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STEROIDAL CONSTITUENTS OF *YUCCA GLAUCA*

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Abstract—From the leaves of *Yucca glauca* Nutt., sarsasapogenin, smilagenin, tigogenin and neo-tigogenin have been isolated, and hecogenin, gitogenin and possibly neo-gitogenin detected. From the rhizomes and roots the same sapogenins were detected, but another compound, probably diosgenin, was found, which was present also in minute quantities in the leaves. In all of three plants studied, the predominant monohydroxy steroids in the extracts of the leaves and roots were 5α-sapogenins, but in the rhizomes, 5β-sapogenins were present in the greater quantity. From the extracts of leaves, rhizomes and roots, sitosterol, stigmasterol and cholesterol were detected. Of ten plants examined at one time, five contained tigogenin as the principal sapogenin in the leaves, three neo-tigogenin and two sarsasapogenin. No significant qualitative changes were noticed in the leaf sapogenins of two plants grown under conditions of high and low humidity combined with either high or low temperature. The total monohydroxy, steroidal sapogenin yield of *Y. glauca* leaves, collected from plants grown in an unheated glasshouse at Portsmouth, showed a steady decrease from January to July, when the yield started to increase rapidly, the increase continuing until January. The monohydroxy, saturated sapogenins from *Y. glauca* leaves did not undergo any significant decomposition on storage.

INTRODUCTION

STEROIDAL sapogenins are of economic importance as precursors of oral contraceptives, sex hormones and other medicinally useful steroids. One source of steroidal sapogenins is the genus *Yucca*. The majority of species in this genus have received some chemical investigation and many different steroidal sapogenins have been isolated or detected in the genus.[1–5] Wall and co-workers[1,2] found that the *Yucca* species growing in the arid areas of the western U.S.A. produced only sarsasapogenin (5β,25β-spirostan-3β-ol), whereas those growing in the moist south-eastern and south-western areas produced mainly tigogenin (5α,25α-spirostan-3β-ol) and gitogenin (5α-25α-spirostan-2α,3β-diol). Controlled experiments to determine the effects of temperature and humidity on *Yucca* constituents have not been recorded, however.

Y. glauca Nutt. is found growing in dry soil in the U.S.A. from Iowa and South Dakota to Montana, south to Missouri, Texas and Arizona.[6] Marker *et al.*[7] showed that the leaf

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[1] M. E. WALL, M. M. KRIDER, C. F. KREWSON, C. R. EDDY, J. J. WILLAMAN, D. S. CORRELL and H. S. GENTRY, *J. Am. Pharm. Assoc., Sci. Ed.* **43**, 1 (1954).

[2] M. E. WALL, C. R. EDDY, J. J. WILLAMAN, D. S. CORRELL, B. G. SCHUBERT and H. S. GENTRY, *J. Am. Pharm. Assoc., Sci. Ed.* **43**, 503 (1954).

[3] M. E. WALL, C. S. FENSKE, J. J. WILLAMAN, D. S. CORRELL, B. G. SCHUBERT and H. S. GENTRY, *J. Am. Pharm. Assoc., Sci. Ed.* **44**, 438 (1955).

[4] M. E. WALL, C. S. FENSKE, H. E. KENNEY, J. J. WILLAMAN, D. S. CORRELL, B. G. SCHUBERT and H. S. GENTRY, *J. Am. Pharm. Assoc., Sci. Ed.* **46**, 653 (1957).

[5] M. E. WALL, J. W. GARVIN, J. J. WILLAMAN, Q. JONES and B. G. SCHUBERT, *J. Pharm. Sci.* **50**, 1001 (1961).

[6] N. L. BRITTON and A. BROWN, *Illustrated Flora of the Northern United States and Canada*, 2nd ed., Vol. 1, p. 512, Charles Scribner's Sons, New York (1913).

[7] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, E. L. WITTBECKER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Am. Chem. Soc.* **65**, 1199 (1943).

contained saponins, which on hydrolysis yielded sarsasapogenin. Sarsasapogenin was isolated also from the leaf by Blunden *et al.*,[8] who showed that the sapogenin yield was dependent on the action of an endogenous enzyme system. *Y. glauca* is listed as one of the best sources of sarsasapogenin[9] and, as it may be possible to grow the plants in areas where land is cheap and at present unproductive, it was considered that further chemical investigation of the species was warranted.

