Phytochemistry, 1976, Vol. 15, pp 211–214. Pergamon Press. Printed in England

ELLAGITANNINS FROM *TELLIMA GRANDIFLORA**

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(*Received* 29 *March* 1975)

Key Word Index—*Tellima grandiflora*; Saxifragaceae; ellagitannins; hydrolysable tannins; polyphenols.

Abstract—Three ellagitannins present in *Tellima grandiflora* have been isolated and partly identified. Two are 2,3-digallyl-4,6-hexahydroxydiphenoyl-β-D-glucopyranose and 1,2,3-trigallyl-4,6-hexahydroxydiphenoyl-β-D-glucopyranose. The third is complex, with five gallyl and two hexahydroxydiphenyl residues; hydrolysis yielded glucose, gallic acid and ellagic acid.

INTRODUCTION

Little taxonomic use has been made of hydrolysable tannins, except for general comments on their presence or absence in various plants [1,2]. This has undoubtedly been due to the difficulties in structural identification, although the situation has changed through the application of NMR methods [3–6]. Since hydrolyzable tannins occur in several genera of Saxifragaceae [7–9] we decided to undertake structural studies with a view to determining their usefulness as taxonomic markers within the family. In connection with our study of *Tellima grandiflora* (Pursh) Dougl. [10,11] we found that hydrolysable tannins constitute the major polyphenols of this plant. Eight compounds were observed using TLC. Three ellagitannins present are described in this paper.

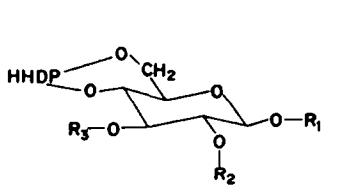
RESULTS AND DISCUSSION

A combination of partition and adsorption chromatography was used to isolate the tannins [12,13]. A partition system using ethyl acetate and petroleum on cellulose gave satisfactory results. The gallotannins were obtained in a single fraction while the major ellagitannins, compounds F and G, appeared as the primary constituents of two other fractions. Sephadex LH-20, eluted with methanol, was used for final purification. Hydrolysis of all three ellagitannins with trifluoroacetic acid gave glucose, gallic acid, ellagic acid, and various intermediates.

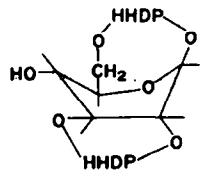
Attempts to prepare per-*O*-methylated derivatives yielded complex mixtures in all cases. The NMR spectral data for the three substances are presented in Table 1. The carbohydrate portion of compound G exhibited the following features. The chemical shifts of protons at C_2, C_3, C_4 and one at C_6 occurred in the region 5·0–6·0 δ, while that of the anomeric proton was 6·17 δ. These results indicate that the compound is totally acylated [4,6]. The large coupling constants observed for the sugar protons ($J_{1,2}$ 8 Hz, $J_{2,3}$ 9 Hz, $J_{3,4}$ 9 Hz, $J_{4,5}$ 9 Hz) indicate that the glucose exists in the pyranose form having the C-1 conformation. Since the J value indicates axial–axial coupling for the protons on C_1 and C_2, the anomeric oxygen is in the equatorial or β-position.

The spectrum of G showed 3 gallate signals (singlets, equivalent to 2 protons each) which account for 3 of the acyl groups. The aromatic region also showed 2 signals (singlets, equivalent to 1 proton each) attributable to hexahydroxydiphenic acid (HHDP). This accounts for 2 acyl functions, which, in combination with the 3 gallates, shows that G is totally acylated.

