectens var. holmii, Barr 33a
6 = S. bigelovii
7 = S. crassulus
8 = S. integerrimus
9 = S. fremontii
10= S. triangularis
11= S. serra

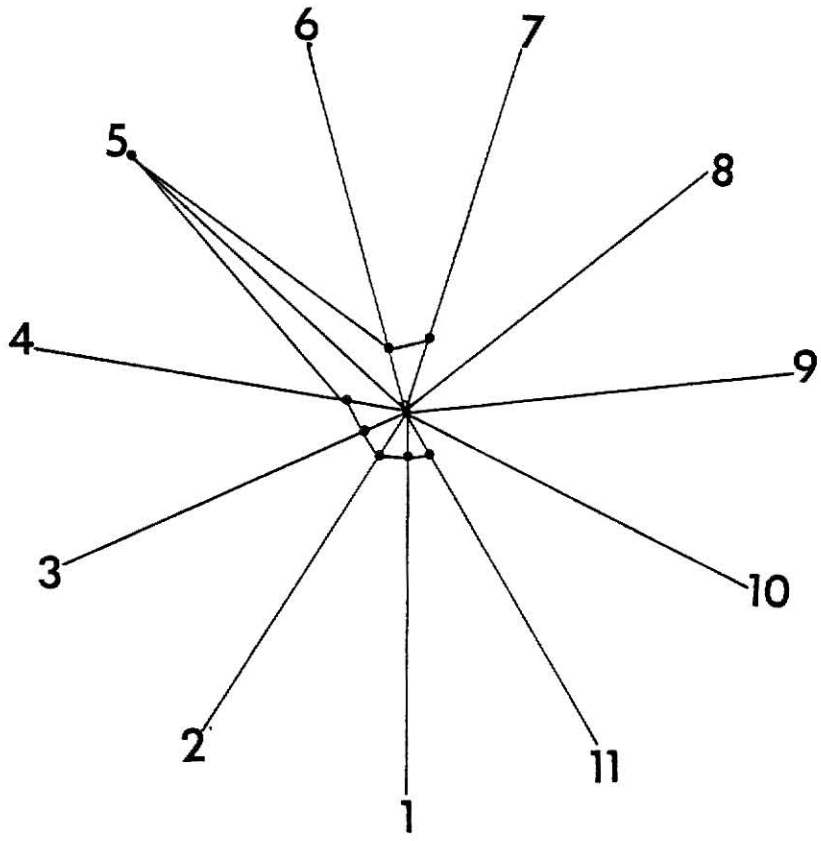


4 S. amplectens var. holmii 23

Figure 46. Diagram of paired affinity indices based on flavonoid data of *Senecio amplectens* var. *holmii*, Barr 33a, compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *S. amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

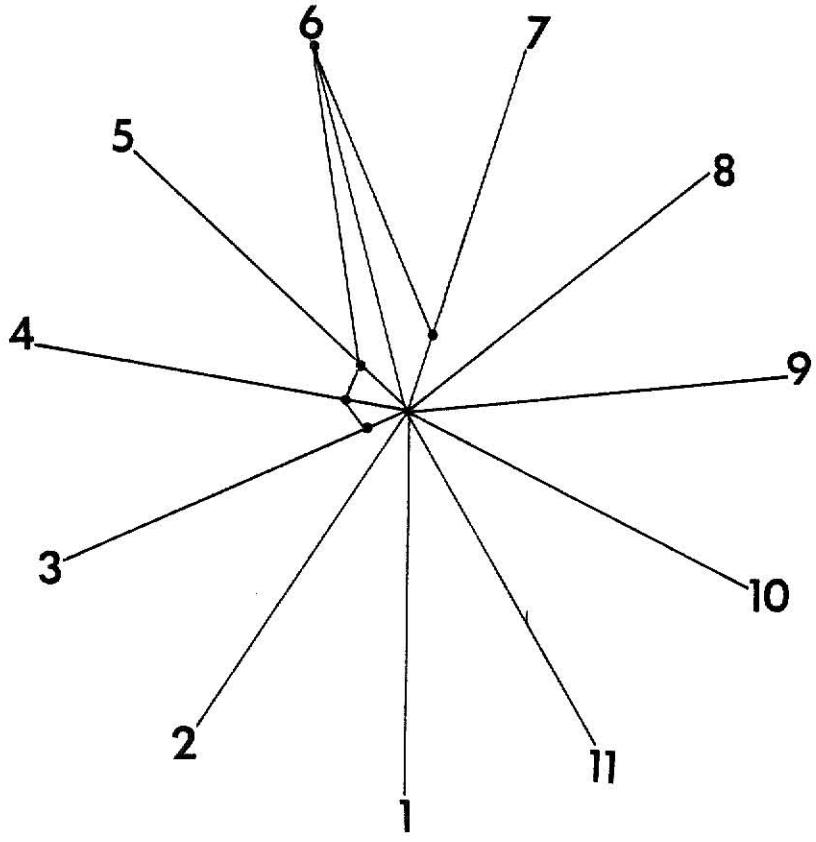


5 S. amplectens var. holmii 33a

Figure 47. Diagram of paired affinity indices based on flavonoid data of *Senecio bigelovii* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *S. amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

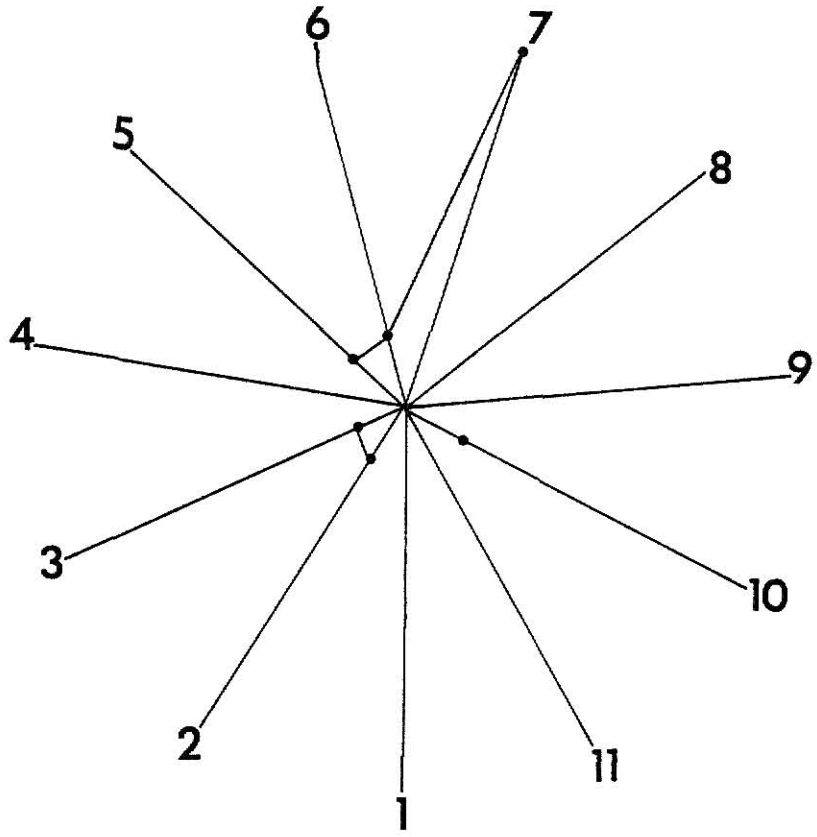


6 S. bigelovii

Figure 48. Diagram of paired affinity indices based on flavonoid data of *Senecio crassulus* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

