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STEROIDAL SAPOGENINS FROM LEAVES OF AGAVEAE SPECIES

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Key Word Index—*Agave*; *Beschorneria*; *Doryanthes*; *Furcraea*; Agaveae; Agavaceae; steroidal sapogenins.

Abstract—The steroidal sapogenins yielded by the leaves of 34 species and 1 cultivar of *Agave*, 1 species of *Beschorneria*, 1 species of *Doryanthes* and 3 species of *Furcraea* have been studied. Steroidal sapogenins were found in extracts of most of the species examined. Smilagenin, sarsasapogenin, diosgenin, yamogenin, tigogenin, neotigogenin, gloriogenin, gentrogenin, hecogenin, sisalagenin, 9-dehydrohecogenin and gitogenin were detected. Gloriogenin was found only in *A. ghiesbrechtii*, yamogenin in *A. horrida* and *A. rigidissima*, neo-tigogenin in *A. horrida* and *A. toneliana* and gitogenin in *A. filifera*, *F. cabua*, *F. gigantea* and *F. selloa* cv marginata. The highest yield of smilagenin was obtained from both *A. haynaldii* and *A. rigidissima*, of sarsasapogenin from *A. attenuata*, of diosgenin from *A. ellemeetiana*, of tigogenin from *A. haynaldii* and of hecogenin from *F. cabua*.

INTRODUCTION

In Hutchinson's classification of the Agavaceae, the tribe Agaveae is composed of the genera *Agave*, *Beschorneria*, *Doryanthes* and *Furcraea* [1]. In this present study, the steroidal sapogenins obtained from the leaves of 34 species and one cultivar of *Agave*, 1 species of *Beschorneria*, 1 species of *Doryanthes* and 3 species of *Furcraea* have been determined.

RESULTS AND DISCUSSION

The steroidal saponins present in the dry, powdered leaf samples of *Agave*, *Beschorneria*, *Doryanthes* and *Furcraea* species were hydrolysed and the resulting sapogenins extracted and examined by TLC against suitable reference compounds before isolation by PLC. Acetates were prepared of the monohydroxy, non-keto sapogenins, which enabled the 25*R*- and 25*S*-epimers, when present, to be separated and isolated by PLC. The monohydroxy keto sapogenins were isolated together, subjected to Wolff–Kischner reduction and their products, both as alcohols and as acetates, were examined by TLC allowing the characterisation of the original keto sapogenin mixture [2]. The mps and IR spectra of the major sapogenins and their acetates from each species were compared with those of reference compounds. For the detection of 9-dehydrohecogenin, the UV spectra of the keto-sapogenin fraction was also recorded.

The steroidal sapogenins detected in the species examined are listed in Table 1, along with the yields of the major compounds. When no yield is quoted, this indicates that the amount of sapogenin present was too small for accurate determination. In none of the species examined did a di- or tri-hydroxy sapogenin form a major steroidal leaf constituent. However, such compounds were detected in many of the extracts, but were present in quantities too small to isolate in pure, crystalline form and most could not be identified chromatographically because of the lack of suitable reference compounds. Chromatographic evidence was obtained, however, for the presence of gitogenin in *A. filifera*, *F. cabua*, *F. gigantea* and *F. selloa*.

Of the 34 species of *Agave* examined, 17 have been previously studied for sapogenin content, literature records of which are given in Table 1. In addition, *A. ferdinandi-regis* was included in a list of species examined by Wall *et al.* [3], but the results were not reported. Of the newly investigated species, an extract of *A. coarctata* revealed a single compound on TLC examination, which from its R_f data appears to be a di-hydroxy sapogenin, but was not further identified.

One interesting feature of the present survey is the frequency in *Agave* species of gentrogenin, a substance previously reported from only two taxa of the genus: *A. sisalana* Perrine [2] and *A. ghiesbrechtii* [6]. Gloriogenin also has been recorded in leaf tissue of *A. sisalana* [2]. However, in this present survey, the compound was found only in *A. ghiesbrechtii*; we have reported earlier the isolation and characterization of gloriogenin in this species [6].

