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Biomechanical Relationships in Astragalus and Oxytropis (Fabaceae)

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This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of The University of New Mexico in partial fulfillment of the requirements for the degree of

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BIOCHEMICAL RELATIONSHIPS IN ASTRAGALUS AND OXYTROPIS (FABACEAE)

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BIOCHEMICAL RELATIONSHIPS IN *ASTRAGALUS* AND *OXYTROPIS* (FABACEAE)

By

Stephan L. Albrecht

B.C., University of New Mexico, 1968

THESIS

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Dried plant material was hydrolyzed in 2 N hydrochloric acid, the hydrolysates were extracted with *n*-amyl alcohol, and chromatographed in two dimensions using thin-layer chromatography (TLC). The patterns and the R_F values of the "spots" separated were recorded and analysed.

The flavonoid patterns for all the plant organs studied were similar and no definite relationships between the flavonoid patterns and morphological characters could be ascertained. The flavonoid patterns exhibited by the members of both taxa indicate that the present means of morphological separation of this plant complex into two genera may not be valid.

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INTRODUCTION

The taxa Astragalus and Oxytropis (Fabaceae) have long presented a problem in generic separation because of their obviously close morphological similarity. This study was an attempt to analyze their relationships on a more fundamental level, and to determine if biochemical evidence may aid in making a sharper deliniation between these two taxa, or in modifying the concept of relationships arrived at previously through morphological studies.

The genus Astragalus (milk-vetch), comprising about 1,500 species, is distributed throughout the northern hemisphere as well as South America and southeast Africa. The genus Oxytropis (loco-weed), including about 300 species, occurs in Europe, Asia, and North America. Both are common in southwestern United States.

Although Astragalus and Oxytropis are generally regarded as separate entities, the apparent lack of distinguishing characteristics points to a need for reevaluation of the taxonomic status of these taxa. These somewhat nebulous generic boundaries have been points of controversy between taxonomists for many years.

The separation of Oxytropis from Astragalus is mainly dependent on a single character, that of the extension of the keel into a beak in Oxytropis in contrast to the beakless keel in Astragalus.

Wooton and Standley (1915) noted that Oxytropis was "like Astragalus in most particulars, but distinguished by the short subulate prolongation or beak of the keel." In their opinion this was sufficient evidence to separate these related species into two separate genera

designated Astragalus and Oxytropis. However, because this characteristic seems not to have been sufficiently clear-cut, all the species of Oxytropis mentioned by Wooton and Standley (1915) have been placed in the genus Astragalus at one time or another.

The majority of authors including Hutchinson (1964), Porter (1951), and Weber (1967) tend to retain the separate generic ranking. Interestingly the descriptions of the two genera by Hutchinson (1964) are practically identical. Kearney and Peebles (1964) also place the two taxa in separate genera although they feel compelled to qualify their arrangement of Oxytropis, saying that "the genus is a very weak one, and is merged with Astragalus by some authorities."

Tidestrom and Kittell (1941) did not consider that the two taxa were sufficiently distinct to warrant generic separation, thus Oxytropis was reduced to the status of one of the subgenera of the genus of Astragalus. Other authors were not convinced of the necessity of generic separation, and Wheeler (1939) reported that Hegi treated Oxytropis only as a synonym of Astragalus.

The division of the two taxa by the sole characteristic of the beaked keel not only results in disagreements between authors, but poses certain difficulties in identification for individuals. Wheeler (1939) stated that in 1895 M. E. Jones found both Astragalus acutirostris and A. nothoxys with acuminate keels, and that Jones was motivated to transfer A. acutirostris to the genus Oxytropis. Apparently Jones was not completely convinced of the correctness of this move because he later retracted this designation.

Some authors consider that a major point of consideration in generic delimitation is the caulescent habit of Astragalus in contrast to the acaulescent characteristic of Oxytropis. Wheeler (1939) believes this method fails in two ways: "Oxytropis pilos (L.) DC. (based on Astragalus pilosus (L.))has long stems. On the other hand Astragalus mollissimus Torr. bears such a strong habitual resemblance to Oxytropis Lambertii Pursh, a species with the habit considered typical of Oxytropis, that it may be more than a coincidence that these two species share the distinction of being the two worst 'loco-weeds'." Wheeler also points to the caulescent habit of Astragalus alpinus L. as bearing a strong resemblance to that of Oxytropis foliolosa Hook.

Biochemical Systematics

Historical

The application of chemistry to taxonomy began almost 160 years ago. In France, A. P. de Candolle gave much attention to the chemical properties of plants as correlated with their morphological characters.

In 1886 Helen C. De S. Abbott published a paper entitled Certain Chemical Constituents of Plants Considered in Relation to Their Morphology and Evolution. This was a novel concept for its time, and its author presented some very modern ideas, one of which advocated using secondary metabolic compounds in studies of relationships rather than basic metabolites, the latter group being presumably very similar in most species. Gibbs (1963) mentions that in 1909 Greshoff used the term "comparative phytochemistry" and suggested using a short chemical description as a part of the formal description of a new genus or species.

Unfortunately little progress was made during these early years, possibly due to the developing interest in genetics and enzymology, the lack of chemical knowledge by early biologists, and the lack of suitable techniques needed for biochemical investigations. Prior to the early 1940's biochemical systematics was a costly and time-consuming affair. In recent years, however, rapid advances in biochemical knowledge and the development of efficient, relatively sophisticated physical techniques have helped make biochemical systematics a useful research tool.

Chromatography

Much of the progress in biochemical systematics has been due to the development of chromatographic methods. Regarded as recent in origin, some of these methods actually are very old. Pliny, circa 70 A. D., used papyrus impregnated with tannins to detect ferrous sulphate. Schonbein's work in "capillary analysis" marked the real beginning of chromatography. Chromatography developed as a research tool about the turn of the century with the studies of Day and Tswett (Gibbs, 1963). Chromatography did not acquire its great popularity until Martin and Synge (Macek and Hais, 1962) published their papers on the separation of amino acids. Since that time chromatography has become a powerful tool in plant studies and has played a large part in the emerging field of biochemical systematics.

The separation of compounds by thin-layer chromatography (TLC), using a cellulose layer as the support medium, depends upon the partition of the compounds between two solvents. The cellulose has a strong affinity for the water phase of the solvent system but little affinity for the

organic liquid fraction. The cellulose acts as an inert medium supporting the stationary aqueous phase. As the solvents travel up the plate on which a solute has been applied, some of the solute will enter the organic phase. When the mobile organic liquid containing solute reaches a part of the plate that contains no solute in the aqueous phase, partitioning of the solute between the aqueous and the organic phases again takes place and some of the solute is transferred to the cellulose-water layer. With continuous solvent flow the effect of the partioning operation is to move the solute from its point of application to a point further up the cellulose layer in the direction of solvent flow. This process can be compared to fractional distillation.

This technique is analagous to paper chromatography. However, Randerath (1963) states that his experiments with TLC using cellulose as the support layer affords a much better resolution of compounds than conventional paper chromatography.

Flavonoids in Biochemical Systematics

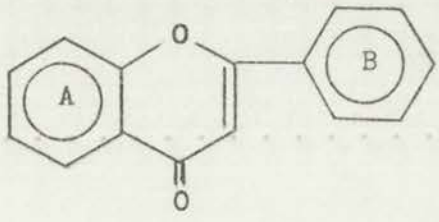
The primary objective of chemical plant taxonomy is to clarify the relationships of plants by studying their chemical compositions. Apparently, not all compounds are useful indicators of taxonomic relationships, for it is obvious that compounds which play a primary role in metabolism of most or all plants will not be as useful to taxonomic study as the more variable so-called secondary products of metabolism.

The plant kingdom contains a great many classes of such secondary chemical constituents, but much is yet unknown about their separation, synthesis, and function. The presence of these compounds should indicate

the nature of the metabolic pathways involved in their development and give some insight into the biochemical processes unique to certain organisms. Harborne (1966) admits that the significance of any change in a pattern of flavonoid synthesis may not be clear; however, he points out that it is probably due to an indirect response to changes in the primary metabolic pathways.