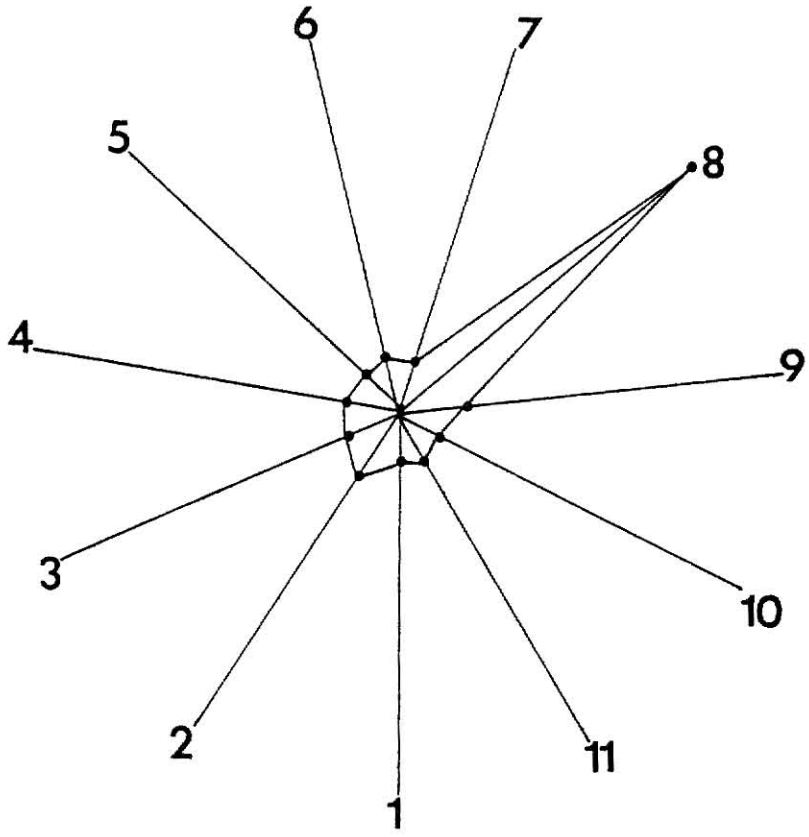


7 S. crassulus

Figure 49. Diagram of paired affinity indices based on flavonoid data of *Senecio integerrimus* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*



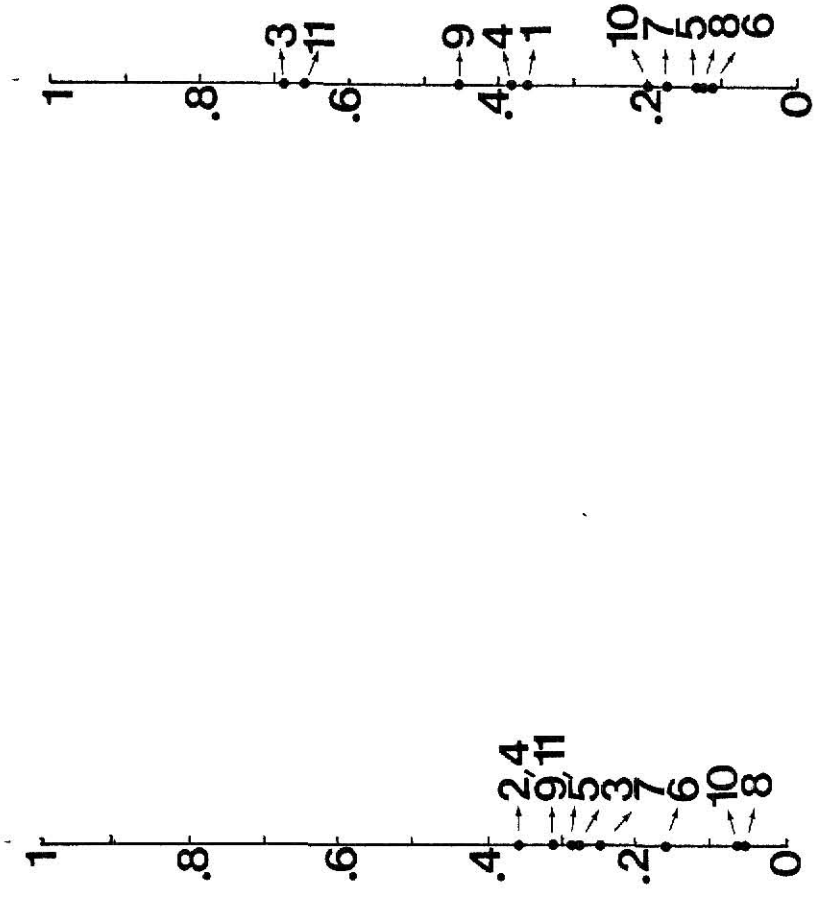
8 S. integerrimus

Figure 50. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of Ligularia altaica compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

Figure 51. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of Ligularia sibirica compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

Key to numbers:

1 = Ligularia altaica
2 = L. sibirica
3 = Senecio amplectens var. amplectens
4 = S. amplectens var. holmii, Barr 23
5 = S. amplectens var. holmii, Barr 33a
6 = S. bigelovii
7 = S. crassulus
8 = S. integerrimus
9 = S. fremontii
10= S. triangularis
11= S. serra



2 L. sibirica

1 L. altaica

Fig. 50 J_{bs} values Fig. 51

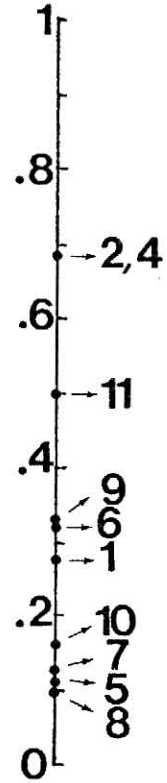
Figure 52. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of Senecio amplectens var. amplectens compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

