selinen-4-ol (juniper camphor), elemol and 4-methyl-4-hydroxy-penten-2-oic acid[1] by direct comparison of retention time in GLC, R_f values on TLC, and by their IR, NMR and MS. Petrol. (60–80°) extract of seeds afforded sitosterol (needles. m.p. 136–136·5°), tricontane and tricontanol. These compounds did not show any depression in their m.m.p. with authentic samples.

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AGAVE GHIESBRECHTII, A NEW SOURCE OF GLORIOGENIN

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From *Furcraea selloa* C.Koch, Marker *et al.*[1] isolated furcogenin, which they claimed to be 5β,25α-spirostan-3β-ol-12-one, but in a later paper, Marker and Lopez[2] showed that furcogenin was a mixture of smilagenin and hecogenin. A compound with the same structure was reported by Marker and Applezweig[3] under the name jimogenin, but no physical or chemical data was given and the plant source was not listed. Gloriogenin was isolated from the leaves of *Yucca gloriosa* L. by Dávila and Panizo[4] and the structure of the compound was established as 5β,25α-spirostan-3β-ol-12-one by Gonzáles *et al.*[5] Gloriogenin has been isolated also from the seeds of *Y. glauca* Nutt. by Stohs *et al.*[6] In connection with our investigations of the steroidal sapogenin content of the family Agavaceae, we now report the isolation of gloriogenin from the leaves of *Agave ghiesbrechtii*.

The saponins of *A. ghiesbrechtii* were hydrolysed to give the sapogenins, which were examined by two-dimensional TLC. Five sapogenin spots were detected with H_2SO_4, four of which co-chromatographed with smilagenin, diosgenin, gentrogenin and hecogenin respectively, in order of decreasing R_f. The fifth spot, labelled A, had an R_f slightly higher than that of gentrogenin and did not co-chromatograph with any of the reference sapogenins available to us. The spot produced a yellow colour with H_2SO_4, indicating that

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[5] GONZÁLES, A. G., FREIRE BARREIRA, R., HERNÁNDEZ GONZÁLES, R., SALAZAR, J. A. and SUAREZ LOPEZ, E. (1972) *Quimica* **68**, 309.
[6] STOHS, S. J., EL-OLEMY, M. M. and SABATKA, J. J. (1973) *Lloydia* **36**, 443.

the compound was saturated.[7] Compound A was isolated along with hecogenin and gentrogenin by PLC. The sapogenin mixture, on Wolff–Kishner reduction, produced smilagenin, tigogenin and diosgenin, these compounds being identified by chromatographic comparison with appropriate reference compounds of the alcohols, as well as of their acetate and trifluoroacetate derivatives. Tigogenin and diosgenin are formed on reduction of hecogenin and gentrogenin respectively and hence smilagenin is formed on reduction of compound A. This evidence indicated that compound A was the 5β-epimer of hecogenin and, as this compound was unknown to us at the time, it was examined further.