The most taxonomically useful substances are the stable end products of metabolism, such as the alkaloids, terpenoids, and the water-soluble plant pigments. Among the secondary metabolites those containing phenolic groups are very common, and seem to be the most widely use taxonomically. Karrer (1958) estimates that 36% of the natural products from plants contain a phenolic group and a large segment of that category includes the flavonoid compounds.

Many researchers in the field use the term flavonoid to include the anthocyanins, flavonols, flavones, flavanones, and flavanonols which possess the heterocyclic configuration of flavone (2-phenylchromone I).



I

Sometimes the closely related compounds: isoflavones, chalkones, aurones, catechins, leuco-anthocyanins and the biflavonyls, are included in the flavonoid group.

The biosynthesis of flavonoids has been under investigation for some time, much of the early work being done by Sir Robert Robinson in

the 1930's. At first the aromatic rings were thought to be derived from the Shikimic acid pathway, accepted by authorities as the principal route in the formation of many phenolic compounds. Birch (1962) points out that as early as 1953 he postulated that the "A" ring of the flavonoid skeleton was formed from acetic acid units, rather than the shikimic acid pathway, an hypothesis now widely accepted. The "B" ring and its three adjacent carbon atoms, however, are apparently formed by the shikimic acid pathway.

Alston and Turner (1963) state that "the inheritance of the flavonoid pigments has been studied more intensively than perhaps any other group of chemical substances in flowering plants." There is a genetic basis for flavonoid formation, and flavonoids appear to be relatively close to the site of gene action, which is important when considering these substances as taxonomic indicators. Geissman and Hinreiner (1952) noted that specific genes control several important aspects of flavonoid chemistry. These genes control the presence or absence of flavonoid, the degree of oxidation or the number of hydroxyl groups present, the extent to which methylation occurs, the number and position of sugar residues, and the pH of the cell sap.

Flavonoids lend themselves very well to use in taxonomic studies because of some rather special properties. They seem to be chemically stable regardless of whether the plant is in a fresh condition or in a state of dehydration for an indefinite length of time. Bate-Smith (1965) compared fresh and dry specimens and noted that the results indicated no qualitative differences in flavonoid constituents, even though some of the specimens "were stored for over 100 years without

either complete deterioration of the constituents themselves or the appearance of interfering products of oxidation."

Furthermore, the environment also seems to have little effect on the presence or absence of flavonoids. Turner (1967) cited a study by McClure and Alston in 1964 on the effect of environmental variables on chromatographic patterns of flavonoids, in which uniclonal-derived populations of Spirodela oligohiza were aseptically cultured in a wide range of environmental conditions. Turner reported that there was no evidence that varied environmental conditions altered the flavonoid pattern, even though the morphology of the plants changed significantly in different environments. Earlier Bate-Smith (1963) had reported that the occurrence of flavonoid compounds in the vegetative tissues of specific taxa was rarely variable. This was apparently his major reason for favoring flavonoid analysis as an important taxonomic criteria.

Swain and Bate-Smith (1962) indicated that flavonoids from the leaves should be used in a taxonomic survey because they considered leaves as more representative of the organism as a whole.

Although flavonoid compounds occur in all parts of the plant, Geissman (1962) believes their ubiquity is not based on the action of natural selection for color. Swain and Bate-Smith (1962) cite examples of the roles played by flavonoids as proposed by several authors, for example: as substrates for oxidation-reduction processes related to those of phenolase; in antibiotic action, particularly against fungi and viruses; in preventing the cross-pollination in two varieties of Forsythia; and as soluble carbohydrate reserves. In a more recent article Bate-Smith (1963) stated that flavonoids can promote or suppress

lignin deposition in the vascular plants. More recently Harborne (1966), in referring to the evolution of flavonoids in the angiosperms, also connected the function of flavonoids to liginification.

The flavonoid compounds are widely distributed in the higher plants and many flavonoids have been reported in the Fabaceae by Geissman and Hinreiner (1952) and Venkataraman (1959) as well as others.

Methods for identification of flavonoids range from simple chemical tests to the use of nuclear magnetic resonance (NMR). Many investigators have used chromatography with success. Harborne (1960) is convinced that chromatographic methods work well in the separation of flavonoids and provide an accurate means for provisional identification based on R_F values and color reactions. He is also of the opinion that confusion of flavonoid spots with those of other pigments or other natural products is unlikely. Most of the flavonoid compounds will fluoresce in ultraviolet light, and the color emitted is an indication of the type of compound present. In addition, most of the flavonoids will produce a characteristic color in either visible or ultraviolet light with various detection reagents, thus providing additional information as to the nature of the compounds present.

MATERIALS AND METHODS

Plant Materials

The samples of dried plant material used were obtained from specimens deposited in the herbarium of the University of New Mexico. When possible as many different organs of the plant were collected as permitted by the individual specimen. These were then segregated into categories of leaves, stems, sepals, petals and seed pods.

The species surveyed in this investigation include Astragalus albulus Woot. & Standl., A. allochrous Gray, A. alpinus L., A. amphioxys Gray, A. bisulcatus (Hook.) Gray, A. calycosus Torr., A. crassicarpus Nutt., A. diphysus Gray, A. drummondii Dougl. ex Hook., A. emoryanus (Rydb.) Cory, A. flavus Nutt., A. flexuosus Dougl., A. giganteous Wats., A. gilensis Greene, A. haydenianus Gray, A. hallii Gray, A. humistratus Gray, A. lentiginosus Doug., A. missouriensis Nutt., A. mollisimus Torr., A. nothoxys Gray, A. nuttallianus DC., A. praelongus Sheld., A. shortianus Nutt., A. tephrodes Gray, A. wootoni Sheld., A. yaquianus Wats., Oxytropis lambertii Pursh, O. oroephilia Gray, O. sericea Nutt., and O. viscida Nutt..

The species were selected as a representative sample from a widely diversified group of organisms.

Extraction of Phenolic Compounds

The extraction procedure used was essentially that of Bate-Smith (1965). Samples of dried plant material weighing between 0.2 to 1.5 g were macerated in a mortar and pestle and transferred to a 250 ml round-bottomed flask. One hundred ml of aqueous 2 N hydrochloric acid was

added and a reflux condenser placed on the flask, and the hydrolysis effected by heating the mixture for 20 minutes in a boiling water bath (see Figure 2). After 20 minutes the water bath was removed and the flask was allowed to return to room temperature. At this time the mixture had undergone a color change, usually becoming red-brown. The plant parts were then removed by filtering the entire mixture through a Büchner funnel.

The filtrate was then transferred to a separatory funnel where the hydrolysates were extracted with 10 ml portions of n-amyl alcohol (1-pentanol), the organic layer becoming colored during this operation. The aqueous acid was then removed from the funnel, and the remaining organic phase was washed several times with small portions of distilled water, and finally washed with a small portion of a saturated sodium chloride solution. The washed organic layer was placed over anhydrous magnesium sulfate until the former became essentially water free. The magnesium sulfate was removed by gravity filtration. This solution was applied immediately to the activated TLC plates and chromatographed.

Chromatography

The thin layer plates were prepared using a Desaga spreader, adjusting the thickness regulator to give a thickness of 300 μ. The plates were first thoroughly scrubbed with Alconox, then rinsed in tap water and finally rinsed in distilled water. The plates were dried overnight at room temperature. Five of the 20 X 20 cm plates were aligned in a Brinkmann Standard Mounting Board. To prevent any sliding during spreading the plates were affixed to the board with a film of distilled

water. A five by 20 cm end plate was positioned at each end of the board to allow for an even and continuous motion of the spreader. The process of coating the guide-bar of the spreader with a film of glycerol allowed a more uniform movement of the spreader and resulted in an even distribution of the cellulose. Ten g of Avicel Micro Crystalline Cellulose were combined with 90 ml of distilled water and mixed in a Sorvall Omnimixer until the cellulose was in complete suspension. The cellulose suspension was then quickly transferred to the spreader and the plates were prepared.

After spreading, the plates were not moved until their surface had acquired a "mat" finish. Any cellulose adhering to the sides or back of the plates was removed at this time, and the coated plates were dried overnight at room temperature. The plates were activated at 105 C and were used immediately.

The solution to be chromatographed was spotted at a point 1.5 cm from the edge of the plate using a 10 μliter micropipette. Spots were made in three of the corners of the TLC plate. (See Figure 1.)