Figure 53. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of Senecio amplectens var. holmii, Barr 23, compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

Figure 54. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of Senecio amplectens var. holmii, Barr 33a, compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

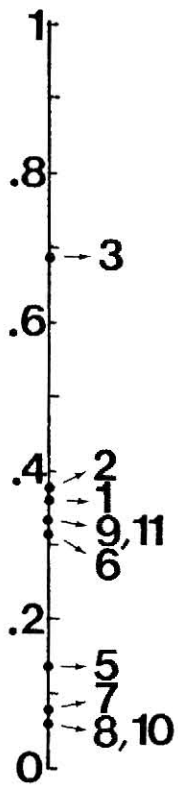
Key to numbers:

1 = Ligularia altaica
2 = L. sibirica
3 = Senecio amplectens var. amplectens
4 = S. amplectens var. holmii, Barr 23
5 = S. amplectens var. holmii, Barr 33a
6 = S. bigelovii
7 = S. crassulus
8 = S. integerrimus
9 = S. fremontii
10= S. triangularis
11= S. serra



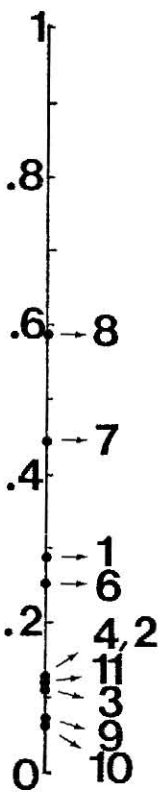
3 S. amplectens
var. amplectens

Fig. 52



4 S. amplectens
var. holmii 23

Fig. 53
J_{bs} values



5 S. amplectens
var. holmii 33a

Fig. 54

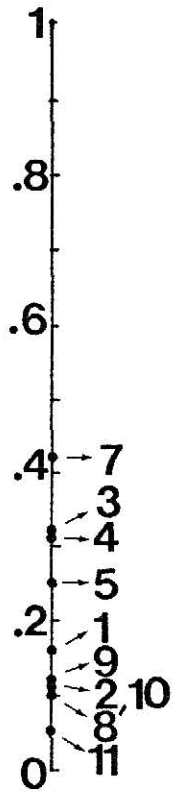
Figure 55. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of *Senecio bigelovii* compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

Figure 56. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of *Senecio crassulus* compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

Figure 57. Plot of biosynthetic-step index values (J_{bs}), based on flavonoid data of *Senecio integerrimus* compared to each of the other taxa studied. Higher values indicate higher similarities. Arrows point to numbers representing the taxa being compared.

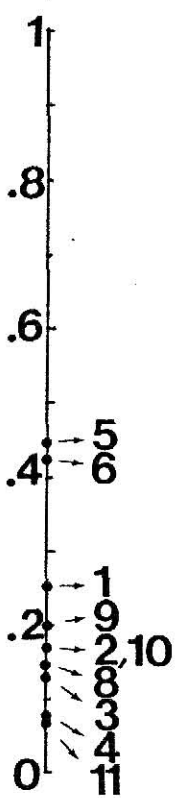
Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*



6 S. bigelovii

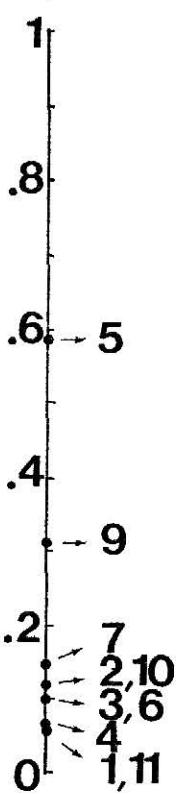
Fig. 55



7 S. crassulus

Fig. 56

J_{bs} values



8 S. integerrimus

Fig. 57

SESQUITERPENE STUDY

Introduction. Terpenoid compounds are one of the many types of systematically useful secondary metabolites which characterize the Compositae. Their synthesis is thought to involve the cyclization and subsequent modification of chains of isoprene units. A single isoprene unit (isopentenyl pyrophosphate) is formed from three acetyl-CoA molecules. Two isopentenyl pyrophosphates join to form geranyl pyrophosphate, which is considered to be the precursor of monoterpenes. Similarly, when three isoprene units join, they form farnesyl pyrophosphate, which is thought to be the precursor of sesquiterpenes. A few representative structures are shown in Fig. 58. There are about 15 types of sesquiterpenes commonly found in the Compositae. The types are all thought to be interrelated along their proposed biosynthetic pathways.

Early studies of sesquiterpenes were hampered by the isolation, separation, and identification techniques, with only those compounds which could be conveniently crystallyzed being reported (Herz, 1977). More recently, improved techniques have shown there may be numerous sesquiterpenes in each species, rather than the one or two previously identified (Bohlmann, 1974).

Certain types of sesquiterpenes seem to characterize certain taxa within the family (Herz, 1977). The genus Senecio is characterized by having almost exclusively the furanoeremophilane type. The genus Ligularia also has sesquiterpenes of the furanoeremophilane type, together with biogenetically related eremophilenolides and bakkenolides (Seaman, pers. comm.).

This study is a preliminary survey of the sesquiterpene chemistries of three species of Senecio sect. Amplectentes and two species of Ligularia, in order to elucidate the possible relatedness of the two groups. Four other Senecio species were also examined for comparison. Non-volatile terpenoids were extracted by a standard technique (Yoshioka et al., 1973), separated by two dimensional thin layer chromatography, and detected using a spray reagent selective for sesquiterpene lactones (Drozdz and Bloszyk, 1978). Spot patterns were then compared using paired affinity indices (Ellison et al., 1962). This approach has the flaw of not taking into account the possible relatedness of the sesquiterpenes along a biosynthetic pathway.

Materials and Methods. Dried stem and leaf material were ground and extracted by the method of Yoshioka et al. (1973), except that only 5 g of plant material from each population was used. This procedure uses nonpolar solvents and a precipitation step and

yields a crude syrup of terpenoid compounds. A 10% solution of this syrup in chloroform was examined by two-dimensional thin layer chromatography using 20 X 20 cm EM pre-coated silica gel 60 plates. Development in the first dimension used chloroform:methanol (100:1) as solvent system, and diethyl ether was used for development in the second dimension. Sesquiterpene lactones were then selectively detected by the $FeCl_3$/H_3PO_4/H_2SO_4 spray reagent of Drozdz and Bloszyk (1978). (This reagent is stated to be selective for sesquiterpene lactones by the above authors but may in fact detect certain other terpenoids as well.) The resulting patterns of spots were compared. The R_f's in each solvent system and the colors following treatment with reagent are listed in Table 7. Table 8 shows the distribution of these spots in the various species. In comparing the two-dimensional spot patterns on different plates spots with similar R_f's and color reactions were assumed to be the same compound. Because many furanoeremophilane, eremophilenolide, and bakkenolide constituents reported from Ligularia and Senecio share similar R_f's, this assumption should be verified by a more exhaustive analysis of the chemical constituents.