Marker and co-workers[10, 11] showed that the steroidal constituents of *Yucca* species underwent seasonal variations. However, to our knowledge, no study has been made showing the quantitative variations from month to month in the monohydroxy sapogenin yields from *Yucca* leaves, although this information would be of great importance if the leaves were to be used commercially.

RESULTS

Leaf samples from seventeen different *Yucca glauca* plants grown under glass in Manitoba were collected in December 1966, powdered, bulked and the saponins hydrolysed to give the sapogenins. The sapogenin extract was examined by two-way TLC and showed four major spots and one minor one with the H_2SO_4 locating reagent. The four major spots were labelled A–D in order of decreasing R_f and the minor spot was labelled E. Spot A gave a purple colour with the locating reagent, co-chromatographed with the common phytosterols such as β-sitosterol (24α-ethyl-Δ^5-cholesten-3β-ol) and stigmasterol (24α-ethyl-$\Delta^{5,22}$-cholestadien-3β-ol) and was concluded to be sterol. Spots B and C gave yellow colours with the reagent, indicating that they were saturated compounds.[12] Spot B co-chromatographed with sarsasapogenin and smilagenin (5β,25α-spirostan-3β-ol) and spot C with tigogenin. Spot D had a low R_f, which indicated that it was probably a dihydroxysapogenin, gave a yellow colour with H_2SO_4, often turning a pinkish colour on further heating and co-chromatographed with gitogenin. Spot E, a trace component, gave a reddish-pink colour with the locating reagent, indicating it to be a Δ^5-unsaturated steroid.[12] Because of its low R_f, it probably contained more than one hydroxyl group, but insufficient reference compounds were available to identify it.

The compounds producing spots A, B, C and D were separated by preparative TLC. The sterols producing spot A were examined by GLC both before and after acetylation. Sitosterol, stigmasterol and cholesterol (Δ^5-cholesten-3β-ol) were detected, sitosterol being the major component. Other peaks were located, including one having the same retention time as a component present in the β-sitosterol reference solution.

Acetates were prepared of the spot B, C and D compounds and these were examined by TLC. Two spots were produced from the B mixture, one co-chromatographing with smilagenin acetate and the other with sarsasapogenin acetate. Similarly, two spots were detected from the C mixture, one of which co-chromatographed with tigogenin acetate. The acetylated material from spot D produced only one spot, which co-chromatographed with gitogenin diacetate. Trifluoroacetates were prepared of the various sapogenin mixtures and these were examined by TLC. Again, spot B compounds produced two spots, one of which

[8] G. BLUNDEN, R. HARDMAN and W. R. WENSLEY, *J. Pharm. Pharmacol.* **17**, 274 (1965).
[9] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, E. L. WITTBECKER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Am. Chem. Soc.* **69**, 2167 (1947).
[10] R. E. MARKER and J. LOPEZ, *J. Am. Chem. Soc.* **69**, 2375 (1947).
[11] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, E. L. WITTBECKER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Am. Chem. Soc.* **69**, 2167 (1947).
[12] R. D. BENNETT and E. HEFTMANN, *J. Chromatogr.* **9**, 353 (1962).

co-chromatographed with sarsasapogenin trifluoroacetate and the other with smilagenin trifluoroacetate. Similarly, one of the two trifluoroacetates from spot C co-chromatographed with the trifluoroacetate of tigogenin. Two spots were produced by the trifluoroacetate mixture of spot D. A reference sample prepared from gitogenin also produced two spots, which co-chromatographed with the two spots formed from spot D.