This initial arrangement of the spots would not only display the movement of the components in two dimensions on the completed chromatogram, but the behavior of the components in one dimension in each solvent system would also be apparent.

The sample was applied with a micropipette by lightly touching the tip of the pipette to the cellulose layer. In order to make the spots as small as possible (not more than 5 mm in diameter) several applications of minute amounts of solution, allowing evaporation of solvent between each application, were made. A small mark was placed

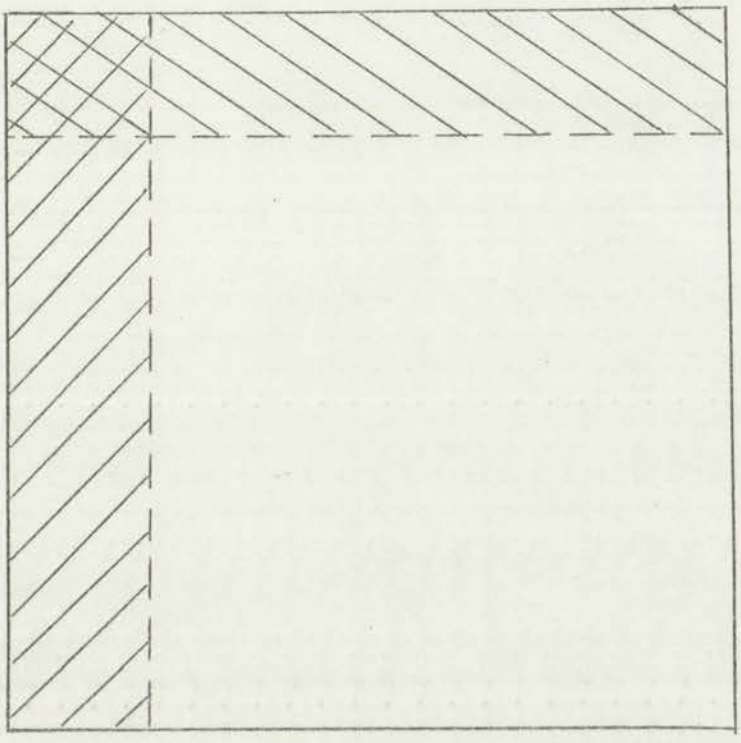


FIGURE 1. Diagrammatic representation of developed chromatogram. ⊠ area not developed. ▨ area developed in one dimension. □ area developed in two dimensions.

at the extreme edge of the cellulose layer and at the same latitude as the spot to show the location of the origin, a point of reference important for the calculation of R_F values.

Once the plates had been spotted they were developed in one dimension by using Forestal solvent (distilled water, hydrochloric acid, and glacial acetic acid in a ratio of 10:3:30) in a Brinkmann or Desaga rectangular tank. Two hundred ml of solvent was used. To get the atmosphere of the tank as fully saturated with solvent as possible, Gelman Solvent Saturation Pads were used and several hours were allowed to elapse in order for the tank to reach a saturation equilibrium. After the solvent had traveled approximately 12 to 15 cm the plates were removed from the tank and air dried at room temperature.

The plates were then developed, at a right angle to the previous direction, in a second tank containing 200 ml of BAW (the top phase only of a mixture of n-butanol, glacial acetic acid, and distilled water in a ratio of 4:1:5). Again the solvent front was allowed to migrate 12 to 15 cm. All of the chromatography was carried out at room temperature (22 to 24 C).

The plates were then viewed under ultraviolet light in a Chromato-vue cabinet, first under long wavelength (3650 A) and then under short wavelength (2537 A). Any spots that were evident either in visible light or under ultraviolet light were outlined with a pencil, their colors under the respective light noted, and the chromatogram placed on a tracing table where the pattern was transferred to paper.

After the initial data were taken, the plates were sprayed with a 1% w/v solution of sodium carbonate. When dry, any spot that had become

visible was noted and the plates were again viewed under the ultraviolet light and any color change of the original spots was noted and any additional spots that had appeared were recorded as before. The plates were then sprayed with a solution of 0.1N $AgNO_3$ and 5N NH_4OH in a ratio of 1:1. When the plates had dried after the final spraying, they were examined for any color changes in previous spots and if any changes appeared they were recorded.

After all the spraying was complete, the R_F values for each spot (the ratio of the movement of the substance to that of the solvent front) were computed.

RESULTS

The compounds that were separated, for the most part, fell into two broad categories: (1) those that fluoresced purple in ultraviolet light and (2) those that fluoresced yellow in ultraviolet light. The characteristics of the compounds, i.e. color reaction in visible light (VL), fluorescence in ultraviolet light (UV), and the color reactions to the spray reagents, along with the mean $\underline{R_F}$ values and the standard errors are given in Table 1. The presence or absence of these compounds in the plant species surveyed is indicated in Table 2.

Although the completed plates were viewed under both long wavelength (3650 A) and short wavelength (2537 A) ultraviolet light, no distinction in color value was made between the two forms; apparently only the intensity of the fluorescence varied (being less intense in the short wavelength).

Evidence presented in Table 1 indicates that only one of the spots was apparent in visible light; however, when sprayed with sodium carbonate solution certain spots became yellow in visible light. After being sprayed with sodium carbonate solution, some spots became more intense in ultraviolet light, and after spraying with ammonical silver nitrate solution some spots became dark brown in visible light.

One of the more striking color changes was produced as a distinct red color, during the refluxing stage in the extraction of the flavonoid compounds from Astragalus lonchocarpus, in contrast to the brown or yellow color produced in the same stage when the other species were extracted. Also A. lonchocarpus was the only species in this study that appeared to have an anthocyanin present in the leaves.

In addition to the spray reagents used all the extracts were treated

with alcoholic ferric chloride in the manner used to test for phenolic compounds. This test invariably gave a dark blue color, characteristic of certain phenolic compounds (Geissman, 1955).

Because the presence of additional compounds, i.e., amino acids and sugars, would tend to affect the reproducibility of the R_F values, tests for these compounds were made. All the tests were negative.

The two genera exhibit no apparent relationships between the morphological characteristic and the flavonoid pattern. In addition flavonoid patterns are not uniform among species of the same genus but may be identical between certain species of different genera. For example, the flavonoid patterns for Oxytropis lambertii and O. sericea are identical to those of Astragalus yaquiensis, A. Praelongus, A. missouriensis, A. haydenianus, A. bisulcatus, and A. allochrous; and the flavonoid pattern of O. viscida is the same as that of A. amphioxys.

With few exceptions, all the spots characteristic of the composite chromatographic pattern of Astragalus also are featured in the composite pattern of Oxytropis.

A control, duplicating the entire procedure, with the exception of using plant parts, was also run. The results were negative, indicating that no contaminants were present in either the apparatus or the reagents used.

TABLE 1. R_F values and color reactions recorded for extracted compounds.

Spot no.	R_F value				Color reactions				
							Na_2CO_3		$AgNO_3$
	BAW	S.E.	F.S.[1]	S.E.	VL	UV	VL	UV	VL
1	0.91	0.008	0.91	0.005	-	P	-	BP	Bn
2	0.84	0.006	0.87	0.005	-	P	-	BP	-
3	0.73	0.007	0.85	0.008	-	P	-	P	-
4	0.73	0.008	0.58	0.008	-	Y	-	Y	-
5	0.63	0.005	0.54	0.004	-	Y	Y	BY	Bn
6	0.51	0.009	0.37	0.010	-	Y	-	Y	Bn
7	0.34	0.010	0.70	0.006	-	Y	-	Y	-
8	0.21	0.039	0.70	0.014	-	Y	-	Y	-
9	0.50	0.105	0.81	0.016	-	Da	Y	Da	-
10	0.86	0.018	0.77	0.032	-	Da	Y	Da	-
11	0.18	0.005	0.24	0.018	R	R	Bl	Bl	Bn
12	0.85	0.017	0.68	0.006	-	P	-	P	-
13	0.80	0.001	0.64	0.004	-	Y	-	Y	-

[1] Explanation of abbreviations: B = bright; Bl = blue; Bn - brown; Da = dark; F.S. = Forestal solvent; P = purple; R = red; UV = ultraviolet light; VL = visible light; Y = yellow. Spot numbers correspond the numbered spots found in the chromatograms in the appendix.