Each taxon was compared to all the others by the paired affinity index of Ellison et al. (1962). The

following formula was used to calculate the paired affinity index (PA) for each species pair:

$$PA = \frac{\#\text{ spots in common}}{\#\text{ spots in species A} + \#\text{ spots in species B}}$$

The resulting values are plotted in Figs. 59-67.

Table 9 lists the plant species and populations used in this study. All plant material was collected and dried in bulk rather than by pressing. The Ligularia species are from the collections of Elias, Weber, and Tomb (KSC) in the Altai Mountain region of the Soviet Union; the Senecio species are from the author's own field collections (KSC) in the Rocky Mountain region of Colorado.

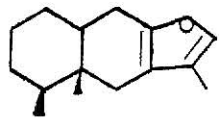
Results and Discussion. Table 8 shows the distribution of compounds among the taxa examined. Only compounds occurring in two or more taxa are shown here. Many compounds occur throughout or randomly distributed among the taxa. However, Table 8 shows a grouping of eight compounds that appear in the species of sect. Amplectentes plus Ligularia altaica which do no occur in the species of sect. Triangulares and Integerrimi examined. Ligularia sibirica has none of these eight compounds. This seems to say that the sect. Amplectentes hangs together as a group; L. altaica fits in rather well with this group, while L. sibirica does not.

When the paired affinity indices were calculated and plotted, some further conclusions were possible. Figs. 61-63 show that the varieties of S. amplectens have rather high affinities for each other, as would be expected. The affinity of the two populations of S. amplectens var. holmii for each other was not as high as might have been predicted, meaning the terpenoid content as detected by this method can vary substantially from population to population. Senecio bigelovii shows affinity for the S. amplectens species also (Fig. 64), but S. crassulus seems to have a rather low affinity for all the other species (Fig. 65); it shows only slightly raised affinity for S. amplectens var. amplectens. It shows no more affinity for S. integerrimus than for anything else (Figs. 65-66). Ligularia altaica also shows rather low affinities for any of the species (Fig. 59); the affinities for one population of S. amplectens var. holmii and for S. serra are slightly above the rest. Ligularia sibirica shows higher affinities all around (Fig. 60) but doesn't show any more affinity for the species of sect. Amplectentes than for any other group. It is interesting to note that the two Ligularia species don't show any particular affinity for each other (Figs. 59-60); the significance of this is not apparent. It is also interesting to note that S. fremontii had quite high affinities for S. amplectens

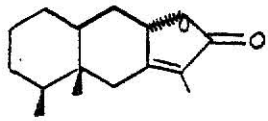
var. amplectens, S. amplectens var. holmii, and L. sibirica (Fig. 67). Senecio fremontii and the varieties of S. amplectens are frequently found growing together on north-facing, gray talus slopes above 12,000 feet in the Colorado Rocky Mountains; and where one is found the other two can nearly always be found also. Thus, their mutual affinities might be expected. The affinity for L. sibirica is more unexpected. However, all taxa except S. crassulus and L. altaica show some raised affinity for S. fremontii.

In conclusion, the results are not striking and definitive, but it may be said that the Amplectentes hang together as a group, with the status of S. crassulus still in question; S. crassulus doesn't seem to fit with the Amplectentes particularly well, but it shows even less affinity for S. integerrimus. Ligularia altaica seems to possess some of the eight compounds that appear to be distinctive to the sect. Amplectentes, but according to paired affinity indices, neither Ligularia species shows much more affinity for species of sect. Amplectentes than for any other Senecio section studied.

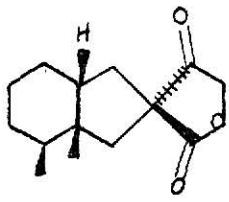
Figure 58. A few representative sesquiterpene structures.



furanoeremophilenolide



eremophilenolide



bakkenolide

TABLE 7

NON-VOLATILE TERPENOIDS

Compound Number	Color (after reagent)	R_f Et_2O	R_f $CHCl_3/CH_3OH$ (100:1)
1	purplish blue	0.15	0.09
2	blue	0.25	0.08
3	yellow	0.21	0.20
4	purple	0.40	0.06
5	yellow-orange	0.35	0.09
6	purple	0.46	0.12
7	blue	0.51	0.15
8	gray	0.62	0.07
9	purple	0.62	0.05
10	purple	0.68	0.07
11	blue	0.69	0.08
12	blue	0.73	0.24
13	red	0.72	0.18
14	purple	0.77	0.16
15	purple	0.76	0.32
16	blue	0.89	0.19
17	red	0.83	0.43
18	purple	0.88	0.42
19	red	0.90	0.47
20	yellow	0.82	0.48

TABLE 7 continued.

NON-VOLATILE TERPENOIDS

Compound Number	Color (after reagent)	R_f Et_2O	R_f $CHCl_3/CH_3OH$ (100:1)
21	light purple	0.85	0.63
22	green	0.93	0.69
23	purple	0.86	0.66
24	yellow	0.92	0.68
25	orange-purple	0.90	0.46

TABLE 8

NON-VOLATILE TERPENOIDS

Taxon	1	2	3	4	5	6	7	9	10	12	14	15	17	19	22	24	25	8	11	13	16	18	20	21	23
L. altaica	+	+		+			+				+	+				+	+	+		+			+		+
L. sibirica	+	+						+				+	+	+											
S. amplectens var. ampl.	+	+	+		+				+			+	+	+	+				+		+	+	+	+	
S. amplectens var. hol. 23	+	+	+		+	+		+	+			+		+								+	+	+	
S. amplectens var. hol. 33	+	+	+		+	+			+	+	+	+		+		+		+			+	+	+	+	+
S. bigelovii	+	+	+							+		+		+					+	+		+			
S. crassulus				+			+					+	+	+	+				+	+	+		+		
S. integerr.	+	+		+		+						+	+	+											
S. fremontii	+	+	+		+			+	+			+	+	+	+										
S. triangul.	+					+	+	+	+	+		+			+		+								
S. serra	+	+	+				+			+	+	+	+	+	+	+									

+ means compound is present.
Numbers refer to compounds as shown in TABLE 7.

