STEROIDS FROM EUPHORBIA AND OTHER LATEX-BEARING PLANTS

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Abstract—Steroids in latices isolated from 15 *Euphorbia* species and four other latex-bearing plants were analysed (in terms of their chemotaxonomy) using computerized GC-MS. Steroid patterns divided these plants into six groupings.

INTRODUCTION

Recently the identification of sterols from plant latices has been given much attention, because they can be used as a tool in the taxonomic grouping of the species concerned. Both isolation and chemical identification of the individual sterols are rather tedious and time-consuming procedures using conventional methods. However, the task can be more conveniently done with the aid of GC-MS. We have analysed a number of *Euphorbia* and some other species for sterols by this method, some of

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which have not previously been reported in the literature $\begin{bmatrix} 1-7 \end{bmatrix}$ and report the results herein.

RESULTS AND DISCUSSION

The GC-MS method has its limitations in that only known compounds can be identified easily. Fortunately, most of the major sterols from the Euphorbiae have already been described [1]. However, as is apparent from Table 1, several sterols were found in the nineteen species examined which, as yet, we have been unable to identify. In spite of this, most of the species (13/19) are easily placed in the six taxonomic groups described by Ponsinnet and Ourisson [1], and only four of the others cannot be assigned to any of these groups (Table 1).

	Relative amount of sterols [‡]												
Species	а	b	с	d	e1	e2	ſ	g	h	i	j1	j2	Group
1 Euphorbia aphylla		0.37		0.10	0.82	~ <u></u>	1.0	0.46		- <u></u>			$\begin{array}{c} C_1 \\ B \\ C_2 \\ C_1 \end{array}$
2 Euphorbia arbuscula	1.0	0.19											Bʻ
3 Euphorbia balsamifera							1.0				0.80		С,
4 Euphorbia characias				0.13	1.0		0.68	0.27					C,
5 Euphorbia coerulescens	1.0	0.10	0.20										A
6 Euphorbia cylindrifolia	1.0	0.25				0.50							?
7 Euphorbia globosa								0.30			1.0	1.0	?
8 Euphorbia ingens	1.0	0.30											В
9 Euphorbia lathyris	0.12			0.80	0.50		0.30	1.0					$\begin{array}{c} C_1 \\ C_2 \\ C_4 \\ (C_1) \end{array}$
10 Euphorbia marlotthii							0.41			0.32		1.0	C_2
11 Euphorbia misera	0.69	0.32		0,66			1.0						C_
12 Euphorbia obtusifolia	0.05				1.0	1.0	0.80	0.50					(Ĉ,)
13 Euphorbia stenoclada	1.0	0.16							0.29	0.17			(B)
14 Euphorbia tirucalli	1.0	1.0											B
15 Euphorbia trigona	1.0						0.61						?
16 Elaeophorbia drupifera	1.0	0.16	0.20										Α
17 Achras sapote									1.0*	0.20*			E
18 Asclepias sp.									1.0*	0.20*			Е
19 Synadenium grantii	0.25	0.20	0.10		0.40	+							?

Table 1. Steroids from plant latex

* Found as acetates.

⁺ The major sterol was found to be a lanosterol isomer different from el and e2.

[‡] The steroids were identified as described in the experimental section, and the amount estimated from the area of the GLC peaks. Key to the steroids: a: euphol, b: tirucallol, c: euphorbol, d: lanosterol, e: lanosterol isomers, f: cycloartenol, g: 24-methylenecycloartanol, h: α -amyrin, i: β -amyrin, j: pentacyclic steroids. The taxonomic groups are defined as follows (see also [1]). A: euphol, tirucallol and euphorbol; B: euphol and tirucallol only; C_1 : cycloartenol, 24-methylenecycloartenol and lanosterol; C_2 : cycloartenol + pentacyclic steroids; C_4 : cycloartenol + euphol and tirucallol; E: α - and β -amyrin without any tetracyclic steroils.