TABLE 2. Distribution of flavonoids.

Species	Spot number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. albulus	+	+	+				+	+					
A. allochrous	+	+	+	+	+	+	+	+					
A. alpinus	+	+	+	+	+	+							
A. amphioxys	+	+		+	+								
A. bisulcatus	+	+	+	+	+	+	+	+	+				
A. calycasus	+	+	+	+	+	+	+		+	+			
A. crassicarpus	+	+	+	+	+	+						+	
A. diphysus	+	+	+	+	+	+							+
A. drummondii	+	+	+	+	+	+							
A. emoryanus	+	+	+		+	+			+	+			
A. flavus	+	+	+	+	+	+						+	
A. flexuosus	+	+	+	+	+	+	+	+	+	+			
A. giganteous	+	+	+	+	+	+							
A. gilensis	+	+	+	+	+	+							
A. hallii	+	+		+	+	+	+	+					
A. haydeniamus	+	+	+	+	+	+	+	+					
A. humistratus	+	+	+	+	+		+	+				+	
A. lentiginosus	+	+	+	+	+								
A. lonchocarpus	+	+			+						+		
A. missouriensis	+	+	+	+	+	+	+	+				+	
A. mollisimus	+	+	+		+		+	+					
A. nothoxys	+		+		+	+	+	+					
A. nuttallianus	+	+	+		+	+	+	+					

TABLE 2. Continued.

Species	Spot number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. praelongus	+	+	+	+	+	+	+	+					
A. shortanius	+	+	+		+	+	+	+					
A. tephrodes	+	+	+	+	+	+							
A. wootoni	+	+	+	+	+	+							
A. yaquianus	+	+	+	+	+	+	+	+					
O. lambertii	+	+	+	+	+	+	+	+					
O. oroephilia	+				+	+							
O. sericea	+	+	+	+	+	+	+	+					
O. viscida	+	+			+	+							

DISCUSSION

Only the flavonoid patterns found in the leaves were presented in the results, primarily because leaves were the only organs present in all specimens. When available pods, stems, sepals, and petals were extracted, chromatographed, and the chromatographs compared with those of the leaves. Typically the flavonoid patterns in all the organs studied were identical. The only notable exceptions were apparent in the species having flowers with purple petals; these apparently contain an anthocyanin not found in the leaves. These results compared favorably with those of other investigators in the field of flavonoid extraction (Swain and Bate-Smith, 1962; Harborne, 1966).

Although none of the compounds chromatographed were conclusively identified, most of the evidence points to the high probability that these are flavonoid compounds. Hais and Macek (1963) state that the "majority of flavonoid substances possess either a characteristic colour, or fluorescence in ultraviolet light." Although only one of the compounds isolated in this study exhibited color in visible light, they all fluoresced in ultraviolet light. In addition when the extracts were treated with alcoholic ferric chloride, they produced a dark blue color. This reaction is indicative of a flavonoid with a 3,4,5-trihydroxy grouping in the "B" ring (Geissman, 1955).

Most authorities believe that fluorescence of the flavonoids yields little information about the precise arrangement of the chemical groups. Many, however, are agreed that unknown and unidentified flavonoids can be arranged in particular classes based on the color values in ultraviolet light as well as the color reaction to certain reagents.

Venkataraman (1959) states that a compound giving a yellow color with alkali indicates the presence of a phenolic substance.

Geissman (1962) mentions that anthocyanins will change from red to blue in alkali, and Harborne (1960) states that anthocyanins appear mauve in visible light. It is probable that the red spot that was isolated from Astragalus lonchocarpus is indeed an anthocyanin. The tests outlined by Hayashi (1962) were applied to this compound, and the results indicate the likelihood of a delphinidin-type anthocyanin being present.

Seikel (1962) describes the flavones and the flavonols as compounds that appear yellow in ultraviolet light and remain yellow or become bright yellow in ultraviolet light after being sprayed with a sodium carbonate solution. Both Hais and Macek (1963) and Harborne (1960) concur with this definition. Therefore, there is a strong possibility that those spots which exhibit these characteristics are either flavones or flavonols. In addition, two groups of flavonoid compounds provide a blue-violet (sometimes described as purple) color under ultraviolet light. They are the 3,5-methoxylated flavonols, as reported by Harborne (1960), and the isoflavones, as reported by Hais and Macek (1963). Because the presence of methoxyl groups increases the R_F values of flavonoids, and because the purple spots in the chromatograms have characteristically high R_F values, added evidence is given for the probability that these spots represent 3,5-methoxylated flavonols (Hais and Macek, 1963).

Since the greater number of phenolic groups on the molecule produce a decrease in the R_F value, it could be assumed that the spots designated numbers 6, 7 and 8 (Table 1) possess more phenolic groups than the other spots present.

Some inferences can be made about the steriochemistry of the compounds isolated. For example, it is strongly probable that none of the molecules worked with are planar in the arrangement of their benzene nuclei. Such planar molecules have been reported to have $\frac{R}{F}$ values very near zero in aqueous solvent systems, and none had these values.

Flavonoid Patterns and Morphological Relationships

Although no definite correlations are apparent between flavonoid patterns and morphological differences, it is interesting that many species shown by Wooton and Standley (1915) as being closely related also exhibited identical flavonoid patterns.

It is, however, apparent by observing Table 2 that the flavonoid patterns in Astragalus and Oxytropis, as shown by this survey, provide no support for the arrangement of these two taxa as separate genera. This survey does present evidence in support of those taxonomists who combine Astragalus and Oxytropis into the single genus Astragalus. The biochemical evidence seems to indicate that these two taxa have not yet diverged sufficiently in relation to their genetic compositions to be considered separate genera, an assumption also borne out by morphological evidence.

This study in no way exhausts all the chemical indicators that can be presented by this group of plants. It is generally considered by most workers in the field of chemical systematics that the number of chemical indicators studied is directly proportional to the usefulness of the study in the evaluation of natural relationships.

Additional work should include an analysis of both alkaloid and

terpenoid components. Also a more recent approach to chemical taxonomy has been the use of protein analysis. Although this is an expensive and complicated field, some very useful information could be obtained from such study if it were applied to the Astragalus-Oxytropis complex.

Conclusions

As shown by this study, the genera Astragalus and Oxytropis possess a variety of flavonoid compounds. These compounds, however, offer little support to the concept of generic separation between these two taxa.

The data collected during this research seems to favor those taxonomists who combine Astragalus and Oxytropis into a single genus. Although relatively few chemical indicators were employed in this study, the evidence presented indicates possible criteria for re-evaluation of the generic status of Astragalus and Oxytropis, and tends to support the rather obvious morphological similarities. This study also indicates that new approaches and possibly additional data, particularly in the realm of biochemistry should be sought out, possibly as a valid adjunct to the existing system of classification.

SUMMARY

1. Fluorescent compounds were extracted from dried specimens of *Astragalus* and *Oxytropis*.
2. The compounds were chromatographed by using two-dimensional thin-layer chromatography (TLC).
3. The compounds were tentatively identified as belonging to the flavonoid group of organic compounds.
4. The chromatographic patterns of the compounds from different taxa do not support the existing generic distinction that is made between *Astragalus* and *Oxytropis*, and this survey suggests that possibly *Astragalus* and *Oxytropis* should be placed in the same genus.