The two sapogenins of spot B and the two of spot C were isolated as their acetates by preparative TLC. They were recrystallized from methanol and examined by mixed m.p. with an appropriate reference compound and by comparison of the i.r. spectra with spectra of reference sapogenin acetates. The acetates were hydrolysed and the sapogenins liberated were recrystallized from acetone and m.ps. were determined. One of the sapogenin acetates produced from spot B was obtained as needle crystals, with a m.p. and mixed m.p. with smilagenin acetate of 150–151° and an identical i.r. spectrum. The sapogenin had a m.p. and mixed m.p. with smilagenin of 181–182°. The second sapogenin acetate derived from spot B produced needle crystals, with a m.p. and mixed m.p. with sarsasapogenin acetate of 144° and an identical i.r. spectrum. The sapogenin had a m.p. and mixed m.p. with sarsasapogenin of 197° and crystallized as needles. One of the two sapogenin acetates derived from spot C produced crystals shaped like elongated prisms, had a m.p. and mixed m.p. with tigogenin acetate of 198–199° and an identical i.r. spectrum. The sapogenin, as prismatic crystals, had a m.p. and mixed m.p. with tigogenin of 201–202°. The second sapogenin acetate, formed as clusters of prisms, had a m.p. of 173–175° (lit. value for neo-tigogenin acetate, 174–176°[13]) and an i.r. spectrum consistent with that of neo-tigogenin (5α,25β-spirostan-3β-ol).[14] The sapogenin, as plate-shaped crystals, had a m.p. of 200–202° (lit. value for neo-tigogenin, 200–203°[13]).

The spot D compounds were isolated as their sapogenins by preparative TLC as needle crystals from acetone. The components of the mixture were not separated by the chromatographic system used, but the i.r. spectrum of the mixture was identical to that of the reference sample of gitogenin. From the spectrum, the band near 922 cm^{-1} was larger than the band near 900 cm^{-1}. After acetylation of both the isolated material and the reference compound, the band near 900 cm^{-1} was larger than that near 922 cm^{-1}. The spectra of the diacetates agreed with that published by Jones *et al.*[14] for gitogenin diacetate.

The steroidal constituents of the roots and rhizomes were compared with those of the leaves. Three plants were used in the study; two, 6 months and 3·5 yr old, were grown in a glasshouse at Portsmouth and were collected in December and a third, 3·5 yr old, was grown in the garden at Portsmouth and collected in February. All three plants produced insufficient stem for chemical examination and, in the case of the 6-month-old plant, the weight of rhizome produced was insufficient for use. The sapogenin contents of the root, rhizome and leaf extracts were examined by two-way TLC and assayed by the densitometric method of Blunden and Hardman.[15] Qualitatively, the constituents of the roots, rhizomes and leaves appeared the same. A new sapogenin spot, labelled F, was detected as a minor component in all three extracts. It gave a greenish-yellow colour with H_2SO_4 and co-chromatographed with hecogenin (5α,25α-spirostan-3β-ol-12-one). Another sapogenin, labelled G, was detected by TLC in the extracts of the roots and rhizomes. This spot, which merged with spot C, but had a slightly higher R_f, produced a purple spot with the H_2SO_4 reagent, indicating it to be a Δ^5-unsaturated steroid.[12] This spot co-chromatographed with diosgenin

[13] L. H. Goodson and C. R. Noller, *J. Am. Chem. Soc.* **61**, 2420 (1939).
[14] R. N. Jones, E. Katzenellenbogen and K. Dobriner, *Natl Research Council Can. N.R.C.* No. 2929 (1953).
[15] G. Blunden and R. Hardman, *J. Chromatogr.* **34**, 507 (1968).

(25α-Δ^5-spirosten-3β-ol) and yamogenin (25β-Δ^5-spirosten-3β-ol). The sapogenin was detected also in the leaf extracts, but only in very small quantities, when large loadings were put on the TLC plates. Quantitatively, the highest yields of monohydroxy, saturated, steroidal sapogenins were found in the leaves and only low yields were recorded in the roots (Table 1). In the leaves and roots, the major monohydroxysapogenins were 5α-compounds, whereas in the rhizomes the 5β-compounds predominated. Another difference was that the spot D components were the principal compounds in the root extracts, from which also spot G was most clearly detected when the extracts were examined by chromatography.

