Tetrahedron. Vol. 26, pp. 3233 to 3244. Pergamon Press 1970. Printed in Great Britain

NEW SOURCES OF STEROID SAPOGENINS—VIII[1]

SCEPTRUMGENIN AND ISOPLEXIGENIN A, B, C AND D FROM ISOPLEXIS SCEPTRUM

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(*Received in the UK* 3 *March* 1970; *Accepted for publication* 16 *March* 1970)

Abstract—Structures are assigned to five new sapogenins of steroid type isolated from *Isoplexis sceptrum*: Sceptrumgenin (I), Isoplexigenin A (IIg), B (IVa), C (IIa) and D (IIk) have been shown by chemical and spectroscopic methods to be closely related to each other.

INTRODUCTION

FOUR species of the genus *Isoplexis* (Scrophulariaceae) have been described; three of them are endemic to the Canary islands and the fourth to Madeira. They are of interest because of their relationship to the European *Digitalis* rich in cardenolides, substances isolated[2-7] from *Isoplexis canariensis* Lindl. and *Isoplexis Isabelliana* W.B.

The hydrolysate of the extract from twigs and leaves of the wild form of *Isoplexis sceptrum* (Linn.) Steudel, collected on the mountains of Madeira, did not yield any cardenolides but instead a series of spirostane sapogenins[8,9] together with other compounds such as triterpenes, anthraquinones, etc. This result indicates a significant difference between *I. sceptrum* and the other species investigated. Four of the twelve spirostane sapogenins isolated were identified as tigogenin, diosgenin (IVe), gitogenin (IId), and $\Delta^{25(27)}$-gitogenin. Another three are being investigated and this paper reports the structure elucidation of the remaining five which have not been described as naturally occurring products in the literature. They are closely related to each other and, considering their origin, we propose to name them *sceptrumgenin*, and *isoplexigenin A*, *B*, *C*, and *D*.

RESULTS AND DISCUSSION

Sceptrumgenin (I), eluted together with tigogenin and diosgenin (benzene-$CHCl_3$, 1:1), was separated from them, as acetates by chromatography on silica gel—$AgNO_3$. The monoacetate, obtained by mild acetylation with acetic anhydride in pyridine, has the empirical formula $C_{29}H_{42}O_4$, m.p. 191–193°, $[\alpha]_D$ − 119°. Its IR spectrum indicates the presence of a methylene group (3080, 1660, 880 cm^{-1}) and a trisubstituted double bond (3030, 2830, 840 cm^{-1}), assignations which are confirmed by the NMR spectrum:* two multiplets at 5·27 (2H) and 4·65 (1H), respectively. The NMR signals for the 13-Me and 10-Me suggest position 5–6 for the double bond (9·22 and

* All chemical shifts are reported in ppm on the τ-scale. See Table 1.

8·96; calculated by the method of Zürcher:[10] 9·22 and 8·96). The 20-Me is observed at the same field (9·03) as the corresponding one in $\Delta^{25(27)}$-gitogenin acetate (9·09) and diosgenin acetate (9·03[11a]), from which we may conclude that the substitution of the 25-Me by a methylene group does not affect the position of the signal for the 20-Me. On the other hand, the NMR spectrum of sceptrumgenin acetate lacks the peak for the 25-Me. This requires as the only possibility, that C_{25} bears a methylene group and is in accord with the fact that the two protons at C_{26} form an AB system and that the region 850–1000 cm^{-1} of the IR spectrum of our acetate is analogous to that given by Takeda *et al.*[11b] for $\Delta^{25(27)}$-gitogenin acetate obtained by us. We therefore conclude that sceptrumgenin must be spirost-5,25(27)-diene-3β-ol (I). The data coincide with those published by Minato and Shimaoka[12] who obtained this substance as a secondary product in the course of the structure determination of narthogenin.

Isoplexigenin C (IIa), by chromagraphy of the hydrolysate eluted with $CHCl_3$—3% MeOH, had m.p. 272·5–273·5°, $[\alpha]_D$ − 62°. The elemental and mass spectral analyses indicated the empirical formula $C_{27}H_{44}O_5$ (Mol. wt. by MS: 448), its IR spectrum showing bands at 3610 and 3580 cm^{-1} (OH). Mild acetylation gave a triacetate (IIb) which in the IR does not possess any OH absorptions. The NMR spectrum of IIb exhibits a multiplet at 6·57 (2H) whose shape and $W_{h/2}$ (12 c/s) are characteristic for protons at C_{26} in a spirostane sapogenin if C_{25} has the configuration *R*.[11a] The spirostane skeleton would also explain the presence of the two non-hydroxylic O atoms. Between 4·8 and 5·75 (4H) there appears a broad set of lines assignable to the three protons situated at the same carbon as the acetyl groups and to the lone proton at C_{16}. The spectrum lacks the signal at 8·40 which in sapogenins with no substituents in the spiroketal side-chain is attributed to the protons at C_{23}. In the region 850–1000 cm^{-1} of the IR spectrum no absorptions appear which are characteristic of spirostane sapogenins without substituents in ring E or F[13–15] According to Takeda *et al.*[16] substitution in these rings has a pronounced effect on the region mentioned. The mass spectrum of IIa shows the base peak at *m/e* 289 (Fig. 1, fragment 1c), constituted by the androstane skeleton with two OH groups, analogous to what is observed in 23-Br-desoxytigogenin, but contrary to what occurs in spirostane sapogenins unsubstituted in the spiroketal side-chain, in whose mass spectrum the base peak is formed by this side-chain.[17] From all this we conclude that one of the OH groups might be located on C_{23} or C_{24}, while the remaining two are found in the androstane skeleton.

The attempted degradation of isoplexigenin C to the pregnane derivative V, following the method used by Minato and Shimaoka,[12] only yielded the starting material because the first reaction step (i.e. isomerization to a $\Delta^{20(22)}$-furostene) did not take place, probably because the OH group in ring F impedes the reaction.[18] On the other hand, degradation of gitogenin (IId) by the same method gave compound V.