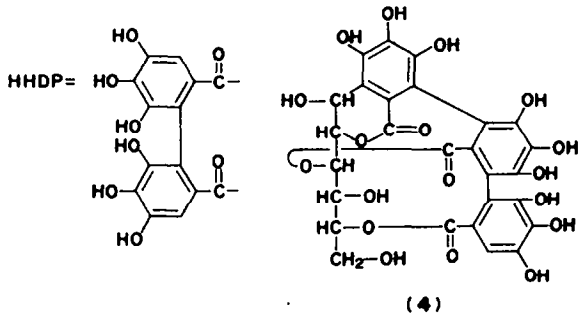
The difference in chemical shift between the C_6 and C'_6 protons establishes C_6 as one of the points of attachment of the HHDP group [4,6]. Since the only HHDP linkage known to permit the C-1 conformation of glucose is the 4,6 linkage it is possible to assign this grouping to G. With the remaining glucose hydroxyls bound to gallates, the total structure must be 1,2,3-trigallyl-4,6-hexahydroxydiphenoyl-β-D-glucopyranose (**1**). The chemical shifts of the gallate protons are consistent with the assignments summarized by Haslam [3] for gallotannins.



(**1**) $R_1 = R_2 = R_3$ = Gallyl
(**2**) R_1 = H; $R_2 = R_3$ = Gallyl



(**3**)



(**4**)

* Part 3 of the series *Chemotaxonomic Studies of Saxifragaceae*. For Part 2 see F. W. Collins, C. K. Wilkins and B. A. Bohm (1975) *Phytochemistry* **14**, 1099.

Table 1. NMR data for

Compound	Aromatic groups at glucose positions 1	2	3	4	6
"F" 2,3-digallyl-4,6-HHDP-glucose	—	7·05 (2)	6·98 (1·2) 6·94 (0·8)	6·65 (1)	6·48 (1·2) 6·44 (0·8)
"F" acetate	—	7·65	7·62 (combined 4)	7·43 (0·8) 7·41 (1·2)	7·32 (0·8) 7·30 (1·2)
"G" 1,2,3-trigallyl-4,6-HHDP-glucose	7·10 (2)	6·95 (2)	6·98 (2)	6·63 (1)	6·45 (1)
"G" acetate	7·75 (2)	7·64 (combined 4)		7·41 (1)	7·31 (1)
"H"	7·17 (2), 7·12 (4), 7·00 (2), 6·86 (2), 6·71 (1), 6·58 (1), 6·55 (1)				
"H" acetate	7·83 (2), 7·74 (2), 7·70 (2), 7·69 (2), 7·62 (2), 7·58 (1), 7·52 (1), 7·35 (1), 7·23 (1)				
"H" acetate (acetone-D_6)	7·89 (2), 7·88 (2), 7·72 (2), 7·70 (2), 7·64 (2), 7·63 (1), 7·46 (1), 7·44 (1), 7·32 (1)				

Compounds F, G, and H were run in acetone-D_6. The acetates, except where noted, were run in $CDCl_3$. Temperature was constant expressed in Hz.

Decoupling experiments utilizing the C'_6 proton signal frequency indicated the approximate location of the signal from the C_6 proton. Similarly, the location of the C_2 proton was established by irradiation at the position of the anomeric proton (see Table 1). Acid hydrolysis of G gave, in addition to glucose, gallic acid and ellagic acid, two compounds which were presumably intermediate hydrolysis products. Both compounds had higher R_f values than G on cellulose TLC using 15% acetic acid. This behaviour is probably attributable to successive loss of acyl groups. One of these products appeared to be identical to unknown F as judged by its chromatographic properties and colour reaction with DQC.

The NMR spectrum of F exhibited signals for an HHDP group and two gallates. The signals for the glucose protons at C_3, C_4, C_5, and C_6 were almost identical in chemical shifts and coupling constants with those from G. Absence of an anomeric proton signal in the region 6·0–6·3 δ indicates that the anomeric hydroxyl group is free. Signals at about 5·5 δ appeared to be due to 2 anomeric protons (combined integral equal to 1 proton) thus indicating the existence of a mixture of both α- and β-anomeric hydroxyl groups. The α- proton signal exhibited a coupling constant of 4 cps while the β-signal showed an 8 cps coupling constant. That both anomers were present was supported by double signals observed for one gallate group, one HHDP proton, the C_5 proton, and the C'_6 proton. All pairs existed in the same ratio, approximately 3:2.