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APPENDIX

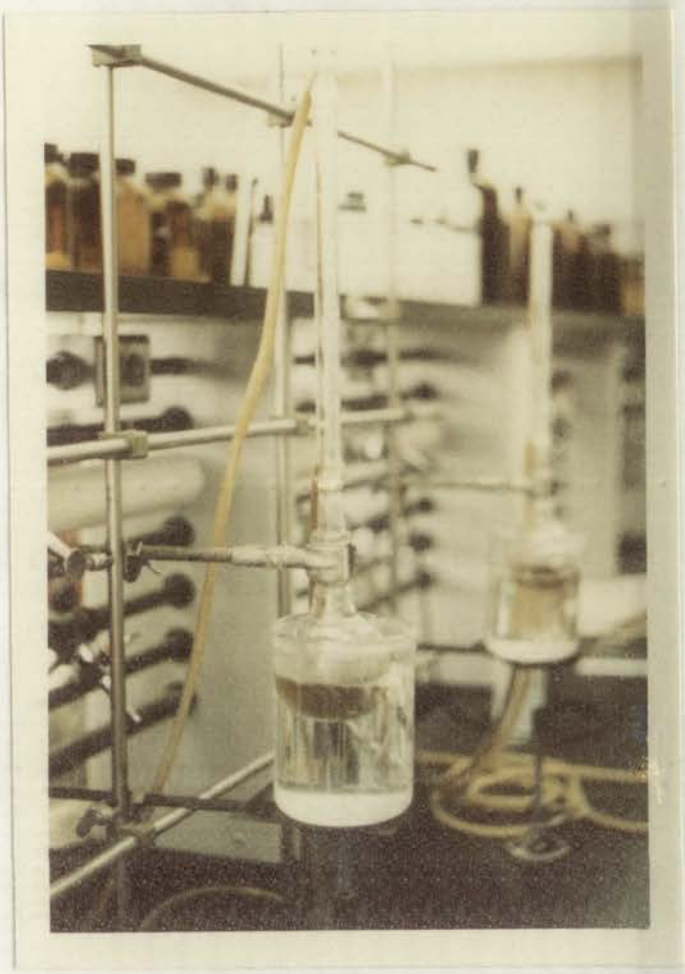

FIGURE 2. Extraction apparatus.

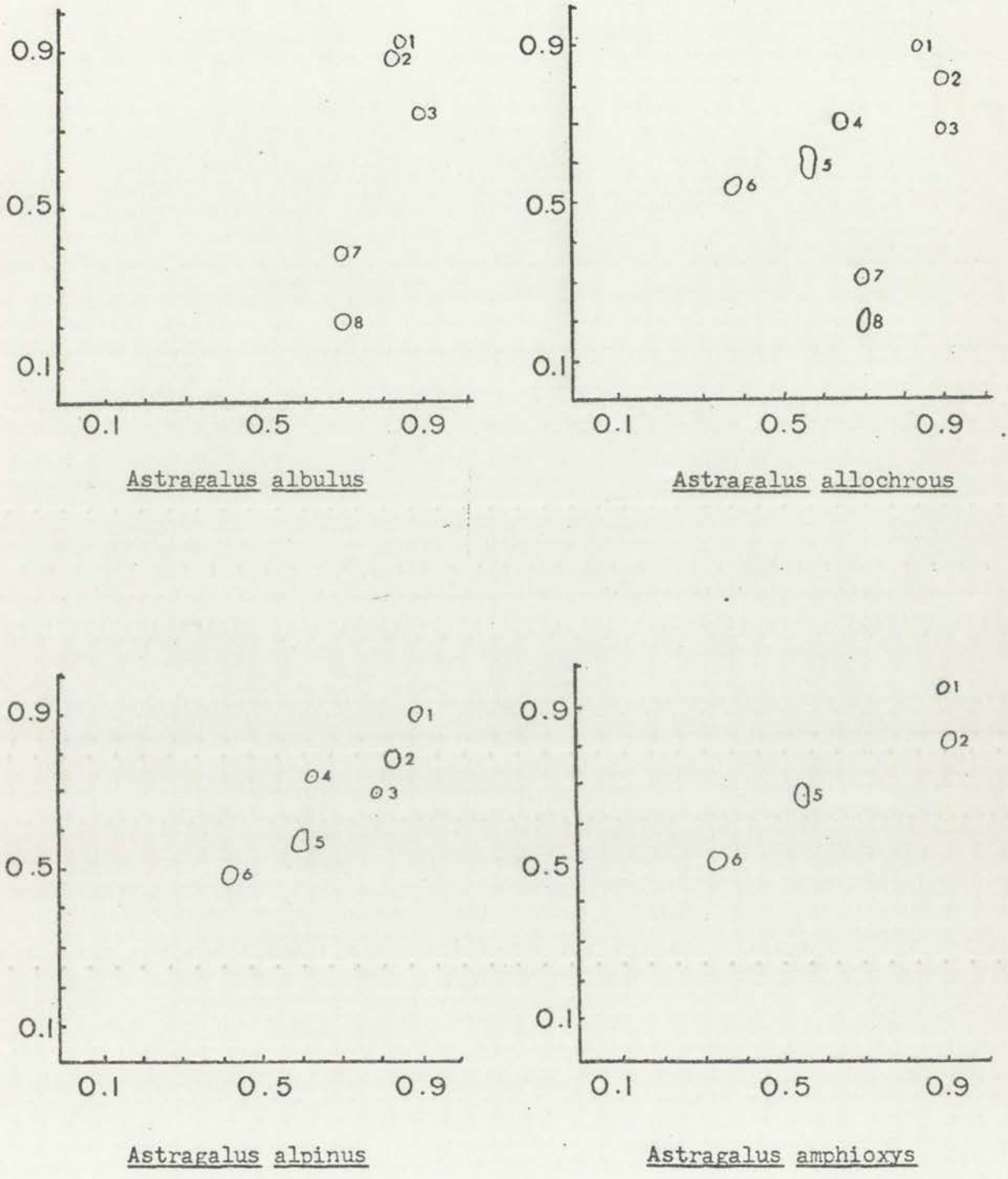

FIGURE 3. Representative chromatograms of four selected species of Astragalus. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.

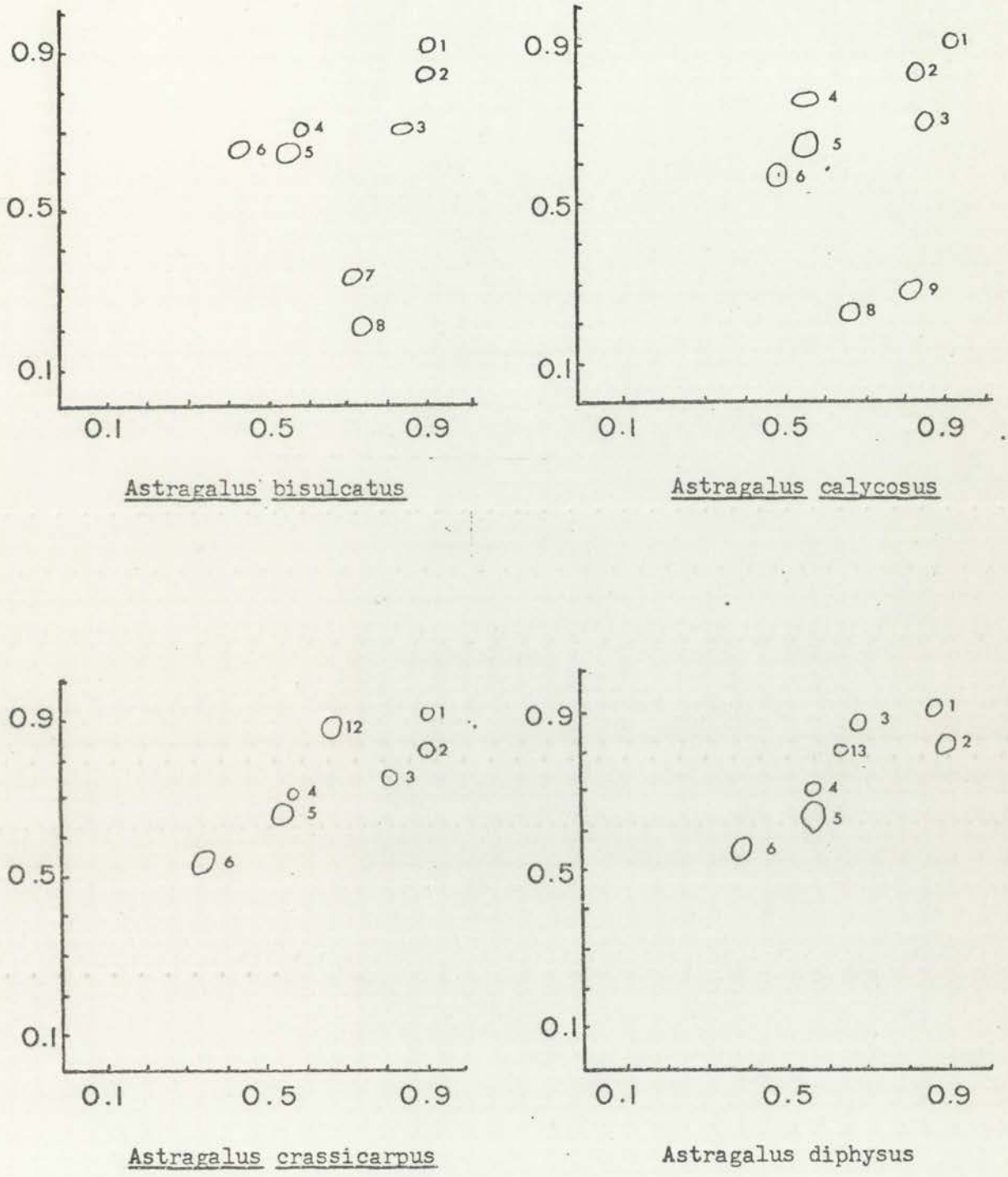

FIGURE 4. Representative chromatograms of four selected species of Astragalus. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.