The structure determination of isoplexigenin C by chemical methods was performed as follows. Partial acetylation of IIa afforded the diacetate IIc whose IR spectrum shows the typical absorptions of an associated OH (3580 cm^{-1}). By oxidation of this alcohol with CrO_3 we obtained the monoketone IIIc in whose IR spectrum the CO band coincides with that of the acetate groups (1740 cm^{-1}), therefore suggesting position C_{23}.[19] The keto group was reduced by the method of Huang-Minlon

TABLE 1. CHEMICAL SHIFTS IN $CDCl_3$ (τ-SCALE)

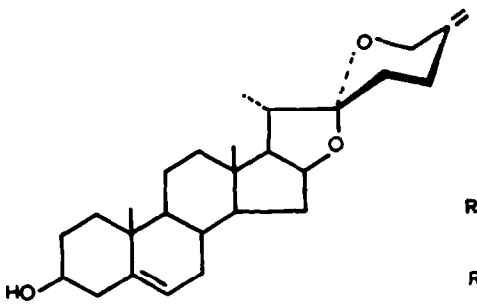
Compound	H—C_6	H—C_2	$H_{(2)}$—C_{23}	H—C_{16}	H_2—C_{26}	H_2—C_{24}	10-Me	20-Me	13-Me	25-Me	=CH_2	OAc
Sceptrumgenin (I) acetate	4·65m				5·60, 5·80[a] 6·10, 6·30		8·96s	9·03d(6)	9·22s		5·27m	8·00s
Isoplexigenin A (IIg)			6·65m	5·60m	6·65m		9·19s	9·06d(7)	9·19s	9·19d(7)		
Isoplexigenin A diacetate (IIh)			5·0–5·8m	5·0–5·8m	6·65m[12]		9·18s	9·09d(7)	9·22s	9·17		8·00s
Isoplexigenin B diacetate (IVb)	4·68m		5·05–5·8m	5·05–5·8m	6·65m [12]		8·97s	9·05d(7)	9·17s	9·17d(7)		7·98s
Isoplexigenin C triacetate (IIb)			4·8–5·75m	4·8–5·75m	6·57m [12]		9·07s	9·07d(7)	9·20s	9·18		7·98s(8H) 8·00s(6H)
2,3-Diacetyl-isoplexigenin C (IIc)		4·8–5·8m	6·65m	4·8–5·8m	6·65m [12]		9·07s	9·06d(7)	9·20s	9·19d(6)		8·01s
Isoplexigenin D triacetate (IIm)			5·10m	5·50m	6·50m [12]		9·10s	8·98d(6)	9·25s	9·23d(6)		7·90s(3H) 8·00s(6H)
2,3-Diacetyl-isoplexigenin D (II)		5·15m	6·65m	5·60m	6·65m		9·09s	8·91d(7)	9·25s	9·22d(6)		8·00s
Gitogenin (IId) diacetate		5·13m	8·42[b]	5·63m	6·60m [12]		9·08s	9·05d(6)	9·25s	9·23d(6)		8·01s
$\Delta^{25(27)}$-Gitogenin diacetate		5·15m			5·60, 5·80[a] 6·10, 6·30		9·09s	9·09	9·22s		5·25m	8·00s
23-Hydroxy-tigogenone (IX)			6·65m	5·55m	6·65m		9·00s	9·08d(7)	9·17s	9·18d(7)		
23-Keto-tigogenone (IIId)				5·45m	6·40m [12]		8·96s	9·06d(7)	9·15s	9·17d(7)		
2,3-Diacetyl-23-keto-gitogenin (IIIc)		4·8–5·6m		4·8–5·6m	6·40m	7·63m	9·09s	9·08d(7)	9·25s			8·03s
23-Acetyl-spirost-2-ene (VI)		4·42m	5·07, 5·15[c] 5·25, 5·34	5·55m	6·62m [12]		9·19s	9·08d(7)	9·23s			7·98s

Coupling constants J in parentheses, $W_{h/2}$ in brackets (both in c/s)

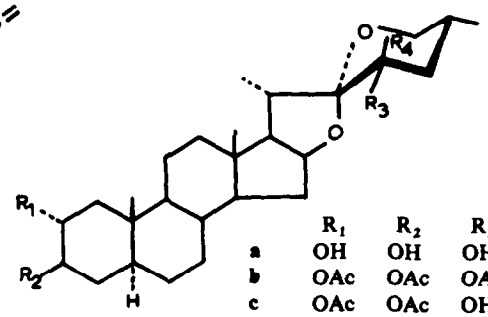
[a] AB system; J = 12 c/s

[b] not resolved multiplet

[c] X part of an ABX system

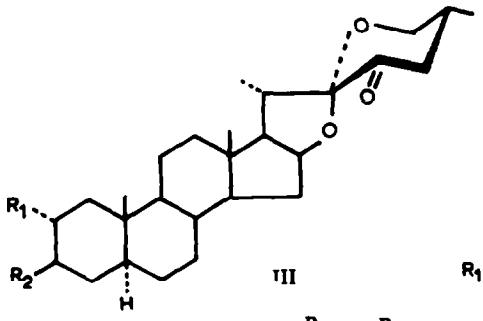


I



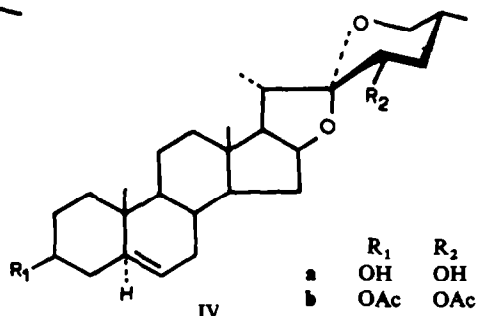
II

	R_1	R_2	R_3	R_4
a	OH	OH	OH	H
b	OAc	OAc	OAc	H
c	OAc	OAc	OH	H
d	OH	OH	H	H
e	OH	OH	OAc	H
f	OMs	OMs	OAc	H
g	H	OH	OH	H
h	H	OAc	OAc	H
i	H	OAc	OH	H
j	H	OH	OAc	H
k	OH	OH	H	OH
l	OAc	OAc	H	OH
m	OAc	OAc	H	OAc