The crude steroidal extracts of the leaves, rhizomes and roots were separated by preparative TLC. The sterol fractions were examined, both as free sterols and as sterol acetates, by GLC and the sapogenin fractions were each divided into two parts, one part being acetylated and the other part being treated with trifluoroacetate anhydride. The sterol chromatograms of leaves, rhizomes and roots showed peaks corresponding to sitosterol, stigmasterol, cholesterol and another peak, present also in the β-sitosterol reference solution. In all cases sitosterol was the major compound. The intensities of the sterol spots on thin-layer chromatograms of the two plants that were 3·5 yr old were greater in the leaf extracts than in either the

TABLE 1. MONOHYDROXYSAPOGENIN YIELDS AS A PERCENTAGE, DRY WEIGHT, IN *Yucca glauca* LEAF, RHIZOME AND ROOT EXTRACTS

Date collected	Yield of monohydroxysapogenins								
	Leaf			Rhizome			Root		
	5α	5β	Total	5α	5β	Total	5α	5β	Total
Dec. 1967	0·57	0·05	0·62	0·04	0·23	0·27	0·06	0·01	0·07
Dec. 1967	0·50	trace	0·50	—	—	—	0·08	0·02	0·10
Feb. 1968	0·82	0·02	0·84	0·02	0·51	0·53	0·04	trace	0·04

rhizome or root extracts, the relative intensities being approximately 2:1·5:1. In the 6-month-old plant, the intensity of the sterol spot was two and a half times greater in the root than in the leaf. The sapogenin acetates and trifluoroacetates prepared from the extracts of leaves, rhizomes and roots behaved as described above in the earlier examination of the leaf extract. The spot F component, after acetylation, produced only one spot on chromatographic examination, this not separating from hecogenin acetate when chromatographed together. Similarly, the trifluoroacetate derivative formed only one spot, which co-chromatographed with hecogenin trifluoroacetate. Spot G, isolated along with the spot C compounds, was acetylated and examined by TLC. Only one purple spot was produced, which co-chromatographed with diosgenin acetate; no spot corresponding to yamogenin acetate was detected.

Leaves from ten different *Y. glauca* plants, all 3·75 yr old and collected in December, were assayed separately by the method of Blunden and Hardman[15] to give the ratio of 5α- to 5β-sapogenins and by the method of Brain *et al.*[16] to give the ratio of 25α- to 25β-sapogenins. Five of the plants had been grown in the Department of Plant Sciences' hothouses at the University of Manitoba and the other five plants in an unheated glasshouse at Portsmouth.

[16] K. R. BRAIN, F. R. Y. FAZLI, R. HARDMAN and A. B. WOOD, *Phytochem.* 7, 1815 (1968).

The results are given in Table 2. The sapogenin extracts were examined also by two-way TLC. Qualitatively, the extracts from all ten plants were similar, but the proportions of the four isomeric, monohydroxy, saturated sapogenins varied considerably. In the leaves of five plants tigogenin predominated, in three plants neo-tigogenin was the principal sapogenin and in two it was sarsasapogenin.

TABLE 2. MONOHYDROXYSAPOGENIN YIELDS AS A PERCENTAGE, DRY WEIGHT, IN *Yucca glauca* LEAVES, COLLECTED IN DECEMBER

Yield of monohydroxysapogenins													
Canadian-grown plants							English-grown plants						
Densitometric results			i.r. results				Densitometric results			i.r. results			
5α	5β	Total	25α	25β	Total	Predominant sapogenin	5α	5β	Total	25α	25β	Total	Predominant sapogenin
0·26	1·14	1·40	0·29	1·15	1·44	Sarsasapogenin	0·57	0·05	0·62	0·12	0·48	0·60	Neo-tigogenin
0·48	0·69	1·17	0·40	0·86	1·26	Sarsasapogenin	0·68	0·04	0·72	0·17	0·57	0·74	Neo-tigogenin
1·08	0·03	1·11	0·92	0·16	1·08	Tigogenin	0·50	trace	0·50	0·40	0·11	0·51	Tigogenin
0·72	0·54	1·26	0·25	1·03	1·28	Neo-tigogenin	0·55	0·04	0·59	0·41	0·19	0·60	Tigogenin
0·66	0·36	1·02	0·98	trace	0·98	Tigogenin	0·76	trace	0·76	0·61	0·13	0·74	Tigogenin

Variations in the total yield of monohydroxy, saturated, steroidal sapogenins during a year were determined by taking leaf samples from two plants at monthly intervals and assaying them densitometrically.[15] The extracts prepared from each sample were examined by two-way TLC. The seasonal variations in the total monohydroxy, saturated, steroidal