Compound A was separated from the other steroidal sapogenins by PLC and, after repeated crystallization from methanol, was obtained as flat prisms, m.p. 178–179°, $[\alpha]_D^{25}$ 23·05 (C, 0·34; dioxane). The sapogenin acetate, as prismatic needles, had a m.p. 186–187°. The IR spectrum showed bands at 866, 900, 920 and 981 cm^{-1} (spiroketal), the absorption at 900 cm^{-1} being stronger than at 920 cm^{-1} (25α), and strong absorptions at 1710 cm^{-1} (carbonyl on six-membered ring) and 3450 cm^{-1} (hydroxyl). The IR spectrum was very similar to that of hecogenin, the differences observed being of the type expected for diastereomers. The MS showed a molecular ion at *m/e* 430, which was 20% of the intensity of the base peak, *m/e* 139. This base peak has been observed in the MS of hecogenin, diosgenin and tigogenin,[8] and showed that the CO is not part of the spiroketal moiety. The NMR spectrum ($CDCl_3$) showed resonances at τ8·95 (6H, C-18, C-19 methyls), τ8·94 (3H, *d*, *J* 7 Hz; C-21 methyl) and τ9·20 (3H, *d*, *J* 7 Hz; C-27 methyl). Using the Tori and Aono[9] additivity rules for predicting the chemical shifts of methyl proton resonances in steroidal sapogenins, the observed values for compound A were found to be in best agreement with those calculated for 5β,25α-spirostan-3β-ol-12-one [C-18, 8·86; C-19, 8·93; C-21, 8·96; C-27(Hα), 9·21]. The ORD curve of compound A showed a strong positive Cotton effect associated with the CO group, which was of similar shape, sign and amplitude to those of ergostan-3β-ol-12-one and hecogenin, but markedly different from those of 6-, 7- and 11-keto steroids.[10] This similarity between the ORD curves of compound A and the 12-keto steroids strongly suggested that the keto group of A was at C-12.

The available data enabled us to conclude that compound A was 5β,25α-spirostan-3β-ol-12-one. The lower m.ps recorded by us for both gloriogenin and gloriogenin acetate in comparison with the values listed by Gonzáles *et al.*[5] are probably due to the presence of impurities in our samples. A full account of the steroidal sapogenins of *A. ghiesbrechtii* and of the other *Agave* species examined in our study will be published shortly. The gloriogenin isolated from *A. ghiesbrechtii* was used as the reference for the identification of the same compound in trace amounts in the leaves of *A. sisalana* Perrine.[11]

EXPERIMENTAL

Agave ghiesbrechtii leaves were obtained from a plant growing in the Royal Botanic Gardens, Kew. The leaves were cut up, dried at 65° for 16 hr, powdered and the sapogenins extracted by the method of Blunden *et al.*[12] The sapogenin extract was examined by 2-D TLC on air-dried layers of silica gel G (Merck), 250 μm, using $CHCl_2$–MeOH–$HCONH_2$ (93:6:1) in the first direction, cyclohexane–EtOAc–H_2O (600:400:1) in the second.

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and locating the sapogenins with 50% H_2SO_4.[7] The keto-sapogenins were isolated by PLC on air dried silica gel G layers, 500 μm, with double development in cyclohexane–EtOAc–H_2O (600:400:1). A sample of the keto-sapogenin mixture was reduced by the Huang-Minlon modification of the Wolff-Kishner method.[13] Acetates of the compounds formed were prepared by refluxing the sapogenins with Ac_2O for 30 min and sapogenin trifluoroacetates were prepared by the method of Bennett and Heftmann.[7] The sapogenin acetates and trifluoroacetates were examined on activated silica gel G layers, 250 μm, using $CHCl_3$–toluene (9:1). The three keto-sapogenins were separated from each other by PLC on silica gel G layers, 500 μm, developing 4× in *n*-hexane–EtOAc (6:1).

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THE ESSENTIAL OIL IN *SCIRPUS AMERICANUS**

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The Mississippi salt marsh is an irregularly flooded estuary dominated by the needle-rush, *Juncus roemerianus* (Juncaceae), the giant cordgrass *Spartina cynosuroides* (Graminae), and *Scirpus americanus* (Cyperaceae). Preliminary studies by Odum[2] include those on primary production and decomposition of *Scirpus americanus*, and the food value of this species to marsh and estuarine organisms. To our knowledge, there is no report of a detailed study on the organic constituents of *S. americanus*. This communication on the essential oil of *S. americanus* is a part[3,4] of a continuing chemoecological study of the Mississippi salt marsh.

An investigation of the essential oil of the marsh grass, *S. americanus*, by combined GC–MS resulted in the identification of 40 compounds that comprise 78·8% of the total oil.

* Part IV in the series "Constituents of Marsh Grass". For Part III, see Ref. 1.
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