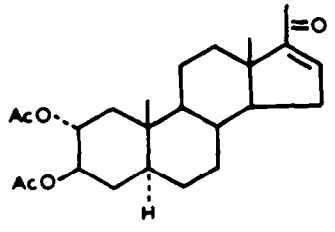
III

	R_1	R_2
a	H	OH
b	H	OAc
c	OAc	OAc
d	H	=O



IV

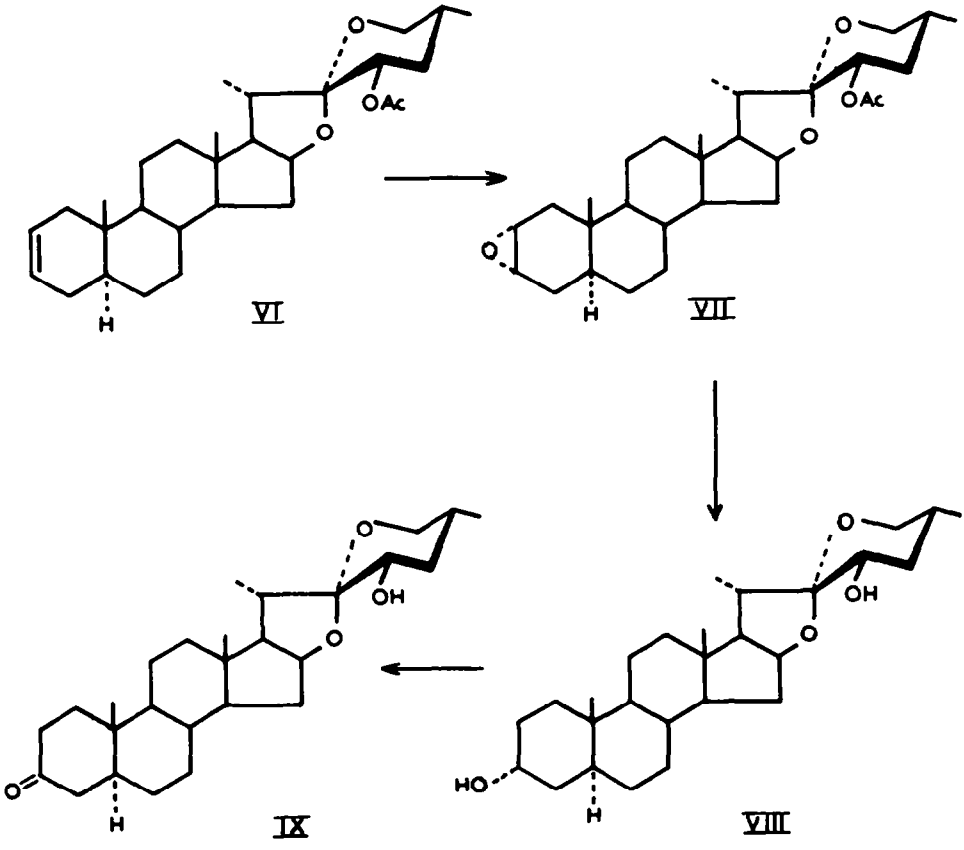
	R_1	R_2
a	OH	OH
b	OAc	OAc
c	OAc	OH
d	OH	OAc
e	OH	H
f	OAc	=O



V

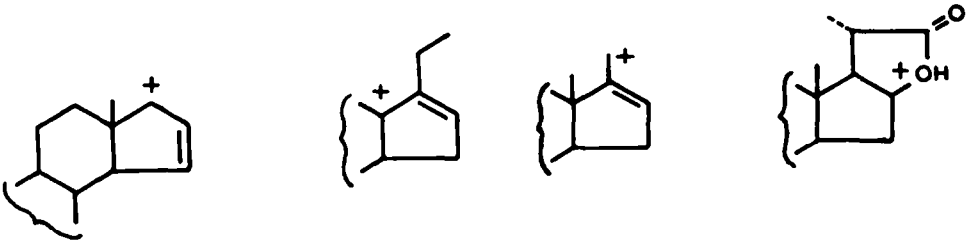
yielding gitogenin (IId). Hence, IIa is a 25*R*-spirostane sapogenin with two OH groups in positions 2α and 3β.

The position of the third OH group was determined by the following reactions. Selective saponification of isoplexigenin C triacetate (IIb) gave the monoacetate IIe. Comparing its behaviour in TLC with that of gitogenin (IId), we assumed that the two free OH groups are those in position 2α and 3β. IIe was converted to the dimesylate IIf, which was then treated with NaI[20] affording the Δ^2-derivative VI. This compound was transformed into the epoxide VII, which by comparison with analogous samples is supposed to have the configuration 2α, 3α. Reduction of VII with LAH gave the 3α-axial [21] dialcohol VIII, which selectively oxidized with NBS forms the 3-keto derivative IX (IR: 1715 cm^{-1}). IX was then reduced with $NaBH_4$, yielding mainly the dihydroxy derivative IIg, with equatorial OH at C_3.



This reduction product IIg proved to be identical with *isoplexigenin A*, m.p. 227°, $[\alpha]_D$ − 61°, which by chromatography of the hydroltsate was eluted with benzene-$CHCl_3$ (1:1) and by analysis and mass spectrum has the empirical formula $C_{27}H_{44}O_4$ (mol. wt. by MS: 432). The *m/e* values of the prominent peaks of its mass spectrum (Fig. 1, fragments 1a and 4a) are 16 units lower than the corresponding ones of isoplexigenin C, pertaining therefore to the androstane skeleton with only one OH group. In the IR it shows two absorption frequencies at 3620 and 3580 cm^{-1} (OH).

Upon dilution, the intensity of the former increases and is therefore assigned to an OH group associated intermolecularly, whereas the latter does not change and is attributed to an intramolecularly associated OH with a H-bond of approximately 2·4 Å.[22] This is only possible if the OH group at C_{23} has the configuration 23*R* or 23*S*, depending on whether H-bonding occurs with the furan or pyran oxygen. The fact that the IR region 850–1000 cm^{-1} of IIg prepared from isoplexigenin C (IIa) as described above, is completely identical to that of IIa indicates that during the course of the reactions no change has occurred in the configuration of the spiroketal side-chain.



a *m/e* 273	a *m/e* 302	a *m/e* 287	a *m/e* 347
b *m/e* 271	b *m/e* 300	b *m/e* 285	b *m/e* 345
c *m/e* 289	c *m/e* 318	c *m/e* 303	c *m/e* 363

a Isoplexigenin A
b Isoplexigenin B
c Isoplexigenin C

FIG. 1

Treatment of isoplexigenin A (IIg) with CrO_3 afforded the diketone IIId whose IR spectrum has two absorptions at 1715 and 1740 cm^{-1}. The latter, compared with the value observed for the compound IIIc, is compatible with a CO group at C_{23}.[19]

By oxidation of tigogenin acetate Callow *et al.*[19] obtained 23-keto-tigogenin (IIIa) in poor yield. This same substance was also prepared from isoplexigenin A by acetylating the OH group at C_3, thus giving IIi, followed by oxidation. Callow *et al.*[19] reduced IIIa with $NaBH_4$ obtaining an alcohol which, although it may be one impure stereoisomer at C_{23} contaminated by a small quantity of the other, has physical constants which coincide with those of isoplexigenin A, the IR spectra being completely superposable.

Isoplexigenin B (IVa), $C_{27}H_{42}O_4$ (mol wt by MS: 430), m.p. 205–207°, $[\alpha]_D$ − 96°, eluted together with isoplexigenin A, could be separated from it as its acetate by chromatography on silica gel with 20% $AgNO_3$. Its IR spectrum, similar to that of isoplexigenin A, exhibits two OH absorptions (3610 and 3580 cm^{-1}) whose intensities upon dilution behave as discussed above. Moreover, the region 850–1000 cm^{-1} is identical for the three acetates IIb, IIh and IVb, indicating that the substitution in the spiroketal side-chain must be the same. The IR spectrum of isoplexigenin B diacetate (IVb) shows clear evidence of unsaturation (3030, 2830, 840 cm^{-1}). Its NMR spectrum confirms this by a multiplet at 4·68 (1H, $W_{h/2}$ = 8 c/s) characteristic of a vinyl proton. It has also a signal at 6·65 (2H, $W_{h/2}$ = 12 c/s) whose position and shape are the expected ones for a spirostane sapogenin of the series 25*R*.[11a]

The chemical shift of the Me groups at C_{13} and C_{10} in the NMR spectrum of IVb suggests the position of the double bond. The signal of the 13-Me is found at 9·17

and that of the 10-Me at 8·97, the values for Δ^5-isoplexigenin A calculated by the method of Zürcher[10] being 9·19 and 8·96, respectively.*

In the mass spectrum of IVa, whose fragmentation is similar to that of isoplexigenin A and other spirostane sapogenins with substituents on ring E or F, the androstane fragments (Fig. 1: 1b, 4b) appear 2 units lower than those of isoplexigenin A.