The NMR spectrum of F acetate showed an α-anomeric signal at 6·46 (J 4 Hz) with an intensity of approximately one half proton. The β-anomeric proton signal probably contributed to the complex series of bands centered at *ca* 5·98. The acetate also showed double signals for one gallate group, both HHDP protons and the C_5 and C'_6 protons. Double signals have also been observed in the spectrum of pedunculagin (3) isolated from *Eucalyptus delegatensis*. The phenomenon was rationalized [6] on the basis that one of the HHDP rings assumed 2 conformations.

Compound F was more mobile than G in absorption chromatographic systems while the reverse was true in partition systems. This behaviour is consistent with the view that F is a degallyl G. The UV extinction coefficients also suggest a reduced number of aromatic systems in F, relative to G. Thus F is 2,3-digallyl-4,6-hexahydroxydiphenoyl-α and β-D-glucopyranose (**2**).

The NMR spectrum of compound H showed signals for 5 gallate groups (5 singlets, each equivalent to 2 protons) and 3 signals attributable to HHDP protons (3 singlets, each equivalent to 1 proton). The unusual behaviour of the HHDP protons did not appear in the acetate where 10 gallate protons and 4 HHDP protons could be accounted for. The chemical shifts of the gallate protons were not consistent with those of either *m*-digallate or *m*-trigallate structures [3]. A possible interpretation of the odd HHDP proton situation might involve a phenol-aldehyde cleavage similar to that observed in castalin (**4**) which Mayer and coworkers obtained from *Castanea sativa* and *Quercus sessiliflora* [5]. The sugar protons of H appeared as uninterpretable envelopes. The chromatographic mobility of H was much lower than either F or G. Since H was excluded from Sephadex G-25 using aqueous acetone its molecular weight must exceed 1000 [15,16]. It seems reasonable to assume that 2 or more glucose units are involved. At least 2 glucoses would be required to accommodate 5 gallate groups and 2 HHDP groups, a total of 9 acylating functions.

EXPERIMENTAL

TLC was carried out with Machery-Nagel Cel-300 and Sil S-HR/UV 254 plates.

Isolation of crude tannin mixture. One kg of washed, aerial portions of *Tellima grandiflora* was blended at high speed with 1·5 l. Me_2CO. The homogenate was filtered, the Me_2CO evaporated and the resulting aq suspension again filtered. Yellow

Tellima ellagitannins

	Glucose protons					
1	2	3	4	5	6	6′
Ca 5·5–5·6	5·0–5·4 *J* 10	5·88 *J* 10, 10	*ca* 5·1 *J* 10, 10	4·66 (1·2) *J* 10, 7 4·26 (0·8) *J* 10, 7	5·0–5·4 *J* 13, 7	3·86 (0·8) *J* 13 3·79 (1·2) *J* 13
6·46 (0·5) *J* 4		5·16–5·80		4·50 4·20	5·16–5·80 *J* 13, 7	3·97 (1·2) *J* 13 3·92 (0·8) *J* 13
6·17 *J* 9	5·64 *J* 9, 9	5·82 *J* 9, 9	5·20 *J* 9, 9	4·51 (1) *J* 9, 6·5	5·20–5·54 *J* 13, 6·5	3·87 (1) *J* 13
6·06 (1) *J* 7·5	5·66 *J* 6 (combined 2)		*ca* 5·33	4·25	5·20–5·54	3·95 (1) *J* 13
	3·4 — 5·8					
	3·8 — 5·8					
	3·84 — 5·9					

ca 30°. Tetramethylsilane was used as the standard. Numbers in parentheses represent integration numbers; *J* is the coupling

filtrate was saturated with NaCl and extracted with EtOAc (180 ml × 4). The combined organic layer was evaporated to give *ca* 17 g of a green–brown glass (EtOAc fraction). The remaining water phase was extracted with 180 ml *n*-BuOH. The BuOH fraction was combined with the MeOH solution of material which was insoluble in either phase in the EtOAc extraction. Evaporation of the solvents yielded 2·5 g of a brown glass (MeOH fraction).

