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STEROLS FROM FOUR SPECIES OF LILIACEAE

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Key Word Index—*Tristagma uniflorum*; *Nothoscordum inodorum*; *Nothoscordum gramineum* var. *Philippianum*; *Nothoscordum montevidense*; Liliaceae; sterols.

Abstract—Fractionation of the sterol mixture from *Tristagma uniflorum*, *Nothoscordum gramineum* var. *philippianum*, *Nothoscordum inodorum*, and *Nothoscordum montevidense* were achieved by means of preparative TLC. Analysis of the fractions by GC and GC-MS allowed the identification of Δ^0, Δ^5, and Δ^7 sterols. The unusually high proportion of cholestan-3β-ol seems to be biogenetically related to the C_{27} steroidal sapogenins contained in those plants.

INTRODUCTION

Liliaceae is one of the Angiospermae families for which approximately 220 genera has been recognized. Cabrera [1] has indicated that the difference among the genera *Nothoscordum*, *Allium*, and *Tristagma* has not been established beyond any doubt.

We have previously reported the steroidal sapogenins present in *Tristagma uniflorum*, *Nothoscordum inodorum*, *Nothoscordum montevidense* and *Nothoscordum gramineum* var. *philippianum* [2–4]. As it has been postulated that the presence of steroidal sapogenins is related to the presence of sterols from which they are biogenetically derived, we have studied the sterol composition of four species of the genera *Tristagma* and *Nothoscordum*. The purpose of this study was to compare the relationship between sterols and steroidal sapogenins as well as to provide additional data for a better classification of the conflicting genera.

RESULTS AND DISCUSSION

The unsaponifiable fraction from a toluene extract of each species was separated by preparative TLC. The sterol fraction was isolated and analysed by GC and GC-MS. The results are presented in Table 1, and a comparison of the sterols from each genus is shown in Table 2.

Although the number of analysed species of each genus is limited, the results show some differences among them. It was found that 24-ethylcholest-5-en-3β-ol (sitosterol) is the main sterol in all the species studied. 24-Methylcholest-5-en-3β-ol (campesterol) was also found, though in much lower proportion. An important sterol in *Nothoscordum* was 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol) but it was presented in low amount in *Tristagma* and only as traces in *Allium* [5]. Other marked differences were the identification of 24-ethylcholesta-7,22-dien-3β-ol (α-spinasterol) only in *Nothoscordum* and 24-ethylcholesta-5,28-dien-3β-ol (isofucosterol) in *Allium* [5]; those sterols could not be detected in *Tristagma*. Another chracteristic of the sterols from *Nothoscordum* was that cholestan-3β-ol appeared as an important component while cholesterol was just a minor one. On the other hand, cholesterol was found in *Allium* in significant amounts (up to 32% in *A. fisulosum*). Itoh *et al.* [5] have indicated that the unusually large proportion of cholesterol in those species of Liliaceae must be as consequence that cholesterol is the main biogenetic precursor of the C_{27} steroidal sapogenins and alkaloids [6, 7]. However, this does not apply to *Nothoscordum* genus which contains steroidal sapogenins [2–4] but a high proportion of cholestanol and only traces of cholesterol. This difference could be explained considering the structures of the steroidal sapogenins found in *T. uniflorum* and in the *Nothoscordum* species which belong to the group of saturated C_{27} steroidal sapogenins. For their formation it can be postulated that the Δ^5 sterols should be reduced to 5α-stanols before being converted into sapogenins. On the other hand, diosgenin was frequently isolated from *Allium* species as a predominant steroidal sapogenin [8]; its Δ^5 spirostenol structure should be derived from cholesterol which, in fact, is present in those plants. From *Nothoscordum* only 5α-spirostanol sapogenins were identified [2–4] and accordingly cholestanol should be their biosynthetic precursor.