The supposed structure of Δ^5-isoplexigenin A (IVa) for isoplexigenin B was confirmed by chemical methods. Catalytic hydrogenation of IVa gave isoplexigenin A (IIg); hence, the OH groups in IVa are situated at C_3 and C_{23}. On the other hand, partial acetylation of IVa yielded IVc, which treated with CrO_3 was transformed into IVf. Reduction of this compound by the method of Huang-Minlon afforded diosgenin (IVe), thus proving position 5–6 for the double bond in IVa.

So far, the stereochemistry of the spirostane sapogenins isoplexigenin A (IIg), B (IVa) and C (IIa) has been determined except for the configuration of the OH group at C_{23} which, nevertheless, must be the same in the three compounds cited.

Isoplexigenin D (IIk), eluted with $CHCl_3$—3% MeOH after isoplexigenin C, clarified the configuration of the OH at C_{23}. It has the empirical formula $C_{27}H_{44}O_5$, m.p. 280–281°, $[\alpha]_D$ − 74°, and mild acetylation forms a triacetate (IIm) whose IR spectrum in the region 850–1000 cm^{-1} differs from those of the acetates of the other three isoplexigenins as well as of sapogenins without substituents on the spiroketal side-chain. The signals at 6·50 (2H, $W_{h/2}$ = 12 c/s) and 5·50 in the NMR spectrum of IIm, which are assigned to the protons at C_{26} and C_{16}, respectively, confirm that the compound is a spirostane sapogenin of the series 25*R*. The peak for the 10-Me appears at 9·10, in accord with that of the corresponding Me group in gitogenin acetate (9·08) and in isoplexigenin C triacetate (9·07). For the 13-Me the chemical shift is identical to that of gitogenin acetate (9·25), but a little displaced with respect to that of isoplexigenin C triacetate IIb (9·20). However, the 20-Me of IIm (8·98) is deshielded in comparison with the corresponding ones in gitogenin acetate (9·05) and IIb (9·07). This seems to indicate a different type of substitution in the ring F, possibly a stereoisomer at C_{23}.

In order to prove this hypothesis, 2,3-diacetyl-23-keto-gitogenin (IIIc) obtained by oxidation of 2,3-diacetyl-isoplexigenin C (IIc), was reduced with Na in n-BuOH, chiefly yielding the 23-hydroxy derivative with *S* equatorial OH which was identified as isoplexigenin C (IIa). Hence, the OH group at C_{23} of the isoplexigenins A, B and C is *S* equatorial. To obtain the C_{23}-stereoisomer of isoplexigenin C, IIIc was catalytically reduced in acid medium, resulting in 2,3-diacetyl-23-hydroxygitogenin (III) as principal component which was different from IIc. Saponification of III and subsequent acetylation gave a trihydroxy sapogenin and its triacetate which were identified as isoplexigenin D and its triacetate (physical constants, TLC; IR and NMR spectra superposable). Isoplexigenin D (IIk) is therefore a C_{23}-epimer of isoplexigenin C, its OH group at C_{23} being *R* axial.

The stereochemistry of the OH at C_{23} in the isoplexigenins was confirmed by applying the method of Horeau[23] to 2,3-diacetylisoplexigenin C (IIc). We obtained levoratatory α-phenylbutyric acid which indicates that the OH group at C_{23} of isoplexigenin C (IIa) must be *S* equatorial.

* $\tau_{\text{diosgenin acetate}} - \tau_{\text{gitogenin acetate}} + \tau_{\text{isoplexigenin A diacetate}}$ = 9·22 − 9·25 + 9·22 = 9·19 for 13-Me and 8·96 − 9·18 + 9·18 = 8·96 for 10-Me.

A further proof of the configuration of the OH at C_{23} is given by the NMR spectrum of compound VI obtained from isoplexigenin C. The shape of the signal attributed to the proton at the same C atom as the acetyl group, is the expected one for the X part of an ABX system if the proton is axial: four small peaks in the region 5·07 to 5·34 ($W_{h/2}$ = 16 c/s).

From all these results we conclude that isoplexigenin A (IIg) must be (23*S*, 25*R*)-5α-spirostane-3β,23-diol, isoplexigenin B (IVa) (23*S*,25*R*)-spirost-5-ene-3β,23-diol, isoplexigenin C (IIa) (23*S*,25*R*)-5α-spirostane-2α,3β,23-triol, and isoplexigenin D (IIk) (23*R*,25*R*)-5α-spirostane-2α,3β,23-triol.

EXPERIMENTAL

The m.ps, determined on a Kofler block, are uncorrected. Optical rotations were measured in $CHCl_3$ soln if not stated otherwise, and with a Perkin–Elmer model 141 polarimeter. IR spectra were recorded on a Perkin–Elmer model 237 spectrophotometer in CS_2 soln unless otherwise specified. The mass spectra were determined on a Perkin–Elmer model RMU-7 mass spectrometer. GLC was performed with a Pye Argon chromatograph with Sr^{90} detector. For TLC silica gel G (Merck) was used, which for the separation of olefins was impregnated with 20% $AgNO_3$. The spray reagents were 4% H_2SO_4 + 16% H_2O in AcOH, and 1% $Ce(SO_4)_2$ in 10% H_2SO_4 aq. Column chromatography was carried out on silica gel 0·2–0·5 mm and dry column chromatography on silica gel 0·05–0·2 mm (Merck). If not otherwise indicated, the acetates were prepared with Ac_2O in pyridine, leaving the mixture at room temp overnight. Solvent used for recrystallizing compounds was MeOH unless otherwise specified. Light petroleum refers to the fraction of boiling range 40–80°.

Isolation of the sapogenins. Air-dried twigs and leaves (16·8 kg) of *Isoplexis sceptrum*, collected in Madeira in July, were finely cut and extracted with EtOH in a soxhlet tube. After filtering the cold alcoholic extract, it was concentrated *in vacuo*, diluted with the same volume of water, and extracted with benzene in a liquid–liquid extractor. The benzene soln is actually being investigated.