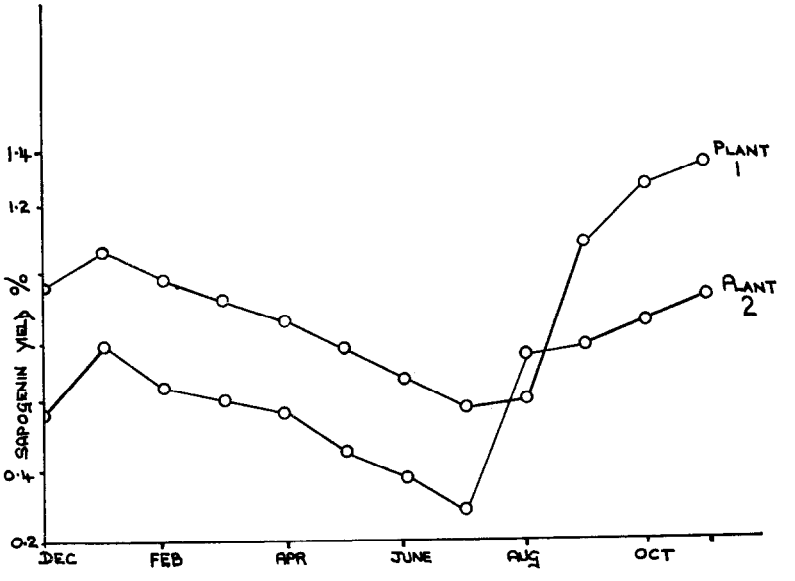

FIG. 1. SEASONAL VARIATIONS IN MONOHYDROXYSAPOGENIN YIELDS AS A PERCENTAGE, DRY WEIGHT, IN *Yucca glauca* LEAVES.

sapogenin yields are shown in Fig. 1. The sapogenin yield decreased steadily from January until July, when the yield started to increase rapidly, the increase continuing until January. No major qualitative changes in the sapogenin contents were observed by TLC.

Two *Y. glauca* plants were grown for 8 weeks in a growth cabinet under four different conditions of temperature and humidity. Leaf samples were taken, which were examined by two-way TLC and then assayed for sapogenin content by the densitometric[15] and i.r. methods.[16] No major qualitative changes were detected by TLC. The ratio of 25α- to 25β-sapogenins varied between the different samples, but in no case was the principal monohydroxysapogenin different (Table 3).

A sample from a batch of powdered *Y. glauca* leaves was assayed by the densitometric method[15] and part of the remainder was stored. Samples were taken after intervals of 1, 2, 3 and 4 months and assayed. From the same batch of powdered leaves, samples were processed until the acid-insoluble residue was obtained. Samples of the acid-insoluble residue were stored in contact with 2 N HCl and others in contact with 2 N Na_2CO_3 and both assayed

TABLE 3 EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE RELATIVE PROPORTIONS, AS PERCENTAGES, OF MONOHYDROXY, SATURATED SAPOGENINS IN *Yucca glauca* LEAF

Growing conditions			Relative proportions of monohydroxysapogenins							
			Plant A				Plant B			
			Densitometric results		I.r. results		Densitometric results		I.r. results	
Temperature	Relative humidity (%)	Month collected	5α	5β	25α	25β	5α	5β	25α	25β
24°	77	June	100	trace	38	62	100	trace	47	53
24°	10	August	100	trace	35	65	100	trace	35	65
12°	78	October	100	trace	47	53	100	trace	40	60
12°	58	December	100	trace	32	68	100	trace	48	52

after intervals of 1, 2, 3 and 4 months. In all cases, the *Y. glauca* leaf stored as powder or as the acid-insoluble residue, in either acidic or alkaline conditions, showed no loss in the yield of monohydroxy, saturated, steroidal sapogenins. Similarly, samples of sarsasapogenin, smilagenin, tigogenin and neo-tigogenin were stored as powders, in $CHCl_3$ solution and as suspensions in 2 N HCl or 2 N Na_2CO_3. Samples were assayed after intervals of 1, 2, 3 and 4 months. In no case was any significant quantitative decomposition recorded.