EXPERIMENTAL

Plant material. Nothoscordum inodorum and *Tristagma uniflorum* were collected near Bahía Blanca and *N. gramineum* var. *philippianum* near Carmen de Patagones (Argentina). A voucher specimen of each plant has been deposited at the Herbarium of the Department of Agricultural Sciences of Universidad Nacional del Sur under nos BB 3788, BB 1637, and BB 3786 respectively. *N. montevidense* was collected near La Plata (Argentina) and a voucher specimen was deposited at the Herbarium Universidad Nacional de La Plata under no. LP 5373.

Isolation and identification of sterols. Dried and powdered bulbs of each species (usually *ca* 1 kg) were extracted with toluene (3 × 800 ml) in a Soxhlet apparatus. Each extract was evapd to dryness and the residue was refluxed with 10% KOH in MeOH. After addition of H_2O and extraction with Et_2O, the extract was taken to dryness and the sterol fraction was separated by prep.

Table 1. Sterols from *Nothoscordum inodorum* (N. i.), *Nothoscordum gramineum* (N. g.), *Nothoscordum montevidense* (N. m.), and *Tristagma uniflorum* (T. u.)

Sterol	RR_t*	MS (main fragments)	Composition (%) N. i.	N. g.	N. m.	T. u.
5α-Cholestan-3β-ol	1.00	388 ([M]$^+$), 373, 370, 355, 275 262, 257, 233, 230, 215	8	15	11	4
Cholest-5-en-3β-ol	1.00	386 ([M]$^+$), 371, 368, 353, 275, 273, 255, 231, 229, 213	2	4	1	1
5α-Cholest-7-en-3β-ol	1.03	386 ([M]$^+$), 371, 368, 353, 273, 255, 246, 231, 229, 213	4	3	—	1
24ξ-Methylcholest-5-en-3β-ol	1.08	400 (M]$^+$), 385, 382, 367, 315, 289, 273, 255, 231, 229, 213	3	1	8	4
24ξ-Ethylcholesta-5,22-dien-3β-ol	1.12	412 ([M]$^+$), 397, 394, 379, 369, 351, 299, 273, 255, 253, 231	16	15	24	5
24ξ-Ethylcholest-5-en-3β-ol	1.16	414 ([M]$^+$), 399, 396, 329, 303, 275, 273, 255, 231, 229, 213	60	62	54	85
24ξ-Ethyl-5α-cholesta-7,22-dien-3β-ol	1.18	412 ([M]$^+$), 397, 394, 369, 314, 300, 273, 271, 255, 253, 231	7	—	3	—

*Relative to 5α-cholestan-3β-ol.

Table 2. Sterols in genera *Nothoscordum*, *Tristagma* and *Allium*

Sterol	*Nothoscordum*	*Tristagma*	*Allium* [5]
5α-Cholestan-3β-ol	+	+	−
Cholest-5-en-3β-ol	trace	trace	+
5α-Cholest-7-en-3β-ol	+	+	+
24-Methylcholest-5-en-3β-ol	+	+	+
24-Ethylcholest-5-en-3β-ol	++	++	++
24-Ethylcholesta-5,22-dien-3β-ol	+	+	trace
24-Ethyl-5α-cholesta-7,22-dien-3β-ol	+	—	—
24-Ethylcholesta-5,28-dien-3β-ol	—	—	+

Trace: less than 1%; +: up to 50%; ++: more than 50%.

TLC (silica gel $HF_{366+254}$, $CHCl_3$–Me_2CO, 95:5). GC was performed with a Hewlett-Packard model(5840A gas chromatograph equipped with a FID detector and a fused silica capillary column (12 m × 0.2 mm) coated with methyl–silicone fluid (Hewlett-Packard). The carrier gas was He and analyses were performed between 200 and 280° at a rate of 8°/min. Relative retention times of the sterols to 5α-cholestan-3β-ol are indicated in Table 1. GC-MS analyses were conducted with a Hewlett-Packard model 5970B GC-MS. Chromatographic conditions were as previously indicated.

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