After adding the same volume of 2N H_2SO_4 to the aqueous soln, it was refluxed for 6 hr and then poured into water, neutralized with $NaHCO_3$, and filtered. The ppt was dissolved in $CHCl_3$ and washed 3 times with 10% NaOH aq. Evaporation of the solvent yielded the crude mixture of sapogenins (180 g; 1·1%) which in TLC ($CHCl_3$-7·5% acetone) gave 6 spots with R_f values 0·44, 0·40, 0·30, 0·09, 0·05, and 0·03. It was separated by column chromatography, using benzene-$CHCl_3$, $CHCl_3$, and $CHCl_3$-MeOH as eluents.

Product with R_f 0·44. GLC (column QF-1, temp 202°, Ar 55 ml/min) showed this product to consist of β-sitosterol (82%), stigmasterol (16%), and cholesterol (2%).

Product with R_f 0·40. TLC of the acetylated product on silica gel with 20% $AgNO_3$ revealed it to be a mixture of three compounds. They were separated on a dry column with 20% $AgNO_3$ and identified as follows:

Tigogenin, m.p. 199–201°, $[\alpha]_D$ −71° (*c*, 1·13). Forms an acetate, m.p. 200–202°, $[\alpha]_D$ −70° (*c*, 1·79). (Found: C, 75·85; H, 10·27. Calc. for $C_{29}H_{46}O_4$: C, 75·94; H, 10·11%). IR spectra of both substances superimposable with those of authentic material.

Diosgenin (IVe), m.p. 206–208°, $[\alpha]_D$ −120° (*c*, 1·18). (Found: C, 78·21; H, 10·21. Calc. for $C_{27}H_{42}O_3$: C, 78·06; H, 10·27%). Forms an acetate, m.p. 193–195°, $[\alpha]_D$ −119° (*c*, 1·10). (Found: 76·26; H, 9·91. Calc. for $C_{29}H_{44}O_4$: C, 76·27; H, 9·71%). IR spectra of both compounds superimposable with those of authentic samples.

Sceptrumgenin (I), obtained in small quantity (20 mg), m.p. 182–184°, $[\alpha]_D$ −122° (*c*, 0·51); ν_{max} 3620 (OH), 880 (CH_2=), 3030, 2830, 840 (Δ^5), 980, 960, 920, 895 cm^{-1} (spirostane ring). Sceptrumgenin acetate, m.p. 191–193°, $[\alpha]_D$ −119° (*c*, 1·75); ν_{max} 3080, 880 (CH_2=), 3030, 2830, 840 (Δ^5), 1740, 1240 (OAc), 980, 960, 920, 895 cm^{-1} (spirostane ring); $\nu_{max}^{CCl_4}$ 1660 cm^{-1} (CH_2=). (Found: C, 76·97; H, 9·68. Calc. for $C_{29}H_{42}O_4$: C, 76·61; H, 9·31%). NMR: see Table 1.

Product with R_f 0·30. This product was a mixture of isoplexigenin A (IIg) and isoplexigenin B (IVa). They were separated as acetates on a dry column with 20% $AgNO_3$ using benzene as eluent.

Isoplexigenin A (IIg), m.p. 227°, $[\alpha]_D$ − 61° (*c*, 1·61); ν_{max} 3620, 3580 (OH), 995, 965, 945, 915, 905, 895 cm^{-1} (spirostane ring). Mass spectrum: *m/e* (%) 432 (2), 347 (40), 329 (10), 273 (100), 255 (15). (Found: C, 75·21;

H, 10·18; Mol wt 432. Calc. for $C_{27}H_{44}O_4$: C, 74·96; H, 10·25%; Mol wt 432). NMR: see Table 1. Diacetate (IIh), m.p. 194–196°, $[\alpha]_D$ −60° (*c*, 1·81); ν_{max} 1740, 1240 (OAc), 980, 965, 950, 930, 920, 885 cm^{-1} (spirostane ring). (Found: C, 72·37; H, 9·32. Calc. for $C_{31}H_{48}O_6$: C, 72·06; H, 9·36%). NMR: see Table 1.

Partial acetylation of isoplexigenin A (IIg). To a soln of IIg (749 mg) in pyridine (18 ml) cold Ac_2O (9·2 ml) was added at 0° and the mixture left at this temp for 1½ hr. Work-up as usual gave a mixture (940 mg) consisting of starting material (IIg), diacetate (IIh), and the two possible monoacetates (IIi and IIj). It was separated by column chromatography.

3-Acetyl-isoplexigenin A (IIi), m.p. 212–214°, $[\alpha]_D$ −63° (*c*, 1·55); ν_{max} 3580 (OH), 1740, 1240 cm^{-1} (OAc). (Found: C, 73·26; H, 9·90. Calc. for $C_{29}H_{46}O_5$: C, 73·38; H, 9·77%).

23-Acetyl-isoplexigenin A (IIj), m.p. 195–197·5° (from EtOAc-light petroleum), $[\alpha]_D$ − 54° (*c*, 1·42); ν_{max} 3620 (OH), 1740, 1240 cm^{-1} (OAc). (Found: C, 73·16; H, 9·94. Calc. for $C_{29}H_{46}O_5$: C, 73·38; H, 9·77%).

23-*Keto-tigogenin acetate* (IIIb) *from* IIi. A soln of CrO_3 (70 mg) in glacial AcOH (11 ml) was added, at room temp and with stirring, to a soln of IIi (109 mg) in glacial AcOH (20 ml). Stirring was continued for ½ hr at room temp and the ketone recovered in the usual way, purifying it by dry column chromatography, m.p. 232–233·5°, $[\alpha]_D$ −58° (*c*, 1·48); ν_{max} 1740 (OAc, O=C_{23}), 1240 (OAc), 970, 920, 900, 860 cm^{-1} (spirostane ring). (Found: C, 73·67; H, 9·20. Calc. for $C_{29}H_{44}O_5$: C, 73·69; H, 9·38%).

Saponification of IIIb with 5% KOH in cold MeOH for 12 hr gave 23-keto-tigogenin (IIIa), m.p. 230–233°, $[\alpha]_D$ −44·4° (*c*, 0·81); ν_{max} 3620 (OH), 1735 cm^{-1} (O=C_{23}).

Our data of IIIa and IIIb coincide perfectly with those published by Callow *et al.*[19]

23-*Keto-tigogenone* (IIId) *from isoplexigenin A* (IIg). IIg (160 mg) was oxidized with an excess of CrO_3 in glacial AcOH as described above, yielding the diketone IIId, m.p. 248–250° (from EtOAc-light petroleum), $[\alpha]_D$ −31·5° (*c*, 1·90); ν_{max} 1740 (O=C_{23}), 1715 (O=C_3), 970, 940, 925, 910, 890, 870 cm^{-1} (spirostane ring). NMR: see Table 1.

