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THE COMPOSITION OF THE PROTEINACEOUS POLYSACCHARIDES EXUDED BY *ASTRAGALUS MICROCEPHALUS*, *A. GUMMIFER* AND *A. KURDICUS*—THE SOURCES OF TURKISH GUM TRAGACANTH*

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Key Word Index—*Astragalus* spp.; Leguminosae; gum tragacanth; amino acids; bassorin; tragacanthin; sugar composition.

Abstract—Gum tragacanth is a variable commodity because commercial samples may legitimately be admixtures, in any relative proportions, of the exudates from Asiatic *Astragalus* spp. Analytical data show that the exudates collected from the three major contributing Turkish spp., *A. microcephalus*, *A. gummifer* and *A. kurdicus*, differ extensively, particularly in terms of their fucose, xylose, galacturonic acid and methoxyl contents and in the relative proportions of their soluble (tragacanthin) and insoluble (bassorin) components. In addition, these three *Astragalus* exudates are shown to be proteinaceous polysaccharides; their amino acid compositions differ, particularly in terms of their hydroxyproline, histidine, aspartic acid and arginine content. In contrast, the amino acid compositions of the soluble and insoluble components of *A. kurdicus* do not differ extensively.

INTRODUCTION

Gum tragacanth, with a history of use extending over some five thousand years [2], is used as an emulsifier, stabiliser and thickening agent in pharmaceuticals and foodstuffs, particularly for stabilising oil-in-water emulsions and acidic salad dressing preparations [3].

At present, gum tragacanth (E413) is accepted only provisionally as a permitted foodstuffs additive. It has not been assigned an acceptable daily intake (ADI) value within the EEC [4] nor by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [5]. Although several of the toxicological demands made [6] have been met, e.g. by a dietary study of gum tragacanth in Man [7] and by data quantifying its immunogenicity [8], international regulatory committees have demanded [9, 10] the submission during 1985 of the further data known to have been obtained in studies undertaken in the U.S.A. in 1981. Gum tragacanth has been classified as generally recognised as safe (GRAS) at the 0.2–1.3% level in foodstuffs [11] in the U.S.A. since 1961. This affirmation will come under cyclical review by the FDA Select Committee; the current GRAS specification states, erroneously, that gum tragacanth contains fructose (a misprint, presumably, for fucose). Structural details ascribed [11] to gum tragacanth's components, based on very early work, require amendment in the light of more recent studies [12]. In addition to further toxicological evaluations, current regulatory criteria for identity and purity require revision [13], e.g. gum tragacanth contains an arabinogalactan, not 'galactoarabans' [4, 5, 14].

Although the EEC [4], JECFA [5], FDA [11], USP [15] and Food Chemicals Codex [16] all define gum tragacanth as 'the dried gummy exudation from *Astragalus gummifer* Labillardière, or other Asiatic species of *Astragalus* (Leguminosae)', the British Pharmacopoeia [17] differs slightly, specifying 'the air-hardened gummy exudate, flowing naturally or obtained by incision from the trunk and branches of *Astragalus gummifer* Lab. and certain other species of *Astragalus* grown in Western Asia'. The precise significance or limitations of 'other Asiatic species', 'certain other species' and 'Western Asia' remain to be specified. If there is to be rigorous and meaningful international regulation and legislation, it should be capable of enforcement.

Clearly a tighter botanical specification is greatly to be desired. *Astragalus*, the largest genus in the Leguminosae, comprising *ca* 2000 spp., grouped into more than 100 subdivisions [18], is cosmopolitan outwith the tropics and Australia, with South-Western Asia the largest centre of distribution [19] and with some 380 spp. within Turkey [20]. There is reasonable agreement between the number and identity of the possible *Astragalus* gum-bearing species [19, 21]. Gentry [21] stated that the major Iranian gum-producing species is not *A. gummifer* [21]. Only 3 or 4 spp. are involved commercially to any extent in Turkey [22], the most important species being cited as *A. microcephalus* [22, 23].

For legislative purposes, more detailed chemical data are also desirable. Commercial gum tragacanth is a very variable commodity. The Turkish and Iranian products differ. Commercial expertise and technical skill are necessary to establish the market value of each consignment in terms of its colour, viscosity, solubility, emulsifying power, etc. Various commercial samples, evaluated in this laboratory over the years, have shown greater differences in chemical composition than might reasonably be attributable to seasonal and/or geographical variations for one particular botanical species. Studies of a wide range of other genera, e.g. *Acacia* [24], *Combretum* [25], *Prosopis*

*Part 76 in the Series "Studies of Uronic Acid Materials". For Part 75, see ref. [1].

