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SHORT COMMUNICATION

ELLAGITANNIN CONTENT OF LEAVES OF *GERANIUM* SPECIES

E. C. BATE-SMITH

A.R.C. Institute of Animal Physiology, Babraham, Cambridge

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Abstract—The ellagitannin content, expressed in terms of hexahydroxydiphenylglucose (HHDPG), of mature leaves of 27 species of *Geranium*, varying from 1 to 20% of the dry wt, are not clearly related to Knuth's arrangement of the genus, but there are indications of a relationship with the chromosome number of the species. *Geranium phaeum* and *G. nodosum* contain a constituent which interferes with the determination of HHDPG.

THE METHOD of determination of ellagitannins (esters of hexahydroxydiphenic acid) recently described[1] has been applied to the leaves of *Geranium* species. This genus is well known for the high tannin content of the tissues of many of its members,[2] as much as 32% of the dry wt of the root of *G. pratense* being reported[3] as consisting of ellagitannin and condensed tannins.

The determinations are carried out in aqueous-methanolic extracts of the leaves by measuring the absorption at 600 nm of the blue colour developed by treatment with nitrous acid in absence of oxygen. The ellagitannin content is expressed as hexahydroxydiphenylglucose (HHDPG) in the dried leaf. Under the conditions specified for the determination, monoesters of HHDPG with glucose have molecular absorptions at 600 nm between 2150 and 2300,[1] the $E^{1\%}_{1\,cm}$ of 2,3-HHDPG, being 51·5.

RESULTS

If, as in Table 1, the species are arranged according to the treatment of Knuth,[4] there is little apparent correlation between the HHDPG content and his subdivision of the genus, the only feature of note being the uniformly high content of the species in section Sylvatica. It was, however, immediately obvious when the chromosome numbers of the species as recorded by Bolkhovskikh *et al.*[5] came to be considered, that a high content of tannin was found in species having the chromosome number most commonly present in, and therefore presumably characteristic of, the genus, viz. $2n = 28$ (or the polyploid numbers 56 or 84). Those species with lower chromosome number mostly had lower ellagitannin contents.

The lowest ellagitannin content was found in spec es in section Columbina having 22 or

[1] E. C. BATE-SMITH, *Phytochem.* **11**, 1153 (1972).
[2] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. 4, pp. 195–197, Birkhauser Verlag, Basel and Stuttgart (1966).
[3] W. BRANDT and F. SCHLUND, *Pharm. Ztg.* **69**, 597 (1924).
[4] R. KNUTH, Geraniaceae, in *Das Pflanzenreich* (edited by A. ENGLER), Vol. IV, p. 129, W. Engelmann, Leipzig (1912).
[5] Z. BOLKHOVSKIKH, V. GRIF, T. MATVEJEVA and O. ZAKHARIJEVA, *Chromosome Numbers of Plants*, Hayka, Leningrad (1969).

26 chromosomes, in *G. lucidum* with 20, *G. phaeum* with 14 or 28, *G. endressii* with 26 and *G. nodosum* with 28. Five of these (indicated in Table 1 with the suffix †) had a constituent, most conspicuously present in *G. phaeum*, which produced a peak at 530 nm during the course of the reaction with nitrous acid, giving rise to a persistent red tinge in the reaction mixture; none of these being in section Sylvatica. The nature of this constituent is unknown, but the circumstances of its formation suggest that it is nearly related to the ellagitannins.

HHDPG contents higher than usual were found in *G. psilostemon* and *G. nervosum* (section Sylvatica) and *G. sanguineum*; the chromosome number of the first has not been recorded, the second has the aneuploid number 52 and the third the polyploid numbers 56 or 84. In these two cases, therefore, *higher* chromosome numbers are associated with *higher* ellagitannin contents; but this is not generally true because *G. ibericum* (section Sylvatica) with 56 has normal HHDPG, and *G. traversii* (section Australiensia) with normal chromosome number has high HHDPG.

Variability. Although the results in Table 1 are no more than representative values of the HHDPG content of the leaves of the species named, whenever more than one deter-

TABLE 1. ELLAGITANNIN CONTENT OF THE LEAVES OF *Geranium* SPECIES ARRANGED ACCORDING TO THE CLASSIFICATION OF KNUTH[4]