DISCUSSION

The main sterols detected in the leaves of *Yucca glauca* were sitosterol, stigmasterol and cholesterol, the same three sterols having been recorded in extracts of the aerial parts of both steroid-containing[17,18] and steroid-free *Dioscorea* species.[18] On examination of the sterol fraction by GLC, both before and after acetylation, peaks were obtained having the same retention times as components present in the reference solutions of β-sitosterol and

[17] D. F. JOHNSON, R. D. BENNETT and E. HEFTMANN, *Science* **140**, 198 (1963).
[18] G. BLUNDEN, C. J. BRIGGS and R. HARDMAN, *Phytochem.* **7**, 453 (1968).

β-sitosterol acetate. These peaks may result from 5α-stigmastanol and its acetate.[19] Other peaks were detected which were not identified.

On the basis of chromatographic evidence of the sapogenins, sapogenin acetates and sapogenin trifluoroacetates, spot B was thought to contain sarsasapogenin and smilagenin and spot C tigogenin and neo-tigogenin. No reference neo-tigogenin was available but, with the solvent systems used, it is known that it does not separate from tigogenin.[12] The acetate and trifluoroacetate derivatives of neo-tigogenin separate from the equivalent derivatives of tigogenin, and the behaviour of the second spot, of the spot C acetate and trifluoroacetate mixtures, was consistent with the position of the neo-tigogenin derivatives.[12] Sarsasapogenin, smilagenin, tigogenin and neo-tigogenin were isolated from the leaves of *Y. glauca* and each was fully characterized.

The trifluoroacetates derived from both spot D and the gitogenin reference material produced two spots on TLC, one of the spots being significantly larger than the other. The chromatographic characteristics of both mixtures were the same, the two major and the two minor components not separating from each other when the two were chromatographed together. Assuming that gitogenin was the major component of the reference material, it was concluded that the principal compound producing spot D was gitogenin. The minor component of spot D and the reference material was probably neo-gitogenin (5α,25β-spirostan-2α,3β-diol). The i.r. spectrum of the spot D components was difficult to interpret. From the data published by Wall *et al.*[20] the spectra of the isolated material and the reference gitogenin sample would indicate that 25β-compounds predominated. However, the spectra of the acetylated materials would be consistent with 25α-compounds predominating. Hydrolysis of the acetates to form the sapogenins and re-examination of the mixtures by i.r. spectroscopy produced spectra identical to those obtained earlier. These results do not fit the published data and cannot be explained by us.

The steroidal constituents of the roots, rhizomes and leaves of the three *Y. glauca* plants examined were qualitatively very similar. From chromatographic examination of the sapogenin, sapogenin acetate and trifluoroacetate, spot F was considered to be hecogenin. Similarly, from the chromatographic data of the sapogenin and sapogenin acetate, spot G was thought to be diosgenin. One interesting quantitative difference between the extracts was the predominance of 5α- over 5β-sapogenins in the leaves and roots and of 5β- over 5α-sapogenins in the rhizome. In extracts from the leaves and rhizomes, monohydroxysapogenins predominated, but in the roots, the dihydroxysapogenins were major.

When the leaves of individual *Y. glauca* plants were examined for steroidal content, they were found to be very similar qualitatively, but the major sapogenin characteristically varied. Tigogenin, neo-tigogenin and sarsasapogenin were each the principal sapogenin in certain plants. The ten plants examined were all collected at the same time of the year and the plants were of the same age. The predominance of different sapogenins has been recorded also for *Y. filamentosa*[1] and it seems that this species and *Y. glauca* probably exist as a number of chemical races.

Growing plants under specified conditions of high and low temperature at high and low relative humidities did not produce any change in the major steroidal component. The ratio of 5α- to 5β-sapogenins did not alter significantly from sample to sample, although there was variation in the ratio of 25α- to 25β-compounds. This variation may have been due to the climatic conditions imposed, but may also have been the result of normal seasonal

[19] C. W. SHOPPEE, *Chemistry of the Steroids*, 2nd edition, p. 76, Butterworths, London (1964).
[20] M. E. WALL, C. R. EDDY, M. L. MCCLENNAN and M. E. KLUMPP, *Anal. Chem.* **24**, 1337 (1952).

variations. Because of the small samples of leaf obtained each month during the seasonal variation studies, it was only possible to study the total monohydroxysapogenin yields and the 5α- to 5β-sapogenin ratios. As a result, possible seasonal variations in the 25α- to 25β-sapogenin ratio were not determined.