Isoplexigenin B (IVa), m.p. 205–207° (from acetone-light petroleum), $[\alpha]_D$ −96° (*c*, 1·38); ν_{max} 3610, 3580 (OH), 3030, 2830, 840 (Δ^5), 990, 965, 945, 925, 910, 900 cm^{-1} (spirostane ring), mass spectrum: *m/e* (%) 430 (2), 345 (80), 327 (45), 271 (100), 253 (55). (Found: C, 75·31; H, 9·92; Mol wt 430. Calc. for $C_{27}H_{42}O_4$: C, 75·31; H, 9·83%; Mol wt 430).

Diacetate (IVb), m.p. 187–190°, $[\alpha]_D$ −97° (*c*, 1·65); ν_{max} 3030, 2830, 840 (Δ^5), 1740, 1240 (OAc), 980, 965, 950, 930, 920, 900, 885 cm^{-1} (spirostane ring). (Found: C, 72·58; H, 9·20. Calc. for $C_{31}H_{46}O_6$: C, 72·34; H, 9·01%). NMR: see Table 1.

Partial acetylation of isoplexigenin B (IVa). Cold Ac_2O (7·5 ml) was added to a soln of IVa (0·6 g) in pyridine (15 ml) at 0° and the mixture allowed to stand at this temp for 2 hr. The product was recovered as usual. Separation by chromatography on silica gel (0·2–0·5 mm) showed it to consist of starting material (IVa), diacetate (IVb), and the two possible monoacetates (IVc and IVd).

3-Acetyl-isoplexigenin B (IVc), m.p. 178–180° (from EtOAc-light petroleum), $[\alpha]_D$ −108° (*c*, 1·45); ν_{max} 3580 (OH), 3030, 2830, 840 (Δ^5), 1740, 1240 cm^{-1} (OAc). (Found: C, 73·75; H, 9·39. Calc. for $C_{29}H_{44}O_5$: C, 73·69; H, 9·38%).

23-Acetyl-isoplexigenin B (IVd), m.p. 207–209°, $[\alpha]_D$ −93° (*c*, 0·20); ν_{max} 3610 (OH), 3030, 2830, 840 (Δ^5), 1745, 1240 cm^{-1} (OAc). (Found: C, 73·68; H, 9·53. Calc. for $C_{29}H_{44}O_5$: C, 73·69; H, 9·38%).

23-*Keto-diosgenin acetate* (IVf) *from* IVc. The oxidation of IVc (120 mg) with CrO_3 (70 mg) in glacial AcOH was carried out as mentioned above for the preparation of IIIb, yielding IVf (114 mg), m.p. 184·5–186·5°, $[\alpha]_D$ −96° (*c*, 1·38); ν_{max} 3030, 2830, 840 (Δ^5), 1740 (OAc, O=C_{23}), 1240 (OAc), 960, 920, 900, 890 cm^{-1} (spirostane ring). (Found: C, 74·19; H, 9·14. Calc. for $C_{29}H_{42}O_5$: 74·01; H, 8·99%).

Diosgenin (IVe) *from* IVf. IVf (80 mg) was reduced by the method of Huang-Minlon, refluxing it for 2 hr with ethylene glycol (5 ml) and hydrazine hydrate (1 ml), the temp of the vapors being 130°. Then, NaOH pellets (0·15 g) were added and boiling continued for ¾ hr, after which the refrigerator was taken off and the temp risen to 180°. At this temp refluxing was continued for 2½ hr. The soln was then poured into water and extracted as usual. The product was percolated on a dry column, yielding IVe (35 mg), m.p. 201–202°, $[\alpha]_D$ −117° (*c*, 1·00), (Found: C, 77·94; H, 10·21. Calc. for $C_{27}H_{42}O_3$: C, 78·21; H, 10·21%), forms an acetate, m.p. 193–195°, $[\alpha]_D$ −111° (*c*, 0·91). Both compounds were identified by direct comparison with authentic samples of diosgenin and its acetate (mixed m.p.; TLC; IR spectra superimposable).

Isoplexigenin A (IIg) *from isoplexigenin B* (IVa). Isoplexigenin B diacetate IVb (175 mg) was dissolved in ether (200 ml) containing glacial AcOH (0·25 ml) and hydrogenated at 3 atm and room temp over PtO_2 for 14 hr. After filtering, the reduction product was percolated on a dry column and crystallized from EtOAc-light petroleum, m.p. 192–194°, $[\alpha]_D$ −59° (*c*, 1·36). (Found: C, 72·18; H, 9·39. Calc. for $C_{31}H_{48}O_6$: C, 72·06; H, 9·36%). Upon saponification it yielded IIg, m.p. 226–227° (from EtOAc-light petroleum),

$[\alpha]_D$ −60° (*c*, 0·90), which was identified by direct comparison with the previously obtained sample (TLC; IR spectra superimposable). (Found: C, 75·01; H, 10·03. Calc. for $C_{27}H_{44}O_4$: C, 74·96; H, 10·25%).

Product with R_f 0·09. TLC on silica gel with 20% $AgNO_3$ showed this product to be a mixture of two compounds. They were separated as acetates on a dry column with 20% $AgNO_3$, eluting with benzene, and identified as gitogenin and $\Delta^{25(27)}$-gitogenin.

Gitogenin (IId), m.p. 268–270°, $[\alpha]_D$ −71° (*c*, 0·29). (Found: C, 74·78; H, 10·27. Calc. for $C_{27}H_{44}O_4$: C, 74·96; H, 10·25%). Forms a diacetate, m.p. 240–240·5°, $[\alpha]_D$ −97° (*c*, 1·87). (Found: C, 72·17; H, 9·37. Calc. for $C_{31}H_{48}O_6$: C, 72·06; H, 9·36%). NMR: see Table 1. IR spectra superimposable with those of authentic samples.

$\Delta^{25(27)}$-Gitogenin, m.p. 252–254°, $[\alpha]_D$ −77° (*c*, 0·31), (Found: C, 75·38; H, 9·83. Calc. for $C_{27}H_{42}O_4$: C, 75·31; H, 9·83%), forms a diacetate, m.p. 218–220°, $[\alpha]_D$ −103° (*c*, 2·12); ν_{max} 3080, 880 (CH_2=), 1740, 1250, 1230 (OAc), 975, 950, 935, 925, 900 cm^{-1} (spirostane ring); $\nu_{max}^{CCl_4}$ 1660 cm^{-1} (CH_2=). (Found: C, 72·34: H, 9·09. Calc. for $C_{31}H_{46}O_6$: C, 72·34: H, 9·01%). NMR: see Table 1.