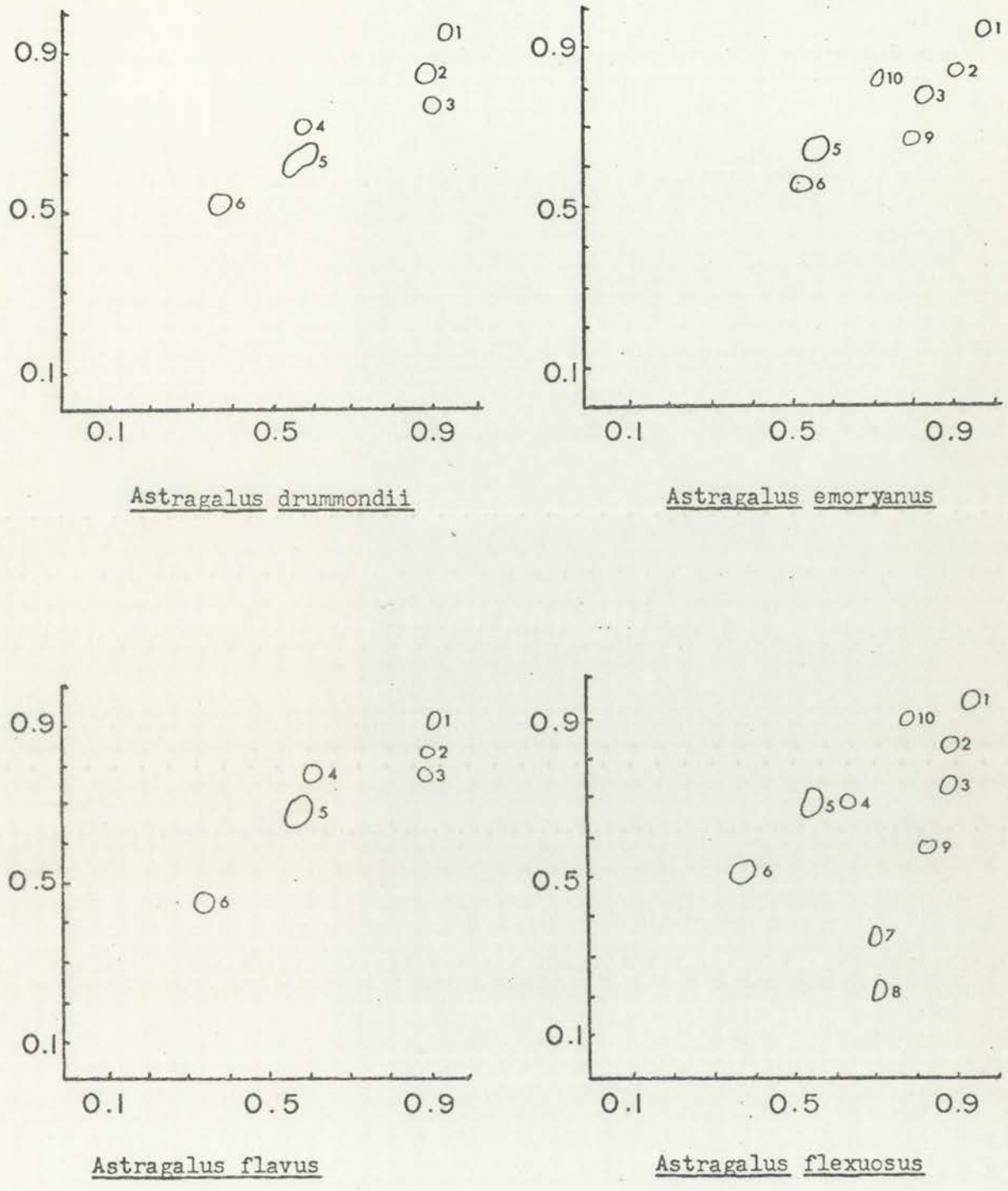

FIGURE 5. Representative chromatograms of four selected species of Astragalus. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.

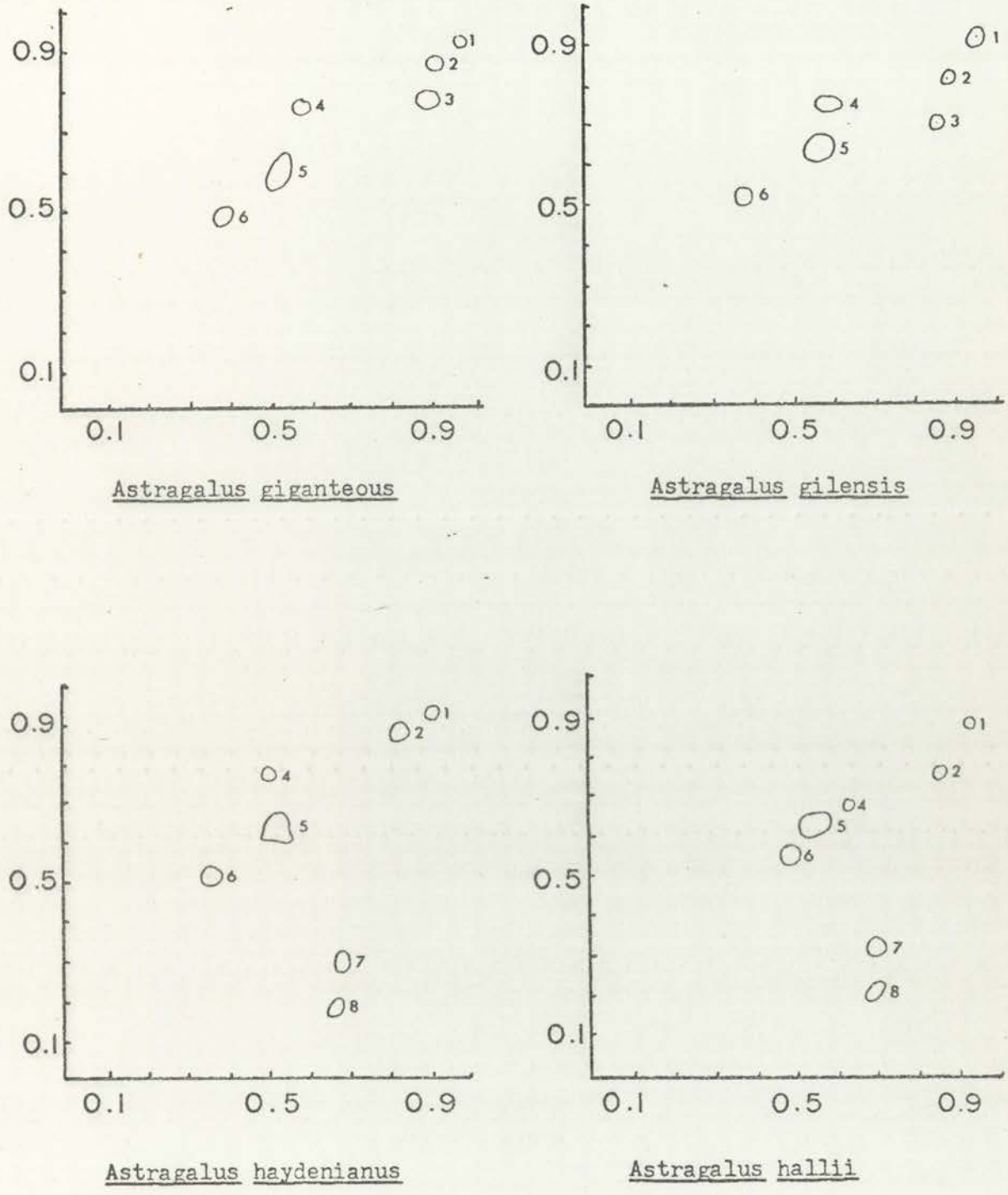

FIGURE 6. Representative chromatograms of four selected species of Astragalus. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.