Qualitatively the extracts from the different *Y. glauca* leaves were very similar, but some differences were noted. Hecogenin was only detected clearly in a few plant extracts and was absent, or not detected, in the others. Most of the leaf extracts showed the presence of 5α- and 5β-sapogenins, but some plants, including one of the two used for the seasonal variation studies, did not contain detectable quantities of 5β-sapogenins. The unidentified spot E components were not always detected in plant extracts. Some extracts occasionally showed the presence of small quantities of unidentified spots on chromatographic examination.

Study of the monohydroxy, saturated, steroidal sapogenin yields of *Y. glauca* leaves collected during the year from plants growing in an unheated glasshouse at Portsmouth showed a steady decrease in the yield, calculated on the dry weight of leaf, from January to some time between July and August. At this time the yield increased rapidly, the increase continuing, although more slowly until January. If the leaves were to be collected for extraction of the sapogenins, the time of harvesting would be of obvious economic importance.

If *Y. glauca* were grown as an economic crop, details of the stability of the sapogenins would be necessary. After harvesting, the plant material may well be stored for several months before being processed and it is important to know whether the sapogenin yield decreases on storage. Processing of the plant material would involve acid hydrolysis of the saponins and possible neutralization of the acid-insoluble material with an alkali. Hence data on the stability of the compounds when stored under acid and alkaline conditions would be useful. The four monohydroxy, saturated, steroidal sapogenins found in *Y. glauca* leaves were found to be stable to both acid and alkaline conditions over a 4-month period. This was in marked contrast to the Δ^5-sapogenin, diosgenin, which underwent considerable decomposition when stored in acidic conditions.[21] Like diosgenin, the saturated sapogenins tested were stable when stored as solids or as solutions in chloroform. It is also shown that no significant decrease in sapogenin yield was obtained when powdered *Y. glauca* leaf was stored for a 4-month period or when the powder was processed to the acid-insoluble residue stage and stored as such in either an acidic or alkaline condition. The care to avoid storage of plant material in an acidic condition needed for diosgenin-containing material is not so necessary for materials containing monohydroxy saturated sapogenins.

EXPERIMENTAL

The *Yucca glauca* plants used in this study were all grown from a batch of seeds collected in October 1963 from western North Dakota. Some plants were grown in the hothouses of the Department of Plant Sciences at the University of Manitoba and others in the garden or glasshouses at Portsmouth College of Technology. For the studies on the constituents of the leaves and the determination of plant-to-plant variations, leaves were cut in December. After collection, the leaves or other plant parts were cut up, and dried in a circulating air oven at 65° for 16 hr. The Canadian-grown leaf samples arrived at Portsmouth as powders. Extraction of the plant material was by the method of Blunden *et al.*,[8] which entailed disintegration in water of a known weight of plant material, of known moisture content, incubation for 24 hr at 37°, refluxing with 2 N HCl for 2 hr, separation of the acid-insoluble material by filtration and washing with water, Na_2CO_3 solution and water until neutral. If the leaf sample were already powdered, disintegration was not necessary, the sample being added to water for incubation. The dried, acid-insoluble residue was extracted with petroleum ether

[21] G. BLUNDEN and C. T. RHODES, *J. Pharm. Sci.* **57**, 602 (1968).

(40–60°) for 24 hr, the extract evaporated to dryness, re-dissolved in $CHCl_3$ and made up to volume. This extract was examined by two-way chromatography on air-dried thin layers of silica gel G (Merck), wet thickness 250 μ, using CH_2Cl_2–methanol–formamide (93:6:1 v/v) in the first direction and cyclohexane–ethyl acetate–water (600:400:1 v/v) in the second.[12] Development was for 15 cm in each direction. The steroidal compounds were located by spraying with 50% H_2SO_4 and heating at 100° until the characteristic colours developed.[12]