The frequent differences in the sapogenins obtained from the leaves of a particular species in the current study when compared with earlier reports of leaf sapogenins could be due to various factors. For example, it has been shown in *A. sisalana* that the hecogenin to tigogenin ratio varies considerably, both with the age of the leaf [2,7] and in different parts of the same leaf, the ratio being highest in the butt end and lowest in the apical regions. Moreover, it has been demonstrated that 9-dehydrohecogenin is found only in the basal areas of the leaves [8]. Variations in the sapogenin contents of several *Agave* species due to differences in age of the plants, time of collection and geographical area have been reported by several workers [7,9–13].

Diosgenin is another sapogenin which was found frequently in small amounts, although it did form a major leaf constituent of *A. ellemeetiana* and was present in assayable quantities in *A. lophantha* and *A. rigidissima*.

Table 1. Distribution of steroidal sapogenins in leaves of *Agave*, *Beschorneria*, *Doryanthes* and *Furcraea* species

Species	Steroidal sapogenin												Yield of major sapogenins (‰, dry wt)					Previously published work on leaf sapogenins
	Smilagenin	Sarsasapogenin	Diosgenin	Yamogenin	Tigogenin	Neo-tigogenin	Gentrogenin	Hecogenin	Sisalagenin	Gloriogenin	9-Dehydrohecogenin	Gentrogenin	Smilagenin	Sarsasapogenin	Diosgenin	Tigogenin	Hecogenin	
Agave albicans H. Jacobsen	–	–	–	–	–	–	–	–	–	–	–	–						
A. amaniensis Trelease & Nowell	–	–	+	–	+	–	–	–	–	–	–	–				0 03		
A americana L.	–	–	–	–	–	–	+	+	–	–	–	–					0 06	H [13,16–27], DH [18,22]
A. americana L. cv aureovariegata	–	–	–	–	–	–	–	+	–	–	–	–					0.40	
A atrovirens Karw.	–	–	–	–	–	–	tr	–	–	–	–	–						H [13,24,28], DH [24], M [16,24]
A attenuata Salm-Dyck	–	+	–	–	–	–	–	–	–	–	–	–		1 76				S [29,30], N [30], Y [30]
A bergeri Trelease	–	–	+	–	+	–	+	+	+	–	tr	–				0.15	0.06	
A. brandegeei Trelease	–	–	–	–	+	–	–	–	–	–	–	–				0.42		H, T [12,28], C [28], M, DM, GI, DH [12]
A. coarctata H Jacobsen	–	–	–	–	–	–	–	–	–	–	–	–						
A. dasyliriοides H Jacobsen & Bouché	–	–	tr	–	–	–	–	–	–	–	–	–						
A. ellemeetiana H. Jacobsen	–	–	+	–	+	–	–	+	–	–	–	–			0.72		0.05	
A. ferdinandi-regis A. Berger	–	–	–	–	–	–	–	–	–	–	–	–						
A. ferox C. Koch	–	–	–	–	–	–	+	+	–	–	–	–					0.07	H [12, 24], M [M[16.24], T, GI, DM, K [24]
A. filifera Salm-Dyck	–	–	–	–	+	–	tr	±	–	–	–	+						T [31], GI [29,31], C [31]
A. fourcroydes Lem.	–	–	+	–	+	–	+	±	–	–	–	–				0 14		H [12,16,32–35], T [12,32,34], C [16,32], M, GI [12]
A franzosinii Nissen	–	–	–	–	–	–	+	+	+	–	–	–					0.46	
A. geminiflora Ker-Gawl	–	–	tr	–	+	–	–	–	–	–	–	–						
A. ghiesbrechtii Lem.	+	–	+	–	–	–	+	±	–	+	–	–	0 40					SM [6,31], GL, D, G, H [6]
A. haynaldii Tod.	+	–	+	–	+	–	–	–	–	–	–	–	1 20			0.52		
A. horrida Lem.	–	–	+	+	+	+	+	–	–	–	–	–						– [12]
A. lecheguilla Torrey	+	–	+	–	+	–	+	–	–	–	–	–	0 50			0 08		SM [16,28,29,36–38], C, GI [16,29]
A. lophantha Schiede	+	–	+	–	+	–	+	tr	–	–	tr	–	0 99		0 14			SM [16,24,28], T [16], H [12], M[16,24]
A macroacantha Zucc	–	–	–	–	tr	–	+	+	–	–	tr	–						– [12], H, T, GI [30]
A mulmanni H. Jacobsen	–	–	tr	–	–	–	tr	–	–	–	–	–						
A potatorum Zucc.	–	–	–	–	–	–	+	+	–	–	+	–					0.15	– [12]
A rigidissima H. Jacobsen	+	–	+	+	–	–	+	±	–	–	–	–	1 20		0.08			
A salmiana Otto	–	–	–	–	–	–	+	+	–	–	+	–					0.12	H [13], M [16]
A. sartori C. Koch	–	–	–	–	–	–	–	–	–	–	–	–						
A. spectabilis Tod	–	–	+	–	+	–	+	+	+	–	tr	–					0 33	
A. stricta Salm-Dyck	–	–	tr	–	–	–	tr	–	–	–	–	–						H, T, GI [16,39]
A tonelıana Baker	+	–	–	–	+	+	–	–	–	–	–	–	0.46			0.37		
A *weberi* Cels ex Poisson	–	–	–	–	+	–	+	+	+	–	–	–				0 07	0.22	
A xylonocantha Salm-Dyck	–	+	tr	–	tr	–	–	–	–	–	–	–		0 15				S, N, GI [31]
A. yuccaefolia DC	+	+	+	–	+	–	–	±	–	–	–	–	0 94			0 11		
A. zapupe Trelease	–	–	–	–	+	–	+	+	+	–	–	–				0.18	0 15	H, T [33]
Beschorneria yuccoides C. Koch	–	–	–	–	tr	–	–	–	–	–	–	–						
Doryanthes palmeri W. Hill	tr	tr	–	–	–	–	–	–	–	–	–	–						
Furcraea cabua Trelease	–	–	–	–	+	–	–	+	–	–	–	+				0 33	0.80	T, GI, M [12]
F gigantea Vent.	–	–	–	–	+	–	–	+	–	–	–	+				0 20	0.33	H [18,40], DH [18], T, C[40]
F selloa C Koch cv marginata	–	–	–	–	+	–	–	+	–	–	–	+				0.20	0.40	H, SM [16,41]