TABLE 9

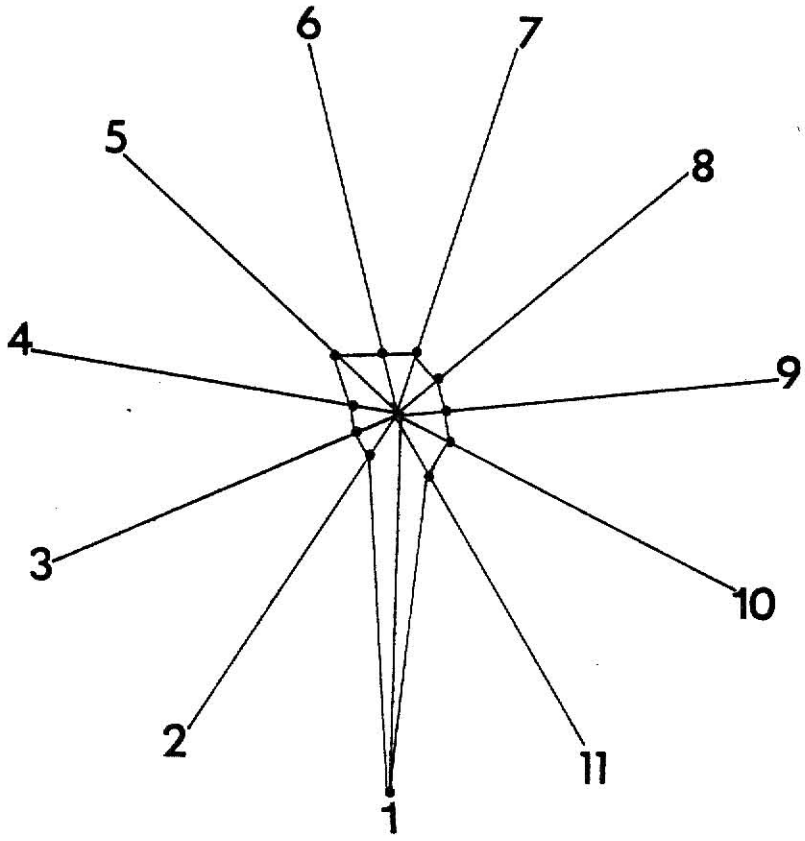
LIST OF TAXA, POPULATIONS, AND VOUCHERS
SESQUITERPENE STUDY

Ligularia altaica DC, Elias, Weber, and Tomb 4748 (KSC)
L. sibirica (L.) Cass., Elias, Weber, and Tomb 4849 (KSC)
Senecio amplectens A. Gray var. amplectens, Barr 33b (KSC)
S. amplectens var. holmii (Greene) Harrington,
Barr 23 (KSC)
Barr 33a (KSC)
S. bigelovii var. hallii A. Gray, Barr 27 (KSC)
S. crassulus A. Gray, Barr 14 (KSC)
S. integerrimus var. exaltatus (Nuttall) Cronquist,
Barr 34 (KSC)
S. fremontii var. blitoides (Greene) Cronquist,
Barr 34 (KSC)
S. triangularis Hooker, Barr 32 (KSC)
S. serra var. admirabilis (Greene) A. Nelson, Barr 31 (KSC)

Figure 59. Diagram of paired affinity indices based on sesquiterpene data of *Ligularia altaica* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

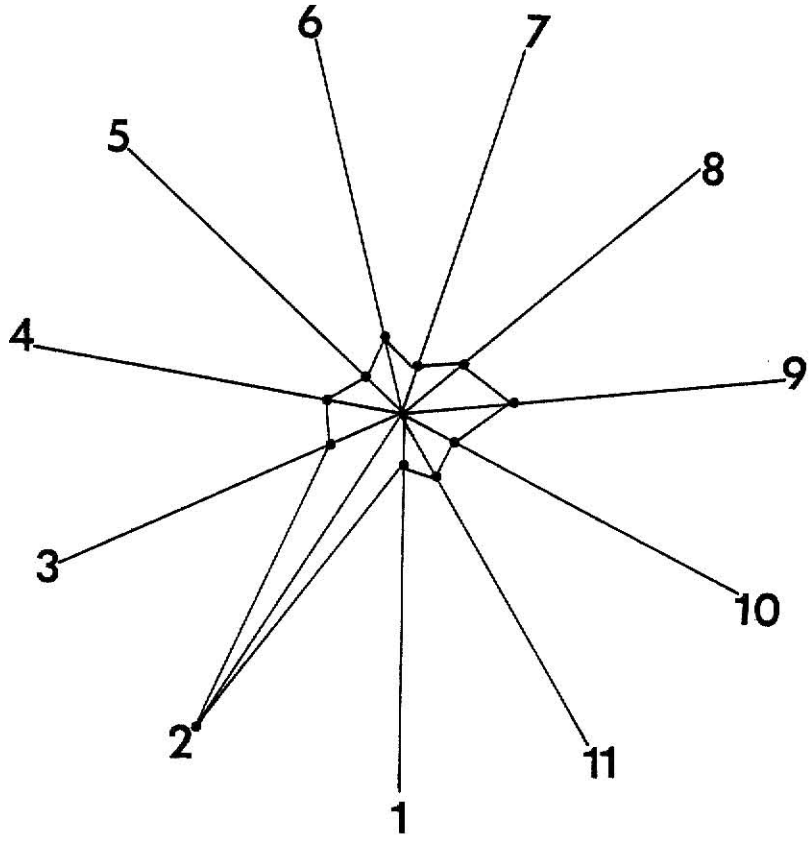


1 L. altaica

Figure 60. Diagram of paired affinity indices based on sesquiterpene data of *Ligularia sibirica* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