Different steroidal components were isolated by preparative TLC on silica gel G layers, wet thickness 500 μ, using six-fold development of the chromatogram with *n*-hexane–ethyl acetate (12:1 v/v). The plates were sprayed with distilled water, which located the sapogenin and sterol bands. The compounds were eluted from the silica gel G by maceration with $CHCl_3$ for 24 hr. The compounds from each band, with the exception of the dihydroxysapogenins, were again treated by preparative TLC and the extracts from identical bands bulked. The sterols and sapogenins were each acetylated by the method of Wall *et al.*[20] Sapogenin trifluoroacetates were prepared by the method of Bennett and Heftmann.[12]

The sterol and sterol acetate solutions were examined by GLC using a Perkin–Elmer F11 Gas Chromatograph, fitted with dual 1·8 m × 6 mm glass columns, filled with silicone gum rubber E-301 on A.W.–DMCS Chromosorb G 80–100 mesh (D.E.—400), 2·5:97·5, injection temperature 240°, oven temperature 230° and carrier gas N_2 55 ml/min. Prior to GLC, the sterol and sterol acetate extracts were tested by TLC to show the absence of sapogenins.

The sapogenin acetates and trifluoroacetates were examined by TLC on silica gel G layers, wet thickness 250 μ, using $CHCl_3$–toluene, 9:1 v/v. Chromatographic examination for diosgenin and yamogenin acetates was done using CH_2Cl_2–ether, 97:3 v/v.[22]

The monohydroxy sapogenins present in the two main bands isolated from the leaves of *Y. glauca* by preparative TLC were acetylated by refluxing with Ac_2O for 30 min. The acetates were purified by band chromatography in $CHCl_3$–toluene, 9:1 v/v. Apart from a small quantity of unchanged sapogenins, each of the acetylated extracts produced two bands, which were further purified. The final sapogenin acetate extracts were evaporated to dryness, the residues recrystallized from methanol and the m.ps and mixed m.ps (uncorrected) with authentic reference compounds and the i.r. spectra from Nujol mulls determined. The sapogenin acetates were hydrolysed by refluxing with saturated methanolic KOH for 15 min. The sapogenins obtained were recrystallized from acetone before m.p. determination.

The major dihydroxysapogenins, isolated by preparative TLC, were further purified by preparative TLC in CH_2Cl_2–methanol–formamide, 93:6:1 v/v. The sapogenin mixture obtained was examined by i.r. spectroscopy as described earlier.

The monohydroxy, saturated, steroidal sapogenin yields were determined by using a Vitatron densitometer[15] after six-fold development of the chromatograms with *n*-hexane–ethyl acetate (12: 1v/v) and visualization with $SbCl_3$ in conc. HCl. Multiple development was only necessary when there were appreciable quantities of 5β-sapogenins present. If there were only trace quantities, single development with *n*-hexane–ethyl acetate, 55:15 v/v, produced satisfactory separation for the assay of 5α-compounds. Some of the extracts were assayed also for their proportions of 25α- and 25β-sapogenins.[16]

Two *Y. glauca* plants were grown under controlled conditions of humidity and temperature in a Weyco climatic cabinet. The plants were illuminated by two banks of 80 W, 5 ft, Atlas warm white delux strip lights (23 in top row and 24 in bottom). All the lights were used for 12 hr a day and each bank of lights separately for 30 min each at both ends of the daylight period as a twilight time. The shelves supporting the plants were 3 ft from the lower bank of lights. Light intensity was recorded using a light-meter calibrated in lm/ft^2. The top bank of lights gave 503 lm/ft^2, the bottom bank 1229 lm/ft^2 and both banks together 1638 lm/ft^2. Leaf samples were taken from both plants before being placed in the cabinet. The plants were grown first at a temperature of 24° and a relative humidity of 77 per cent, followed by a temperature of 24° and a relative humidity of 10 per cent. The temperature was then dropped to 12° and the plants were first grown at a relative humidity of 78 per cent, followed by a relative humidity of 58 per cent. The plants were grown for 8 weeks under each set of conditions, watering of the plants only being done when necessary. Leaf samples were assayed by both the densitometric and i.r. spectroscopic methods.

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[22] R. D. Bennett and E. Heftmann, *Phytochem.* **4**, 577 (1965).