Table 2.	Identification	of steroids	from latex*

Compound	Relative retention time	Characteristic <i>m/e</i> (relative intensity)			
Euphol Tirucallol	0.85 0.94	468(30), 453(80), 408(10), 393(65), 301(10), 69(100), 43(70)			
Euphorbol	1.04	482(30), 467(100), 422(10), 383(15), 323(20), 301(25), 69(60), 43(30)			
Lanosterol Lanosterol isomer 1 (e1) Lanosterol isomer 2 (e2)	$\left. \begin{array}{c} 1.00 \\ 1.05 \\ 0.93 \end{array} \right\}$	468(10), 453(40), 408(5), 393(45), 301(8), 69(100), 43(80)			
Cycloartenol	1.18	468(5), 453(5), 408(25), 393(25), 365(20), 339(20), 286(15), 69(100), 43(90)			
24-Methylenecycloartenol	1.29	482(4), 467(3), 422(20), 407(20), 379(0)1, 353(6), 300(7), 69(100), 43(90)			
α-Amyrin	1.19	468(15), 453(6), 408(10), 393(6), 218(100), 203(35), 189(50)			
β-Amyrin	1.07	468(4), 453(3), 408(4), 398(3), 218(100), 203(70), 189(40)			
Taraxerol	1.02	468(2), 208(19), 393(20), 269(40), 418(20), 204(100), 189(45), 69(90)			
Lupeol	1.22	468(10), 408(35), 393(10), 365(20), 297(10), 229(10), 218(35), 203(30), 189(55), 43(100)			
Multiflorenol	1.32	468(7), 453(7), 408(20), 393(15), 365(10), 339(4), 241(20), 229(25), 218(15), 69(20), 43(100)			

*Relative retention times and characteristic mass fragments (first peak is M⁺) of steroid acetates.

Although the species 17 and 18 are not Euphorbiae, their analyses agree with group 'E' (Table 1). It is noteworthy that five of the sterols—lanosterol, euphol. tirucallol and the lanosterol isomers e_1 and e_2 —have identical mass spectra, and these can therefore only be distinguished by their GLC retention-times. The MS of the steroids j_1 and j_2 show that they are pentacyclic, but by comparing with standards, we can conclude that they are not taraxerol, multiflorenol, or lupeol. Cycloartanol and 24-methylenelophenol are not shown in the table. but were found in *E. balsamifera*.

This work has just begun, but the GC-MS method seems to be a convenient way to expand our knowledge of the chemotaxonomy of Euphorbiae and other latexbearing plants. A report on the mass spectral fragmentation mechanism of Euphorbiaceae sterols will be presented elsewhere.

EXPERIMENTAL

Plant sources. Euphorbia coerulescens and E. tirucalli were obtained from the Botanic garden, Univ. of Calif. at Los Angeles; E. lathyris, E. trigona and Achras sapote were obtained from the Botanic garden, Univ. of Calif. at Berkeley; E. balsamifera and E. ingens were obtained from T. J. Bahlman. P.O. Box 82, Frankfort 9830, South Africa; the un-named Asclepias sp. was collected near the Tri-City Airport. Michigan, 1977; the remaining 11 species listed in Table 1 were obtained from the Botanic garden, Univ. of Calif. of Davis.

The plant latices were obtained by tapping specimens from the collection at the Department of Botany, University of California, Davis. The sterols were extracted from the latex with hot Me₂CO and acetylated in Ac₂O/Py (4:1). The acetylated sterols were subjected to analysis by GLC (3% OV-17 at 280° isothermally) and computerized GC–MS as described elsewhere [6] using a stainless steel column packed with 3% OV-17 coated on Gas Chrom Q. The sterols were identified by coinjection or comparison of R_i s and MS with those of standard compounds (Table 2). Wherever ambiguity occurred, coinjections were performed.

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REFERENCES

- 1. Ponsinnet, G. and Ourisson, G. (1968) Adansonia Ser. 2, 8, 227.
- Atallah, A. M. and Nicholas, H. J. (1972) Phytochemistry 11, 1860.
- 3. Starrett, A. N. (1973) Phytochemistry 12, 23.
- Misra, D. R., Naskar, D. B., Ray, T. K. and Khastgir, H. N. (1973) *Phytochemistry* 12, 1819.
- Gonzalez, A. G., Fraga, B. M., Gonzalez, P. and Ravelo, A. G. (1976) *Phytochemistry* 15, 427.
- Nishimura, H., Philp, R. P. and Calvin, M. (1977) Phytochemistry 16, 1048.
- Nielsen, P. E., Nishimura, H., Otvos, J. W. and Calvin, M. (1977) Science 198, 942.