[26] and *Grevillea* [27] have established that the gum exuded by a particular species has a unique chemical composition. The chemical differences between closely related species can be extensive [28, 29]. Physicochemical differences [30] and qualitative chemical differences in sugar compositions [23] have been reported for some *Astragalus* spp.

It therefore appeared to be desirable for more extensive, quantitative, data to be obtained for the exudates from authenticated botanical sources. In the interests of securing both botanical and chemical data of value for regulatory purposes, the International Natural Gums Association for Research (INGAR) recently sponsored a botanical field survey of the Turkish tragacanth producing areas. The objectives were to identify the major *Astragalus* spp. involved and to secure reference gum specimens from them for determinations of their polysaccharide and proteinaceous components, which have been shown recently to be of potential interest for specification purposes and structural considerations [1].

This paper reports the analytical data obtained for Turkish specimens of the gums from *Astragalus microcephalus*, *A. gummifer* and *A. kurdicus*.

RESULTS

Analytical data for the polysaccharide parameters of the exudates are shown in Table 1; data for the amino acid compositions of their proteinaceous components are shown in Table 2.

DISCUSSION

The survey of the major producing areas for gum tragacanth in Turkey was undertaken in July and August 1984 by experts in the identification of Turkish *Astragalus* spp., Dr. Musa Dogan and Dr. Tuna Ekim. They concluded [personal communication] that by far the major proportion of good quality Turkish gum tragacanth is secured by the tapping of *Astragalus microcephalus*; in some districts a small proportion of gum is collected from that species as a result of natural exudation. The natural exudate is distinguishable from tapped gum in terms of the colour, texture and characteristic shape of the pieces. In other districts, minor amounts of natural exudate gum are collected from *A. gummifer*; as this exudate is darker in colour and of inferior quality, it has a much smaller market value and there is little financial incentive for its collection. Similarly, only minor amounts of natural exudate gum are collected from *A. kurdicus*; as its exudate is characteristically different in appearance, it is sold as a separate commodity by reputable merchants. Of the many other *Astragalus* spp. in Turkey, very few yield gum of marketable quality in commercially viable quantities; 100 acres of *A. condensatus* shrubs yielded only 500 g of gum on one occasion.

Thus the recent field survey has confirmed earlier Turkish reports [22, 23, 30] that the major gum-producing species is *A. microcephalus*, not *A. gummifer*, and that these two species, with possibly *A. kurdicus*, are the sources of modern commercial Turkish gum tragacanth. The gum specimens studied here, collected at Bitlis (Hizan), are backed by botanical voucher reference specimens lodged at ANKA. Many other gum specimens from *A. microcephalus* were collected in other areas; their chemical analysis will present a formidable task but it is hoped to publish data in due course to provide evidence concerning geographical variations in gum composition.

The data presented (Table 1) show extensive differences in the analytical parameters for the polysaccharide components of the exudates from *A. microcephalus*, *A. gummifer* and *A. kurdicus*. Previous qualitative indications [31] that there were differences in the proportions of xylose and fucose are confirmed. In addition, there are

Table 1. Analytical data for the exudates from Turkish *Astragalus* species

	Astragalus				
			Kurdicus		
	Microcephalus	*Gummifer*	Whole gum	Soluble component	Insoluble component
Loss on drying, 105°, %	12.7	9.9	13.1	(7.4)‡	(6.9)‡
Total ash, 550°, %*	3.2	2.9	2.0	3.9	1.6
Nitrogen, %†	0.58	0.46	0.46	0.35	0.46
Hence protein, %†	3.65	2.84	2.88	2.2	2.88
Methoxyl, %†	3.3	0.9	0.7	1.2	0.3
Ratio of soluble/insoluble components	65/35	40/60	30/70		
Sugar composition after hydrolysis, %†					
Galacturonic acid	11	3	2		
Galactose	14	23	28		
Arabinose	37	63	64		
Xylose	22	5	3		
Fucose	12	2	Trace		
Rhamnose	4	4	4		

*Corrected for moisture content.

†Corrected for moisture and ash contents.

‡Freeze-dried product.