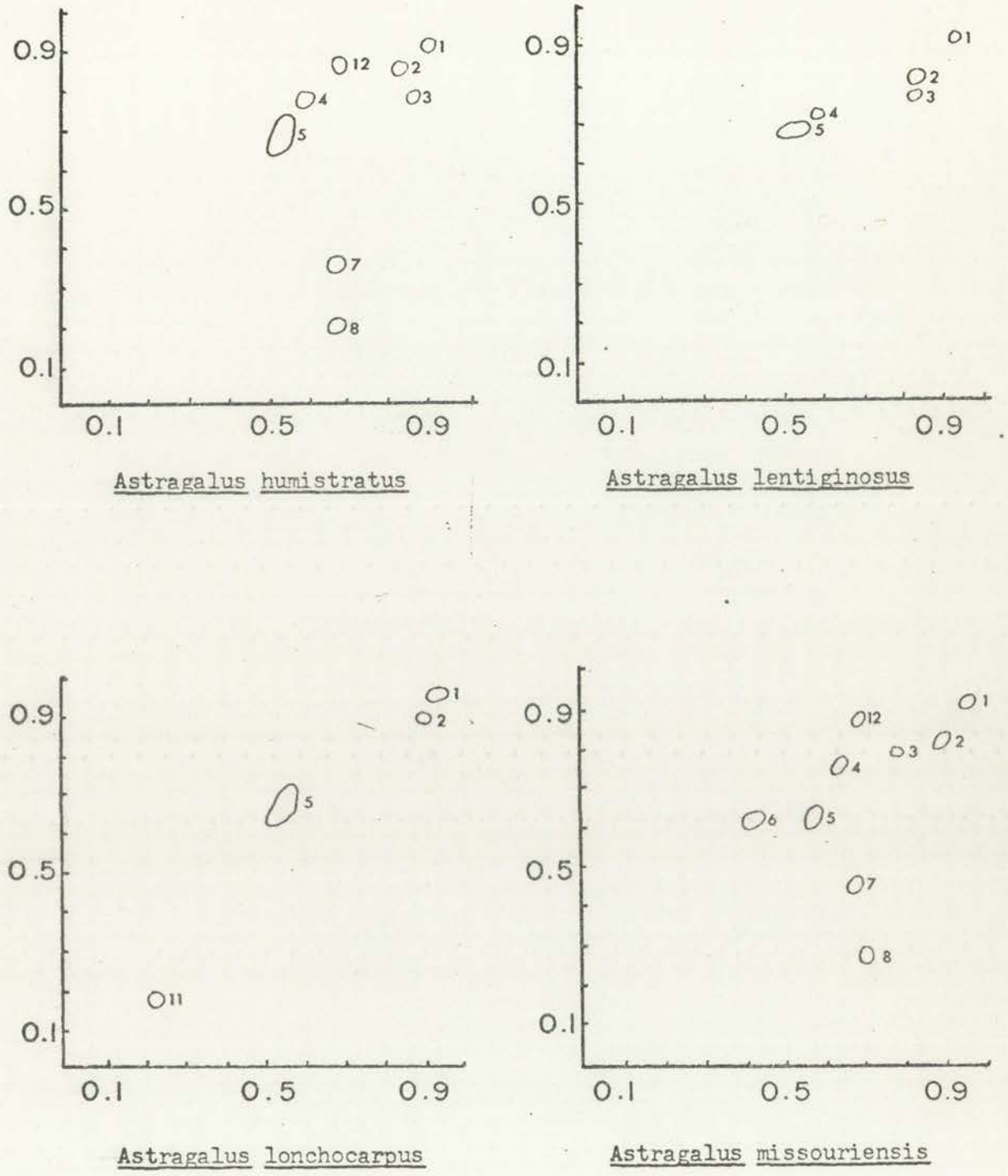

FIGURE 7. Representative chromatograms of four selected species of Astragalus. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.

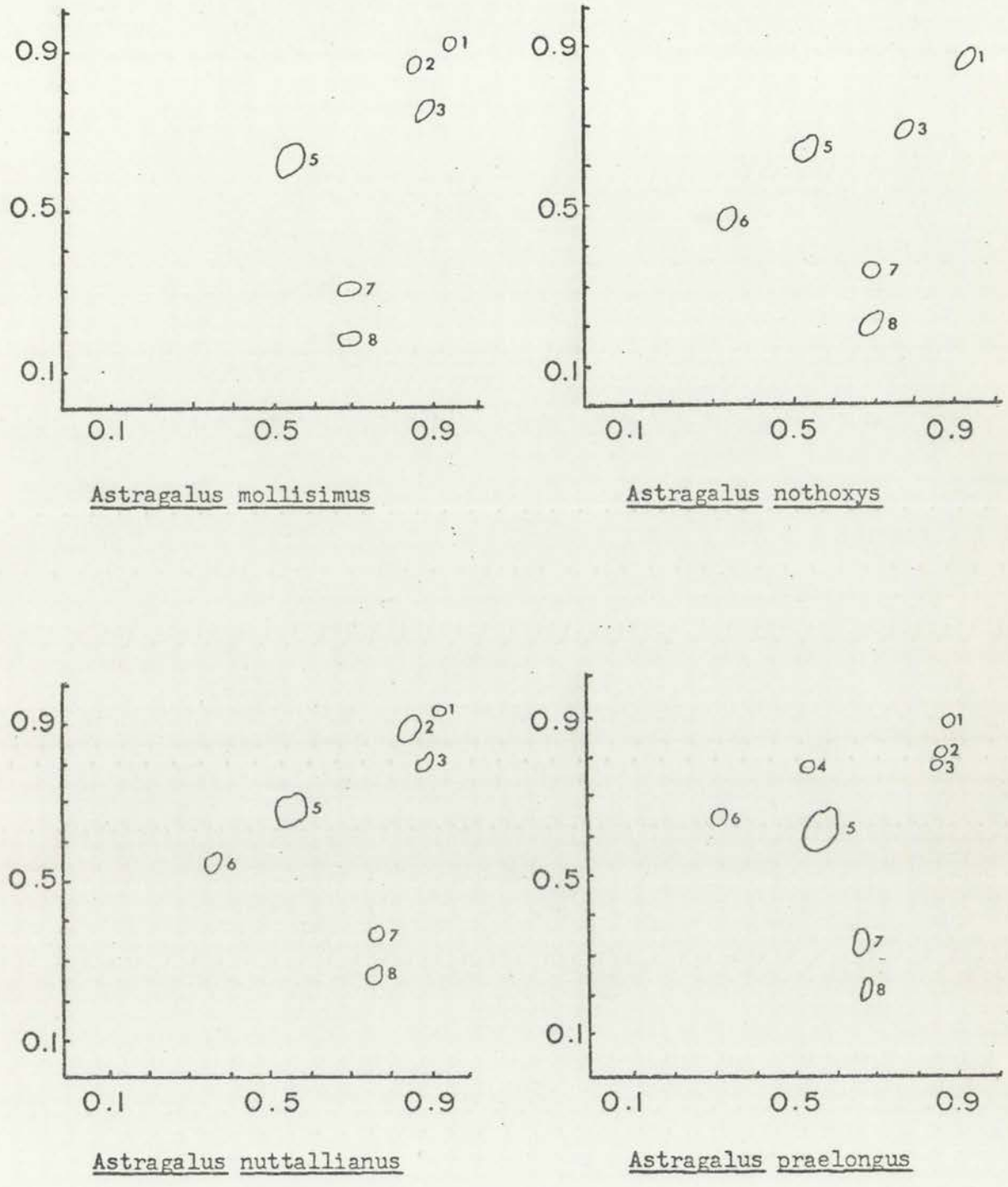

FIGURE 8. Representative chromatograms of four selected species of Astragalus. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.