Product with R_f 0·05 consisted only of *isoplexigenin C* (IIa), m.p. 272·5–273·5° (from acetone), $[\alpha]_D$ −62° (*c*, 1·26); $\nu_{max}^{CHCl_3}$ 3610, 3580 (OH), 985, 970, 960, 945, 920, 910, 890, 870 cm^{-1} (spirostane ring); mass spectrum: *m/e* (%) 448 (2), 363 (40), 345 (5), 327 (7), 289 (100), 271 (10), 253 (8). (Found: C, 72·42; H, 9·80; Mol wt 448. Calc. for $C_{27}H_{44}O_3$: C, 72·28; H, 9·89%; Mol wt 448).

Triacetate (IIb), m.p. 210–213°, $[\alpha]_D$ −83° (*c*, 1·38); ν_{max} 1740, 1240 (OAc), 980, 960, 945, 930, 920, 905, 895 cm^{-1} (spirostane ring). (Found: C, 68·84; H, 8·85. Calc. for $C_{33}H_{50}O_8$: C, 68·96; H, 8·77%), NMR: see Table 1.

Attempted degradation of isoplexigenin C (IIa) *to a pregnane derivative.* A soln of IIa (0·5 g) and pyridine hydrochloride (0·5 g) in Ac_2O (5 ml) was refluxed for 3 hr, cooled to room temp, and diluted with AcOH (1 ml) and H_2O (2 ml). Then CrO_3 (300 mg), dissolved in AcOH (3 ml), was added under stirring at room temp, and stirring was continued for 3 hr, whereupon formaldehyde (1 ml) and NaOAc (0·5 g) were added. This mixture was heated on a steam bath for 1 hr, poured onto crushed ice and the product recovered as usual. It showed to be identical to isoplexigenin C triacetate (IIb) (physical constants, TLC; IR spectra superimposable).

Gitogenin (IId), undergoing the same reaction, yielded compound V, m.p. 184–186° (from EtOAc-light petroleum); ν_{max} 3060 ($\Delta^{16(17)}$), 1740, 1250, 1230 (OAc), 1670 cm^{-1} (α,β-unsaturated ketone); λ_{max}^{EtOH} 243 nm (ε 6157).

2,3-*Diacetyl-isoplexigenin C* (IIc). To a soln of IIa (5 g) in pyridine (125 ml) cold Ac_2O (62 ml) was added at 0° and the soln allowed to stand at this temp for 20 min. The mixture of partial acetates (5·85 g) was recovered in the usual way and chromatographed on silica gel 0·2–0·5 mm (375 g), obtaining IIc, m.p. 245–247°, $[\alpha]_D$ −89° (*c*, 1·82); ν_{max} 3580 (OH), 1745, 1245, 1230 cm^{-1} (OAc). (Found: C, 69·88; H, 9·34. Calc. for $C_{31}H_{48}O_7$: C, 69·89; H, 9·08%), NMR: see Table 1.

2,3-*Diacetyl-23-keto-gitogenin* (IIIc). IIc (264 mg) in glacial AcOH (50 ml) was oxidized with CrO_3 (170 mg) in the same manner as for the preparation of IIIb, obtaining IIIc, m.p. 271·5–272°, $[\alpha]_D$ −73° (*c*, 1·26); ν_{max} 1740 (OAc, O=C_{23}), 1250, 1235 (OAc), 985, 965, 945, 925, 905, 895 cm^{-1} (spirostane ring), NMR: see Table 1.

Gitogenin (IId) *from* IIIc. IIIc (110 mg) was reduced by the method of Huang-Minlon as described for IVe from IVf. The recovered product was percolated on a dry column, yielding IId (50 mg), m.p. 260–262°, $[\alpha]_D$ −63° (*c*, 1·57), (Found: C, 74·55; H, 10·32. Calc. for $C_{27}H_{44}O_4$: C, 74·96; H, 10·25%), forms a diacetate, m.p. 238–240°, $[\alpha]_D$ −97° (*c*, 1·17). (Found: C, 72·17; H, 9·60. Calc. for $C_{31}H_{48}O_6$: C, 72·06; H, 9·36%). The compounds were compared with authentic samples of gitogenin and its diacetate and shown to be identical in all respects.

23-*Acetyl-isoplexigenin C* (IIe). Isoplexigenin C triacetate IIb (4·5 g) was dissolved in MeOH (40 ml) satd with Na_2CO_3 and left at 0° for 7½ hr, determining the end of the reaction by TLC. The product, which contained a small quantity of IIa, was chromatographed on silica gel (0·2–0·5 mm), $CHCl_3$ – 1·5% MeOH eluted IIe, m.p. 217–218° (from acetone), $[\alpha]_D$ −54° (*c*, 0·72); $\nu_{max}^{CHCl_3}$ 3610 (sh), 3590 (OH), 1740, 1250 cm^{-1} (OAc).

2α,3β-*Dimesyl-23-acetyl-isoplexigenin C* (IIf). To a soln of IIe (1·66 g) in dry pyridine (16 ml) mesyl chloride (1·7 ml) was added at 0° and the mixture left at this temp for 16 hr, after which it was poured onto crushed ice and the product recovered as usual. IIf crystallized from acetone-light petroleum, m.p. 230–232°, $[\alpha]_D$ −60° (*c*, 1·86); ν_{max} 1750, 1240 cm^{-1} (OAc). (Found: C, 57·37; H, 7·87; S, 9·99. Calc. for $C_{31}H_{50}O_{10}S_2$: C, 57·28; H, 7·70; S, 9·85%).

23-*Acetyl-spirost-2-ene* (VI). IIf (1·3 g) and NaI (6 g) were dissolved in acetone (55 ml) and heated to

105–110° for 24 hr in a pressure reaction apparatus. The reaction product was poured into water and extracted with $CHCl_3$. After washing the extract with $Na_2S_2O_3$ aq, the product was recovered in the usual way and purified by column chromatography, obtaining VI, m.p. 176–178°, $[\alpha]_D$ −25·8° (*c*, 1·31); ν_{max} 3060, 3020 (Δ^2), 1740, 1240 cm^{-1} (OAc). (Found: C, 76·28; H, 9·83. Calc. for $C_{29}H_{46}O_4$: C, 76·21; H, 9·71%), NMR: see Table 1.

2α,3α-Epoxy-23-acetyl-spirostane (VII). To a soln of VI (0·6 g) in $CHCl_3$ (20 ml) perbenzoic acid (0·425 g) in $CHCl_3$ (25 ml) was added and the mixture kept at room temp for 15½ hr. Then it was extracted with $CHCl_3$, successively washed with solns of KI, $Na_2S_2O_3$, and $NaHCO_3$, and the product recovered as usual, m.p. 196–198°, $[\alpha]_D$ −46·2° (*c*, 1·32); ν_{max} 1740, 1240 cm^{-1} (OAc). (Found: C, 73·45; H, 9·53. Calc. for $C_{29}H_{46}O_5$: C, 73·69; H, 9·38%).