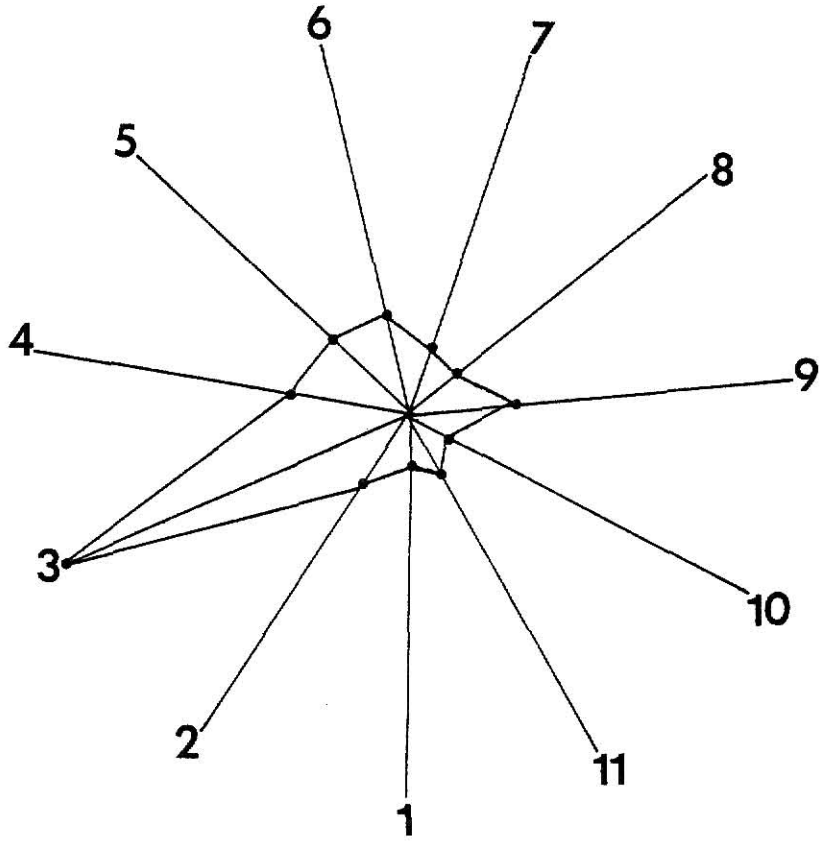


2 L. sibirica

Figure 61. Diagram of paired affinity indices based on sesquiterpene data of *Senecio amplectens* var. *amplectens* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

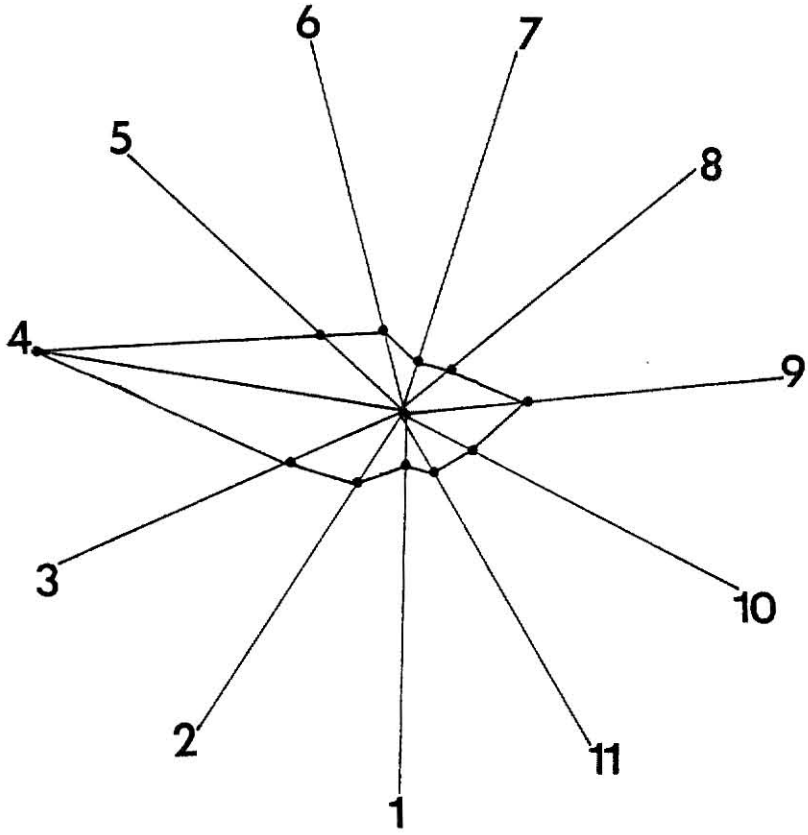


3 S. amplectens var. amplectens

Figure 62. Diagram of paired affinity indices based on sesquiterpene data of Senecio amplectens var. holmii, Barr 23, compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = Ligularia altaica
2 = L. sibirica
3 = Senecio amplectens var. amplectens
4 = S. amplectens var. holmii, Barr 23
5 = S. amplectens var. holmii, Barr 33a
6 = S. bigelovii
7 = S. crassulus
8 = S. integerrimus
9 = S. fremontii
10= S. triangularis
11= S. serra

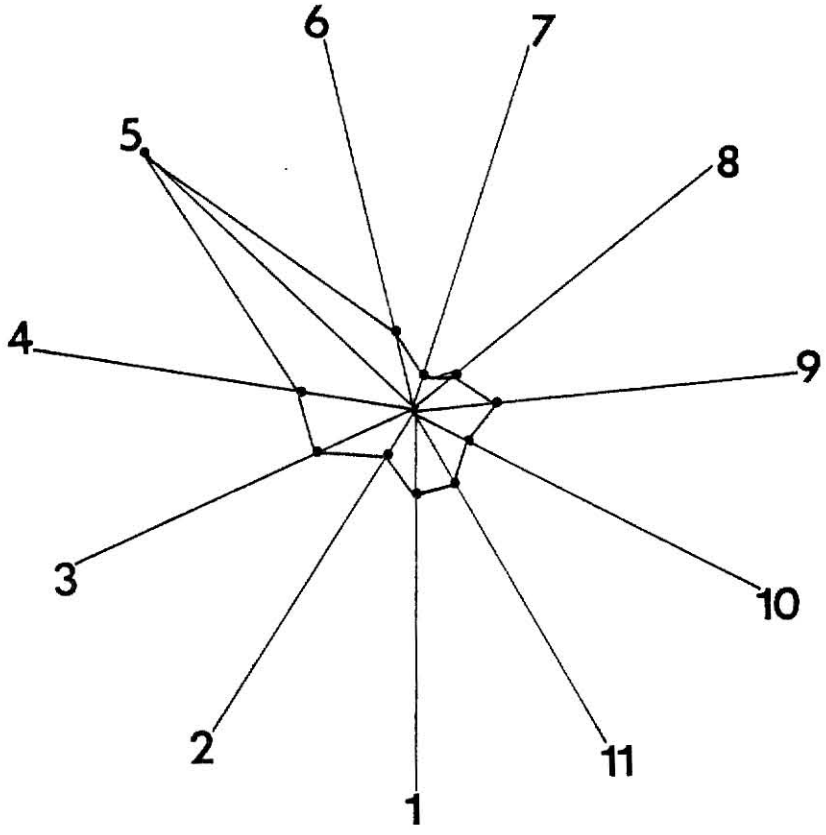


4 S. amplectens var. holmii 23

Figure 63. Diagram of paired affinity indices based on sesquiterpene data of *Senecio amplectens* var. *holmii*, Barr 33a, compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