Table 2. The amino acid composition of the exudates from Turkish *Astragalus* species

	Astragalus				
			Kurdicus		
	Microcephalus	*Gummifer*	Whole gum	Soluble component	Insoluble component
% N	0.58	0.46	0.46	0.35	0.46
Alanine	32	66	53	55	54
Arginine	3	25	9	10	13
Aspartic acid	46	103	84	82	79
Cystine	1	1	1	1	1
Glutamic acid	29	64	49	45	46
Glycine	25	59	52	56	48
Histidine	126	52	75	95	87
Hydroxyproline	268	96	179	189	187
Isoleucine	20	46	35	30	36
Leucine	30	67	47	34	47
Lysine	48	44	38	32	34
Methionine	3	8	4	1	2
Phenylalanine	13	35	26	20	23
Proline	88	66	60	68	60
Serine	96	96	100	98	96
Threonine	37	55	53	54	55
Tyrosine	50	31	40	33	36
Valine	84	87	97	100	97

large differences, not only in the proportions of galactose, arabinose, and galacturonic acid, but also in the methoxyl contents. Previous investigations [12, 32, 33] reported inconsistencies in the relative proportions of the soluble (tragacanthin) and insoluble (bassorin) components of commercial samples which appear invariably to have been used, without even indications of the country of origin, in all earlier studies. Although the earliest analytical study [34] reported differences between three 'best white leaf' tragacanth samples and two yellow/brown samples, it is clear that these simply represented commercial trade samples of different qualities and not gum tragacanth samples from different botanical origins.

Table 2 shows that the amino acid compositions of *A. microcephalus*, *A. gummifer* and *A. kurdicus* differ, particularly in respect of their relative proportions of hydroxyproline, histidine, aspartic acid and arginine. Gum tragacanth must therefore be regarded as a proteinaceous polysaccharide, with a protein content of *ca* 3–4 %. This is in keeping with recent observations for several *Acacia* gums [28, 29], commercial gum arabic [1], and exudates from other plant genera [36]. In addition to the amino acid variations (Table 2), which may be helpful as additional analytical parameters for distinguishing individual *Astragalus* spp., the separation of *A. kurdicus* exudate into fractions soluble and insoluble in cold water [12] has shown that they retain proteinaceous components virtually identical, in terms of their amino acid compositions, to that present in the original whole gum.

Thus the exudates from three Turkish *Astragalus* spp. differ extensively in terms of their polysaccharide and proteinaceous components. A comparable survey of the Iranian *Astragalus* spp. is desirable; only then may a much more precise regulatory statement of identity become possible. As long as the present definitions remain, commercial gum tragacanth can legitimately comprise the widest possible range of exudates from *Astragalus* spp. This may not be toxicologically satisfactory now that the extent of the inter-species variations in composition has been established. These variations are adequate to explain the well-established variation in compositions and properties, e.g. the huge range of viscosity [37] shown by commercial samples. It is unfortunate that the comparatively modern, comprehensive structural studies of gum tragacanth [12, 33] were made on commercial grade material, without reference, so far as can be ascertained, even being made to geographical origins. Thus the possibility remains that the large number of different chemical fractions isolated [12, 33] may have arisen, at least in part, because the material studied was a mixture of gums from different *Astragalus* species. The situation nevertheless remains that the exudates from each of the three *Astragalus* spp. studied here, comprising soluble and insoluble proteinaceous polysaccharides based on galacturonic acid and five neutral sugars, represent heterogeneous chemical systems as complex as any natural product known. Further progress towards a satisfactory regulatory specification will only become possible when separate structural studies of at least the major contributing *Astragalus* exudates have been undertaken.

EXPERIMENTAL

Origin of gum specimens. Gum samples from *Astragalus microcephalus* Willd. and *A. gummifer* Labill. were collected at Hizan, Bitlis Province, East Anatolia in August 1984 by Dr. Dogan and Dr. Ekim. Reference vouchers are lodged at Ankara University Herbarium (ANKA). The sample of gum from *A. kurdicus* Boiss. was provided by Mr. Salvador Taranto, Istanbul.

Analytical methods. The standard analytical methods for polysaccharide components [38] and for amino acid analyses of the proteinaceous components [1] have been described. The separation of the water-soluble and insoluble components of the *Astragalus* gums was carried out by a procedure similar to that used previously [12].

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