+ = positive, tr = trace, ± = uncertain, – = negative

C = chlorogenin; D = diosgenin; DH = 9-dehydrohecogenin; DM = 9-dehydromanogenin; G = gentrogenin; GI = gitogenin; GL = gloriogenin; H = hecogenin; K = kammogenin; M = manogenin; N = neo-tigogenin; S = sarsasapogenin; SM = smilagenin; T = tigogenin; Y = yuccagenin

Yamogenin, however, was detected in only *A. horrida* and *A. rigidissima*. Similarly, although tigogenin and hecogenin were isolated from over half the species examined, their 25β-epimers, neo-tigogenin and sisalagenin, were detected in only 2 and 5 species, respectively.

Small amounts of both smilagenin and sarsasapogenin were found on chromatographic examination of extracts of the leaves of *Doryanthes palmeri*. Sarsasapogenin has been recorded for the roots and crown of this species [4]. The detection of sapogenins in *D. palmeri* is of interest as the taxonomic position of the genus both in the Agaveae and the Agavaceae [5] is uncertain.

EXPERIMENTAL

Plant materials. Leaves of all the species examined were supplied by the Royal Botanic Gardens, Kew, from plants growing in the Gardens. The identity of *Agave fourcroydes*, *A. geminiflora* and *Doryanthes palmeri* have been verified, but the remaining species are subject to verification. The leaf anatomy of all the species studied has been published earlier [5].

Extraction, examination and isolation of sapogenins. The recently harvested leaf samples were cut up, dried in a circulating air oven at 65° for 16 hr and powdered. The methods of extraction, chromatographic examination, isolation and acetylation of the sapogenins have been described earlier [2]. IR spectra were measured as KBr discs. Keto sapogenins were reduced by the Huang–Minlon modification of the Wolff–Kischner procedure [14].

Estimation of sapogenin content. Sapogenin yields were determined, when appropriate, using the densitometric TLC method of Blunden and Hardman [15]. The separation of 5α keto and non-keto monohydroxy sapogenins was satisfactory on Si gel layers using CH_2Cl_2–MeOH–formamide (93:6:1) as development solvent. When 5α and 5β monohydroxy sapogenins occurred together, 2-fold development in cyclohexane–EtOAc–H_2O (600:400:1), followed by 4-fold development in *n*-hexane–EtOAc (6:1) was used. The sapogenins were visualised by

spraying with 300% $SbCl_3$ in conc HCl and heating at 100° in a circulating air stream until the spots were a blue-black colour.

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