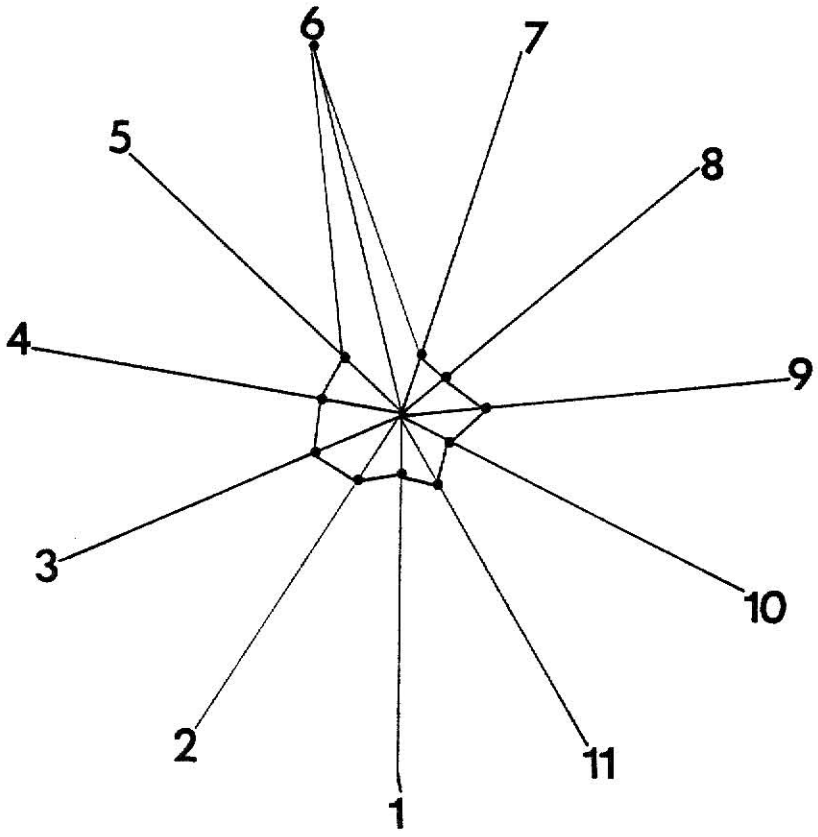


5 S. amplectens var. holmii 33a

Figure 64. Diagram of paired affinity indices based on sesquiterpene data of *Senecio bigelovii* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

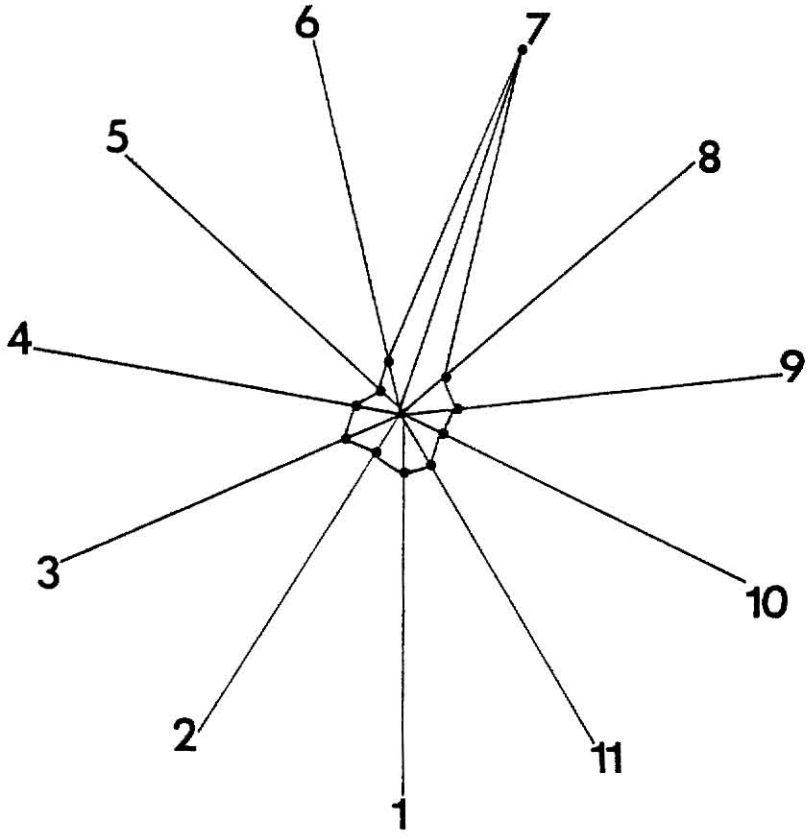


6 S. bigelovii

Figure 65. Diagram of paired affinity indices based on sesquiterpene data of *Senecio crassulus* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

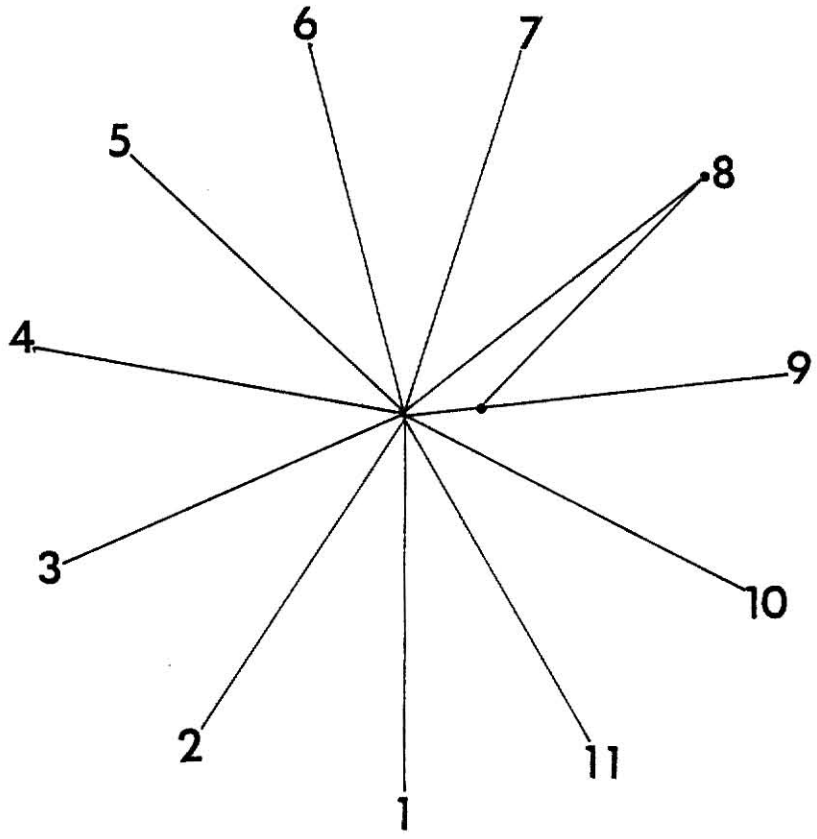


7 S. crassulus

Figure 66. Diagram of paired affinity indices based on sesquiterpene data of *Senecio integerrimus* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*