Partition separation of EtOAc fraction. A partition column 5·0 cm diam. consisting of 100 g Avicel microcrystalline cellulose treated with 50 ml H_2O was prepared in 60% EtOAc in petrol (bp 65–110°). Six g of the EtOAc fraction was deposited onto 12 g Avicel and placed on top of the column. The column was eluted with EtOAc in petrol in the following proportions: 1·5 l. of 60%, 1·5 l. of 70%, 1·5 l. of 80%, 1·0 l. of 90%. These were followed by 0·5 l. EtOAc and 1·2 l. *n*-BuOH–EtOAc (1:3). Fractions of *ca* 70 ml were monitored on cellulose TLC using 15% AcOH. Pooling of similar fractions and evaporation of solvent gave fractions: I—0·3 g, II—1·3 g, III—0·3 g, IV—0·35 g and V—4·0 g. Fraction I contained compounds A–E, II A. B and F, III and IV F and G, and V F, G and H.

Sephadex column isolation of ellagitannins. Ellagitannins were isolated in purified form by Sephadex LH-20 column chromatography of fractions II to V, using MeOH as eluent. Appropriate fractions were combined to give pure tannins F, G and H, purity being judged by the lack of contaminating materials on TLC. Yields of tannins per kilogram of fresh plant were approx: F, 2·8 g; G, 5·1 g; and H, 2·2 g. R_f (× 100) data in 15% HOAc, BAW (4:1:5) and WAB (water-acetic acid–*n*-BuOH; 90:5:5) were as follows: F 67, 66 and 64; G 57, 69 and 44; H 43, 61 and 21. They absorbed purple in UV and gave a pink changing to brown colour in visible light following NH_3 treatment. They gave a gray-pink colour with DQC and a pink colour with Fast Blue Salt B [14].

Acetylation of tannins F, G and H. This was carried out in Me_2CO with Ac_2O–NEt_3 at 4° overnight. F gave an off-white acetate from $CHCl_3$–EtOH, which softened at 164° and melted 169–178°. R_f 0·55 on Si gel in EtOH–EtOAc (1:1). G gave an off-white crystalline acetate from EtOAc–EtOH, which softened at 179° and had mp 180–184° (dec), R_f 0·15. H gave an off-white crystalline acetate from EtOAc–EtOH which softened at 203° and had mp 204–208° (dec). R_f 0·39 in EtOAc.

Hydrolysis of tannins F, G and H. Ten mg of each tannin in 1 ml H_2O with 3 drops of trifluoroacetic acid were heated at 100° for 3 hr. F gave gallic acid, ellagic acid, glucose, and an intermediate with R_f 0·74 (15% HOAc on cellulose) which gave DQC colour reactions identical to F and G. G yielded gallic acid, ellagic acid, glucose and 2 intermediates, R_f's 0·72 and 0·65 (conditions as above) which gave identical DQC colour reactions to F and G. R_f F 0·67. H yielded gallic acid, ellagic acid, glucose, and many intermediates (R_f 0·16–0·53) which gave green–gray colour with DQC.

UV and CD data for the tannins. λ_{max} in nm. F: 275 (ε = 2·6 × 10^4), 218 (ε = 5·8 × 10^4). G: 280 (ε = 4·5 × 10^4), 218 (ε = 9·7 × 10^4). H: 276 ($A_{1\,cm}^{1\,0\,ppm}$ = 0·40), 218 ($A_{1\,cm}^{1\,0\,ppm}$ = 0·85). λ_{max} in nm (ΔE) or (H in cm) at 25°. F: 325 (−0·6), 287 (+9·1), 264 (−9·5), 237 (+14·9). G: 323 (−0·6), 284 (+4·3), 263 (−4·7), 238 (+9·2). H: 317 ($H_{1=1\,cm}^{1\,0\,ppm}$ = −0·40), 282 ($H_{1=1\,cm}^{1\,0\,ppm}$ = +2·6), 262 ($H_{1=1\,cm}^{1\,0\,ppm}$ = −2·0); S = 2 × 10^{-3}, K = 3 × 10^{-2}.

Acknowledgements—We thank the National Research Council of Canada for financial support; Prof. L. D. Hayward, Chemistry Dept., UBC for help with CD determinations; Dr. Bob Bose, Environment Canada for use of the NMR spectrophotometer; and Dr. Elijah Tannen, of this laboratory, for making unpublished results available to us.

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