Section	Species	HHDPG % dry wt	Chromosome No.
Columbina	*G. bohemicum* L.	13	28, 56
	G. columbinum L.	11	18
	G. dissectum L.	2·5†	22
	G. lanuginosum Lam.	15	48
	G. molle L.	5†	26
	G. pusillum Burm. f.	14	26, 34
	G. rotundifolium L.	1·3†	26
Lucida	*G. lucidum* L.	4·5	20
Robertiana	*G. robertianum* L.	5	32, 64
Unguiculata	*G. cataractarum* Coss.	12	36*
	G. macrorrhizum L. 'Bevan's' var.	10	46
Subacaulis	*G. cinereum* Cav.	11·5	28,56
Tuberosa	*G. malviflorum* Boiss. et Reut.	14	—
Anemonifolia	*G. palmatum* Cav.	13	68
Sylvatica	*G. ibericum* Cav.	8–12	28, 56
	G. maculatum Leder.	14	—
	G. nervosum Rydb.	20	52
	G. pratense L.	9–11	28, 56
	do 'Kashmir form'	16·5	
	G. platypetalum Fisch. et Mey.	14·5	28, 42
	G. psilostemon Leder.	20	—
	G. sylvaticum L.	13	—
Reflexa	*G. phaeum* L.	2†	14, 28
Polyantha	*G. polyanthes* Edgew. et Hook. f.	14·5	28, 56
Sanguinea	*G. sanguineum* L.	20	28, 56, 84
	G. soboliferum Komarov	12·5	—
Australiensia	*G. traversii* Hook, f.	20	28, 56
Pyrenaica	*G. albanum* Marsch. Bieb.	11	28, 56
Incana	*G. incanum* Burm. f.	9	—
Palustria	*G. endressii* J. Gay	3–4†	26
Striata	*G. nodosum* L.	3–7†	28, 56

* Unpublished data from Dr. P. F. Yeo.
† Absorption at 530 nm.

mination has been carried out on the mature leaves of any species the results have usually agreed very closely. This is not always the case, however, with 'rosette' (radical) and 'inflorescence' (cauline) leaves. In several instances, recorded in Table 1 by a range of HHDPG values, the ellagitannin contents differed considerably between leaves harvested from these two positions. In the case of *G. ibericum* the inflorescence leaf had the lower content, in *G. endressii* and *G. nodosum* the higher content. In *G. dissectum* and *G. robertianum* the results for the two positions were identical.

As regards maturity, Liefertova *et al.*[6] remark that in *G. sanguineum* the tannin content of the aerial growth diminishes steadily during the growing period. The only relevant observation made in the course of the present work was that the HHDPG content of very young seedlings of *G. lucidum* was 2·5%, while that of the mature leaves was 4·5%.

EXPERIMENTAL

Material. Most of the material was obtained from plants growing in the Cambridge University Botanic Garden. *G. molle, dissectum, robertianum, sanguineum* and *phaeum* were growing wild in the vicinity.

Preparation of extracts. Weighed amounts of fresh or dried leaves were covered with 50% aq. methanol and boiled gently for 5–10 min. The extract was decanted and the leaves macerated repeatedly with sand and small vols of solvent which were poured through a cotton wool filter and added to the main extract. Estimation of tannin in the residue showed that extraction was from 93% (in the case of low-tannin species) to 99% (in the case of high-tannin species) efficient.

Determination of ellagitannin in extracts. 0·5 ml of extract (= 20 mg fresh leaf) is measured in a 1-cm dia. test-tube drawn into a capillary. 1·5 ml of 50% aq. MeOH and 0·16 ml of 6% aq. AcOH are added, the temperature is adjusted to 25–30° as convenient, and N_2 bubbled for 15 min; 0·16 ml of 6% aq. $NaNO_2$ is then added, N_2 passed for a further 0·25 min, and the capillary sealed off. Spectrophotometric measurements are begun immediately. The first peak to appear is at 500 nm which reaches a maximum in 2–3 min; the blue reaction (600 nm) and a yellow reaction (400–430 nm) develop more slowly. At 30° the blue reaction reaches a max in 30–40 min and the absorptivity at this time is used for calculation of HHDPG ($E^{1\%}_{1cm} = 51{\cdot}5$). The blue colour fades to *ca.* 0 at 24 hr.

Ellagitannin content of residue. After repeated extraction with aqueous MeOH, any unextracted ellagitannin is determined by measuring the absorption at 430 nm after standing with HNO_2 (from 1 ml of 6% $NaNO_2$, 1 ml of 6% HOAc and 15 ml MeOH) for 24 hr using a suitable blank. This is not strictly quantitative, but the amount of bound ellagic acid is usually about 1–7% of that extracted. The average $E^{1\%}_{1cm}$ (430 nm) of HHDPG esters so treated is 72.

Detection of the 'phaeum factor'. The red reaction product with λ_{max} 530 nm is especially prominent in *G. phaeum* and *G. nodosum*, both of which have low HHDPG. Liefertova *et al.*[7] note that the UV absorption of extracts of these two species differs from that of all other species examined. The red product is formed more slowly than the blue and is more persistent; it can be detected after standing for 24 hr when the blue has faded.

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[6] I. Liefertova and H. Bučkova, *Preslia* **40**, 60 (1958).
[7] I. Liefertova, H. Bučkova and L. Natherova, *Preslia* **37**, 413 (1965).

Key Word Index—Geranium; Geraniaceae; ellagitannin content; correlation with chromosome number.