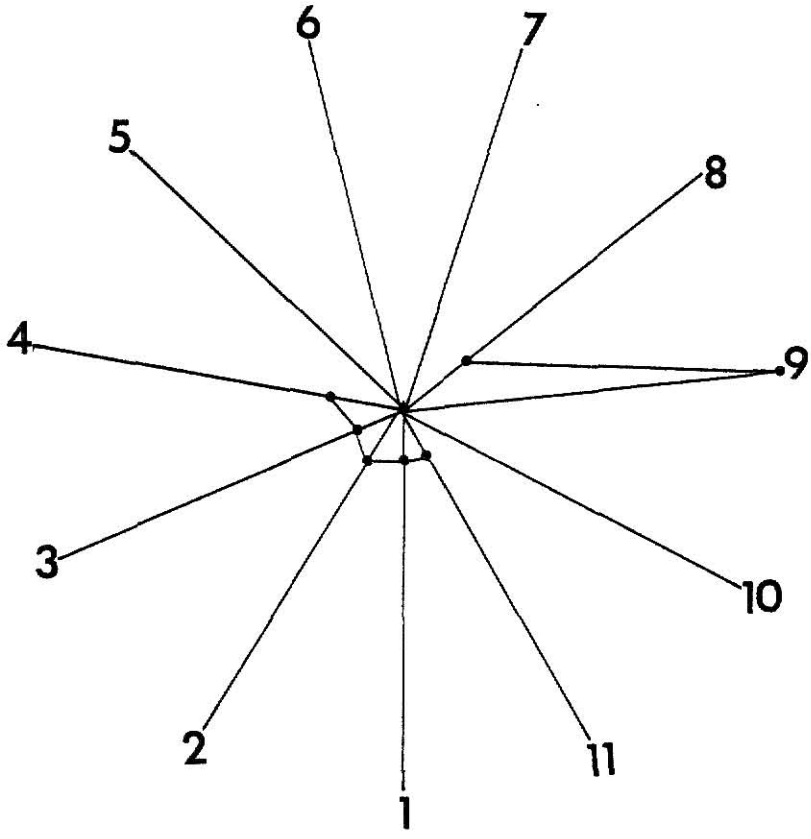


8 S. integerrimus

Figure 67. Diagram of paired affinity indices based on sesquiterpene data of *Senecio fremontii* compared to each of the other taxa studied. Center represents 0% affinity; ends of lines represent 100% affinity. Numbers refer to taxon being compared.

Key to numbers:

1 = *Ligularia altaica*
2 = *L. sibirica*
3 = *Senecio amplectens* var. *amplectens*
4 = *S. amplectens* var. *holmii*, Barr 23
5 = *S. amplectens* var. *holmii*, Barr 33a
6 = *S. bigelovii*
7 = *S. crassulus*
8 = *S. integerrimus*
9 = *S. fremontii*
10= *S. triangularis*
11= *S. serra*



9 S. fremontii

CONCLUSIONS

In this study, flavonoid composition, sesquiterpene composition, external pollen morphology, and certain floral microstructures were examined to make clearer the relative taxonomic positions of certain questioned species of *Senecio* sect. *Amplectentes* and *Ligularia*.

External pollen morphology showed minor variations in grain size, spine length, and pattern of perforations around spine bases, but these variations did not appear in any systematic pattern among the examined species of sects. *Amplectentes*, *Triangulares*, and *Integerrimi*, and *Ligularia*. Thus, external pollen morphology did not elucidate the relative taxonomic positions. Subsequent analysis of the pollen internal wall structure might prove more revealing.

The floral microcharacters examined (stigmatic areas, anther collars, and anther endothecial cells) did distinguish *Senecio amplectens* from the *Ligularia* species examined, and indicated the questioned species of sect. *Amplectentes* properly belong in *Senecio*.

The meaning of the patterns of flavonoid distribution is not clear. The flavonoid distributions did not follow expected taxonomic lines and provided no clear basis for any taxonomic re-alignments. Flavonoids may not vary with taxonomic relatedness but with other

unknown factors. The very dissimilar flavonoid compositions of the two varieties of Senecio amplectens which were growing intermixed may indicate some interesting differentiation perhaps similar to the well-established phenomenon of character displacement in animals (Brown and Wilson, 1956). A population-by-population analysis of flavonoid composition of Senecio amplectens in the Rocky Mountains would be worthwhile to establish the factors affecting flavonoid composition and to investigate this possible occurrence of character displacement.

The distribution of sesquiterpenes likewise provided no clear basis for any taxonomic re-alignments. It is possible clearer patterns might appear if sesquiterpene structures and biosynthetic pathways were determined.

On the basis of the floral microstructure data, and in the absence of any contrary data from the other studies, it is suggested that the questioned species of sect. Amplectentes (Senecio amplectens var. amplectens, S. amplectens var. holmii, and S. bigelovii) properly belong in Senecio and are distinct from Ligularia. However, a more extensive survey of the floral microstructures, a study of internal pollen wall structures, and a thorough population-by-population study of the chemo-taxonomic characters might reveal additional distinctions.

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CHEMOTAXONOMIC AND MICROCHARACTER COMPARISONS OF SELECTED SPECIES OF *LIGULARIA* AND *SENECIO* SECTION *AMPLECTENTES*

by

ROBIN REED BARR

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CHEMOTAXONOMIC AND MICROCHARACTER COMPARISONS OF SELECTED SPECIES OF *LIGULARIA* AND *SENECIO* SECTION *AMPLECTENTES*

ABSTRACT

The morphological similarities between certain species of *Ligularia* (a genus of the highlands of Central Asia) and certain species of *Senecio* section *Amplectentes* (which are found in the southern Rocky Mountains of North America) have suggested these two groups of plants are more related than traditionally supposed and may suggest that at one time there was a connection between the floras of these two regions. Flavonoid compositions, sesquiterpene compositions, external pollen morphology, and briefly, stigmatic areas, anther collars, and anther endothecial cells were examined to see if these characters also showed marked similarities between the two groups of plants. No marked similarities were found using any of these characters; in fact, the differences in the floral microstructures examined indicated the two groups were distinct.