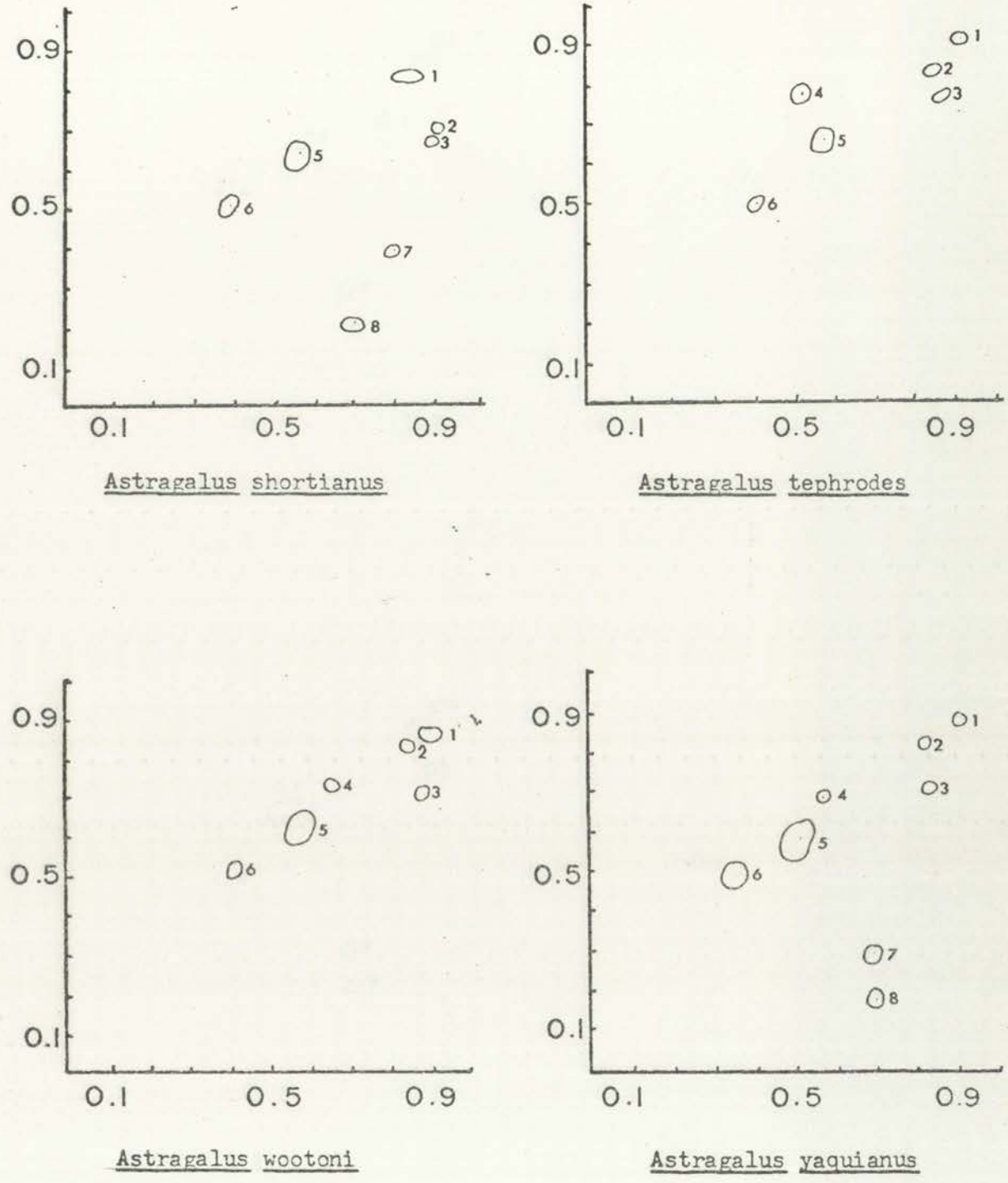

FIGURE 9. Representative chromatograms of four selected species of Astragalus. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.

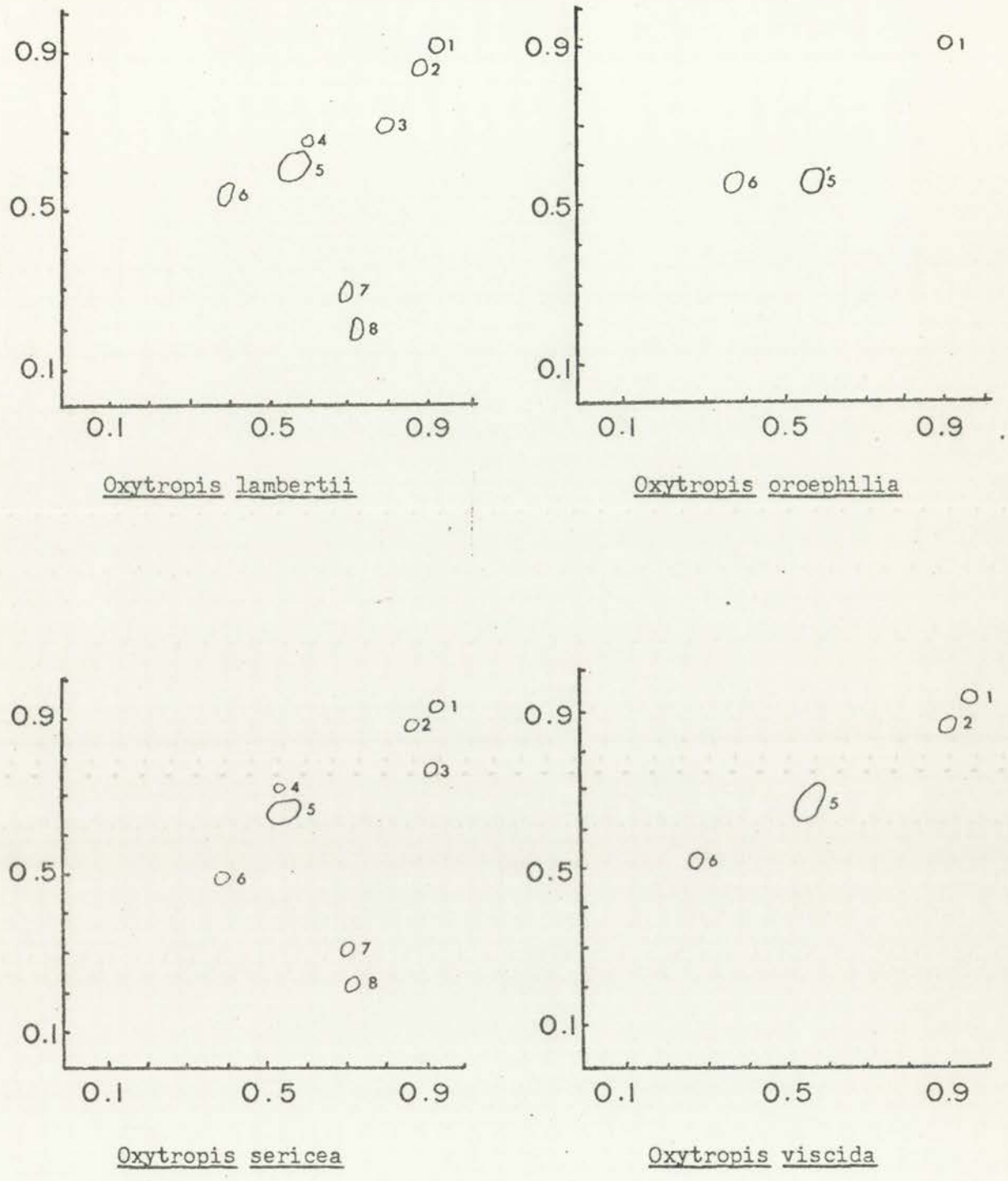

FIGURE 10. Representative chromatograms of four selected species of Oxytropis. Vertical axis is R_F value in BAW. Horizontal axis is R_F value in Forestal solvent.