3-Epi-isoplexigenin A (VIII). A soln of VII (0·5 g) in dry ether (17 ml) was added to a suspension of LAH (0·25 g) in the same solvent (13 ml). After refluxing for 2 hr, H_2O and dil H_2SO_4 were added, the filtered soln extracted with ether and the product recovered as usual. VIII crystallized from acetone-light petroleum, m.p. 221–222°, $[\alpha]_D$ −64° (*c*, 0·90); ν_{max} 3620, 3580 cm^{-1} (OH).

Acetylation of VIII yielded a diacetate, m.p. 170–172°, $[\alpha]_D$ −48° (*c*, 1·10); ν_{max} 1740, 1240 cm^{-1} (OAc). (Found: C, 71·86; H, 9·39. Calc. for $C_{31}H_{48}O_6$: C, 72·06; H, 9·36%).

23-Hydroxy-tigogenone (IX). VIII (300 mg) was dissolved in acetone (45 ml) containing H_2O (2 ml), NBS (300 mg) and AcOH (0·3 ml) and the reaction mixture left at room temp for 40 min. It was then poured into water, extracted with ether and washed with $NaHCO_3$ soln and dil HCl. IX (273 mg) crystallized from acetone-light petroleum, m.p. 210–212°, $[\alpha]_D$ −44° (*c*, 1·25); ν_{max} 3580 (OH), 1715 cm^{-1} (O=C_3). (Found: C, 75·05; H, 9·96. Calc. for $C_{27}H_{42}O_4$: C, 75·31; H, 9·83%), NMR: see Table 1.

Isoplexigenin A (IIg) *from isoplexigenin C* (IIa). A soln of IX (156 mg) and $NaBH_4$ (30 mg) in EtOH (15 ml) was kept at room temp for 2 hr. The excess of reducing agent was removed by adding AcOH, and the product recovered in the usual manner. IIg crystallized from EtOAc-light petroleum, m.p. 225–226°, $[\alpha]_D$ −59° (*c*, 1·07), (Found: C, 74·64; H, 10·34. Calc. for $C_{27}H_{44}O_4$: C, 74·96; H, 10·25%), forms a diacetate (IIh), m.p. 194–195° (from EtOAc-pet ether), $[\alpha]_D$ −57° (*c*, 1·40). (Found: C, 72·17; H, 9·46. Calc. for $C_{31}H_{48}O_6$: C, 72·06; H, 9·36%). Both compounds were identified by comparison with the previously obtained samples of isoplexigenin A and its diacetate (mixed m.p., TLC; IR spectra superimposable).

Product with R_f 0·03. TLC in the system $CHCl_3$-15% acetone showed this product to be a complex mixture of substances with very similar R_f values. Upon acetylation, two spots were observed (benzene-10% EtOAc), one consisting of IIm and the other, more polar one, of three compounds which are being investigated. Chromatography on a dry column, eluting with benzene-5% EtOAc, yielded pure isoplexigenin D triacetate (IIm).

Isoplexigenin D (IIk), m.p. 280–281°, $[\alpha]_D$ −74° (*c*, 0·12); $\nu_{max}^{CHCl_3}$ 3620, 3580 (OH), 990, 970, 960, 925, 910, 895, 870 cm^{-1} (spirostane ring). (Found: C, 72·04; H, 10·12. Calc. for $C_{27}H_{44}O_5$: C, 72·28; H, 9·89%).

Triacetate (IIm), m.p. 198–200°, $[\alpha]_D$ −91° (*c*, 0·29); ν_{max} 1740, 1240 (OAc), 980, 960, 940, 925, 905, 885, 865 cm^{-1} (spirostane ring). (Found: C, 68·82; H, 8·75. Calc. for $C_{33}H_{50}O_8$: C, 68·96; H, 8·77%), NMR: see Table 1.

Isoplexigenin D (IIk) *from isoplexigenin C* (IIa). 2,3-Diacetyl-23-keto-gitogenin IIIc (144 mg), prepared earlier from IIc, in glacial AcOH (20 ml) was hydrogenated over PtO_2 (50 mg) at room temp under atm press, obtaining mainly III (with axial OH). Dry column chromatography of the product afforded pure III (20 mg), m.p. 229–231°, $[\alpha]_D$ −97° (*c*, 0·122); ν_{max} 3580 (OH), 1740, 1250, 1230 cm^{-1} (OAc). (Found: C, 69·72; H, 9·14. Calc. for $C_{31}H_{48}O_7$: C, 69·86; H, 9·08%), NMR: see Table 1.

Total acetylation of III yielded the triacetate IIm, m.p. 197–199°, $[\alpha]_D$ −88° (*c*, 0·094). (Found: C, 69·02; H, 8·88. Calc. for $C_{33}H_{50}O_8$: C, 68·96; H, 8·77%). Saponification of IIm gave IIk, m.p. 280–281°, $[\alpha]_D$ −66° (*c*, 0·082). Both compounds were compared with previously obtained samples of isoplexigenin D and its triacetate and shown to be identical in all respects.

Reduction of 2,3-diacetyl-23-keto-gitogenin (IIIc) *with* Na *in* n-BuOH. To a soln of IIIc (10 mg) in n-BuOH (5 ml) Na was added until the reaction was complete (TLC). The soln was then washed 5 times with water and concentrated *in vacuo*. The reaction product consisted of IIa and IIk, the former in greater proportion. They were separated and identified as acetates (TLC, IR).

Method of Horeau. A soln of IIc (55·1 mg) and racemic α-phenylbutyric anhydride (135·3 mg) in pyridine (3 ml) was left at room temp for 16 hr. The excess anhydride was destroyed by adding H_2O (1 ml) and again leaving the soln for 6 hr. It was then extracted with ether and the extract successively washed with H_2O, sat $NaHCO_3$ aq, and H_2O. The combined aqueous extracts were washed with $CHCl_3$, acidified with dil HCl, and extracted with $CHCl_3$. After drying over Na_2SO_4 anhyd, the solvent was evaporated *in vacuo*,

affording α-phenylbutyric acid (87·9 mg) with $[\alpha]_D$ −0·96° (benzene; *c*, 1·758). For a 100% optical yield the acid obtained ought to have $[\alpha]_D$ −13·2°. Hence, the optical yield in our case is 7·2%.

The neutral fraction extracted with ether was successively washed with H_2O, dil HCl, and H_2O. The residue left after distilling the solvent did not contain any starting material (TLC), which signifies that the esterification was complete.

Acknowledgement—The authors are indebted to Dr. Callow for the gift of a sample of 23-hydroxy-tigogenin, and to Dr. Rui Viera, Madeira. They also thank Dr. J. L. Bretón for running the NMR spectra, and Dr. Calderón of the Centro Nacional de Química Orgánica, Madrid for carrying out the elementary analyses.

This study was performed with the financial supports "Manuel Aguilar" of Aguilar S.A. de Ediciones (A.G.G.), and "Fomento a la Investigación en la Universidad" of the Ministry of Education and Science (R.F.).

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